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Screening of Medicinal Plants for Secondary Metabolites

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Abstract: The traditional medicine involves the use of different plant extracts or the bioactive constituents. This type of study provides the health application at affordable cost. Secondary metabolites are responsible for medicinal activity of plants. Hence in the present study phytochemical screening of some important medicinal plants was carried out. Qualitative phytochemical analysis of these plants confirm the presence of various phytochemicals like saponins, terpenoids, steroids, anthocyanins, coumarins, fatty acids, tannins, leucoanthocyanins and emodins. The results suggest that the phytochemical properties for curing various ailments and possess potential antioxidant and leads to the isolation of new and novel compounds.

Key words: Phytochemical screening · Medicinal plants · Secondary metabolites · Tannins · Steroids · Coumarins

INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases [1]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [2]. In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha [3]. The study of plants continues principally for the discovery of novel secondary metabolites. Around 80% of products were of plant origin and their sales exceeded US $65 billion in 2003 [4].

Annona reticulata (Annonaceae) is small tree. Fruits are astringent, sweet and useful in blood complaints. It is also used as anti-dysenteric and anti-helminthic [5]. *Annon squamosa* (Annonaceae) is small tree. Traditionally used for the treatment of epilepsy, dysentery, cardiac problems, fainting, worm infestation, constipation, hemorrhage, diarrhoea, fever, thirst, malignant tumours, ulcers [6]. *Artabotrys hexapetalus* (Annonaceae) is climbing or straggling shrub. Flower oil used in perfumes and *Bixa orellana* (Bixaceae) is a small evergreen tree. The pulb gives a beautiful flesh colour largely used in dyeing silks. Astringent and slightly purgative also a good remedy for dysentery and kidney diseases [7]. *Cadaba indica* (Capparaceae) is shrubs common in scrub jungles and wastelands. The leaves are used to eczema, swelling and constipation [8]. *Capparis zeylanica* (Brassicaceae) is thorny stout climbing shrub used as antidote to snake bite, to cure swelling of testicle, small pox, boils, cholera, colic, hemorrhage, neuralgia, sores, pneumonic and pleurisy [9]. *Clematis gouriana* (Ranunculaceae) is climbing glabrous shrub, Bruised leaves and stems are used for killing of lice [7]. *Cleome viscosa* (Cleomaceae) is erect viscous glandular herb. Seed paste taken orally with hot water in antihelminthic and liver complaints [10]. *Cochlospermum religiosum* (Cochlospermaceae) is deciduous tree. The oral administration of gum powder about 20g mixed with ghee works as an aphrodisiac [11]. *Cocculus hirsutus* (Menispermaceae) is a struggling scandent shrub with softly villous young parts. The leaves are useful in eczema, gonorrhoea, prurigo, impetigo cough, ophthalmia, cephalalgia and neuralgia [12]. *Cyclea peltata* (Menispermaceae) is a slender twining shrub. The roots and leaves are used in anti-inflammatory, cough, bronchitis, helminthiasis, diarrhoea, dropsy, painful swellings, skin diseases, leprosy, fever strangury, ulcers, wounds, vomiting, hyperdipsia and cardiac disorders [12]. *Dillenia indica* (Dilleniaceae) is evergreen trees. The leaves and fruits are used to astringent, laxative, fever and diarrhoea and *Nymphaea nelumbo* (Nelumbonaceae) is wide spreading rhizomatous aquatic herbs.

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The whole plant used to haemorrhage, sterility, skin disease, ulcers and thirsty [13]. *Polyalthia longifolia* and *Polyalthia pendula* (Annonaceae) are evergreen trees. The stem bark are used to febrifuge, rheumatism, menorrhagia, scorpion sting and diabetes; Rheumatism, constipation, worm infestation, polyuria, skin disorders and fever respectively [13]. *Tinospora cardifolia* (Menispermaceae) is woody climber. The stem bark and roots are used in diarrhoea and dysentery. Munda tribe use starch (extracted from stem) to woman after delivery as diet for vitality [7]. *Cissampelos pareira* (Menispermaceae) is dioecious, tomentose, climbing shrubs. The roots are used to antiperiodic, diuretic, purgative, dropsy, urinary disorders, febrifuge, stomachic and diabetes and *Maerua oblongifolia* (Capparaceae) is unarmed stragglers, leaves and roots are used to diabetes, stimulant and alterative [13].

