

Original Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTI-MICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS OF FRUITS OF *ANNONA RETICULATA* AGAINST STANDARD PATHOGENIC STRAINS

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ABSTRACT

Objective: To evaluate the anti-microbial activity of ethanolic extract of fruits of *Annona reticulata* (family-Annonaceae) in conjugation with phytochemical analysis.

Methods: The ethanolic extract of fruits of *Annona reticulata* (family-Annonaceae) was prepared by Soxhlet extraction and analysed for phytochemical constituents using standard methods. The anti-microbial activity of the plant extract was examined against bacterial strains and fungal strains using disc diffusion method.

Results: The present investigation shows the phytochemical analysis, anti-microbial activity of the ethanolic extract of the plant *Annona reticulata*. Various phytochemical analyses revealed the presence of alkaloids, saponin, flavonoids, carbohydrates, glycosides, steroids, proteins and amino acids and tannins. The anti-microbial activity of the ethanolic extract of the plant showed significant result against all the of the test organisms.

Conclusion: The present study concluded that ethanolic extract of fruits of the *Annona reticulata* contain high presence of phytochemicals. The ethanolic extract of the plant was found to possess promising anti-microbial activity when compared with the standards.

Keywords: *Annona reticulata*, Anti-microbial activity, Disc diffusion method, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*

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INTRODUCTION

Beginning from the history, there has been a continual battle between humans and the multitude of microorganisms which are basis of infectious disease. The crucial fact about the treatment of bacterial infections is the ability of bacteria to develop resistance to antimicrobial agents which is variable in different geographic areas and it has been correlated with the consumption of antibiotics in the general population [1].

Antibiotics are designed to inhibit/kill the infecting organism and to have no/minimal effect on the recipient. They are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less effective against certain bacteria as they produced resistance due to lack of target site or metabolic process which is affected by the particular drug or due to mutation/gene transfer and thereby increases chances of chronic infection and risk of morbidity. The drug resistance also increased expenditure on patient management and implementation of infection control measures [2]. Antimicrobial agents principally act by the following mechanism-interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of a metabolic pathway and disruption of bacterial membrane structure [3]. Microbial infection may also increase generation of highly reactive oxygen species (ROS) which causes significant damage to cell structures due to environmental stress [4]. And the similarity of the eukaryotic fungal cell with human cell is the basis of toxicity with the antifungal drugs [5]. Prolong use of standard antifungal drugs such as Amphotericin-B and Fluconazole may be led to several marked adverse effects [6]. Therefore, it is essential to investigate newer drugs with minimal resistance and increased spectrum of activity with desirable properties. Drugs derived from natural sources play a significant role in the prevention and treatment of many human diseases. The plant based technique of medicine being natural does

not cause any serious side effects or problems and so are popularized worldwide [7]. North Eastern part of India comes under one of the world biodiversity hotspots which are very rich in traditional herbal medicine [8, 9].

As plants are a rich source of secondary metabolites and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice.

Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Now a days, there is a revival of interest with herbal-based medicine due to the increasing realization of the health hazards associated with the indiscriminate use of modern synthetic medicine and now the herbal drug industries is the fastest growing sector in the international market. But unfortunately, India has not done well in this international trade of herbal industry due to lack of scientific input in herbal drugs.

Medicinal plants represent a rich source of antimicrobial agents [10]. Plant-derived antimicrobials have a long history of providing the much-needed novel therapeutics [11]. Plants of the *Annona* genus are the notable source of potential therapeutic agents. The plant *Annona reticulata* Linn. belonging to family Annonaceae, commonly known as Custard apple, Bullock's heart, and Ramphal is found in India and cultivated in Thailand and originates from the West Indies and South America. Leaves are membranous, oblong or narrow-lanceolate, 10-20 cm long, 2-5 cm wide with conspicuous veins. Flowers are fragrant, slender, with 3 outer fleshy, narrow petals 2-4 cm long; light-green externally and pale-yellow with a dark-red or purple spot on the inside at the base and fruits are symmetrically heart-shaped, irregular, or nearly round, or oblate, with a depression at the base. The covering or skin of this fruit is

quite thin and when ripe, it generally changes to yellowish or slightly brownish. Beneath the covering or the skin lies a dense, creamy white layer of flesh that is granular and akin to that of custard. This succulent encases small black solid seeds.

