Journal of Medicinally Active Plants

Volume 5
Issue 2 *Vol 5 Issues 1-4*

October 2017

Characterization and Antimicrobial Activity of Essential Oils of Stachytarpheta indica (Linn.) Vahl and Mariscus alternifolius Vahl.

Emmanuel E. Essien
University of Uyo, emmaflowus1@yahoo.co.uk

Tameka M. Walker
University of Alabama in Huntsville

Jennifer S. Newby
University of Alabama in Huntsville

William N. Setzer
University of Alabama - Huntsville, wsetzer@chemistry.uah.edu

Olusegun Ekundayo
University of Ibadan

Follow this and additional works at: http://scholarworks.umass.edu/jmap

Part of the Plant Sciences Commons

Recommended Citation

Essien, Emmanuel E.; Tameka M. Walker; Jennifer S. Newby; William N. Setzer; and Olusegun Ekundayo. 2016. "Characterization and Antimicrobial Activity of Essential Oils of Stachytarpheta indica (Linn.) Vahl and Mariscus alternifolius Vahl.." *Journal of Medicinally Active Plants* 5, (2):47-52.
http://scholarworks.umass.edu/jmap/vol5/iss2/7

This Research Note is brought to you for free and open access by ScholarWorks@UMass Amherst. It has been accepted for inclusion in Journal of Medicinally Active Plants by an authorized editor of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.

Characterization and Antimicrobial Activity of Essential Oils of *Stachytarpheta indica* (Linn.) Vahl and *Mariscus alternifolius* Vahl.

Emmanuel E. Essien[1], Tameka M. Walker[2], Jennifer Schmidt Newby[2], William N. Setzer[2*], and Olusegun Ekundayo[3]

[1]Department of Chemistry, University of Uyo, Akwa Ibom State 520101, Nigeria
[2]Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA
[3]Department of Chemistry, University of Ibadan, Ibadan 200284, Nigeria

***Corresponding author**: wsetzer@chemistry.uah.edu

Manuscript received: February 1, 2016.

Keywords: Analysis, antimicrobial, hydrodistillation, medicinal plant, volatile oils.

ABSTRACT

Essential oils obtained from hydrodistilled *Stachytarpheta indica* and *Mariscus alternifolius* plants were characterized by GC-MS. The extracted oil from *S. indica* contained 61 identified constituents, representing 99.2% of the oil, and the extracted oil from *M. alternifolius* contained 45 identified constituents, representing 94.3% of the oil Palmitic acid (39.2%), linoleic acid (13.7%), *p*-cymene (6.7%) and 1,8-cineole (4.1%) were the main components of *S. indica* oil, while *M. alternifolius* oil was dominated by β-turmerone (14.3%), *ar*-turmerone (9.9%), (*E*)-phytol (8.5%), α-turmerone (7.9%) and 6,10,14-trimethyl-2-penta-decanone (7.9%). The essential oils demonstrated potent *in-vitro* antimicrobial activity (39-625 μg/mL) against the pathogens (*Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa*, *Escherichia coli, Candida albicans* and *Aspergillus niger*) employ-ed in the assay. No appreciable cytotoxic activity, however, was observed on human breast (Hs 578T) or prostate carcinoma cells (PC-3). The volatile oil constituents of *S. indica* and *M. alternifolius* and biological activities are reported for the first time.

INTRODUCTION

Stachytarpheta indica (Linn.) Vahl (Verbenaceae), commonly known as snake weed and rat's tail, is a multi-branched herb, 0.5-1.0 m tall with long narrow spikes, and deep blue flowers with a white center. As a plant considered to have originated in the New World tropics, *S. indica* currently has a pan-tropical distribution (Burkill, 1997). The plant has been used in local growing areas as an abortifacient and for management of asthma, headaches, alopecia, bronchitis, bruises, constipation, diarrhea, skin sores, dysentery, dysmenorrhea, fevers, inflammations, liver disease, poisoning, tumor, venereal diseases, cataracts, sedative, anti-fertility and rheumatism (Watt and Breyer-Brandwijk, 1962; Kumar *et al*., 2012).

While earlier studies have not characterized the essential oil of *S. indica*, the composition of other plants in the genus *Stachytarpheta* have been analyzed with reported antimicrobial activity (Kumar *et al*., 2012; Mohammed *et al*., 2012; Silva *et al*., 2012; Christian *et al*., 2013; Ruma and Zipagang, 2015).

