Analysis of the Phytochemical Composition of the Leaves of *Chromolaena odorata* King and Robinson by Gas Chromatography-Flame Ionization Detector.

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ABSTRACT

The phytochemical composition of the leaves of *Chromolaena odorata* was investigated with gas chromatography-flame ionization detector. Thirty-eight known alkaloids were detected, consisting mainly of akuammidine (44.74%), voacangine (24.51%) and echitamine (11.30%). Twenty-three known flavonoids were detected, consisting mainly of kaempferol (19.63%) and (-)-epicatechin (16.63%). Five known carotenoids were detected, consisting mainly of lutein (48.30%) and carotene (33.30%), anthera-xanthin. Four known benzoic acid derivatives were detected, consisting mainly of 4-hydroxybenzaldehyde (36.63%), ferulic acid (26.45%), 4-hydroxybenzoic acid (19.67%) and vanillic acid (17.25%). Seven known lignans were detected, consisting mainly of galgravin (59.39%) and retusin (16.61%). Two known phytosterols were detected, consisting of stigmasterol (66.22%) and sitosterol (33.78%). Two known hydroxycinnamic derivatives were detected, consisting of p-coumaric acid (53.48%) and caffeic acid (46.52%). Only tannic acid was detected in the tannin extract. Four known saponins were detected, consisting mainly of avenacin A1 (61.92%) and avenacin B1 (36.53%). Five known terpenes were detected, consisting mainly of β-amyrin (31.12%), lupeol (21.88%), bauerenol acetate (20.91%) and taraxerol (16.58%). The above result highlights the potential of the leaves of *Chromolaena odorata* as sources of nutraceuticals and other bioactive agents.

(Keywords: alkaloids, *Chromolaena odorata*, flavonoids, gas chromatography, tannic acid)

INTRODUCTION

Chromolaena odorata (family Asteraceae), previously called *Eupatorium odoratum*, is native to South and Central America, but is presently found throughout the tropics, Nigeria inclusive (Fosberg and Sachet, 1980; State of Queensland, 2007). It is commonly called Awolowo, independence weed, Siam weed, triffid weed, bitter bush, or Jack-in-the-bush (Okon and Amalu, 2003). The Ibo people of South Eastern Nigeria call it “ahihia eliza” or “obiara kara”. It suppresses young plantations, agricultural crops and smothers vegetation as it possesses allelopathic potentialities and growth inhibitors (Onwugbuta – Enyi, 2001; Nandi and dev Mandal. 2010; Suwal et al., 2010; Hu and Zhang, 2013).

In traditional health care practice it is popular for its antispasmodic, anti-protozoal, antifungal, anti-trypanosomal, antibacterial, antihypertensive, anti-inflammatory, astringent, diuretic and hepatotropic activities (Phan et al., 2001; Akinmoladun et al., 2007). In the southern part of Nigeria and in Vietnam, fresh leaves or a decoction of *C. odorata* are used for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection, dento-alveolitis, and to stop bleeding (Phan et al., 2001). The hepatoprotective (Alisi et al., 2011), anti-cholesterolemic (Ikewuchi and Ikewuchi, 2011), hemostatic (Akah, 1990), hypotensive (Ikewuchi et al., 2012), nitric oxide scavenging (Alisi and Onyeze, 2008), wound healing (Phan et al., 2001; Alisi and Onyeze, 2008), antihelmintic (Panda et al, 2010) antibacterial, insecticidal, and insect repellant (Inya-Agha et al., 1987; Bouda et al., 2001; Suksamrarn et al., 2004; Cui et al., 2009) activities have been investigated.

The possibility of using the leaves in the formulation of rabbit feed (Bamikole et al., 2004), as well as its potential as a manure (Nawaz and Sansamma, 2004) and bioremediative agent (Akonye and Onwudiwe, 2004; Offor and Akonye, 2006) have also been investigated.

The leaves are rich in fiber and a high quality protein (Igboh et al., 2009), biotin, ascorbic acid, vitamin K, calcium, iron and sodium (Ikewuchi and Ikewuchi, 2009 a,b). Previous investigations of the leaves and stems revealed the presence of N-oxides of five pyrrolizidine alkaloids (Biller et al., 1994), essential oils (Inya-Agha et al., 1987; Lamaty et al., 1992; Bamba et al., 1993; Chowdhury, 2002), steroids (Ikewuchi et al., 2012), flavonoids (Barua et al., 1978; Metwally et al., 1981; Hai et al., 1991, 1995; Triratana et al., 1991; Wollenweber et al., 1995; Wollenweber and Roitman, 1996; Suksamrarn et al., 2004; Yuan et al., 2007; Ikewuchi et al., 2012). This study, investigated the detailed phytochemical profile of the leaves of *Chromolaena odorata* by gas chromatography-flame ionization detector.

MATERIALS AND METHODS

Collection of Plant Samples

Samples of fresh *Chromolaena odorata* were collected from within the Choba and Abuja Campuses of University of Port Harcourt, Nigeria. Their identity was confirmed by Dr. M.C. Dike of Taxonomy Unit, Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria; and Mr. J. Ibe, the Herbarium Manager of the Forestry Department, National Root Crops Research Institute (NRCRI), Umuahia, Nigeria. They were rid of dirt and the leaves were removed, oven dried at 55 °C and ground into powder, which was stored in an air tight container.

