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Investigation of Biologicaly Active Phytoconstituents present in selected Plants Material of Verbenaceae, Lamiaceae and Fabaceae family

I.P. Tripathi[1], Ruchita Tripathi[2*] and Amit Tiwari[3]

[1]Dean, Department of Biosciences, M.G.C.G.V., Chitrakoot, Satna (M.P.), India
[2]Research Scholar, Department of Biotechnology, M.G.C.G.V., Chitrakoot, Satna (M.P.), India
[3]HOD, Department of Zoology & Biotechnology, Govt T.R.S. (Darbar) College, Rewa (M.P.), India

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Abstract

The present investigation represent to qualitative analysis of available parts of six medicinal plants of three different families found in Chitrakoot region. Selected plant materials were extracted with methanol, petroleum ether and water by cold and hot extraction methods and screened for the presence of carbohydrates, alkaloids, flavonoids, proteins, rasin, anthocynin and betacynin, saponin, steroids, starch, tannins, starch, glycosides, phenol, phlobatannins and terpenoids. We found that the selected plants are good source of various phytochemicals. This study revealed the presence of various biologically active secondary metabolites which could be helpful in the prevention of systemic infectious diseases.

Keywords: *Phytochemicals, medicinal plants, fungal diseases, systemic infection, qualitative property, yield.*

Introduction

The emergence of several fungi as opportunistic pathogens has reawakened interest in chemotherapeutic and prophylactic agents for mycoses. During the past decades significant advances have been made in the development of novel antimycotic agents for treatment of systemic mycoses. It correlates with the modernization in medicine and surgery as also increasing incidence of AIDS and neoplasms. Though a battery of antimycotic agents is available for clinical use and significant advances have been made in antimycotic therapy, yet the management of deep mycoses poses a formidable challenge. There is indeed urgent need to redouble efforts both in the academia and industry to develop new antimycotic agents and pursue novel strategies to cope with diagnosis and treatment of fungal infections [1]. Such resistant fungi are a frequent cause of morbidity and even death in immune-compromised and immune-competent patient hence are challenges to both the physicians and mycologist. Hence, resistance ability and some adverse effect of antimycotic allopathic medication, forced for alternative or modern therapy.

Plants are best source for developing alternative drugs, extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer, antibacterial, antifungal, antidiabetic, antioxidant, hepatoprotective, hemolytic, larvicidal and anti-inflammatory activity**.** Developing as well as developed countries use traditional plant medicines which possess phytochemicals (bioactive compounds) can be derived from barks, leaves, flowers, roots, fruits and seeds. These isolated phytochemicals include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids, phlobatannins, starch, glycosides, phytosterols and saponinetc, which provide certain physiological action on the human body as prevent to chronic infectious diseases and also possess other biochemical properties like free radical scavenging activity or antioxidant activity [2].

Chitrakoot situated in the northern region of Satna district of Madhya Pradesh, has a very rich wealth of medicinal plants which has also been described in our epics like Ramayana. The place is well known for its beautiful hill ranges, historical caves, perennial streams and diverse fauna and flora. Vast variety of herbs, shrubs, trees, climbers, having different flowers, fruits, roots are available [3].This work is intended to screen different phytoconstituents found in some selected six medicinal plants parts of Chitrakoot area.

Material and method

Six plants of family Verbenaceae, Lamieaceae, Fabaceae were collected from different regions of Chitrakoot, and washed with 70% methanol. These were shade dried in room temperature and grinded using a grinder. Powdered sample was kept in air tight container.

Preparation of plant extracts &Yeild (%) of selected plant extracts (by 80% methanol)

Dried plant materials were extracted with water, methanol (hot extraction) and petroleum ether (cold extraction). Methods of extract preparation were adapted from Pandey S. (2014), [4] with some modifications.

Preparation of water and methanol extract: 10 gm of dried sample was taken for maceration and dissolved in 50ml of water (to prepare water extract), and 50ml (90%) of methanol (for methanol extract) and kept in rotary shaker for 1 h. Then it was filtered through whatman No. 1 filter paper and the filtrate was used for the screening. Preparation of petroleum ether extract: 10 gm of dried powdered was taken and soaked in 50 ml petroleum ether and kept in refrigerator for 1 hour. After 1h, it was filtered through Whatman No. 1 filter paper and the filtrate was used for analysis.