Mariscus alternifolius Vahl. (Cyperaceae) is a tufted plant with a short woody rhizome composed mostly of primary stem bases. The *M. alternifolius* plant is characterized by purple leaf sheaths and variable inflorescences with rays more or less well developed and bearing spikes of small greenish to reddish one or two-flowered spikelets. This monocot plant, which is common in damp grassy places, is distributed throughout tropical regions (Egunjobi, 1969), and has been used locally.

The rhizome is aromatic and has been used as a food flavoring (Irvine, 1952). The chewed stem is bandaged on a cut or wound for healing and the crushed swollen stem bases are used for gonorrhea (Burkill, 1985; Williamson, 1970). The phytochemical components and anti-microbial activity of whole plant extract from *M. alternifolius* has been documented (Adeniyi *et al*., 2014). Rabelo *et al*. (2014) reported the composition, antinociceptive, anti-inflammatory, and redox properties of *M. pedunculatus* (syn. *Remirea maritima*) rhizome essential oil. Garo *et al*. (1996) also published findings on the isolation and characterization of five flavans from *M. psilostachys*.

The present study was undertaken to provide information on the composition, antimicrobial and cytotoxicity potential of the essential oils of *S. indica* and *M. alternifolius* due to the current lack of research data on these plants and to determine if folkloric claims for the effective treatment of wounds and some venereal diseases could be scientifically validated.

MATERIALS AND METHODS

Plant materials. Plant samples of *S. indica* and *M. alternifolius*), consisting of roots, shoots, and inflorescences, were collected within the vicinity of the Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. The collected plant tissues were authenticated by Mr. F. Usang of FRIN, Ibadan, and voucher specimens were deposited at FRIN under FHI 407413 and 407414 for *S. indica* and *M. alternifolius*, respectively.

Essential oil extraction. Replicate plants from each species were air-dried, weighed, and subjected to hydrodistillation for four hours to extract the oil from the plant tissues, using a Clevenger-type apparatus in accordance with the British Pharmacopoeia (1980). The extracted oils were dried over sodium sulfate and, after estimation of percentage oil from each species, stored under refrigeration (4 ºC).

Essential oil analysis. The essential oil of *S. indica* and *M. alternifolius* were analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec), and an Agilent ChemStation data system. The GC column was an HP-5m fused silica capillary with a (5% phenyl) polymethylsiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Inlet temperature was 200 °C and interface temperature was 280 °C.

The GC oven temperature was programed for 40°C initial temperature, held for 10 min; increased at 3 °C/min to 200 °C; increased 2 °C/min to 220 °C. A 1% w/v solution of the sample in CH_2Cl_2 was prepared and 1 µL was injected using a splitless injection technique. Identification of oil constituents was achieved based on retention indices as determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007) and stored on the MS library [NIST database (G1036A, revision D.01.00)/Chem- Station data system (G1701CA, version C.00.01.08)].

Antimicrobial screening. Essential oils were screened for antibacterial activity against the Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213), and the Gram negative bacteria, *Pseudomonas aeruginosa* (ATCC No. 27853) and *Escherichia coli* (ATCC No. 254922) as described earlier (Setzer *et al.*, 2003). Minimum inhibitory concentration (MIC) was determined using a microbroth dilution technique (Sahm and Washington, 1991). Dilutions of the oils were prepared in cation-adjusted Mueller Hinton Broth (CAMHB) beginning with 50 µL of 1% w/w solutions of essential oils in DMSO plus 50 µL CAMHB. The oil solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5 x 10^8 colony forming units (CFU)/mL were added to each well. Plates were incubated at 37 °C for 24 h.

The final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Gentamicin was used as a positive control while DMSO was used as a negative control. Antifungal activity was determined as described above using *Candida albicans* (ATCC No. 10231) in a yeast nitrogen base growth medium with approximately 7.5×10^7 CFU/mL. Antifungal activity against *Aspergillus niger* (ATCC No. 16401) was determined as above using potato dextrose broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control. Both *A. niger* and C. *albicans* were maintained at 25°C and 32°C, respectively, for 24 h.

Cell culture media. Human Hs 578T breast ductal carcinoma cells (ATCC. No. HTB-129) (Hackett *et al.*, 1977) were grown in 3% CO_2 environment at
37°C in DMEM (Dulbecco's Modified Eagle's Media) with 4500 mg glucose per liter of medium, supplemented with 10% fetal bovine serum, 10 µg bovine insulin, 100,000 units penicillin, and 100 mg streptomycin per liter of medium and then buffered with 44 mM $NaHCO_3$, pH 7.35. Human PC-3 prostatic carcinoma cells (ATCC No. CRL-1435) (Kaighn *et al.*, 1979) were grown in 3% CO_2 environment at 37°C in RPMI-1640 medium with L-glutamine, supplemented with 10% fetal bovine serum, 100,000 units penicillin and 10.0 mg streptomycin per liter of medium, and

buffered with 15 mM HEPES buffer and 23.6 mM $NaHCO_3$.

