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Antibacterial Activity and Phytochemical Screening of *Garcinia pedunculata* Roxb. ex Buch. - Ham. fruit extract by HPLC–ESI-MS

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Abstract

Disc and well diffusion methods were used to test the antibacterial activity of methanol extract and ethanol extract of the selected ethnomedicinal plant viz. *Garcinia pedunculata* Roxb. ex Buch.-Ham. Both the methanol extract and ethanol extract were subjected to antibacterial activity assay against the six clinical isolates. Antibiotic sensitivity test of the test bacteria against standard antibiotics were also determined. All the bacterial pathogens *(Staphylococcus aureus, Enterococcus faecalis, Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae)* were exposed to the plant extract in triplicates. The investigation revealed the potency of *G. pedunculata* extract as an effective antibacterial agent against both Gram-positive bacteria (GPB) and Gram-negative bacteria (GNB). *G. pedunculata* are evaluated to be bactericidal against the tested bacteria. The antibacterial activity may be due to an individual compound or synergistic effect of more than one compound present in the medicinal plant extract. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the plant extracts were also investigated by using the broth microdilution method. HPLC-MS of the methanol extract of *G. pedunculata* fruit revealed the presence of Hydroxy Citric Acid Lactone (MW-190), Garcinone-E (MW-464), α-Mangostin (MW-410), β–Mangostin (MW-424), and γ-Mangostin (MW-396).

Keywords: *Garcinia pedunculata,* phytochemicals, antimicrobial activity, Mueller Hinton Agar, Resazurin, Retention time

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INTRODUCTION

Garcinia pedunculata Roxb. ex Buch.-Ham. is well recognized as medicinal plants amongst the tribals of North Eastern States of India. *G. pedunculata* fruit are used in the treatment of stomachache, diarrhea and urinary tract disorder.[1] Medicinal plants contain diverse array of secondary metabolites which have significant antioxidant and antibacterial properties.

There are more than 200 species of Garcinia (family: Clusiaceae), distributed in tropical regions of the world, mainly in Asia, Africa and Polynesia. They are medium size evergreen trees. There are around 35 species in India, many of which are endemic, with significant economic importance and great medicinal value.[2] In India, different species of Garcinia are found in Gujarat, West Bengal, Jaintia Hills, Khasi Hills, Assam, Konkan region of Maharashtra, Goa, Karnataka and Kerala coastal areas. It grows widely in the semi-wild state in the evergreen forests. *G.kydia, G.cowa, G. pedunculata* and *G.Lancifolia* are the most important species in northeast India.[3] Many species of Garcinia have edible arils and are consumed locally.[4]

The current study was undertaken to determine the bioactive compounds present as well as the antibacterial activity of methanol and ethanol extracts of *G. pedunculata* pericarp in vitro.

The antibacterial activities of methanol and ethanol extract of *G. pedunculata* pericarp were tested against the most common causative agent for UTI (Urinary Tract Infection) viz. uropathogenic *E.coli* (UPEC), *K. pneumoniae, S.aureus, E. faecalis, P. aeruginosa* and *E. cloacae*, and then compare the results obtained with that of the effect of the standard antibiotics.

MATERIALS AND METHODS

Collection and identification of plant

The plant sample (Fig. 1) was collected from its natural habitat and identified at Botanical Survey of India, Eastern Regional Centre, Shillong. *G. pedunculata* voucher specimen (Accession No. 96682) has been deposited at Assam Herbarium, BSI, Shillong, India.

Preparation of plant extract

The clean dried pericarp of *G. pedunculata* were grounded by motor-driven grinder into powder form. Ten (10) gms of powdered plant material was weigh and extracted in methanol and ethanol solvent in Soxhlet Hot Extraction Apparatus. The crude extract was subjected to filtration using Whatmann filter paper no. 1. The collected solution was dried in a rotary shaker (150 rpm; 50° C) and stored at 4°C until further use.

The reconstituted working methanol (MEGP) and ethanol (EEGP) extracts were prepared by dissolving the required amount of dry extract in their respective solvents and are employed directly without any adjustment of pH.

Preparation of standardized inoculum

Each of the bacterial isolates were grown on Mueller-Hinton Agar (MHA, HiMedia) plate for 18–24 hrs. at 37 ± 2°C. Well-isolated colonies were inoculated into sterile Mueller-Hinton Broth (MHB, HiMedia) and the turbidity was adjusted against the 0.5 McFarland standard to yield approximately 1.5 x 10^8 CFU/ml.

