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Original Article

POTENTIAL ANTIMICROBIAL, ANTHELMINTIC AND ANTIOXIDANT PROPERTIES OF ARECA CATECHU L. ROOT

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ABSTRACT

Introduction: *Areca catechu L* (Arecaceae) root is commonly used in the traditional systems of medicine against various ailments like urinary tract disorders, skin irritations, worm disturbances, as a component in health tonic preparation etc.

Objectives: The present study gives first insight of anti-microbial, anthelmintic, anti-oxidant properties and preliminary phytochemical analysis of the root of *A.catechu.*

Methodology: All the analysis was done according to standard protocols.

Results: The ethanolic extract produced significant anti-bacterial, anti-fungal and anthelmintic properties in a dose-dependent manner. DPPH free radical scavenging assay exhibited IC 50 value of 65.7 ± 1.53 and super oxide anion scavenging assay showed 201.7 ± 0.76, IC 50 value. Preliminary phytochemical screening revealed the presence of alkaloids, steroids, flavonoids, terpenoids, cardiac glycosides, quinone, phlobatanins, tannins and phenols that may be the reason for its biological properties. Yield in different solvent fractions of the crude extract showed maximum bulk in the aqueous fraction, in which the alkaloids present.

Conclusion: This paper first reporting the medicinal property of *A. catechu* root and the further procedures of identification and isolation of active principles are in progress.

Keywords: Areca, Antimicrobial, Anthelmintic, Antioxidant, Phytochemistry.

INTRODUCTION

Areca catechu L, belongs to the family Arecaceae is commonly called as Betel nut, grows in much of the tropical pacific, Asia and parts of East Asia. It is one of the most commonly used drug in the world containing alkaloids, tannins, polyphenols, sugars and lipids that have anthelmintic, antibacterial, antifungal, anti-inflammatory and anti oxidant activities[1].The plant is reported to have multiple therapeutic properties like, masticatory, anthelmintic, aphrodisiac [2], antihypertensive [3; 4], wound healing [5], antimycobacterial [6], hypoglycemic [7; 8], antidepressant [9] and anti-HIV [10]. Its use has been reported to produce sense of well being, a hot sensation in the body, increased sweating, salivation[11], heightened awareness, prevention of hunger and an increased capacity to work [12;11]. In the previous studies, natural antioxidants from the nut and ripened pericarp were identified [13;14] and extensive studies were there in the nut and pericarp. *Areca catechu* is using in the traditional medicine of Kerala. Root is effective against various ailments like urinary tract disorders, skin irritations, worm disturbances and as a component in health tonic preparation . Young leaf sheath is using in the treatment of migraine [15]. To the best of our knowledge medicinal properties of *Areca* root is not yet reported. Therefore, the present study was planned to evaluate its biological properties including antimicrobial, anthelmintic and antioxidant properties, identification of the various secondary metabolites present in it and fractionation of the crude extract according to their polarity for determining the yield in different solvent fractions.

MATERIALS AND METHODS

Collection of plant material and preparation of extract

The fresh roots of *A.catechu* were collected in the month of January 2014 from Manna mangalam village of Thrissur District and shade dried for several days. The dried plant material was ground to a course powder and 50g of the powdered plant material was soaked in 95% ethanol (1:5) for 72 hours [16]. The solvent was then removed by rotary evaporation. The dried extract was stored in refrigerator for further studies.

Phytochemical Screening

The preliminary phytochemical analysis of the plant extracts was performed using standard protocol given by Harborne, then the crude extract was fractionated according to their polarity and yield in each fraction were determined [17].

Antimicrobial assay

a. Organisms and culture media: The pathogenic strains of bacteria and fungus were obtained from the laboratory, Department of Microbiology, St.Mary's College, Thrissur. Organisms used were *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger* and *Penicillium notatum.* The bacterial cultures were maintained on nutrient agar (NA), while fungal cultures on Sabouraud dextrose agar (SDA).

b. Antibacterial and antifungal activity of the plant extract: Well diffusion assay [18] on nutrient agar and Sabouraud dextrose agar plates were used to determine the antibacterial and antifungal properties respectively. Bacteria were inoculated into nutrient broth (NB), while fungus into Sabouraud dextrose broth (SDB) and incubated at 37 ^{0} C for 6 hours. The turbidity of the resulting suspensions was diluted with NB and SDB to obtain a transmittance of 74.3% (absorbance of 0.132) at 600 nm. The percentage is found spectrophotometrically comparable to 0.5 McFarland turbidity standards. This level of turbidity is equivalent to approximately 1.5 × 10 ^{8} CFU/mL [19]. Then the bacterial cultures were inoculated on the surface of Nutrient agar (NA) plates and fungal cultures on SDA plates. Subsequently, wells of 6 mm diameter was prepared on NA and SDA plates using sterile cork borer and 25 µL sample in different concentrations (100 µg/ml, 250µg/ml & 500µg/ml) were loaded in each well. Antibiotics were used as positive control (Chloramphenicol for bacteria and Fluconazole for fungus) [20].

