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IN VITRO SCREENING OF *Annona reticulata* L. PERICARP FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Unripe and ripe pericarp extracts of *Annona reticulata* Linn. fruits were investigated for their *in vitro* antimicrobial properties against three gram-positive bacteria (*Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus*), gram-negative bacteria (*Eschericia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) and three fungal strains (*Aspergillus flavus*, *Aspergillus niger* and *Penicillium chrysogenum*). All the extracts showed activity against at least one strain of bacteria and fungi and the result shows there is significant differences ($P<0.05$) between the activities of extract on microorganism. The ethanol extract of unripe pericarp exhibited the highest inhibition activity against the growth of *Eschericia coli* (19.67 mm) which was higher than the zone of inhibition produced by Ampicillin standard (15.33 mm). Also MIC for ethanol extract was the lowest (1.0 mg/ml) compared with other extracts. From the investigation carried out it was seen that the ethanolic extract of unripe pericarp could be a possible source of obtaining new and effective herbal medicines to treat infections.

KEYWORDS: ***antimicrobial activity, disc diffusion method, MIC, Inhibition zone***

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INTRODUCTION

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine.[1] Since time immemorial, many plant species have been reported to have pharmacological properties due to the presence of various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes and hence they can be used to combat the disease causing pathogens.[2,3,4] With the advancement in science and technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs .[5] Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. But, only one third of the infectious diseases are known to have been treated from these synthetic products.[6] This is because of the emergence of resistant pathogens due to the indiscriminate use, incessant and misuse of antibiotics.[7,8] One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. [9,10] It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens.[11]The active principles present in the plants appear to be one of the important alternatives, when compared to many synthetic medicines, because of their less or no side effects and better bioavailability.[12] Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs.[13,14] *Annona reticulata* L. has been traditionally used in Indian folk medicine as vermifuge, antiinflammatory agent, in wound healing, as antimalarial agent and in the treatment of diarrhoea and dysentery. [15] Various scientific studies reported the astringent, anti-anxiety, anti-stress, anti-mutagenic and spasmolytic activities.[16]To best of our knowledge, there is no antibacterial activity study of unripe and ripe pericarp of A. reticulata against pathogenic bacteria and fungal species has been reported. Hence the present research was set up to evaluate the antimicrobial effectiveness of different extracts of unripe and ripe pericarp *of Annona reticulata* fruits against some pathogenic bacteria and fungi.

MATERIALS AND METHODS

Collection of plant material

Mature and immature fruits of *Annona reticulata* Linn. used for this study were collected from Kumarakoil, Kanya kumari district. The taxonomical identity of the plant material was confirmed by the taxonomist of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram, India. The voucher number was 76877. The pericarp (both unripe and ripe) of fruits was separated from the seeds and shade dried. The shade dried plant materials (100 gms each) were powdered and subjected to percolation process using solvents (300ml) like hexane, ethyl acetate, acetone, ethanol and water for 72 hours at room temperature. The filtrates were concentrated under reduced pressure at 40°C and stored in a refrigerator at 2–8°C for further use. [17]

Culture and maintenance of microorganisms

The bacterial and fungal cultures were procured from the culture collection in the Microbiology division of Sree Chithra Thirunal Institute of Medical Science and Technology, Tiruvananthapuram. These include *Bacillus subtilis* (MTCC 739), *Enterococcus faecalis* (MTCC 2912), *Staphylococcus aureus* (MTCC96), *Eschericia coli* – used were *Aspergillus fumigatus* (MTCC 277), *Aspergillus niger* (MTCC 2425) and *Penicillium chrysogenum* (MTCC160). The pure bacterial cultures were maintained on Muller Hinton agar medium and fungal culture on potato dextrose agar (PDA) medium.[18] Each bacterial and fungal culture was further maintained by subculturing regularly on the same medium and stored at 4°C before use in experiment.

