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Research Article

PHYTOCHEMICAL ANALYSIS OF *ANNONA RETICULATA L.* LEAF EXTRACTS

Sangeetha V.S[1]; Michael Babu[2], Beena Lawrence[3]
[1]Asst. Professor, Noorul Islam College of Arts and Science, Thuckalay, Tamil Nadu India,
[2]Asso. Professor, Centre for Marine Science and Technology, Rajakkamangalam, Tamil Nadu, India
[3]Asso.Professor, Women's Christian College, Nagercoil, Tamil Nadu, India

*Corresponding Author: V.S.Sangeetha; Email: sangee_vs@yahoo.com

Abstract: Leaf extracts of *Annona reticulata* were screened for the presence of phytochemical constituents like terpenoids, phenols, flavanoids , saponins and others. The medicinal activity of the plants is due the presence of these phytochemicals. For this purpose, extracts of the leaves were prepared in different solvents like water, methanol, acetate, ethyl acetate and hexane and concentrated. The results showed the rich presence of majority of phytochemical constituents which can be correlated with the possible significant medical potential of the plant.
Key words: *Annona reticulata*, phytochemicals, percolation

INTRODUCTION

The family Annonaceae commonly known as the "sour sop family" has been long utilized by communities in forest areas where it is found[1]. The *Annona* genus (Annonaceae) consists of about 119 species, most of which are shrubs and trees widely distributed in the tropical and subtropical regions, including the Southeast Asia countries. In Indian folk medicine, various species of *Annona* have been used as vermifuges, anti-inflammatory agents, in wound healing, as antimalarial agents and in the treatment of diarrhoea and dysentery[2]. *Annona reticulata Linn*, belonging to family *Annonaceae* is a small ever green tree and is cultivated throughout India for its fruits and different parts of this plant are used in folkloric medicine for the treatment of various diseases[3]. *Annona reticulata* is possibly a native of the Carribean and Central America, cultivated and neutralized in many parts of the world. It is found in the wild and cultivated throughout India upto an altitude of 900m. It is found gregariously and widely in the hilly tracts as well as waste lands. In Indian folk medicine, various species of Annona have been used as vermifuges, anti-inflammatory agents, in wound healing, as antimalarial agents and in the treatment of diarrhoea and dysentery[2]. The bark of *Annona reticulata L*. is a powerful astringent and given as tonic. The plant has also been used as anti-anxiety, anti-stress, anti-mutagenic and spasmolytic agent. Leaf and stem extract shows inotropic, positive chronotropic and spasmolytic activities[4]. All these activities are due to the presence of phytochemical substances. Previously reported phytochemical constituents from the plant are anonaine, roemerine, carvone, corydine, linalool, bullacin B, norcorydiene, samoquasine A, motrilin[5,6] etc…

Phytochemicals are naturally occurring biologically active chemical components in plants. The prefix "phyto" is from a Greek word meaning plant. In plants, phytochemicals act as natural defence system for host plants and provide colour, aroma and flavor. More than 4000 of these compounds have been discovered to date and it is expected that scientists will discover many more. Phytochemical aspects of most medicinal plants have been known and used since time memorial. Etanobotanical advantages conferred by these plant-based products have surpassed the chemical counterparts owing to their lesser side effect and more potent therapeutic effect. Natural products continue to play the most significant role in the drug discovery and development process. Hence it is a demanding need to study the various pharmacologically valuable aspects of these medicinal plants.

MATERIALS AND METHODS

Chemicals

The chemicals used include acetone (BP 56 – 57^{0}C), ethyl acetate (BP 77.1^{0}C), methanol (BP 65^{0}C), hexane (BP 68.5 – 69.1^{0}C), benzene (BP 80.1^{0}C), ethanol (BP 78.37^{0}C), chloroform, acetic anhydride, sulphuric acid and hydrochloric acid (Qualigens). All the chemicals used were of analytical grade.

Methodology

Preparation of extracts

The leaves of *Annona reticulata* were collected from Kanya kumari district, Tamilnadu state. The organic extracts of the leaves were prepared with water, acetone, ethyl acetate, methanol and hexane by cold percolation method[7]. The extracts were then stored at 4^{0}C for further tests to be carried out.

Qualitative phytochemical tests

The different qualitative tests can be performed to establish the profile of five extracts for its chemical composition. *Annona reticulata* leaves (SL) aqueous extract (SL-Aq), methanol extract (SL-MOH), acetone extract (SL – Ac), ethyl acetate extract (SL-EA) and hexane extract (SL-Hx) were analysed for the presence of various

phytoconstituents by following standard phytochemical tests.