Determination of the Phytochemical Content Calibration, Identification and Quantification

Standard solutions were prepared in methanol for alkaloids, flavonoids and simple phenolics, acetone for carotenoids and lignans, methylene chloride for phytosterols, ethanol for hydroxycinnamic acids. The linearity of the dependence of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound, using the standards. Sample chromatograms are shown in Figures 1-5.

Determination of Alkaloid Composition

The extraction was carried out according to the method of Tram et al. (2002). The sample was extracted with methanol and subjected to gas chromatographic analysis. Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 [1206] software, to quantify and identify compounds. The column was a capillary DB-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. The carrier gas was nitrogen. The hydrogen and compressed air pressures were 28 psi and 38 psi. The oven program was: initial temperature at 60 °C for 5 min. First ramping at 10 °C/min for 20 min was followed by a second ramping at 15 °C/min for 4 min.

Determination of Carotenoid Composition

The extraction was carried out by a modification of the method of Rodriguez-Amaya and Kimura (2004). The carotenoids were successively extracted with acetone and a (1:1) mixture of diethyl ether and petroleum ether, then concentrated and re-dissolved in acetone before saponifying and re-extracting with a (1: 1) mixture of diethyl ether and petroleum ether. The resultant extracts were dried and re-dissolved in petroleum ether and subjected to gas chromatography analysis. Flame ionization detector (FID; range scanned, 300 to 600 nm) and capillary column, ZP-5 Column (30m × 0.32 mm × 0.25 µm film thickness) was used. Temperature was programmed at 45°C, held for 6 min, before programming at 38°C/min to 250°C. Initial column head pressure was 3.47 psi.

Determination of Flavonoid Composition

The extraction was carried out according to the method of Millogo-Kone et al. (2009). The sample was extracted with methanol and the resultant extract was subjected to gas chromatographic

analysis. Flame ionization detector (FID) and capillary column, HP INNOWax Column (30 m × 0.25 mm × 0.25 µm film thickness) were used. The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. The carrier gas was nitrogen. The hydrogen and compressed air pressures were 22 psi and 35 psi. The oven program was: initial temperature at 50°C, first ramping at 8°C/min for 20 min, maintained for 4 min, followed by a second ramping at 12°C/min for 4 min, maintained for 4 min.

Assay of Hydroxycinnamic Acid Derivatives' Composition

The extraction was carried according to method of Ortan et al. (2009). The sample was extracted thrice with methanol, and the extracts were pooled, concentrated and subjected to gas chromatographic analysis. Flame ionization detector (FID) and column, HP-5 (30m×0.32mm × 0.25 µm film thickness) was used. The samples were introduced via an all-glass injector working in the split mode, with nitrogen as the carrier gas, at a flow rate of 1mL/min. The injection and detector temperatures were 260°C and 300°C, respectively. The oven temperature was programmed from 170°C to 250°C at 5°C/min.

Determination of the Lignan Composition

The extraction was carried out according to the method of Chapman et al. (2006). The sample was extracted with methanol, and a hexane/dichloromethane mixture, the resultant extract was rid of water and subjected to gas chromatography. Flame ionization detector (FID; range scanned, 350 to 400 nm) and column, ZP-5 (30m×0.32mm×0.25µm film thickness), detected at 300 nm were used. One microliter of sample was injected. The conditions for the GC were initial oven temperature of 40°C, injector 250°C, transfer line 280°C, a solvent delay of 2.00 min, the temperature was ramped at 10°C/min to a final temperature of 230°C and held for 1.00 min.

Assay of Benzoic Acid Derivatives' Profile

The extraction was carried out according to the method of Ndoumou et al. (1996). The sample was extracted with methanol, and after removing the pigments with petroleum ether, were re-extracted with ethyl acetate, before gas chromatography analysis. Detection was achieved with a flame ionization detector (FID). The column was a capillary HP 1 Column (30 m × 0.25 mm × 0.25 µm film thickness). The inlet and detection temperatures were 250 and 320°C. Split injection was adopted with a split ratio of 20:1. The carrier gas was nitrogen, at a pressure of 30 psi. The hydrogen and compressed air pressures were 28 psi and 32 psi. The oven program was: initial temperature at 60°C for 5 min, first ramping at 15°C/min for 15 min, maintained for 1 min, followed by a second ramping at 10°C/min for 4 min.

Determination of Phytosterol Composition

Extraction of oil was carried out according to AOAC method 999.02 (Association of Official Analytical Chemists, 2002), while the analysis of sterols was carried out according to AOAC method 994.10 (Association of Official Analytical Chemists, 2000). This involved extraction of the lipid fraction from homogenized sample material, followed by alkaline hydrolysis (saponification), extraction of the non-saponifiables, clean-up of the extract, derivatisation of the sterols, and separation and quantification of the sterol derivatives by gas chromatography (GC) using a capillary column. Flame ionization detector (FID) and column, HP INNOWax Column (30m×0.25mm×0.25µm film thickness) was used. The inlet and detection temperatures were 250 and 320°C. Split injection was adopted with a split ratio of 20:1. The carrier gas was nitrogen. The hydrogen and compressed air pressures were 22 psi and 35 psi. The oven program was: initial temperature at 60°C, first ramping at 10°C/min for 20 min, maintained for 4 min, followed by a second ramping at 15°C/min for 4 min, maintained for 10 min.

