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Full Length Research Paper

Phytochemical and *in-vitro* antibacterial evaluation of the extracts, portions and sub-portions of the ripe and unripe fruits of *Nauclea latifolia*

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The petroleum ether and methanol extracts of the ripe and unripe fruits, the partitioned-soluble portions of the ripe fruits and the water-insoluble sub-portions of both the ripe and unripe fruits of *Nauclea latifolia* revealed the presence of alkaloids, flavonoids, steroidal nucleus, saponins, coumarins and tannins. Antibacterial activity of the extracts (100 mg/ml), portions (50 mg/ml) and sub-portions (50 mg/ml) in comparison with standard drugs (1 mg/ml) against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and four Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi*) using the agar-well diffusion method revealed higher zones of inhibition, higher calculated percent activities (A%), higher calculated bacterial susceptibility (BSI), lower minimum inhibition concentrations (MICs) and minimum bactericidal concentrations (MBCs) for the extract/sub-portions of the unripe fruits than the extracts/portions/sub-portions of the ripe fruits, an indication that the unripe fruits exhibited better antibacterial efficacy against the tested strains. The diethyl ether (MuiD) and ethyl acetate (MuiE) sub-portions of the unripe fruits displayed broad spectrum activity than chloramphenicol and tetracycline supporting their use in the treatment of dysentery and diarrhea.

Key words: *Nauclea latifolia*, ripe/unripe fruits, extracts/portions/sub-portions, standards, phytoconstituents, antibacterial.

INTRODUCTION

Medicinal plants are of great importance because they provide drugs that help widen the therapeutic arsenal and equally help bridge the gap between the availability and the demand for modern medicines (Cordell, 2000). *Nauclea latifolia* Smith (Family Rubiaceae) is a straggling shrub with sweet-scented white or white-yellow flowers. The fruits which are usually fleshy are red when ripe, resembling hard strawberry and yellow when unripe. Embedded within the fruits are numerous small brownish seeds surrounded by a pink, edible, sweet-sour pulp (Iwu, 1993). The dried fruit is used traditionally in the treatment of dysentery, diarrhea and piles (Abbiw, 1990; Iwu et al., 1999). The fruit extract of the plant has been shown to be active against human immune deficiency virus (Hussein et al., 1999). The charred roasted succulent ripe fruits are used to treat measles (Lawal et al., 2010a), while eating of the fruits in Cameroon is said to enhance indigestion (Jiofack et al., 2010). Non-medicinally, the plant is regarded as a source of food in Northern Nigeria (Aiyela'agbe et al., 1996). In Sudan, ripe fruits are eaten and soft drinks are prepared from these fruits while over-ripe fruits are sometimes dried and a powder is prepared from them and used as a base for soft drinks (Abdelmuti, 1991). The fruits of the plant are reportedly rich in vitamin C and this has made them a good source of fruit juice (Amoo and Lajide, 1999). The plant is generally a Savannah-woodland plant, native to tropical Africa and Asia and is well distributed in many parts of Nigeria (Igoli et al., 2005). Although, much work

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has been carried out on other organs of the plant, a review of the literature reveals no/little work has been carried out on portions/sub-portions of both the ripe and unripe fruits of the plant. This work therefore presents the results of the phytochemical and antibacterial potentials of extracts/portions/sub-portions of both the ripe and unripe fruits of *N. latifolia* against a range of bacteria in comparison with three commercial antibiotics.

MATERIALS AND METHODS

Collection of plant

The ripe and unripe fruits of *N. latifolia* were collected from a farmland in Maikunkele area of Bosso Local Government Area, Minna, Niger State, Nigeria, in the months of October, 2009 and January, 2010, respectively. The fruits were authenticated by Mallam Musa Gallah, of the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen was deposited (Number 40768).