Antibacterial Susceptibility Test (AST) of UTI clinical bacterial isolates against standard antibiotics by Disc Diffusion Method

The susceptibility of the clinical bacterial isolates to twelve (12) commonly employed different antibiotics viz. Ceftazidime CAZ 30mcg/disc, Gentamicin GEN 10mcg/disc, Piperacillin PI 100mcg/disc, Amikacin AK 30mcg/disc, Cefepime CPM 30mcg/disc, Aztreonam AT 30mcg/disc, Cefoperazone CPZ 75mcg/disc, Ciproflaxacin CIP 5mcg/disc, Levoflaxacin LE 5mcg/disc, Imipenem IPM 10mcg/disc, Meropenem MRP 10mcg/disc and Piperacillin/Tazobactam PIT 100/10mcg/disc was assessed by disc diffusion method using HiMedia's Dodeca Disc for easy and relevant comparison and to ascertain the effectiveness of the extract of *G. pedunculata*.

Antibacterial activity of methanol and ethanol extracts of *G. pedunculata* towards UTI clinical bacterial isolates by Disc Diffusion Method

Antibacterial activity of methanol and ethanol extract of *G. pedunculata* towards different clinical isolates were assessed in terms of diameter of inhibition zone using disc diffusion method as stated by Kirby - Bauer et.al[5] It is the recommended method of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and National Committee for Clinical Laboratory Standard (NCCLS),USA .

The isolates to be tested were, firstly, inoculated/spread on the surface of MHA using sterile spreader. Sterile 10-mm diameter blank discs (HiMedia) impregnated with 250 µl of different plant extracts stock solution (200 mg/ml) were place on the inoculated MHA plate as test disc. Blank disc impregnated with 250 µl methanol and ethanol were used as negative control for methanol and ethanol plant extract respectively. Place the discs in an empty sterile petri dish and then apply the solution to the discs in an installment of 50 µl, 100 µl and 100 µl. Allow 15–30 minutes for the discs to absorb and dry at 40°C in an Oven before applying every installment. Disc of ampicillin (10 mcg) and methicillin (5mcg) antibiotics (HiMedia) were used as reference antibiotics. All test plates were incubated at 37 ± 2°C for 18 to 24 hours and diameter of zones of inhibition produced by the plant extracts were measured. The experiment was repeated three times for each combination of extract and microbe.

Antibacterial activity of methanol and ethanol extracts of *G.pedunculata* towards UTI clinical bacterial isolates by Well Diffusion Method

The susceptibility of different bacterial clinical isolates to the methanol and ethanol extract of *G. pedunculata* was determined in terms of diameter of zone of inhibition using agar well diffusion assay. Firstly, the MHA plates were spread with the inoculum and wells (8mm diameter) were cut out using a sterilized stainless steel well borer. The wells were then filled with 250 µl of the plant extracts (200 mg/ml). Wells filled with 250 µl methanol and ethanol were used as negative control for methanol and ethanol plant extract respectively. Disc of ampicillin (10 mcg) and methicillin (5 mcg) antibiotics (HiMedia) were used as reference antibiotics. The bacteria-inoculated plates were incubated at 37± 2° C for 18 to 24 hours, and the diameter of any resulting zone of inhibition was measured. The experiment was repeated three times for each combination of extract and bacterial strain.

MIC Determination by Broth Microdilution Method supplemented with Resazurin

The MIC of the methanol extract (MEGP) was determined by using broth microdilution method, as described previously by Wiegand et al[6] supplemented with Resazurin dye.[7] MHB was used as the diluent in a two-fold serial dilution of plant extract. Negative control (inoculum and MHB only) was also maintained. Solvent blanks and positive controls were also included.

The bacterial inoculum was prepared in the same manner as described above for the disc-diffusion method. Fifty (50) µl of the adjusted inoculum were mixed into each Eppendorf tube containing 200 µl of MHB and 250 µl of each plant extract dilution in the dilution series. In an ambient air incubator, the capped microdilution vials were incubated at 37± 2°C for 18 to 24 hours.

After incubation for 18 to 24 h at 37 ± 2°C, resazurin (0.015 %) was added to all the Eppendorf tubes (150 µl per vial), and further incubated for 2–4 h for the observation of color change. On completion of the incubation, the Eppendorf tubes with the lowest concentration of the extract that shows no color change (blue resazurin color remained unchanged) is scored as the MIC value. The experiment was repeated three times.

