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Isolation and Characterisation of Essential oils of Mango (*M. indica*) Leave

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Abstract

This research work aimed at isolation and characterisation of essential oils of *Mangifera. indica* (*M. indica*) leaves. Phytochemical screening was carried out to determine the bioactive components present in the leaf sample as well as the antimicrobial activity of the crude ethanolic extract. The percentage yield of essential oils (colourless) of the leaf was 0.19%. The phytochemical screening revealed the presence of alkaloid, glycoside, flavonoid, saponin and tannin. Steroids was absent in the extract. The antimicrobial evaluation of the essential oil of *M. indica* leaf revealed a significant activity (zone of inhibition). The percentage (%) of Diphenyl picryl hydrazine (DPPH) scavenged of antioxidant activities for the essential oils of *M. indica* at different concentration of 5µl, 10µl, 25µl and 100 µl showed a significant antioxidant activity. In concluded results, it's clearly observed that, when the concentration of both ethanolic extract and essential oils leaf sample increases, the antimicrobial and antioxidant properties increases when compared to the standard (Amoxicillin) and that of essential oils has a significant property than other extracts. Considering the results of phytochemical screening, it's also a potential source of cost-effective food supplements, nutritive ingredients and antibacterial agent for improving human health and curing acute and chronic diseases. The GC-MS analysis of the essential oils for the leaf sample revealed fifty two (52) different chemical compounds which include Alloaromadendrene, Humulene, Bicyclo[4,4,0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene, Bicyclo[7.2.0]undec-4-ene,4,11,11,-trimethyl 8-methylene-,[1R-(1R*,4Z,9S*)].

Keywords: Alkaloids; antioxidant; characterisation; essential oils; phytochemicals.

INTRODUCTION

Mango (*Mangifera indica*) leaf ascribed to the family Anacardiaceae has been adjudged as the vital traditionally significant and one of the most economically important tropical fruit crop globally (Barreto, et al; 2008). The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct. People are now choosing to visit traditional medicine practitioners because they are disillusioned and dissatisfied with conventional medicine. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. Mango leaf is a good source of supplementary protein, vitamins, and minerals. These can be utilized as an alternative source of livestock feeding in developing countries for alleviating food shortage for livestock. Proximate composition of MLs showed 13.6% of Crude Protein (CP) in dauphine Mauritian variety, 20.38% CP in Nigerian variety, and 6.90% CP in Laos variety (Laulloo, et al., 2018).

Its well-known mango-producing countries include China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria, and Philippines (Kumar et al., 2021). Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). Besides fruit, mango leaves can also be used as a food supplement and a good fodder as it has a good amount of minerals, vitamins, and protein (Kumar et al., 2021; Jhaumeer et al., 2018). Some compounds from mango can be used in the pharmaceutical industry, such as steroids, gallic acid that has antifungal and antiviral activity, flavonoids that have antioxidant activity, and tannin as a diarrhea remedy (Ali et al. 2020). Mangiferin is a natural miracle biologically active compound against lifestyle-related disorders. Some studies have shown that mangiferin treats COVID-19 (Umar et al. 2021). Mangiferin has a binding affinity for the Mpro of COVID-19. Mpro is a key enzyme that plays a vital role in viral replication and transcription. Therefore, mangiferin can inhibit viral replication and transcription (Umar et al. 2021).

Mangifera indica has attracted to be studied to determine new biomolecules from different parts of plants like fruits, leaves, stems, and seed kernels. Its therapeutic importance is well established and has been used as a traditional remedy for treating several diseases (Jhaumeer et al. 2018). The aim of this research was to determine the essential oils, antimicrobial and antioxidant properties of *M. indica* leave.

MATERIALS AND METHODS

Sample Collection and Preparation

The *M. indica* leaves sample was collected in Sangere Ward, Girei Local Government Area of Adamawa State, Nigeria located at coordinates 9^{0}22'0" N and 12^{0}33'0"E in DMS (Degrees Minutes Seconds).

The fresh leaves samples was dried at room temperature and blended into fine powder. Eighty grams (80g) of the powdered plant leaves was dispense in 800ml of ethanol and then keep for two weeks with shaking at regular intervals after which the content was filtered using whatman filter paper and the filtrates was concentrated at 30 ^{0}C (Fatope *et al.,* 1993).

