IJPSR (2023), Volume 14, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 March 2023; received in revised form, 08 May 2023; accepted 31 May 2023; published 01 November 2023

ANTIMICROBIAL ACTIVITY IN METHANOLIC SEED EXTRACT OF *ANNONA SQUAMOSA*

Anshuman Bhattacharya [1], Anindita Pal [1], Soumya Datta [* 2], Souvik Biswas [2], Arijit Das [2], Pallakhyi Chanda [2], Dipan Roy [3], Sarbani Roy [4], Mrinmoy Nag [5], Souvik Chattopadhyay [6] and Amartya Sen [7]

BCDA College of Pharmacy and Technology [1], Hridaypur, Barasat, Kolkata - 700127, West Bengal, India.
Bharat Technology [2], Jadurberia, Uluberia, Howrah - 711316, West Bengal, India.
Department of Pharmaceutical Sciences and Technology [3], Birla Institute of Technology, Mesra, Ranchi - 835215, Jharkhand, India.
Mata Gujri College of Pharmacy [4], Kishanganj - 855107, Bihar, India.
NEF College of Pharmaceutical Education and Research [5], Haibargaon, Nagaon - 782001, Assam, India.
Pandaveswar School of Pharmacy [6], Pandaveswar - 713346, West Bengal, India.
ADAMAS University [7], Adamas Knowledge City, Barasat, Barrackpore Road, Kolkata - 700126, West Bengal, India.

Keywords:

Sugar apple, Epilepsy, Dysentery, Anti-tumor activity, CNS depressant, Antimicrobial activity, *Staphylococcus aureus*, *Salmonella typhi*

Correspondence to Author:
Mr. Soumya Datta

Associate Professor,
Bharat Technology, Jadurberia, Uluberia, Howrah - 711316, West Bengal, India.

E-mail: dattasoumya86@gmail.com

ABSTRACT: *Annona squamosa* (sugar apple) is commonly cultivated in tropical South America occasionally in southern Florida as well as in Jamaica, Puerto Rico, Barbados, and dry regions of Australia. It was growing in Indonesia early in the 17th century and has been widely adopted in southern China, tropical Africa, Egypt, and the lowlands of Palestine. This plant is a small well-branched tree or shrub that bears edible fruits called sugar-apples, species of the genus Annona and a member of the family Annonaceae is traditionally used for the treatment of epilepsy, dysentery, cardiac problem, warm infection, constipation, hemorrhage, antibacterial infection and also has anti-tumor activity. Especially the seeds have another important effect they are medically useful to show many therapeutic activities such as anticancer, and CNS depressant. Hence the present study was taken to test the antimicrobial activity of seeds of Annona squamosa and microbes such as *Staphylococcus aureus* and *Salmonella typhi*. The evaluation of the antimicrobial activity of the seed extracts of *Annona squamosa* was done with methanol using the soxhlation method followed by steam distillation to perform phytochemical screening.

INTRODUCTION: Herbal medicines can treat diseases where chemicals and other drugs have failed. These include common illnesses, which lead to drowsiness and other side effects when treated through regular medicines.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(11).5340-48

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(11).5340-48

Annona squamosa (Sugar apple) is commonly cultivated in tropical South America but not often in Central America, very frequently in southern Mexico, West Indies, Bahamas, Bermuda, and occasionally southern Florida as well as in Jamaica, Puerto Rico, Barbados, and in dry regions of Australia.

It was growing in Indonesia early in the 17th century and has been widely adopted in southern China, tropical Africa, Egypt, and the lowlands of Palestine. Cultivation is most extensive in India where the tree is exceedingly popular [1].

The sugar apple is one of the most important fruits in the interior of Brazil. Sugar apple tree ranges from 10 to 20 ft (3–6 m) in height with an open crown of irregular branches and zigzag twigs. A branch tips opposite the leaves and the fragrant flowers are borne singly or in groups of 2 to 4. The fruit is nearly round, oval or conical, long its thick rind composed of knobby segments, separating when the fruit is ripe and revealing a conically segmented, creamy white delightfully fragrant juice sweet delicious flesh. Crushed leaves of the plant are used in India to overcome hysteria and faint spell while leaf decoction is used in the case of dysentery [2].

