COMPARATIVE PHYTOCHEMICAL ANALYSIS OF SOME CLIMBERS IN AND AROUND MEJ RIVER

मेज नदी क्षेत्र के निकट कुछ आरोही पादपों का तुलनात्मक पादपरसायन विश्लेषण

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To the
University of Kota

By

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2019

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ABSTRACT

The Mej river which is an important tributary of the Chambal, has it catchment area spread over four districts of Rajasthan. Bundi district situated in the southern part of the state accommodates most of the catchment area of the Mej river. The area in and around the river is known for floristic diversity. In a preliminary study 27 climber species belonging to various plant families were recorded.

Present study was undertaken for comparative qualitative and quantitative phytochemical analysis of four climber species namely, *Cayratia trifolia, Coccinia grandis, Cocculus hirsutus* and *Pergularia daemia*. Leaf, stem and fruit extracts of each plant species were prepared by Soxhlet apparatus using alcohol as solvent. Qualitative phytochemical analysis revealed presence of four primary metabolites (carbohydrates, reducing sugar, proteins and fats) and six secondary metabolites (alkaloids, phenols, flavonoids, glycosides, saponins and terpenoids) by different tests. Quantitative analysis of the extracts showed presence of carbohydrate (primary metabolites) in different quantities. Similarly, estimation of total phenol content also revealed variation in different plant extracts.

CANDIDATE'S DECLARATION

I, hereby, certify that the work, which is being presented in the thesis, entitled **''Comparative phytochemical analysis of some climbers in and around Mej river**'' in partial fulfilment of the requirement for the award of the Degree of Doctor of Philosophy, carried out under the supervision of Dr O. P. Sharma, Associate Professor, Department of Botany, Govt. College Bundi and submitted to the University of Kota, Kota represents my ideas in my own words and where others ideas or words have been included I have adequately cited and referenced the original sources. The work presented in this thesis has not been submitted elsewhere for the award of any other degree or diploma from any Institutions.

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ABBREVIATIONS

Fig.	:	Figure
g	:	Microgram
gm.	:	Gram
mg	:	Milligram
g	:	Gram
SD	:	Standard deviation
CT	:	*Cayratia trifolia*
CG	:	*Coccinia grandis*
CH	:	*Cocculus hirsutus*
PD	:	*Pergularia daemia*
S E	:	Standard Error
S (in plates)	:	Sample
T (in plates)	:	Test
GE	:	Glucose equivalent
CE	:	Catechol equivalent

CHAPTER 1

INTRODUCTION

Plants are the invaluable gift by nature, as the green cover on the planet earth cannot be imagined without plant kingdom. All organisms found in nature make biosphere and excellent ecological relations of plants and animal in the biosphere is foundation of biodiversity in various ecosystems. In fact, different plant species form a unique group of living things which is capable of fulfilling the basic and other needs of animals and human beings. They are capable of trapping light of the Sun, through photosynthesis. This energy is stored in the form of carbohydrates, hence, plants are known as primary producer in food chains, serving the energy needs of different consumers. Apart from this, plant products are important in many ways such as paper, rubber, liquor, dyes, coal, food, wax, green fodder, pulses, vegetables, beverages, spices and fruits, etc. Therefore, it can be said that the plant products are foundation of human life and welfare.

Phytochemistry encompasses the study of phytochemicals, particularly the secondary metabolites produced and stored in plants. It takes into account the structural composition biosynthetic pathways and functions in the living organisms including plants. Phytochemistry is important part of a number of disciplines such as systematic, botany, nutrition, biochemistry, medicine, pharmacy, biotechnology, environmental sciences and remediation, microbiology, etc. It can be said to fall into a subfield of botany or chemistry. This branch of science is very important for the determination of the active ingredients of medicinal plants, their quantification and analysis of the beneficial and harmful effects on human health. It also deals with methods of obtaining these active ingredients, their classification according to the functional organic chemical groups to which they belong, and the analytical methods to verify quality. It is chemistry or chemical analysis of primary and secondary metabolites of plants.

Plants produce many chemical compounds which play essential roles to survival and existence of their life in unfavourable environmental conditions.

Phytochemicals (from the greek word phyto meaning plant) are biologically active, naturally occurring chemical compounds found in plants which provide health benefits for human beings further than those attributed to macronutrients and micronutrients (Hasler *et al.*1999). They protect plant from disease and damage. They made the plant coloured, flavoured and aromatic. (Gibson *et al*. 1998; Mathai, 2000)

Metabolic substances are important part of plant life, without which biological processes cannot be imagined in plants. In plant cells, biochemical processes occur in the coordinated and balanced form and the products of these pathways or bio-molecules are called metabolites. The metabolites can be mainly divided into two groups i.e., primary metabolites and secondary metabolites.

The primary metabolites are essential for the survivals of the plants and are produced in cells in sufficient quantity. Sugars, proteins, amino acids, fatty acids, fats, pyridine and purines are examples of primary metabolites. Primary metabolites obtained from plants are also used as industrial raw materials.

Secondary metabolites are non-essential for basic life processes of plants. The secondary metabolic pathways result in production of secondary metabolic products, such as alkaloids, phenols, glycosides, terpenes, gums, antibiotics, etc. They work in plants for safety, energy, antibiotic and many functions which are yet to be ascertained. They do not make special contributions to the life processes of plants but make any plant species special. These product derived from different parts of plants are very useful in therapeutic uses. Plants, the most wonderful gift from nature have been used as drugs. Various types of drugs are obtained from them. These types of plants are known as medicinal plants (Yadav *et al.* 2010).

Among primary metabolites carbohydrates constitute major group of macromolecules in plants. It is an important group of organic compounds which is found stored mainly in vegetative tissues. It is one of the three main ingredients of food and is important in organic and industrial perspective. Under this group there is useful substance in the form of monosaccharides, disaccharides, oligosaccharides and polysaccharides. These are produced during photosynthesis

in plants. The most important function of these substances is to give energy by undergoing oxidation in the respiratory processes.

Carbohydrates are a group of organic compounds consisting of carbon, hydrogen, and oxygen usually in the ratio of 1:2:1 and include such well known compounds as sugar, starch, cellulose, etc. (Ernst *et al.* 2000). These biomolecules are represented by the common formula $C_2 (H_2O)_n$ and are technically hydrates of carbon.

The term "total available carbohydrate" may be defined as all those carbohydrates which can be used in the plant body as a source of energy or as building material, either directly or indirectly, after having been broken down by enzymes. In most ordinary higher green plants the bulk of available carbohydrate is composed of sugars, fructosans, dextrin and starch, whereas hemicelluloses and true cellulose act merely as structural materials and as cannot further be utilized in the same way as the former (Weinmann,1947). In present work, monosaccharides are studied for qualitative and quantitative analysis.

Lipids are also prominent primary metabolites found in plants and animals. In each living cell, these substances are abundant in the form of oils and fats. The term was first coined by German biochemist Bloor in 1946. According to him lipids are naturally occurring compounds, which are not water-soluble, but are soluble in one or more organic solvents like benzene, chloroform, ether, acetone, etc., called fat solvents. Hydrolysis of lipids yields fatty acids. Lipids are found in every plant species, but their content and composition differ widely, depending on the type and part of the plant. The main lipids include triacylglycerols, diacylglycerols, monoacylglycerols, phospholipids, glycolipids, sterols, free fatty acids, vitamins soluble in fat and waxes. Lipids, such as oil in oilseeds contain the highest amount of triacylglycerols (95-98%), in the form of fatty acid esters with glycerol (Kamal-Eldin, A. 2005). The concentration and quality of plant lipids have an impact particularly on their application (edible oils, bio-fuels and industrial oils) and processing conditions (Sacchetti *et al*. 2005).

Being insoluble in aqueous solution, the lipids are found stored in both plants and animals. In plants, lipids are stored mainly in seeds and some fruits, while in animals these are stored in adipose cells. At the time of germination of seeds, the accumulated fat is converted into re-soluble monosaccharide by enzymes. Many oils such as castor, mustard, olive, coconut, etc. are useful in the form of medicines. Edible oils and fats are also used in cooking.

Protein has the most important role among various types of components present in living cells of plants and animals. Chemically, proteins are complex nitrogenous organic substances that have higher molecular weight. All of the activities of the plants occur in the presence of specific protein enzymes. About 20 types of amino acids are obtained from hydrolysis of different types of proteins. Proteins are complex nitrogenous macromolecules present in protoplasm of all unicellular and multicellular organisms. The term protein was used by Mulder in 1939 at the suggestion of Berzelius (derived from Greek word proteios). Different types of amino acids form protein biopolymers on binding by peptide bond. From structure and chemical point of view, the proteins are divided into three groups namely, simple, compound and derived proteins. Due to the presence of independent amino and carbonyl groups they react with both acids and alkalis hence their nature is amphoteric.

Secondary metabolic products, such as alkaloid, phenol, glycoside, terpenes, gums, antibiotics, etc. are produced as a result of the secondary metabolic pathways. They work in plants only for safety, accumulation of food, energy, and resistance against various pathogens. Some of the products derived from the plants are very useful economically.

Alkaloids are heterocyclic nitrogen compounds which form large group of secondary plant products. The name “Alkaloids” (German: Alkaloide) was introduced by German chemist Carl Friedrich Wilhelm Meibner in 1819. Ledenburg defined Alkaloids as “Naturally occurring basic, complex, nitrogenous organic, heterocyclic ring compound of plant origin with physiological and pharmacological properties.” Their study began in the 19^{th} century as morphin was the first alkaloid isolated in 1805 from Opium poppy (*Papaver somniferum*)

by German chemist Friedrich Serturner. The first complete synthesis of alkaloid was done in 1886 by the German chemist Albert Ladenburg. More than 12000 Alkaloids have been identified so far.

Most of the alkaloids are crystalline, solid, bitter and very poisonous. Some of these are liquid such as conine, nicotine etc. Liquid alkaloids are dissolved in water, the remaining alkaloids are quick soluble in certain solvents like ether, chloroform, benzene, etc. Due to alkaline nature these biochemicals make salts in solution with inorganic acids. Alkaloids protect the plants against insects and other animals. Nicotine sulphate and azadirachtin which are derived from tobacco and neem, respectively, are used as insecticides and pesticides. Their role in protein synthesis as a reservoir of N is also proposed by some scientists. Some of the alkaloids like morphine, ergotine, quinine and emetine are used as medicines.

Terpenes are functionally diverse group of secondary metabolites. Being chief constituents of essential oils, many of terpenes are integral to primary metabolism e.g. hormones, electron carriers, terpene–dervied compounds and photosynthetic pigments. The terpenoid are divided into many types such as monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, tetraterpenoids (carotenoids) and polyterpenoids (Raaman, 2006). Some example as lycopene, a red pigment of tomato, is a tetraterpenoids. Steroids are triterpenes or triterpenoids found both in gymnosperms and angiosperms. Tetraterpenoids and carotenoids are commonly found in fruits. Terpenoids, mainly found in leaf glandular trichomes, bud exudates and bark resins act as antioxidants, attractants of pollinators, growth hormone and reserve food materials. It also helps in wound healing of the plant.

Phenolic compounds constitute a class of compounds with one or more benzene ring and hydroxyl groups. These secondary metabolites are ubiquitous in plants and are an essential part of the human diet. With antioxidant properties phenolics show potential against oxidative damage diseases. In plants these compounds are essential for process of growth and reproduction. These are also produced against pathogens as a part of defence mechanism. Phenol (C_6H_5OH,),

commonly known as carbolic acid, is the first member of this class of secondary metabolites. All other members of phenolic groups are derivatives of phenol. Flavonoids, lignin, anthocynin, flavonals, tannins, quinine are few of the phenolic compounds with diverse functions.

Flavonoids are phenolic substances which are derived from the C_{15} body of flavons. The degree of oxidation of their central pyran ring distinguished them from other phenolic compound. This varied degree of oxidation alters their biological properties. Barring some classes e.g. flavonones, member of other classes are known as pigment that colour different parts of a plant body. These secondary metabolites are water soluble. These plants products consist of carbohydrates portion combined with either hydroxyl compound or carbohydrates combined with other non-carbohydrate molecules such as glucose+phenolic compound. These compounds originate in plants an animal that upon hydrolysis yields glycone (a sugar compound) and aglycone (non-sugar compound). Flavonoids biomolecules exhibit remarkable physiological effects in plants. Glycosides with foaming characteristics are termed saponins. Chemically they are made of sugars attached to polycyclic aglycones known as sapogenins. The sapogenins is triterpenes or steroids.

Climbing plants are important group of plants whose structural support does not come entirely from its own tissue with original rooting position in the soil or a surface close to the soil and whose climbing efforts could take its foliage and reproductive organs into tree canopies (Burnham, 2009). Medicinal climbing plants have been treated as ambiguous growth forms in most floristic studies in arid ecosystems, leading to poor or difficult to obtain knowledge about their abundance (Rundel and Franklin, 1991). About 5000–10,000 species of climbers have been recorded within angiosperms (Caballe, 1993). India hosts about 265 climber species of which 125 are woody and the rest are herbaceous. About 100 species are medicinal in nature (Chaudhuri, 2007). Some species are widely distributed across the globe and others restricted to one country only, while a few are found in very small areas with particular environments (Bongers *et al*. 2005). Climbers are interesting group of plants but sufficient research of many species

has not been carried out. Various climbers are found in the catchment area of the Mej river. A total of 27 species of climbers of various plant families have been recorded in the study area. Present work deals with the study of primary and secondary metabolites in four climbers i.e., *Cayratia trifolia, Coccinia grandis, Cocculus hirsutus and Pergularia daemia*. There is no attempt made so far for such type of study in the area.

The objectives of the present study are following:-

I. To conduct a preliminary survey for preparation of a list of climbers species of Mej river locality.
II. Preparation of herbarium sheets of collected plant species.
III. To prepare ethanol extracts of different plant parts of selected climbers.
IV. Phytochemical screening of the extracts to evaluate their biochemical constituents in selected taxa of area.
V. To record bioactive constituents present in the taxa.
VI. Comparative study of the estimated phytochemicals among the selected taxa.

The present work has been divided into seventh chapters. The first chapter is an introduction to the work. This chapter includes concept of primary and secondary metabolites, climbers, plan of work and significance of the study. General account, profile and flow area of Mej river have been given in the second chapter. Locations of river and villages around it have been shown through the maps. Location, catchment area and name of villages in and around Mej river have also been described in this chapter. In the third chapter review of literature has been mentioned.

Fourth chapter contains floristic diversity of the study area and methodology adopted during phytochemical estimation. List of climbers identified during visit of area, systematic enumeration of selected climbers, herbarium preparation and various methods for primary and secondary metabolites analysis have been described in this chapter. For the phytochemical screening of the selected plants various tests have been conducted. Results of qualitative and quantitative analysis of various plant parts (Stem, leaves and fruit) of four selected

climbers of area have been mentioned in fifth chapter. The phytochemical analysis of these climbers has been depicted through various tables and figures.

Comparative analysis of the results of quantitative and qualitative estimations of different plant parts has been discussed in sixth chapter. Seventh chapter incorporates summary of the whole work. Conclusion of the whole research which includes significant findings and future aspects of the study is given in this chapter. At the end of the thesis bibliography is given which is followed by copies of published research papers and list of research papers presented in seminars and conferences. Photographic plates, tables, figures, etc. are also put inside the concerning chapters.

CHAPTER 2

STUDY AREA

Rajasthan, being the largest state of India, shares about 10.4% geographical area of the country. It is situated in the north-western part of India between 23^0 30' to 30^0 12' north latitudes and 69^0 29' to 78^0 17' east longitudes. The dimension of the state is 869 kilometres from east to west and 826 kilometres from north to south. Rajasthan is rich in geographical, historical, economic and social diversity. About 9.4 percent of the total geographical area of Rajasthan is recorded as forest.

The south-eastern part of Rajasthan, known as Hadoti, consists of four districts, namely Kota, Bundi, Jhalawar and Baran. Hadoti is the northern part of the Malwa plateau which is composed of many physical variations with average height upto 300 meters from the sea level. The slope of this region is from south west to north-east. The central part of it is drained by the Chambal and its tributaries. The southern part, eastern plateau part and the south western part are of high land with various high altitude characteristics. Bundi and Mukandra mountain ranges are situated in the middle of the region. In this area, the Vindhyan range is present in the form of the Mukundra mountain ranges and hills of the Aravalli range are situated near Bundi district. The region is also known as the Hadoti plateau and is marked by black fertile soil and deciduous type of forests. This region can be further subdivided depending on the height, structure, drainage system, geomorphology, mountain ranges and catchment area of important rivers, etc.

Water is the basis of the biological system and rivers are an important source of water. The Hadoti plateau of this region also plays an important role in the construction of the runoff system. The southern part of the state is traversed by a number of rivers. However, the Chambal is the only perennial river in the state which originates near the Janapao hills, about 24 km south-west from Mhow in Indore district in Madhya Pradesh (Saksena *et al.* 2008). After flowing through Jhalawar district of Rajasthan it meets the Yamuna, draining south–eastern part of the state. Banas, Kalishind, Parvati, Mej, etc. are some of the tributaries of the Chambal. Being only permanent duct of the Hadoti region the Chambal is also called the Life River of drainage system. The major tributaries of Chambal are Kali Sindh, Perven, Parvati and the Mej. In these tributaries, small streams meet. All the surface flows of this region get absorbed in Yamuna which in turn meets

the Bay of Bengal. In fact, this is an example of impermeable flow control. Natural vegetation and forests are an important place in natural resources. This region is rich in natural vegetation and dense forests because of availability of favourable conditions. Natural vegetations include mixed deciduous forest of *Anogeissus pendula*, *Acacia catechu*, *Bueta monosperma,* etc.

The Mej river, stretched south-west to north-east direction over a length of approximately 144 km is a main left bank tributary of the Chambal which forms an oval shaped basin over an area of 5500 square km (Sharma and Padmaja, 1982) extended over four districts of Rajasthan, namely Bhilwada, Bundi, Tonk and Kota. The catchment basin of the Mej river falls under semi arid zone of India and supports rich floral and faunal diversity. Some parts of Ramgarh Wild Life Sanctuary which is extended over Bundi district, harbouring a number of plant species of medicinal, religious and economical importance also share drainage area of the Mej. Moreover, the river traverses hilly areas in some parts of the sanctuary with spectacular meanders.

The Mej River originates in Dhanwada village in Bhilwara district, about 6 km east of Shyampura Railway Station, located in east Malwa plateau of Mandlgarh tehsil. This village is situated on and around a hill. According to information received from the local residents of the village, there is a natural source of water behind the hill which is known as Khatyadi Kund, from where the main stream of the Mej river flows continuously. During the rainy season, all the water of Malwa plateau is drained by this main stream and forms the Mej river. This stream is the main stream of the Mej River and in the same region two more streams namely Adanala stream and Laxmipura stream also discharge their water in the main stream. Adanala stream originates from Sita Kund, a natural water source (pond), located 4 km away from Dhanwada village in the eastern side. This area is surrounded by dense forest of *Diospyros melanoxylon, Ficus religiosa, Butea monosperma, Syzygium cumini, Phoenix dactylifera,* etc. Laxmipura stream originates from Padaliya dam located in Laxmipura, the western part of the village. This main stream goes beyond the village of Dhanwada passing through Rooparlel Gaon, Shakhti ji ka Kheda and enters Bundi district near Negad town.

Further, passing by the Shakargarh town it discharges water into the Gudha dam built near Nehri village.

Gudha dam is the largest dam on this river and situated about 35 km away from the district headquarters of Bundi. The capacity of this dam is 34.50 feet. This dam supplies water to the wildlife throughout the year. Apart from this, the dam is the lifeline for the surrounding local residents including nomadic and tribal peoples. In the rainy season the dam becomes a tourist spot. The surrounding area of the dam is full of many medicinal plants including some climber plant species. Near Samela village, the Kurel river merges into the Mej and further it becomes a lifeline for animals and tribal communities residing in and around the famous Ramgarh Vishdhari Wildlife Sanctuary in Bundi district.

Ramgarh Vishdhari Wild life Sanctuary was declared a sanctuary on 25.5.1982 vide Govt. Notification No.DF1/a/ RajB/75 under the Indian Wild life Protection Act, 1972. The forest falls under the subsidiary edaphic type of tropical dry deciduous forest. This is the only habitat in Haroti region which provides abode to tigers. It has been reported that tigers from Ranthambore tiger project area (Swaimadhopur district) frequently move into the Ramgarh Vishdhari Wild life Sanctuary via Kamleshwar Mahadev closed area corridor. Earlier this sanctuary was known as Shikargarh of Bundi State. The sanctuary lies in the south-eastern part of Rajasthan between 24º59' and 25º53'11" north latitude and 75º19'30" to 76º49'30" east longitudes. The sanctuary is stretched between continuous double lines of Vindhyan hills from south-west to north-east direction approximately over a length of 45 km. Mej river coming from northern side of Ramgarh-Piplia block enters the sanctuary area and another river called Machali also enters from north-east part of the sanctuary. The Machali river meets the Mej near Ramgarh palace. Now the Mej river comes out of the sanctuary at Khatkar village. The river traverses some parts of the famous Ramgarh Vishdhari Wild Life sanctuary and after flowing through Khatkhar, Jhaliji Ka Barana, Lakheri, Papadi and Basawada villages meets the Chambal near Pali village.

The oval shaped basin of the Mej is transitional zone between the north-western lobe of the great vidhyan basin and south-eastern fringe of the Aravalli and climatically falls under semi-arid zone of India. Broadly, the Mej river basin

can be divided into three topographic units–Hills, Plateaus and Plains. The basin is divided into two equal parts by backbone like hilly stretch extended from south-west to north-west. The northern area to the north of these hills at as average elevation of 330 meters is a plateau. Kota plateau extends over south- western portion of the basin. The plain, with an average height of 210 meters, in south-eastern part of the basin, is overlain by sediments deposited by the Mej and its tributaries (Sharma and Padamaja, 1982). Main tributaries of the Mej river are Banjad, Kural, and Mangali. About 240 villages (Table 1) are situated in the basin of the Mej which are inhabitated by a number of tribal, ethnic and nomadic communities such as Bhil, Meena, Kanjar, Sansi, Bhat, Mogya, Kalbeliya, Banjara, etc. The catchment areas of this river possess rich floristic diversity of angiospermic species. Out of these species in and around Mej river four climbers were selected for phytochemical study. One of the objectives of the present work includes comparative phytochemical analysis of these climbers of the study area.

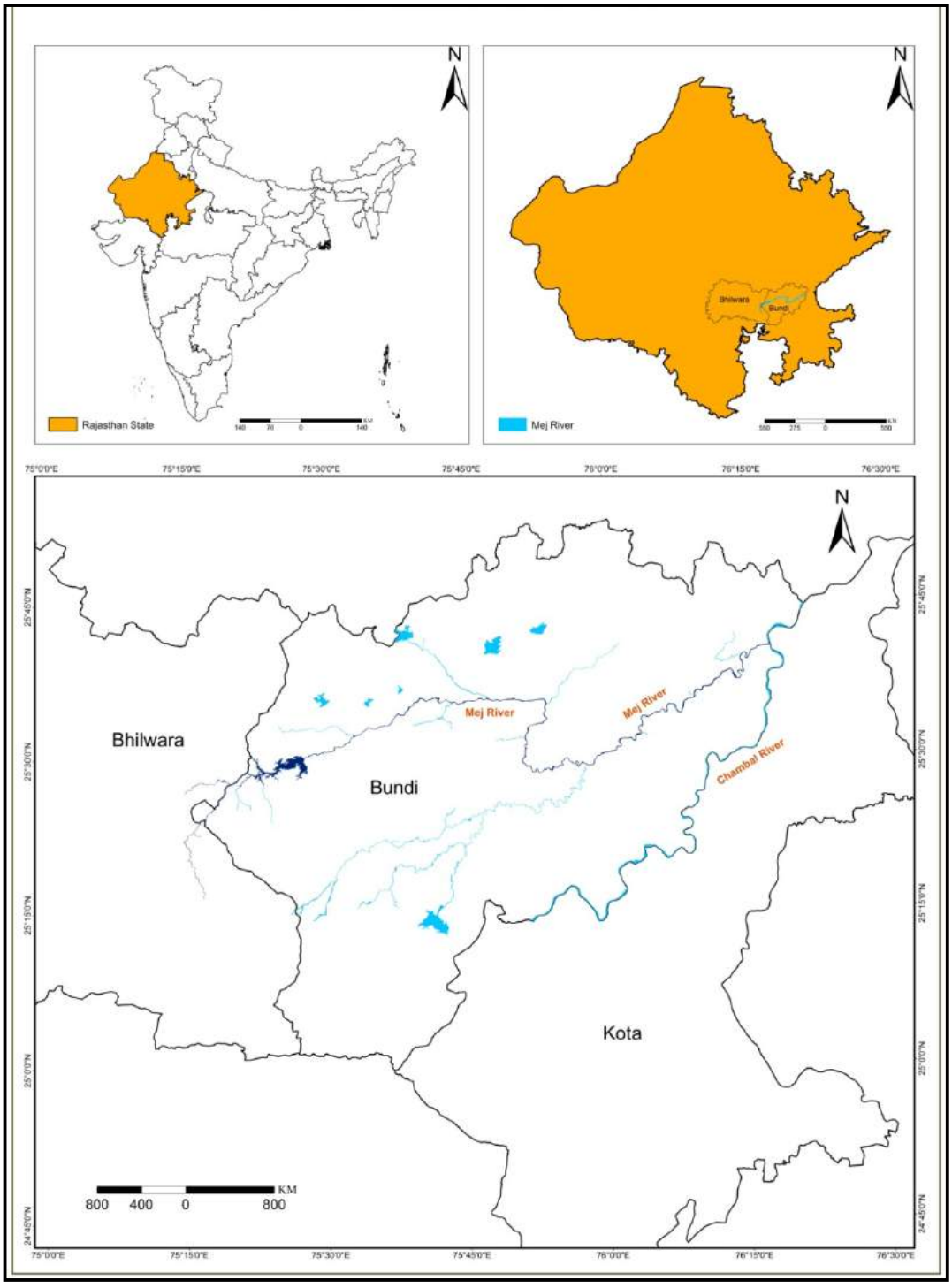


FIGURE 1 MAP OF THE MEJ RIVER AREA

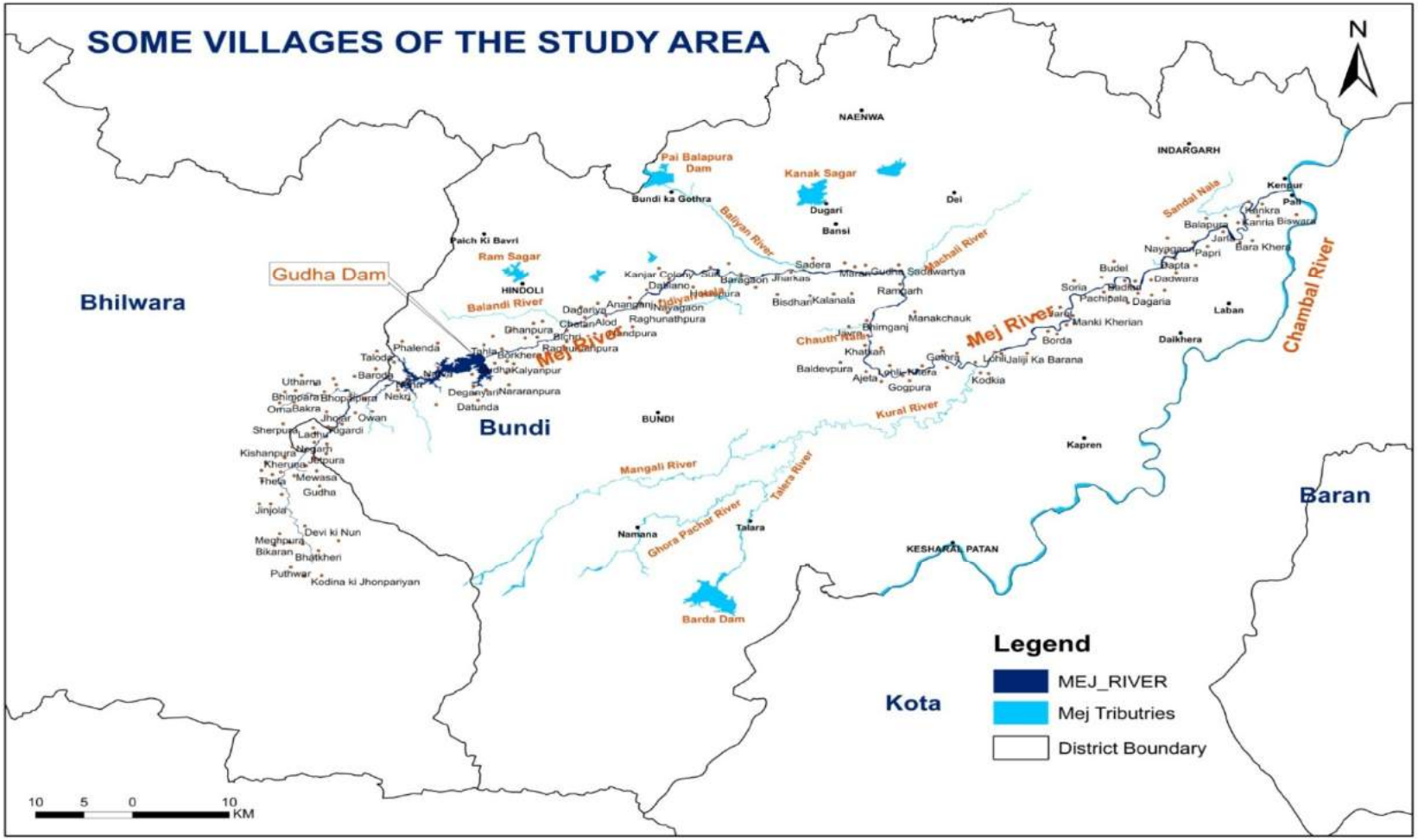


FIGURE 2 IMPORTANT VILLAGES IN AND AROUND MEJ RIVER CATCHMENT AREA

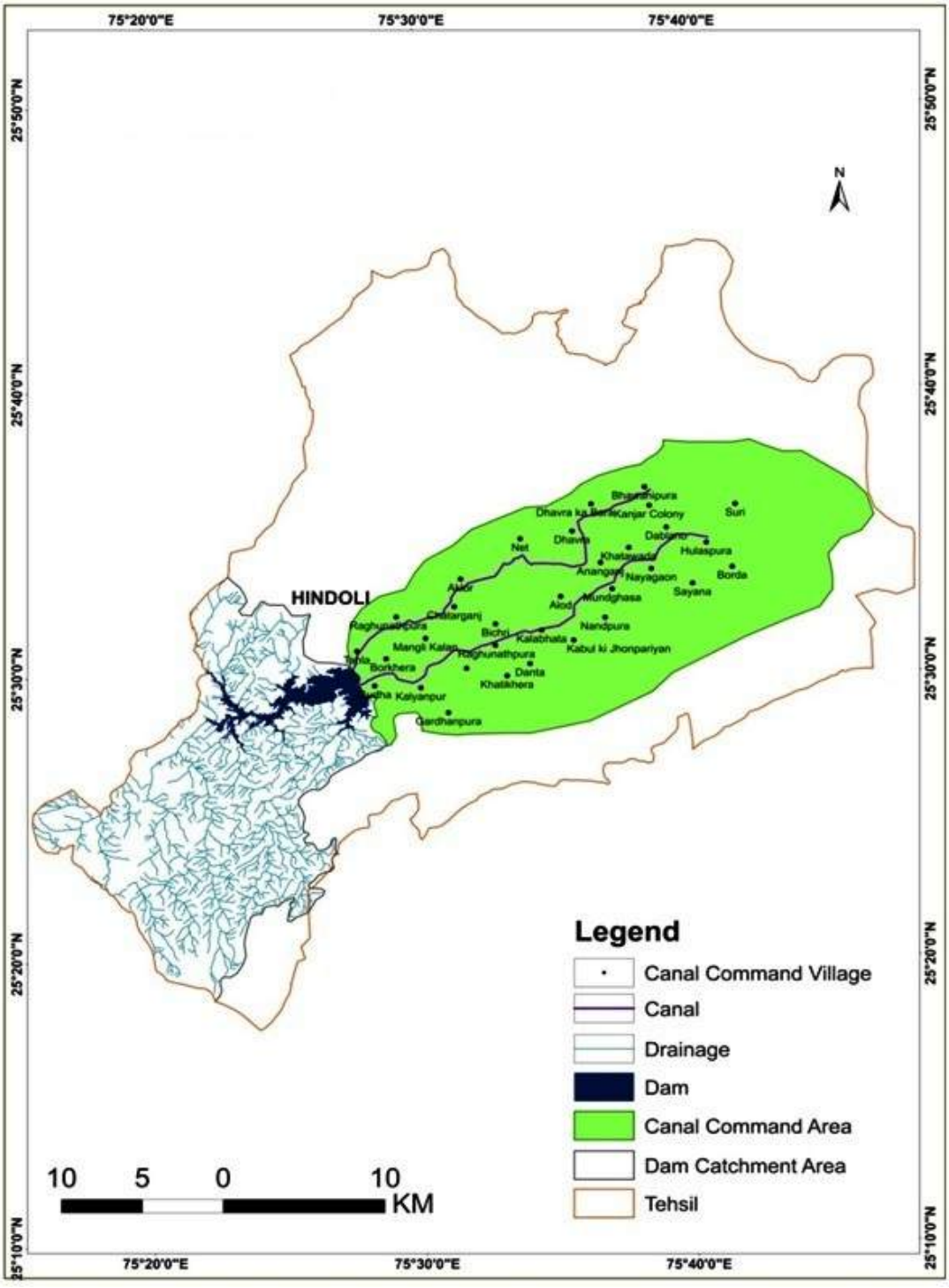


FIGURE 3 GUDHA DAM AND COMMAND AREA

Table 1 List of important villages around Mej river

1	Lakshmipura	41	Kishanpura	81	Melwa
2	Dhanwara	42	Bhawanipura	82	Delunda
3	Shyampura	43	Negarh	83	Gogpura
4	Kodina ki Jhonpariyan	44	Bandha ka Khera	84	Ajeta
5	Kasnaji ki Jhanpariyan	45	Padal	85	Barj ki Dungari
6	Jalam ki Jhonpariyan	46	Ladhu	86	Karwala ki Jhonpariyan
7	Puthwar	47	Amarpura	87	Rampura
8	Ukhali ka Khera	48	Bolapura	88	Nehri
9	Bhuwanisingh ka Khera	49	Rigardi	89	Lakshmipura
10	Bhatkheri	50	Sherpura	90	Riana
11	Bikaran	51	Basoli	91	Utharna
12	Sultanpura	52	Sheipura	92	Taloda
13	Kalyanpura	53	Gurujanya	93	Janajpuriyan
14	Malka Khera	54	Sukhbilas	94	Kodkia
15	Meghpura	55	Bakra	95	Banadarpuriya
16	Devi ki Nun	56	Jhojar	96	Pipalda
17	Jinjola	57	Owan	97	Ajeta
18	Achlaji ka Khera	58	Sujanpura	98	Gardhanpura
19	Ruparet	59	Bandi ka Khera	99	Khyavada
20	Bharji ka Khera	60	Orna	100	Bagli

Contd.....

