

ANTIBACTERIAL AND ANTIFUNGAL POTENTIAL OF METHANOL STEM-BARK EXTRACT OF MANGIFERA INDICA

Kerenhappuch Isaac Umaru¹, Akafa Andes Tansaba², Egeonu Stephen Ugoeze³,
Chakfa Nanmar⁴, Isaac John Umaru⁵

^{2,3,4,5}Federal University Wukari, Taraba State, Nigeria

¹University of Higher Institute Buea, South West Cameroon, Cameroon

umaruisaac@gmail.com

Article Info:

Submitted:	Revised:	Accepted:	Published:
Jun 13, 2024	Jun 29, 2024	Jul 11, 2024	Jul 16, 2024

Abstract

Medicinal plants have served through the ages, as a constant source of medicaments for the exposure of variety of diseases. The history of herbal medicine is as old as human civilization. The plants are known to provide a rich source of botanical anthelmintic, antibacterial and insecticides. *Mangifera indica* (MI), popularly known as mango belong to the genus *Mangifera* and family *Anacardiaceae*. The leaves, stem bark and roots are used as herbal medicines worldwide. In antibacterial analysis, higher growth inhibition rate was observed at 400 ug/ml for *Klebsiella pneumonia* with $20.55 \pm 1.26 \mu\text{g/mL}$ indicating that the extract has effect at a higher concentration than when treated at a lower concentration. In blood glucose level analysis, administration of 100 mg/kg body weight of *Mangifera indica* stem bark methanolic extract showed reduced blood glucose level in day 14 with $5.11 \pm 0.14a$ of treatment when compared to normal control.

Keywords: Antibacterial, Antifungal, Methanol, Stem- Bark Extract, *Mangifera Indica*

INTRODUCTION

Mangifera indica L. (family Anacardiaceae)), a popular tropical fruit, originated from the Indian sub-continent and then started becoming popular around the World. Around fifth and fourth centuries BC, cultivation of mango started in Southeast Asia, and then in East and West Africa around 10th century AD, and finally in Brazil, Mexico, Spain, Canary Islands and Portugal, West Indies and Caribbean Islands (United Nations Conference of Trade and Development, 2016; Imran, Arshad and Butt, 2017; Shah, Patel, and Patel, 2010; Acevedo, Raya and Martinez-Moreno, 2017; Okwu and Ezenagu, 2008). Today, India, China, Thailand and Mexico are the largest producers and exporters of mango (United Nations Conference of Trade and Development, 2016; Shah, Patel and Patel, 2010). Mango trees, flowers, fruits, seeds, leaves and bark have been reported to exhibit diverse medicinal properties and health benefits. Scientific literature demonstrates that different parts of mango tree and its integral constituents exhibit a number of health benefits including the reduction of chronic inflammatory conditions, anti-viral and anti-bacterial effects, immunomodulatory, anti-spasmodic, gastrointestinal well-being as well as metabolically-mediated chronic diseases (Shah, Patel and Patel, 2010; Acevedo, Raya and Martinez-Moreno, 2017; Okwu, and Ezenagu, 2008).

It is a medicinal plant that served through the ages, as a constant source of medicaments for the exposure of variety of diseases. The history of herbal medicine is as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides (Satyavati, Raina, and Sharma, 1976; Lewis and Elvin-Lewis, 1977).

The stem-bark are used as astringent, acrid, refrigerant, styptic, anti-syphilitic, vulnerary, anti-emetic, anti-inflammatory and constipating. They are useful in vitiated conditions of diabetes mellitus, pitta, metrorrhagia, calorrhagia, pneumorrhagia, leucorrhoea, syphilis, uteritis, wounds, ulcers and vomiting. The juice of fresh bark has a marked action on mucous membranes, in menorrhoea, leucorrhoea, bleeding piles and diarrhoea. (Jouad et al., 2001).

The chemical constituent in *Mangifera indica* L. cannot be over emphasised. The phytochemical compounds of mango are mainly phenolic compounds and these compounds can be obtained from various parts of the plant such as fruit, kernel (stone),

leaves, and bark (Ajila et al., 2007; Dorta et al., 2012). These results corroborate previous information that mangoes are a good source of many phytochemicals (Barreto et al., 2008).

