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Screening of Antibacterial, Antioxidant and Phytochemical of Leaf, Stem and Root extracts of *Annona squamosa* L. against Pathogenic Bacteria

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ABSTRACT: In India, *Annona squamosa* L. is commonly used as a traditional medicine to treat various illnesses. The antibacterial properties of extracts from *Annona squamosa* leaves, stems, and roots were investigated using two strains of gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two strains of gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The antibacterial components were extracted sequentially using solvents such as hexane, chloroform, ethyl acetate, methanol, and an aqueous solution. The antimicrobial properties of the extracts were determined using the agar well diffusion method. It was found that the root extracts of *A. squamosa* exhibited higher antibacterial activity compared to the leaf and stem extracts. Specifically, the chloroform extracts of *A. squamosa* roots were found to be effective against *E. coli* and *B. subtilis*. In this study, the antioxidant capacity of the leaf, stem, and root extracts of A. *squamosa* was also tested using the DPPH free radical scavenging method. The aim of this study is to provide a comprehensive summary of the chemical components present in the leaf, stem, and root extracts of *A. squamosa*, as well as the wide range of phytochemicals described in the study. Due to the increasing prevalence of multi-drug resistant pathogens, these findings support the therapeutic applications of this plant in traditional medicine, enhancing its therapeutic value.

Keywords: Anti-bacterial, *Annona squamosa*, Agar well diffusion method, Traditional medicine.

INTRODUCTION

Annona squamosa L., commonly known as custard apple, belongs to the *Annonaceae* family and is a small tree (Salman and Senthilkumar 2015). The term "*anon*" in Latin means "yearly produce," which is the origin of the name "*Annona*," referring to the numerous fruit-bearing species within this genus, which comprises over 2300 species (Leatemia and Isman 2004). *A. squamosa* specifically refers to the species, describing the fruit's knobby appearance (Vyas *et al.*, 2012). The custard apple is an edible tree or large shrub that typically reaches a height of three to five feet, but can grow up to six or eight meters under optimal conditions (Shashirekha *et al.*, 2008). It is cultivated in gardens for its aesthetic appeal and also serves as a valuable source of fuel (Wayne, 2002). This tree is grown extensively in India and other tropical countries primarily for its delicious heart-shaped fruits, weighing around 150g. The ripe fruit has a creamy and extremely sweet pulp, which can be consumed fresh or used to flavour milk and ice cream (Vanitha *et al*, 2010). The pulp can be used to create various delightful dishes such as jelly, squash, and even wine (Al-Nemari *et al.*, 2020) and (Nagy *et al.*, 1990).

Custard apples have a wide range of therapeutic and non-therapeutic uses. In herbal medicine, various parts of the plant are utilized to treat different illnesses, including heart disease, diabetes, hyperthyroidism, and tumours (Shirwaikar *et al.*, 2004). Traditionally, custard apple has been employed to treat conditions such as dysuria, sickness, hunger, ulceration, ringworm, dysentery, nausea, bleeding, painful urination, and as an abortion inducer. The root of *A. squamosa* was believed to possess strong purgative properties, and root bark scrapings were used for toothaches (Raj Sobiya *et al.*, 2009) and (Yang *et al.*, 2008). Internally, the roots were used to treat spinal conditions and depression. The stem is known for its highly effective astringent properties.

A. squamosa leaves are believed to be effective in treating prolapses in children. In cases of hysteria and fainting spells, crushed leaves are sniffed. A leaf decoction is taken to alleviate dysentery (Gajalakshmi *et al.*, 2011). Poultices made from the leaves are applied to boils and ulcers. The mature fruits of this plant are used to treat cancerous tumours and aid in the process of suppuration. In Ayurveda, the fruits are considered a good tonic, enriching the blood and increasing muscle strength, among other benefits. The powder derived from dried unripe fruits is used as a pesticide. However, it should be noted that the seeds of *A. squamosa* are poisonous and acrid. Powdered seeds are used to create fish poison and insecticides. A paste made from seed powder is applied to the head to eliminate lice. It is also

utilized to kill worms in cattle wounds (Parvin *et al.*, 2003).

The seeds of *A. squamosa* can be used to extract high-quality oil, which contains fatty acids such as oleic, linoleic, palmitic, and stearic acids (Mariod *et al.*, 2010). These acids have various applications in the production of alkyds, soap making, and the plasticizer industry (Ahmad *et al.*, 2006). The seed cake can be utilized as a fertilizer (Khan *et al.*, 1983), while the non-edible seed oil serves as an insect repellent (Kamble and Soni 2010). Additionally, it was found that the alkaloidal extract of the plant effectively inhibits the corrosion of C38 steel in a standard hydrochloric acid medium (Lebrini *et al.*, 2010).

The increasing interest in consuming natural ingredients for a healthier lifestyle aligns with the use of plants as a source of traditional medicine (Solikhah *et al.*, 2021). Plants are readily available in our surroundings, making them accessible for medicinal purposes (Safira *et al.*, 2021). The World Health Organization reports that approximately 80% of the global population has utilized herbal ingredients in healthcare.

By exploring the pharmacological properties of *A. squamosa*, researchers have uncovered its potential as a medicinal plant (khairullah *et al.*, 2021). This knowledge can contribute to the development of natural remedies and therapeutic interventions (kalidindi *et al.*, 2015). It is worth noting that further research and clinical studies are necessary to fully understand the efficacy, safety, and specific applications of *A. squamosa* in various aspects of healthcare.

Overall, the interest in natural ingredients for a healthier lifestyle, combined with the pharmacological effects of *A. squamosa*, highlights its potential as a valuable medicinal plant. Continued research and exploration of its properties can lead to the development of new treatments and contribute to the well-being of individuals worldwide.