Preparation of the inoculum

A loopful of inoculum was taken from a pure culture of respective bacteria/fungi inoculated into 10 ml of Muller Hinton broth respectively. The broth suspension was then incubated at 37°C for 3 hours. The growth so obtained was used as inoculum for the sensitivity assay. [19]

Preparation of paper discs impregnated with crude extract

Commercially available sterile paper discs of 6mm diameter were used in the study. Each disc was impregnated with 100 µl each of the crude extracts (10mg/ml concentration) to give a final concentration of 1 mg/disc. The discs were used after drying them in an incubator at 37°C to remove any residual solvent, which might interfere with the determination.[20]

Screening of extracts for antimicrobial activity-Disc-diffusion assay

The disc diffusion method was used to study the antimicrobial activity. [21] Muller – Hinton agar (for bacteria) and Potato Dextrose agar (for fungi) plates were seeded with bacterial and fungal inoculums using sterile cotton. The discs impregnated with plant extracts were then placed on the seeded agar plates. Also sterile discs impregnated with solvents alone were used as control. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. An inhibition zone of 14mm or greater (including diameter of the disc) was considered as high anti-bacterial activity. Streptomycin (1mg/disc) was used as positive control for gram-positive bacteria, ampicillin (1mg/disc) for gram-negative bacteria and fluconazole (1mg/disc) for fungal strains. The experiment was performed in triplicates for each organism under strict aseptic conditions and at the end the mean diameter of zone of inhibition (mm) was determined. The Activity Index (AI) for each extract was calculated by using the following formula

AI = Inhibition zone of the sample/ Inhibition zone of the standard

Determination of minimum inhibition concentration (MIC) of the extract with maximum antimicrobial activity

The MIC of the unripe pericarp extracts was determined according to the micro broth dilution technique. [22] The MIC value was studied for the test pathogens, which are determined as most sensitive to each plant extract in well diffusion assay. The inoculum of microorganisms was prepared from 18 h nutrient broth cultures. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 2000µg/ml (stock concentration) in propanol and serially diluted (two-fold) to a working concentration ranging from 2000µg/ml to 31.25µg/ml using Muller Hinton broth and added in 96 – well microtitre plates. Thereafter, a 100µl inoculum of standard size was added to each well. The positive control was broth with standard drug (Streptomycin for gram-positive bacteria, ampicillin for gram-negative bacteria and fluconazole for fungi) while the bacterial and fungal inoculums in broth served as negative control. [23] The microtitre plates were incubated at 37 ± 2°C for 24 hours for bacteria, 27 ± 2°C for five to seven days for fungi. After incubation at 37°C, the microtitre wells were observed for turbidity. The turbidity in the wells was interpreted as a visible growth of microorganisms. The least concentration of the extracts that showed no turbidity after incubation was noted as the MIC value.

Determination of MBC/MFC

The minimum bacterial/fungicidal concentration (MBC/MFC) was determined by subculturing 50 µl from each well showing no apparent growth. [24] The least concentration of extract showing no visible growth on subculturing was taken as MBC/MFC.

STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation (SD) of triplicates. Two-way analysis of variance (ANOVA) using the Statistical Package for the Social Science (SPSS) program (SPSS Statistics 22.0) was used to analyze the effect of different solvents on antimicrobial activity. A statistically significant difference was considered at $p < 0.001$.

RESULTS

In the present investigation, the inhibitory effect of different extracts (Hexane, chloroform, ethyl acetate, ethanol and water) of the unripe and ripe pericarp of fruits of *Annona reticulata* were evaluated against fungal and bacterial strains. The activity was quantitatively assessed on the basis of inhibition zones (IZ) and their activity index (AI) along with MIC. The results (zone of inhibition) were compared with the activity of the standards, namely streptomycin and ampicillin (1mg/disc) for bacteria and flucanazole (1mg/disc) for fungi.

