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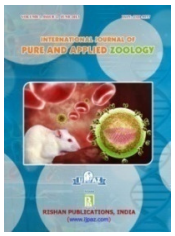

STUDY OF THE PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *DODONAEA VISCOSA*

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ABSTRACT

Ethanol extracts of *Dodonaea viscosa* were used traditionally in India for the treatment of skin diseases. The present study was investigated for *in vitro* antimicrobial activity against pathogens namely *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa, Klebsiella pnemonia, Aspergillus níger, Trichoderma viride* and *Candida albicans* using the agar well diffusion method. A parallel study was performed to identify the distribution and the concentration of the phytocompounds in the roots and leaves of this plant. For this purpose alcoholic extracts were prepared from each part of the plant and studied them separately. Among the leaf extracts of *D. viscosa* possess the highest inhibitory activity then the root extracts. The results revealed that the plant leaf extract possessed the highest inhibitory activity against the bacteria and fungi.

Key words: *Dodonaea viscosa,* leaf extracts, root extracts, antimicrobial activity, phytochemical analysis.

INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi and Mehrotra, 2002).

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine. Various medicinal plants have been used for daily life to treat disease all over the world. They have been used as a source

of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Boominathan and Ramamurthy, 2009).

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge in modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. The worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care.

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Boominathan and Ramamurthy, 2009).

Dodonaea viscosa is used as a traditional medicine in different countries. Stem or leaf infusions were used to treat sore throats, root infusion to treat colds and seeds (in combination with other plants) used to treat malaria. The leaves are used to treat itching, digestive system disorders, including indigestion, ulcers and diarrhea; and the powdered leaves were given to expel round worms. The plant is also used as antibacterial (Rojas *et al*., 1992; Thring *et al*., 2007) and has insecticidal activity (Malarvannan *et al*., 2008).

D. viscose popularly known as aliar and vilayatimehandi in India is an evergreen shrub abundantly growing in Western Ghats of Karnataka, India. This species has been used in traditional ayurvedic system of medicine to heal simple ulcer, fracture (Perry and Metzge, 1980), soreness, and snakebite pain, relief of gum and teeth pain (Kirtikar and Basu, 1993). Experimental studies have demonstrated antimicrobial, anti-inflammatory (Getie *et al*., 2003), anti-ulcer (Veerapur *et al*., 2004), wound healing (Joshi *et al*., 2003), local anaesthetic and smooth muscle relaxant activities of the title plant

D. viscosa inhibited the adherence of *Candida albicans* to oral epithelial cells, which is the initial step of colonization in the infection process and this plant has a therapeutic potential at subinhibitory concentration (Patel *et al*., 2009). The crude ethanol extract and n-hexane, dichloromethane, ethyl acetate, n-butanol and aqueous fractions of *D. viscosa* were analyzed for antibacterial potential against four Gram positive bacteria: *Bacillus subtilis, B. cereus, Micrococcus luteus, Staphylococcus aureus, and three Gram negative bacteria: Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa.* Screening showed inhibition against *Staphylococcus aureus, Micrococcus luteus, Escherichia coli* and *Pseudomonas aeruginosa* (Khurram *et al*., 2009).

D. viscosa, a medicinal plant commonly used for skin diseases in Ethiopia was subjected to a systematic dermatotoxicity study. To this effect, the dermatotoxicity of an 80% methanol extract of the leaf was investigated in animals following standard procedures for irritation, sensitization, acute toxicity and repeated toxicity tests. *D. viscosa*is not associated with any toxicologically relevant effects and the data could provide satisfactory preclinical evidence of safety to launch a clinical trial on a standardized formulation of the plant extracts (Kefale *et al*., 2009). Against this background information and appreciating the knowledge of medicinal plants an effect has been made in this study to evaluate

the antimicrobial efficacy of *Cassia alata* medicinal plants and also characterizing them by screening preliminary by phytochemical analysis. The study also pertains to inculcate the subject about the utilization of natural flora as therapeutic agents.

MATERIALS AND METHODS

D. viscose Jacq belongs to the family Sapindaceae was collected from Thanjavur District, Tamil Nadu, India and identified by the special key given Cambell flora. The leaf and root of *D. viscose* were washed with sterile distilled water. After, the leaves were shade dried and powdered by using pestle and mortar. Twenty five gram of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator and preserved at 5^{o}C in airtight bottle until further use. All the extracts were subjected to phytochemical analysis and antimicrobial activity assay.

Phytochemical Analysis

The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered leaves (50 gm) were extracted in a Soxhlet apparatus with ethanol and water successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc.

Chemical tests were carried out on the ethanol and aqueous extracts using procedures to identify the phytochemicals as described by Sofowara (1993), Trease and Evans (1983) and Harborne (1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Antimicrobial Assay: The following organisms were employed for this study as test organisms: Bacteria such as *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa* and *Klebsiellapnemonia.* Fungisuch as *Aspergillus níger, Trichodermaviride* and *Candida albicans*. The test microbial pathogen cultures were obtained from the stock cultures maintained in specific agar medium.

