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A comparative study of phytochemical screening of wild variety of *Annona reticulata* L. and *Annona squamosa* L.

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Abstract- Plant based medicines have been a part of traditional healthcare in most parts of the world for thousands of years. Its civilization is very ancient and the country as a whole has long been known for its rich resources of medical plants. The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicines. There is an increasing demand for the herbal drug treatment of various ailments and many plant drugs from ayurvedic system are being explored globally. Studying the plants used in folklore medicine promises to yield commendable results as investigating their medicinal properties has led to a better understanding of the use of traditional medicines as potential drugs in addition to contemporary drugs. *Annona squamosa* L. is extensively studied for its medicinal properties by advanced scientific techniques and a variety of bioactive compounds have been isolated from the different parts of the plant and were analysed pharmacologically. In our present investigation, the comparative phytochemical screening between *Annona squamosa* and *Annona reticulata* leaf extracts revealed the presence of various bioactive compounds. Leaf extracts of *Annona reticulata* and *Annona squamosa* 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, and ethanol. 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: Methanolic extract, Secondary metabolite, *Annona squamosa*, *Annona reticulata*

INTRODUCTION

The family Annonaceae belongs to the family of flowering plants consisting of trees, shrubs, or rarely lianas, commonly known as the custard apple family^{1,2} or soursop family. This family includes 108 accepted genera and about 2400 known species, it is known as the largest family in the order Magnoliales. The plant *Annona squamosa* and *Annona reticulata* belongs to this family.

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Most of the species of genus *Annona* are mostly known for their edible fruits.^{3,4} The species of *A. squamosa* are generally found in Thailand, West Indies and South America. It is cultivated in over all part of the world because its testy edible fruits. The leaves of this plant considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is considered as a drastic purgative.⁵ The crushed leaves are used to overcome hysteria fainting spells and in case of dysentery also.⁶ Apart from this, leaves are also used to treat boils and ulcers. The powder of

unripe fruit is also used to destroy vermin and seeds are poisonous in nature. Seed powder is used in killing fish and insects and also applied on head to kill lice and wound worm of cattle.⁷ Both primary and secondary metabolites are found in this plant. Primary constituents comprises of proteins, fats, carbohydrates, amino acids where as secondary metabolites constitutes phenols, tannins, alkaloids, terpenoids, saponins and phenols.⁸ It has been proved by many literature that every part of *A. squamosa* posses medicinal properties.⁹⁻¹²

The another plant *Annona reticulata* also belongs to the family Annonaceae. This family, commonly known as “sour- soup family” has long been utilized by native indigenous people of that area where it is found.^{13,14} This plant has been used as a anti-inflammetry agent, vermifuges, treatment in wound healing, and as a anti-malarial agent in the treatment of dysentery and diarrhea.¹⁵ *A. reticulata* is a small evergreen tree found in overall India and cultivated for its fruits and different parts which are used in traditional folkloric medicine for the treatment of various disease.¹⁶ Bark of this plant is a powerful astringent and given as a tonic. Plants are used as anti-anxiety, anti-stress and anti- mutagenic. Leaves are used in inotropic, positive chronotropic and spasmolytic activities.¹⁷ All the above activities are occurring due to presence of various types of secondary metabolites. These metabolites are anonaine, roemerine, carvone, corydine, linalool, bullacin B, norcorydiene, samoquasine A, motrilin etc.^{18,19} The chemical Annonaceous acetogenins are anti-neoplastic agents isolated from Annonaceae plants.²⁰ Recently, Annonaceous acetogenins showed activity against anti-cancer drugs for multidrug resistant (MDR) cancers. The Phytochemicals are exclusively found in plants commonly known as secondary metabolic compounds. The major function of this compounds are known to provide protection against insect attacks and plant diseases, stimulation of the immune system, hormone modulation metabolism and antibacterial and antiviral activity.^{21,22} The most important bioactive constituents of plants are Alkaloids, Tannins, Flavonoids, Cardiac glycosides, Steroids and Saponins.^{22,23}

MATERIAL & METHODS

Plant materials

The plant materials of both species of *Annona* (*A. reticulata* & *A. squamosa*) were collected from the

Morabadi, Ranchi. The healthy and fungus free leaves were plucked carefully and washed properly in running water. Leaves were dried and they were ground with the help of mixture grinder into powder form. The powder was stored in dry place until used for further experiment.²⁴

Extraction of plant material

The leaf extracts of both the species were prepared with water, acetone and methanol. The prepared extract of both species of *Annona* were stored in an air tight container at 4 degree for further study.²⁵ The organic extracts of the leaves were prepared with, acetone, methanol and ethanol by cold percolation method.²⁶

Phytochemical screening

Phytochemical screening can be performed by establishing the profile of both plant extract with its various chemical composition. These all were analysed for the presence of various phytochemicals with the help of following standard phytochemical test.