Extraction of Essential Oils

One kg of the pulverized sample was subjected to steam distillation in a steam distiller, according to the British Pharmacopoeia (BP) method. The time taken for the extraction of the oil was $2^{1}/_{2}$ hours.

Phytochemical Screening of the Crude Extract

The freshly prepared extract was subjected to standard phytochemical analysis according to the method adopted by (Jigna *et al.*, 2006).

Antimicrobial Activity for Essential Oils

The Antimicrobial activity of the essential oils from the leave of *M. indica* was tested against seven bacteria Multidrug Resistant Acinetobacter (MDRA), *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Methicillin Resistant Streptococcus Aureus* (MRSA), *Proteus vulgaris and Staphylococcus epidermis*)

Antimicrobial potentials of leaf sample against actively growing broth cultures of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Methicillin Resistant Streptococcus Aureus* (MRSA) as well as *proteus vulgari* bacteria were tested by well diffusion of the method following Ghosh *et al.*, (2020) and Majumder *et al.*, (2022b). A nutrient agar (NA) medium was used for this test. Following the pour plate method, 0.0125 µL of bacterial broth culture was first poured into each sterile Petri dish and then sterilized. The plates were left for a few minutes at room temperature for the media to be solidified. Following solidification, a sterile steel cork borer was used to cut out circular wells (8 mm in diameter). Next, 0.25 µL of each leaf extract sample was poured into the well. This process was done under the sterile condition in a laminar airflow cabinet. The plates were then incubated at 37°C for 24 hours in a non-inverted position.

Determination of Anti-oxidant activity

The free radical scavenging activity of different concentrations of crude extracts of leaves and standard ascorbic acid was evaluated by using DPPH radical scavenging method as per reported method (Alhakmani *et al*, 2013). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and superoxide dismutase (SOD)-like activity had shown that *M. indica* Leaves serve as a moderate antioxidant with an IC_{50} value of ~9 and 117 µg/mL (Itoh *et al*, 2020)

RESULTS AND DISCUSSION

RESULTS

Percentage Yield of Essential Oils of *M. indica* leaves

Table 1 shows the percentage yield of Essential Oils of *M. indica* leaves.

The percentage yield was calculated using the equation, $RO_U = (M/Bm) \times 100$ where M is the mass of the extracted oil in grams and Bm is the initial plant Biomass in grams. Or Percentage (%) yield of oil = Weight of Oils/ Weight of Samples x 100

Table 1. Percentage Yield of Essential Oils of *M. indica* leaves

Plant	Weight of Essential oils, in (g)	Colour of Oils	Weight of Plants, in (g)	% Yield of the Oils
M. indica	1.25	Colourless	654.93	0.19%

Phytochemical Screening of *M. indica* Leave Extract

The results of the phytochemical screening of the crude leaf extract of *M. indica* is shown in Table 2.

Table 2. Phytochemical Screening of Crude Leave Extract of *M. indica*

Phytochemical	Tests	Result
Alkaloid	Dragendoff's Test	+
Glycoside	Fehling's tests	+
flavonoid	Conc. H_2SO_4 test	+
Saponin	Foam tests	+
Tannin	$FeCl_3$ tests	+
Steroids	Libermann-buchard test	-

Keys: (+) = Present, (-) = Absent

Antimicrobial Activity of the Crude Leave Extracts of *M. indica* Leave

The result of antibacterial activities of the *M. indica* crude extract is shown in Figure 1.

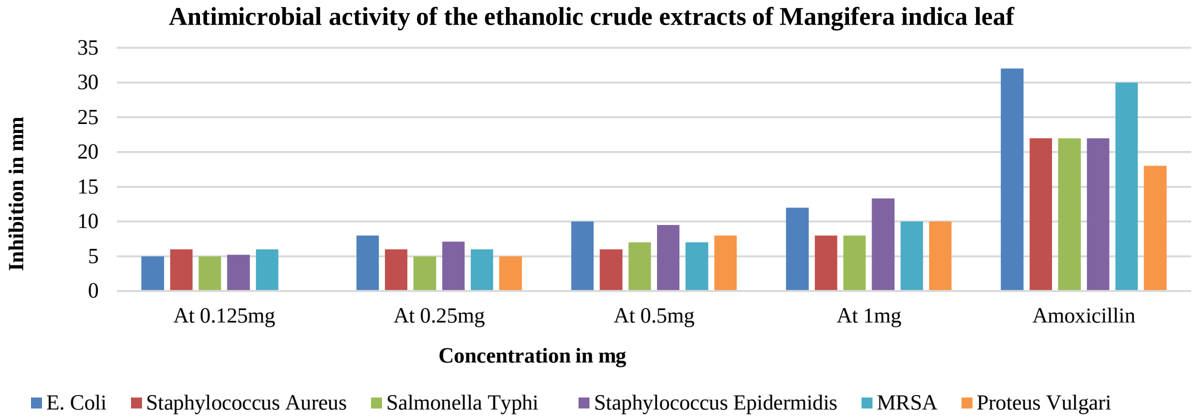

Figure 1. Antimicrobial activity of the ethanolic crude extracts of *M. indica* leave.