Fig. 1. *Garcinia pedunculata*

Table 1. Diameter of zone of inhibition (mm) of standard antibiotics against UTI clinical bacterial isolates - Disc Diffusion Assay

No.	Standard antibiotics	*S.aureus*	*E.faecalis*	*E.cloacae*	*E.coli*	*P.aeruginosa*	*K.pneumoniae*
1	Ceftazidime CAZ 30mcg	16.0±0.9	19.0±0.9	0	25.7±0.5	25.3±1.0	10.3±0.5
2	Gentamicin GEN 10mcg	30.3±0.5	23.0±0.9	18. 7±0.5	19.7±0.5	26. 7±0.5	23.0±0.9
3	Piperacillin PI 100mcg	45.0±0.9	25.7±0.5	11.3±0.5	27.0±0.9	15. 7±0.5	10.3±0.5
4	Amikacin AK 30mcg	42.3±0.5	23.0±0.9	18.3±0.5	18.7±0.5	41.0±0.9	25.3±1.0
5	Cefepime CPM 30mcg	42.3±0.5	23.0±0.9	0	36.7±0.5	30.3±0.5	23.7±0.5
6	Aztreonam AT 30mcg	20.7±0.5	14.3±0.5	0	28.7±0.5	25.7±0.5	13.3±0.5
7	Cefoperazone CPZ 75mcg	41.0±0.9	29.3±0.5	11.3±0.5	34.7±0.5	27.7±1.0	13.7±0.5
8	Ciproflaxacin CIP 5mcg	43.7±0.5	29.3±0.5	39.7±0.5	40.0±0.9	51.3±1.0	31.7±0.5
9	Levoflaxacin LE 5mcg	59.7±0.5	40.0±0.9	26.7±0.5	38.3±1.0	52.0±0.9	39.7±0.5
10	Imipenem IPM 10mcg	35.0±0.9	36.3±0.5	22.3±0.5	33.7±0.5	31.3±1.0	24.3±1.0
11	Meropenem MRP 10mcg	30.0±0.9	31.0±0.9	22.3±0.5	33.7±0.5	29.7±0.5	23.3±0.5
12	Piperacillin/Tazobactam PIT 100/10mcg	23.3±0.5	32. 7±0.5	15. 67±0.5	28.0±0.9	19.7±0.5	10.3±0.5

Footnote : Diameter of zone of inhibition (mm) are given as mean ± SD; (n = 3)

Table 2. Antibacterial activity of *G.pedunculata* extracts and some of the standard antibiotics against UTI bacterial isolates

Bacteria	Diameter of zone of inhibition (mm) are given as mean ± SD; (n = 3)					
	EEGPDD	MEGPDD	MEGPWD	EEGPWD	AMP	MET
S.aureus	14±0	16.7±0.5	30±0.9	16.7±0.5	24.3±0.5	11.6±0.5
E.faecalis	16±0.9	19±0	34±0.9	23.7±0.5	26.3±0.5	13.3±0.5
E.cloacae	11±0	10.7±0.5	25.7±0.5	17±0.9	0	0
E.coli	10±0	13±0.9	26.7±0.5	22±0.9	17.7±0.5	0
P.aeruginosa	10.3±0.5	12.7±1	29.3±1.0	20.7±0.5	0	0
K.pneumoniae	10.3±0.5	10.7±0.5	23.3±0.5	14±0.9	0	0

Footnote : MEGPDD = Methanol Extract of *G. pedunculata* (Disc Diffusion); EEGPDD = Ethanol Extract of *G. pedunculata* (Disc Diffusion); MEGPWD = Methanol Extract of *G. pedunculata* (Well Diffusion); EEGPWD = Methanol Extract of *G. pedunculata* (Well Diffusion); AMP = Ampicillin 10 mcg; MET = Methicillin 5 mcg.

Minimum Bactericidal Concentration (MBC)

The MBC was determined by directly plating the contents of the Eppendorf tube at a concentration higher than the MIC value. The lowest concentration of extract that did not allow any growth was taken to MBC.

LC-MS Instrumentation and Experiment

The methanol extract of *G. pedunculata* (MEGP) was subjected to LCMS analysis. The LC/MS was performed on Waters ACQUITY UPLC-TQD Mass spectrometer model ACQ-TQD#QBB1152 with Waters 2424 ELSD detector. The HPLC Column HYDROSPHERE C18, 12nm, 5µm, 250 x 4.6 mm, maintained at temperature of 35° C was used for separation. The solvents used were of LC-MS grade. The sample injection volume was 20.0 µl. A continuous gradient system was followed using mobile phase composed of acetonitrile (ACN) and formic acid (0.1 % FA, v/v in water) for 40 mins. At a flow rate of 1.5 mL/min, the extract was investigated using the following eluent mixtures:

1. 0-10 min 5:95 → 30:70 (ACN : FA, v/v)
2. 10-16 min 30:70 → 60:40 (ACN : FA, v/v)
3. 16-24 min 60:40 ---80:20 (ACN : FA, v/v)
4. 24-35 min 80:20---5:95 (ACN : FA, v/v)
5. 25-40 min 5:95 → 5:95 (ACN : FA, v/v)

The capillary temperature was 350˚C and the ESI voltage was 2.5 kV. The detection was carried out in negative ion mode over a mass range of 150.0-750.0 m/z.