21	Devpura	61	Bakra	101	Baroda
22	Saktaji ka Khera	62	Datunda	102	Chhaparda
23	Gudha	63	Hakumpura	103	Narva
24	Joraji ka Khera	64	Manri	104	Khera
25	Arimalji ka Khera	65	Chavandiya	105	Lohli
26	Thela	66	Saripura	106	Lal ka Khera
27	Gulji ka Khera	67	Shakergarh	107	Kalyanpur
28	Jodpara	68	Achia ki Jhonpatiyan	108	Gudha
29	Mewasa	69	Babaji ka Khera	109	Baldevpura
30	Bachlakhera	70	Bagh ka Jhonpara	110	Bhainskhera
31	Sharngarh	71	Bhopalpura	111	Ralayata
32	Shobhaj ka Khera	72	Bhimpara	112	Turkon
33	Bhilon ki Jhonpai	73	Nekri	113	Bara Nayagaon
34	Thithora	74	Madhdpura	114	Menoli
35	Kararkheri	75	Dotana	115	Jaliji Ka Barana
36	Sitla ka Khera	76	Kishangarh	116	Morkhundana
37	Rampuriya	77	Deganyari	117	Khatikhera
38	Kheruna	78	Bir ka Jhonpara	118	Baldevpura
39	Jetpura	79	Nararanpura	119	Lohli
40	Bhutpuriya	80	Mal ka Khera	120	Chautra Khera
121	Taloda	161	Manki Kherian	201	Jharkas

Contd.....

122	Gothra	162	Gendoli Ki Jhoparian	202	Kanjar Colony
123	Jhalji Ka Barana	163	Chancholian Ki Jhoparian	203	Suri
124	Kundla	164	Chetan ka Jhonpara	204	Maran
125	Mujra Borda	165	Raghunathpura	205	Kharaita
126	Borkhera	166	Laharpura	206	Fatehpura
127	Sorajpura	167	Manakchauk	207	Gudha Sadawartya
128	Kesarpura	168	Jarol	208	Dadwara
129	Gopalpura	169	Chetan ka Khera	209	Kalyanpura
130	Kharibaran	170	Aklor	210	Shankarpura
131	Nalpura	171	Mundghasa	211	Mundli
132	Khatkan	172	Dagariya	212	Budel
133	Tahla	173	Lakshmipura	213	Dapta
134	Jagannathpura	174	Nayagaon	214	Bhawanipura
135	Vijaigarh	175	Chhapanpura	215	Sadera
136	Bhatiyan ki Jhonpriyan	176	Ananganj	216	Thakron ki Jhanpariyan
137	Raghunathpura	177	Raibarpura	217	Akoliya
138	Phalenda	178	Bisdhari	218	Kakavta
139	Bhim Ka Khera	179	Dabeta Khurd	219	Kherli
140	Mangli Kalan	180	Kalanala	220	Bhamar
141	Mangli Khurd	181	Dabeta Kalan	221	Gujra Khera
142	Dola	182	Phalsthuni	222	Nimod

Contd.....

143	Deroli	183	Dagaria	223	Papri
144	Bata Dhundhlaji	184	Pachipala	224	Bara Khara
145	Borda	185	Kalporiya	225	Nayagaon
146	Kalabhata	186	Pratapgarh	226	Bara Khera
147	Dhingsi	187	Khatawada	227	Haripura
148	Raghunathpura	188	Sambharwari	228	Aeria
149	Bichri	189	Lodha ka Jhonpara	229	Jarla
150	Nandpura	190	Hulaspura	230	Bargaon
151	Javra	191	Paprli	231	Kishanganj
152	Badarpura	192	Ramgarh	232	Balapura
153	Jalera	193	Barian	233	Hathi Dumba
154	Dhanpura	194	Soria	234	Kanria
155	Durjan Ki Kherian	195	Badian	235	Bhandgawar
156	Chatarganj	196	Mararia	236	Biswara
157	Bhimganj	197	Dablano	237	Kankra
158	Kargajpur	198	Dangaheri	238	Sadhavda
159	Chetan	199	Baragaon	239	Kenpur
160	Alod	200	Dadwara	240	Pali

DHANWADA HILLS

KHATYADI KUND

CATCHMENT AREA OF MEJ

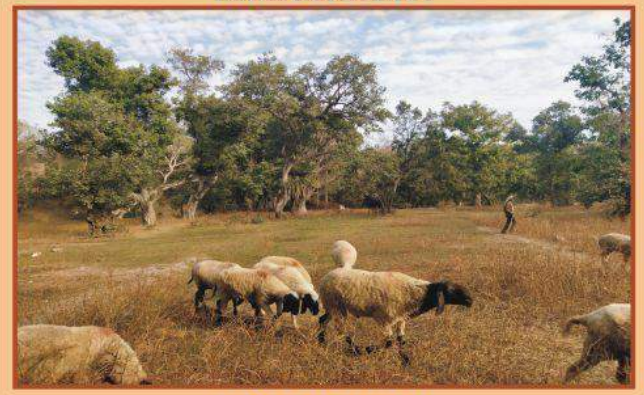
NATURAL WATER SOURCE

PLATE 1 VIEW OF THE ORIGIN SITES OF THE MEJ RIVER

LAXMIPURA STREAM-1

LAXMIPURA STREAM-2

SITAKUND



TENDUPATTA FOREST

PLATE 2 VIEW OF THE ORIGIN SITES OF THE MEJ RIVER

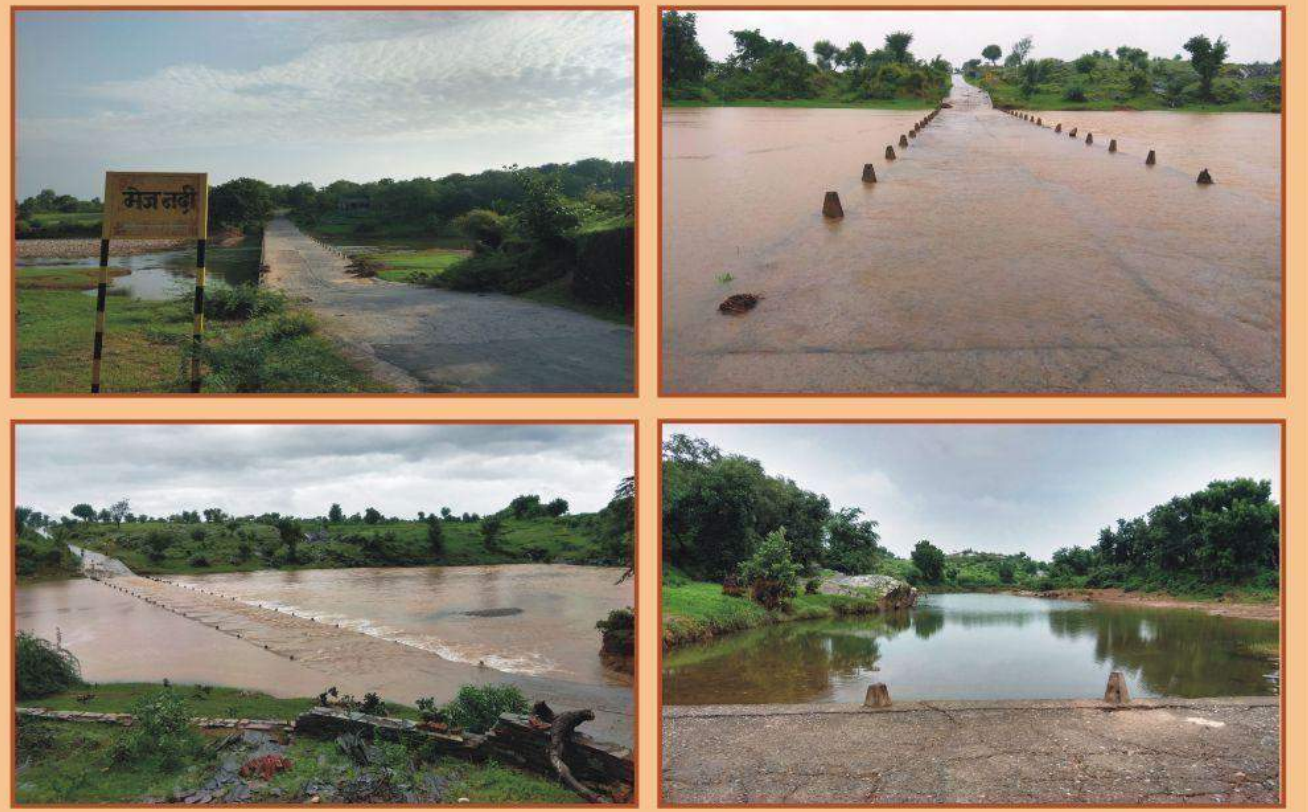
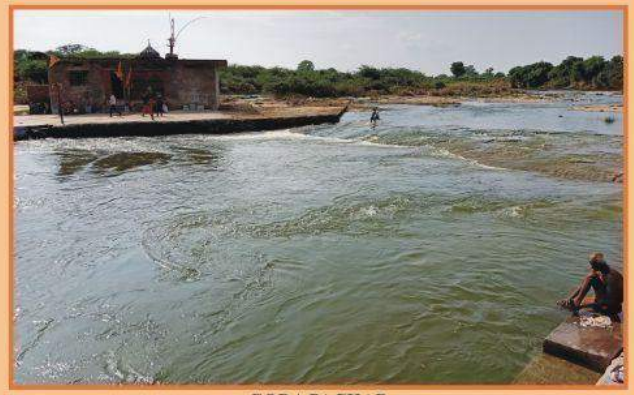


PLATE 3 VIEW OF THE MEJ RIVER IN BHILWARA DISTRICT



GODA PACHAR

KUREL

MANGLI

MACHALI RIVER IN RAMGARH WILD LIFE SANCTUARY

PLATE 4 TRIBUTARIES OF MEJ RIVER

PLATE 5 VIEW OF THE GUDHA DAM ON THE MEJ RIVER

SURVEY OF GUDHA DAM

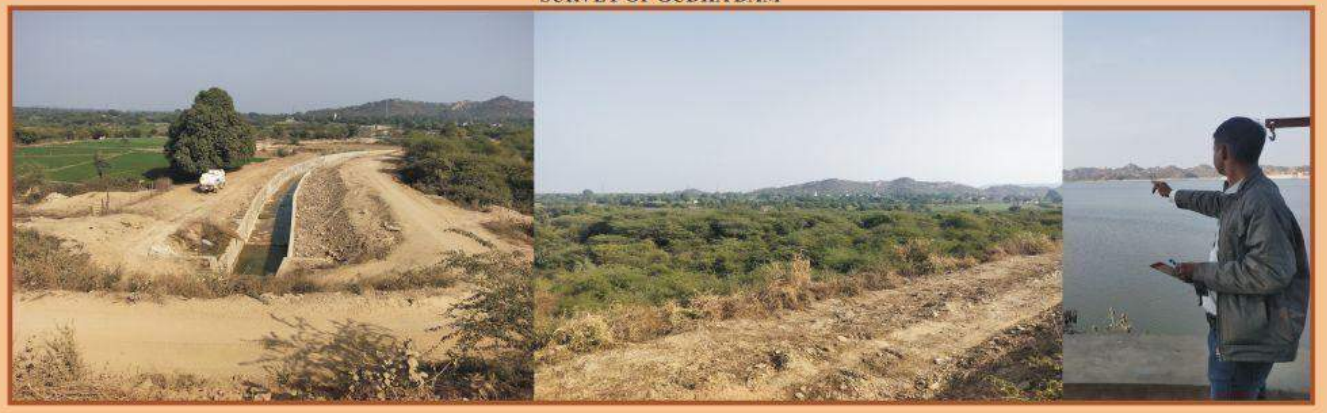
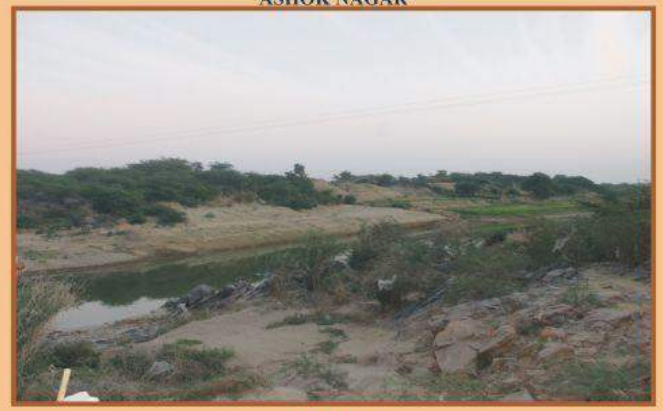


PLATE 6 VIEW OF THE GUDHA DAM ON THE MEJ RIVER

PLATE 7 CLIMBER BIODIVERSITY IN AND AROUND MEJ RIVER

ASHOK NAGAR

SHAKARGARH



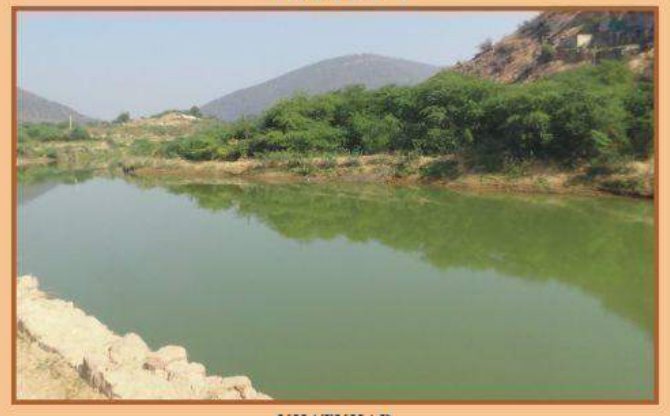
NEGADH

KODKIA BALAGI

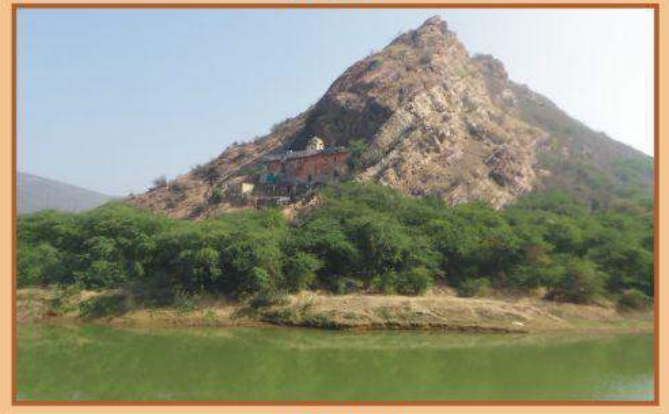
PLATE 8 MEJ RIVER AT VARIOUS LOCALITIES IN HINDOLI AND K.PATAN BLOCK

SADAWARTYA

JAHLERA



KHATKHAD

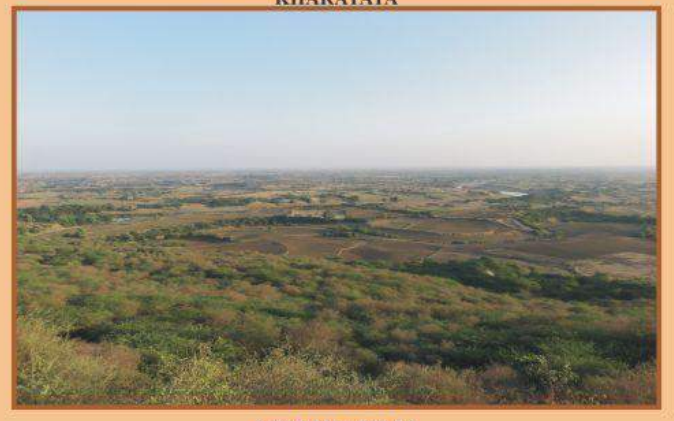


DUNDALA JI

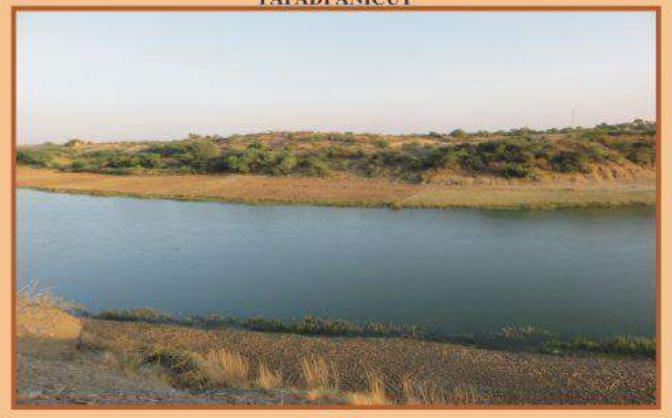
PLATE 9 MEJ RIVER AT VARIOUS LOCALITIES IN RAMGARH WILD LIFE SANCTUARY

KHARAYATA

PAPADI ANICUT



SHANPUR HILLS

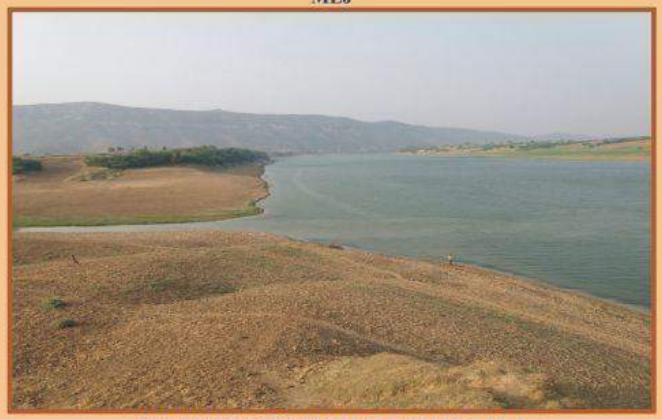


PALI

PLATE 10 MEJ RIVER AT VARIOUS LOCALITIES IN LAKHERI BLOCK

MEJ

CHAMBAL



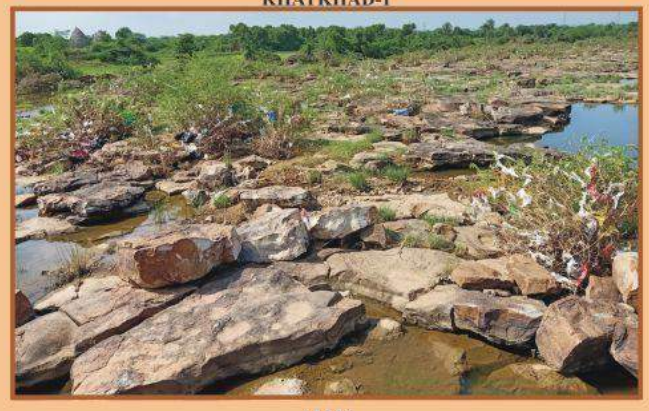
CONFLUENCE OF MEJ AND CHAMBAL-1

CONFLUENCE OF MEJ AND CHAMBAL-2

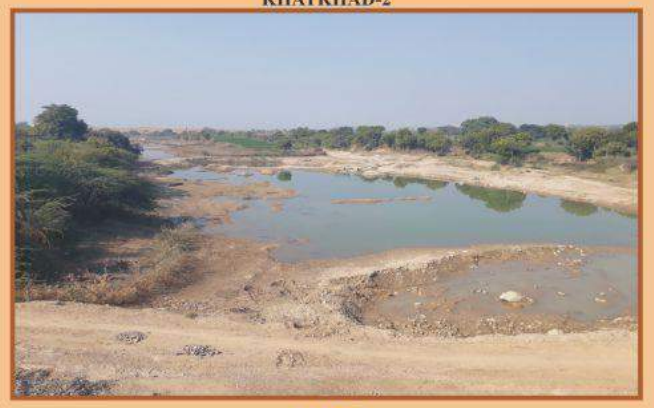
PLATE 11 SITE OF THE MEJ RIVER NEAR CONFLUENCE POINT WITH THE CHAMBAL

KHATKHAD-1

KHATKHAD-2



ALOD



ALOD

PLATE 12 IMPACT OF ANTROPOGENIC ACTIVITIES IN THE MEJ RIVER

CHAPTER 3

REVIEW OF LITERATURE

Ancient man depended on plant primarily for food and shelter but with development of cognitive powers he explored other aspects of plants to address new challenges. Phytochemicals in the form of primary and secondary plant metabolites have potential role in human life. This chapter deals with the review highlighting works of researchers pertaining to exploration of phytochemicals in various plant species and their use in many ways.

Antioxidant activities of hydromethanolic extract of the *Coccinia grandis* L. have been studies by Umamaheswari and Chatterjee in the year 2008. The antioxidant activity of the extract was assessed with nine *in vitro* assays with some standard antioxidants. They observed free radicals scavenging activity, H-donor activity and inhibition of β-carotene bleaching. However, the antioxidant property was found to be does dependent.

In the year 2009, Satheesh *et al*. carried out a study to assess anti-nutrients and nutrients of *Coccinia indica* with *Trichosanthes bracteata*. They revealed significantly high phenolic content, i.e., 0.5 mg/g fr.wt. to 0.7 mg/g fr.wt. with analytical methods and reverse phase high performance liquid chromatography. The same species of *Coccinia* was studied for bioactive compounds of its fruits for their anti-bacterial activities by Shaheen *et al.* (2009). The organic extracts were found to be more potent in comparison of the aqueous extract against the test bacteria. They also reported the extract to possess some bioactive phytochemical like phenols, alkaloids, flavonoids, tannins, glycosides, etc.

In an assessment of phytochemical and antibacterial properties of *Coccinia indica* leaf extract, Hussain *et al*. (2010) observed potent antibacterial properties against some test bacteria. They found ethanol and aqueous extract more potent than the petroleum ether and chloroform extracts. Sutar *et al*. (2010) studied medicinal properties of the same plant species and oral administration of the ethanolic extract of *Coccinia indica* leaves to reduce blood glucose and certain fatty acids. The study was conducted on the basis of several parameters, i.e.,

phytochemical analysis, microscopic characters, and physical examinations of the *Coccinia indica* leaves.

Pradhan *et al*. (2013) carried out study to evaluate presence of phytochemicals among *Tinospora cordifolia* stems of varied diameter. The study revealed that their phytoconstituents have correlation with the diameter of the stem as except alkaloid content remaining phytoconstituents increased with the diameter of *Tinospora cordifolia* stem. They also reported variation in phytoconstituents among the plant material collected from different locations.

Leaf extract of *Tinospora cordifolia* was tested for its antioxidant and antimicrobial properties by Priti and Rani (2017). They observed that among the extract prepared in different solvents the methanolic leaf extract expressed presence of highest number of phytochemicals which may be attributed to the comparatively higher solubility of the phytoconstituents in methanol as compared to the other solvents, viz., diethyl ether, chloroform, ethyl acetate and water.

Patra *et al*. (2016) did preliminary phytochemical and pharmacognostic study of *Tinospora cordifolia* plants climbing on six different tree species namely, *Ficus religiosa*, *Anthocephalus cadamba*, *Cassia fistula*, *Ficus rumphi*, *Mangifera indica* and *Terminalia bellirica*. They observed that the plant materials varied in their microscopical features, however, morphologicaly the materials were similar.

In a study Jijith *et al*. (2016) evaluated alcoholic extract of stem bark of *Anamirta cocculus*, a member of family Menispermaceae. They reported presence of various phytoconstituents in the extract. Another member of the family Menispermaceae, i.e., *Cissampelos pareira* was studied by Patel *et al*. (2014) using different pharmacognostical parameters. Preliminary screening and qualitative chemical analysis was done to assess different phytochemicals.

Chellappan *et al*. (2011) did antiulcer, antioxidant and pharmacognistical screening of *Cyclea peltata*, which belongs to the family Menispermaceae. They observed significant gastric protection against the gastric ulcer in rats. They

reported the root extract of the plant to be devoid of toxicity in the test animals when treated with the extract upto 2g/kg by oral route.

Calotropis procera is commonly found xerophytic shrub. Stem of this plant is used in curing various ailments. Rajesh *et al.* (2014) carried out phytochemical investigation on the stem of this plant species. Similar study has been done by Gupta and Singh (2015) as they worked on phytochemical screening of hydro alcoholic leaf and stem extracts of *Calotropis procera*. Preliminary phytochemical analysis of *Calotropis gigantea* leaf obtained from petroleum ether and methanol extract were examined by Singh *et al.* (2014). *Calotropis gigantea* commonly known as *Safed Aak* is well known medicinal and religious plant.

Dey (2011) reported on pharmacological and phytochemical aspects of *Alstonia scholaris*. This is evergreen tree and is known as Devil's tree and belongs to family Apocynaceae.

Aegle marmelos (family Rutaceae) is the most widely distributed medicinal as well as religious plants of India. Varughese and Tripathi (2013) studied *Aegle marmelos* fruits at different stages of ripening and confirmed suitable solvent extract for this from phytochemically evaluation of different solvent extracts. *Aegle marmelos* possess free radical and hydroxyl radical scavenging activity and antioxidant activity *in vitro* (Gupta *et al.* 2012). They revealed that several phytochemical constituents present in hydromethanolic extract of *Aegle marmelos* are responsible for antioxidant activity of the plant.

Physiochemical traits value and phytochemical evaluation of leaves of *Callicarpa macrophylla* was carried out by Anjum *et al.* (2013). Different extracts (petroleum ether, chloroform, methanol and aqueous extracts) were used for phytochemical analysis of various bioactive compounds of *Callicarpa macrophylla* leaves. Ashwagandharistha (prepared by traditional and modern methods) showed a rich of total phenol and flavonoids and also showed dose dependent antioxidant activities (Tiwari and Patel, 2013). Both types of Ashwagandharistha were studied for their antioxidant potential on two differents

in vitro model as super-oxide radical scavenging activity and lipid per oxidation assay by authors.

Temilselvan *et al*. (2011) in review of pharmocognosy reported different parts of *Coccinia grandis* used in traditional medicine to cure several diseases like ulcer, skin disease, asthma, diabetes, jaundice etc. They informed that the leaf and its constituents have hypolipidemic, antioxidants and hypoglycaemic activities. Deshpande *et al*. (2011) studied antioxidant activities of another part of the same plant species, i.e., fruits. Three *in vitro* assays were used to assess antioxidant property of methanlolic fruit extract. They used butylated hydroxyanisole (BHA) as a standard antioxidant.

In the year 2011, Hussain *et al*. carried out anatomical, macroscopical and physiochemical studies on *Coccinia indica*. They assessed the fresh and dried crude leaves with fluorescence analysis, TLC finger print profiling as well as quantitative and qualitative parameters. This species of *Coccinia* was also reported to be used for treatment of skin disease, wounds, diabetes, inflammation, etc. The study highlights the difference of leaves of this species of the genus with the other species of the same genus. Chatterjee and Chatterjee (2012) carried out anti-inflammatory and phytochemical analysis of 60% methanolic, petroleum ether and aqueous extracts of the whole plant of the same plant species. In the study they observed that only methanolic extract exhibits maximum anti-inflammatory property with respect to the remaining plant extract using the standard drug diclofenac sodium.

Dhiman *et al.* (2012) in a review reported fruits or seeds of certain members of family Cucurbitaceae to have antihelmintics, purgative and emetic properties. These properties are considered due to presence of the Cucurbitacin content, in these plant species which constitute a class of triterpenoids. The substances are phytochemicals with Cucurbitacin skeleton.

Antioxidant activity of hydromethanolic root extract of *Coccinia grandis* was investigated by Bhadauria *et al*. (2012). They reported antioxidant properties of the fractions to be concentration dependent. This activity of the extract was

reported to be due to presence of flavonoids and phenolic contents in the hydromethanolic extract.

Arora *et al*. (2013) studied presences of phytochemicals and pharmacognostical properties of *Cissampelos pareira*. They registered many secondary plant metabolites which may be explored for their medicinal properties.

Behera *et al*. (2019) in a study screened phytochemicals of 20 traditional medicinal plants belonging to 19 families. They recorded presence of terpenoids, alkaloids, tannins, steroid, leucoanthocyanin, coumarin, phenol, etc. Among the studied 20 plant species alkaloid was present in all plants whereas phenol and tannins were registered only in 18 plant species.

Siddika *et al.* (2013) studied chemical composition and activities of certain enzymes at different maturity levels of *Coccinia cordifolia* fruits. They reported that immature and matured fruits are rich in starch and minerals, respectively. However, ripe fruits of this plant species possess highest amount of total sugars, reducing sugar, β-carotene, vitamin C, B, and B2 as well as protein.

Chunduri (2013) in a study carried out nutritional and antioxidant analysis of *Momardica charantia, Momardica dioica, Trichosenthes dioica* and *Coccinia indica.* They reported high concentration of phosphorus (24.11 mg/ 100g) in *Coccinia indica*. DPPH and FRPA assays with high total phenol concentration (> 50.0 X 10 mg GAE/g) suggest antioxidants properties of these vegetables.

According to the observation made by Irulandi and siva (2018), *Atalantia racemosa* and *Coscinium fenestratum* possess a number of valuable phytoconstituents.

Onkar *et al*. (2012) in a study evaluated antioxidant activity of hydro alcoholic extract of the *Curculigo orchioides* and the traditional Ayurvedic formulation Giloy satva (*Tinospora cardifolia*) promising free radical scavenging activity in comparison with the standard.

Shalini *et al*. (2014) tested antioxidant activity and presence of phytochemicals in ripe fruits of *Coccinia grandis*. The results of the study revealed presence of primary and secondary metabolites. Among the five extracts prepared in different solvents, ethyl acetate and chloroform extracts were recorded to possess higher free radical scavenging activity. The same extracts were found to contain high flavonoid and phenolic contents.The same plant species was studied for antimitotic, cytotoxic and qualitative analysis by Alamagir *et al*. (2014). They observed leaf extract to exhibits inhibitory effect on wheat seed germination and growth of seedling root. The extract also exhibited inhibitory effect on tumors on potato discs without lethality on the tumor inducing agent *Agrobacterium tumefaciens*.

Ramachandran *et al*. (2014) in a review highlighted chemoprotective properties of *Coccinia indica* against cyclophosphamide used to treat cancer. They reported use of this plant species to cure number of ailments. The medicinal properties of this plant species are considered to be due to presence of many phytochemicals in this member of the family Cucurbitaceae. The leaf extract of the plant is also reported to have xanthine oxidase inhibitory, antipyretic, hyperglycemic, anti-hepatotoxic, anti-insecticidal and hyperglycemic activities. Kishore *et al*. (2014) also reported medicinal values of this plant species in a review. This plant is used in different systems of medicines such as Chinese, Ayurveda, Sidda and Unani. Biological and pharmacological activities of this medicinal plant are extensively worked upon in last few years.

The presence of various phytoconstituents was correlated with medicinal potential of four species of Wrightia plants by qualitative phytochemical analysis (Sharma *et al.* 2017). Wrightia is most common plant of deciduous forest. Comparative phytochemical analysis of *Wrightia coccinea*, *Wrightia tinctoria*, *Wrightia mollissima* and *Wrightia tomentosa* have been investigated by these authors.

Rahman *et al*. (2015) evaluated cytotoxic antibacterial and antioxidant properties of *Coccinia cordifolia* along with its phytochemical analysis. They reported the plant to show positive results for lipids, carbohydrates, proteins,

phenol, vitamin, flavonoids, etc. The antibacterial activity was assessed using ethanol and methanol extract of this plant against certain Gram positive bacteria and Gram negative bacteria. By DPPH scavenging assay antioxidant potential was also tested. They reported the extract to exhibit significant antioxidant activity.

Cell proliferative, antibacterial and antioxidant properties of *Coccinia grandis* were assessed by Sakharkar and Chouhan (2017). They observed that hot and cold extracts prepared in the acetone and ethanol solvents exhibited inhibition of bacterial growth. The same extract revealed antioxidant properties in comparison with Trolox. All the studied plant extracts also exhibited promoting effects on cell proliferation.

Sood *et al*. (2012) tested phytochemical and antimicrobial activity of five plant species of family Cucurbitaceae, namely *Cucumis sativa, Cucurbita pepo, Momcodica charantia, Lagenaria siceraria* and *Praecitrullus fistulosus*. The investigation revealed presence of phytochemicals such as resins, saponins, carbohydrates, cardiac glycosides, tannins and phytosteroids.

Trichosanthes cucumerina was studied by Stellus and Nair (2015) for antioxidant activity and phytochemistry using extracts of this plant prepared in five different solvents, namely petroleum ether, chloroform, methanol, benzene and distilled water. The methanolic extract of this plant was registered to have phenols, flavonoids, tannins and alkaloids. The same extract was found to exhibit DPPH radical scavenging properties which increased with the concentration level of the extract. This plant species is known for certain medicinal properties such as anthelmintic, antifertility, anti HIV and cardioproctetive.

Deshmukh and Gaikwad (2014) reviewed phytochemistry, ethanobotany pharmacology and taxonomy of *Basella alba*. This plant species has anti-iflammatory, antiulcer, antidiabetic, antioxidant, wound healing, hepatoprotective and CNS depressant properties. The plant is known to be used for curing a number of ailments such as anemia, leprosy, whooping cough, headache, etc. Karthishwaran and Mirunalini (2010) also highlighted medicinal properties of the

same plant species. This plant is reported to be traditionally used in treatment of various diseases such as infantile, diarrhea, jaundice, etc. Presence of alkaloids, cardenolides, triterpenes, and saponins has also been studied in this plant species along with a number of pharmacological activities.

Antioxidant activity and phytochemical constitution of methanolic leaf extract of five plant species, i.e., *Trigonella foenum-graecum*, *Mentha spicata*, *Spinacia oleracea* and *Gamelina arborea* was studied by Soni and Sosa (2013). The quantitative analysis revealed that *Gmelina arborea* possesses highest alkaloids (5.56%) and flavonoids (22.80%), *Metha spicata* possesses highest phenolic (18.41%) and *Trigonella foenum-graecum* has highest saponins (50.12%) contents. They also found *Mentha spicata* extract to exhibits promising antioxidant properties.

