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Research Article

BIOACTIVITIES OF SELECTED EDIBLE FRUITS OF WESTERN GHATS REGION OF KARNATAKA

Komala H. P.*[1], Deepashree C. L.[2], Shubha Gopal[2], Devi Prasad A. G.[1]

[1]*Department of Studies in Environmental Science, University of Mysore, Manasagangotri, Mysuru, Karnataka, India*

[2]*Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysuru, Karnataka, India*

**Corresponding author: komalapanchuhp@gmail.com*

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ABSTRACT

Medicinal plants are well known for their therapeutic potential. The present study explored some of the selected medicinal plants for their bioactive potential. Fruits of selected plants were evaluated for their antibacterial and antioxidant activities. These plants extract were also subjected for the determination of functional groups present. All the test extracts showed good antibacterial and antioxidant properties. Methanolic extract of *Bridelia scandens* fruit was found to be more potent in inhibiting all the test cultures used, with a better zone of inhibition. It also revealed to possess stronger antioxidant ability by scavenging 87.62 % of DPPH radicals, 80.12 % of superoxide radical and by inhibiting 77.27% of lipid peroxidation process compared to other test extracts. FTIR studies of these species have shown the common and uncommon functional groups. Fruits of *Bridelia scandens* appeared to be a promising source compared to other test plants in the further isolation of bioactive compounds for determining bioactivities.

Keywords: Medicinal plants, Antibacterial activity, Antioxidant activity, FTIR.

1. INTRODUCTION

Plants are the potential source of medicine and have been exploited in folk medicine for centuries by most of the populations throughout the world. Plant-based drugs can be extracted from any part of plant-like barks, leaves, fruits, seeds, etc. [1]. The fruits of numerous plants have found their considerable importance in treating various diseases like epilepsy, chlorosis, migraines, eczema, psoriasis [2], arthritis, heart diseases, muscle aches, drug addiction, etc. [3]. These therapeutic properties lie in their secondary metabolites and their extraction facilitates pharmacological studies leading to a synthesis of a more potent drug with a reduced toxicity [4, 5]. The ability of various fruit extracts have been tested and have been reported for their various bioactivities by a larger group of researchers, but still numerous fruits are under the shade without their scientific exposure. This opens up an avenue for their investigation.

Western Ghats region of Karnataka harbours many medicinal plant species bearing fruits with their biological history. The ethnomedicinal values of most of these species are unexplored. Documentation of the traditional medicinal practices and systematically determining their therapeutic properties, offers scope for scientific validation and bioprospection. In view of this, an attempt has been made to evaluate the bioactivities of fruit of medicinal plants species namely, *Garcinia xanthochymus, Garcinia indica, Bridelia scandens, Carissa carandus* and *Flacourtia inermis* collected from the Western Ghats region. Along with this, the functional groups present in the test samples were identified using FTIR spectroscopic technique. This is a high-resolution method and plays the key step in the process of determining the chemical constituents present [6].

2. MATERIAL AND METHODS

2.1. Plant material and extraction

The Western Ghats region of Shimoga and North Canara was explored, where fruit-yielding plant species with medicinal importance were collected and were identified by Mr. Shivamurthy G R, Visiting Professor, Department of Botany, University of Mysore, Mysuru, Karnataka, India. The details of these species and their potentialities are shown in table 1. The fresh fruits of *Garcinia xanthochymus, Garcinia indica, Bridelia scandens, Carissa carandus* and *Flacourtia inermis* were collected and washed

thoroughly under running tap water. The washed fruits were chopped into pieces, dried under shade and were subjected to methanolic extraction using the Soxhlet apparatus. The obtained extracts of these plants were concentrated and were used further for various analyses.