A. squamosa seeds produce wax and latex, which function as detergents, and their powder can be used as an effective hair shampoo when combined with gram flour. In sustainable agriculture, the use of pesticides derived from plants like *A. squamosa* can greatly benefit pest management. These pesticides have regenerative properties and pose relatively less danger to humans, non-target organisms, and natural predators. The objective of this study is to investigate the antibacterial properties of crude leaf, stem, and root extracts of *A. squamosa*.

MATERIALS AND METHODS

A. Collection of plant material

Fresh leaves, stems, and roots of fully grown *Annona squamosa* were collected from Munnukalimoodu in Thiruvananthapuram district.

B. Preparation of plant extracts

The leaves, stems, and roots were thoroughly cleaned and then dried in the shade before being finely powdered. Using a Soxhlet apparatus, the dried and powdered leaves, stems, and roots were extracted with five different solvents: hexane, chloroform, ethyl acetate, methanol, and an aqueous solution. After the solvent evaporation process, concentrated extracts were obtained. These extracts were stored in tightly sealed containers in a freezer at -40°C for future use.

C. Antibacterial activity

Antibacterial activity was assessed against two selected gram-positive bacteria, namely *B. subtilis* and *S. aureus*, as well as two gram-negative bacteria, *E. coli* and *P. aeruginosa*. The strains used in the investigation were provided by the Microbial Type Culture Collection (MTCC) of the Institute of Microbial Technology in Chandigarh. The Agar well diffusion method was employed to determine the minimum inhibitory activity of the plant parts (leaves, stems, and roots) in order to evaluate their biological utility and potential. Each of the bacterial strains, including *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, was inoculated on 20 millilitres of Muller Hinton Agar Medium (MHA) in petri plates and allowed to grow overnight. Wells with a diameter of approximately 6 mm were made using a cork borer, and 20µl of each sample extract (leaves, stems, and roots) from a stock concentration of 1 mg/1 ml was added to the respective wells. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zones that formed around the wells in millimetres (Wayne, 2002). As a positive control, ciprofloxacin, a commonly used antibacterial drug at a dosage of 5 µg/1 ml, was used.

D. Antioxidant activity

DPPH free radical scavenging assay. A modified technique based on (Kokate *et al.*, 2003) was used to measure the scavenging potential of the extracts using the DPPH free radical scavenging assay. A solution of DPPH (0.1m/M) was prepared by dissolving 1.1829g of DPPH in methanol and bringing the volume up to 30ml with methanol. The solution was then left in the dark for 30 minutes to complete the reaction. Various extract concentrations (0.2, 0.4, 0.6, 0.8, and 1mg/ml) were mixed with 22µl of the DPPH solution and allowed to sit at room temperature for 30 minutes. The mixture was spectrophotometrically measured at 517 nm. The free radical scavenging activity was estimated using the following formula:

$$\% \text{ Inhibition} = (Ac - At) / Ac \times 100$$

where Ac represents the absorbance of the test sample and *At* represents the absorbance of the control. Ascorbic acid testing was conducted using extract sample concentrations as a standard (Jamkhande *et al.*, 2014). The antioxidant activity of the sample was expressed by the IC50 value, which is defined as the concentration of the sample that inhibits the generation of DPPH radicals by 50% (Mulla *et al*, 2010) and (Chew *et al.*, 2012).

E. Preliminary phytochemical Screening

Preliminary phytochemical screening was conducted to confirm the presence of primary chemical constituents

in the newly obtained extracts. The following conventional tests were performed:

Alkaloid Test. A small amount of the extract (mg) was warmed in 2% sulfuric acid for 2 minutes in a separate test tube. Afterwards, a few drops of Dragendorff's reagent were added after filtration in a different test tube. The formation of orange or red precipitates indicated the presence of alkaloids.

Phenol Test. Ferric chloride with 5% alcohol content was added to the substance in water. The presence of phenols was indicated by the appearance of a dark blue or green colour.

Flavonoid Test. Substance in alcohol was treated with 10% NaOH or ammonia. The presence of flavonoids was indicated by the development of a dark yellow colour.

Saponin Test. Few milligrams of the extract were thoroughly mixed with distilled water. The presence of saponins was confirmed when foam formed.

Glycoside Test. The substance was treated with concentrated anthrone and sulphuric acid. Heating the mixture over a water bath revealed the presence of glycosides, indicated by the emergence of a green hue.

Terpenoid Test. Concentrated H_2SO_4 was added to a few milligrams of the extract in chloroform. The presence of terpenoids was indicated by the formation of a dark brown precipitate.

Steroid Test. In a dry test tube, 2 ml of chloroform were added to a few milligrams of the extract. After heating, a few drops of concentrated sulphuric acid and two drops of acetic anhydride were added following a few drops of acetic acid. The presence of steroids was indicated by the appearance of a green coloration.

Protein and Amino Acid Test. A few drops of a diluted (1%) copper II sulphate solution were added to the sample solution in a test tube, followed by sodium hydroxide solution. Gentle stirring was performed to combine the substances. The presence of proteins was indicated by the purple colour.

Reducing Sugar Test. 5ml of Fehling's solutions 1 and 2 (in a 1% ratio) were added to 2ml of the plant extract and heated for 5 minutes. The presence of reducing sugar was confirmed by the formation of red precipitates.

RESULT AND DISCUSSION

A. Agar well diffusion method

The antibacterial activity of *Annona squamosa* leaf, stem, and root extracts, dissolved in DMSO, was assessed in vitro using the agar well diffusion method against microbial strains including *B. subtilis, S. aureus, E. coli* and *P. aeruginosa*. The following tables present the significant antibacterial activity observed against gram-positive (*B. subtilis* and *S. aureus*) and gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains using the leaf, stem, and root extracts of A. squamosa in combination with different solvents. Table 1 illustrates the zone of inhibition produced by the leaf extracts of *A. squamosa* in combination with five different solvents against the bacterial strains on Muller Hinton Agar. Table 2 illustrates the zone of inhibition created by the stem extracts of *A. squamosa* in combination with five different solvents against the bacterial strains on Muller Hinton Agar. Table 3 illustrates the zone of inhibition generated by the root extracts of *A. squamosa* in combination with five different solvents against the bacterial strains on Muller Hinton Agar.