Throughout tropical America, a decoction of leaves is imbibed as a tonic cold remedy, digestive or to clarify urine whereas the crushed ripe fruit mixed with salt is applied on tumors while the bark and root are both highly astringent [3]. The traditional claim that concoctions of *A. squamosa* can be used in the treatment of bacterial diseases needs to be substantiated with scientific facts that could either support or negate this claim which necessitates the need for this study. This study was aimed at extracting the plant material and evaluating the presence of secondary metabolites as well as the potency of the extracts on some clinical bacterial isolates [4]. From the beginning of history, disincentive diseases like tuberculosis malaria, human immunodeficiency virus, or acquired immunodeficiency syndrome can affect a portion of the human population causing significant morbidity and mortality [5].

The crucial fact about the treatment of bacterial infections is the ability of bacteria to develop resistance to an antimicrobial agent that has been correlated with the consumption of antimicrobials in the general population. An antimicrobial agent like penicillin-induced allergic responses with the broad spectrum antibiotics kills the normal flora of the body that impairing normal body infection. The antimicrobial agent interferes with cell wall synthesis, inhibition protein synthesis, interferes with nucleic acid synthesis and inhibition of metabolic pathways, and disruption of bacterial membrane structure. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in chemical substances that produce a definite physiological action on the human body. Medicinal plants represent a rich source of antimicrobial agents. This plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problem, warm infection, constipation, hemorrhage, and antibacterial infection. It also has anti-tumor activity [6]. Especially the seeds have another important effect they are medically useful to show much therapeutic activity as an anticancer, and CNS depressant Seeds yield oil and resin that act as a detergent. Hence the present study was taken to test the antimicrobial activity of the seed of *Annona squamosa* and microbes such as *Staphylococcus aureus* and *Salmonella typhi* [7].

Types of Annona Genus Plants [8]: Out of varied genus 42 different species were found in the Annona genus, some of them are:

- *Annona glabra* - Pond apple
- *Annona muricata* - Soursop
- *Annona squamosa* - Sugar apple
- *Annona reticulata* - Custard apple
- *Annona diversitolia* - Lama
- *Annona accuminata*- Annona

Traditional Usages of Annona Genus Plants [9]:

- The bark, leaves, and roots of some species are used in folk medicines.
- The strong bark is used for carrying burdens in the Amazon Rainforest and for wooden implements, such as tool handles and pegs. The wood is valued as firewood, Yellow and brown dyes.
- *Annona glabra*: A recent study suggests that the alcoholic seed extract contains anti-cancer compounds.
- *Annona diversitolia*: Leaf extract was possessed to have an anti-nociceptive effect. Roots were found to have an anticonvulsant effect.
- *Annona purpurea*: In Mexico, juice is used for chills and fever. *Annona craciljlora* pulp was found to have mutagenic properties.

- *Annona muricata*: Fruit and fruit juice are taken for worms and parasites, to cool fevers, to increase the mother's milk after childbirth, and as an astringent for diarrhea and dysentery. The crushed seeds are used against internal and external parasites, head lice, and worms. Roots containing acetogenins proved to have anticarcinogenic effects by inhibiting DNA synthesis.

- *Annona squamosa*: Leaves are used to treat hysteria and fainting spells. Leaf decoction is used in the treatment of colds, coughs, intestinal infections, and acidity conditions. Bark decoction is used in diarrhea. Roots are used in dysentery. Fruit is used in the making of ice creams and milk beverages.

Taxonomical Characterisation and Description [10]:

Taxonomical Characterisation of *Annona squamosa:*

Kingdom: Plantae

Order: Magnoliales

Family: Annonaceae

Genus: Annona

Species: Squamosa

Vernacular Names:

English: Custard apple, sugar-apple, sweetsop

Hindi: Sitafal

Telugu: Seethaphalam

Description: *Annona squamosa* is a small well-branched tree or shrub that bears edible fruits called sugar-apples, species of the genus Annona and a member of the family Annonaceae more willing to grow at lower altitudes than its relatives *Annona reticulata* and *Annona cherimola* [10].

