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Qualitative Phytochemical Screening of *Annona squamosa* Leaf Extract and *Terminalia arjuna* Bark Extract

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Abstract
Annona squamosa and Terminalia arjuna are medicinal plants used to treat various ailments. *A. squamosa* leaf extract and *T. arjuna* bark extract studied for presence of Phytochemical constituents in chloroform, benzene, methanol, ethanol, and aqueous extracts prepared through Soxhlet extraction method. Various methods were used to determine the presence of phytochemicals. Carbohydrates, alkaloids, flavonoids, tannins, steroids, saponins, glycosides, phenols and terpenoids were found in both extract of *A. squamosa* and *T. arjuna* whereas protein and starch were found in *A. squamosa* but not in *T. arjuna*. This study is useful for further use of plants or plant parts for antimicrobial studies and biomedicine.

Keywords
Phytochemical, Biomedicine, Antimicrobial, Qualitative

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1. INTRODUCTION
From time immemorial, human depended on plants for medicine. The plants are rich source of organic compounds and primary and secondary metabolites. Many plants have been used for medicinal and therapeutic purposes and could serve as lead for the development of novel agents due to good efficacy in treating various pathological disorders (Khan et al., 2014). Plants are still an independent source of medication in the health care delivery system. Their role is crucial in the development of medicines for various disorders and served as a important part for the development of drugs, modern medicines, food supplements, folk medicines and various pharmaceutical intermediates (Hammer et al., 1999). In the recent past there has been a tremendous increase in the use of plant products in developing as well as developed countries. World Health Organization has researched to identify all medicinal plants used globally for medicinal purpose and listed more than 20,000 species worldwide (Tijani et al., 2008). According to the WHO more than 80% of the population in the world relies on traditional herbal medicine for their primary health problems (Pandey et al., 2008). Recently much attention has directed towards plant extracts and biologically active compounds isolated from 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 and presence of useful phytochemical constituents (Nataraj et al., 2014). *Annona squamosa*, commonly known as custard apple, sugar apple, sweet sop, sweet custard apple, and sitaphal, is a member of the *Annonaceae* family, which includes over 135 genera and 2300 species (Raj et al., 2009; Srivastava et al., 2011). Plants having a long legacy in ethno-medicine are rich source of active Phyto-constituents, providing therapeutic and health advantages for a variety of maladies and diseases (Li et al., 2015; Xiao

et al., 2015). *Annona squamosa* is one of these plants having a long history of use. It is considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is considered as a drastic purgative (Raj et al., 2009). An infusion of the leaves is considered beneficial in prolapsus ani of children. The crushed leaves are used to overcome hysteria and fainting spells. Leaf decoction was taken in the case of dysentery (Gajalakshmi et al., 2011). Leaves are used as poultice over boils and ulcers. The ripe fruits are used for malignant tumors to hasten suppuration. The dried unripe fruit powder is used to kill vermin. *Terminalia arjuna L. (Combretaceae*) is a large perennial deciduous tree (often known as Arjuna) that grows to a height of 20-25 meters in India. The stem, fruits, bark, and leaves of *Terminalia arjuna* is used in biomedical and pharmaceutical field. Renewed leaf juice is used to alleviate relief in earaches, while root paste is used to treat headaches. In south India, fruit paste is applied topically as a traditional healer (Muthu et al., 2006). The bark has been the most important portion utilized in Ayurveda and allopathy to treat many diseases. Snakebite and scorpion stings are treated with bark ash (Jain et al., 2009). It acts as an astringent, cardiotonic, antidysentery, urinary astringent, hypertension, antidysentery hemorrhage, blood-related diarrhea, liver cirrhosis, hypertension inflammation, and skin disorder *(Soni et al., 2019).*

2. MATERIALS AND METHODS

2.1 Collection and Identification of plant material

In the present study leaves of *Annona squamosa* and bark of *Terminalia arjuna* is used for the study. Fresh leaves of *Annona squamosa* and bark of *Terminalia arjuna* were collected from Sajjan Niwas bagh, Udaipur between June to august and air dried.