Antimicrobial activity of unripe pericarp extracts

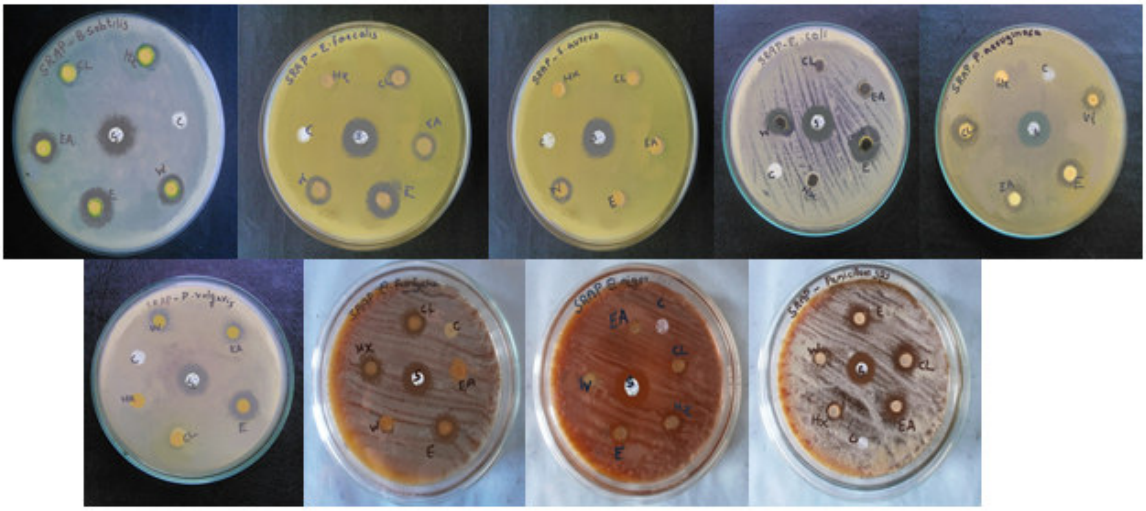
Hexane extract of unripe pericarp, showed resistance against all the tested bacteria. The highest inhibition zone was recorded for ethanol extract against the growth of *E. coli* (IZ 19.67± 0.57, AI 1.283), even higher than the inhibition zone produced by the standard antibiotic, ampicillin (15.33±1.52 mm). Water extract recorded maximum inhibitory activity against *E. coli* (IZ 13.33±0.57, AI 0.870).The water extract showed no activity against both fungal strains *(A. fumigatus* and *A. niger)*. Ethyl acetate, chloroform and hexane extracts showed no activity against both *S. aureus* and *E. coli*. All the organisms, except *S. aureus* showed susceptibility to the ethanol extract. Among the tested solvent extracts, ethanol extract was found to be the most effective against all the tested microorganisms and *S. aureus* was found to be the most resistant organism in the present investigation (Table 1 and Figure 1).

Table 1

Antimicrobial activity of unripe pericarp extracts of Annona reticulate

MO	Activity	Hexane	Chloroform	Ethyl acetate	Ethanol	Water	Standard
BS	IZ (mm)	-	-	10.33±1.52	14.33±1.52	8.66±0.57	22.67±1.52[a]
	AI	-	-	0.456	0.632	0.382	
EF	IZ (mm)	-	6.0±1.73	8.67±1.15	18.67±1.15	9.33±0.57	17.67±0.57[a]
	AI	-	0.34	0.491	1.057	0.528	
SA	IZ (mm)	-	-	-	-	7.33±0.57	19.33±0.57[a]
	AI	-	-	-	-	0.379	
EC	IZ (mm)	-	-	-	19.67±0.57	13.33±0.57	15.33±1.52[b]
	AI	-	-	-	1.283	0.87	
PA	IZ (mm)	-	7.33±0.57	7.33±1.52	16.33±1.52	7.66±1.52	21.33±0.57[b]
	AI	-	0.344	0.344	0.766	0.359	
PV	IZ (mm)	-	-	10.33±1.53	17.67±1.15	11.67±1.15	16.33±0.57[b]
	AI	-	-	0.633	1.082	0.715	
AF	IZ (mm)	9.66±0.57	10.0±1.73	-	9.67±0.57	-	18.33±1.52[c]
	AI	0.527	0.546	-	0.528	-	
AN	IZ	5.67±0.57	-	-	6.0±1.00	-	16.67±0.57[c]
	AI (mm)	0.34	-	-	0.36	-	
PC	IZ	-	6.33±0.57	7.67±1.15	8.33±1.52	7.33±0.57	19.67±1.15[c]
	AI (mm)	-	0.322	0.39	0.423	0.373	