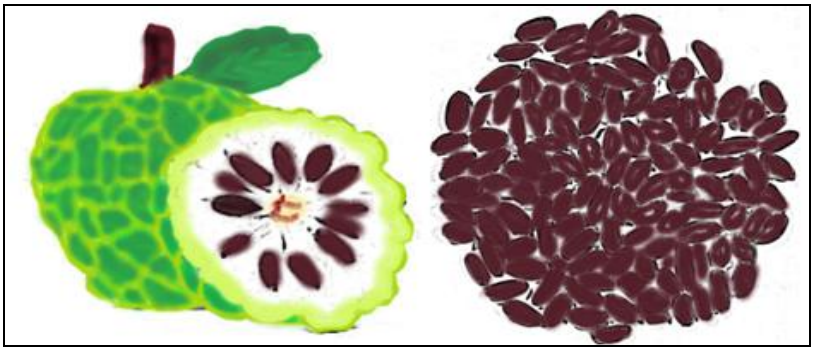

FIG. 1: SUGAR APPLE AND SEED OF SUGAR APPLE

Screening of *Annona squamosa* Extracts: *Annona squamosa* seeds powder was extracted with methanol using the Soxhlation method.

MATERIALS AND METHODS:

Collection of Plant Materials: Seeds were collected from the Annona tree and dried.

Collection and Preparation of Seed Extract: The seeds were milled into a coarse powder using mechanical grinders. Material (50.1 gm) was extracted with methanol by the Soxhlet apparatus. Then the extracted product was distilled out from the methanolic extract by steam distilled apparatus. The extract was concentrated under a vacuum and dried to produce an oily extract that was used for the study. Then the extract was prepared in different concentrations.

Soxhlet Extractor: A Soxhlet extractor Typically, a Soxhlet extraction is only required where the desired compound has limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent, then a simple filtration can be used to separate the compound from the insoluble substance. Normally a solid material containing some of the desired compounds is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid.

The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material the chamber containing the solid material slowly fills with warm solvent. Some of the desired compounds will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles, the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically using a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble and is usually discarded [11-12].

Steam Distillation: After Soxhlation, the methanolic extract was placed in a steam distillation to get a more concentrated product.

Phytochemical Screening:

Alkaloids: Approximately 50 mg of extract was dissolved in 5 ml of distilled water. Further 2M hydrochloric acid was added until an acid reaction occurred and filtered. The filtrate was tested for the presence of alkaloids as detailed below [13].

Dragendorff's Test: At first 2 ml of the filtrate was added to 1 ml of Dragendorff's reagent along the side of the test tube. The formation of orange or orange-reddish brown precipitate indicated the test as positive.

Hager's Test: At first l ml of test solution or filtrate, and a drop or two drops of Hager's reagent were added. The formation of yellow precipitate indicated the test as positive.

Cardiac Glycosides

Keller - Killiani Test: At first 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution were added to a little dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully. The formation of blue colour in the acetic acid layer confirmed the test [14].

Saponin Glycoside:

Foam Test: 5 ml of the test solution was taken in a test tube. It was shaken well for 5 minutes. The formation of stable foam confirmed the test [15].

Steroids:

Salkowski Test: Approximately 2 mg of dry extract was shaken with 1 ml of chloroform and a few drops of concentrated sulfuric acid were added along the side of the test tube. A red-brown color formed at the interface indicated the test as positive for triterpenoids [16].

Liebermann-Burchard's Test: The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled, and then 1 ml of concentrated sulfuric acid was added along the side of the test tube. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols [16].

Tanin:

Ferric Chloride Test: Added a few drops of 5% ferric chloride solution to 2 ml of the test solution. The formation of blue color indicated the presence of hydrolyzable tannins.

