

## ANTIBACTERIAL AND ANTIOXIDANT CAPACITY OF METHANOL ROOT EXTRACT OF MANGIFERA INDICA

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### Abstract

Recent years have witnessed enhanced research work reported on plants and plant products. In this regard, plants with traditional therapeutic usage are being screened more efficiently to be considered as a substitution or as a better alternative agent for Human Pathogens. Antioxidant activity, antibacterial, antifungal properties, colour, phytochemical and FT-IR spectral analysis of flowers belonging to Hibiscus moscheutos was determined. Radical scavenging activity of sample extracts were determined based on the percent inhibition of DPPH and ferric reducing antioxidant power (FRAP) assays. Total phenolics were estimated based on the Folin–Ciocalteu method, while, vanillin–HCl and aluminum chloride methods were employed to estimate total tannins and flavonoids in the sample extracts, respectively. To determine total flavonols and anthocyanin contents, spectrophotometric method was employed. For antibacterial activities, modified agar disk diffusion method was adopted. Results: The crude extracts of hexane, dichloromethane, chloroform, ethyl acetate and methanol, extracts with concentration levels at 100, 200, 300 and 400 mg/mL were shown to significantly affect the inhibition of bacterial selected. The presence of phytochemicals compounds possesses the high

antibacterial activities of this crude extracts. This study proved that *Mangifera Indica* crude extracts could be useful for inhibiting the selected pathogens which has an antioxidant potential as well as a natural bacterial agent.

**Keywords:** Antibacterial, Antioxidant, Methanol, Root Extract, *Mangifera Indica*

## INTRODUCTION

The genus *Mangifera* belongs to the family Anacardiaceae. Genus *Mangifera* approximately contains 69 different species with *M. indica* being the most common species in the same genus. *M. indica* plant is an evergreen broad canopy tree which grows to a height of 8–40 m. *M. indica* bark is a thick brown-grey colour and is superficially cracked. Leaves are 15–45 cm in length with variable sizes. Leaf petiole has a variable length from 1–10 cm. *M. indica* leaves possess different shapes (lanceolate, ovate-lanceolate, linear-oblong, roundish-oblong, oval, and oblong). Green, red, and yellow leaves are seen in some mango varieties and upper leaf surfaces are normally shiny. In the case of *M. indica* flowers, male and hermaphrodite flowers are produced in the same panicle; their size can vary from 6–8 mm in diameter. There are about 4000–5000 small flowers in panicles with red/purple spots on the petals (Nurul-Huda et al., 2015). Even though a large number of flowers are present in panicles, very few will be developed as fruits. Flowering season is mainly from January to April and most of the flowers are subsessile and have a sweet smell. *M. indica* fruit is a drupe with different sizes, shapes, and colours. Fruit peel is green, yellow, red, or orange. Seeds are ovoid- or oblong-shaped covered with a hard endocarp having a woody fibre covering (Sivakumar et al., 2011).

### Phytochemicals Present in *M. indica*

A large variety of chemical compounds have been reported in *M. indica*. Among these, polyphenols (flavonoids, xanthones, and phenolic acids) are the most abundant compound types in *M. indica* (Berardini et al., 2005). Mangiferin, gallic acid, catechins, quercetin, kaempferol, protocatechuic acid, ellagic acids, propyl and methyl gallate, rhamnetin, and anthocyanins are the major polyphenolic compounds found in *M. indica* (Nayan et al., 2017). Mangiferin is a well-known polyphenolic compound which has been extensively studied for its numerous biological properties. The quantities of different polyphenols in

mango depend on the part and variety of mango (Ma et al., 2011). Antioxidant properties are the main biological property of almost all the *M. indica* polyphenols. Ascorbic acid and dehydroascorbic acid (oxidized form of ascorbic acid) are two other common polyphenols found in *M. indica* (Rocha Ribeiro et al., 2006).

The number of polyphenols is high in many parts of *M. indica*. Thus, a pure compound alone has been proven to be less effective than crude drugs, implying that the synergism of many *M. indica* polyphenols is essential for optimum biological activities (Martin and He, 2009). Carotenoids are another class of natural compounds found in plants. They are considered natural organic pigments. The bright yellow colour of *M. indica* fruit peel and flesh is due to the presence of carotenoids. Biologically they are very good free radical scavengers. It has been reported that carotenoids in *M. indica* are biosynthesized in the fruit and carotenoid concentration rises upon ripening.  $\beta$ -carotene, luteoxanthin, violaxanthin, neoxanthin, zeaxanthin, and cryptoxanthin are the main carotenoids found in *M. indica* fruit flesh and peel. Among these,  $\beta$ -carotene is the most abundant. Terpenoids are a class of lipids, similar to terpenes, commonly found in the plant kingdom (Chen et al., 2011).