Plant material was labeled, numbered, noted with the date of collection, locality, and their medicinal uses were recorded. These plant samples were subjected to qualitative Phyto-chemical screening of metabolites. For the authentication and validation of plants herbarium specimen were prepared with taxonomical affiliations.

2.2 Preparation of plant Materials

Healthy and non-infected leaves of *Annona squamosa* and bark of *Terminalia arjuna* were washed thoroughly with running tap water and twice with autoclaved distilled water to remove dust particles. Plant parts were then air dried at room temperature for at least 7 days in shade. Then dried plant materials of all plants were then ground with the help of mixer grinder to a fine powder. The ground material was passed through sieve of mesh size 60 mm to obtain a fine powder and stored in glass bottles with proper labeling.

2.3 Preparation of Plant Extracts

2.3.1 Aqueous Extraction

For preparation of aqueous extract 20 g dried plant samples of *Annona squamosa, Terminalia arjuna* were soaked in 100 ml sterile distilled water in Two separate conical flasks. These flasks were kept for water bath at 80° C for 10 min. Then the solutions were filtered using whatman filter paper no.1 followed by vacuum filtration using vacuum filtration unit with filter paper of 0.2µm diameter (Pandey et al., 2014). The filtrate thus obtained were weighed and stored at 4ºC for further phytochemical analysis.

2.3.2 Soxhlet Extraction

Different partially purified organic constituents were successively separated from dried powder of plant samples by reflux method of solvent extraction (Harborne, 1984). The solvents are arranged in a series from non-polar to polar nature in a manner mentioned below: Benzene → Chloroform →Ethanol →Methanol →Water

50 gm of leaf powder was placed in porous cellulose thimble. The thimble was then placed in an extraction chamber above the collection flask and the flask were filled with 250 ml Benzene solvent. The solvent was heated and evaporate with the adjustment of boiling temperature of the solvent. The extraction process lasted for 6 to 8 hours and the flask containing the solvent and extract was removed. The leaves residue remained was dried in an oven below 50°C and used for extraction with next solvent in series. Fractions obtained at every step including aqueous fraction were vacuum dried in a rotary evaporator and the dried residue was used as extract. The extracts thus obtained were weighed and stored at 4ºC for further phytochemical analysis. The DMSO (Dimethyl sulfoxide) is act as dissolved solvents for these extracts. The dried extract was used for calculation of percent extractive value which was calculated by using formula mentioned below:

$$\textbf{Percent extractive value} = \frac{\textbf{weight of dried extract}}{\textbf{weight of dried plant material}} \times 100$$

2.4 Preliminary Phytochemical screening

Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. secondary constituents include the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrates (Marslin et al., 2018). Phytochemical analysis was carried out for the extract as per standard methods (Evans, 2000).

2.4.1 Test for carbohydrates: Detection of reducing sugar (Fehling test)

1 gm of each plant extract was added in 5 ml of distilled water taken in separate test tubes. Then 1 ml of ethanol was mixed in each plant extract. To this mixture 1 ml of Fehling solution A and 1 ml of Fehling solution B was added and the mixture was observed for the color reaction to occur. The appearance of a brownish red precipitate indicates the presence of reducing sugars in the samples.

2.4.2 Detection of protein (Biuret Test)

The detection of protein was primarily done by Biuret test. 0.1 gm of each extract was treated with 1 ml of 10% NaOH solution and two drops of 0.1% copper sulphate solution. The mixture was then observed for the formation of violet/pink color that indicates the presence of protein in the sample.

2.4.3 Test for Alkaloids

- **Mayer's test:**

About 1ml of aqueous plant extracts of each plant was taken in separate test tubes. Then Few drops of Mayer's reagent were added. Formation of White or pale-yellow precipitation indicated the presence of alkaloids.