Cytotoxicity screening. Hs-578T cells (Sigma-Aldrich) were plated into 96-well cell culture plates at 1.0×10^5 cells per well and PC-3 cells at 1.9×10^4 cells per well. The volume in each well was 100 µL for both cell types. After 48 h, supernatant fluid was removed by suction and replaced with 100 µL growth medium containing 1.0 or 2.5 µL of a DMSO solution of oil (1% w/w in DMSO), making final concentrations of 100 or 250 µg/mL, respectively, for each cell line. The PC-3 cells were tested at a concentration of 100 µg/mL and the Hs-578T cells were tested at a concentration of 250 µg/mL. Tests on the PC-3 and Hs-578T were each replicated four times. Media and DMSO controls were 10 µL and 25 µL DMSO/mL, respectively. Tingenone (100 and 250 µg/mL) was used as a positive control (Setzer *et al*., 1998).

After the addition of compounds, plates were incubated for 48 h at 37°C. The medium was then removed by suction and 100 µL of fresh medium was added to each well. To establish percent kill rates, a cell Titer 96® AQueous Non-Radioactive Cell Proliferation Assay was used (Promega Technical Bull., 1996). Colorimetric readings were recorded in quadruplicate using a molecular Devices SpectraMAX Plus microplate reader, 490 nm. Average absorbance, standard deviations, and percent kill ratios ($\% \ kill_{cmpd}/\% \ kill_{DMSO}$) were calculated.

RESULTS

GC-MS analysis. Chemical analysis of the essential oil of *S. indica* indicated the presence of 61 constituents accounting for 99.2% of the oil (Table 1). Relative concentrations of the essential oil constituents were identified and quantified according to their elution order. Oxygen-containing compounds made up 84.4% of the oil constituents in *S. indica* oil, while 21 hydrocarbons constituted the remaining oil fraction (14.8%), including aldehydes, alcohols, ketones, ethers, acids, phenolics, terpenes, and other hydrocarbons.

Chemical analysis of *M. alternifolius* essential oil identified 45 volatile constituents, representing 94.3% of the oil (Table 2). The primary constituents in the oil were β-turmerone (14.3%), *ar*-turmerone (9.9%), (*E*)-phytol (8.5%), α-turmerone (7.9%) and 6,10,14-trimethyl-2-pentadecanone (7.9%). Other components detected in significant quantity included 1,8-cineole, α-phellandrene, spathulenol, β-caryophyllene,

Table 1. Essential oil composition of *Stachytarpheta indica*..