Various extracts of different parts of this plant have shown anti-hyperglycemic [12], cytotoxic and recombinant caspase inhibitory activity [13], anti-nociceptive [14], analgesic and CNS depressant [15], analgesic and anti-inflammatory [16], tumor inhibitor [17], and anti-proliferative [18] effects. Traditionally it is used for various purposes like-

- Crushed leaves/paste is used in internal and external wounds and boils, leaf decoction is used in gastritis.
- Leaf juice is used as vermifuge.
- Unripe dried fruits used in diarrhoea and dysentery treatment.
- Root bark is used in tooth ache.
- Seeds, leaves, young fruits have got insecticidal activity [19].

It is one of the most popular nutritional and medicinal plant which is rich in a variety of secondary metabolites including polyphenols [20]. Pinene, Myrcene, Limonene, Terpinen-4-ol, Germacene D are the bioactive metabolites commonly present in fruits. Other species of plant reported to have antibacterial potential, but *A. reticulata* is still not confirmed for antimicrobial potential. Therefore, the objective of the present study was to investigate the antimicrobial activity of *A. reticulata* fruits using different pathogenic strains of micro-organism.

MATERIALS AND METHODS

Collection of plant material

The fruits of the plant *Annona reticulata* were collected from Dibrugarh district of Assam in January 2017 and the fruits were thoroughly washed with water, dried in shade for 2 w and finally dried in a Tray drier at a considerably low temperature not exceeding 30 °C for 24 h.

Authentication of plant material

The plant was authenticated by Prof. Dr. P. P. Baruah, Department of Botany, Gauhati University. A voucher specimen (Acc-18384, Dated: 23/11/2017) was kept in Department of Botany, Gauhati University for future reference.

Chemicals and reagents

Petroleum Ether (PE), Ethanol (ET), dimethyl sulfoxide (DMSO), hydrochloric acid, Dragendorff reagent, Mayer's reagent, Wagner's reagent, Benedict's reagent, sulphuric acid, lead acetate, Molisch's reagent, Fehling solution A and B, sodium citrate, copper sulphate, ferric chloride, sodium hydroxide, glacial acetic acid, benzene, chloroform, ammonia, nitric acid, potassium nitrite, gelatine, Beef extract, Peptone and agar. All the chemicals and solvents used were of standard analytical grades.

Preparation of the plant extracts

The fruits of the plant materials were collected washed, shade dried and then kept at tray drier at 37 °C for 48 h. The dried plant materials were grinded to a fine powder using an electric grinder. The dried and powdered plant material was defatted by using petroleum ether and then was subjected to Soxhlet extractor using the solvent ethanol. The ethanolic extract was concentrated by using water bath and stored in the refrigerator for further analysis.

The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material using the formula:

$$\% \text{Yield} = (\text{Dry weight of the extract} / \text{Dry weight of leaf sample}) \times 100.$$

Qualitative phytochemical screening

The plant extracts were analysed for the presence of alkaloids, saponin, flavonoids, phenol, carbohydrates, proteins, cardiac glycosides, steroids, anthraquinone and terpenoids using the standard methods.

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test-Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test-Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendorff's Test-Filtrates were treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Detection of saponins

Foam Test-0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of flavonoids

Lead acetate Test-Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test-Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test-Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test-Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified bortrager's test

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides

Detection of phytosterols

Salkowski's test Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpene.

Detection of proteins and amino acids

Xanthoproteic test-The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of tannins

Gelatin test-To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins [21].

Antimicrobial activity

Microbial strains

Three bacterial strains [*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922)] and one fungi [*Candida albicans*]

(ATCC 10231)] were used. Stains were obtained from Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati.

