PHYTOCHEMICAL ANALYSIS OF SEED OF MEDICINAL HERB, *ARECA CATECHU LINN.* AND EVALUATION OF *IN-VITRO* ANTHELMINTIC ACTIVITY OF ARECOLINE IN AQUEOUS AND ORGANIC EXTRACTS

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ABSTRACT

The objective of the study was to extract the active constituent of *Areca catechu Linn* (Arecaceae) and evaluate the anthelmintic effect of arecoline. Liquid liquid extraction was used to extract the arecoline from areca nut. Polar and nonpolar solvents were used for extraction. The anthelmintic activity was performed by *in vitro* studies using adult earthworm (*Pheretima posthuma*). The anthelminitic test was performed for both extracts and the results compared by using piperazine citrate as reference standard. Water and Hexane there used as polar and non polar solvent for extraction. The hexane extract produced maximum anthelmintic activity with minimum concentration.The hexane and aqueous extracts of nuts of areca catechu exhibited significant anthelmintic activity, as evidenced by decreased paralyzing time and death time. The results support the use of areca catechu as an anthelmintic agent.

Keywords: Anthelmintic, Areca catechu , arecoline, liquid –liquid Extraction

INTRODUCTION

A number of medicinal plants have been used to treat parasitic infections in human being and animals (Nadkarni, 1954; Chopra et al., 1956, 1958; Said, 1969; Akhtar, 2000)[1-4] Synthetic anthelmintics are the only sole source for the control of the gastrointestinal nematode by means of continuous and intensive use in recent decades. However, certain constraints like the high cost of these drugs and the usual development of nematode-resistant populations, along with the risk of contamination of the animal products and environment have lead to the search for alternatives[5]. Increasing problems of development of resistance in helminths against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity[6].

Areca catechu L. (Arecaceae), widely distributed in South and Southeast Asia, is a popular traditional herbal medicine that can be chewed for the purpose of dispersing accumulated fluid in the abdominal cavity and killing worms. Arecoline is an alkaloid obtained from the betel nut *(Areca catechu)*, fruit of the palm tree. It is an agonist at both muscarinic and nicotinic acetylcholine receptors.The present study was conducted to evaluate the anthelmintic activity of *Areca catechu* by *in vitro* studies.

MATERERIALS AND METHODS

Collection of crude drug

The commercially dried areca nut seeds were collected from local super market, washed thoroughly in tap water and dried under the sun for seven days and ground into fine powder using an electric grinder. Then the powdered mass obtained was stored in clean sterile bottles at room temperature and used for further extraction procedures.

Extraction by using LLE[7]

The arecoline was extracted from areca nut by liquid liquid extraction. We have used both polar and non polar solvents. Water and hexane extraction was selected for further process.

Preparation of aqueous extract

100 g of areca nut powder was weighed and soaked in 500mL of double distilled water for 24 h. The content was filtered and concentrated in a water bath at 100°C until a semisolid residue was obtained. The extract was stored in refrigerator at suitable temperature in order to avoid microbial contamination

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Preparation of non aqueous extract

100 g of areca nut powder was weighed and soaked in 250 mL of cyclohexane for 2 days. Then, it was filtered using muslin cloth and the filtrate was concentrated by keeping it in a water bath at 60° - 80° C. The concentrated extract was further evaporated to obtain semisolid extract. The extract was stored in suitable containers in order to avoid contamination.

Phytochemical screening [8-10]

The phytochemical screening of both alcohol and aqueous extracts of *Areca catechu* was performed by using standard methods like Mayer's test, alkaline reagent test, Total Phenol content test, ferric chloride test and killer killani test.

Identification of arecoline by TLC method

The arecoline was identified by thin layer chromatography. Different ratios of ethanol and dichloromethane were used for this process.