*M. indica* is reported to contain several terpenoids, including careen, ocimene, terpinolene, myrcene, or limonene. These terpenoids are volatile and responsible for the aroma of *M. indica* (Lalel et al., 2005). Lupeol and lupeol linoleate are two other common triterpenoids found in mangoes (Ruiz-Montanez et al., 2016). Gallotannins (hydrolyzable tannins) are another class of chemical compounds found in *M. indica* bark, leaves, kernel, and fruit pulp (Engels et al., 2009). The presence of tocopherols in *M. indica* has also been reported. Alpha-tocopherol, betatocopherol, and gamma-tocopherol are commonly found tocopherols in *M. indica* fruit peel and flesh (Ornelas-Paz et al., 2007). Resorcinolic lipids (phenolic lipids) are another class of natural compounds found in *M. indica*. The isolation of a wide range of resorcinolic lipids with different biological properties has been reported from *M. indica* fruit peels, flesh, and bark. The isolation of a novel resorcinolic lipid from the bark of *Mangifera zeylanica* (endemic Sri Lankan mango) with anticancer effects has been presented in a study carried out by us (Ediriweera et al., 2017).

It was thought that halogenated compounds are limited only to marine plants and microorganisms. However, the occurrence of halogenated compounds in the bark of *M. indica* has been reported in a study conducted in India (Singh et al., 2015). A recent study

carried out by us also reported the isolation of two novel halogenated compounds (chloromangiferamide and bromomangiferic acid) from the bark of *M. zeylanica* (Ediriweera et al., 2016). Quercetin and mangiferin are most commonly found in *M. indica*. As these two compounds containing food items (including mango fruits) are very common in the human diet, studies on their safety and toxicity have been well-documented (Jyotshna et al., 2016; Harwood et al., 2007). Moreover, kaempferol, another well-known mango compound, has also been subjected to various safety and toxicity studies to validate its uses in the human diet (Calderon-Monta et al., 2011; Ribeiro et al., 2008).

### **Phytochemicals in Different Parts of *M. indica***

#### **Leaves**

Amino acids include alanine, glycine, valine, tyrosine, leucine, and  $\gamma$ -aminobutyric acid. Polyphenols and phenolic acids include protocatechuic acid, gallic acid, hyperin, catechin, quercetin, mangiferin, kainic acid, ethyl digallate, ellagic acid, and shikimic acid. Alcohols include methyl, ethyl, and isobutyl alcohols. Terpenes include  $\alpha$ -pinene,  $\beta$ -pinene,  $\delta$ -elemene, taraxerol,  $\beta$ -elemene,  $\alpha$ -cubebene, camphene,  $\gamma$ -cadinene, lupeol, friedelin, linalool,  $\beta$ -bulnesene,  $\alpha$ -guaiene, humulene,  $\alpha$ -farnesene, myrcene, car3-ene, limonene,  $\beta$ -ocimene,  $\gamma$ -terpinene, and  $\alpha$ -terpinolene. Phenylpropenes include estragole, methyl eugenol and elemicin. Sterols include  $\alpha$ ,  $\beta$ , and  $\gamma$ -sitosterol (Kabir et al., 2017; Ribeiro and Schieber, 2010; Rai et al., 2007).

#### **Fruit Peel and Flesh**

Triterpenes and triterpenoids include cycloartenol,  $\alpha$ -amyirin,  $\beta$ -amyirin, ocotillol, 3 $\beta$ -hydroxycycloart-24-en-26-al, 24-methylene-cycloartan-3 $\beta$ ,26-diol, dammarenediol II, and psi-taraxastane-3 $\beta$ . Polyphenols and phenolic acids include ascorbic acid, quercetin, mangiferin, quercetin 3-ara, quercetin 3-rha, isomangiferin gallate, mangiferin gallate, methyl mangiferonate, methyl mangiferolate, tetra-O-galloylglucose, hexa-O-galloylglucose, methyl isomangiferolate, caffeic acid, ferulic acid, gallic acid, cinnamic acid, vanillin, rhamnetin-3-O-galactoside, kaempferol, and kaempferol-hexose. Resorcinolic lipids include 5-(11-Z-heptadecenyl)-resorcinol and 5-(8-Z,11-Z-heptadecadienyl)-resorcinol. Carotenoids include  $\beta$ -carotene, cis-violaxanthin, neochrome, cis-neoxanthin, luteoxanthin, zeaxanthin, and 9- or 9-cis-lutein. Long-chain fatty acids include oleic acid, linoleic acid,

linolenic acid, and n-pentacosanol (Kabir et al., 2017; Ribeiro and Schieber, 2010; Rai et al., 2007).

### **Root**

Triterpenes and triterpenoids include friedelin, friedelan-3b-ol,  $\alpha$ -amyrin,  $\beta$ -amyrin, and cycloartenol. Sterols include  $\beta$ -sitosterol and 3-methoxy-2-(4-methyl benzoyl)-chromone (Kabir et al., 2017; Ribeiro and Schieber, 2010).