Preparation of media

- Beef Extract, peptone were accurately weighted out and in 1000 ml of distilled water beef extract, peptone are added.
- pH was checked by using pH meter (pH 7.4), and finally Agar was added to the flask.
- Boil the media to dissolve and allow it to cool for few minutes.
- The media is placed in an autoclave for sterilization, while the agar is still warm, but not hot for 20 min.
- Then the media is being taken from the autoclave and pour it is into the Petridish with labeled as test, control and standard.
- The antimicrobial activities of the test agents were determined by measuring the diameter of the zone of inhibition.

Antibacterial activity

Disc diffusion method

The antimicrobial action of the plant extract was measured by agar well diffusion technique as described in NCCLS, 1993. 1 ml of bacteria suspension was uniformly spread on the sterile nutrient agar media. Amikacin (30 µl) and DMSO were used as positive and negative controls respectively. Fruits extracts were prepared in DMSO (stock: 1 mg/ml DMSO). The plant extracts of 25, 50 and 100 µg/ml concentrations were added. Sterile filter paper disc (Whatman no.1, diameter 6 mm) was soaked with the extract solution and the solvent allowed drying. The disc was placed on the

nutrient agar media Petri dish inoculated with bacteria suspension to allow the extracts to diffuse into the media. The Petri dish was then placed in an incubator for 24 h at 37 °C. At the end of the incubation period, the inhibition diameter was measured using calipers and expressed in millimeters. Positive antibacterial was established by the presence of measurable zones of inhibition.

Antifungal activity

The potato dextrose agar plates were prepared and inoculated with a fungal culture. Wells of approximately 10 mm was bored using a well cutter. Plant extracts were prepared in DMSO (stock: 1 mg/ml DMSO). The plant extracts of 25, 50, and 100 µl concentrations were added. The plates were then incubated at 37 °C for 48 h. The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact with the test organisms in the freshly seeded plate. The antifungal activity was reported after 48 h of incubation and compared with that of standard antifungal (Fluconazole) (10 µg/ml) which was used as positive control and DMSO as the negative control [22].

RESULTS

The present investigation shows the phytochemical analysis, antimicrobial activity of the ethanolic extract of the plant *Annona reticulata*. The yield % of the extraction of ethanol was 3.26%. It was a light green solid powder (ET) in appearance.

Phytochemical analysis

Different photochemical investigations with the ethanolic plant extract were demonstrated the presence of alkaloids, saponin, flavonoids, carbohydrate, glycosides, steroids, amino acids, tannins (table 1).

Table 1: Phytochemical screening of ethanolic (ET) extract of fruits parts of *Annona reticulata*

S. No.	Phytochemical test	Reagent used	Observation	Result
1	Alkaloids	Mayer's test	Creamy white precipitate	+
		Wagner's test	Reddish brown precipitate	+
		Dragendorff's test	Orange brown precipitate	+
2	Saponin	Foam Test	Froth formation	+
		LeadAcetate test	Yellow precipitate	+
3	Flavanoid	Molisch'stest	Formation of violet ring	+
		Benedict'stest	Formation of orange	+
		Fehling's test	Red precipitate	+
4	Carbohydrate	Modified Borntrager's test	Formation of pink colour	+
		Salkowski's test	Formation of green colour	+
5	Glycosides	Xanthoproteic test	Formation of yellow colour	+
		Gelatin test	Formation of green colour	+

+sign indicates presence and –sign indicates absence.

Antimicrobial activity

Plant extract is generally rich in antimicrobial compounds. The *in vitro* antimicrobial activity of the ethanolic extract and petroleum

ether extract of fruits of *Annona reticulata* fruit under different concentration with the DMSO and standard was determined against bacterial and fungal strains and recorded as zone of inhibition. The results are appeared in table 2.

