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Antibacterial Efficacy of the Butanol-Soluble Portion of De-pigmented Methanol Leaf Extract of *Strychnos spinosa*

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With 7 tables and 29 references

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ABSTRACT

Background: The presence of pigments, such as chlorophyll, in plant extracts, has reportedly masked or interfered with the antibacterial property of some extracts. This work was designed to compare the anti-bacterial efficacies of both the pigmented and de-pigmented extracts of the leaves of *Strychnos spinosa*.
Methods: De-pigmentation of extracts, phytochemical screening of extracts and their partitioned-soluble portions were carried out using standard methods. The butanol-soluble portion was further purified to obtain fractions and sub-fractions. All extracts/portions/fractions/sub-fractions were tested against a range of Gram-positive and Gram-negative bacteria strains in comparison with chloramphenicol using the agar-well diffusion method.
Results: The de-pigmented crude methanol extract (Mss-C) revealed stronger presence of phenolic compounds than the pigmented crude methanol extract (Mss). *In vitro* antibacterial assay of Mss-C and its butanol-soluble portion (Mss-Cb) revealed appreciable broad spectrum inhibitory activities against Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, and Gram-negative *Pseudomonas aeruginosa*, better than that exhibited by chloramphenicol. Mss-C (21.3 mm ± 1.00) and Mss-Cb (25.1 mm ± 0.71) displayed significant inhibitory activities against *Staph. aureus* than chloramphenicol (16.1 mm ± 1.05). Fractions and sub-fractions obtained from Mss-Cb displayed lesser inhibitory activities than Mss-Cb, while a combination of the two most active fractions of Mss-Cb gave rise to a mixture that produced a stronger broad spectrum inhibitory activity than Mss-Cb.
Conclusion: The above findings suggest that the butanol-soluble portion of the de-pigmented methanol extract of the leaves of *Strychnos spinosa* possess significant antibacterial activity.

Key words: Antibacterial, butanol, de-pigmented, fractions, methanol, phytochemical, sub-fractions, *Strychnos spinosa*
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Introduction

Strychnos spinosa (family Loganiaceae) is locally known as 'Alako' (Yoruba) and `Kookiyar' (Hausa). It is a thorny savannah shrub of about 1 - 9 m in height and widespread in tropical Africa. The ethnobotanical claims of the plant includes its use as an anti-snake poison, an analgesic and a purgative [Mann *et al.*, 2003]. It has also been reported useful in the treatment of colds, wounds, diarrhoea, stomachic pains, eye troubles and earaches [Kokwaro, 1976]. The plant has also been reported to posses the ability to stimulate breast milk production [Lockett

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and Grivetti, 2000]. The antimalarial [Asase et al., 2005], antitrypanosomal [Itoh et al., 2005; Hoet et al., 2007] and antifungal [Kone and Atindehou, 2008] properties of the plant have also been reported. Although, much work has been carried out on the crude extracts of the plant, a review of the literature reveals that no work has been carried out on the de-pigmented butanol-soluble portion of the de-pigmented methanol extract of the leaves of this plant and its fractions/sub-fractions. This is necessary as the development of microbial resistance to antibiotics makes it pertinent to constantly search for new, active and safe compounds as alternatives but effective against pathogenic bacteria.

Materials and Methods

Collection and identification of plant material

Fresh leaves of *Strychnos spinosa* were collected from a farmland in Maikunkele Area of Bosso Local Government Area of Niger State, Nigeria, in the month of June, 2010. The plant was duly identified and deposited at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

Extraction procedures

One kilogram of air-dried leaves of *S. spinosa* was defatted by macerating it with 2.5 l of petroleum ether (60 - 80°C) for several days until the extracting solvent had become colourless. The resulting solution was filtered and the filtrate concentrated *in vacuo* using a rota-vapour. The extract was further dried over a water bath and labelled Pss (3.52%). Dried marc was again macerated with 3.5 l of methanol and subjected to same procedure as above. The dried extract was labelled Mss (6.37%). The pigmented extracts were subsequently subjected to antibacterial testing.

De-pigmentation of crude extracts.