RI[a]	RI[b]	Compound[c,d]	Area (%)	QI ([e]%)
855	854	(*E*)-2-Hexenal	0.1	95
940	939	α-Pinene	1.0	94
979	980	β-Pinene	0.6	96
982	978	1-Octen-3-ol	3.1	90
1003	1005	α-Phellandrene	1.4	94
1026	1026	*p*-Cymene	6.7	95
1031	1033	1,8-Cineole	4.1	97
1088	1088	Terpinolene	1.0	97
1102	1098	Linalool	1.0	92
1176	1177	Terpin-4-ol	0.2	94
1190	1189	α-Terpineol	0.3	91
1194	1190	Methyl salicylate	1.0	95
1218	1218	β-Cyclocitral	0.5	91
1260	1261	(*E*)-2-Decenal	0.3	90
1291	---	Theaspirane B	0.1	92
1313	---	Theaspirane A	0.1	92
1319	1314	(*E,E*)-2,4-Decadienal	0.1	91
1338	---	2,5-Epoxy-6,8 megastigmadiene	1.1	86
1348	1351	α-Longipinene	0.1	98
1365	1368	Cyclosativene	0.1	96
1375	1376	α-Copaene	0.2	99
1385	1380	(*E*)-β-Damascenone	0.9	97
1392	1391	β-Elemene	0.3	90
1397	1398	Cyperene	0.1	95
1408	1401	Methyl eugenol	0.3	95
1419	1418	β-Caryophyllene	2.8	99
1428	1426	α-(*E*)-Ionone	0.2	92
1436	1418	α-(*E*)-Bergamotene	0.1	91
1452	1454	α-Humulene	0.9	99
1455	1453	Geranyl acetone	0.3	90
1477	1477	α-Muurolene	0.1	96
1480	1480	Germacrene D	0.4	84
1484	1485	*ar*-Curcumene	0.7	98
1486	1485	β-(*E*)-Ionone	0.8	83
1494	1494	α-Selinene	0.4	96
1500	1499	[illegible]-Muurolene	0.2	99
1511	1508	(*E,E*)-α-Farnesene	1.4	91
1523	1524	δ-Cadinene	0.7	98
1542	1542	α-Calacorene	0.2	89
1564	1564	(*E*)-Nerolidol	0.3	62
1576	1576	Spathulenol	0.2	90
1580	1581	Caryophyllene oxide	1.1	90
1612	1611	Tetradecanal	0.5	87
1648	1649	β-Eudesmol	0.2	96
1653	1652	α-Eudesmol	0.5	95
1666	1664	*ar*-Turmerone	0.5	95
1669	---	β-Turmerone	0.4	91
1673	1674	Cadalene	0.2	90
1688	1685	Acorenone	0.1	96
1705	---	α-Turmerone	0.4	86
1714	1714	Pentadecanal	0.8	99
1757	1759	Drimenol	0.3	95
1772	---	Myristic acid	0.6	97
1842	1838	6,10,14-Trimethyl-2 pentadecanone	2.4	91
1914	1914	(*E,E*)-Farnesylacetone	0.3	94
1921	1927	Methyl palmitate	0.2	97
1942	1944	Isophytol	0.2	98
1967	1963	Palmitic acid	39.2	99
2111	2111	(*E*)- Phytol	1.9	94
2164	2164	Linoleic acid	13.7	99
2174	---	Stearic acid	1.3	91

[a]RI, Calculated retention indices;
[b]RI, Retention index from published literature;
[c]Elution order on HP-5ms capillary column;
[d]Identification by comparison of mass spectrum and retention index data;
[e]QI, 'Quality index' reflecting comparison of experimental mass spectrum and NIST library mass spectrum.

limonene, cyperene and (*E,E*)-farnesyl acetone. Oxygenated components constituted 67.6% of the oil while 26 constituents were hydrocarbons.

Identified oxygenated aliphatic compounds in the extracted oil were two aldehydes (1.1%), eight ketones (44.3%) and nine alcohols (21.5%). The *M. alternifolius* oil was composed primarily of 32 sesquiterpenoids (61.7%) with lesser amounts of the monoterpenoids (15.6%), diterpenoids (9.2%), and aliphatics (8.8%). The oil also contained five monoterpene hydrocarbons (9.2%), five oxygen-containing monoterpenes (6.4%), 22 sesquiterpene hydrocarbons (19.4%), 10 oxygenated sesquiterpenes (42.3%) and two diterpenoids (9.2%). The *M. alternifolius* oil also contained two uncommon sesquiterpene hydrocarbons, α-ylangene and sativene.

Antimicrobial screening and cytotoxicity. The essential oils of *S. indica* and *M. alternifolius* exhibited a broad spectrum of antibacterial and anti-fungal activity against the test microorganisms (Table 3). *Aspergillus niger* was most susceptible to the inhibitory effects of *S. indica* oil at 39 µg/L. *Bacillus cereus*, *P. aeruginosa*, and *C. albicans* were mostly tolerant of the essential oil of *S. indica*, but the growth of *S. aureus* and *E. coli* were inhibited at concentrations of 78 µg/mL and 156 µg/mL, respectively. *M. alternifolius* oil displayed relatively strong anti-bacterial activity against *S. aureus* (39 µg/mL). The antifungal assay indicated that the filamentous fungus, *A. niger* was more susceptible to the inhibitory effects of the oil (156 µg/mL) than *C. albicans* (625 µg/mL). An antibacterial test indicated the growth of gram-negative bacteria *E. coli* and *P. aeruginosa* were less inhibited (625 µg/mL) as compared with the growth of *B. cereus* (312 µg/mL). The cytotoxicity evaluation *S. indica* and *M. alternifolius* essential oils showed no appreciable inhibitory activities against Hs 578T and PC-3 carcinoma cells.

Table 2. Essential oil composition of *Mariscus alternifolius*.

