



ORIGINAL RESEARCH PAPER

Life Sciences

PHYTOCHEMICAL SCREENING OF AQUEOUS AND ETHANOLIC, STEM EXTRACT OF ANNONA SQUAMOSA LINN.

KEY WORDS: Annona squamosa, Phytochemical, Qualitative analysis

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ABSTRACT

Annona squamosa is an evergreen shrub belonging to the family Annonaceae. These plants have medicinal importance, widely used in preparation of anti head like medicines. It has anticancerous activity, used as antioxidants. The Phytochemical present in these plants also shows the antimicrobial, larvicidal and insecticidal activity. Plant chemical also inhibit the growth of pathogenic bacteria and fungi. In present study, we focused on the Phytochemicals in stem bark of A. squamosa plant. For their extraction simple soxhlet extraction (Clevenger apparatus) was carried out by using different solvent that is aqueous and ethanol. In qualitative analysis alkaloids, proteins, carbohydrates, steroids, tannins, oxalate, quinine, phenols, amino acid were found in ethanolic extract except saponin and flavonoid while in aqueous extract all Phytochemicals were present but phenol and tannins are absent.

INTRODUCTION

In our surroundings there are different types of plant species present which widely used as a medicinal treatment and natural remedies from ancient time. The nature provides us lots of variety of plant species which have essential components called as Phytochemicals. (J.Ashok kumar et al, 2010)

The best example is A.squamosa, the fruit of plant commonly known as the custard apple which is eatable. (Orwa, C et al, 2009). This plant belongs to the Annonaceae family. It is small, evergreen , straggling shrub commonly occurring in India. According to the world health organization (WHO) survey ,80% population living in developing countries like India are widely used the traditional medicines for their primary treatments. (Yadav, R. N. S., et al, 2011). The Annona squamosa are having the different types of essential components, which makes it beneficial for cardiac disease, diabetes, hyperthyroidism and cancer. (Vanitha, V et al, 2011). The plant contains glycosides, alkaloid, saponin, flavonoid, tannin, carbohydrates, protein, phenolic compound, polysterol and amino acids (Biba, V.S., et al, 2013).

MATERIAL AND METHODS

Collection and Identification

The stem bark of A.squamosa were collected from campus of Swami Ramanand Teerth Marathwada University, Nanded (MH). The identification and authentication of material was carried out at Dept. of Botany at S.R.T.M.U. Nanded (MH), India.

Extraction of Phytochemicals

The stem bark of Annona squamosa was dried under shade and prepared the fine powder by using the electrical / mechanical grinder. Soxhlet extraction was used for the isolation of plant chemical. In that stem bark powder was subjected for successive extraction with aqueous and ethanolic solvents and run the cycle for 3-4 hours. After completion the plant extract was evaporated at 40°-50°C and preserve in dried form for further use. A.Squamosa aqueous and ethanolic extract was then subjected for qualitative analysis

Test for Alkaloids:

Crud extract was treated with 3 drops of Wagner's reagent and the formation of reddish brown precipitate show the presence of alkaloid.

Test for proteins:

Ninhydrin test Crude extract boiled with 2 ml of 0.2% solution of ninhydrin reagent which turned to violet color appeared/suggesting the presence of Amino acid and protein.

Millon's reagent test

Crude extract mixed with 2 ml of million's reagent white precipitate turned into red upon gentle heating that confirmed the presence of protein

Test for carbohydrate

Benedict's test

Crude extract when mixed with 2 ml of Benedict's reagent and boiled it, a reddish brown precipitate formed which indicated the presence of the carbohydrate.

Fehling's test

2 ml of reagent added in crude extract and gently boiled .A brick red precipitate appeared at the bottom of the test tube indicate the presence of reducing sugars.

Iodine test

Crude extract when mixed with 2 ml of iodine solution. A dark blue or purple coloration indicates that presence of carbohydrate.

Molsich's test

Crude extract mixed with 2 ml of Molsich's reagent and the mixture was shaken properly. Then 2 ml of conc.H2SO4 was added carefully along with the side of test tube. Appearance of violet ring at the interphase indicated the presence of carbohydrate.

Test for Phenol and tannin

Crude extract mixed with 2 ml of 2 % solution of FeCl₃ .A blue green or black coloration indicates the presence of phenol and tannin.

Test for flavonoids

Alkaline test

Crude extract mixed with 2 ml of 2% solution of NaOH .An intense yellow color was formed which tuned colorless or addition of few drop of dilute acid which indicated the flavonoids

Test for Saponin

Crude extract was mixed with 5ml of d/w in a tube and it was shaken vigorously .The formation of stable foam was taken as

indication for the presence of saponin.

Test for Glycoside

Salkwaski's test

Crude extract mixed with 2 ml of chloform. Then 2ml of conc.H₂SO₄ was added carefully. A reddish brown color indicated presence of steroidal ring that is glycogen portion of glycosides

Keller-Kilani Test

Crude extract mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl₃. The mixture was then poured into another test tube containing 2ml of conc. H₂SO₄. A brown ring at the interphase indicated the presence of cardiac glycoside.

Test for steroid

Crude extract mixed with 2ml of chloroform and conc.H₂SO₄ was added sidewise .A red /brown color produced in lower chloroform layer indicated that presence of steroids.

Test for terpenoid

Crude extract dissolve in 2ml of chloroform and evaporation to dryness 2 ml of conc.H₂So₄ was added and heated for about 2 min. A grayish color indicated presence of terpenoid.

Test for Phlobatannin

2ml of extract boiled with 1ml of 1% aqueous HCl acid, then deposited the red precipitate at bottom of the test tube it shows the presence of phlobatannin.

Test for quinone

Extract treated with conc.HCL and formation of yellow precipitate the presence of quinone.

Test for oxalate

Take 3ml portion of extract added a few drops of glacial acetic acid. A grayish black coloration indicates the presence of oxalate.

RESULT AND DISCUSSION

In present study, the Phytochemicals in *A.squamosa* have the great efficiency. The extract contains different biologically active compounds which contributed major role in medicinal applications.

Phytochemical analysis of *Annona squamosa* Table no. 1

Sr no.	Name of the test	Aqueous Extract	Ethanolic Extract
1	Test for Amino acids and Protein	+	+
2	Test for Carbohydrates	+	+
3	Test for Phenols	-	+
4	Test for Tannins	-	+
5	Test for Glycoside	+	+
6	Test for Flavonoid	+	-
7	Test for Saponin	+	-
8	Test foe Phlobatannin	-	-
9	Test foe Steroid	+	+
10	Test for Terpenoid	+	+
11	Test for Quinone	+	+
12	Test for Oxalate	+	+

The extraction of Phytochemical in *A.squamosa* was carried out by using the soxhlet extraction process. The aqueous and ethanolic further tested for qualitative analysis in that ethanolic plant extract contains alkaloid, protein, carbohydrates, steroid, tannin, oxalate, quinone, phenol, amino acid etc but we found that saponin and flavonoid is absent.

Likewise, in aqueous all above Phytochemicals is present except phenol and tannin. This test shows the conformation

for presence of various essential components which make it a good and effective against microbes, larva, insect etc.

CONCLUSION

The active compound at highly acceptable level shows application towards natural pesticides .The stem bark extract have the efficiency in preventive medicines, economical and agronomical point of view.

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