Antimicrobial activity of the Essential Oils of *M. indica* Leave

The result of antimicrobial activities of the essential oils of *M. indica* leave is shown in Figure 2.

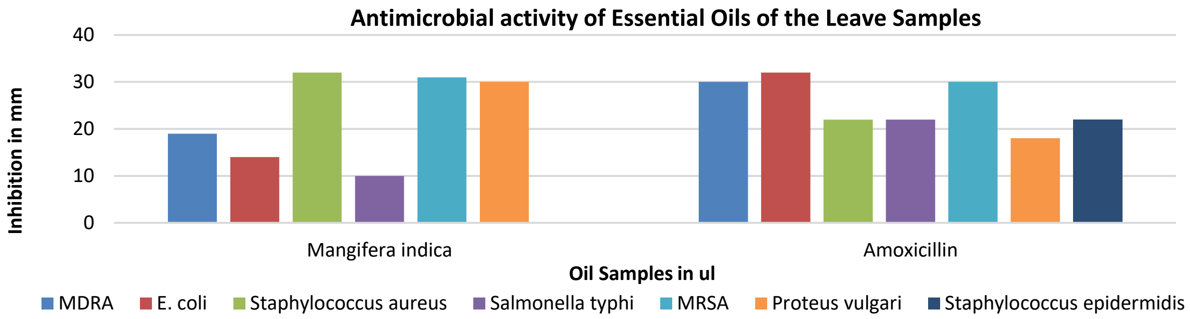

Figure 2. Antimicrobial activity of Essential Oils of *M. indica* Leave (as measured by level of microbial inhibition in mm).

Antioxidant activity (DPPH)

Antioxidant activity of *M. indica* ethanolic leaf extract is shown in Figure 3. Results were expressed as the percentage of DPPH inhibition (%) that occurred due to the exposure of samples. The radical scavenging activity and antioxidant potential of the plant extracts were determined by the ability of plant extracts to scavenge the stable free radical DPPH and convert into Diphenyl picryl hydrazine.

$$Free\ radical\ scavenging\ activity\ (\%)\ =\ \frac{Absorbance\ of\ control - Absorbance\ of\ sample\ \times\ 100}{Absorbance\ of\ control}$$

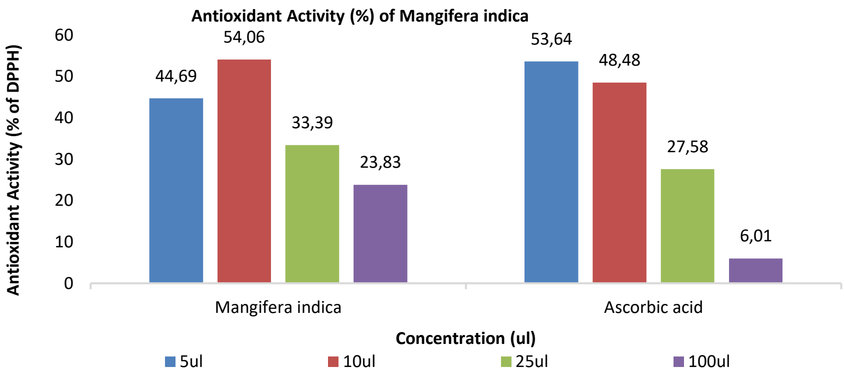

Figure 3. Antioxidant activity (%) of *M. indica* ethanolic leaf extracts.

Chemical Composition of Essential oil of *M. indica* Leave

Table 3. GC-MS Analysis of the Essential oil of *M. indica* Leave.

