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RADICAL SCAVENGING COMPOUNDS FROM THE AERIAL PARTS OF *SOLENOSTEMON MONOSTACHYS* BRIQ (LAMIACEAE)

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Abstract

Background: *Solenostemon monostachys* Briq. (Lamiaceae) is a weed widely used in ethno-medicine to treat infections and inflammatory conditions but the active compounds are yet to be identified. This study isolated and identified the radical scavenging compounds from *S. monostachys* crude extract using diphenyl-1, 1-picryl hydrazyl (DPPH) to monitor the separation.
Materials and Methods: The crude extract of the aerial parts of the plant was evaluated for antimicrobial activity using the agar diffusion test, anti-inflammatory test using carrageenan induced oedema of the rat paw and radical scavenging test using diphenyl-1, 1-picryl hydrazyl (DPPH). Repeated chromatographic separation of the ethyl acetate and *n*-butanol fractions on silica gel and Sephadex LH-20, monitored by DPPH bio-autographic assay resulted in the isolation of seven active compounds, which were identified using spectroscopic methods and comparison with literature data.
Results: The ethyl acetate and *n*-butanol fractions were the most active fractions and contained apigenin, apigenin glucuronide, luteolin, caffeic acid, methyl caffeate, rosmarinic acid and methyl rosmarinate as the radical scavenging compounds with EC_{50} values of 26.67 ± 0.31, 185.89 ± 1.02, 5.35 ± 0.31, 3.92 ± 0.06, 13.41 ± 0.18, 4.99 ± 0.10, 5.97 ± 0.08 µg/ml respectively compared with 2.32 ± 0.08 µg/ml for quercetin as the reference standard.
Conclusion: The isolated compounds are reported for the first time in this plant. The presence of the compounds in *S. monostachys* P. Beauv. (Briq.) (Lamiaceae) may provide justification for some of the ethnomedicinal uses of the plant in infections and inflammatory conditions.

Key words: Flavonoids, caffeic acid derivatives, radical scavenging ability, Lamiaceae.

Introduction

Solenostemon monostachys (P. Beauv.) Briq (Lamiaceae) is an annual or perennial weed occurring in a wide range of habitats and widespread in West and Central Africa with numerous medicinal uses. The leaf sap is considered sedative and stomachic and is used to treat colic, convulsions, fever, headache and cough, especially in children, and externally against eyesight troubles and aphthae (Burkill, 1985). Also, the leaves are used to treat dysmenorrhoea, haematuria, female sterility, rheumatism, foot infections and snakebites. The roots are used to treat onchocerciasis (river-blindness). The plant has many ritual uses, especially related to pregnancy (Lemmens, 2004). It is eaten in Ghana as a leafy vegetable, while in South Western Nigeria; it is commonly employed in the recipe of many herbal medicines especially for infections and inflammatory diseases (Burkill, 1985). The dichloromethane and methanol extracts of the aerial parts of *S. monostachys* have been reported to have antibacterial and antioxidant activities (Ekundayo and Ezeogu, 2006; Jacques et al., 2010; Baba and Onanuga, 2011). The only constituents reported in literature are coleons, unusually rearranged diterpenes, from the aerial parts of the plant (Miyase et al., 1980).

As part of our ongoing investigations on antioxidants and antimicrobial agents from plants (Adeloye et al. 2005, Taiwo et al. 2006, *Idowu* et al. 2003; 2010), we report the isolation of apigenin, apigenin glucuronide, luteolin, caffeic acid, methyl caffeate, rosmarinic acid and methyl rosmarinate for the first time from the aerial parts of *S. monostachys*.

Materials and Methods

General

Solvents used for extraction and chromatography were redistilled before use. Adsorption column chromatography was performed using Accelerating Gradient Chromatography (AGC). This is a modification of conventional medium pressure liquid chromatography (Dunstan, 1995). The ascending mode was employed using silica gel 60 (ASTM 230–400 mesh, Merck). The equipment for the AGC work station was from Baeckstrom Separo AB, Lidingo, Sweden. Size exclusion column chromatography was achieved using Sephadex LH-20 (Pharmacia) pre-swollen in the specified solvent before loading onto the column. All Thin Layer Chromatography (TLC) analysis were performed at ambient temperature using analytical silica gel 60 $GF_{254+366}$ pre-coated aluminum backed plates (Merck, 0.25 mm thick). The resulting spots on TLC plates were visualized under UV light (254 nm) and detected by the use of vanillin/H_2SO_4, anisaldehyde-sulphuric acid and DPPH spray reagents as appropriate. The UV spectra of isolated compounds were obtained in methanol on a Cecil UV/visible spectrophotometer (Cecil Instruments Ltd., Cambridge). ^{1}H NMR spectra were recorded at 200 MHz and ^{13}C NMR at 50 MHz, respectively, on a Varian 200MHz spectrometer at the Obafemi Awolowo University. Chemical shifts are expressed in ppm and referenced to the residual solvent signals.