Determination of Tannin Composition

Extraction was carried out according to the method of Luthar (1992). The sample was extracted with methanol followed by gas chromatographic analysis. Flame ionization detector (FID) and column, HP 5 Column (30 m × 0.25 mm × 0.25 µm film thickness) was used. The inlet and detection temperatures were 250 and 320°C. Split injection was adopted with a split ratio of 20:1. He carrier gas was nitrogen. The hydrogen and compressed air pressures were 28

psi and 40 psi. The oven program was: initial temperature at 120°C, followed by ramping at 10°C/min for 20 min.

Inter-conversion of Weights

The compositions per dry weight of the determined parameters were derived from the compositions per wet weight and vice versa, using the following formula, adapted from Health Canada Official Methods (1999).

$$\text{Composition per wet weight (\%)} = \frac{\text{Composition per dry weight (\%)} \times \text{Dry matter content(\%)}}{100}$$

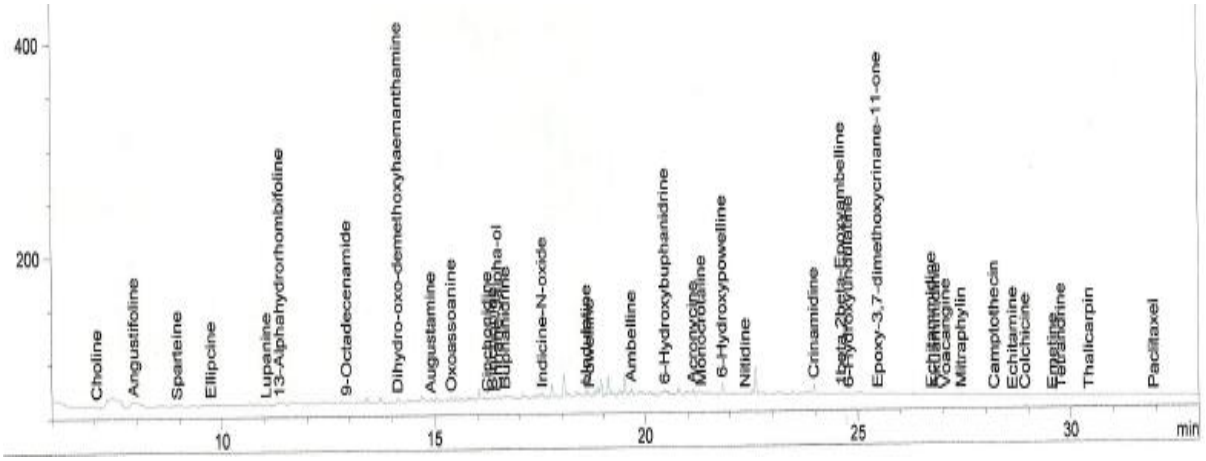

Figure 1: The Chromatogram of the Alkaloids Fraction of the Leaves of *Chromolaena odorata.*

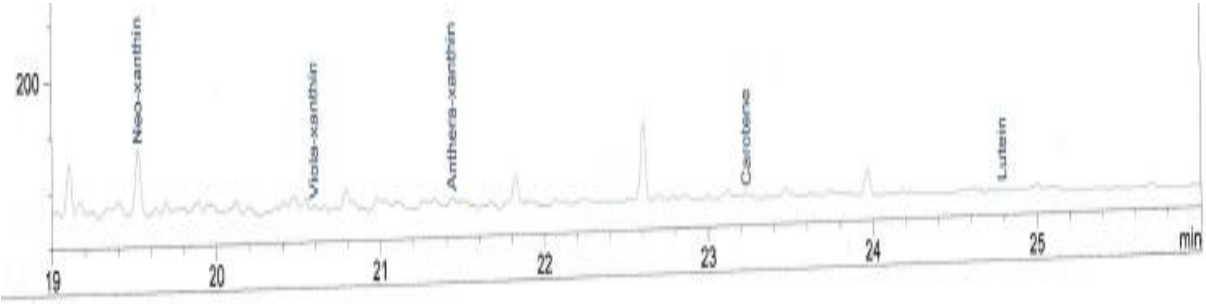

Figure 2: The Chromatogram of the Carotenoids Fraction of the Leaves of *Chromolaena odorata.*

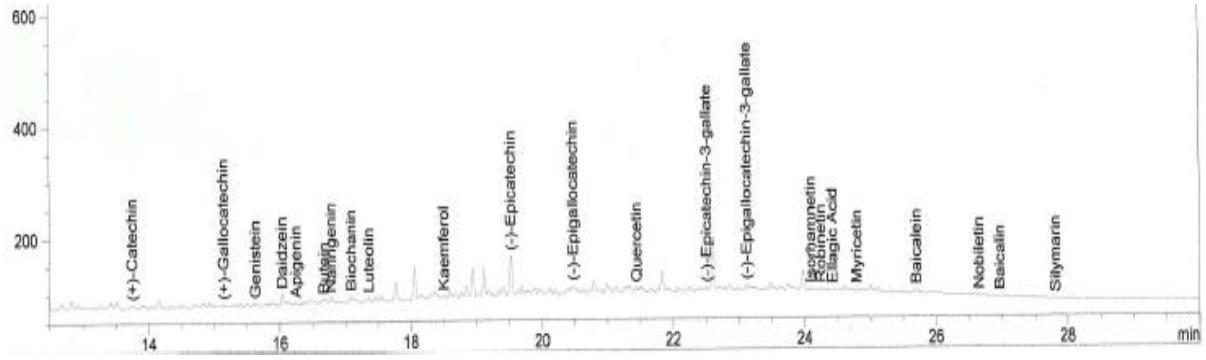

Figure 3: The Chromatogram of the Flavonoids Fraction of the Leaves of *Chromolaena odorata.*

