IJPSR (2016), Vol. 7, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 June, 2015; received in revised form, 01 September, 2015; accepted, 06 November, 2015; published 01 January, 2016

STUDIES ON EVALUATION OF *IN-VITRO* ANTFUNGAL ACTIVITIES OF *MELIA AZEDARACH* L. BARK

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Keywords:

Agar well diffusion, Poison food method, Antifungal activity, *Melia azedarach* L

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ABSTRACT: The present investigation was attempted in order to evaluate the antifungal potential of successively extracted n-hexane and methanolic extracts of *Melia azedarach* L. bark. The fungi-toxic efficacy of the extracts were tested against both yeast like fungi [*Candida albicans* (MTCC-3017), *Candida krusei*, *Candida tropicalis*, *Cryptococcus marinus* (MTCC-1029)] as well as mycellial like fungi such as *Aspergillus niger* (MTCC-9933) and *Rhizopus oryzae* employing well diffusion and poison food method respectively. The study revealed that n-hexane extract exhibited high inhibition against *Candida krusei*, whereas *Candida albicans* and *Candida tropicalis* showed no response and *Cryptococcus marinus* responded moderately. The methanolic extract showed high inhibition against *Candida krusei*, whereas *Candida albicans* and *Candida tropicalis* showed no response and moderate effect against *Cryptococcus marinus*. The n-hexane and methanolic extracts of this plant showed better zone of restriction against *Rhizopus oryzae* than *Aspergillus niger*. Fluconazole and clotrimazole was taken as reference antifungal. The result clearly indicated that bark of *Melia azedarach* L. contains phytochemicals which are responsible for inhibition of fungal growth and manifestation.

INTRODUCTION: Medicinal plants play an important role in medicine or veterinary practice for therapeutic and prophylactic purposes [1]. According to the traditional system of Ayurvedic treatment, medicines made up of plant products, either singly or in combination with others are considered to be less toxic and free from side effects as compared to the synthetic drugs [2, 3]. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(1).331-35

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (1).331-35

Many plants posses pharmacological properties because they consist of various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, triterpenes which should be utilized to combat the disease causing pathogens [4-6].

Plants have been exploited for treatment of human diseases by different ethnic groups in different parts of the world since the dawn of civilization. But the traditional cultures without proper scientific evidence are not able to ponder the importance of plant species for treatment of human diseases. Therefore, to ascertain the medicinal value of the phytochemicals, pharmacological studies have been carried out by different workers world over [7]. The phytochemicals from medicinal plants also serve as lead compounds in drug discovery and design [8].

Melia azedarach L. (Meliaceae) is a moderate sized deciduous tree (5-15 m in height) [9]. It is native to tropical Asia but widespread and naturalized in most of the tropics and subtropical countries [10, 11]. In India it is widely distributed in Himalayas region between the altitudes of 700 - 1000m [9]. *Melia azedarach* has many medicinal values which have been used for various medicinal purposes [12, 13]. Kernel extracts of this plant have already been studied and shows important anti-fungal activity [14-16]. Bark extract used for curing many diseases of skin conditions, such as eczema, ulcerative wounds, syphilitic ulcer, leprosy, scrofula etc. in the form of lotion, ointment or poultice. Systematically it is used as an emetic, cathartic, anthelminthic, antipyretic, expectorant, and diuretic [17-24]. *Melia azedarach* is being used in skin diseases, against intestinal disorders, stomach ache, intestinal worms, uterine illnesses, cystitis, as diuretic and febrifuge [25, 26]. This plant has also antimalarial, anthelminthic and cytotoxic activities [27]. Methanol, ethyl acetate and aqueous extracts of this plant showed significant inhibition against tested bacteria [28].

According to various scientific studies this plant is reported to have anticancer [29], antimalarial, analgesic and anti-inflammatory activity [30]. In the earlier studies, ethanolic leaf extract of *Melia azedarach* showed activity against fever, nausea, thirst and skin diseases [31-33]. Methanol, ethyl acetate and aqueous crude leaf extracts of this plant showed significant inhibition against some gram positive and gram negative bacteria [28]. Its n-hexane and methanolic bark extract showed good antibacterial and antioxidant potential [33].

