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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *ANNONA MURICATA* STEM EXTRACTS

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ABSTRACT

The present study aimed at *in vitro* study of the antioxidant and antibacterial activities of methanol, acetone and hexane extracts of *Annona muricata* stem by cold and hot methods. The antioxidant activity of these extracts were determined by using DPPH (1,1-Diphenyl -2-picryl hydroxyl) radical scavenging method. It was found that the crude hot extract of methanol solvent of *Annona muricata* stem had significantly highest antioxidant activity as compare to all other crude extracts. All extracts showed lower antioxidant activity than the standard gallic acid. The antibacterial activity of these extracts were determined by using agar well diffusion method. DMSO was used as a solvent to dissolve the extracts, Ampicillin was taken as a positive control. It was found that the bacteria (*B.licheniformis* and *E.coli)* were largely inhibited by methanol hot extract. The present study thus suggested that the use of this medicinal plant may be exploited for health supplements.

KEYWORDS: **DPPH (1,1-Diphenyl -2-picryl hydroxyl) radical scavenging, Gallic acid, agar well diffusion, DMSO (dimethyl sulphoxide), Ampicillin, *Annona muricata*.**

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INTRODUCTION

Plants are potential source of natural antioxidants. It produces various anti-oxidative compound to counteract reactive oxygen species (ROS) in order to survive. ROS, which include free radicals such as superoxide anion radicals, hydroxyl radicals (OH) and non free-radicals species such as H_2O_2 and singlet oxygen (0_2), are various from of activated oxygen . The safety of synthetic antioxidant, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are now in doubt[1]. Thus attention is now increasingly paid to the development and utilization of more effective and non-toxic antioxidant of naturals origin. A great number of natural medicinal plant have been tested for their antioxidant activity and results have shown that the raw extracts or isolated pure compounds from them were more effective antioxidants *in vitro* than BHT or vitamin E.[2] There has been an increasing incidence of multiple resistances in human pathogenic micro-organisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infection disease. This has forced scientist to search for new antimicrobial substance from various source like the medicinal plants so, for new antimicrobial agent search should be continued by screening many plant families. Recent work revealed the potential of several herbs as source of drugs[3]. The *Annona muricata* refers to plants in the Annonaceae family, Which is a member of order Magnoliales. The objective of the research was to evaluate the antioxidant and antibacterial activities of different stem extracts (Methanol, Acetone and Hexane) of *Annona muricata* and also to compare the hot and cold extraction method.

MATERIALS AND METHODS

Annona muricata stem was collected from the local nursery of Bangalore. *Annona muricata* were washed under the water and stem were separated from the plant and cut into small pieces for the extraction. Extraction was done by two methods and the solvents used were methanol, acetone and hexane.

Preparation of extracts

Soaking method

150 gm of plant material was suspended in the solvent (methanol, acetone and hexane) using different conical flasks. Then it was filtered, concentrated to dryness under reduced pressure. The condensed extracts were stored in refrigerator.[4]

Soxhlet method

150 gm of powdered dried plant material was submitted to extraction for 72 hours in each solvent, using soxhlet extraction method. The extracts were collected, concentrated to dryness under pressure. The condensed extracts were stored in refrigerator.[5]

Antioxidant activity by DPPH radical scavenging assay

Different dilution of extract (20,40,60 and 80 µg/ml) was prepared. DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml methanol then 2 ml of extract from each dilution was added ethanol into 2ml DPPH solution. Gallic acid was used as standard. The mixture was shaken vigorously and left to stand in the dark for 30 minute. The absorbance of the resulting solution was measured spectrophotometerically at 517 nm. The scavenging activity of the extract was calculated using the formula: scavenging activity % = 100 (1-AE/AD), Where AE is absorbance of the solution, when extracts has been added at a particular level and AD is the absorbance of the DPPH solution, without extract (control) [6].

Antibacterial activity by Agar well diffusion method

Agar well diffusion method was followed in the present work[7].