Table 2: Antimicrobial activity of *Annona reticulata* extract using disc diffusion method

Name of the compounds with concentration	Anti-bacterial activity diameter of zone of inhibition (mm)		Anti-fungal activity diameter of zone of inhibition (mm)
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Amikacin (30 µl)	23	21	-
Fluconazole (10 µl)	-	-	13
DMSO	-	-	-
Eth. extract (25 µl)	10	6	11
Pet. ether extract (25 µl)	9	5	10
Eth. extract(50 µl)	12	8	12
Pet. ether extract (50 µl)	11	7	11
Eth. extract (100 µl)	13	10	13
Pet. ether extract (100 µl)	12	8	11

Zone including 5 mm of paper diameter. Eth. = Ethanolic extract, Pet. = Petroleum ether extract, DMSO = Dimethylsulfoxide.

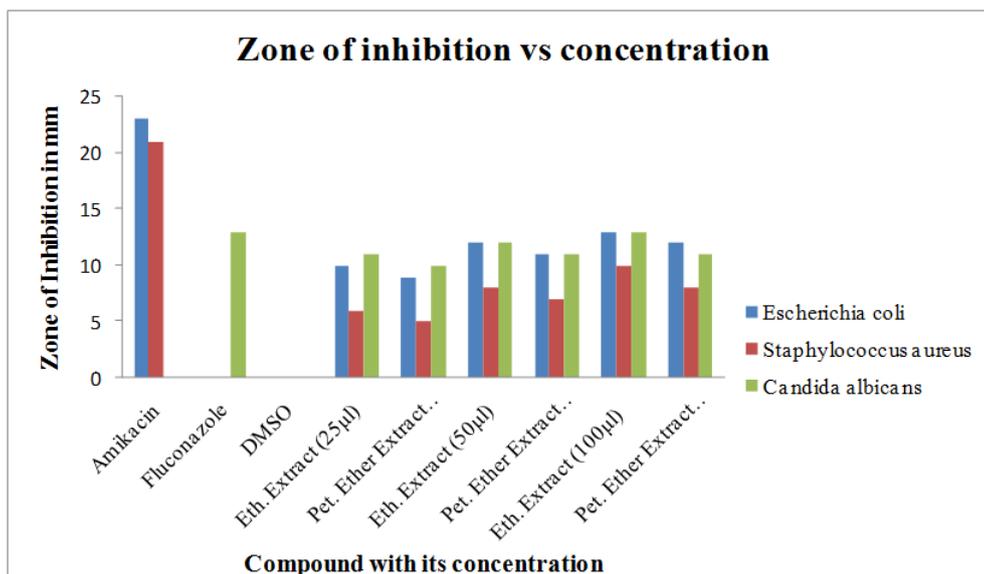


Fig. 1: Zone of inhibition vs concentration

The zone of inhibition of ethanoic extract was against the strains of bacteria were in the range of 6-13 mm and that of petroleum ether extract was 5-12. The ethanolic and petroleum ether extract exhibited potent inhibitory activity against *E. coli*.

The zone of inhibition of ethanolic and petroleum ether extracts against fungal strain was found within the range 10-13.

DISCUSSION

In recent years, multiple drug resistance against pathogenic microorganisms developing due to indiscriminate use of commercial antimicrobial drugs. This situation has necessitated a search of effective antimicrobial compounds. Medicinal plants can produce a variety of secondary metabolites with antimicrobial activity that are active against pathogens [23]. Every part of plant individually or in combination show antimicrobial properties. A significant part of the chemical diversity produced by plants if thought to protect plants against pathogenic microbes.

Current research is focusing over plants as a potential source of medicine, as phytochemicals are generally safe with no or few side effects. *A. reticulata* is a most popular possessing nutritional and medicinal value. The antibacterial and antifungal effect of ethanolic extract of *A. reticulata* against different pathogenic bacterial and fungal strains had supported traditional claims of plant as a medicine. The potent inhibitory effects of ethanolic extract against the bacterial strains are attributed due to the presence of phenolic and polyphenolic compounds in the extract. It is already reported that polyphenolic compounds are biologically active and possess antimicrobial property [24] suggesting that antibacterial and antifungal activity of ethanolic extract may be due to the presence of some phenolic compounds. There are several mechanisms that explained the antimicrobial effect of polyphenols that mainly includes ability of polyphenols to neutralize bacterial toxins, biofilm inhibition, and reduction of host ligands adhesion, enzyme inhibition and interaction with eukaryotic DNA and membrane disruption [25]. The antimicrobial activity of fruits of the plant has been assessed. Phyto-constituents exhibit in plants to be specific alkaloids, flavonoids, tannins and triterpenoids are delivering an energizing open door for expansion of modern therapies against an extensive variety of microorganisms.