In the present investigation, n-hexane and methanolic extracts of *Melia azedarach* L. bark have been evaluated for their antifungal activity.

MATERIALS AND METHODS:

Collection and identification of plant material:

The plant *Melia azedarach* was collected from the Chandaka reserve forest area near Bhubaneswar, Odisha in the month of April 2014. Identification of the voucher specimen was done by following the Flora of Orissa [34]. The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar. The bark were collected in bulk amount, washed in running tap water, dried under shade and made to coarse powder form.

Processing of plant material and preparation of extract:

The collected bark which was shade dried and ground to form a coarse powder and had been successively extracted with the solvent n-hexane and methanol by soxhlet apparatus [35] and the extract was recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.

In vitro antifungal activity:

i) Standard drugs used and preparation of doses for antifungal assay:

Fluconazole and Clotrimazole were used as Reference Antifungal (RA). The stock solutions of RA were prepared in 10 % dimethylsulphoxide (DMSO) to give a concentration of 1.56 mg / ml for RA respectively.

ii) *In vitro* antifungal assay:

The fungal strains were sub-cultured in Sabouraud's Dextrose Agar (HiMedia, Mumbai) medium. The fungal susceptibility test was determined by measurement of zone of inhibition (ZI) by agar well diffusion assay as described below against some yeast like fungal strains. However, the antifungal activity of the extracts against some mycelial fungi was determined by poison food method and expressed in terms of zone of restricted growth [36].

Agar well diffusion assay:

Agar well diffusion method [35] was followed to determine the zone of inhibition of microbes in Sabouraud's Dextrose Agar (SDA, HiMedia Laboratories Ltd., Mumbai). Plates were swabbed (sterile cotton swabs) with 8 hr old broth culture of fungi. Wells (8 mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. Stock solution of plant extracts were prepared at a concentration of 3 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 hrs. Control treatments comprising inoculums without plant extract were set up. The plates were incubated at 28 °C for 48

hrs for fungal pathogens. Triplicates were maintained and the diameter of the zone of inhibition (mm) was measured and statistical analysis was carried out.

Poison food method:

Poison food method [36] was followed to determine the zone of restriction of fungus. It is used for that fungus having high mycellial spreading. For those strains of fungus, the agar plates are prepared by mixing the Sabauraud's Dextrose Agar (SDA, HiMedia Laboratories Ltd., Mumbai) with the plant extract at the concentration of 3 mg/ml. Then a disc of the fungal mycelium was taken out using the sterile cork borer and placed by inverting the disc so that the portion of the fungal disc touches the SDA medium and kept in the centre of the plate. These plates were kept in the room temperature (28 °C) for 2-3 days for measuring the zone of restriction.

RESULT AND DISCUSSION: The bark extract of this plant was subjected to antifungal screening against some yeast like as well as mycelial like fungi. The results of antifungal activities were expressed in terms of zone of inhibition and zone of restriction respectively. The result indicated that n-hexane and methanolic extracts of *Melia azedarach* exhibited highest zone of inhibition against *C. krusei* (24.16 ± 0.62 mm) and (21.03 ± 0.25 mm) respectively. The extracts showed least activity against *Cryptococcus marinus* (n-Hexane: 17 ± 0.88 mm) and (Methanolic: 19.93 ± 0.30 mm) respectively while did not respond to *C. albicans* and *C. tropicalis*. The n-hexane and methanolic extracts of *Melia azedarach* also showed highest zone of restriction against *R. oryzae* (n-Hexane: 3.76 ± 0.58 mm) and (Methanolic: 4.1 ± 0.26 mm) respectively, least against *Aspergillus niger* i.e. (n-Hexane: 4.06 ± 0.30 mm) and (Methanolic: 10.47 ± 0.37 mm*)* respectively. (**Table 1, Fig. 1)**

TABLE 1: *IN-VITRO* ANTIFUNGAL ACTIVITY (ZONE OF INHIBITION OR RESTRICTION IN MM) OF VARIOUS PLANT EXTRACTS OF *MELIA AZEDARACH* L.

