Original Research Article

A comparative evaluation of antifungal activity of medicinal plant extracts and chemical fungicides against four plant pathogens

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ABSTRACT

Plants historically have served as models in drug development. The production of medicines and the pharmacological treatment of diseases began with the use of herbs. Leaves extract of *Pongamia pinnata*, *Calotropis procera*, *Nerium indicum* and *Curcuma longa* were taken in aqueous solution, tested against plant pathogenic organisms - *Aspergillus fumigatus*, *Alternaria solani*, *Helminthosporium* spp. and *Fusarium solani*. The antifungal activities of these medicinal plants were investigated. Antifungal activities of various plant extracts were compared with commercially available antibiotics. The antimicrobial potential of the above plant extracts were seen against the test organism using agar gel diffusion technique. The minimum inhibitory concentration was determined. It was observed that the latex of the plant had a broad spectrum antifungal activity against all the fungi in each of the tested extracts. These findings supported the traditional uses of the plants in the treatment of different infections.

Keywords

Minimum Inhibitory Concentration, broad spectrum antifungal activity, *Pongamia pinnata*, *Calotropis procera*, *Nerium indicum* and *Curcuma longa*

Introduction

The use of synthetic chemicals as antimicrobial for the management of plant diseases has undoubtly increased crop protection but with considerable deterioration of environmental quality and human health (Cutler and Cutler 1999). An attempt was made to employ plant extract against number of plant pathogens. A large number of plants have been reported to possess fungi toxic properties against plant pathogens which could be exploited commercially with practically no residual or toxic effect on ecosystem (Kumar et al., 2008). The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. A large number of plants have been reported by various researchers and practitioners of traditional medicine for the treatment of skin diseases (Bohlmann et al., 1964). Medicinal plants have nodoubt remained the major sources of traditional medicine worldwide. Accordingly, attention of scientists and researchers have been attracted towards developing new antibiotics that will curtail the increasing drug resistance among
microorganisms (Edith et al., 2005). Schimmer et al. (1994) reported that plants used for traditional medicine generally contain a number of compounds which may be a potential natural natural antimicrobial combination and which may serve as an alternative, effective, cheap and safe antimicrobial agents for treatment of common microbial infections (Mathur and Goyal, 2011).

*Calotropis procera* belongs to the family *Asclepiadaceae* and is a soft wooded, evergreen perennial shrub. It is a xerophytic erect shrub, growing widely throughout the tropical and subtropical regions of Asia and Africa. This plant is popularly known because it produces large quantity of latex. This plant has potential antimicrobial properties against microbial infections (Mathur and Goyal, 2011).

Traditionally, *Calotropis* is used alone or with other medicines to treat common diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea. The leaves and the flowers *Nerium indium* (kanner) are cardio tonic, diaphoretic, and diuretic, emetic expectorant and sternutatory. A decoction of the leaves is applied externally in the treatment of scabies and to reduce swellings. This is a very poisonous plant, containing a powerful cardiac toxin and should only be used with extreme caution. The root is powerfully resolving. Because of its poisonous nature it is only used externally. Oil prepared from the root bark is used in the treatment of leprosy and diseases of scaly nature. The plant is used as a rat poison, parasitic idée and an insecticide (Kavitha et al., 2000). *Pongamia pinnata* (karanji) exhibits many pharmacological attributes. First, anti-Plasmodial characteristics make *P. pinnata* important to treat malaria caused by *Plasmodium falciparum*. Next, the leaves of the Pongam tree exhibit anti-inflammatory qualities as well as anti-diarrheal activity; also, the leaf extracts are antioxidants. *Pongamia* prevents ulcers by protecting damage from aspirin. It may also provide diabetic patients with a safer anti-hyperglycemic drug.

