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PHYTOCHEMICAL SCREENING OF SOLVENT EXTRACTS OF SOME MEDICINAL PLANTS

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ABSTRACT

The phytochemical profiles for alkaloids, saponins, glycosides, flavonoids, tannins and terpenoids are the same in all solvent extracts of respective plant species. The crude drugs which possess phytochemicals are evidenced in phytochemical analysis and the potential drug samples and revealed by local ethnic people were selected for antimicrobial studies. The micromolecules play an important role in plant species defence against herbivores, pathogenic microorganism, to combat environmental stress, fire, etc. These plant components have been used as drugs for millennia. Therefore, the screening for phytochemicals serves as the initial step in predicting the types of potentially bioactive compounds. The phytochemical profile revealed the therapeutic potential of crude drugs and also supports the folk claims. Further, the purification and characterization of active principles is being carried out in the laboratory to understand the molecular basis of pharmacological property of the crude drugs.

INTRODUCTION

From times immemorial, plants have provided the principal sources for the sustenance and over all development of Homo sapiens. It is no wonder, therefore, that plant resources have attracted attention throughout the world. In recent years, there has been considerable debate on the efficacy of synthetic and natural compounds not only in alleviating human sufferings but also in meeting the human needs. The natural sources are still the store-house of most effective compounds, capable of combating hitherto unconquered diseases. The rapid

advances in this direction have often led to over exploitation of the valuable resources and driving some of them to the verge of extinction. The chemical investigation of plants on scientific lines started from 1800 AD. The former a German pharmacist discovered morphine from the plant *Papaver somniferum* and the latter discovered quinine from Cinchona bark that was universally recognized as a cure for malaria. They are considered to have laid foundation for the school of phytochemistry that resulted in the discovery of many active principles of plants in the alleviation of human diseases (Farooqi and Sreeramulu, 2001; Vinod, 2004).

The preliminary phytochemical screening of hydro alcoholic and aqueous extracts of the stem of *Tinospora cordifolia* confirmed the presence of alkaloids, carbohydrates, glycosides, steroids, proteins, saponins, gums and mucilages (Javeed *et al.,* 2011). The phytochemical profile prepared for alkaloids, saponins, glycosides, flavonoids, tannins and terpenoids was the same for methanol and acetone extracts of some Indian folklore medicines (Bharat and Farzin, 2011). Three quinolinone alkaloids, two acridone alkaloids and a flavone glycoside were isolated from the aerial parts of *Glycosmis mauritiana* (Intekhab *et al.,* 2011). The ethanol extract of *Ailanthus excelsa* contains triterpenoids, alkaloids, phenolic compounds, amino acids, saponins, tannins and reducing sugars. (Velmurugan. 2011). Glycosides, saponins, flavonoids and triterpenoids are present in *Alstonias cholaris, Achyranthes aspera, Moringa oleifera, Tinospora cordifolia* and *Ericostemma hyssopifolium*. Chaurasia *et al.* (2011) isolated the napthopyrone glycoside from the n-butanol extract of the seeds of *Sennatora.* Alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, saponins and reducing sugars were present in most of the tested extracts of leaf and *in vitro* grown callus of *Centella asiatica* (Arumugam *et al.,* 2011). Qualitative phytochemical screening of hydro alcoholic extract of *Abutilon indicum* bark showed the presence of carbohydrate, steroid, glycoside flavonoids, alkaloid and phenol compound (Chandan *et al.,* 2012). Phytochemical investigation and evaluation of the antibacterial and antioxidant activities of leaf bud exudate of *Tarenna asiatica* (Ramabharathi *et al*., 2013). Conversely, the phytochemical investigations of ethnomedicinal plants revealed the presence of active principles.

MATERIAL AND METHODS

Different ethnomedicinal plant parts such as leaves, bark, seeds, fruits, rhizomes or sometimes the whole plant having active constituents we collected carefully and shade dried. The barks were generally collected in spring or early summer when the cambium is active and it is easy to detach them.

Processing of plant material

On the basis of scarce distribution and potential therapeutic properties of plant drugs listed during the present study 20 different plant parts of 15 species belonging to 14 genera and 10 families of flowering plants were selected for preliminary phytochemical studies.The whole plant was used in *Aerva lanata, Andrographis paniculata, Senna occidentalis,* roots in *Abutilon indicum, Achyranthes aspera, Toddalia asiatica,* stem bark in *Albizia lebbeck, Soymida febrifuga, Tinospora cordifolia*and leaves in *Clitoria ternatea*. Leaves and root of *Alangium salviifolium, Elytraria acaulis, Glycosmis mauritiana,* seeds and roots in *Senna tora* and stem bark and leaves in *Chloroxylon swietenia* were used in the present study.