RESULT

Antibacterial Susceptibility Test of UTI clinical bacterial isolates against standard antibiotics by Disc Diffusion Method

The result of the antibacterial susceptibility test is given in Table 1. The different cultures of the clinical bacterial isolates responded to standard antibiotics in a variable manner resulting in various size of zones of inhibition. The size of zone of inhibition range from 10.3±0.5 (Piperacillin/Tazobactam PIT 100/10mcg against *K.pneumoniae*) to 59.7±0.5 (Levoflaxacin LE 5mcg against *S.aureus*).

According to the 31st edition of CLSI performance standard for antimicrobial susceptibility testing, *E.coli* was susceptible to all the 12 standard antibiotics tested; *E.cloacae* was resistant to CAZ, PI, CPM, AT, CPZ and PIT but susceptible to GEN, AK, CIP and LE; *K.pneumoniae* was susceptible to GEN, AK, CIP, LE, IPM and MRP but resistant to CAZ, PI, CPM, AT, CPZ and PIT; *P.aeruginosa* was susceptible to CAZ, GEN, AK, CPM, AT, CIP, LE and IPM : *S.aureus* was susceptible to GEN, CIP and LE: *E.faecalis* was susceptible to CIP and LE.

Antibacterial activity of methanol and ethanol extracts of *G. pedunculata* against UTI clinical bacterial isolates - Disc Diffusion Assay

To evaluate the antibacterial activity of *G. pedunculata* fruit extracts, two different solvents were used to determine which of them

might offer optimal activities against UTI clinical bacterial isolates. The disc diffusion method was used to determine the presence of antibacterial activities. All the methanol and ethanol negative control discs did not produce any zone of inhibition against any of the tested clinical isolates.

Table - 2 and Fig. - 2 & 3 shows the diameter of the zones of inhibition produced by the methanol and ethanol extracts (Disc diffusion method) on the various test bacteria. The data presented in this paper pertains to extracts of crushed plant parts without any pH adjustment. Both the extracts produced very significant antibacterial activities against all the bacterial strains tested. The ethanol extract demonstrated zones of inhibition ranging from 10 mm (*E.coli*) to 16 mm (*E.faecalis*). The methanol extract showed zones of inhibition ranging from 10.7 mm (*E. cloacae, K.pneumoniae*) to 19 mm (*E. faecalis*).The results were compared with those of ampicillin and methicillin as standard antibiotics. Both the solvent extracts (200mg/ml) of *G. pedunculata* were not as potent as ampicillin (10 mcg) but are quite effective as methicillin (5 mcg) antibiotics or even better.

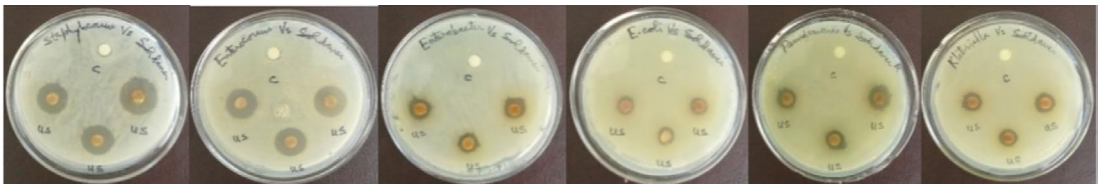

Fig. 2. Plates of Disc diffusion assay showing antibacterial activity of *G. pedunculata* ethanol extract on UTI clinical bacterial isolates.(C=Control disc impregnated with ethanol; US=Disc impregnated with EEGP).

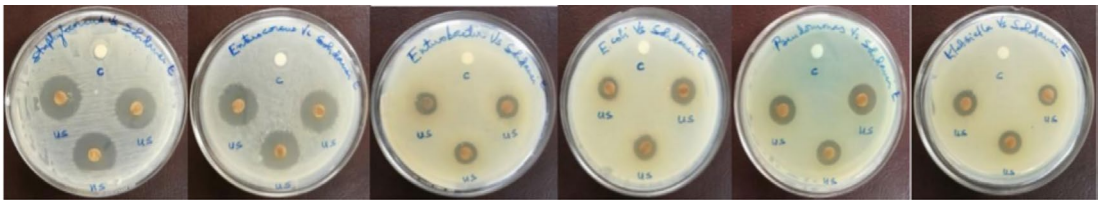

Fig. 3. Plates of Disc diffusion assay showing antibacterial activity of *G. pedunculata* methanol extract on UTI clinical bacterial isolates. (C=control disc impregnated with methanol; US=disc impregnated with MEGP).

