

Phytochemical Screening of Selected Medicinal Plants for Secondary Metabolites

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ABSTRACT- Systemic and Scientific investigation was carried out for identification, selection, characterization of popularly grown medicinal plants in and around Patna and their phytochemical analysis was carried out to assess their medicinal value potentialities. The traditional medicine involves the uses of different plant extracts or the phytochemical bioactive constituents, which provides the health application at an affordable cost. Secondary metabolites are responsible for the medicinal activity of plants. Qualitative phytochemical analysis of these plants confirm the presence of various phytochemicals like saponins, terpenoids, steroids, anthocyanins, coumarins, fatty acids, tannins, leucoanthocyanins and emodins etc. The result suggests that the phytochemical properties for curing various ailments and possess potent antioxidant, which may lead to the isolation of new and novel secondary compounds for generation of new drugs. Knowledge of the phytochemical constituents of plants are desirable because such information will be of value for the synthesis of complex chemical substances. Out of 50 medicinal plants subjected to phytochemical screening 11 were found to be highly potent, 18 moderately potential and 21 to be least potential on the basis of presence of phytochemicals in the leaf extract of secondary metabolites.

Key-words- Phytochemical screening; Medicinal plants; Secondary metabolites

INTRODUCTION

In recent year, interest has been revived in the study and use of traditional medicine in different parts of the world. As a result, countries have sought cooperation in identifying and using safe positive components of traditional medicine in their national health systems.^[1]

Since ancient times, people have been exploring nature particularly plants, in search of new drugs, and this has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases^[2]. In India, almost 95% of the prescriptions have been reported to be plant based on the traditional systems of Unani, Ayurveda, Homeopathy and Siddha.^[3-4] Plants produce primary and secondary metabolites with divergent functions.^[5] The primary metabolites, amino acids, simple sugars (glucosides), proteins and lipids are involved in cellular processes. Secondary metabolites are chemically active compounds i.e. (flavonoids, alkaloids, terpenoids,

steroids, saponins, etc.), which are produced in response to stress with complexity in structure and more restriction in distribution than the primary metabolites.^[6] Plants can produce different kind of secondary metabolites also known as natural products as they elicit effects on other organisms.^[7]

Plant products have been part of phytomedicines since time immemorial. The study of the distribution, diversity and utilization of herbal flora of J & K state (Rajouri) revealed that many ethanomedicinal plants are popularly in used by locals such as Gujjar, Bakarwals & Paharies for cure from various ailments.^[8] Similar results were also reported for M.P region. Total 35 plants species from Madhya Pradesh were reported, out of which 23 plant species were effective in treatment of diseases.^[9] These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc. i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants are desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screenings of various plants are reported by many workers. The qualitative phytochemical analysis of 10 medicinal plants of M.P were found to be the source of secondary metabolites like alkaloids, phytosterols, glycosides, phenol, flavonoids and diterpenoids.^[10] Secondary Metabolites or Phytochemicals from plants have

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eminent pharmacological activities such as anti-oxidative, anti-allergic, antibiotic, hypoglycaemic and anti carcinogenic.^[11] In the present work, qualitative phytochemical analysis was carried out in medicinal plants abundantly found in and around capital city of Patna, Bihar

(Latitude: 25° 11' N & Longitude: 85° 32' E). A total number of 50 species of various families known for their medicinal properties were screened for phytochemical characteristics.

MATERIALS AND METHODS

Fresh leaves of 50 different plant species were collected in and around A. N College campus in Patna during 2013- 2016 and from different locations (N= 6) of Patna district in Bihar, India. Taxonomic identification of plants was carried out and also compared with the herbarium present in the Department.

Extraction: The leaves of 50 selected Plants listed in Table 1 were washed thoroughly 2-3 times with running tap water, leaf material was then air dried under the shade.

After complete shade drying, the plant material was grinded in mixer, the powder was kept in small plastic bags with paper labeling. The grinded leaves material of 5 gm weighed using an electronic balance and crushed in 25 ml of sterile water, boiled at 50–60°C for 30 minutes on water bath and it was filtered through whatman No. 1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottle at 5°C for further use. The plants studied are listed below in Table 1.

