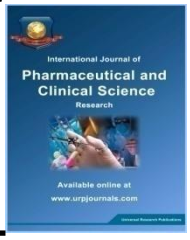
Available online at http://www.urpjournals.com

International Journal of Pharmaceutical and Clinical Science



ISSN 2277– 7202

Original Article

Antifungal Activity of *Areca catechu* L

Reena R Nelson Anthikat [a], A.Michael [a], V.A. Kinsalin [b] and S Ignacimuthu [b]*

a- Department of Microbiology, PSG College of Arts and Science,
Coimbatore 641014, Tamil Nadu.

b*- Entomology Research Institute, Loyola College, Chennai 600034, Tamil Nadu.

*Corresponding author; Dr. S. Ignacimuthu, Entomology Research Institute, Loyola College, Nungambakkam, Chennai – 600 034, Tamil Nadu, India. Email: eriloyola@hotmail.com, Tel: 044 2817 8348 Fax: 044 2817 5566.

Received 11 August 2014; accepted 25 August 2014

Abstract

The purpose of this investigation was to study the antifungal activity of hot water extract of *Areca Catechu* nuts collected from coastal region of Kerala. The fungi (*Mucor sp, Aspergillus niger, Cladosporium sp* and *Candida albicans*) were inoculated on Sabouraud's Dextrose Broth (100ml) and kept at room temperature for overnight incubation. 0.1 ml of this O/N culture was incubated along with different concentrations of the extract (10-1000µg/ml) for eight hours, in 3 ml of nutrient broth. The absorbance was read at 530 nm at different intervals. The experiment was done in triplicate. The Arecanut extract did not inhibit the growth of mycelial fungal forms such as *Mucor sp, Aspergillus niger* and *Cladosporium sp* but the growth of unicellular fungus *Candida albicans* was inhibited. The concentration needed for 100% inhibition was 16.67 µg/ml concentration. Disc diffusion method was used to evaluate the zone of inhibition against fungi by taking Nystatin disc as standard. The largest zone of inhibition was observed against *Candida albicans*. The most effective concentration needed for 85% inhibition of the production of aflatoxin by *Aspergillus flavus* was between 100-250 µg/ml.

KEYWORDS:-*Areca catechu*; Sabouraud's Dextrose Broth (SDB); Antifungal activity; *Candida albicans*; Aflatoxin

1. Introduction

Arecanut, which is also known as betelnut, is the seed of arecanut palm, which is an ornamental plant. Arecanut chewing is practiced in India as well as in many Asian Countries from time immemorial. In traditional practice it is used for cleansing the oral cavity [1]. A common practice among Asian elders is the chewing of betel quid commonly known as "*nganga*", a combination of betel nut, betel leaves with lime or tobacco. Chewing betel quid is considered a past time and is a cultural tradition in different countries like India, Indonesia, and Arabian Peninsula, some of the Pacific islands such as Micronesia, Fiji, Solomon Islands and the Philippines [1]. *Areca catechu* L. is a straight solitary tree that has annular leaf scars. The leaves have leaflets that measure up to 4 cm. The ovoid fruit turns red or orange when ripe and has a fleshy pericarp and a fibrous mesocarp Seeds are called as nuts; they are used for mastication [2]. The husks are used as an alternative to toothbrush and it is also used as a vermifuge in some parts of China [3]. However this property of betel nut chewing has not been scientifically proven. The antimicrobial effect of ethanol extract of *Areca catechu* L. seeds against mixed-oral flora and certain-gram negative clinical isolates were tested using agar-well diffusion method by [4]. Their work demonstrated positive antimicrobial properties of *Areca catechu* L. nut against *Candida albicans* but not against Gram-negative bacetria. Arecanut is reported to have antiinflammatory and antiproliferative properties. These activities are due to the presence of alkaloids in Arecanut. Four major alkaloids have been found in *Areca catechu*; they are arecoline, arecaidine, guvacoline, and guvacine. Arecoline is an oily-liquid that is soluble in water, alcohols and ether; this lipophilic nature can have an entry to the brain and intracellular spaces to give stimulatory effects. We carried out this work with a view to assess the antifungal activity of *Areca catechu* nuts. Since not much work has been found in the literature.

2. Materials and Methods:

2.1 Chemicals and media

Dimethyl sulphoxide (DMSO), Mueller Hinton Agar (MHA), Sabouraud dextrose agar (SDA), the antifungal agents Nystatin, Amphotericin B and Aflatoxin were obtained from Himedia, Mumbai, India. Methanol was obtained from Rankem Company, India.