In vitro analysis of anthelminitic activity [12-13]

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al. The assay was performed *in vitro* using adult earthworm (*Pheretima posthuma*) as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings, for preliminary evaluation of anthelmintic activity. Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity *in vitro*. Earthworms were collected from moist soil and washed with normal saline to remove all faecal matter and used for the anthelmintic study. The earthworms of 3-6 cm in length and 0.1-0.2 cm in width were used for the experimental protocol. Test samples of the extract were prepared at concentrations, of 50, 100 and 150 mg/mL in distilled water and one worm i.e. *Pheretima posthuma* of approximately equal size (same type) was placed in each nine cm Petri dish containing 15 mL of above test solution of extract. Piperazine citrate (10 mg /mL) was used as reference standard and double distilled water as control.

The first Group served as control and received only normal saline; Second Group served as Test-1 and received different concentrations of (50,100,150 mg/mL) water extract of sample 1 (aqueous extract). The third Group served as Test-2 and received different concentrations of (50, 100,150 mg/mL) hexane extract of sample 2. Fourth Group served as standard and receive standard drug Piperazine citrate (50,100, 150 mg/mL). All the test solutions and standard drug solution were prepared freshly before starting the experiments. Observations were made by noting the time taken for paralysis when no movement of any sort could be observed. The worms time for death was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (100^{0}C). Paralysis was said to occur when the worms dit not react even in normal saline.

RESULT AND DISCUSSION

The preliminary phytochemical screening has showed the presence of, alkaloids, tannins, phenols and flavonoids in both the aqueous and hexane extracts of *Areca catechu*. The presence of phytoconstituents present in *Areca catechu* was identified by following test. The presence of alkaloid was identified by Mayer's test. The aqueous and hexane extracts produce white precipitate with Mayer's reagent. The flavanoidis were confirmed by Alkaline reagent test, it gives yellow fluorescence indicating the presence of flavonoids.The total phenol content by was determined ferric chloride test. A dark green color indicates the presence of phenol content. The terpienoids were confirmed by Keller-killani test, it forms red violet color.The results are shown in Table I and Fig. 1 and 2.

Table I: Presence of Phytochemical constituents in *Areca catechu* seed extract

Phytochemical compound	Aqueous extract	Hexane extract
Alkaloids	+	+
Saponins	+	+
Flavanoids	-	-
Phenols	+	+
Acids	-	+
Protein	+	+
Terpenoids	-	-
Tannins	+	+
Phytosterols	-	-

(+ = Present and - = absence of Phytoconstituents)

Identification of Arecoline by TLC

The arecoline was identified by thin layer chromatography. Different ratios of ethanol and dichloromethane were used. The (23:77)% V/V ratio