Molisch's test for carbohydrates

The extracts were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml conc.H_2SO_4 was added carefully along the sides of the test tube. Formation of dull violet/red ring at the interphase indicates the presence of carbohydrates[8].

Test for acids

To 1 ml of extract I ml of sodium bicarbonate solution was added. Formation of effervescence indicates the presence of acids.

Test for betacyanins

To 2 ml of plant extract, 1 ml of 2N NaOH was added and heated for 5 minutes at 100° C. Formation of yellow colour indicated the presence of betacyanins[9].

Test for quinones

To 1 ml of extract, 1 ml of conc.H_2SO_4 was added. Formation of red colour indicated the presence of quinones[10].

Test for coumarins

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

Mayer's test for alkaloids

The extracts were treated with Mayer's reagent (1.36 g mercuric chloride and 5 gms of potassium iodide was dissolved in 100 ml distilled H_2O). The formation of a yellow cream precipitate indicates the presence of alkaloids[11].

Ninhydrin test for aminoacids

To the extract 0.25% Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acids[12].

Biuret test for proteins

Extracts were treated with 1 ml of 10% NaOH solution & heated. To this a drop of 0.7% $CuSO_4$ solution was added. Formation of purplish violet colour indicates the presence of proteins[13].

Benedicts test for reducing sugars

The extracts were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars[14].

Steroids/ terpenoids - Liebermann-Burchard reaction

Freshly prepared LB reagent (5 ml acetic anhydride and 5 ml conc.H_2SO_4) was added to the various extracts. Presence of terpenoid was determined by the development of pink colour whereas green colour showed the presence of steroids[15].

Stain test for fixed oils and fats

Small quantities of the extracts were pressed between 2 filter papers. Formation of an oily stain on the filter paper indicates the presence of fixed oils and fats.

Ferric chloride test for flavanoids

The extract was treated with a few drops of $FeCl_3$ solution. Formation of a blackish red colour indicates the presence of flavanoids[16, 17].

Gums and mucilages

About 5 ml of the extract was slowly added to 5 ml of absolute alcohol under constant stirring. The appearance of precipitation indicates the presence of gums and mucilages[18].

Test for steroids

2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. Change in colour from violet to blue or green indicates the presence of steroids[19].

Test for tannins

To 1 ml of the solvent extract, few drops of 1% $FeCl_3$ solution were added. The appearance of a blue, black, green or blue green precipitate indicated the presence of tannins[20].

Acetone-H_2O test for resins

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

Test for phlobatannins

About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatannins[9].

Salkowski test for terpenoids

To 1 ml of the solvent extract, 2 ml of chloroform was added. Then 3 ml of conc. H_2SO_4 was added carefully to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids[11].

Ferric chloride test for phenols

To 1 ml of solvent extracts, 3 ml of distilled H_2O 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[21].

Foam test for saponins

About 2 ml of distilled H_2O and 1 ml of solvent extract were mixed and shaken vigorously. Formation of a stable persistent froth indicated the presence of saponins[22].

Keller-Killani test for cardiac glycosides

The **e**xtract was dissolved in glacial acetic acid containing traces of $FeCl_3$. The tube was then held at an angle of 45° and 1 ml of conc. H_2SO_4 was added along the sides of the tube. Formation of a purple ring at the interface indicates the presence of cardiac glycosides[23].

Borntrager's test for anthraquinones

Small portion of the extract was shook well with 10 ml benzene and filtered. 5 ml of 10% ammonia solution was added to the filtrate and stirred. The production of a pink red or violet colour indicates the presence of free anthroquinones[8, 24].

Test for volatile oils

To 1 ml of the extract, 1 ml of 90% ethanol was added, followed by the addition of a few drops of $FeCl_3$ solution. Formation of a green colour indicated the presence of volatile oils in the given sample[20].

Test for emodols

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

Test for starch

To 1 ml of the extract 10 ml of saturated $NaCl_2$ solution was added. It was then heated. After heating, starch reagent was added. Formation of a blue-purplish/pink colour is a positive test for the presence of starch[25].

Test for fatty acids

0.5 ml of extract was mixed with 5 ml 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 indicates the presence of fatty acids[26].

