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Phytoconstituents and Antibacterial efficacy of Mango *(Mangiferaindica)* leave extracts

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Abstract
The palm wine and ethanol extracts of Mangifera indica leaves revealed the bioactive compound from the phytoconstituents analysis to include alkaloids, carbohydrate, anthranol glycosides, triterpenes, phenol, flavonoids and amino acid as shown and saponin was only found in the palm wine extract. Screening the two extracts for antibacterial activity against *Shigella flexneri*, *Pseudomonas fluorescens*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus spp* showed that palm wine extract was more effective than ethanol extract. Palm wine extract showed the highest antibacterial activity on all the five test organisms with zones of inhibition ranging from 18 to 24 mm at a concentration of 25 and 12.5 $mgml^{-1}$ and showed no sensitivity at concentration 6.25, 3.125 and 1.5625 $mgml^{-1}$. Ethanol extract showed no inhibitory effect on *Staphylococcus aureus* and weak inhibitory effects against the other four organisms. Gentamycin which was used as a positive control showed a strong inhibitory effect against all the tested organisms compared to all the leave extracts. The result showed that the MIC of palm wine and ethanol extract was 12.5 $mgml^{-1}$ for all the organisms tested. The palm wine extract was found to be bactericidal to all the test bacteria, while the ethanol extract was found to be bacteriostatic to the test bacteria with the exception to *Staphylococcus aureus* which showed no inhibition. The concentration of the extract is directly proportional to the inhibitory activity exerted on the bacterial isolates. *Shigella flexneri* was the most susceptible organism to the palm wine leave extract, while *Pseudomonas aeruginosa* was the least susceptible organism. *Shigella flexneri* was the most susceptible to the ethanol leave extract and *Escherichia coli* was the least susceptible organism with the exception of *Staphylococcus aureus* with no susceptibility but resistance.

Keywords: Phytoconstituents, Palm wine, Ethanol, Bacteria, *Mangifera indica*.

1. Introduction
Medicinal plants occupy an important position of being the paramount sources of the discovery of pharmacology in recent year [1]. Medicinal plants contain biologically active components which over the years have been exploited in the traditional medical practices for the treatment of various ailments [2]. For a long period of time, plants have been a valuable source of natural products for maintaining well-being in the last decade, with more increasing studies for natural therapies.

Medicinal plants might be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, containing compounds isolated from medicinal plants [3]. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [4].

Many plants have been used because of their antimicrobial properties, which are due to compounds produced in the secondary metabolism of the plant. These compounds are identified by their active substances, for instance, the phenolic compounds which are part of the essential oils [5], also as in tannin [6]. According to the WHO, medicinal plants would be the greatest source to obtain a wide range of drugs with antimicrobial properties [7]. Ethnobotanical surveys carried out in Nigeria and elsewhere in the world have reported a number of plants that are used in the treatment of infectious diseases, including *Mangifera indica* [8-9].

Mangifera indica is a large evergreen tree, with a heavy, dome-shaped crown. It belongs to the family Anacardiaceae. It is found in the tropics where it is used as a horticultural and medicinal plant [10]. Fruits contain protein, fat, carbohydrate, minerals, vitamins A, B and C and amino acids. The fruits also yield a resin that is said to contain mangiferin, mangiferic acid, resinol and maniferol and others [11-12]. The leaves contain the glucoside mangiferin.

The bark of the mango tree contains 16–20% tannin [11-12]. The leaves have been reported to contain glycosides, sterols, polyphenols, tannins, euxanthin acid, saponins, mangiferin, mangin, and so on. The ashes obtained from the leaves are used to treat burns, scalds, sores, cough and diarrhoea in South America and other parts of the world [11, 13]. The leave, bark and root are used to treat Oral candidiasis, Malaria, Skin infection, dysentery, diarrhoea, thrush and shingles reported in a number of ethnobotanical survey [8,14]. This study is aimed at determining the phytoconstituents and antibacterial efficacy of palm wine and ethanol leave extracts of *Mangifera indica*.

2. Materials and Methods

2.1 Media, Chemicals and Solvents used

Media used include: Muller-Hinton Agar (MHA; Zayo-Sigma Chemical Ltd, Germany) and Nutrient broth (NB; Zayo-Sigma Chemical Ltd, Germany). The media were prepared in accordance with manufacturer's instructions. The media used was sterilized at 121 ^{0}C for 15minutes using autoclave and the glasswares were dried at 60 ^{0}C using hot air- oven.