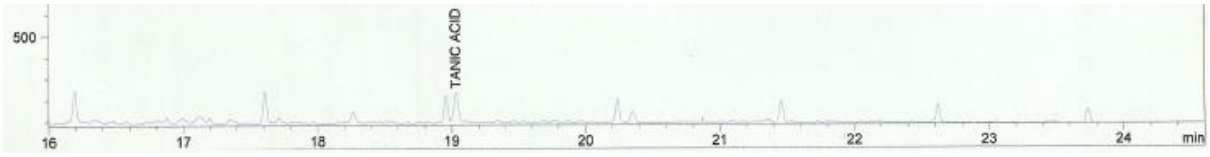

Figure 4: The Chromatogram of the Tannins Fraction of the Leaves of *Chromolaena odorata.*

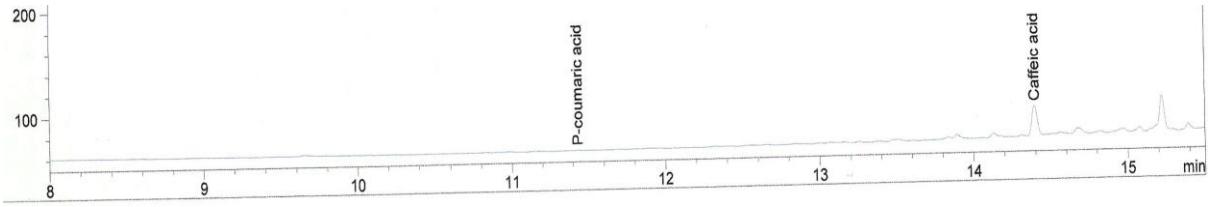

Figure 5: Chromatogram of Hydroxycinnamic Acid Derivatives Fraction of the Leaves of *Chromolaena odorata.*

RESULTS

The alkaloids composition of the leaves of *Chromolaena odorata* is shown in Table 1. Thirty-eight known alkaloids were detected, consisting mainly of akuammidine (44.74%), voacangine (24.51%), echitamine (11.30%), angustifoline (3.61%), lupanine (3.58%), echitamidine (3.29%), augustamine (2.36%) and crinamidine (1.21%).

The carotenoids composition of the leaves of *Chromolaena odorata* is presented in Table 2. Five known carotenoids were detected, consisting mainly of lutein (48.30%), carotene (33.30%), antheraxanthin (8.95%), neoxanthin (7.50%) and violaxanthin (1.95%).

Table 3 shows the flavonoids composition of the leaves of *C. odorata*. Twenty-three known flavonoids were detected, consisting mainly of kaempferol (19.63%), (-)-epicatechin (16.63%), robinetin (8.75%), baicalein (7.66%), biochanin (5.15%), ellagic acid (4.41%), naringenin (4.24%), daidzein (3.99%), (-)-epigallocatechin (3.91%), myricetin (3.34%), butein (3.27%), (+)-catechin (2.90%), quercetin (2.83%), (-)-epigallocatechin-3-gallate (2.29%), apigenin (1.77%), genistein (1.68%), isorhamnetin (1.66%), baicalin (1.64%), luteolin (1.46%) and nobiletin (1.33%).

The lignans composition of the leaves of *C. odorata* is presented in Table 4. Seven known lignans were detected, consisting mainly of galgravin (59.39%), retusin (16.61%), dehydroabietic acid (11.07%), sakuranin (9.42%), epieudesmin (3.51%), apigenin-4',7-dimethyl ether (0.004%) and (9E,12E,15E)-9,12,15-octadecatrien-1-ol (0.000008%).

The benzoic acid derivatives' composition of the leaves of *C. odorata* is shown in Table 5. Four known benzoic acid derivatives were detected, consisting mainly of 4-hydroxybenzaldehyde (36.63%), ferulic acid (26.45%), 4-hydroxybenzoic acid (19.67%) and vanillic acid (17.25%).

Table 6 shows the saponins and terpenes composition of the leaves of *C. odorata*. Four known saponins were detected, consisting mainly of Avenacin A1 (61.92%), Avenacin B1 (36.53%), Avenacin B2 (1.36%) and Avenacin A2 (0.19%). Five known terpenes were detected, consisting mainly of β-amyrin (31.12%), lupeol (21.88%), bauerenol acetate (20.91%), taraxerol (16.58%) and α-amyrin (9.51%).

The phytosterols, hydroxycinnamic acid derivatives' and tannins composition of the leaves of *C. odorata* is presented in Table 7. Two known phytosterols, stigmasterol (66.22%) and sitosterol (33.78%); and two known hydroxycinnamic acid dervatives, p-coumaric acid (53.48%) and caffeic acid (46.52%), were detected. Only tannic acid was detected in the tannin fraction.

