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Antimicrobial Activity of Hydroalcoholic Extract of *Annona Squamosa*

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ABSTRACT: The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles. Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today. Historically, plants have provided a source of inspiration for novel drug Compounds, as plant derived medicines have made large contributions to human health and well-being. In this study, hydro alcoholic extracts of *Annona squamosa* samples with different concentration were screened for antimicrobial activity against two Gram positive bacteria and two Gram negative bacteria by agar well diffusion assay.

KEYWORDS: Antimicrobials, *Annona squamosa*, Gram positive bacteria, Gram negative bacteria and Infection.

I. INTRODUCTION

The widespread Annonaceae, is a large family which comprising about 130 genera over 2000 species; the most important genera having a largest number of species of *Annona*, with 120 species, from genera. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles [1]. Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today. [2] Historically, plants have provided a source of inspiration for novel drug Compounds, as plant derived medicines have made large contributions to human health and well-being. [3] The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. The medicinal plants around the world contain many compounds with antibacterial activity.[4] Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds. [5]The use of botanical medicines is generally on the rise in many parts of the world [6]. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents. [7-8 -9]Numerous experiments have been carried out to screen natural products for antimicrobial property. [10-11] Considering the above, it can be stated that plants are valuable sources for new compounds and should receive special attention in research strategies to develop new antimicrobials urgently required in the near future. [12-13]. *Annona squamosa* (family: Annonaceae) commonly known as “Sugar Apple or Custerd Apple” and locally known as “Sitaphal” is cultivated throughout India, Mainly for its edible fruit. *Annona squamosa* is a large Semi- deciduous shrub or small tree about 4 to 5 meters tall. Leaves are simple, oblong, lanceolate or elliptic, glabrous above and alternately arranged. Flowers are fragrant, creamy yellow to greenish, seen solitary or in groups of up to four in leaf opposed cymes. The fruit is globose or ovoid, yellowish green when ripe, embossed with prominent scales with soft creamy pulp. Seeds are

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hard, brownish black and smooth in texture. [14].The objective of present study screening of hydro alcoholic extract of leaves of *Annona squamosa*.

II. RELATED WORK

MEDICINAL USES OF ANNONA SQUAMOSA IN AYURVEDA: The species of *Annona squamosa* is well known for its traditional medicinal properties. [14] *Annona squamosa* Leaves, shoots, bark and roots have been reported to have medicinal properties. The unripe fruit is astringent, and the root is a drastic purgative, the seed of the plants is believed to have antifertility activity. The roots are used as a drastic purge. The leaves are used as a vermicide, for treating cancerous.

CHEMICAL CONSITUTENTS OF ANNONA SQUAMOSA: *Annona squamosa* consists of steroids, alkaloids, saponins, terpenes, tannins, phenolic substance, carbohydrate, volatile oil and mucilage. . Quantitative estimations revealed the presence of 2% of volatile oil in fresh leaves and 15.26% of total mucilage content in the dried leaf powder .Compounds were isolated from *Annona squamosa*, (-)-kauran-16 alpha-ol-19-oic acid; (2,4-cis and trans)-Mosinone A; (2,4-cis and trans)squamoxinone; (2,4-cis and trans)-squamolinone; (2,4-cis and trans)-9-oxoasimicinone; 1HCycloprop(e)azulene; 4-deoxyannoreticuin; 16 alpha-methoxy(-)-kauran-19-oic acid; 16 beta,17-dihydroxy(-)-kauran-19-oic acid; annonin I; anonin VI; annonacin; annonacin A; annonastatin; annomosin A; anonaine; annosquamosin C; annosquamosin D; annosquamosin E; annosquamosin F; annosquamosin G; annotemoyin-1; annotemoyin-2; bisabolene; bisabolene epoxide; bullacin B; bullatacin; bullatacinone; caryophyllene oxide; cholesteryl glucopyranoside; mosin C; motrilin; moupinamide; reticulatain-2; samoquasine A; squamocenin; squamocin; squamocin-B; squamocin-I; squamocin-O; squamosamide;; squamostatin-E; squamostanin-A; squamostanin-B; squamotacin.

III. MATERIAL & METHDS

EXTRACTION & CHEMICALS: In present study extraction was performed using continuous hot percolation 'Soxhlation'. Chemicals and standard antibiotics used in this work were purchased from Hi-Media, Mumbai, India.

TEST MICRO-ORGANISMS: The reference bacterial strains used in this study were obtained from Pinnacle Biomedical Lab Bhopal, India. These included; Gram positive bacteria *Staphylococcus aureus* MTCC No. 87), *Bacillus subtilis* (MTCC No. 441)and Gram negative bacteria *Escherichia coli* (MTCC No. 739), *Pseudomonas aeruginosa* (MTCC 741).

PREPARATION OF MEDIA (NUTRIENT AGAR MEDIA (1L))

All glassware's were sterilized by autoclaving. Agar media were prepared from a commercially available Nutrient agar (Hi-media, M012), with 1% peptone according to the manufacturer's instructions. One liter of nutrient agar media was prepared by dissolving 35.0 g of commercially available nutrient agar medium (Hi-Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

STANDARD DRUG USED

Ofloxacin (100µg/ml)

PREPARATION OF SUSPENSION OF BACTERIA

Approximately 4 to 5 well isolated colonies of the bacterial strain are inoculated into 5 ml of Nutrient broth and incubated at 37°C.

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SCREENING OF ANTIMICROBIAL ACTIVITY OF ANNONA SQUAMOSA.

After standardization of bacterial suspension, sterile cotton swap was immersed in it and the swap was rotated several times, with firm pressure on the inside wall of the tube to remove excess fluid. Nutrient agar media plate was prepared with a depth of 4mm. Dried surface of nutrient agar plate was inoculated the by streaking the swab 3 times over the entire agar surface. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the sample which were labeled accordingly. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48 hours in upright condition. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along two axis i.e. 90° to each other and the mean of the four reading were then calculated included 5mm well.

IV. EXPERIMENTAL RESULTS

**TABLE1: ANTIBACTERIAL ACTIVITY HYDROALCOHOLIC EXTRACT OF ANNONA SQUAMOSA
PARAMETER - ZONE OF INHIBITION (MM):**

In this study, we tested the hydroalcoholic extract of *Annona squamosa* for their antimicrobial activity. The plant extract showed antimicrobial activity against all four of the types of micro-organisms tested, as exhibited by disc diffusion assay (Table1).

**ANTIBACTERIAL ACTIVITY HYDROALCOHOLIC EXTRACT OF ANNONA SQUAMOSA:
PARAMETER - ZONE OF INHIBITION (mm)**

S. No.	Organism	Zone of Inhibition				Standard (ofloxacin) 100 µg/ml
		100 µg/ml	200mg/ml	300 mg/ml	400 mg/ml	
1	Staphylococcus aureus	16.00±0.816	18.75±0.957	22.25±0.957	26.50±1.291	39.75±0.645
2	Bacillus Subtilis	17.50±1.291	22.00±0.816	26.00±0.816	30.75±0.957	38.75±0.645
3	Escherichia Coli	16.50±1.291	21.00±1.633	25.00±1.826	30.25±1.708	36.88±0.854
4	Pseudomonas aeruginosa	18.00±1.155	20.75±0.957	23.50±0.577	27.00±0.816	37.63±0.750

V. CONCLUSION

The hydroalcoholic extract of *Annona squamosa* could be possible source of to obtain new and effective herbal medicine to treat infection caused diseases. However, it is necessary to determine the toxicity of active constituent, their side effects and pharmacokinetics properties. In above table 400mg/ml dose showing good result.

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