Chemicals and Solvent used include: Palm wine (Angwan Lambu, Keffi, Nigeria), Ethanol, Hager's reagent (saturated picric acid solution), Molisch's reagent (alcoholic alpha naphthol solution), HCl, Ferric chloride solution, benzene, ammonia, sodium nitroprusside, pyridine, sodium hydroxide, distilled water, chloroform, Conc. Sulphuric acid, lead acetate and nitric acid.

2.2 Collection of plant materials

Fresh leaves of *Mangifera indica* L. were collected from Nasarawa State University, Keffi, Nigeria in the month of June, 2014. The Keffi Local Government Area of Nasarawa State is located between latitude 8^{0}5^{l}N of the equator and longitude 7^{0}5^{l}E of the Greenwich meridian at the altitude of 850 m above sea level [15], and approximately 58km away from F.C.T, Abuja and 128 km away from Lafia, the State capital.

2.3 Preparation of Plant Materials

The freshly collected leaves were spread to dry under shade at normal room temperature for seven days in the shade. Upon drying, the plant materials were pounded using mortar and pestle into smaller and the powdered extract is stored in airtight containers and kept under normal room temperature (28 ± 2 ^{0}C) until required.

2.4 Extraction Procedure

25 g of powdered sample was soaked in 250 ml solvent (Palm wine and Ethanol) contained in a 250 ml sterile conical flask. The flask was covered with cotton plug and then wrapped with aluminium foil and shaken vigorously for 24 hrs at room temperature. The soaked sample was then filtered using wool and then Whatman no.1 filter paper, the filtrate was evaporated to dryness using water bath maintained at 40 ^{0}C and the dried substance was stored in airtight bottles until required.

2.5 Preparation of Test Organisms

The test organisms used were pure culture of *Shigella flexneri*, *Pseudomonas fluorescens*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus spp*. These organisms were obtained from the Laboratory Unit of Microbiology, Biological Science Department, Nasarawa State University Keffi. The said test organisms were inoculated onto nutrient broth for a period of 24 hours and at growth temperature of 37 °C in order to ascertain their viability.

2.6 Phytochemical Screening

The phytochemical screening was carried out using the method previously described [16] with modifications. The phytoconstituents assayed were Alkaloids, Anthranol glycosides, Cardiac glycosides, Saponins, Triterpenes, Phenol and Flavonoids.

2.7 Susceptibility Test of organisms

The inhibitory test was determined by the method of Owuna *et al*. [17] with some modifications. Briefly, Plates of Mueller-Hinton agar media were prepared. The plates were inoculated with the test organisms in duplicates by spread plate method. A cork borer of width 6 mm in diameter was flamed using a Bunsen burner and was used to bore wells on the inoculated plates. The wells were then filled with the extracts at different concentrations, that is 25, 12.5, 6.25, 3.125 and 1.5625. mg/ml. Standard drug Gentamycin was used as control at concentration of 4 mg/ml. The extracts were allowed for 30 minutes to diffuse into the agar plates and then incubated at 37 °C for 24 hours. The potency of the extracts was determined by the clear zones of inhibition around the wells and these respectively measures at nearest millimetre as diameter zones of inhibition.

2.8 Minimum Inhibitory Concentration (MIC)

The MIC was determined by the method of Owuna *et al*. [17] with some modifications. Briefly, a weight of 0.125g (125 mg) of extract was dissolved in 4 ml nutrient broth. Subsequently, two fold serial dilutions were made from the original stock of 4ml containing 250 mg to obtain the following concentrations, 6.25, 3.125, 1.563, and 0.7815 mg/ml^{-1}. Standardized inoculums of each test organism was introduced into the mixture and incubated at 37 °C for 24 hours. The lowest concentration of the extract that inhibited the test organisms was considered as the minimum inhibitory concentration (MIC).

2.9 Minimum Bactericidal/Bacteriostatic Concentration

The minimum bactericidal/Bacteriostatic concentration (MBC) was determined by the method of Owuna *et al*. [17] with some modifications. Briefly, all minimum inhibitory concentration (MIC) tubes that showed no microbial growth after 24 hours of incubation were sub cultured by streaking onto the surface of freshly prepared Mueller-Hilton agar plates and incubated at 37 °C for 24 hours. The MBC was taken as the least concentration of the extract that did not allow any visible bacterial growth on the nutrient agar plate.