Table 1: Selected medicinal plants for their bioactivity

Medicinal plants	Family	Vernacular name	Ethno medicinal uses
Garcinia xanthochymus	Clusiaceae	Devanige/Nelamaavu	Diarrhoea, dysentery and removing food toxin [7]
Garcinia indica	Clusiaceae	Punarpuli	Tumours, diarrhoea, dysentery, infected wounds [8]
Bridelia scandens	Euphorbiaceae	Bisilubaale	Diarrhoea, inflammation, jaundice [9]
Carissa carandus	Apocynaceae	Kouli	Constipation, diarrhoea, scabies, oral inflammation [10]
Flacourtia inermis	Flacourtiaceae	Sampige	Cancer, stroke, diabetes [11]

2.2. Microorganisms

Both Gram-positive bacterium *Staphylococcus aureus* (ATCC 12600) and Gram-negative bacteria such as *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 11775), *Vibrio parahaemolyticus* (ATCC 17802), *Pseudomonas aeruginosa* (ATCC 10145) and *Klebsiella pneumoniae* (ATCC 10031) were obtained from the Department of Microbiology, University of Mysore, Mysuru, Karnataka, India and were used in the present study to determine their sensitivity to these plants extract.

2.3. Chemicals

1,1-diphenyl-2-picrilhydrazyl(DPPH) radical, Butylated -hydroxy anisole (BHA), Nitro Blue Tetrazolium (NBT), Nicotinamide Adenine Dinucleotide reduced (NADH), Phenazine Methosulfate (PMS), Potassium ferricyanide, Tri Chloro Acetic acid (TCA), Thio Barbituric acid (TBA) were obtained from HiMedia, Mumbai, India.

2.4. Evaluation of antibacterial activity

Antibacterial activities of the plant's extracts were determined by the well diffusion method [12]. Sterile nutrient agar plates were prepared and inoculated with different test bacterial cultures by spread plate method. 6mm wells were made in the nutrient agar plates and were loaded with 20 µl of different test samples (250mg/ml). The loaded plates were incubated at 37°C for 24 hrs. Antibacterial activities of test extracts were evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as negative control and 10µg gentamicin was used as a positive control. After incubation, the inhibition zone formed around the wells was measured in millimetres.

2.5. Evaluation of antioxidant activity

2.5.1. DPPH radical scavenging assay

The method described by Smith [13] was followed for measuring DPPH radical scavenging ability of the plant extracts. Different concentration (20, 40, 60, 80, 100 µg/ml) of samples were treated with 300 µM of DPPH solution in 96-well microtiter plates, incubated at room temperature for 30 min in dark and the absorbance was measured spectrophotometrically at 515 nm. Ascorbic acid was used as a positive control. The radical scavenging percentage of the extracts was calculated using the following formula.

DPPH radical scavenging effect (%) = [(Absorbance of control - Absorbance of sample)/Absorbance of control] X 100,

where, Absorbance of control is the absorbance of DPPH radical + methanol and Absorbance of sample is the absorbance of DPPH radical + test sample/ standard.

2.5.2. Reducing power assay

The method described by Yen and Chen [14] was followed for determining the total reducing power ability of the test extracts. Different concentration (20, 40, 60, 80, 100 µg/ml) of plant samples were mixed with 0.2 M phosphate buffer (pH 6.6) and potassium ferricyanide (1%). The reaction mixture was incubated for 20 min at 50°C, followed by the addition of trichloroaceticacid (10%) and centrifugation at 3000 g for 10 min. The upper layer of the solution was mixed with ferric chloride (0.1%) and 1.5ml of distilled water. The absorbance was measured at 700 nm in a spectrophotometer. BHA was used as a positive control.

2.5.3. Superoxide radical scavenging assay

Superoxide radicals scavenging assay was performed [15]. PMS-NADH system was used to create superoxide radicals and was assayed by the reduction of NBT. Superoxide anion as generated in 100 mM of 3mL Tris-HCl buffer of pH 7.4 containing 300 µM NBT solution, 936 µM NADH solution, and different concentrations

(20, 40, 60, 80, 100 µg/ml) of the samples; 120 µM of PMS was added to initiate the reaction. The mixture was incubated at room temperature for 5 min and the absorbance was measured at 560 nm in a spectrophotometer. BHA was used as a positive control. The super oxide anion scavenging activity was calculated using the formula:

Scavenging (%) = [(Absorbance of control - Absorbance of sample) / Absorbance of control] X 100,

Where, absorbance of control is the absorbance of tris-HCl buffer containing NBT -NADH-PMS and absorbance of samples is the absorbance of tris-HCl buffer containing NBT -NADH-PMS+ test plant extracts/standard (positive control).