MO- Microorganisms; BS- B. subtilis; EF - E. faecalis; SA – S. aureus; EC – E. coli; PA – P. aeruginosa; PV – P. vulgaris; AF – A. fumigatus; AN – A. niger; PC – P. chrysogenum; IZ – Inhibition Zone (in mm) includes the diameter of the disc (6mm); ([a]) – Streptomycin (1.0 mg/disc), ([b]) – Ampicillin (1.0 mg/disc), ([c]) –Flucanazole (1.0 mg/disc); AI-Activity Index; Values are mean of triplicate readings (mean ±SD) ; The data analysed statistically by Two-way ANNOVA shows significant difference among the activity of the five solvent extracts tested (F-value = 18.80 > F crit = 2.44)



S- Standard; Hx-Hexane; CL – Chloroform; EA-Ethyl acetate; E-Ethanol; W-Water; C – Control; SRAP-Raw pericarp extract

Figure 1
Antimicrobial activity of unripe pericarp extracts of Annona reticulate

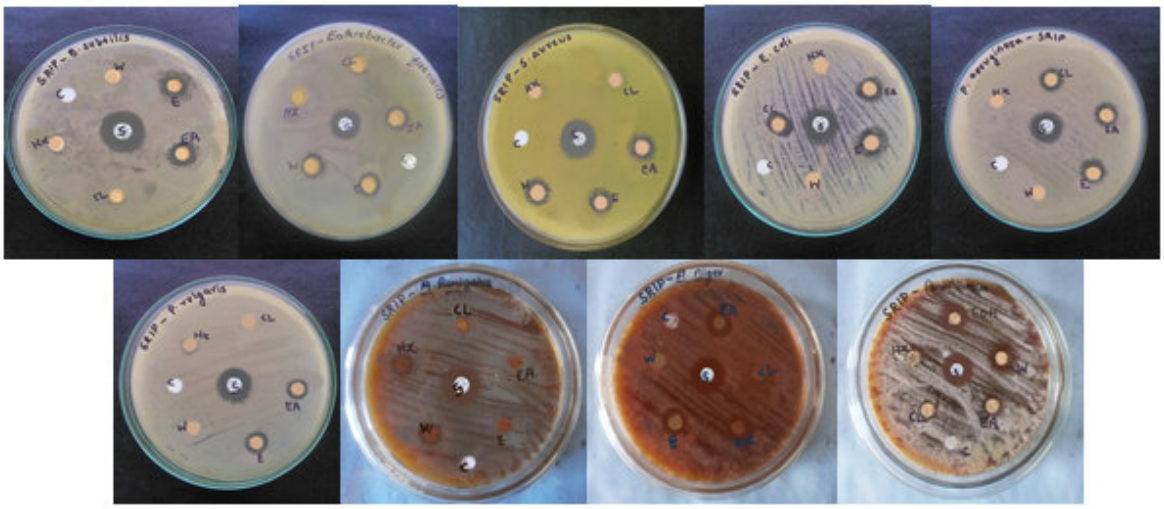
Antimicrobial activity of ripe pericarp extracts

Moderate inhibitory effect was observed against both gram-positive and gram-negative bacteria in ethyl acetate and ethanol extracts of ripe pericarp of *Annona reticulata.* Water extract showed no activity against all the bacteria except *E. faecalis* (IZ 7.33±0.57 AI 0.423) and *S. aureus* (IZ 8.67±1.15 AI 0.441). Feeble activity was determined against *E. coli* and *P. aeruginosa* by chloroform, ethyl acetate and ethanol extracts. All the other organisms showed resistance towards both hexane and chloroform extracts. All the tested fungi showed resistance towards chloroform extract, while the other extracts showed feeble activity towards the tested fungal species as shown in Table 2 and Figure 2