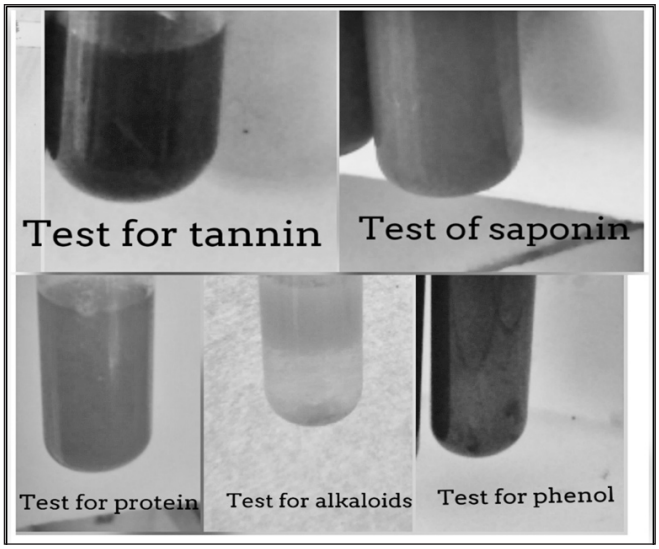

Fig. 1: Phyto-chemical screening of aqueous extract

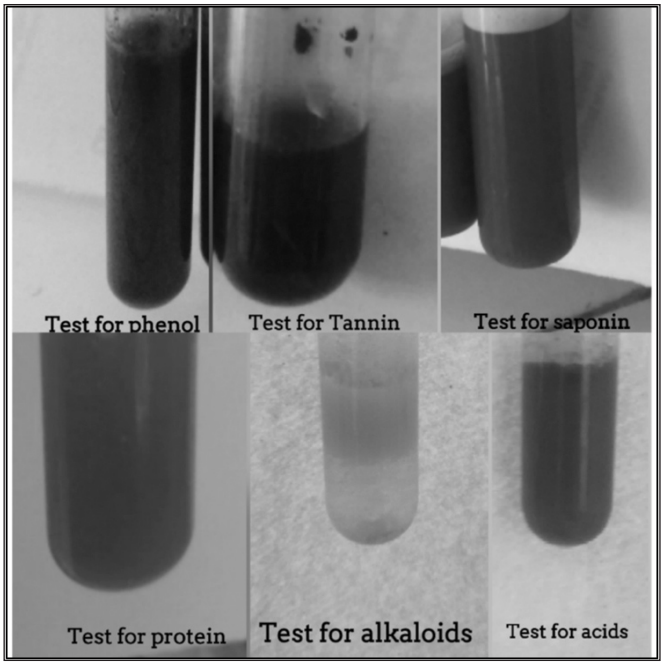

Fig.2: Phytochemical screening of hexane extract

was found to give clear and ideal Rf value (Rf: 0.54) for arecoline. The fingerprint of Arecoline and TLC report are shown in Fig. 3 and 4.

Anthelminitic Activity of *Areca catechu* by *In vitro* studies

In vitro anthelmintic activity was performed and the paralysis time and lethal time were recorded. Statistical evaluation of the data was performed by one-way ANOVA.

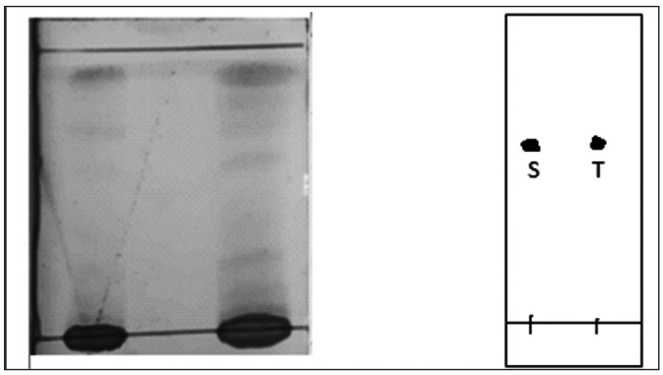

Fig.3 Fingerprint and TLC report of Arecoline in *Areca catechu* extract.

The results were expressed as mean ± SD using Graph Pad Instat 3 (n = 6).