Fig. 4. Plates of Well diffusion assay showing antibacterial activity of *G. pedunculata* methanol extract on UTI clinical bacterial isolates.(C=control well filled with methanol; US=well filled with MEGP).

Fig. 5. Plates of Well diffusion assay showing antibacterial activity of *G. pedunculata* ethanol extract on UTI clinical bacterial isolates.(C=control well filled with ethanol; US=well filled with EEGP; Met = methicillin disc; Amp=ampicillin disc).

Well Diffusion Assay

In order to counter check and corroborate the result of the disc diffusion assay, the antibacterial activity of the methanol extract and ethanol extract of *G. pedunculata* was also determined by the well diffusion method. It was observe that both the organic solvent extracts of *G. pedunculata* were inhibitory to all the six bacterial isolates and the diameter of the zones of inhibition were relatively greater than the corresponding zones of inhibition observed in disc diffusion assay. Though the same volume of the extract were used against the six bacteria, the bigger zone of inhibition is expected in the well diffusion assay as the size of the wells (8 mm) are comparatively greater than the size of the disc (6mm). All the methanol and ethanol negative control discs did not produce any zone of inhibition against any of the tested clinical isolates (Table - 2, Fig. - 4 & 5)

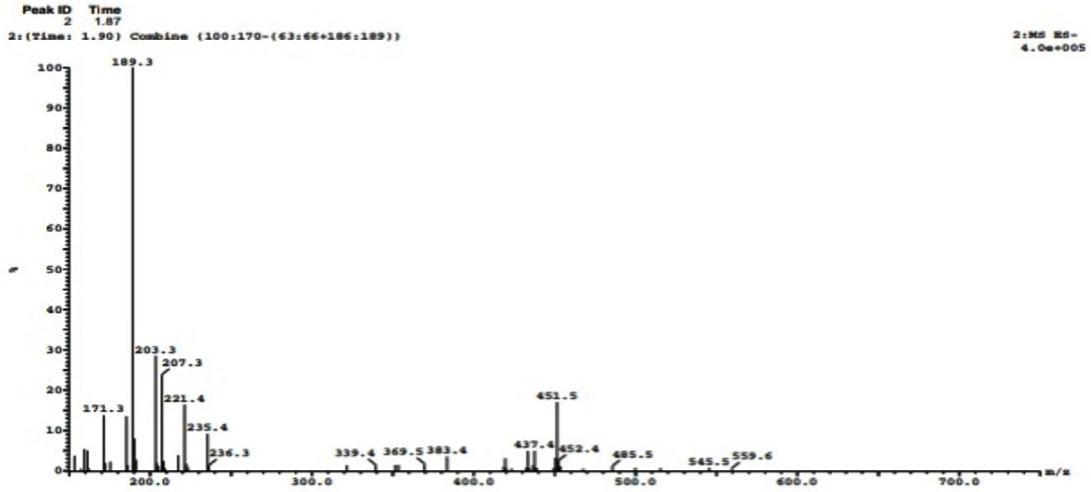

Fig. 6 (i). Mass spectrum at the retention time 1.87.

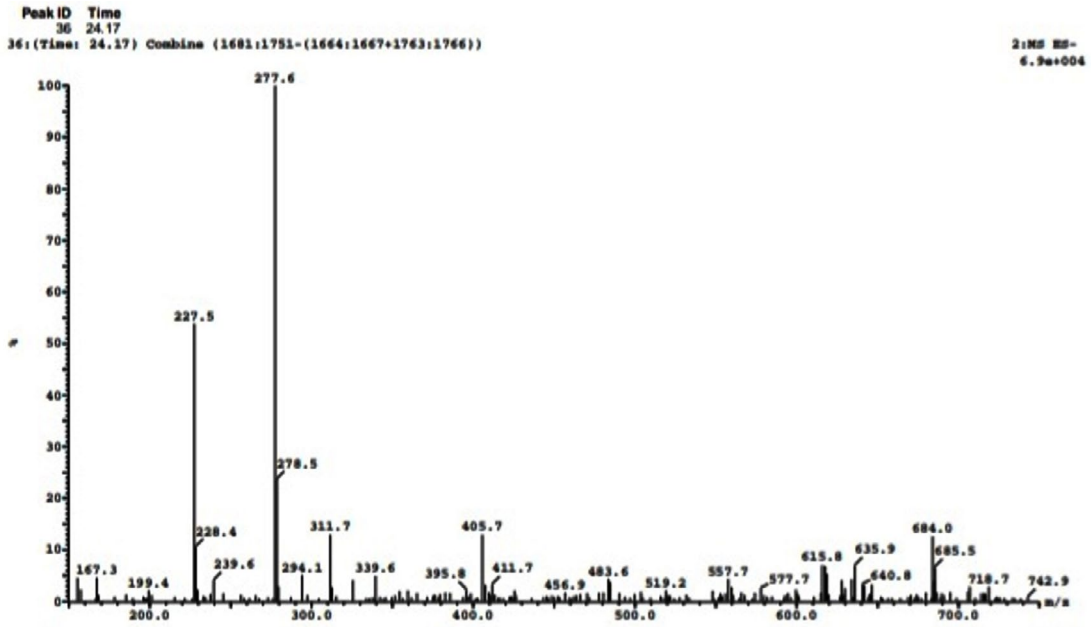

Fig. 6 (ii). Mass spectrum at the retention time 24.17.