Antibacterial activity of leaf extract. However, it should be noted that the ethyl acetate leaf extracts demonstrated a zone of inhibition measuring 10 mm against *Pseudomonas aeruginosa*. On the other hand, the leaf extracts combined with five different solvents exhibited no antibacterial activity against *Bacillus subtilis, Staphylococcus aureus*, and *Escherichia coli.* In a study published in the journal "International Journal of Pharma and Bio Sciences," researchers investigated the antimicrobial potential of *Annona squamosa* leaf extract against various pathogenic bacteria. The study reported that the leaf extract exhibited significant inhibitory effects against a range of bacteria, including *Escherichia coli, Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa*. The antimicrobial activity was attributed to the presence of alkaloids and flavonoids in the leaf extract (Neethu *et al.*, 2016).

Antibacterial activity of stem extract. The stem extracts combined with five different solvents exhibited no antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. However, the hexane, chloroform, and ethyl acetate stem extracts showed zones of inhibition measuring 7 mm, 7 mm, and 9 mm respectively against *Bacillus subtilis*. Furthermore, the ethyl acetate stem extracts demonstrated a zone of inhibition measuring 13 mm against *Pseudomonas aeruginosa*.

Kachhawa and his colleague conducted a study where they found that a methanolic extract of the stem bark of *Annona squamosa* exhibited antimicrobial activity against both gram-positive and gram-negative strains of *Bacillus* coagulants and *Escherichia coli* in vitro (Kachhawa *et al.*, 2012).

Table 1: Illustrates the zone of inhibition produced by the leaf extracts of *A. squamosa* in combination with five different solvents against the bacterial strains on Muller Hinton Agar.

Test organisms	Zone of inhibition of leaf extracts					Positive control
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous solution	
B. subtilis	-	-	-	-	-	20
S. aureus	-	-	-	-	-	20
P. aeruginosa	-	-	10	-	-	20
E. coli	-	-	-	-	-	30

Similarly, Padhi and his co-workers reported positive results for the plant's antibacterial properties when tested against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Vibrio alginolyicus* (Padhi *et al.*, 2011). In another study, Chavan and his team (Chavan *et al.*, 2010) isolated a compound called 18-acetoxy-ent-kaur-16-ene from a petroleum-based extract of *A. squamosa* bark, which demonstrated analgesic and anti-inflammatory properties.

Antibacterial activity of root extract. The root extracts combined with five different solvents did not exhibit any antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However, the hexane and chloroform root extracts showed zones of inhibition measuring 7 mm and 14 mm respectively against *Bacillus subtilis*. Additionally, the chloroform root extract produced a 6 mm zone of inhibition against *Escherichia coli*, while the hexane root extract exhibited a 12 mm zone of inhibition against the same strain. In his study Vidyasagar said that, by analysing the crude extracts of *A. squamosa* root, researchers may have identified specific phytochemicals responsible for the observed antimicrobial properties. These phytochemicals could include compounds such as alkaloids, flavonoids, tannins, phenols, or other secondary metabolites known for their antimicrobial activity (Vidyasagar, 2012).

Table 2: Illustrates the zone of inhibition created by the stem extracts of *A. squamosa* in combination with five different solvents against the bacterial strains on Muller Hinton Agar.

Test organisms	Zone of inhibition of stem extracts					Positive control
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous solution	
B. subtilis	7	7	9	-	-	20
S. aureus	-	-	-	-	-	20
P. aeruginosa	-	-	-	-	-	20
E. coli	-	-	13	-	-	30

Table 3: Illustrates the zone of inhibition generated by the root extracts of *A. squamosa* in combination with five different solvents against the bacterial strains on Muller Hinton Agar.

Test organisms	Zone of inhibition of root extracts					Positive control
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous solution	
B. subtilis	7	14	-	-	-	20
S. aureus	-	-	-	-	-	20
P. aeruginosa	-	-	-	-	-	20
E. coli	6	12	-	-	-	30

They investigated the bactericidal activity of five different solvents (hexane, chloroform, ethyl acetate, methanol, and aqueous solution) at a concentration of 1 mg/1 ml. The chloroform root extracts showed the highest antibacterial activity against *B. subtilis* and *E. coli* at the given concentration. These findings suggest the potential of further research in this area.

Antioxidant activity. The DPPH free radical scavenging assay was employed to assess the antioxidant properties of the extracts. This assay is commonly used to determine the ability of compounds to neutralize free radicals and evaluate their antioxidant potential. The extracts were tested for their ability to scavenge DPPH radicals, which are highly reactive and stable free radicals. The scavenging activity was measured spectrophotometrically at 517 nm, and the percentage of inhibition was calculated using the absorbance values of the test sample and the control. Ascorbic acid was used as a standard for comparison. The IC50 value, which represents the concentration of the sample required to scavenge 50% of the DPPH radicals, was used to evaluate the antioxidant activity of the extracts. This assay provides valuable information about the antioxidant potential of the tested extracts and their ability to protect against oxidative stress.