The antibacterial activity of crude extracts of *Annona muricata* was evaluated by using agar well diffusion method. The Nutrient Agar plates were prepared by pouring 15 ml of molten media into sterile petri plates. Wells of 5mm size were made with sterile borer into agar plates containing the bacterial inoculums. 10µl of the microbial broth culture was spread on the surface of nutrient agar plates. 30µl volume of plant extract of different concentration (1.0, 2.0, 3.0, and 4.0 mg/ml) was poured into the well of inoculated plates separately. Ampicillin of similar concentration as of plant extract was used as a positive control which was introduced into the well instead of plant extract. The plates thus prepared and left at room temperature for 10 minutes allowing the diffusion of the extract into the agar [8]. After incubation for 24 hours at 37^0C, the plates were observed. Antibacterial activity was indicated by an inhibition zone surrounding the well containing the plant extract. The zone inhibition was measured and expressed in millimeters. Antibacterial activity was recorded if the radius of zone of inhibition was greater than 4 mm[9]. The antibacterial activity results were considered as inactive if < 4.5mm: 4.5-6mm as partially active while 6.5-9 mm as active and greater than 9mm as very active[10].

RESULTS AND DISCUSSION

Antioxidant activity

Extracts of *Annona muricata* stem possess antioxidant activity. Stem extracts were studied by free radical scavenging assay method. This is based on UV visible absorption spectrophotometric method. With maximum absorption wavelength 517 nm. Among all the extracts of *Annona muricata* stem, in cold extraction method the methanol extract of stem had highest % inhibition 71.56% followed by 64.47% acetone and least 51.97% of hexane at 80µg/ml as given in the table 1. Similarly among all the extracts of *Annona muricata* stem in hot extraction method the methanol extract of stem showed highest % inhibition 76.05% followed by 64.70% of acetone extract and least 56.67% of hexane extract. On comparing the activities of different extracts it was found that the activity obtained in the hot extraction method (soxhlet) was similar the result obtained earlier. similar result were reported in literature[11]. It was observed that methanol stem extract showed better result in both the extraction methods. This may be due to the more polarity of methanol than acetone and hexane. Polarity of solvents indirectly played a vital role in extraction process. DPPH free radical scavenging assay method of *A. muricata* showed that soxhlet method was more

effective in comparison to soaking method because in soxhlet extraction method, bioactive compound is extracted with solvent and once it is extracted, it did not come in contact with the mother impure solid, only solvent vapors move from the mother impure solid. In soaking extraction method, extract always remain in the contact of mother impure solid as compared to soxhlet[12]. So soxhlet extract showed better antioxidant activity in comparison to soaking extract. Results are shown in terms of percent inhibition and readings are calculated by ANNOVA software.

Table 1
DPPH Free Radical Scavenging Assay (%) Of Different stem Extracts of Annona muricata (Soaking Method) and Gallic Acid

Conc. (µg/ml)	methanol	% Inhibition	acetone	% Inhibition	hexane	% Inhibition	Gallic acid	% Inhibition
20	0.685 ± 0.056	48.97	0.756 ± 0.016	43.56	0.973 ± 0.202	27.33	0.132 ± 0.015	91.85
40	0.545 ± 0.016	59.06	0.68 ± 0.014	49.19	0.838± 0.017	37.42	0.145 ± 0.008	94.25
60	0.465 ± 0.016	64.83	0.557 ± 0.038	59.28	0.762 ± 0.034	41.66	0.117 ±0.009	94.85
80	0.339 ± 0.035	71.56	0.440 ± 0.032	64.47	0.639 ± 0.015	51.97	0.098 ± 0.005	97.45
Result	S		S		S		S	
S. Ed. (±)	0.009		0.010		0.017		0.014	
C.D. at 5%	0.019		0.020		0.037		0.030	

Table 2
DPPH Free Radical Scavenging Assay (%) Of Different stem Extracts of *Annona muricata* (Soxhlet Method) and Gallic Acid