The quantities and characteristics of different *Mangifera indica* phenolics differ in the different plant parts, besides being affected by the geographic location of the plants. The chemical composition of mango varies with the location of cultivation, variety, and stage of maturity (Manthey and Perkins-Veazie, 2009). Abdalla et al., (2007) characterized the phenolic compounds in Egyptian mango seed kernels. The components included tannins, gallic acid, coumarin, ellagic acid, vanillin, mangiferin, ferulic acid, cinnamic acid, and unknown compounds. Cheema and Sommerhalter (2015) reported that crude extracts of Ataulfo exhibited polyphenol oxidase activity with pyrogallol, 3-methylcatechol, catechol, gallic acid, and protocatechuic acid. During different stages of ripening, besides skin color changes, total phenolic acids and flavonoids concentration of mango fruit also changes.

Early studies showed that the main phenolics found in *Mangifera indica* are leucocyanidin, catechin, epicatechin, chlorogenic acid, quercitrin, and quercetin (Schieber et al., 2000). Mango also contains significant amounts of pigments, including chlorophylls and carotenoids (Grundhofer et al., 2001). Gallic acid is one of the most abundant phenolic compounds detected in mango fruit (Palafox-Carlos et al., 2012). Gallic acid has been identified as the major polyphenol present in mango fruits, followed by 6 hydrolysable tannins and 6 minor compounds, for example p-OH-benzoic acid, m-coumaric acid, p-coumaric acid, and ferulic acid (Kim et al., 2009). Schieber et al., (2000), identified 20 polyphenols in mango puree, and these compounds included gallic acid, caffeic acid, protocatechuic acid, p-coumaric acid, mangiferin, quercetin 3-ara-glc (peltatoside), kaempferol (hexose), six quercetin derivatives, gallotannin, and others. The most abundant phenolics in *Mangifera indica* were gallic acid, p-hydroxybenzoic acid, p-coumaric acid, sinapic acid, quercetin, ellagic acid, and catechin (Robles-Sánchez et al., 2009a). Other reports have indicated that the major phenolics in mango are leucocyanidin, catechin, epicatechin, chlorogenic acid, and quercetin (Robles-Sánchez et al., 2009b).

Mangiferin

The natural C-glucoside xanthone, mangiferin, chemically known as 2-C- β -D-glucotyranosyl-1,3,6,7-tetrahydroxyxanthen-9-one CAS 4773-96-0 (Formula C₁₉H₁₈O₁₁; Molecular Weight: 422.35; melting point 271°C). As indicated earlier, mangiferin is

abundant in the mango leaves, fruits, stem bark, heartwoods and roots (Imran, Arshad, and Butt, 2017; Shah, Patel, and Patel, 2010; Acevedo, Raya, and Martinez-Moreno, 2017). Mangiferin, a light yellow color crystalline powder, is slightly soluble in ethanol, sparingly soluble in methanol and water and practically insoluble in diethyl ether, acetone, and n-hexane (Acosta et al., 2016). Mangiferin is a novel antioxidant and exhibit pro-hypoglycemic activity by modulating glucose metabolism, ameliorating insulin resistance, lowering cholesterol synthesis, and inhibiting the expression of the TNF α and inducible nitric oxide synthase (Imran, Arshad, and Butt, 2017; Shah, Patel, and Patel, 2010; Acevedo, Raya, and Martinez-Moreno, 2017; Okwu, and Ezenagu, 2008; Zhao et al., 2017). The acute oral toxicity of mango leaf extract was found to be greater than 5,000 mg/kg body weight p.o. Both gross anatomy check and macroscopic evaluation were conducted and no signs of gross toxicity were observed till 14 days of treatment. Another study demonstrated no signs of gross or organ toxicity up to 18.4 gms mango leaf extract/kg bodyweight p.o. In another independent sub-chronic, repeated dose sub-chronic toxicity study using either 0, 100-, 300- or 900 mg mango leaf extract/kg body weight p.o. doses over a period of 90-consecutive days using exhibited no signs of toxic manifestations were observed, except a lower K⁺ level was detected in female rats in the high dose group (Zhang et al., 2014; Prado et al., 2015). In an independent investigation, Prado et al., demonstrated that acute and subchronic toxicities of mangiferin (p.o.) are low (Prado et al., 2015). A detailed anti-mutagenic investigation was conducted by Gold-Smith et al., (2016), which affirms the non-mutagenic potential of magniferin.