2.5.4. Lipid peroxidation inhibition assay

The method described by Baskar [16] was followed for determining lipid peroxidation inhibition abilities of the test extracts. Fresh goat liver was processed in phosphate buffered saline (pH 7.4) and the homogenate obtained was filtered. The filtered liver homogenate was added with 1 ml of different concentrations (20, 40, 60, 80, 100 µg/ml) of test plant extracts. To this mixture, 15 mM ferrous sulphate solution was added and this initiates the lipid peroxidation. After 30 min of incubation, 1 ml of this reaction mixture was added to 10 % TCA, incubated for 10 min, centrifuged and the supernatant was separated. The supernatant was mixed with 0.67 % TBA in 50 % acetic acid, heated for 30 min in a boiling water bath and absorbance was measured at 535 nm. BHA was used as the positive control. Lipid peroxidation inhibition abilities of the test plant extracts were calculated using the following formula.

Lipid peroxidation inhibition % = [(Absorbance of control - Absorbance of samples)/Absorbance of control] X 100,

Where, the absorbance of control is the absorbance of liver homogenate + ferrous sulphate + TCA and TBA and absorbance of samples is the absorbance of liver homogenate + ferrous sulphate + TCA and TBA + test plant extracts /standard (positive control).

2.6. FTIR Spectroscopic Analysis

Fourier transform infrared spectroscopic method (ATR method) was used for the identification of functional groups present in each plant extract. The extract of each fruit material was loaded in FTIR spectroscope (Perkin Elmer), with a scan range from 600 to 4000 cm^{-1} with a resolution of 4cm^{-1}.

2.7. Statistical analysis

Results were expressed as mean ± SD. The statistical comparison among the groups was performed with one way ANOVA at $p<0.05$ significant level (SPSS software version - 16).

3. RESULTS AND DISCUSSION

3.1. Antibacterial activity

Antibacterial activities of test extracts determined showed moderate to better activities and are tabulated in table 2.

Table 2: Antibacterial activity of test extracts (diameter - zone of inhibition in mm)

	G. x	***G. i***	***B. s***	***C. c***	***F. i***	**Gen.**
S. aureus	12.33±0.577	25.66±0.577	25.00±1.00	24.33±0.577	10.66±0.577	20.330.±0.577
S. typhimurium	-	15.00±0.00	14.33±0.577	15.33±0.577	-	24.66± 0.577
E. coli	11.66±0.577	21.00±0.00	19.33±0.577	-	-	25.00±0.00
V. parahaemolyticus	11.33±0.577	15.33±0.577	16.33±0.577	14.33±0.577	-	22.33±0.577
P. aeruginosa	6.33±0.577	18.66±0.577	22.00±1.00	23.00±0.00	11.33±0.577	14.33 ±0.577
K.pneumoniae	12.33±0.577	17.33±0.577	22.00±0.00	22.33±0.577	18.00 ±1.00	24.00 ±0.00

**Values are expressed as mean±S.D*

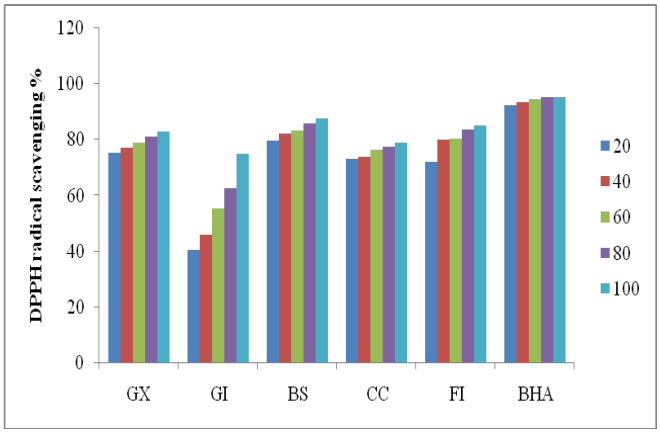
G.x: Garcinia xanthochymus, G.i: Garcinia indica, B.s: Bridelia scandens, C.c: Carissa carandus and *F.i: Flacourtia inermis, Gen.: Gentamicin, S.a: Staphylococcus aureus; S.t: Salmonella typhimurium; E.c: Escherichia coli; V.p: Vibrio parahaemolyticus; P.a: Pseudomonas aeruginosa* and *K.p*: *Klebsiella pneumoniae.*