Table 1: Alkaloids composition of *Chromolaena odorata* leaves.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
Choline	7.054	87.010	35.239
Trigonelline	7.641	0.000	0.000
Angustifoline	7.918	4160.913	1685.170
Sparteine	8.938	150.900	61.114
Ellipcine	9.742	195.043	78.992
Lupanine	11.042	4123.120	1669.864
13-α-Hydrorhombifoline	11.354	185.288	75.042
9-Octadecenamide	12.935	178.615	72.339
Dihydro-oxo-demethoxyhaemanthamine	14.149	250.778	101.565
Augustamine	14.917	2724.698	1103.503
Oxoassoanine	15.394	233.089	94.401
Cinchonidine	16.244	401.626	162.659
Cinchonine	16.367	219.048	88.715
Crinane-3α-ol	16.487	557.340	225.723
Buphanidrine	16.668	267.189	108.212
Indicine-N-oxide	17.545	200.099	81.040
Undulatine	18.586	98.313	39.817
Powelline	18.660	94.374	38.221
Ambelline	19.677	145.460	58.911
6-Hydroxybuphanidrine	20.466	347.458	140.721
Acronycine	21.124	274.985	111.369
Monocrotaline	21.323	324.801	131.544
6-Hydroxypowelline	21.817	727.675	294.709
Nitidine	22.357	226.512	91.737
Crinamidine	23.964	1396.115	565.427
1β,2β-Epoxyambelline	24.610	398.842	161.531
6-Hydroxyundulatine	24.788	294.084	119.104
Epoxy-3,7-dimethoxycrinane-11-one	25.477	48.554	19.664
Echitamidine	26.733	3788.677	1534.414
Akuamidine	26.829	51580.800	20890.220
Voacangine	27.060	28260.600	11445.540
Mitraphylin	27.427	0.495	0.200
Camptothecin	28.197	117.330	47.519
Echitamine	28.633	13028.079	5276.372
Colchicine	28.921	60.929	24.676
Emetine	29.576	35.465	14.363
Tetrandrine	29.759	79.040	32.011
Thalicarpin	30.425	11.443	4.634
Paclitaxel	31.944	9.082	3.678
Total		83385.600	33771.170

Table 2: Carotenoids Composition of *Chromolaena odorata* Leaves.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
Neoxanthin	19.522	1.4016	0.5677
Viola-xanthin	20.594	0.3644	0.1476
Anthera-xanthin	21.438	1.6718	0.6771
Carotene	23.231	6.2194	2.5188
Lutein	24.790	9.0213	3.6536
Total		18.6785	7.5648

Table 3: Flavonoids Composition of *Chromolaena odorata* Leaves.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
(+)-Catechin	13.754	2584.891	1046.881
(+)-Gallocatechin	15.150	618.683	250.566
Genistein	15.630	1500.223	607.590
Daidzein	16.038	3564.475	1443.612
Apigenin	16.246	1579.728	639.790
Butein	16.673	2918.199	1181.871
Naringenin	16.784	3781.739	1531.604
Biochanin	17.091	4599.896	1862.958
Luteolin	17.361	1306.990	529.331
Kaempferol	18.502	17521.099	7096.045
(-)-Epicatechin	19.524	14845.421	6012.396
(-)-Epigallocatechin	20.479	3494.504	1415.274
Quercetin	21.444	2530.172	1024.720
(-)-Epicatechin-3-gallate	22.513	88.894	36.002
(-)-Epigallocatechin-3-gallate	23.121	2047.607	829.281
Isorhamnetin	24.095	1478.441	598.769
Robinetin	24.244	7810.409	3163.216
Ellagic acid	24.422	3937.663	1594.753
Myricetin	24.794	2984.677	1208.794
Baicalein	25.696	6837.714	2769.274
Nobiletin	26.649	1182.939	479.090
Baicalin	26.965	1465.756	593.631
Silymarin	27.811	587.353	237.878
Total		89267.463	36153.323

Table 4: Lignans Composition of *Chromolaena odorata* Leaves.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
2-Allyl-5-ethoxy-4-methoxyphenol	11.377	0.00000	0.00000
(9E,12E,15E)-9,12,15-Octadecatrien-1-ol	14.097	0.00009	0.00004
Apigenin-4',7-dimethyl ether	16.274	0.04301	0.01742
Dehydroabietic acid	18.620	131.33640	53.19124
Retusin	19.459	197.03010	79.79719
Galgravin	20.495	704.61600	285.36948
Epieudesmin	22.323	41.67550	16.87858
Sakuranin	24.003	111.81670	45.28576
Total		1186.51770	480.53967

Table 5: Benzoic Acid Derivatives' Composition of *Chromolaena odorata* Leaves.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
4-Hydroxybenzaldehyde	8.933	85.483	34.62145
4-Hydroxybenzoic acid	12.359	45.903	18.591
Vanillic acid	15.234	40.247	16.300
Ferulic acid	18.049	61.717	24.995
Total		233.349	94.506

