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Research Article

Antimicrobial activity of hydro alcoholic extract of Areca catechu

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The purpose of this investigation was to prepare hydro alcoholic extract of Areca Catechu which was screened for antimicrobial activity against bacteria (Escherichia Coli and Staphylococcus aureus) and fungi (Candida albicans and Bacillus subtilis) at 200mg/ml & 100mg/ml concentration. Disc diffusion method is used to evaluate zone of inhibition against bacteria and fungi by taking amoxicillin disc as standard. The largest zone of inhibition is (against hydro alcoholic extract of Candida albicans) observed at 200mg/ml concentration. The hydro alcoholic extract of Areca catechu used for treatment of human skin infected with fungus and bacteria and it improves the drawbacks of the available extract in the market. This extract is based upon the plant products having synergistic effect hence enhancing the antifungal and antibacterial activity with negligible side effects or toxicity.

Keywords: Hydro alcoholic extract, Areca catechu, antimicrobial activity, Disc Diffusion Method, Amoxicillin disc, zone of inhibition

INTRODUCTION

In the last few decades there has been an exponential growth in the field of herbal medicines, owing to their natural origin and lesser side effects. The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage. Today, the world over, there is a great deal of interest in the Ayurvedic System of Medicine and thus the demand for various commonly used medicinal plants in the production of Ayurvedic medicines is ever increasing. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards

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maintaining human health. The use of herbal medicine due to toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufactures.

Herbal medicines are currently in demand and their popularity is increasing day by day. In the healthcare sector WHO recommends and encourages the use of traditional herbs/remedies because huge amount of raw material is easily available. They are comparatively safe because of their low toxicities. However, plants are very complex in their composition and their therapeutic activity depends on their chemical constituents, these according to age, geographical location and harvesting processes.. At present it is very difficult to identify the presences of all the ingredients as claimed in a formulation. Hence the first important task is to envolve such parameter by which the presence of the entire ingredient can be identified, various chromatographic and spectrophotometer methods Wherever possible these methods can be applied for quantitative estimation of bioactive group of compounds like alkaloids, flavonoids, polyphenolic components or estimation of particular compound. Natural products from plant, animal and minerals have been the basis of the treatment of human disease.

Areca catechu (supari) Areca catechu trees can be found growing in parts of Arabia, China, East Africa, Egypt, India, Indochina, Indonesia, Madagascar, Malaysia, Maldive Islands,Pakistan, Sri Lanka, and Taiwan. Areca nut is aromatic and astringent and is said to intoxicate when first taken. The natives chew these nuts all day. Areca nut is made into a dentrifrice on account of its astringent properties. Its flowers are very sweet-scented and in Borneo are used in medicines as charms for the healing of the sick. The action of Arecaine resembles that of Muscarine and Pilocarpine externally, internally used it contracts the pupils.

MATERIALS AND METHODS:

Test organisms and Inoculums

Escherichia coli (NCTC-6571) and Staphylococcus aureus (NCTC-10418)Candida albicans (NCIM NO.3557) and Bacillus subtilis (NCIM NO.513) were obtained from the Department of Microbiology, Swami Vivekanand College of Pharmacy, (Indore).

Standard solution

Amoxicillin disc of the concentration of 30 µg/ disc were obtained from the Department of Microbiology, Swami Vivekanand College of Pharmacy, (Indore).

Test solution

The test solutions of the extracts were prepared at a concentration of 100 mg/ml, 200 mg/ml.

Media

a) Dehydrated Nutrient Agar Media was used and was prepared in distilled deionized water. The composition and preparation of the media was according to Indian pharmacopoeia.

b) Media MGYP (Malt extract, Glucose, Yeast extract, Peptone) media: Composition and preparation of the media was according to Indian pharmacopoeia.

Sterilization of both media

The conical flask containing the nutrient agar medium was plugged with the help of a non- absorbent cotton bung. The mouth of the conical flask and the cotton bung were properly covered with aluminum foil. The medium was then sterilized by autoclaving at 15 lbs per square inch pressure for 20 minutes.

Procedure for both activities:

In the present study, antimicrobial and antifungal screening was carried out by the disc diffusion method. The extract diffuses from the disc through a solidified agar layer in a petridish or plate to an extent, so that the growth of added microorganism is inhibited entirely in a circular area or zone around the cavity containing the solution of known quantity of hydro alcoholic extract. The activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader.