Most of the test extracts, determined for their anti-bacterial activities, were found to be active in inhibiting the used test cultures at determined concentrations. Among the test extracts, methanol extract of *Bridelia scandens* and *Garcinia indica* were found to be active against all the bacterial strains used and were more potent against *Staphylococcus aureus. Escherichia coli* were found to be resistant to methanol extract of *Carissa carandus* while other test cultures were susceptible. Except for *Salmonella typhimurium,* all the bacterial

strains were inhibited by the methanolic extract of *Garcinia xanthochymus*. Methanolic extract of *Flacourtia inermis* was active only against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The observed antibacterial effect of different test extracts could be attributed to their secondary metabolites/ bioactive compounds present in them [17, 18]. These bioactive compounds may vary with different test plants and thus might have produced varying zones of inhibition, indicating the antibacterial potency of test plants.

3.2. Antioxidant activity

The test extracts evaluated for their antioxidant potential resulted in an extract of *Bridelia scandens* fruit to possess higher DPPH radical scavenging ability (87.62%) as compared to standard (95.40%) followed by methanolic extract of *Flacourtia inermis* (84.95%), *Garcinia xanthochymus* (82.84%) and *Carissa carandus* (78.97%). Lower radical scavenging activity was observed in *Garcinia indica* among the test extracts (Figure 1).

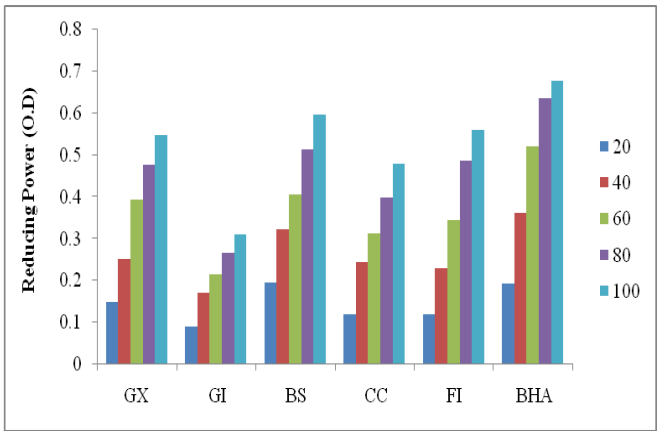


**GX: Garcinia xanthochymus, GI: Garcinia indica, BS: Bridelia scandens, CC: Carissa carandus and FI: Flacourtiainermis, BHA: Butylated hydroxyl anisole, p<0.05 significant level*

Fig. 1: DPPH radical scavenging ability

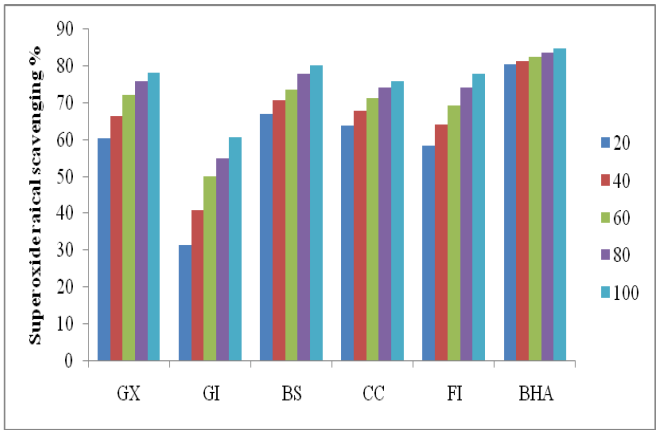
The obtained varying DPPH radical scavenging property of test extracts is may be due to the presence of antioxidant molecules [19, 20]. These compounds cause the reduction of DPPH radical to its 2,2-diphenyl-1-hydrazine form by donating hydrogen atoms [21]. It has been reported that there is a direct correlation between antioxidant activities and the reducing power of plant extract [22]. The reducing power of test extracts determined showed that the extract of *Bridelia scandens* show better reducing power followed by *Flacourtia inermis*, *Garcinia xanthochymus*, *Carissa carandus* and by *Garcinia indica* (Figure 2). The ability of test plant extracts to reduce Fe^{3+} to Fe^{2+} showed an increase with the increase in concentration. This ability is due to the presence of reductants/antioxidants and varied with test extracts. *B. scandens* proved to be more potent among the test extracts.