RESULTS AND DISCUSSION

Plants have been used to treat or prevent illness from ancient times. *Annona reticulata* have also gained organic chemists's and biochemist's attention because of the presence of novel compounds with wide range of bioactivities. A root decoction is taken as a febrifuge, while fragments of the root bark are packed around the gums to relieve toothache. The bark is very astringent and the decoction is taken as a tonic and also as a remedy for diarrhea and dysentery. Crushed leaves may be applied to boils, abscesses and ulcers. The unripe dried fruit is employed against diarrhea and dysentery[27].The leaf extracts were evaluated for their phytochemical constituents. Among the four Hexane < Ethyl acetate < Acetone < Methanol < Aqueous) leaf extracts, the ethyl acetate and methanolic leaf extracts were found to contain major phytochemicals. Phenoloc compounds, flavanoids, tannins and emodols were abundantly present in methanol, acetone and ethyl acetate leaf extracts. Betacyanin was present in all the extracts of *Annona reticulata* leaves. Aqueous extracts recorded less separation of compounds from the leaf extracts. On the other hand, other components like carbohydrates, starch, quinones, cardiac glycosides, alkaloids, saponins, volatile oils and terpenoids were also found to be present in varying amounts in the different extracts of leaves (Table.1).

Table: 1 Preliminary phytochemical analysis of the leaves of *Annona reticulata L.*

Sl.No	Tests	Different solvent extracts of leaves (SL)				
		SL-Aq	SL-MOH	SL-Ac	SL-EtAc	SL-Hx
1.	Carbohydrates	-	-	-	-	-
2.	Starch	-	-	-	+++	++
3.	Reducing sugar	-	+++	+++	+++	-
4.	Amino acids	-	-	-	-	-
5.	Proteins	-	-	-	-	-
6.	Acid	-	-	-	-	-
7.	Quinones	-	+	-	-	-
8.	Coumarins	-	+++	++	++	-
9.	Gums and mucilages	-	-	-	-	-
10	Steroids	-	++	++	+++	+
11	Tannins	-	+++	+++	+++	+
12.	Phlobatannins	-	-	-	-	-
13.	Phenols	-	+++	+++	+++	-
14.	Cardiac glycosides	-	-	-	-	+
15.	Alkaloids	++	-	-	++	+
16.	Anthraquinones	-	-	-	-	-
17.	Betacyanins	++	+	+	+++	+++
18.	Emodols	++	+++	+++	+++	-
19.	Saponins	+	+	-	-	-
20.	Volatile oils	-	++	+	+++	-
21.	Flavanoids	++	+++	+++	+	-
22.	Terpenoids	-	-	-	-	+
23.	Resins	-	-	-	-	-
24.	Fixed oils and fats	-	-	-	-	-

(+) ------ Low levels
(++) ------ Moderate levels
(+++) ------ High levels

Kamaruz Zaman and Kalyani Pathak, 2013[28] reported the presence of maximum number of active constituents in the methanol extract of leaves of this plant when compared with petroleum ether, acetone, chloroform and aqueous extracts. Their studies on leaf extracts also revealed the presence of carbohydrate, fats and oils, terpenoids, flavonoids, amino acids, tannins and phenolic compounds, alkaloids, glycosides and steroids.

These secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. Plants in all facets of life have served as a valuable starting material for drug development. For example, saponins have hypotensive and cardio depressant properties[29]. Saponins cause hemolysis of red blood cells[30]. Tannins and flavonoids are thought to be responsible for antidiarrheal activity[31]. Tannins have been widely used topically to sprains, bruises and superficial wounds as such[32]. These groups of phenolic compounds have been found to form irreversible complexes with proline rich proteins resulting in the inhibition of cell protein synthesis[33]. Tannins also possess free radical scavenging activity[34]. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia[35]. Betacyanins were identified by *in vitro* methods as antioxidant[36] which may protect against oxidation of low-density lipoproteins[36]. Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes[37]. Their consumption can reduce the cancer risk[38, 39]. Proteins contributed to the structure and functions of the living cell, they occur as independent units as well as in combination with lipids, nucleic acids, carbohydrates and many other compounds[40]. Terpenoids are attributed for their analgesic and anti-inflammatory activities and flavonoids are have been reported to possess many useful properties, including antiinflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumour activity[41]. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical functions[42]. Steroids are partly responsible for the anti-diarrheal activity[43]. Therefore, the presence of appreciable to moderate amounts of these phytochemicals can be correlated with the possible significant medicinal potential of the plant.

CONCLUSION

The present study demonstrated that different leaf extracts of *Annona reticulata* are excellent source of bioactive phytocompounds like quinones, coumarins, steroids, tannins, phenols, cardiac glycosides, alkaloids, flavanoids and terpenoids. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. These findings can form the basis of further studies to isolate, identify, characterize and elucidate the structure of the bioactive compounds, to find new therapeutic principles. Thus the phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery.

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