	Zone of Inhibition (in mm)		
Test organism (Yeast like fungi)	**n-hexane extract** (3 mg/ml)	**Methanol extract** (3 mg/ml)	**Reference Antifungal** (RA) **Fluconazole** (1.56 mg/ml)
Candida krusei	24.16 ± 0.62	21.03 ± 0.25	13.7 ± 1.32
Cryptococcus marinus	17 ± 0.88	19.93 ± 0.30	14.7 ± 0.72
Candida albicans	--	--	21.6 ± 0.5
Candida tropicalis	--	--	19.2 ± 0.2
	Zone of Restriction (in mm)		
Test organism (Mycelial fungi)	**n-hexane extract** (3 mg/ml)	**Methanol extract** (3 mg/ml)	**Reference Antifungal** (RA) **Clotrimazole** (1.56 mg/ml)
Rhizopus oryzae	3.76 ± 0.58	4.1 ± 0.26	17.6 ± 0.8
Aspergillus niger	4.060.30	10.47 ± 0.37	15.4± 0.45

Results expressed as mean ± S.D. of three determinations, (-) denotes no zone of inhibition

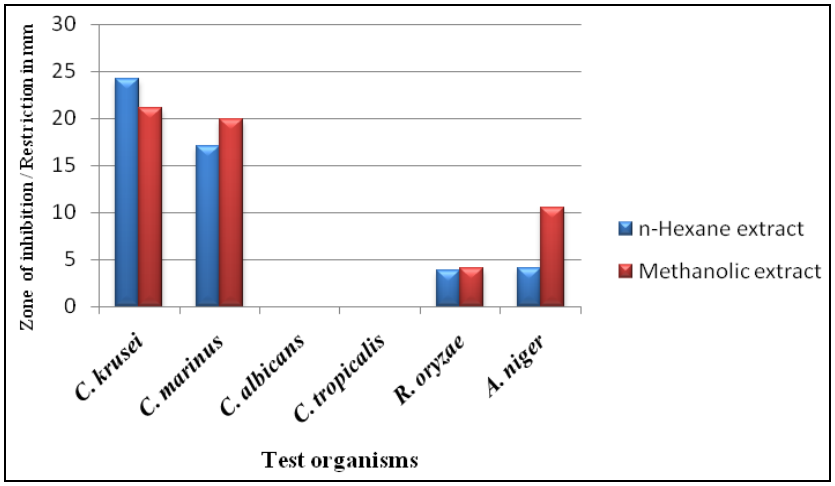

FIG. 1: *IN - VITRO* ANTIFUNGAL ACTIVITY (ZONE OF INHIBITION OR RESTRICTION IN MM) OF VARIOUS PLANT EXTRACTS OF *MELIA AZEDARACH* L.

CONCLUSION: It was found that *Melia azedarach* L. has toxic effect against fungal strains. The n-hexane extracts of this plant has higher antifungal activity against yeast like and mycelial fungi than methanolic extract. The fungitoxic potential of the extract of *Melia azedarach* against pathogenic fungi under study indicated that further studies are required for the identification of the bioactive molecules for drug development.

ACKNOWLEDGEMENT: The authors are thankful to the Head, Post Graduate Department of Botany, Utkal University, Bhubaneswar, Odisha for providing necessary laboratory facilities to conduct the study. The financial assistance received from the University Grants Commission, Govt. of India, New Delhi in the form of Maulana Azad Fellowship to the first author is deeply acknowledged.