In the present study, antimicrobial activity was studied in ethanol, petroleum ether fruit extract of *Annona reticulata*. One Gram-positive, one Gram-negative bacterium and one fungal strains were chosen start with *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* for screening antimicrobial impact of the extract to see antimicrobial spectrum.

To study the antimicrobial properties disc diffusion method was used to observe the growth of tested microorganism. Table 2 showed that the ethanolic extract of the fruits *Annona reticulata* had greater zone of inhibition than the petroleum ether extract. All the extracts are active against all the tested pathogens. *E. coli* was strongly influenced by fruit extract with the inhibition zone of 10 mm, 12 mm, 13 mm for ethanolic extract of 25,50,100µl respectively and zone of inhibition of 9 mm, 11 mm, 12 mm for petroleum ether extract respectively. And zone of inhibition of 23 mm for standard (Amikacin). Against *Staphylococcus aureus* zone of inhibition of 6 mm, 8 mm, 10 mm for the ethanolic extract of 25, 50,100µl respectively and zone of inhibition of 5 mm, 7 mm, 8 mm for petroleum ether extract of 25,50,100µl respectively. And zone of inhibition of 21 mm for standard (Amikacin). Among the various concentration of the ethanolic extract concentration 25 µl exhibit minimum zone of inhibition whereas 100 µl exhibit maximum zone of inhibition against bacterial strain of *Escherichia coli* and *Staphylococcus aureus*. And Gram-negative bacteria were more susceptible than Gram-positive bacteria.

Against the fungal strain *Candida albicans*, zone of inhibition was found to be 11 mm, 12 mm, 13 mm for ethanolic extract of 25,50,100µl respectively and zone of inhibition of 10 mm, 11 mm, 11 mm for petroleum ether extract of 25,50,100µl respectively. And zone of inhibition of 13 mm for standard (Fluconazole). In case of fungal strain concentration 25 µl exhibit minimum zone of inhibition whereas 100 µl exhibit maximum zone of inhibition against fungal strain of *C. albicans*. The exhibit of antimicrobial activity against both Gram-positive and Gram-negative bacteria and on various fungal strains might be demonstrative of the presence of broad spectrum antibiotic compounds in the extract.

CONCLUSION

On the basis of data obtained by the study, it can be concluded that tested fruit extract has beneficial effect on tested pathogens and leaves of *A. reticulata* could be source of bioactive antimicrobial components. Ethanolic leaf extract possessed strong antimicrobial activity. Different classes of phenolics such as flavonoids, steroids and saponin present in tested extract are most likely to the active substance inhibiting the growth of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. This study reveals potential use of these fruits for developing new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. Finally, the results obtained in the present study demonstrated that *Annona reticulata* possesses good antimicrobial activity. Be that as it may, additionally studies are required to toss light on the biological activity of *Annona reticulata*

and its bioactive compounds against various diseases and also to explore precise mechanisms and component underlying these beneficial biological effects.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

