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SCREENING OF DIFFERENT PARTS OF *ANNONA SQUAMOSA* EXTRACT FOR ANTIBACTERIAL ACTIVITY

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Abstract

Annona squamosa belongs to the family Annonaceae, commonly known as custard apple. A comparative antibacterial activity of methanolic leaf and seed cotyledon extracts of Annona squamosa were evaluated against three bacterial strains namely Bacillus subtilis, Escherichia coli, staphylococcus aureus using agar well diffusion method. Maximum inhibition was found with 40 mg/ml concentration of methanolic leaf and seed cotyledon extracts against all the tested organisms under investigation. The minimum inhibitory concentrations were determined by disk diffusion method. To find the compound responsible for antibacterial activity thin layer chromatography was done.The FTIR analysis was performed represented the presence of various functional groups. The phytochemical analysis was performed. The study suggests that the leaf and seed cotyledon extracts of Annona squamosa are promising the development of phytomedicine for antimicrobial properties.

Keywords:*Annona squamosa,* Bacterial strains, Discdiffusion, Methanolic extract.

Introduction

Plants are a rich source of secondary metabolites with biological activities. In general, these secondary metabolites are a beneficial source with a variety of structural

arrangements and properties [1].A properties of compounds include flavonoids, Steriods, phenols, phenolic glycosides, saponins and cyanogenic glycosides [2,3].

According to the WHO survey 80% populations living in the developing countries dependon traditional medicine for their primary health care needs. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition, herbs have provided us some important life saving drugs used in the modern medicine. However among theestimated 2,50,000-4,00,000 plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically[4]

Natural products from bacterial sources have been the initial source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important.[5] There are various reasons that people use plants for medication. This includes improvement of health after herbal treatment, low cost of the drugs, non availability of synthetic drugs particularly in the rural areas, where available were either fake or expired drugs and in some cases the people are more comfortable with traditional healing [6]

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* contains medicinal property.[7]

The plant is traditionally used for the treatment ofepilepsy, dysentery, cardiac problem, worm infection, constipation,hemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also

has antifertility, antitumor activity[8,9]Sugar apple tree ranges from 10 to 20 ft (3 – 6m) inheight with open crown of irregular branches and zigzagtwigs. A branch tips opposite the leaves, the fragrantflowers are borne single or in groups of 2 to 4. The fruitis nearly round, oval or conical, long its thick rindcomposed of knobby segments, separating when thefruit is ripe and revealing a conically segmented,creamy – white delightfully fragrant juice sweetdelicious flesh [10]

The crushed leaves are sniffed to overcome hysteria & fainting spells, they are also applied on ulcer & wounds. Roots are employed internally in depression of spirits and spinal diseases. Bark is known to be a powerful astringent [11]. The leaves and stems also gave alkaloids dopamine, salsolinol and coclaurine[12].Powdered seeds are used to kill head-lice and fleas.Two acerogenins, annoreticuin and isoannoreticuin, isolated from the leaves, were found to be selectively cytotoxic to certain human tumors. The seeds are said to be abortifacient, seed yields oil and resin which acts as detergent and their powder, is mixed with gram-flour, is a good hair wash. Seeds are powerful irritant of conjunctiva and produce ulcers in the eye [13].

The seeds are toxic, and have been used as an insecticide. Seeds are, however, high in oil, which can be used in soap manufacture or, if treated to remove the toxic alkaloids, as a cooking oil. Leaves, unripe fruits, and extracts of bark and root, all rather astringent, have been used in traditional medicine to treat fevers, rheumatism etc[14].Leaves contains 4-(2-nitroethyl)- 1-((6-O-ß-D-xylopyranosyl-ß-D-glucopyranosyl) oxy) benzene, Anonaine, Benzyltetrahydro-isoquinoline, Borneol, Camphene, Camphor, car-3-ene, Carvone, ß-Caryphyllene, Eugenol, Farnesol, Geraniol, 16-Hentriacontanone, Hexacontanol, Higenamine, Isocorydine, Limonine, Linalool, Linalool acetate, Menthone, Methyl anthranilate, Methyl salicylate, Methylheptenone, p- (hydroxybenzyl)-6,7-(2-hydroxy,4-hydro) isoquinoline, n-

Octacosanol, á-Pinene, ß-Pinene, Rutin, Stigmasterol, ß-Sitosterol, Thymol and n-Triacontanol [15]

Due to uniqueness of leaves and seeds property in curing of different ailments, this parts were selected for the study. This paper describes the presence of some phytochemicals in *Annona squamosa* which are responsible for antibacterial activity especially against the enteric pathogens.