The yield of resultant plant extrats were found by following formula:

(yeild of plant extract/wight of sample)* 100

All the obtained extracts were then subjected to different qualitative tests to find out the presence of specific phytochemicals.

(i) Test for Carbohydrates

Molicsh test: 1 ml of sample is placed in a test tube and two dropes of Molisch reagent was added. 2ml solution of concentrated H_2SO_4 was added in test tube. Formation of Red violet ring in the interface gave the positive Molisch test.

Fehling test: 2ml solution of Fehling A and Fehling B were taken in a test tube then dropwise sample were added. The mixture was shaken well and kept in a water bath for 10-15 minuts at 100 ^oC. A rusty brown or brick red colour precipitate confirms the presense of carbohydrates in the sample.

Anthron test: 2 ml of anthron reagent was added to 500 µl of extract. Formation of green blue colour gives a positive anthron test.

(ii) Test for Alkaloids

Mayer's test: 1ml of sample was added to a few drops of Mayer's reagent. Formation of white or pale yellow precipitate indicates the presence of alkaloids in the sample.

Wagner's test: 1.5% of HCl was added in 1 ml of extract and a few drops of Wagner's reagent were added to it. Appearance of yellow/ brown precipitate indicates the presence of alkaloids.

Dragondrof test: 5ml of distilled water was added to the the 2 ml of sample, then 2M HCl and 1 ml of Dragondrof's reagent was added. Orange / orange red precipitate indicates the presence of alkaloids.

(iii) Test for Flavonoids

H_2SO_4 test: A fraction of the extract was taken and treated with concentrated H_2SO_4 and observed for the formation of orange colour.

(iv) Test for Proteins

Biuret test: 1% of NaoH was added to 1 ml of extract and few drops of 1% $CuSO_4$ were then added. Blue/ purple or violet/ pinkish colour indicates the presence of proteins.

Millon's test: 1 ml of test extract was mixed with H_2SO_4 then Millon's reagent was added dropwise. White/ yellow precipitate appears which turns into red colour precipitate, after heating the mixture. This indicates the presence of proteins.

Ninhydrin's test: 2 drops of freshly prepared Ninhydrin reagent (0.1% in n- butanol) is added to 1ml of extract and heat and observed for blue or red orange colour.

(v) Test for Rasins

1ml of ethanolic extract was dissolved in acetone and then 1 ml of distilled water is added. Turbidity indicates the presence of rasin.

(vi) Test for Tannins

To 1 ml of the extract, 2ml of 5% $FeCl_3$ is added which gives dark blue or greenish black colour and a positive tannin test.

(vii) Test for Steroids

Salvoski test: 1 ml of test sample was dissolved in 1 ml of chloroform and equal amount of concentrated H_2SO_4. Formation of Bluish red to cherry colour in chloroform layer shows the presence of steroids.

(viii) Test of Saponin

Foam test: A small amout of extract was shaken with water and observed for the presence of foam.
Sodium Bicarbonate test: Few drops of Sodium bicarbonate was added to 1 ml of plant extract. If honeycomb like structure forms, it confirms saponin.

(ix) Test for Anthocyanin and Betacynin

1 ml of plant extract was treated with 1 ml of 2N NaOH then heated. Formation of bluish –green colour indicated the presence of Anthocynin while yellow colour indicated the presence of betacynin.

(x) Test for Starch

1 ml of I_2 solution is mixed in 1ml of extract, formation of blue colour indicated the presence of starch in the extract.

(xi) Test for Glycosides

To 1 ml of plant extract, 1 ml $Fecl_3$ (5%), and equal amount of acetic acid is added, then few drops of H_2SO_4 is added to the mixture. Greenish blue colour indicates the presence of glycosides.

(xii) Test for phenols

1ml of plant extract, when treated with few drops of $FeCl_3$ solution; it gives blue green colour and confirms the presence of phenols.

(xiii) Test for Phlobatannins

1ml of plant extract was treated with 1 ml of 1% HCl and heat. Red colour precipitate indicates the presence of Phlobatannins in the sample.

