Phytochemical Analysis and Antimicrobial Activity of Hildegardia Populifolia (Roxb.) Schott & Endl. (Sterculiaceae)

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I. INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world with regard to genetic resources of medicinal plants (Martins et al., 2001). In recent years, Phytochemical, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju et al., 2005). Thus, it is anticipated that Phytochemical with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin et al., 1985).

Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Bisignano et al., 1996; Hammer et al., 1999). Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is very important.

The aim of this study is to investigate the antimicrobial activity of extracts from a medicinal plant Hildegardia populifolia (Roxb.) Schott & Endl. of the family Sterculiaceae used in folk medicine. Antimicrobials are used worldwide in human medicine, food, agriculture, livestock and household products. The increasing use of household antibacterial products and agricultural antimicrobials fosters resistance to drugs specific for human therapy, and may have huge consequences for particularly children and elderly (Levy, 2001). Phytochemical study plays a significant role in giving the solutions to systematic problems on one hand (chemotaxonomy) and in the search for additional resources of raw materials for pharmaceutical industry on the other hand. Plants synthesize a wide variety of chemical compounds numbering well into the hundreds of thousands, perhaps even millions. These compounds can be classified into two major groups based on their biosynthetic origin and functional groups i.e., primary metabolites and secondary metabolites. Primary metabolites consists of compounds such as carbohydrates, lipids, proteins, chlorophylls and nucleic acids, which make up the physical integrity of the plant cell and are involved in the primary metabolic process of building and maintaining living cells. Secondary metabolites have been defined as naturally occurring substances that do not seem to be vital to the immediate survival of the organism that produces them and are not an essential part of the process of building and maintaining living cells.

It is necessary to isolate secondary metabolites from traditionally used medicinal plants, because they have the following features:

1. Provide a scientific complement to traditional approaches
2. To understand functional similarities among different drugs
3. Provide better comprehension of toxicology
4. Establish correlation between scientific approach and traditional herbal practice
5. Provide tools to communicate with researchers and other practitioners

6. Provide tools for understanding scientific literature.

Hildegardia populifolia (Roxb.) Schott & Endl. is a medium sized deciduous tree of the family Sterculiaceae confined to tropical forests of Tamil Nadu and Andhra Pradesh in India. According to IUCN list of threatened species, H. populifolia is critically endangered. This tree is easily recognizable by its pale green bark. They are distributed in dry deciduous forest of Anantapur, Kadapa and Chittoor districts. This tree is growing up to 20 meters tall. It has lobed leaves and panicles of flowers with red sepals and no petals. Most trees produce both male and bisexual flowers.

In the traditional medical practice of Tamil Nadu and Andhra Pradesh, the stem bark and leaves of these plants are used for the treatment of dog bite and malaria (Varaprasad et al., 2009). Saradha and Paulsamy (2012) concluded that methanolic extracts of both leaf and stem bark of H. populifolia can be considered as a new potential source of natural antioxidants for pharmaceutical industries.

II. Materials & Methods

Preliminary Phytochemical Screening

Leaves of selected species were washed with water, chopped into small fragments and shade dried and then at 600C in a hot air oven. The dried samples were grounded into powder and stored in polythene containers at room temperature. These samples were used for further screening of secondary metabolites. 25g. of powder was soaked in each 250ml. of Hexane, Ethyl acetate, Ethanolic and aqueous solvents and were kept in dark for one day. These extracts were concentrated under reduced pressure to one third volumes and used for testing of 9 components namely Steroids, Triterpenes, Saponins, Alkaloids, Carbohydrates, Flavonoids, Tannins, Glycosides, and Polyphenols respectively. Preliminary Phytochemical analysis was under taken using standard quantitative methods as described by Amarasingham et al. (1964), Das and Battacharjee (1970), Brain and Turner (1975), Horborne (1984) and Venkata Raju (1996).

Assessment of Anti Microbial activity

The present investigation was attempted to evaluate antimicrobial activity with ethonolic leaf extracts of H.Populifolia of the family Sterculiaceae which is considered as potential medicinal plant, using some pathogenic bacteria and phytopathogenic fungal species. Extracts obtained from leaf parts of the plants were tested against Staphylococcus aureus (gram positive), Pseudomonas aeruginosa (gram negative) and fungal species Aspergillus niger.

The plant leaves were collected in bulk quantities and shade dried, powdered and subjected to extraction using organic solvent depending on the polarity. The extracts were collected evaporated and the residues were used for antimicrobial activity. All of the above plant leaves are extracted in Ethonolic solvent only.

The Muller Hinton Agar (MHA) media plates are prepared according to the manufacturers recommendations. They are dried in an incubator at 37oC for 30 minutes. The standard working inoculums of three different organisms are also taken. The bacteria are inoculated on to this media plates. Potato dextrose sugar media is used for the growth of a fungus. All the inoculated plates are left to dry at room temperature for about 10 minutes with closed lid. Then the impregnated filter paper discs with different concentrations of crude leaf extracts are placed in the inoculated plates.