Table 6: Saponins and Terpenes Composition of *Chromolaena odorata* Leaves.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
Saponins			
Avenacin A1	21.476	4072.189	1649.237
Avenacin B1	23.073	2402.751	973.114
Avenacin A2	24.842	12.382	5.015
Avenacin B2	26.303	89.524	36.257
Total		6576.846	2663.622
Terpenes			
Taraxerol	19.525	27.188	11.011
α-Amyrin	20.542	15.601	6.319
β-Amyrin	21.441	51.040	20.671
Lupeol	23.234	35.876	14.530
Bauerenol acetate	24.794	34.294	13.889
Total		163.999	66.420

Table 7: Phytosterols, Hydroxycinnamic Acids Derivatives' and Tannins Composition of *Chromolaena odorata* Leaves.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
Phytosterols			
Cholesterol	20.596	0.000	0.000
Cholestanol	21.439	0.000	0.000
Ergosterol	22.442	0.000	0.000
Campesterol	22.986	0.000	0.000
Stigmasterol	23.273	2138.640	866.149
5-Avenasterol	24.169	0.000	0.000
Sitosterol	24.821	1090.800	441.774
Total		3229.440	1307.923
Tannins			
Tannic acid	19.031	9637.926	3903.360
Total		9637.926	3903.360
Hydroxycinnamic acids derivatives			
p-Coumaric acid	11.433	5263.806	2131.842
Caffeic acid	14.128	4578.304	1854.213
Total		9842.111	3986.055

DISCUSSION

Voacangine exhibits cardiovascular toning, central nervous system depressant, anti-convulsive, anti-pyretic, analgesic, local anesthetic and anti-leishmanial activities, potentiation of barbiturates hypnotic and anti-cholinesterase effects (Carroll and Starmer, 1967; Okuyama et al., 1992; De Smet, 1996; Andrade et al., 2005; Pratchayasakul et al., 2008; Pereira et al., 2011). The pharmacological activities of echitamine include hypotensive activity in normotensive anaesthetized animals, diuretic and anti-tumor activities, inductions of negative chronotropic and inotropic responses in isolated atrial muscle strips, relaxation of isolated vascular and extra-vascular smooth muscles, inhibition of electrically-provoked and agonist-induced contractions or relaxations of isolated smooth muscle preparations, paralysis of electrically-evoked skeletal muscle twitches (Chandrasekaran and Nagarajan, 1981; Ojewole, 1984; Kamarajan et al., 1991; Jagetia et al., 2005).

Akuammidine is a hypotensive, anti-plasmodial, anti-depressant and skeletal muscle relaxant, with a local analgesic activity that is about 3 times as potent as cocaine (Dhingra and Sharma, 2006; Hirasawa et al., 2009). It acts selectively as a sympatholytic, unaccompanied by para sympatholytic effects. It inhibits the irritability of the sympathetic nervous

system and opposes akuammine (Nyunaï and Njifutié, 2006). Epoxyambelline both alone and in 1:1 combination with ambelline, produces moderate to pronounced activation of mouse spleen lymphocytes (Ghosal et al., 1984). This probably may be the basis of the immune-modulatory activity of the leaves.

Lutein has antioxidant, photo-protective and anti-cancer activities (Sertie et al., 1990; Dillard and German, 2000; Pintea et al., 2003; Tinoi et al., 2006). Carotenes have pro-vitamin A, antioxidant and anti-cancer activities (Dillard and German, 2000; Tinoi et al., 2006).

Stigmasterol is a brassinosteroid, which are growth regulator and signaling molecules essential for normal plant growth (Rao et al., 2002; Ayad et al., 2009). As plant hormones, steroids have regulatory function in cell elongation and division, vascular differentiation and other diverse developmental processes (Priti, 2003; Sasse, 2003). Brassinosteroids have the ability to confer resistance to plants against various abiotic stresses (Priti, 2003). Plant sterols modulate the activity of ATPase (Piironen et al., 2000). Cholesterol and stigmasterol stimulate export of H+ at low concentrations, whereas all other sterols act as inhibitors. In animals, phytosterols have anti-inflammatory, hypocholesterolemic and anti-cancer (Dillard and German, 2000; Piironen et al., 2000; Tinoi et al., 2006) activities. β-Sitosterol has anti-inflammatory, anti-neoplastic, hypoglycemic, atheroprotective, hepatoprotective, immune-modulating, anti-pyretic and hypocholesterolemic activities (Ivorra et al., 1988; Nan-Lin and Pin Tome, 1988; Dillard and German, 2000; Piironen et al., 2000; Beta-sitosterol Monograph, 2001; Berger et al., 2004). It also has hypotensive properties with little effect on heart rate (Ogundaini et al., 1983, cited in Ogundaini et al., 2005). Tannic acid is an antioxidant, hypoglycemic, hepatoprotective and hypocholesterolemic agent (Liu et al., 2005; Basu et al., 2007; Mittal et al., 2010).

The leaves have higher quercetin (73 mg/kg, US Highbush Blueberry Council, Summer 2005; 17-24 mg/kg, Hakkinen et al., 1999), myricetin (26 mg/kg, US Highbush Blueberry Council, Summer 2005; 23-26 mg/kg, Hakkinen et al., 1999) and epicatechin (11.1 mg/kg, US Highbush Blueberry Council, Summer 2005) contents than blueberry.