Methods of preparation of test organisms:

The test organisms were maintained on slants of medium and transferred to a fresh slant once a week. The slants were incubated at 37°C for 24 hours. Using 3 ml of saline solution, the organisms were washed from the agar slant on to a large agar surface (medium) and incubated for 24 hours at 37°C. The growth from the nutrient surface was washed using 50ml of distilled water. A dilution factor was determined gave 25% light transmission at 530 nm. The amount of suspension to be added to each 100 ml agar or nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

Temperature Control for both activities:

Thermostatic control is required in several stages of a microbial assay when culturing a microorganism and preparing its inoculums and during inoculation in a plate assay.

Disc diffusion method for checking activities:

Agar (20–25 mL) was poured into 90 mm sterile Petri dishes to give a depth of 3–4 mm. Before inoculation, the surface of the agar was dried to remove excess moisture. Organism suspensions or overnight broth cultures were initially adjusted with sterile distilled water to a density equivalent to

the 0.5 McFarland standards. A further dilution in sterile distilled water of 1:100 subtilis, enterococci for antibacterial and antifungal respectively or 1:10 Candida, staphylococci and pneumococci for antibacterial and antifungal respectively was made before the organism suspension was swabbed on to the surface of the agar plate. A disc containing 30µg of Amoxicillin was placed on the surface of the agar and the plates were incubated (stacking of plates more than six high in the incubator was avoided) using the atmospheric conditions detailed previously. After incubation, zone diameters (in millimeters) were measured using a ruler.

Observations and Evaluations

A. Morphological Characters

The morphological characters of resulting media after activity are as follows:

Table 1: Morphological Characteristics

External Colour	**Brown**
Size	1-2cm. in diameter
Shape	Conical
Surface	Rough
Odour	Odourless
Taste	Bitter
Fracture	Hard and brittle

B. Extractive Values

Extractive values were obtained using cold maceration process observations are as follows

Table 2: Extractive Values

S. No.	**Weight of drug (gm)**	**Weight of china dish (gm)**	**Weight of china dish + Wt. of extractable matter (gm)**	**Weight of extractable matter (gm)**	**% Extractable matter**
1.	10.003	55.031	56.107	1.076	10.756
2.	10.007	55.112	56.178	1.066	10.654
3.	10.005	55.097	56.140	1.043	10.424
Mean	**10.005**	**55.08**	**56.141**	**1.275**	**10.611**

Table 3: C.1 Determination of Total ash

S. No.	**Weight of drug (gm)**	**Weight of Crucible (gm)**	**Weight of crucible + Wt. of ash (gm)**	**Weight of ash (gm)**	**% Total ash**
1.	1.005	16.633	16.680	0.055	5.470
2.	1.008	16.567	16.634	0.067	6.646
3.	1.001	16.789	16.843	0.054	5.394
Mean	**1.004**	**16.663**	**16.719**	**0.058**	**5.836**

Table 4: C.2 Determination of Acid insoluble ash

S. No.	Weight of drug (gm)	Weight of Crucible (gm)	Weight of crucible + Wt. of ash (gm)	Weight of ash (gm)	% Total ash
1.	1.005	16.635	16.64	0.013	1.29
2.	1.008	16.776	16.79	0.014	1.38
3.	1.001	16.555	16.65	0.104	10.38
Mean	**1.004**	**16.655**	**16.69**	**0.043**	**4.35**

Table 5: C.3 Determination of Water soluble ash

S. No.	Weight of drug (gm)	Weight of crucible (gm)	Weight of crucible + Wt. of ash (gm)	Weight of ash (gm)	Total ash- Wt. of ash (gm)	%Total ash
1.	1.004	16.659	16.68	0.034	0.021	2.10
2.	1.001	16.789	16.89	0.034	0.033	3.29
3.	1.007	16.554	16.58	0.029	0.025	2.48
Mean	**1.004**	**16.667**	**16.71**	**0.032**	**0.026**	**2.62**

C. Phytochemical Screening

Phytochemical screening was done using test tube method change in color indicated presence of constituents in hydro alcoholic extracts.

Table 6: Phytochemical screening

Extract/Constituents	Hydroalcholic extract
Alkaloids	+
Carbohydrates	+
Anthraquinone Glycosides	+
Phenolic compounds &	+
Flavonoid	+
Proteins and amino-acids	+
Saponins	-
Acidic compounds	+
Mucilage	+
Resins	+
Lipids / Fats	+

(- Absent; + Present)

D. DETERMINATION OF pH

pH was measured by digital pH meter

Table 7: Determination of pH

S. No.	Sample	pH
1.	pH of 1% solution	5.86
2.	pH of 10% solution	5.07

Results and discussions:

The antibacterial activity of the aqueous-ethanolic extract of Areca catechu was studied by taking two different concentrations of 100 mg/ ml and 200mg/ ml against S.aureus. (Gm +ve) E.coli, (Gm –ve), B.subtilis & C.albicans. The standard used against this was Amoxicillin at the conc. of 30 µg/ ml.