Extraction procedures

Two kilograms each of air-dried, pounded and sieved ripe and unripe fruits of *N. latifolia* were individually exhaustively extracted with methanol by maceration at room temperature for 3 weeks. The resulting solutions were separately combined, filtered and the filtrates were concentrated *in vacuo* using a rotatory evaporator. Extracts were further dried over a water bath and defatted with petroleum spirit (60 to 80°C) by maceration at room temperature for a week. The petroleum ether-soluble extracts were coded Pr and Pu, while the residual defatted extracts were coded Mr and Mu for the ripe and unripe fruits, respectively.

Partitioning of crude methanol extracts (Mr and Mu)

457 g of Mr and 420 g of Mu were separately suspended in 1 L of distilled water. The mixtures were shaken vigorously and allowed to stand for 2 h after which they were filtered to give a clear reddish-brown filtrate for the ripe fruits, while the unripe fruits extract (Mu), yielded no significant filtrate; the extract was practically water-insoluble. The water-insoluble portions (residue) were set aside, weighed and coded MrWi and MuWi for the ripe and unripe fruits, respectively. To the filtrate of the ripe fruits in a separatory funnel, 200 ml×6 portions of $CHCl_3$ was added, shaken gently and allowed to settle. The organic phase was removed, concentrated *in vacuo*, dried, weighed and coded $CHCl_3$-soluble portion of partitioned methanol extract of ripe fruits of *N. latifolia* (MrC). The residual water-soluble portion was again successively and exhaustively partitioned with 200 ml×7 portions of EtOAc and 200 ml×10 portions of n-BuOH, respectively. The resulting organic portions were concentrated, dried, weighed and coded EtOAc-soluble (MrE) and BuOH-soluble (MrB) portions of partitioned methanol extract of ripe fruits of *N. latifolia*, respectively. The residual aqueous portion was concentrated, dried, weighed and coded MrR (Figure 1).

Fractionation of water-insoluble portions of methanol extracts (MrWi and MuWi)

212 and 418 g of air-dried MrWi and MuWi were individually successively and exhaustively extracted with 200 ml×10 portions each of Et_2O, $CHCl_3$, EtOAc and Me_2CO, respectively. The resulting filtrates were concentrated under reduced pressure, dried, weighed and coded Et_2O-soluble (MriD and MuiD), $CHCl_3$-soluble (MriC and MuiC), EtOAc-soluble (MriE and MuiE) and Me_2CO-soluble (MriA and MuiA) sub-portions of water-insoluble portion of methanol extract of ripe (r) and unripe (u) fruits of *N. latifolia*, respectively. Their residual water-insoluble sub-portions were concentrated, weighed and coded MriR and MuiR, respectively (Figure 1).

Preliminary phytochemical screening

All extracts/portions/sub-portions were screened for the presence of various secondary metabolites using standard methods (Sofowora, 1993; Evans, 1996) (Table 1).

Bacterial cultures

All extracts/portions/sub-portions were individually tested against overnight cultures of two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and four Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi*). All organisms were obtained from the Department of Microbiology, Federal University of Technology, Minna, Nigeria. Organisms were standardized by sub culturing into nutrient broth at 37°C for 18 h. Organisms were maintained in agar slants at 4°C and sub cultured 24 h before use.

Bacterial susceptibility testing

The agar-well diffusion method was employed (Perez et al., 1990; Dall'Agnol et al., 2003). Standardized inoculum containing 10^6 cfu/ml 0.5 ml McFarland standards were evenly streaked onto the surface of sterile agar plates for each organism. 8 mm wells were bored into the solidified agar using sterile cork borer at equidistance. 100 µl of each extract (100 mg/ml), portions (50 mg/ml) and sub-portions (50 mg/ml) were introduced into different wells individually. Negative (methanol) and positive (1 mg/ml each of chloramphenicol, Ningbo Shuangwei, China; Erythromycin, Falma Laboratories, India and Tetracycline, La Tetra-250, Me-Cure Industries, Ltd., Lagos, Nigeria) control wells were also set up. Agar plates were incubated aerobically at 37°C for 24 h. Zones of inhibition around the wells were measured to the nearest millimeter using a meter rule. Experiments were carried out in triplicates and results analyzed for statistical significance (Table 2).