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Collection

The aerial parts of *S. monostachys* P. Beauv. (Briq.) (Lamiaceae) were collected from the Obafemi Awolowo University campus, in October, 2006. The plant was identified by Mr. Oladele A.T. and a voucher specimen with number UHI 16,399 was deposited at IFE Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. The plant materials were air-dried at room temperature and powdered.

Extraction and Isolation

The powdered plant material (1.5 kg) was extracted successively with dichloromethane (4 L) followed by extraction of the marc with ethyl acetate (4 L) at room temperature and lastly *n*-butanol (2.5 L). The crude dichloromethane and ethyl acetate extracts were each concentrated to dryness *in vacuo*. The dichloromethane, ethyl acetate and *n*-butanol fractions were 17.6 g, 11.7 g and 6.8 g respectively.

The ethyl acetate extract (9.5 g) was fractionated on silica gel using a gradient of hexane, ethyl acetate and methanol to give six fractions coded SME l-SME 6 with fractions SME 4 and SME 6 having spots which tested positive in the DPPH autographic assay. Fraction SME 4 (1.2 g, eluted with 100 % EtOAc) was subjected to further fractionation on a silica gel column using a gradient of *n*-hexane, ethyl acetate and methanol to give fractions SME4a-c. Fraction SME4b (0.5 g, eluted with 20% MeOH in EtOAc) with radical scavenging spots was further fractionated on silica gel with a gradient of *n*-hexane, ethyl acetate and methanol of increasing polarities to give five fractions SME4bi-v. SME4bv **1** (62 mg) was obtained as a pure compound. SME4bii (90 mg) and SME4biii (0.1 g) were each further purified on open column on silica gel mesh 70-230 using isocratic elution with dichloromethane-ethyl acetate (70:30) and dichloromethane-ethyl acetate (1:1) respectively. SME4biii afforded **2** (34 mg) and **3** (27 mg) while SME4bii afforded **4** (30 mg). Fraction SME 6 (2.1 g, eluted with MeOH : EtOAc (2:98) was repeatedly fractionated on silica gel with a gradient of hexane, ethyl acetate and methanol to give four fractions SME6a-d. Fraction SME6a (490 mg) with DPPH active spots was subjected to a column of Sephadex LH-20 previously swollen in EtOAc:Hex (3:7). The column was eluted with the following solvent gradient: Hex:EtOAc (3:7, 2:8, 1:9), EtOAc (100%), MeOH: EtOAc (1:9, 2:8, 4:6 and 5:5) leading to the isolation of compound **5** (27 mg) and compound **6** (21 mg).

The *n*-butanol fraction (6 g) was eluted on silica gel on open column with 200 ml each, of 100% ethyl acetate, ethyl acetate-methanol gradient (9:1, 8:2, 7:3, 5: 5) and 100% methanol to give fractions coded SMB1-SMB3. Fraction SMB3 (2.3 g) was subjected to a Sephadex LH-20 column using the following solvent mixtures ethyl acetate, ethyl acetate-methanol mixtures (9:1, 8:2, 7:3, 5:5), methanol and methanol-water (4:1) to give fractions SMB3a-SMB3c. SMB3b was purified on a Lobar RP-18 column with the following mixtures of methanol-water (3:7, 4:6, 5:5, 6:4) and methanol (100%) to give compound **7** (38 mg).