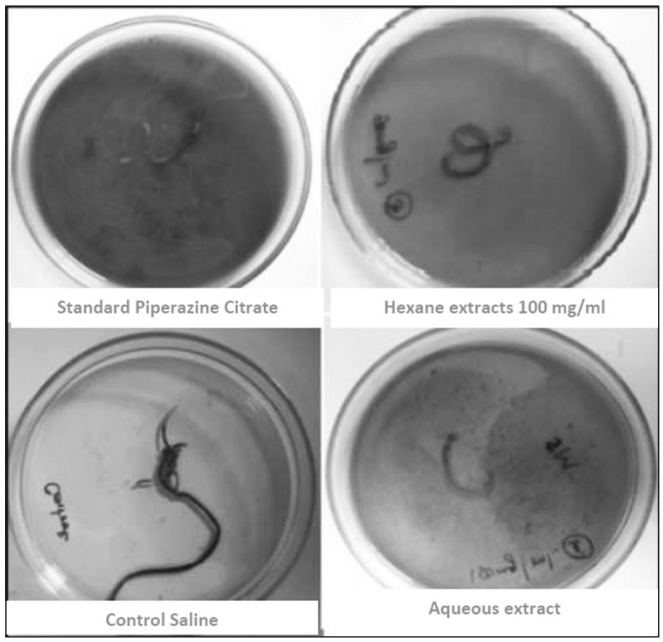

Fig. 4: Observation for anthelmintic activity

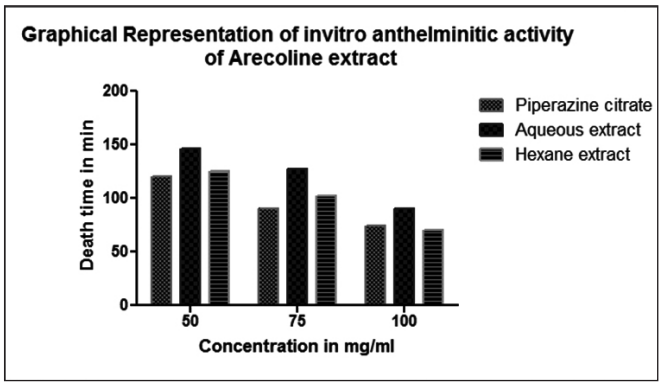

Fig. 5: Anthelmintic activity of aqueous and hexane extracts of areca catechu seed and piperazine citrate as standard

Table II: Anthelmintic activity of aqueous and hexane extracts of *Areca catechu* seed

Sr. No.	Sample	Concentration (mg/mL)	Mean time taken for paralysis (min)	Mean time taken for death (min)
1.	Control (saline solution)	-	-	-
2.	Areca catechu (Aqueous extract)	50	82±3.24min	146 ± 2.828 min
		75	50±3.2 min	127.5 ± 6.75 min
		100	36±2.5 min	90.66 ± 3.266 min
3.	Areca catechu (Hexane extract)	50	73±4.5min	125.83 ± 5.23 min
		75	50±2.5min	102.33 ± 5.87 min
		100	30±4.5min	70.2 ± 1.75 min
4.	Piperazine citrate (standard)	50	72±2.5 min	120.83 ± 5.99 min
		75	53±5.2min	90.5 ± 6.84 min
		100	34±5.6min	73.83 ± 5.167 min

The results show that for the 50 mg/mL concentration, piperazine citrate showed the best activity for death time (120.83 ± 5.99 min) and the aqueous extract and hexane extract showed a death time of 146 ± 2.828 min and 125.83 ± 5.23 min, respectively. Also, for the 75 mg/mL concentration, piperazine citrate showed the highest activity against the worms (90.5 ± 6.84 min) and the aqueous and hexane extract, of *Areca catechu* showed a death time of 127.5 ± 6.75 min and 102.33 ± 5.87 min, respectively. For the 100 mg/mL concentration, piperazine citrate showed the least death time of 73.83 ± 5.167 min, and the aqueous and hexane extract of *Areca catechu* extract showed a death time of 90.66 ± 3.266 min and 70.2 ± 1.75 min, respectively. The paralysis and death times of both the extract along with the standard are given in Table II. The study revealed that both the extracts had significant activity at higher concentration (100 mg/mL). The hexane extract has shown better activity than aqueous extract at a higher concentration (100 mg/mL) compared with the standard, piperazine citrate (100 mg/mL). The results are shown in Fig.4. The comparison of the death time of both extracts in different concentrations with respect to the standard is given in Fig. 5.

From the results it is concluded that the aqueous and hexane extracts of seeds of *Areca catechu* possess intrinsic anthelmintic properties. The extracts showed concentration related anthelmintic activities with all the worms used in the study. The 100mg/mL gave shortest time of paralysis and death for all the worm types. The results from the figure showed that the hexane extract of the areca catechu extract exhibited a higher activity than the aqueous extract.

CONCLUSION

The anthelmintic activity of non-polar cyclo hexane extract shows that it is effective against parasitic infections of humans. It is necessary to identify and isolate the possible active phytoconstitutents responsible for the anthelmintic activity and study their pharmacological actions.

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