J. Res. Educ. Indian Med., April - June, 2012 Vol. XVIII (2) : 107-111 **ISSN 0970-7700**

ANTIMICROBIAL ACTIVITY OF AN INDIAN MISTLETOE, THE HEMIPARASITE *DENDROPHTHOE FALCATA* L. F. (LORANTHACEAE)

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Abstract: *Dendrophthoe falcata linn*. is reported to contain biologically active substances such as flavonoids, ß-sitosterol, ß-amirin, oleonolic acid, chlorophyll, steroids and terpenoids. The present study was under taken to evaluate the antimicrobial and antifungal potential of extracts of this plant. The extracts of *Dendrophthoe falcata linn.* leaves, showed significant antimicrobial activity against Gram positive and Gram-negative test organisms. *Dendrophthoe falcata linn*.is an hemiparasite plant belonging to Loranthaceae, Synonyms are *Loranthus longifluorous* also known as banda, vandal vrikshabhaksha and bandgul. This parasitic plant is one of the most widespread plants in the forests of India, usually occurring in deciduous forests of Western Ghats of India.

Keywords: *Dendrophthoe falcata linn.*, leaves, Cup and Plate method, Antibacterial, Antifungal

Introduction

Even though pharmaceutical industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. This fact has also been verified in other clinics around all over world.

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

Even though some literature on the medicinal properties of Loranthaceae plants is found, but information on genus *Dendrophthoe* is rare.[1-7] Traditionally, the whole plant is used in indigenous system of medicine as aphrodisiac, astringent, diuretic, narcotic and in treatment of pulmonary asthma, menstrual disorders, and as antiviral herbal drug.[8-13]

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Materials and methods

Plant material

The leaves of *Dendrophthoe falcata* parasitic on *Mangifera indica* (Anacardiaceae) were collected from Western Ghat region of Maharashtra (India) in February 2005. The plant specimen was authenticated from Botanical Survey of India, Pune (Voucher specimen no. PSH-1).

Reagents and Materials

The media used for antimicrobial testing was Muller Hinton agar and the media for anti-fungal testing was Potato dextrose agar. The medias were purchased from Hi-media labs, India. Other solvents used in the test were of analytical reagent grade purchased from Rankem, India. The various strains of microorganisms were obtained from NCIM, Pune, India.

Preparation of extracts

The leaves were shade dried and coarsely powdered. The powdered leaves were extracted by soxhlet extraction by successive extraction method and the extractive yield for each of the extracts was calculated. Petroleum ether**,** Benzene, Chloroform, Acetone, Methanol, Ethanol, Ethylacetate, Butanol, and Aqueous extracts were prepared. The prepared extracts were then subjected to preliminary phytochemical investigations to estimate the presence of various phytoconstituents. The image of ***Dendrophthoe falcata*** **L. f. is** depicted in **Figure 1.**

Figure 1. Photograph of *Dendrophthoe falcata* L. f.

Evaluation of Anti-microbial activity

The size of Inoculums for Anti-microbial tests were 1 X 10^8 bacteria per ml and the concentration of extracts used in the assays was 5mg/ml.

Antimicrobial assessment of the extracts using Agar Well Diffusion method

Preparation of inoculums

About 20ml of Muller Hinton agar medium for bacteria and Czapek Dox for fungi were allowed to set in empty sterile Petri plate. About 0.1ml of fungal inoculums was made in Petri plates preset for spore count, cell density and bacterial inoculums in respective medias. The cups of 6 mm diameters were bored on the agar media and were then filled with 0.5ml of plant extracts. The plates were then incubated at30^{o}C for 48 hours and 37^{o}C for 24 hours respectively for fungi and bacteria. The zone of inhibition produced was read after incubation.

Culture medium

All the solutions used for testing were prepared in DMSO as a solvent.

Microorganisms

Antibacterial activity of extract was tested *in vitro* against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli***,** *Salmonella typhi*_*(Gram*-negative) and *Bacillus subtilis* (Gram-positive), *Shigella sonnei.*

Antimicrobial Agent

The standard compound (Gentamycin) was dissolved in dimethyl sulfoxide to give a concentration of 5x10^{-2} mm/ml.