DPPH free radical scavenging assay. The DPPH assay is a commonly used method to evaluate the antioxidant activity of samples. DPPH is a stable free radical that absorbs UV-Vis light at 517 nm. When an antioxidant is present in the sample, it reduces the DPPH radical, leading to a decrease in its absorbance. The degree of inhibition is used to assess the antioxidant capacity of the sample, and the concentration required to achieve 50% inhibition is represented by the IC50 value. Table 4 illustrates the percentage inhibition of ascorbic acid, a standard antioxidant, using the DPPH assay method. Tables 5, 6 and 7 show the percentage inhibition of DPPH by methanolic extracts of the leaf, stem, and root of *A. squamosa*, respectively. These tables provide information about the scavenging activity of the extracts and their ability to inhibit DPPH radicals. The results indicate that the methanolic extracts of the leaf, stem, and root possess proton-donating capacity and exhibit strong DPPH radical inhibition. Fig. 1, 2, 3 and 4 depict the scavenging activity of the extracts compared to the standard ascorbic acid, visually representing their antioxidant potential.

Antioxidant activity of leaf extract. Despite the mean percentage of the leaf extract being lower than that of ascorbic acid, the standard antioxidant, it exhibited a similar concentration-dependent free radical scavenging effect. The scavenging activity of the leaf extract was particularly pronounced at concentrations of 0.8mg and 1mg. By using linear regression analysis, the IC50 values for ascorbic acid (0.24mg/ml) and leaf extract (0.27mg/ml) were determined. Both ascorbic acid and leaf extract demonstrated an IC50 value for their ability to scavenge free radicals, as depicted in Fig. 1 and 2, as well as Table 4 and 5. The findings from the research conducted by Neha and her friend justify the antioxidant activity of *A. squamosa* leaf extract (Neha and Dushyant 2011).

Antioxidant activity of stem extract. Despite the mean percentage of the stem extract being lower than that of ascorbic acid, it exhibited a similar concentration-dependent pattern of free radical scavenging activity. The scavenging activity of the stem extract was particularly pronounced at concentrations of 0.8mg and 1mg. Using linear regression analysis, the IC50 values for ascorbic acid (0.24mg/ml) and stem extract (0.47mg/ml) were determined. Fig. 1 and 3, as well as Tables 4 and 6, illustrate the IC50 values and the free radical scavenging activity of both ascorbic acid and the stem extract. Neha and her colleague explained that the extract of Annona squamosa Linn stem bark exhibited a significant scavenging effect on the DPPH free radical in their research work (Neha and Dushyant 2011).

Antioxidant activity of root extract. Although the mean percentage of the root extract was lower than that of ascorbic acid, a standard antioxidant, it exhibited a similar concentration-dependent pattern of free radical scavenging activity. The scavenging activity of the root extract was particularly prominent at concentrations of 0.8mg and 1mg. Using the linear regression equation, the IC50 values for ascorbic acid (0.24mg/ml) and root extract (0.60mg/ml) were determined. Fig. 1 and 4, as well as Tables 4 and 7, illustrate the IC50 values and the free radical scavenging activity of both ascorbic acid and the root extract. Abdalbasit and colleagues explained in their research that the root of *Annona squamosa* has higher antioxidant activity compared to the root of *Catunaregam nilotica* (Abdalbasit *et al*, 2012).

Table 4: Illustrates the percentage inhibition of ascorbic acid, a standard antioxidant, using the DPPH assay method.

Sr. No.	Conc. (mg/ml) of leaf extract	% inhibition	IC $_{50}$
1	0.2	48.40	**0.24mg/ml**
2	0.4	59.64	
3	0.6	68.14	
4	0.8	80.75	
5	1	95.60	

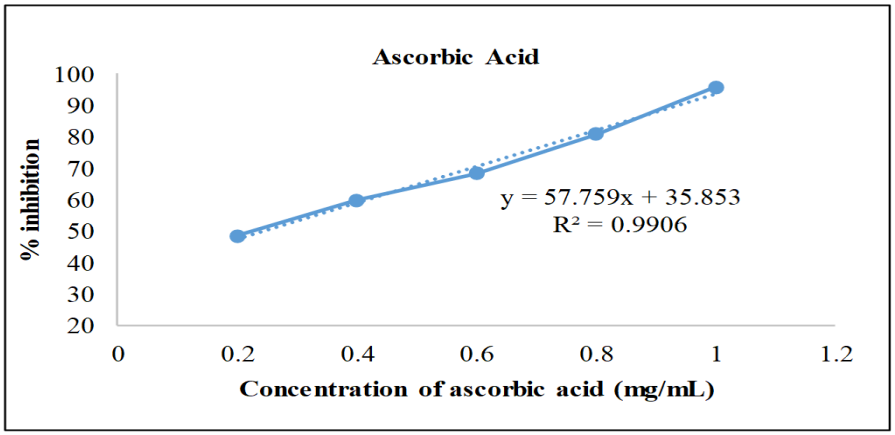

Fig. 1. Depict the scavenging activity of the standard ascorbic acid, visually representing their antioxidant potential.

Table 4: Illustrates the percentage inhibition of leaf extract, a standard antioxidant, using the DPPH assay method.

Sr. No.	Conc. (µg/ml) of leaf extract	% inhibition	IC $_{50}$
1	0.2	44.45	**0.27mg/ml**
2	0.4	57.08	
3	0.6	68.06	
4	0.8	87.05	
5	1	89.57	

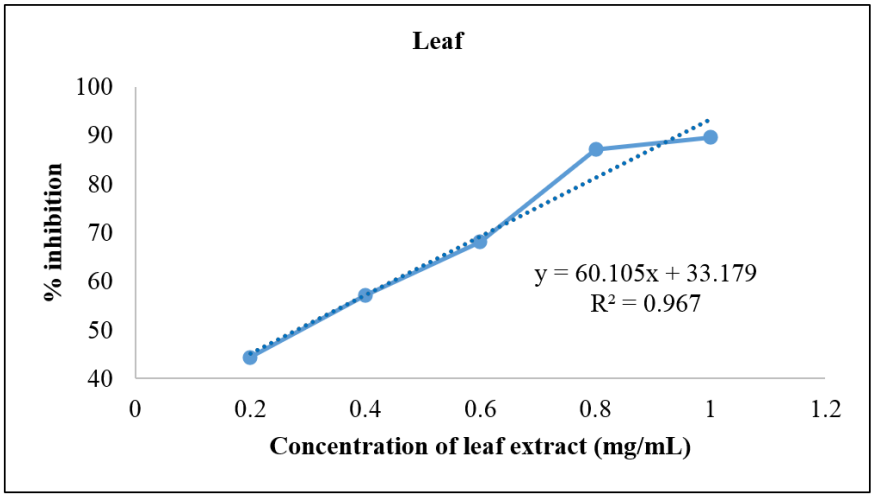

Fig. 2. Depict the scavenging activity of the leaf extracts compared to the standard ascorbic acid, visually representing their antioxidant potential.

