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Phytochemical Investigation of Artocarpus Heterophyllus Fruit

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Abstract:
Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Traditional use of medicine is recognized as a way to learn about potential future medicine which was derived from ethno medical plant sources. Herbal medicines are derived from plants or some other natural sources. Plant kingdom is an unlimited resource of extra ordinary variety of compounds which are commonly called as primary and secondary metabolites. Their importance in making medicinal drugs flavours and industrial materials on commercial scale is well established. The use of plants as medicines is a valuable source in human history. Phytochemistry is mainly concerned with enormous varieties of secondary plant metabolites which are biosynthesized by plants. The plant kingdom represents a treasure trove of structurally diverse bioactive molecules. Most of the best plant medicines are the sum of their constituents. The beneficial physiological and therapeutic effects of plant materials typically results from the combinations of these secondary products present in the plant. The information on the constituents of the plant clarifies the uses of the plants but only a small percentage have been investigates for their phytochemicals and only a fraction has undergone biological or pharmacological screening. As more phytoconstituents are being identified and tested, traditional uses of the plants are being verified. In phytochemical evaluation the powdered leaves were subjected to phytochemical screening for the detection of various plant constituents, characterized for their possible bioactive compounds which have been separated and subjected to detailed structural analysis.

Keywords: Artocarpus heterophyllus, fruit extract, phytochemical

MATERIALS AND METHODS

Phytochemical screening:

Collection of plant material

Fruits of *Artocarpus heterophyllus* were collected in the month of March 2014 from Kanyakumari District. The edible parts of fruit were air dried, powdered and stored in a air tight container for future use.

Preparation of plant power:

Fresh plant parts were collected ,washed thoroughly and air dried in shade until dried completely. The drying process was continued until the moisture content was decreased. After drying, the plant material was macerated using mixer grinder. Then the powder was stored in air tight containers and kept in refrigerator for future use.

Preparation of plant extracts (Parekh and Chanda, 2007)

For percolation process, the macerated plant powder was soaked in solvents such as aqueous, methanol, ethyl acetate, acetone and hexane individually. Extraction was done by soaking one part of plant powder to three parts of liquid solvent in 1:3 ratio and kept for percolation process overnight. Then the crude extracts were filtered using whatmann No-1 filter paper, evaporated and concentrated into solid extracts under room temperature.

Qualitative phytochemical test

The extracts of each solvent was used to analyze the presence of different phytochemical constituents. The method employed to analyze the phytochemical constituents are described below.

Molisch's Test for carbohydrates (Sofowora, 1993)

The extracts were treated with 2 drops of alcoholic alpha-napthol solution in a test tube and 2 ml concentrated H_2SO_4 was added carefully along the sides of the test tubes. Formation of dull violet or red ring at the interphase indicated the presence of carbohydrates.

Test for acids

To 1ml of each extract, 1ml of sodium bicarbonate solution was added. Formation of effervescence indicated the presence of acids.

Test for betacyanins (Harborne, 1973)

To 2ml of each plant extract, 1ml of 2N NaOH was added and heated for 5 minutes at 100^{o}C. Formation of yellow colour indicated the presence of betacyanin.

Test for quinones (Evans, 1996)

To 1ml of each solvent extract 1ml of conc. H_2SO_4 was added. Formation of red colour indicated the presence of quinones.

Test for coumarins

A few drops of ammonia were added on a filter paper. To this a drop of different extract was added and paper was observed for fluorescence.

Test for alkaloids (Evans, 1997)

The extracts were treated with Mayer's reagent (1.36 gm mercuric chloride and 5 gm of potassium iodide was dissolved in 100ml distilled water). The formation of yellow cream predicts the presence of alkaloids.

Ninhydrin test for amino acids (Yasuma and Ichikawa, 2000)

To each of the solvent extract 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicated the presence of amino acids.

Biuret test for proteins (Bralin and Turner, 1975)

The extracts were treated with 1ml of 10% NaOH solution and heated. To this extract a drop of 0.7% $CuSO_4$ solution was added. Formation of purplish violet colour indicated the presence of proteins.

Benedict's test for reducing sugars (Tiwari *et al*., 2011)

The extracts were treated with Benedict's reagent and heated on a water bath. Formation of orange red colour precipitate indicated the presence of reducing sugars.

Stain test for fixed oils and fats

Small quantities of extracts were pressed between two filter papers. Formation of an oily stain on the filter paper indicated the presence of fixed oils fats.

Ferric chloride test for flavonoids (Raman, 2006)

The extracts were treated with few drops of $FeCl_3$ solution. Formation of a blackish red colour indicated the presence of flavonoids.

Test for gums and mucilages (Whistler and Bemiller, 1993)

About 5ml of the each extract was slowly added to 5ml of absolute alcohol under constant stirring. The appearance of precipitation indicated the presence of gums and mucilages.

Test for steroids (Kokate, 1994)

2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml H_2SO_4. Change in colour from violet to blue or green indicates the presence of steroids.

Test for tannins (Trease and Evans, 1989)

To 1ml of solvent extract, a few drops of $FeCl_3$ solution were added. The appearance of a blue, black, green or blue green precipitate indicated the presence of tannins.