The method of Hostettmann *et al.* [1998] was adopted for the de-pigmentation of the crude extract. Thirty grams of the petroleum ether leaf extract of *S. spinosa* (Pss) was solubilized in 750 ml of petroleum ether and thoroughly mixed with 150 g of activated charcoal until a right consistency was achieved. This was tightly sealed and kept aside for 72 h. The mixture was filtered and the residue washed severally with petroleum ether to ensure a chlorophyll-free extract. The filtrate was concentrated *in vacuo*, dried and labelled Pss-C (40%). For the methanolic-based extract, 60 g of the methanol leaf extract (Mss) was solubilized in 1 l of methanol and mixed thoroughly with 350 g of activated charcoal. The same procedure as above was repeated and the extract labelled Mss-C (58.7%). The de-pigmented extracts were subsequently subjected to antibacterial testing.

Preliminary phytochemical screening of the extracts.

All extracts (Pss, Pss-C, Mss and Mss-C) were screened for the presence of various phytoconstituents using standard methods [Sofowora, 1993; Evans, 1996].

Partitioning of crude de-pigmented methanol extract (Mss-C)

Thirty-five grams (35 g) of Mss-C was suspended in 500 ml of distilled water, shaken vigorously and the mixture allowed to stand for 2 h after which it was filtered and the filtrate in a separatory funnel, partitioned with 100 ml × 3 portions of petroleum ether. The organic phase was removed, concentrated *in vacuo*, dried, weighed and coded petroleum ether-soluble portion of the partitioned de-pigmented methanol extract of *S. spinosa* (Mss-Cp; 1.43%). The residual water-soluble portion was again successively and exhaustively partitioned with 100 ml × 6 portions of chloroform, 100 ml × 7 portions of ethyl acetate and 100 ml × 9 portions of n-butanol. The resulting organic portions were concentrated, dried, weighed and coded $CHCl_3$-soluble (Mss-Cc; 10.6%), EtOAc-soluble (Mss-Ce; 14.6%) and BuOH-soluble (Mss-Cb; 32.9%) portions of partitioned methanol extract of *S. spinosa*, respectively. The residual aqueous portion was concentrated, dried, weighed and coded (Mss-Cr; 35.7%). All the soluble portions were subsequently subjected to antibacterial testing.

Preliminary phytochemical screening of the partitioned-soluble portions

All partitioned-soluble portions of the crude de-pigmented methanol extract of *S. spinosa* (Mss-Cp, Mss-Cc, Mss-Ce, Mss-Cb and Mss-Cr) were screened for the presence of various phytoconstituents using standard methods [Sofowora, 1993; Evans, 1996].

Fractionation of butanol-soluble portion (Mss-Cb)

Ten grams of Mss-Cb was subjected to fractionation using column chromatography. Silica gel (60 - 120 mesh) was used as the stationary phase, while varying proportions of increasing polarity of chloroform and chloroform-methanol was used as the mobile phase. Collected fractions were pooled using TLC and the developed chromato-

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grams examined under sunlight, UV light (254 nm and 366 nm) and iodine crystals in an iodine chamber. The pooled fractions (Mss-Cb1 to Mss-C5) were subsequently each subjected to antibacterial testing.

Further fractionation of fraction Mss-Cb4.

A 4.2 g of fraction Mss-Cb4 was subjected to further purification by column chromatography using Sephadex LH-20 and methanol as stationary and mobile phases, respectively. The pooled sub-fractions (Mss-Cb4a to Mss-Cb4c) were also subjected to antibacterial testing.

Thin layer chromatography (TLC)

TLC of all column fractions and sub-fractions were carried out by using oven-baked pre-coated aluminium plates as the stationary phase and various solvent systems as the mobile phase.

Antibacterial testing of the extracts/portions/fractions/sub-fractions

Source of bacteria

Five bacterial strains, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* (clinical strain) in overnight cultures at 37°C in nutrient broth were used in this study. All organisms were obtained from the Microbiology Laboratory, Federal University of Technology, Minna, Nigeria.