(xiv) Test for Terpinoids

To 1ml of plant extract, 2ml of chloroform and 3ml of conc. H_2SO_4 was added. Areddish brown precipitate at thr interface, confirmed the presence of terpenoids.

Result and Discussion

After collection and authentification, collected plant parts extracted with 80% methanol with maceration extraction method. Table 1.1 represents the results of Yeild (%) of selected plant extracts of three family Verbeaceae, Lamiaceae and Fabaceae (by 80% methanol). Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form [5].

In present study we found highest yield in *Vitex negundo* (Flower) (22.34%), *Lantana camara*(Leaf) (16.32%) and *Acacia catachue*(Stem) (16.35%) of extract. Repot that represent, yield of the methanol extract was highest then other solvents. Polar extracts extracted with methanol gave the highest yield is because methanol is a universal solvent that dissolves all types of compounds capable of either polar, semi-polar and non-polar. In the extraction process, composition, color, aroma and the resulting yield will be influenced by the type, size and level of maturity of raw materials, the type of solvent, temperature, extraction time and extraction method [6]. Percentage weight secondary metabolites is strongly influenced by the type of plants and parts of plants, (fruits, seeds, stems, bark, wood, flowers, leaves) but is generally less than 10% [7]. Alo,M.N. *et al*., (2012), indicated that ethanol and methanol are better solvent than water for the extraction of the active ingredients of plants which is responsible for potent antibacterial activity [8]. Jignaparekh & Sumitrachanda (2007), reported that methanolic extract of medicinal plant showed highest antifungal activity against some yeast [9]. M. Veljic*et al*., (2010), reported that methanolic extract of liverwort, *ptilidiumpulcherrimum* showed strong antifungal activity. The best antifungal activity was achieved against *Trichoderma-viride* compared to the synthetic fungicide bijonazol[10]. Meharobiedat (2012), reported methanol leaf extract produced broad-spectrum of antimicrobial activity. Interestingly, methanol extraction method was found to be the most effective extraction method of anticandidal agents [11]. The reports of Hertog*et al.* (1993) and Yen *et al.* (1996) showed that methanol is a widely used and effective solvent for extraction of antioxidants and phenolic compounds [12, 13].

Phytochemical Screening

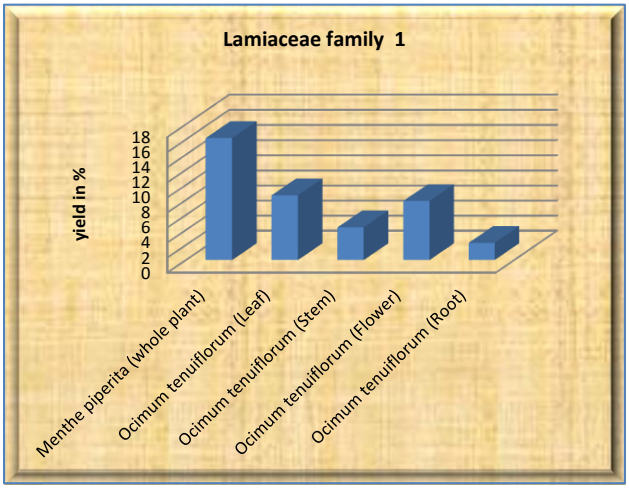
Table 1.2-1.5 represents the results of preliminary phytochemical screening of different parts of the selected plant extracts. For phytochemical screening, water, methanol and petroleum ether extracts were used. Preliminary phytochemical tests indicate the presence or absence of various phytoconstituents in a given plant sample. Screening results indicated that all the plants are rich in diverse advantageous phytoconstituents, like phenols, flavonoids, alkaloids, anthocyaninsrasins, saponin, steroids, tannins, starch, glycosides, terpenoids as well as proteins and carbohydrates. Except phlobatannins, all these bioactive compounds were obtained to be present in almost all the studied samples. Slightest amount of phlobatannins was shown by *P. cineraria* stem extracts in our preliminary screening studies.

