Original Article

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS BY QUALITATIVE AND QUANTITATIVE ANALYSIS OF A HYDROALCOHOLIC EXTRACT OF TENDER *Areca catechu* L. CONTRIBUTING FOR MEDICINAL PROPERTY

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Abstract

Introduction: Arecanut is the chief component of the betel quid commonly used for chewing. Ayurvedic preparations containing *Areca catechu* were used to treat a variety of ailments. The *Areca catechu* plant was believed to possess many phytoactive components, which contributed to its medicinal use.

Objective: The present study aimed at evaluating the phytochemical constituents of the hydroalcoholic extract of tender areca nut (6-7 months) by preliminary qualitative and quantitative estimation by the high-performance thin layer chromatography (HPTLC) method.

Methods: The tender arecanut was collected from the coastal region of Karnataka and removed from adulterants, then dried and sliced. The extract was prepared by the cold maceration method at a 1:1 concentration of water and ethanol. The extract was analysed for its preliminary phytochemical analysis using standard procedures. Quantitative analysis of the active phytochemical was done using the HPTLC method to separate the various active components based on their Rf values, and densitogram analysis was done using a CAMAG TLC scanner 3 linked to WINCAT software to detect the concentration of each phytoconstituent.

Results: The preliminary qualitative methods of analysis showed the presence of many phytoactive constituents. There were nine active phytoconstituents separated by HPTLC, and concentrations were estimated as percentages.

Conclusion: The nine different phytoactive constituents separated from the plant's extract support the medicinal property of the plant.

Keywords: Hydroalcoholic; Phytochemical, Phytoactive, Chromatography, Densitogram.

1. Introduction

Herbal medicines are widely used and accepted in all countries. Herbal plants product is easily available and commonly used in traditional medicine like Ayurveda and Yunani since from ancient days. The phytoactive constituents of the plants are contributing the variety of medicinal properties. The phytoactive components of the plants brings changes in the physiological functions in the humans to prevent the pathological changes [1]. Ethnobotany and ethnomedicine are the two scientific disciplines that provide the majority of the identification and discovery of new active medicines derived from plant sources [2]. The different parts of the plant consist of primary and secondary metabolites, which contribute to its biological activities. The phytoactive components posses less adverse effects with better patient compliance [3].

The primary plant metabolite was very important for the plant's growth, self-defense, development and adaptation to various conditions [4]. The major plant primary metabolites were carbohydrates, nucleic acids, amino acids, vitamins and polysaccharides, etc. Primary metabolites are utilized as the nutritional requirements of the plant rather than for the medicinal use [5]. Secondary metabolites are chemical in nature and are produced by the cell metabolism pathway from the primary metabolite. Major metabolites are not utilized by the cells and production is very high during stress. The secondary plant metabolites are phenols, flavonoids, alkaloids, tannins, saponins, lipids, etc. These secondary metabolites contribute to its medicinal properties like antioxidants, antimicrobials, antivirals, antimalarials, etc. This biological activity explains the scientific use of plant products in traditional medicine [6]. The Areca catechu fruit possesses antischizophrenic [7], antioxidant [8], anti-inflammatory, hepatoprotective [9], antipyretic, antidiarrheal, hypoglycemic, and hypoglycemic [10]. The phytoconstituents of the plants are depending on the solvent and methods used for its extraction [11-12]. Hence, hydroalcoholic extract of tender *Areca catechu* L. fruit obtained from cold maceration method was evaluated for its different phytoconstituents.

2. Materials and methods

2.1. Requirements

Dried and powdered *Areca catechu* fruits, Distilled water, Ethanol, Stoppered glass flask, Water bath, Desiccator, weighing balance, Air oven, Test tubes, Test tube holder, Bunsen burner, Silica gel, TLC plates, Hamilton syringe, Glass chamber, Camag TLC scanner connected to WINCAT software, Deuterium and tungsten lamp, the chemicals and reagents required are procured from the authorized dealers.

2.2. Areca catechu fruit collection and hydroalcoholic extract preparation

Areca catechu fruit 6-7 months old was collected from Dakshina Kannada, Karnataka, and identified at Department of Pharmacognosy, SDM Centre for Research, Kuthpady, Udupi. Fruit was cleaned, and the husk was removed from the kernel. The fruit was sliced and sun-dried (Figure 1).