Medicinal herbs have been use in one form or another under indigenous systems of medicine. [14] mentioned that the complete phytochemical investigations of medicinal plants of India should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant. Number of plants were screened for secondary metabolites for their medicinal values by [15] in *Cichorium intybus*, [16] in *Eclipta alba* and *Morinda citrifolia*, [17] in *Mangifera indica*, [18] in *Andrographis neesiana*, [19] in *Indigofera heterantha*, [20] in *Boswellia ovalifoliolata*, [21] in *Nerium oleander* and *Momordica charantia* and [22] in *Naringi crenulata.*

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc [23] i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers [24-26]. In the present work, qualitative phytochemical analysis was carried out in 18 plants.

MATERIALS AND METHODS

Plant Material: Fresh leaves of 18 different plant species free from diseases were collected during the month of December, 2010 from Tirumala hills and different locations of Chittoor District. Taxonomic identification of the plants were carried out with the help of Gamble, [27] and also compared with the herbarium present in Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Extraction: The leaves were washed thoroughly 2-3 times with running tap water, leaf material was then air dried under 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 5gm weighed using an electronic balance and were 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 bottles at 5°C for further use [28].

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

Steriods: 1 ml of the extract was dissolved in 10 ml 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 [29].

Terpenoids: 2 ml of extract was added to 2 ml of acetic anhydride and concentration of H_2SO_4. Formation of blue, green rings indicate the presence of terpenoids [30].

Fatty Acids: 0.5 ml of extract was mixed with 5 ml of ether. These extract was allow it for evaporation on filter paper and dried the filter paper. The appearance of transparence on filter paper indicates the presence of fatty acids [30].

Tannins: 2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins [31].

Saponins: 5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins [32].

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 [33].

Leucoanthocyanins: 5 ml of aqueous extract added to 5 ml of isoamyl alcohol. Upper layer appears red in colour indicates for presence of leucoanthocyanins [33].

Coumarins: 3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates the presence of coumarins [34].

Emodins: 2 ml of NH_4OH and 3 ml of Benzene was added to the extract. Appearance of red colour indicates the presence of emodins [34].

RESULTS AND DISCUSSION

The phytochemical screening and qualitative estimation of 18 medicinal plants studied showed that the leaves were rich in anthocyanins, coumarins, fatty acids, emodins, leucoanthocyanins, tannins, terpinoids, steroids and saponins (Table 1). Anthocyanines are present only *Cleome viscose,* anthocyanins helps the human immune system to work more efficiently to protect against viral infections. It is little bit more complex, specific types of anthocynins may have a direct effect in decreasing influenza viruses infectivity by decreasing the ability of the virus itself to get into the human cell or to be related from infected cells or by having a viricide effect [35], coumarins are found in *Cleome viscose, Cochlospermum* and *Polyalthia longifolia*. Various studies have been demonstrated that coumarin is a potential antioxidant and its antioxidant activity is due to its ability to scavenge free radicals and to chelate metal ions [36]. Fatty acids are present only *Tinospora cardifolia*. Emodin compounds are present in *Annona reticulata, Clematis gouriana* and *Polyalthia longifolia*. 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 [37]. Leucoanthocyanine substances are found in *Artabotrys hexapetalus, Cappris zeylanica, Cleome viscose, Cochlospermum* and *Cissampelos pareira*. Tannin compounds are present in *Cocculus hirsutus* and *Dillenia indica*. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins [38]. Terpinoids are found in 9 medicinal plants out of 18 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 [39]. Saponins are present in *Artabotrys, Cadaba* and *Cocculus* species. Traditionally saponins have been extensively used as detergents, as piscicides and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects [40]. Steroids compounds are found in 14 plants out of 18 medicinal plants. It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with sex hormones [21]. Steroids and terinoids are found to be rich in most of the medicinal plants for the present study: the presence of bioactive compounds indicate the medicinal value of the plants. Antioxidants and antimicrobial 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