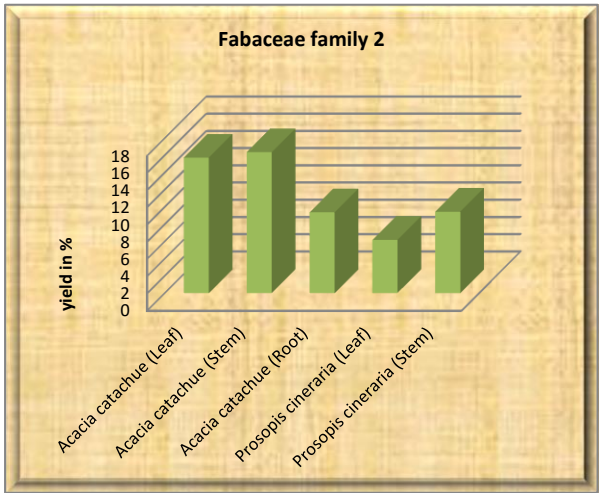
Natural compounds that can be used as antifungal agent have become a renewed source of interest. Phenolic, flavonoid, terpenoid, glycosides, saponins, anthocyanin and steroid compounds were extracted from medicinal herbs, play the vital role in different biological and pharmacological activity, reduced infection caused by microorganisms [14, 15]. Phenolic compound affect to C*andida albicans* by targeting on its dimorphic transition (dimorphic transition play an important role for survival of pathogen in host) [16, 14]. All the members of family verbeaceae (*Vitex negundo*; leaves, stem, flower and root & *Lantana camara*; leaves, stem, flower, fruit, and root), Lamiaceae (*Mentha piperita*; whole herb &*Ocimum tenuiflorum*; leaves, flower, stem and root) and Fabaceae (*Acacia catacheu*; leaves, stem and root & *Prosopis cineraria*; leaves and stem) exhibited polyphenolic compounds in our phytochemical screening.

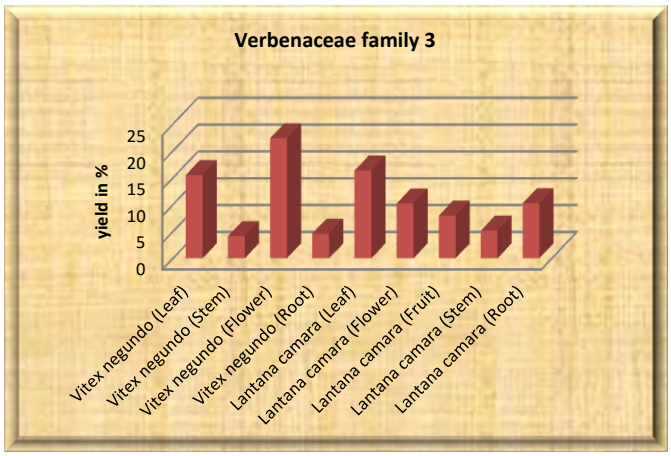
Present investigation demonstrates the presence of flavonoid compounds in all the studied samples. Methanolic, Petroleum ether and aqueous extracts of all the plants display flavonoids in phytochemical screening. Several alkaloids are still in use such as caffeine (Psychostimulant), codeine (Anti tussive agent that suppresses the coughing reflex), cocaine (local anaesthetic), morphine (analgesic) and quinine (antipyretic) [17]. All the samples were found to possess moderate or high amount of alkaloids, except methanolic & water extracts of *Vitex negundo* leaves and stem, methanolic extract of leaves and stem of *Lantana camara*.

Table 1.1: Yeild (%) of selected plant extracts (by 80% methanol)

S. No.	Name of Plant (Part)	Yield (%)
Verbenaceae Family		
1	*Vitex negundo* (Leaf)	15.50
2	*Vitex negundo*(Stem)	4.11
3	*Vitex negundo* (Flower)	22.34
4	*Vitex negundo* (Root)	4.58
5	*Lantana camara*(Leaf)	16.32
6	*Lantana camara*(Flower)	10.21
7	*Lantana camara*(Fruit)	7.96
8	*Lantana camara*(Stem)	5.14
9	*Lantana camara*(Root)	10.23
Lamiaceae Family		
10	*Menthe piperita*(whole plant)	16.18
11	*Ocimum tenuiflorum* (Leaf)	8.62
12	*Ocimum tenuiflorum* (Stem)	4.35
13	*Ocimum tenuiflorum* (Flower)	7.86
14	*Ocimum tenuiflorum* (Root)	2.28
Fabaceae		
15	*Acacia catachue*(Leaf)	15.70
16	*Acacia catachue*(Stem)	16.35
17	*Acacia catachue*(Root)	9.35
18	*Prosopis cineraria* (Leaf)	6.16
19	*Prosopis cineraria* (Stem)	9.43