Table 2
Antimicrobial activity of ripe pericarp extracts of Annona reticulate

MO	Activity	Hx	CL	EA	E	W	S
BS	IZ (mm)	-	-	7.33±1.15	8.00±1.73	-	24.33±0.57[a]
	AI	-	-	0.456	0.632	-	
EF	IZ (mm)	-	-	7.67±1.52	6.66±0.58	7.33±0.57	17.33±0.57[a]
	AI	-	-	0.443	0.384	0.423	
SA	IZ (mm)	-	-	7.00±1.00	8.67±0.57	8.67±1.15	19.67±1.15[a]
	AI	-	-	0.356	0.441	0.441	
EC	IZ (mm)	-	7.33±0.57	8.67±1.15	8.33±1.52	-	16.33±0.57[b]
	AI	-	0.449	0.531	0.51	-	
PA	IZ (mm)	-	8.67±1.15	8.67±0.57	8.67±1.15	-	22.33±0.57[b]
	AI	-	0.388	0.388	0.388	-	
PV	IZ (mm)	-	-	8.00±1.73	7.33±0.57	-	16.67±1.15[b]
	AI	-	-	0.48	0.44	-	
AF	IZ (mm)	7.67±1.15	-	-	-	8.00±1.73	17.33±0.57[c]
	AI	0.443	-	-	-	0.462	
AN	IZ (mm)	7.33±0.57	-	9.67±0.57	6.33±0.57	-	15.33±0.57[c]
	AI	0.476	-	0.631	0.413	-	
PC	IZ (mm)	-	-	6.67±1.15	8.33±1.52	7.33±1.15	19.33±0.57[c]
	AI	-	-	0.345	0.431	0.379	

MO- Microorganisms; BS- B. subtilis; EF - E. faecalis; SA – S. aureus; EC – E. coli; PA – P. aeruginosa; PV – P. vulgaris; AF – A. fumigatus; AN – A. niger; PC – P. chrysogenum; IZ – Inhibition Zone (in mm) includes the diameter of the disc (6mm); ([a]) – Streptomycin (1.0 mg/disc), ([b]) – Ampicillin (1.0 mg/disc), ([c]) –Flucanazole (1.0 mg/disc); AI-Activity Index; Values are mean of triplicate readings (mean ±SD; The data analysed statistically by Two-way ANNOVA shows there is significant difference among the activity of the five solvent extracts tested (F-value = 30.38 > F crit = 2.44)



S- Standard; Hx-Hexane; CL – Chloroform; EA-Ethyl acetate; E-Ethanol; W-Water; C – Control; SRIP - Unripe pericarp extract

Figure 2
Antimicrobial activity of ripe pericarp extracts of Annona reticulate

Among the various unripe pericarp extracts, a maximum activity index of 1.283 and 1.057 was recorded for *E. coli* and *E. faecalis* respectively by the ethanol extract which proves the maximum antibacterial property of these extracts. In case of ripe pericarp extract, highest AI was observed in ethyl acetate extract (0.531) against *E. coli*. However this was far lower than the activity observed in unripe pericarp extracts

Determination of MIC, MBC and MFC

MIC and MBC/MFC values were evaluated for the plant extract, which showed good activity in disc diffusion assay. Low MIC values were observed in the ethanol extract which ranged from 1.0 – 3.0 inhibiting the growth of all test organisms except S. *aureus*. The least MBC value of 1.5 and 2.0 mg/ml was observed in the ethanol extract against *E. coli* and *E. faecalis* respectively. The same extract was found to have at least some meager activity against the growth of the three fungal strains producing MFC values ranging from 3 - 3. The MIC values indicated that the unripe pericarp extracts of *A. reticulata* were more potent against bacteria than fungi. Also, these extracts are more potent against gram-positive bacteria than gram-negative bacteria (Table 3 and 4).

Table 3
Determination of MIC of the unripe pericarp extracts of Annona reticulata

Unripe pericarp extracts	Conc (mg/ml)	Presence/absence of growth								
		Bacterial strains								
		BS	EF	SA	EC	PA	PV	AF	AN	PC
Water	3	-	-	-	-	-	-	+	+	-
	2.5	-	-	+	-	+	-	+	+	+
	2	+	+	+	-	+	-	+	+	+
	1.5	+	+	+	-	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+
Ethyl acetate	3	-	-	+	+	-	-	+	+	-
	2.5	-	-	+	+	+	-	+	+	+
	2	-	+	+	+	+	-	+	+	+
	1.5	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+
Ethanol	3	-	-	+	-	-	-	-	-	-
	2.5	-	-	+	-	-	-	-	+	-
	2	-	-	+	-	-	-	+	+	+
	1.5	+	-	+	-	-	-	+	+	+
	1	+	-	+	-	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+

BS- B. subtilis; EF - E. faecalis; SA – S. aureus; EC – E. coli; PA – P. aeruginosa; PV – P. vulgaris; AF – A. fumigatus; AN – A. niger; PC – P. chrysogenum