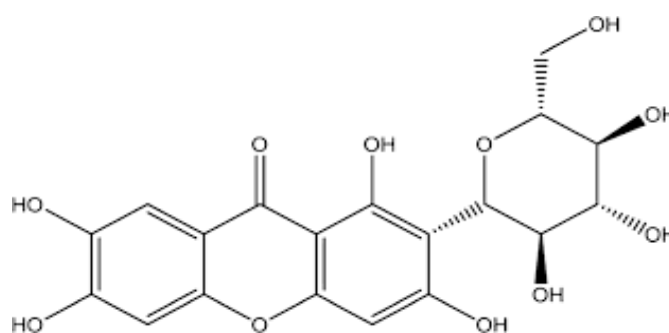


Figure 1: Chemical Structure of Mangiferin

Mangifera Indica L. Stem Bark

Mangifera indica L. stem bark extract (MSBE) has been developed as a bioactive ingredient for nutraceutical, cosmeceutical, and pharmaceutical formulations due to its antioxidant,

anti-inflammatory, and analgesic effects (Nuñez-Selles et al., 2007). The MSBE's major component is a xanthone (mangiferin, 2- β -D-glucopyranosyl-1,3,6,7-tetrahydroxyl-9H-xanthen-9-one, hereafter MF), which has been intensively studied as a promising candidate to be developed for neurodegenerative diseases treatment (Nuñez Selles, Daglia and Rastrelli, 2016), besides its use as antioxidant in food formulations against lifestyle disorders (Imran et al., 2017). Our first work about the chemical composition of the MSBE led to the isolation of seven phenolic components: gallic acid and its methyl and propyl esters, MF, (+)-catechin, (-)-epicatechin, benzoic acid and its propyl ester, and 3,4-dihydroxybenzoic acid; four sugars: glucose, galactose, arabinose, and fructose; and 3 polyols: sorbitol, myoinositol, and xylitol (Nuñez et al., 2002).

Health Benefits of *Mangifera Indica* Stem Bark

The bark of the *Mangifera indica* stem is something many people find useful, especially in this part of the world. While it is not eaten or consumed like fruit, it is often used in the preparation of herbs, and it has proven effective over the past centuries. Sometimes it is mixed with parts of other plants and sometimes it is used alone. According to HealthGuide.ng (as reviewed by Collins Nwokolo, 2021), the following importance are ascribed to mango stem bark:

1. Treating Boils

One thing you can use the *Mangifera indica* stem bark for is to treat and suppress boils. This can be done by making a paste out of the bark and applying it directly to the boils, particularly the ones that are yet to show fully. The mango bark contains vitamin C and ascorbic acid that would help to suppress the boils, healing it from the inside.

2. Diarrhoea Treatment

Mangifera indica bark is also very effective in treating diarrhoea. This can be done by boiling the bark and extracting the fresh juice. It can also be used to treat menorrhoea, leucorrhea, and bleeding piles. A 2015 study found out that the phytoconstituents of mango stem bark had powerful anti-malarial and antibacterial properties, which can be useful in treating diarrhoea.

3. Stopping Blood in Stool

If you are experiencing blood in your stool, then drinking juice extracted from mango stem bark would help relieve it. Just be sure to boil the mango bark in some milk and take it with honey two times a day. While this is optional, it helps to make the juice taste a lot better.

4. Cure for Sore Throat

Gargling *Mangifera indica* bark extract can also help you find relief from a sore throat. Gargle the juice for some seconds and spit out. Repeat this process two or three times twice a day. Just be careful not to swallow the juice after gargling.

5. Anticancer Properties

According to studies, the bark of the *Mangifera indica* stem contains anticancer such as isomangiferin gallate, mangiferin, mangiferin gallate, quercetin 3-O-galactoside, and quercetin-3-O-arabinopyranoside.

6. Anti-inflammatory Properties

It has also been observed that the *Mangifera indica* stem bark is rich in anti-inflammatory properties, such as flavonoids and polyphenols, making it very effective against inflammation, especially intestinal inflammation, such as ulcerative colitis and irritable bowel syndrome.

Antibacterial Assay

Preparation of test samples

The crude extracts of *Mangifera indica* was used in antibacterial assay, that is; the methanol crude extracts. The crude extracts were tested by disc diffusion method on nutrient agar medium as described by (Umaru et al., 2018). 3mg of the crude sample was dissolved homogenously in 3mL of methanol giving a stock solution of 1000µg/mL. Different volumes from the stock solution were taken, amounting to 100, 200, 300 and 400µg/mL 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°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 five sections by making a line, marking on the outside surface of the plate. The sections were for each test samples namely the 100, 200, 300, 400 µg/mL samples, tetracycline 30 µg (positive control) and methanol (negative control). The plate was sealed using parafilm and kept chilled at 4°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 Central 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. After which, 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 100, 200, 300, 400 µg/mL was each put 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 and 30 µg of tetracycline used 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. 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.