Superoxide radicals are formed by the cellular oxidation reaction. These are usually formed first and its effect is magnified as it produces other damaging ROS [15]. These radical initiates lipid peroxidation leading to many diseases [23]. Thus, superoxide radical inhibition becomes necessary to avoid free radical-mediated diseases. In the present study, it was recorded that the test extracts of *Bridelia scandens* showed scavenging of radicals (80.12%) followed by other species (Figure 3).



**GX: Garcinia xanthochymus, GI: Garcinia indica, BS: Bridelias candens, CC: Carissa carandus and FI: Flacourtiainermis, BHA: Butylated hydroxyl anisole, p<0.05 significant level*

Fig. 2: Reducing power ability



**GX: Garcinia xanthochymus, GI: Garcinia indica, BS: Bridelia scandens, CC: Carissa carandus and FI: Flacourtiainermis, BHA: Butylated hydroxyl anisole, p<0.05 significant level*

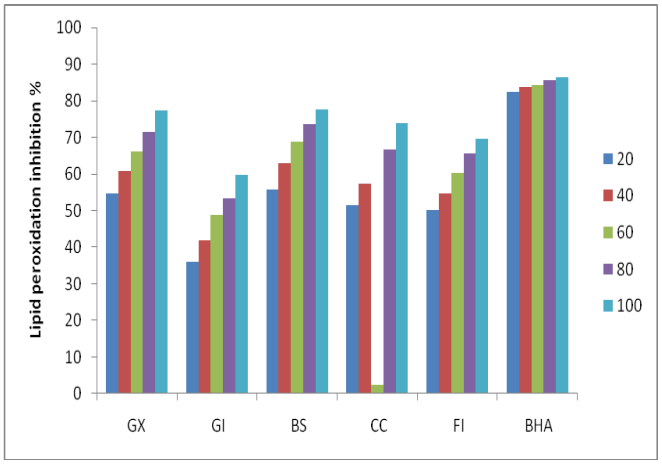
Fig. 3: Superoxide radicals scavenging ability

This ability to scavenge varied in different plant extracts as it is dependent on the concentration of extract, type of antioxidant molecule present and their potency to donate a hydrogen atom. The test extracts can directly or indirectly inhibit various ROS by inhibiting superoxide radicals. The obtained inhibitory effects of test extracts on superoxide anion formation as reported in the present work possibly make these to be a promising source of antioxidants.

The exposure of lipids to oxygen results in its peroxidation and is a natural phenomenon [24]. This lipid peroxidation leads to membrane damage [25], genomic and mtDNA damage [26] and results in various pathological conditions [24]. Thus, their inhibition becomes very much essential. In the present study, the test extracts determined for their lipid peroxidation inhibition ability were in the order of *Bridelia scandens*> *Garcinia xanthochymus*> *Carissa carandus*>*Flacourtia inermis* > *Garcinia indica* extracts (Figure 4). The test samples may inhibit lipid peroxidation either by binding to ferrous ions or by scavenging hydroxyl radical (a major cause of peroxidation of lipids). The diversity in radical scavenging shown in these assays may also be due to factors like stereo-selectivity of the radicals or the differential solubility of the extracts in the testing systems [27].

3.3. FTIR analysis

The functional groups present in each test extracts were identified based on the peaks obtained in infrared spectrum. The FTIR spectrum profiles of each extracted sample are presented in figures. 5-9. All five samples studied showed almost the same peak values indicating similar functional groups. Functional groups such as hydroxyl group, alkanes [28], alkenes, halogen compounds, carboxyl groups [29] were present in all the tests samples. A peak at 1518 cm^{-1} indicates the presence of nitro compounds in *Garcinia xanthochymus*. The band at 1788 cm^{-1} represents the presence of acid anhydrides in *Garcinia indica* [30]. Vibrations at 1449 cm^{-1} show the presence of Nitrosamine in *Bridelia scandens*. A peak at 1146 cm^{-1} in *Garcinia indica* represents aryl conjugated group [31].