Figure 1: Areca catechu row fruit and dried sliced fruit

Figure 2: Hydroalcoholic Extract of *Areca catechu* (HAEAC)

2.3. Extraction methodology

The cold maceration method with a 1:1 concentration of ethanol and water was used as an extraction solvent in this extraction method. The extraction yields similar constituents to the original as there was no heating, hence less degradation and thermolabile constituents were also extracted in stable condition. Both water and alcohol soluble phytoconstituents are easily extracted. This method also increases the shelf life of the extract. 100 g of the sample was placed in a glass stoppered flask. Added 200 ml of alcohol (approximately 95%) and water (1:1). Kept for 6 hours between shakings. The solvent mixture was allowed to stand for 18 hours. Filtered quickly without wasting any solvent. The filtrate was allowed to dry in a water bath. Then it was placed in an air oven at 105^0C for 6 hours, cooled in desiccator and weighed to calculate the yield. The % yield of the extract was approximately 11.83% [13] (Figure 2).

2.4. Preliminary phytochemical analysis [14]

2.4.1. Test for alkaloids

In a test tube, a small amount of the extract was stirred with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested for alkaloids with the following reagents.

a. Dragendroff's reagent

The reagent solution consists of basic bismuth nitrate, tartaric acid, and potassium iodide, and when alkaloids are present in the extract, DR produces an orange red precipitate.

b. Hager's reagent

Hager's reagent is a saturated solution of picric acid, which, when reacting with alkaloids, gives a yellow coloured precipitate.

c. Wagner's reagent

Wagner's reagent, consisting of iodine and potassium iodide in distilled water, reacts with plant alkaloids to give a reddish-brown precipitate.

2.4.2. Test for carbohydrates

A small amount of extract was dissolved in 5 ml of distilled water and the collected filtrate was tested for carbohydrates.

a. Molisch's test

In a test tube, the filtrate was placed, and 2-3 drops of Molisch's reagent (α -naphthol in methanol) and concentrated sulphuric acid were added to form a purple coloured ring between the two layers, confirming the presence of carbohydrates.

b. Fehling's test

The Fehling's A and B reagents was added to extract taken in a test tube. Heated in a water bath for 5–10 minutes. Reddish brown precipitate indicates the presence of carbohydrates.

c. Barfoed's test

Barfoed's reagent, consisting of copper acetate and acetic acid, reacts with the carbohydrates present in the extract when heated in a water bath for 5–10 minutes, giving a reddish precipitate that confirms the presence of carbohydrates.

2.4.3. Test for phytosterols

In a test tube containing a few drops of acetic acid, 1gm of extract was dissolved. 3 ml of acetic anhydride and 2-3 drops of concentrated sulphuric acid were added. The phytosterols react with the reaction mixture, causing it to turn blue-green.

2.4.4. Test for glycosides

In a test tube, a solution of the extract, glacial acetic acid, and a few drops of 5% ferric chloride were added. The formation of a reddish-brown coloration at the junction of two layers and a blueish-green coloration in the upper layer indicated the presence of glycosides.

2.4.5. Test for fixed oils and fats

a. Paper test: The filter paper turning in to translucent in light when a small quantity of extract was placed in between filter paper and pressed indicates the presence of fixed oil.

b. In a test tube containing a small amount of extract, 3–4 drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein were added and heated on a water bath for 1-2 hours. The presence of fixed oil and fats is indicated by the formation of soap or partial alkali neutralization.

2.4.6. Test for Gums

The presence of gums can be detected by the formation of red colour when 1 ml of extract was treated with 3 ml dilute HCl drop by drop.

2.4.7. Test for triterpenoids

In a test tube, 5 mg of the extract was dissolved in 2 ml of chloroform, and 1 ml of each acetic acid and concentrated sulphuric acid were added to it. A reddish violet colour indicates the presence of triterpenoids.

2.4.8. Test for tannins and phenolic compounds

A small amount of the extract was dissolved in separate test tubes for tannin and phenolic compound identification. Each test tube added with following reagents to get respective colours in the presence of phenolic compounds and tannins.

a. Dilute ferric chloride solution (5%) – violet colour

b. 1% solution of gelatin with10% NaCl – white precipitate

c. 10% lead acetate solution – white precipitate

2.4.9. Test for anthraquinone

Borntrager's test: The solution containing extract and dilute sulphuric acid was boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added and shaken well. The organic layer was separated and an equal volume of dilute ammonia solution was added to the organic layer. The upper layer did not turn pink showing the presence of anthraquinone.