Table 1 Effect of *Arecanut* extract on *Candida albicans*

Concentration + strain	Percent Inhibition					
	1h	2h	3h	4h	5h	6h
10 µg/ml A.E + *C albicans*	0	0	0	0	0	0
20 µg/ml A.E + *C albicans*	0	0	0	0	0	0
50 µg/ml A.E + *C albicans*	100	100	100	100	100	100
100 µg/ml A.E + *C albicans*	100	100	100	100	100	100

Note: Concentration is expressed per 3 ml of medium; there was no inhibition against *Mucor sp, Aspergillus niger, Cladosporium sp.*

Table 2 Antifungal Activity of *Arecanut* extract

Organism	Diameter of growth inhibition zone (mm)		
	Arecanut extract	Standard Amphotericin B	Nystatin
Mucor sp.	12	16	-
Aspergillus niger	14	20	-
Cladosporium sp.	13	16	18
Candida albicans	18	16	20

All values are an average of three determinations. The concentrations of extracts employed were a 1000 µg/ml. Diameter of cups was 6 mm.

Table 3 Inhibition of Aflatoxin production by Arecanut

Concentration of Arecanut/ml	Concentration of Aflatoxin (ppb)	Percent Inhibition
Control	35 ppb	-
10-50 µg/ml	20 ppb	42.85%
100-250 µg/ml	5 ppb	85.71%

2.2 Extraction of *Areca catechu* L

Arecanuts were procured from the coastal areas of Kerala. Hot water extract of the arecanut were prepared by boiling 100g nuts in 500 ml of distilled water for one hour. The extract was then concentrated by evaporation. The yield of the extract was found to be 6.4%. The extract was then resuspended in distilled water and diluted to the desired concentration. The extract was stored at 4˚C until further use.

2.3 Determination of antifungal activity of the extract:

2.3.1 Preparation of Inoculum

The filamentous fungi were grown in Sabouraud's 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's dextrose broth (SDB) at 28˚C for 48 h.

2.3.2 Effect of *Arecanut* extract on *Candida albicans* by Tube method

A loopful of fungi were inoculated in Sabouraud's Dextrose Broth (100 ml) and kept at room temperature for overnight incubation. 0.1 ml of this O/N culture was incubated in the various concentrations of the extract (10-1000µg/ml) for eight hours, in 3 ml of nutrient broth. The absorbance was read at 530 nm at various time intervals. The experiment was done in triplicate.

2.3.3 Antifungal activity of *Arecanut* extract by disc diffusion method

The antifungal activity was screened by disc diffusion method [5]. The Sabouraud's dextrose agar plates were inoculated with the fungal culture (72hr culture). The following fungal pathogens were used for the experiments: *Mucor sp*, *Aspergillus niger*, *Cladosporium sp* and *Candida albicans*. Sabouraud Dextrose Broth cultures were prepared in test tubes. Using sterile cotton swab, the surface of Sabourauds Dextrose Agar plates were swabbed to prepare lawn cultures. 5 min after the agar surface had dried, wells were dug using cork borer aseptically. Wells were saturated with arecanut extract, concentrations ranging from 100µg/ml- 1000 µg/ml. The plates were incubated at 28ºC for 24-48 hrs. The diameters of zones of inhibition were measured using a scale to the nearest millimeter [6].

2.3.4 Determination of the effect of Arecanut extract on Aflatoxin production by *Aspergillus flavus*.

A loopful of *A. fl*avus culture, which was originally grown on Sabouraud's Dextrose Agar, was uniformly suspended in 5 ml of glucose ammonium nitrate medium containing the mineral supplements. It was incubated at 37 ºC for six days. 100 µl of this suspension was inoculated in test tubes containing 2 ml of glucose ammonium nitrate medium with different concentrations of arecanut extract, ranging from 10-250 µg/ml. All the tubes were kept in triplicates and incubated at 30ºC for six days. Afaltoxin was extracted using Modified Pons' method [7]. The toxin concentrations were estimated in correlation with the intensity of fluorescence of standard aflatoxin on TLC plates [8].

3. Results and Discussion

The antifungal activity of *Arecanut* hot water extract was studied. [9] Used the tube method to study the growth

characteristics of fungi by inoculating the culture in different Medias at different temperature, pH and NACL concentration. In another study, [10] reported the inhibition of *C. albicans* by germ tube assays in different medicinal plants namely such as *Piper bredemeyeri* Jacq and *Lippia origanoides* oils. In the present study the tube method was used to see the effect of Arecanut extract against fungi by inoculating the cultures in nutrient broth and incubating at various concentrations for eight hours. There was 100% inhibition of growth at 50 and 100µg/ml from the first itself for Candida albicans. The growth of other fungi were not inhibited (Table 1). In the present study the plate method was employed for *Mucor sp*, *Aspergillus niger*, C*ladosporium sp* and *Candida albicans* to assess the zones of inhibition. Arecanut extract showed minimum zone of inhibition against *Mucor sp* (12mm), *Aspergillus niger* (14mm) and *Cladosporium sp* (13mm) but it showed good zone of inhibition against *Candida albicans* (18mm) at 16.67 µg/ml concentration (Table 2). In another study, [11] evaluated the antimicrobial activity of Arecanut seed extract against *S.mutans* and found 100% inhibition at 100µg/ml. [4] demonstrated the inhibitory effects of *Areca catechu* L against *Candida albicans* with zones of inhibition measuring 6 mm in diameter in MHA agar using 50 µg/ml concentration. [12] Reported that *Areca catechu* root ethanol extract showed antifungal activity against *Aspergillus niger* with zone of inhibition of 28 mm at 500 µg/ml. [13] reported that the acetone extract of *Aloe vera* leaves in concentration of 2000µg/ml significantly inhibited (100%) the growth of *A. flavus* and at the lowest concentration (2µL), the growth inhibition was 51.72%. In the present study the inhibition of aflatoxin by arecanut extract was 85.71% at the concentration of 100-250µg/ml (Table 3). [14] Arecanuts had collected from different regions of South Asia. Among them Indonesian and Sri Lankan samples inhibited Aflatoxin production in the range between 3.3–39.2 and 6.5–103.4 µg kg−1 respectively. This study gives scientific evidence for the antifungal property of Arecanut extract.