Statistical analysis

Data were represented as mean ± standard error of the mean (SEM). Comparisons between groups were performed using two-way analysis of variance (ANOVA) on statistical software package-Statistical Package for Social Sciences (SPSS 15.0 for Windows, 2006 version) with Ryan-Einot-Gabriel-Welsch F Post hoc tests for separation of means. A plant extract is considered 'active' when it has an inhibition zone of ≥ 14 mm (Mothana and Linderquist, 2005).

Determination of antibacterial percent activity (A%) of the test compounds and bacterial susceptible index (BSI) of the test organisms

These parameters were determined using the following equations (Eloff, 2004; Mahlke et al., 2009) (Figures 2 and 3):

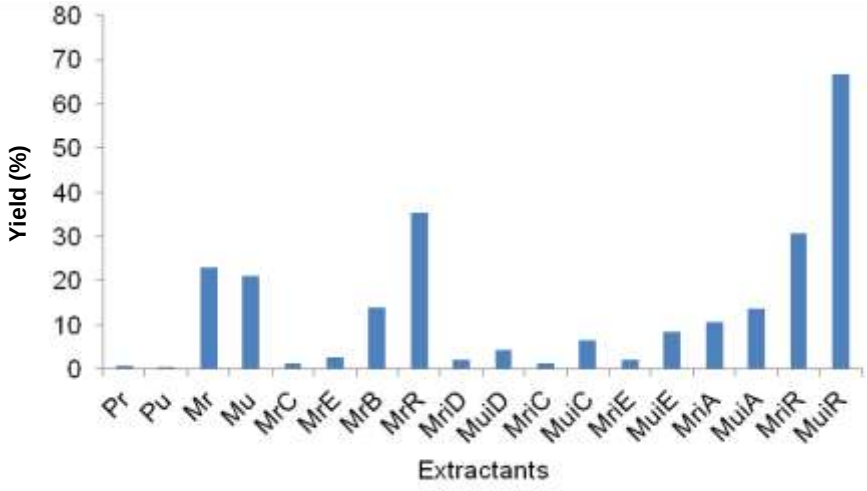

Figure 1. Yield percentage of extracts (Pr-Mu), portions (MrC-MrR) and sub-portions (MriD-MuiR) extracted from 2 kg of ripe (r) and unripe (u) fruits of *N. latifolia.*

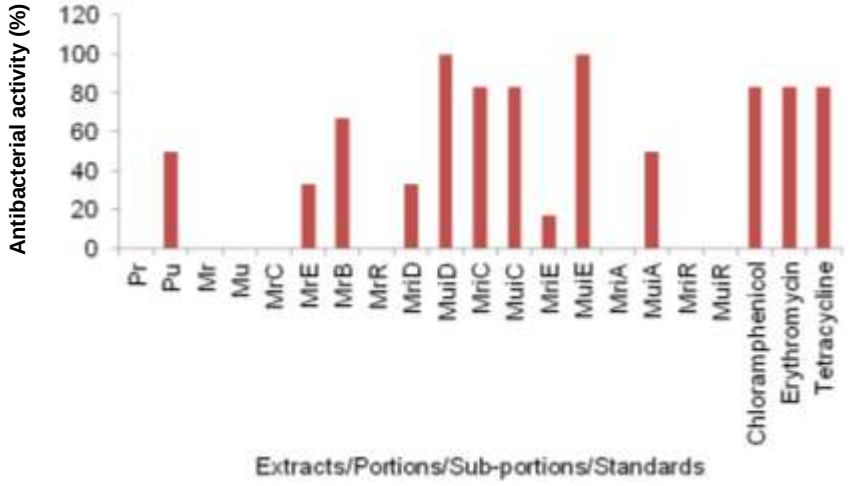

Figure 2. Percent antibacterial activities (A%) of the extracts (Pr-Mu), portions (MrC-MrR) and sub-portions (MriD-MuiR) ripe and unripe fruits of *N. latifolia* in comparison with the standard drugs.