The tests were carried out in triplicates. The plates were incubated at 37^{0} C for 24 hours. At the end of incubation, zones of inhibition were measured with a transparent ruler. Zones of clearing greater than 6 mm were considered susceptible to the extracts.

Anthelmintic property

The standard Albendazole (25mg /ml) and the test solutions of *A. catechu* (25,50, 100 mg/ml) were evaluated for anthelmintic activity with Indian adult earthworm *Pheretima posthuma*. Observations were made for the time taken for paralysis and death of individual worms up to four hours of test period. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water of 50 ^{0} C.

Antioxidant property screening

a. DPPH Radical scavenging assay

Free radical scavenging activity of the plant extract was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH), by a modified method [21]. The diluted working solutions of the test extracts (10 µg/ml -1000 µg/ml concentration) and 6.34 µM solution of DPPH were prepared in methanol, and 100µl of drug to be tested, 100µl DPPH solution and 800µl of methanol was taken in a test tube and mixed well. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (900µl) with DPPH solution (6.34µM,100µl) was taken as control and methanol as blank. The optical density was recorded and % of inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = A-B/A × 100

Where A = optical density of the control and B = optical density of the sample.

b. Super oxide radical scavenging assay

In-vitro super oxide radical scavenging activity was measured by NBT reduction method [22]. This method is based on the generation of super oxide radical by auto oxidation of riboflavin in presence of light. The super oxide radical reduces NBT to a blue colored formazon that can be measured at 590 nm.

100 µL riboflavin solution, 200 µL EDTA, 200 µL ethanol 100 µL NBT solution was mixed in a test tube and diluted up to 3 ml with phosphate buffer. The absorbance of solution was measured at 590 nm using phosphate buffer as blank after illumination for 15 minutes. This was taken as control reading. For screening of test sample along with the above solutions added 100 µL sample of varying concentrations (10 µg/ml -1000 µg/ml) and finally the volume was made up to 3 ml using phosphate buffer and the reading was taken after 15 minutes of illumination. % of inhibition was calculated using the formula given below:

Percent (%) inhibition = A-B/A × 100

Where A = optical density of the control and B = optical density of the sample.

RESULTS AND DISCUSSION

Phytochemical screening of *A. catechu* root extract

The preliminary Phytochemical screening of *A.catechu* root showed the presence of secondary metabolites like alkaloids, steroids, flavonoids, terpenoids, cardiac glycosides, quinones, phlobatanins, tannins and phenols.

The crude extract was fractionated into different classes according to their polarity and the yield in different fractions was noted to know the quantity of different compounds present in it. The aqueous fraction represents ~ 30% of the bulk, which denotes the alkaloid fraction. Other fractions show less than 1% yield. In addition the presence of many different valuable secondary metabolites, alkaloid fraction constitutes the major percentage.

Antibacterial and antifungal activity of *A. catechu* root extract

The results of the study showed that the ethanolic extract of *Areca* root, had prominent antimicrobrial activity against the human pathogenic bacteria and fungi studied (Tables 1 and 2). It is highly effective against the bacterial species *Pseudomonas aeruginosa* with zone of growth inhibition 15.4±0.92 mm at 500µg/ml concentration and it was least active against *Staphylococcus aureus* with 12.2±0.57 mm zone of growth inhibition at the same concentration. In case of antifungal screening, the highest activity was noted in case of *Aspergillus niger* with zone of inhibition 28.3 ±0.57 mm in the concentration 500 µg/ml and least activity in case of *Penicillium notatum* with 20 ±1 mm zone of clearing in the same concentration. *C.albicans* was resistant to Fuconazole but it showed promising activity with root extract with maximum zone of inhibition at 500 µg/ml concentration (24 ± 1 mm).

Table 1: It shows Antibacterial property of *Areca catechu* root ethanol extract.

S. No.	Organism	Zone of inhibition [MM]			
		Chloramphenicol (25 µg)	100 µg	250 µg	500 µg
1	*Klebsiella pneumoniae*	18.7±1.2	10.6±.1.2	12.6±0.57	12.7±1.2
2	*Salmonella typhi*	16.6 ±0.57	10.7±1.15	12.7±1.15	14.6±0.57
3	*Pseudomonas aeruginosa*	9.3±1.2	12.6 ± 0.57	14.6±0.57	15.4 ± 0.92
4	*Bacillus cereus*	16.7± 1.15	10.7± 1.15	12	14
5	*Streptococcus pyogenes*	17.6±2.5	10.7±0.57	11.6±1.2	12
6	*Staphylococcus aureus*	14.6±0.72	8.6±0.57	10.6±0.57	12.2±0.57

Table 2: It shows Antifungal property of *Areca catechu* root ethanol extract.