Assay of antibacterial activity

The agar-well diffusion method was employed [Perez *et al.*, 1990; Dall'Agnol *et al.*, 2003]. Standardized inoculums containing 10^6 cfu/ml and 0.5 ml McFarland standards were evenly streaked onto the surface of sterile agar plates for each organism. Eight millimetre wells were bored into the solidified agar using a sterile cork borer at equidistant. Samples were separately reconstituted to give concentrations of 50 mg/ml [extracts/portions/fractions/sub-fractions] and 0.5 mg/ml chloramphenicol (Ningbo Shuangwei, China). A 0.5 ml of each extracts/portions/fractions/sub-fractions/drug was introduced into the wells with the aid of a Pasteur pipette individually. Plates were incubated aerobically at 37°C for 24 h and zones of inhibition around the wells were measured to the nearest millimetre using a meter rule. The experiments were carried out in triplicate and results analyzed for statistical significance. 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. Differences were considered significant, if $p<0.05$. A plant extract is considered `active', when it has an inhibition zone of ≥ 14 mm [Mothana and Linderquist, 2005].

Results and Discussion

Preliminary phytochemical screening of the pigmented and de-pigmented petroleum ether (Pss and Pss-C) and methanol (Mss and Mss-C) extracts as shown in Table 1, revealed that both Pss and Pss-C showed a strong presence of steroidal sapogenins, while, both Mss and Mss-C revealed a strong presence of alkaloids, saponins (sapogenins/triterpenoidal genins) and cardiac glycosides. Tannins and flavonoids were more prominent in Mss-C than Mss. Different solvents have been reported to have the ability to extract different phytoconstituents which depends on their polarity and solubility in the solvents [Marjorie, 1999]. The butanol-soluble portion (Mss-Cb) followed by the ethyl acetate-soluble portion (Mss-Ce) of the five soluble portions obtained from partitioning of Mss-C revealed the highest number of plant constituents (Table 2). This shows that the phytoconstituents of the de-pigmented methanol extract were of mid-polar/polar compositions. Partitioning between solvents is an adequate approach for the preliminary separation of complex plant matrices, because the procedure permits discrimination of activities between the polar and non-polar fractions [Mahlke *et al.*, 2009].

Table 1. Phytochemical constituents in the crude pigmented (Pss, Mss) and de-pigmented (Pss-C, Mss-C) extracts of *S. spinosa* leaves

Constituents	Pss	Mss	Pss-C	Mss-C
Alkaloids	-	+++	-	+++
Saponins	-	+++	-	+++
Tannins	-	++	-	+++
Flavonoids	-	++	-	+++
Steroidal nucleus	+++	+++	+++	+++
Cardiac glycosides	+	+++	+	+++
Carbohydrates	-	+++	-	++

Key: +++ = highly present, ++ = moderately present, + = fairly present, - = absent

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Table 2. Phytochemical constituents in the crude partitioned-soluble portions of de-pigmented methanol extract of *S. spinosa* leaves (Mss-C)

Constituents	Mss-Cp	Mss-Cc	Mss-Ce	Mss-Cb	Mss-Cr
Alkaloids	-	-	+++	+++	-
Saponins	-	-	-	+++	+
Tannins	-	-	+	+++	-
Flavonoids	-	+	+++	+++	-
Steroidal nucleus	++	+++	++	++	-
Cardiac glycosides	-	-	++	+++	-
Carbohydrates	-	-	-	++	++

Key: +++ = highly present, ++ = moderately present, + = fairly present, - = absent.