Minimum Inhibitory Concentration (MIC) of *G. pedunculata*

MIC is used to determine the potency of the antimicrobial compounds towards the susceptible bacteria. An antimicrobial agent with lower MIC value is considered better as less compound is required to inhibit growth of the bacteria. The MEGP showed antibacterial activities against both GPB and GNB. The inhibitory effect of the extract increased with increasing concentration (1.56 – 100 mg/ml). The MEGP is approximately equally potent against *S.aureus, E.faecalis* and *P.aeruginosa*, with MIC being 12.5 mg/ml. Against *E.cloacae, E.coli* and *K.pneumoniae*, the MIC of the

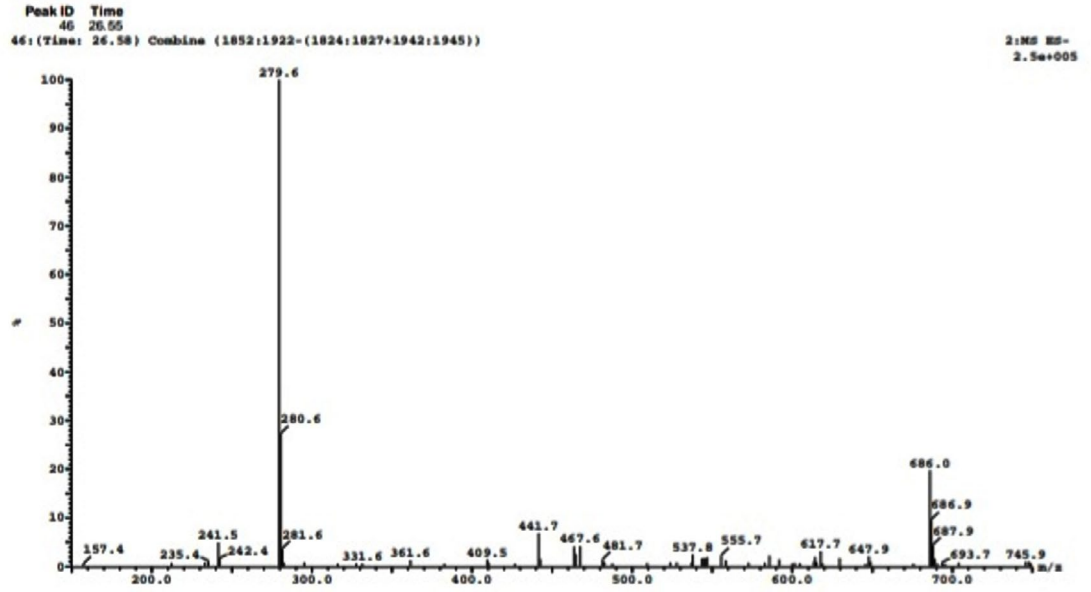

Fig. 6 (iii). Mass spectrum at the retention time 26.55.