Table 1: Secondary metabolites of medicinal plants used to treat different ailments

S. No.	Name of the Species	Name of the secondary metabolites								
		Antho-cyanines	Steroids	Terpinoids	Coumarins	Fatty acids	Tannins	Saponins	Leuco anthocyanins	Emodins
1.	*Annona reticulta* L.	-	+	-	-	-	-	-	-	+
2.	*Annona squamosa* L.	-	+	+	-	-	-	-	-	-
3.	*Artabotrys hexapetalus* (L.f) Bhandari.	-	+	+	-	-	-	+	+	-
4.	*Bixa orellana* L.	-	-	-	-	-	-	-	-	-
5.	*Cadaba indica* Lam.	-	+	-	-	-	-	+	-	-
6.	*Capparis zeylanica* L.	-	+	+	-	-	-	-	+	-
7.	*Clematis gouriana* Roxb. ex DC.	-	+	-	-	-	-	-	-	+
8.	*Cleome viscosa* L.	+	+	+	+	-	-	-	+	-
9.	*Cochlospermum religiosum* (L.) Alston.	-	+	-	+	-	-	-	+	-
10.	*Cocculus hirsutus* (L.) Diers.	-	+	-	-	-	+	+	-	-
11.	*Cyclea pelteta* (Lam.) Hook.f. and Thoms.	-	+	+	-	-	-	-	-	-
12.	*Dillenia indica* L.	-	-	+	-	-	+	-	-	-
13.	*Maerua oblongifolia* (Forsk.) A.Rich.	-	+	-	-	-	-	-	-	-
14.	*Nymphaea nelumbo* L.	-	-	-	-	-	-	-	-	-
15.	*Polyalthia pendula* Hook. and Thoms.	-	-	+	-	-	-	-	-	-
16.	*Polyathia longifolia* (Sonner.) Thw.	-	+	+	+	-	-	-	-	+
17.	*Cissmpelos pareira* L.	-	+	-	-	-	-	-	+	-
18.	*Tinospora cardifolia* (Willd.) ex Hook.f. and Thoms	-	+	+	-	+	-	-	-	-

Note: '+' indicates presence and '-' absence

antimicrobials with natural ones [41]. Preliminary qualitative test according to [42] is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development. [43] analyzed 53 medicinal plants for phytochemical characterization.

According to previous studies, roots of *Strychnons potatorum* [42], leaves of *Bauhinia recemosa* [44], methanolic extract of roots and leaves of *Hyptis suaveolens* [45], ethanolic extract of *Thymus fontanesii* and *Laurus nobilis* [46] and *Rumex vesicarius* [47], aqueous extracts of *Echiumpynanthum pommel* [48], *Cardiosperumum halicacabum* [49], root tuber of *Curculigo* [50], leaves of *Nerium* and *Momordica* [21] and leaves, bark, root and galls of *Pistacia* [51].

In order to promote Indian herbal drugs, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO guidelines [52]. [4] mentioned that 30% of the world wide sales of drugs is based on natural products. Traditional indigenous medicine is limited to small tribal and geographical areas called "little traditions" are an excellent repository of knowledge about medicinal properties of botanical sources. [53] stated that the bioactive extract should be standardized on the basis of phytochemical compounds. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent steps for screening of plants for secondary metabolites. The present communication attempt to assess the status of phytochemical properties in leaves of medicinal plants to improve the health status of people and also to use in pharmaceutical and nutraceutical products of commercial importance.

CONCLUSION

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, antianalgesic and antidiuretic can be attributed to their high steroids, tannins, terpenoids and saponins. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification.

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Secondary metabolites also called Specialized Metabolites, secondary products or Natural Products are organic compounds produced by bacteria, fungi, or plants which are not directly involved in the normal growth, development, or reproduction of the organism. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in a long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. Specific