$$A\% = \frac{\text{Susceptible strains to a specific extract/portion/sub-portion}}{\text{Total number of bacterial strains tested}} \times 100$$

$$BSI = \frac{\text{Number of extracts/portions/sub-portions effective against each bacterial strain}}{\text{Total number of extracts/portions/sub-portions}} \times 100$$

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and MBC/MIC ratios

The serial tube dilution as described by Andrews (2001) was adopted for MIC. Active test compounds were diluted to various concentrations ranging from 3.125 to 100 mg/ml in methanol. 1 ml of each of this preparation was added to 1 ml of nutrient broth and mixed thoroughly. 1 ml of the content of each was transferred to the next tube up to the last tube. 10 µl containing 10^6 cells/ml of standard inoculum was added to each of the tubes and control tubes were maintained simultaneously. All tubes were incubated aerobically at 37°C for 24 h.

The lowest concentration of test compounds that produced no visible bacterial growth (no turbidity) when compared with control tube was considered as the MIC. MBC was determined by sub-culturing the no turbidity tubes onto fresh agar plates and was incubated for 24 h. The lowest concentrations that yielded no single bacterial colony were taken as the MBC. The MBC/MIC ratios were calculated to determine whether the observed antibacterial effects of the test compounds were bacteriostatic or bactericidal (Agnese et al., 2001) (Table 3).

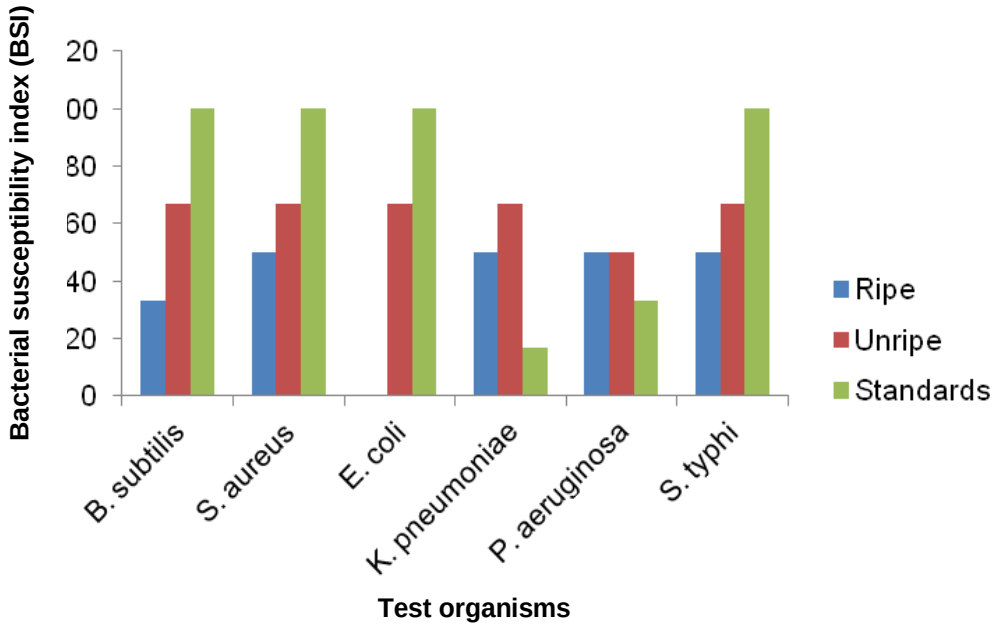

Figure 3. Bacterial susceptible index (BSI) of test organisms to the extracts/portions/sub-portions of ripe and unripe fruits of *N. latifolia* in comparison with the standard drugs.

Table 1. Result of preliminary phytochemical screening of the ripe (r) and unripe (u) fruits.