Fresh parts of each plant species, viz. bark, leaf, root, rhizome and whole plants were collected in bulk during the exploration trips and thoroughly washed with running waters, followed by sterile distilled water. Proper care to remove some moisture content, to improve its quality. The fresh plant materials collected are cut into small twigs and shade dried. Then the dried plant material is granulated or size reduced using a blender and sieved (no.60) to get uniform particles. Then, the fine powder is used for the extraction.

Screening for phytochemical constituents: The chemical tests for various phytoconstituents in the extracts were carried out following the standard works as described below.

1. Test for Alkaloids (Smolenski test)

The methanol, ethanol and water extracts were tested for the presence of alkaloids. A portion of the extract was concentrated and residue was digested with 1.5 ml of 2% HCL. The resulting acidic solution was divided into three portions. Of these, two portions were tested for alkaloids by adding Mayer's reagent and Wagner's reagent respectively, while third served as blank. The formation of a faint turbidity or precipitation and addition of the above reagents indicated the presence of alkaloids. A portion of ethanol extract was digested with 1.5 ml of 2% HCL, filtered, neutralized with 10% ammonium hydroxide. The ether soluble portion was tested for alkaloids as the ether extract. A portion of water extract was basified with 10% ammonium hydroxide and extracted with ether. The ether solution was extracted with 10% HCL and the acidic aqueous solution was tested for alkaloids as in the ether extract (Smolenski *et al.,* 1972).

Ethanol extract was tested for the presence of indole alkaloids. A few ml of the ethanol extract was treated with 1ml of Ehrlich's reagent (5% p-dimethylaminobenzaldehyde). The development of a violet color indicated the presence of indole alkaloids. Ethanol extract was tested for the presence of Quinolizidine alkaloids. Dragendorff's reagent was added to few ml

of ethanol extract. The development of a precipitate indicated the presence of Quinolizidine alkaloids.

2.Anthocyanins and Anthacyanidins (Bancroft-Rutzler test)

The ethanol extract was tested for the presence of anthocyanins and anthacyanidins. Red colour in acidic aqueous acidic solution of ethanol extract at pH 3-4 indicates the presence of anthocyanins and the change of colour with pH modification (pH 8-9) indicates the presence of anthocyanidins (Bancroft and Rutzler, 1938).

3.Anthracene glycosides and Emodins (Peyer's test)

The extracts were tested for the presence of anthracene glycosides. Solutions of ethanol, methnol and water extracts were treated with 25% ammonium hydroxide. The formation of red colour indicates the presence of anthracene glycosides (Peyer, 1931).The extract was tested for the presence of emodins by Borntrager's reaction. When the alkaline aqueous solution was red in color, a portion of the ethereal solution was evaporated and the residue dissolved in benzene followed by the addition of 25% ammonium hydroxide. The development of red colour indicated the presence of emodins.

4. Detection of Anthraquinones: Fresh plant material was tested for the presence of anthraquinones. The plant material was extracted with 0.5% potassium hydroxide. To the alkaline extract were added 1ml of hydrogen peroxide, 1 ml of acetic acid and 1.0 ml of benzene. The mixture was treated with an equal amount of dilute ammonia. The appearance of red colour in the ammonia layer indicates the presence of anthraquinones.

5. Acubins and iridoids (Wieffering test)

The plant material was choapped and treated with 5 ml of 1% aqueous HCL. After 3-6 hours, the extract was treated with 1ml of Trim-Hill reagent (10ml of acetic acid, 1ml of 0.2% copper sulphate in water and 0.5 ml of concentrated HCL) and heated on water bath. The appearance of blue color indicates the presence of aucubins (diterpenoids) while green and red colours indicate presence of iridoids (monoterpenoids) (Wieffering, 1966).

6. Carotenoids (Goodwin test)

The extract was tested for the presence of carotenoids by Carr-Price's reaction. Half volume of the ethereal solution was evaporated and the residue dissolved in Antimony chloride

followed by the addition of concentrated H_2SO_4. The development of blue /green color indicated the presence of carotenoids (Goodwin, 1955).

7. Coumarins (Casparis-Manella test)

Methanol, Ethanol and water extracts were tested for the presence of coumarins. The ethereal solutions of the three extracts were evaporated and dissolved in water separately. UV fluorescene (at 254 nm) of the aqueous solution was tested and the increase in intensity after the addition of 10% ammonium hydroxide indicates the presence of coumarins (Casparis and Manella, 1944).