Anti-Fungal Assay

Preparation of Fungal Agar Plate

Potato dextrose agar medium (PDA) was prepared from commercially available dehydrated base according to the manufacturer instructions as described by Mayuri et al. [11]. About 39 g of PDA was mixed with 1 L of distilled water, autoclaved at 121°C for 15 min and was allowed to cool at 37°C to 40°C in the autoclave. The freshly prepared and cooled medium was taken to the laminar flow and 20 mL was poured into the plastic flat bottomed Petri dishes with uniform depth and cover. The agar medium was allowed to solidify in the laminar flow at room temperature for 30 min. Plates stored in a refrigerator were used within 7 days after preparation to avoid waste.

Preparation of Fungal Broth

Fungus used in this study (*Aspergillus niger*, *Aspergillus flavin*, *Candida tropicalis*, and *Fusarium oxysporum*) were obtained from the stock culture provided by Microbiology Laboratory, Modibbo Adama University Yola, Nigeria. The nutrient broth was prepared according to manufacturer's instruction. Fluconazole (Diflucan 30 µg/mL) was used as standard antifungal. All stock cultures were maintained on PDA slants and subculture. The slants were incubated at 25°C for 7 days and then stored at 4°C.

Plate Inoculation

Fungal isolates were maintained on Sabouraud's dextrose agar SDA at 4°C. A Loopful of the fungal spores was streaked on potato dextrose agar (Himedia) plates and incubated at 37°C for 2-3 days. All fungus plates were maintained at 4°C in refrigerator until further use.

Preparation of Antifungal Disc Diffusion Assay

Disc diffusion assay was used to assess the inhibitory effect of antifungal agents on the activation of fungi (*Candida tropicalis*, *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus flavin*) as reported by Fickers et al. [12]. The spore suspensions were prepared by flooding fungal culture plates with 3 mL of sterile distilled water and a sterile loop was used to agitate colonies. One hundred microliters of each spore suspension were used to

inoculate PDA plates which were left to dry at room temperature for 15 min. Inoculated PDA plates were sectioned and 30 μL of each of the extracts were pipetted onto an autoclaved Whatman filter paper disc 6 mm in diameter and placed in the middle of each section. Fluconazole (Diflucan 30 $\mu\text{g}/\text{mL}$) was used as a positive control for fungal growth inhibition and dimethylsulfoxide (DMSO) was used as a negative control. Plates were sealed with Parafilm and incubated in the laminar flow (incubator) 37°C for 5-6 days' observation for fungal growth and formation of inhibition zones around disks. Each test was repeated a minimum of 3 times for each extract. An extract was categorised as having antifungal activity when the diameter of the inhibition zone was 0.5 mm larger than the diameter of the paper disc. This value serves as a frame of reference against which the antifungal susceptibility of the fungi can be compared. The larger the concentric area of inhibited growth, the greater the efficacy of the antifungal agent. Antifungal efficacy was determined by measuring the mean perpendicular diameter of the inhibition zone [13].

METHODS

The data was analyzed using One-Way Anova and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the mean $\pm$ standard deviation. Significance level for the differences was set at $p < 0.05$.

RESULTS

Antibacterial Results

Table1: Effect of stem-bark of *Mangifera Indica* 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>	20.63 $\pm$ 0.01	16.13 $\pm$ 1.15
	<i>Staphylococcus aureus</i>	21.22 $\pm$ 0.12	17.21 $\pm$ 0.13
	<i>Klebsiella pneumonia</i>	21.12 $\pm$ 0.16	14.18 $\pm$ 0.16
200 $\mu\text{g}/\text{mL}$	<i>Escherichia coli</i>	21.11 $\pm$ 0.23	20.15 $\pm$ 0.19
	<i>Staphylococcus aureus</i>	21.17 $\pm$ 0.17	19.14 $\pm$ 0.14
	<i>Klebsiella pneumonia</i>	21.27 $\pm$ 0.19	20.32 $\pm$ 0.22

300µg/mL	<i>Escherichia coli</i>	21.36 ± 0.18	18.23 ± 0.13
	<i>Staphylococcus aureus</i>	21.33 ± 0.21	15.19 ± 0.16
	<i>Klebsiella pneumonia</i>	21.39 ± 0.11	20.13 ± 0.11
400µg/mL	<i>Escherichia coli</i>	21.22 ± 0.16	17.17 ± 0.18
	<i>Staphylococcus aureus</i>	21.19 ± 0.13	18.18 ± 0.12
	<i>Klebsiella pneumonia</i>	21.17 ± 0.27	20.55 ± 1.26