Antioxidant Tests

TLC autography assay (Qualitative) was conducted as follows. A small amount of the sample was dissolved in an appropriate organic solvent and spotted on silica gel sheet, dried and developed using a suitable solvent system. The dried plate was sprayed with 0.2% methanolic solution of stable radical diphenyl picryl hydrazyl hydrate (DPPH). Zones that turned yellow immediately after spraying were deemed to be strongly antioxidant; zones that turned yellow within 15 min of spraying were categorized as moderately antioxidant, while those that turned yellow within 30 min of spraying were categorized as weakly antioxidant (Burits and Bucar, 2000). Quantitative hydrogen donating or radical scavenging properties of the isolated compounds was determined using the stable radical DPPH- (2, 2- diphenyl-2-picrylhydrazyl) as described by Menzor et. al. (2001). To 1 ml of different concentrations (10, 8, 6, 4, 2 and lg/ml) of the isolated compounds or standard (Quercetin: 6, 4, 2, and 1 µg/ml) in a test tube was added 1 ml of 0.3 mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 min. After 30 min, the absorbance (Ab) values were measured at 517 nm and converted into the percentage antioxidant activity. The 50% inhibition concentration (IC_{50}) was obtained from a linear regression plots of concentration of each of the test compounds (M) against the mean percentage of the antioxidant activity (% inhibition) obtained from three replicate assays.

Statistical Analysis

Results are expressed as S.E.M. The results were analysed using the one-way analysis of variance (ANOVA) followed by the student Newman Keul-Test for comparison between the isolated and reference compounds. The level of significance was set at $^{*}P \leq 0.05$ (5%) for all measurements carried out compared to the reference compound.

Agar-diffusion Method

Antibacterial activity tests were carried out using agar-diffusion (hole-in-plate) method (Adesina et al., 2000) with 18 h broth cultures of the following organisms: *Escherichia coli* NCTC 10418, *Staphylococcus aureus*, NCTC 6571; *P. aeruginosa* ATCC 10145, *B. subtilis* NCTC 8236 and *Candida pseudotropicalis* NCYC 6. A stock solution concentration of 20.0 mg/ml in methanol-water (1:1) was used except otherwise stated. Streptomycin (l.0 mg/ml) and the solvent, aqueous MeOH (50%) were used as controls. Each sample was tested in duplicate. The diameters of the zones of inhibition were measured to the nearest mm.

Anti-inflammatory Test

The method used was the carrageenan induced oedema of the rat paw (Winter et al., 1962). The level of inflammation was assessed by measuring the volume of the paw, using a plethsysmometer. The paw volume was measured at time 0, 0.5, 1, 2, 3, 4, 5 and 6 hours after the carrageenan injection. The acute inflammatory activities in the animals that received the crude extract (100 mg/ kg body weight) were compared with those of the vehicle and indomethacin (5 mg / kg body weight). The percentage inhibition of edema was normalized with the vehicle control animals.

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Results and Discussion

Repeated chromatography of the ethyl acetate fraction of *S. monostachys* on silica gel and Sephadex LH-20 yielded compounds **1-7** (Fig. 1). Compound **1** was isolated as an amorphous brown solid with an AB coupling pattern characteristic of a *trans*-stilbenoid skeleton in the ^{1}H NMR. The compound was identified as rosmarinic acid by comparison of the recorded spectra with those reported for it in literature (Wang et al., 2000; Mehrabani et al., 2005). The spectra of compound **2** were almost the same with those of **1** except for a singlet at 3.60 ppm for a methoxy group in the ^{1}H NMR spectrum and an additional signal observed at 51.6 ppm in the ^{13}C NMR spectrum. This signal is characteristic of a methyl group of an ester. On comparison of the spectral data with literature compound **2** was identified as methyl rosmarinate. (Wang et al., 2000, Mehrabani et al., 2005).

Compounds **3** and **4** showed the presence of an aryl ABX system and *trans*-olefinic protons identified by the large J values (16 Hz) at 7.50 and 6.25 ppm, with the occurrence of a signal at 167.8 ppm for an αβ unsaturated carbonyl carbon suggestive of the existence of a caffeoyl group with an additional signal at 3.8 ppm in the proton spectrum and 52.13 ppm in the ^{13}C NMR indicative of an ester methyl group in compound **4**. Comparison of the spectral data of compounds **3** and **4** with those in the literature (Wang et al., 2000), enabled the compound to be identified as caffeic acid and methyl caffeate respectively.

Compounds **5-7** are flavones and their structures were established from UV shift reagent studies, ^{1}H and ^{13}C NMR spectroscopic data, inclusive of 2D NMR data and comparison with those reported in literature. Compounds **5**, **6** and **7** were identified as apigenin (Xiao et al., 2006), luteolin (Markham, 1982) and apigenin-7-O-β-D-glucuronide and respectively.