8. Fatty acids (Eckey test)

The extract was tested for the presence of fatty acids. A portion of the ethereal solution was evaporated on a piece of filter paper. The observation of a translucent spot on the filter paper indicated the presence of fatty acids.

9. Flavonoids (Shinadow's test)

Three extracts were evaporated and the residues dissolved in 50% methanol separately on a sand bath. On the addition of magnesium powder and concentrated HCL, the development of yellow or red color indicated the presence of flavonoids.Ethanol extract was tested for the presence of different flavonoids and inferred by their colour reactions with different reagents

10. Detection of Gallic-tannins and catecholic compounds:

The extract was tested for the presence of gallic-tannins and catecholic compounds. 0.5 ml of ethanol extract was diluted with 1.0 ml of water and 2-3 drops of dilute ferric chloride solution was added. The development of a blue black color indicated the presence of gallic-tannins, while a green black color indicated catecholic compounds.

11. Detection of Lignans:

The extract was tested for the presence of lignans. 5 ml of the extract was treated with 1ml of concentrated HCL and 2% furfuraldehyde. The development of red color indicated the presence of lignans.

12. Detection of Polyoses:

2ml of the extract was evaporated and the residue was treated with 2-3 drops of concentrated H_2SO_4 followed by 3-4 drops of alcoholic Thymol. The development of the red color indicated the presence of polyoses.

13. Detection of Polyuronoids:

2.0 ml of the extract was admixed with 10ml of alcohol or acetone followed by the addition of 4-5 drops of hematoxylin. The mixture was filtered and the precipitate was washed with alcohol. A violet precipitate indicated the presence of poly-uronoids.

14. Detection of Reducing compounds:

About 0.5 ml of the extract was diluted followed by the addition of 5-8 drops of Fegling's reagent and the mixture was heated. The development of a brick red colour precipitate indicated the presence of reducing compounds.

15.Saponins (Cambie test)

Water extract was tested for the presence of saponins. Two ml of extract was shaken for ten seconds and allowed to stand. The formation of a persistent honey-comb like froth indicated the presence of saponins (Cambie *et al.,* 1961).

16. Sterols andTriterpenoids (Libermann-Burchard test)

The three extracts were evaporated and the residues dissolved in 0.5 ml of acetic anhydride followed by the addition of 0.5 ml of chloroform and 0.5 ml of concentrated HCL separately. The development of green colour indicated the presence of sterols while red-violet colour triterpenoids (Harborne, 1991).

17. Detection of Volatile oils:

Ether extract was tested for the presence of volatile oils. 2.0 ml of the extract was evaporated on a porcelain tile; aromatic smell of the residue indicated the presence of volatile oils.

The presence of the compounds was recorded by the plus (+) sign for present and (-) for no reaction.

RESULTS AND DISCUSSION

The pharmacological activity of the crude drug and therapeutic uses are due to the therapeutically active constituents, the biodynamic compounds. Hence, the phytochemical screening received pronounced importance because the crude drugs possess varied secondary metabolites.

The research work on medicinal plants needs the coordination of different disciplines such as Botany, Chemistry, Pharmacology and Clinical medicine. Phytochemical screening is important in identifying new sources of therapeutically and industrially important compounds

of medicinal importance like alkaloids, coumarins, flavonoids, saponins, steroids and triterpenoids etc. This helps to establish the therapeutic efficacy of drug yielding plants used in ethno or folklore medicine for centuries, but also to establish scientific base on the use of plant resources in traditional.

Based on the wide usage by the local ethnic people and their antimicrobial activity, the 15 plant species belonging to 10 families were selected for experimental analysis. The crude extracts were screened to evaluate the composition and distribution of different groups of secondary metabolites. The preliminary phytochemical analysis was conducted on the crude extracts, obtained from different polar solvents like methanol, ethanol and water. The extracts were subjected to different qualitative tests and recorded the observations. The frequency of distribution and richness of chemical compounds found are dipicted in Table. 1(Fig. 1)

Table: 1 List of compounds with their frequency of distribution.

	compound	plant extracts	% of compounds
1	Alkaloids	19	95
2	Anthraquinones	17	85
3	Triterpenoids	17	85
4	Coumarins	16	80
5	Flavones	15	75
6	Anthracene glycosides	13	65
7	Reducing compounds	13	65
8	Indole alkaloids	12	60
9	Saponins	12	60
10	Emodins	11	55
11	Flavonoids	11	55
12	Volatile oils	11	55
13	Carotenoids	10	50
14	Flavonones	10	50
15	Gallic-tannins	10	50
16	Polyoses	10	50
17	Lignans	9	45
18	Polyuronoids	8	40
19	Anthocyanidins	7	35
20	Dihydro-chalcones	7	35
21	Quinolizidine alkaloids	7	35
22	Catecholic compounds	6	30
23	Aucubins	5	25
24	Iridoids	5	25
25	Steroids	5	25
26	Anthocyanins	4	20
27	Flavonols	4	20
28	Fattyacids	2	10