Gelatin Test: Added five drops of 1% gelatin containing 10% sodium chloride to 1 ml of the test solution. The formation of white precipitates confirmed the test [17].

Thin-Layer Chromatography: Chromatography is the separation of two or more compounds or ions by the distrıbution between two phases, one which is moving and the other which is stationary. These two phases can be solid-liquid, liquid-liquid, or gas-liquid. Although there are many different variations of Chromatography, the principles are essentially the same Thin-layer chromatography, or TLC is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents [18]. Samples are added to one end of the sheet of paper and dipped into the liquid or mobile phase. The solvent is drawn through the paper by capillary action and the molecules are distributed by partition between the mobile and stationary phases. Different inks and dyes, depending on their molecular structures and interactions with the paper and mobile phase, will

adhere to the paper more or less than the other compounds allowing a quick and efficient separation [19].

Rf Values: In addition to qualitative results, TLC can also provide a chromatographic measurement known as an R_f value. The R_f value is the "retardation factor" or the "ratio-to-front" value expressed as a decimal fraction [19-20].

R_f = Distance spot travels / Distance solvent travels

This number can be calculated for each spot observed on a TLC plate. Essentially it describes the distance traveled by the individual components.

Antimicrobial Activity of Seed Extract on *Annona squamosa:*

Microbial Strains: Stock cultures of microbes used for this study were obtained from the Bharat Technology Laboratory, Uluberia, Howrah. The subculture was prepared from the stock culture using a nutrient broth medium then, the subculture was used for the antimicrobial study. Muller Hinton agar media and Nutrient agar media were used as media [21].

Material Composition for Muller Hinton Agar Media: 0.1 gm Beef extract, 0.875 gm casein hydrolysate, 0.075 gm starch, and 0.85 gm Agar were taken and dissolved in 100 ml of distilled water, and pH was adjusted to neutral (7.2) at 25°C [22].

Methods for Microbial Strains [23-24]:

1. At first NaOH solution was prepared by taking 0.4 gm of NaOH salt dissolved in 100 ml of distilled water and placing this solution at the side.
2. Then 0.1 gm beef extract, 0.875 gm of casein hydrolysate, and 0.075 gm starch were taken in an l00 ml conical flask and made up the volume of 90 ml with distilled water.
3. After that NaOH solution was added for the pHadjustment at 7.2 and the volume was made up to 100 ml with distilled water.
4. From 100 ml preparation, 50 ml was separated for a liquid subculture that was poured into Mccartney's bottle, and another 50 ml was added with 0.85 gm agar to prepare a solution.
5. Then the conical was placed in a hot water bath to melt the agar.
6. After getting clear preparation, the media is distributed in a test tube with cotton plugging, and sterilization was done in an autoclave at 121°C for 15 minutesthenthe test tube containing liquid media was kept in an incubator for 24 hours at 37°C.
7. In next day the sub-culturing process was done, from stock culture organisms that were supplied by laboratories.
8. With the culturing loop, the *Salmonella typhi* and *Staphylococcus aureus* organisms were collected and transferred in slunt or liquid media in an aseptic condition.
9. Then all the media were stored in the autoclave for subculturing.

Material Composition for Nutrient Agar Media: Beef extract (0.3 gm), Peptone (0.5 gm), NaCl (0.5 gm), and Agar (1.5 gm) were used to prepare Nutrient agar media. These ingredients were dissolved in l00 ml distilled water and boiled for 15 minutes to ensure they were mixed and sterilized them. They were cooled and then the organisms were collected from the surface of the slant and transferred in the nutrient agar media that was present in the bijou bottle. After that, the agar media was poured into the Petri dishes which are covered immediately. This transfer was made under aseptic conditions. Agar media containing the Petri dish was solidified and incubated at 37°C for 24 hours [25].

Assay for Antimicrobial Testing by Bore Method: The antimicrobial activities of the seed extract were tested by the boring method. Using a borer on each agar plate. 4 bores were done (S1, S2, S3, S4) for 4 different concentrations of seed extract of antimicrobial activity. Pre sterilized pasture pipette was used to add one drop of the different products as per the numbering [26].