Table 6: Illustrates the percentage inhibition of stem extract, a standard antioxidant, using the DPPH assay method.

Sr. No.	Conc. (µg/ml) of stem extract	% inhibition	IC $_{50}$
1	0.2	34.36	**0.47mg/ml**
2	0.4	44.08	
3	0.6	59.06	
4	0.8	70.91	
5	1	75.67	

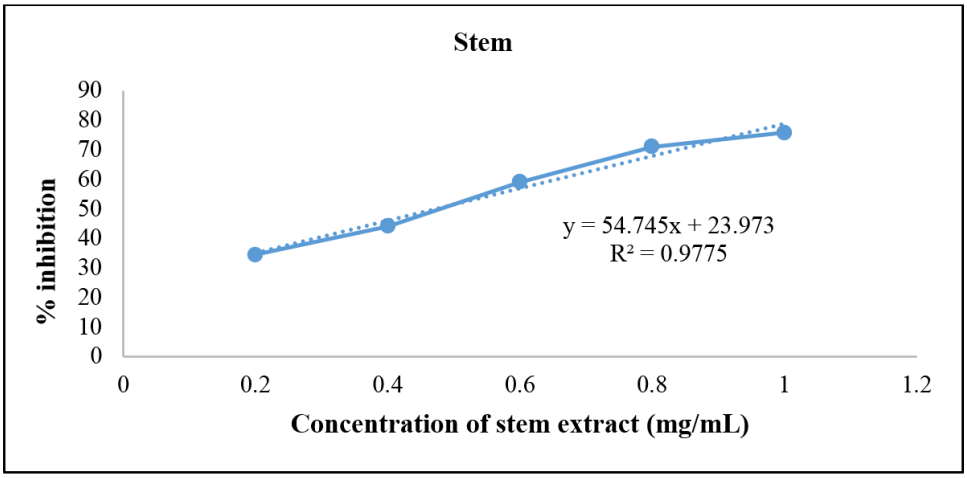

Fig. 3. Depict the scavenging activity of the stem extracts compared to the standard ascorbic acid, visually representing their antioxidant potential.

Table 7: Illustrates the percentage inhibition of root extract, a standard antioxidant, using the DPPH assay method.

Sr. No.	Conc. (µg/ml) of root extract	% inhibition	IC $_{50}$
1	0.2	31.09	**0.60mg/ml**
2	0.4	36.21	
3	0.6	48.40	
4	0.8	57.98	
5	1	74.36	

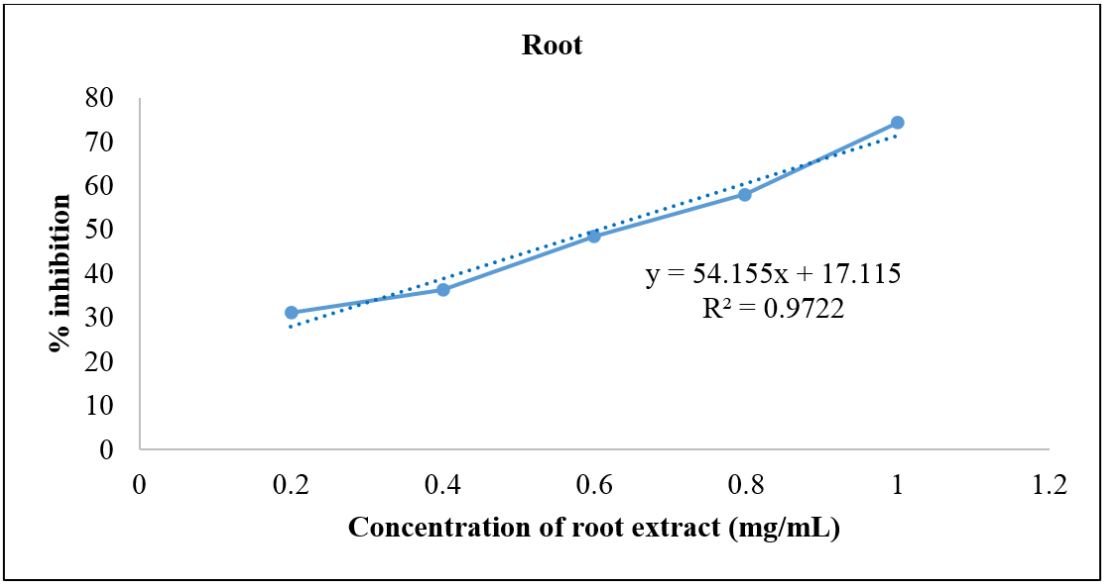

Fig. 4. Depict the scavenging activity of the root extracts compared to the standard ascorbic acid, visually representing their antioxidant potential.