Turmeric is an ancient spice derived from the rhizomes of *Curcuma longa*, which is a member of the ginger family (*Zingiberaceae*). Also known as ‘Golden Spice of India’ turmeric has been used in India for medicinal purposes for centuries. Turmeric, derived from the rhizomes of *Curcuma longa*, (family- *Zingiberaceae*) is a perennial plant having short stem with large oblong leaves, and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in colour (Rathaur et al. 2012). *Curcuma longa* (Turmeric) is taken as the blood purifier and is very useful in the common cold, leprosy, intermittent, affections of the liver, dropsy, inflammation and wound healing. The rhizome of the turmeric plant is highly aromatic and antiseptic. It is even used for contraception, swelling, insect stings, wounds, whooping cough, inflammation, internal injuries, pimples, injuries, as a skin tonic. Sweetened milk boiled with the turmeric is the popular remedy for cold and cough. It is given in liver ailments and jaundice. The most important fungi causing post-harvest diseases include: *Helminthosporium* spp, *Aspergillus* spp, *Alternaria* spp, *Fusarium* spp. Many fruits are prone to damage caused by insects, animals, early splits, and during mechanical harvesting. This damage predispose the fruits to the wound invading pathogen *Aspergillus fumigatus*, and other fungi, that causes decay on stored citrus fruits. *Aspergillus fumigatus*
can pose a health problem; especially it produces aflatoxin, a group of toxic, carcinogenic compounds (Diener et al., 1987). *Alternaria solani* is a fungal pathogen that produces a disease in tomato and potato plants called early blight. *Alternaria solani* infects stems, leaves and fruits of tomato (*Solanum lycopersicum* L.), potato (*S. tuberosum*), eggplant (*S. melongena* L.), bell pepper and hot pepper (*Capsicum* spp.), and other members of the *Solanum* family. Distinguishing symptoms of *A. solani* include leaf spot and defoliation, which are most pronounced in the lower canopy. In some cases, *A. solani* may also cause damping off. *A. fumigatus* the second most common agent of aspergillosis, the first being *Aspergillus fumigatus*. *A. fumigatus* may invade arteries of the lung or brain and cause infarction. Neutropenia predisposes to *Aspergillus* infection. *Aspergillus fumigatus* also produces a toxin, aflatoxin, which is one of the aetiological agents for hepatocellular carcinoma. In humans whose immune systems are weakened in a particular way, (neutropenia, i.e., very low neutrophils count), aggressive fusarial infections penetrating the entire body and bloodstream (disseminated infections) may be caused by members of the *Fusarium solani* complex (Bruce and Miller, 2003). *Helminthosporium* attack essentially all turf grasses as well as numerous pasture, wild and weed grasses, and small grains. This work reports the antimicrobial effect of *Curcuma longa*, *Pongamia pinnata*, *Nerium indicum*, *Calotropis procera* and their various combinations on some bacterial species. This is in pursuance of the efforts to search for drugs from plants and the verification of the scientific basis of some known practices in traditional medicine. A few reports have focused on the immense potential of this plant which has antimicrobial, wound healing, anti-inflammatory and immuno-modulatory properties.

Potato (*Solanum tuberosum* L.) is the world's fourth kept in dark between the filter papers at room temperature important food crop after wheat, rice and maize because of till completely dry. Each plant sample was individually its great yield potential and high nutritive value for grounded into powder for preparation of extract. The extract was allowed to internationally important disease of potato resulting in stand for some time and decanted off into the flask and about 25 to 60% loss in yield in different countries and final volume was raised to 100ml by adding boiled distilled attempts have been made to manage the disease by water. The supernatant was used for assay. The treating with chemical compounds, biological agents as antifungal activity of each plant part extract was reported by Wharton and Kirk. Therefore, the investigation was preceded with the objective of antifungal activity determination of the selected medicinal value plants against plant pathogens and compare with the chemical fungicides.

**Materials and Methods**

**Place of sample collection**

The plant sample of *Calotropis procera*, *Pongamia pinnata*, *Curcuma longa* and *Nerium indicum* was collected from Sam Higginbottom Institute of Agriculture and Sciences, Allahabad.

**Procurement of Microorganism**

The plant pathogens *Alternaria solani*, *Fusarium solani*, *Aspergillus fumigatus* and *Helminthosporium* spp. and antifungal disc of Bavistin and Mancozeb was procured.
from the Microbial Culture Collection (MCCB), Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture and Science, Allahabad.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>0207</td>
</tr>
<tr>
<td>Alternaria solani</td>
<td>0079</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>0068</td>
</tr>
<tr>
<td>Helminthosporium spp.</td>
<td>0243</td>
</tr>
</tbody>
</table>

Extraction of Plant Material

Fresh flower of Nerium indicum and leaves of Calotropis procera, Pongamia, Pinnata, Curcuma longa were collected, washed under tap water, then in distilled water, and then kept between folds of filter paper to remove excess of water from external surface. On drying 10g of fresh leaves of Curcuma longa, Pongamia pinnata, Calotropis procera and Nerium indicum, was chopped well, and 100 ml of distilled water was added separately (1:1 w/v) respectively. These were ground to fine powder and paste respectively, soaked for 24 h. Soaked materials were then filtered through muslin clothes and leaf extract of Curcuma longa, Pongamia pinneta, Calotropis procera and Nerium indicum, was collected with the help of funnel in separate conical flask. Finally filtrate obtained was used against plant pathogens.

