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A Comparative in Vitro Antimicrobial Activity of Annona Squamosa on Gram Positive & Gram Negative Microorganism

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ABSTRACT

Annona squamosa (L) is a multipurpose tree with edible fruits & is a source of the medicinal & industrial products. It is used as an antioxidant, antidiabetics, hepatoprotective, cytotoxic, genetoxic, anti-tumor, anti-lice agent etc.*Annona squamosa* (L) belongs to the family *Annonaceae* commonly known as custard apple. Antimicrobial activity of combined methanolic leaf & seed extract of *A.squamosa* were evaluated against four bacteria: *Bacillus subtilis*, *Styphaloccocus aureus* (gram positive) & *E.coli, Pseudomonas aerogenosa* by using cup & plate method. Maximum inhibition was found with 20mg/ml concentration of combined extract as compare to separate leaf & seed extract against all the tested organism under investigation. The study suggest that maximum antibacterial activity was observed against gram negative organism i.e., *E.coli* & *P.aerogenosa*.

KEYWORDS: *Annona squamosa L, antimicrobial activity, cup & plate method.*

INTRODUCTION

A microorganism or microbe is an organism that is unicellular or lives in a colony of cellular organisms. The study of microorganisms is called microbiology, a subject that began with Anton Van Leuwenhoek's discovery of microorganisms in 1976, using a microscope of his own design. Microbes are important in human culture & health in many ways, serving to ferment foods, treat sewage, produce fuel, enzymes & other bioactive compounds. They are essential tools in biology as model organisms. They are a vital component of fertile soils. In the human body microorganisms make up the human microbiotica including the essential gut flora.

They are pathogens responsible for many infectious diseases [1]. Bacteria are unicellular, free living, microscopic microorganisms capable of performing all the essentials of life. They posses both DNA & RNA. [2] Gram positive Bacteria are bacteria that give a positive result in the gram stain test, they take up the crystal violet stain used in the test and then appear to be purple coloured when seen through a microscope. This is because the thick peptidoglycon layer in the bacterial cell wall retains the stain after it is washed away from rest of the sample, in the decolourization stage of the test. Gram negative bacteria cannot retain the stain after the decolourization step; alcohol used in this stage degrades the outer membrane of gram negative cells making the cell wall, more porous & incapable of retaining the crystal violet stain. Their peptidoglycon layer is much thinner & sandwiched between an inner cell membrane & a bacterial outer membrane, causing them to take up the counter stain (safrannin or fuchsine) & appear red or pink. Gram positive bacteria are more receptive to antibiotics than gram negative due to the absence of the outer membrane.

An antimicrobial is an agent that kills microorganism or stop their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For ex. antibiotics are used against bacteria & antifungal are used against fungi.

The main classes of antimicrobial agents are disinfectants (non- selective antimicrobials such as bleach), which kill a wide range of microbes on non- living surfaces to prevent the spread of illness, antiseptics and antibiotics.

Antimicrobial use is known to have been common practice for at least 2000 years. Ancient Egyptians & ancient Greeks used specific moulds & plant extracts to treat infection.

In 1928 Alexander Fleming became the first to discover a natural antimicrobial fungus known as Penicillin Ruben's & named the extracted substance penicillin which in 1942 was successfully used to treat *Streptococcus* infection [3].

The species of *Annona squamosa* is a small evergreen tree reaching 6-8 meters (20-60ft) tall is commonly found in deciduous forests, cultivated throughout India & other countries. It is commonly called as custard apple; it is native of West Indies. It is mainly grown in gardens for its fruits and ornamental value. The taste of pulp of the fruit is really sweet because of its higher sugar content about 58% of dry mass, and hence it is found clear that fruit pulp possess a high calorie value.