The disc diffusion method is followed to assess the growth of colonies. The crude extract of sample dissolved in dimethyl sulfoxide (DMSO) and the concentrations of 10 mg/1 ml of sample is applied to sterile Whatmann filter paper discs. All these plates are incubated for 18 hrs. in an incubator at 37oC. After 18 hrs. the zone of inhibition is measured with the help of a ruler and the data is tabulated. Antimicrobial activity of crude extract is assessed by measuring the diameter of the growth inhibition zone in millimeters.

Preparation of Plant extract

The shade dried plant material was taken and powdered in a mechanical grinder. The powder (25.0 g) of the plant material was initially defatted with ethyl alcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatmann filter paper

(No.1). While hot and concentrated it is filtered in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccators. The ethyl alcoholic extract yields a dark greenish solid residue. The extract was then kept in sterile bottles, under refrigerated conditions, until further use. The extract was preserved at 2 to 4°C. This crude extract of ethyl alcohol was used for further investigation for potential of antimicrobial properties.

III. RESULTS & DISCUSSION

Phytochemical screening was carried out with four extracts – Hexane, Ethyl acetate, Ethonolic and Aqueous extracts. The results of phytochemical analysis of the present study are given in the following table - 6.

The table showing that steroids, alkaloids, carbohydrates, flavonoids, tannins, glycosides and Polyphenols are present in all the four studied extracts. Triterpenes are present in ethyl acetate and ethonolic leaf extracts. Saponins are present in hexane and aqueous leaf extracts. Tri terpinoidal saponins are not found in any extracts. Sixty percent of the plant samples gave positive reactions for a number of secondary metabolities indicating that the plants of tropical origin are rich in phytochemical diversity as reported by Atal and Kapoor, (1982), Sinha and Dogra, (1985).

Table -6: Showing the results of Phytochemical analysis

S.No	Secondary metabolites	Hexane	Ethyl acetate	Ethonolic	Aqueous
1	Steroids	+	+	+	+
2	Triterpenes	-	+	+	-
3	Saponins	+	-	-	+
4	Tri terpinoidal saponins	-	-	-	-
5	Alkaloids	+	+	+	+
6	Carbohydrates	+	+	+	+
7	Flavonoids	+	+	+	+
8	Tannins	+	+	+	+
9	Glycosides	+	+	+	+
10	Polyphenols	+	+	+	+

Alkaloids, flavonoids, saponins, steroids, tri terpenoids, tannins, carbohydrates, glycosides, Polyphenols were observed, whose presence may be attributed to the medicinal properties of plants (Kapoor et al., 1969; Chhabra et al., 1993). The physiological activity of alkaloids renders them important as potential drugs and exhibited a variety of biological activities in controlling recurrent fevers, in ophthalmology, in prevention of motion sickness, in the treatment of high blood pressure, leukemia and anticancer effects (Atal and Kapoor, 1982). Tannins are known to have antimicrobial properties (Chhabra et al., 1984) and also known to produce anthelmintic activities (Niezen et al., 1995). Flavonoids, known to posses antiviral, antifungal and arthritic properties (Fairbairn, 1959; Tripathi and Rastogi, 1981). Triterpenoids are known to possess anti inflammatory, lipolytic activities (Chhabra et al., 1984, Chawla et al., 1987) Polyphenols have many health beneficial functions including antimutagenicity, anti carcinogenicity and anti-aging.

Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Bisignano et al., 1996; Hammer et al., 1999). In this concern, ethanolic leaf extract of

the studied plant is showed higher inhibition zone against Staphylococcus aureus, a gram + ve bacteria and is active against a gram –ve bacteria, Pseudomonas aeruginosa and a fungi, Aspergillus niger.

Cyanogenic glucosides and glucosinolates play a major role in the ecosystem as defense compounds.

Antimicrobial activity:

H. populifolia extract exhibited higher zone of inhibition in Pseudomonas aeruginosa – ve (2.4 mm) when compared to Staphylococcus aureus + ve (1.6 mm) and Aspergillus niger (1.7 mm). Krishna et al. (2013) attributed its antibacterial and antifungal activity to the presence of soluble Polyphenols and phenols.

IV. CONCLUSION

As there is no phytochemical and antimicrobial work on record of this critically endangered plant, the present work was taken up with a view to lay down standards, which could be useful to detect the authenticity of this medicinally useful plant and found various secondary metabolites in all the extracts studied. The antimicrobial and antifungal activity of this crude drug is also proved. It is important to both the research institutes and pharmaceuticals companies to know the phytochemical constituents of medicinal plants for the manufacturing of the new drugs for treatment of various diseases and have commercial importance. Thus we hope that the important phytochemical properties identified by our study will be helpful in the copping different diseases.

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