### **Bark**

Polyphenols and phenolic acids include protocatechuic acid, catechin, mangiferin, benzoic acid, kainic acid, gallic acid, shikimic acid, and kaempferol. Triterpenes and triterpenoids include cycloart-24-en-3b,26-diol, 3-keto dammar-24 (E)-en-20S,26-diol, friedelin, mangocoumarin, manglupenone, manghopanal, cycloartan-3 $\beta$ -30-diol cycloartan-3b,24,27-triol, mangoleanone, mangiferolic acid ethyl ester, mangiferolate A and mangiferolate B, and 29-hydroxymangiferonic acid. Halogenated amide includes 3-chloro-N- (2-phenylethyl) propenamide. Long-chain hydrocarbons include N-triacontane, N-tetracosane, and 9,12-tetradecadiene1-ol-acetate. Terpenoid saponins include indicoside A and B. Amino acids include alanine, glycine, and  $\gamma$ -aminobutyric acid (Kabir et al., 2017; Singh et al., 2015; Kalita, 2014).

### **Seed and Kernel**

Long-chain hydrocarbons and fatty acids include stearic acid, eicosanoic acid, linoleic, linolenic, oleic acid, arachidonic acid, and palmitic acid. Sterols include stigmasterol, sitosterol, and campesterol. Triterpenes and triterpenoids include  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, and limonene. Polyphenols and phenolic acids include ascorbic acid, mangiferin, quercetin, and gallic acid (Kabir et al., 2017; Shah et al., 2010; Ribeiro and Schieber, 2010).

### **Flowers**

Amino acids include threonine, valine, alanine, and tryptophan. Polyphenols and phenolic acids include gallic acid, mangiferin, quercetin, and ellagic acid. Triterpenes and triterpenoids include  $\beta$ -pinene, nerol, limonene,  $\alpha$ -phellandrene, and  $\alpha$ -pinene (Kabir et al., 2017; Shah et al., 2010; Ribeiro and Schieber, 2010).

## **MATERIALS AND METHODS**

### **Sample Collection**

The root of *Mangifera indica* will be locally sourced in Wukari Local Government Area of Taraba State, Nigeria.

### **Extraction of Plant Material**

The plant material will be extracted according to the method described by Omotayo et al., (2015). The roots will be cut into small pieces, air-dried under the shade pulverized into coarse powder using a wooden pestle and mortar and stored until required for use. 100g of powdered *M. indica* root will be taken into two different beakers. The samples will be soaked with 500 ml of methanol and boiled distilled water respectively. The methanol root extract of the mango tree will be concentrated to a small volume by the use of a rotary evaporator and dried at 50°C in a water bath. The extract containing the bioactive compounds will be stored at - 20°C until the period of analysis.

### **Screening of Antibacterial Activities**

#### **Punch Hole Diffusion Method**

The method described by Chidozie et al. (2014) and Bauer et al. (1966) will be used to prepare an inoculum containing 10<sup>8</sup> colony-forming units (CFU) per millilitre of various clinical isolates of gram-positive and gram-negative bacteria. This included five types of gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella*, and *Salmonella typhi*. Additionally, *Staphylococcus aureus* and *Streptococcus faecalis* (gram-positive bacteria) will be utilized. Nutrient agar plates will be inoculated with these isolates and dried in an incubator at 37°C. Seven holes will be made on each plate using a 6mm sterile cork borer. Molten nutrient agar will be placed in these holes to seal them. The holes will be then filled with various concentrations of crude extracts corresponding to their labels, ensuring no overflow. Negative controls with sterile distilled water and a positive control with ciprofloxacin (10 mg/ml) will be included. The plates will be aerobically incubated at 37°C for 24 hours, and the diameters of the zones where bacterial growth will be inhibited will be measured in millimetres (mm) and recorded.

#### **Determination of Minimum Inhibitory Concentration (MIC)**

The determination of the Minimum Inhibitory Concentration (MIC) will be conducted following the method described by Chidozie et al. (2014). 9ml of freshly prepared nutrient

broth will be dispensed into sterile test tubes labelled 2-6. Subsequently, 1ml each of the plant extracts at concentrations of 200mg/ml, 400mg/ml, 600mg/ml, 800mg/ml, and 1000mg/ml will be added to tubes 2-6, respectively. Tube 1 served as the positive control, containing 10ml of nutrient broth. 50 $\mu$ l of the test organism will be added to each of the tubes using a sterile pipette. After thorough mixing, the tubes will be incubated at 37°C for 24 hours. Following incubation, the tubes will be visually examined for turbidity, indicating bacterial growth. The minimum concentration at which no turbidity will be observed (indicating inhibition of bacterial growth) will be considered as the Minimum Inhibitory Concentration

### **Determination of Minimum Bactericidal Concentration**

The Minimum Bactericidal Concentrations (MBCs) of the crude extract preparations will be assessed using the agar diffusion method as described by Nakamura (1999). Subsequently, 10 $\mu$ l of suspension from each negative tube in the MIC assays and the positive growth control tubes underwent aerobic incubation at 37°C for 24 hours. MBCs will be determined as the lowest concentration of the extract that resulted in negative subcultures.