Graph no 1, 2 & 3: Represent extracted yield of selected plant parts of Lamiaceae, Fabaceae and Verbenaceae family

Table 1.2: Phytochemical Screening of Verbeaceae family plants (1)

S. No.	Phytochemical	*Vitex negundo leaves*			*Vitex negundo Stem*			*Vitex negundo flower*			*Vitex negundo root*		
		W	M	PE	W	M	PE	W	M	PE	W	M	PE
1.	Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+
2.	Alkaloid	-	-	+	-	-	+	+	+	+	+	+	+
3.	Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+
4.	Protein	+	+	+	+	+	+	+	+	+	+	+	+
5.	Resin	-	-	-	-	-	-	-	-	-	-	-	-
6.	Anthocyanin	-	-	-	-	-	-	-	-	-	-	-	-
7.	Saponin	+	+	-	+	+	-	+	+	-	-	-	-
8.	Steroid	-	-	+	-	+	+	+	+	+	+	+	+
9.	Tannin	+	+	+	+	+	+	+	+	-	-	-	-
10.	Starch	-	-	-	-	-	-	-	-	-	-	-	-
11.	Glycoside	+	-	-	+	-	-	+	-	-	+	-	-
12.	Phenol	+	+	+	+	+	+	+	+	+	-	+	+
13.	Phlobatanin	-	-	-	-	-	-	-	-	-	-	-	-
14.	Terpenoid	+	+	+	+	+	+	+	+	+	+	+	+

W = water extract, PE = Petroleum ether extract, M = Methanolic extract

Table 1.3: Phytochemical Screening of Verbeaceae family plants (2)

S. No.	Phytochemical	*Lantana camara* leaves			*Lantana camara* stem			*Lantana camara* flower			*Lantana camara* root			*Lantana camara* Fruit		
		W	M	PE	W	M	PE	W	M	PE	W	M	PE	W	M	PE
1.	Carbohydrate	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
2.	Alkaloid	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+
3.	Flavonoid	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	Protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5.	Resin	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
6.	Anthocyanin	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-
7.	Saponin	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-
8.	Steroid	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
9.	Tannin	+	+	-	-	+	+	+	+	-	+	-	+	+	-	-
10.	Starch	-	-	+	-	-	+	-	-	+	-	-	+	+	-	-
11.	Glycoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12.	Phenol	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
13.	Phlobatanin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14.	Terpenoid	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+

W = water extract, PE = Petroleum ether extract, M = Methanolic extract

Table 1.4: Phytochemical Screening of Lamiaceae family plants

S. No.	Phytochemical	Mentha piperita Whole plant			*Ocimum tenuiflorum* leaves			*Ocimum tenuiflorum* stem			*Ocimum tenuiflorum* flower			*Ocimum tenuiflorum* root		
		W	M	PE	W	M	PE	W	M	PE	W	M	PE	W	M	PE
1.	Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	Alkaloid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	Protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5.	Resin	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
6.	Anthocyanin	-	-	+	+	-	-	+	-	-	+	-	-	+	-	-
7.	Saponin	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
8.	Steroid	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
9.	Tannin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.	Starch	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
11.	Glycoside	-	+	+	+	+	-	+	+	-	+	+	-	+	+	-
12.	Phenol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13.	Phlobatanin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14.	Terpenoid	+	+	-	+	+	+	+	+	-	+	+	-	+	+	-