The previous phytochemical investigations made on the plant have proved that they possess a wide variety of

compounds like acetogenins which are responsible for anti-feedant, anti-malarial, cytotoxic & the immunosuppressive activities. Diterpenes which was isolated from the *Annona squamosa* possess the Anti-HIV principle and the anti-platelet aggregation activity. The partially purified flavonoids were reported from the same source as the responsible agent for the anti-microbial and other pesticidal activities [4].The leaves of the plants have been used as insecticide, anthelmintic ,styptic, externally used as suppurant.Unripe and dried fruit work as antidysentric. Bark is used as powerful astringent, antidysentric and vermifuge.

Figure No:-1- Whole Plant of *Annona squamosa*

Figure No:-2 Leaves of *Annona squamosa*

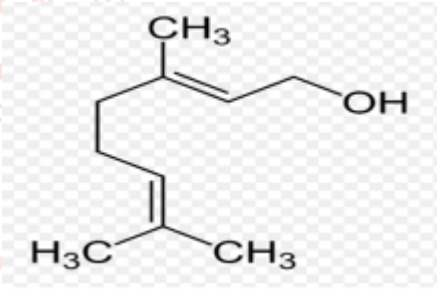
Figure No:-3 Flowers of *Annona squamosa*

Figure No:-4 Fruits of *Annona squamosa*

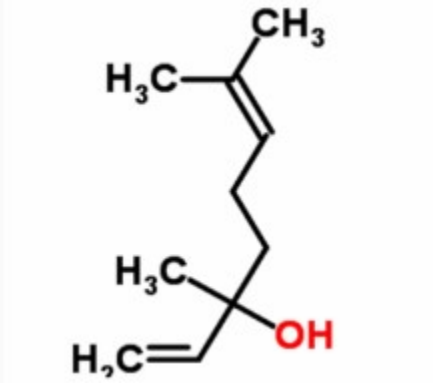
Chemical Constituents-

It contains alkaloids, glycosides, saponins, phenolic compounds, flavanoids, oils, steroids, and triterpins. The diterpenoid atisine is the most abundant alkaloid in the roots. It also contains alkaloids oxophoebine, reticuline, isocorydine & methylcorydaldine & the flavanoid quercetin-3-O-glucoside.It also contain linalool, geraniol, borneol, eugenol, farnesol, acetogenin. It also contains annotemoyin, squamosin & glucopyranoside [8, 9].

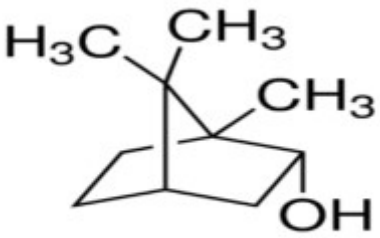
Structure:



Geraniol



Linalool



Borneol

MATERIALS AND METHODS

Collection and Authentication-

The fully matured fresh leaves and seeds of *Annona squamosa* were collected from Pawarwadi in Satara district. These are authenticated by Deshpande mam, Dept. of Botany, Y.C .Institute of science, Satara.

Extraction of Plant Material by Soxhlet Apparatus:-

In the present study, initially leaves and seeds of *Annona squamosa* were collected and dried under sunlight. After drying, the crude materials were reduced to fine powder by using mixer grinder.12.5 gm of shade dried leaves powder and 12.5 gm of shade dried seed powder were weighed.Both were mixed and 25 gm of powder extracted successively with 250ml of methanol in soxhlet extractor for 48hr. The methanol extracts was concentrated& preserved in refrigerator in airtight bottle for further antimicrobial assay.

Figure No:-5 Methanolic Leaf & Seed extract of *Annona squamosa*

Phytochemical Screening:-

Qualitative Phytochemical Analysis of methanolic extract of *Annona squamosa* leaf & seed was carried out using standard protocol to assess, different types of bioactive constituents present in the plant using different chemical tests. Screening was carried out for Alkaloid, Glycoside, reducing sugar, flavonoids, sterols & phytosterols. [11, 12]

1. Test for Alkaloid:

To 0.1 ml extract, 2-3 drops of Dragendroff's reagent added.
Observation- Orange red precipitate with turbidity.