There is no conflict of interest

REFERENCES

1. Francesco VD, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, et al. Pylori antibiotic resistance: a systematic review. J Gastrointest Liver Dis 2010;19:409-14.
2. Woodford N, Livermore DM. Infections caused by gram-positive bacteria: a review of the global challenge. J Infect 2006;59:1-16.
3. Tripathi KD. Antimicrobial drugs. Essentials of medicinal pharmacology. Jaypee Brothers Medical Publishers; 2008. p. 668-9.
4. Dubovskiy IM, Martemyanov VV, Vorontsova YL, Rantala MJ, Gryzanova EV, Glupov VV. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of *Galleria mellonella* L. Larvae (Lepidoptera, Pyralidae). Comp Biochem Physiol Part C: Toxicol Pharmacol 2008;148:1-5.
5. Willey JM, Sherwood LM, Woolverton CM. Microbiology (7th ed.) McGraw-Hill, New York; 2008.
6. Hong LS, Ibrahim D, Kassim J, Sulaiman S. Gallic acid: an anticandidal compound in hydrolysable tannin extracted from the barks of *Rhizophora apiculata* Blume. J Appl Pharm Sci 2011;1:75-9.
7. Abirami S, Kannan N, Poonkothai M. Antimicrobial activity and phytochemical screening of the leaf extracts of *Eucalyptus globules*. Int J Curr Pharm Res 2017;9:85-9.
8. Farnsworth NR. Ethno pharmacology and future drug development: The North American experience. J Ethnopharmacol 1993;38:145-52.
9. Houghton PJ. The role of plants in traditional medicine and current therapy. J Alter Complement Med 1995;1:131-43.
10. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World J Agric Sci 2008;4(S):839-43.
11. Janakia A, Kaleena K, Elumalai D, Hemalatha P, Velu K, Sudha R. Phytochemical screening, antioxidant and antibacterial activities of *Millingtonia hortensis*. Int J Curr Pharm Res 2017;9:162-7.
12. Rahman SM, Rashedul MI, Rahman S, Mosaib T, Ahmed R, Khatun F, et al. Antihyperglycemic studies with methanol extract of *Annona reticulata* L. (Annonaceae) and *Carissa carandas* L. (Apocynaceae) leaves in Swiss Albino mice. Adv Nat Appl Sci 2011;5:218-22.
13. Mondal SK, Mondal NB, Mazumder UK. *In vitro* cytotoxic and human recombinant caspase inhibitory effect of *Annona reticulata* leaves. Indian J Pharmacol 2007;39:253-4.
14. Islam MR, Rahman SM, Ahmed M, Das PR, Islam MT, Kabir MH, et al. Antinociceptive activity studies with methanol extract of *Annona reticulata* L. (Annonaceae) and *Carissa carandas* L. (Apocynaceae) leaves in swiss albino mice. Adv Nat Appl Sci 2012;6:1313-8.
15. Bhalke RD, Chavan MJ. Analgesic and CNS depressant activities of extracts of *Annona reticulata* Linn. bark. Phytopharmacology 2011;1:160-5.
16. Chavan MJ, Kolhe DR, Wakte PS, Shinde DB. Analgesic and anti-inflammatory activities of kaur-16-en-19-olic acid from *Annona reticulata* L. bark. Phytother Res 2011;26:273-6.
17. Suresh HM, Shivakumar B, Shivakumar SI. Inhibitory potential of the ethanol extract of *Annona reticulata* Linn. against melanoma tumor. J Nat Pharm 2011;2:168-72.
18. Suresh HM, Shivakumar B, Shivakumar SI. Phytochemical potential of *Annona reticulata* roots for antiproliferative activity on human cancer cell lines. Adv Life Sci 2012;2:1-4.
19. Morton J. Sugar apple. Fruits Warm Climate; 1987. p. 69-72.
20. Ogunwande IA, Ekundayo E, Olawore NO, Kasali AA. Essential oil of *Annona reticulata* L. leaves from Nigeria. J Essent Oil Res 2006;18:374-6.
21. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Int Pharm Sci 2011;1:98-106.
22. Balouiri M, Sadiki M, Ibsouda SK. Methods for *in vitro* evaluating antimicrobial activity: a review. J Pharm Anal 2016;6:71-9.
23. Ushimaru PI, Da Silva MTN, Stasi LCD, Barbosa L, Fernandes A JR. Antibacterial activity of medicinal plant extracts. Braz J Microbiol 2007;38:717-9.
24. Bansal S, Choudhary S, Sharma M, Kumar SS, Lohan S, Bhardwaj V, et al. Tea: a native source of antimicrobial agents. Food Res Int 2013;53:568-84.
25. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiol Rev 1999;12:564-82.