Table 1: Medicinal Plants Selected For Phytochemical Study

S. No	Botanical name	Common name	Family
1.	<i>Murraya koenigii</i> L	Curry leaf	Rustaceae
2.	<i>Moras alba</i> L	White mulberry	Moraceae
3.	<i>Heliotropium indicum</i> L	Indian heliotrope	Boraginaceae
4.	<i>Momordica charantia</i> L	Bitter gourd	Curcubitaceae
5.	<i>Alstonia scholaris</i> L	Devil tree	Apocynaceae
6.	<i>Althea rosea</i> L	Hollyhock	Malvaceae
7.	<i>Solanum nigrum</i> L	Mokai	Solanaceae
8.	<i>Weddella vidacea</i> L	Weddella	Asteraceae
9.	<i>Treme orientales</i> L	Mustard	Ulmaceae
10.	<i>Cardiospermum halicacabum</i> L	Kanputi	Bapindaceae
11.	<i>Cannabis sativa</i> L	Bhang	Cannabinaceae
12.	<i>Ipomoea hederacea</i> Jacq.	Ivy- morning glory	Convolvulaceae
13.	<i>Alternanthera philoxeroides</i> L	Alligator weed	Amaranthaceae
14.	<i>Oxalis corniculata</i> L	Yellow wood sorrel	Oxalidaceae
15.	<i>Blumea mollis</i> (D. Don) Merr.	Blumea	Asteraceae
16.	<i>Achyranthis aspera</i> L	Chaff-flower	Amaranthaceae
17.	<i>Vernonia cinerea</i> L	Dandotapala, sadodi	Asteraceae
18.	<i>Parthenium hysterophorus</i> L	Congress grass	Asteraceae
19.	<i>Gnephallium indicum</i> L	Indian cudweed	Asteraceae
20.	<i>Vicia faba</i> L	Favabean	Fabaceae
21.	<i>Vicia hirsute</i> L	Wildpea	Fabaceae
22.	<i>Vitex negundo</i> L	Five leaved chaste tree	Verbenaceae
23.	<i>Cassia fistula</i> L.	Golden shower	Caesalpinaceae
24.	<i>Cassia corymbosa</i> Lam.	Flowery senna	Caesalpinaceae
25.	<i>Polyalthia longifolia</i> (Sonn) Thwaites	False ashoka	Anonaceae
26.	<i>Euphorbia nivulea</i> Ham.	Leafy milk hedge	Euphorbiaceae
27.	<i>Coccinia indica</i> L	Lvy gourd	Cucurbitaceae
28.	<i>Sagittaria sagittaeifolia</i> L	Arrow head leaf	Alismataceae
29.	<i>Crinum annum</i> L	River lily	Amaryllidaceae
30.	<i>Terminalia catappa</i> Linn.	Indian almond leaf	Combretaceae
31.	<i>Nicotiana plumbaginifolia</i> Viv.	Jangli tambakoo	Solanaceae
32.	<i>Cyperus flabelliformis</i> Rottb.	Umbrella plant	Cyperaceae

33.	<i>Trigonella foenum</i> Linn.	Greek hayseed	Fabaceae
34.	<i>Duranta plumieri</i> Jacq.	Golden dew drop	Verbenaceae
35.	<i>Mellettia ovalifolia</i> Kurz.	Poonga oil tree	Fabaceae
36.	<i>Malvaviscus arboreus</i> Cav.	Firecracker hibiscus	Malvaceae
37.	<i>Thevetia peruviana</i> (Pers.) K. Schum.	Luckynut	Apocynaceae
38.	<i>Teramnus indicum</i> Linn.	Indian mallow	Fabaceae
39.	<i>Sizygium cuminum</i> L	Jamun	Myriaceae
40.	<i>Bauhinia acuminata</i> L	White orchid tree	Caesalpinaceae
41.	<i>Ficus religiosa</i> L	Peepal tree	Moraceae
42.	<i>Catharanthus roseus</i> L	Sadabahar	Apocynaceae
43.	<i>Lathyrus sativa</i> L	White pea	Fabaceae
44.	<i>Sonchus asper</i> L	Spiny sowthistle	Asteraceae
45.	<i>Basella alba</i> L	Indian spinach or poi	Basellaceae
46.	<i>Lantana camara</i> L	Lantana or wild saga	Verbenaceae
47.	<i>Nymphae nelumbo</i> L	Indian lotus	Nelumbonaceae
48.	<i>Erythrina variegata</i> Linn.	Indian coral tree	Fabaceae
49.	<i>Plectranthus amboinicus</i> (Lour.) Spreng.	Ajwain patta	Lamiaceae
50.	<i>Tinospora cardifolia</i> (Willd.) Miers.	Giloy	Menispermaceae