Antifungal activity of Medicinal plant extract and Chemical fungicides against plant pathogens

Sabouraud dextrose agar was taken in culture tubes and broth culture was mixed in tubes by vortex mixture and poured into the plates. The plates were allowed to solidify for an hour. Wells (10 mm diameter) was made with the aid of flamed cork borer on the surface of the agar plates. 0.1ml of the leaf extracts of the plants and chemical fungicides suspension (Bavistin and Mancozeb) filled in the wells and the plates were incubated at 25°C for 5 days to observed zone of inhibition.

Determination of Minimum Inhibitory Concentration

To measure the Minimum Inhibitory Concentration values, micro-broth dilution method was used. The reconstituted extract was serially diluted 2-fold in SDA broth medium to obtain various concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 µl/ml) of the stocks and were assayed against the reconstituted organisms. The minimum inhibitory concentration is defined as the lowest concentration of extract which inhibited the growth of microorganism (Prescott et al., 1999).

Results and Discussion

Antifungal activity of Curcuma longa

The antifungal activity of aqueous extract of Curcuma longa was determined against Aspergillus fumigatus, Alternaria solani, Fusarium solani and Helminthosporium spp. at concentration of 100µl and zone of inhibition was found as a clear function of concentration. The antifungal activity of Curcuma longa was determined on subbouraud dextrose agar medium. The aqueous extract of Curcuma longa showed better activity against Aspergillus fumigates, Fusarium solani, Alternaria solani, Helminthosporium spp. Similar study conducted by Fairs, et al. (2002) had reported zone of inhibition of aqueous extract of Curcuma longa against Aspergillus spp. with recorded inhibition
zones of 58.2%, 73.4%, 79.3%, and 90.1% at similar plant extract concentrations respectively. The inhibition zones observed in Fusarium spp. were 46.5%, 64.7%, 71.7% and 88.9% respectively. Rathaur et al. (2012) reported anti-inflammatory, antioxidant, anticarcinogenic, anti-mutagenic, anticoagulant, antifertility, anti-diabetic, antibacterial, antifungal, antiprotozoal, antiviral, anti-fibrotic, anti-venom, antilucer, hypotensive and hypocholesteremic activities of Curcuma longa. Kumar et al. (2008) concluded that Curcuma longa gives zone of inhibition of 25 mm to 30 mm against Aspergillus fumigatus. Antifungal activity of Nerium indicum

The antifungal activity of Nerium indicum was determined by agar well diffusion at the concentration of 100 µl against the organisms (Aspergillus fumigatus, Alternaria solani, Fusarium solani and Helminthosporium spp. Nerium indicum showed high activity against Aspergillus fumigatus (20mm), Alternaria solani (22mm) but did not show activity against Fusarium solani and Helminthosporium spp. Chauhan et al. (2013) concluded the inhibitory effect of Nerium indicum (19 mm) against some bacterial spp. Parastoo et al. (2012) concluded that the methanol extract of Nerium indicum shown inhibition of Bacillus sp., Escherichia coli, Yersinia sp., Staphylococcus sp. at 500ppm and 1000ppm, whereas no inhibitory effect was observed against Pseudomonas sp., Lactobacillus sp., Enterococcus sp., and Klebsiella sp.

Antifungal activity of Pongamia pinnata

The antifungal activity of aqueous extract of Pongamia pinnata determined at concentration of 100 µl against Aspergillus fumigatus, Alternaria solani, Fusarium solani and Helminthosporium spp. Pongamia pinnata showed better activity against Aspergillus fumigatus (20mm) and Alternaria solani (21mm), but did not showed activity against Helminthosporium spp. and Fusarium solani. Sharma et al. (2012) reported that the Pongamia pinnata leaf extracts (Chloroform) produced zone of 22, 18, 16, 15 mm against T. mentagrophytes, M. fulvum, T. rubrum, T. tonsurans and M. gypseum, and methanol extracts produces maximum zones of 17, 16, 12, 12 and 11mm for M. fulvum, T. mentagrophytes.