S/n	Constituents	Retention Time (min)	Area %
1	Elemene isomer	5.325	1.03
2	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	5.821	5.96
3	1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	5.858	6.97
4	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	6.021	1.52
5	Isocaryophillene	6.154	8.00
6	Humulene	6.265	12.46
7	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	6.384	8.98
8	Alloaromadendrene	6.450	13.65
9	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	6.658	10.80
10	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	6.843	1.05
11	.alpha.-Guaiene	6.954	2.26
12	Valerena-4,7(11)-diene	7.013	2.62
13	Aromandendrene	7.169	4.91
14	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]-	7.465	5.66
15	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	7.761	2.25
16	Isocaryophillene	7.976	2.12
17	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	8.376	0.44
18	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	8.398	0.60
19	3-Chloropropane-1,2-diol, bis(tert-butyldimethylsilyl) ether	8.509	1.06
20	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	8.732	0.11
21	Valerena-4,7(11)-diene	8.828	0.60
22	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	8.939	0.60
23	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	9.087	0.46
24	N-Benzyl-N-ethyl-p-isopropylbenzamide	9.191	0.33
25	Spiro[4.5]decan-1-one	9.509	0.10
26	Cyclohexadecane	9.583	0.19
27	2-Heptadecanol	9.813	0.39
28	13-Tetradece-11-yn-1-ol	9.887	0.14
29	Octadecane, 1-(ethenyloxy)-	10.006	0.20
30	Cyclohexaneethanol, 4-methyl-.beta.-methylene-, trans-	10.131	0.11
31	2-Pentadecanol	10.391	0.02
32	Tetradecane, 1-chloro-	10.472	0.11
33	1-Formyl-2,2,6-trimethyl-3-(3-methyl-but-2-enyl)-6-cyclohexene	10.717	0.01
34	1-Octadecene	10.880	0.08
35	1-Pentadecene	11.279	0.17
36	Phthalic acid, 2-cyclohexylethyl propyl ester	11.479	0.20
37	1-Nonadecene	11.657	0.08
38	2-Dodecen-1-yl(-)succinic anhydride	11.761	0.03
39	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	11.916	0.00
40	2-Piperidinone, N-[4-bromo-n-butyl]-	12.035	0.08
41	2-Dodecen-1-yl(-)succinic anhydride	12.146	0.00
42	Oleic Acid	12.183	0.01
43	9-Tricosene, (Z)-	12.242	0.02
44	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	12.287	0.01
45	Cyclotetradecane	12.398	0.04
46	cis-9-Hexadecenal	12.442	0.04
47	22-Tricosenoic acid	12.502	0.04
48	9-Octadecenamide, (Z)-	12.716	3.38
49	2-Methyl-Z,Z-3,13-octadecadienol	13.524	0.03
50	trans-13-Docosenamide	13.553	0.05
51	Erucic acid	13.650	0.01
52	E-11-Hexadecenal	13.687	0.01

DISCUSSION

Table 1 shows that, *M. indica* essential oil is colourless with the percentage yield of 0.19%. The percentage yield of essential oils varies with the factors like site of collection, time of collection, part and form of plant used as well as the method employed among others. Phytochemical screening revealed the presence of glycoside, flavonoids, saponins, tannins, steroids and alkaloids in the leaf crude extract of *M. indica* but steroid was completely absent. The phytochemical content in the mango leaf extracts is responsible for the antibacterial, anti-inflammatory and antioxidant activity (Jhaumeer *et al.,* 2018). Previous studies on Mango leaf extracts have been reported by many workers to show the presence of active functional groups such as xanthones (mangiferin), flavonols (quercertin), benzophenones and terpenoids (Laulloo, *et al.*,2018). Kumar et al. (2021) stated the major phytochemicals responsible for the antimicrobial activity were phenolics, alkaloids, glycosides, tannins, terpenes, and saponins

It was showed that, at the Concentration of 0.125 µL, *E. coli* has no effect. At 0.25 µL, 0.5 µL, 1 µL was 8mm, 10mm, and 12mm respectively, from *Staphylococcus aureus*, coming to *Salmonella typhi, Staphylococcus epidermidis, Methicillin Resistant Streptococcus Aureus* (MRSA) as well as *proteus vulgari*, as the concentration increases the antimicrobial activities also increases including the Standard (STD) Amoxicillin (AM). Several studies have reported that the different parts of mango plants, such as leaves, bark, stems and seeds, exhibited antimicrobial activity against some multi drug resistant (MDR) microbes (Kumar *et al*., 2021). From Figure 1, it can be seen that, the ethanolic crude extracts of *M. indica* leaf sample showed a slight increase in antimicrobial property against all the tested organisms. It's clearly observed that, when the concentration of the leaf sample increases the antimicrobial activity increases when compared to the standard antibiotics.