Result is Mean + SD. N = 3

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

Concentration of standard is 30 µg/mL of tetracycline, Conc = Concentration

Table 2. Antifungal activity of *Barringtonia asiatica* leaves extracts on *Aspergillus niger*, *Aspergillus flavin*, *Candida tropicalis* and *Fusarium oxysporium* in millimetre (mm)

Solvent	Organism	Fluconazole	Concentration (µg/mL)				
			100	200	300	400	500
	<i>Aspergillus niger</i>	25.67 ± 0.11	9.14 ± 0.04	11.17 ± 0.05	13.31 ± 0.11	13.58 ± 0.08	14.77 ± 0.77
Hexane	<i>Aspergillus flavin</i>	24.40 ± 0.05	8.65 ± 0.05	10.93 ± 0.05	12.12 ± 0.06	12.28 ± 0.08	13.42 ± 0.13
	<i>Candida tropicalis</i>	24.10 ± 0.08	7.67 ± 0.03	12.57 ± 0.05	13.23 ± 0.06*	13.02 ± 0.08	14.23 ± 0.14*
	<i>Fusarium oxysporium</i>	24.20 ± 0.10	8.31 ± 0.08	12.47 ± 0.05	11.61 ± 0.09	11.78 ± 0.04	13.93 ± 0.12
	<i>Aspergillus niger</i>	24.67 ± 0.11	9.11 ± 0.04	13.87 ± 0.05	13.31 ± 0.13	13.58 ± 0.08	14.77 ± 0.11*
Dichloromethane	<i>Aspergillus flavin</i>	23.40 ± 0.05	9.12 ± 0.05	11.93 ± 0.05	12.14 ± 0.07	12.38 ± 0.08	12.42 ± 0.23
	<i>Candida tropicalis</i>	24.10 ± 0.08	9.43 ± 0.05	12.57 ± 0.05	10.73 ± 0.03	12.02 ± 0.08	13.23 ± 0.11
	<i>Fusarium oxysporium</i>	24.20 ± 0.10	9.31 ± 0.05	10.17 ± 0.05	14.64 ± 0.07*	14.78 ± 0.04	16.12 ± 0.17*
	<i>Aspergillus niger</i>	24.67 ± 0.11	9.23 ± 0.05	12.68 ± 0.04	13.13 ± 0.06	13.50 ± 0.06	13.77 ± 0.14
	<i>Aspergillus flavin</i>	23.40 ± 0.05	9.51 ± 0.08	11.30 ± 0.06	12.45 ± 0.06	13.07 ± 0.08	13.18 ± 0.12
Chloroform	<i>Candida tropicalis</i>	22.10 ± 0.08	10.12 ± 0.05	11.35 ± 0.05	11.53 ± 0.07	12.00 ± 0.06	12.45 ± 0.14
	<i>Fusarium oxysporium</i>	24.20 ± 0.10	9.53 ± 0.08	12.17 ± 0.05	13.62 ± 0.05*	14.15 ± 0.12	14.37 ± 0.16*

	<i>Aspergillus niger</i>	24.67 ± 0.11	11.15±0.05	14.40 ± 0.09*	13.45 ± 0.07	13.48 ± 0.08	13.92 ± 0.22
Ethyl acetate	<i>Aspergillus flavin</i>	24.40 ± 0.05	10.35±0.05	12.38 ± 0.04	12.62 ± 0.11	13.13 ± 0.05	14.30 ± 0.12*
	<i>Candida tropicalis</i>	23.10 ± 0.08	9.13 ± 0.05	11.30 ± 0.06	11.78 ± 0.07	12.92 ± 0.08	13.98 ± 0.15
	<i>Fusarium oxysporium</i>	24.20 ± 0.10	10.17±0.05	13.20 ± 0.06*	12.57 ± 0.03	12.67 ± 0.05	13.83 ± 0.16
	<i>Aspergillus niger</i>	24.67 ± 0.11	11.23±0.05	12.27 ± 0.08	13.56 ± 0.07*	12.58 ± 0.08	13.63 ± 0.11
	<i>Aspergillus flavin</i>	24.40 ± 0.05	10.17±0.05	11.13 ± 0.08	13.47 ± 0.07	12.55 ± 0.05	13.63 ± 0.12
Methanol	<i>Candida tropicalis</i>	24.10 ± 0.08	11.17±0.05	12.23 ± 0.05	12.31 ± 0.08	13.42 ± 0.04	14.55 ± 0.16*
	<i>Fusarium oxysporium</i>	24.20 ± 0.10	10.48±0.04	12.17 ± 0.05	12.23 ± 0.09	14.37 ± 0.10	14.60 ± 0.17*