Phytochemical screening: Preliminary qualitative phytochemical screening was carried out with the following methods:

Steroids: 1ml of the extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.^[12]

Terpenoids: 2 ml of extract was added to 2ml of acetic anhydride and concentration of H₂SO₄. Formation of blue, green ring, indicates the presence of terpenoids.^[13]

Fatty acids: 0.5 ml of extract was mixed with 5 ml of ether. These extracts were allowed for evaporation on filter paper and dried the filter paper. The appearance of transpance on filter paper indicates the presence of fatty acids.^[13]

Tannins: 2 ml of the extract was added a few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.^[14]

Saponins: 5ml of extract was mixed with 20 ml distilled

water and then agitated in a graduated cylinder for 15 min. Formation of foam indicates the presence of saponins.^[15]

Anthocyanins: 2 ml of aqueous extract is added to 2 ml of 2N HCL and ammonia. The appearance of pink-red turns blue-violet indicates the presence of anthocyanins.^[16]

Leucoanthocyanins: 5 ml of aqueous extract added to isoamyl alcohol. Upper layer appears red in colour indicates for presence of leucoanthocyanins.^[16]

Coumarins: 3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow color indicates the presence of coumarins.^[17]

Emodins: 2 ml of NH₄OH and 3 ml of Benzene was added to the extract. The appearance of red color indicates the presence of emodins.^[17]

RESULTS AND DISCUSSION

Preliminary screening of secondary metabolites

The phytochemical screening and qualitative estimation of 50 medicinal plants study showed the leaves which are rich in Anthocyanins, coumarins, fatty acids, emodins, leucoanthocyanins, tannins, terpinoids, sterods and saponins (Table 2).

Table 2: Result of the Phytochemical Analysis of 50 Selected Plants

S. No	Plants	Antho cyanines	Steroids	Terpi noids	Coum arins	Fatty acids	Tannins	Saponins	Leuco Antho	Emodins
1	<i>Murraya koenighii</i>	+	+	+	+	+	+	+	-	-
2	<i>Moras alba</i>	-	+	+	-	+	-	-	-	-
3	<i>Heliotropium indicum</i>	-	+	+	-	+	+	+	-	-
4	<i>Momordica charantia</i>	+	+	+	+	-	+	+	-	+
5	<i>Alstonia schol- aris</i>	-	+	-	-	-	-	-	-	-
6	<i>Althea rosea</i>	-	-	-	-	-	-	-	-	-
7	<i>Solanum nigrum</i>	-	+	+	-	-	+	+	-	-
8	<i>Wedellea vida- cea</i>	-	+	-	-	-	-	-	-	-
9	<i>Treme orien- tales</i>	-	-	-	-	-	-	-	-	-
10	<i>Cardiospermum halicacabum</i>	-	-	+	-	-	+	+	-	-
11	<i>Cannabis sativa</i>	+	+	+	-	-	-	-	-	-
12	<i>Ipomoea hede- racea</i>	-	+	+	-	-	-	+	-	-
13	<i>Alternanthera philoxeroides</i>	-	-	-	-	-	-	+	-	-
14	<i>Oxalis cornicu- lata</i>	-	-	-	-	+	-	-	-	-
15	<i>Blumea mollis</i>	-	+	+	-	-	+	-	-	-
16	<i>Achyranthis aspera</i>	+	+	+	-	+	+	+	-	-
17	<i>Vernonia ciner- ea</i>	-	+	+	-	-	-	-	-	-
18	<i>Parthenium hysterophorus</i>	+	+	+	-	+	+	-	-	-
19	<i>Gnephallium indicum</i>	-	+	-	-	-	-	-	-	+
20	<i>Vicia faba</i>	-	+	-	-	-	+	+	-	-
21	<i>Vicia hirsutus</i>	-	-	-	-	-	-	-	-	-
22	<i>Vitex negunda</i>	-	+	-	-	+	+	+	-	-
23	<i>Cassia fistula</i>	-	-	-	-	-	+	+	-	-
24	<i>Cassia corym- bosa</i>	-	+	+	+	-	+	+	-	+
25	<i>Polyalthia lon-</i>	-	+	+	+	-	-	-	-	-