Irimpan *et al.* (2011) in an investigation obtained positive results for sterols, terpenoids, flavonoids, alkaloids, hydrolysable and tannins. They found Ca/Mg ratio for stem and leaf to be 3 and 3.7, however, K/Na ratio was 17.5 and 7.9, respectively. Methanolic and ethanolic leaf extracts of this plant were registered to exhibit antibacterial activity against certain Gram positive and Gram negative bacteria.

Psychopharmacological profiling and phytochemical screening of *Pergularia daemia* crude petroleum ether extract carried out by Sravani *et al*. (2012) revealed significant decrease in exploratory behavior pattern in mice by the Y-maze and head dip test. The extract also exhibited significant decrease in skeletal muscle relaxant activity.

Krishnamoorthy *et al*. (2018) explored antioxidant capacity of methanolic extract of *Coscinium fenestratum*. They predicted a total of 30 phytochemical compounds with their structure, molecular formula and activities. Bis (2, 4, 6-triisopropylphenyl) phosphinicazide (6.70%) was registered to be the most prevalling heterocycle compound.

Similar studies encompassing antimicrobial and antioxidant potential of *Pergularia daemia* was done by Jain *et al*. (2012). They evaluated extracts of

whole plant prepared in acetone, methanol, chloroform, hexane, pet ether and dichloromethane solvents and isolated lupeol, β- sitosterol, α-, β-amyrin, quercetin, acetate, oleanolic acid and kaempferol with lowest IC_{50} value. The methanol extract exhibited higher antioxidant activity among all the extracts.

Karthishwaran and Mirunalini (2012) in a study tested antioxidant efficacy of methanolic extract of *Pergularia daemia* through *in vitro* and *in vivo* experiments on 7, 12-dimethybenz (a) anthracene (DMBA) induced hamster buccal pouch carcinogenesis. They observed that pre-treatment of BMBA exported test animals with the methanolic extract (200mg/kg bw) significantly increased ($P<0.05$) level of erythrocyte lysate and plasma vitamin C, vitamin E, and GSH. In buccal tissue the level of vitamin E and GSH also increased in comparison with DMBA alone induced hamster.

In a study Upadhyay *et al.* (2013) examined the total phenolic content and antioxidant effects of *Tinospora cordifolia* ethanolic and methanolic stem extracts. The study revealed ethanolic stem extract to possess highest phenol content (66.28±0.82 mg/g). The same extract exhibited higher antioxidant activity when compared to the antioxidant activity of ethanolic stem extract.

Savitha *et al.* (2014) also evaluated phytochemical and antimicrobial potency against selected pathogens and reported presence of terpenoids, tannins, saponins, alkaloids and glycosides in *Pergularia daemia*.

Jangir *et al.* (2016) in a review provided updated comprehensive information of pharmalogical properties and phytochemistry of *Cocculus pendulus*. This medicinal plant in used to cure leprosy, helminthic, syphilis, fever, menstrual disorders, etc.

In vitro antioxidant potential of *Cocculus hirsutus* methanolic extract was evaluated by Meena *et al*. (2014). They recorded methanolic extract of stem to show highest DPPH radical scavenging activity which was followed by methanolic extract of leaf and callus respectively. The activity however, was registered to be less than that of standard ascorbic acid.

Umamaheshwari and Sangeetha (2014) studied anti-inflammatory and antioxidant activities of *Cocculus hirsutus* ethanolic leaf extract, DPPH, nitric oxide radical generation and reducing power assay to assess antioxidant properties. However, human red blood cell assay was used for anti-inflammatory activity. They reported anti-inflammatory activity to be due to presence of flavonoids.

Patil *et al*. (2014) in a phytochemical and aphrodisiac study of *Cocculus hirsutus* areal parts (stem and leaf) extracts observed the extract to have promotory effects on spermatogenesis and performance of accessory reproductive organs in albino rats. In immature test animals, alcohol extract was observed to show potent androgenic activity, however in mature rats the same extract showed highly stimulant spermatogenic activity. Alcoholic extract also exhibited positive test for phenolic compounds tannins, steroids, saponins, oils and fats.

Kalirajan *et al*. (2012) tested wound healing and antimicrobial properties of methanol and aqueous extract of *Cocculus hirsutus*. They reported wound healing potential of the medicinal plant to be better than neomycin (a commercial antibiotic). Against *Vibrio cholerae* (18mm) and *Staphylococcus aureus* (17mm) the methanolic extract was comparatively more effective whereas the aqueous extract of the same plant species showed potency against *Klebsiella pneumonia* (14mm) and *vibrio cholerae* (13mm). The study also ascertained presence of important phytochemicals. In a study Rakkimuthu *et al*. (2012) undertook study of antioxidant activity and qualitative phytochemical analysis of acidified fruit extract of the same plant species and reported anthocyanin, phenol and flavonoid content to be 0.788 ± 0.236mg/g, 326.66 ± 3.05mg/g and 260 ± 20mg/g, respectively. The IC_{50} value of metal chelating, inhibition of lipid peroxidase in egg yolk and reducing power assay was recorded to be 200.27 ± 1.85, 107.6 ± 0.48 and 97.03 ± 0.88 ug/ml, respectively.

Quantification of *Clerodendrum serratum* containing poly herbal formulation by HPTLC and *in vitro* antioxidant activities was carried out by Jayaprakasam, *et al*. (2013). Phan and Nguyen (2014) investigated preliminary

phytochemical analysis of different solvent extracts of *Scoparia dulcis* L. In a study Panda *et al*. (2011) assessed antioxidant properties of extract of areal parts of the same plant species prepared in alcohol solvent using 1,1-diphenyl-2 picryl hydrazyl, nitric oxide, superoxide and hydroxyl radicals. They reported that the extract in a concentration dependent manner exhibited antioxidant properties which may be attributed to the presence of phenol compounds and antioxidant vitamins. The workers also reported that as the free radicals are implicated for many diseases, the antioxidant properties of naturally occurring phytochemicals have gained due importance.

For an anti-arthritic study of *Cocculus hirsutus* methanolic and aqueous root extracts, Bothara *et al*. (2011) used Freund's adjuvant arthritis model in Wistar albino rats. They observed that treatment with the methanolic extract corrected the body weight loss caused by the induced arthritic condition in the right hind paw of the experimental animal.

Savithramma *et al*. (2011) screened fresh leaves of 20 different medicinal plants for secondary metabolites by qualitative phytochemical analysis and reported presence of various secondary metabolites including triterpenoids, steroids, saponins, tannins, phenols, flavonoids, etc.

Nayak and Singhai (2003) examined antimicrobial properties of roots of *Cocculus hirsutus* using ethanloic and petroleum ether extracts. The test microbes *Psedomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* were used to assess antimicrobial activity with agar disc diffusion method. They observed that crude alkaloids fraction and ethanolic extract have significant antimicrobial activity against the test bacteria.

Semwal *et al*. (2010) presented medicinal values of Stephania species a member of the same plant family. They highlighted phytochemical constituents and pharmacology of the plant species with recent developments and scope of research activities of various phytochemicals in future.

Pharmacological and Ethno-medicinal activities of *Sphenocentrum jollyanum* have been presented in a review by Olorunnisola *et al*. (2017). The

species which is native to the tropical forest zones of West Africa used to cure a number of ailments including high B.P., breast tumor, cough, constipation, etc. The medicinal plant is rich source of secondary metabolites which show many biological activities.

Antimicrobial and phytochemical activity of *Synclisia scabrida* n-hexane leaf extract was assessed by Fredrick *et al*. (2013). They reported certain phytochemicals such as flavonoids, saponins, glycosides, alkaloids, etc. in the extract.

Sharma and Singh (2013) demonstrated comparative analysis of the phytochemicals present in different extracts of *Operculina turpethum.*

Chand *et al.* (2014) studied the biochemical profile of *Eichhornia crassipes.* Sharma *et al.* (2012) described the antifungal activity of *Duranta erecta* against some phytopathogenic fungi. Macled (1963) demonstrated several transaminase enzymes in *Cuscuta reflexa*.

Zahin *et al.* (2009) studied *in vitro* antioxidant activity and total phenolic content of four Indian medicinal plants. The percent decrease of 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) standard solution was recorded maximum for *Hemidesmus indicus* (77.0%) followed by *Plumbago zeylanica* (73.41%), *Acorus calamus* (20.88%) and *Holarrhena antidysenterica (*20.06%) extracts at a concentration of 100 g/ml. Phytochemical analysis revealed the presence of major phytocompounds like alkaloids, glycosides, phenolics and saponins. Moreover, total phenolics concentration equivalents to gallic acid was found in the range of 59.50 to 109.0 mg/g of plant extracts, which was correlated with antioxidant activity.

Yadav and Agarwala (2011) investigated the presence of phytochemicals to determine the total phenolic and flavonoid contents of the selected medicinal plants.

Tyagi *et al.* (2010) studied *in vitro* antioxidant activity of methanolic and aqueous extracts of *Flacourtia indica* Merr. Total antioxidant capacity of extract

was found to be 260 g/ml and 180 g/ml ascorbic acid for methanolic and aqueous extracts, respectively. The results indicate that both the extracts clearly have strong antioxidant effects. The freshly prepared extracts were subjected to preliminary phytochemical screening for various phytoconstituents.

Bhandary *et al.* (2012) investigated the presence of various phytochemicals from the ethanolic, aqueous and chloroform extracts of *Punica granatum* peel, whole fruit and seeds. The three different extracts from peel were found to contain triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrates and vitamin C. The three different extracts from whole fruit were found to contain triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrates and vitamin C.

Prasad (2016) described the ethanomedicinal properties of a total of 12 plants used to cure various diseases by tribals and nomadic groups of the Mej river catchment area.

Prasad and Sharma (2018) qualitatively assessed ethanolic extracts of different parts of the *Coccinia indica* and revealed that they contain of primary metabolites such as carbohydrates, proteins and fats and fixed oils.

Prasad and Sharma (2019) assessed of ethanolic extracts of different parts of the *Coccinia indica* for qualitative analysis and revealed that the extracts contain alkaloids, phenols, flavonoids, saponin, terpenoids and glycosides.

The floristic study of south-eastern Rajasthan has been worked out by many authors. Prasad (2018) highlighted plant biodiversity in the Government post graduate college campus Bundi. The study resulted in identification and documentation of 77 plant species belonging to 34 families.

Sharma (1986) has described the taxonomical and phytosociological studies of vegetation of Jhalawar and its environs. Sharma (1999) studied ecology and phytogeography of vegetation of Bundi district with special reference to Pteridophytes. The notable contribution of taxonomic and ethanobotanical work from Haroti plateau includes Gupta (1966), Singh (1979),

Majumdar (1980), Khan (1993), Sharma (2002), Shekhawat (2005), Joshi (2009), Prasad (2014), Sharma and Prasad (2015), Nawar (2015) and Sharma (2017). However, the studies related to present work in study area are not available. Therefore climbers of this area were selected for comparative phytochemical analysis.

CHAPTER 4

FLORISTIC DIVERSITY

AND

METHODOLOGY

The study area harbours rich plant diversity including various medicinal climbers found in the catchment area of the Mej river. Climbers are plants with weak stem so they climb on other plant with help of some special structures to get access of sun light for photosynthesis. On the basis of climbing mechanisms, these plants are further grouped as twining plant, leaf climber, tendril bearers, and root climbers and hook climbers by Darwin (1865).

By definition, a plant which cannot withstand on itself due to weak stem and takes support of other plants or objects to continue its growth and ascending up to trap the solar energy is by and large considered as a climber. There are over 2,500 species of climbing plants worldwide belonging to 90 families. A few families such as Dioscoreaceae, Cucurbitaceae and Vitaceae are represented by almost climbing plants exclusively. Rubiaceae, Fabaceae, Celastraceae and Apocynaceae are some of the climber species rich families with each one representing more than 50 climber species (Gentry, 1991; Schnitzer and Bongers, 2002). It is believed that the climbing habit apparently evolved independently in a diverse array of taxa including the gymnosperms (Gnetaceae), monocots (Palmae) and dicots.

Many climbers grow invariably in and around the Mej river area. These species belong to different ecological habitats of the locality. In a preliminary survey, a total of 27 species of climbers of various plant families have been recorded. The recorded climbers of the area were identified and a list of these plant species is given in table 2. Out of these climbers four were selected for phytochemical analysis namely, *Cayratia trifolia, Coccinia grandis, Cocculus hirsutus* and *Pergularia daemia.*

Table 2 Climbers in and around Mej river

S.No.	Name of Plant Species	Family
1.	*Abrus precatorius* L.	Fabaceae
2.	*Ampelocissus latifolia* (Roxb.) Planch	Vitaceae
3.	*Asparagus racemosus* Willd.	Liliaceae
4.	*Blastania garcinii* (Burm.f.) Cogn	Cucurbitaceae
5.	*Butea superba* Roxb.	Fabaceae
6.	*Cayratia trifolia* (L.) Domain.	Vitaceae
7.	*Celastrus paniculatus* willd.	Celastraceae
8.	*Cissampelos pareira* auct. Non.L	Menispermaceae
9.	*Coccinia grandis* (L.) J.O.Voigt	Cucurbitaceae
10.	*Cocculus hirsutus* (L.) Diels	Menispermaceae
11.	*Cryptolepis buchananii* Roem. & Schult.	Periplocaceae
12.	*Cryptostegia grandiflora* R.Br.	Periplocaceae
13.	*Cuscuta reflexa* Roxb.	Cuscutaceae
14.	*Hemidesmus indicus* (L.) R.Br.	Periplocaceae
15.	*Ichnocarpus frutescens* (L.) R.Br	Apocynaceae
16.	*Ipomoea nil* (L.) Roth	Convolvulaceae

17.	*Ipomoea pes-tigridis* L.	Convolvulaceae
18.	*Ipomoea sindica* Stapf.	Convolvulaceae
19.	*Ipomoea sinensii* (Desr.) Choisy	Convolvulaceae
20.	*Maerua arenaria* (DC.) Hook.f.&Thoms.	Capparaceae
21.	*Mucuna pruriens* (L.) DC	Fabaceae
22.	*Pergularia daemia* [Forsk.] Chiov	Asclepiadaceae
23.	*Pueraria tuberosa* [Roxb.ex Willd.] DC.	Fabaceae
24.	*Quamoclit phoenicea* (Roxb.) choisy	Convolvulaceae
25.	*Rhynochosia minima* (L.) DC.	Fabaceae
26.	*Tinospora cordifolia* (Willd.) Miers.	Menispermacaceae
27.	*Trichosanthes bracteata* [Lam.]Voigt	Cucurbitaceae

SYSTEMATIC ENUMERATION OF SELECTED TAXA

1. *Cayratia trifolia* (L.) Domain

Cayratia trifolia (L.) Domin in Biblioth. Bot. 89: 371. 1927. *Vitis trifolia* L. Sp. Pl. 203. 1753; Duthie, Fl.Gangetic Plain 1:174.1903.*Cissus carnosa* Lam. Encycl. 1: 31. 1789. *Vitis carnosa* (Lam.) Wight & Arn. Prodr.127.1834; Wight, Ic. 1: t. 171. 1839; Lawson in Hook. f. Fl. Brit. India 1: 654. 1875. *Cayratia carnosa* (Lam.) Gagnep. in Not. Syst. 1: 347. 1911. *'Char'* (Hindi).

Family: Vitaceae

Description: Slender, herbaceous climbers, somewhat woody at base. Leaflets ovate-elliptic or obovate, thinly pubescent, glaucous green. Flowers in 4-6 cm broad, branched cymes, greenish white. Berries obovoid-globose, shining, dark-purple to black.

Flowering: July - September

Fruiting: September - December.

Occurrence: Common in wastelands, outskirts of forests, boundaries of fields and Farms.

2. *Coccinia grandis* [L.] J.O. Voigt

Coccinia grandis (L.) J.O. Voigt, Hort. Suburb. Calc. 59. 1845; Chakravarty in Fl. India Fasc. 11: 24. f. 1-9. 1982. *Bryonia grandis* L. Mant. Pl. 1: 126. 1767. *Coccinia indica* Wight & Arn. Prodr. 347. 1834; Duthie, Fl. Gangetic Plain 1: 376. 1903. *Cephalandra indica* (Wt. & Arn.) Naud. in Ann. Sci. Nat. ser. 5.5: 16. 1856; Clarke in Hook.f. Fl. Brit. India 2: 621. 1879, *excl. syn. 'Kundru'* (Hindi).

Family: Cucurbitaceae

Description: Perennial, scandent, dioecious herbs. Leaves 3-10cm long, entire to palmately lobed, bright green above, with few glistering glands beneath, margins minutely denticulate. Petioles 1.5 cm long. Male flowers white, pedunculate. Female flowers pedunculate; ovary 10.0-12.5x 2.0-3.5

mm, stigma c. 5 mm long, densely papillose. Fruits 2.5 - 5.0 x 2-3 cm, subglabrous, fusiform-ellipsoidal, streaked with white when immature, bright scarlet and fleshy when mature. Seeds 5-7 x 2.5-3.5mm, oblong, compressed, rounded at the apex, notched at the base, yellowish.

Flowering and Fruiting: Almost throughout the year.

Occurrence: Most common climber on the hedges of fields among clumps of trees and shrubs in wastelands and outskirts of forests.

3. *Cocculus hirsutus* [L.] Diels

Cocculus hirsutus (L.) Diels in Engl. Pflanzenr. 46: 236. 1910. *Menispermum hirsutum* L. Sp. Pl. 341. 1753. *Cocculus villosus* DC.Syst. Nat. 1: 525. 1817; Hook. F & Thomas. In Hook.f. Fl. Brit. India 1: 101. 1872; Duthie, Fl. Gangetic Plain 1: 28: 1903. "*Chhireta, Bajar-bel"* (Hindi).

Family: Menispermaceae

Description: Scandent, dioecious shrubs; younger parts softly and densely villous. Leaves alternate, 1.5-8.0 x 0.7-4.5 cm, ovate-oblong, cordate-lanceolate or subdeltoid. Flowers minute, yellowish green. Male flowers in small axillary, cymose panicles; sepals 6 in 2 series; petals 6, membranous, emarginate, embracing the stamens. Female flowers in axillary clusters, rarely racemose. Drupes smooth, reddish purple. Seeds transversely rugose, black or dark purple.

Flowering and Fruiting: September - April.

Occurrence: Common on the frings of forests and in wastelands.

4. *Pergularia daemia* [Forssk.] Chiov.

Pergularia daemia (Forsk.) Chiov. In result. Sci. Miss. Stefan.-Paoli Somal. Ital. 1:115 1916. *Asclepias daemia* Forsk. Fl. Aegypt.–Arab. 51. 1775. *Cynanchum extensum* Jacq. Misc. Aust. Bot. 2:353.1781.*Daemia extensa* (Jacq.) R. Br. In Mem.Wern. Soc. 1 : 50. 1811; Wight, Ic. 2[3]: 7. t. 596. 1842 ; Hook. f. Fl. Brit. India 4: 20. 1883; Duthie, fl. Gangetic Plain 2:52. 1911. '*Gandaria ki bel'* (Hindi).

Family: Asclepiadeaceae

Description: Hispid, twining undershrubs. Leaves 3 - 9 x 2 5 – 6 0 cm, ovate, acuminate, cordate at base. Flowers in drooping, lateral cormbosecymes, pale yellowish green. Basal staminal corona of 5–lobed membrane; upper of 5, laterally compresed lobes adnate to the anthers. Follicles in pairs, 5-8 x 1.0–1.5cm, reflexed, slightly curved, softly echinate all over. Seeds 7 x 5 mm, Ovate, truncate at apex, dentate- margined, densely valvety pubescent; coma 3-4 cm long. White, hairy.

Flowering and Fruiting: March – December.

Occurrence: Common among bushes on the edges of fields, fringes of forests and wastelands.

COLLECTION OF PLANT MATERIALS

The Study area was regularly visited on seasonal basis many times during the study period, especially during winter (Dec-Jan), summer (April-May) and rainy season (Aug-Sept). Selected Climbers were collected from the catchment area of the Mej River, during the visits. The collected plant species were duly identified, with the help of standard literature such as Flora of Rajasthan (*Shetty* and Singh, vol I-III 1987-1993) and confirmed by Botanical survey of India, Jodhpur centre (Hebarium sheets and certificate attached). The Mej river catchment area was surveyed during 2015 to 2018 for documentation of climbing angiospermic floristic diversity.

PREPARATION OF HERBARIUM

The herbarium sheets were prepared according to the standard method suggested by Jain and Rao (1977). The prepared herbarium sheets of all the climbers were preserved and deported in the Herbarium Chamber Department of Botany Government College Bundi. All the observed plant species were recorded according to their systematic position and vernacular names. These climbers were kept as herbarium specimen for future reference.

PLATE - 13 HERBARIUM (*CAYRATIA TRIFOLIA*)

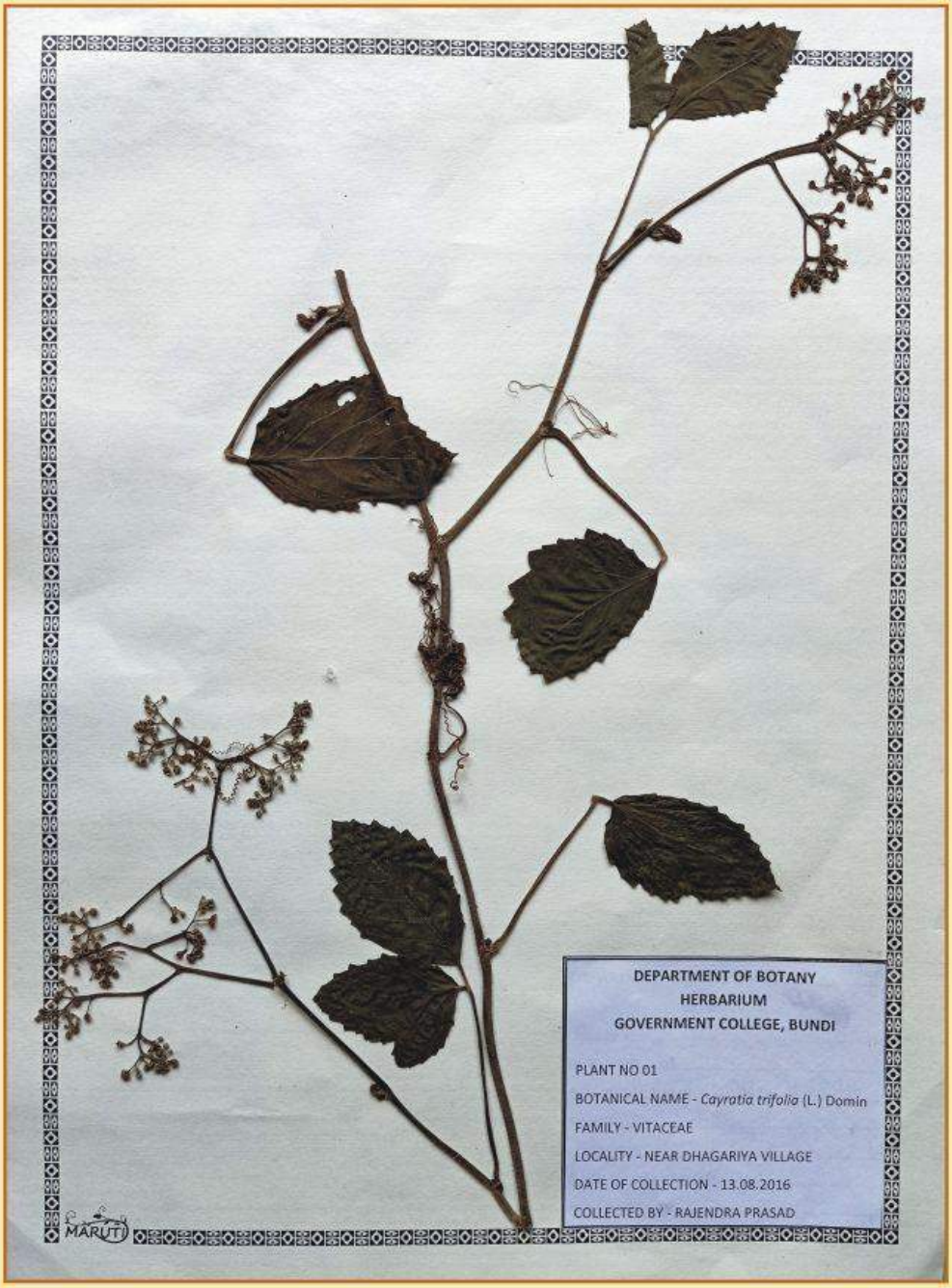


PLATE - 14 HERBARIUM (*COCCINIA GRANDIS*)

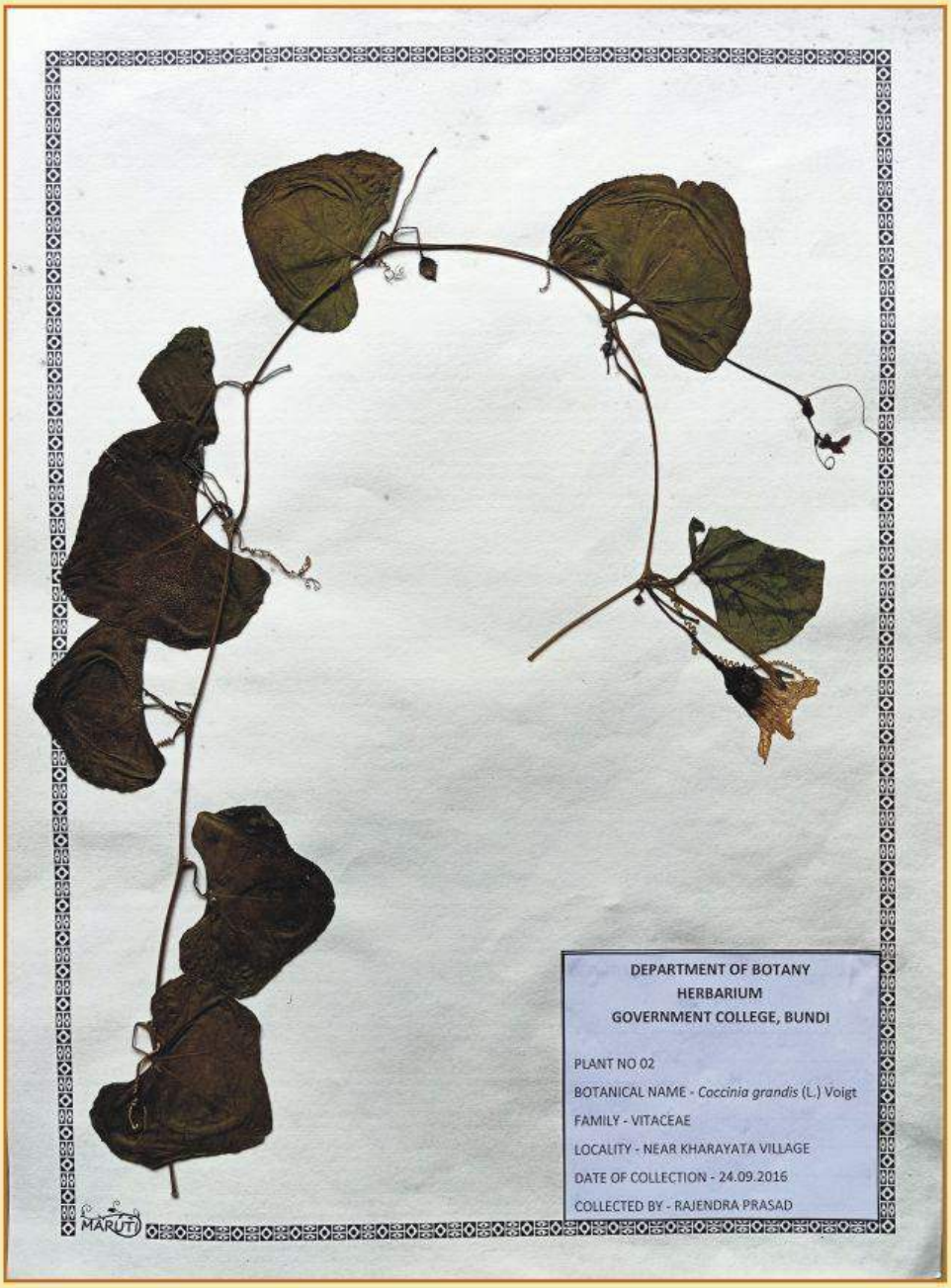


PLATE - 15 HERBARIUM (*COCCULUS HIRSUTUS*)

PLATE - 16 HERBARIUM (*PERGULARIA DAEMIA*)

PLATE - 17 IDENTIFICATION CERTIFICATE ISSUED BY BOTANICAL SURVEY OF INDIA

भारत सरकार / Government of India

पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय / Ministry of Environment Forests & Climate change

भारतीय वनस्पति सर्वेक्षण / Botanical Survey of India

शुष्क अंचल क्षेत्रीय केंद्र / Arid Zone Regional Centre

खेने का कुआँ के पास, पाल–बासनी केनाल लिंक रोड / Near Kheme Ka Kuan, Pal-Basni Canal Link Road

सुभाष नगर–II, पी.ओ.– पाल/ Subhash Nagar-II, P.O.- Pal

जोधपुर –342008(राजस्थान) /Jodhpur-342008 (Raj.)

No.: BSI /AZRC / I.12012/ Tech. / 2019-20- (Pl. Id.)/259 Date: 14 / 08 / 2019

CERTIFICATE

This is to certify that the plant specimens brought to this Regional Centre by **Mr. Rajandra Prasad Meena,** Assistant Professor, Botany, Government College, Bundi, Rajasthan are identified as follows:

Sr. No.	Field No. / Specimen No.	Botanical Name	Family
1.	01	*Cayratia trifolia* (L.) Domin	Vitaceae
2.	02	*Coccinia grandis* (L.) Voigt	Cucurbitaceae
3.	03	*Cocculus hirsutus* (L.) W.Theob.	Menispermaceae
4.	04	*Pergularia daemia* (Forssk.) Chiov.	Asclepiadaceae

(यह सत्यापित किया जता है कि श्री राजेंद्र प्रसाद मीणा, असिस्टेंट प्रोफेसर, वनस्पति विज्ञान, गवर्नमेंट कॉलेज, बूंदी, राजस्थान, के पादप पहचान किया ।)

(Vinod Maina)

Scientist-D & H. o.O.

वैज्ञानिक "डी" एवं कार्यालय अध्यक्ष
Scientist "D" & Head of Office
भारतीय वनस्पति सर्वेक्षण (शु.अ.क्षे.के.)
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PHYTOCHEMICAL STUDIES

Selected plants were evaluated for their phytochemical profiling. Various parameters included in the study were qualitative and quantative methods for primary and secondary metabolites. Quantitative methods included estimation of total carbohydrates for primary metabolites and estimation of total phenols for secondary metabolites. Qualitative methods were used for detection of carbohydrates, reducing sugar, proteins, fats & fixed oils, alkaloids, phenols, flavonoids, glycosides, saponins, and terpenoids.

CHEMICALS

All the chemicals used in the analytical methods and reagent preparation were of analytical grade with maximum available purity. mercuric chloride, potassium iodide, iodine, picric acid, hydrocholric acid, acetone, ethanol, a-naphthol (1-naphthol), sulfuric acid, copper sulphate, potassium sodium tartarate, sodium hydroxide, ammonium hydroxide, phenophthalein, pyridine, sodium nitroprusside, sodium hydroxide, lead acetate, ferric chloride, potassium hydroxide pellets, mercury, fuming nitric acid, chloroform, catechol, folin ciocalteau reagent, phenol, potassium sodium tartrate (rochelle salt), sodium carbonate have been used in present investigation.

INSTRUMENTS

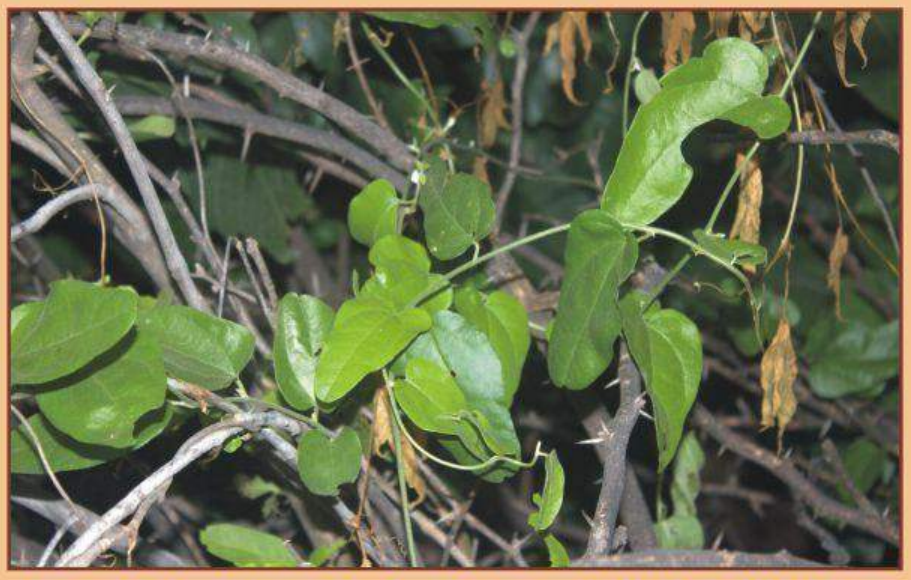
Apparatus and instruments used in different analylitical methods are as follows-Soxhlet apparatus, Water bath, Test tube shaker, Digital balance (Minimum .01gm), Centrifuge, Hot air oven, Electric mixer grinder, Refrigerator, etc.

PREPARATION OF PLANT EXTRACT

Freshly collected leaves, stems and fruits of the four climbers were washed thoroughly under tap water and were dried in hot air oven at 40-50º c for a week. The dired leaves, stems and fruits of each climber were pulverized, using a sterile electric grinder to obtain a fine power. Soxhlet apparatus was used for preparation of extract in this study. 60gm of dried powder was extracted for 24 hours in 300

ml solvent, i.e., ethanol (Raaman, 2006). Repeated extraction was done with the some solvent till colourless solvent was obtained which indicates culmination of the extraction process. The condensed extract was stored as stock and was later used for screening of primary and secondary metabolites. Different extracts were prepared for different parts (leaves, stems and fruits) of the same climber species.

PLATE 20 *Cocculus hirsutus* (L.) W. Theob.

