

# Antifungal Activity of Some Plant Extracts Against Decay Fungi From Palmyrah Leaf Handicrafts

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## ABSTRACT

Fungi associated with decaying handicrafts made from palmyrah leaf were isolated from two different sites of the Jaffna peninsula and characterized as *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* sp. These were used as test fungi for screening antifungal activity of neem leaf, neem seed, neem bark, omum (Thyme) and turmeric powder obtained from local market were used. The hot water extracts of above plants parts were evaluated against the isolated fungi on PDA. During screening of antimicrobial activity, tests and controls were set to determine MIC (minimum inhibitory concentration) and Percentage of Growth Inhibition (PGI). Hot water extracts of neem leaf, neem seed, neem bark and turmeric powder recorded no significant ( $0.05 > P$ ) antifungal activity while omum showed significantly different PGI for all fungal species compared with control and showed lowest MIC was at 15ml/dl. Therefore omum was selected for further study and active component of omum as thymol was compared with the omum hot water extract. There was no significant difference in PGI of thymol at the concentrations of 0.5, 1 and 2 ml/dl and 15ml/dl of omum hot water extracts, against all fungal species. Therefore omum could be used for further study to develop a new environmental friendly antifungal agent for the preservation of leafy handicrafts. Further formulation, field experiments are necessary to achieve this target.

**Key words:** Antifungal activity, hot water extract, neem and omum

## 1. INTRODUCTION

The presence and growth of fungi on palmyrah leaf handicrafts may cause damage during rainy season and result in a reduction in quality such as discoloration and degradation. Fungi produce a variety of secondary metabolites as products of their metabolism; mycotoxins

are metabolites that have deleterious effects on other organisms. Synthetic fungicides are currently used as the primary means for the control of fungi. However, the alternative control techniques that are cheap and environmentally safe to eliminate or reduce the occurrence of fungi are great importance, so the search for substances meeting these needs is an important research issue.

Extracts obtained from neem (*Azadirachta indica*) is one of the most important plant products which inhibit the growth of microorganism and mycotoxin production. It comprises several parts, such as fruit, seed, leaf and oil with several active compounds [1, 2, 3]. Neem leaf and its constituents have been demonstrated to exhibit, antifungal and antibacterial properties [4].

Several studies have shown that thyme oils, particularly those of *Thymus vulgaris* and *Thymus zygis* [5, 6, 7], possess antimicrobial activity, those of the phenol type being the most active. The limited occurrence of these phenols in nature is one of the reasons why *Thymus* oils containing thymol and carvacrol have been of great interest for some time. Klaric *et al.* [8] reported that thyme oil contains p-cymene (36.5%), thymol (33.0%) and 1, 8-cineole (11.3%).

The main objective of this work was to illustrate the invitro antifungal activities of some plant parts crude extracts on different fungus species isolated from Palmyrah leaf handicrafts.

## 2. MATERIALS AND METHODS

### Collection of plant materials

Turmeric powder, bark and seeds of neem (*Azadirachta indica*) and flowers of omum/ thyme (*Thymus vulgaris*), were used for this study. They were collected from the local market in Jaffna, Sri Lanka and thymol was obtained from Sigma Aldrich.

### Culture and Maintenance of fungus

Pure cultures of fungi such as *Aspergillus niger*, *Aspergillus flavus* and *Penicillium sp* that were isolated from affected Palmyrah leaf handicraft. The pure cultures were maintained on potato dextrose agar (PDA) slants. Each fungal culture was sub-cultured regularly on the same medium and stored at 4°C before use in experiments.

### Preparation of plant extract

Plant materials collected were washed 2-3 times with tap water and finally with distilled water and allowed to dry at 50° C overnight and ground to a coarse powder. Cool, hot water extraction was used for this study. For hot water extraction, powdered plant material (6g), was added into a conical flask containing 30ml of water separately and boiled for 30 min. This was then allowed to cool and filtered through sterile muslin cloth.

### Antifungal screening

Agar plate dilution test was used to determine the Percentage of Growth Inhibition (PGI) and Minimum Inhibitory Concentration (MIC) of the extracts and the antifungal agent.

### Media preparation and sterilization

Potato Dextrose Agar (3.9 g) was dissolved in 40 mL of distilled water in a boiling water bath and total volume was made up to 100mL and the medium was sterilized at 121°C and 15lb/in<sup>2</sup> for 15 min. The medium was allowed to cool to 50°C until use.

### Determination of the minimum inhibitory concentration (MIC)

PDA medium with 1, 5, 10 and 15 (ml/dl) concentrations of the plant extracts such as Leaves, bark and seeds of neem, flowers of thyme, Rhizome of turmeric and 0.1, 0.5, 1.0 & 2.0 ml/dl of thymol were prepared. About 15 mL of the medium was poured into each petridish and allowed to solidify. Nine mm mycelia discs of 5 day old culture of the test fungi from the margin of the colony were placed at the center of the medium and the plates were incubated at room temperature for 4 days. After incubation, the colony diameter was measured in millimeter. For each treatment replicates were maintained. PDA medium without the plant extracts served as control. Growth zones were measured at 4<sup>th</sup> and 7<sup>th</sup> days of incubation. The fungi-toxicity of the extracts in terms of percentage of growth inhibition of colony growth was calculated by using the formula

$$\text{Growth inhibition (\%)} = \frac{dc - dt}{dc} \times 100$$

Where dc = Average increase in mycelial growth in control,

dt = Average increase in mycelial growth in treatment [9].