RI[a]	RI[b]	Compound[c,d]	Area%	QI[e]%
940	939	α-Pinene	1.3	96
977	980	β-Pinene	1.0	94
1003	1005	α-Phellandrene	3.6	92
1026	1031	Limonene	2.4	97
1031	1033	1,8-Cineole	4.2	98
1087	1088	Terpinolene	0.9	95
1218	1218	β-Cyclocitral	0.2	96
1365	1368	Cyclosativene	1.2	99
1370	1372	α-Ylangene	0.7	92
1375	1376	α-Copaene	1.4	99
1388	---	Sativene	0.3	93
1392	1391	β-Elemene	0.9	91
1397	1398	Cyperene	2.4	94
1415	1415	(*Z*)-α-Bergamotene	0.2	92
1418	1418	β-Caryophyllene	2.5	99
1428	1426	α-(*E*)-Ionone	0.2	94
1435	1436	(*E*)-α-Bergamotene	0.3	93
1444	1443	(*Z*)-β-Farnesene	0.2	93
1452	1454	α-Humulene	0.6	98
1456	---	Neryl acetone	0.7	99
1459	1458	(*E*)-β-Farnesene	0.5	94
1473	---	δ-Selinene	0.4	98
1476	1470	α-Amorphene	0.6	95
1479	1476	γ-Muurolene	0.6	89
1484	1483	*ar*-Curcumene	1.7	96
1486	1485	(*E*)-β-Ionone	1.1	90
1493	1494	α-Selinene	0.7	95
1496	1495	α-Zingiberene	0.8	90
1504	1503	Germacrene A	0.7	97
1509	1509	β-Bisabolene	0.8	94
1512	1514	Cubebol	0.3	86
1515	1517	7-*epi*-α-Selinene	0.5	95
1523	1524	β-Sesquiphellandrene	1.4	90
1548	1549	Elemol	1.4	91
1564	1564	(*E*)-Nerolidol	0.4	90
1580	1576	Spathulenol	3.2	90
1596	1595	Guaiol	1.8	95
1652	1652	Kongol	0.8	93
1666	1664	*ar*-Turmerone	9.9	92
1669	---	β-Turmerone	14.3	95
1705	---	α-Turmerone	7.9	98
1713	1714	Pentadecanal	0.9	91
1840	1838	6,10,14-Trimethyl-2-pentadecanone	7.9	91
1914	1914	(*E,E*)-Farnesylacetone	2.3	96
1942	1944	Isophytol	0.7	95
2111	2111	(*E*)-Phytol	8.5	99

[a]RI, Calculated retention indices.
[b]RI, Retention index from published literature.
[c]Elution order on HP-5ms capillary column;
[d]Identification by comparison of mass spectrum and retention index data;
[e]QI, 'Quality index' reflecting comparison of experimental mass spectrum and NIST library mass spectrum.

Table 3. Minimum inhibitory concentration of essential oil for antimicrobial activity of *S. indica* and *M. alternifolius*.

Essential oil source	*Bacillus cereus*[b] (µg/mL)	*Staphylococcus aureus*[c] (µg/mL)	*Escherichia coli*[d] (µg/mL)	*Pseudomonas Aeruginosa*[e] (µg/mL)	*Candida Albicans*[f] (µg/mL)	*Aspergillus niger*[g] (µg/mL)
Stachytarpheta indica (µg/mL)	625	78	156	625	625	39
Mariscus alternifolius[a] (µg/mL)	312	39	625	625	625	156
Positive controls	1.22*	0.61*	2.44*	1.22*	0.61**	0.61**

a = whole plant; b = ATCC No. 14579; c = ATCC No 29213); d = ATCC No. 25922; e = ATCC No. 27853; f = ATCC No. 10231); g = ATCC No. 16401. *= Gentamicin sulfate; **= Amphotericin B; the negative control (DMSO) had no effect within the assay.

DISCUSSION

In this study, a greater number of constituents were identified in *S. indica* essential oil as compared with the ten constituents originally reported earlier in *S. gesnerioides* by Silva *et al.* (2012) and in identifying ten constituents representing 68.5% of the leaf essential oil. The primary components of the *S. gesnerioides* oil were guaiol (56.5%) and β-sesquiphellandrene (4.72%). The hexane fraction of *S. gesnerioides* also differed from the *S. indica* plant leaves the by containing higher levels of guaiol (56.5%) and α-pinene (16.09%), than that detected in *S. indica* oil. Indeed, several other dominant compounds in *S. gesnerioides* were not detected in *S. indica*, indicating significant chemical differences within the genus.