Preliminary phytochemical Screening

Phytochemical analysis of leaf extract. Qualitative tests were conducted on the leaf extract of *A. squamosa* dissolved in five different solvents (hexane, chloroform, ethyl acetate, methanol, and aqueous solution) to analyse its phytochemical composition. The results revealed the presence of alkaloids and glycosides in all tested solvents. Methanol and the aqueous solution also showed the presence of phenols, while hexane and chloroform extracts contained steroids. Reducing sugars were detected only in the aqueous solution. However, flavonoids, terpenoids, proteins, and amino acids were not found in any of the leaf extracts, as indicated in Table 8.

In their study, Narasimharaju and his colleagues revealed the presence of glycosides, flavonoids, phenols, tannins, saponins, alkaloids, carbohydrates, and steroids in different extracts through preliminary phytochemical analysis of *Annona squamosa* leaf extracts (Narasimharaju *et al.*, 2015).

Phytochemical analysis of stem extract. Based on the information provided, the *A. squamosa* stem extract was analysed using five different solvents: hexane, chloroform, ethyl acetate, methanol, and an aqueous solution. The tests conducted on the stem extracts from all five solvents revealed the presence of alkaloids. Here's a summary of the chemical components found in each solvent, hexane extract contains terpenoids. Chloroform extract has no specific information was given regarding its chemical composition. Ethyl acetate extract contains terpenoids. Methanol extract contains phenol and terpenoids. Aqueous solution extract contains saponin, glycosides, and reducing sugar. Based on Table 9, the *A. squamosa* stem extract was found to be lacking in the following chemical components: flavonoids, steroids, proteins, and amino acids. These components were not detected in any of the solvent extracts tested.

Neha and her co-worker explained that the phytochemical screening revealed that the major constituents of the ethanolic extract of *Annona squamosa* Linn stem bark were phenolic compounds, glycosides, alkaloids, etc. These constituents may be responsible for the antioxidant activities (Neha and Dushyant 2011).

Table 8: Illustrating the phytochemical present in the leaf extracts of *A. squamosa.*

Sr. No.	Phytochemicals	Solvents				
		Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous solution
1.	**Alkaloids**	+	+	+	+	+
2.	**Phenols**	-	-	-	+	+
3.	**Flavonoids**	-	-	-	-	-
4.	**Saponins**	-	-	-	-	+
5.	**Glycosides**	+	+	+	+	+
6.	**Terpenoids**	-	-	-	-	-
7.	**Steroids**	+	+	-	-	-
8.	**Proteins**	-	-	-	-	-
9.	**Amino acids**	-	-	-	-	-
10.	**Reducing sugar**	-	-	-	-	+

Table 9: Illustrating the phytochemical present in the stem extracts of *A. squamosa.*

Sr. No.	Phytochemicals	Solvents				
		Hexane	chloroform	Ethyl acetate	Methanol	Aqueous solution
1.	**Alkaloids**	+	+	+	+	+
2.	**Phenols**	-	-	-	+	+
3.	**Flavonoids**	-	-	-	+	+
4.	**Saponins**	-	-	-	-	+
5.	**Glycosides**	-	-	+	+	+
6.	**Terpenoids**	-	-	-	-	-
7.	**Steroids**	-	-	-	-	-
8.	**Proteins**	-	-	-	-	-
9.	**Amino acids**	-	-	-	-	-
10.	**Reducing sugar**	-	-	-	-	-

Phytochemical analysis of root extract. The phytochemical components of *A. squamosa* root extract were analysed using five different solvents: hexane, chloroform, ethyl acetate, methanol, and an aqueous solution. Qualitative tests were conducted to determine its chemical composition. Alkaloids were found in all five solvent extracts. Hexane and aqueous solution extracts showed the presence of saponins, glycosides, and reducing sugar. The methanolic extract contained phenol, while hexane, chloroform, and methanol extracts contained terpenoids. Flavonoids, steroids, proteins, and amino acids were not detected in the *A. squamosa* root extract, as indicated in Table 10.

No phytochemical screening of *Annona squamosa* root extract has been conducted as of the present study, indicating that this is a new research endeavour.

Table 10: Illustrating the phytochemical present in the root extracts of *A. squamosa.*

Sr. No.	Phytochemicals	Solvents				
		Hexane	chloroform	Ethyl acetate	Methanol	Aqueous solution
1.	**Alkaloids**	+	+	+	+	+
2.	**Phenols**	-	-	-	+	+
3.	**Flavonoids**	-	-	-	+	+
4.	**Saponins**	-	-	-	-	+
5.	**Glycosides**	-	-	+	+	+
6.	**Terpenoids**	-	-	-	-	-
7.	**Steroids**	-	-	-	-	-
8.	**Proteins**	-	-	-	-	+
9.	**Amino acids**	-	-	-	-	+
10.	**Reducing sugar**	-	-	-	-	-

CONCLUSION

Traditional plants have long been recognized for their beneficial effects on human health, attributed to the presence of active phytochemical constituents. Based on the findings of the current study, it can be inferred that *Annona squamosa* is rich in antibacterial, antioxidant, and phytochemical characteristics. The study evaluated the antimicrobial, antioxidant, and phytochemical properties of leaf, stem, and root extracts from multiple samples.

The results indicated that certain solvents triggered activity in some extracts, while others did not. Specifically, chloroform root extracts exhibited significant antibacterial activity, while methanolic root extracts showed high antioxidant properties. Additionally, most of the tested phytochemicals were found to be present in the root extracts.

The primary focus of this study was to explore the potential use of *A. squamosa* extracts from leaves, stems, and roots as a source of antibacterial agents for the treatment of digestive diseases. The investigation demonstrated that *A. squamosa* roots possess strong antibacterial and antioxidant properties, along with various phytochemical constituents. This suggests that further research on *A. squamosa*, such as its potential antidiabetic action, may unveil additional beneficial properties and mechanisms.