Materials and Methods

Collection & Extraction of Plant Materials

The leaves and seeds of the fruit *Annona squamosa* were collected from Solapur area. The leaf and seeds were washed thoroughly with tap water followed with sterilized distilled water and shade dried for 8-10 days and then powered with the help of blender. For cold extraction, 25g of the plant powder was taken in 50ml methanol. It was kept for 3-4 days and then filtered by using Whatman filter paper No. 1. Extracts were evaporated and made powder. Both the extracts were stored in the refrigerator at 4°C for future use. The extracted powder was dissolved in 10 % dimethyl sulfoxide (DMSO) for the further use.

Test microorganisms

Thethree bacterial cultures *Bacillussubtilis, Escherichia coli, Staphylococcus aureus*were used in the presentstudy. Bacterial cultures were grown in nutrient broth (HI media, M002) at

37°C and maintained on nutrient agar slants at 4o°C.

Phytochemical Analysis

The prepared plant extracts were analyzed for the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, phenolic compounds, tannins, flavonoid.[16]

Antibacterial Activity

The selected standard strains of bacteria such as *Bacillussubtilis, Escherichia coli, Staphylococcus aureus*were inoculated into 10ml of sterile nutrient broth, and incubated at 37°C for 16-18 hours. A 1% of the standard inoculums of the test bacterial strain were spreadby sterile cotton swab on Nutrient Agar. Then, 6 mm diameter wells were bored in the Nutrient agar plates. Using a micropipette, 25µl (40mg/ml) of the plant extracts were added to different wells in the plate. Dimethyl sulfoxide (DMSO) is used as the negative control. Standard antibiotics were used as positive control. The plates were incubated at 37°C for 24 hours. The diameters of inhibition of zones were measured in millimeter and the results were recorded.

Thin Layer Chromotography

The silica gel G-60 PF-254 mixture (25g/50ml H2O) is poured on TLC glass plates in a thin layer and is allowed to dry at room temperature for 1 day. It is then activated at 120ºC for 30 minutes. The prepared plant extract is spotted on the plate with a capillary tube on a marked spot. Then, the solvent must be in contact with stationary phase. The solvent system were used Chloroform: Methanol. The FTIR analysis was studied.

Result and Discussion

In the present investigation the phytochemical analysis of *A.squamosa* leaves and seed extract were studied. The phytochemical analysis containspresence of alkaloids, carbohydrates, glycosides, saponins, proteins, phenolic compounds, tannins, flavonoid, which are showed in Table no. 1 and 2.

Table no.1 : Phytochemical analysis of A.squamosa leaves extract

Compound	Test	Methanolic Extract
Alkaloid	Mayer's	-
Carbohydrates	Fehling's	+
Glycosides	Lugol's	+
Saponins	Saponin	-
Proteins	Ninhydrin	-
Phenols	Ferric chloride	+
Tannins	Lead acetate	+
Flavonoids	Magnesium	+

Table no.2 : Phytochemical analysis of A.squamosa seed extract

Compound	Test	Methanolic Extract
Alkaloid	Mayer's	-
Carbohydrates	Fehling's	+
Glycosides	Lugol's	+
Saponins	Saponin	+
Proteins	Ninhydrin	-
Phenols	Ferric chloride	+
Tannins	Lead acetate	+
Flavonoids	Magnesium	+

Three bacterial species cultures were tested to determine the antibacterialactivity of methanolic extract of *A. squamosa*. The values given in table no.3 the mean of the three observations.

Table no.3: Antibacterial activity of methanolic extracts of A. squamosa leaves and seed cotyledon

Sample	**Diameter of inhibition zone in mm**		
	Bacillus subtilis	***Staphylococcus aureus***	***E.coli***
Positive control	18	12	16
Negative control	3	7	2
Leaf extract	14	6	13
Seed extract	16	8	14