Table 1. Results of Antibacterial activity (Gram positive strains)

Bacteria (Gram +)	*Bacillus pumilis*	*Staphylococcus aureus*	*Proteus vulgaris*
Control	-	-	-
Pet. ether	13	-	10
Chloroform	12	10	-
Benzene	-	-	-
Acetone	12	-	9
Methanol	10	10	11
Ethanol	-	-	12
Ethyl acetate fraction	11	11	11
Butanol fraction	10	-	-
Aqueous extract	-	9	13
Standard (40µg/well)	14	13	19

* Standard for bacteria = Gentamycin; '-' means no zone of inhibition

Table 2. Results of Antibacterial activity (Gram negative strains)

Bacteria (Gram -)	*Escherichia coli*	*Shigella sonnei*	*Pseudomonas aeruginosa*
Control	-	-	-
Pet. ether	14mm	-	-
Chloroform	12mm	12	9
Benzene	12	11	-
Acetone	-	10	9
Methanol	13	12	12
Ethanol	12	-	13
Ethyl acetate fraction	12	14	12
Butanol fraction	-	-	-
Aqueous extract	12	13	13
Standard* (40µg/well)	16	20	20

* Standard for bacteria = Gentamycin; '-' means no zone of inhibition

Table 3. Results of Anti-fungal activity

Fungi	*Aspergillus niger*	*Candida albicans*
Control	-	-
Pet. ether	14	-
Chloroform	13	9
Benzene	-	-
Acetone	11	11
Methanol	12	12
Ethanol	13	13
Ethyl acetate fraction	11	13
Butanol fraction	-	-
Aqueous extract	12	12
Standard*(40µg/well)	17	18

Standard for fungi =Griseofulvin; '-' means no zone of inhibition

Table 4. Results of measurement of Zone of inhibition

Extract	I	II	III	IV	V	VI
Control	-	-	-	-	-	-
Pet. ether	14mm	13	-	-	10	-
Chloroform	12mm	12	10	12	-	9
Benzene	12	-	-	11	-	-
Acetone	-	12	-	10	9	9
Methanol	13	10	10	12	11	12
Ethanol	12	-	-	-	12	13
Ethyl acetate fraction	12	11	11	14	11	12
Butanol fraction	-	10	-	-	-	-
Aqeous extract	12	-	9	13	13	13
Standard* (10µg/well)	16	14	13	20	19	20

* Standard for bacteria = Gentamycin; '-' means no zone of inhibition

The concentrations of various extracts were made to obtain a concentration of 1mg/ml. Cup plate agar diffusion method was used to determine the zone of inhibition of various extracts.

Preparation of test solution

The concentration of various extracts mentioned above were made in Dimethyl sulfoxide (DMSO) to give concentration of 200 mg/ml.

Determination of zone of inhibition by cup plate method

The antibacterial activity of the extracts was performed using Agar cup-plate method. About 20ml of sterile nutrient agar medium was poured into sterile petri-dishes and allowed to solidify. The petri dishes were incubated at 37ºC for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile top agar (4 ml) contained 1 ml culture. Bores were made on the medium using sterile borer. Dried extracts were dissolved in Dimethyl sulfoxide (DMSO) to obtain different concentrations and sterilized by filtration through a Whatman filter paper no. 1, and 0.1 ml of the different concentrations of extracts were added to the respective bores. The plates were incubated overnight at 37ºC with appropriate positive and negative controls.

The petri-dishes were kept in refrigerator at 4ºC f o r 30 min for diffusion. After diffusion the petri-dishes were incubated at 37ºC for 24 hours and zone of inhibition were observed and measured. Dimethyl sulfoxide was used as the control.

Determination of anti-fungal activity

Antifungal activity of extracts against was assessed with *Candida albicans* (MTCC 227) and *Aspergillus niger* (NCIM 545). The evaluation was performed similar to antibacterial activity by use of potato dextrose agar (PDA) as media for assay. The fungal sensitivity of extracts was evaluated for 7 days. Amphotericin B (20 micg./ml) was used as a standard for this activity.

Results and Discussion

Extraction of plant material

Various extracts of plant material were prepared by soxhlet extraction and the extractive yield were calculated. The extractive yields of Petroleum ether**,** Benzene, Chloroform, Acetone, Methanol, Ethanol, Ethylacetate, Butanol and Aqueous are 3.99%, 1.26%, 2.10%, 2.23%, 4.16%, 2.21%, 3.2%, 1.25% and 9.4%$^{w}/_{w}$ respectively.

Preliminary phytochemical investigation

The preliminary phytochemical investigations were carried out to reveal the presence of various phytoconstituents. Petroleum ether and $CHCl_3$ extract gave positive tests for steroids, EtOAc and MeOH extracts revealed presence of glycosides and flavonoids.

Antibacterial and antifungal activity

The antibacterial studies confirmed that the extracts had zone of inhibition, but the MIC of extracts ensured no prominent action on the tested bacterial strains. The antifungal studies confirmed that the extracts had a effective zone of inhibition against the tested organisms. The results of antibacterial and antifungal extracts are shown in **Table 1-4.** In future one can formulate the active constituents into a topical dosage form with antimicrobial and antifungal effect.

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504_2012 09702541624