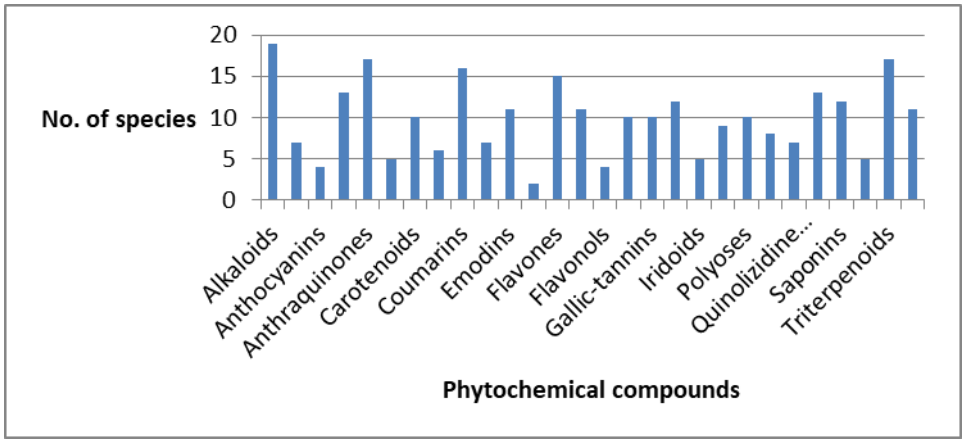

Fig. 1.Phytochemical compounds with their frequency of distribution.

Table. 2. Plant parts showing the richness of secondary metabolites.

Name of the plant	Plant part	Compounds	Percentage
Abutilon indicum	root	18	64.28
Achyranthesaspera	root	19	67.85
Aervalanata	whole plant	14	50.00
Alangiumsalviifolium	leaf	10	35.71
	root	18	64.28
Albizialebbeck	stem bark	16	53.14
Andrographispaniculata	whole plant	14	50.00
Chloroxylonswietenia	stem bark	17	60.71
	leaf	10	35.71
Clitoriaternatea	leaf	15	53.57
Elytrariaacaulis	leaf	07	25.00
	root	14	50.00
Glycosmismauritiana	leaf	10	35.71
	root	16	53.14
Sennaoccidentalis	whole plant	09	32.14
Sennatora	seed	15	53.57
	root	11	39.28
Soymidafebrifuga	stem bark	16	53.14
Tinosporacordifolia	stem	15	53.57
Toddaliaasiatica	root	12	42.85

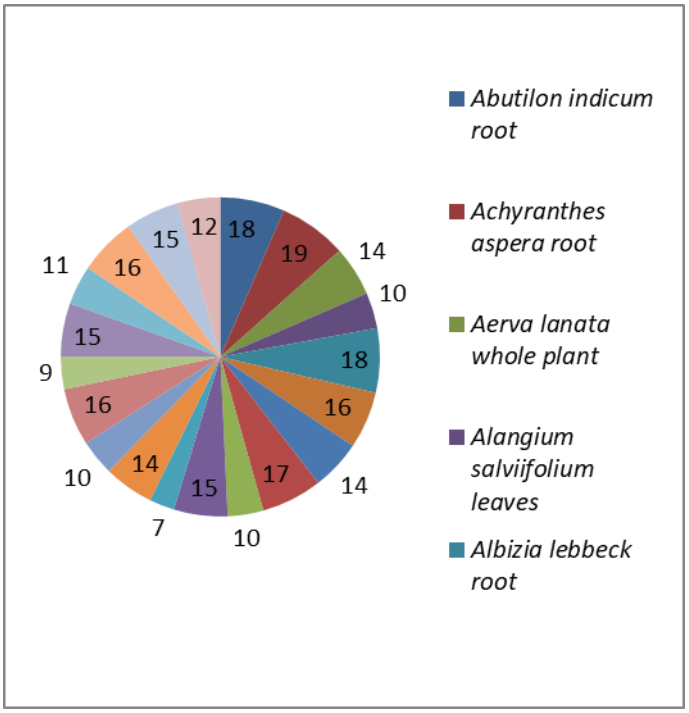

Fig. 2: Plant parts showing the richness of secondary metabolites.