Molisch's test for carbohydrates

The plant extracts were treated with 2 drops of alcoholic α -naphthol solution in a test tube. After that 2 ml conc. H_2SO_4 was added carefully along the sides of the test tube. A violet/red ring will formed at the interphase indicates the presence of carbohydrates.²⁷

Mayer's test for alkaloid

For the test of alkaloids the plant extracts were treated with Mayer's reagent (1.36 g mercuric chloride and 5 gm of potassium iodide was dissolved in 100 ml distilled H_2O). A yellow cream precipitate was formed, indicates the presence of alkaloids.²⁸

Ninhydrin test for aminoacids

To check the presence of amino acid 0.25% Ninhydrin reagent was added in plant extracts and boiled for a few minutes. Appearance of blue colour indicates the presence of amino acids.²⁹

Biuret test for proteins

Plant Extracts were treated with 1 ml of 10% NaOH solution & heated, after that a drop of 0.7% $CuSO_4$ solution was added. Purplish violet colour was formed indicates the presence of proteins.³⁰

Salkowski test for terpenoids

Take 1 ml of the solvent extract and 2 ml of chloroform was added in it. 3 ml of conc. H_2SO_4 was added carefully, a layer will formed. Reddish brown coloration is the indication of the presence of terpenoids.²⁸

Ferric chloride test for flavanoids

The plant extracts were treated with a few drops of FeCl₃ solution. Formation of a blackish red colour is the indication of the presence of flavanoids.^{31,32}

Test for steroids

Take 2 ml of acetic anhydride and it was added to 0.5 g plant extract of each sample with 2 ml H₂SO₄. Colour changing from violet to blue or green indicates the presence of steroids.³³

Test for tannins

Take 1 ml of the solvent extract and add a few drops of 1% FeCl₃ solution. Appearance of a blue, black, green or blue green precipitate indicated the presence of tannins.³⁴

Test for phlobatannins

About 2 ml of plant aqueous extract was added to 2 ml of 1% HCl. The mixture was boiled. A red precipitate was formed, indicates the presence of Phlobatannins.³⁵

Ferric chloride test for phenols

Take 1 ml of solvent extracts and 3 ml of distilled H₂O was added in it. Neutral 5% FeCl₃ solution was added in it in a drop wise manner. Formation of a dark green colour indicated the presence of phenol.³⁶

Foam test for saponins

About 2 ml of distilled H₂O and 1 ml of solvent extract were taken and mixed and shaken vigorously. Formation of a stable persistent froth indicated the presence of saponins.³⁷

Keller-Killani test for cardiac glycosides

The plant extracts was dissolved in glacial acetic acid containing traces of FeCl₃. Then tube was held at an angle of 45°. 1 ml of conc. H₂SO₄ was added along the sides of the tube carefully. Formation of a purple ring at the interface indicates the presence of cardiac glycosides.³⁸

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.³⁹

RESULT & DISCUSSION

Both the species of *Annona* have been used from ancient times as traditional medicine. This is because of the presence of noval compounds with wide range of bio active compounds. The leaf extract were evaluated for their phytochemical estimation, methanolic extract were found to contain major phytochemicals as compared to

other solvents. The secondary metabolites and other chemical constituents of medicinal plants are used for their medicinal value. This represents the potential medicinal value present in these plants. Powdered extract of both species of *Annona* (*A. reticulata* & *A. squamosa*) were subjected to various qualitative tests for the determination of phytochemicals using specific reagents. The results are tabulated below.

Table 1- Phytochemical Screening of *A. squamosa* leaves in various extracts.

Phytochemicals	Aqueous	Methanol	Acetone
Carbohydrate	+	+	-
Alkaloids	+	+	+
Aminoacids	+	+	+
Proteins	+	+	-
Tarpenoids	-	+	-
Flavonoids	+	-	+
Steroids	+	-	-
Tannins	+	+	+
Phlobatannins	+	+	+
Phenols	+	+	+
Saponins	-	+	+
Cardiac glycosides	-	+	-
Fatty acids	+	-	-

‘+’ present, ‘-’ absent

Table 2- Phytochemical Screening of *A. reticulata* leaves in various extracts.

Phytochemicals	Aqueous	Methanol	Acetone
Carbohydrate	-	-	-
Alkaloids	+	+	-
Aminoacids	-	-	-
Proteins	-	-	-
Tarpenoids	-	-	-
Flavonoids	+	+	+
Steroids	+	+	+
Tannins	-	+	+
Phlobatannins	-	-	-
Phenols	-	+	+
Saponins	-	+	+
Cardiac glycosides	-	-	-
Fatty acids	+	-	-

‘+’ present, ‘-’ absent

CONCLUSION

The present study demonstrated that the leaf extract of both species prepared from different solvent having

excellent source of bioactive compound like alkaloids, flavonoids, phenol, tannins etc. the phytochemical analysis is very important because its help in identifying new therapeutically and industrially important compounds. These research may perform as a platform for further studies about the isolation, identification, characterization and structural studies of these bioactive compounds. Apart from these screening tests may also helpful in detection of bioactive compounds and also useful in drug industry. Thus from present study we finally concluded that among three solvent maximum bioactive compounds were found in the methanolic extract in both species of *Annona*. Minimum bioactive compounds were found in aqueous extract. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs, multipurpose medicinal drug, further clinical etc..

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