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Qualitative Phytochemical and Antibacterial Screening of *Annona squamosa* L. of Akot tehsil in Akola District (M.S.)

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ABSTRACT –

Plants are one of the most important sources of medicines from ancient time. The importance of herbal medicine is increases day by day. the chemical constituents present in the crude leaf extracts of *Annona squamosa* L. with special emphasis on their pharmacological actions. Qualitative phytochemical screening was carried out using the crude leaf extracts in four different solvents such as water, ethanol, acetone and petroleum ether. Phytochemical screening of the leaf extracts Shows the presence of glycosides, alkaloids, oils, saponins and flavonoids. the dried leaf extracts of *Annona squamosa* L. were evaluated against *Escherichia coli* and *Pseudomonas aeruginosa*. The leaf extracts of *Annona squamosa* L. shows the highly antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. These results shows that the leaves are a rich source of primary and secondary metabolites exhibiting the antimicrobial activity

INTRODUCTION

From the ancient times, people have been inspecting the plants in search of novel drugs which has resulted in the use of numerous medicinal plants for the cure of various diseases (Verpoorte, 1998).
According to World Health Organization (WHO, 2008) 80% populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs of which most involve the use of plant extracts (Sandhya et al., 2006). *Annona squamosa* L. belonging to the family Annonaceae is a small ever green tree commonly found in India and originates from West Indies and South America. Different parts of *Annona squamosa* L. are used in traditional medicine for the treatment of numerous diseases (Suresh et al., 2006). It is mainly grown in gardens for its fruits and ornamental value. This plant is commonly called custard apple in English, sharifa in Hindi, Shitafal in Marathi and sitaphalam in telungu in india (Raj Sobiya et al., 2009). The discovery of novel secondary metabolites or phytochemicals which are the non-essential nutrients derived from plants exhibiting a number of protective functions for human consumers. It is considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is considered as a drastic purgative (Raj Sobiya et al., 2009). A leaf decoction was taken in the case of

dysentery (S. Gajalakshmi et al., 2011). Leaves are used as plaster over boils and ulcers. The ripe fruits of this plant are applied to malignant tumors to accelerate suppuration. The dried unripe fruit powder is used to destroy insect. The seeds are pungent and poisonous. Powdered seeds serve as fish poison and insecticides. A paste of seed powder has been applied to the head to kill lice. It is also used for destroying worm in the wound of cattle's (Parvin et al., 2003). Phytochemical screening is a method in which we expose the properties readily available in plants for bio-activity or ethno-medical applications. Thuse it is expected that phytochemicals with adequate antibacterial efficiency can be used for the treatment of bacterial infections (Balandrin et al., 1985). The medicinal plants play an important role in the development of newer drugs because of their effectiveness, less side effects and relatively low cost when compared with synthetic drugs (Raj et al., 2011).
The present study aims in exploring the phytochemical constituents, antibacterial properties of the crude leaf extracts of *Annona squamosa* L.

MATERIALS AND METHODS -

Collection and Identification – The dust and infections free leaves and of *Annona squamosa* L. were collected from its natural habitat in khatkali region of Akot tehsil in District Akola. Maharashtra, India. During January – February of 2023. Taxonomic identification of plants was carried out by using published floras.

Extraction: The leaves and stem of *Annona squamosa* L. were thoroughly washed 3-4 times in running tap water, and then the leaves and stem are shade dried. After complete shade drying, the leaves and stem were grinded in mixer; the powder was stored in small polyethene bags. 30 gm of powder was taken for preparation of plant extract in solvents like acetone (300 ml), Petroleum ether (300 ml) and Ethanol (300 ml) by using Soxhlet extractor. Then the extracts become concentrated by evaporating solvent and stored in sterile bottle in deep freezer at 5ºC for further use. For the preparation of aqueous extract, the powdered material of 5 gm was taken by weighing balance and crushed in 50 ml of distilled water then boiled at 50-60˚ C for 30 – 40 minutes in water bath and filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 2800 rpm for 10 minutes then the filtrate was stored in sterile bottle in deep freezer at 5ºC for further use.