Luteolin has antibacterial, anti-inflammatory, anti-mutagenic, antioxidant and immune-modulating activities (Dillard and German, 2000). Catechins have anti-carcinogenic, antimicrobial, antioxidant and hypocholesterolemic activities (Tapas et al., 2008). They protect LDLs from oxidation and prevent cardiovascular disease (Kang et al., 1999; Demeule et al., 2000; Chu et al., 2004). They also protect neurons, enhance resistance of red blood cells to oxidative stress and inhibit ultraviolet radiation-induced oxidative stress in the skin (Chu et al., 2004). Kaempferol is known for its strong antioxidant and anti-inflammatory properties, and has antibacterial, anti-cancer, anti-fungal, cardioprotective, hepatoprotective, hypocholesterolemic, hypo-glycemic, hypotensive and immune-modulatory activities (Ahmad et al., 1993; Song et al., 2003; De-Sousa et al., 2004; Oh et al., 2004; Lim, et al., 2007; Lau, 2008; Lin et al., 2011). Over the years, studies have shown that it can help in the prevention and treatment of cancers, neuron disorder, and cardiovascular, heart, spinal cord, and brain disease (Lau, 2008). It inhibits both oxidative susceptibility of LDL in vitro, and platelet aggregation (Kowalski et al., 2005; Lau, 2008). Its rutinoside causes remarkable decrease in systolic, diastolic, mean arterial blood pressure and heart rate (Ahmad et al., 1993).

Apigenins have antioxidant, hypoglycemic, hepatoprotective, diuretic, hypotensive, smooth muscle relaxation enhancing, anti-tumor, anti-phlogistic, anti-spasmodic, anti-inflammatory, anti-proliferative, antibacterial, oxygenase inhibitory, and apoptosis inducing activities (National Cancer Institute, n.d.; Dillard and German, 2000; Martini et al., 2004; Abate et al., 2005; Hougeea et al., 2005; Skerget et al., 2005; Wang et al., 2005; Zheng et al., 2005; Saeed et al., 2006; Tajdar and Sarwat, 2006; Panda and Kar, 2007). The monomer apigenin is fit into a pharmacophore model for ligands binding to the GABA receptor benzodiazepine site (Svenningsen et al., 2006). It acts as an inhibitor of IL-4 synthesis and CD40 ligand expression by basophils. It also has protective effect on radiation-induced chromosomal damage in human lymphocytes (Kanokporn Noy et al., 2005; Saeed et al., 2006). It inhibits motility and invasion of prostate carcinoma and melanoma cells, disrupts actin cytoskeleton organization, and inhibits FAK/Src signalling (Caltagirone et al., 2000; Franzen et al., 2009).

Naringenin has antioxidant, hepatoprotective, hypocholesterolemic, gastroprotective, anti-cancer, cardioprotective and anti-ulcer activities (Parmar and Parmar, 1998; Lee et al., 1999; Raj Narayana et al., 2001; Lee et al., 2004; Pari and Gnanasoundari, 2006; Ganapathy et al., 2008; Wei-wei et al., 2009). Naringin has hypoglycemic activities (Jung et al., 2004), while epicatechin has antioxidant, hypoglycemic and immune-modulatory activities (Chakravarthy et al., 1982; Vinardell and Mitjans,

2008). Diadzein and genistein, lower total cholesterol, LDL-cholesterol and blood pressure, reduces coronary and generalized thrombosis, and exhibits oestrogen-like properties (Houston, 2002, 2005, 2007; Dixon and Ferreira, 2002; Pereira et al., 2009). They inhibit tyrosine kinase activity, which decreases vascular smooth muscle contraction and lowers blood pressure, and inhibit oxidation activity in the blood vessel (Houston, 2002, 2005, 2007). Genistein also has hypoglycemic activity (Lee, 2006). Nobiletin has anti-fungal activity (Tapas et al., 2008), while silymarin has antioxidant and hepatoprotective activities (Suja et al., 2004). Luteolin has antibacterial, anti-inflammatory, anti-mutagenic and antioxidant activities (Dillard and German, 2000). Myricetin exhibits hypoglycemic (Ong and Khoo, 1996), antibacterial, anti-gonadotropic and antioxidant activities (Dillard and German, 2000).

