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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACTS OF FIVE INDIGENOUS MEDICINAL TREE SPECIES FROM SEMI ARID REGIONS OF KATTAKADA, KERALA, INDIA.

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Abstract

The present study reports 15 leaf extracts using three different solvents (such as Water, Acetone and chloroform) derived from commonly occurring five native medicinal tree species belonging to different families collected from the semi-arid regions of Kattakada area, Kerala, India. The plants selected for the study were *Annonasquamosa*, *Thespesiapopulnea*, *Murrayakoenigii*, *Glycidia sepium* and *Moringaoleifera*. Most of these plants were reported to treat a variety of diseases in traditional system of medicine. Qualitative phytochemical screening was carried out on aforesaid extracts which revealed the presence of glycosides, phytosterols, alkaloids, phenols, oils, saponins and flavanoids. The phytochemical studies of all the extracts in three different solvents conclude that acetone and water extracts of leaf samples had more positive results for glycosides, oils, saponins and flavanoids. The results suggest that the leaves are a rich source of valuable primary and secondary metabolites.

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Introduction:-

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Martins *et al.*, 2001). Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (Westhet *et al.*, 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandowet *et al.*, 2003). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.*, 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia 2004). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo *et al.*, 1996 and Iwuet *et al.*, 1999).

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnarajuet *et al.*, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrinet *et al.*, 1985). Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments (Tanaka *et al.*, 2002). Knowledge of the chemical constituents of plant is desirable because such information will be of great value for the synthesis of complex chemical substances (Ashok Kumar *et al.*, 2010). In

the present work, qualitative phytochemical analysis was carried out in the leaf extracts of five tree species from kattakada area.

Materials and methods:-

Collection and Identification of Plant Material:-

Fresh plant/plant parts were collected randomly from the semi arid regions of Kattakada, Thiruvananthapuram, Kerala, India. The plants and the parts screened together with their families and vernacular names, are given in Table 1. The taxonomic identities of these plants were confirmed by Dr. Neethu S Kumar, at Post Graduate Department and Research centre of Botany, Mahatma Gandhi College, Kesavadasapuram, Kerala University. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

Extraction of Plant Material:-

The dried powdered leaf samples were extracted with three different solvents such as water, acetone and chloroform. For aqueous extraction, ten grams of the powdered leaves were mixed with 100ml distilled water, boiled for two hours and filtered. Whereas acetone and chloroform extracts were prepared by mixing ten grams of powdered leaf samples with 100ml of each solvent separately in a mechanical shaker for 48 hours at room temperature. Extracts were filtered, concentrated, dried and stored in the refrigerator at 4°C for further use.

Preliminary Phytochemical Analysis:-

The prepared plant extracts were analyzed for the presence of alkaloids, glycosides, saponins, fixed oils, phytosterols, phenols, flavonoids, gum, mucilages etc (Harborne J.B. 1998). The presence of phytochemicals extracted in different solvents was confirmed by standard protocols.

Test for glycosides and phytosterols:-

Salwaski Test:-

The extract, 0.5 ml was dissolved in 2 ml of chloroform. To this, H₂SO₄ was carefully added in drops to form a layer. A reddish colour formation indicates the presence of steroidal rings.

Phytosterols:-

Mix 2 ml of extract in concentrated H₂SO₄. Shake it well and allow to stand for five minutes. Appearance of a golden yellow colour indicates the presence of sterols and phytosterols.

Alkaloids:-

Two ml of extract was stirred with a few drops of dilute HCl and was filtered. Two ml filtrate was treated with Mayer's and Wagner's reagent. A creamy precipitate will appear for Mayer's reagent and reddish brown precipitate for Wagner's reagent.

Fixed oils:-

Two drops of extract was poured in between two filter papers. Appearance of oil on the paper indicates the presence of oils.

Saponins:-

Foam Test

Two ml of extract was added to 20 ml of distilled water and was shaken well. Froth like appearance indicates the presence of saponins.

Phenolic compounds:-

FeCl₃ Test

Two ml of the extract was added to dilute FeCl₃. Appearance of a violet colour indicates the presence of phenolic compounds and tannins.

Flavonoids:-

Two ml of extract was added to a few drops of concentrated H₂SO₄. Appearance of a yellowish orange colour indicates the presence of flavanoids.

Results and discussion:-

Our approach involved the collection, identification, extraction and phytochemical evaluation of extracts derived from commonly occurring native plants growing in the semi arid regions of Kattakada, Thiruvananthapuram, Kerala. In this study, 5 tree species belonging to 5 different families were collected. Most of these plants were reported to treat a variety of diseases in traditional system of medicine. Local names (in Malayalam) and the family names of these tree species were reported in Table 1. (Fig 1,2,3, 4,& 5). The powdered leaf extracts have been screened for phytochemical constituents in three different solvents such as acetone, chloroform and water. Preliminary phytochemical analysis revealed the presence of seven compounds such as alkaloids, flavanoids, glycosides, oils, saponins, phenolics, phytosterols, gum, mucilage etc and the results are reported in Table2.