Antibacterial assay of the extracts showed that the de-pigmented crude methanol extract (Mss-C) exhibited significant inhibitory activity against the test organisms. The extract was most active against Gram-positive *Staph.*. *aureus* (21.3 mm ± 1.00) and least active against Gram-negative *S. typhi* (13.7 mm ± 2.08) as shown in Table 3. Mss-C exhibited an inhibitory activity that was better than that displayed by chloramphenicol against Gram-positive *Staph. aureus, B. subtilis* and Gram-negative *P. aeruginosa*. The pigmented and de-pigmented crude petroleum ether extracts expressed practically no activity against the test organisms. This is probably because of the absence of most phytoconstituents in these extracts (Table 1) or it may be the extracts contained 'inactive substances' which probably antagonized/reduced the antibacterial action of the each phytoconstituent [Ebi and Ofoefule, 1997] or probably, sometimes, the amount of active components in crude extracts from medicinal plants may be small or too diluted [Ndip *et al.*, 2009]. The observed appreciable broad spectrum activity of Mss-C against both Gram-positive and Gram-negative bacteria in comparison with Mss, could probably be due to the removal of the chlorophyll pigment which is sometimes assumed to act as an inhibitory or masking substance [Iriyama *et al.*, 1974; Khackik *et al.*, 1986] which sometimes interferes with the antibacterial property of some extracts [Khan and Saeed, 1998] although, chlorophyll, has been reported to possess lots of biological importance [Indrajith and Ravindran, 2009].

Table 3. Antibacterial activity of crude pigmented and de-pigmented extracts of *S. spinosa* leaves at 50 mg/ml in comparison with chloramphenicol at 0.5 mg/ml against some bacterial strains.

Test compound	Diameter of zones of inhibition of test organisms (mm)*				
	B. subtilis	*Staph. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
Pss	11.2 ± 1.53^{c2}	10.0 ± 0.00^{e3}	11.3 ± 0.58^{d1}	5.7 ± 0.58^{e4}	-
Pss-C	10.0 ± 0.71^{d3}	11.3 ± 1.53^{d1}	10.2 ± 1.00^{e2}	7.3 ± 1.15^{d4}	6.3 ± 1.53^{d5}
Mss	6.3 ± 0.58^{e5}	11.5 ± 1.67^{c3}	19.3 ± 0.71^{a1}	8.2 ± 1.53^{c4}	15.5 ± 0.58^{b2}
Mss-C	19.0 ± 0.25^{a3}	21.3 ± 1.00^{a1}	18.7 ± 0.58^{c4}	19.3 ± 0.58^{a2}	13.7 ± 2.08^{c5}
Chloramphenicol	15.5 ± 0.38^{b4}	16.1 ± 1.05^{b3}	19.2 ± 2.00^{b1}	11.6 ± 2.00^{b5}	18.2 ± 1.41^{a2}

– = no measurable zone of inhibition; *= mean values of triplicates 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

Antibacterial assay of the partitioned soluble portions of Mss-C (Table 4) revealed that the butanol-soluble portion (Mss-Cb) was most active against all the test organisms, a broad spectrum activity that was better than that exhibited by Mss-C and chloramphenicol. For example, activity displayed by Mss-Cb, Mss-C and chloramphenicol against *Staph. aureus* was 25.1 mm ± 0.71, 21.3 mm ± 1.00 and 16.1 mm ± 1.05, respectively (Tables 3 and 4). This is noteworthy, because crude plant preparations have reportedly exhibited lower antimicrobial activity than pure antibiotics [Iroegbu and Nkere, 2005]. The ethyl acetate-soluble portion (Mss-Ce) was also moderately active, especially against the Gram-positive bacteria. This could be as a result of these portions being richer in various phytoconstituents (Table 2). Compounds like tannins, saponins, alkaloids and flavonoids have been linked to, or suggested to be involved with antimicrobial activity [Palombo, 2006]. Further purification of the active Mss-Cb portion using column chromatography (silica gel as stationary phase) gave rise to 5 major fractions (Mss-Cb1 to Mss-Cb5) of which fractions Mss-Cb4, followed by Mss-Cb5 exhibited moderate activity against the test organisms at 50 mg/ml (Table 5). Further purification of fraction Mss-Cb4 using column chromatography (Sephadex LH-20 as stationary phase) gave rise to 3 major sub-fractions (Mss-Cb4a to Mss-Cb4c) of which sub-fractions Mss-Cb4b and Mss-Cb4c were mildly active against the test organisms at 50 mg/ml (Table 6). It was observed that the