S. No.	Organism	Zone of inhibition (mm)			
		Fluconazole(15 µg)	100 µg	250 µg	500 µg
1	*Aspergillus niger*	12.5 ± 0.76	21.7 ± 1.5	24.6 ± 0.57	28.3± 0.57
2	*Aspergillus flavus*	14.3 ± 0.64	16 ± 1.2	17.6± 0.57	22.7 ± 1.2
3	*Penicillium notatum*	8.6 ± 0.57	14 ± 1	16 ± 1	20 ± 1
4	*Candida albicans*	R	16.7 ± 1.5	18.7 ± 0.58	24 ± 1

R- resistant

Table 3: It shows Anthelmintic property of *Areca catechu* root ethanol extract.

	Distilled water	Albendazole (25mg/ml)	Drug (25 mg/ml)	Drug(50 mg/ml)	Drug(100 mg/ml)
Time taken for paralysis (min)	-	32.4± 2	24±1	11 ± 1	4.5± 0.5
Time taken for death (min)	-	-	36.7± 2.08	14 ±1	6.3 ± 0.58

Due to the reported development of resistance by bacteria and fungi to avarious commercially available antimicrobial agents, the plants extracts are potential sources of new compounds which may be developed as effective drugs against microorganisms. Further, the use of this plant may offer a new source of antifungal agent against the pathogenic fungus like *Candida albicans*, *Aspergillus niger, A.flavus* and *Penicillium notatum* all these fungal species were inhibited by the crude drug in dose dependent manner. In the lower concentration itself (100µg/ml) *Candida albicans*, *Aspergillus niger, A.flavus* and *Penicillium notatum* showed 16.7±1.5, 21.7± 1.5, 16± 1.2 and 14±1 mm of growth inhibition respectively. *C. albicans* is not easily inhibited by other drugs.

Anthelmintic property of *A.catechu* root extract.

It was seen that the ethanolic extract of *A.catechu* root possess dose dependent antihelmintic activity as compared to a standard drug Albendazole. The mean paralyzing time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 24±1, 11 ± 1 and 4.5± 0.5minutes respectively. Albendazole in the concentration of 25 mg/ml takes almost 32.4± 2 minutes for getting paralysis. The mean death time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml root extract were found to be 36.7 ± 2.08,14 ±1 and 6.3 ± 0.58 minutes respectively. In the case of Albendazole at a dose of 25 mg/ml cause paralysis only no death was observed during the experimental period of 4 hours. (Table: 3).

Antioxidant property screening of *Areca catechu* root

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [23]. Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same [24].

DPPH Radical scavenging assay

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The percentage of DPPH radical scavenging activity of *A.catechu* root ethanol extract is presented in Table 4. *A.catechu* root extract exhibited a maximum DPPH scavenging activity of 95 % at 1000 µg/ml concentration with IC 50 value 65.7 ± 1.53.

Superoxide radical scavenging assay

The super oxide radical scavenging assay also shows significant radical scavenging property with IC 50 value 201.7 ± 0.76 .The activity was increasing with the increasing concentrations of test solution and shows 93% of inhibition at 1000 µg/ml concentration (table :4).

Table 4: It shows Antioxidant property of *Areca catechu* root ethanol extract.

S. No.	Concentration of plant extract(µgL^{-1})	Percentage of inhibition	
		DPPH	NBT
1.	10	15.85 ± 1.11	5.06 ± 1.27
2.	15	28.95 ± 0.69	14.3 ± 0.27
3.	25	35.87 ± 0.45	27.46 ± 1.36
4.	50	43.25 ± 0.40	34.54 ± 2.7
5.	75	54 ± 0.95	40 ± 1.10
6.	100	68.73 ± 0.063	44.30 ± 0.68
7.	250	75.21 ± 0.24	52.53 ± 0.72
8.	500	80.01 ± 0.85	65.06 ± 0.40
9.	750	86.67 ± 1.51	77.9 ± 1.27
10.	1000	94.49 ± 0.70	91.8 ± 1.11
IC 50 value		65.7 ± 1.53	201.7 ± 0.76

The present study indicates that *A.catechu* root extract could inhibit the oxygen radicals as seen from scavenging super oxide and DPPH radicals, and it could reduce the oxygen radicals and subsequently reduce the harmful effects. The literature supports that phytoconstituents such as polyphenolic compounds in drugs are responsible for the antioxidant potential [25, 26]. Further, phenolic compounds are effective hydrogen donors, which make them antioxidant [27]. The observed activity may be mainly due to flavonoids, tannins and phenolic content of the plant extract. Further studies are in progress to identify the antioxidant principle of this plant.

CONCLUSION

The present study reveals that the crude drug posses prominent antimicrobial, anthelmintic and anti-oxidant properties, which analyses its folk claim. Phytochemical studies portray the presence of several biologically active secondary metabolites. Therefore there is no doubt that this plant is a reservoir of potentially useful chemical compounds which serve as drugs, provide newer leads and clues for modern drug design.

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