PROXIMATE ANALYSIS, PRELIMINARY PHYTOCHEMICAL SCREENING AND IN VITRO ANTI-INFLAMMATORY ACTIVITY OF PREMATURE ARECA NUTS (ARECA CATECHU)

Hemand Aravind*, 1Krishnaja Mohan, 2Anisree P A

1Department of Biotechnology, Navajyothisree Karunakara Guru Research Centre for Ayurveda and Siddha, Uzhavoor, Kottayam Kerala-686 634, INDIA
2Department of Botany and Biotechnology, C.M.S College, Kottayam, Kerala, 686001, INDIA

Abstract

Areca catechu L. (Palmaceae), commonly known as Areca nut in English, is a perennial tree occurring throughout the Indian subcontinent and used traditionally for several medicinal purposes. The present study was expected to evaluate phytochemical contents using methanol extract of A. catechu nut. The phytochemical analysis of A. catechu nut established the presence of quinines, sterols, tannins, carbohydrate, glycosides and phenols. Evaluation of Nutritional values revealed that the methanol extract contains significant amount of carbohydrate, protein and crude fiber. Anti-inflammatory studies with In vitro models showed that areca catechu posses considerable protein stabilization and HRBC membrane stabilization activity.

Keywords: Areca catechu, proximate analysis, phytochemical screening, HRBC membrane stabilization.

Corresponding Author:

Hemand Aravind
Department of Biotechnology,
Navajyothisree Karunakara Guru Research Centre for Ayurveda and Siddha,
Uzhavoor, Kottayam Kerala-686634, INDIA
Email: hemantharavind@gmail.com
Phone: +91-9847808780
INTRODUCTION

*Areca catechu* L. is a palm species plant commonly termed as Betal palm or Betal nut tree. It is an angiosperm, it belongs to the family Arecaceae, order Arecales. Areca nut is the seed of areca palm. In Indian community chewing areca nut with betel leaf is found common and is considered as a commercially important seed crop. Areca nut is not be used as a stimulant rarely it is used in some ayurvedic preparations and traditional drugs. In Asia areca nut is known as one of the oldest masticatories [1].

Various medicinal properties of areca nut were cited, specially antibacterial and antiviral activity [2-4]. Traditional medicine of Kerala mentioned about various uses of *Areca catechu*. Its root is effective against various ailments like urinary tract disorders, skin irritations, worm disturbances and as an ingredient in tonic preparation. Tender leafs have curative effect in the treatment of migraine [5].

A number of phytocomponents were isolated from seeds of areca nut mostly catechin, tannins (15%), Gallic acid fat, gum and alkaloids like arecoline (0.07%), arecaine (1%). Arecaidine and guvacoline, guvacine and choline are present in trace amount. Among these, arecoline is the most important alkaloid [2,6,7].

*Areca catechu* extract has Central Nervous System stimulant effect which is mostly due to presence of these alkaloids [7-9].

In this study an effort was taken to screen phytoconstituents and analysis anti-inflammatory activity of premature areca nut.

MATERIALS AND METHODS

Premature areca nuts were collected from local farms of Uzhavoor and the seeds were separated from husk. Washed thoroughly with water then grind the areca seeds in form of paste and allow them to shade drying for 1 day. Later the dried material was powdered using motor and pestle and prepare the extract was prepared by plant tissue homogenization method using methanol [10].

Nutritional analysis

The successive extract, as mentioned above, was subjected to proximate analysis and qualitative phytochemical tests for standardization and screening of chemical constituents present in premature areca nut. Samples were analyzed for carbohydrate, protein, crude fibre, acid insoluble ash, water soluble ash and moisture content were determined using specified quality tests, as per procedures described by AOAC [11] and Indian Herbal Pharmacopoeia (IHP) [12].

Available online: www.ijipsr.com
Preliminary phytochemical screening

Preliminary phytochemical screening of the methanol extract of (MEAF) was done to find out the presence or absence of different phyto-constituents [13].