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fractions/sub-fractions displayed lower inhibitory activities than the crude butanol-soluble portion. This is in agreement with the findings of Kafaru [1994] and Okoli and Iroegbu [2004], that crude extracts could sometimes exhibit higher efficacy than their fractions/sub-fractions. This may be attributed to lack of possible synergistic effect(s) between the phytoconstituents present in the fractions/sub-fractions, which is sometimes required for enhanced antibacterial effect [Paulo *et al.*, 1997]. It could also be that the active components are present in trace or dilute amount [Dall'Agnol *et al.*, 2003] and their activity could probably be enhanced at increased concentrations of the fractions/sub-fractions. Antibacterial assay of the combined two active fractions of Mss-Cb obtained from column chromatography (Mss-Cb4 + Mss-Cb5) revealed an enhanced significant broad spectrum inhibitory activity against the test organisms at 50 mg/ml (Table 7). This may be attributed to the presence of some phytoconstituents which may be acting synergistically with one another or with other constituents [Aliyu *et al.*, 1998; Doughari and Obidah, 2008].

Table 4. Antibacterial activity of crude partitioned-soluble portions of de-pigmented methanol extract of *S. spinosa* leaves (Mss-C) at 50 mg/ml against some bacterial strains.

Test compound	Diameter of zones of inhibition of test organisms (mm)*				
	B. subtilis	*Staph. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
Mss-Cp	–	–	–	–	–
Mss-Cc	7.3 ± 1.52^{c5}	9.8 ± 1.41^{c1}	9.2 ± 2.00^{c2}	7.4 ± 1.05^{c4}	7.4 ± 1.53^{c3}
Mss-Ce	16.5 ± 1.00^{b2}	18.2 ± 1.15^{b1}	12.4 ± 0.58^{b4}	11.2 ± 1.53^{b5}	12.8 ± 2.08^{b3}
Mss-Cb	20.4 ± 0.38^{a2}	25.1 ± 0.71^{a1}	15.6 ± 1.00^{a4}	12.3 ± 1.41^{a5}	16.3 ± 1.67^{a3}
Mss-Cr	–	–	–	–	–

- = no measurable zone of inhibition; *= mean values of triplicates 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 5. Antibacterial activity of column fractions of crude butanol-soluble portion of de-pigmented methanol extract of *S. spinosa* leaves (Mss-Cb) at 50 mg/ml against some bacterial strains.

Test compound	Diameter of zones of inhibition of test organisms (mm)*				
	B. subtilis	*Staph. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
Mss-Cb1	2.7 ± 1.53^{e2}	2.1 ± 2.33^{e3}	3.3 ± 1.71^{e1}	–	0.9 ± 1.00^{e4}
Mss-Cb2	7.3 ± 1.52^{d5}	9.8 ± 1.41^{d1}	9.2 ± 2.00^{d2}	7.4 ± 1.05^{d4}	7.4 ± 1.53^{d3}
Mss-Cb3	9.5 ± 1.00^{c3}	10.2 ± 1.15^{c1}	9.4 ± 0.58^{c4}	10.1 ± 1.53^{b2}	8.6 ± 2.08^{c5}
Mss-Cb4	15.2 ± 0.38^{a2}	17.1 ± 0.71^{a1}	12.3 ± 1.00^{a4}	10.3 ± 1.41^{a5}	12.4 ± 1.67^{a3}
Mss-Cb5	12.2 ± 1.00^{b1}	11.9 ± 1.15^{b2}	10.5 ± 0.58^{b4}	9.2 ± 1.53^{c5}	10.8 ± 2.08^{b3}

- = no measurable zone of inhibition; *= mean values of triplicates 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 6. Antibacterial activity of sub-fractions of an active column fraction of butanol-soluble portion of de-pigmented methanol extract of *S. spinosa* leaves (Mss-Cb4) at 50 mg/ml against some bacterial strains.