### **Determination of Antioxidant Capacity**

#### **a. Free radical scavenging activity (DPPH)**

The free radical scavenging assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH) followed the method described by Rodríguez-García et al. (2019). Distilled water served as the control, and ascorbic acid will be the standard. Free radical scavenging activity, presented as the percentage of inhibition, will be determined using the formula

$$\% \text{ Inhibition} = ((A_0 - A_1) / (A_0 - 100))$$

where A<sub>0</sub> represents the absorbance of the control measured at 517 nm, and A<sub>1</sub> is the absorbance of the sample.

The antioxidant potential will be assessed through a regression analysis of the percentage of free radical scavenging against the concentration of phenolic compounds in the methanol extract. This analysis produced an IC<sub>50</sub> value, indicating the number of antioxidants required to reduce the initial DPPH radical concentration by 50%.

## **b. Ferric Reducing Antioxidant Power (FRAP)**

FRAP will be assessed to determine the total antioxidant power of MO using the protocol adapted from Benzie and Strain (1996). In addition, 10  $\mu\text{L}$  of standard (L-Ascorbic acid) will be added followed by the addition of 10  $\mu\text{L}$  of extract, 300  $\mu\text{L}$  of FRAP reagent, which included (10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), 300 mM acetate buffer at pH 3.6 in 0.1 M HCl, 20 mM Iron (III) Chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) and 6.6 mL distilled water, which produced a straw colour. All reagents will be incubated for 30 min before the reading will be taken. Absorbance will be measured at 593 nm. The results will be expressed as  $\mu\text{M}$  ascorbic acid equivalent per g dry weight ( $\mu\text{M}$  AAE/g DW).

## **Antibacterial**

### **Preparation of test sample**

The crude extracts of *Mangifera Indica* was used in antibacterial assay, the methanol crude extracts. The crude extracts were tested by disc diffusion method on nutrient agar medium as described by (Umaru et al., 2018). Exactly 3 mg of the crude sample was dissolved homogeneity in 3 mL of methanol giving a stock solution of 1000  $\mu\text{g}/\text{mL}$ . Different volumes from the stock solution were taken, amounted to 50, 100, 250, 500 ppm each, and dissolved in 5 mL of methanol to make final concentration respectively

### **Preparation of agar plates**

Preparation of agar plates was performed based on method described by (Umaru et al., 2018). Nutrient agar was prepared according to manufacturer's instruction with 14 g of dried agar dissolved in 500 mL distilled water. The agar solution was heated until boiling followed by sterilization in autoclave at  $121^\circ\text{C}$ . The agar solution was then poured into a sterile petri plate and allowed to cool down and forming a gel. The plate was divided into eight sections by making a line marking on the outside surface of the plate. The eight sections were for each test samples namely the 50, 100, 250, ppm samples, tetracycline 30  $\mu\text{g}$  (positive control) and methanol (negative control). The plate was sealed using parafilm and keep chilled at  $4^\circ\text{C}$  upon bacteria inoculation.

### **Preparation of bacteria broth**

Several selected bacteria were used to evaluate the antibacterial activities of the crude extracts of *Mangifera Indica* as obtained from the stock culture provided by Microbiology Laboratory, Federal University Wukari. The nutrient broth was prepared according to



manufacturer's instruction, with 2.6 g of the dried broth dissolved in 200 mL distilled water followed by sterilization in autoclave at 121°C. The bacterial was sub-cultured in a 10 mL of broth, each in universal glass bottle for 16 hours inside an incubator equipped with shaker at 37°C (Malesh and Satish, 2008). After 16 hours incubation, turbidity (optical density/OD) of the bacterial broth was measured by using UV mini spectrophotometer (model 1240 of Shimadzu brand), comparable to that of nutrient broth standard tube for further use. Measurement was performed at wavelength 575 nm and the bacterial broth was ready to be used when its turbidity was between OD 0.6 to 0.9. Nutrient broth was used to adjust the turbidity until the desired value was obtained.

### **Plate inoculation**

Inoculation of the bacteria was carried out in a biohazard cabinet and the procedure was based on method described by (Umaru et al., 2018). Approximately 1 mL of the ready bacterial broth were transferred into mini centrifuge tubes. A sterile cotton swap was dipped into the mini centrifuge tube containing bacteria broth and streaked over entire of the agar plate surface, performed in 4 different directions. The agar plate was then left for 5-10 minutes before applying the test samples. The disc used was 6 mm diameter. A volume of 10 µL of the test samples of concentration 25, 50, 100, 250, ppm was each pupated onto the discs and placed onto the agar plate by using sterile forceps and gently pressed to ensure contact. Next to be placed on the agar plate was the disc pupated with methanol as negative control, followed by 30 µg of tetracycline as standard antibacterial agent (positive control). The plates were left at room temperature for 10 minutes to allow the diffusion of the test samples and the standards into the agar. Each crude extract was tested in triplicate for each bacterium used. The plate samples were then incubated at 37°C for 24 hours before the inhibition zone around every sample disc being examined. The inhibition zone was measured in diameter to indicate the presence of antibacterial activity for each sample, as compared to the positive control.