FUTURE SCOPE

1. Identification and characterization of novel bioactive compounds: Further investigation can be carried out to identify and isolate specific bioactive compounds responsible for the antimicrobial and antioxidant properties of *A. squamosa*. This could involve advanced techniques such as chromatography, spectroscopy, and mass spectrometry to elucidate the chemical structures and mechanisms of action.

2. Mechanisms of antimicrobial action: Understanding the underlying mechanisms of the antimicrobial activity exhibited by *A. squamosa* can contribute to the development of new antimicrobial

agents or the enhancement of existing ones. Studies could focus on elucidating the specific targets and modes of action of the active compounds against different microorganisms.

3. Synergistic effects and combination therapies: Investigating the potential synergistic effects of *A. squamosa* extracts or bioactive compounds with existing antimicrobial agents could lead to the development of more effective and efficient therapeutic strategies. Combination therapies have the potential to enhance antimicrobial activity, reduce the development of resistance, and broaden the spectrum of action.

4. Antioxidant and free radical scavenging activity: Further research can explore the antioxidant potential of *A. squamosa* and its bioactive components. This can involve assessing the ability to scavenge free radicals, measuring antioxidant enzyme activities, and evaluating the protective effects against oxidative stress-related diseases.

5. Preclinical and clinical studies: Conducting preclinical studies using animal models and eventually progressing to clinical trials can help evaluate the safety, efficacy, and potential therapeutic applications of *A. squamosa* extracts or isolated compounds. This research can provide valuable insights into the potential use of *A. squamosa* in the prevention and treatment of microbial infections and oxidative stress-related disorders.

6. Formulation development: Developing novel formulations, such as nan formulations, microencapsulation, or delivery systems, can enhance the stability, bioavailability, and targeted delivery of *A. squamosa* extracts or bioactive compounds. This can facilitate their incorporation into various pharmaceutical, cosmetic, and food products.

7. Bioinformatics and molecular studies: Utilizing bioinformatics tools and molecular techniques, such as transcriptomic and proteomics, can aid in understanding the gene expression patterns and molecular pathways associated with the antimicrobial and antioxidant activities of *A. squamosa*. This knowledge can provide valuable insights into the regulatory mechanisms and facilitate targeted modifications or enhancements of bioactive components.

By pursuing these avenues of research, scientists can further explore the potential of *A. squamosa* as a source of antimicrobial and antioxidant agents, leading to the development of new therapeutic options and functional products with broad applications in healthcare and related industries.

Author's contributions. K R Beula Rani: Conceptualization. Gama Mohan M G: Materials and Methodology, Field exploration, data curation and analysis, and writing - original draft. K R Beula Rani: Writing- review & editing. Both authors have verified and approved the final version of the manuscript.

Conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCE

Ahmad, S., Naqvi, F., Sharmin, E., & Verma, K. L. (2006). Development of amine– acid cured *Annona squamosa* oil epoxy anticorrosive polymeric coatings. *Progress in organic coatings*, *55*(3), 268-275.

Al-Nemari, R., Bacha, A. B., Al-Senaidy, A., Arafah, M., Al-Saran, H., Abutaha, N., & Semlali, A. (2020). Selective cytotoxic effects of *Annona Squamosa* Leaves against breast cancer cells via apoptotic signaling proteins. *Preprints*.

Chavan, M. J., Wakte, P. S., & Shinde, D. B. (2010). Analgesic and anti-inflammatory activity of Caryophyllene oxide from *Annona squamosa* L. bark. *Phytomedicine*, *17*(2), 149-151.

Chew, A. L., Jessica, J. J. A., & Sasidharan, S. (2012). Antioxidant and antibacterial activity of different parts of *Leucas aspera*. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), 176-180.

Gajalakshmi, S., Divya, R., Deepika, V. D., Mythili, S., & Sathiavelu, A. (2011). Pharmacological activities of *Annona squamosa:* A review. *International Journal of Pharmaceutical Sciences Review and Research*, *10*(2), 24-29.

Jamkhande, P. G., Wattamwar, A. S., Pekamwar, S. S., & Chandak, P. G. (2014). Antioxidant, antimicrobial activity and in silico PASS prediction of *Annona reticulata* Linn. root extract. Beni-Suef University *Journal of Basic and Applied Sciences*. *3*(2), 140-148.

Kachhawa, J. B. S., Sharma, N., Tyagi, S., & Sharma, K. K. (2012). Screening of stem bark methanol extract of *Annona squamosa* for antibacterial activity. *Int J. Curr. Pharm. Res*., *4*(1), 48-50.

Kalidindi, N., Thimmaiah, N. V., Jagadeesh, N. V., Nandeep, R., Swetha, S., & Kalidindi, B. (2015). Antifungal and antioxidant activities of organic and aqueous extracts of *Annona squamosa* Linn. leaves. *Journal of food and drug analysis*, *23*(4), 795-802.

Kamble, K. J., & Soni, S. B. (2010). Effect of steam blanching on quality of custard apple pulp. *Journal of Horticulture and Forestry*, *2*(5), 104-107.

Khairullah, A. R., Solikhah, T. I., Ansori, A. N. M., Hidayatullah, A. R., Hartadi, E. B., Ram, S. C., & Fadholly, A. (2021). Review on the pharmacological and health aspects of apium graveolens or celery: An update. *Systematic Reviews in Pharmacy*, *12*(2), 595-601.

Khan, M. W. Y., Ahmad, F., Ahmad, I., & Osman, S. M. (1983). Nonedible seed oils as insect repellent. *Journal of the American Oil Chemists' Society*, *60*, 949-950.

Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2003). *Text book of Pharmacognosy*. *Pune: Nirali Prakashan*, *8*(66), 1-624.

Leatemia, J. A., & Isman, M. B. (2004). Insecticidal activity of crude seed extracts of *Annona* sp., *Lansium domesticum* and *Sandoricum koetjape* against lepidopteran larvae. *Phytoparasitica*, *32*, 30-37.