Table 1: Antimicrobial activities of the crude extract of *S. monostachys*.

	Organisms				
Samples	EC	SA	PA	BS	CP
S. monostachys crude extract	-	++	-	++	-
Streptomycin (1 mg/ml)	+	++	-	+	NT
50% aqueous methanol	-	-	-	-	-

Keys: - No activity; (+) Weak activity; + Strong activity

EC = *Escherichia coli*, **SA** = *Staphylococcus aureus*, **PA** = *Pseudomonas aeruginosa*, **BS** = *Bacillus subtilis*, ***CP* =** *Candida pseudotropicalis*. **NT=**Not tested. Test conc. of extract. = 20 mg/ml in 50% aqueous methanol.

Table 2: Anti-inflammatory activities *S. monostachys* expressed in mean paw volume and percentage inhibition of oedema formation at the 3rd hour**.**

Test Samples	Dose (mg/kg)	PVBC (ml)	PVAC (ml)	Inhibition of oedema (%)
S. monostachys	100	2.22± 0.2	2.82 ± 0.4	68.09 ± 0.3
Indomethacin	5	2.06± 0.3	2.42 ± 0.3	80.85 ± 0.2
Tween 80	10 ml	1.92± 0.2	3.80± 0.5	-

Keys: PVBC= Paw volume before carrageenan. PVAC= Paw volume after carrageenan.

Table 3: DPPH radical scavenging activity of compounds isolated from *S monostachys*

Comp. No	Compound Name	EC_{50} (µg/ml)
1	Rosmarinic acid	4.99 ± 0.10
2	Methyl rosmarinate	5.97 ± 0.08
3	Caffeic acid	3.02 ± 0.06
4	Methyl caffeate	13.41 ± 0.18
5	Apigenin	26.67 ± 0.21
6	Luteolin	5.35 ± 0.31
7	Apigenin glucuronide	185.89 ± 1.02
	Quercetin	**2.32 ± 0.08**

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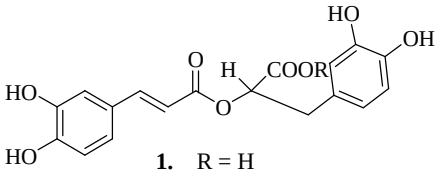
Biological Activity

The crude extract showed activity against G +ve organisms only, as shown in Table 1 and demonstrated anti-inflammatory activity in the carrageenan induced oedema of the rat paw compared with indomethacin (reference compound) at 5 mg/kg (Table 2). The crude extract also showed spots with strong radical scavenging activity in the rapid DPPH thin layer autographic assay used in the preliminary screening and the process was employed to monitor the isolation of the compounds. The EC_{50} values for the isolated pure compounds from the plant are as shown in Table 3. The compounds (**1-7**) conformed to the structural requirements for strong radical scavenging activity as described by Seyoum et al., (2006). Flavonoids have been reported in literature to have good anti-inflammatory activities (Cody et al., 1986; Middleton and Kandaswami, 1992; Gonzalez-Gallego *et al.,* 2007). Caffeic acid derivatives especially rosmarinic acid (RA) and methyl rosmarinate (MR) have also been reported to have a number of remarkable biological activities e.g antiviral, antibacterial, anti-inflammatory and antioxidant activities (Peake et al., 1991, Sahu et al., 1999, Abedini et al., 2013, Inatani et al., 1983, Kuhnt et. al., 1995, Dyke et al., 1986).

This study reported, for the first time in the plant, the presence of luteolin, apigenin, apigenin glucoronide, caffeic acid, methyl caffeate, rosmarinic acid and methyl rosmarinate as compounds responsible for the radical scavenging activity of *S. monostachys.* The literature reports on the anti-inflammatory and antibacterial activity activities of these compounds may additionally justify the ethno-medicinal uses of this plant species in infections and inflammatory conditions.