**GX: Garcinia xanthochymus, GI: Garcinia indica, BS: Bridelia scandens, CC: Carissa carandus and FI: Flacourtiainermis, BHA: Butylated hydroxyl anisole, p<0.05 significant level*

Fig. 4: Lipid peroxidation inhibition ability

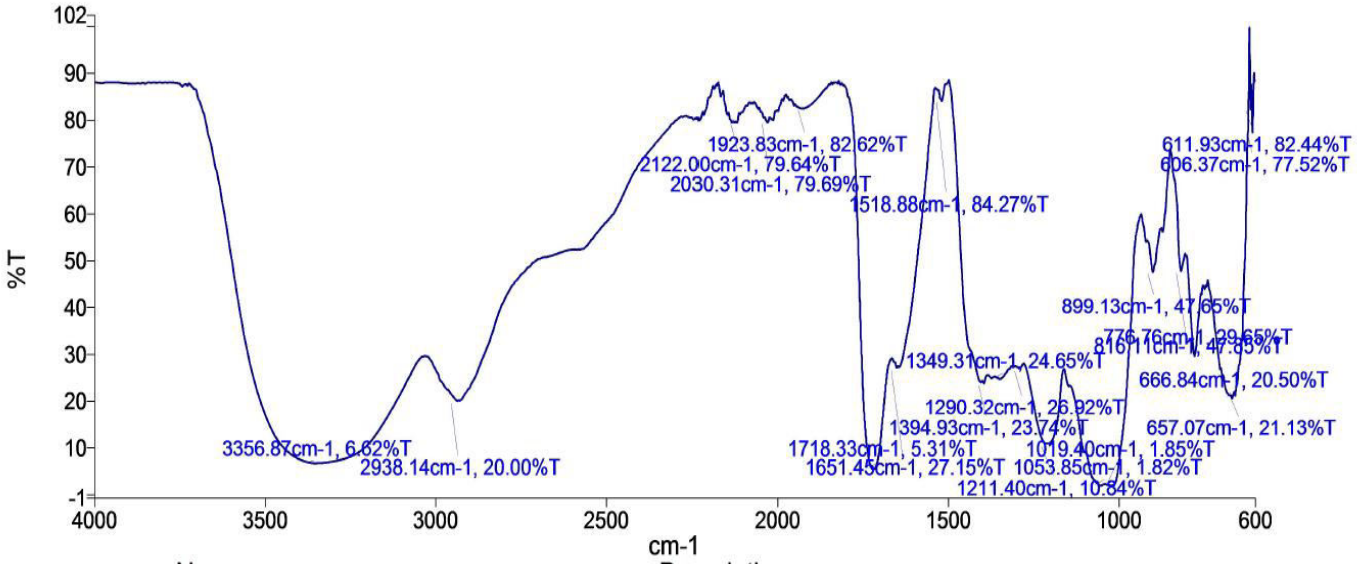


Fig. 5: FTIR spectrum of *Garcinia xanthochymus*

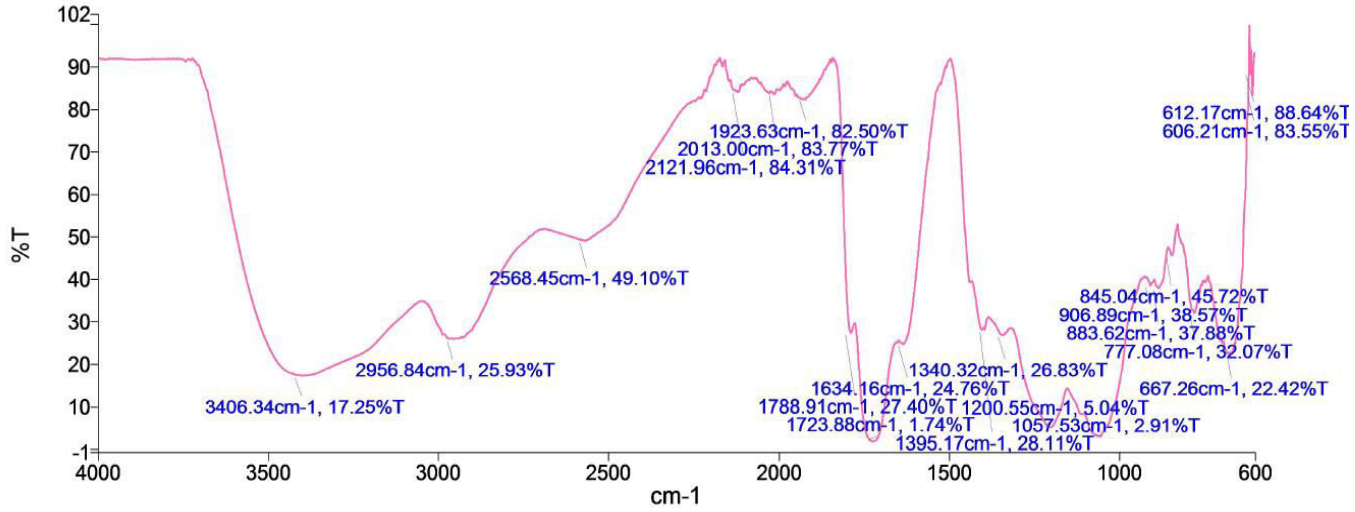


Fig. 6: FTIR spectrum of *Garcinia indica*

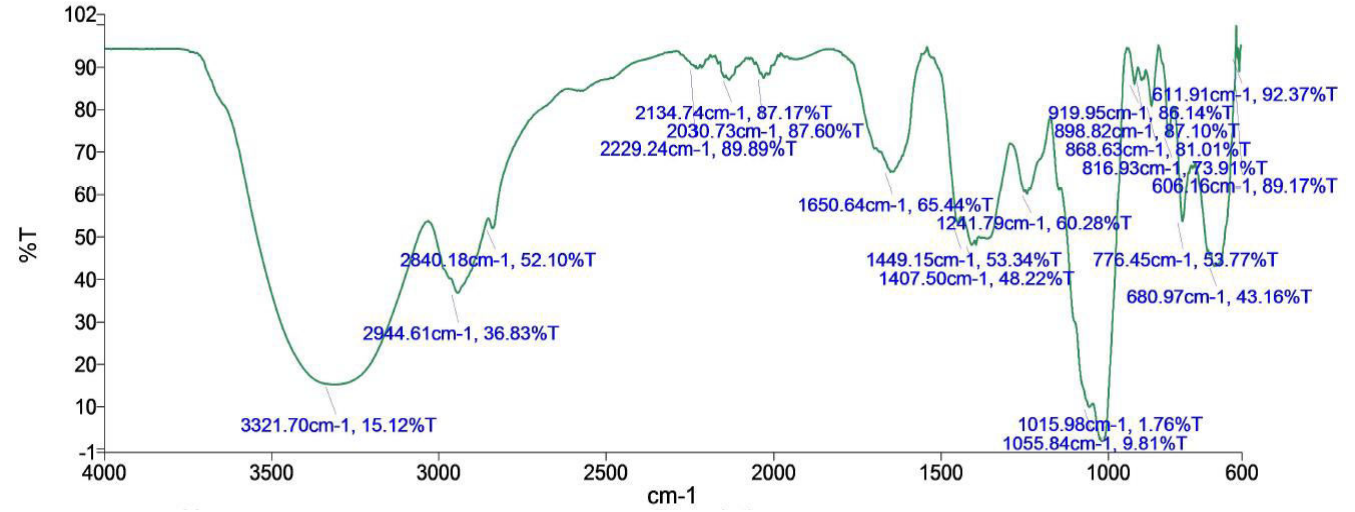


Fig. 7: FTIR spectrum of *Bridelia scandens*