Phytoconstituents	Pr	Pu	Mr	Mu	MrC	MrE	MrB	MrR	MriC	MuiC	MriD	MuiD	MriE	MuiE	MriA	MuiA	MriR	MuiR
Carbohydrates	−	−	+++	+++	++	++	+++	+++	−	−	−	+	+	++	+	++	+	++
Tannins	−	−	++	+++	+	++	+++	−	−	+	−	++	+	+++	+	++	+	−
Phlobatannins	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Saponins	−	−	+++	+++	++	+++	+++	+	++	−	++	++	++	+++	++	++	+	−
Flavonoids	−	−	+++	+++	++	+++	+++	+	++	++	+++	+++	++	+++	++	+++	−	−
Cardiac glycosides	+++	+++	+++	+++	++	++	+++	−	+++	+++	+++	+++	+++	+++	+++	++	−	−
Anthraquinones	−	−	++	−	++	++	+	−	−	−	−	−	−	−	−	−	−	−
Coumarins	++	++	+++	++	+	++	++	−	++	+++	+++	+++	++	++	++	++	+	+
Tetraterpenoids	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Alkaloids	−	−	+++	+++	++	++	++	+	−	++	+	+++	++	+++	++	++	−	−

+ = Low concentration; ++ = moderate concentration; +++ = high concentration. Extracts (Pr-Mu), portions (MrC-MrR) and sub-portions (MriD-MuiR) of the ripe and unripe fruits of *N. latifolia.*

Table 2. Antibacterial activity of extracts/portions/sub-portions of ripe and unripe fruits of *N. latifolia* against test organisms.

Test compound	Diameter of zones of inhibition against test organisms (mm)*					
	B. subtilis	***S. aureus***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***S. typhi***
Pr	−	−	−	−	3.11^{q2}±1.15	6.67^{r1}±0.88
Pu	12.1^{k4}±0.58	−	15.0^{f2}±1.00	14.6^{g3}±0.95	−	15.3^{i1}±0.36
Mr	7.05^{n4}±0.67	11.0^{j3}±0.00	−	12.3^{j1}±2.52	5.33^{o5}±0.58	12.2^{l2}±2.00
Mu	−	−	12.3^{i2}±1.18	12.0^{k4}±0.00	13.3^{i1}±2.06	12.1^{m3}±0.82
MrC	−	−	6.33^{m4}±1.53	12.7^{i1}±3.06	7.67^{m3}±2.52	11.5^{n2}±1.00
MrE	8.25^{m5}±0.58	−	11.3^{k4}±1.53	17.6^{b1}±0.83	12.2^{k3}±1.00	17.3^{e2}±0.58
MrB	12.2^{j5}±0.38	14.7^{i4}±2.08	−	15.3^{d3}±0.58	16.4^{e2}±1.00	17.3^{e1}±2.33
MrR	−	−	−	7.33^{q2}±1.15	−	9.22^{o1}±0.82
MriD	17.5^{b2}±0.36	21.2^{c1}±1.00	11.7^{j6}±0.95	16.1^{c5}±1.18	16.7^{d3}±1.53	16.3^{g4}±1.53
MuiD	17.3^{c4}±0.46	17.5^{d3}±0.92	18.4^{d1}±1.18	15.3^{d3}±1.62	18.3^{a2}±0.94	16.1^{h5}±0.74
MriC	14.2^{h4}±0.38	17.1^{e1}±0.16	−	9.14^{o5}±1.15	12.3^{j3}±2.08	12.7^{k2}±1.15
MuiC	14.8^{g4}±0.83	16.0^{h2}±0.00	13.1^{h6}±1.30	15.0^{e3}±0.00	14.2^{h5}±0.63	17.2^{f1}±0.46
MriE	10.7^{l3}±0.25	7.33^{k6}±1.15	7.36^{l5}±1.53	11.7^{l2}±1.53	15.3^{f1}±1.53	7.67^{p4}±1.15
MuiE	20.4^{a2}±0.82	21.5^{b1}±0.92	18.3^{e3}±1.24	17.7^{a4}±0.42	17.3^{b6}±0.76	17.6^{d5}±0.64
MriA	−	−	−	10.1^{m1}±1.00	7.00^{n3}±2.00	7.33^{q2}±0.58
MuiA	14.1^{i3}±0.15	16.5^{g1}±0.68	14.2^{g2}±0.46	12.8^{h5}±0.95	12.3^{j6}±0.98	13.1^{j4}±0.42
MriR	−	−	−	−	3.33^{p2}±0.58	7.33^{q1}±1.53
MuiR	4.20^{o2}±0.45	3.82^{l3}±1.18	−	2.48^{r4}±0.64	−	4.33^{s1}±0.58
Chloramphenicol	15.5^{e4}±1.05	16.6^{f3}±0.32	20.2^{c1}±2.00	14.8^{f5}±2.00	9.60^{l6}±0.45	19.3^{c2}±0.00
Erythromycin	15.1^{f5}±2.05	22.2^{a3}±0.24	22.6^{a2}±0.56	9.60^{n6}±1.06	17.1^{c4}±0.62	26.2^{a1}±1.41
Tetracycline	17.2^{d3}±1.00	16.6^{f4}±0.20	22.0^{b1}±0.00	8.50^{p6}±0.83	14.3^{g5}±0.70	19.8^{b2}±0.52