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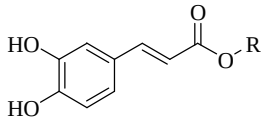
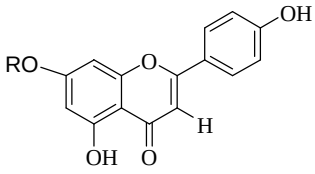
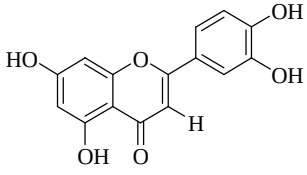


3. R = H
4. R = CH_3



5 R = **H**



6

7 R =

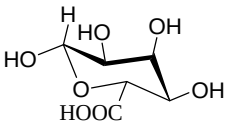


Chart showing the structures of the isolated compounds from *S. monostachys*

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Spectroscopic Data of Isolated Compounds

Rosmarinic acid : ^{1}HNMR (200MHz) (Acetone-d_6) δ: 7.61, [1H, d, (16.1 Hz)-H-3], 7.18 [1H, d, (1.8 Hz) H-5], 7.08 [1H, dd, (1.8, 8.1 Hz) H-9], 6.89 [1H, d, (8.1 Hz) H-8], 6.82 [1H, dd, (1.8, 8.1 Hz) H-5'] 6.58 [1H,d, (8.1 Hz) H-6'], 6.61 [1H, d, (1.8 Hz) H-9'], 6.34 [1H, d, (16.1 Hz) H-4] 5.23 [m, H-2'].

Methyl rosmarinate: ^{1}HNMR (200MHz)(Acetone-d_6) 7.56 [1H, d, (16.1 Hz)-H-3], 7.08 [1H, d, (1.8 Hz) H-5], 7.06 [1H, dd, (1.8, 8.1 Hz) H-9], 7.05 [1H, d, (8.1 Hz) H-8], 6.83 [1H, dd, (1.8, 8.1 Hz) H-5'] 6.81 [1H,d, (1.8 Hz) H-9'], 6.75 [1H, d, (8.1 Hz) H-6'], 6.30 [1H, d, (16.1 Hz) H-4] 5.23 [m, H-2'], 3.65[3H, s, OCH_3], 3.16 [2H, m, H-3'a, 3'b] . **^{13}C NMR** (50 MHz, $CDCl_3$) δ; 167.8 (s, C-9) δ. 168.9, s (C-1), 147.0, s (C-6) 145.8, s (C-7), 144.3, d (C-13), 127.4, d, (C-2), 122.7, s, (C-4), 115.7, d, (C-5); 115.0, d, (C-9) , 114.5, d, (C-8); 52.1, q, ($O\underline{C}H_3$)

Metthyl caffeate. ^{1}H NMR (200 MHz, $CDCl_3$) δ; 7.56 [1H, d, J = 15.8 Hz (H-2)], 7.57, [1H, d, J = 1.8 Hz (H-9)], 6.98 [1H, d, J = 8.1 Hz (H-6)], 6.86 [1H, dd, J= 8.1, 1.8 Hz (H-5)], 6.28 [1H d, J = 15.8 Hz (H-3)], 3.80 [3H, q, (OCH_3)]. **^{13}C NMR** (50 MHz, $CDCl_3$), ppm, 168.9 (C-1), 147.0 (C-6), 145.8 (C-2), 144.3 (C-7), 127.4 (C-4), 122.9 (C-3), 115.7 (C-5), 115.0 (C-8), 114.5 (C-9), 52.1 (C´-1).

Caffeic acid. ^{13}C NMR (50MHz) ($CDCl_3$) δ; 167.8 (s, C-9), 148.1 (s, C-3), 145.1 (s, C-4) 145.4 (d, C-7), 127: (s, C-1), 121.9 (d, C-8), 115.7 (d, C-5) 115.1 (d, C-6), 114. 5(d, C-2).

Apigenin. ^{1}H NMR (200 MHz, DMSO-d_6). δ: 7.987 (2H, d, J=8.3 Hz, H-3' and H-5'), 6.926 (1H, s, H-3), 6.905 (2H, d, J=8.3 Hz, H-2' and H-6'), 6.455 (1H, d, J=0.977 Hz, H-8), 6.174 (1H, d, J=0.977 Hz, H-6). **^{13}C NMR** (50 MHz, DMSO-d_6), δ: 164.82 (C-2), 103.52(C-3), 182.43(C-4), 161.85(C-5), 99.53(C-6), 162.15(C-7), 94.65(C-8), 158.00(C-9), 104.39(C-10), 121.87(C-11), 116.64(C-12), 129.14(C-13), 164.43(C-14), 129.14(C-15), 116.64(C-16).