(a) Test for Flavanoids:
Shinodas Test (Mg/HCl): Dissolved a small amount of the extract in methanol/ethanol, a few magnesium turnings and few drops of 5 M HCl were also added. Development of deep red or majenta colour indicates the presence of flavonones and dihydroflavonols.

(b) Test for Coumarins:
A little amount of extract was dissolved in methanol/ethanol and 3-4 ml of alcoholic KOH or NaOH was added. Formation of a yellow colour which disappears on adding conc. HCl indicated the presence of coumarins.

(c) Test for Tannins:
Ferric chloride test: A few drops of ferric chloride was mixed to a little amount of the extract. The development of green colour revealed the presence of tannin.

(d) Tests for alkaloids
(i) Mayer’s Test: One or two drops of Mayer’s reagent was mixed to the acidified plant extract. A white precipitate indicates the presence of alkaloids.
(ii) Dragendorff’s Test: A little amount of extract was mixed to this reagent; appearance of brown precipitate revealed the presence of alkaloids.
(iii) Wagner’s Test: Alkaloids gave brown flocculent precipitate with Wagners reagent.

(e) Detection of steroids/terpenoids
Salkowski test: A few drops of concentrated sulphuric acid were mixed to a little amount of extract and was shaken for few minutes; the development of red or brown colour indicated the presence of sterols.

(f) Test for Phenols
A few drops of alcoholic ferric chloride solution was mixed to the extract dissolved in alcohol or water. Formation of violet, bluish green or bluish black colour indicated the presence of phenols.

(g) Test for Quinines
To the extract, sodium hydroxide was added. Formation of blue, green or red colour indicated the presence of quinines.
(h) Test for Anthroquinones
Borntrager’s test: The extract was shaken with aqueous ammonia or caustic soda. Formation of pink, red or violet colour in the aqueous layer indicated the presence of anthroquinones.

(j) Test for the detection of Glycoside
Benedict’s reagent test: The extract was mixed with Benedict’s reagent in equal amount and the mixture was heated for 2 min. The appearance of brown to red colour indicated presence of glycoside.

(k) Protein detection
(i) Xanthoprotein test: A small amount of the extract was mixed with 0.5 ml of concentrated HNO₃. Appearance of white or yellow precipitate revealed the presence of protein.
(ii) Biuret test: An aliquot of the extract was added to 0.5 ml of 4 % sodium hydroxide solution followed by a drop of 1 % copper sulphate solution. The development of violet to pink colour indicated the presence of protein.

(l) Reducing sugar detection test
(i). Benedict’s test: The extract was mixed with equal amount of Benedict’s reagent and the mixture was heated for 2 min. The appearance of brown to red indicates presence of reducing sugar.

(m) Test for detection of Resin
A little amount of extract was dissolved in 5 ml of alcohol and added 2 ml of distilled water and petroleum ether respectively. The development of white turbidity indicated the presence of resin.

In vitro anti-inflammatory activity:
(a) Determination of albumin denaturation assay: According to method of Sakat et al [14], with minor modification test solution containing different concentrations of methanol extract of Areca catechu (100μg/ml, 150μg/ml, 200μg/ml) and reference standard Sodium dichlofenac with 1ml of bovine albumin solution (1mM) and incubated at 37 ±1° C for 15 min. Denaturation was induced by keeping the reaction mixture at 51°C in a water bath for 10 min. After cooling the turbidity was measured spectrophotometrically at 660 nm [15]. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was carried out in triplicate and the average was taken.

\[
\% \text{inhibition} = \left( \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \right) \times 100
\]
(b) Determination of membrane stabilization activity: The HRBC suspension method as prescribed by Sakat [14], with a slight modification was used for the estimation of anti-inflammatory activity. Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution. Blood solution was centrifuged at 3000rpm and the packed cells were separated. The packed cells were washed with isosaline solution and 10% (v/v) suspension was made with isosaline. Different concentrations of extract (100μg/ml, 150μg/ml) and reference standard sodium diclofenac and control were separately mixed with 1ml of phosphate buffer (10mM, pH 7.4), 2ml of hyposaline and 0.5 ml of HRBC suspension. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000rpm for 20 minutes. The supernatant was decanted and the haemoglobin content was estimated by spectrophotometrically at 560nm. The assay was performed in triplicate. All the experiments were performed in triplicates and the results were expressed as mean ± SE.