- **Wagner's test:**

About 1ml of plant extract is taken and then few drops of 5% HCl was added in extract and a few drops of Wagner's reagent were added to it. Appearance of yellow/brown precipitate indicates the presence of alkaloids.

2.4.5 Test for Flavonoids:

Extracts taken in separate tubes were treated with 2ml of sodium hydroxide solution. On addition of sodium hydroxide, if an intense yellow colour is formed, which disappears on addition of dilute acid indicates the presence of flavonoids.

2.4.6 Test for tannins (Braymer'sTest):

1ml of the extracts suspended in different tubes, 1ml of 0.1% Ferric chloride solution was added and observed for the formation of colour. Formation of a dark blue or greenish black colour product indicates the presence of tannins.

2.4.7 Test for Steroids:

Crude extract of each plant was taken in separate test tube and then 10 ml of chloroform was added, shaken well and filtered through Whatman filter paper. The filtrate was used as a test solution. To 2 ml of test solution few drops of acetic anhydride were added. The mixture was then boiled and cooled. Concentrated H_2SO_4 was then added from the sides of the test tubes and observed for the formation of a brown ring at the junction of two layers. Green colored layer indicates a positive test for presence of steroids.

2.4.8 Test of Saponins:

0.5 gm of extract taken in different tubes was shaken with 2 ml of water. This led to the formation of foam that if persists for around ten minutes, indicates the presence of saponins.

2.4.9 Test for Anthrocyanin and Betacynin:

About 1 ml plant extract was treated with1ml of 2N NaOH then heated. Formation of bluish green colour confirms anthrocyanin while yellow color indicates the presence of betacy.

Test for Glycosides: 1ml of plant extract, 1ml of $FeCl_3$ with equal amount of acetic acid was added. Then few drops sulphuric acid was added to the mixture. Green blue colour indicates the presence of glycosides.

2.4.10 Test for phenols: Few drops of $FeCl_3$ added to 1 ml of plant extract which give blue green colour, indicates the presence of phenol.

2.4.11 Test of Resins: 1 ml ethanolic plant extract was dissolved in acetone and then 1ml of deionised water is added. Turbidity indicates the presence of resin.

2.4.12 Test for starch: 1 ml of plant extract mixed with 1 ml of iodine solution, blue colour was formed which indicates the presence starch.

2.4.13 Test for Terpinoids: The aqueous plant extracts (2ml) were taken in clean test tubes. 2ml of acetic acid was added and followed by few drops of concentrated sulphuric acid along with the samples. The development of deep red color indicated the presence of terpenoids.

2.4.14

3. OBSERVATION

Table 1: Percent extractive values of different solvent extracts of selected plant

Solvent	*A. squamosa*	*T. arjuna*
Ethanol	12.56%	13.73%
Benzene	2.81%	0.97%
Chloroform	0.76%	1.16%
Methanol	16.06%	20.68%
Water	12.30%	19.04%

Table 2: Phytochemical screening of different solvent extracts of *Annona squamosa*

Phytochemicals	Ethanol	Benzene	Chloroform	Methanol	Aqueous extract
Carbohydrates	+	+	–	+	+
Alkaloids	+	+	–	+	+
Flavonoids	+	+	+	+	+
Proteins	+	–	–	–	+
Tannins	–	–	+	+	+
Steroids	+	–	+	–	+
Saponins	–	–	–	+	+
Anthrocyanin	–	–	–	–	–
Betacynin	–	–	–	–	–
Glycosides	–	–	+	+	+
Phenols	+	–	+	+	+
Resins	–	–	–	–	–
Starch	–	–	–	–	+
Terpinoids	–	–	–	–	+

*(+) = presence of phytochemicals; (–) = absence of phytochemicals

Table 3: Phytochemical screening of different solvent extracts of *Terminalia arjuna*

