INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

PHYTOCHEMICAL SCREENING OF *ANNONA SQUAMOSA* (L.) SEED EXTRACTS: A POTENTIAL SOURCE OF ETHNOMEDICINE

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ARTICLE INFO

Article history
Received 23/01/2017
Available online
30/04/2017

Keywords
Medicinal Plants,
Annona Squamosa,
Phytochemical Screening,
Bioactive Compounds.

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. In India, more than 7000 medicinal plant species are known. 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, phytochemical screening of *Annona squamosa* seed extracts revealed the presence of various bioactive compounds. The results lend credence to the folkloric use of this plant and show that *Annona squamosa* seeds could be exploited therapeutically for novel potent drugs.

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*Please cite this article in press as **Vanitha Thangaraj** et al. Phytochemical Screening of Annona Squamosa (L.) Seed Extracts: A Potential Source of Ethnomedicine. Indo American Journal of Pharmaceutical Research.2017:7(04).*

INTRODUCTION

From time immemorial, man depended on plants as medicine. From a historical perspective, it is evident that the fascination for plants is as old as mankind itself. The plant kingdom represent a rich store house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years [1]. Plants are still an independent source of medication in the contemporary health care delivery system. Their role is twofold in the development of medicines and served as a natural blue print for the development of new drugs, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [2]. In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species [3]. According to the WHO more than 80% of the world's population relies on traditional herbal medicine for their primary health care [4]. Recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their traditional uses [5].

Annona squamosa L., the plant of Annonaceae family, also known as custard apple, is commonly found in deciduous forests, also cultivated in wild in various parts of India. It is a native of West Indies; now cultivated throughout India and other tropical countries. Literatures of many research works prove that every parts of *A. squamosa* possess medicinal property [6-9]. It is considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is considered as a drastic purgative [10]. An infusion of the leaves is considered efficacious in prolapsusani of children. The crushed leaves are sniffed to overcome hysteria and fainting spells. A leaf decoction was taken in the case of dysentery [11]. Leaves are used as poultice over boils and ulcers. The ripe fruits of this plant are applied to malignant tumors to hasten suppuration. The dried unripe fruit powder is used to destroy vermin. The seeds are acrid and poisonous. Powdered seeds serve as fish poison and insecticides. A paste of seed powder has been applied to the head to kill lice. It is also used for destroying worm in the wound of cattles [12]. The medicinal value of the plants lies in some active chemical substances called phytochemicals that produce a definite physiological action on the human body. Phytochemicals are divided into two groups, which are primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, aminoacids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, flavonoids, tannins, phenolic compounds [13]. In view of its medicinal value, the present study is aimed to screen the pharmaceutically important bioactive substances from *A. squamosa* seeds that greatly contribute the nutraceutical and drug research.

MATERIALS AND METHODS

Collection and identification of plant material

The specimen was collected from Coimbatore and authenticated by Botanical Survey of India, Coimbatore, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SRC/5/23/2017/Tech.2189). The seeds of *A. squamosa* were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter. After complete drying, young leaves were powdered well using a mixer. Then the powdered material was weighed and kept in air tight container and stored in a refrigerator for future use. About 10g of this powdered sample was refluxed with petroleum ether, methanol and water in the ratio of 1:10 (w/v). The crude extracts were collected in amber coloured sample bottles and stored. All chemicals and reagents used including the solvents were of analytical grade.

Phytochemical Screening

Phytochemical screening was carried out in the petroleum ether, methanol and aqueous extracts of *A. squamosa* seeds using standard procedures [14-16].

Test for alkaloids

Dragendroff's test

To 5 mL of the extract few drops of Dragendroff's reagent was added for the formation of orange coloured precipitate.

Mayer's test

To 5 mL of the extract few drops of Mayer's reagent was added for the formation of cream coloured precipitate.

Wagner's test

To 5 mL of the extract few drops of Wagner's reagent was added for the formation of reddish brown coloured precipitate.