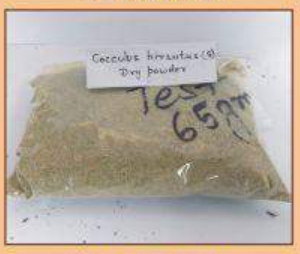


Family- Manispermaceae B.N.- *Cocculus hirsutus* (L.) W. Theob. V.N.- *Chhireta*

DRY LEAVES

DRY STEMS

FRUITS



LEAF POWDER

STEM POWDER

FRUIT POWDER

LEAF EXTRACT

STEM EXTRACT

FRUIT EXTRACT

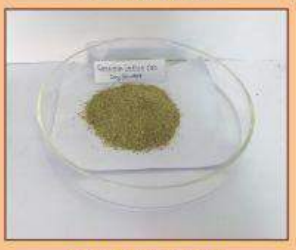
PLATE 19 *Coccinia grandis* (L.) Voigt

Family- ***Cucurbitaceae*** **B.N.-** ***Coccinia grandis*** **(L.) Voigt V.N.-** ***Kandru***

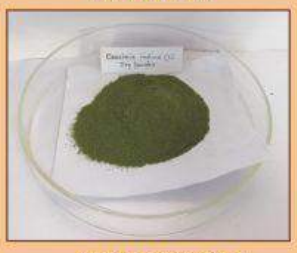
DRY LEAVES

DRY STEMS

FRUITS



LEAF POWDER



STEM POWDER

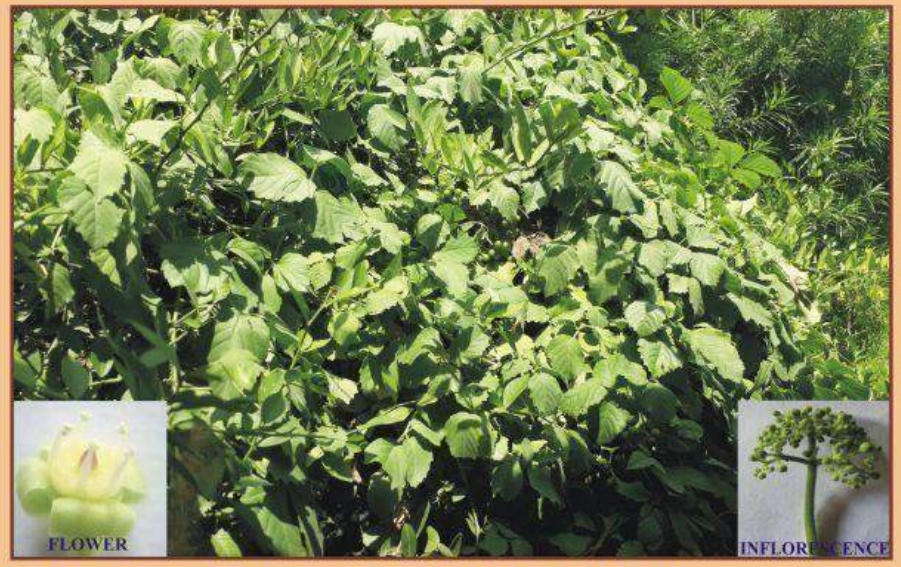
FRUIT POWDER

LEAF EXTRACT

STEM EXTRACT

FRUIT EXTRACT

PLATE 18 *Cayratia trifolia* (L.) Domin



Family- *Vitaceae* B.N.- *Cayratia trifolia* (L.) Domin. V.N.- *Char*

DRY LEAVES

DRY STEMS

FRUITS

LEAF POWDER

STEM POWDER

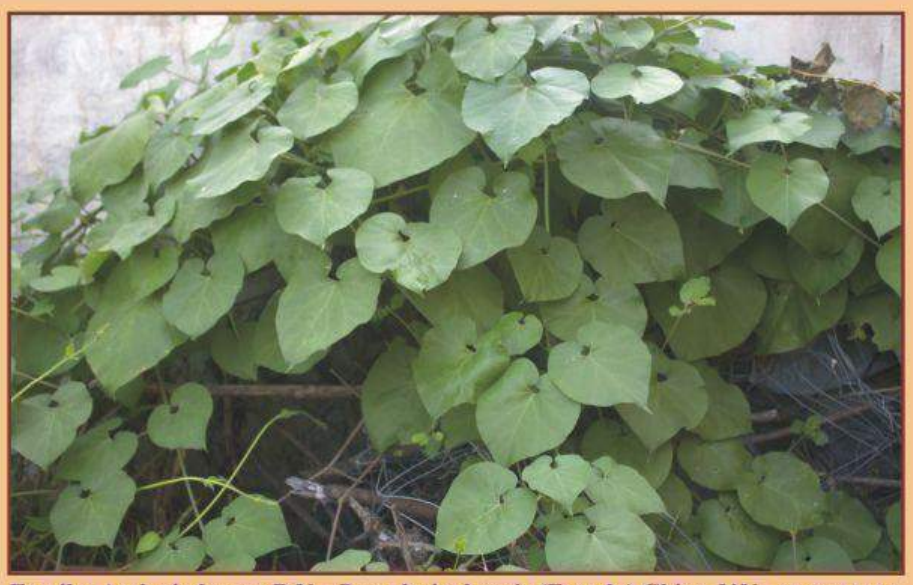
FRUIT POWDER

LEAF EXTRACT

STEM EXTRACT

FRUIT EXTRACT

PLATE 21 *Pergularia daemia* (Forssk.) Chiov.



Family- Asclepiadaceae B.N.- *Pergularia daemia* (Forssk.) Chiov. V.N.- *Gadaria ki bel*

DRY LEAVES

DRY STEMS

FRUITS

DRY POWDER

DRY POWDER

DRY POWDER

LEAF EXTRACT

STEM EXTRACT

FRUIT EXTRACT

QUALITATIVE PHYTOCHEMICAL SCREENING

The extracts of selected plant species were subjected to qualitative chemical investigation to test the presence of various phytochemicals in the ethanolic extracts.

ANALYSIS OF PRIMARY METABOLITES

Tests for Carbohydrates

Molisch's Test

The test was carried out following the method by Ramakrishnan *et al.* (1994). 2 ml of aliquot of the extract was treated with 2 drops of Molisch's reagent. After shaking and holding test tube in slanting position, 2 ml of concentrate sulphuric acid was added along the side of the test tube. The reddish violet ring at the junction of two solutions indicates presence of carbohydrates.

Preparation of Molish's Reagents: - 5gm of α-naphthol dissolved in 100 ml of ethanol.

Test for Reducing Sugars

Benedict's test

The test was carried out following the method by Ramakrishnan *et al.* (1994). 1 ml aliquot of the extract was treated with 3 ml of Benedict's solution and were mixed and heated in boiling water bath for 10 minutes on cooling of the solution an appearance of green, yellow or red precipitate indicates the presence of reducing sugars.

Preparation of Benedict's reagent: -

Sodium citrate (173 gm) and sodium carbonate (100 gm) dissolved in 800 ml of distilled water and boiled to make a clear solution. Copper sulphate (17.3 gm) dissolved in 100 ml distilled water is added to it.

Tests for Proteins

Millon's Test

The test was carried out following the method by Fisher (1968) and Ruthmann (1970). 2 ml of aliquot of the extract was treated with 2 drops of

Millon's reagent in a test tube. A white creamy precipitate appeared which changed to brick red on heating. It indicates the presence of proteins.

Biuret Test

The test was carried out following the method by Gahan (1984). An aliquot of 2 ml of filtrate is treated with few drops of copper sulphate solution. To this, 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Violet or pink colour in the ethanolic layer indicates the presence of proteins.

Test for Fats and Fixed Oils

Saponification Test

The test was carried out following the method by Kokate (1999). A few drops of 0.5 N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

ANALYSIS OF SECONDARY METABOLITES

Tests for Alkaloids

Dried powder, 50 mg was stirred with few ml of dilute hydrochloric acid and the solution was filtered. The filtrate was tested carefully with various alkaloidal reagents as follows:

Mayer's Test

The test was carried out following the method by Evans (1997). An aliquot of 4 ml of filtrate was treated with few drops of Mayer's reagent added by the side of the test tube. An appearance of white or creamy precipitate indicates the test as positive.

Preparation of Mayer's reagent

Mercuric chloride (1.358 gm) is dissolved in 60 ml of water and potassium iodide (5.0 gm) is dissolved separately in 10 ml water. The solutions were mixed and made up to 100 ml with water.

Wagner's Test

The test was carried out following the method by Wagner (1993). 4 ml of aliquot of the extract was treated with 1 ml of Wagner's reagent. A reddish brown precipitate confirms the test as positive.

Preparation of Wagner's reagent:-

Iodine (1.27 gm) and potassium iodide (2 gm) are dissolved in 5 ml of water and made up to 100 ml distilled water.

Hager's Test

The test was carried out by following the method Wagner *et al*. 1996. 4 ml of aliquot of the extract was treated with 1 ml of Hager's reagent. A prominent yellow precipitate indicates the test as positive.

Preparation of Hager's reagent: - 1 gm of picric acid dissolved in 100 ml of water.

Tests for Tannins and Phenols

Ferric Chloride Test

The test was carried out following the method by Mace (1963). An aliquot of 3 ml of filtrate was treated with 1ml of 5% ferric chloride solution in a test tube. The Bluish black colour in the ethanolic layer indicates the presence of tannins and phenols.

Lead Acetate Test

An aliquot of 3 ml of filtrate was treated with 1 ml of 10% lead acetate solution. A bulky white precipitate indicates the presence of phenolic compound.

Test for Flavonoids

Alkaline Test

An aliquot of 3 ml of filtrate was treated with few drops of 10% sodium hydroxide solution. Formations of intense yellow colour, which disappears on further addition of dilute acid, indicates the presence of flavonoids or an aliquot of 3 ml of filtrate was treated with few drops of 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Test of Saponins

Froth Test

1ml ethanolic aliquot mixed with, 50 mg sodium carbonate and 1.5 ml distilled water added to the mixture, shaken vigorously up to 5 minutes. Formation of honey comb like froth shows the presence of saponins. The froth is stable for 15 minutes for a positive result.

Test of Terpenoids

Horizon's Test

1 ml of ethanolic extract was added to 2 ml of trichloroacetic acid. The formation of yellow to red precipitate shows the presence of terpenoids.

Test of Glycosides

Legal Test

1 ml of peridine and 1ml of sodium nitroprusside were added to 2ml of extract. The change in colour to pink or red indicates the presence of glycosides.

QUANTATIVE PHYTOCHEMICAL SCREENING

ESTIMATION OF TOTAL CARBOHYDRATES

Phenol-sulfuric acid method is the easiest and most reliable method among the quantitative assays for carbohydrate estimation (Masuko *et al*. 2005).

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a yellow brown coloured product with phenol and has absorption maximum at 490 nm.

Chemicals

(A) Hydrochloric acid (HCL): 2.5N

(B) Phenol 5%: Redistilled (reagent grade) phenol (50 g) dissolved in water and diluted to one liter.

(C) Sulphuric acid 96% analytical reagent.

(D)Standard Glucose: Stock–100 mg glucose is dissolved in little amount of distilled water and the volume is made upto 100 ml with distilled water. From this solution, 10 ml taken and diluted to make 100 ml with distilled water. The concentration of this solution was 100 ug/ml.

(E) Sodium carbonate

Procedure

(a) Extraction of total carbohydrates from sample

1. 100 mg of sample powder was transferred to a boiling tube.
2. The powder was hydrolysed by keeping it in boiling water bath for 3 hours with 5 ml of 2.5 N-hydrochloric acid.
3. The boiling tube with digested sample was let cool to room temperature and neutralized with solid sodium carbonate until the effervescence ceases.
4. The content of the boiling tube made up to 100 ml with distilled water.
5. The homogenate was transferred to centrifuge tube and centrifuged for 10 minute at room temperature.

6. Thereafter, the supernatant was collected and used for further estimation.

(b) Estimation of total carbohydrates from sample

1. Initially, 0.2, 0.4, 0.6, 0.8 and 1ml aliquots of working standard solution pipetted out into the series of test tubes marked S1, S2, S3, S4 and S5, respectively.
2. Then, 0.5 ml and 1 ml of plant sample was taken into two other test tubes marked T1 and T2, respectively.
3. Contents of all the test tubes made up to 1 ml with distilled water.
4. Anthor test tube marked 'B' with 1 ml of distilled water served as the blank.
5. 1ml of phenol solution added to each test tube.
6. 5 ml of 96% sulphuric acid added to each test tube and shaken well with vortex.
7. After 10 minutes the content in the test tubes were shaken and placed in a water bath at 25-30°C for 20 minutes.
8. When the test tubes were cooled, the absorbance was recorded at 490 nm using spectrophotometer. The absorbance was found to increase with intensity of colour developed in the test tubes, i.e., yellow to brown against the reagent blank.
9. A standard graph was drawn by plotting different concentrations of glucose on x-axis and respective absorbance on the y-axis.
10. Finally, the amount of total carbohydrates in the sample was calculated and expressed as mg glucose equivalents/100 g sample.

(C) **Statistical analysis**

All the experiments of estimation of total carbohydrates content in selected climbers were carried out in triplicates and the data recorded were subjected to mean ± standard deviation with the help of MS Office Excel 2007. Calculation of linear correlation coefficient and correlation analysis were also carried out. The linear regression equation for a straight line is, $Y = mx + c$ where, Y = absorbance of extract, m= slope of the calibration curve, x = concentration of extract, c = intercept. Total

carbohydrates content was calculated with the help of the regression equation, from the calculated values of concentration of each extract.

ESTIMATION OF TOTAL PHENOLS

Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. The simplest form of phenol, i.e., catechol is not largely found and the most abundant phenolic compound is hydroquinone.

Principle

In alkaline medium, phenols react with folin-ciocalteu phenol reagent and give rise to blue coloure which is measured by spectrophotometer.

Chemicals

(A) Ethanol 80% in distilled water (W/V)

(B) folin-ciocalteu phenol reagent: 1N

(C) Standard phenol solution: 100 mg catechol is dissolved in little amount of distilled water and the volume is made upto 100 ml with distilled water. From this solution, 10 ml taken and diluted to make 100 ml with distilled water. The concentration of this solution will be 100ug/ml.

(D) Sodium carbonate solution: 20% in distilled water (W/V)

(a) Extraction of total phenols from sample

1. 1 gm of sample powder ground with a pestle and mortar in 8 to 10 time volume of 80% ethanol.
2. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was preserved and the residue rectified with five times the volume of 80% ethanol. the ethanol was evaporated in a water bath
3. The residue was dissolved in distilled water and made up to a 100 ml stock solution.

(b) Estimation of total phenols from sample

1. Initially, 0.2, 0.4, 0.6, 0.8 and 1ml aliquots of working standard solution pipetted out into the series of test tubes marked S1, S2, S3, S4 and S5, respectively.
2. Then 0.5 ml and 1 ml of plant extract of sample was taken into two other test tubes marked T1 and T2, respectively.

3. Contents of all the test tubes were made up to 1 ml with distilled water.
4. Anthor test tube marked 'B' with 1 ml of distilled water served as the blank.
5. 0.5 ml of folin-ciocalteu phenol reagent was added to each test tube.
6. After 3 minutes, 2 ml of 20% sodium carbonate solution was added to each test tube.
7. After mixing thoroughly the test tubes were placed in boiling water for exactly one minute. After cooling, the absorbance was recorded at 650 nm using spectrophotometer. The absorbance was found to increase with intensity of colour developed in the test tubes, i.e., light blue to dark blue against the reagent blank.
8. A standard graph drawn by plotting different concentrations of catechol on x-axis and respective absorbance on y-axis.
9. Finally, the amount of total phenols in the sample was calculated and expressed as mg catechol equivalents/100 g sample. The total phenolic contents were expressed in mg of catechol equivalents (GAE)/g dry weight of extract (Bray and Thorpe, 1954).

(c) Statistical analysis

All the experiments of estimation of total phenols content in selected climbers were carried out in triplicates and the data recorded were subjected to mean ± standard deviation with the help of MS Office Excel 2007. Calculation of linear correlation coefficient and correlation analysis were carried out. The linear regression equation for a straight line is, $Y = mx + c$ where, Y = absorbance of extract, m= slope of the calibration curve, x = concentration of extract, c = intercept. Total phenols content was calculated with the help of the regression equation, from the calculated values of concentration of each extract.

Standard Deviation (SD)

It was calculated according to the following formula:

$$\mathrm{SD} = \sqrt{\frac{\sum |x - \bar{x}|^2}{n}}$$

Whaere,

SD = Sample standard deviation

Σ = Sum of

$\bar{x}$ = Sample mean

n = Number of scores in sample

CHAPTER 5

PHYTOCHEMICAL STUDY

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Various tests have been conducted for qualitative analysis of selected climber species. The results of these tests have been presented under primary and secondary metabolites headings.

1. *Cayratia trifolia* (L.) Domin

I. PRIMARY METABOLITES

Table 3 exhibits the results of different tests for qualitative analysis to ascertain presence of primary metabolites (carbohydrates, reducing sugar, proteins and Fats and fixed oils) in leaf, fruit and stem extracts of *Cayratia trifolia*.

(1) Carbohydrates

Molisch's test for carbohydrates showed the higher degree of precipitation **(+++)** in leaf and fruit extract whereas lesser degree of precipitation **(+)** has been recorded in the stem extract.

Benedict's test showed the presence of reducing sugars with higher degree of precipitation **(+++)** in the fruit extract. However, the same test showed moderate degree of precipitation **(++)** for leaf extract and lesser degree of precipitation **(+)** in stem extract.

(2) Proteins

All of three extract namely, leaf, stem and fruit extracts showed higher degree of precipitation **(+++)** of protein content in Millon's test, whereas results of Biuret test confirmed varied degree of precipitation in different parts of the same plant species ranging from moderate to higher degree of precipitation.

(3) Fats and Fixed Oils

Saponification test indicates the presence of fats and fixed oils with higher degree of precipitation **(+++)** in fruit extract whereas the same test resulted in lesser degree of precipitation **(+)** for the leaf and stem extract of *Cayratia trifolia*.

II. SECONDARY METABOLITES

(1) Alkaloids

Hager's test reveals the presence of alkaloids with higher degree of precipitation **(+++)** in leaf and stem extract of *Cayratia trifolia* whereas the same test resulted in lesser degree of precipitation **(+)** for the fruit extract. Leaf, stem, and fruit extracts showed the presence of alkaloids with lesser degree of precipitation **(+)** when the extracts were subjected to Mayer's and Wagner's test. (Table 4)

(2) Phenols and Tannins

The preliminary analysis of *Cayratia trifolia* leaf, stem and fruit extracts revealed the presence of phenols and tannins with high degree of precipitation **(+++)** for ferric chloride test and lead acetate test (Table 4).

(3) Flavonoids

Flavonoids analysis by sodium hydroxide test and alkaline reagent test resulted in high degree of precipitation **(+++)** in the fruit, stem and leaf extracts.

(4) Saponins

The phytochemical observations on the basis of froth test showed that this climber species contains saponins in all three extracts. However, the fruit extract has been recorded to possess more saponin which is evident by higher degree of precipitation **(+++)** as compared to leaf and stem extracts that showed moderate degree of precipitation (++) (Table 4).

(5) Terpenoids

Qualitative analysis of terpenoids by Horizon test resulted in higher degree of precipitation **(+++)** for the fruit extract of *Cayratia trifolia*. However, the same test when used to assess presence of terpenoids in leaf and stem extract of the same plant species resulted in lesser degree of precipitation **(+)** (Table 4).

(6) Glycosides

The Table 4 also shows that Legal test applied to reveal presence of glycosides resulted in lesser (+), moderate (++) and higher degree of precipitation **(+++)** for the stem, leaf and fruit extracts, respectively.

Table 3 Qualitative analysis for primary metabolites in alcoholic leaf, stem and fruit extracts of *Cayratia trifolia*

S.No.	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Carbohydrates	Molisch's Test	Leaf	+++
			Stem	+
			Fruit	+++
2.	Reducing sugars	Benedict's Test	Leaf	++
			Stem	+
			Fruit	+++
3	Proteins	Millon's Test	Leaf	+++
			Stem	+++
			Fruit	+++
		Biuret Test	Leaf	+++
			Stem	++
			Fruit	+++
4	Fats and Fixed Oils	Saponification Test	Leaf	+
			Stem	+
			Fruit	+++

Table 4 Qualitative analysis for secondary metabolites in alcoholic leaf, stem and fruit extracts of *Cayratia trifolia*

S.No.	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Alkaloids	Mayer's Test	Leaf	+
			Stem	+
			Fruit	+
		Wagner' Test	Leaf	+
			Stem	+
			Fruit	+
		Hager's test	Leaf	+++
			Stem	+++
			Fruit	+
2	Phenols & Tannins	Ferric Chloride Test	Leaf	+++
			Stem	+++
			Fruit	+++
		Lead Acetate Test	Leaf	+++
			Stem	+++
			Fruit	+++
3	Flavonoids	Sodium Hydroxide Test	Leaf	+++
			Stem	+++
			Fruit	+++
		Alkaline Reagent Test	Leaf	+++
			Stem	+++
			Fruit	+++
4	Saponins	Froth Test	Leaf	++
			Stem	++
			Fruit	+++
5	Terpenoids	Horizon Test	Leaf	+
			Stem	+
			Fruit	+++
6.	Glycosides	Legal Test	Leaf	++
			Stem	+
			Fruit	+++

2. *Coccinia grandis* (L.) Voigt

I. PRIMARY METABOLITES

Table 5 shows the results of qualitative analysis of primary metabolites namely, carbohydrates, proteins and fats and fixed oils in fruit, stem and leaf extracts of *Coccinia grandis*.

(1) Carbohydrates

The Molisch's test for leaf, stem and fruit extracts of *Coccinia grandis* reveals the presence of carbohydrates with higher degree of precipitation **(+++)**. In ethanolic leaf extract, Benedict's test for reducing sugar showed higher degree of precipitation **(+++)** in comparison of stem and fruit extracts, which showed lesser degree of precipitation**(+)**.

(2) Proteins

The results of presence of protein content in the leaf stem and fruit extracts are presented in Table 5. It is interesting to note that presence of protein has been ascertained with higher degree of precipitation **(+++)** in all these part's extracts by Millon tests. However, in Biuret test the test resulted in higher degree of precipitation **(+++)** in stem and fruit extracts as compared to the leaf extract which showed lesser degree of precipitation **(+)**.

(3) Fats and Fixed Oils

In stem and fruit extracts, fat and fixed oils showed higher degree of precipitation **(+++)** in saponification test followed by the leaf extract giving moderate degree of precipitation **(++)** (Table 5).

I. SECONDARY METABOLITES

The results of qualitative analysis of the secondary metabolites like alkaloids, phenols, tannins, saponins, terpenoids and glycosides have been presented in table 6.

(1) Alkaloids

Presence of alkaloids contents has been investigated through different tests [Mayer's test, Wagner's test and Hager's test]. The results of Mayer's test and Wagner's test for alkaloids presence revealed the lesser degree of precipitation **(+)** in ethanolic extract of leaves, stem and fruits of *Coccinia grandis*. However, the Hager's test for alkaloids contents varies in degree of precipitation in the different extracts, with higher degree of precipitation **(+++)** in stem and fruit extracts compared to lesser degree of precipitation in the leaf extract (Table 6).

(2) Phenols and Tannins

Ferric chloride test and lead acetate test to ascertain presence of phenol and tannins resulted in the higher degree of precipitation **(+++)** in all plant extracts namely, leaf, stem and fruit (Table 6).

(3) Flavonoids

Presence of flavonoids was assessed on the basis of results of sodium hydroxide test and alkaline reagent test. For fruit extract, sodium hydroxide test revealed higher degree of precipitation **(+++)** followed by lesser degree of precipitation (+) in leaf and stem extracts. However, the alkaline reagent test resulted in lesser degree of precipitation (+) for all the plant extracts (Table 6).

(4) Saponins

Ethanolic leaf, stem and fruit extract of *Coccinia grandis* revealed the presence of saponins with higher degree of precipitation **(+++)** in froth test.

(5) Terpenoids

Horizon test shows higher degree of precipitation **(+++)** of terpenoids in fruit extract and lesser degree of precipitation **(+)** in leaf and stem extract.

(6) Glycosides

Legal test revealed the presence of glycoside with higher degree of precipitation **(+++)** in fruit extract followed by moderate (++) and lesser degree of precipitation **(+)** in leaf and stem extracts, respectively (Table 6).

Table 5 Qualitative analysis for primary metabolites in alcoholic leaf, stem and fruit extracts of *Coccinia grandis*

S.No.	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Carbohydrates	Molisch's Test	Leaf	+++
			Stem	+++
			Fruit	+++
2.	Reducing sugars	Benedict's Test	Leaf	+++
			Stem	+
			Fruit	+
3	Proteins	Millon's Test	Leaf	+++
			Stem	+++
			Fruit	+++
		Biuret Test	Leaf	+
			Stem	+++
			Fruit	+++
4	Fats and Fixed Oils	Saponification Test	Leaf	++
			Stem	+++
			Fruit	+++

Table 6 Qualitative analysis for secondary metabolites in alcoholic leaf, stem and fruit extracts of *Coccinia grandis*

S.No.	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Alkaloids	Mayer's Test	Leaf	+
			Stem	+
			Fruit	+
		Wagner's Test	Leaf	+
			Stem	+
			Fruit	+
		Hager's Test	Leaf	+
			Stem	+++
			Fruit	+++
2	Phenols & Tannins	Ferric Chloride Test	Leaf	+++
			Stem	+++
			Fruit	+++
		Lead Acetate Test	Leaf	+++
			Stem	+++
			Fruit	+++
3	Flavonoids	Sodium Hydroxide Test	Leaf	+
			Stem	+
			Fruit	+++
		Alkaline Reagent Test	Leaf	+
			Stem	+
			Fruit	+
4	Saponins	Froth Test	Leaf	+++
			Stem	+++
			Fruit	+++
5	Terpenoids	Horizon Test	Leaf	+
			Stem	+
			Fruit	+++
6.	Glycosides	Legal Test	Leaf	++
			Stem	+
			Fruit	+++

3. *Cocculus hirsutus* (L.) W.Theob.

I. PRIMARY METABOLITES

Results of qualitative analysis of primary metabolites (carbohydrates, proteins and fats and fixed oils) have been presented in Table 7.

(1) Carbohydrates

The results of the preliminary analysis of *Cocculus hirsutus* show that the primary metabolites are present in fruits, leaves and stems. The Molisch's test to ascertaine presence of carbohydrates for leaf and fruit extracts revealed higher degree of precipitation (+++), whereas the same test for stem extract resulted in moderate degree of precipitation (++).

Benedict's test for reducing sugars showed moderate degree of precipitation (++) in stem and fruit extracts followed by lesser degree of precipitation (+) in leaf extract.

(2) Proteins

Leaf and fruit extracts showed higher degree of precipitation (+++), when subjected to Millon's test. The ethanolic extract of stem however, showed lesser degree of precipitation (+) in the same test. Biuret test resulted in higher degree of precipitation (+++) in all three plant extracts, namely, leaf, stem and fruit.

(3) Fats and Fixed Oils

Saponification test showed the presence of fats and fixed oils with moderate degree of precipitation **(++)** in fruit and leaf of extracts whereas lesser degree of precipitation **(+)** in stem extract has been recorded for the same plant species.

II. SECONDARY METABOLITES

The qualitative phytochemical screening of leaf, stem and fruit extracts of *Cocculus hirsutus* exhibited in Table 8 indicates the presence of multiple phytoconstituents. It shows the presence of alkaloids, phenols flavonoids, saponins, terpenoids, and glycosides in the ethanolic extracts in varied degree of precipitation like copious, slight and moderate in all tested extracts.

(1) Alkaloids

Presence of alkaloids has been investigated through different tests [Mayer's test, Hager's test and Wagner's test]. All three tests for alkaloids revealed the lesser degree of precipitation **(+)** in ethanolic extracts of leaves, stems and fruits of *Cocculus hirsutus*.

(2) Phenols and Tannins

Ferric chloride test and lead acetate test shared similar results as the both tests ascertained the presence of phenols with higher degree of precipitation **(+++)** in leaves and stems extracts compared to lesser degree of precipitation **(+)** in fruit extract.

(3) Flavonoids

Presence of flavonoids contents has been investigated through different tests [sodium hydroxide test and alkaline reagent test]. The results of sodium hydroxide test, for flavonoids revealed the higher degree of precipitation **(+++)** in ethanolic extracts of leaves, and fruits of *Cocculus hirsutus* whereas, the same test resulted in moderate degree of precipitation (++) in stem extract. Alkaline reagent test showed moderate degree of precipitation (++) in leaf and fruit extracts while stem extract showed slight precipitation (+) in the same test.

(4) Saponins

Saponins in stem, leaf and fruit extracts showed higher degree (+++), moderate degree (++) and lesser degree of (+) precipitation, respectively with froth test.

(5) Terpenoids

Presence of terpenoids contents has been investigated through Horizon test. The results of this test revealed lesser degree of precipitation **(+)** in ethanolic leaf, stem and fruit extracts of *Cocculus hirsutus*.

(6) Glycosides

Glycosides in fruit, leaf and stem extracts showed higher degree of (+++), moderate degree (++) and lesser degree (+) of precipitation, respectively with legal test.

Table 7 Qualitative analysis for primary metabolites in alcoholic leaf, stem and fruit extracts of *Cocculus hirsutus*

S.No.	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Carbohydrates	Molisch's Test	Leaf	+++
			Stem	++
			Fruit	+++
2.	Reducing sugars	Benedict's Test	Leaf	+
			Stem	++
			Fruit	++
3	Proteins	Millon's Test	Leaf	+++
			Stem	+
			Fruit	+++
		Biuret Test	Leaf	+++
			Stem	+++
			Fruit	+++
4	Fats and Fixed Oils	Saponification Test	Leaf	++
			Stem	+
			Fruit	++

Table 8 Qualitative analysis for secondary metabolites in alcoholic leaf, stem and fruit extracts of *Cocculus hirsutus*

S.No	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Alkaloids	Mayer's Test	Leaf	+
			Stem	+
			Fruit	+
		Wagner' Test	Leaf	+
			Stem	+
			Fruit	+
		Hager's Test	Leaf	+
			Stem	+
			Fruit	+
2	Phenols & Tannins	Ferric Chloride Test	Leaf	+++
			Stem	+++
			Fruit	+
		Lead Acetate Test	Leaf	+++
			Stem	+++
			Fruit	+
3	Flavonoids	Sodium Hydroxide Test	Leaf	+++
			Stem	++
			Fruit	+++
		Alkaline Reagent test	Leaf	++
			Stem	+
			Fruit	++
4	Saponins	Froth Test	Leaf	++
			Stem	+++
			Fruit	+
5	Terpenoids	Horizon Test	Leaf	+
			Stem	+
			Fruit	+
6.	Glycosides	Legal Test	Leaf	++
			Stem	+
			Fruit	+++

4. *Pergularia daemia* (Forssk.) Chiov.

I. PRIMARY METABOLITES

Table 9 shows results of qualitative analysis of primary metabolites in the ethanolic extracts of three parts of *Pergularia daemia* namely, leaf, stem and fruit.

(1) Carbohydrates

Moderate degree of precipitation (++) showed by leaf and fruit ethanolic extracts of *Pergularia daemia* with Molisch's test for phytochemical screening of carbohydrates whereas stem extract exhibited higher degree of precipitation (+++). Benedict's test showed lesser degree of precipitation (+) with leaf and stem extracts for reducing sugars while the same test resulted in moderate degree of precipitation (++) with the fruit extract.

(2) Proteins

The results of protein content in the leaf stem and fruit extracts are represented in Table 9. It is interesting to note that presence of protein was observed by higher degree of precipitation **(+++)** in all three part's extract when screened through Millon's test and Biuret test.

(3) Fats and Fixed Oils

In leaf and fruit extracts fats and fixed oils showed moderate degree of precipitation **(++)**, followed by the stem extract with lesser degree of precipitation **(+)** in saponification test.

II. SECONDARY METABOLITES

The results of phytochemical screening for important secondary metabolites namely, alkaloids, phenols, tannins, flavonoids, saponins, terpenoids and glycosides are present in Table 10 which shows presence of different phytochemicals ascertained by varied degree of precipitation.

(1) Alkaloids

Among the selected climbers *Pergularia daemia* showed best results for alkaloids. Fruit extract showed higher degree of precipitation (+++) with Mayer's test and stem extract showed higher degree of precipitation (+++) with Wagner's test. Moderate degree of precipitation (++) has been reported for Wagner's test with fruits extract and stem extract with Hager's test. However, lesser degree of precipitation **(+)** was observed in leaf and stem extracts in Mayer's test, leaf extract in Wagner's test as well as leaf and fruit extracts in Hager's test.

(2) Phenols and Tannins

Ferric chloride test and lead acetate test conducted to reveal the presence of phenols resulted in higher degree of precipitation **(+++)** in leaf, stem and fruit extracts for lead acetate test. Whereas ferric chloride test resulted in moderate degree of precipitation (++) in stem extract while leaf and fruit extracts showed higher degree of precipitation **(+++).**

(3) Flavonoids

The higher degree of precipitation **(+++)** was identified in fruit extract for both the sodium hydroxide test and the alkaline reagent test. In the alkaline reagent test the leaf extract presented lesser degree of precipitation (+). Moderate degree of precipitation **(++)** was observed in leaf and stem extracts with sodium hydroxide test.

(4) Saponins

Saponins in fruit, leaf and stem extracts showed higher degree **(+++)**, Moderate degree (++) and lesser degree (+) of precipitation, respectively with froth test.

(5) Terpenoids

The higher degree of precipitation of **(+++)** was observed in leaf and stem extracts in Horizon test whereas fruit extract showed slight (+) presence of the precipitate.

(6) Glycosides

Leaf extract showed higher degree of precipitation (+++), whereas lesser degree of precipitation (+) was exhibited in stem and fruits ethanolic extract in legal test.