	<i>gifolia</i>									
26	<i>Euphorbia nivu- lea</i>	-	-	-	-	-	-	-	-	-
27	<i>Coccinia indica</i>	-	-	+	+	-	+	+	+	-
28	<i>Sagittaria sagit- taefolia</i>	-	-	-	-	-	-	-	-	-
29	<i>Crinum annu- um</i>	-	-	-	-	-	-	-	-	-
30	<i>Terminalia catappa</i>	-	+	-	-	+	+	+	+	-
31	<i>Nicotiana plum- baginifolia</i>	-	-	+	-	-	+	-	-	-
32	<i>Cyperus flabel- liformis</i>	-	-	-	-	-	+	-	-	-
33	<i>Trigonella foe- num</i>	-	+	+	-	+	+	+	-	-
34	<i>Duranta plumier</i>	+	+	+	-	-	+	+	-	-
35	<i>Melletia ovali- folia</i>	-	-	-	-	-	-	-	-	-
36	<i>Malvaviscus arboreus</i>	-	+	+	-	+	+	-	-	-
37	<i>Thevetia peru- viana</i>	-	+	+	-	-	-	-	+	+
38	<i>Teramnus indi- cum</i>	-	-	+	-	-	+	-	-	-
39	<i>Sizygium cum- inum</i>	-	+	+	-	-	+	+	-	-
40	<i>Bauhinia acuminate</i>	+	+	-	-	-	+	+	-	-
41	<i>Ficus religiosa</i>	-	-	+	-	-	+	+	+	-
42	<i>Catharanthus roseus</i>	-	+	+	+	-	+	+	+	-
43	<i>Lathyrus sativa</i>	-	+	-	-	+	-	-	-	-
44	<i>Sonchus asper</i>	-	+	+	-	-	+	+	-	-
45	<i>Basella alba</i>	-	-	+	-	-	+	+	-	-
46	<i>Lantana camara</i>	-	+	-	-	-	+	+	-	-
47	<i>Nymphae nelumbo</i>	-	-	-	-	-	-	-	-	-
48	<i>Erythrina variegata</i>	-	-	-	-	-	+	-	-	-
49	<i>Plectranthus amboinicus</i>	-	+	+	-	-	+	+	-	-
50	<i>Tinospora car- difolia</i>	-	+	+	-	+	-	-	-	-

 (+)= Present & (-)= Absent

Anthocyanins are present in 7 plants i.e *Murraya koenighii*, *Momordica charantia*, *Cannabis sativa*, *Achyranthis aspera*, *Parthenium hysterophorus*, *Duranta plumier* and *B. auhinia acuminata*.^[18] Anthocyanins help the human immune system to work more efficiently to protect against viral infections. It is little bit more complex, specific types of anthocyanins may have a direct effect in decreasing the influenza viruses infectivity by decreasing the ability of the virus itself to get into human cell or to be related from infected cells or by having a viricide effect.^[19] Coumarins are found in 6 plants i.e *Murraya koenighii*, *Momordica charantia*, *Cassia corymbosa*, *Polyalthia longifolia*, *Coccinia indica* and *Catharanthus roseus*. Various studies have been demonstrated that coumarins is a potential antioxidant and its antioxidant activity is due to its ability to scavenge free radicals and to chelate metal ions.^[20]