The antifungal agent nystatin added to the agar plates (final concentration of 1.0 mg/l) served as a positive control for *Aspergillus niger*, *A. flavus* and *penicillium sp*. Diameter of the colony were measured at the end of the incubation period. The MIC was taken to be the lowest dilution inhibiting the growth of the organism.

**Statistical Analysis:** MIC and percentage of inhibition were analyzed by SAS package and the mean separation was done by LSD at p=0.05.

### 3. RESULTS AND DISCUSSION

The antifungal activities of different extracts of Turmeric powder, neem leaf, neem seed, neem bark and thyme flowers were determined against fungi isolated from palmyrah leaf handicraft. Of different extracts tested, only the hot water extracts inhibited the growth of the organisms with highest GI percentage for all the fungi species.

The level of GI of *A.niger*, *A.flavus* and *Penicillium* by hot water extracts of turmeric powder, neem leaf, neem seeds and neem bark of all concentrations were not significantly different (p<0.05) whereas the sensitivity of fungi increased with the increase of the concentration of extracts of neem seed, neem bark and thyme.

The results showed that hot water extract of thyme plant materials caused 100% growth inhibition of all species of fungi at 25 ml/dl of concentration. Therefore we used lower concentrations (1, 5, 10 and 15 ml/dl) to determine the MIC of each plant extract on these fungi. The hot water solution of thyme showed significantly (p<0.05) higher (100%) GI for all fungi species at 15 ml/dl of concentration (Table 1) than the controls while the hot water extracts of plant material have not showed significant difference on GI and *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* showed 76.41, 77.01 and 75.41% of GI at 10 ml/dl of concentration respectively. This plant hot water extract exhibited toxicity to a broad spectrum of fungi toxicity by inhibiting the mycelial growth of all fungi tested. The MIC of hot water extract of thyme was same for the all three fungal species. *A. flavus* and *Penicillium* were less sensitive than *A. niger* to the hot water extract of thyme.

**Table 1:** Percentage of growth inhibition with different concentrations of hot water extracts of Neem seed, Neem bark and Thyme

Plant part Concentration (ml/dl)	Aspergillus niger	Aspergillus flavus	Penicillium
Neem seed 1	13.1 <sup>e</sup>	0.8 <sup>e</sup>	5.3 <sup>c</sup>
Neem seed 5	14.8 <sup>e</sup>	26.2 <sup>d</sup>	4.8 <sup>c</sup>
Neem seed 10	12.3 <sup>e</sup>	28.4 <sup>d</sup>	7.3 <sup>c</sup>
Neem seed 15	12.3 <sup>e</sup>	26.7 <sup>d</sup>	7.3 <sup>c</sup>
Neem bark 1	6.3 <sup>f</sup>	0.5 <sup>e</sup>	2.3 <sup>f</sup>
Neem bark 5	7.8 <sup>f</sup>	1.3 <sup>e</sup>	3.8 <sup>e</sup>
Neem bark 10	7.0 <sup>f</sup>	1.4 <sup>e</sup>	4.3 <sup>d</sup>
Neem bark 15	6.8 <sup>f</sup>	1.8 <sup>e</sup>	5.8 <sup>c</sup>
Thyme 1	40.7 <sup>d</sup>	2.0 <sup>e</sup>	7.0 <sup>d</sup>
Thyme 5	50.1 <sup>c</sup>	2.3 <sup>e</sup>	7.3 <sup>c</sup>
Thyme 10	77.0 <sup>b</sup>	76.4 <sup>b</sup>	75.4 <sup>b</sup>
Thyme 15	100.0 <sup>a</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>

Based on this study thyme extract was selected and compare with pure thymol with 0.1, 0.5, 1.0 & 2.0 ml/dl of concentration. All the concentrations of pure thymol showed 100% of GI of *A.niger*, *A.flavus* and *Penicillium* 4 days after incubation. There was no significant difference ( $p<0.05$ ) between the growth at inhibition concentration of 0.5, 1 and 2 ml/dl thymol on 7<sup>th</sup> day incubation and the inhibition caused at 15 ml/dl of thyme, of all fungi species, was, however, significantly higher compared with other concentrations.

GI of thymol at the concentration of 10 ml/dl was 76.9, 76.4 and 75.3% for *A.niger*, *A.flavus* and *Penicillium* respectively while thymol with the concentration of 0.1ml/dl showed 66.9, 59.3 and 66.9% GI for above fungi respectively.

At low concentrations, phenolic lipophilic compounds such as thymol altered the microbial cell permeability permitting the loss of macromolecules. The mode of action of plant extracts on test fungi has not been determined, but they may inactivate essential enzymes, react with cell membrane proteins or disturb genetic material functionality [2]. From this point of view, essential oil of thyme, which is rich in thymol and other antifungal components, could be used for disinfection of fungi in low concentration. Considering the results, we recommend the use of thymol selected for development of new and safe fungicides. Further formulation and field experiments are necessary to achieve this target.

#### 4. CONCLUSION

The results showed that hot water extracts of omum showed a significant GI ( $p<0.05$ ) of all selected fungi compared with other plant extracts such as turmeric powder, neem leaf neem seeds and neem bark. There

was no significant different between 0.5, 1 and 2 ml/dl thymol and 15 ml/dl of omum hot water extracts, for all fungi species. Therefore these have potential to control of some palmyrah leaf article decay fungi and could be considered for developing new antifungal agent.

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