Table 9 Qualitative analysis for primary metabolites in alcoholic leaf, stem and fruit extracts of *Pergularia daemia*

S.No	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Carbohydrates	Molisch's Test	Leaf	++
			Stem	+++
			Fruit	++
2.	Reducing sugars	Benedict's Test	Leaf	+
			Stem	+
			Fruit	++
3	Proteins	Millon's Test	Leaf	+++
			Stem	+++
			Fruit	+++
		Biuret Test	Leaf	+++
			Stem	+++
			Fruit	+++
4	Fats and Fixed Oils	Saponification Test	Leaf	++
			Stem	+
			Fruit	++

Table 10 Qualitative analysis for secondary metabolites in alcoholic leaf, stem and fruit extracts of *Pergularia daemia*

S.No	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Alkaloids	Mayer's Test	Leaf	+
			Stem	+
			Fruit	+++
		Wagner's Test	Leaf	+
			Stem	+++
			Fruit	++
		Hager's Test	Leaf	+
			Stem	++
			Fruit	+
2	Phenols & Tannins	Ferric Chloride Test	Leaf	+++
			Stem	++
			Fruit	+++
		Lead Acetate Test	Leaf	+++
			Stem	+++
			Fruit	+++
3	Flavonoids	Sodium Hydroxide Test	Leaf	++
			Stem	++
			Fruit	+++
		Alkaline Reagent Test	Leaf	+
			Stem	+++
			Fruit	+++
4	Saponins	Froth Test	Leaf	++
			Stem	+
			Fruit	+++
5	Terpenoids	Horizon Test	Leaf	+++
			Stem	+++
			Fruit	+
6.	Glycosides	Legal Test	Leaf	+++
			Stem	+
			Fruit	+

PLATE 22
Phytochemical screening of primary metabolites of *Cayratia trifolia*

Detection of Carbohydrates

Molisch's Test - Leaf

Molisch's Test - Stem

Molisch's Test - Fruit

Detection of Reducing Sugars

Benedict's Test - Leaf

Benedict's Test - Stem

Benedict's Test - Fruit

Detection of Proteins

Million's Test - Leaf

Million's Test - Stem

Million's Test - Fruit

Biuret Test - Leaf

Biuret Test - Stem

Biuret Test - Fruit

Detection of Fats and Fixed Oils

Saponification Test -Leaf

Saponification Test -Stem

Saponification Test -Fruit

PLATE 23
Phytochemical screening of secondary metabolites of *Cayratia trifolia*

Detection of Alkaloids

Mayer's Test - Leaf

Mayer's Test - Stem

Mayer's Test - Fruit

Wagner's Test - Leaf

Wagner's Test - Stem

Wagner's Test - Fruit

Hager's Test - Leaf

Hager's Test - Stem

Hager's Test - Fruit

Detection of Phenols

Ferric chloride Test -Leaf

Ferric chloride Test -Stem

Ferric chloride Test -Fruit

Lead acetate Test - Leaf

Lead acetate Test - Stem

Lead acetate Test - Fruit

PLATE 24
Phytochemical screening of secondary metabolites of *Cayratia trifolia*

Detection of Flavonoids

Alkaline Reg. Test -Leaf

Alkaline Reg. Test -Stem

Alkaline Reg. Test -Fruit

Alk. Reg. (NH4OH) Test -Leaf

Alk. Reg. (NH4OH) Test -Stem

Alk. Reg. (NH4OH) Test -Fruit

Detection of Saponins

Foam Test -Leaf

Foam Test -Stem

Foam Test -Fruit

Detection of Terpenoids

Horizon Test - Leaf

Horizon Test - Stem

Horizon Test - Fruit

Detection of Glycosides

Legal Test -Leaf

Legal Test -Stem

Legal Test -Fruit

PLATE 25
Phytochemical screening of primary metabolites of *Coccinia grandis*

Detection of Carbohydrates

Molisch's Test - Leaf

Molisch's Test - Stem

Molisch's Test - Fruit

Detection of Reducing Sugars

Benedict's Test - Leaf

Benedict Test's - Stem

Benedict Test's - Fruit

Detection of Proteins

Million's Test - Leaf

Million's Test - Stem

Million's Test - Fruit

Biuret Test - Leaf

Biuret Test - Stem

Biuret Test - Fruit

Detection of Fats and Fixed Oils

Saponification Test-Leaf

Saponification Test -Stem

Saponification Test-Fruit

PLATE 26
Phytochemical screening of secondary metabolites of *Coccinia grandis*

Detection of Alkaloids

Mayer's Test - Leaf

Mayer's Test - Stem

Mayer's Test- Fruit

Wagner's Test - Leaf

Wagner's Test - Stem

Wagner's Test - Fruit

Hager's Test - Leaf

Hager's Test - Stem

Hager's Test - Fruit

Detection of Phenols

Ferric chloride Test-Leaf

Ferric chloride Test-Stem

Ferric chloride Test-Fruit

Lead acetate Test - Leaf

Lead acetate Test - Stem

Lead acetate Test - Fruit

PLATE 27
Phytochemical screening of Secondary Metabolites of *Coccinia grandis*

Detection of Flavonoids

Alkaline Reg. Test - Leaf

Alkaline Reg. Test - Stem

Alkaline Reg. Test-Fruit

Alk. Reg. (NH4OH) Test - Leaf

Alk. Reg. (NH4OH) Test - Stem

Alk. Reg. (NH4OH) Test - Fruit

Detection of Saponins

Foam Test -Leaf

Foam Test -Stem

Foam Test -Fruit

Detection of Terpenoids

Horizon Test -Leaf

Horizon Test -Stem

Horizon Test -Fruit

Detection of Glycosides

Legal Test -Leaf

Legal Test -Stem

Legal Test -Fruit

PLATE 28
Phytochemical screening of primary metabolites of *Cocculus hirsutus*

Detection of Carbohydrates

Molisch's Test - Leaf

Molisch's Test - Stem

Molisch's Test - Fruit

Detection of Reducing Sugars

Benedict's Test - Leaf

Benedict's Test - Stem

Benedict's Test - Fruit

Detection of Proteins

Million's Test - Leaf

Million's Test - Stem

Million's Test - Fruit

Biuret Test - Leaf

Biuret Test - Stem

Biuret Test - Fruit

Detection of Fats and Fixed Oils

Saponification Test - Leaf

Saponification Test - Stem

Saponification Test - Fruit

PLATE 29
Phytochemical screening of secondary metabolites of *Cocculus hirsutus*

Detection of Alkaloids

Mayer's Test - Leaf

Mayer's Test - Stem

Mayer's Test - Fruit

Wagner's Test - Leaf

Wagner's Test - Stem

Wagner's Test - Fruit

Hager's Test - Leaf

Hager's Test - Stem

Hager's Test - Fruit

Detection of Phenols

Ferric chloride Test -Leaf

Ferric chloride Test -Stem

Ferric chloride Test -Fruit

Lead acetate Test - Leaf

Lead acetate Test - Stem

Lead acetate Test - Fruit

PLATE 30
Phytochemical screening of secondary metabolites of *Cocculus hirsutus*

Detection of Flavonoids

Alkaline Reg. Test -Leaf

Alkaline Reg. Test -Stem

Alkaline Reg. Test -Fruit

Alk. Reg. (NH4OH) Test -Leaf

Alk. Reg. (NH4OH) Test -Stem

Alk. Reg. (NH4OH) Test -Fruit

Detection of Saponins

Foam Test -Leaf

Foam Test -Stem

Foam Test -Fruit

Detection of Terpenoids

Horizon Test -Leaf

Horizon Test -Stem

Horizon Test -Fruit

Detection of Glycosides

Legal Test -Leaf

Legal Test -Stem

Legal Test -Fruit

PLATE 31
Phytochemical screening of primary metabolites of *Pergularia daemia*

Detection of Carbohydrates

Molisch's Test - Leaf

Molisch's Test - Stem

Molisch's Test - Fruit

Detection of Reducing Sugars

Benedict's Test - Leaf

Benedict's Test - Stem

Benedict's Test - Fruit

Detection of Proteins

Million's Test - Leaf

Million's Test - Stem

Million's Test - Fruit

Biuret Test - Leaf

Biuret Test - Stem

Biuret Test - Fruit

Detection of Fats and Fixed Oils

Saponification Test -Leaf

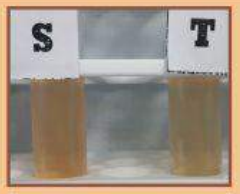
Saponification Test -Stem

Saponification Test -Fruit

PLATE 32
Phytochemical screening of secondary metabolites of *Pergularia daemia*

Detection of Alkaloids

Mayer's Test - Leaf



Mayer's Test - Stem

Mayer's Test - Fruit

Wagner's Test - Leaf

Wagner's Test - Stem

Wagner's Test - Fruit

Hager's Test - Leaf

Hager's Test - Stem

Hager's Test - Fruit

Detection of Phenols

Ferric chloride Test -Leaf

Ferric chloride Test -Stem

Ferric chloride Test -Fruit

Lead acetate Test - Leaf

Lead acetate Test - Stem

Lead acetate Test - Fruit

PLATE 33
Phytochemical screening of secondary metabolites of *Pergularia daemia*

Detection of Flavonoids

Alkaline Reg. Test -Leaf

Alkaline Reg. Test -Stem

Alkaline Reg. Test -Fruit

Alk. Reg. (NH4OH) Test -Leaf

Alk. Reg. (NH4OH) Test -Stem

Alk. Reg. (NH4OH) Test -Fruit

Detection of Saponins

Foam Test -Leaf

Foam Test -Stem

Foam Test -Fruit

Detection of Terpenoids

Horizon Test -Leaf

Horizon Test -Stem

Horizon Test -Fruit

Detection of Glycosides

Legal Test -Leaf

Legal Test -Stem

Legal Test -Fruit

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

In the quantitative phytochemical analysis, total carbohydrates content has been assessed as the primary metabolite whereas total phenol content has been estimated among the secondary metabolites in the three parts, i.e., leaf, stem and fruit of the selected climber species found in the catchment area of the Mej river.

A. PRIMARY METABOLITES

Estimation of total carbohydrates content

Quantitative estimation of the primary metabolite carbohydrates present in the four selected climbers, i.e., *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* and *Pergularia daemia* was done by phenol sulphuric acid method using glucose as the standard. The calibration curve was prepared by mixing distilled water in solution of glucose then added 5% phenol with 5 ml sulphuric acid and the absorbance was measured at 490 nm. The absorbance values obtained at the different concentrations of glucose was used for the construction of the standard calibration curve (Fig. 4).

Total carbohydrate content present in the different parts namely, leaf, stem and fruit of the selected four climbers was calculated with the regression equation of calibration curve $Y=0.011x+0.004$ with a coefficient of $R^2=0.999$ and expressed as g glucose equivalents (GE) per gram of sample in dry weight (mg/g). Experiments were carried out in triplicate for each of the selected three parts of all the four climber species. The results have been presented in Table 11, 13, 15, 17.

In the quantitative analysis of total carbohydrates in the powdered leaves of the selected plant species namely, *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* and *Pergularia daemia,* total, carbohydrate content was recorded to be 96 ± 1.6 mg, 80 ± 1.2 mg, 223 ± 4.24 mg and 69 ± 0.78 mg GE/g sample, respectively. These data exhibit that the maximum concentration of total carbohydrate content (223 ± 4.24 mg/g) has been reported in the powdered leaves of *Cocculus hirsutus* however, the minimum concentration (69 ± 0.78 mg/g) of the same primary metabolites has been reported in the *Pergularia daemia*. Moderate concentrations of total carbohydrate content, i.e., 96 ± 1.6mg, 80 ± 1.2mg have

been reported in powdered leaves of *Cayratia trifolia* and *Coccinia grandis*, respectively (Fig 5).

Similarly, quantitative analysis of total carbohydrates in powdered stems of the four selected plant species namely, *Cayratia trifolia*, *Coccinia grandis, Cocculus hirsutus* and *Pergularia daemia* revealed 155 ± 0.08mg, 150 ± 1.02 mg, 196 ± 3.28 mg and 158 ± 0.86 mg GE/g carbohydrate content, respectively. The data show that the maximum concentration of total carbohydrate content (196 ± 3.28mg) has been reported in the powdered stem of *Cocculus hirsutus* compared to the minimum concentration (150 ± 1.02mg) of the same primary metabolites reported in the stem of *Coccinia grandis.* Moderate level of concentration of total carbohydrate content, i.e., 155 ± 0.08mg and 158 ± 0.86 mg has been reported in powdered stems of *Cayratia trifolia* and *Pergularia daemia*, respectively (Fig 5).

Quantitative analysis of total carbohydrates of powdered fruits of *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* and *Pergularia daemia* revealed total carbohydrate content to be 163 ± 1mg, 151± 0.73mg, and 245 ± 0.26mg and 141 ± 1.13mg GE/g sample, respectively. These data show that the maximum concentration of total carbohydrate content (245 ± 0.26mg) has been reported in the powdered fruits of *Cocculus hirsutus* whereas the minimum concentration (141 ± 1.13 mg) has been reported in the powered stem of *Pergularia daemia.* Moderate concentrations of total carbohydrate content (163 ± 1mg, 151 ± 0.73mg) have been reported in stem powder of remaining two climber species namely, *Cayratia trifolia* and *Coccinia grandis* (Fig 5).

The results clearly indicate that among the studied climbers, *Cocculus hirsutus* possesses maximum concentration of total carbohydrates content, in per gram of leaf, stem and fruit powder, i.e., 223mg, 196mg and 245 mg, respectively.

B. SECONDARY METABOLITES

Estimation of total phenol content

Quantitative analysis of phenol present in four selected climbers, i.e., *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus*, and *Pergularia daemia* was carried out with the folin-ciocalteau reagent method using catechol as the standard. The calibration curve of catechol was prepared in different concentration (10-100 g/ml). The absorbance values obtained at the different concentrations of catechol was used for construction of the standard calibration curve and the amount of phenol was determined and expressed as mg CE (catechol equivalent) of the studied climber parts (Fig 6).

Total phenol content of the different parts namely, leaf, stem and fruit of the selected climbers was calculated with the regression equation of calibration curve Y=0.004x-0.01 with a coefficient of R^2=0.998 and expressed as g catechol equivalents (CE) per gram of sample in dry weight (mg/g). Experiments were carried out in triplicates for all three parts of the selected climber species. The results have been presented in Table 12, 14, 16 and 18.

Estimation of total phenol content of powdered leaves of the selected climbers namely, *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* and *Pergularia daemia* revealed 11 ± 0.12mg, 3.86 ± 0.07 mg, and 13 ± 0.1mg and 8.5 ± 0.1 mg CE/g sample total phenol content, respectively. These data show that the maximum concentration of total phenol content (13 ± 0.1mg) has been reported in the powdered leaves of *Cocculus hirsutus* however, the minimum concentration of the same secondary metabolite (3.86 ± 0.07 mg) has been reported in the powered leaves of *Coccinia grandis*. In the powdered leaves of *Cayratia trifolia* and *Pergularia daemia,* total phenol content recorded was 11 ± 0.12mg and 8.5 ± 0.1 mg, respectively (Fig 7).

With the same method quantitative estimation of total phenol in powdered stems of *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* and *Pergularia daemia* revealed 3 ± 0.07mg, 3.76 ± 0.01mg, 5.7 ± 0.1mg and 4.8 ± 0.1 mg CE/g sample total phenol content, respectively. The data show that maximum concentration of total phenol content (5.7 ± 0.1mg) has been reported in the powdered stem of *Cocculus hirsutus* whereas the minimum concentration (3 ±

0.07mg) of the same secondary metabolites has been reported in the stem of *Cayratia trifolia*. Moderate concentrations of total phenol content has been reported in powdered stems of *Coccinia grandis* and *Pergularia daemia*, which have been reported to contain 3.76 ± 0.01mg, 4.8 ± 0.14 mg quantity of the assessed secondary metabolite, respectively (Fig 7).

Quantitative analysis of total phenol content in powdered fruits of *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus*, and *Pergularia daemia* exhibited 18 ± 0.11mg, 5.2 ± 0.02mg, and 4.8 ± 0.5mg and 4.15 ± 0.07 mg GE/g sample, quantity of the total phenol content, respectively. These results show that maximum concentration of total phenol content (18 ± 0.11mg) has been reported in the powdered fruit of *Cayratia trifolia* whereas the minimum concentration (4.15 ± 0.07 mg) of the same phytochemical has been reported in *Pergularia daemia*. The data also reveal that moderate concentrations of total phenol content, i.e., 5.2 ± 0.02mg and 4.8 ± 0.5mg have been reported in powdered stems of *Coccinia grandis* and *Cocculus hirsutus*, respectively (Fig 7).

Table 11 Quantitative analysis for total carbohydrate content in leaf, stem and fruit powder of *Cayratia trifolia*

S.No.	Absorbance (490nm)	Concentration in mg/100mg	Concentration in mg/gram sample
A. Leaf powder			
1.	0.514	9.23	92.3
2.	0.579	10.41	104.1
3.	0.522	9.38	93.8
	Mean	9.67	96.7
	S. D.	0.52	5.2
	S.E.	0.16	1.6
	Result	**9.6 ± .16**	**96 ± 1.6**
B. Stem powder			
1.	0.864	15.58	155.8
2.	0.861	15.52	155.2
3.	0.864	15.58	155.8
	Mean	15.56	155.6
	S. D.	0.025	0.254
	S.E.	.008	.08
	Result	**15.56 ± .008**	**155 ± .08**
C. Fruit powder			
1.	0.886	15.9	159
2.	0.915	16.50	165.04
3.	0.922	16.63	166.3
	Mean	16.3	163
	S. D.	0.319	3.19
	S.E.	0.10	1
	Result	**16.34 ± .10**	**163 ± 1**

S.D. = Standard deviation
S.E. = Standard error

Table 12 Quantitative analysis for total phenol content in leaf, stem and fruit powder of *Cayratia trifolia*

S.No.	Absorbance (650nm)	Concentration in mg/100mg	Concentration in mg/gram sample
A. Leaf powder			
1.	0.115	1.10	11.05
2.	0.11	1.06	10.6
3.	0.121	1.15	11.5
	Mean	1.10	11.0
	S. D.	0.039	0.39
	S.E.	0.01	0.1
	Result	**1.15 ± .01**	**11 ± .12**
B. Stem powder			
1.	0.02	0.26	2.68
2.	0.024	0.30	3.03
3.	0.027	0.32	3.29
	Mean	0.30	3.00
	S. D.	0.02	0.25
	S.E.	0.007	0.07
	Result	**.30 ± .15**	**3 ± .07**
C. Fruit powder			
1.	0.21	1.94	19.4
2.	0.2	1.85	18.5
3.	0.205	1.89	18.9
	Mean	1.89	18.9
	S. D.	0.036	0.36
	S.E.	0.011	0.114
	Result	**1.8 ± .01**	**18 ± .11**

S.D. = Standard deviation
S.E. = Standard error

Table 13 Quantitative analysis for total carbohydrate content in leaf, stem and fruit of powder of *Coccinia grandis*

S.No.	Absorbance (490nm)	Concentration in mg/100mg	Concentration in mg/gram sample
A. Leaf powder			
1.	0.432	7.75	77.5
2.	0.478	8.58	85.8
3.	0.43	7.71	77.1
	Mean	8.01	80.1
	S. D.	0.40	4.02
	S.E.	0.12	1.27
	Result	**8 ± .12**	**80 ± 1.2**
B. Stem powder			
1.	0.822	14.8	148
2.	0.828	14.9	149
3.	0.862	15.5	155
	Mean	15.0	150
	S. D.	0.319	3.19
	S.E.	0.101	1.01
	Result	**15.09 ± .10**	**150 ± 1.02**
C. Fruit powder			
1.	0.863	15.5	155
2.	0.831	14.9	149
3.	0.827	14.9	149
	Mean	15.1	151
	S. D.	0.291	2.91
	S.E.	0.092	0.92
	Result	**15.17 ± .10**	**151 ± .73**

S.D. = Standard deviation
S.E. = Standard error

Table 14 Quantitative analysis for total phenol content in leaf, stem and fruit powder of *Coccinia grandis*

S.No.	Absorbance (650nm)	Concentration in mg/100mg	Concentration in mg/gram sample
A. Leaf powder			
1.	0.03	0.35	3.5
2.	0.037	0.41	4.1
3.	0.034	0.39	3.9
	Mean	0.38	3.8
	S. D.	0.02	0.2
	S.E.	0.007	0.07
	Result	**.38 ± .14**	**3.86 ± .07**
B. Stem powder			
1.	0.032	0.37	3.7
2.	0.033	0.38	3.8
3.	0.032	0.37	3.7
	Mean	0.37	3.7
	S. D.	0.004	0.04
	S.E.	0.001	0.013
	Result	**.37 ± .02**	**3.76 ± .01**
C. Fruit powder			
1.	0.051	0.54	5.4
2.	0.049	0.52	5.2
3.	0.049	0.52	5.2
	Mean	0.52	5.2
	S. D.	0.008	0.08
	S.E.	0.002	0.026
	Result	**.52 ± .05**	**5.2 ± .02**

S.D. = Standard deviation
S.E. = Standard error

Table 15 Quantitative analysis for total carbohydrate content in leaf, stem and fruit powder of *Cocculus hirsutus*

S.No.	Absorbance (490nm)	Concentration in mg/100mg	Concentration in mg/gram sample
A. Leaf powder			
1.	0.124	21.6	216.6
2.	0.121	21.12	211.2
3.	0.138	24.2	242
	Mean	22.3	223
	S. D.	1.34	13.4
	S.E.	0.424	4.24
	Result	**22.4 ± .42**	**223 ± 4.24**
B. Stem powder			
1.	0.121	21.12	211.2
2.	0.108	18.76	187.6
3.	0.11	19.12	191.2
	Mean	19.6	196
	S. D.	1.03	10.3
	S.E.	0.328	3.28
	Result	**19.6 ± .32**	**196 ± 3.28**
C. Fruit powder			
1.	0.139	24.38	243.8
2.	0.14	24.56	245.6
3.	0.14	24.56	245.6
	Mean	24.5	245
	S. D.	0.084	0.848
	S.E.	0.0268	0.26
	Result	**24.5 ± .02**	**245 ± .26**

S.D. = Standard deviation
S.E. = Standard error

Table 16 Quantitative analysis for total phenol content in leaf, stem and fruit powder of *Cocculus hirsutus*

S.No.	Absorbance (650nm)	Concentration in mg/100mg	Concentration in mg/gram sample
A. Leaf powder			
1.	0.14	1.32	13.2
2.	0.13	1.23	12.3
3.	0.134	1.27	12.7
	Mean	1.27	12.7
	S. D.	0.036	0.36
	S.E.	0.011	0.11
	Result	**1.27 ± .01**	**13 ± .1**
B. Stem powder			
1.	0.06	0.62	6.2
2.	0.058	0.60	6.0
3.	0.047	0.50	5.06
	Mean	0.57	5.7
	S. D.	0.05	0.50
	S.E.	0.015	0.159
	Result	**.57 ± .01**	**5.7 ± .1**
C. Fruit powder			
1.	0.029	0.34	3.4
2.	0.029	0.35	3.5
3.	0.074	0.74	7.4
	Mean	0.48	4.8
	S. D.	0.185	1.8
	S.E.	0.058	0.585
	Result	**.48 ± .05**	**4.8 ± .5**

S.D. = Standard deviation
S.E. = Standard error

Table 17 Quantitative analysis for total carbohydrate content in leaf, stem and fruit powder of *Pergularia daemia*

S.No.	Absorbance (490nm)	Concentration in mg/100mg	Concentration in mg/gram sample
A. Leaf powder			
1.	0.374	6.69	66.9
2.	0.378	6.77	67.7
3.	0.405	7.26	72.6
	Mean	6.90	69.09
	S. D.	0.24	2.49
	S.E.	0.078	0.78
	Result	**6.9 ± .07**	**69 ± .78**
B. Stem powder			
1.	0.859	15.49	154.9
2.	0.876	15.79	157.9
3.	0.896	16.16	161.6
	Mean	15.81	158.1
	S. D.	0.273	2.73
	S.E.	0.086	0.865
	Result	**15.88 ± .08**	**158 ± .86**
C. Fruit powder			
1.	0.8	14.42	144.2
2.	0.759	13.67	136.7
3.	0.802	14.45	144.5
	Mean	14.18	141.8
	S. D.	0.359	3.59
	S.E.	0.11	1.13
	Result	**14.18 ± .11**	**141 ± 1.13**

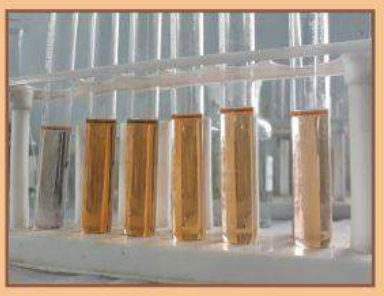
S.D. = Standard deviation
S.E. = Standard error

Table 18 Quantitative analysis for total phenol content in leaf, stem and fruit powder of *Pergularia daemia*

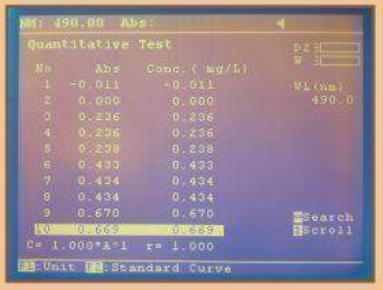
S.No.	Absorbance (650nm)	Concentration in mg/100mg	Concentration in mg/gram sample
A. Ethanolic Leaf extract			
1.	0.086	0.84	8.4
2.	0.093	0.91	9.1
3.	0.082	0.81	8.1
	Mean	0.85	8.5
	S. D.	0.040	0.4
	S.E.	0.012	0.12
	Result	**.85 ± .01**	**8.5 ± .1**
B. Ethanolic Stem extract			
1.	0.05	0.53	5.32
2.	0.046	0.49	4.97
3.	0.038	0.42	4.26
	Mean	0.48	4.85
	S. D.	0.044	0.44
	S.E.	0.01	0.1
	Result	**.48 ± .01**	**4.8 ± .1**
C. Ethanolic Fruit extract			
1.	0.04	0.44	4.4
2.	0.033	0.38	3.8
3.	0.037	0.41	4.1
	Mean	0.41	4.1
	S. D.	0.025	0.25
	S.E.	0.007	0.079
	Result	**.41 ± .007**	**4.15 ± .07**

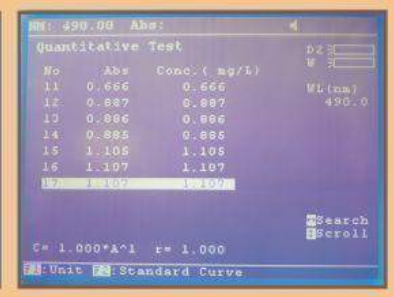
S.D. = Standard deviation
S.E. = Standard error

PLATE - 34
Preparation of spectroscopy for phytochemical analysis



Different concentration of glucose for standard curve





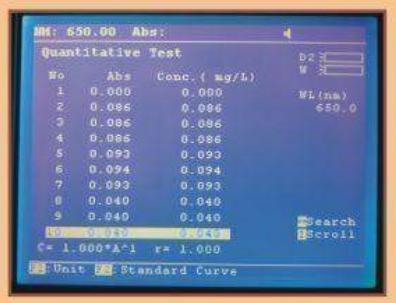
Abosrbance of plant samples in triplicate

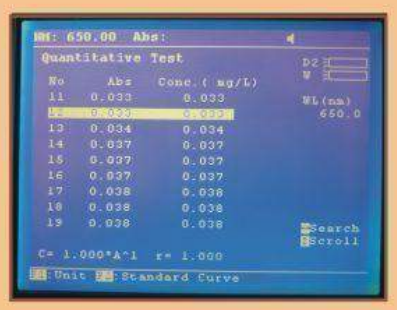
Triplicate experiments of samples

PLATE 35
Preparation of spectroscopy for phytochemical analysis

Different concentration of catechol for standard curve

Triplicate experiment of samples (Phenol)