Conc. (µg/ml)	Methanol	% Inhibition	Acetone	% Inhibition	Hexane	% Inhibition	Gallic acid	% Inhibition
20	0.558 ± 0.015	64.01	0.809 ± 0.018	47.89	0.948 ± 0.031	37.80	0.132 ± 0.015	91.85
40	0.510 ± 0.014	67.21	0.757 ± 0.018	51.22	0.857 ± 0.038	43.82	0.145 ± 0.008	94.25
60	0.436 ± 0.009	72.03	0.696 ± 0.017	55.04	0.752 ± 0.018	51.47	0.117 ±0.009	94.85
80	0.367 ± 0.012	76.05	0.543 ± 0.016	64.70	0.645± 0.032	56.67	0.098 ± 0.005	97.45
Result	S		S		S		S	
S. Ed. (±)	0.021		0.029		0.032		0.0032	
C.D. at 5%	0.045		0.061		0.067		0.0068	

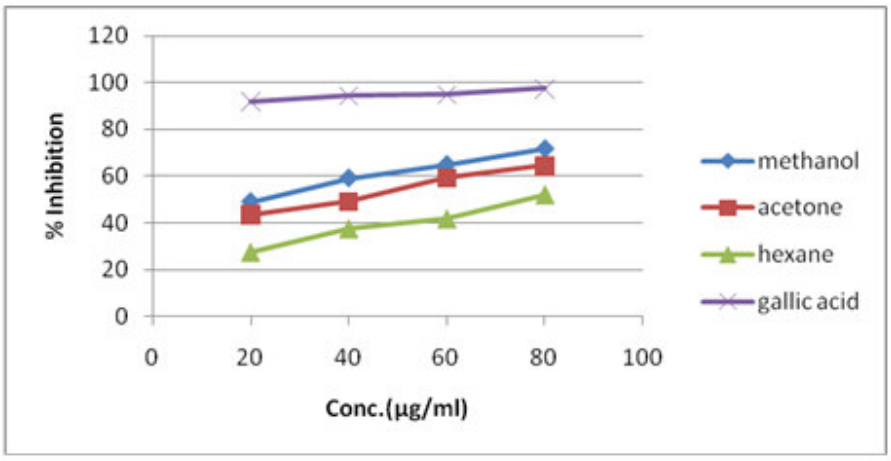

Figure 1
DPPH free radical scavenging activity of stem extract of Annona muricata by soaking method of extraction

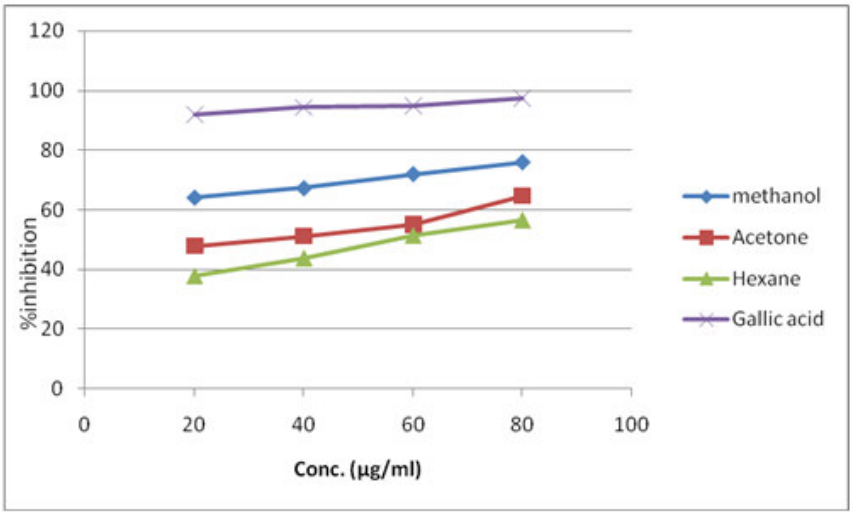

Figure 2
DPPH free radical scavenging activity of stem extract of Annona muricata by soxhlet method of extraction