Phytochemical studies of all the three different extracts conclude that acetone and aqueous extracts of leaf samples had more positive results for glycosides, alkaloids, flavanoids, phytosterols, saponins, oils etc. Interestingly, the plants belonging to all families tested were given maximum positive indications for glycosides, oils, and saponins. In *Moringaoleifera* leaf extracts, almost all the phytochemicals were present. In *Annonasquamosa* glycosides, oils and saponins were present in all the three extracts. Where as in *Thespesiapopulnea* glycosides, oils, saponins and phenols were present while flavanoids was found to be absent in all extracts. *Murrayakoenigii* leaves are an important source of phytochemicals where Saponins, oils and phenols were found to be present in all extracts. In *Glyricidiasepium* glycosides, oils, saponins, alkaloids and flavanoids were present.

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. Primarily phenolic compounds are of great importance as cellular support material because polymeric phenols form the integral part of the cell wall structure (Guptha *et al.*, 2010). Traditionally saponins have been extensively used as detergents, pesticides as well as molluscicides, in addition to their industrial application such as foaming, surface active agents etc and also found to have beneficial health effects (Arunasalam J.K. 2004). Previous studies by various other workers prove that flavanoids provide health benefits through cell signaling pathways and antioxidant effects.

Bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may lead to many diseases including cancer, cardiovascular problems and ageing (Robard *et al.*, 1999).

The aqueous extract of the leaves of these plants were found to be suitable in the elucidation of bioactive components which could be used effectively in the treatment of several ailments. It would not be surprising therefore to use the plant samples to cure certain types of illness in humans and animals. This obtained information will be helpful as a primary platform for further phytochemical and pharmacological studies.

Table 1: Taxonomic name, Common name, Family name and uses of plant species.

Taxonomic name	Common name in Malayalam	Family name	Uses
<i>Annonasquamosa</i>	Seethapazham	Annonaceae	Used in the treatment of epilepsy, dysentery, cardiac problems, fainting, constipation etc...
<i>Thespesiapopulnea</i>	Cheelanthi	Malvaceae	In ayurveda, fruits are used for control of diabetes, barks possess astringent, hepato protective and anti oxidant activity.
<i>Murrayakoeniggi</i>	Curry leaf tree	Rutaceae	Wound healing capacity, memory improving effect, antioxidant activity.
<i>Glyricidiasepium</i>	Sheemakonna	Papilionaceae	Wound dressing, treatment of dysentery, anti bacterial and anti fungal activity. Used as primary health care.
<i>Moringaoleifera</i>	Muringa	Moringaceae	Leaves and roots are used as astringent and to relieve pain in gut. Used against arthritis, cancer, gastro vascular and heart diseases.

Fig: 1*Annonasquamosa***Fig: 2***Thespesiapopulnea***Fig: 3***Murrayakoenigii***Fig: 4***Glyricidasepium***Fig: 5***Moringaoleifera***TABLE 2: Phytochemical Screening of Leaf Extracts of Tree Species**

Plant name	Leaf Extracts	Phytochemical Screening						
		Glycosides	Phytosterols	Alkaloids	Oils	Sapponins	Phenols	Flavanoids
<i>Annonasquamosal</i>	Water	+	—	+	+	+	—	+
	Acetone	+	+	—	+	+	—	+
	Chloroform	+	+	—	+	+	—	—
<i>Thespesiapopulnea</i>	Water	—	+	—	+	+	+	—
	Acetone	—	+	+	+	+	+	—
	Chloroform	—	—	+	+	+	—	—
<i>Murrayakoenigii</i>	Water	—	—	+	+	+	+	+
	Acetone	+	—	—	+	+	+	+
	Chloroform	+	—	—	+	+	+	—
<i>Glyricidasepium</i>	Water	+	—	+	+	+	—	+
	Acetone	+	—	+	+	+	—	—
	Chloroform	+	—	+	—	+	+	+
<i>Moringaoleifera</i>	Water	+	+	—	+	+	+	+
	Acetone	+	+	+	+	+	+	+
	Chloroform	+	—	+	+	+	+	—

+: Present - : Absent

Summary and conclusion:-

Medicinal plants were the potent source of human health due to the active phytochemical compounds that is responsible for its various pharmacological activities. On the basis of the results obtained in the present study conclude that, plants are rich in phytochemical constituents. The results of the phytochemical screening of the leaf

extracts of the samples varied, while some of the components were present, some were absent. It was observed that most of the components were present in water extracts.

In *Annonasquamosaglycosides*, oils and saponins were present in all solvents whereas in *Thespesiapopulnea*, oils and saponins were present. Oils, saponins and phenols were present in both *Murrayakoenigii* and *Moringaoleifera*. In *Glyricidiasepium*, glycosides, alkaloids and saponins were found. Interestingly, leaf extracts of all the investigated plants showed the presence of saponins. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, saponins, phenols and flavanoids were believed to exhibit the antibiotic properties of leaves of these plants.

The present work highlights the possible use of the leaf extracts of these tree species as a source of antioxidants and as antimicrobial agents that can be used to prevent enteric diseases. The study reveals that the results of extraction yield, total phenol and flavanoid compounds and bioactivity tests varied depending upon the type of solvent being used. Hence it can be concluded that the leaves of these trees would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin. Therefore future research should be addressed on the application of using leaves of the aforesaid trees as natural remedied and to protect against infectious diseases.

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