Result is Mean ± SD. N = 3

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

Concentration of standard is 30 µg/mL of Fluconazole

DISCUSSION

Antibacterial Test

Result of screening plant extracts for antibacterial activity as shown in Table 4.1 shows that most of the organisms were sensitive to the extract. In this study, different concentrations of *M. Indica* stem-bark methanolic extracts 100, 200, 300 and 400 µg/mL respectively were tested against three isolated organisms and were sensitive to inhibition at different concentrations.

The activity of *Mangifera indica* stem-bark methanolic extract against selected bacterial was significant when compared to the test control at all the concentration tested, at concentration 100 µg/mL, the extract did not show significant activity. The antibacterial activity is dose dependent; therefore, optimum activity is at higher dose (Hugo W.B.1998). Higher growth inhibition rate was observed at 200ug/mL, but for 300 and 400 ug/ml, the growth inhibition was more on gram negative bacteria *Klebsiella pneumonia* with 20.13 + 0.11 and 20.55+ 1.26 respectively. The antibacterial effect of this plant extract could be attributed to the presence of some bioactive compounds like mangiferin (Masibo M. and He Q. 2009) and some phytochemical compounds like the flavonoids and resins. However,

these secondary metabolites are widely known for their antibacterial activities (Qudsia et al, 2009).

In the nutshell, the extract has active inhibitory effect on both the gram positive and gram-negative bacteria; *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* respectively. However, at higher concentrations, the extract tends to have more inhibitory effect than when it is administered a lower concentration.

Antifungal Test

Related literature has shown that several authors have reported on the effectiveness of medicinal plants in different solvents against the pathogenic bacterial strains [14,15,16,17,18,19]. However, the effect of *Mangifera indica* stem bark extract against antifungal strain was reported in Table 2.

Table 2 shows the mean value of the inhibition zone of the antifungal activity of *Mangifera indica* stem bark extract. The stem-bark extract exhibited considerable activity against the pathogens *Aspergillus niger*, *Aspergillus flavin*, *Candida tropicalis* and *Fusarium oxysporium*. Significant inhibition of hexane extract was observed on *Aspergillus niger* at 500 µg/mL with an inhibition zone of 14.77 ± 0.11 mm. Dichloromethane crude stem-bark extract exhibited higher growth inhibition against *Fusarium oxysporium* with an inhibition zone of 16.12 ± 0.17 *mm. Lower growth inhibition was observed with hexane crude at 25 µg/mL on *Candida tropicalis* with a growth inhibition zone of 7.67 ± 0.03 mm when compared to the fluconazole used as a control with an inhibition zone of 24.10 ± 0.08 mm.

The result obtained from this study revealed that the leaf extracts of *Mangifera indica* stem bark extract contains potential antifungal agents, this shows the plant can be a valuable natural source for the treatment and discovery of novel phytochemicals that could be effective against antimicrobial infections and drug resistant microorganism.

CONCLUSION

The study demonstrated the antifungal effects of *Mangifera indica* stem bark and showed efficacy in all the polar and non-polar solvent extracts, from the result it was observed that the plant has the potential value in treating clinical diseases caused by fungi, as well as bacteria, this confirms the acclaimed information by traditional medical practitioner of its activity against diseases and ailment.

In this research, *Mangifera indica* stem bark extract have been studied to contain Mangiferin which is a major chemical for anti-fungal and anti-bacterial activities. The result of this study shows that the stem bark extract possessed higher anti-fungal and antibacterial agents compared to leaf and root extracts as carried out by other researchers. These can serve as possible source of raw material for pharmaceutical products. However, the extract is found not to be harmful to the liver and kidney. We suggest that this extract could be acknowledged by the pharmaceutical industry to cuttle menace of resistant pathogens.

Recommendation

More investigations as regards toxicological studies and purification of active components should be considered in novel drug development. The plant should be considered a traditional drug for health remedy. The extract is a promising source for therapeutic agent that can be used in combating infectious diseases caused by drug-resistant bacteria, antibacterial activities against selected bacterial strains, further studies should be carried out for the isolation and identification of individual bioactive compounds which are responsible for this therapeutic activity and the investigation of their mechanism(s) of action is also recommended.