### **Statistical Analysis**

Data will be subjected to analysis using SPSS software. The results will be expressed as mean  $\pm$  SD.

## RESULTS

**Table 1:** Effect of *Mangifera Indica* methanol Root-bark crude extract ( $\mu\text{g}/\text{mL}$ ) on Gram positive and Gram-negative bacteria in millimetre (mm)

Conc. ( $\mu\text{g}/\text{mL}$ )	Organism	Tetracycline (30 $\mu\text{g}/\text{mL}$ )	Methanol
100 $\mu\text{g}/\text{mL}$	<i>Escherichia coli</i>	20.62 $\pm$ 0.11	<b>13.33 <math>\pm</math> 1.16</b>
	<i>Staphylococcus aureus</i>	20.24 $\pm$ 0.14	15.41 $\pm$ 0.15
	<i>Klebsiella pneumonia</i>	20.16 $\pm$ 0.19	14.25 $\pm$ 0.19
200 $\mu\text{g}/\text{mL}$	<i>Escherichia coli</i>	20.12 $\pm$ 0.14	17.13 $\pm$ 0.16
	<i>Staphylococcus aureus</i>	20.11 $\pm$ 0.13	18.21 $\pm$ 0.15
	<i>Klebsiella pneumonia</i>	20.23 $\pm$ 0.16	19.24 $\pm$ 0.18
300 $\mu\text{g}/\text{mL}$	<i>Escherichia coli</i>	20.30 $\pm$ 0.14	19.22 $\pm$ 0.17
	<i>Staphylococcus aureus</i>	20.35 $\pm$ 0.16	19.26 $\pm$ 0.15
	<i>Klebsiella pneumonia</i>	20.31 $\pm$ 0.13	20.23 $\pm$ 0.12
400 $\mu\text{g}/\text{mL}$	<i>Escherichia coli</i>	20.23 $\pm$ 0.17	20.20 $\pm$ 0.14
	<i>Staphylococcus aureus</i>	20.20 $\pm$ 0.14	19.22 $\pm$ 0.16
	<i>Klebsiella pneumonia</i>	20.19 $\pm$ 0.15	<b>20.27<math>\pm</math> 0.45</b>

Result is Mean  $\pm$  SD. N = 3

\*= significant activity was observed when compared to the control ( $p < 0.05$ ).

Concentration of standard is 30  $\mu\text{g}/\text{mL}$  of tetracycline, Conc=concentration

**Table 2:** Antioxidant compound and antioxidant activities of extracts of Hexane, DCM, Chloroform, Ethyl acetate, and Methanol.

Parameter	Mangifera Indica of Polar and Non-Polar Solvents Extracts				
	Hexane	DCM	Chloroform	Ethyl acetate	Methanol
% DPPH inhibition	84.11 $\pm$ 0.2a	95.66 $\pm$ 0.77c	97.23 $\pm$ 0.5d	87.78 $\pm$ 0.7b	89.09 $\pm$ 0.3a
FRAP values	2451.12 $\pm$ 179.3 ab	2997.35 $\pm$ 223. 5c	2313.17 $\pm$ 343. 6b	2057.01 $\pm$ 12. 8a	2459.04 $\pm$ 242.4 ab
Total phenolics	4650.27 $\pm$ 79.6 4	5532.44 $\pm$ 166.8 9b	2569.36 $\pm$ 512. 4d	4776.23 $\pm$ 186. 2c	4896.16 $\pm$ 121. 3a
Total tannins	2916.66 $\pm$ 115.1 2c	4378.86 $\pm$ 112. 4d	1766.99 $\pm$ 140. 2b	2987.45 $\pm$ 124. 3c	2954.34 $\pm$ 116. 8b
Total flavonoids	2091.22 $\pm$ 129. 2b	2843.36 $\pm$ 179. 6c	3753.85 $\pm$ 159. 4d	2244.56 $\pm$ 177. 4b	2243.12 $\pm$ 131. 3b
Total flavanols	579.11 $\pm$ 2.1c	350.87 $\pm$ 3.3b	1278.77 $\pm$ 49. 2d	578.12 $\pm$ 2.6b	661,02 $\pm$ 1.4c
Total anthocyani	177.29 $\pm$ 4.7c	223.75 $\pm$ 2.9d	79.06 $\pm$ 3.4b	156.7 $\pm$ 6.7c	178.17 $\pm$ 4.4c

## DISCUSSION

Table 1 and 2 shows results obtained for the antibacterial antioxidant activities of crude extract from five different solvent; hexane, dichloromethane, chloroform, ethyl acetate and methanol extract of *Mangifera Indica* against Gram-positive and Gram-negative pathogenic bacteria. It shows that at the concentration of 100, 200, 300 and 400 mg/mL were observed to inhibit the growth of the pathogen (*Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia*) a Gram-negative bacterium at various values. Higher inhibition was observed with methanol crude extract on *Klebsiella pneumonia* with inhibition value of  $22.27 \pm 0.45$  mg/mL at 400mg/mL and lower inhibition was observed with 100mg/mL of  $13.33 \pm 1.16$  mg/mL on *Escherichia coli*.