3. Results

3.1 Phytoconstituents of *Mangifera indica* leaves

All plant extracts were subjected to phytochemical screening methods that revealed the presence of different classes of compounds including as alkaloids, anthranol glycosides, saponins, triterpenes, phenol and flavonoids as shown in Table 1.

Table 1: Phytoconstituents of different *Mangifera indica* leaves extracts

Phytoconstituents	Palm wine extracts	Ethanol extract
Alkaloids	++	+
Anthranol glycoside	+	++
Cardiac glycosides	-	-
Saponins	+	-
Triterpenes	++	+
Phenol	++	+
Flavonoids	+	++

-= Absent; += Present; ++: Strongly present

3.2 Inhibitory activity of *Mangifera indica* leaves extracts

The results of the antibacterial assay of the palm wine and ethanol leave extracts of *Mangifera indica* indicated that the plant exhibited antibacterial activity against the tested microorganisms at concentrations of 25 and 12.5 $mgml^{-1}$ and did not produce any effect at concentrations of 6.25, 3.125 and 1.5625 $mgml^{-1}$. The potential sensitivity of the extract was obtained against all the microorganisms tested and the zone of inhibition was recorded and presented in the table shown below (Table 2 and 3).

Table 2: Inhibitory activities of Palm wine leaves extract of *Mangifera indica* against some bacterial isolates

Zones of inhibition (mm)/Concentration ($mgml^{-1}$)							
S/No	**Organisms**	**25**	**12.5**	**6.25**	**3.125**	**1.5625**	**G(4mg/ml)**
1	*Shigella flexneri*	24	22	-	-	-	29
2	*P. fluorescens*	21	18	-	-	-	28
3	*E. coli*	24	20	-	-	-	25
4	*S. aureus*	23	19	-	-	-	20
5	*Bacillus spp*	21	19	-	-	-	22

-: No inhibition; G: Gentamycin

Table 3: Inhibitory activities of Ethanol leaves extract of *Mangifera indica* against some bacterial isolates

Zones of inhibition (mm)/Concentration ($mgml^{-1}$)							
S/No	**Organisms**	**25**	**12.5**	**6.25**	**3.125**	**1.5625**	**G(4mg/ml)**
1	*Shigella flexneri*	18	13	-	-	-	29
2	*P. fluorescens*	19	11	-	-	-	28
3	*E. coli*	9	8	-	-	-	25
4	*S. aureus*	-	-	-	-	-	20
5	*Bacillus spp*	14	9	-	-	-	22

-: No inhibition; G: Gentamycin

Table 4: Minimum inhibitory concentration (MIC) of Palm wine leaves extract of *Mangifera indica* against some bacterial isolates

Concentration ($mgml^{-1}$)							
S/No	**Organisms**	**12.5**	**6.25**	**3.125**	**1.5625**	**0.7813**	**G 4mg/ml)**
1	*Shigella flexneri*	-	+	+	+	+	124
2	*P. fluorescens*	-	+	+	+	+	124
3	*E. coli*	-	+	+	+	+	124
4	*S. aureus*	-	+	+	+	+	124
5	*Bacillus spp*	-	+	+	+	+	124

-: No growth; +: Growth

Table 5: Minimum inhibitory concentration (MIC) of ethanol leaves extract of *Mangifera indica* against some bacterial isolates

Concentration ($mgml^{-1}$)							
S/No	**Organisms**	**12.5**	**6.25**	**3.125**	**1.5625**	**0.7813**	**G (4mg/ml)**
1	*Shigella flexneri*	-	+	+	+	+	124
2	*P. fluorescens*	-	+	+	+	+	124
3	*E. coli*	-	+	+	+	+	124
4	*S. aureus*	-	+	+	+	+	124
5	*Bacillus spp*	-	+	+	+	+	124

3.3 Minimum inhibitory concentration (MIC) of *Mangifera indica* leaves

The results of the Minimum inhibitory concentration (MIC) of the palm wineand ethanol leave extract of *Mangifera indica* indicated that these microbes except *S. aureus* in ethanol extract showed no growth activity at the concentration 12.5 $mgml^{-1}$ of the plant extract and showed growth activity at concentrations of 6.25, 3.125, 1.5625 and 0.7813 $mgml^{-1}$. The potential sensitivity of the extract was obtained against all the microorganisms tested and the MIC was recorded and presented in the table shown below (Table 4 and 5).

3.4 Minimum Bactericidal Concentration and/or Bacteriostatic Concentration (MBC) of *Mangifera indica* leave extracts

The results of the Minimum Bactericidal and/or Bacteriostatic Concentration (MBC) of the palm wine and ethanol leave extract of *Mangifera indica* obtained against all the microorganisms tested is presented in the table shown below (Table 6).