\[
\% \text{Protection} = \left( \frac{100 - \text{Abs. sample}}{\text{Abs. control}} \right) \times 100
\]

RESULT AND DISCUSSION

Proximate analysis of Areca catechu seed:

Results obtained showed that (Table 1) the seed contained 0.95% carbohydrate, 0.16% protein, 0.12% crude fiber, 10% acid-insoluble ash content, 7% water soluble ash, 25% moisture content by loss on drying method. Significant amount of various nutritional compounds supports its energy boosting capacity.

Phytochemical screening of Areca catechu seed:

The preliminary phytochemical screening of A. catechu nut showed the presence of secondary metabolites like steroids, quinines, tannins, glycosides, resin, carbohydrate and phenols. Expression of various secondary metabolites signed its medicinal importance.

In-vitro anti-inflammatory activity of Areca catechu seed:

Inhibition of albumin denaturation

Denaturation of protein is considered to be an important reason of inflammation. In search of anti-inflammatory activity methanol extract of Areca catechu was investigated for its prevention ability of protein denaturation. It was found that methanol extract of premature areca seed showed maximum inhibition of 96.73% at a concentration of 200mg/ml (Table 2). Diclofenac a
nonsteroidal anti-inflammatory drug as standard showed a maximum inhibition of 79.73% at 200mg/ml.

**HRBC membrane stabilization test**

Membrane stabilization test of HRBC was further conducted to confirm the anti-inflammatory property of *Areca catechu*. Methanol extract of areca nuts successfully prevent heat induced hemolysis of HRBC at different concentration. Maximum inhibition was found to be 58.22% at a corresponding concentration of 150mg/ml (Table-2) compared with standared drug diclofenac 70% at 150mg/ml. These results provide evidence for membrane stabilization as a confirmatory test for anti-inflammatory effect.

<table>
<thead>
<tr>
<th>Table 1: Nutritional values of methanol extract of premature <em>Areca catechu</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional values</td>
</tr>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>Crude fiber</td>
</tr>
<tr>
<td>Carbohydrate</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Water soluble ash</td>
</tr>
<tr>
<td>Acid soluble ash</td>
</tr>
</tbody>
</table>

*Values are the Mean ± Standard deviations of triplicate*

<table>
<thead>
<tr>
<th>Table 2: In vitro anti-inflammatory activity of <em>Areca catechu</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
</tr>
<tr>
<td>Areca catechu</td>
</tr>
<tr>
<td>150mg/ml</td>
</tr>
<tr>
<td>200mg/ml</td>
</tr>
<tr>
<td>Diclofenac</td>
</tr>
<tr>
<td>150mg/ml</td>
</tr>
<tr>
<td>200mg/ml</td>
</tr>
</tbody>
</table>

*Values are expressed in Mean ± Standard deviations of three consecutive tests*

**CONCLUSION**

The present work reveals that methanol extract of *Areca catechu* posse’s significant amount of carbohydrate, protein and crude fiber. Phytochemical profiling of premature areca nuts showed the presence of various bioactive phytocomponents. *In vitro* anti-inflammatory studies carried out with protein denaturation and HRBC membrane stabilization method proved that methanol extract having promising level of anti-inflammatory activity. Therefore there is no doubt that this crop
plant is a pool of potentially useful chemical compounds. The above findings may help the crop to relabel it as a useful material for versatile products.

ACKNOWLEDGEMENTS

The authors are gratefully acknowledges Dept of Biotechnology, Navajyothi Sree Karunakara Guru Research Center for Ayurveda and Siddha, Uzhavoor, Kerala, India for providing the lab facilities.

REFERENCE