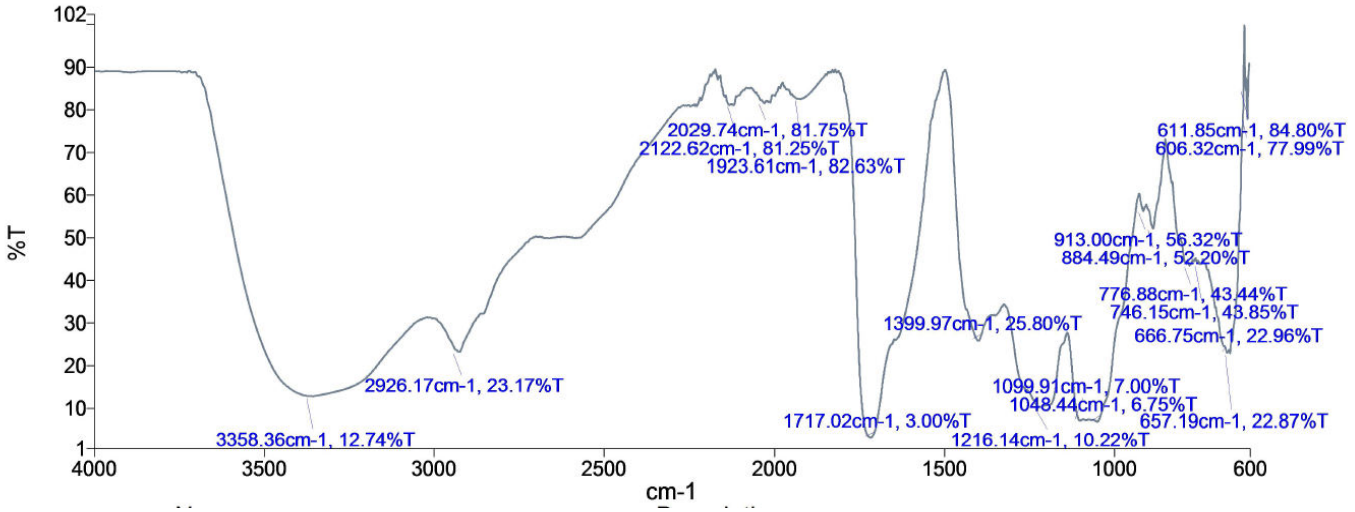


Fig. 8: FTIR spectrum of *Carissa carandus*

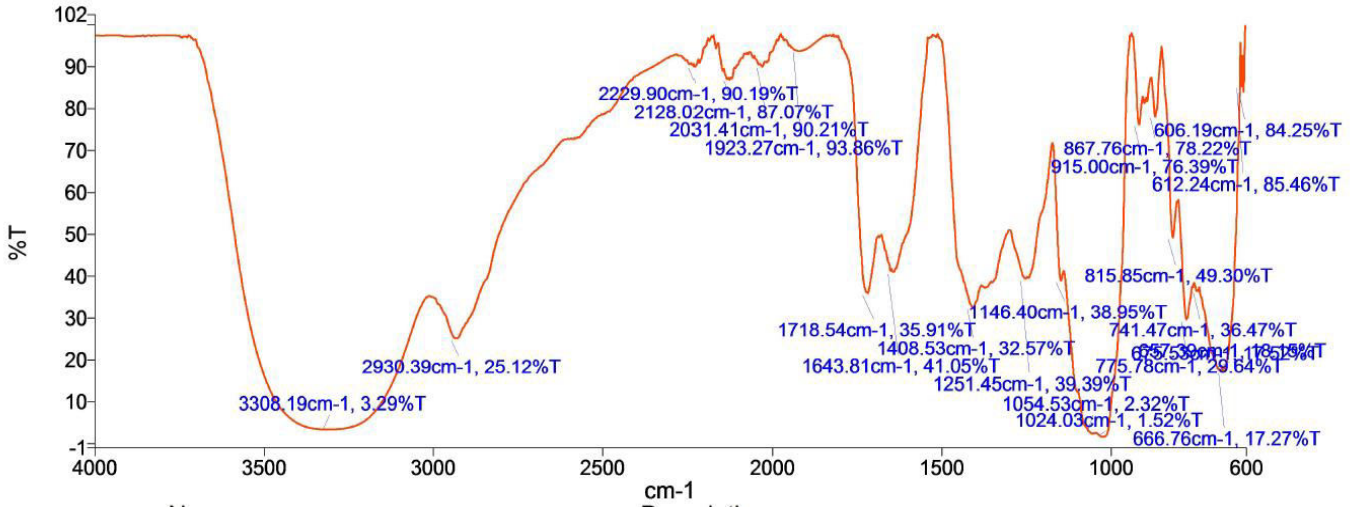


Fig. 9: FTIR spectrum of *Flacourtia inermis*

4. CONCLUSION

The findings and observations of the present study would support that the medicinal plants could be the source of alternative medicine with stronger antibacterial and antioxidant activities. Among the fruit extracts of the plant species investigated, *Bridelia scandens* showed greater inhibition of the test bacterial strain and was also found to be a better radical scavenger. Thus, this species could be a promising source for bioprospection of compounds, especially for antibacterial and antioxidant activities.

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Conflict of interest

The authors declare that they have no conflict of interest.

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None declared

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