Antimicrobial Activity of Some Medical Plants Against
Pythium debaryanum (Hesse)

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ABSTRACT

Bacterial and fungal infections were some of the most serious global health issues of the present century. Chilli is an very important vegetable crops, suffer from many diseases called by fungi bacteria, viruses, nematodes. Pythium debaryanum are soil borne pathogenic fungi which causes damping off of disease in chilli. Pythium debaryanum parasitizes seedlings of many plants and caused a destructive disease known as damping off. Several plants were known to possess medicinal value including antimicrobial properties. Totally three solvents were used for the present work. Out of three solvents used (Methanol, n-butanol, and aqueous) the methanolic extract should maximum activity in controlling the growth of pathogen. In the present study methanolic extracts of various plants such as Murraya Koenigii (Karuveppilei), Pithecellobium dulce (Kodukkapuli), Vitex negundo (Karunocci), Aleo vera (kattalai) individually for antimicrobial activity by agar well diffusion method were investigated against Pythium debaryanum. Antimicrobial effect was measured by the zone of inhibition. The methanolic extracts of vitex negundo showed considerably high activity against Pythium debaryanum than other plant extracts.

INTRODUCTION

In the tropical countries such as India, Conjunctival and corneal conditions are the most important opthalmic problems. The climate conditions (humidity, temperature) are favourable for the growth and proliferation of fungi. It has been expressed about the rising prevalence of pathogenic microorganisms, which are resistant to the newer or modern antibiotics that have been produced in the last three decades [1, 2]. Also, the problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in disease treatment found more especially in the developing countries were
emphasized [3]. Coincidentally the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds therapies or herbal medicine [2, 4].

For over thousands of years now, natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents.

\textit{Vitex negundo} every parts of this plant is valuable in medicine and various skin dieases , antibacterial [5] anti inflammatory, anti androgenic [6].

**MATERIALS AND METHODS**

**Plants collection**
The plants were collected from the non-irrigated cultivated lands in and around Thanjavur (Dt), Tamilnadu. Medicinal plants species such as \textit{Aloe vera} Mil, \textit{Alternanthera sessilis} L, \textit{Murraya Koenigii} L, \textit{Pithecellobium dulce} Benth. \textit{Vitex negundo} L, were collected from Botanical garden of A.V.V.M Sri Pushpam College , Poondi, Thanjavur, Tamilnadu for the study period.

\textit{Aleo vera}
A coarse perennial with short stem and shallow root system; Leaves fleshy in rosettes ,sessile, often crowded with horny prickles on the margins convex below, 45-60 cm long tapering to a blunt point, surface pale green with irregular white blotches; flowers yellow or orange in receives; fruits loculicidal capsule. It is also cultivated (or) seen wild in the drier part of India. Propagation is usually carried out by suckers. The leaves contain barbaloin, chrysophanol glycoside and the aglycone, aloe-emodin.

\textit{Alternanthera sessilis}. 
A much branched prostrate herb, branches often purplish, frequently rooting at the lower; leaves simple, opposite, somewhat fleshy, lanceolate, oblanceolate or linera-oblong, obtuse or subacute, sometimes obscurely denticate, glabrous, shortly petiolate; flowers small, white, in axillary clusters; fruits compressed obcordate utricles, seeds suborbicular. It is also found in throughout India, in moist places upto 2,100 m. chemical constituents of \(\beta\)-sitosterol, stigmasterol, campesterol, \(\alpha\)-spinasterol, oleanolic acid rhamnside, 24-methylene cycloartenol, cycloeucalenol, lupeol, 5-\(\alpha\)-stigmasta-7-enol and its palmitate.

\textit{Murraya Koengii}
An aromatic shrup or small tree;leaves pinnate, Leaflets mostly ovate, crenate-denate; flowers white in corybose cymes, berries purplish black. It is also cultivated in India. Propagation is usually carried out by seeds and vegetative method. The major aroma constituents in the oil and \(b\)-Caryophyllen, \(b\)-gurjunene, \(b\)-elemene and \(b\)-phallandrene.

\textit{Pithecellobium dulce}
The plant is a small to medium sized, evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plants of India and in the Andamans . It is known as ‘Vilayati Babul’ in Hindi and ‘Kodukkapuli’ in Tamil. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it s also useful in dermatitis and eye inflammation.
The leaves have been reported to possess astringent, emollient, abortifiacient and antidiabetic properties. The presence of steroids, saponins, lipids, phospholipids, glucosides, glycolipids and polysaccharides have been reported in the seeds.

**Vitex negundo**

An aromatic large shrub or small tree about 3m in height with quadrangular branches; Leaves opposite, exstipulate, long petioled and digitately 3-5 foliolate, all leaflets with petiolules, the middle one longer, flowers bluish purple in panicles up to 3cm long; fruits globose or ovoid (or)obovoid, four seeded drupe black when ripe. It is also found in throughout India , on waste lands up to 1500m. propagation is carried by seeds and vegetative methods. Leaves contain two alkaloid nishindine and hydrocotylene. Fresh leaves yield a pale greenish yellow oil.

