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Comparative Antimicrobial Studies of *Dioscorea Hamiltonii* hook.f.tubers with *Azadirachta Indica* Stem

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Abstract

Antimicrobial activity of tuber extract of *Dioscorea hamiltonii* Hook.f. and stem extracts of *Azadirachta indica* was comparatively studied against *Aspergillus niger*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Lactobacillus delbrueckii subsp lactis*, *Penicillium chrysogenum*, *Proteus Vulgaris*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Streptococcus pyogenes*, *Streptococcus thermophilus and Vibrio parahaemolyticus.* Methanolic and Ethyl acetate leaf extracts from *D.hamiltonii* and *A.indica* has shown good antimicrobial activity against tested microbes (9 to 25mm zone diameter). The extract has shown good activity against Gram positive bacteria (14 to 25mm), when compared with Gram negative bacteria (12 to 23) and fungi (9 to 15mm, except *S.cerevisiae*-17 to 24mm). Higher concentrations of extracts are required to retard the growth of fungi.

Key words: *Dioscorea hamiltonii*, *Azadirachta indica*, Antimicrobial activity.

INTRODUCTION

Medicinal plants are playing an important role in protecting against dreadful and dangerous microbial species. These plants are being used in various traditional systems due to having immune potential and better activity against numerous diseases. The medicinal activity may be slow with the plant extracts but have permanent cure against various diseases. One such plant *Dioscorea hamiltonii* Hook.f., tubers are presently using for the treatment of gastro intestinal disorder (Dysentery) [1].

Dioscorea hamiltonii is a monocotyledonous angiosperm belongs to the order Liliflorea and Family Dioscoreaceae (**Figure 1**).

This plant is commonly known as *adavi pemdalam* or *karu kamda* in Telugu and *venni kalasu* in Malayalam (local vernacular). It is a rare plant especially grows in interior evergreen and moist deciduous forests of Himalaya (Nepal to Bhutan), Assam, NE India, Burma and Indochina[2]. The general characters of the plant are tuberous, climber, leaves 4-8 x 2-5 cm, ovate or lanceolate, truncate to deep cordate, arrow shaped; male spikes 1-4 nate or axillary bracklets and capsular reinform. Since times immemorial, medicinal plants have occupied a place of prime importance especially *Azadirachta indica* (Common name: neem – Anti-inflammatory, Anti arthritic, Anti pyretic, Hypoglycaemic, Anti gastric ulcer, spermicidal, Anti fungal, Anti bacterial, Diuretic, Anti malarial, Anti tumour, Immunomodulatory) [3.] Neem or morgosa is a botanical cousin of mahogany belongs to family Meliaceae. Neem has multidirectional therapeutic uses have been known in India since the Vedic times[4]. This is an indigenous plant possessing several medicinal properties, available widely in India and Burma. The leaves and stems mainly yield Quercetin (a poly phenolic flavonoid) is known to have antibacterial and antifungal properties[5].

The present investigation has been carried out to evaluate the antimicrobial activity of *Dioscorea hamiltonii* against bacteria and fungi. The extractants used were methanol, ethanol, ethyl acetate and water. The antimicrobial activity of *Dioscorea hamiltinii* is also compared with *Azadirachta indica* (an antimicrobial plant[6,7, 8]), for antimicrobial activity. This is in pursuance of the efforts to search for new drugs from plants.

Figure 1 : *Dioscorea hamiltonii* plant and tuber

MATERIALS AND METHODS

Collection of plant materials

The fresh tubers of *Dioscorea hamiltonii* were collected from Burna west of srikakulam district during rainy season. Fresh neem stems are collected locally.

Preparation of plant extracts

Freshly collected tubers of *Dioscorea hamiltonii* and *Azadirachta indica* stems are thoroughly washed with distilled water separately. 100 g of each plant material were separately homogenized with 100 ml of Methanol, Ethanol, Ethyl acetate and Distilled water. The homogenates were thoroughly squeezed through three layers of cheesecloth to remove larger particles and then centrifuged at 10,000 xg at 4°C for 20 min. The supernatant was collected and the centrifugation process repeated for 3 times at 10,000 xg at 4°C for 20 min. The final supernatant was adjusted to the concentration of 25mg/ml .The obtained concentrates were filter sterilized by passing through 0.22μm Millipore filters. The filtrates are kept at -20°C before use for maximum 24 hours.