2.4.10. Test for amino acids

Few drops of 40% NaOH and 10% of lead acetate were added to 5ml of test sample solution and boil the solution. The presence of amino acid was indicated by the formation of black precipitate.

2.4.11. Test for proteins

The extract was dissolved in water and tested using following reagents.

a. Millon’s reagent: Formation of red colour indicates the presence of the proteins and free amino acids.

b. Biuret test: 5% solution of NaOH and 1% $CuSO_4$ were added in equal volume. Formation of pink or purple colour indicates the presence of proteins and free amino acids.

2.4.12. Test for flavonoids

Shinoda’s test – the extract was dissolved in ethanol, warmed and filtered. To that a piece of magnesium and followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of orange or pink colour indicates the presence of flavonoids.

2.5. Quantitative analysis of HAEAC by HPTLC

The HPTLC plate consists of precoated silica gel (G 60 F 254) and a Hamilton syringe was used for the application of the sample. A small amount of extract was used for dissolving in the respective solvent, and with the help of a linomat applicator, extract was applied to the precoated plates. The standard solvent optimized for HPTLC was used. An aliquot of the sample solution was applied as a 5 mm band at 8.00 mm from the base of the plate on a 2.8 x 10 cm TLC plate. Then it was developed in a twin trough glass chamber up to 75.00 mm using suitable mobile phases. The developed plate was dried in air, and subsequent photographs at UV 200–360 nm were taken [15].

The chromatogram was run for 80mm length. Chromatogram was dried in an oven at 100^0C for 15 minutes and then initially detected by visualization. The layers are air dried for 30 minutes and then densitometric analysis was done using CAMAG TLC scanner 3 linked to WINCATS software which was set at 366 nm, after multi-wavelength scanning between 250 and 400 nm in the absorption mode had first been tried. The sources of radiation were deuterium and tungsten lamps. The slit dimension was kept at 6.00 × 0.45 mm and the scanning speed used was 20 mm s-1. The chromatogram of many compounds in HAEAC was recorded. The peak chromatogram was noted using the software WINCATS 1.3.4 version [16-17].

Table I: Preliminary phytochemical evaluation of HAEAC

Sl. No	**Phytochemicals**	**HAEAC**
1.	Alkaloids	+
2.	Saponins	+
3.	Diterpenes	+
4.	Triterpenoids	+
5.	Amino acids	+
6.	Phenolic compounds	++
7.	Flavonoids	++
8.	Tannins	++
9.	Phytosterols	+
10.	Glycosides	-
11.	Carbohydrates	+
12.	Anthraquinones	-
13.	Gums	-
HAEAC: Hydroalcoholic extract of *Areca catechu*		

