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Antifungal activity of *Vitex agnus castus-in vitro* study

G. MOHANDASS*, K. SUGUNA DEVI[1] and AMALORPAVARAJ[2]

*Sathyabama University, Chennai, (India).
[1]Prince Dr K. Vasudevan College of Engineering and Technology, Chennai (India).
[2]St. Joseph's College (Autonomous), Trichy, Tamil Nadu (India).

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ABSTRACT

In the present study agnuside was isolated from *Vitex agnus castus* by bio-assay guided fractionation and subjected to antifungal assay. Agnuside showed potent activity against tested fungi namely *E. floccosum* (31.25 µg/ml), *T. simii* (62.5 µg/ml), *C. lunata* (250 µg/ml), *T. rubrum* (62.5µg/ml), *C. albicans* (125 µg/ml) and *Scopulariopsis sp* (500 µg/ml). This study report the potency of agnuside as antifungal agent developed from plant source.

Key words: Agnuside, antifungal, dermatophytes, MIC.

INTRODUCTION

Pathogenic fungi, dermatophytes have the ability to invade keratinized tissues of animals, humans and cause a disease, dermatophytosis, which is the commonest human contagious fungal disease (Esquenazi et al 2004; Sidat et al 2006). The antimicrobial properties of certain Indian medicinal plants were reported based on traditional use (Perumal Samy et al 1998, 1999), and a few attempts were made on inhibitory activity against certain pathogenic fungi. Due to the increasing development of drug resistance in human pathogens as well as negative effects of certain antimicrobial agents, there is a need to search for new antifungal agent without toxicity and side effect. In this study we focused on the *in vitro* screening of antifungal activity of *Vitex agnus castus* (agnuside) against dermatophytes and opportunistic pathogens.

MATERIAL AND METHODS

Plant material

Leaves of *Vitex agnus castus* were collected from botanical garden, University of Madras, Maduravoil (Chennai, Tamil Nadu, India) during June-July 2007. A specimen was deposited at department herbarium, Sathyabama University. Collected plant material was air-dried under shade at room temperature, ground with an electric grinder into fine powder and stored in airtight containers.

Extraction and isolation

Air-dried powdered of *V. agnus* (1.5 kg) was extracted with ethyl acetate (2 x 2l) for 48 h at room temperature (± 25°C). The ethyl acetate crude extract was filtered and evaporated under reduced pressure. The total concentrated (160 gm) was chromatographed on a silica gel column (Merck 70–230 mesh, 800 gm, 3.5 i.d.x60 cm) and successively eluted with stepwise gradient of hexane-ethyl acetate system (0%, 5%, 10%, 20%, 30%, 50%, 70% and 100%). Eleven fractions were collected and each fraction was spotted on a precoated Silica gel 60 F254, 0.25mm thick TLC plate (Merck) and eluted in hexane: ethyl acetate (4:6) and fractions with similar Rf values in TLC pattern were pooled together. Fraction-7 (7.3 g) showed signiûcant antibacterial (MIC) and DPPH free radical inhibition. For further separation bioactive substance was chromatographed on a silica gel column and eluted

with a stepwise gradient of Isoproponal alcohol–methanol (8:2) solvent system, and an active isolate of 3.5 g was obtained. This active isolate was subjected to spectral analysis. ^{1} H and ^{13} C NMR spectra were recorded with a JEOL 300MHz FT NMR spectrometer (H1) 75, MHz (13C) and chemical shifts were given in ppm. IR spectra were taken on a Perkin Elmer FT-IR spectrophotometer and mass spectra on a JEOL GC-MASS spectrometer.

Phytochemical analysis

The presence of phytochemicals alkaloids (Draggendorff's), flavonoids (Shibat'as reaction), saponins (Frothing test), tannins (5% ferric chloride), terpenoids (2,4-dinitro-phenyl hydrazine), glycosides (Fehling's solution), steroids (Liebermann's Burchard test) were evaluated according to the methods described by Edeoga et al. (2005).

Fungal strains

The following fungi were used for experiments: *Trichophyton rubrum* MTCC 296, *T. mentagrophytes* 66/01, *T. simii* 110/02, *Epidermophyton floccosum* 73/01, *Scopulariopsis* sp. 101/01 *Aspergillus niger* MTCC 1344, *Botyritis cinerea, Curvularia lunata* 46/01, *Magnaporthe grisea* and *Candida albicans* MTCC 227.

Preparation of fungal spore

The filamentous fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on Sabouraud Dextrose Broth (SDB) at 28°C for 48 h.

Antifungal assays

The antifungal activity was performed according to the standard reference method (NCCLS, 2002). The extracts were dissolved in 2% dimethyl sulfoxide (DMSO). The initial concentration of extract was 1mg/ml. The initial test concentration was serially diluted two-fold. Each well was inoculated with 5µl of suspension containing 10^4 spore/ml of fungi. The antifungal agent Fluconazole was included in the assays as positive controls; the plates were incubated for 24h up to 9 days at 27°C for dermatophytes strains. MIC was defined as the lowest extract concentration, showing no visible fungal growth after incubation time.