Antifungal activity of Calotropis procera

The antifungal activity of aqueous extract of Calotropis procera was determined against Aspergillus fumigatus, Alternaria solani, Fusarium solani and Helminthosporium spp. at concentration of 100 µl and zone of inhibition was found to be clear function of concentration. The antifungal activity of Calotropis procera was determined on subouraud dextrose agar medium. The aqueous extract of Calotropis procera showed better activity against Aspergillus fumigatus (21mm) and Alternaria solani (30mm), but not showed activity against Fusarium solani and Helminthosporium spp. Saadabiet et al. (2012) reported that Calotropis procera showed inhibition of 25 mm against Aspergillus fumigatus. Velmurugan et al. (2011) examined the highest zone of inhibition (21mm) by Calotropis procera against fungal pathogens was noted at the concentration of 100 µl (50mg/1ml). Analysis of variance revealed that the
calculated value of f-test due to organism was greater than the tabulated value of F on 3,9 degree of freedom and at 5 % probability level, therefore it can be conclude from the data that there was significant effect of different organism on zone of inhibition. The average value due to Curcuma longa was highest followed by the average value of Calotropis procera and Nerium indicum. Minimum value was recorded due to Pongamia pinnata. But the calculated value of F due to extract was smaller than its table value at 5% level of significance; hence there was no significant difference.

**Antifungal activity of chemical fungicides against plant pathogens**

The antifungal susceptibility test was determined by suspension of Bavstin and Mancozeb against (Fusarium solani, Aspergillus fumigatus, Alternaria solani and Helminthosporium spp.) both showed high activity against Fusarium solani, Aspergillus fumigatus, Alternaria solani, and Helminthosporium spp. Shirurkar and Wahegaonkar (2012) studied the antifungal activity of the eight different plant derived oils and two known antifungal synthetic chemicals available in market tested against the eight fungal species Aspergillus flavus, A. niger, A. terreus, A. oryzae, A. fumigatus, Fusarium moniliforme., F. solani, Penicillium sp. isolated from maize grains. The results revealed that Bavstin, Mancozeb and some of the oils tested exhibited different degrees of antifungal activity against different seed borne fungi of maize. Kiran et al. (2011) studied the antifungal activity of aqueous and solvent extract of seeds of P. corylifolia against five seed borne fungi of maize viz., Curvularia lunata, Dreschlerahalodes, Alternaria alternata, Cladosporium cladosporioide and Rhizopus sp. were tested in vitro. In aqueous extract, maximum inhibition was observed in A. alternata and recorded 95.4% inhibition at 50% concentration followed by C. lunata (86.0%), Rhizopus sp. (82.3%), D. halodes (68.0%) and C. cladosporioide (57.7%). Significant activity was also observed in 10, 20, 30 and 40% concentration. In solvent extracts tested at 250, 500, 750 and 1000 µl concentration, maximum inhibition was observed in petroleum ether extract and moderate activity was observed in methanol extract. Compared to synthetic fungicide Bavastin and Thiram, complete inhibition was observed against all the test fungi tested at 2% recommended concentration.

**Comparative analysis of antifungal activity of plant extract/chemical fungicides against plant pathogens**

After comparing the antifungal activity of plant extract with chemical fungicides against plant pathogens showed that the aqueous extract of plant leaves were more susceptible then the chemical fungicides. In plant extract the leaves of Curcuma longa were more active against pathogenic fungi.

**Determination of Minimum Inhibitory Concentration**

Further using themacro-dilution broth method, the aqueous extract of the leaves of Curcuma longa, Nerium indicum, Pongamia pinnata and Calotropis procera were subjected to MIC determination. The extracts were serially diluted to obtain a decreasing concentration of 200 to 1.56 µl of the SDA broth.

The aqueous extract of Curcuma longa exhibited inhibition at the concentration of
6.25 µl/ml against Aspergillus fumigatus, at a concentration of 12.5 µl/ml against helminthosporium spp. The final MIC value was found to be two-fold higher than the initial MIC value.