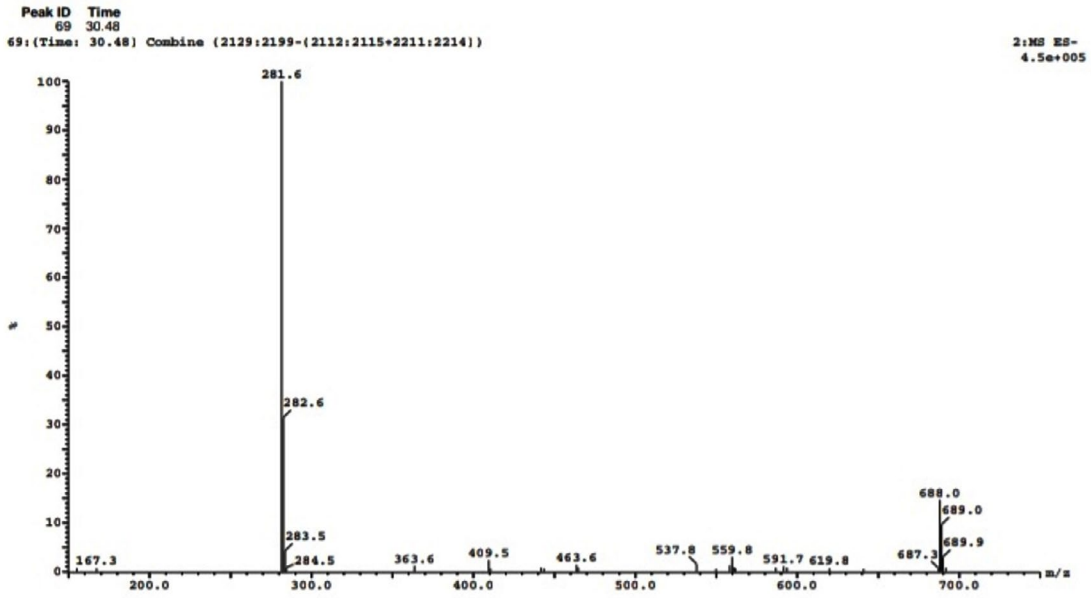

Fig. 6 (iv). Mass spectrum at the retention time 30.48.

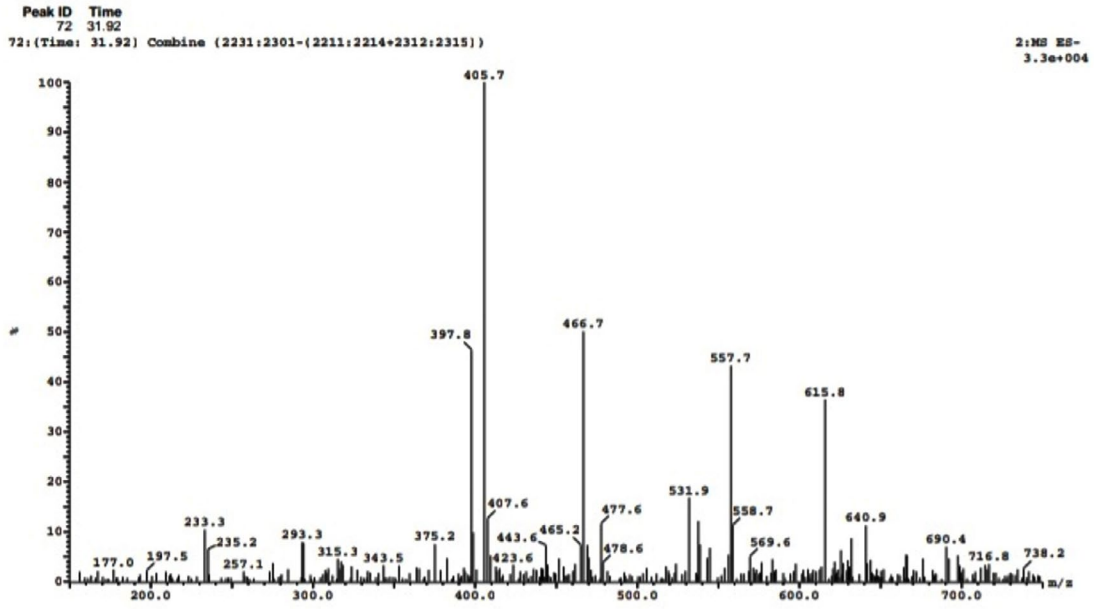

Fig. 6 (v). Mass spectrum at the retention time 31.92.

MEGP was observed to be approximately 25 mg/ml. This result also suggest that the MIC of the MEGP is bacterial genus/species dependent.

Minimum Bactericidal Concentration (MBC) of *G. pedunculata*

MEGP showed antibacterial activity against all the tested Gram-negative bacteria as well as Gram-positive bacteria. The MBC values was found to be approximately 25 mg/ml against *S.aureus, E.faecalis* and *P.aeruginosa* while 50 mg/ml was the observed MBC against *E. cloacae, E.coli* and *K.pneumoniae*.

LC-MS Result

The methanol extract of *G. pedunculata* (MEGP) was selected for LCMS analysis over ethanol extract (EEGP) since it was observed that it gives larger zone of inhibition against the bacteria tested. It implies that methanol may be a better organic solvent for extraction of antibacterial compounds from *G. pedunculata* fruit. LC-MS of the methanol extract of *G. pedunculata* fruit revealed presence of several compounds. Five of these compounds were identified as Hydroxy Citric Acid Lactone (MW-190), Garcinone E (MW-464), α-Mangostin (MW-410), β–Mangostin (MW-424), and γ-Mangostin (MW-396). Identification were carried out based on molecular weight, using the mass spectra of the LC-MS. Retention times, UV and mass spectral data of compounds were compared to literature data and to those of authentic standards, where available, for unambiguous identification. The mass spectra of Hydroxy Citric Acid Lactone, Garcinone E, α-Mangostin, β–Mangostin, and γ-Mangostin are shown in Fig. 6 (i-v).

