***Short Communication***

# ANTIFUNGAL STUDIES OF *WITHANIA COAGULANS* AND *TAMARIX APHYLLA*

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**ABSTRACT:**
*In vitro* studies were carried out to evaluate the antifungal activity of methanolic , pet ether and dichloromethane extract of aerial parts of *Withania coagulans* and *Tamarix aphylla* against seven fungal strains (*Trichoderma viridis* ,*Aspergillus flavus* , *Fusarium laterifum* , *Aspergillus fumigatus* , *Candida albicans* , *Trichophyton mentogrophytes* and *Microsporum canis*). Assessment revealed that all extracts of *Withania coagulans* caused significant inhibition in biomass production of all test fungi (0.5µg/ml), while dichloromethane extract of *Tamarix aphylla* showed significant results (1.0µg/ml) against the tested fungal strains only.

***Key words*****:** *Withania coagulans****,*** *Tamarix aphylla,* Antifungal activity

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## INTRODUCTION:

Parasitic diseases remain a major public health problem affecting hundreds of millions of people, particularly in tropical developing countries. The limited availability and affordability of pharmaceutical medicines means that the majority of the world's population depends on traditional medical remedies, and it is estimated that some 20 000 species of higher plant are used medicinally throughout the world. Many well-known drugs listed in the modern pharmacopoeia have their origins in nature, including, for example, quinine from the bark of the *Cinchona* tree for the treatment of malaria, which has been followed by the subsequent development of the synthetic derivatives chloroquine, amodiaquine, primaquine and mefloquine.

The plant kingdom has contributed to the health problems since the time immemorial. A WHO study reveals that around 90% of the world population in the developing world relies on herbal remedies for their basic healthcare needs. A number of compounds isolated from plants have medicinal properties [Goldstein A., Aronow L.,Kalman S.M., (1974)] [Tyler V.E., Brady L.R.,

Robbers J.E. (1988)] [Kinghom A.D., (1993)] [Lenaz, Defuria, (1993)] [Klayman, (1984)] [Klayman, (1985)] [Nair, (1986)] [De Souza, (1983)].

Hammer.K.A studied the activity of 52 plant oils extracts against *Acinetobacter baumanii, Aeromonas veronii*, Candida *albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* subsp. *enterica* serotype *typhimurium, Serratia marcescens and Staphylococcus aureus*, using an agar dilution method. Lemongrass, Oregano and Bay inhibited all organisms at concentrations of ≤2·0% (v/v). Six oils did not inhibit any organisms at the highest concentration, which was 2·0% (v/v) oil for Apricot kernel, Evening primrose, Macadamia, pumpkin, Sage and Sweet almond. Twenty of the plant oils and extracts were re investigated, using a broth microdilution method, for activity against *C. albicans*, *Staph. aureus* and *E. coli*. The lowest minimum inhibitory concentrations were 0·03% (v/v) thyme oil against *C. albicans* and *E. coli* and 0·008% (v/v) Vetiver oil against *Staph. aureus*. Plant essential oils and extracts may have a role as pharmaceuticals and preservatives.

Magro.A (2007) evaluates fungistatic activity of six aqueous extracts (*Lavandula stoechas* L., *Zingiber officinale* Roscoe, *Malva sylvestris* L., *Origanum vulgare* L., *Tabebuia impetiginosa* and *Rosmarinus officinalis* L.) They were tested against *Aspergillus candidus, A. niger, Penicillium* sp. and *Fusarium culmorum.* These extracts are showed good fungistatic activity.