The extract of Pongamia pinnata exhibited at the concentration of 6.25 µl against Alternaria solani and Aspergillus fumigatus and even the lowest concentration the MIC was not found against Fusarium solani and Helminthosporium spp. The final MIC value was found to be two-fold higher than the initial MIC value.

The extract of Nerium indicum exhibited against Alternaria solani at the concentration of 12.5 µl, and for Aspergillus fumigatus it exhibited at the concentration of 6.25 µl. but MIC was not found against Helminthosporium spp. and Fusarium solani even at the lowest concentration. The final MIC value was found to be two-fold higher than the initial MIC value.

The extract of Calotropis procera exhibited against Alternaria solani at the concentration of 12.5 µl, for Aspergillus fumigatus it exhibited at the concentration of 3.125 µl but no MIC was found even at the lowest concentration Helminthosporium spp. and Fusarium solani. The final MIC value was found to be two-fold higher than the initial MIC value. Similer study was shown by Saadabi et al. (2012), who determined the minimum inhibitory concentration of the different extracts of Calotropis procera showed that the highest activity was recorded against Aspergillus fumigatus, Candida albicans and Bacillus subtilis (10mg ml⁻¹) in chloroform extract of the latex. The lowest activity obtain against Candida albicans (25mg ml⁻¹) in methanol and in aqueous (25mg ml⁻¹) against Escherichia coli. It was clearly noticed that the latex of the plant have a broad spectrum antimicrobial activity against all of the bacteria and fungi in all of the tested Extract. Lalsangluai et al. (2013) determined the minimum inhibitory concentration (MIC) of the oil of Curcuma longa was recorded at 1.9 mg/ml against T. mentagrophytes and 2.1 mg/ml against T. rubrum. However, it was fungicidal at 2.4 mg/ml against T. mentagrophytes and 2.5 mg/ml against T. rubrum respectively. The effective concentration contains heavy doses of inoculums (25 discs of 5 mm each).

When modern drugs of choice become ineffective in the treatment of various diseases, it is anticipated 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 for the treatment. It will also help in alleviating the health problems of the people especially living in far flung areas having no access to modern health care systems.

Further this study indicates that the leaf extracts using solution in water were more effective supporting the use of “teas” to treat intestinal disease caused by worm parasites. Curcuma longa is primarily an ornamental plant, although it has been used in traditional medicine to cure some
**Table 4.1** Antifungal activity of plant extracts against plant pathogenic fungi

<table>
<thead>
<tr>
<th>Extract</th>
<th>Aspergillus fumigatus</th>
<th>Alternaria solani</th>
<th>Fusarium solani</th>
<th>Helminthosporium spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Curcuma longa</em></td>
<td>22</td>
<td>20</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td><em>Nerium indicum</em></td>
<td>20</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Pongamia pinnata</em></td>
<td>20</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Calotropis procera</em></td>
<td>21</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Due to Organism $f_{(cal)}=6.611>f_{(tab)}=3.86$ at 5%, Significant C.D. = 11.56
Due to Extract $f_{(cal)}=2.932<f_{(tab)}=3.86$, Non significant

**Fig. 4.2** Antifungal activities of chemical fungicides against plant pathogens

![Antifungal activities of chemical fungicides against plant pathogens](image)

**Table 4.3** Comparative analysis of antifungal activity of plant extract/chemical fungicides against plant pathogens

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antifungal activity of plant extract/chemical fungicides against plant pathogens (Zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Curcuma longa</em></td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>22</td>
</tr>
<tr>
<td>Alternaria solani</td>
<td>20</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>23</td>
</tr>
<tr>
<td>Helminthosporium spp.</td>
<td>25</td>
</tr>
</tbody>
</table>
Fig.4.3 Comparative analysis of antifungal activity of plant extract/chemical fungicides against plant pathogens

![Graph showing comparative analysis of antifungal activity of plant extract/chemical fungicides against plant pathogens.]

...diseases. Recent reports of various pharmaceutical properties of this plant established it as a valuable source of medicinal compounds. In this study, aqueous extract of *Neriumindicum.* leaves possessed broad spectrum antimicrobial activity against various clinical isolates of fungi. It is concluded that aqueous extract of *Calotropisprocera* leaves is a good source of antimicrobial compounds and in future, the bioactive molecule can be isolated and characterized using advanced analytical techniques.

**References**


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