Phytochemical screening –
Preliminary phytochemical screening (qualitative) for to detect the bioactive compounds like phenols, alkaloids, glycosides, tannins, flavonoids, steroids, saponins, was carried out by using following methods (Mirge et al., 2022):
Test for proteins – Take 2 ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO4 solution was added. A peptide linkage in molecules is indicated by the violet color.
Test for amino acids - Take 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acid in the sample.
Test for reducing sugars - Take 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of conc. H2SO4 was added drop by drop on the sides of test tube. A reddish violet ring form at the junction of two layers which indicated the presence of carbohydrates.
Test for Alkaloids - Solvent free extracts, 50 mg was stirred with few ml of dilute HCL and filtered. The filtrate is tested with various alkaloidal reagents as follows:
Mayer's test- Few ml of filtrate and a drop or two of Mayers reagent were added by the side of the test tube. A white or creamy ppt indicates the presence of alkaloids.
Wagner's test - Take a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish- brown ppt confirms the presence of alkaloids.
Hager's test - Take a few ml of filtrate, 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent yellow ppt indicates the presence of alkaloids.
Test for phenolic compound - Lead acetate test - The extract (50mg) was dissolved in distilled water and to this; 3ml of 10% lead acetate solution was added. A bulky white precepted indicates the presence of phenolic compounds.

Test for Tannins - Take a 0.5g of the plant extract was added in 10 ml of water in test tube and filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or blue- black coloration

Test for glycoside - Each extract was hydrolyzed with HCL and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added to each mixture. Formation of red precepted indicates the presence of glycosides.

Test for Flavonoids

(a) 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCL were added. A yellow solution that turn colorless indicates the presence of flavonoids

(b) To 2 ml of test solution, 0.5ml alcohol was mixed. Then a bit of magnesium and 1 or 2 drops of con. HCL were added and heated. The mixture was analyzed for reaction

Test for Phenols - To 2 ml of test solution, alcohol and then few drops of neutral ferric chloride solution was added. A dark green color indicated the presence of phenolic compound.

Terpenoids: 2ml of leaf extract was added to 2ml of acetic anhydride and concentrated H_2SO_4. The blue, green ring is form which shows the presence of terpenoids.

Steroids: 1ml of leaf extract was dissolved in 10ml of concentrated sulphuric acid and equal volume of chloroform was added by sides of the test tube. The upper layer turns red in color and sulphuric acid layer showed the yellow with green fluorescence. Which shows the presence of steroids

Test for Resins - To the 0.2 g of each extract, 10 ml of glacial acetic acid was added then heated and cooled. A drop of conc. H_2SO_4 was added. Purplish red color shows the presence of resins

Saponins: 5ml of leaf extract was mixed with 20 ml dist. water and continuously shake it for 15 min. the foam is form which shows the presence of saponins.

Antibacterial Activity –

Antibacterial activity was carried out against *Escherichia coli* and *Pseudomonas aeroginosa*. In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by well diffusion method. Petriplates containing 20ml of Nutriant agar medium were seeded each with 24hr old culture of bacterial strains such as *E. coli* and *P. aeroginosa*. Wells of approximately 10 mm diameter was bored using a well cutter and 25 µl, 50 µl and 100µl of the extracts were added to the well from a stock concentration of 0.1g /1ml. The plates were then incubated at 37 °C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition in millimeters formed around the wells.