Extracts (Pr-Mu), portions (MrC-MrR) and sub-portions (MriD-MuiR). − = No measurable zone of inhibition; *= mean values of three replicates with standard error shown as ± Mean values on the same column/row with same superscript (letters/numbers) are not significantly different from each other ($p>0.05$) while those with different superscript (letters/numbers) are significantly different from each other ($p<0.05$), respectively.

Table 3. MICs, MBCs and *MBC/MIC values of active extract/portions/sub-portions of ripe and unripe fruits of *N. latifolia* against test organisms.

Test compound	MIC, MBC and MBC/MIC values of test compounds against test organisms					
	B. subtilis	***S. aureus***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***S. typhi***
Pu	ND	ND	12.5;100;8.00	25;100;4.00	ND	25;100;4.00
MrE	ND	ND	ND	12.5;12.5;1.00	ND	12.5;12.5;1.00
MrB	ND	25;50;2.00	ND	12.5;25;2.00	12.5;12.5;1.00	12.5;12.5;1.00
MriD	12.5;12.5;1.00	6.25;6.25;1.00	ND	ND	25;50;2.00	25;25;1.00
MuiD	12.5;12.5;1.00	12.5;12.5;1.00	12.5;12.5;1.00	25;50;2.00	12.5;12.5;1.00	25;25;1.00
MriC	12.5;50;4.00	12.5;12.5;1.00	ND	ND	ND	ND
MuiC	12.5;50;4.00	12.5;12.5;1.00	ND	25;50;2.00	12.5;50;4.00	25;50;2.00
MriE	ND	ND	ND	ND	25;50;2.00	ND
MuiE	6.25;6.25;1.00	6.25;6.25;1.00	12.5;12.5;1.00	12.5;12.5;1.00	12.5;12.5;1.00	12.5;12.5;1.00
MuiA	12.5;50;4.00	12.5;25;2.00	12.5;50;4.00	ND	ND	ND
Chloramphenicol	0.40;0.30;0.75	0.30;0.20;0.66	0.30;0.20;0.66	0.40;0.20;0.50	ND	0.30;0.20;0.66
Erythromycin	0.40;0.30;0.75	0.20;0.20;1.00	0.30;0.30;1.00	ND	0.30;0.30;1.00	0.20;0.20;1.00
Tetracycline	0.30;0.20;0.66	0.30;0.20;0.66	0.20;0.20;1.00	ND	0.50;0.30;0.60	0.30;0.30;1.00

ND = Not Determined. *MBC/MIC ratios >1= Bacteriostatic effect; < 1= Bacteriocidal effect.