Phytochemical tests	Ethanol	Benzene	Chloroform	Methanol	Aqueous extract
Carbohydrates	+	+	–	+	+
Alkaloids	–	–	+	+	+
Flavonoids	+	–	+	+	+
Proteins	–	–	–	–	–
Tannins	+	+	–	+	+
Steroids	+	–	–	+	+
Saponins	+	+	–	+	+
Anthrocyanin	–	–	–	–	–
Betacynin	–	–	–	–	–
Glycosides	+	–	–	+	+
Phenols	+	–	–	+	+
Resins	–	–	–	–	–
Starch	–	–	–	–	–
Terpinoids	+	–	–	+	+

*(+) = presence of phytochemicals; (–) = absence of phytochemicals

4. RESULTS

The percent extractive values of different solvent extracts of *Annona squamosa and Terminalia arjuna* was calculated (Table 1) as highest yield for *A. squamosa* was observed in aqueous extract and minimum yield was noted for chloroform extract. On the other hand, maximum yield for *T. arjuna* was showed in ethanolic extract and lowest yield was found in benzene extract.

The preliminary phytochemical screening of leaf extract of *Annona squamosa* showed the occurrence of several phytochemical compounds (Table 2). The leaf extract of *Annona squamosa* have a number of phytochemical compounds, including carbohydrates, alkaloids, flavonoids, proteins, tannins, steroids,

saponins, glycosides, phenols, starch, and terpinoids, while phytochemical compounds such as anthrocyanin, betacynin and resins were not detected in the aqueous leaf extract of *Annona squamosa*.

The preliminary phytochemical screening of bark extract of *Terminalia arjuna* showed the occurrence of several phytochemical compounds (Table 3). The bark extract of *Terminalia arjuna* have a number of phyto-chemical compounds, such as carbohydrates, alkaloids, flavonoids, tannins, steroids, saponins, glycosides, phenols and terpinoids, whereas some phytochemical compounds such as proteins, anthrocyanin, betacynin, resins and starch were not identified in the aqueous bark extract of *Terminalia arjuna*.

The leaf extract of *Annona squamosa* have a number of phytochemical compounds, including carbohydrates, alkaloids, flavonoids, proteins, tannins, steroids, saponins, glycosides, phenols, starch, and terpinoids, while phytochemical compounds such as anthrocyanin, betacynin and resins were not detected in the aqueous leaf extract of *Annona squamosa*. The bark extract of *Terminalia arjuna* have a number of phyto-chemical compounds, such as some phytochemical compounds such as proteins, anthrocyanin, betacynin, resins and starch were not identified in the aqueous bark extract.

5. DISCUSSION

Plant derived substances have recently gain great interest due to their various application in different fields (Hossain et al., 2011). Medicinal plants are very good bio-resource of drugs of traditional system of medicine, pharmaceutical intermediates and chemical entities for drug synthesis (Kokate, 1997). Results of phytochemical screening revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, tannins, phenols, glycosides, saponins and terpenoids. Of all the extracts, the aqueous extract of the *A. squamosa* leaves showed the presence of maximum bioactive compounds. Methanolic extract of *Terminalia arjuna* bark show the presence of maximum bioactive compounds. All these secondary metabolites components showed antioxidant and antimicrobial properties through different mechanism. Normally these secondary metabolites components were isolated from the polar plant extract (Hossain et al., 2011).In the present study, the preliminary phytochemical screening of the various extracts revealed the presence of major bioactive compounds which may have a wide range of efficacy. The antibacterial activity of the bark and leaves of the respective plants revealed the presence of various active principles. Several authors already reported on flavonoids groups exhibited a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-allergic (Ayoola et al., 2008). Further studies are also needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs. Thus, *A. squamosa* leaf extract and *Terminalia arjuna* bark extract is quite promising as a multipurpose medicinal agent so further clinical trials should be performed to prove its efficacy.

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