Figure 3: HPTLC plate of HAEAC

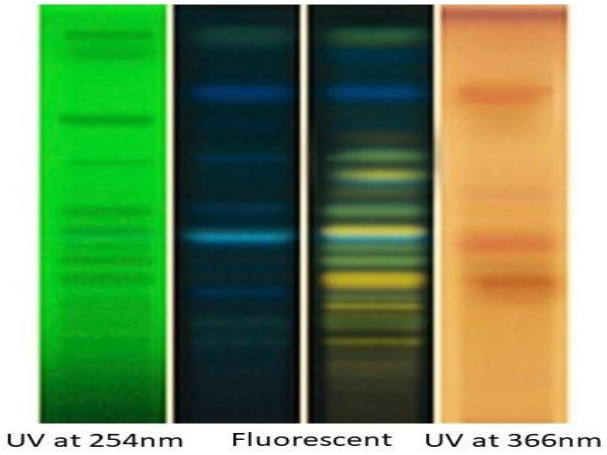

Figure 4: HPTLC peak of HAEAC

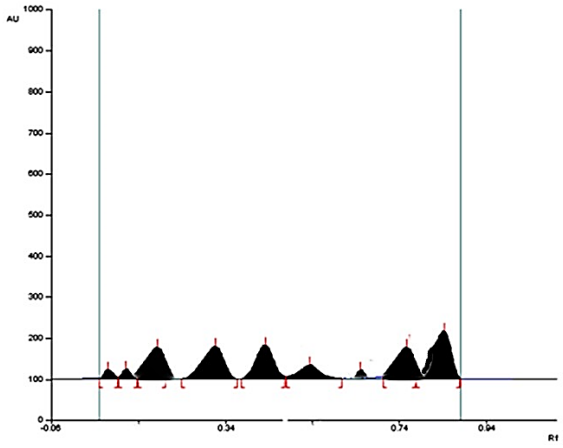

Table II: Quantitative phytochemical evaluation of HAEAC by HPTLC

Sl. No	**Phytochemicals**	**Rf Values**
1.	Alkaloids	0.09
2.	Saponins	0.11
3.	Diterpenes	0.18
4.	Triterpenoids	0.32
5.	Amino acids	0.43
6.	Phenolic compounds	0.69
7.	Flavonoids	0.73
8.	Tannins	0.79
9.	Phytosterols	0.91

HAEAC: Hydroalcoholic extract of *Areca catechu,* HPTLC: High performance thin layer chromatography

Table III: Peak table profile of HPTLC densitogram of HAEAC

Peak	**Start Rf**	**Max Rf**	**Max Height**	**Height %**	**End Rf**	**Area %**	**Assigned substance**
1	0.03	0.05	114.6	2.41	0.09	1.13	Alkaloids
2	0.07	0.09	115.9	2.46	0.12	1.09	Saponins
3	0.14	0.16	120.7	4.89	0.23	2.12	Diterpenes
4	0.26	0.31	121.4	4.94	0.34	3.89	Triterpenoids
5	0.36	0.43	128.6	5.45	0.42	4.18	Amino acids
6	0.43	0.54	98.7	3.14	0.61	2.98	Phenolic compounds
7	0.67	0.69	108.6	1.23	0.70	1.02	Flavonoids
8	0.72	0.74	120.5	2.79	0.84	9.86	Tannins
9	0.85	0.89	127.9	3.15	0.92	9.99	Phytosterols

HAEAC: Hydroalcoholic extract of *Areca catechu,* HPTLC: High performance thin layer chromatography

Results and discussion

The preliminary qualitative analysis detected the presence of alkaloids, flavonoids, triterpenoids, tannins, phenolic compounds, phytosterols, saponins, carbohydrates, amino acids. The glycosides, gums, anthraquinones were absent in the hydroalcoholic extract of *Areca catechu* L. fruit (Table I).

The quantitative analysis by HPTLC method revealed the presence of nine different compounds with different Rf values of 0.09, 0.11, 0.18, 0.32, 0.43, 0.69, 0.73, 0.79, 0.91. The Rf values in the HPTLC peak obtained from the densitogram corresponds to alkaloids, saponins, diterpenes, triterpenoids, amino acids, phenolic compounds, flavonoids, tannins, phytosterols respectively. The concentration of each phytochemical was calculated from the area under the curve in densitogram. The concentration of each phytochemical from the area percentage was alkaloids-1.13%, saponins-1.09%, diterpenes-2.12%, triterpenoids-3.89%, amino acids-4.18%, phenolic compounds-2.98%, flavonoids-1.02%, tannins-9.86%, phytosterols-9.99% respectively (Table II & III; Figure 3& 4).

According to several studies, saponins [18], diterpenes, triterpenoids [19], phenolic compounds [20], flavonoids [21], and phytosterols [22] are the primary plant antioxidants, while amino acids [23], tannins [24], are secondary antioxidants. Except for alkaloids, all other phytoconstituents act as antioxidants, and can prevent oxidative stress induced cellular damage. Alkaloids are contributing for improved memory in the several neurological disorders like improvement in Alzheimer's disease, Parkinsonian disease [25].

Conclusion

In conclusion, the hydroalcoholic extract of tender *Areca catechu* L. fruit by cold maceration method possessed alkaloids, flavonoids, triterpenoids, tannins, phenolic compounds, phytosterols, saponins, carbohydrates, and amino acids in the preliminary analysis. The HPTLC separated nine different components based on their Rf values. All these phytoconstituents contribute to its medicinal properties, which were used in traditional medicine since ancient times.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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