2. Test for reducing sugar:

To 2ml of crude plant extract, 5mlof distilled water was added &filtered. The filtrate was boiled with 3-4 drops of Fehling's solution A and Fehling solution B in excess (1-2ml) for 2min.
Observation- Formation of the orange red precipitate.

3. Test for Glycoside:-

10ml of 50% H_2SO_4 was added to 1ml of the filtrates in separate test tubes & the mixtures heated for 15mins. Followed by addition of 10 ml of Fehling solution & boiled.
Observation- A brick red precipitate.

4. Test for Steroids & Phytosterols:-

2ml of acetic anhydride was added to 0.5 ml of the plant extract of each sample with 2ml of H_2SO_4.
Observation- The colour change from violet to blue green

5. Test for Flavonoids:-

A portion of plant extract was separately heated with 10ml of ethyl acetate in a water bath for 3 min. The mixture was filtered & 4 ml of filtrate was shaken with 1ml of dilute ammonia solution

Observation- Yellow colour observed

Antimicrobial Assay:-

Step 1-Sterilization of equipments
Petri plates, glass spreader, corn borer and pipette were sterilized in oven at 120^0c for 1 hr.

Step 2-Preparation of nutrient agar medium

Ingredients	Quantity
Beef extract	10gm
Peptone	10gm
Sodium chloride	5gm
Agar	20gm
Distilled Water	1000ml

Table.No-1 Composition of nutrient agar medium

Procedure-

All ingredients were dissolved in 900ml Distilled Water & then make up volume up to 1000 ml. Heated with agitation and boiled for few min to dissolve the powder. The pH of the medium was determined 6.8-7. Media was sterilized by Autoclaving at 121^0c for 15min.

Step-3

Preparation of bacterial suspension-

Using the 24hrs old growth of the test bacteria from the slant. Then suspension of the bacteria was made separately in sterile normal saline solution or (0.85% Nacl in distilled water) in aseptic condition, to get moderate turbidity.

Cup & Plate method:-

1. Cup and plate method was adopted for the evaluation of antimicrobial activity of leaf and seed extract of *Annona squamosa* [2].Nutrient agar was prepared and autoclaved at 15 Ibs pressure for 20 mins and cooled for 45^0c.The cooled media were poured on to a sterile petriplate & allowed for solidification. The plates with media were seeded with respective microbial suspension using sterile L-rod. Wells were bored on nutrient agar media with the help of corn borer. The extracts were diluted with Dimethyl sulfoxide at a concentration with 20mg /ml. Extracts were poured in the wells of petridishes along with the control. Ciprofloxacin antibiotic was used as a standard drug control for bacterial pathogens. The plates were kept on deep refrigerator for 1hr for penetration. After, these plates were kept on incubator for incubation for 48 hrs at 37^0c.After the incubation period the zone of inhibition (mm) was measured.

RESULT AND DISCUSSION:

Table No:-2-Phytochemical Screening

Sr. No	Test	Observation	Inference
1.	**Test for Alkaloid:** To 0.1 ml extract, 2-3 drops of Dragendroff,s reagent added.	Orange red precipitate with turbidity	Alkaloids are present.
2.	**Test for Reducing sugar:** To 2ml of crude plant extract, 5mof distilled water was added &filtered. The filtrate was boiled with 3-4 drops of Fehling's solution A and Fehling solution B in excess (1-2ml) for 2min.	Formation of the orange red precipitate.	Reducing sugars are present.
3.	**Test for Glycoside:-** 10ml of 50% H2SO4 was added to 1ml of the filtrates in separate test tubes & the mixtures heated for 15mins. Followed by addition of 10 ml of Fehling solution & boiled.	A brick red precipitate.	Glycosides are present
4.	**Test for Steroids & Phytosterols:-** 2ml of acetic anhydride was added to 0.5 ml of the plant extract of each sample with 2ml of H2SO4.	The colour change from violet to blue green.	Steroids and sterols are present.
5.	**Test for Flavonoids:-** A portion of plant extract was separately heated with 10ml of ethyl acetate in a water bath for 3 min.The mixtures was filtered & 4 ml of filtrate was shaken with 1ml of dilute ammonia solution.	Yellow colour observed.	Flavonoids are present.