Fatty acids are present in 12 plants, i.e., *Murraya koenighii*, *Moras alba*, *Heliotropium indicum*, *Oxalis corniculata* etc. Emodin compounds are present in 4 plants i.e, *Momordica charantia*, *Gnephallium indicum*, *Cassia corymbosa* & *Thevetia peruviana*. Emodin isolated from a great deal of herbs is an effective constituent with many effects. Lots of pharmaceutical studies have demonstrated that emodin has many biological effects, such as anticancer, antimicrobial and anti inflammatory effects.^[21] Leucoanthocyanins are found in only 5 plants i.e., *Coccinia indica*, *Terminalia catappa*, *Thevetia peruviana*, *Ficus religiosa* and *Catharanus roseus*. Tannins compounds are present in 29 plants out of 50 medicinal plants. The growth of many fungi, yeast, bacteria and viruses were inhibited by tannins.^[22] Terpenoids are found in 28 medicinal plants out of 50 plants selected. Terpenoids and tannins are attributed for analgesic and anti inflammatory activities. Apart from this tannins contribute property of astringency i.e faster the healing of wounds and inflamed mucous membrane^[23]. Saponins are present in 24 plants out of 50 plants. Traditionally saponins have been extensively used as detergents, as pesticides and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects.^[24] Steroid compounds are found in 31 plants out of 50 medicinal plants. It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with sex hormones.^[25] Steroids and Terpenoids along with tannins and saponins are found to be rich in most of the medicinal plants for the present study. The presence of bioactive compounds indicates the medicinal value of the plants. Antioxidants and antimicrobial/ antibacterial properties of various extracts from many plants have recently been of great interest in both research and the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants and antimicrobials with natural ones.^[26]

Preliminary qualitative test^[27] is useful in detection of bioactive principles and subsequently may lead to drug

discovery and development.^[28] Analyzed 53 medicinal plants for phytochemical characterization in order to promote Indian herbs, there is an urgent need to evaluate the therapeutical potential of drugs as per WHO guideline.^[29] Scientific botanical data mentioned that 30% of the worldwide sales of drugs are based on natural products. Traditional indigenous medicine is limited to small tribal and geographical are called "Little traditions" are an excellent repository of knowledge about medicinal properties of botanical sources. Phytochemical screenings of medicinal plants are very important in identifying new sources of therapeutically and industrially important compounds. It is also used in pharmaceutical and nutraceutical products of commercial importance. Encouragement for conservation and cultivation of herbal fauna can play an important role in livelihood enhancement of rural population.^[30]

A medicinal plant of Genus *Impatiens* was studied in depth for its Phytochemical and Pharmacological significance and detailed discussion on the structural activity relationship of different compounds and the scope for future research was presented.^[31]

CONCLUSIONS

Plants that are rich in secondary metabolites, called medicinal plants are widely used in traditional medicine to combat and cure various ailments. Several plants studied are used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The different extracts of leaves of medicinal plants contained many bioactive chemical constituents, including anthocyanins, steroids, terpenoids, coumarins, fatty acids, tannins, saponins, leucoanthocyanins and emodins. The anti-inflammatory, antispasmodic, antianalgesic and diuretic effects can be attributed to the high steroids, tannins, terpenoids, saponins and glycosides present in medicinal plants. It has been used as an aphrodisiac, neuroprotective, liver tonic, astringent, and to treat bronchitis, asthma, ulcers, emaciation, insomnia, and senile dementia. While medicinal plants have been used successfully in Ayurvedic medicine for centuries, more clinical trials should be conducted to support its therapeutic use. Out of 50 medicinal plants subjected to phytochemical screening 11 were found to be highly potential (presence of 5 or more phytochemicals), 18 moderately potential presence of 3 or 4 phytochemicals) and 21 to be least potential (presence of 0-2 phytochemicals) on the basis of presence of phytochemicals in the leaf extract of secondary metabolites.

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