Acetone - water test for resins

The extract was treated with acetone. A small amount of water was then added and shaken. Appearance of turbidity indicated the presence of resins.

Test for phlobatannins (Harborne, 1973)

About 2ml of aqueous extract was added to 2ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatannins.

Test for terpenoids (Evans, 1997)

To 1ml of the solvent extract, 2ml of chloroform was added. Then 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

Ferric chloride test for phenols (Mace, 1963)

To 1ml of solvent extract 3ml of distilled water was added. To this, a few drops of neutral 5% $FeCl_3$ solution was added. Formation of a dark green colour indicated the presence of phenolics.

Foam test for saponins (Kumar *et al*.,2009)

About 2ml of distilled water and 1ml of each solvent extract were mixed and shaken vigorously. Formation of a stable persistent froth indicated the presence of saponins.

Keller - Killani test for cardiac glycosides (Sofowora, 1984)

The extract was dissolved in glacial acetic acid containing traces of $FeCl_3$. The tube was then held at an angle of 45^oC and 1ml of conc. H_2SO_4 was added along the sides of the tube. Formation of a purple ring at the interphase indicated the presence of cardiac glycosides.

Borntrager's test for anthroquinones (Sofowora, 1993& Harborne, 1984)

Small portion of the different extract was shaken well with 10ml Benzene and filtered. 5ml of 10% common solution was added to the filtrate and stirred. The production of a pink, red or violet colour indicated the presence of free anthroquinones.

Test for volatile oils (Trease and Evans 1989)

To 1ml of each extract, 1ml of 90% ethanol was added followed by the addition of few drops of $Fecl_3$ solution. Formation of green colour indicated the presence of volatile oils.

Test for emodols

The dry extract was added to 25% ammonia solution. The formation of cherry red solution indicated the presence of emodols.

Test for starch (Harborne, 1998)

To 1ml of each extract, 10ml of saturated $NaCl_2$ solution was added. It was then heated. After heating the extract starch reagent was added. Formation of a blue purplish or pink colour is a positive test for presence of starch.

Test for fatty acids (Ayoola *et al*., 2008)

0.5ml of different extract was mixed with 5ml of ether. This mixture was allowed to evaporate on the filter paper and then the filter paper was dried. The appearance of transparent areas on filter paper indicated the presence of fatty acids.

RESULTS

Phytochemical analysis was carried out in *Artocarpus heterophyllus* to find the presence of medically important bioactive compounds. The presence of phytochemicals in *A. heterophyllus* was evaluated in ripe fruits by successive

solvent method using different solvents such as aqueous, methanol, acetone, ethyl acetate and hexane. The secondary metabolites were acids, quinones, alkaloids, tannins, gums and mucilages, phenols, steroids, saponins, flavanoids, terpenoids, anthroquinones, betacyanin, resins, phlobatannins, volatile oils, cardiac glycosides, fixed oils and fats, coumarins, reducing sugar and emodols. The present investigation revealed the presence of various phytochemical constituents in different extracts of *A. heterophyllus* ripe fruits. The results observed are tabulated in Table 1. In aqueous extract, tannins and coumarins could be detected. Among these two constituents tannins were observed in moderate levels and coumarins in low levels. All the other phytochemicals such as acids, quinones, alkaloids, gums and mucilages, phenols, steroids, saponins, flavanoids, terpenoids, anthroquinones, betacyanin, resins, phlobatannins, volatile oils, cardiac glycosides, fixed oils and fats, reducing sugar and emodols were not in aqueous extract. The methanolic extract of *A. heterophyllus* fruit revealed the presence of maximum number of phytochemical constituents such as quinones, alkaloids, tannins, gums and mucilages, steroids, phenols, saponins, flavanoids, terpenoids, betacyanin, volatile oils, fixed oils and fats, coumarins and emodols.(Table 1). Among the above phytoconstituents alkaloids, tannins, phenols, saponins, flavanoids and terpenoids were recorded in high levels. While, quinones, gums and mucilages, steroids and betacyanin in moderate levels and volatile oils, fixed oils and fats, coumarins and emodols seen in low levels (Table 1). The phytochemicals such as acids, aminoacid, anthroquinones, resins, phlobatannins, and cardiac glycosides could not be detected in methanol extract.

The phytochemical constituents observed in acetone extracts were steroids, terpenoids and volatile oils. Among the above three phytoconstituents screened, steroids exhibited moderate intensity and low in the case of volatile oils and terpenoids. The phytochemicals such as acid, quinones, alkaloids, tannins, gums and mucilages, phenols, saponins, flavanoids, aminoacid, anthroquinones, betacyanin, resins, phlobatannins, cardiac glycosides, fixed oil and fats, coumarins and emodols could not be detected in acetone extract of *A. heterophyllus.* The phytochemicals such as acids, quinones, alkaloids, tannins, steroids, phenols, saponins, flavanoids, aminoacid, terpenoids, resins, phlobatannins, volatile oils, cardiac glycosides, coumarins and emodols could not be detected, instead the ethanolic extract of *A. heterophyllus* fruits indicated the presence of gums and mucilages, anthroquinones, betacyanin, fixed oils and fats in low intensities. (Table 1). The hexane extract of *A. heterophyllus* exhibited alkaloids and coumarins. The former in high intensity and the later at low intensity. While many phytochemicals such as acid, quinones, tannins, gums and mucilages, steroids, phenols, saponin, flavanoids, aminoacid, terpenoids, anthroquinones, betacyanin, resin, phlobatannins, cardiac glycosides and emodols could not be detected in the extract.