Antibacterial and antifungal activity of above mentioned extracts were tested using the agar diffusion method described by Collins and Lyne, (1970). All the above-mentioned bacteria were inoculated into nutrient agar medium and fungi inoculated to potato dextrose agar medium. The well of 8 mm diameter was punctured in the culture medium using sterile cork borer. Different extracts were administered to fullness in each well. Culture plates were incubated at 37^{o}C for 24 h in bacteria and incubated at 37^{o}C for 4 days in fungi. Bioactivity was determined by measuring diameter of inhibition zones in mm. Solvents used for extraction served as control.

RESULTS AND DISCUSSION

Qualitative phytochemical analyses for alkaloids, carbohydrates, tannins, phenols, gums and mucilage, fixed oils and fats, saponins, proteins, volatile oils, flavonoids and steroids were screened in ethanolic extracts of the selected medicinal plants *D. viscosa* . The screening of the extract indicated the presence of alkaloids, tannins and saponin in the ethanolic extracts of leaves (Table 1). The chemical test of hydroalcoholic extract of *D. viscosa* revealed that it contains alkaloids tannins and saponins, this observation was accordance with the earlier phytochemical reports on this plant. Incidentally many of that alkaloids other plant sources have been identified to impair release of aetocoids in inflammation. Previous study in the naturally the ethanolic extracts of *Cassia alata* were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids, and steriods, but reducing sugars, carbonyl (aldehyde) and phlobatanin show negative results (Makinde *et al*., 2007).

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers (Sofowara, 1993). In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type

of metabolites present in the *D. viscosa*. Boominathan and Ramamurthy (2009) reported that the phytochemical analysis of the *H. indicum*and *C. procumbens* extractsshowed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

Ethanolic extracts were tested against bacteria and fungi. Among the extracts, the leaf extract of *D. viscosa* were effective against bacteria and fungi. The antibacterial activity crude extract is shown in Table 2. The extracts showed maximum activity against *Staphylococcus aureus, Streptococcus pyogens, Klebsiella pnemonia*and *Pseudomonas aurogonosa*. These data revealed that leaf extracts of *D. viscose* exhibited significant antimicrobial activity. In testing, inhibition zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavanoids (Tsuchiya *et al.,* 1996), phenolics and polyphenols (Mason and Wasserman, 1987), tannins (Ya *et al.,* 1988), terpenoids (Scortichini and Pia Rossi, 1991), sesquiterpenes (Goren, *et al.,* 1996), etc. are effective antimicrobial substances against a wide range of microorganisms.

The extracts showed maximum activity against *E. coli, Enterobacter aerogenes* and *Alcaligenes faecalis*. These data revealed that extracts of *R. tetraphylla* exhibited significant antibacterial activity (Suresh *et al*., 2008). Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect. Medicinally, this is important for the treatment of inflamed or ulcerated tissues (Mota *et al.,* 1985). Tannins have important roles such as stable and potent antioxidants (Trease and Evans, 1983). Herbs that have tannins as their main component are astringent in nature and used for treating intestinal disorders such as diarrhoea and dysentery, thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plant is the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications (Raffauf, 1996).

Table 1. Qualitative Phytochemical screening on extracts of *Dodonaea viscosa.*

S. No	Name of Test	Test applied / Reagent used	Leaf extract	Root extract
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]) Dragndorff's test	+ + + +	+ + + +
2	Flavanoids	HCl and magnesium turnings	+	+
3	Carbohydrate	Molisch's test	+	+
4	Tannins & Phenols	A] 10% Lead acetate B] $Fecl_3$	+ +	+ +
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	+ +	+ +
6	Gums &Mucilages	Alcoholic Precipitation	-	-
7	Fixed oil & Fats	Spot test	+	+
8	Saponins	Foam test	-	-
9	Phytosterols	LB test	+	+
10	Volatile oils	Hydro distillation method	+	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+ + +	+ + +

- absents; + present.

Table 2. Antimicrobial efficacy of *Dodonaeaviscosa.*

S. No.	Organism	Zone of inhibition in mm	
		Leaf	Root
	Bacterial species		
1	*Staphylococcus aureus*	21	7
2	*Bacillus subtilis*	14	6
3	*Streptococcus pyogens*	24	8
4	*Pseudomonas aurogonosa*	18	9
5	*Klebsiellapnemonia*	20	7
	Fungal species		
6	*Aspergillusniger*	12	6
7	*Trichodermaviride*	10	5
8	*Candida albicans*	15	9

*H. indicum*and *C. procumbens* are used for the treatment of inflammation, wound healing, antitumor and antianelgesic, hence different formulations could be prepared for clinical trials (Boominathan and Ramamurthy, 2009).

Conclusion

It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Studies are in progress to further evaluate the mechanisms of action *D. viscosa* extracts on some organisms associated with human diseases. Hence, the present study suggests that pathogenic microorganisms may become resistant to existing drugs. Moreover, this study shows that some plants show much promise in the development of phytomedicines having antimicrobial properties. In this endeavour, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serve further global needs. The drugs derived from herbs may have the possibility of use in medicine because of their antibacterial activity. With onset of scientific research in Ayurvedic system of medicine, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20% of the plant flora has been studied and 60% of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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