Table 4
Determination of MIC of the unripe pericarp extracts of Annona reticulata

MO	Activity (mg/ml)	Water	Ethyl acetate	Ethanol
BS	MIC	2.5	2	2
	MBC	>3	3	3
EF	MIC	2.5	2.5	1
	MBC	3	>3	2
SA	MIC	3	-	-
	MBC	>3	-	-
EC	MIC	1.5	-	1
	MBC	2	-	1.5
PA	MIC	3	3	1.5
	MBC	>3	>3	2.5
PV	MIC	2	2	1.5
	MBC	3	2.5	2
AF	MIC	-	-	2.5
	MFC	-	-	>3
AN	MIC	-	-	3
	MFC	-	-	>3
PC	MIC	3	3	2.5
	MFC	>3	>3	3

BS- ***B. subtilis*****; EF -** ***E. faecalis*****; SA –** ***S. aureus*****; EC –** ***E. coli*****; PA –** ***P. aeruginosa*****; PV –** ***P. vulgaris*****; AF –** ***A. fumigatus*****; AN –** ***A. niger*****; PC –** ***P. chrysogenum; MIC – Minimum inhibitory concentration; MBC – Minimum bactericidal concentration; MFC – Minimum fungicidal concentration***

DISCUSSION

Vast number of bacteria and their associated infections are the major challenge in modern medicine. Rapid ability of bacteria to develop resistance to antimicrobial agents produces subsequent failure of most of standard antimicrobial drug treatment thereby increasing chances of chronic infection and risk of mortality.[25] The number of multi drug resistant strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This situation encouraged the search for new, safe and effective antimicrobial agents of herbal origin. It is important to investigate scientifically these plants which have been used in traditional medicines as potential source of novel antimicrobial compounds. The first step towards this goal is the *in vitro* antimicrobial activity assay. The antibacterial activity observed in this study may be attributed to the phytochemical constituents of the extracts. Phytochemicals are secondary metabolites produced by plants that fight with microorganisms in their environment.[26, 27] Among the two plant parts tested, unripe pericarp caused high inhibition of test bacteria. The antimicrobial studies conducted on the leaf, ripe and unripe pericarp extracts of *Polyalthia longifolia* also yielded similar results. Also, unripe pericarp extract suppressed the growth of test fungus to high extent in a similar manner. [28] It has also been experimentally shown that the unripe pericarp extracts of *Polyalthia longifolia*, *Anaphalis lawii* and *Gnidia glauca* displayed stronger inhibition of uropathogenic bacteria and fungus (*Candida capsici*) followed by ripe pericarp and leaf extracts.[29] The most sensitive organism to unripe pericarp extract was *P. aeruginosa* and *E. faecalis* followed by *S. aureus, P. vulgaris, E. coli* and *B. subtilis*. The present study with unripe and ripe pericarp revealed a controversy report that both gram-negative and gram-positive bacteria were equally susceptible to the tested extracts. This may indicate a broad spectrum of activity and their phytochemical analysis revealed the presence of many phytoconstituents. High inhibitory activity towards both gram-positive and gram-negative organisms was also reported in methanolic stem bark and root extracts of *Annona muricata.*[30, 31] This observation is very significant because of the possibility of developing therapeutic substances that will be active against multi-drug resistant organisms. Literature representing the studies on the antimicrobial activity of selected parts of *Annona reticulata* under study was scanty. The demonstration of antimicrobial activity against both gram-positive and gram-negative bacteria was an indication that *Annona reticulata* was potential source of drugs with broad spectrum of activity. [32] Although the mechanism of action of these plant constituents is not yet fully known, it is clear that the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provided a more powerful antimicrobial activity, as compared to the aqueous extracts. [33] Similar results showing that the alcoholic extract had the best antimicrobial activity were also reported in *Anethum graveolens* and *Leucas aspera*, *Holarrhenaanti dysenterica.* [5, 34] The results of study of Kaladhar *et al.,* [35] also showed that *Annona reticulata* raw fruit peel methanol extract showed good activity against *Pseudomonas aeruginosa*, *Eschericia coli*, *Enterococcus faecalis*, *Bacillus subtilis* and *Aspergillus niger*. Ethanolic extract of aerial parts of Annona reticulata plant was studied for its Nephro-protective activity in animal experimental models. [36] These variations in these results might be due to differences in the extraction method, media used, environmental conditions, metabolic stress on plant, physiological age of plant, seasonality and cultivar type. MBC/MFC values were found higher than the MIC values of the extracts against the microorganisms tested. This indicates the bacteriostatic/fungistatic effects of the extracts. Also for most of the extracts, MIC values recorded were very low, indicating the strong