Different mechanisms of action of phytochemicals have been suggested. They may inhibit microorganisms, interfere with some metabolic processes or may modulate gene expression and signal transduction pathways (Kris – Etherton *et al.,* 2002). Phytochemicals may either be used as chemotherapeutic or chemo preventive agents with chemoprevention referring to the use of agents to the use of agent have to inhibit reverse or retard tumorigenisis. In this sense chemo preventive phytochemicals are applicable to cancer therapy, since modular mechanisms may be common to both chemoprevention and cancer therapy (Sarkar and Li., 2006). Plant extracts and essential oils may exhibit different modes of action against bacterial strains such as interference with the phospholipids bilayer of the cell membrane which has a consequence a permeability increase and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components (Kotzekidou *et al.,* 2008).

This research work was initiated to evaluate the bioactivities of *A. heterophyllus* plant in order to study their potential role as an antioxidant agent. Since, the concentration of phytochemicals varies in different solvents extract of the plant were selected for the analysis of phytochemicals. Phytochemicals are natural compounds found in plants that are responsible for the colour, taste and aroma of foods. As well as these pleasant attributes, they protect us from environmental and ingested carcinogens by arming antioxidant enzymes. There is a wide range of dietary phytochemicals but one of the largest well known groups is the polyphenols. The average dietary intake of polyphenol is reported to be over 1gm/day, which is upto ten times higher than all other classes of phytochemicals are known as dietary antioxidants. (Bauer *et al.,* 2012). Many plant extracts are reported to have health beneficial properties due to secondary metabolites such as acids, quinones, alkaloids, tannins, gums and mucilages, phenols, steroids, saponins, flavanoids, terpenoids, anthroquinones, betacyanin, resins, phlobatannins, volatile oils, cardiac glycosides, fixed oils and fats, coumarins, reducing sugar and emodols. These bioconstituents are known for their versatile biological effects and are implicated in treatment of variety of diseases. Literature reveals that lot of pharmacological investigations have been carried on Artocarpus species. The reported pharmacological uses are the leaves of A. heterophyllus are used to treat fever, boils, ulcers, wounds and skin diseases.

From the above five extracts, the methanolic extract of *A. heterophyllus* ripe fruit contained the maximum number of phytochemicals and most of the phytochemicals were with high intensities (+++) in methanolic extract when compared to all other extract used in the present investigation.

S.NO	PHYTOCHEMICAL TEST	DIFFERENT SOLVENT EXTRACT OF ALF				
		Aqueous	Methanol	Acetone	Ethyl acetate	Hexane
1	Acid	-	-	-	-	-
2	Quinones	-	++	-	-	-
3	Alkaloids	-	+++	-	-	+++
4	Tannins	++	+++	-	-	-
5	Gums and mucilages	-	++	-	-	-
6	Steroids	-	++	++	-	-
-7	Phenols	-	+++	-	-	-
8	Saponins	-	+++	-	-	-
9	Flavanoids	-	+++	-	-	-
10	Aminoacid	-	-	-	-	-
11	Terpenoids	-	+++	+	-	-
12	Anthroquinones	-	-	-	+	-
13	Betacyanin	-	++	-	+	-
14	Resins	-	-	-	-	-
15	Phlobatannins	-	-	-	-	-
16	Volatile oils	-	+	+	-	-
17	Cardiac glycosides	-	-	-	-	-
18	Fixed oils and fats	-	+	-	+	-
19	Coumarins	+	+	-	-	+
20	Emodols	-	+	-	-	-

\+ Low intensity
++ Moderate intensity
+++ High intensity

CONCLUSION

Phytochemicals can be defined in the strictest sense, as chemicals produced by plants. Phytochemicals are plant or fruit derived chemical compounds. Phytonutrients refer to phytochemicals or compounds that come from edible plants. These phytochemicals give plants their colour, flavor, smell and texture. These constituents are obtained from highly reactive molecules of oxygen and a number of environmental hazards such as viruses and fungi that could affect their chance for survival (Diplock *et al.*, 1998). It is estimated that there are 250,000 – 500,000 species of plants on earth. Among that 1-10% of phytoconstituents are used as food and medicines and claimed to have medical and antimicrobial properties. Phytochemicals include large families of phenolic metabolites and other nitrogen containing plant constituents. Many of these plant metabolites are not considered as essential nutrients for life. However Phytonutrients such as carotenoids, vitamin E and C and other phenolic compounds had been shown to help, protect the human body against damage by reactive oxygen and nitrogen species that contribute to the diseases such as cancer, heart and neurodegenerative diseases (Halliwell, 1997). Phenolics and terpenoids are reported to exert inhibitory action against microorganisms (Lambert *et al.*, 2001).

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