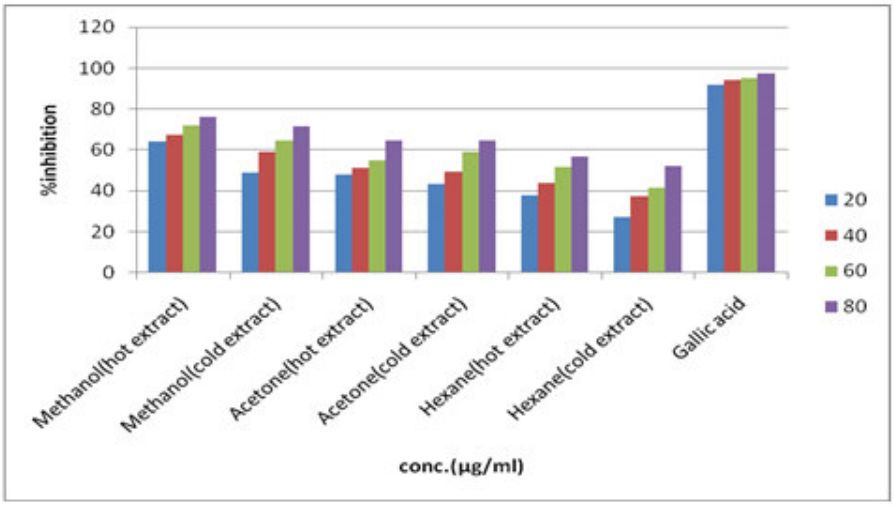

Figure 3
DPPH radical scavenging activity of stem (Cold and Hot) extracts of Annona muricata at different concentration

Antibacterial activity

In all the hot stem extracts it was observed that methanol hot extract of *A. muricata* stem showed better antibacterial activity against gram positive and gram negative bacteria in comparison to the acetone and hexane extracts. The highest antibacterial activity inhibition zone of 23mm was shown by methanolic hot extract of *A.muricata* stem against *B.licheniformis* in comparison to the acetone and hexane extracts that showed an inhibition zone 17mm and 14mm. The lowest antibacterial activity of inhibition zone of 12mm was shown by hexane hot extract of *A.muricata* against *E.coli*, which is also in accordance to the reported literature.[13] Among the cold stem extracts the highest antibacterial activity of inhibition zone of 19mm was shown by methanol cold extract of *A. muricata* stem against *B.licheniformis* in comparison to the acetone and hexane extracts which showed 16mm and 11mm respectively. The lowest antibacterial activity of inhibition zone of 9mm was showen by hexane cold extract of *A. muricata* against *E.coli,* all the results obtained are in accordance to the result reported in literature.[14]

Table 3
Antibacterial activity of stem extracts (Cold and Hot extracts) of A. muricata at higher (4mg/ml) concentration

Name of bacteria	Zone of inhibition (mm) Soxhlet method			Zone of inhibition (mm) Soaking method		
	Methanol	Acetone	Hexane	Methanol	Acetone	Hexane
Bacillus licheniformis	23	17	14	19	16	11
Escherichia coli	17	15	12	14	13	9

CONCLUSION

On the basis of results obtained, it can be concluded that soxhlet (hot) extraction is more preferable than soaking (cold) extraction and the stem of *Annona muricata* possess potent antioxidant and antibacterial activities due to which, it inhibits production of free radicals and the growth of micro organisms. Further the potential of this plant can be explored more and more, in order to develop an alternative therapy for treatment of various diseases. The present studies also suggest that the use of this medicinal plant may be exploited for health supplements. Thus the present work justifies its traditional use.

CONFLICT OF INTEREST

Conflict of interest declared none.

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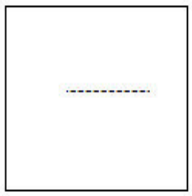

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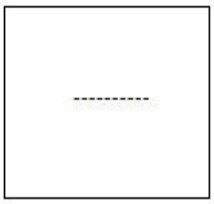

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