REFERENCES:

1. Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, French M, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA and Inge KE: Health benefits of herbs and species: the past, the present, the future. Medical Journal of Australia 2006; 185 (4 Suppl): S4 - S24.
2. Latha U, Rajesh MG and Latha MS: Hepatoprotective activity of an Ayurvedic Medicine. Indian Drugs 1999; 36:470.
3. Agarwal M, Lincy MP and Mohan VR: Hepato protective and Antioxidant effect of *Polygala rosmarinifolia* Wight & Arn against CCl_4 induced hepatotoxicity in rats. Journal of Pharmacognosy and Phytochemistry 2013; 2 (1): 118-124.
4. Kamali HH EL and Amir MYEL: Antibacterial Activity and Phytochemical Screening of Ethanolic Extracts Obtained from selected Sudanese Medicinal plants. Current Research Journal of Biological Science 2010; 2 (2): 143-146.
5. Lalitha P, Arathi KA, Shubasmini K, Sripathi, Hemalatha S and Jayanthi P: Antimicrobial Activity and Phytochemical Screening of an Ornamental Foliage plant, *Pothosaurea* (Linder ex Andre). An International Journal of Chemistry 2010; 1 (2): 63-71.
6. Hussain H, Badawy A, Elshazly A, Elsayed A, Krohn K and Riaz M: Schulz B Chemical Constituents and Antimicribial Activity of Salix subserrate. Records of Natural Products 2011; 5 (2): 133-137.
7. Prusti A, Mishra SR, Sahoo S, Mishra SK and Prusti A: Antimicrobial Activity of Some Indian Medicinal Plants. Ethnobotanical Leaflets 2008; 12:227 – 230.
8. Ebi GC and Ofoefule SI: Antimicrobial Activity of *Pterocarpus osun* stems. Fitoterapia 2000; 71: 433 – 435.
9. Evans WC: Trease and Evans Pharmocognosy. ELBS publications New Delhi, Fifteenth Edition 2002.
10. Asolker LV, Kakkar KK and Chakra OJ: Second supplement to glossary of Indian medicinal plants with active principles. Publication And Information Division (CSIR), New Delhi 1992; part 1 (A-K).
11. Ramya S, Jepachanderamohan PJ, Alaguchamy N, Kalayanasundaram M and Jayakumararaj R: *in vitro* Antibacterial Prospective of Crude Leaf Extracts of *Melia azedarach* Linn. Against Selected Bacteial Strains. Ethnobotanical Leaflets 2009; 13: 254-58.
12. Harbourn JB: Phytochemicals methods – a guide to modern techniques of plant analysis. Harsted Press New York, Reprint 1976.
13. Kumudhavalli MV, Jayakar B, Chandira RM, Kumar M and Saravanan C: Phytochemical and Pharmacological studies on leaves of *Hiptage bengalensis* (L) Kurzz. International Journal of Pharm Tech Research 2010; 2(1): 1017-1020.
14. Carpinella MC, Herrero GW, Alonso RA and Palacios SM: Antifungal activity of *Melia azedarach* fruit extract. Fitoteropia 1999; 70: 269 -291.
15. Carpinella MC, Giroda LM and Palacious SM: Antifungal effects of different organic extracts from Melia azedarach L. on phytopathogenic fungi and their isolated active components. Journal of Agricultural and food Chemistry 2003; 51: 2506-2511.
16. Mohammed Ashif KK, Suresh A and Hashim KM: *in vitro* Pharmacological studies and evaluation of phytochemical constituents from the bark of *Melia azedarach*. Asian Journal of Plant Science and Research 2014; 4(4): 25-30.
17. Watt and Brandwijk B: The Medicinal and Poisoness Plants of Southern and Eastern Africa E.S. 1962 Edition.
18. Lewu FB and Afolayan AJ: Ethnomedicine in South Africa: The role of weedy species. African Journal of Biotechnology 2009; 8(6): 929-934.
19. Dymock W. Hamdard Pharmacographics, India.The institute of Health and Tibbi research, Pakisthan, Vol.I, 1890: 542 -544.
20. Watt G. Dictionary of economic product of India. Office of the superintended government of India, Calcutta 1893.
21. Chopra IC: Indigenous drugs of India. U. N. Dhar and sons (Private) ltd, Calcutta 1958: 363 – 364.
22. Mallick C, Chatterjee K, GuhaBiswas M and Ghosh D: Antihyperglycemic Effects of Separate and Composite Extract of Root of *Musa Paradisiaca* and Leaf of *Coccinia Indica* in Streptozotocin-Induced Diabetic Male Albino Rat. African Journal of Complementary and Alternative medicines 2007; 4(3): 362-371.
23. Baquar SR: Medicinal and Poisoness planys of Pakistan. Printhas Karachi, Pakistan, Edition 1, 1989: 118-121.
24. Khan T, Khan IA and Rehman A: A composite review of Berberies in service of humanity-in-trouble: an ethno pharmacological and pharmacognosic perspective. Journal of Biodiversity and Environmental Science 2015; 6(5): 21-33.
25. Nathan SS, Chung PG and Murugan K: Effect of Bio pesticides applied separately or together on nutritional indices of the rice leaf folder *Cnaphalorocis medinalis (Lepidoptera : pyralidae).* Phyparasitica 2005; 33(2): 187.
26. Alche LE, Barquero AA, San Juan NA and Coto CE: An antiviral principle present in purified fraction from *Melia azedarach* leaves aqueous extracts resistant herpes simples virus type 1 propagation. Phytother Res 2002; 16 – 348.
27. Khan MR, Kihara M and Omoloso AD: Antimicrobial activity of Horsfieldia helwigi and *Melia azedarach*. Fitoterapia 2001; 72- 423.
28. Ramya S, Jepachanderamohan PJ, Alaguchamy N, Kalayanasundaram M and Jayakumararaj R : In vitro Antibacterial Prospective of Crude Leaf Extracts of *Melia azedarach* L. against Selected Bacterial Strains. Ethnobotanical Leaflets 2009; 13: 254-58.

