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Antimicrobial Properties and Phytochemical Constituents of *Rheo discolor* Hance.

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Abstract

Rheo discolor Hance is extensively used in Indian traditional and folklore medicines to cure various human ailments. The preliminary phytochemical screening of the leaves revealed the presence of Alkaloids, Flavonoids, Saponins, Tannins & Phenolic compounds, Cardiac glycoside and Terpenoids. In vitro antibacterial studies on the leaf extracts were carried out on eight medically important bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus citrus, Serratia sp. Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia*, *Proteus vulgaris* which were procured from Department of Microbiology, RVS Hospital, Sulur, Coimbatore, Tamilnadu, using agar disc diffusion method. The results of our antibacterial assay revealed that the extract showed good inhibitory activity against all the tested pathogens compared with standard antibiotics like chloromphenicol.

INTRODUCTION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use (Rojas R, et al., 2003). Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (mer ERT.RK1 et al 2006). World Health Organization (WHO) described plant as a plant with one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The results of investigation performed in the late 19 and 20 century and the advent of streptomycin and other antibiotics provide the ground for experimentation of a vast number of plants for antibiotic or antimicrobial activities that are useful to man (Asirvatham Doss and Rangasamy Dhanabalan., 2008). Rheo discolor Hance belongs to the family Commelinaceae. It is commonly grown in gardens, and is usually known as *Tradescantia*. They are weakly upright to scrambling plants, growing to 30-60cm tall, and are commonly found individually or in clumps in wooded areas and fields. The leaves are large, imbricated, green above and purple beneath. The study is aimed at investigating the anti bacterial properties Rheo discolor leaves as well as to identify the active ingredients of the plant.

MATERIALS AND METHODS

Collection of plant materials

Mature leaves were collected from the healthy plant at Nilgiris, Tamilnadu, India. The collected plant materials were botanically authenticated by Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu.

Preparation of plant extract

The leaves were washed in tap water, shade dried for 10 days and made into a fine powder of 40 mesh size using the laboratory mill. Following that, 100g of the powder was extracted with different organic solvents viz, Benzene, Chloroform, methanol, Petroleum ether and Water and it was stand for over night. The extract was filtered through Whatman no.1 filter paper to remove all un extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure.

Test organisms

The extract was tested on the following three Gram positive bacteria: *Staphylococcus aureus*, *Staphylococcus citrus, Bacillus subtilis* . Five Gram negative bacteria were also tested, including *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Serratia sp.*, *Proteus vulgaris* . All the strains were procured from Department of Microbiology, RVS Hospital, Coimbatore, Tamilnadu.

Phytochemical screening

Phytochemcial analysis of the extract was conducted by Trease and Evans.,1989 : Harborne (1998). By this analysis, the presence of several phytochemicals like Sugar, Protein, Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycoside, Terpenoids and Lipids were tested.

Anti bacterial assay

The three different concentrations of the leaf extracts were tested for antibacterial activity using agar disc diffusion assay according to the method of Bauer et al., 1966. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37^0 C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37^0 C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10µg/disc) was used as standards.

RESULTS

The preliminary phytochemical analysis of the leaf extract revealed the presence of Alkaloids, Flavonoids, Steroids, Saponins, Cardiac glycoside, Terpenoids, Tannin & Phenolic compounds and Oil as presented in Table 1. The results obtained from the disc diffusion assay showed that there has been an increasing effect on bacterial growth inhibition with increasing concentration of the extract. And the extract showed good inhibitory activity on almost all the bacteria tested. It has been found that among all the tested organisms, the Gram negative bacterial strain, *K.pneumoniae* was found to be more susceptible to the plant

extract by showing inhibition zone ranging from 11 mm and the gram positive strains were least susceptible with the inhibition zone ranging from 10 mm. The antibacterial activity in terms of zone of inhibition was presented in Table 2. The observed activity may be due to the presence of potent phyto constituents in the leaf extracts.

DISCUSSION AND CONCLUSION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade (Patrick Ekong Ebong, et al., 2008). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Veeramuthu Duraipandiyan et al., 2006).According to World Health Report of Infectious diseases 2000; overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management.

The antibacterial activity of *Rheo discolor* is reported for the first time. No previous report on the antibacterial activity of this plant species could be found in the literature. In the present study, *Rheo discolor* showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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Table 1. Phytochemical screening of *Rheo discolor*.

S.No	Secondary Metabolites	P.ether	Benzene	Chloroform	Methanol	Water
1	Alkaloids	+	-	-	-	-
2	Flavanoids	-	-	+	+	-
3	Cardiac glycosides	-	-	-	+	-
4	Steroids	-	-	+	+	-
5	Saponins	-	-	-	+	+
6	Terpenoids	-	-	-	+	-
7	oil	+	+	-	-	-
8	Tannin & Phenolic compounds	-	-	-	+	-

Table 2. Antimicrobial activity of *Rheo discolor*.

S.No	Microorganism	Zone of Inhibition (mm)														
		P.ether (mg/ml)			Benzene (mg/ml)			Chloroform (mg/ml)			Methanol (mg/ml)			Water (mg/ml)		
		10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5
1	*S. aureus*	10	9	7	9	7	-	9	7	-	9	8	-	9	7	-
2	*S. Citrus*	9	8	-	7	-	7	-	-	-	-	-	-	-	-	-

3	*B.subtilis*	10	7	-	9	8	-	-	-	-	-	-	-	-	-	-
4	*Serratia*	8	9	8	9	8	9	9	7	-	10	7	-	9	7	-
5	*P.vulgaris*	10	9	8	8	7	-	10	9	7	9	8	-	10	7	-
6	*K. pneumoniae*	11	9	8	8	7	-	-	-	-	-	-	-	-	-	-
7	*S. Typhi*	9	8	-	8	7	-	10	9	-	10	9	7	10	9	7
8	*P.aeruginosa*	9	7	-	8	-	-	9	8	7	9	8	7	9	8	7