Hence, LC-MS analysis confirmed that Hydroxy Citric Acid Lactone (MW-190), Garcinone E (MW-464), α-Mangostin (MW-410), β-Mangostin (MW-424), and γ-Mangostin (MW-396) are an important phytochemical content of the methanol extract of *G. pedunculata* fruit. Garcinone E, α-Mangostin, β–Mangostin and γ-Mangostin are important phytochemical compound belonging to the group of xanthones, which are well known for their antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and antitumor activities. All these phytochemical compounds have been reported from several Garcinia species including *G.indica, G.cambogia, G.atrovirdis and G. cowa* and *G.travancorica.*[8-10]

DISCUSSION

The antimicrobial activity of different species of *Garcinia* had been investigated by

Table 3. Selected List of compounds Identified by LC-MS in the Methanol Extract of *G.pedunculata* fruit

Peak ID	Retention Time	Name of Compounds	IUPAC Name	Molecular Formula	Molecular Weight	m/z ESI-ve
2	1.87	Hydroxy Citric Acid Lactone	(2S,3S)-3-hydroxy-5-oxooxolane-2,3-dicarboxylic acid	$C_6H_6O_7$	190	189.3
36	24.17	γ-Mangostin	1,3,6,7-tetrahydroxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one	$C_{23}H_{24}O_6$	396	395.8
46	26.55	α-Mangostin	1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one	$C_{24}H_{26}O_6$	410	409.5
69	30.48	Garcinone E	2,3,6,8-tetrahydroxy-1,4,7-tris(3-methylbut-2-enyl)xanthen-9-one	$C_{28}H_{32}O_6$	464	463.6
72	31.92	β–Mangostin	1,6-dihydroxy-3,7-dimethoxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one	$C_{25}H_{28}O_6$	424	423.6

various researchers with an encouraging result till date. Negi et al[11] reported that certain foodborne pathogens and spoilage bacteria, including *B.cereus, B.coagulans, B.subtilis, S.aureus,* and *E.coli*, are sensitive to crude hexane and chloroform extracts of *Garcinia cowa* and *Garcinia pedunculata* fruit rinds.[11] Torrungruang et al[12] also observed that *G.mangostana* Pericarp extract exert antibacterial activity against cariogenic *Streptococcus mutans.*[12]

Priya et al[13] study revealed the antibacterial activity of Pericarp extract of *G.mangostana* against *Staphylococcus aureus, Staphylococcus albus* and *Micrococcus luteus*.[13] Mangosteen pericarp extract has long been known for its broad-spectrum antibacterial activity against a number of GPB and GNB, particularly those linked to skin infections, diarrhoea, TB, and acne. α-mangostin, one of the xanthone derivatives derived from mangosteen extract, has been shown to have the strongest antibacterial action.[14-19]

The present investigation revealed the potency of both methanol and ethanol extracts of *G. pedunculata* as an antibacterial agent. *G. pedunculata* is effective as an inhibitory agent against both GPB as well as GNB. The antibacterial activity can be attributed to its phytochemical constituents.[20] The antibacterial activity may be due to an individual compound or synergistic effect of more than one compound present in the medicinal plant extract. Heymsfield et al[8], Kumar et al[9] and Aravinda et al[10] reported xanthones, biflavonoids, benzophenones, benzoquinones, and triterpenes are bioactive chemicals found in Garcinia species that exhibit antibacterial, antifungal, antioxidant, and cytotoxic properties.[8-10]

The antibacterial activity of MEGP was quantitatively evaluated by determining its minimum inhibitory concentration (MIC) values and minimum bactericidal concentration (MBC). As the bigger zone of inhibition was observed with methanol extract of *G. pedunculata* (MEGP) than that of ethanol extract of *G. pedunculata* (EEGP), MEGP was preferred to EEGP for determination of MIC and MBC. The MEGP is considered to be bactericidal rather than bacteriostatic since its MBC/MIC ratio is ≤ 4. An antimicrobial agent is considered bactericidal if the MBC is not more than fourfold higher than the MIC.[21]

CONCLUSION

Our research concluded that *G. pedunculata* exhibited broad-spectrum antibacterial activity. Further investigation leading to the isolation of pure compounds and antibacterial assay is highly recommended. Nevertheless, *G. pedunculata* have been in use for many years as ethnomedicinal plants to treat various ailments. This paper establishes a scientific foundation for the use of *G. pedunculata* in the treatment of UTI infections. Further research could lead to their adoption as safe antibacterial alternatives to synthetic medications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both the authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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