Table 2 of Antioxidant compound and antioxidant activities of extracts of Hexane, DCM, Chloroform, Ethyl acetate, and Methanol. The table shows the result obtained for antioxidant compounds and antioxidant assays produced red coloured extracts, respectively.

Thus, determining the antioxidant activity of *Mangifera Indica* roots extract in this present study the extract of the polar and non-polar solvents exhibited rich scavenging effects on DPPH. The overall comparisons showed that the chloroform extract of  $97.23 \pm 0.5$  mg/mL to exhibit stronger scavenging effects on DPPH radicals, while hexane extract of  $84.11 \pm 0.2$  mg/mL had the lowest. Higher radical scavenging activity might be attributed to the presence of high phenolic, tannins or flavanols in the sample extracts.

The plant-based phenol compounds were reported to have exhibit rich antioxidant activity by scavenging the free radicals generated during the normal metabolism process. This group encompasses a wide diversity of compounds, which mainly includes: flavonoids and proanthocyanins. (Shahidi et al., 2004)

In the present study, the amount of total phenolics significantly varied between the solvent's extracts (hexane, dichloromethane, chloroform, ethyl acetate and methanol) thus, total phenols ranged between  $4650.27 \pm 79.64$  to  $2569.36 \pm 512.4$  dmG GAE/100 g. from the Table 2 it was observed that Dichloromethane extract exhibited the highest value of  $5532.44 \pm 166.89$  mg.

The amount of Tannin in all the solvent extract differed significantly and ranged between  $4378.86 \pm 112.4$  to  $1766.99 \pm 140.2$  mg CE/100g. The tannin value was observed to be

higher in Dichloromethane extract of  $4378.86 \pm 112.4$  mg CE/100g and lower in chloroform extract of  $1766.99 \pm 140.2$  mg CE/100g.

The presence of tannins in adequate amounts can be advantageous as they are able to quench free radicals very effectively, which in turn depended on the number of aromatic rings, molecular weight, and nature of the hydroxyl group substitution. (Cai et al., 2006) With regard to total flavonoid or bio-flavonoid content, chloroform extract showed high value  $3753.85 \pm 159.4^d$  mg CE/100 g and the least was found in hexane extract of  $2091.22 \pm 129.2^b$  mg CE/100 g. it was reported by Rice-Evan and Miller, (Rice-Evans and Miller, 1997) Flavonoids possess rich antioxidant properties and are produced as natural secondary metabolites in plants that encompass 6 sub-classes such as: isoflavones, flavonols, flavones and anthocyanins which vary in their structural characteristics. These flavonoids are capable of effectively interact and scavenge free radicals, which damage cell membranes and biological molecules.

The total flavanols, which are the most widespread sub-class of flavonoids in plant-based food-stuffs significantly varied between the solvent root's extracts. High flavanol content was recorded in chloroform extracts of  $1278.77 \pm 49.2$  mg QE/100 g), while the lowest value was observed in Dichloromethane of  $350.87 \pm 3.3^b$  mg QE/100 g) thus, indicating that chloroform to be more suitable for extracting flavanols compared to the other solvents.

With regard to total anthocyanin content, between the polar and non-polar solvent of the roots extracts Dichloromethane extract exhibited a higher value of anthocyanin  $223.75 \pm 2.9^d$  mg c-3-gE/100 g when compared to the other solvent extracts. It was in report that anthocyanins are becoming increasingly important not only due to their antioxidant properties, but also because of their antibacterial properties and use as a natural food colorant. (Naz et al., 2007).

Presence of high level of total phenols, flavanols, flavonoids, and anthocyanins has been reported in different plant parts and their extracts. (Cai et al., 2004, Gouveia et al., 2013, Yang et al., 2012, Wijekoon et al., 2011). In this study it was observed that different solvent extraction systems can contribute significantly to differences in the antioxidant activities of the extracts, this clearly illustrated that phenols including flavonoids, tannins, flavanol and anthocyanins are most probably the major contributor to the observed antioxidant properties in *Mangifera Indica* roots extracts.

## CONCLUSION

In conclusion, results of this study showed *Mangifera Indica* roots extracts to encompass significant amount of antioxidant compounds, with the extracts exhibiting rich antioxidant activities. In addition, the *Mangifera Indica* roots extracts also possessed antibacterial activity against various selected Gram-positive and Gram-negative bacterial pathogens. This study provides evidence that *Mangifera Indica* roots extracts of solvents (hexane, Dichloromethane, Chloroform, Ethyl acetate and methanol) at 250 and 500 mg/mL had the highest activities on the selected Gram positive and Gram-negative bacteria through in vitro and in vivo bioassays. The treatment altered the bacteria morphology and inhibited pathogens growth. The chemical constituents in this plant extract have the potential to be a natural antibacterial agent. Results on antioxidants activity indicate the prospective of utilizing *Mangifera Indica* roots extracts as a mode of natural agents for such pathogens. Thus, we propose an alternative disease management strategy using *Mangifera Indica* roots extracts to control disease and infections either plant or human to cuttle the menace of this pathogens. However, further research is necessary to elucidate the mechanism of action and develop the formulation to improve its efficacy and stability for use in disease control.