Quercetin is frequently used therapeutically in allergic conditions, including asthma and hay fever, eczema, and hives, due to its ability to mediate production and manufacture of pro-inflammatory compounds (Yoshimoto et al., 1983; Kim et al., 1998; Thorne Research Inc., 1998; Thornhill and Kelly, 2000; Lakhanpal and Kumar, 2007). Additional clinical uses include treatment of gout, pancreatitis and prostatitis, which are also, in part, inflammatory conditions (Lakhanpal and Kumar, 2007). It has antibacterial, anti-anemic, antiviral, anti-carcinogenic, anti-cataractogenic, anti-diabetic, antioxidant, anti-hypertensive, anti-inflammatory, anti-allergic, gastro-protective, hepatoprotective and hypocholesterolemic activities (Yoshimoto et al., 1983; Kim et al., 1998; Thorne Research Inc., 1998; Arai et al., 2000; Caltagirone et al., 2000; Lamson and Brignall, 2000; Peres et al., 2000; Thornhill and Kelly, 2000; Duarte et al., 2001; Houston, 2002, 2005, 2007; Lee et al., 2003; Pavanato et al., 2003; Vessal et al., 2003; Sen et al., 2005; Lakhanpal and Kumar, 2007; Lim et al., 2007; Rigano et al., 2007; Tapas et al., 2008; Chen, 2010). It inhibits calmodulin dependent enzymes that influence membrane permeability and secretions of histamine from mast cells (Bennett et al., 1981; Middleton et al., 1981; Havsteen, 1983; Pearce et al., 1984; Middleton and Drzewiecki, 1985; Fox et al., 1988; Otsuka et al., 1995; Thornhill and Kelly, 2000; Lakhanpal and Kumar, 2007). It has also been shown to limit the function of adhesion molecules on endothelial cells; chelate ions of transition metals such as iron, thereby preventing them from initiating the formation of oxygen free radicals; directly inhibit lipid peroxidation; and inhibit platelets aggregation (Sorata et al., 1984; Afanas'ev et al., 1989; Middleton and Anne, 1995; Ferrali et al., 1997; Osman et al., 1998; Lakhanpal and Kumar, 2007). Its intake protects against coronary heart disease, caused by oxidized LDL (bad cholesterol) (Hertog et al., 1993, 1995; Thorne Research Inc., 1998; Lakhanpal and Kumar, 2007). Quercetin inhibits aldose reductase, the first enzyme in the polyol pathway, and so, may be beneficial in the nutritional management of diabetes, and the prevention of long-term diabetic complications such as cataracts, nephropathy, retinopathy and neuropathy (Lakhanpal and Kumar, 2007). It may also provide beneficial effects in people with diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity. Quercetin also spares vitamin C, stabilizes cell membranes including those of mast cells, and helps in maintaining lens transparency after an oxidative insult.

The caffeic acid content is comparable to those reported for thyme, aniseed, caraway and rosemary (> 1000 mg/kg) (IARC, 1993). p-Coumaric acid has antioxidant and anticancer properties (Kikugawa et al., 1983; Ferguson et al., 2005; Oksana et al., 2012). It represses the expression of T3SS genes of the plant pathogen Dickeya dadantii, suggesting that plants can also defend against bacterial pathogens by manipulating the expression of the type III secretion system (Yan et al., 2009; Oksana et al., 2012). Lignans are one of the major classes of phytoestrogens and also act as antioxidants.

In general, phenolics are important for cell structure, signaling and pigmentation (Adyanthaya, 2007). Phenolic acids are known to act as allelochemicals, protect plants against environmental and biological stress such as high energy radiation exposure, bacterial infection or fungal attacks, cold stress, hyperthermia and oxidative stress (Dillard and German, 2000; Tüzen and Özdemír, 2003; Yoshioka et al., 2004; Adyanthaya, 2007). Thus, the present result suggests a likely allelopathic potential of *Chromolaena odorata*.

4-Hydroxybenzoic acid esters (also called parabens), are widely used as antimicrobial agents in a large variety of food, pharmaceutical, and cosmetic products (Valkova et al., 2001). 4-hydroxybenzoic acid has antifungal, antimicrobial, anti-mutagenic, anti-sickling and estrogenic activities (Pugazhendhi et al., 2005; Chong et al., 2009; Khadem and Marles, 2010; Oksana et al., 2012). In plants, p-hydroxybenzoic acid increases the impermeability of the cell wall, leading to increased resistance against pathogen infection (Tan et al., 2004; Horváth et al., 2007). It is synthesized de novo in stems and petioles in response to a mobile signal (Smith-Becker et al., 1998). It also has a growth stimulation effect (Kamaya et al., 2006). Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is used as a

flavoring agent. It has anti-inflammatory, anti-sickling, anthelmintic, hepatoprotective and immune-modulating activities (Chiang et al., 2003; Itoh et al., 2009, 2010; Khadem and Marles, 2010; Oksana et al., 2012). It also inhibits snake venom 5'-nucleotidase (Dhananjaya et al., 2009; Khadem and Marles, 2010; Oksana et al., 2012). 4-Hydroxybenzaldehyde is used as a flavor and fragrance agent. Ferulic acid protects against coronary disease, increases sperm viability and has hypocholesterolemic activity (Shiyi and Kin-Chor, 2004).

Saponins are reported to have broad range of pharmacological properties (Soetan, 2008). They have hypoglycemic properties (Soetan, 2008). Avenacins have antimicrobial properties (Armah et al., 1999; Mert Türk et al., 2005; Soetan, 2008). Avenacin A-1 has potent anti-fungal activity (Armah et al., 1999), and is believed to confer resistance against a range of soil fungi (Field et al., 2006). Ouabain is a cardiotonic steroid (Dmitrieva and Doris, 2002).

Terpenes are used as flavor enhancers in food, fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy (Kappers et al., 2005; Zwenger and Basu, 2008). They have anti-cancer, antimicrobial, antioxidant, cardiotonic and insecticidal activities (Dillard and German, 2000; Islam et al., 2003; Dewick, 2004; Soetan, 2008). Limonoids have anti-cancer properties (Dillard and German, 2000; Islam et al., 2003). Limonene promotes glutathione-S-transferase activity and cancer cell apoptosis. In a bioremediative study by Suttinun et al. (2004), limonene and pinene were found to increase the uptake and subsequent degradation of trichloroethylene by bacteria.

CONCLUSION

These results show that the leaves are a rich source of alkaloids, tannins, flavonoid, saponins, hydroxycinnamic acids and lignans.

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