Acknowledgement

The author thanks the support of laboratory staff of Federal University Wukari

Competing Interests

Authors have declared that no competing interests exist.

REFERENCES

- Shah, K.A., Patel, M.B., Shah, S.S., Chauhan, K.N., Parmar P.K. and Patel, N.M. (2010) Antihyperlipidemic activity of *Mangifera indica* L. leaf extract on rats fed with high cholesterol diet. *Der Pharmacia Sinica*. 1, 156–161.
- Y. Prado, N. Merino, J. Acosta, J.A. Herrera, Y. Luque, I. Hernández, E. Prado, G. Garrido, R. Delgado, I. Rodeiro. (2015). Acute and 28-day subchronic toxicity studies of mangiferin, a glucosyl xanthone isolated from *Mangifera indica* L. stem bark. *Journal of Pharmacy and Pharmacognosy Research*. 3: 13-23.
- Satyavati, G.V., Raina, M.K., Sharma, M. (1976). *Medicinal Plants of India*, Indian Council of Medical Resea. Sharmarch. 1: 201–206.

- Acevedo, L.M, Raya, A.I., Martinez-Moreno, J.M. (2017). Mangiferin protects against adverse skeletal muscle changes and enhances muscle oxidative capacity in obese rats. *PLoS One*; 12(3): e0173028.
- Lewis, W.H. and Elvin- Lewis, M.P.H. (1977). *Medicinal Botany Plants Affecting Man's Health*. John Wiley and Sons, New York.
- Jouad, H., Haloui, M. and Rhouiouani, H. (2001). Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez Boulemane). *Journal of Ethnopharmacology*. 77(2-3):175–182
- Ajila, C.M., Naidu, K.A., Bhat, S.G. and Prasada Rao, U.J.S. (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry*, 105, 982–988.
- Dorta, E., Lobo, M.G. and Gonzalez, M. (2012). Reutilization of mango by-products: Study of the effect of extraction solvent and temperature on their antioxidant properties. *Journal of Food Science*, 71, 80–88.
- Barreto, J.C., Trevisan, M.T.S., Hull, W.E., Erben, G., de Brito, E.S. et al. (2008). Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). *Journal of Agricultural and Food Chemistry*, 56, 5599–5610.
- Abdalla, A.E.M., Darwish, S.M., Ayad, E.H.E. and El-Hamahmy, R.M. (2007). Egyptian mango by-product: Compositional quality of mango seed kernel. *Food Chemistry*, 103, 1134–1140.
- Cheema, S. and Sommerhalter, M. (2015). Characterization of polyphenol oxidase activity in Ataulfo mango. *Food Chemistry*, 171, 382–387.
- Schieber, A., Ullrich, W. and Carle, R. (2000) Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. *Innovative Food Science and Emerging Technologies*, 1, 161–166.
- Grundhofer, P., Niemetz, R., Schilling, G. and Gross, G. (2001). Biosynthesis and subcellular distribution of hydrolyzable tannins. *Phytochemistry*, 57, 915–927.
- Palafox-Carlos, H., Yahia, E. Islas-Osuna, M.A., Gutierrez-Martinez, P., Robles- Sánchez, M. and González-Aguilar, G.A. (2012) Effect of ripeness stage of mango fruit (*Mangifera indica* L., cv. Ataulfo) on physiological parameters and antioxidant activity. *Scientia Horticulturae*, 135, 7–13.
- Kim, Y., Lounds-Singleton, A.J. and Talcott, S.T. (2009). Antioxidant phytochemical and quality changes associated with hot water immersion treatment of mangoes (*Mangifera indica* L.). *Food Chemistry*, 115, 989–993.
- Robles-Sánchez, R.M., Islas-Osuna, M.A., Astiazaran-Garcia, H., Vazquez-Ortiz, F.A., Martin-Belloso, O. et al. (2009a). Quality index consumer acceptability, bioactive compounds, and antioxidant activity of fresh-cut Ataulfo mangoes (*Mangifera indica* L.) as affected by low-temperature storage. *Journal of Food Science*, 74, S126–S134.
- Robles- Sánchez, R.M., Rojas-Grau, M.A., Odriozola-Serrano, I., Gonzalez-Aguilar, G.A. and Martin-Belloso, O. (2009b) Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut “Kent” mango (*Mangifera indica* L.). *Postharvest Biology and Technology*. 51, 384–390.