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## Conflicts of Interest

The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## REFERENCES

- Sivakumar, D., Jiang, Y. and Yahia, E. M. (2011). Maintaining Mango (*Mangifera indica* L.) Fruit Quality During the Export Chain. *Food Research International*, 44(5), 1254–1263.
- Berardini, N., Fezer, R., Conrad, J., Beifuss, U., Carle, R. and Schieber, A. (2005). Screening of mango (*Mangifera indica* L.) cultivars for their contents of flavonol O- and xanthone C-glycosides, anthocyanins, and pectin. *Journal of agricultural and food chemistry*, 53(5), 1563-1570.
- Nayan, V., Onteru, S. K. and Singh, D. (2018). *Mangifera indica* flower extract mediated biogenic green gold nanoparticles: Efficient nanocatalyst for reduction of 4-nitrophenol. *Environmental Progress & Sustainable Energy*, 37(1), 283-294.

- Ma, X., Wu, H., Liu, L., Yao, Q., Wang, S., Zhan, R. and Zhou, Y. (2011). Polyphenolic compounds and antioxidant properties in mango fruits. *Scientia Horticulturae*, 129(1), 102-107.
- Rocha Ribeiro, S. M., Queiroz, J. H., Lopes Ribeiro de Queiroz, M. E., Campos, F. M. and Pinheiro Sant'Ana, H. M. (2007). Antioxidant in mango (*Mangifera indica* L.) pulp. *Plant foods for human nutrition*, 62, 13-17.
- Martin and He, 2009
- Chen, F., Tholl, D., Bohlmann, J. and Pichersky, E. (2011). The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *The Plant Journal*, 66(1), 212-229.
- Lalel, H. J., Singh, Z. and Tan, S. C. (2003). Aroma volatiles production during fruit ripening of 'Kensington Pride' mango. *Postharvest Biology and Technology*, 27(3), 323-336.
- Ruiz-Montañez, G., Ragazzo-Sánchez, J. A., Calderón-Santoyo, M., Velazquez-De La Cruz, G., De León, J. R. and Navarro-Ocaña, A. (2014). Evaluation of extraction methods for preparative scale obtention of mangiferin and lupeol from mango peels (*Mangifera indica* L.). *Food chemistry*, 159, 267-272.
- Engels, C., Knodler, M., Zhao, Y. Y., Carle, R., Gänzle, M. G. and Schieber, A. (2009). Antimicrobial activity of gallotannins isolated from mango (*Mangifera indica* L.) kernels. *Journal of agricultural and food chemistry*, 57(17), 7712-7718.
- Ornelas-Paz, J. D. J., Yahia, E. M. and Gardea-Bejar, A. (2007). Identification and quantification of xanthophyll esters, carotenes, and tocopherols in the fruit of seven mexican mango cultivars by liquid chromatography– atmospheric pressure chemical ionization– time-of-flight mass spectrometry [LC-(APCI+)-MS]. *Journal of agricultural and food chemistry*, 55(16), 6628-6635.
- Ediriweera, M. K., Tennekoon, K. H., Samarakoon, S. R., Adhikari, A., Thabrew, I. and De Silva, E. D. (2017). Isolation of a new resorcinolic lipid from *Mangifera zeylanica* Hook. f. bark and its cytotoxic and apoptotic potential. *Biomedicine & Pharmacotherapy*, 89, 194-200.
- Ediriweera, M. K., Tennekoon, K. H., Adhikari, A., Samarakoon, S. R., Thabrew, I. and De Silva, E. D. (2016). New halogenated constituents from *Mangifera zeylanica* Hook. f. and their potential anti-cancer effects in breast and ovarian cancer cells. *Journal of Ethnopharmacology*, 189, 165-174.
- Jyotshna, Khare, P. and Shanker, K. (2016). Mangiferin: A review of sources and interventions for biological activities. *BioFactors*, 42(5), 504-514.
- Harwood, M., Danielewska-Nikiel, B., Borzelleca, J. F., Flamm, G. W., Williams, G. M. and Lines, T. C. (2007). A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. *Food and chemical toxicology*, 45(11), 2179-2205.
- Calderon-Montano, J., Burgos-Morón, E., Pérez-Guerrero, C. and López-Lázaro, M. (2011). A review on the dietary flavonoid kaempferol. *Mini reviews in medicinal chemistry*, 11(4), 298-344.