RESULTS

Crude extraction carried out on both the ripe and unripe fruits of *N. latifolia* using a non-polar and polar solvent, revealed that the methanol extracts of both fruits contained more of the extractives than the petroleum

ether extracts, with the ripe fruits giving a higher percent yield. Successive partitioning of the crude methanol extract of the ripe fruits (portions) showed that the phytoconstituents were extracted more into butanol and water.

Fractionation of the water-insoluble portions of both the ripe and unripe fruits (sub-portions) revealed that the extracting solvents extracted more of the phytoconstituents in the unripe than the ripe fruits (Figure 1).

Phytochemical screening carried out on the crude extracts/portions/sub-portions of the ripe (r) and unripe (u) fruits showed that they revealed the presence of almost the same phytoconstituents, with the ripe fruits revealing the presence of anthraquinones, while the unripe fruits were richer in tannins (Table 1).

Antibacterial screening of the test compounds (Table 2) revealed that the petroleum ether extract of the unripe fruits (Pu) exhibited better inhibitory activity against the test bacterial strains than all other extracts tested. The ethyl acetate (MrE) and butanol (MrB) portions exhibited better antibacterial activity than the crude, Mr. Both portions displayed significant activity against *S. typhi*, an activity quite close to that displayed by chloramphenicol. For the sub-portions, the diethyl ether sub-portion of the ripe fruits (MriD) significantly inhibited the growth of Gram-positive *S. aureus* (21.2±1.00) better than chloramphenicol (16.6±0.32) and tetracycline (16.6±0.20) and similar to erythromycin (22.2±0.24). Gram-positive *B. subtilis* (20.4±0.82) and *S. aureus* (21.5±0.92) were the most susceptible to ethyl acetate sub-portion of the unripe fruits (MuiE), with an activity better than that displayed by chloramphenicol and tetracycline. The calculated percent activity (A%) as shown in Figure 2, shows that of all the extracts tested, only petroleum ether extract of the unripe fruits (Pu) expressed a 50% activity against the test organisms.

For the portions, MrB (66.7%) was the most active, followed by MrE (33.3%), while for the sub-portions, MuiD and MuiE expressed a 100% activity against the test organisms, better than that expressed by MriC, MriD, MriE, MriA and the standard drugs (83.3%). The calculated BSI as shown in Figure 3, revealed that the test organisms were more susceptible to the unripe extract/sub-portions (50 to 66.7%), an activity lesser than that exhibited by the standards (16.7 to 100%), but better than that exhibited by the ripe fruits portions/sub-portions (0 to 50%).

The MIC, MBC and MBC/MIC values (Table 3) ranged from 12.5 to 25 mg/ml, 100 mg/ml and 4.00 to 8.00 for the crude extract (Pu); 12.5 to 25 mg/ml, 12.5 to 50 mg/ml and 1.00 to 2.00 for the portions of ripe fruits (MrE and MrB); 6.25 to 25 mg/ml, 6.25 to 50 mg/ml and 1.00 to 4.00 for the sub-portions of the ripe fruits (MriD, MriC and MriE); and 6.25 to 25 mg/ml, 6.25 to 50 mg/ml and 1.00 to 4.00 for the sub-portions of the unripe fruits (MuiD, MuiC, MuiE and MuiA).

DISCUSSION

The methanol extracts of both fruits was of higher percent yield than the petroleum ether extracts, because methanol has reportedly been a better solvent for more consistent extraction of phytoconstituents from medicinal plants when compared with other solvents such as petroleum ether, chloroform, ethanol and water (Masoko et al., 2008). Partitioning of the crude methanol extract of the ripe fruits, revealed that the phytoconstituents were more of polar than non-polar composition. Partitioning between solvents is an adequate approach for the preliminary separation of complex matrices, because the procedure permits discrimination of activities between the polar and non-polar fractions (Mahlke et al., 2009). Generally, polarity of solvents will affect the quantity and types of phytoconstituents eluted from extracts; more polar solvents most often elute more active molecules (Eloff, 1998b). The presence of diverse phytoconstituents such as alkaloids, flavonoids, sterols, glycosides, etc., has been reported in different parts of *N. latifolia* (Karou et al., 2011).