4. Conclusion

Areca Catechu nuts possess antifungal activity. The present investigation indicated that the hot water extract of *Areca catechu* exhibited antifungal activity against *C. albicans*, *Mucor sp*, *Aspergillus niger*, *Cladosporium sp*. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity.

References

1. G.W. Staples and R.F. Bevaqcua. *Areca catechu* (betel nut palm): www.traditionaltree.org; (2006). Pg: 1-17.
2. D.J. Rummel. Useful Plants of the Philippines: Vol. 1.Quezon City, Philippines. C and E Publishing Inc; (2005). ISBN 9789715844178.
3. W.M. Zhang, J. Wei, W.X. Chen, H.D. Zhang. The Chemical Composition and Phenolic Antioxidants of Areca (*Areca catechu L.*) Seeds: http://www.ier-institute.org; 2160-0589/abe1/v1- 2/016.pdf; (2011).
4. M.B Cyriac, V. Pai, I. Varghese, M. Shantaram, M. Jose. Antimicrobial Properties of *Areca catechu* (*Areca nut*) Husk Extracts against Common Oral Pathogens: International Journal of Research in Ayurveda and Pharmacy (2012); 3(1): 81-84.
5. NCCLS: Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A. Wayne, Pa: National committee for clinical laboratory standards (2002).
6. L.M. Prescott, J.P. Harley, D.A. Klein. Antimicrobial Chemotherapy, Microbiology: 2 nd Edn., (1993),328-329.
7. W.A. Pons, A.F. Cuculia, L.S. Lee, J.A. Robertson, S.O. Franz and L.A. Goldblatt. Determination of aflatoxin in products; Use of aqueous acetone for extraction. J Assoc.Agri.Chem, (1966),67, 554.
8. S.L. Hung, Y.W. Lin, Y.H. Wang, Y.T. Chen, C.Y. Su, L.J. Ling. Permeability of *Streptococcus mutans* and *Actinobacillus* actinomycetemcomitans through guided tissue regeneration membranes and their effects on attatchment of periodontal ligament cells: J Periodontology, (2002). 73(8): 843-851.
9. Apoorva Pahadia, Rakhi Gawde, Shikha Agrawal. Antimicrobial activity of hydro alcoholic extract of *Areca catechu*: International Journal of Pharmaceutical Erudition. www.pharmaerudition.org May (2013), 3(1), 18-25. ISSN: 2249-3875.
10. Veronica Tangarife Castanol, Julieth Correa Royero, Bibiana Zapata Londono, Camilo Duran, Elena Stanshenko, Ana Cecilia Mesa Arango. *Anti-Candida albicans* activity, cytotoxicity and interaction with antifungal drugs of essential oils and extracts from aromatic and medicinal plants: Asociacion Colombiana de Infectologia Infectio. (2011); 15(3): 160-167.
11. A. Reena, A. Michael. Study on *areca nut* for its antimicrobial properties: J young Pharmac (2009); 1 Suppl 1:42-46.
12. Alby Alphons Baby and K. Regi Raphael. Potential Antimicrobial, Anthelmintic and Antioxidant properties of *Areca catechu L.* Root: International Journal of Pharmacy and Pharmaceutical Sciences. Innovare Academic Sciences. Int J Pharm Pharm Sci., Vol 6, Issue 6, 486-489. (2014) ISSN- 0975-1491.
13. A. Babaei1, M. Manafi and H. Tavafi. Study on Effect of *Aloe vera* Leaf Extracts on Growth of Aspergillus flavus: Annual Review & Research in Biology 3(4): (2013) Science Domain international www. sciencedomain.org. 1091-1097.
14. Muhammad Asif Asghara, Javed Iqbala, Aftab Ahmeda, Mobeen Ahmed Khana and Zuzzer Ali Shamsuddina. Aflatoxin B1 in betel nuts (Areca catechu L.) imported to Pakistan from different regions of South Asia. Food Additives & Contaminants: Part B: Surveillance. Volume 7, Issue 3, (2014), pages 176-181.

Source of support: Nil; Conflict of interest: None declared