Hager's test

To 3 mL of the extract few drops of Hager's reagent was added for the formation of prominent yellow precipitate.

Test for flavonoids

To 3 mL of the extract few magnesium ribbons are dipped and conc. HCl was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoids.

Test for proteins

Biuret test

To 3 mL of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple color. On addition of alkali, it becomes dark violet.

Millon's test

To 3 mL of the extract few drops of Millon's reagent was added for the formation of red colour.

Test for carbohydrates

Molisch's test

To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 mL of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

Fehling's test

The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugar.

Test for tannins

A fraction of the extract was dissolved in water and then it was subjected to water bath at 37°C for 1 hour and treated with ferric chloride solution and observed for the formation of dark green colour.

Test for sterols

Liebermann-Burchard test

To a small amount of the extract few drops of chloroform, acetic anhydride and H_2SO_4 was added along the sides of the test tube to observe the formation of dark red or pink colour.

Test for glycosides

Baljet's Test

To 5 mL of the extract few drops of sodium picrate was added to observe yellow to orange colour.

Keller-Killiani test

To 5 mL of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides.

Test for phenols

Ferric chloride test

A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

Test for saponins

Foam test

To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam was observed.

Test for terpenoids

Chloroform test

To 5 mL of the extract few drops of chloroform and conc. H_2SO_4 was added carefully along the sides of the test tube to form a layer and observed for the presence of reddish brown colour.

RESULTS AND DISCUSSION

Phytochemical Screening

Powdered *A. squamosa* seed extracts were subjected to various qualitative tests for the identification of phytochemical constituents includes tests for alkaloids (Dragendroff's test, Mayer's test, Hager's test, Wagner's test), saponins, glycosides (Baljet's test, Kellar-Killiani test), carbohydrates (Molisch's test, Fehling's test), proteins (Biuret test, Xanthoprotein test, Millon's test), tests for tannins, flavonoids, steroids (Liebermann-burchard test), phenols, terpenoids were performed using specific reagents and results are tabulated in Table 3.

Table 3: Preliminary Phytochemical Screening of *A. squamosa* seeds in various extracts.

Phytochemicals	Aqueous	Methanol	Petroleum ether
Alkaloids	+	+	+
Flavonoids	+	-	+
Proteins	+	+	-
Carbohydrates	+	+	-
Tannins	+	+	+
Sterols	+	-	-
Glycosides	-	+	-
Phenols	+	+	-
Saponins	-	-	-
Terpenoids	-	+	-

'+' present, '-'absent.

Phytochemical screening results of the powdered sample of *A. squamosa* seeds extracted in water and methanol showed the presence of all the constituents whereas the petroleum ether extract showed the presence of very few bioactive compounds. Chemical investigation on the different parts of the plant has resulted in the isolation of a large number of novel and interesting metabolites [17].

CONCLUSION

Plant derived substances have recently become great interest due to their multiple application. Medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicine pharmaceutical intermediates and chemical entities for synthetic drugs. Researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs. Results of phytochemical screening revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, tannins, phenols, glycosides, saponins and terpenoids. Of all the extracts, the methanolic extract of the *A. squamosa* seed showed the presence of maximum bioactive compounds. The phytochemical screening of the present study showed favorable effects for the standardization parameters of plant parts. In the present study, the preliminary phytochemical screening of the various extracts revealed the presence of major bioactive compounds which may retain a wide range of actions. The antibacterial activity of the young leaves may be due to the presence of various active principles in their leaves. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs. Thus, *A. squamosa* seeds are quite promising as a multipurpose medicinal agent so further clinical trials should be performed to prove its efficacy.

ACKNOWLEDGEMENT

The authors are grateful to the Management, Hindusthan Educational and Charitable Trust and Principal, Staff of Hindusthan College of Arts and Science, Behind Nava India, Coimbatore, Tamil Nadu, India for the use of facilities and encouragement.

CONFLICTS OF INTEREST

None declared.

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