In the present study from figure 3 Where the extract showed the significant maximum zone of inhibition against *Staphylococcus aureus*(32mm) followed by Methicillin Resistant Streptococcus Aureus (MRSA) (31mm), *Proteus vulgaris* (30mm), MDRA (19mm), *Escherichia coli* (14mm), *Salmonella typhi* (10mm), and lastly no activity on *Staphylococcus epidermis* (none). The essential oils remarkably inhibited the growth of all tested microorganism, but it was cleared from the present results obtained, *M. indica* leaf extract exhibited pronounced activity against all the tested bacteria excluded *Salmonella typhi with* the weaker value of 10mm as showed in Figure 2. Therefore, it's revealed that, the essential oils of *M. indica* leaf has a significant antimicrobial property.

From Figure 3, Antioxidant activity (DPPH) of *M. indica* leaves, showed that, an increased in concentration of the extract possess increased in scavenging activity. Mango leaves have been reported to have antioxidant activity mostly from phenolic compounds (Ali et al., 2020). In some study the free radical scavenging capacity of mango leaf extract was determined by the DPPH assay performed on petroleum benzene, acetone, and methanol leaf extract of all samples, following the protocol of Majumder et al., (2022a) and Ghosh et al., (2020). Mango leaf were reported to have antioxidant capacity due to the presence of phenolics and flavonoids in different studies (Kumar et al., 2020). From figure 3, it's clearly observed that *M. indica* leaf possess high antioxidant properties compared with the control group (i.e ascorbic acid).

The GC-MS analysis of the essential oil obtained from the leaves of Mango leave (*M. indica*) revealed the presence of fifty two (52) compounds, as shown in Table 3 of the gas chromatography and mass spectra. The major compounds were Alloaromadendrene (13.65%), Humulene (12.46%), Bicyclo[4,4,0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene- (10.80%), Bicyclo[7.2.0]undec-4-ene,4,11,11,-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]- (8.98%), Isocaryophillene (8.00%), 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]- (6.97%), 1H-Cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1aR (1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]- (5.96%), Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]- (5.66%), Aromandendrene (4.91%), 9-Octadecenamide, (Z)- (3.38%), Valerena-4,7(11)-diene (2.62%), .alpha.-Guaiene (2.26%), (1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene (2.25%), Isocaryophillene (2.12%), 2H-2,4a Methanonaphthalene,1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-,(2S)-(1.52%), 3 Chloropropane-1,2-diol, bis(tert-butyldimethylsilyl) ether (1.06%), Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]- (1.05%), Elemene isomer (1.03%). Total of 18 compounds contained 94.68%, the remaining bulk of thirty four (34) compounds covers only 5.32%. Another result of the constituents of the essential oil of Mango leaf obtained by other researcher were identified using GC–MS spectrometry and showed the presence of several biologically active compounds, such as humulene-4-hydroxy-4-methyl-2-pentanone, elements, and trans-caryophillene, which was common in all five cultivars of mango (Ouf, et, al; 2020).

CONCLUSIONS

In conclusion, it's clearly observed that, when the concentration of both ethanolic and essential oils leaf sample increases, the antimicrobial and antioxidant properties increases when compared to the standard

(Amoxicillin) and that of essential oils has a significant property than other extracts. Mango Leaves exhibit exceptional biological, medicinal, and metabolic properties. Considering phytochemical screening it's also a potential source of cost-effective food supplements, nutritive ingredients and antibacterial agent for improving human health and curing acute and chronic diseases. There are numerous vital chemical compounds present in mango leaves, which are instrumental to performing various metabolic, bacteriostatic, and antimicrobial activities. Some of these include Alloaromadendrene, Humulene, Bicyclo[4,4,0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene,Bicyclo[7.2.0]undec-4-ene,4,11,11,-trimethyl 8-methylene-,[1R-(1R*,4Z,9S*)]- Isocaryophillene, 1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, and so on.

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***Author's Contribution*:** Auwal Abubakar wrote the manuscript and carried out laboratory work, Mohammed Hassan Shagal analyzed the data and Charles Milam designed the study. All authors read and approved the final version of the manuscript.

***Competing Interest*:** The authors declare that there are no competing interests.

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