29. Ntalli NG, Cottiglia F, Bueno CA, Alche LE, Leonti M, Vargiu S, Bilfulco SUM, Caboni P : Cytotoxic Tirucallane Tritepenoid from *Melia azedarach* Fruits, Molecules 2010; 15: 5866-5877.
30. Vishnukanta RAC. Evaluation of Hydroalcoholic Extracts of *Melia azedarach* L. Roots for Analgestic and Anti-inflammatory Activity. International Journal of Phytomedicine 2010; 2: 341-344.
31. Sultan S, Akhtar N, Ashif HA: Phytochemical screening and antipyretic effects of hydro methanol extract of *Melia azedarach* leaves in rabbits, Bangladesh Journal of Pharmacology 2013; 8: 214-217.
32. Chatterjee A and Pakrashi: Treatise on Indian Medicinal Plants. Publications and information Directorate, New Delhi, 1994: 80-82.
33. Khatoon A, Jethi S, Nayak SK, Sahoo S, Mohapatra A and Satapathy KB: Evaluation of *in vitro* Antibacterial and Antioxidant Activities of *Melia azedarach* L. Bark. IOSR Journal of Pharmacy and Biological Sciences 2014; 9(6) : 14-17.
34. Saxena, H.O. and Brahmam, M: The Flora of Orissa. Orissa Forest Development Corporation, Bhubaneswar, 1996: 1-4.
35. Abdul vigar K, Khan AA and Shukla I: *in vitro* antibacterial potential of *Melia azedarach* crude leaf extracts against some human pathogenic bacterial strains. Ethnobotanical leaflets 2008; 12: 439-445.
36. Sahoo S, Panda PK, Tripathy, S, Dash, SK and Nayak SK: Antifungal activity of *hybanthus enneaspermum* on selected Human Pathogenic Fungi. Indian Drugs 2007; 44(5): 352-357.

How to cite this article:
Khatoon A, Mohapatra A and Satapathy KB: Studies on Evaluation of *In-Vitro* Antfungal Activities of *Melia Azedarach* L. Bark. Int J Pharm Sci Res 2016; 7(1): 331-35.doi: 10.13040/IJPSR.0975-8232.7(1).331-35.