Antibacterial screening of the test compounds showed that the sub-portions of the unripe fruits displayed broad spectrum activity than the sub-portions of the ripe fruits. Generally, the sub-portions were more active than the portions and crude extracts. This may be attributed to the presence/increased level of synergism between the phytoconstituents of the sub-portions (Doughari and Obidah, 2008). Sometimes, because plant extracts are complex mixtures, which contain many constituents, the biological activity of a given extract probably reflects contributions from a number of the constituents (Ndip et al., 2009).

Calculated A% showed that the unripe fruits (extract/sub-portions) were more active than the ripe fruits (portions/sub-portions), while calculated BSI showed that the unripe fruits exhibited a broad spectrum activity against the test organisms than the ripe fruits. A% and BSI are useful tools which may help to choose the better extract or fraction of a medicinal plant to be studied further (Eloff, 2004; Masoko et al., 2008; Mahlke et al., 2009). The difference in the antibacterial activities of ripe and unripe fruit extracts of *N. latifolia* could probably be attributed to the fact that the unripe fruits was found to be richer in tannins and alkaloids which are known to be cytotoxic to bacterial cells (Jones et al., 1994). Herbs that have tannins are astringent in nature and are used for the treating of intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). Also, ripening or over ripening of fruits could probably have caused disintegration reactions that led to the production of some non-active/less active phytoconstituents, since ripe fruits are most often prone to microbial attacks (Levey et al., 2007). The unripe fruit is said to be medicinally better than the ripe fruit (Rajasekaran et al., 2009).

Generally, most of the sub-portions exhibited significant

activity against Gram-positive *S. aureus*. This is not unusual, because *S. aureus* is affected by most antimicrobials (Omwenga et al., 2009). However, this finding is still noteworthy, since food intoxication is caused by *S. aureus*, an organism that causes diarrhea (Timbury et al., 2002). The inhibitory activities of some of the test compounds against Gram-negative *S. typhi,* another bacterial cause of diarrhea is also noteworthy. Sub-portions of the unripe fruits also exhibited significant inhibitory activities against Gram-negative *E. coli*, an organism which is a normal inhabitant of the human and animal intestine and a common cause of diarrhea (not all strains) and urinary tract infections (Timbury et al., 2002). This probably explains why the plant has found its use as an antidysenteric and antidiarrheal in most African countries (Abbiw, 1990; Iwu et al., 1999). The observed antibacterial activities of both the ripe and unripe fruits of *N. latifolia* can be attributed to the strong presence of phytochemical constituents, such as alkaloids, saponins, flavonoids, tannins, steroidal compounds, coumarins and cardiac glycosides which are known to be biologically active and thus aid antibacterial/antimicrobial activities of medicinal plants.

MIC and MBC determination for the active test compounds showed that the sub-portions of both ripe and unripe inhibited the growth of pathogens used at lower concentration. Low MIC and MBC values are an indication of the efficacy of a plant extract/portion/sub-portion and may serve as veritable sources for compounds with therapeutic potency (Fabry et al., 1998). The calculated MBC/MIC ratios showed that the extract/portions/sub-portions were all bacteriostatic, while most of the standards were bactericidal at the tested concentrations (Agnese et al., 2001).

Conclusion

This investigation revealed that the pathogens used in this study were most susceptible to the unripe fruits than the ripe fruits of *N. latifolia*, thus supporting the use of these fruits in the treatment of dysentery and diarrhea. These fruits, especially, the sub-portions of the unripe fruits, could make good candidates for the isolation of broad spectrum antimicrobials.

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