Lebrini, M., Robert, F. & Roos, C. (2010). Inhibition effect of alkaloids extract from *Annona squamosa* plant on the corrosion of C38 steel in normal hydrochloric acid medium. *International Journal of Electrochemical Science*, *5*(11), 1698-1712.

Mariod, A. A., Abdelwahab, S. I., Elkheir, S., Ahmed, Y. M., Fauzi, P. N. M., & Chuen, C. S. (2012). Antioxidant activity of different parts from *Annona squamosa*, and *Catunaregam nilotica* methanolic extract. *Acta Scientiarum Polonorum Technologia Alimentaria*, *11*(3), 249-258.

Mariod, A. A., Elkheir, S., Ahmed, Y. M., & Matthäus, B. (2010). *Annona squamosa* and *Catunaregam nilotica* seeds, the effect of the extraction method on the oil composition. *Journal of the American Oil Chemists' Society*, *87*(7), 763-769.

Mulla, W. A., Kuchekar, S. B., Thorat, V. S., Chopade, A. R., & Kuchekar, B. S. (2010). Antioxidant, antinociceptive anti-inflammatory activities of ethanolic extract of leaves of *Alocasia indica* (Schott.). *Journal of Young Pharmacists*, *2*(2), 137-143.

Nagy, S., Shaw, P. E., & Wardowski, W. F. (1990). Fruits of tropical and subtropical origin: composition, properties, and uses. *Florida Science Source*.

Padhi, L. P., Panda, S. K., Satapathy, S. N., & Dutta, S. K. (2011). In vitro evaluation of antibacterial potential of *Annona squamosa* L. and *Annona reticulata* L. from Similipal Biosphere Reserve, Orissa, India. *Journal of agricultural Technology*, *7*(1), 133-142.

Pandey, N., & Barve, D. (2011). Phsytochemical and pharmacological review on *Annona squamosa* Linn. *International Journal of research in pharmaceutical and biomedical sciences*, *2*(4), 1404-1412.

Pandey, N., & Brave, D. (2011). Antioxidant activity of ethanolic extract of *Annona squamosa* Linn Bark. *Int J Biomed Pharmaceut Sci*., *2*, 1692-1697.

Parvin, S., Islam, E. M., Rahman, M., & Haque, E. (2003). Toxicological evaluation of annotemoyin-1isolated from *Annona squamosa* from long Evan's rat. *Pakistan Journal of Biological Science*, *6*(18), 1593-1596.

Raj Sobiya D, Vennila Jannet J, Aiyavu C, Panneerselvam (2009). The heapatoprotective effect of alcoholic extract of *Annona squamosa* leaves on experimentally induced liver injury in Swiss albino mice. *International Journal of Integrative Biology*, *5*(3), 182-18.

Safira, A., Savitri, S. L., Putri, A. R. B., Hamonangan, J. M., Safinda, B., Solikhah, T. I., ... & Puspitarani, G. A. (2021). Review on the pharmacological and health aspects of Hylocereus or Pitaya: An update. *Journal of Drug Delivery and Therapeutics*, *11*(6), 297-303.

Safira, A., Widayani, P., An-Najaaty, D., Rani, C. A. M., Septiani, M., Putra, Y. A. S., ... & Raharjo, H. M. (2022). A Review of an Important Plants: *Annona squamosa* Leaf. *Pharmacognosy Journal*, *14*(2).

Salman, H. A., & Senthilkumar, R. J. A. J. P. C. R. (2015). Antibacterial activity of *Annona squamosa* L. and *Annona reticulata* L. against clinical isolates of mutans *Streptococci* the causative agents of dental caries. *Asian J Pharm Clin Res*., *8*(4), 152A155.

Santhoshkumar, R., & Kumar, N. S. (2016). Phytochemical analysis and antimicrobial activities of *Annona squamosa* (L) leaf extracts. *Journal of Pharmacognosy and phytochemistry*, *5*(4), 128-131.

Shashirekha, M. N., Baskaran, R., Rao, L. J., Vijayalakshmi, M. R., & Rajarathnam, S. (2008). Influence of processing conditions on flavour compounds of custard apple (*Annona squamosa* L.). *LWT-Food Science and Technology*, *41*(2), 236-243.

Shirwaikar, A., Rajendran, K., & Kumar, C. D. (2004). In vitro antioxidant studies of *Annona squamosa* Linn. leaves. *Indian J Exp Biol*., *42*(8), 803-7.

Solikhah, T. I., Solikhah, G. P., & Susilo, R. J. K. (2021). Aloe vera and Virgin Coconut Oil (VCO) accelerate healing process in domestic cat (*Felis domesticus*) suffering from scabies. *Iraqi J Vet Sci*., *35*(4), 699-704.

Vanitha, V., Umadevi, K. J., & Vijayalakshmi, K. (2010). *In vitro* assessment of alcoholic leaf extracts of *Annona squamosa* and *Aegle marmelos*. *Bioscan*, *5*(2), 225229.

Vidyasagar, G. M. (2012). A comparative antimicrobial activity of methanolic root, leaf, seed cotyledon extracts of *Annona squamosa* L.

Vyas, K., Manda, H., Sharma, R. K., & Singhal, G. (2012). An update review on *Annona squamosa*. *IJPT*. *3*(2), 107-118.

Wayne, P.A. National committee for clinical laboratory standards. *Performance standards for antimicrobial disc susceptibility testing*. (2002); *12*, 01-53.

Yang, Y. L., Hua, K. F., Chuang, P. H., Wu, S. H., Wu, K. Y., Chang, F. R., & Wu, Y. C. (2008). New cyclic peptides from the seeds of *Annona squamosa* L. and their anti-inflammatory activities. *Journal of agricultural and food chemistry*, *56*(2), 386-392.

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