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Luteolin. **^{1}H NMR** (200 MHz, DMSO-d_6). δ 7.343 (1H, d, J=8 Hz, H-5ˈ), 6.89 (1H, dd, J=8 and 1.5 Hz, H-6ˈ), 6.748 (1H, d, J=1.5 Hz, H-2ˈ), 6.642 (1H, s, H-3), 6.42 (1H, d, J=1.8Hz, H-8), 6.17 (1H, d, J=1.8Hz, H-6). **^{13}C NMR,** (50 MHz, DMSO-d_6), δ: 164.3 (C-2), 103.3(C-3), 182.1(C-4), 161.9(C-5), 99.3(C-6), 164.6(C-7), 94.3(C-8), 157.7(C-9), 104.1(C-10), 122.9(C-11), 113.8(C-12), 146.2(C-13), 150.2 (C-14), 116.5 (C-15), 119.4(C-16).

Apigenin glucoronide. **^{1}H NMR** (200 MHz, DMSO-d_6), δ: 7.846 (2H, d, J=8 Hz, H-2ˈ and H-6ˈ), 6.876 (2H, d, J=8 Hz, H-3ˈ and H-5ˈ), 6.766 (1H, s, H-3), 6.766, (2H, d, J=1.8 Hz), 6.393 (1H, d, J= 1.8 Hz). **^{13}C NMR** (50 MHz, DMSO-d_6). δ: 164.74 (C-2), 103.17 (C-3), 182.37 (C-4), 161.39 (C-5), 100.00 (C-6), 162.23 (C-7), 95.03 (C-8), 157.36(C-9), 105.18(C-10), 120.33(C-11), 116.54(C-12), 128.84(C-13), 162.34(C-14), 128.84 (C-15), 116.54(C-16), 99.8 (C-1″), 74.7 (C-2″), 77.1 (C-3″),73.1 (C-4″),76.9 (C-5″), 170.8 (C-6″).