bioefficiency of the selected plants. [37] Determination of MIC is important in diagnostic laboratories, as it helps to confirm the resistance of the microorganism to an antimicrobial agent, and it monitors the activity of new antimicrobial agents. Also antimicrobial activity detected at various concentrations of extracts was found to be a linear function of concentration. The traditional healers usually make use of water primarily as solvent, but the results obtained suggests that ethanol extracts of unripe pericarp were much better, powerful and possess strong antibacterial efficacy. This could be because of the better solubility of the active constituents in organic solvents. [38, 39] This is in agreement with the fact that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram-positive and gram-negative bacteria. [40] Antimicrobial activity of medicinal plants lies in the various chemical constituents (secondary metabolites) present in it. Bonjar *et al.*, [41] reported that the secondary metabolites of plants serve as a defense mechanism against predation by microorganisms, insects and other herbivores. The results of phytochemical screening of ethanol, ethyl acetate, chloroform, hexane and water fractions of *Annona reticulata* plant parts also revealed the presence of various secondary metabolites. Most of these metabolites have been reported to possess antimicrobial activity. [33] Also many phytochemicals acting as antimicrobial agents in the plant's defense are likewise active against human pathogenic microorganisms, and various studies reported on the antimicrobial activities of crude plant extracts. [42] In particular, the flavonoids inhibit the microbial growth by inhibiting its nucleic acid biosynthesis and other metabolic processes. [43, 44] The basic character of tannins allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane. [45] Polyphenols act against the microbes by inhibiting the hydrolytic enzymes or other interactions that inactivate microbial adhesins, cell envelope transport proteins and nonspecific interactions with carbohydrates. [46] The antifungal and antibacterial activity of phenolic and flavonoid compounds has been reported previously. [47,48,49] Also Cowan, 1999[50] reported that the phenolic compound synthesis in plants is for self-protection and it works in response to microbial infections. Antimicrobial activity of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cells. [51] Steroids specifically associate with membrane lipid and exert its action by causing leakages from liposomes. [52] Extract of seeds of *Vitexagnus castus* were reported to possess antimicrobial activity which was associated with its alkaloids, saponins, tannins, flavonoids and glycosides content. [53] It was also reported that the family Annonaceae contains a large number of pharmacologically active substances which are both antibacterial and antifungal. [54, 55] They are used as counter medicine to treat a number of bacterial diseases. [56, 57] In the present study, the MIC value of the active plant extracts obtained in this study were lower than the MBC/MFC values suggesting that the unripe pericarp extracts were bacteriostatic at a lower concentration, but bactericidal at a higher concentration. [37] Thus the study ascertains the value of plants used in ayurvedha which could be of considerable interest in the development of new drugs.

CONCLUSION

The result of present study offer a scientific basis for the traditional use of solvent extracts of the selected medicinal plants and ascertains the value of these plants to be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant microbes. A marked inhibitory potential of ethanolic unripe pericarp extracts of *Annona reticulata* Linn. against gram-positive and gram-negative bacteria and fungi were observed in this study. The zone of inhibitions varied suggesting the varying degree of efficacy and different phytochemical constituents of *Annona reticulata* on the target organism. The antimicrobial potential of extracts could be ascribed to the presence of secondary metabolites detected in the extracts. The unripe pericarp extracts can be the potential candidate for the development of agents active against human pathogenic bacteria and fungi.

CONFLICT OF INTEREST

Conflict of interest declared none

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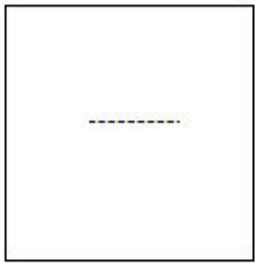

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