TABLE 1: CONCENTRATION OF DIFFERENT SEED EXTRACT

Numbering	Concentration (mg/ml)
S1	200
S2	150
S3	100
S4	50

After the extracted product on the agar media plate was incubated at 37°C for 24 hours. The antimicrobial activity was reported by measuring the width of the zone of inhibition around each disc [27].

Minimum Inhibitory Concentration on Antimicrobial Activity of Seed Extract:

Microbial Strains: Stock cultures of microbes used for this study were obtained from the Bharat Technology Laboratory, Uluberia, Howrah. The subculture was prepared from the stock culture using a nutrient broth medium. Then, the subculture was used for the antimicrobial study.

Media:

1. Muller Hinton agar media
2. Nutrient agar media

Material Composition for Muller Hinton Agar Media: At first, 0.1 gm Beef extract, 0.875 gm casein hydrolysate, 0.075 gm starch, and 0.85 gm Agar were dissolved in 100 ml of distilled water. Then, pH was adjusted to neutral (7.2) at 25°C [28].

Methods for Microbial Strains [23-24]:

1. At first NaOH solution was prepared by taking 0.4 gm of NaOH salt dissolved in 100 ml of distilled water and placing this solution at the side.
2. Then 0.1 gm beef extract, 0.875 gm of casein hydrolysate, and 0.075 gm starch were taken in a 100 ml conical flask and made up the volume of 90 ml with distilled water.
3. After that NaOH solution was added for the pH adjustment at 7.2 and the volume was made up to 100 ml with distilled water.
4. From 100 ml preparation, 50 ml was separated for a liquid subculture that was poured into Mccartney's bottle, and another 50 ml was added with 0.85 gm agar to prepare the solution.
5. Then the conical was placed in a hot water bath to melt the agar.
6. After getting the preparation the media was distributed in a test tube with cotton plugging and sterilization was done in an autoclave at 121°C for 15 minutes thenthe test tube containing sterile media was kept in an incubator for 24 hours at 37°C.
7. In next day the subculturing process was done, from stock culture organisms that were supplied by laboratories.
8. With the culturing loop, the *Salmonella typhi* and *Staphylococcus aureus* organisms were collected and transferred in slunt or liquid media in an aseptic condition.
9. Then all the media were stored in the autoclave for subculturing.

Material Composition for Nutrient Agar Media: Beef extract (0.3 gm), Peptone (0.5 gm), NaCl (0.5 gm), and Agar (1.5 gm) were used to prepare Nutrient agar media. These ingredients were dissolved in l00 ml distilled water and boiled for 15 minutes to ensure they were mixed and sterilized them. They were cooled and then the organisms were collected from the surface of slunt and transferred in the nutrient agar media that is present in the bijou bottle. After that, the agar media was poured into the Petri dishes which are covered immediately. This transfer was made under aseptic conditions. Agar media containing the Petri dish was solidified and incubated at 37°C for 24 hours [29].

Assay for Antimicrobial Testing by Bore Method: The antimicrobial activities of the seed extract were tested by a simple agar plate method. At first, 10 squares were drawn on the back side of the small Petri dishes using a marker. In 10 ml, the concentration was decreased 10 times. So, in a 9 ml agar solution, different concentrations of seed extracts were taken. It is given below [26-27].

TABLE 2: CONCENTRATION OF DIFFERENT SEED EXTRACTS FOR MIC STUDY

Numbering	Concentration (mg/ml)
S1	20
S2	15
S3	10
S4	5

1. After giving the extract into agar media, the agar solution was kept in 4 marked small Petri dishes and waited for its solidification.
2. After solidification, the inoculation was done with every 10 microorganisms in 10 small

squares of each plate, and plates were kept in an incubator at 37°C for 1 day.

3. After 1 day, all the plates were taken out to check antimicrobial activity [30-31].

RESULTS AND DISCUSSION:

Phytochemical Screening: The result of phytochemical screening was given below. Different types of phytochemical tests were done.