References

1. Abedini, A., Roumy, V., Mahieux, S., Biabany, M., Standarert-Vitse, A., Riviere, C., Sahpaz, S., Bailleul, F., Neut, C. and Hennebelle, T. (2013). Rosmarinic acid and its methyl esters as antimicrobial components of the hydromethanolic extract of *Hyptis atrorubrens* Poit (Lamiaceae). Evidenced based Compl. and Alternative Med. Vol 2013 article 604536 ;1-12.
2. Adeloye, A.O., Aderogba, M.A., Idowu, T.O., Obuotor, E.M. and Ogundaini A.O. (2005). Investigation of the antioxidant activity of *Alchornea laxiflora* (BENTH) and its constituents *J. Food Tech.* 3 (3):365–369.
3. Adesina S.K, Idowu O, Ogundaini A.O, Oladimeji H, Olugbade T.A, Onawunmi G.O, Pais M.(2000). Antimicrobial constituents of the leaves of *Acalypha wilkesiana and Aacalypha hispida*. *Phytother Res.* 14(5):371-374.
4. Baba, H. and Onanuga, A. (2011). Preliminary phytochemical screening and antimicrobial evaluation of three medicinal plants used in Nigeria. Afr J Tradit Complement Altern Med. 8(4): 387–390
5. Burits, M. and Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14:323-328.
6. Burkill, H.M. (1985). The useful plants of West Tropical Africa. (Families J-L). Ithaka Harbors Inc. New York p 369.
7. Cody, V., Middleton, E. and Harborne, J.B. (eds) (1986). *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships*. Alan R. Liss, Inc., New York. p 78
8. Dunstan, C.A., (1995). Isolation and structure elucidation of pharmacologically active compounds from *Aliphitonia zizyphoides* (Spreng.) A. Gray, with emphasis on inflammation, Acta Universitatis Upsaliensis 138: 8.
9. Dyke, T.E., Braswell, L. and Offenbaclar, S. (1986) Inhibition of gingivitis by topical application of ebelen and rosmarinic acid. Agents and Actions19: 376-377
10. Ekundayo, E.O. and Ezeogu, L.I. (2006). Evaluation of antimicrobial activities of extract of five plants used in traditional medicine in Nigeria. Internat. J. of Tropical Med. 1:93-96.
11. Idowu, T.O., Onawunmi, G.O., Ogundaini, A.O. and Adesanya, S.A. (2003). Antimicrobial constituents of *Chrysophyllum albidum* seed cotyledons. Nig. J. Nat. Prod. and Med.**7:**33-36.
12. Idowu, T. O., Ogundaini A. O., Salau A. O., Obuotor E. M, Bezabih M., Abegaz B. M. (2010). Doubly linked, A-type proanthocyanidin trimer and other constituents of *Ixora coccinea* leaves and their antioxidant and antibacterial properties Phytochemistry 71, 2092-2098
13. Inatani, R.N., Nakatani, N. And Fuwa, H. (1983). Antioxidant effect of constituents of Rosemary. (*Rosemarinus officinalis*) and their derivatives. Agric. Biol. Chem. 47: 521-528.
14. Jacques, D., Fidele K. and Michel, O.A. (2010). Acute toxicity and antioxidant activity of the hydroethanolic extract of *Solenostemon monostachys* P. Beauv. Leaves. J. Complem. Integrat. Med. DOI: 10.2202/1553-3840.1492
15. Kuhnt, M., Probsttle, A., Rimpler, H., Bavier, R. and Heinrich, M. (1995) Biological and Pharmacological activities and further constituents of *Hyptis verticallat.* Planta Med. 61:227-232.
16. Lemmens, R.H.M.J. (2004) *Solenostemon monostachyus* (P.Beauv.) Briq. In: Grubben, G.J.H. and Denton, O.A. (Eds). PROTA 2: Vegetables/Légumes. PROTA, Wageningen p 507.
17. Markham, K.K. (1982). Ultraviolet-visible absorption spectroscopy, In: Techniques of flavonoids identification. Academic Press. London pp 36-71.
18. Mehrabani, M., Ghassemi, N., Sajjadi, E., Ghannadi, A. and Ardakani, M. (2005). Main phenolic compound of petals of Echium Amoenum (Fisch and MEY), a famous medicinal plant of Iran. *DARU* 13(2):65-69
19. Menzor, L.L, Menezes, F.S., Leitao, G.G., Reis, A.S., Santos, T. Coube, C.S. and Leitao, S.G. (2001). Screening of Brazilian plant extract for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15:127-130.
20. Middleton, E. and Kandaswami, C. (1992). Effects of flavonoids on immune and inflammatory cell function. Biochem. Pharmacol. 43: 1167–1179.
21. Miyase, T., Ruedi, P. and Eugester, C.H. (1980). Structures of six coleons from *Solenostemon monostachys* (P.Beauv.) Briq. (Labiateae). Helv. Chim. Acta 63(1):95-101.
22. Peake, P.W., Pussell, B.A., Martyn, P., Timmermans, V. and Charlesworth, J.A. (1991) The inhibitory effect of rosmarinic acid on complement involves C5-convertase. Int. J. Immunopharmacol. 13:853-857.
23. Sahu A, Pawal N and Pangburn MK (1999).Inhibition of complement by covalent attachement of Rosmarinic acid to activated C3b. Biochem Pharmacol. 57:1439-1446
24. Seyoum, A., Asres, K. and El-Fiky, K.F. (2006). Structure –radical scavenging activity relationships of flavonoids. Phytochemistry 67**:** 2058-2070.
25. Taiwo, B.J., Aderogba, M.A. and Ogundaini, A.O. (2006). Antioxidant lignans from the leaves of *Ficus exasperata.* Nig. J. Nat. Prod. and Med. 10:111-113.
26. Wang, Z. J., Zhao, Y.Y., Wang, B., Ai, T. M. and Chen, Y.Y. (2000). Depsides from *Prunella vulgaris*. Chin. Chem. Lett.11:997-1000.
27. Winter, C.A., Risley, E.A. and Nuss, G.W. (1962). Carragenin-induced oedema in hindpaw of the rat as an assay for anti-inflammatory drugs. Proceedings of Society for Experimental Biology and Medicine 111:544-547.
28. Xiao J.B., Ren F.L. and Xu M (2006). Flavones from *Marchantia convolute*: Isolation of apigenin-7-*O*-*β*-D-glucuronide and 5-hydroxyl-7-methoxyl-2-methyl chromene. J. Pharm. and Allied Sci. 3(1): 310-313.