A comparison of *M. alternifolius* to *M. pedunculatus* (syn. *Remirea maritima*, Cyperaceae) rhizome essential oil demonstrated that main oil constituents in the two species differed significantly. Indeed, the main volatile components of *M. alternifolius* oil were mostly not detected in *M. pendunculatus* oil. For example, cyperene (13.8%) occurring in a relatively high amount in *M. pendunculatus*, was a minor component in *M. alternifolius* oil (2.4%). Other minor constituents common to the essential oils of both species were 1,8-cineole, α-copaene, β-elemene, β-caryophyllene and α-humulene.

A large variation in essential oils constituents within the same genus has been noted in other studies (Rabelo et al., 2014; Silva, et al., 2012; N'goka *et al.*, 2014). Whether the differences in constituents in the current analysis could be related to differences in genotypes or differences in growth environments will require more research into these species.

The essential oils of both *S. indica* and *M. alternifolius* exhibited a broad spectrum of constituents with bioactive activities. The antibacterial and antifungal actions expressed by the essential oil extracts suggest that some oil constituents could have practical use as folk medicines in areas where these plants grow. As the antimicrobial study was limited and showed no effects on carcinomas, further research and clinical trials will be necessary to determine if the oil or oil constituents have practical applications in modern medicine.

ACKKNOWLEDGEMENTS

William N. Setzer is grateful to an anonymous private donor for the gift of the GC-MS instrumentation.

REFERENCES

Adams, R.P. 2007. *Identification of Essential Oil Components by Gas Chromatography /Mass Spectrometry*, 4th Ed. Allured Publishing, Carol Stream, Illinois, USA.

British Pharmacopoeia. 1980. Vol. 2. H. M. Stationary office: London. p.109.

Burkill, H.M. 1985. *The Useful Plants of West Tropical Africa*. Vol. I. Royal Botanic Gardens, Kew. 635 p.

Burkill, H.M. 1997. *The Useful Plants of West Tropical Africa*. Vol. 4. Royal Botanic Gardens, Kew. 555 p.

Christian, A.G., U.P. Monday, U.E. Charles, and O.O. John. 2013. Antibacterial potential of the methanol stem bark extract of *Stachytarpheta indica*. Asian Journal Medical Sciences 4(4):5-10.

Egunjobi, J.K. 1969. *Some Common Weeds of Western Nigeria.* Bull Research Division, Ministry of Agriculture and Natural Resources. Western State, Nigeria. pp. 23-31.

Garo, E., M. Maillard, S. Antus, S. Mavi, and K. Hostettmann. 1996. Five flavans from *Mariscus psilostachys.* Phytochemistry 43:1265-1269.

Hackett, A.J., H.S. Smith, E.L. Springer, R.B. Owens, W.A. Nelson-Res, J.L. Riggs, and M.B. Gardner, 1977. Two syngeneic cell lines from human breast tissues: The aneuploid mammary epithelial (Hs 578T) and the diploid myoepithelial (Hs 578BsT) cell lines. Journal of the National Cancer Institute 58:1795-1806.

Silva, P.S., E.Y. Suzuki, A.P. Moreira, N.R.B. Raposo, T.M.A. Alves, and L.F. Viccini. 2012. *Stachytarpheta gesnerioides* Cham.: Chemical composition of hexane fraction and essential oil, antioxidant and antimicrobial activities. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 11(6):542-548.

Sahm, D.H. and J.A. Washington. 1991. Antimicrobial susceptibility tests dilution methods. In: A. Balows, W.J. Hausher, K.L. Herrmman, H.D. Isenberg and H.J. Shamody, eds., *Manual of Clinical Microbiology*. American Society for Microbiology. Washington DC, USA.

Setzer, W.N., M.C. Setzer, A.L. Hopper, D.M. Moriarity, G.K. Lehrman, K.L. Niekamp, S.M. Morcomb, R.B. Bates, K.J. McClure, C.C. Stessman, and W.A. Haber. 1998. The cytotoxic

activity of a *Salacia liana* species from Monteverde, Costa Rica, is due to a high concentration of tingenone. Planta Medica 64:583.

Setzer, M.C., D.M. Moriarity, R.O. Lawton, W.N. Setzer, G.A. Gentry, and W.A. Haber. 2003. Phytomedicinal potential of tropical cloudforest plants from Monteverde, Costa Rica. Revista de Biologia Tropical 51(3):647-674.

Watt, J.M. and M.G. Breyer-Brandwijk. 1962. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. 2nd ed. Livingstone, London. 568 pp.

Williamson, K. 1970. Some food plant names in the Niger Delta. International Journal of American Linguistics 36:156-67.