TABLE 3: PHYTOCHEMICAL SCREENING RESULT

Test For	Test Performed	Pet. ether extract	Ethanol extract
Alkaloids	Dragendroff's Test	-Ve / +Ve	-Ve
	Hager's Test	+Ve	-Ve
Cardiac Glycosides	Killer-Killani Test	+Ve	+ve
	Raymond's Test	+Ve	+Ve
	Baljet's Test	+ve	+Ve
Saponin Glycosides	Foam Test	+Ve	+Ve
Steroids	Salkowski Test	+Ve	-Ve
	Liberman-Buchardt Test	+Ve	-Ve
Tanin	Ferric Chloride Test	+ve	-Ve
	Gelatin Test	+Ve	-Ve

Table 3 depicted the various results of the phytochemical screening study. '+Ve' confirmed that it was present and '-Ve' confirmed that it was absent.

Thin-Layer Chromatography: Table 4 depicted that various solvents were used to perform the TLC. From the upper table, measurements of solvent, as well as a solute, were found.

From the **Table 4**, it was found that the Rf value of Flavonoids is better than the other compounds.

TABLE 4: SOLVENT AND SOLUTE RUNNING

Name of the constituents for the TLC test	Name of the solvent and its appropriate ratio	Measurement of solvent running	Measurement of solute running
Flavonoids	Chloroform: Methanol (15:1)	1. 5.5	1. 4.6
		2. 5.5	2. 4.7
Saponins	Chloroform: Methanol: Water (7:3:1)	1. 5.4	1. 4.2
		2. 5.4	2. 4.1
Glycosides	Ethylacetate: Methanol: Water (10:13.5:1)	1. 5.4	1. 4.4
		2. 5.4	2. 4.5
Alkaloids	Methanol: Ammonia (9.9:0.1)	1. 5.4	1. 4.3
		2. 5.4	2. 4.5

Visualization: *Annona squamosa* seed extract was coloured, so it is visualized by the eye.

Antimicrobial Activity Results: Table 5 depicted the Zone of Inhibition of various strains of Gram-Positive and Gram-Negative bacteria where **S1 =** 200 mg/ml, S2 = 150 mg/ml, S3 = 100 mg/ml, and S4 = 50 mg/ml. It was seen in all the strains of Gm Positive as well as Gm Negative bacteria that as the concentration of seed extract is higher then the Zone of Inhibition (cm) is more.

When the concentration of seed extract is lower then the Zone of Inhibition is lesser than the previous one. It was seen in **Table 5**.

TABLE 5: ANTIMICROBIAL ACTIVITY OF SEED EXTRACT OF *ANNONA SQUAMOSA*

	Name of Microorganism (Stain No.)	Zone of Inhibition			
		S1 (cm)	S2 (cm)	S3 (cm)	S4 (cm)
Gram Positive	*Staphylococcus aureus* (ATCC25923)	1	1	0.83	0.76
	Staphylococcus aureus (ML351)	1.56	1.46	1.33	1.16
	Staphylococcus aureus (ATCC29157)	1.03	1	0.86	0.73
	Staphylococcus aureus (ML366)	1	0.93	0.86	0.80
Gram Negative	*Salmonella typhi* (BE860)	1.23	1.16	1.06	1
	Salmonella typhi (BE672)	1	0.96	0.90	0.80
	Salmonella typhi (D1604)	1.06	1	0.90	0.76
	Salmonella typhi (TY259)	1.1	1.1	1	0.8

Minimum Inhibitory Concentration: Table 6 depicted the minimum inhibitory concentration. It was shown in the upper table by using the symbol 'In' and 'G' where "In" = Growth inhibited and "G" = Growth of microorganism. In **Table 6**, it was observed that different concentrations of seed extract were given in various strains of Gm Positive as well as Gm Negative bacteria. No inhibition was found in the number '7', '9', and '10'. It was depicted in **Table 7** that numbers '7', '9', and '10' are the various strains of *Salmonella typhi.* So, it is noted that the MIC of *Staphylococcus aureus*is better than the MIC of *Salmonella typhi.* The minimum inhibitory concentration is 5 mg/ml.