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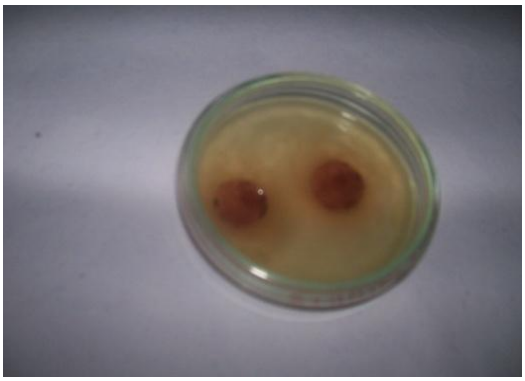
Table 8 : Antibacterial Activity

S. No.	Organism	200 mg/ml		100 mg/ml	
1.	S. aureus	17mm	20mm	15mm	15mm
2.	E. coli	18mm	20mm	---	---
3	B. subtilis	15mm	15mm	---	---
4	C. albicans	24mm	22mm	21mm	22mm

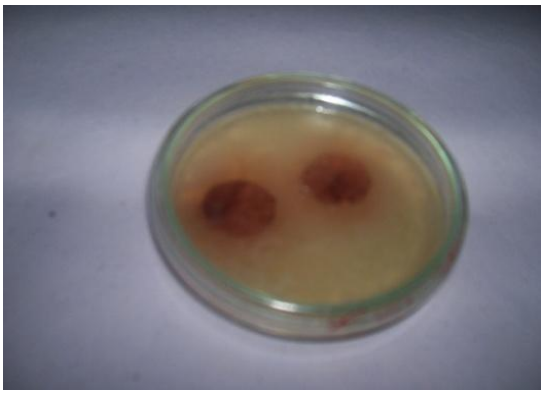
1 S. Aureus (200mg/ml)

Aqueous ethanolic extract at 200mg/ml is active against S. aureus while at 100mg/ml concentration it is moderately active.



2 E. Coli (200mg/ml)

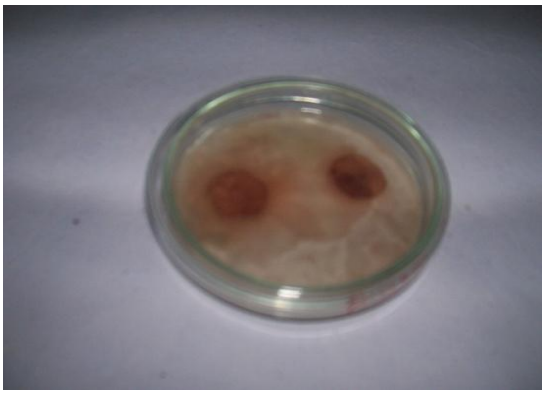
Similarly, aqueous ethanolic extract at 200mg/ml is active against E.coli while



at 100mg/ml concentration it is inactive i.e. as compared to the standard at 100 mg/ml concentration.

3 Candida Albicans (200mg/ml)

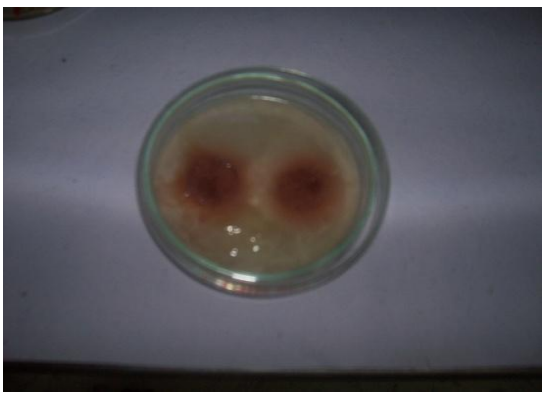
Aqueous ethanolic extract at 200mg/ml is very active against C. albicans while at



100mg/ml concentration it is moderately active.

4 Bacillus Subtilis (200mg/ml)

Aqueous ethanolic extract at 200mg/ml is moderately active against B. subtilis while at 100mg/ml it is totally inactive**.** Hence the hydroalcoholic extract of Areca catechu at 200mg/ml



concentration is mainly responsible against antifungal activity but not at 100mg /ml concentration.

Conclusion:

Areca Catechu is a herb possessing antimicrobial activity, in the present investigation indicated that the fruit extract of areca catechu exhibited antimicrobial activity against S. aureus, E.coli, C.albicans and B. subtilis. The findings of investigations suggested that Areca Catechu has potential against antimicrobial activity.

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