W = water extract, PE = Petroleum ether extract, M = Methanolic extract

Table 1.5: Phytochemical Screening of Fabaceae family plants

S. No.	Phytochemical	*Acacia catechue* leaves			*Acacia catechue* stem			*Acacia catechue* root			*Prosopis cineraria* leaves			*Prosopis cineraria* Stem		
		W	M	PE	W	M	PE	W	M	PE	W	M	PE	W	M	PE
1.	Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	Alkaloid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	Protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5.	Resin	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+
6.	Anthocyanin	+	-	+	+	+	-	+	+	-	+	-	+	+	-	-
7.	Saponin	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
8.	Steroid	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
9.	Tannin	+	+	+	-	-	-	-	+	+	-	+	-	-	+	-
10.	Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11.	Glycoside	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+
12.	Phenol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13.	Phlobatanin	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
14.	Terpenoid	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+

W = water extract, PE = Petroleum ether extract, M = Methanolic extract

Our findings indicated that terpenoids were present in all the extracts except petroleum ether extract of *Lantana camara* leaves and stem, *Mentha piperita*, Acacia catechu leaves, *Prosopis cineraria* leaves and stem. steroids were absent in petroleum ether extracts of *Lantana camara* leaves, *Prosopis cineraria* leaves, water extract of *Vitex negundo* leaves and methanol extracts of *Vitex negundo* leaves, *Lantana camara* leaves and *Mentha piperita* herb. Saponins are high molecular weight compounds in which a sugar molecule is combined with triterpene or steroid aglycon, so there are two major groups of saponins; triterpenesaponins and steroid saponins. These are therapeutically important as they show hypolipidemie and anticancer activity of cardiac glycosides [18].

Preliminary screening revealed that petroleum ether extract of all plants did not show saponins. Methanol extract of *Vitex negundo* root, *Lantana camara* fruit and water extract of *Vitex negundo* root and *Lantana camara* fruit were found to be devoid of saponins. methanolic extract of *Vitex negundo* root, *Lantana camara* fruit, water extract of *Vitex negundo* root, *Lantana camara* stem, *Acacia catechu* stem and petroleum ether extract of *Vitex negundo* flower and root, *Lantana camara* leaves, flower and fruit, *Acacia catechu* stem and *Prosopis cineraria* leaves and stem showed negative results for tannins instead of all the sample were shown positive result for tannins. In our investigation, anthocyanins were found to be present in water extract of *Lantana camara* leaves, stem, flower, fruit and root. As demonstrated by our screening results. It was found that *Mentha piperita* did not exhibit the presence of anthocyanins in water and methanolic extracts but found in Petrolium extract. water extract of *Ocimum sanctum* leaves, stem, flower and root possess positive result for anthocyanins.

Glycosides were present in all the samples except water extracts of *Vitex negundo* leaves, stem, flower and root, *Lantana camara* leaves, stem, flower, fruit and root, and methanolic extract of *Acacia catechu* leaves, stem, root and *Prosopis cineraria* leaves and stem. Resins were absent in all the three extracts of *Vitex negundo* leaves, stem, flower and root. Petroleum ether extracts of *Lantana camara* leaves, stem, flower, fruit and root, *Mentha piperita*, *Ocimum tenuiflorum* leaves, stem, flower and root, *Acacia catechu* leaves and *Prosopis cineraria* leaves and stem. Starch were absent in all three extracts of *Vitex negundo* leaves, stem, flower and root. Water and mehanolic extracts of *Lantana camara* leaves, stem, flower, fruit and root, *Ocimum tenuiflorum* leaves, stem, flower and root and *Mentha piperita* herb. All three extracts of fabaceae family member showed negative result for starch. Phlobatanins were absent in all the studied samples, except *Prosopis cineraria* stem. Presence of carbohydrate was observed in all the members of family verbeaceae, lamiaceae and fabaceae except petroleum ether extract of *Lantana camara* leaves, stem, flower, fruit and root. Methanol and petroleum ether extracts of selected plants of verbenaceae family contain proteins.

Conclusion

From the present investigation, it can be concluded that all the studied, selected plants of different family, are potent source of diverse biologically active phytochemicals. Methanol, petrolium ether and aqueous extracts revealed the presence of various secondary metabolites such as phenolic compounds, flavonoids alkaloids, carbohydrates, proteins, saponins, steroids, tannins, starch, glycosides, resins, anthocyanins and terpenoids in varying amounts. The test plants shows absence of phlobatanins, except *Procopis cineraria.* Further studies are needed to reveal some more medicinally important facts about these phytochemicals.

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