- Acosta J, Sevilla I, Salomon S, Nuevas L, Romero A, Amaro D. (2016). Determination of magniferin solubility in solvents used in the Biopharmaceutical University. *Journal of Pharmaceutical Pharmacognosy. Research.* 4(2): 49-53
- Okwu, D. E. and Ezenagu, V. (2005). Evaluation of the Phytochemical Composition of Mango (*Mangifera indica* Linn) Stem Bark and Leaves. *International Journal of Chemical Sciences.* 6(2):705–716
- Zhao, Y., Wang, W., Wu, X., Ma, X., Qu, R., Chen, X., Liu, C., Liu, Y., Wang, X., Yan, P., Zhang, H., Pan, J., Li, W. (2017). Mangiferin antagonizes TNF- α -mediated inflammatory reaction and protects against dermatitis in a mice model. *International Immunopharmacology.* 45: 174-179.
- Zhang, Y., Li, J., Wu, Z., Liu, E., Shi, P., Han, L., Guo, L., Gao, X., Wang, L. (2014). Acute and long-term toxicity of mango leaves extract in mice and rats. *Evidence-Based Complementary and Alternative Medicine.* 2014, Article ID 691574 <http://dx.doi.org/10.1155/2014/691574>.
- Gold-Smith, F., Fernandez, A., Bishop, K. (2016). Mangiferin and cancer: Mechanisms of action. *Nutrients.* Jun 28; 8 (7). pii: E396. doi: 10.3390/nu8070396
- Umaru, I. J., Badruddin, F. A., & Umaru, H. A. (2018). Gastroprotective Effect of *Leptadenia Hastata* (Pers.) Decne and Histopathological Changes on Indomethacin-Induced Gastric Ulcer in Rats. *International Journal of Pure and Applied Biosciences.* 6(5), 20-27.
- Hugo W.B. (1998). Bacteria. In: *Pharmaceutical microbiology.* Hugo, W. B. and Russell, A. D. (eds.). Oxford, Blackwell Scientific Publications
- Masibo M. and He Q. (2009). In vitro antimicrobial activity and the major polyphenol in leaf extract of *mangifera indica* L. *Malaysian Journal of Microbiology.* Vol 5(2) p.73-80
- Qudsia, K., Ishtiaq, H. and Arshad, J. (2009) Flavonoids from Mango Leaves with Antibacterial Activity. *Journal of the Serbian. Chemical Society.* 74 (12) 1389–1399.11. Mayura AK, Suparma MB, Pratima K. Role of antioxidants and nutrition in oxidative stress. *International Journal of Pure and Applied Chemistry.* 2015; 7:1-4.
- Ficker CE, Arnason JT, Vindas PS, Alvarez LP, Akpagana K, Gbeassor M, Smith ML. (2003). Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts. *Mycoses.*46(1-2):29-37.
- Umaru IJ, Badruddin FA, Umaru HA, Umaru KI. (2018b). Antifungal potential of some medicinal plants on selected pathogenic fungi. *MOJ Proteomics & Bioinformatics.*7(5):271–276.
- Boyan B, James H, Judicael P. (2005). Principles of accessing bacterial susceptibility to antibiotics using agar diffusion method. *Journal of Antimicrobial Chemotherapy.*61:1295- 1301.
- Prashanth VK, Chauhan NS, Padh H, Rajani M. (2006). Search for antibacterial and antifungal agents from selected Indian medicinal plants. *Journal of Ethnopharmacology.* 107(2):182-188.
- Runyoro DK, Matee MI, Ngassapa OD, Joseph CC, Mbwambo ZH. (2006). Screening of Tanzanian medicinal plants for anti-candida activity. *BMC Complementary and Alternative Medicine.*6(1):11.

- Apu AS, Muhit MA, Tareq SM, Pathan AH, Jamaluddin ATM, Ahmed M. (2010). Antimicrobial activity and brine shrimp lethality bioassay of the leaves extract of *Dillenia indica* Linn. *Journal of Young Pharmacists*. 2(1):50-53.
- Opawale BO, Onifade KA, Ogundare AO. (2011). Antimicrobial, free radical scavenging and cytotoxic activity of *Khaya grandifolia* C.CD extracts. *Malaysian Journal of Microbiology*.12(3):233-238.
- Ihsan-Ul-Haq A, Ahmed I, Hussain I, Jamil M, Mirza B. (2012). Antibacterial activity and brine shrimp toxicity of *Artemisia dubia* extract. *Pakistan Journal of Botany*. 44(4):1487-1490.