RESULTS

Isolation of active compound

One active compound was obtained by using bio-assay guided fractionation (Fig. 1). Structural determination of this compound was done using different spectral technique and confirmed as agnuside (Fig. 2). It had IR absorptions at 3429 (br, hydroxyl), 1700 (Carbonyl), 1637 (olefinic region) and 1274 (C-O) Cm-^{1}. ^{1}H NMR (CDCL3, 300 MHZ) spectrum showed the peaks at 6.88-7.88 ppm,

Table 1: Antifungal activity of isolated compounds (MIC, µg/ml)

Tested Fungi	Agnuside	Flu
Trichophyton mentagrophytes 66/01	-	<12.5
Epidermophyton floccosum 73/01	31.25	<12.5
T. simii 110/02	62.5	<12.5
Curvularia lunata 46/01	250	<12.5
Aspergillus niger MTCC 1344	-	<12.5
Botrytis cinerea	-	NT
T. rubrum MTCC 296	62.5	<12.5
Magnaporthe grisea	>500	NT
C. alicans	125	<12.5
Scopulariopsis sp. 101/01	500	<12.5

NT- Not test; Flu- Fluconazole (standard)

Fig. 1: TLC of Agnuside (Single spot)

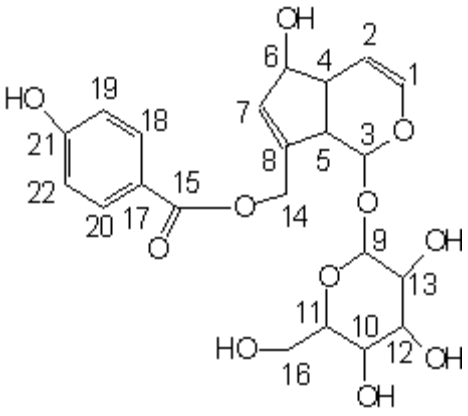

Fig. 2: Agnuside

which corresponds to the aromatic proton. The peak appears in the range in between 3.40 to 5.20 ppm assigned to carbon connected to oxygen atom. ^{13}C NMR spectrums showed the peaks at 115.85 to 162.52 ppm correspond to the aromatic carbon. The carbon connected oxygen atom appears in the range between 62.52 to 96.25 ppm. The carbonyl peaks are assigned to 165.23 ppm. The EI-MS (70 eV) of the compound showed molecular ion peaks at 464 (M) +.

Phytochemical analysis

Phytochemical analysis revealed the presence of triterpenoids, flavonoids and glycosides

Antifungal assay

Agnuside showed potent activity against tested fungi (Table 1) namely *E. floccosum* (31.25 µg/ml), *T. simii* (62.5 µg/ml), *C. lunata* (250 µg/ml), *T. rubrum* (62.5µg/ml), *C. albicans* (125 µg/ml) and *Scopulariopsis sp* (500 µg/ml).

DISCUSSION

Screening of antimicrobial activity provided the required preliminary observation to select crude plant extracts with potentially useful properties for further chemical and pharmaceutical investigation.

CONCLUSION

Results of the present work indicate that the plant species assayed possess antifungal properties. This explains the use of these plants in folk medicine for the treatment of various diseases whose symptoms might involve fungal infections, and underline the importance of the ethno botanical approach for the selection of plants in the discovery of new bioactive compounds. We found significant antifungal activity of agnuside of *Vitex agnus-castus* (leaves).

REFERENCES

1. Edeoga, H.O., Okwu, D.E., and Baebie, M., Phytochemical constituents of some Nigerian medicinal plants. *Afri.J. Biotechnol.* **4**: 685-688 (2005).
2. Esquenazi, D., Alviano, C.S., DeSouza, W., Rozental, S., The influence of surface carbohydrates during in vitro infection of mammalian cells by the dermatophyte Trichophyton rubrum. *Research in Microbiology,* **155**: 144-153 (2004).

3. National Committee for Clinical Laboratory Standards., Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A, Wayne, Pa **(**2002).
4. Perumal Samy, R., Ignacimuthu, S., and Sen, A., Screening of 34 Indian medicinal plants for antibacterial properties, *J. Ethnopharmacol.,* **62**: 173-181 (1998).
5. Perumalsamy, R., Ignacimuthu, S., Patric Raja, D., Preliminary screening of ethnomedicinal plants from India. *Journal of Ethnopharmacology*, **66**: 235-240 (1999).
6. Sidat, M.M., Correia, D., Buene, T.P., Tinea capitis among rural school children of the district of Magude in Maputo province, Mozambique. *Mycoses,* **49**: 480-483 (2006).