Table 6: Minimum Bactericidal and/or Bacteriostatic Concentration (MBC) concentration of Palm wine and ethanol leave extract of *Mangifera indica* against some bacterial isolates

Concentration ($mgml^{-1}$)			
S/No	**Organisms**	**Palm wine extract**	**Ethanol extract**
1	*Shigella flexneri*	Bactericidal	Bacteriostatic
2	*P. fluorescens*	Bactericidal	Bacteriostatic
3	*E. coli*	Bactericidal	Bacteriostatic
4	*S. aureus*	Bactericidal	Bacteriostatic
5	*Bacillus spp*	Bactericidal	Bacteriostatic

Bactericidal: when the extract concentration completely inhibited bacterial growth after 48hrs
Bacteriostatic: when the extract concentration partially inhibited bacterial growth after 48hrs

4. Discussion

In the present study, the two extracts of *M. indica* revealed the bioactive compound from the phytoconstituents analysis to include alkaloids, anthranol glycosides, triterpenes, phenol and flavonoids as shown and saponin was only found in the palm wine extract. Triterpenes and alkaloids which are strongly positive in palm wine extract is reported somewhere to possess antibacterial activity [18]. This suggests that the antibacterial activity might be due to different classes of these compounds.

The antibacterial activity of *Mangifera indica* was determined by the view to know the degree of efficacy and potency of palm wine and ethanol extract of leaves of *Mangifera indica* for the treatment of bacterial infection. Palm wine extract showed the highest antibacterial activity on all the five test organisms – *Shigella flexneri*, *Pseudomonas fluorescens*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus spp*– with zones of inhibition ranging from 18 to 24 mm at a concentration of 25 and 12.5 $mgml^{-1}$ and showed no sensitivity at concentration 6.25, 3.125 and 1.5625 $mgml^{-1}$. Ethanol extract showed no inhibitory effect on *Staphylococcus aureus* and weak inhibitory effects against the other four organisms. This effect is not in agreement with the previous work of Bbosa *et al*. [10] which reported that the ethanol extract has the highest inhibitory activity against the entire tested organism including *Staphylococcus aureus*. Gentamycin which was used as a positive control showed a strong inhibitory effect against all the tested organisms compared to all the leave extracts. The result showed that the MIC of palm wine and ethanol extract was at the concentration 12.5 $mgml^{-1}$ for all the organisms tested. The minimum bactericidal concentration of the palm wine and ethanol extract was determined and the result showed that at the concentration of 12.5 $mgml^{-1}$ there was no growth and partial growth after sub culturing onto nutrient agar plate which was referred to bactericidal and bacteriostatic in the Table 6 respectively. Arekemase *et al*. [19] reported that the ethanol extracts of plant were more effective than the aqueous extracts and hypothesised that it might be due to the ability of the solvent to extract more of the active ingredients from the plant materials. This study reported the effect of *Mangifera indica* using palm wine and ethanol as the solvent of extraction and found that the palm wine extract has more active ingredients and that the test organisms, are sensitive to it (Palm wine extract) than the ethanol extract. The above observation suggests that the active ingredients from the leaves of *Mangifera indica* are more soluble in palm wine than in ethanol. This report is in agreement with reports by some scientists in several studies on medicinal plants that organic solvent is extensively used for extracting crude constituents before being re-extracted to obtain purified active compounds [19-20]. The higher potency of palm wine extracts might be connected with the extraction solvent. This report suggests that palm wine has greater extractive power than ethanol.

5. Conclusion

The finding revealed that, *Mangifera indica* contain bioactive compound that are effective against the test organisms, thus the crude extracts could be used directly for chemotherapy or treatment of infections caused by these organisms. The palm wine and ethanol extract of *Mangifera indica* showed zones of inhibition against the test organisms used for this research work, hence stronger activity was observed in palm wine extract.

6. Recommendation

This result showed that leave extract of *Mangifera indica* had antibacterial activity and can be used as a source for developing broad spectrum antibiotics, which further validates its use in traditional herbal medicine to treat a variety of caused by bacteria. However, attempts should be made to conduct *in vivo* studies with the extract so as to confirm the present *in vitro* findings as the diameter of the zone of inhibition is not only affected by sensitivity of the microorganisms alone but concentration of the extract used, solvent of extraction and its rate of diffusion in the media as well.

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