Absorbance of plant samples in triplicate

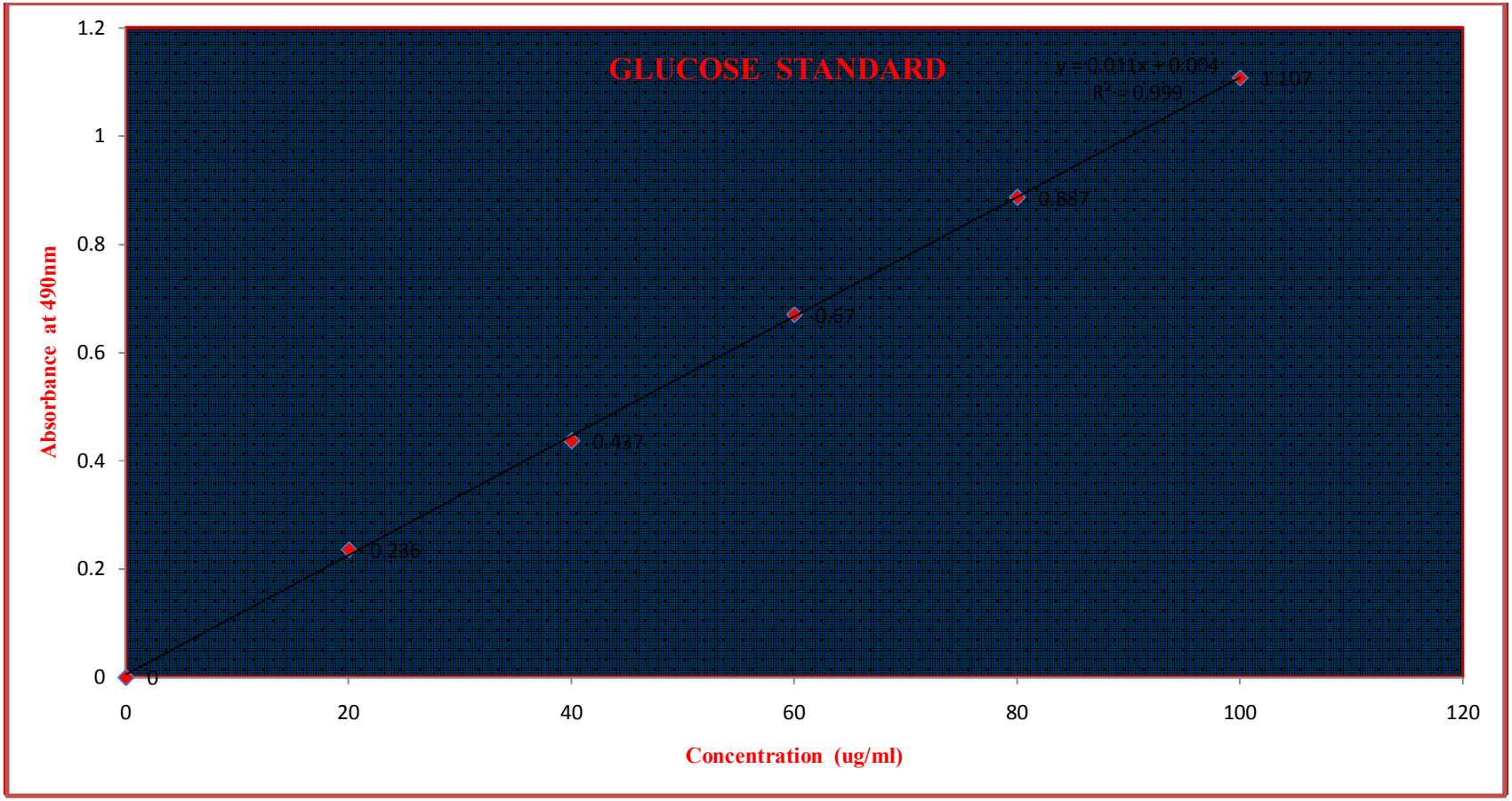


FIGURE 4 STANDARD CALIBRATION CURVE OF CARBOHYDRATES ESTIMATION

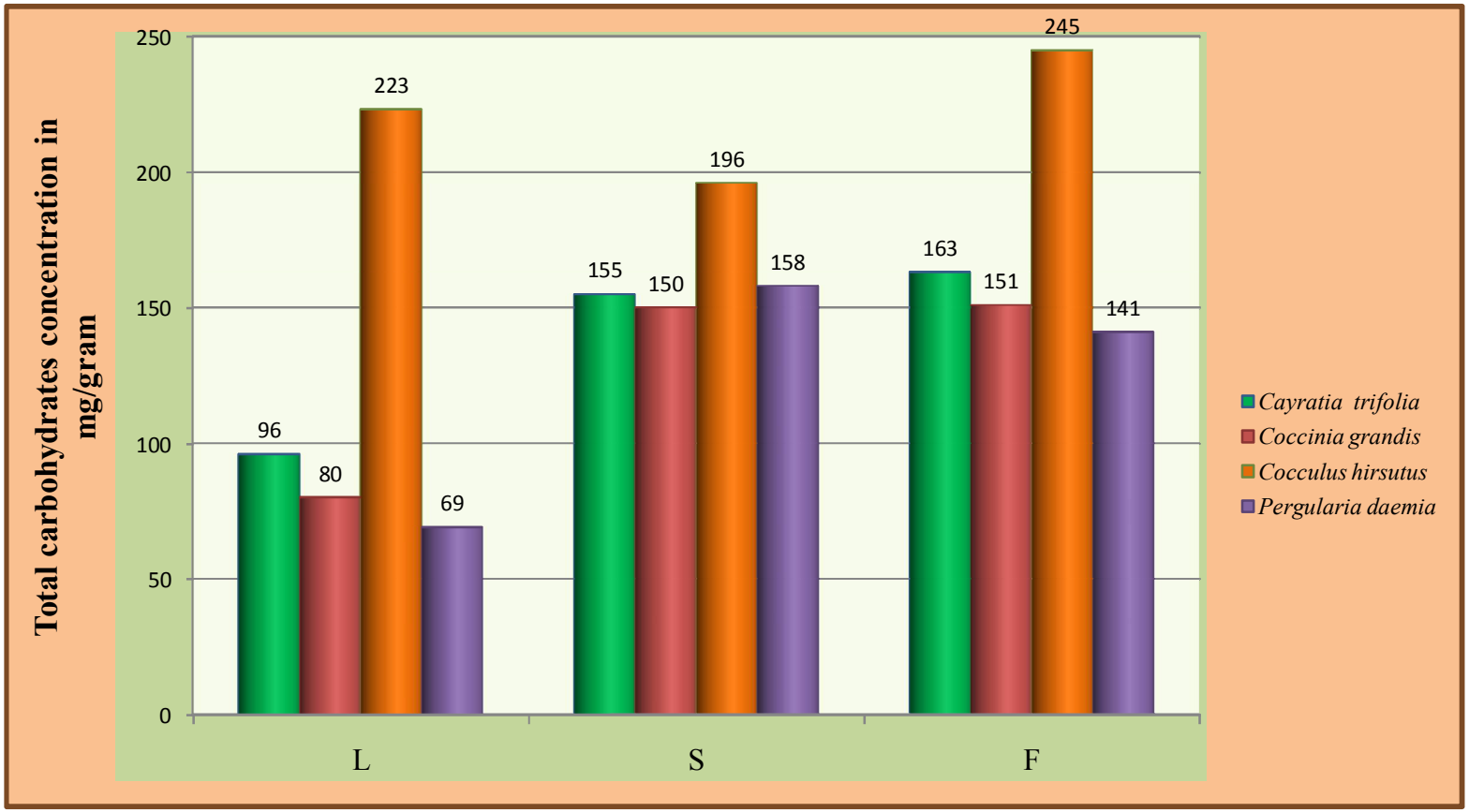


FIGURE 5 TOTAL CARBOHYDRATES CONCENTRATION IN LEAF, STEM AND FRUIT POWDERS OF THE SELECTED CLIMBERS

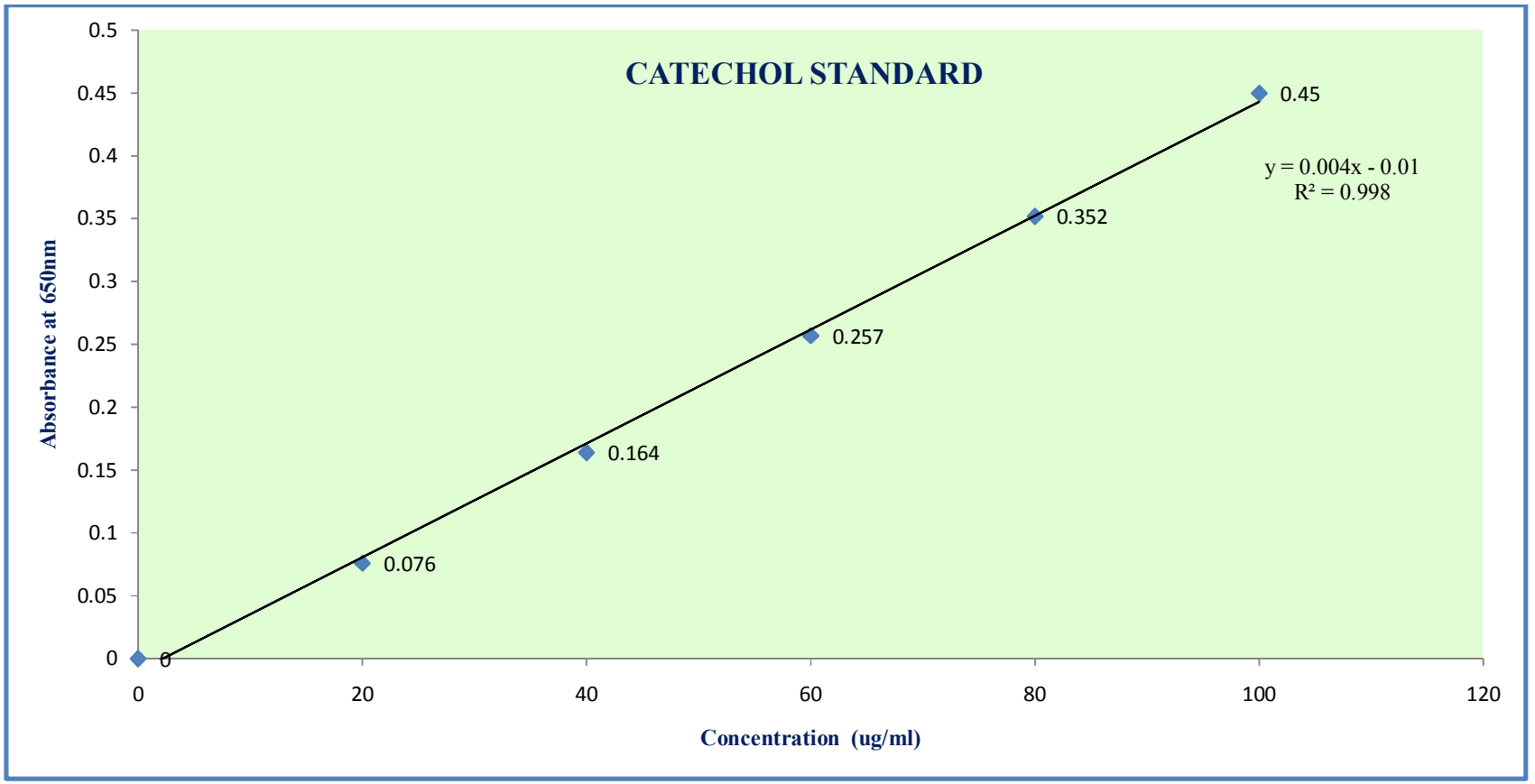


FIGURE 6 STANDARD CALIBRATION CURVE OF PHENOL ESTIMATION

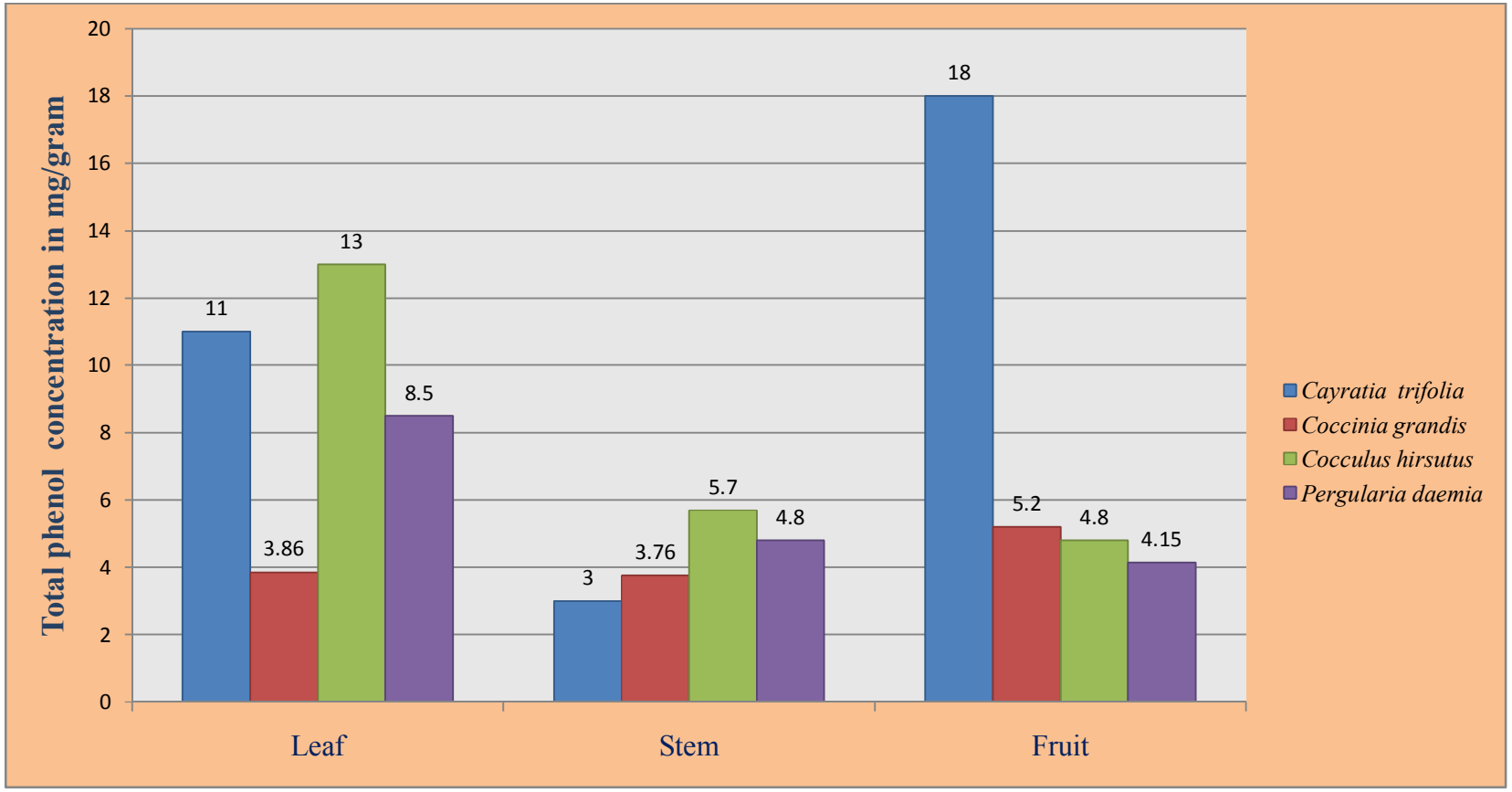


FIGURE 7 TOTAL PHENOL CONCENTRATIONS IN LEAF, STEM AND FRUIT POWDER OF THE SELECTED THE CLIMBERS

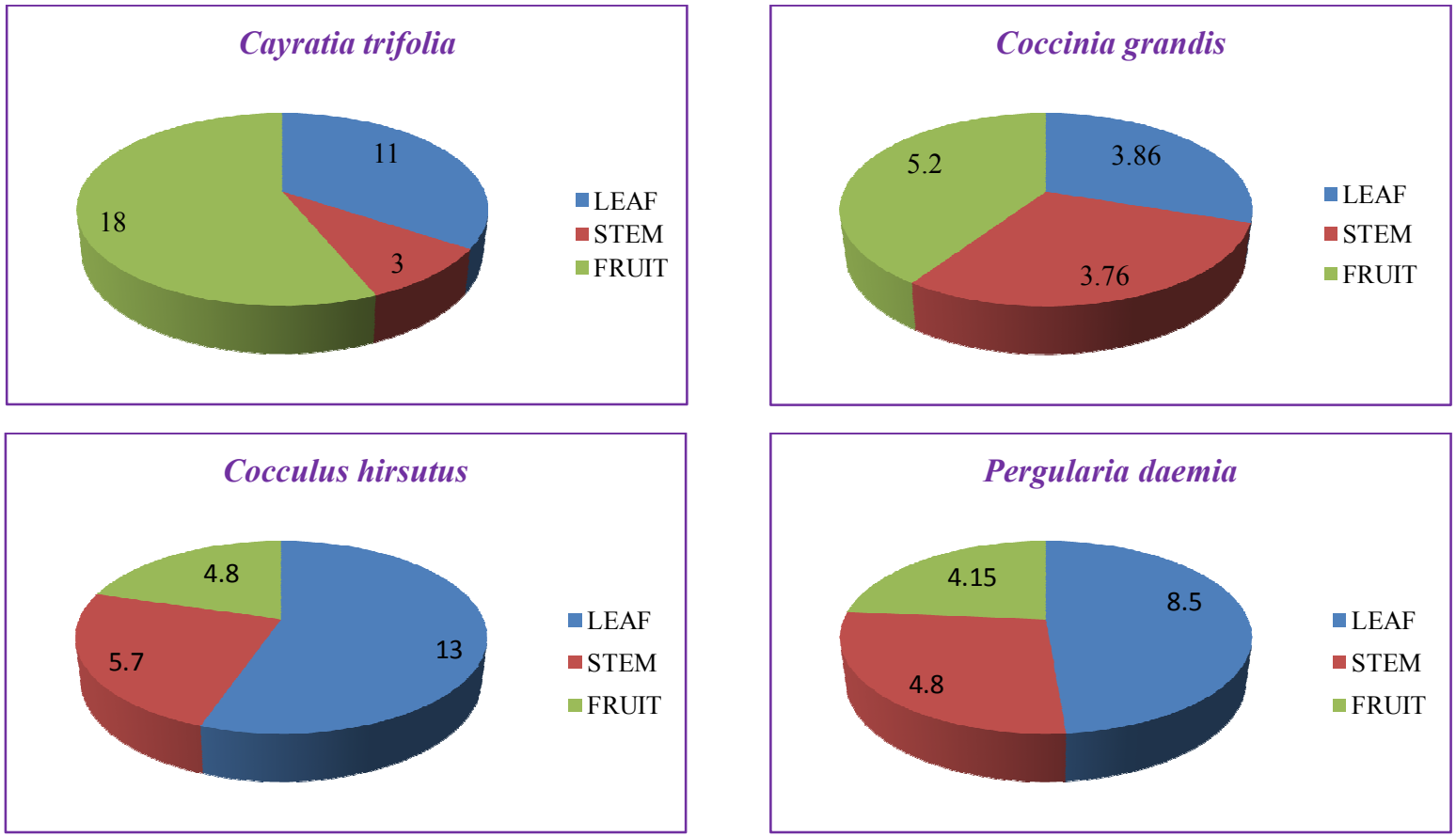


FIGURE 8 RESULTS OF TOTAL PHENOL CONTENT IN LEAF, STEM ANF FRUIT POWDER OF THE SELECTED CLIMBERS (mg/gram)

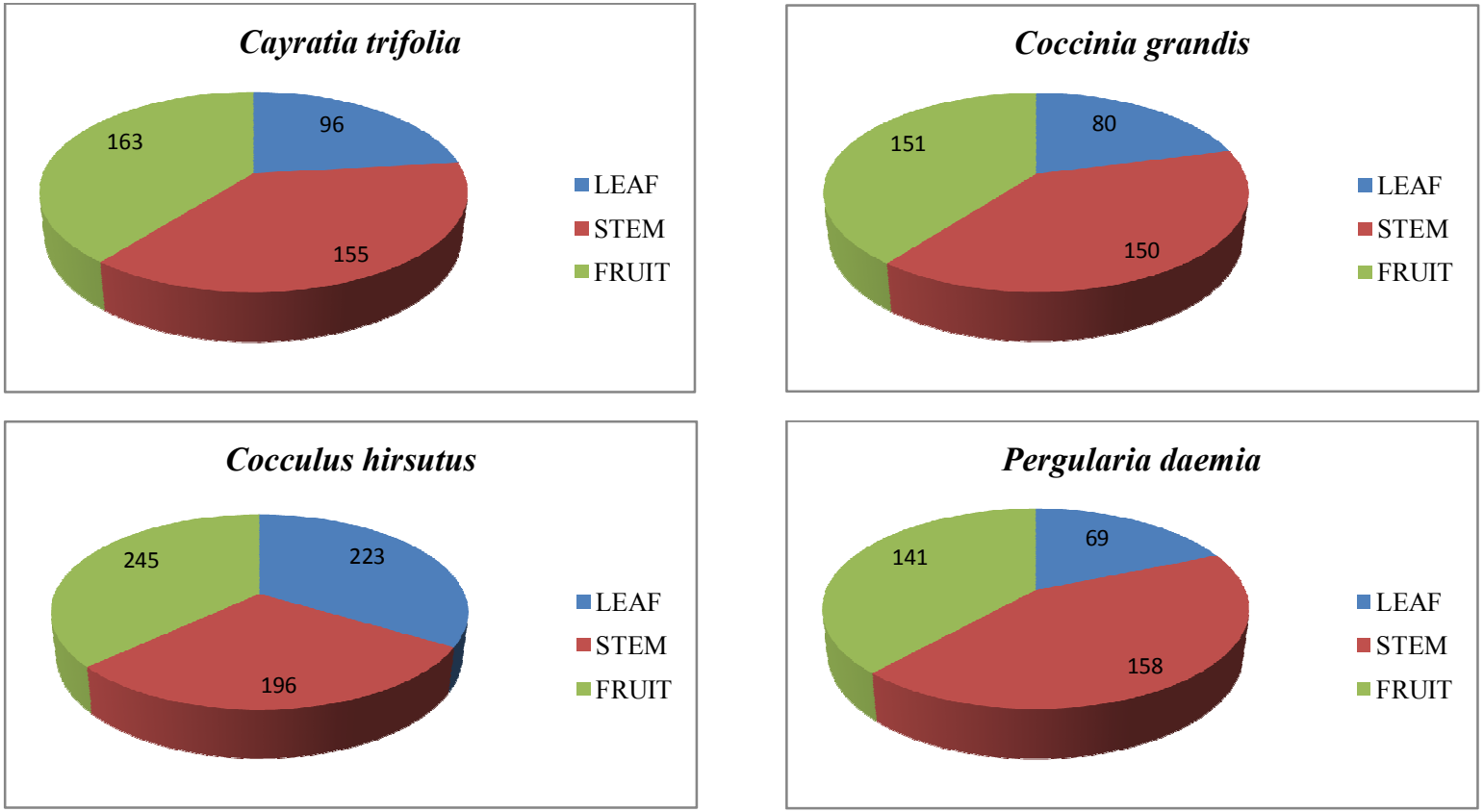
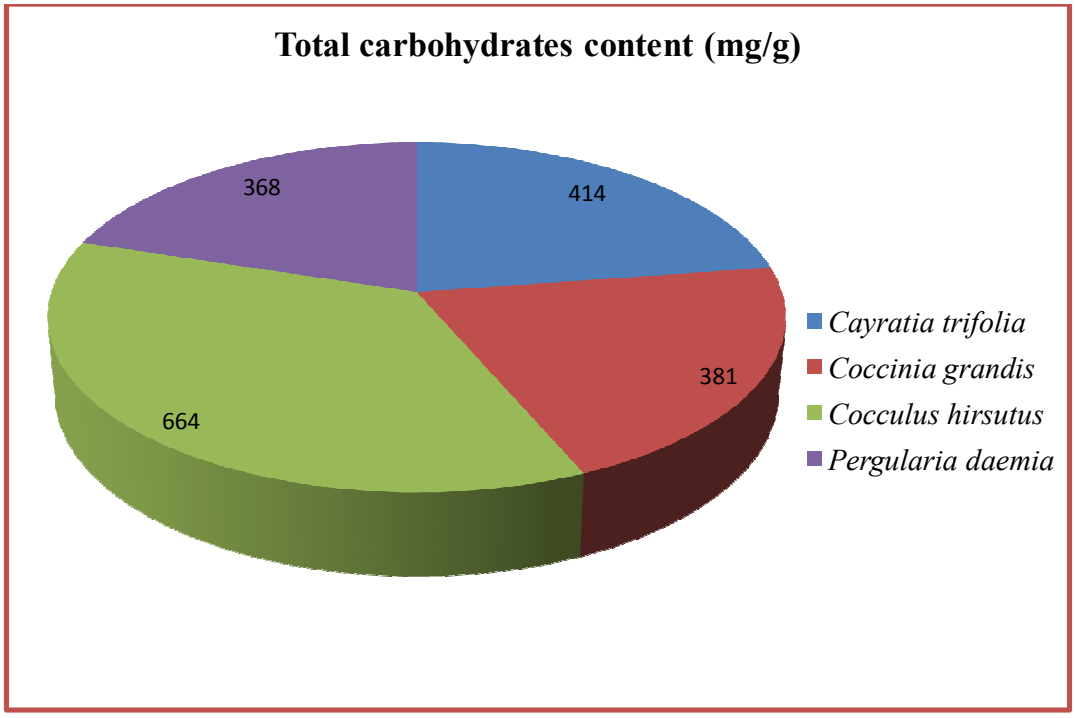


FIGURE 09 RESULTS OF TOTAL CARBOHYDRATES IN LEAF, STEM ANF FRUIT POWDER OF THE SELECTED CLIMBERS (mg/gram)



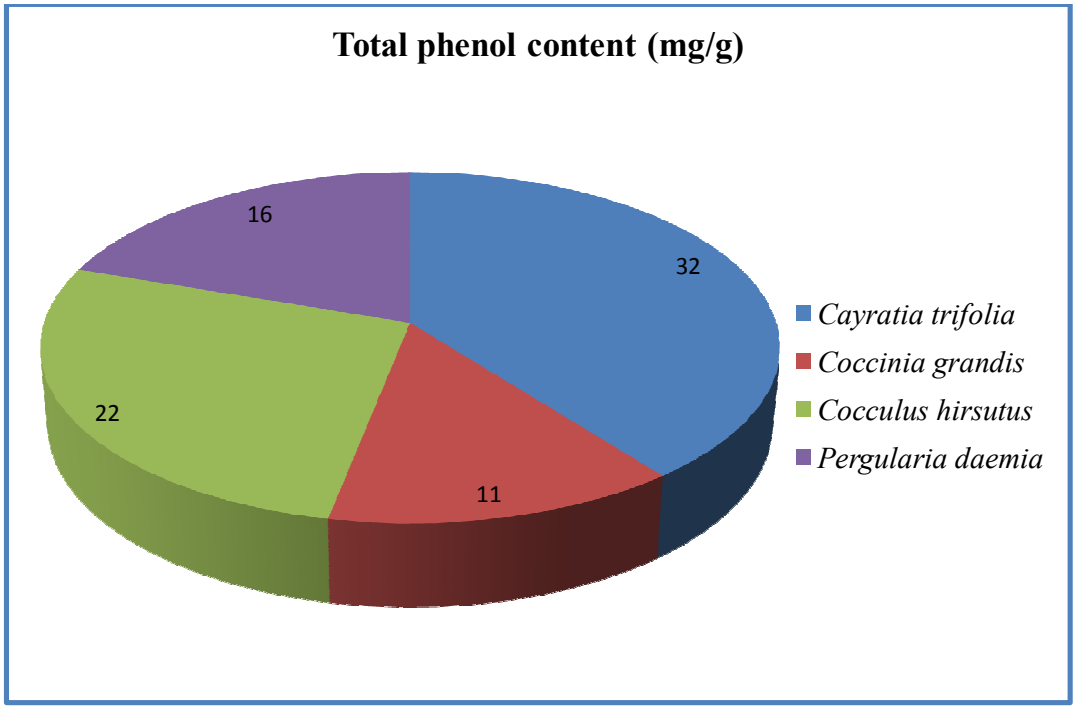


FIGURE 10 RESULTS OF TOTAL CARBOHYDRATES AND PHENOL CONTENT IN THREE PARTS OF SELECTED CLIMBERS.

CHAPTER 6
DISCUSSION

Comparative qualitative analysis of the selected climber species is given in table 19. It indicates results of the tests for primary metabolites in terms of degree of precipitation. Molish's test for carbohydrates resulted in lesser degree of precipitation only in alcoholic stem extract of *Cayratia trifolia*. The same test resulted in intermediate degree of precipitation in three samples, i.e., stem extract of *Cocculus hirsutus*, leaf extract of *Pergularia daemia* and fruit extract of the same climber species. However, the remaining eight extracts namely, leaf and fruit extracts of *Cayratia trifolia* leaf, stem and fruit extracts of *Cocculus hirsutus* as well as stem extract of *Pergularia daemia* showed higher degree of precipitation.

Benedicts's test to ascertain presence of reducing sugars resulted in higher degree of precipitation only in two plant extracts of distinct climber species, i.e., fruit extract of *Cayratia trifolia* and leaf extract of *Coccinia grandis*. Four plant extracts namely, leaf extract of *Cayratia trifolia*, stem and fruit extract of *Cocculus hirsutus* as well as fruit extracts of *Pergularia daemia* showed moderate degree of precipitation. However, six plant extracts namely, stem extract of *Cayratia trifolia,* stem and fruit extract of *Coccinia grandis,* leaf extract of *Cocculus hirsutus* as well as leaf and stem extracts of *Pergularia daemia* responded to the same test with lesser degree of precipitation.

Millon's test for proteins resulted in lesser degree of precipitation only in one extract, i.e., stem extract of *Cocculus hirsutus* whereas, the same test resulted in higher degree of precipitation in remaining eleven extracts namely, leaf, stem and fruit extracts of *Cayratia trifolia,* leaf, stem and fruit extracts of *Coccinia grandis,* leaf and fruit extracts of *Cocculus hirsutus* as well as leaf, stem and fruit extracts of *Pergularia daemia*. It is noteworthy that no plant extract exhibited moderate degree of precipitation for this test.

Biuret test to know presence of proteins in alcoholic extracts gave lesser and moderate degree of precipitation in one extracts each, i.e., leaf extract of *Coccinia grandis* and stem extract of *Cayratia trifolia,* respectively. In the remaining extracts, i.e., leaf and fruit extracts of *Cayratia trifolia,* stem and fruit extracts of *Coccinia grandis* leaf, stem and fruit extracts of *Cocculus hirsutus,* as

well as leaf, stem and fruit extracts of *Pergularia daemia,* biuret test resulted in higher degree of precipitation.

Presence of lipids was ascertained by the saponification test which resulted in lesser degree of precipitation in four extracts namely, leaf and stem extracts of *Cayratia trifolia,* stem extract of *Cocculus hirsutus* as well as stem extract of *Pergularia daemia*. However, fruit extract of *Cayratia trifolia* as well as stem extract of *Coccinia grandis* resulted in higher degree of precipitation.

Table 20 exhibits results of comparative qualitative analysis of secondary metabolites of these climbers. Results of Mayer's test present striking similarity, in all extracts except the fruit extract of *Pergularia daemia.* In eleven extracts, i.e., all three extracts (leaf, stem and fruit) of *Cayratia trifolia, Coccinia grandis* and *Cocculus hirsutus* as well as leaf and stem extracts of *Pergularia daemia* the test resulted in lesser degree of precipitation while in fruit extract of *Pergularia daemia* the test resulted in higher degree of precipitation.

Wagner's test to ascertain presence of alkaloids also gave similar result, i.e., lesser degree of precipitation in ten extracts namely, leaf, stem and fruit extract of *Cayratia trifolia, Coccinia grandis*, *Cocculus hirsutus* and leaf extract of *Pergularia daemia.* However, for stem and fruit extracts of *Pergularia daemia* the test resulted in higher and moderate degree of precipitation, respectively.

Hager's test for alkaloids gave rise to lesser degree of precipitation in all three extracts of *Cocculus hirsutus,* fruit extract of *Cayratia trifolia,* leaf extract of *Coccinia grandis* as well as leaf and fruit extract of *Pergularia daemia.* Only one extract, i.e., stem extracts of *Pergularia daemia* resulted in moderate degree of precipitation. Remaining four extract, i.e., leaf and stem extracts of *Cayratia trifolia* and stem and fruit extracts of *Coccinia grandis* presented moderate degree of precipitation.

Ferric chloride test to know presence of phenols and tannins culminated in lesser and moderate degree of precipitation only in one extract each, i.e., fruit extract of *Cocculus hirsutus* and stem extract of *Pergularia daemia.* In remaining ten extracts, viz., leaf, stem and fruit extracts of *Cayratia trifolia, Coccinia*

grandis leaf and stem extracts of *Cocculus hirsutus* as well as leaf and fruit extracts of *Pergularia daemia,* the test gave similar results, i.e., higher degree of precipitation.

Lead acetate test for phenols and tannins presented uniform results in all the extracts barring fruit extracts of *Cocculus hirsutus* in which the test gave rise to lesser degree of precipitation. In the remaining eleven extracts the test culminated in higher degree of precipitation.

To ascertain presence of flavonoids in the extracts, sodium hydroxide test was also conducted. The results of this test shows that only leaf and stem extracts of *Coccinia grandis* exhibited lesser degree of precipitation compared to moderate degree of precipitation for stem extract of *Cocculus hirsutus* and leaf and stem extracts of *Pergularia daemia.* All three parts of *Cayratia trifolia,* fruit extracts of *Coccinia grandis*, leaf and fruit extract of *Cocculus hirsutus* as well as fruit extract of *Pergularia daemia* presented relatively higher degree of precipitation.

Results of alkaline reagent test for flavonoids presented lesser degree of precipitation for all three extracted parts of *Coccinia grandis* and one part each of *Cocculus hirsutus* and *Pergularia daemia,* i.e., stem and leaf, respectively. Further, all three parts of *Cayratia trifolia* as well as stem and fruit extracts of *Pergularia daemia* gave rise to comparatively higher degree of precipitation.

Presence of saponins was ascertained by froth test which resulted in mixed response by the studied extracts. Only two extracts, i.e., fruit extract of *Cocculus hirsutus* and stem extract of *Pergularia daemia* gave lesser degree of precipitation. Moderate degree of precipitation was exhibited by leaf and stem extracts of *Cayratia trifolia*, leaf extract of *Cocculus hirsutus* as well as leaf extract of *Pergularia daemia*. In this test, comparatively higher degree of precipitation was observed for fruit extracts of *Cayratia trifolia*; leaf, stem and fruit extracts of *Coccinia grandis*; stem extract of *Cocculus hirsutus*, as well as fruit extract of *Pergularia daemia*.

Results of horizon test showed that the studied extracts present either lesser degree of precipitation or higher degree of precipitation but, no extract

showed moderate degree of precipitation. Among the extracts presenting lesser degree of precipitation are leaf and stem extracts of *Cayratia trifolia* as well as same extract of *Coccinia grandis* all three extracts of *Cocculus hirsutus* as well as fruit extract of *Pergularia daemia*. Four extracts namely, fruit extracts of *Cayratia trifolia* and *Coccinia grandis* as well as leaf and stem extracts of *Pergularia daemia* presented higher degree of precipitation.

Legal test for glycosides also gave mixed results as stem extracts of *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* as well as stem and fruit extracts of *Pergularia daemia* presented lesser degree of precipitation. Three extracts, i.e., leaf extract of *Cayratia trifolia*, *Coccinia grandis* and *Cocculus hirsutus* exhibited moderate degree of precipitation compared to higher degree of precipitation for fruit extracts of *Cayratia trifolia, Coccinia grandis, Cocculus hirsutus* and leaf extract of *Pergularia daemia*. These data show that *Cayratia trifolia, Coccinia grandis* and *Cocculus hirsutus* have striking similarity in terms of the degree of precipitation in relation to extracted parts as in these plants some plant parts gave similar degree of precipitation.

Quantitative estimation of primary and secondary metabolites (carbohydrates and phenols) of selected climbers are given in Table 21 and Table 22, respectively. Comparative analysis of carbohydrates and phenols among *Cayratia trifolia, Coccinia grandis, Cocculus hirsutus* and *Pergularia daemia* is presented in Table 23.

By results of quantitative analysis of the primary and secondary metabolites it is clear that among the studied climber species highest amount of carbohydrate is found in all three parts (leaf, stem and fruit) of *Cocculus hirsutus* and the highest amount of phenol compound is found in the leaves and stems of the same plant species. One climber species, i.e., *Cayratia trifolia* is found to possess highest quantity of phenol in fruits Table 23.

The Figure 12 and Table 20 shows that among studied four climber species fruit powder of *Cayratia trifolia* possesses highest quantity of the assessed secondary metabolite phenol, i.e., 18 mg/gm. However, stem powder of the same

part of the same climber was reported to possess minimum quantity of the total phenol content, i.e., 3 mg/gm.

The same figure reveals that the total phenol content reported in descending order is 13, 11, 8.5, 5.7, 5.2, 4.15, 3.86 and 3.76 (mg/gm) present in leaf powder of *Cocculus hirsutus,* leaf powder of *Cayratia trifolia*, leaf powder of *Pergularia daemia*, stem powder of *Cocculus hirsutus*, fruit powder of *Coccinia grandis*, fruit powder of *Pergularia daemia*, leaf powder of *Coccinia grandis,* stem powder of *Coccinia grandis,* respectively. Fruit powder of *Cocculus hirsutus* and stem powder of *Pergularia daemia* were found to possess equal quantity of the total phenol content, i.e., 4.8 mg/gm.

Table 23 and figure 11 show that the assessed primary metabolites, i.e., carbohydrates contents was present in highest quantity in fruit of the three plant species, i.e., *Cayratia trifolia, Coccinia grandis and Cocculus hirsutus*. However among these three plant species the carbohydrates content in fruit in descending orders is as follows, *Cocculus hirsutus* > *Cayratia trifolia* > *Coccinia grandis,* i.e., 245 ± 0.26*,* 163 ± 1 and 151 ± 0.73 mg/g carbohydrates contents, respectively.

The leaf of *Cayratia trifolia* and *Coccinia grandis* exhibited similar trend as in these two plant species leaf extract was found to possess minimum carbohydrates contents among the three parts. Similarly, the stem extracts of same two plant species were found to contain intermidiate quantity of the carbohydrates content. However, despite having higest quantity of carbohydrates content in fruit *Cocculus hirsutus* was found to vary in term of presence of minimum quantity of carbohydrates content as in contrast to *Cayratia trifolia and Coccinia grandis* where leaf possess minimum carbohydrates content in *Cocculus hirsutus* minimum carbohydrates content was found to present in stem whereas, leaf of this plant species was found to possess intermediate quantity of the studied primary metabolites.

Data presented in Table 23 and Figure 12 shows that the phenol content was found to present in all samples of the studied climber species, i.e., *Cayratia trifolia, Coccinia grandis, Cocculus hirsutus* and *Pergularia daemia*. However, in each plants species their relative quantity did not show a definite pattern. In

Cayratia trifolia and *Coccinia grandis* similar trend of presence of total phenol was observed. In these two plants species the presence of phenol content among leaf, stem and fruit in desending order is Fruit > Leaf > Stem.

Among these two plant species though the stem were observed to possess almost similar phenol content, i.e., 3 and 3.76 mg/g, respectively. However, in fruit extract of these two plant quantity of total phenol varied prominently as the same two plant species were observed to possess 18 ± 0.11 and 5.2 ± 0.02 mg/g phenol content in their fruit, respectively. The difference of total phenol content in the leaf of the same two plant species, i.e., *Cayratia trifolia* and *Coccinia grandis* being 11 ± 0.12 and 3.86 ± 0.07 was prominent.

The same table also showed that in *Cayratia trifolia* the total phenol content varied considerably in leaf, stem and fruit. However, in case of *Coccinia grandis* phenol content was almost similar in leaf and stem, i.e., 3.86 ± 0.7 and 3.76 ± 0.1mg/g, respectively. Though the fruit of this plant species when compared to leaf and stem, was observed to contain slightly higher (5.2 ± 0.2 mg/g) quantity of the total phenol content yet the difference is not so prominent as compared to *Cayratia trifolia*.

Similar trend of order in which phenol content in present is three parts of *Cocculus hirsutus* and *Pergularia daemia* is evident by a perusal of the same table. In these two plant species total phenol content in descending order is leaf > stem > fruit. Among these two climbers, only leaf showed considerable difference in total phenol contents, i.e., 13 ± 0.1 and 8.5 ± 0.1 for *Cocculus hirsutus* and *Pergularia daemia*, respectively. However, the remaining two parts of the same two plant species did not show considerable variation in terms of total phenol contents. Further, fruit extract of *Cocculus hirsutus* and stem of *Pergularia daemia* were observed to possess same quantity of the studied secondary metabolite, i.e., 4.8 mg/g.

Table 23 also shows comparative results of phytochemical screening of carbohydrate and phenol contents in all three parts of the selected four plant species. The table clearly indicates that in all plant species and in three parts of

them, i.e., leaf, stem and fruit, carbohydrate content is significantly higher than the phenol content. Further, the difference in quantity of these two metabolites is highest in fruit of *Cocculus hirsutus* which possesses 4.8 ± 0.5mg/g total phenol content in comparison of 245 ± 0.26 mg/g carbohydrate content. In this plant species, leaf and stem also exhibited higher difference between studied primary and secondary metabolites in comparison of remaining three plant species, i.e., *Cayratia trifolia, Coccinia grandis* and *Pergularia daemia*. The same table also shows that the minimum difference between primary and secondary metabolite contents is in leaf powder of *Pergularia daemia* which possesses 69 ± 0.80 and 8.5 ± 0.1 mg/g carbohydrate and phenol contents, respectively. The data indicate that fruit of *Cocculus hirsutus* contains highest quantity of the primary metabolite carbohydrate, however the fruit of *Cayratia trifolia* possess highest quantity of the assessed secondary metabolite, i.e., phenol.