The ethanol extracts of clove (*Eugenia caryophyllus* Bullock & Harrison) and sweet flag (*Acorus calamus* Linn.) were investigated for their antifungal activity in comparison with eugenol and amphotericin B (AmB) by using the National Committee for Clinical Laboratory Standards (NCCLs) M27-P broth microdilution method. Two medicinal plant extracts, eugenol and amphotericin B were used to determine their minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) against 28 clinical isolates of *Candida albicans* and 25 clinical isolates of *Cryptococcus neoformans*. The MICs of clove, sweet flag, eugenol and AmB against *C. albicans* were 17.41±8.64 mg/ml, 28.8±16.32 mg/ml, and 12.16±4.53 mg/ml and 0.23±0.1 µg/ml respectively. The MFCs were 67.5±15.39 mg/ml, >75 mg/ml, 15.4±6.47 mg/ml and 0.47±0.21 µg/ml respectively. The same extracts and antifungal drugs which were tested against C. albicans were also tested against C. neoformans. The MICs were 2.43±0.95 mg/ml, 3.02±1.97 mg/ml, 6.28±3.4 mg/ml and 0.28±0.15 µg/ml, respectively. The MFCs were 22.22±12.71 mg/ml, 30.82±27.11 mg/ml, 10.06±4.9 mg/ml and 0.51±0.25 µg/ml respectively. The results showed that *C. albicans* was significantly ($p<0.01$) more susceptible to the extract of clove than sweet flag, whereas *C. neoformans* was significantly susceptible to the clove extract ($p>0.05$). Moreover, the extract of clove showed significantly ($p<0.01$) more potent inhibitory activity against *C. neoformans* than eugenol, while it showed significantly ($p<0.01$) less inhibitory activity against *C. albicans* than eugenol. AmB, the drug of choice for invasive infection treatment, remains as one of the most effective antifungal drugs. These data indicate that the extracts of clove and sweet flag were potential fungistatic agents against yeasts, whereas AmB and eugenol showed fungicidal effects. [Thirach. S, 2001]

Berrin O, *et al.,* (2005) studied the antibacterial, antifungal, and antiviral properties of 15 lipohylic extracts obtained from different parts (leaf, branch, stem, kernel, shell skins, seeds) of *Pistacia vera* were screened against both standard and the isolated strains of *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Candida albicans* and

*C. parapsilosis* by microdilution method. *Herpes simplex* (DNA) and *Parainfluenza* viruses (RNA) were used for the determination of antiviral activity of the *P. vera* extracts by using Vero cell line. The extracts showed little antibacterial activity between the ranges of 128–256 µg/ml concentrations whereas they had noticeable antifungal activity at the same concentrations.

## MATERIAL AND METHOD

Antifungal activity of plants extract was determined by the agar tube dilution method. (Atta, *et.al.,* 1999). Seven fungal strains were slected for antifungal strains. *Trichoderma viridis* (FCBP# 642) (T.viridis) *Aspergillus flavus* (FCBP# 647) (*A.flavusi*), *Fusarium laterifum* (FCBP# 624) (*F.laterifum*), *Aspergillus fumigatus* (FCBP# 474) (*A.fumigatus*) *Candida albicans* (FCBP# 478) (*C.albicans*) were obtained from the department of Mycology and Plant Patholgy, University of the Punjab, Quaid-e-Azam Campus, Lahore. Two identified fungal strains *Trichophyton mentogrophytes* and *Microsporum canis* were obtained from the Main Microbiology Laboratory of Mayo Hospital, Lahore.

Test samples of plant extracts were dissolved in sterile DMSO to serve as stock solution. Different concentrations were prepared from the stock solution of the plant crude extracts. Sabouraud dextrose was used to grow the fungal strains in test tube . Each tube was inoculated with a seven day old culture of fungi. All culture containing tubes were inoculated with 105(CFU)/ mL-1 fungal spore suspensions at optimum temperature of 28-30°C for growth for 7-10 days. Humidity (40% - 50%) was controlled by plane an open pan of water in the incubator. Pure solvents were used as control as negative control. Other wells were supplemented with reference compounds i.e. Ketoconazole, Econazole, Nystatin, Amphotericin, Clotrimazole and Miconazole as positive control. After the incubation for 7-10 days the test tubes with no visible growth of the microorganism was taken to represent the zone of inhibition of the test sample which was expressed in µg/ml. The test was carried out in triplicate and their means were recorded.

### Statistical Analysis

The results of zone of inhibition were analyzed by analysis of variance (ANOVA) with completely randomized block design. The significant difference between extracts and standard discs against seven fungal strains were analyzed by statistically taking the level of significance at 0.05.