Sr. No.	Phytochemical Component	Acetone	Petroleum ether	Ethanol	Aqueous
01	Protein	+	-	+	-
02	Amino Acid	-	-	-	-
03	Reducing Sugar	+	+	-	+
04	Alkaloids				
	Mayer's	-	-	-	+
	Wagner's	+	+	+	-
	Hager's	+	+	+	+

05	Phenolic compounds Lead acetate	-	+	+	+
06	Tannins	+	+	-	-
07	Glycosides	+	-	+	+
08	Flavonoids	+	-	+	+
09	Phenol	-	+	+	+
10	Terpenoids	+	-	-	+
11	Steroid	+	+	-	-
12	resins	-	-	-	+
13	saponins	+	-	-	+

Table 1: Phytochemical analysis of *Annona squamos*a L. leaf extracts

Table 2 - Qualitative antimicrobial activity of different solvent extract of *Annona squamosa* L. **against test organisms**

Sr. No.	**Test organism**	**Acetone**	**Petroleum ether**	**Ethanol**	**Aqueous**
01	*E. Coli*	+	-	+	-
02	*P. aeroginosa*	+	-	-	+

Table 3: Zone of inhibition of acetone leaf extract of *Annona squamosa* L.

Test organism	**Zone of inhibition**		
	Concentration of leaf extracts		
	25 µl	50 µl	100 µl
E. Coli	-	-	12 mm
P. aeroginosa	-	15mm	18 mm

Table 4: Zone of inhibition of ethanol leaf extract of *Annona squamosa* L.

Test organism	**Zone of inhibition**		
	Concentration of leaf extracts		
	25 µl	50 µl	100 µl
E. Coli	-	12 mm	17 mm

Table 5: Zone diameter of inhibition of Aqueous leaf extract of *Annona squamosa* L.

Test organism	**Zone of inhibition**		
	Concentration of leaf extracts		
	25 µl	50 µl	100 µl
P. aeroginosa	-	-	18 mm

Results and Discussion

Phytochemical Analysis - Table 1 represent the various phytochemical constituents present in the leaf extracts of *Annona squamosa* L. The phytochemical studies of extract in all the four solvent are as follows acetone shows positive result for protein, reducing sugar, alkaloid, tannin, glycoside, flavonoid, steroid, terpenoids and saponin. Petroleum ether shows positive result for reducing sugar, alkaloid, phenolic compound, tannin, phenols, and steroids. Ethanol shows positive for protein, alkaloid, phenolic compound, glycoside, flavonoid, and phenol. Aqueous extract shows positive result for reducing sugar, alkaloid, phenolic compound, glycoside, flavonoids, phenols, terpenoids, resin and saponins. From the above result it is concluded that the acetone and aqueous shows more positive results.

Antibacterial activity- Table – 2 represent the qualitative antibacterial activity of leaf extract against *E. coli* and *P. aeroginosa* which show positive and negative result. In table 3 shows the zone of inhibition in acetone leaf extract exhibited no activity in 25µl and 50µl but produced 12mm zones of inhibition in 100µl concentrations for E.coli. and for the *P. aeroginosa* showed no activity in 25µl but produced a 15mm &18mm inhibition zone in 50µl and100µl concentration respectively. In table 4 shows the zone of inhibition in ethanol leaf extract exhibited no activity in 25µl but shows 12mm and 17 mm zone of inhibition in 50µl and 100µl respectively for E.coli. In table 5 shows the zone of inhibition in Aqueous leaf extract exhibited no activity in 25µl and 50µl but show 18mm zone of inhibition in 100µl for *P. aeruginosa.*
Antibacterial activity was expressed at varying degrees with the difference in concentration. Higher concentration of the leaf extract shows highest antibacterial activity.

Conclusion - Medicinal plants were the potent source of human health due to the presence of active phytochemical compounds that is responsible for its various pharmacological activities. On the basis of the results obtained in the present study conclude that *Annona squamosa L.* is rich in phytochemical constituents and showed antibacterial properties. The results of the phytochemical screening of the leaf extracts of samples varied, while some of the components were present, some were absent. It was observed that most of the components were present in acetone and aqueous extracts. The present study highlights the possible use of *Annona squamosa* L. leaf extracts as a source of antioxidants and antibacterial agents that can be used to prevent gastrocolic diseases.

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