The statistical analysis of secondary metabolites among 20 different plant parts of 15 potential drugyielding plant species revealed 28 major groups of compounds (Table.2& Fig. 2). Methanol, ethanol and water extracts yielded positive results for the presence of alkaloids (95 %), followed by anthraquinones and triterpenoids (85 %), coumarins (80 %) and flavones (75%).

A maximum number of compounds were observed in *Achyranthes aspera* root (19 (67.85 %)), followed by *Alangium salviifolium* root and *Abutilon indicum* root (18 (64.28 %)), *Chloroxylonswieteniastem* bark (17(60.71)), *Albizia lebbeck* (16 (53.14 %)), and least in *Elytraria acaulis* leaves (7 (25%)). Alkaloids, anthracene glycosides, coumarins, flavonoids, saponins, triterpenoids and volatile oils were observed in majority of species. Their presence may be attributed to the medicinal properties of plants. Alkaloids that are reported to have dramatic physiological activities and act mainly on central nervous system were observed in 19 (95%) of 20 different plant parts screened. Anthocyanidins known to possess healing and toxic properties (Fairbaim, 1959) were observed in seven species (35%). Anthocyanins which decrease the capillary permeability in inflammatory conditions of blood vessels were found in four species (20%). Anthraquinones and volatile oils known to have antimicrobial activities were recorded in 17 (85%) and 11 species (55%) respectively. Coumarins reported to have anticoagulation, eastrogenic, vasodialation, antibacterial and anthelmintic properties were recorded in 16 species (80%). Saponins, well known for their expectorant, spasmolytic

and antitissue activities were observed in 12 species (60%). Steroids and triterpenoids known to possess anti-inflammatory, lipolytic and anticholesteremic activities (Chawla *et al.,* 1987) in five species (25 %) and 17 species (85 %) were recorded respectively.

The preliminary phytochemical studies are of importance for validating the efficacy of the crude drugs. Alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars have significant pharmacological application against human pathogens including those that cause infections and are reported to have curative properties against several pathogens and therefore support their use in the treatment of various human diseases (Hassan *et al.,* 2004; Aniel *et al.,* 2015).

The phytochemical profiles for alkaloids, saponins, glycosides, flavonoids, tannins and terpenoids are the same in all solvent extracts of respective plant species. The crude drugs which possess phytochemicals are evidenced in phytochemical analysis and the potential drug samples and revealed by local ethnic people were selected for antimicrobial studies (Owk and Lagudu, 2016).

The micromolecules play an important role in plant species defense against herbivores, pathogenic microorganism, to combat environmental stress, fire, etc. These plant components have been used as drugs for millennia. Therefore, the screening for phytochemicals serves as the initial step in predicting the types of potentially bioactive compounds. (Chew *et al.,* 2004).

Alkaloids, saponins, anthraquinones, glycosides, phenolics, terpenoids and flavonoids have been documented in this study. These principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, antimicrobial, antitumor and antihelmintic activity has reported oils, alkaloids and anthraquinones associated with plants to have medicinal value. The others are triterpenoids which include cardiac glycosides, sterols, saponins and diterpene. The mode of action of compounds present in the extracts indicates that, these plants have the potential of solving the multi-drug resistance problem. Therefore, the presence of these secondary compounds validates the use of the plants as herbal drugs. However further research is called for work out the actual active compounds for commercialization.

The review of literature suggests that phenolic compounds and flavonoids may be responsible for antibacterial and antifungal activity (Du *et al*., 2009). The result of the present phytochemical screening indicates the presence of anthraquinones, saponins, sterols, flavonoids, resins, alkaloids, terpenes, glycosides, carbohydrates and balsam. The presence of these metabolites suggests great potential of the plant as a source of useful phytomedicines.

For instance, the presence of flavonoids and resins might be responsible for its use as anti-inflammatory recipe in Chinese folklore medicine as some flavonoids has anti-inflammatory effect on both acute and chronic inflammation (Kunle and Egharevba, 2009). Some plants which possess alkaloids are known for decreasing blood pressure and balancing the nervous system in case of mental illness. The presence of tannins shows that it is an astringent helping wound healing and antiparasitic. The presence of terpenes suggests its possible use as antitumor and antiviral agent as some terpenes are known to be cytotoxic to tumor cells. Alkaloids are known to possess antimalarial property; hence the plant may be a good source of antimalarial drug for which it is traditionally used locally (Ronan *et al*., 2009). The saponins are believed to have antioxidant, anticancer, anti-inflammatory and anti-viral properties.

CONCLUSIONS

The phytochemical profile revealed the therapeutic potential of crude drugs and also supports the folk claims. Further, the purification and characterization of active principles is being carried out in the laboratory to understand the molecular basis of pharmacological property of the crude drugs.

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