**Sterilization of plant materials**

The disease free and fresh plants were selected. About 2 g of fresh and healthy leaves were taken for each solvent extraction. They were washed with distilled water for three times. Then surface sterilized with 0.1% mercuric chloride for 20 seconds. Again the leaves were washed thoroughly with distilled water (three times).

**Preparation of plant extracts**

Two grams of sterilized plant leaves were kept in the 10 ml organic solvent such as methanol, n-butanol and aqueous. Then they were ground well with the help of mortar and pestle. The plant material was subjected to centrifugation, for 15 minutes in at 10000 rpm. Again, it was filtered through whatmann. No. 1 filter paper. The supernatant was collected and made to known volume, by adding sterile distilled water and stored for further antimicrobial screening purposes.

**Chilli Pathogen**

*Pythium debaryanum* is a soil-borne plant pathogenic fungi which causes damping off of disease in chilli. These plant pathogen were isolated from chilli field soil, Thiruvarur district, Tamilnadu and stored in refrigerator for further study.

**Potato dextrose agar (PDA) medium (pH – 6.7) (g/l)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>Potato</td>
<td>250</td>
</tr>
<tr>
<td>Dextrose</td>
<td>15</td>
</tr>
<tr>
<td>Agar</td>
<td>18</td>
</tr>
<tr>
<td>Water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

The potato tubers were peeled off and weighed for about 250g tubers were chopped in to small pieces in to the sterile conical flask. After boiling the supernatant were collected and dextrose (15g) with agar (18g) to dissolve the ingredients. The medium was observed and adjusted to 6.5 pH. Finally the medium was sterilized at 120°C for 20 minutes.

**Screening for antimicrobial assay**

**Antifungal activity test**

The antifungal properties with organic solvent (methanol, n-butanol and aqueous) extracts from the leaves were investigated against pathogen. The sterilized potato dextrose agar medium was allowed to cool and added streptomycin sulphate for preventing bacterial contamination. Then it was poured in to each petri plate and allowed to solidify using sterile cotton swabs. Each fungal
isolate (3 days old broth culture) was individually swabbed on the surface of the medium with the plant extracts obtained from the leaves using paper disc (10μ/disc). These discs were kept in the reserved place for the evaporation of solvents. Then the discs were positioned on the top layer of the PDA. All the plated were incubated at 27°C for 48 h. The zone of inhibition was measured (each disc) along with the control plates maintained by using available standard antifungal agents.

RESULTS AND DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step of in vitro antibacterial activity assay [7]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [8]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

Table1. Antimicrobial activity of some plant leaf extract against *pythium debaryanum*

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Solvents</th>
<th>Zone of inhibition (Diameter in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aloe vera</em></td>
<td>n-butanol methanol aqueous</td>
<td>10mm 10mm -</td>
</tr>
<tr>
<td><em>Alternanther a sessilis</em></td>
<td>n-butanol methanol aqueous</td>
<td>- 10mm -</td>
</tr>
<tr>
<td><em>Murraya koenigi</em></td>
<td>n-butanol methanol aqueous</td>
<td>- 10mm 15mm</td>
</tr>
<tr>
<td><em>Pithecellobium dulce</em></td>
<td>n-butanol methanol aqueous</td>
<td>- 15mm -</td>
</tr>
<tr>
<td><em>Vitex negundo</em></td>
<td>n-butanol methanol aqueous</td>
<td>- 30mm -</td>
</tr>
</tbody>
</table>
However, not many reports are available on the exploitation of antifungal (or) antibacterial property of plants for developing commercial formulation for application in crop protection. In the present study, the methanol leaf extracts of Aloe vera Alternanthera sessillis, Murraya koenigii, Pithecellobium dulce and Vitex negundo, showed the activity against pythium debayanum and plant based products have been effectively proven for their utilization as source for antimicrobial compounds.

The results reveal that methanolic extracts of Aloe vera posses zone of inhibition was 10mm against pythium debayanum followed by Alternanthera sessilis was 10 mm, Murraya Koenigii was 10 mm, pithecellobium dulce was15mm, Vitex negundo was 30 mm, were observed. The maximum zone of inhibitory effect on Vitex negundo (30 mm) against pythium debayanum with other plant extracts (Table 1).

Some researches report that these is a relationship between the chemical structures of the most abundant compounds in the tested extracts (or) essential oils and the antimicrobial activity [9,10]. Aromatic phenolic compounds which have been found to have antimicrobial properties [11].

Several studies have been conducted in the past three decades that focused on the antimicrobial properties of herbs, spices and their derivates such as essential oils, extracts and decoctions [12,13,14,11]. The methanolic extract of the roots of chelidonium majus revealed a high resistance to Fusarium [15].

The knowledge of extend and inhibition of specific compounds which are present in plant extracts may like fungal and bacterial diseases [16].

**REFERENCE**