Microorganisms and their maintenance

All 9 Bacterial and 3 fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and preserved in deep freezer at Dept. of Biochemistry and Bioinformatics, GITAM University, Visakhapatnam, India. The cultures used for experimentation are *Enterococcus faecalis* (MTCC No. 439), *Escherichia coli* (MTCC No.118), *Proteus Vulgaris* (MTCC No. 426), *Pseudomonas aeruginosa* (MTCC No. 424) and *Vibrio parahaemolyticus* (MTCC No 451) of Gram Negative Bacteria, *Bacillus subtilis* (MTCC No.121), *Lactobacillus delbrueckii subsp lactis* (MTCC No. 911), *Streptococcus pyogenes* (MTCC No. 442), *Streptococcus thermophilus* (MTCC No. 1938) of Gram Positive Bacteria, and *Aspergillus niger* (MTCC No.281) *Penicillium chrysogenum* (MTCC No. 161) and *Saccharomyces cerevisiae* (MTCC No. 1791) of Fungi.

The above Bacterial cultures were maintained on Muller-Hinton Agar (MHA; Himedia-Inda) and Fungal cultures were maintained on Sabouraud Dextrose Agar (SDA; Himedia-India) at 4°C temperature until used for the study. Before use, the Bacterial and Fungal cultures were revived in Muller Hinton Broth (MHB; Himedia-India) for Bacteria and yeast (*Saccharomyces cerevisiae*) and Sabouraud Dextrose Broth (SDB; Himedia-India) for Fungi.

Zone Method

Zone Method is carried out for antimicrobial assay. MHA (for bacterial growth) and SDA (for fungal growth) was weighed and mixed in distilled water based on the composition. The media was autoclaved for 20 min. at 121°C (15 lbs pressure) and cooled to 45°C. The bacterial and fungal cultures with optical density of 0.6 were taken and 50 ml of inoculum was added per 500ml of MHA for bacteria and yeast, SDA for fungi. 20 ml of the media was poured in each plate and was kept for solidification. By using gel puncture, 8 mm diameter wells had been made in the plate for the addition of plant extract at 25 mg/ml concentration. 10mg/ml of Griseofulvin, A*mpicillin* and Penicillin are used as standards. Fresh leaf extract was added into the well and incubated for 24-48 hours for bacteria and yeast and 2 days for fungi. The zone of inhibition can be calculated based on the results obtained by scale reading. With the scale, the zone formed on the plate was calculated and was tabulated.

RESULTS AND DISCUSSION

India has about 45000 plant species where several thousands have been claimed to possess medicinal properties[9]. Various parts of plants such as leaves, roots, barks, tubers and seeds are employed in ethanomedicine[10]. A considerable part of this indigenous knowledge was documented from the past into the organize systems of medicines such as Ayurveda, Yunani, Sidha or other systems. Some of these are having activity against microbial species, which are causing dreadful diseases. One such plant *Dioscorea hamiltonii*, a monocotyledonous angiosperm (belongs to family Dioscoreaceae) found to be having antimicrobial activity by present investigation.

Dioscorea hamiltonii is a vulnerable medicinal plant source rarely available and becoming extinct due to climatic and other factors. This plant has medicinally important compounds. The medicinal activities and secondary metabolite products have not been extensively documented.

Table 1: Antimicrobial activity of Methanolic leaf extract on *Dioscorea hamiltonii* showing zone of inhibition against selected microbes

Microorganism	Zone of growth inhibition (including 8 mm well size)								Standard
	D.hamiltonii				*A. indica*				
	A	B	C	D	A	B	C	D	
E.Coli	19	15	17	13	20	17	19	12	17Amp
V.parahaemolyticus	18	15	19	13	23	16	18	15	18 Amp
E.faecalis	19	17	18	12	19	18	19	14	20 Amp
P.aeruginosa	15	15	17	13	21	18	17	16	17 Amp
P.vulgaris	18	17	16	14	20	18	16	14	20 Amp
S.pyogenes	24	21	20	17	24	23	22	20	24 Pen
B.subtilis	23	19	22	14	25	19	21	18	25 Pen
S.thermophilus	21	20	20	16	22	23	22	19	23 Pen
L.delbrueckii	24	22	22	13	15	14	15	17	14 Pen
A.niger	11	10	10	9	13	12	15	14	14 Gri
P.chrysogenum	11	9	9	9	15	12	14	12	13 Gri
S.cerevisiae	20	19	20	17	24	23	23	21	19 Gri

A–*Methanolic extract;* ***B–****Ethanolic extract;*
C–*Ethyl acetate extract;* ***D–****Aqueous extract; Gri- Griseofulvin; Amp-ampicillin; Pen-Penicillin*

The plant extracts were analyzed for antimicrobial activity against test microorganisms. All the prepared extracts have shown good antimicrobial activity. The results of antimicrobial activity of plant extracts were presented in **Table 1**.

Methanolic and Ethyl acetate leaf extracts from *D.hamiltonii* and *A.indica* has shown good antimicrobial activity against tested microbes (9 to 25mm zone diameter). The extract has shown good activity against Gram positive bacteria (14 to 25mm), when compared with Gram negative bacteria (12 to 23) and fungi (9 to 15mm, Except *S.cerevisiae* -17 to 24mm).

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