ANTIMICROBIAL ACTIVITY

Figure No:-6 *Bacillus subtilis.*

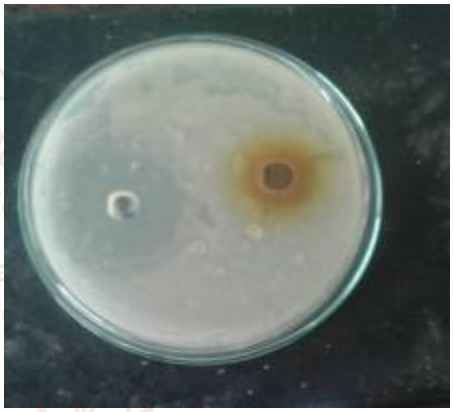


Figure No:-9 *Pseudomonas aerogenosa*

Figure No:-7 *Staphylococcus aureus.*

Sr. no	Microorganism	Zone of ambition	
		Extract	Antibiotic
1	Escherichia. Coli	30 mm	35mm
2	Pseudomonas aerogenosa	25mm	30mm
3	Staphylococcus aureus	24mm	29mm
4	Bacillus subtilis	20mm	26mm

Table no: - 3 Antimicrobial Activity

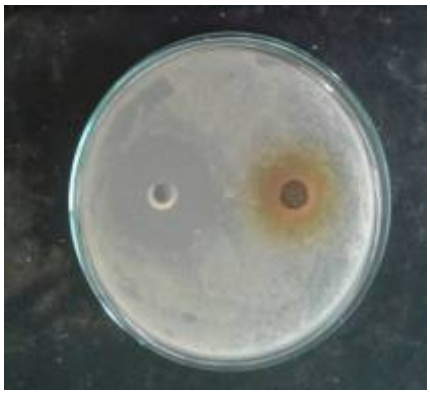


Figure No:-8 *E.coli*

Comparative Antimicrobial Activity:-[13]

Sr. no	Microorganism	Leaf Extract	Seed Extract	Combined Leaf & Seed Extract
1	Escherichia coli	7 mm	9 mm	30 mm
2	Pseudomonas aerogenosa	8mm	9mm	25mm
3	Staphylococcus aureus	6mm	7mm	24mm
4	Bacillus subtilis	5mm	6mm	20mm

Table No:-4 at 20 mg /ml concentration (methanolic extract)

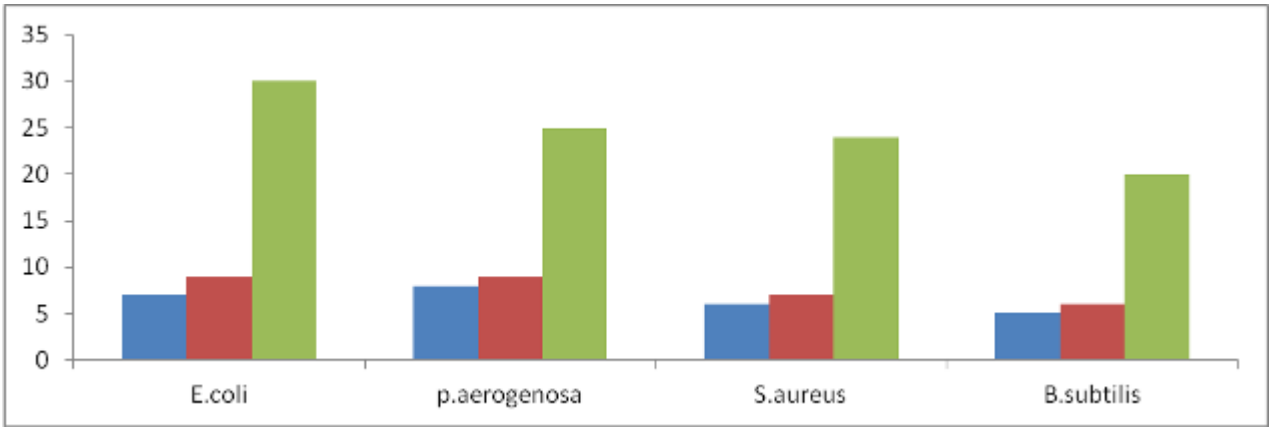


Figure No:-10 Statistical representation of Comparative antibacterial activity of methanolic extracts of *Annona squamosa* Leaf, seed, combined leaf & seed extract.