Test compound	Diameter of zones of inhibition of test organisms (mm)*				
	B. subtilis	*Staph. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
Mss-Cb4a	–	–	–	–	–
Mss-Cb4b	10.1 ± 1.52^{b4}	8.2 ± 1.41^{b5}	10.3 ± 2.00^{b3}	10.5 ± 1.05^{b2}	11.3 ± 1.53^{b1}
Mss-Cb4c	11.3 ± 1.41^{a4}	13.2 ± 1.15^{a1}	12.4 ± 0.71^{a3}	11.2 ± 1.41^{a5}	12.5 ± 1.53^{a2}

- = no measurable zone of inhibition; *= mean values of triplicates 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

Generally, phytoconstituents from medicinal plants are known to play important roles in the bioactivity of medicinal plants, producing definite physiological actions on human body, which implies that the medicinal values of medicinal plants lie in these phytochemical compounds [Akinpelu *et al.*, 2008]. The presence of some phyto-

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constituents in the butanol-soluble portion of the de-pigmented methanol extract of the leaves of *Strychnos spinosa* which were probably masked by pigments, could probably be responsible for the promising broad spectrum inhibitory activity of the plant, suggesting that the removal of plant pigments (such as chlorophyll), the partitioning and fractionation of such extracts, could make a medicinal plant an effective source of antibacterial substances. Further work will aim at isolation, characterization and biological assay of the biologically active constituents responsible for the observed effects.

Table 7. Antibacterial activity of 2 combined active fractions of column fractions of partitioned-soluble portions of methanolic extract of *S. spinosa* leaves (Mss-Cb4 + Mss-Cb5) at 50 mg/ml against some bacterial strains.

Test compound	Diameter of zones of inhibition of test organisms (mm)*				
	B. subtilis	*Staph. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
Mss-Cb4 + Mss-Cb5	22.5 ± 1.00^b	26.7 ± 1.15^a	21.4 ± 0.58^c	19.8 ± 1.53^e	22.1 ± 2.08^d

*= mean values of triplicates with standard error shown as ±; mean values with different superscript (letter) are significantly different from each other ($p<0.05$)

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References

Akinpelu, D. A., Adegboye, M. F., Adeloye, O. A. and Okoh, A. I. (2008). Biocidal activity of partially purified fractions from methanolic extracts of *Garcinia kola* (Heckel) seeds on bacterial isolates. *Biol. Res.* **41:** 277-287.

Aliyu, A. B., Musa, A. M., Abdullahi, M. S. and Oyewole, A. O. (2008). Phytochemical and antimicrobial properties of *Ludwigia suffruticosa* (Willd.) Oliv. Ex. O. Ktze (Onagraceae). *Int. J. Pure Appl. Sci.* **2(4):** 1-5.

Asase, A., Oteng-Yeboah, A. A., Odamtten, G. T. and Simmonds, M. S. J (2005). Ethnobotanical studies of some Ghanaian anti-malaria plants. *J. Ethnopharmacol* **99:** 273-279.

Dall'Agnol, R., Ferraz, A., Bernardi, A. P., Albring, D., Nor, C., Sarmento, L., Lamb, L., Hass, M. von Poser, G. and Schapoval, E. E. S. (2003). Antimicrobial activity of some *Hypericum* species. *Phytomed.* **10:** 511-516.

Doughari, J. H. and Obidah, J. S. (2008). Antibacterial potentials of stem bark extracts of *Leptadenia lancifolia* against some pathogenic bacteria. *Pharmacol. online* **3:** 172-180.

Ebi, G. C. and Ofoefule, S. I. (1997). Investigations into the forkloric antimicrobial activities of *Landolphia owerrience*. *Phytother Res.* **11:**149-151.

Evans, W. C. (1996). *Trease and Evans' Pharmacognosy,* 14th ed. W. B. Saunders Company Ltd., London.: pp. 227, 245, 251.

Hoet, S., Pieters L., Muccioli, G. G., Habib-Jiwan, J. L, Opperdoes, F. R. and Quetin-Leclercq, J. (2007). Antitrypanosomal activity of triterpenoids and sterols from the leaves of *S. spinosa* and related compounds. *J. Nat. Prod.* **70(8):** 1360-1363.

Hostettmann, K., Marston, A. and Hostettman, M. (1998). *Preparative Chromatography techniques: Applications in Natural Product Isolation*. Springer, London. pp. 9-11.

Indrajith, A. and Ravindran, K. C. (2009). Antioxidant potential of Indian medicinal plant - *Phyllantus amarus* L under supplementary UV-B radiation. *Recent Res. Sci. Tech.* **1(1):** 034-042.