- Ribeiro, S. M. R., Barbosa, L. C. A., Queiroz, J. H., Knödler, M. and Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Food chemistry*, 110(3), 620-626.
- Kabir, Y., Shekhar, H. U. and Sidhu, J. *Processing Technology and Nutrition*.
- Ribeiro, S. M. R. and Schieber, A. (2010). Bioactive compounds in mango (*Mangifera indica* L.). In *Bioactive foods in promoting health* (pp. 507-523). Academic Press
- Rai, S., Basak, S., Mukherjee, K., Saha, B. P. and Mukherjee, P. K. (2007). Oriental medicine mangifera indica. *Advances in Traditional Medicine*, 7(1), 1-10.
- Singh, R., Singh, S. K., Maharia, R. S. and Garg, A. N. (2015). Identification of new phytoconstituents and antimicrobial activity in stem bark of *Mangifera indica* (L.). *Journal of pharmaceutical and biomedical analysis*, 105, 150-155.
- Kalita, P. (2014). An overview on *Mangifera indica*: importance and its various pharmacological action. *PharmaTutor*, 2(12), 72-76.
- Shah, K., Patel, M., Patel, R. and Parmar, P. (2010). *Mangifera indica* (mango). *Pharmacognosy Reviews*, 4(7), 42-48.
- Omotayo, M. A., Ogundare, O. C., Oluwafunmilayo, A., and Longe, S. A. (2015). Hepatoprotective effect of *Mangifera-indica* stem bark extracts on paracetamol-induced oxidative stress in albino rats. *European Scientific Journal*, 11(24).
- Chidozie, V. N., Adoga, G. I., Chukwu, O. C., Chukwu, I. D. and Adekeye, A. M. (2014). Antibacterial and toxicological effects of the aqueous extract of *Mangifera indica* stem bark on albino rats. *Global Journal of Biology, Agriculture and Health Sciences*, 3(3), 237-245.
- Bauer, A.W., Kirby, W.M. and Turk, M. (1966). Antibiotic susceptibility testing by standard single disc method. *American Journal of Clinical Pathology*, 45: 493-496
- Nakamura C.V., Nakamura T.V. and Bando E. (1999). Antibacterial activity of *Ocimum gratissimum* L. essential oil. *Memorias do Instituto Oswaldo Cruz*. 94, 675-678.
- Rodríguez-García, C. M., Ruiz-Ruiz, J. C., Peraza-Echeverría, L., Peraza-Sánchez, S. R., Torres-Tapia, L. W., Pérez-Brito, D. and Ortiz-Vázquez, E. (2019). Antioxidant, antihypertensive, anti-hyperglycemic, and antimicrobial activity of aqueous extracts from twelve native plants of the Yucatan coast. *PLoS One*, 14(3), e0213493.
- Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical biochemistry*, 239(1), 70-76
- Umaru, I. J., Badruddin, F. A., and Umaru, H. A. (2018). Gastroprotective Effect of *Leptadenia hastata* (Pers.) Decne and Histopathological Changes on Indomethacin-Induced Gastric Ulcer in Rats. *Int. J. Pure App. Biosci*, 6(5): 20-27.
- Umaru, I. J., Yakubu, O. E., Ahmed, M. U., Ezekiel, I., Umaru, K. I., Abu, M. S., and Abah, M. (2022). GC-MS, radicals scavenging capacity and antidiabetic effect of senna alata seed extract in type ii-induced diabetes mellitus in rats. *Research Journal of Medicinal Plants*, 16(2): 49-58.
- Shahidi, F., Naczki, M., 2004. Phenolics in Food and Nutraceuticals. CRC Press, Boca Raton, FL, 1-576.

- Cai, Y.Z., Sun, M., Xing, J., Luo, Q., Corke, H., 2006. Structure- radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Science*, 78: 2872-2888.
- Rice-Evans, C.A., Miller, N.J., 1997. Structure-antioxidant activity relationships of flavonoids and isoflavonoids. In: Rice-Evans, C.A., Packer, L. (Eds.), *Flavonoids in Health and Disease*. Marcel Dekker, New York.
- Naz, S., Siddiqi, R., Ahmad, S., Rasool, S.A., Sayeed, S.A., 2007. Antibacterial activity directed isolation of compounds from *Punica granatum*. *Journal of Food Science*, 72: 341-345.
- Cai, Y., Luo, Q., Sun, M., Corke, H., 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, 74: 2157–2184.
- Gouveia, S., Gonçalves, J., Castilho, P.C., 2013. Characterization of phenolic compounds and antioxidant activity of ethanolic extracts from flowers of *Andryala glandulosa* ssp. *varia* (Lowe ex DC.) R.Fern. an endemic species of Macaronesia region. *Industrial Crops and Products*, 42: 573–582.
- Yang, D.J., Chang, Y.Z., Chen, Y.C., Liu, S.C., Hsu, C.H., Lin, J.T. (2012). Antioxidant effect and active components of litchi (*Litchi chinensis* Sonn.) flower. *Food and Chemical Toxicology*, 50: 3056- 3061.
- Wijekoon, M.M.J.O., Bhat, R., Karim, A.A., 2011. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bungakantan (*Etlingera elatior* Jack.) in florescence. *Journal of Food Composition and Analysis*, 24: 615–619.