Table 19 Comparative qualitative analysis for primary metabolites in ethanolic extracts of the selected climbers

S.No	Name of Test	*Cayratia trifolia*			*Coccinia grandis*			*Cocculus hirsutus*			*Pergularia daemia*		
		Leaf	Stem	Fruit	Leaf	Stem	Fruit	Leaf	Stem	Fruit	Leaf	Stem	Fruit
1.	Molisch's	+++	+	+++	+++	+++	+++	+++	++	+++	++	+++	++
2.	Benedict's	++	+	+++	+++	+	+	+	++	++	+	+	++
3.	Millon's	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+++
4.	Biuret	+++	++	+++	+	+++	+++	+++	+++	+++	+++	+++	+++
5.	Saponification	+	+	+++	++	+++	+++	++	+	++	++	+	++

Table 20 Comparative qualitative analysis for secondary metabolites in ethanolic extracts of the selected climbers

S.No	Name of Test	*Cayratia trifolia*			*Coccinia grandis*			*Cocculus hirsutus*			*Pergularia daemia*		
		Leaf	Stem	Fruit	Leaf	Stem	Fruit	Leaf	Stem	Fruit	Leaf	Stem	Fruit
1.	Mayer's	+	+	+	+	+	+	+	+	+	+	+	+++
2.	Wagner's	+	+	+	+	+	+	+	+	+	+	+++	++
3.	Hager's	+++	+++	+	+	+++	+++	+	+	+	+	++	+
4.	Ferric Chloride	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	++	+++
5.	Lead Acetate	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++
6.	Sodium Hydroxide	+++	+++	+++	+	+	+++	+++	++	+++	++	++	+++
7.	Alkaline Reagent	+++	+++	+++	+	+	+	++	+	++	+	+++	+++
8.	Froth	++	++	+++	+++	+++	+++	++	+++	+	++	+	+++
9.	Horizon	+	+	+++	+	+	+++	+	+	+	+++	+++	+
10.	Legal	++	+	+++	++	+	+++	++	+	+++	+++	+	+

Table 21 Quantitative estimation of primary metabolites (carbohydrates) in the four climbers

S.No.	Name of climber species	Total carbohydrates mg/g		
		Leaf	Stem	Fruit
1.	***Cayratia trifolia***	96 ± 1.6	155 ± .08	163 ± 1
2.	***Coccinia grandis***	80 ± 1.2	150 ± 1.02	151 ± .73
3.	***Cocculus hirsutus***	223 ± .24	196 ± 3.28	245 ± .26
4.	***Pergularia daemia***	69 ± .78	158 ± .86	141 ± 1.13

Table 22 Quantitative estimation of secondary metabolites (phenols) in the four climbers

S.No.	Name of climber species	Total Phenol mg/g		
		Leaf	Stem	Fruit
1.	***Cayratia trifolia***	11 ± .12	3 ± .07	18 ± .11
2.	***Coccinia grandis***	3.86 ± .07	3.76 ± .01	5.2 ± .02
3.	***Cocculus hirsutus***	13 ± .1	5.7 ± .1	4.8 ± .5
4.	***Pergularia daemia***	8.5 ± .1	4.8 ± .14	4.15 ± .07

Table 23 Comparative analysis of Carbohydrates & Phenols among four climbers

S.No.	Climber	Leaf		Stem		Fruit	
		Carbohydrates	Phenols	Carbohydrates	Phenols	Carbohydrates	Phenols
1.	*Cayratia trifolia*	96 ± 1.6	11 ± .12	155 ± .08	3 ± .07	163 ± 1	18 ± .11
2.	*Coccinia grandis*	80 ± 1.2	3.86 ± .07	150 ± 1.02	3.76 ± .01	151 ± .73	5.2 ± .02
3.	*Cocculus hirsutus*	223 ± 4.24	13 ± .1	196 ± 3.28	5.7 ± .1	245 ± .26	4.8 ± .5
4.	*Pergularia daemia*	69 ± .78	8.5 ± .1	158 ± .86	4.8 ± .14	141 ± 1.13	4.15 ± .07

Cayratia trifolia is the important medicinal climber of family Vitaceae. Many researchers worked on family Vitaceae and related various climbers. Hanaa *et al*. (2015) carried out study to assess the proximate composition, phytochemical screening, total phenolic compounds, total flavonoids and antioxidant activities of selected solvent extracts of *Vitis vinifera* L. family Vitaceae. Prabhavathi *et. al*. (2016) reported various phytochemical of different parts of *Cissus quadrangularis*. Rasale (2014) attempted to highlight phytochemicals, various traditional uses as well as pharmacological reports on *Cissus quadrangularis* L. Similar, studies have been carried out in other species of *Cayratia* such as *Cayratia pedata* (Rajmohana and Sudhakaran, 2014) which also correlates with the present results.

Studies conducted by Satheesh *et al*. (2009) also support present findings of phenolic contents in *Coccinia grandis*. They found that two wild climbing plant species of Cucurbitaceae, i.e., *Coccinia grandis* and *Trichosanthes bracteata* possess significantly high phenolic content (0.5mg/g fr.wt. to 0.7mg/g fr.wt.). In the same work they also found positive and strong correlation between phenol and phenolic acids such as cinnamate, coumarate, caffeate, chlorogenate, ferulate, gallic and hydroxyl benzoic acids among the taxa. However, thypsin inhibitor and amylase inhibition contents were comparatively lower in *Coccinia* than *Trichosanthes*.

In an updated review Alagarraja *et al*. (2017) stated that fruits of *Coccinia grandis* have rich value of anti-diabetic properties compared to other parts of the same plant species. It suggests that pharmacological properties of this plant species can be attributed to the significant quantity of certain secondary metabolites.

A significant correspondence established by Dash *et al.* (2017) through their work on phytochemical and biochemical characterizations from leaf extracts from *Azadirachta indica*. Romila *et al.* (2010), reviewed the work on antidiabetic plants used by the people of Manipur characterized by hypoglycemic activity.

Phytochemical studies were conducted by Saxena and Saxena (2012) of different plants namely, *Acorus calamus* and *Lantana camara*. Phytochemical test of the plant extracts of *Acorus calamus* and *Lantana camara* showed the presence of glycoside, carbohydrates, phenolic compound, alkaloids, flavonoids and tannins, saponins, steroids and tritrepenoids as major phytochemical groups.

In phytochemical screening and antimicrobial activity of *Coccinia cordifolia,* Khatun *et al*. (2012) prepared extract of this medicinal plant and observed that the methanolic extract contains phenols and other bioactive constituents such as tannins, saponins, flavonoids and terpenoids. The study also suggests that these bioactive constituents in methanolic extract exhibit highest activity against certain gram positive and gram negative bacteria.

Tiwari and Rana (2015) in a review highlighted economic and medicinal value of plant secondary metabolites. In another work by Deokate and Khadabadi (2012) various phytoconstituents of *Coccinia grandis* like cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A and B, stigma -7-en-3-one, taraxerone and taraxerol have been reported. They also found that terpenoids are responsible for antidiabetic activity of this medicinal plant. Since in present study also presence of terpenoids has been ascertained in ethanolic leaf, stem and fruit extracts of the climbers in lesser and higher degree of precipitations, their study supports the present findings.

Sharma and Batra (2016) have reported on primary metabolic profiling of *Tinospora cardifolia*. They analyzed quantity of chlorophyll, starch, protein and total phenol from various plant parts of *Tinospora cardifolia*. Rajan, *et al.* (2011) studied antifungal activity of *Aegle marmelos* leaf extract on dermetophytes. Havsteen (2002) explained the biochemistry and medical significance of the flavonoids.

A considerable amount of work on phytochemical analysis of various plant species has appeared (Talreja, 2011; Vijayvergia and Kumar, 2007; Sagwan *et al*., 2010, Shekhawat, 2002; Paulraj *et al.,* 2011).

Phytochemical analysis of *Senecio biafrae* leaf by Ajiboye *et al*. (2013) revealed presence of alkaloids, tannins, photobatannins, phenol, glycosides, steroids, terpenes, cardenolides, flavonoids and chalocones in the plant species. The study registered that the presence of these active compounds may be responsible for the medicinal purpose of the plant.

A similar study of preliminarily phytochemical analysis was done by Sowmya *et al.* (2015) in which they extracted stem, leaf and fruit of *Cayratia trifolia* using successive solvents like petroleum ether, chloroform, ethyl acetate, ethanol and water. They observed that the stem ethanolic extracts possesses more number of phytochemicals in comparison of leaf and fruit ethanolic extracts which justifies the selection of ethanol for extraction of different parts of the selected plant species. The presence of secondary metabolites also gives this plant species good free radical scavenging property which establishes its importance as therapeutic agents in preventing oxidative stress related diseases like cancer, inflammation and diabetes mellitus.

Adarsh Krishna *et al.* (2013) carried out a study to determine phytochemical constituents in ethanolic leaf extract of Tetrastigma leucostophylum, a member of Vitaceae from which also comes *Cayratia trifolia*. The results show that like leaf extract of *Cayratia trifolia* the leaf extract of this plant also possesses alkaloids, phytosteriods, saponins, glycosides, carbohydrate, etc. which supports the present finding about the member of the family Vitaceae.

Rakkimuthu *et al*. (2012) in a quantitative phytochemical analysis of *Cocculus hirsutus* observed that acidified, methanol extract of fruits of this plant possesses secondary metabolites like flavonoids and phenol in substantial quantity which is comparable with present study as in the present qualitative analysis of ethanolic fruit extract of the same plant species flavonoids and phenol contents have been detected. In the aqueous extract of the areal parts of the same plant species Iyer *et al.* (2011) in an advanced study isolated b-sitosterol (1) and 28-acetyl betulin (F1). They extracted compound F1 for the first time in this plant species. This suggests that advanced studies with modern biochemical and

analytical techniques more active ingredient can be identified in extracts of this plant prepared in various solvents.

In a phytopharmacognostical evaluation of leaves of *Cocculus hirsutus* Jethva *et al.* (2016) observed presence of phytoconstituents like flavonoids, saponins and steroids in ethanolic and 70% hydro-alcoholic extract which also supports present findings.

Jain *et al.* (2004) conducted preliminary phytochemical studies on the roots of *Cocculus hirsutus* which was not covered in present study. They found that the extracts prepared in different solvent such as pet-ether, chloroform, benzene, methanol and water gave different result in the different tests. Alcoholic extracts of leaf, stem and fruit studied in present study also have comparable results of the phytochemicals.

In a phytochemical screening Jogi and Akkewar (2012) extracted leaf powder of *Pergularia daemia* in five solvents namely, methanol, ethyl acetate, chloroform, hexane and water. They found maximum phytochemicals in methnolic extract which is also comparable with present study in which different secondary metabolites have been identified in ethanolic extract of leaf powder of the same plant species. The study of Doss and Anand (2012) signifies the medicinal use of *Pergularia daemia*. In the study they screened methanolic and aqueous extracts of *Asteracantha longifolia* and *Pergularia daemia* which were found to contain alkaloids, phenolic compounds, tannins and flavonoids. However, contrary to present study, the methanolic and aqueous extracts of dried leaves of *Pergularia daemia* were found to lack in saponin which is reported in the ethanolic leaf extract in present findings. In similar study karthishwaran *et al*. (2010) reported crude methanolic extract of the leaves of same plant species to contain alkaloids, flavonoids, tannins, terpenoids, carbohydrates and proteins which also supports present findings. They also found that this climber species is an important source of a number of active compounds with diverse chemical structure that exhibit diverse pharmacological activities. According to Bhaskar and Balakrishnan (2009) this medicinal plant has been traditionally used as anthelmintic, laxative, antipyretic and expectorant by various tribal communities

in Western Ghats of India. In another study Kumar and Mishra (2008) demonstrated hepatoprotective properties of ethanol extract of areal parts of the same plant species which suggests promising medicinal values of this climber species .

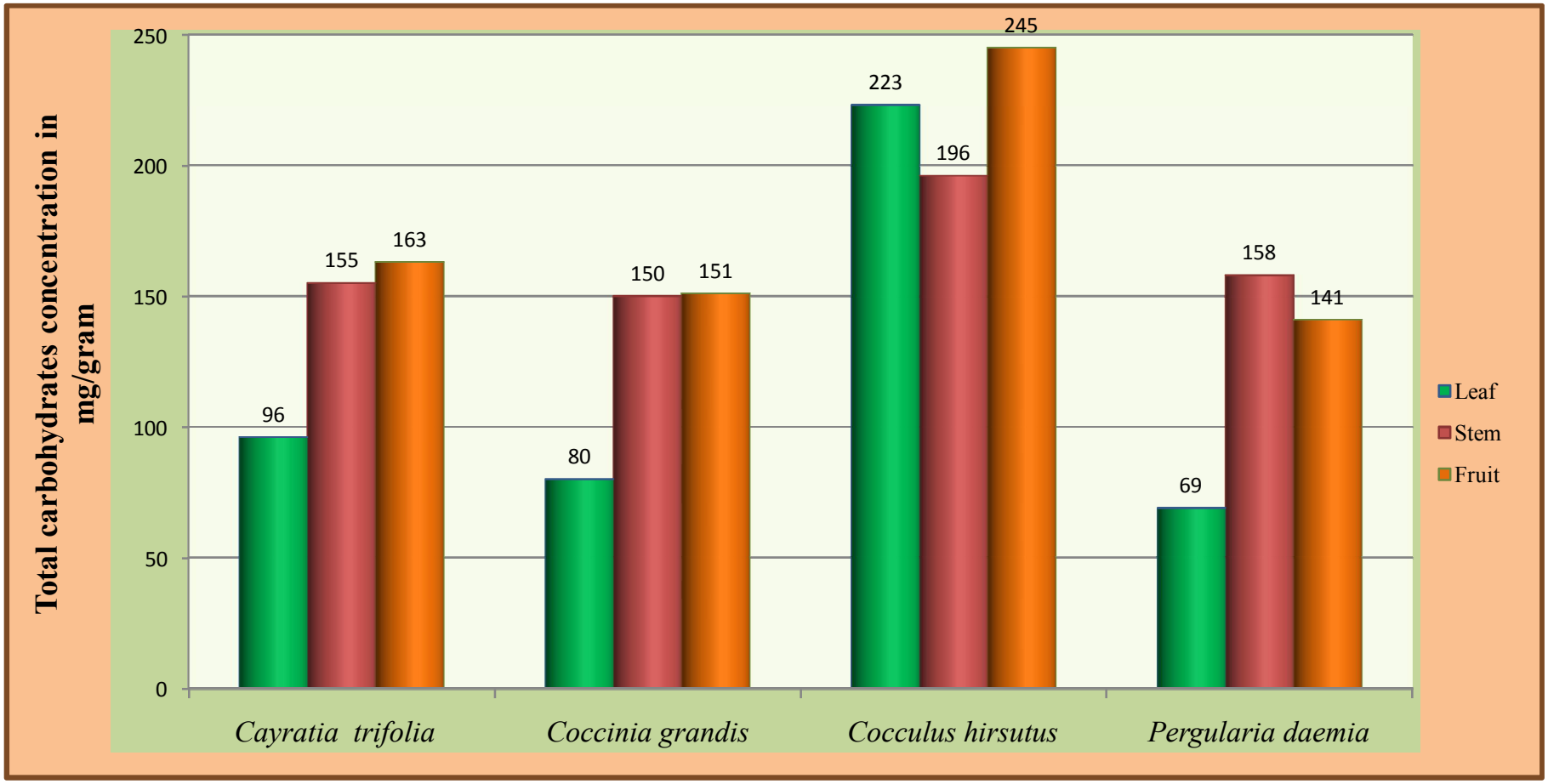


FIGURE 11 COMPARATIVE RESULTS OF TOTAL CARBOHYDRATES IN LEAF, STEM AND FRUIT POWDER OF THE SELECTED CLIMBERS.

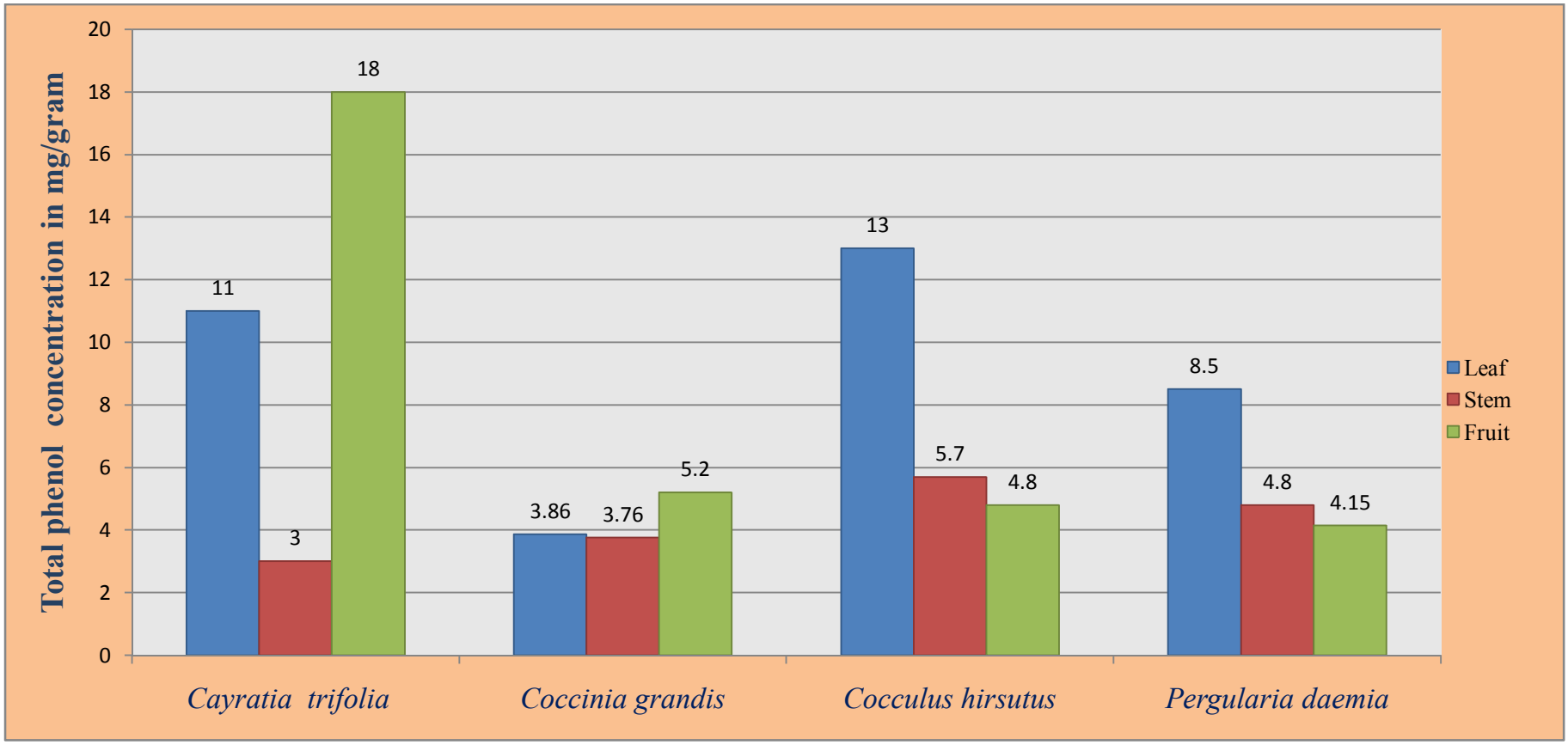


FIGURE 12 COMPARATIVE RESULTS OF TOTAL PHENOL IN LEAF, STEM AND FRUIT POWDER OF THE SELECTED CLIMBERS

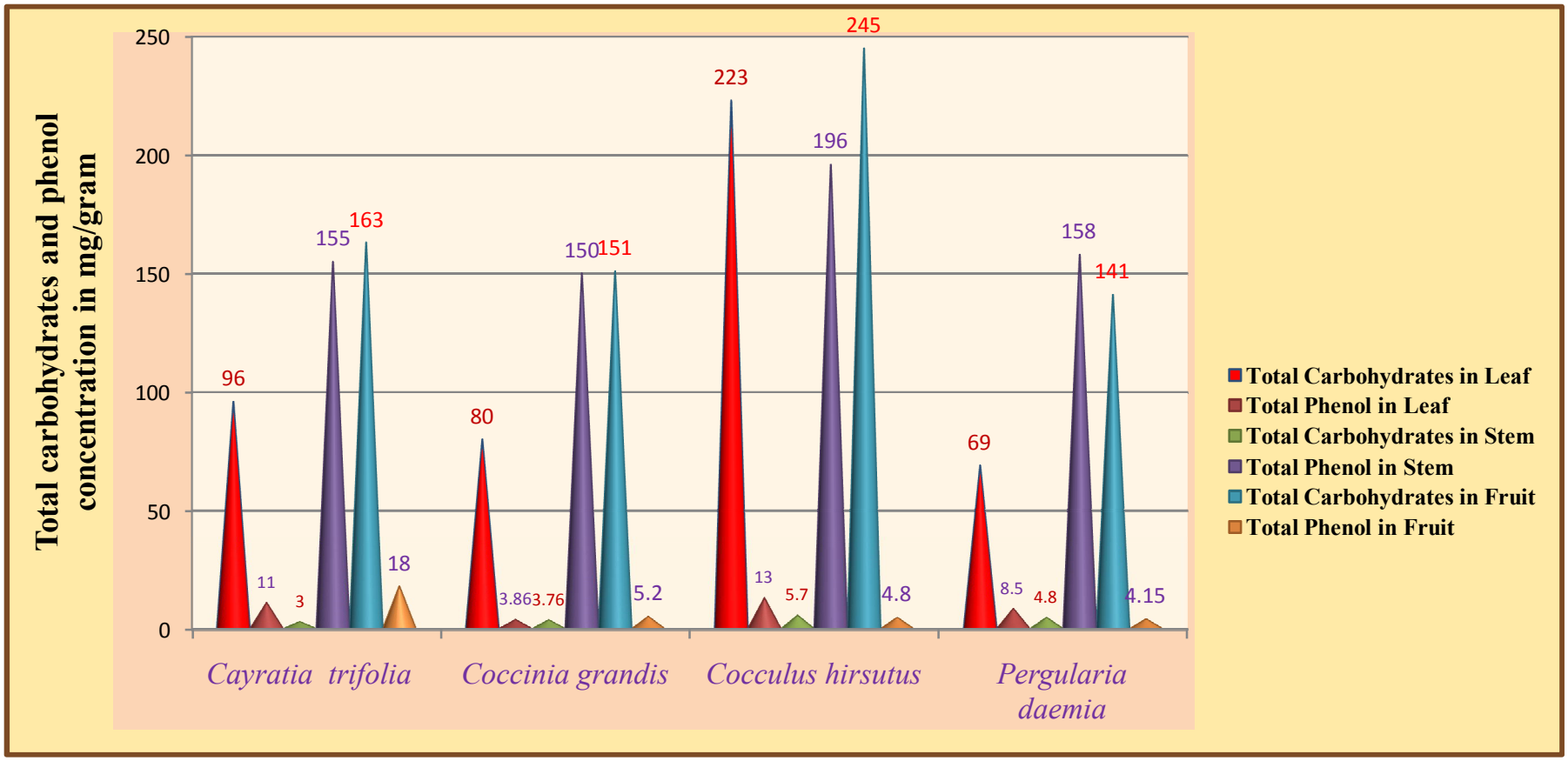


FIGURE 13 COMPARATIVE RESULTS OF TOTAL CARBOHYDRATES AND TOTAL PHENOLS IN LEAF, STEM AND FRUIT OF THE SELECTED CLIMBERS.

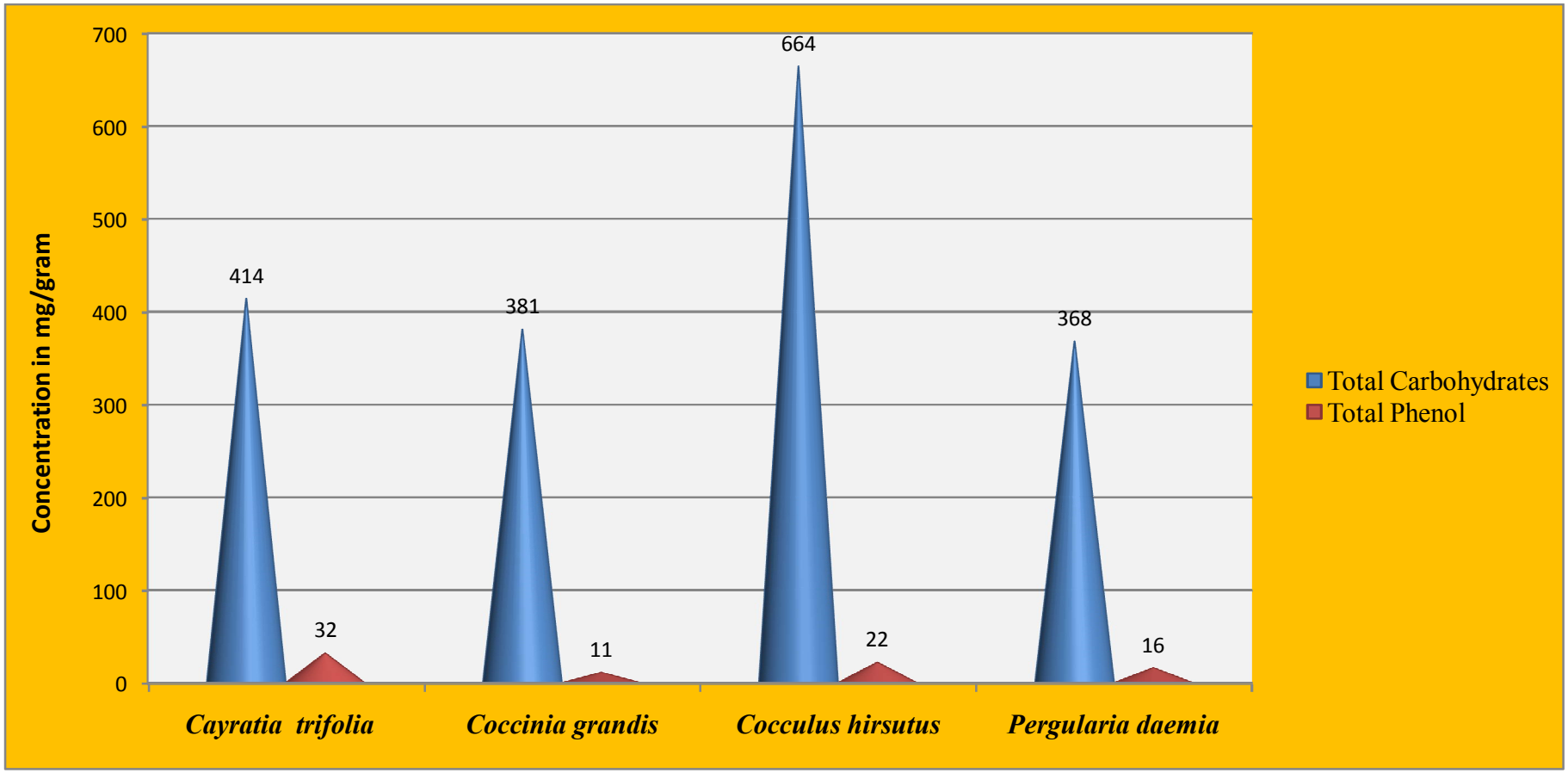


FIGURE 14 COMPARATIVE RESULTS OF TOTAL CARBOHYDRATES AND TOTAL PHENOL IN THE SELECTED CLIMBERS

CHAPTER 7

SUMMARY

Phytochemicals are produced and stored by plant species. However, they vary in different plant species. These biochemicals can be mainly divided into two groups, i.e., primary metabolites and secondary metabolites. The primary metabolites are fundamental biomolecules essential for the survival of the plants. Carbohydrates, proteins, amino acids, fatty acids, fats and fixed oils, pyridine and purines are examples of primary metabolites. They are produced in cells in good quantity. Secondary metabolites are non–essential for basic life processes in plants. The secondary metabolic pathways result in production of secondary metabolic products such as alkaloid, phenol, glycoside, terpenes, gum, antibiotics, etc. They work in plants for safety, energy, antibiotic and many functions which are yet to be ascertained. Though, they do not make special contributions to the life processes of plants yet make any plant species special. Many secondary metabolites derived from that plant are very useful economically and in therapeutic uses.

Among primary metabolites, carbohydrates constitute major group of macromolecules in plants. It is an important group of organic compounds which is found stored mainly in vegetative tissues. It is one of the three main ingredients of food and is important in organic and industrial perspective. In this group there are useful substances in the form of sugars, starch, cellulose and stored food. These are produced during photosynthesis in plants. Lipids are also prominent primary metabolites found in plants and animals. In each living cell, these substances are abundant in the form of fats and oils. Lipids are found in every plant, but their content and composition differ widely, depending on the type and parts of the plant. The main lipids include *triacylglycerols, diacylglycerols, monoacylglycerols,* phospholipids, glycolipids, sterols and free fatty acids. Protein has the most important role among various types of components present in living cells of plants and animals. Chemically, proteins are complex nitrogenous organic substances that contain higher molecular weight. All of the activities of the plants occur in the presence of specific protein enzymes. About 20 types of amino acids are obtained from hydrolysis of different types of proteins.

Alkaloids are heterocyclic nitrogenous compounds which form large group of secondary plant products. Most of the alkaloids are crystalline solid bitter and are very poisonous. Alkaloids protect the plants against insects and other animals. Terpenes are functionally diverse group of secondary metabolites. Being chief constituents of essential oils, many of terpenes are integral to primary metabolism, e.g., hormones, electron carriers, terpene–dervied compounds and photosynthetic pigments. Terpenoids, mainly found in leaf glandular trichomes, bud exudates and bark resins act as antioxidants, attractants of pollinators, growth hormone and reserve food material. It also helps in healing a wound of the plant. In plants, phenolic compounds are essential for growth and process of reproduction. These are also produced against pathogens as a part of defence mechanism. More examples of secondary metabolites are flavonoids, glycosides and saponins which play a number of roles in plants.

The Mej river is a tributary of the Chambal which is a perennial river of the Rajasthan. The catchment area of the Mej river is spread over four districts of the state namely, Bhilwara, Bundi, Tonk and Kota. However, most of the area lies in the Bundi district which is located in the southern part of Rajasthan and is inhabitated by tribal and ethnic communities like Bhil, Meena, Kanjar, Sansi, Bhat, Mogya, Kalbeliya, Banjara, etc. These groups have been living for long in harmony with the nature which full-fills most of their requirements such as food, fodder, medicine, fuel, etc. Further this interaction with nature has generated knowledge of medicinal properties of many plant species which are locally available in the catchment area of the Mej river. Hence, present research has been undertaken to carry out phytochemical study in the catchment area in and around Mej river with respect to the selected climber species.

Various medicinal climbers are found in the catchment area of the Mej river. A total of 27 species of climbers of various plant families have been recorded during a preliminary survey. The main climbers among these such as *Cayratia trifolia, Coccinia grandis, Cocculus hirsutus and Pergularia daemia* were selected for the phytochemical study. The primary objective of this work was to carry out comparative phytochemical analysis of these climbers.

Selected climbers were collected from in and around catchment area of Mej river, during the visits which were made into herbarium specimens for future reference. The prepared herbarium sheets of all the climbers were preserved and deported in the Herbarium Chamber of Department of Botany Government College Bundi. Three of the parts, i.e., leaf, stem and fruit of each of selected climber species were dried and pulverized with the help of an electric grinder. The extraction was done separately for each part with ethanol solvent. Soxhlet apparatus was used for preparation of extracts in this study. The condensed extracts were stored as stock solution which was further used for screening of primary and secondary metabolites.

Selected plants were evaluated for their phytochemical profile. The parameters included in the study were qualitative and quantitative methods for primary and secondary metabolites. Qualitative analysis was done to ascertain presence of primary and secondary metabolites of carbohydrates, reducing sugar, proteins, fats and fixed oils, alkaloids, phenols, flavonoids, glycosides, saponins, as well as terpenoids, etc. Quantitative methods included were estimation of total carbohydrates for primary metabolites and estimation of total phenols for secondary metabolites. All the experiments were carried out in triplicates and data reported were subjected to mean, standard deviation, standard errors, calculation of linear correlation coefficient and correlation analysis using MS Office Excel 2007.

Qualitative analysis of the four primary and six secondary metabolites by fifteen tests showed positive results in all the twelve extracts (three extracts for each plant species). However, the degree of precipitation in different tests ranged from lesser to higher. Molish's test for carbohydrates and Benedict's test for reducing sugar resulted in higher degree of precipitation in eight and two extracts, respectively. Millon's test and biuret test to ascertain presence of proteins gave higher degree precipitation of in eleven and ten extracts, respectively. For fat and fixed oils, saponification test showed higher degree of precipitation in three extracts. Mayer's test and Wagner's test for alkaloids presented higher degree of precipitation only in one extract each. However, Hager's test for the same secondary metabolites showed higher degree of precipitation in four extracts. Presence of phenol was ascertained by ferric

chloride and lead acetate tests which showed higher degree of precipitation in ten and eleven extract, respectively. Sodium hydroxide and alkaline reagent tests for flavonoids exhibited higher degree of precipitation in seven and five extracts, respectively. Froth test for saponins resulted in higher degree of precipitation only in six extracts. Presence of glycosides was exhibited by horizon test with higher degree of precipitation only in four extracts. Legal test for terpenoids also gave higher degree of precipitation in four extracts.

In the quantitative analysis of the selected plant species namely, *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* and *Pergularia daemia,* total carbohydrate content (total of leaf, stem and fruit) was recorded to be 414 mg, 381 mg, 664 mg and 368 mg glucose equivalent per gram sample, respectively. The maximum concentration of total carbohydrate content (664mg/g) has been reported in the whole part of *Cocculus hirsutus.* However, the minimum concentration of the same primary metabolites has been reported in the *Pergularia daemia*. Moderate concentrations of total carbohydrate content, i.e., 414 mg and 381mg have been reported in whole climber of *Cayratia trifolia* and *Coccinia grandis*, respectively.

Estimation of total phenol content of leaf, stem and fruit of selected climbers namely, *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* and *Pergularia daemia* revealed 32 mg, 11 mg, 22 mg and 16 mg catechol equivalent per gram sample total phenol content, respectively. These data show that the maximum concentration of total phenol content (32 mg) has been reported in *Cayratia trifolia* however, the minimum concentration of the same secondary metabolite (11 mg) has been reported in *Coccinia grandis*. In the whole part of *Cocculus hirsutus* and *Pergularia daemia,* total phenol content recorded was (22 mg and 16 mg), respectively.