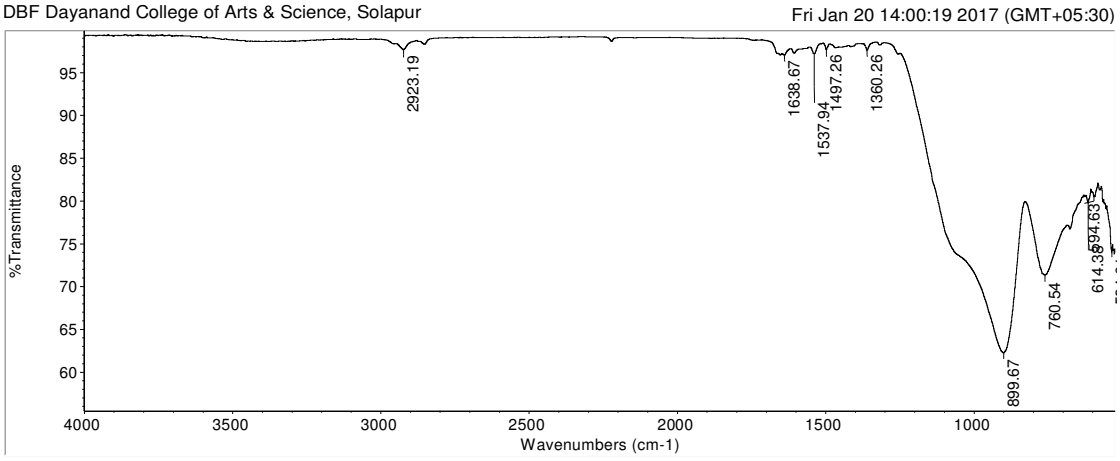
Methanolic leave extract showed maximum of 14 mm inhibition in*Bacillus subtilis* at 40mg/ml followed by *Escherichia coli* (13mm),and *Staphylococcus aureus*(6mm). Maximum inhibition in seed extract was alsoobserved at 40mg/ml concentration against *Bacillus subtilis* (16mm). followed by *E. coli* (14mm) and *Staphylococcus aureus*(8mm). The standard oflaxicin at 40mg/ml showed highest activity against *E. coli* (16mm), followed by *Bacillus subtilis* (18mm), *Staphylococcus aureus*(12mm).Among the plant materials used seed extracts showed

maximum antibacterial activity. The negative control used DMSO showed maximum of 7mm inhibition in *Staphylococcus aureus* at 40mg/ml followed by*Bacillus subtilis* (3mm)and .*E. coli* (2mm)bacterial strains. The phytochemical analysis of leave extract exposing positive results of carbohydrates, flavonoids, phenols and tannins, negative in alkaloids and saponins. The Seedcotyledon extract exposing positive results of tanins,

steroids, and saponins, negative in alkaloids and protein.Our study shows that methanol seed extracts inhibited the growth of *Bacillus subtilis*at 40mg/ml concentration.

The FTIR analysis of leaves and seed cotyledon extracts of *A. squamosa* were estimated. From the FTIR spectra of leave extract (Figure no.1), it can be concluded that, transmittance at 899.67cm-1is due to –C-H bending alkynes group, transmittance at 2923.19cm-1 is due to -OH stretching, transmittance at 1638 cm-1is due to NO2 asymmetric.

From the FTIR spectra of seed cotyledon extract (Figure no.2), it can be concluded that, transmittance at 899.67cm-1is due to –C-H bend alkynes group, transmittance at 2851cm-1 is due to -OH stretching, transmittance at 1465cm-1isC-H bending alkane group.



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(GMT+05:30)

Fri Jan 20 14:00:06 2017 (GMT+05:30)
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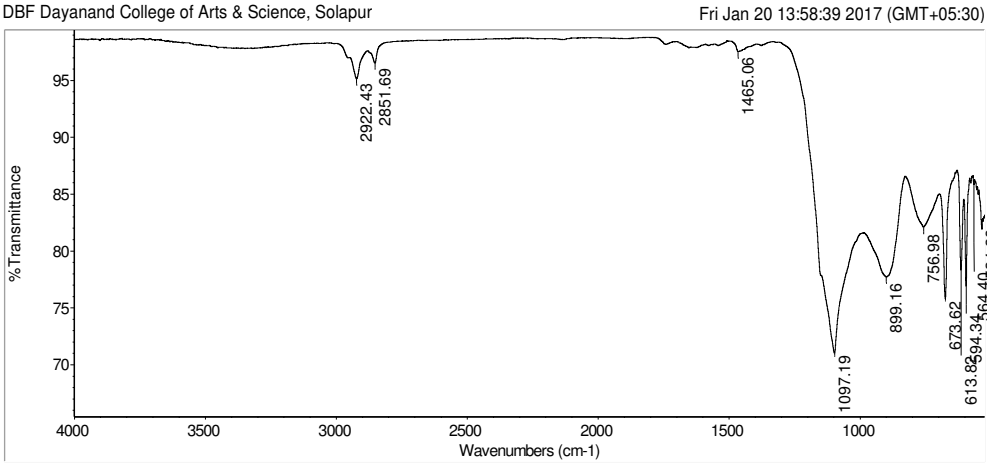
Absolute threshold: 98.315
Sensitivity: 64
Peak list:

Position:	534.61	Intensity:	73.977
Position:	594.63	Intensity:	80.465
Position:	614.38	Intensity:	79.713
Position:	760.54	Intensity:	71.295
Position:	899.67	Intensity:	62.220
Position:	1360.26	Intensity:	97.592
Position:	1497.26	Intensity:	97.672
Position:	1537.94	Intensity:	97.191
Position:	1638.67	Intensity:	97.021
Position:	2923.19	Intensity:	97.647

Figure no.1 FTIR analysis of leaf methanolic extract of A.squamosa



Collection time: Fri Jan 20 13:58:05 2017

Fri Jan 20 13:58:26 2017 (GMT+05:30)
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Region: 4000.12 525.03
Absolute threshold: 97.779
Sensitivity: 64
Peak list:

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Position:	564.40	Intensity:	85.789
Position:	594.34	Intensity:	76.881
Position:	613.82	Intensity:	78.312
Position:	673.62	Intensity:	75.785
Position:	756.98	Intensity:	82.086
Position:	899.16	Intensity:	77.699
Position:	1097.19	Intensity:	70.996
Position:	1465.06	Intensity:	97.534
Position:	2851.69	Intensity:	96.531
Position:	2922.43	Intensity:	95.154

Figure no.2: FTIR analysis of seed cotyledon methanolic extract of A.squamosa

Conclusion

In the present study, based on previous reports we have found that among the all extracts of *A squamosa*, seed cotyledon extract showed wide range of antibacterial activity.Further investigations should be carried out in finding otheractivities of the extract of seed cotyledon.

References

1. Audu JA. Studies on the effective of medicinal Herbs Used As Antihelminitic by traditional medical Practitioners in South of Bauchi State Two. Journal of Econamic Taxanomic Botany 19 ,1995; 653-661

2. Asholkar LV, Kakkar KK, and Chakre OJ,. Glossary of Indian Medicinal plants with active Principle. Publication and information Directorate. New Delhi 1992;72-73.

3. Balsndrin MF, Klocke JA, Wretele ES, and Boilinger WH. Content and purity of extract solasodine in some available species of solanum. Sciences and culture; 56 (5) ,1985;pp 214-216.

4. C. Chandrashekar and Kulkarni V.R. Isolation characterization and Antimicrobial activity of *Annona Squamosa* leaf, *Journal of Pharmacy Research.*,4(6), 2011;1831-1832.

5. C.P. Khare Kirtikar KR and Basu BD. .Indian medicinal plants, An Illustrated Dictionary, Springer publication ,1999.

6. D. Raj Sobiya, J. Vennila Jannet, C. Aiyavu and Panneerselvam. The heapatoprotective effect of alcoholic extract of Annona squamosaleves on experimentally induced liver injury in swiss albino mice, *International Journal of Integrative Biology*, *5(3)*, 2009;182-186.

7. Hallihan B, Delph S, Antimicrobial activity in medicinal plants of the *Scrophulariaceae* and *Acanthaceae*. 1996.

8. Jayshree D. Patel and Vipin Kumar.*Annona squamosa* L.:Phytochemical analysis and Antimicrobial Screening, *Journal of Pharmacy Research*, *1(1),*2008; 34-38.

9. Morton, Julia F. Soursop *Annona muricata.* Fruits of warm climates,1987;p. 75-80.

10. Ranveer ST, Siddharaj SS. Antidiabetic Activity of *Annona squamosa* L. In Experimental Induced Diabetic Rats. International , 2012.

11. Raaman N.*Phytochemical Techniques*,2006 ;pp 1-275.

12. Shahidi F . Antioxidant factors in plant foods and selected oilseeds. BioFactors 13(1-4),2000;179-185.

13. Shahidi F, McDonald J, Chandrasekara A, Zhong Y. Phytochemicals of foods, beverages and fruit vinegars: chem) ,2008.

14. Sukirti Upadhyay, A. K. Ghosh and Vijender Singh. Anti-lice activity of *Abrus Precatorius* (FAM -Fabacae) seeds oil. *Egyptian Dermatology Online Journal,7(2),* 2011;1-6.

15. Veeramuthu Duraipandiyan, Muniappan Ayyanar and Savarimuthu Ignacimuthu.Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *International Journal of Integrative Biology*, *3 (3)*, 2006;162.

16. Wealth of India. A Dictionary of India raw material and Industrial products. Vol V. Publication and information directorate. CSIR. New Delhi 1959.