## RESULT:

Antifungal activity of *Withania coagulans* and *Tamarix aphylla* extracts were checked by using serial dilution tube method [Atta et. al. (2001)] against seven different fungal strains i.e. *Trichoderma viridis, Aspergillus flavus, Fusarium laterifum, Aspergillus fumigatus, Trichophyton mentogrophytes, Microsporum canis* and *Candida albicans.* The zones of inhibitions were measured and statistical analysis was applied on the results of antifungal assay. Methanolic, pet ether and Dichloromethane extract of *Withania coagulans* and *Tamarix aphylla*

, at the concentration of 25µg/ml were used against each of the seven fungal strains. The fungal strains were checked against the following standards Ketoconazole, Econazole, Nystatin, Amphotericin, Clotrimazole and Miconazole as positive control. (**Table -1**)

The pet ether, methanolic and dichloromethane extract of *Withania coagulans* showed highest activity against all the tested fungal strains *Trichoderma viridis, Aspergillus flavus, Fusarium laterifum, Aspergillus fumigatus, Trichophyton mentogrophytes, Microsporum canis* and *Candida albicans.*Their caculated F-vaules were greater then the F-table value. (2.67, 3.98 and 2.99) (**Table -2**)

The methanolic extract of *Tamarix aphylla* showed only the best activity against the fungal strains (F-vaue-2.67) while rest of the other extracts showed insignificant results. (**Table 1 & 2**)

**Table -1: In vitro antifungal activity of different extracts of ethnomedicinal plants**

| **Fungal Strains** | **Zone of inhibition in mm** | | | | | |
|---|---|---|---|---|---|---|
| | **Methanol Extract** | | **Pet ether Extract** | | **Dichloromethane Extract** | |
| | *Tamarix aphylla* | *Withania coagulans* | *Tamarix aphylla* | *Withania coagulans* | *Tamarix aphylla* | *Withania coagulans* |
| *A. flavus* | 14 | 21 | 15 | 21 | 15 | 21 |
| *F. laterifum* | 17 | 21 | 18 | 20 | 18 | 22 |
| *A .fumigatus* | 18 | 29 | 17 | 21 | 17 | 22 |
| *C. albicans* | 17 | 22 | 23 | 25 | 23 | 23 |
| *T. mentogrophytes* | 19 | 22 | 22 | 24 | 22 | 20 |
| *Microsporum canis* | 21 | 20 | 17 | 24 | 17 | 23 |
| *T. viridis* | 22 | 30 | 18 | 21 | 18 | 21 |
| **Control** | | | | | | |
| Ketoconazole | 15 | 21 | 15 | 21 | 21 | 15 |
| Econazole | 21 | 20 | 21 | 20 | 20 | 21 |
| Nystatin | 24 | 17 | 24 | 17 | 17 | 24 |
| Amphotericin | 21 | 18 | 21 | 18 | 18 | 21 |
| Clotrimazole | 22 | 19 | 22 | 19 | 19 | 22 |
| Miconazole | 15 | 21 | 15 | 21 | 21 | 15 |

**Table-2 Ethno medicinal Plant extracts comparison with other antifungal drugs using ANOVA Critical value $F.05_{(6, 36)} = 2.38$**

| Plant Extracts | | Pet ether | Methanol | Dichloromethane |
|---|---|---|---|---|
| *Tamarix aphylla* | **F-value** | 1.97 | 2.67 | 1.67 |
| | **Conclusion** | Insignificant | Significant | Insignificant |
| *Withania coagulans* | **F-value** | 2.67 | 3.98 | 2.99 |
| | **Conclusion** | Significant | Significant | Significant |

## DISCUSSION

*Withana coagulans* and *Tamarix aphylla* are ethnomedicinal plants of Pakistan. These plants are widely used by the local practioners of the different areas of Pakistan. These plants are widely available in the different parts of country as a wild crop. Fungal strains taken under consideration are the major cause of fungal allergies and other infections. These plants have anti fungal potential. These plants are safe and have no lethality.

These plant extract now further taken for pharmacological activity.

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