TABLE 6: RESULT OF MINIMUM INHIBITORY CONCENTRATION

	1	2	3	4	5	6	7	8	9	10
5 mg/ml (S4)	In	In	In	In	In	In	G	In	G	G
10 mg/ml (S3)	In	In	In	In	In	In	G	In	G	G
15 mg/ml (S2)	In	In	In	In	In	In	G	In	G	G
20 mg/ml (S1)	In	In	In	In	In	In	G	In	G	G

TABLE 7: NAME OF THE BACTERIAL STRAIN ALONG WITH NUMBER

Number	Bacterial Strain
1	*Staphylococcus aureus* (ATCC 25923)
2	Staphylococcus aureus (8531)
3	*Staphylococcus aureus* (8531)
4	*Staphylococcus aureus* (ATCC 29157)
5	*Staphylococcus aureus* (ML 366)
6	*Salmonella typhi* (C 145)
7	*Salmonella typhi* (BE 860)
8	*Salmonella typhi* (BE 672)
9	*Salmonella typhi* (D 1604)
10	*Salmonella typhi* (TY 259)

Table 7 depicted the name of the bacterial strain along with the number.

CONCLUSION: After the study, it was concluded that flavonoids are the main chemical constituent present in the *Annona squamosa* seeds which were obtained by phytochemical screening. After performing the TLC, it was found that flavonoid has a good Rf value. So, it was concluded that using this solvent system (chloroform: methanol) flavonoids can be isolated properly. It was also concluded that *Annona squamosa* seeds have antimicrobial activity and the minimum inhibitory concentration is 5 mg/ml. It also has a better minimum inhibitory concentration in Gm Positive bacteria.

ACKNOWLEDGEMENTS: The authors are thankful to Mr. Anshuman Bhattacharya, Assistant Professor at BCDA College of Pharmacy and Technology, Hridaypur, Barasat, Kolkata – 700127, West Bengal, India for the support while conducting this work.

Source of Funding: The authors declare that they have no source of funding.

CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES:

1. Natural Resources Conservation Service (NRCS). Plants profile *Annonas quamosa* L. Uniu States Department of Agriculture 2008; 4-17.
2. Germplasm Resources Information Network (GRIN). Taxonomy for Plants. USDA, ARS, National Genetic Resources Program1997; 7-11.
3. Richard Wunderlin and Bruce Hansen: "Synonyms of Annona squamosa" Atlas of Flor1da Vascular Plants 2008; 24-27.
4. Missouri Botanical Garden. *Annona squamosa* L. Tropicos 1753; 14-17.
5. Current name: Annona squamosa'". AgroForestryTree Database 2006; 13-19.
6. Ithaka Harbors and Aluka: *Annona squamosa* L. [Family: Annonaceae]. African Planis 2008; 41-47.
7. Warrington and Lan J: "Annonaceae". Apples: Botany, Production and Uses. CABI Publishing 2003; 04-20.
8. Jonathan H. Crane, Carlos F. Balerdi and Lan Maguire: "Sugar Apple Growing inthe Florida Home Landscape 1994; 4: 4-19.
9. "Factsheet No. 5. Annona" (PDF). Fruits for the Future 2002; 3: 04-20.
10. Cochrane CB, Nair PK, Melnick SJ, Resek AP and Ramachandran C: Anticancer effects of Annona glabra plant extracts in human leukemia cell lines. Anticancer Res 2008; 28(2): 965-71. PMID: 18507043.
11. Lowell J and Fuglie NY: The miracle Tree: *Moringa oleifera*, Natural nutrition for tropics. Church World Service Darkar 1999; 68.
12. Mishra G, Singh P, Verma R, Kumar S, Srivatsav S, Jha KK and Khosa RL: Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. Der Pharmacia Littre 2011; 3(2): 141-164.
13. Fahey JW: *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1 Trees for life Journal 2005; 1(5): 5-20.
14. Dahiru D, Onubiyi JA and Umaru HA: Phytochemical screening and Antiulcerogenic effect of *Moringa oleifera* aqueous leaf extract. Afr J Trad CAM 2006; 3(3): 70-75.
15. Kasolo JN, Gabriel S, Bimenya, Lonzy O, Joseph O and Jasper W. Ogwal- Okeng: Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. Journal of Medicinal Plants Research 2010; 49: 753-757.