Present research highlights the presence of primary metabolite like carbohydrates, protein and lipid and secondary metabolites like alkaloids, phenols, flavonoids, glycosides, saponins, and terpenoids in climbers of *Cayratia trifolia*, *Coccinia grandis*, *Cocculus hirsutus* and *Pergularia daemia*. In this study, comparative analysis for primary and secondary metabolites in ethanolic extract and dry powder of these climbers has been done. Carbohydrates and phenol have

been selected for quantitative analysis as primary and secondary metabolites respectively. Comparative quantitative estimation of carbohydrates and phenol is also included in this study. These results of comparative analysis have been depicted through tables and figures.

CONCLUSION

In the present investigation an attempt has been made to evaluate the chemical constitutes in *Cayratia trifolia, Coccinia grandis, Pergularia daemia* and *Cocculus hirsutus* of Mej river locality. The present research work greatly helped to understand the phytochemical constitutes of these climbers of the study area. After completion of the present research pertaining to the preliminary survey and phytochemical analysis of the selected climbers found in and around the catchment area of the Mej river, it is found that-

- Phytochemical analysis of the four selected climber species shows that *Cocculus hirsutus* has highest amount (664mg/g) of carbohydrates collectively in the leaf, stem and fruit extracts.
- *Cayratia trifolia* has been recorded to possess highest amount (32mg/g) of phenol content collectively in the leaf, stem and fruit extracts.
- Leaf, stem and fruit ethanolic extracts of the selected climber species have positive results for the primary metabolites.
- Dry leaf, stem and fruit powder of the studied climber species possess phenol contents. However, the quantity varies in different climber species.
- This region is on the verge of destruction as deforestation, illegal mining, industrialization, pollution and encroachment and other anthropogenic activities severely affects the flow of the river.

All small streams discharging their water in the Mej river are being encroached upon by the residents of the area for residential and agricultural use. Gudha dam was constructed for irrigation, but the construction of this dam has interrupted the natural flow of the river. Due to the change in its natural flow, the

aquatic system of the river and the plant community are negatively affected. Among the many reasons for shrinkage of Mej river area and fast depletion of vegetation, anthropogenic disturbance is considered as the most important one. It is urgent need to conserve the river area and natural vegetation through the involvement of rural societies, tribal peoples and nomadic groups living there.

It is clear from the results that bioactive molecules play a major role in characterizing the plant body. Phytochemical constitutes also play an important role in maintaining ecological status, phytophysiology and stress tolerance of plant body. Tabular presentation of data in this study will be useful as future reference. Qualitative analysis of remaining primary and secondary metabolites and studies of their bioactivity may be carried out in further studies to characterize and isolate active biomolecules for therapeutic and other uses.

The Mej river is the life line for the fauna and flora of the surrounding area hence, the area should be monitored to check mining and other illegal anthropogenic activities. Dense plantation should be done in the catchment area of the river to check soil erosion. Natural course of the streams discharging in the river should be free of illegal encroachment so that flooding of the area could be avoided.

FUTURE ASPECTS

- Present study recorded highest amount of carbohydrates content collectively in the leaf, stem and fruit extract of *Cocculus hirsutus*. Further study may be undertaken to ascertain chemical structure and properties of the primary metabolites.
- Phenol content has been recorded to be highest in *Cayratia trifolia* which may be tasted for its antioxidant properties using different assays.
- Quantitative estimation of the primary and secondary metabolites may be extended to other parts of the same plant species, i.e., flowers, bark, root, etc.
- Quantitative estimation in further studies may encompass estimation of other secondary metabolites in the studied plant species such as alkaloids, saponins, flavonoids, etc.

- Qualitative and quantitative analysis of primary and secondary metabolites in other plant species may also be undertaken for their further use in medicinal uses.
- Chemotaxonomy is a promising branch which is very popular in the field of taxonomy. It is an important tool used for the determination of systematic position of flowering plants. Phytochemical analysis of the four selected climbers in and around Mej river done in the present work could also be used in chemo taxonomy.
- The work is also related with stress physiology. The physiochemical behaviour of plants can be determined through this investigation. Ecological niche of plant species of different habitats could be predicated by the phytochemical screening of vegetation of the area.
- Extensive work in further studies may be done to correlate phytochemicals of the plants with the ecological condition of the plants residing in the study area.

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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Qualitative Analysis of Ethanolic Extract of Cayratia Trifolia (Medicinal Climber) For Primary Metabolites

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Abstract

Cayratia trifolia is perennial medicinal climber plant belonging to family Vitaceae. Qualitative analysis of primary metabolites from the ethanolic extract of different parts of this plant has been done in this work. The study revealed that they contain sufficient amount of Carbohydrates, Proteins and Lipids.

Keywords: Metabolites, Alkaloid, Phenol, Glycoside, Turpenes.

Introduction

Metabolic substances are an important part of plant life, without which biological processes cannot be imagined. In plant cells, biochemical processes occur in the coordinated and balanced form. The bio molecules produced by these pathways are termed metabolites. The metabolites can be mainly divided into two types such as primary metabolites and secondary metabolites. The primary metabolites are essential for the survival of the plants life. There products is result of the primary metabolic pathways, which include sugars, proteins, amino acids, fatty acids, fats, pyrimidines and purines. These cells are produced in large amounts. Secondary metabolites are non–essential for basic biochemical and survival of plants process. Secondary metabolic products, such as alkaloid, phenol, glycoside, turpenes, and gums antibiotics and so on are produced as a result of the secondary metabolic pathway. They work in plants only for safety, accumulation of food, energy, and resistance against various pathogens. They do not make special contributions to the life processes of plants but make any plant species special. Some of the products derived from that plant are very useful economically in therapeutic practices for human and animals. Plants products are most wonderful gift from nature has been used as drugs. Some plant species which are across different ethnic groups various types of drugs are obtained from are known as medicinal plants (Yadav *et al*, 2010).

Cayratia trifolia (Family Vitaceae) is climbing or prostrate, much branched, perennial herb commonly known as fox grape in English and Amalbel in Hindi. It also possess medicinal properties. *Cayratia trifolia* is a weak herbaceous climber contains trifoliated leaves with (2-3 cm), long petioles and ovate to oblong-ovate leaflets. Flowers are tiny greenish white brown in colour. It is distributed in both wild and cultivated states on the plains of India. The present research paper deals and qualitative test of the ethanolic extracts of climber *Cayratia trifolia* for primary metabolites.

Aim of the study

1. To prepare extract of different parts (leaves, stem, fruit) of *Cayratia trifolia* on organic solvent ethanol.
2. Identification of primary metabolite in the extract to facilitate further study for human welfare.

Material and Methods

Plant Collection

Cayratia trifolia was collected from in and around catchment area of Mej River. The identity of the plant species was established by Herbarium chamber Government College, Bundi by author department of botany.

Preparation of Plant Extract

Fresh leaves stem and fruit of *Cayratia trifolia* were washed thoroughly tap water and were dried in hot air oven at 40-50°c for a week. 60gm of dried powder was extracted for 24 hours in 300 ml solvent (ethanol 99%). Repeated extraction was done with the some solvent till colourless solvent was obtained. The condensed extract was used for

screening of primary metabolites. Soxhlet equipment was used in this study. Powdered plant material (60 g) was extracted with organic solvents (300 ml) such as n-hexane, ethyl acetate methanol and ethanol in Soxhlet apparatus (Raaman, 2006)

Primary Metabolites analysis

Test for carbohydrates

Molisch test

The test was carried out by following the method Ramakrishanan *et al.* 1994. 2 ml of aliquot of the extract was treated with 2 drops of Molisch reagent. After shaking and holding test tube in slanting position 2 ml concentrate Sulphuric acid along the side of the test tube. The reddish violet ring at the junction of two solutions indicates presence of Carbohydrates.

Benedict's test:-

1 ml of aliquot of the extract was treated with 3 ml of Benedict's reagent in a test tube and boiled for 10 minutes. Bluish or yellowish orange precipitate indicates the presence of reducing sugar.

Test for Proteins

Millon's test

The test was carried out by following the method Fisher, 1968; Ruthmann, 1970. 2 ml of aliquot of the extract was treated with 2 drops of Millon's reagent in a test tube. The test tube a white creamy precipitate appeared which changed to brick red on heating. It indicates the presence of Proteins.

Biuret test

The test was carried out by following the method Gahan, 1984. An aliquot of 2 ml of filtrate is treated with few drops of copper sulphate solution. To this, 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicates the presence of Proteins.

Test for Fats and Fixed Oils

Saponification Test

The test was carried out by following the method Kokate, (1999). A few drops of 0.5 N alcoholic potassium hydroxide solutions are added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on water bath for 2 hours. Formation of Soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Result and Discussion

Table displays result of qualitative analysis of ethanolic extract of different part of *Cayratia trifolia* which reveal that all the extracted plant material (Leaves, Stem, Fruit) of *Cayratia trifolia* possess carbohydrates. The presence of carbohydrates was ascertained by Molisch test. The result reveals that fruits and leaves extract has more quantity of carbohydrates as it exhibited higher degree of precipitation (+++). However the stem extracts showed lesser degree (+) of precipitation.

The presence of reducing sugars was ascertained by Benedict test. Fruit extract exhibited presence of reducing sugars with higher degree of red precipitation (+++). However leaf extract showed presence of reducing sugars with moderate degree of precipitation (++) and stem extract showed lesser degree of precipitation (+).

The presence of protein in the extract was ascertained by Millon test and Biuret test. Fruits and Leaves extract exhibited presence of protein with higher degree of precipitation (+++) in both Millon and Biuret test. However stem extract showed presence of protein with higher degree of precipitation (+++) in Millon test and stem extract showed moderate degree of precipitation (++) in Biuret test.

Saponification test indicates the presence of Fats and fixed oils with high degree of precipitation (+++) in fruit extract whereas the same test resulted in lesser degree of precipitation (+) for the leaves and stem extract.

Present findings are supported by work of a number of researchers who also carried out phytochemical analysis studies of *Cayratia trifolia*. Sowmya et al. (2015) were investigated the presence of phytoconstituents in the different parts stem, leaf and fruit of *Cayratia trifolia*. Singh et al. (2012) was carried out to establish the pharmacognostical studies, physico-chemical parameters along with preliminary phytochemical screening of petroleum ether, chloroform, methanolic and aqueous extracts of *Cayratia trifolia* (Linn.) Kumar et al. (2012) were presented a detailed pharmacognostic study of the leaf of *Cayratia trifolia*. Present finding are supported similar research worked by Prasad & Sharma (2018), Bhaduria *et al*,.(2012), Rahman *et al*,. (2015) and Deokate & Khadabadi, (2012). They evaluated different climber species which also support present research work.

Table -1

S.No.	Phytochemical	Name of test	Plant part	Observation
1	Carbohydrates	Molisch test	Leaves	+++
			Stem	+
			Fruit	+++
		Benedict's test	Leaves	++
			Stem	+
			Fruit	+++
2	Proteins	Millon test	Leaves	+++
			Stem	+++
			Fruit	+++
		Biuret test	Leaves	+++
			Stem	++
			Fruit	+++
3	Fats and fixed oils	Saponification test	Fruit	+++
			Leaves	+
			Stem	+

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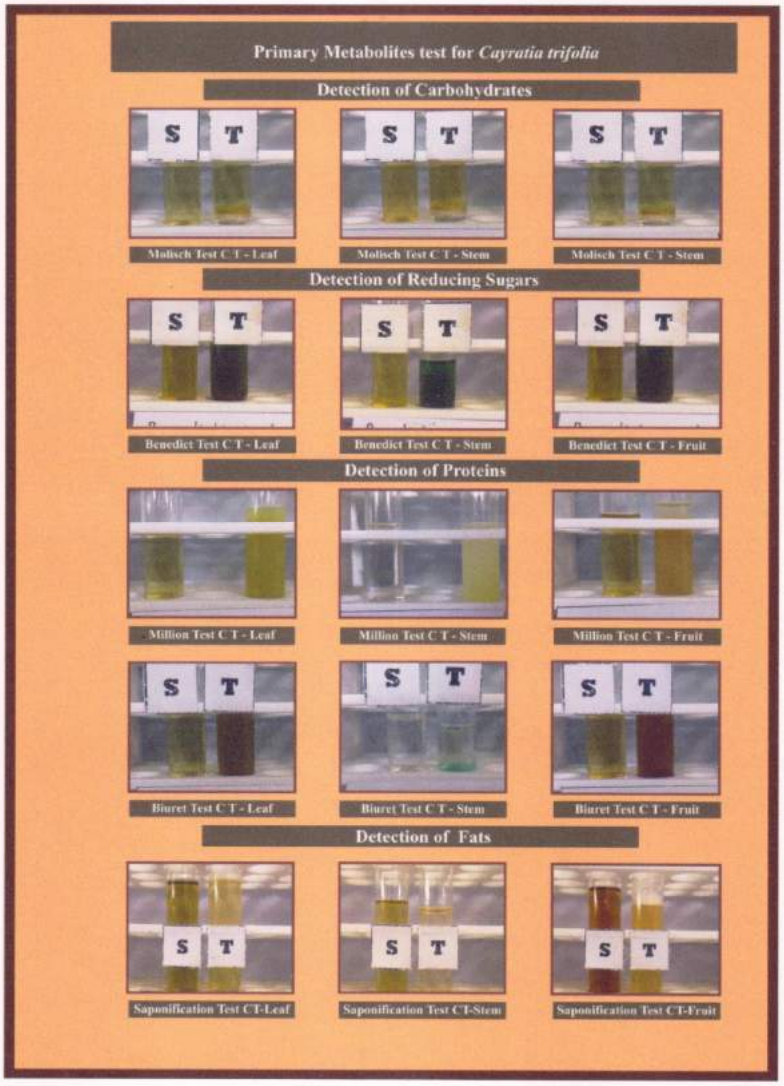

Conclusion

Present research highlights the presence of primary metabolite like carbohydrates, protein and lipid in *Cayratia trifolia*. Beneficial properties could be done in further study by qualitative assessment of this climber.

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Qualitative test on ethanolic extract of *Coccinia indica* for profiling Secondary metabolites

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Abstract

Coccinia indica is climber, perennial medicinal plant belonging to family Cucurbitaceae. A qualitative assessment of ethanolic extract of different parts of the plant species revealed that they contain protein, carbohydrates, and lipids, Alkaloids, Phenols, Flavonoids, Saponin, Terpenoids and Glycosides.

Keywords:-*Metabolites, Alkaloid, Phenol, Glycoside, Turpenes, Antibiotic.*

Introduction

Metabolic substances are an important part of plant life, without which biological processes cannot be imagined. In plant cells, biochemical processes occur in the coordinated and balanced form. The bio molecules produced by these pathways are termed metabolites. The metabolites can be mainly divided into two types such as primary metabolites and secondary metabolites. The primary metabolites are essential for the survival of the plants life. There products is result of the primary metabolic pathways, which include sugars, proteins, amino acids, fatty acids, fats, pyrimidines and purines. These cells are produced in large amounts. Secondary metabolites are non–essential for basic biochemical and survival of plants process. Secondary metabolic products, such as alkaloid, phenol, glycoside, turpenes, and gums antibiotics and so on are produced as a result of the secondary metabolic pathway. They work in plants only for safety, accumulation of food, energy, and resistance against various pathogens. They do not make special contributions to the life processes of plants but make any plant species special. Some of the products derived from that plant are very useful economically in therapeutic practices for human and animals. Plants products the most wonderful gift from nature has been used as drugs. Some plant species which are across different ethnic groups various types of drugs are obtained from are known as medicinal plants [1].

Coccinia indica (synonym *Coccinia grandis*) Wight and Arn (Family Cucurbitaceae) which is a climbing or prostrate, much branched, perennial herb commonly known as *kundri* is also a medicinal plant. It is distributed in both wild and cultivated states on the plains of India. The present research paper deals with and qualitative test of the ethanolic extract of climber *Coccinia indica* for Secondary metabolites.

Aim of the study

1. To prepare extract of different parts (leaves, stem, fruit) of *Coccinia indica* on organic solvent ethanol.
2. Identification of Secondary metabolite in the extract to facilitate further study for human welfare.

Material and Methods:-

Plant collection *Coccinia indica* was collected from in and around catchment area of Mej River. The identity of the plant species was established by Herbarium chamber Government College, Bundi by author department of botany (see plate no-1).

Preparation of plant extract:-

Fresh leaves stem and fruit of *Coccinia indica* were washed thoroughly tap water and were dried in hot air oven at 40-50° c for a week. 30gm of dried powder was extracted for 24 hours in 300 ml solvent (ethanol 99%). Repeated extraction was done with the some solvent till colourless solvent was obtained. The condensed extract was used for screening of primary and secondary metabolites.

Secondary Metabolites analysis:-

Extracts were tested for the presence of active principles. Following standard procedures were used [2, 3].

Test of Alkaloids

Mayer's test:-

4 ml of aliquot of the extract was treated with 1 ml of Mayer's reagent (Potassium mercuric iodide). White or Creamy Precipitate in the ethanolic layer indicates the presence of Alkaloids.

Wagner's test:-

4 ml of aliquot of the extract was treated with 1 ml of Wagner's reagent (iodine in Potassium iodide). A reddish brown precipitate confirms the test as positive.

Hager's test:-

4 ml of aliquot of the extract was treated with 1 or 2 ml of Hager's reagent (saturated aqueous of picric acid). Prominent yellow precipitate in the ethanolic layer indicates the presence of Alkaloids.

Test for Phenols

Ferric chloride test:

The test was carried out by following the method Mace, 1963. An aliquot of 3 ml of filtrate is treated with 1ml of 5% Ferric chloride solution in a test tube. The Black-Blue colour in the ethanolic layer indicates the presence of Tannins and Phenols.

Lead acetate test:

An aliquot of 3 ml of filtrate is treated with 1 ml of 10% Lead acetate solution. A bulky white precipitate indicates the presence of phenolic compound.

Test of Flavonoids

Alkaline reagent test

An aliquot of 3 ml of filtrate is treated with few drops of 10% Sodium hydroxide solution. Formations of intense yellow colour, which colour become colourless on further addition of dilute acid, indicate the presence of Flavonoids or an aliquot of 3 ml of filtrate is treated with few drops of 10% Ammonium hydroxide solution. Yellow fluorescence indicates the presence of Flavonoids.

Test of Saponins

Forth test

Take 1ml ethanolic aliquot and mix 50 mg sodium carbonate with 1.5 ml distilled water. It mixtures, shaken vigorously up to 5 minutes and formation of honey comb like froth was formed which showed the presence of Saponins. Which is stable for 15 minutes for a positive result.

Test of Terpenoids

Horizon test:-

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To 1 ml of ethanolic extract, 2 ml of trichloroacetic acid was added. The formation of yellow to red precipitate shows the presence of Terpenoids.

Test of Glycosides

Legal test:-

To 2ml of extract, 1 ml of Peridine and 1 ml of Sodium nitroprusside were added. The change in colour pink or red indicates the presence of glycosides.

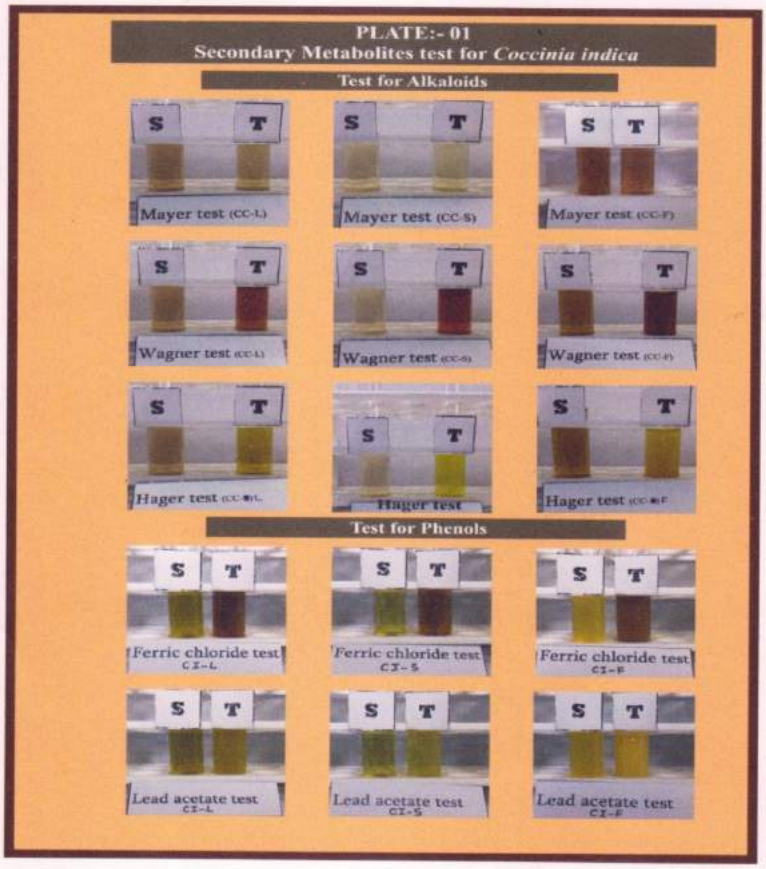

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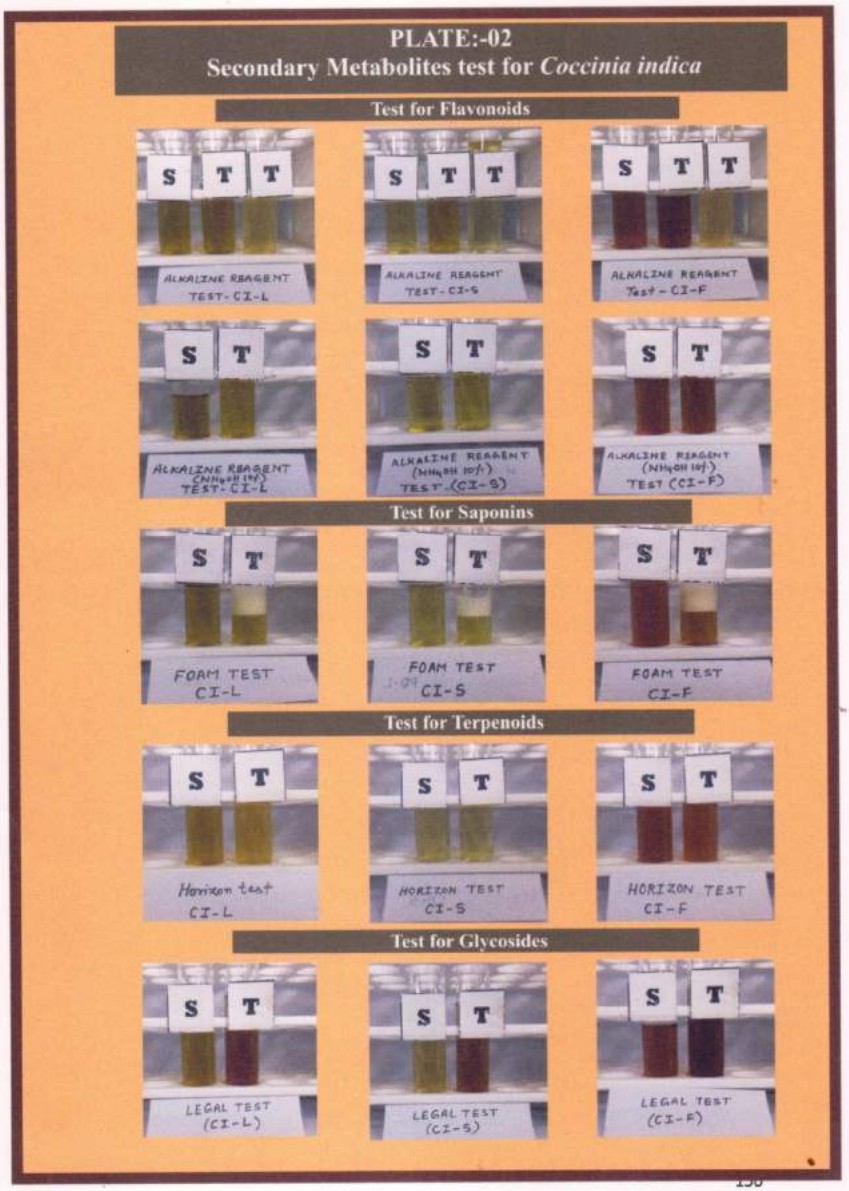
PLATE:-02
Secondary Metabolites test for *Coccinia indica*



Result and discussion:-

Table 1 and figures 1, 2 displays results of qualitative analysis of ethanolic extract of different part of *Coccinia indica* which reveal that all the extracted plant material (Leaves, Stem, Fruit) of *Coccinia indica* possess various secondary metabolites. The presence of alkaloids was ascertained by three tests namely, Mayer test, Hager test and Wagner test. The results reveal that stem and fruit extracts have more quantity of alkaloids as they exhibited higher degree of precipitation (+++) for Hager test, however, for the same test extract of leaves showed lesser degree (+) of precipitation. For all three extracts Mayer and Wagner's test resulted in lesser degree (+) of precipitation.

Ferric Chloride test and Lead Acetate test for testing presence of phenol compounds and tannins resulted in higher degree (+++) of precipitation for all three extracts. Sodium Hydroxide test and alkaline reagent test conducted for ascertaining presence of flavonoids showed presence of the same by varying degree of colouration.

Foam test for ascertaining presence of saponin resulted in formation of foam which shows presence of saponin in all three extracts. Fruit extract showed higher degree (+++) of yellow to red precipitate in Horizon test however stem and leaf extract showed lesser degree (+) of the precipitation. Legal test for presence of glycosides showed increasing intensity of colour for stem, leaves and fruit extract, respectively.

Present finding are supported by studies of Khatun et al. (2012) [4]. who found *Coccinia indica* contain bioactive constituents such as Tannins, Saponins, Phenols, Flavonoides and terpenoides. Yadav *et al.*, (2010)[2] , Shalini *et al*,.(2014)[5], Bhaduria *et al*,.(2012)[6], Rahman *et al*,. (2015)[7], Deokate & Khadabadi,(2012)[8], Sakharkar & Chauhan, (2017)[9] also evaluated different species of Coccinia which also support present research work.

Table -1

S.No.	Phytochemical	Name of test	Plant part	Observation
1	Alkaloids	Mayer's test	Leaves	+
			Stem	+
			Fruit	+
		Wanger's test	Leaves	+
			Stem	+
			Fruit	+
		Hager's test	Leaves	+
			Stem	+++
			Fruit	+++
2	Phenolics &Tennins	Ferric Chloride test	Leaves	+++
			Stem	+++
			Fruit	+++
		Lead Acetate Test	Leaves	+++
			Stem	+++
			Fruit	+++
3	Flavonoids	Sodium Hydroxide Test	Leaves	+
			Stem	+
			Fruit	+++
		Alkaline reagent test	Leaves	+
			Stem	+
			Fruit	+
4	Saponins	Foam Test	Leaves	+++

			Stem	+++
			Fruit	+++
5	Terpenoids	Horizon Test	Leaves	+
			Stem	+
			Fruit	+++
6.	Glycosides	Legal Test	Leaves	++
			Stem	+
			Fruit	+++

Conclusion

Present research highlights the presence of primary metabolite like carbohydrates, protein and lipid. *Coccinia indica* a medicinal plant species and further study may prove beneficial for human welfare.

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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Qualitative Test of Ethanolic Extract of Climber *Coccinia indica* for Primary Metabolites

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Abstract

Coccinia indica is climber, perennial medicinal plant belonging to family Cucurbitaceae. A qualtitative assessment of ethanolic extract of different parts of the plant species revealed that they contain protein, carbohydrates and lipids

Keywords: Metabolites, Alkaloid, Phenol, Glycoside, Turpenes, Antibiotic.

Introduction

Metabolic substances are an important part of plant life, without which biological processes cannot be imagined. In plant cells, biochemical processes occur in the coordinated and balanced form. The bio molecules produced by these pathways are termed metabolites. The metabolites can be mainly divided into two types such as primary metabolites and secondary metabolites. The primary metabolites are essential for the survival of the plants life. There products is result of the primary metabolic pathways, which include sugars, proteins, amino acids, fatty acids, fats, pyrimidines and purines. These cells are produced in large amounts. Secondary metabolites are non–essential for basic biochemical and survival of plants process. Secondary metabolic products, such as alkaloid, phenol, glycoside, turpenes, and gums antibiotics and so on are produced as a result of the secondary metabolic pathway. They work in plants only for safety, accumulation of food, energy, and resistance against various pathogens. They do not make special contributions to the life processes of plants but make any plant species special. Some of the products derived from that plant are very useful economically in therapeutic practices for human and animals. Plants products the most wonderful gift from nature has been used as drugs. Some plant species which are across different ethnic groups various types of drugs are obtained from are known as medicinal plants (Yadav *et al*, 2010).

Coccinia indica (synonym *Coccinia grandis*) Wight and Arn (Family Cucurbitaceae) which is a climbing or prostrate, much branched, perennial herb commonly known as *kundri* is also a medicinal plant. It is distributed in both wild and cultivated states on the plains of India. The present research paper deals with and qualitative test of the ethanolic extract of climber *Coccinia indica* for primary metabolites.

Aim of the Study

1. To prepare extract of different parts (leaves, stem, fruit) of *Coccinia indica* on organic solvent ethanol.
2. Identification of primary metabolite in the extract to facilitate further study for human welfare.

Material and Methods

Plant Collection

Coccinia indica was collected from in and around catchment area of Mej River. The identity of the plant species was established by Herbarium chamber Government College, Bundi by author department of botany.

***Coccinia indica* Whole Plant**

Preparation of Plant Extract

Fresh leaves stem and fruit of *Coccinia indica* were washed thoroughly tap water and were dried in hot air oven at 40-50° c for a week. 30gm of dried powder was extracted for 24 hours in 300 ml solvent (ethanol 99%). Repeated extraction was done with the some solvent till colourless solvent was obtained. The condensed extract was used for screening of primary metabolites.

Dried Leaves

Powder of dried Stem

Dried Fruit

Primary Metabolites Analysis

Test for Carbohydrates

Molisch Test

The test was carried out by following the method Ramakrishanan *et al.* 1994. 2 ml of aliquot of the extract was treated with 2 drops of Molisch reagent. After shaking and holding test tube in slanting position 2 ml concentrate Sulphuric acid along the side of the test tube. The reddish violet ring at the junction of two solutions indicates presence of Carbohydrates.

Test for Proteins

Millon's test

The test was carried out by following the method Fisher, 1968; Ruthmann, 1970. 2 ml of aliquot of the extract was treated with 2 drops of Millon's reagent in a test tube. The test tube a white creamy precipitate appeared which changed to brick red on heating. It the indicates the presence of proteins.

Biuret Test

The test was carried out by following the method Gahan, 1984. An aliquot of 2 ml of filtrate is treated with few drops of copper sulphate solution. To this, 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicates the presence of proteins.

Test for Fats

Spot Test

The test was carried out by following the method Kokate, 1999. A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

Result and Discussion

Table displays result of qualitative analysis of ethanolic extract of different part of *Coccinia indica* which reveal that all the extracted plant material (Leaves, Stem, Fruit) of *Coccinia indica* possess carbohydrates. The presence of carbohydrates was ascertained by Molisch test. The result reveals that fruit extract has more quantity of carbohydrates as it exhibited higher degree of precipitation (+++). The stem and leaves extract however showed moderate degree of precipitation.

The presence of protein in the extract was ascertained by Millon test and Biuret test. Fruit extract exhibited presence of protein with higher degree of precipitation (+++) in both Millon and Biuret test. However leaf extract showed presence of protein with higher degree of precipitation (+++) in Millon test but lasser degree of precipitation (+) in Biuret test. The variation in degree of precipitation was also evident in both test for stem extracted where the Millon test exbitied moderate degree of precipitation (++) as compared to the Biuret test which showoed lasser degree (+) of precipitation.

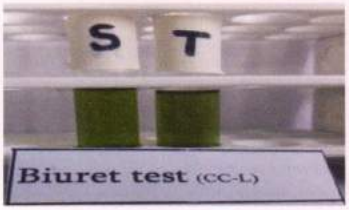
Soap test was used to ascertained presence of lipids in all these ethonolic extract (leaf, stem, fruit). The test showed presence of lipids in fruit leaves and stem of *Coccinia indica*.

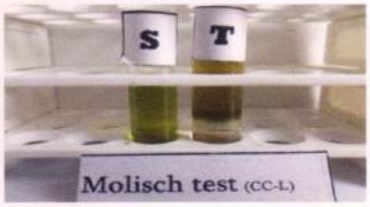
Present finding are supported by studies of Khatun et al. (2012) who found *Coccinia indica* contain bioactive constituents such as Tannins, Saponins, Phenols, Flavonoides and terpenoides. Yadav *et al.*, (2010).

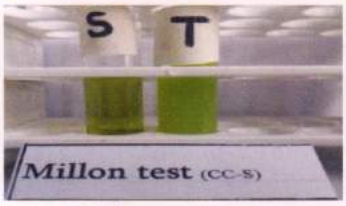
Shalini *et al*,.(2014), Deshpande *et al*,.(2011), Bhaduria *et al*,.(2012), Rahman *et al*,. (2015), Deokate & Khadabadi,(2012), Sakharkar & Chauhan, (2017) also evaluated different species of Coccinia which also support present research work.

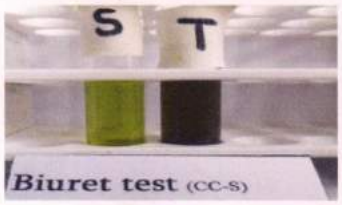
Table -1

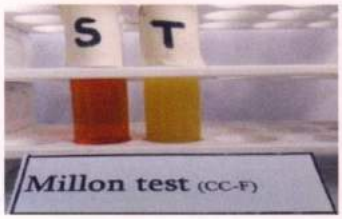
S.No.	Phytochemical	Name of Test	Plant Part	Observation
1	Carbohydrates	Molisch test	Leaves	++
			Stem	++
			Fruit	+++
2	Proteins	Millon test	Leaves	+++
			Stem	++
			Fruit	+++
		Biuret test	Leaves	+
			Stem	+
			Fruit	+++
3	Fats	Soap test	Leaves	+++
			Stem	+
			Fruit	+++

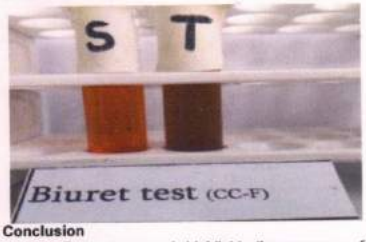












Conclusion

Present research highlights the presence of primary metabolite like carbohydrates, protein and lipid. *Coccinia indica* a medicinal plant species and further study may prove beneficial for human welfare.

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