DISCUSSION:

Antimicrobial activity of combined leaf & seed extract of *Annona squamosa* (Methanolic extract) was assayed in vitro by cup and plate method against gram positive organism i.e., *Bacillus subtilis, Staphylococcus aureus* and gram negative organismi.e., *E.coli, Pseudomonas aerogenosa*.The table no.3 shows the microbial growth inhibition of methanolic leaf & seed extract of *Annona squamosa* along with positive control (Ciprofloxacin).The table no.4 shows the zone of inhibition formed by separate leaf and seed extract and shows maximum antimicrobial activity of combined leaf & seed extract of *Annona squamos*a at the same concentration i.e.,20mg/ml.Maximum inhibition in combined leaf & seed extract of 20mg/ml was observed against *E.coli* (30mm) followed by *P.aerogenosa* (25mm), *S.aureus* (24mm) & *B.subtilis* (20mm).

CONCLUSION:

Thus it is concluded that Sensitivity of the test organisms to *Annona squamosa* extract were indicated by observation & measurement of inhibition zones. In present study, we have found that the combined leaf and seed extract of *Annona squamosa* showed maximum antimicrobial activity than the individual leaf and seed extract. The extract showed maximum activity against gram negative organism i.e., *Escherichia coli* & *Pseudomonas aerogenosa* than the gram positive organism i.e.,*Bacillus subtilis* & *Staphylococcus aureus.*

REFERENCES:

[1] Microorganism wikipedia http://en.m.wikipedia.org

[2] Dr. chandrakant R. Kokare: Pharmaceutical microbiology principles and application, page no- 4.1-4.3, 17.12.

[3] www.microbiologyonline.org

[4] S. Gajalakshmi, R. Divya, V. Divya Deepika, S. Mythili, A. Sathiavelu International Journal of pharmaceutical Sciences Review & Research vol10. Page no-24-29. www.globalreasearchonline.net

[5] Rajsekhar saha; International Journal of Pharmacy and life sciences.vol.2.page.no-1183-1189.

[6] C. Babu, P. Rama Devi, P. Kombish International Journal of biological research, 2(11)-2014. Page no-18. www.sciencepubco.com/index.php/IJBR.

[7] http://www.spicesmedicinalherbs.com/*Annona squamosa*.html.

[8] Neethu Simon K, Santhoshkumar R & Neethu S.Kumar. Journal of Pharmacognosy & Phytochemistry 2016:5(4) page.no-130. www.phytojournal.com

[9] Jayshree et al: Journal of Pharmacognosy & phytochemistry 2016 vol.1.page.no-36.

[10] Soxhlet extractor.wikipedia.

[11] M. Yasha u, D. W. Taura, B. Bello, N. Abdullahi International Research of pharmacy & pharmacology vol.1 (9) Page. No -238.

[12] Samidha M. Pawaskar et al /J Pharm.sci & Res.vol9 (5), 2017.pg.no.-618-619.

[13] Vidyasagar et al. Int. J Pharm sci, vol4 suppl 5, page.no.-289-290

[14] Khan JA & Joshi. D. International Journal Of Biology, Pharmacy & Allied Sciences July, 2015 pg.no-4899-4900

[15] Neha Pandey, Dushyant Barve. International Journal Of Research in Pharmaceutical & Biomedical Sciences Vol 2.Oct-Dec 2011 pg.no.-1692-1694

[16] www.irjpas.com

[17] www.sciencepublishinggroup.com/j/ijpc

[18] www.scienceandnature.org

[19] www.ijcrbp.com