16. Koruthu DP, Manivarnan NK, Gopinath A and Abraham R: Antibacterial evaluation, reducing power assay and phytochemical screening of *Moringa oleifera* leaf extracts, Inte J of Pharma Scien and Research 2011; 2(11): 2991-95.
17. Shahriar M. Hossain MI, Bahar AN M, Akhter S, Haque MA and Bhuiyan MA: Priliminary phytochemical screening, *in-vitro* antioxidant and cytotoxic activity of five different extracts of *Moringa oleifera* leaf. Journal of Applied Pharmaceutical Science 2012; 02(05): 65-68.
18. Manaheji H, Jafari S, Zaringhalam J, Rezazadeh S and Taghizadfarid R: Analgesic effects of methanolic extract of the leaf or root of *Moringa oleifera* on complete Freund's adjuvant induced arthritis in rats. J Chinese Integrative Medicine 2011; 9(2): 216-219.
19. Harborne JB: Phytochemical method, A Guide to Modern technique of Plant Analysis. 3rd Edition, Chapman and Hall. New York 198; 1998.
20. Kokate CK: A text book of Practical pharmacognosy. 5th Edition, Vallabh Prakashan, New Delhi 2005; 107-111.
21. Woodford N: Livermore DM infections caused by gram positive bacteria: a review of the global challenge. Journal of Infection 2006; 59: 1-16.
22. Tripathi KD: Antimicrobial drugs. Essentials of medicinal pharmacology. Jayplee Brothers Medical Publishers.
23. Hill AF: Economic botany, A textbook of useful plants and plant products. McGraw Hill book company Inc, New York 2013.
24. Gajalakshmi S, Divya R, Divya V, Deepika, Mythili S and Sathiavelu S: Pharmacological activities of Annona squamosa; a review. International Journal of Pharmaceutical Sciences Review and Research 2011; 10(14); 24-29.
25. Ushimaru PI, Da silva MTN, Stasi LCD, Barbosa L and Fermandes AJR: Antibacterial activity of medicinal plant extracts. Brazilian Journal of Microbiology 2007; 38: 717-719.
26. Ncube N, Afolayan SAJ and Okoh AI: Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African Journal of Biotechnology 2008; 7: 1797-1806.
27. Arulselvan P and Umamaheswari A: Therapeutic approaches for diabetes with natural antioxidant. In: Medicinal plants as antioxidant agents: Understanding their mechanism of action and therapeutic efficacy. Research Signpost 2012; 237-66.
28. Gowdhami M, Sarkar BL and Ayyasamy PM: Screening of Phytochemicals antibacterial activity of *Annona squamosa*. extracts. International Journal of Pharmaceutical Science Invention 2014; 3: 30-39.
29. Laurence M. Harwood and Christopher J. Moody: Experimental organic chemistry: Principles and Practice (Ilustrated Edition Ed.). 122-125. ISBN 978-0632020171.
30. Soxhlet F: Die gewichtsanalytische Bestimmung des Milchfettes. Dinglers Polytechnisches Journal 1879; 232: 461-465.
31. William B. Jensen: The Origin of the Soxhlet Extractor. Journal of Chemical Education 1913 2007; 84: 12.

How to cite this article:
Bhattacharya A, Pal A, Datta S, Biswas S, Das A, Chanda P, Roy D, Roy S, Nag M, Chattopadhyay S and Sen A: Antimicrobial activity in methanolic seed extract of *Annona squamosa*. Int J Pharm Sci & Res 2023; 14(11): 5340-48. doi: 10.13040/IJPSR.0975-8232.14(11).5340-48.