Iriyama, K., Ogura, N. and Takamiya, A. A. (1974). Simple method for extraction and partial purification of chlorophyll from plant material using dioxane. *J. Biochem* **76(4):** 901-904.

Iroegbu, C. U. and Nkere, C. K. (2005). Evaluation of the antibacterial properties of *Piccralima nitida* stem bark extracts. *Int. J. Mol. Med. Adv. Sci.* **1(2):** 182-189.

Itoh, A., Oya, N., Kawaguchi, E., Nishio, S., Tanaka, Y., Kawachi, E., Akita, T., Nishi, T. and Tanahashi, T. (2005). Secoiridoid glucosides from *Strychnos spinosa. J. Nat. Prod.*. **68(9):** 1434-1436.

Kafaru, E. (1994). *Immense Help from Nature's Workshop,* Elika Health Sciences Ltd. Academic Press Plc., Lagos, Nigeria. pp 1-27.

Khachik, F., Beecher, G. R. and Whittaker, N. F. (1986). Separation, identification and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. Agric. Food Chem.* **34(4):** 603-616.

Khan, F. Z. and Saeed, M. A (1998). Phytochemical and antimicrobial studies of *Salvia splendens* Sello. *Pakistan J. Pharm. Sci.* **11(2):** 13-21.

Kokwaro, J. O. (1976). *Medicinal Plants of East Africa.* East Africa Literature Bureau, Kampala, Uganda.

Kone, W. W and Atindehou, K. K. (2008). Ethnobotanical inventory of medicinal plants used in traditional veterinary medicine in Northern Cote d' Ivore (West Africa). *South Afr. J. Bot.* **74:** 76-84.

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Lockett, C. T and Grivetti, L. E. (2000). Food related behaviour during drought: A study of rural Fulani, North Eastern Nigeria. *Int. J. Food Sci. Nutr.* **51 (2):** 91-1007.

Mahlke, J. D., Bologon, A. A., Machado, M. M., Spader, T. B., Alves, S. H., do Canto-Dorow, T. and Athyde, M. L. (2009). *In vitro* antimicrobial and antioxidant activities of a crude extract and fractions form *Buddleja thyrsoides* Lam. leaves. *Quim. Nova* **32(2):** http://dx.doi.org.

Mann, A., Gbate, M. and Umar, A. N. (2003). *Medicinal and Economic Plants of Nupeland,, Bida, Nigeria:* Jube Evans Books and Publications, Bida, Nigeria. 145pp.

Marjorie, M. C. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12(4):** 564-582.

Mothana, R. A. and Linderquist, U. (2005). Antimicrobial activity of some medicinal plants of Island Soqotra. *J. Ethnopharmacol* **96:** 177-181.

Ndip, R. N., Ajonglefac, A. N., Wirna, T., Luma, H. N., Wirmum, C. and Efange, S. M. N. (2009). *In-vitro* anti-microbial activity of *Ageratum conyzoides* (Linn.) on clinical isolates of *Helicobacter pylori*. *Afr. J. Pharm Pharmacol.* **3(11):** 585-592.

Okoli, A. S. and Iroegbu, C. (2004). Evaluation of extracts of *Anthodersta djalonesis, Nauclea latifolia* and *Uvaira afzalii* for activity against bacterial isolates from cases of non-gonococcal urtheritis. *J. Ethnopharmacol* **94:** 135-144.

Palombo, E. A. (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: Modes of action and effects on intestinal function. *Phytother. Res.* **20(9):** 717-724.

Paulo, A., Gomes, E. T., Duarte, A., Perret, S. and Houghton, P. J. (1997). Chemical and antimicrobial studies on *Crytolepsis obtuse* leaves. *Fitoterapia* **68:** 558-559.

Perez, C., Pauli, M. and Bazerque, P. (1990). An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp.* **15:** 113-115.

Sofowora, E. A. (1993). *Medicinal Plants and Traditional Medicine in Africa.* University Press, Ife, Nigeria. pp. 12-23.

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