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Gas chromatographic characterization of the flavonoids, alkaloids, saponins, and tannins isolated from *C. dolichopentalum* leaves

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ABSTRACT

This study subjected the flavonoids, alkaloids, saponins and tannins isolated from Combretum dolichopentalum to Gas Chromatographic-Flame Ionization Detector (GC-FID) analyses. Keampferol (295915±18219.31 x10^{-4} mg/100g), akuamidine (41.452±1.339 x10^{-3} mg/100g) and sapogenin (6153.76±602.44 x10^{-2} mg/100g) showed the highest concentration of flavonoid, alkaloid and saponin respectively. The phytochemicals identified and quantified in C. dolichopentalum indicate bioactive compounds with pharmacological potentials. The plant constituents could act as antioxidants and used as anti-inflammatory, hypotensive, anticholesterolemic, antiarrythmic agent in the treatment and/or management of diseases and disease conditions.

Keywords: *C. dolichopentalum*, Gas chromatography, Keampferol, Akuamidine, Sapogenin, Tannic acid.

INTRODUCTION

Due to growing drug discovery from natural products, researchers and pharmaceutical industries have increasing interest in traditional health practices used around the world. This interest has been rekindled for decades due to systemic demonstration that plants are the richest source of drugs for traditional system of medicine, modern medicines, nuturaceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. The use of medicinal plants as fundamental components of the African traditional health care system is perhaps the oldest and the most assorted of all the therapeutic systems [2]. In many parts of Africa, medicinal plants are the most easily accessible and affordable healthcare resources available to the local communities.

Medicinal plants are used and marketed worldwide as herbal drugs or as single active ingredients over centuries. Besides their popular consumption to treat and cure human illness, plant derived natural products play important roles as a source of pharmacological tools to enable the understanding of the biochemical pathways and the aetiology of diseases [3]. Plants are sources of potential therapeutic agents against various diseases due to their biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites [4]. The use of medicinal plants in the management of diseases is an important alternative therapy widely employed in developing countries. Several investigations have yielded compounds with properties useful for the development of modern synthetic drugs for the management of several diseases [5]. It is estimated that 80 % of metabolites/plant extracts used as drugs and sold worldwide are derived from natural products and that over 100 new natural product-based lead drugs are in clinical development [6,7]. Aspirin, Atropine, artemisinin, colchine, digoxin, ephedrine, reserpine, taxol, tubocurarine, vincristine and vinblastine are few important examples of what medicinal plants have given us in the past [8]. The pharmaceutical effects of plants are due to the presence of phytoconstituents called phytochemicals which provide health benefits for humans further than those attributed to macronutrients and micronutrients [9]. *Combretum dolichopentalum* belonging to the family of combretaceae, and commonly known as 'food for the small bird' in Nigeria by the Ibos, is used as herbal therapy in both Africa (Nigeria) and India for treating disease of the alimentary tract. Hot aqueous extract of the plant leave is prepared as soup for drinking [10].

The aqueous extract is also consumed by women after parturition for reconditioning of the uterus after delivery in Mbaise, Imo state of Nigeria [11]

EXPERIMENTAL SECTION

Preparation of plant sample

Fresh leaves of *C. dolichopentalum* were harvested from a farm in Obinze in Owerri West Local Government Area of Imo State, Nigeria. The plant was identified by Mr. A. Ozioko, of the Bioresource Development and Conservation Program (BDCP), Research Centre at Nsukka, Enugu State, Nigeria. The fresh leaves were plucked from their stems, washed with distilled water and allowed to dry at room temperature. The dried samples were pulverized (using electric blender) and stored in an airtight container kept in a dark cupboard.

Phytochemical screening by Gas Chromatography (GC)

Extraction and Determination of Saponin Content; Extraction was done as outlined by Guo et al. [12]. The pulverized samples (10 g) of *C. dolichopentalum* were extracted thrice with 20 ml of methanol for 20 min with ultra-sonication. The combined extract were concentrated to syrup under reduced pressure, and then suspended in water. The suspension was extracted with petroleum ether, chloroform and 1-butanol saturated with water, successively, to give the respective extract after removal of the solvent. The 1-butanol-soluble part was subjected to a D101 macro-porous absorption resin column and the column was successively eluated with distilled water and ethanol. The ethanol eluates were collected and some portions of the residues were concentrated to 1 ml in a vial for the gas chromatographic analysis. One microliter (1 µ) was injected into the injection port of the gas chromatography equipment. The extract obtained was subjected to gas chromatography on a capillary DB-225ms column with column dimensions; 30 m × 0.25 mm × 0.25 µm. The inlet and detection temperatures were 250˚C and 320˚C. The equipment was run on split injection, with 20:1 split ratio, and utilised nitrogen as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 40 psi respectively. The oven temperature was run initially at 60˚C for 5 min. The first ramping was at 12˚C/min for 18 min. The second ramping was at 15˚C/min for 5 min. The GC analysis of saponin extract was done in duplicate.

Extraction and Determination of Flavonoid Content; Extraction was done as outlined by Millogo-kone et al [13]. Fifty grams (50 g) of the pulverized sample were transferred to a Stoppard flask and treated with ethanol until the powder was fully soaked. The flask was shook every hour for the first six hours and then kept aside and shook after 24 hours. This process was repeated for three days and then the extract was collected and evaporated to dryness using nitrogen stream. Exactly 0.5 g of the concentrate was weighed into 250 ml conical flask with the addition of 100 ml of de-ionized water and boiled for 10 minutes. The flavonoid extract was obtained by pouring 100 ml of boiling methanol:water (70:300 v/v) onto the materials. The homogenate was allowed to macerate for about 4 hours and then filtered through a Whatman No. 1 filter paper. The filtrate was derivatized for volatility in gas chromatography analysis. The extract obtained was subjected to gas chromatography on a HP INNOWax column with column dimensions; 30 m × 0.25 mm × 0.25 µm. The inlet and detection temperatures were 250˚C and 320˚C. The equipment was run on split injection, with 20:1 split ratio, and utilised nitrogen as the carrier gas. The hydrogen and compressed air pressures were 22 psi and 35 psi respectively. The oven temperature was run initially at 50˚C. The first ramping was at 80˚C/min for 20 min and maintained for another 4 min. The second ramping was at 12˚C/min for 4 min and maintained for another 4 min. The GC analysis of flavonoid extract was done in duplicate.

Extraction and Determination of Alkaloid Content; Extraction was done as outlined by Ngounou et al. [14]. One gram (1 g) of the pulverized sample was macerated in 10 ml hexane for 72 hours. This was filtered and the residue air dried and treated with 10 % aqueous ammonia and macerated in chloroform for 24 hours. After the filtration and evaporation at reduced pressure, the resultant crude extract was treated with 7.5 ml of 5 % aqueous HCl. The aqueous phase was made alkaline with aqueous ammonia and extracted thrice with chloroform. The chloroform fraction was washed with water and the extract recovered with the aid of rotary evaporator. The extract was concentrated by using anhydrous sodium sulphate to eliminate water. The extract was subjected to gas chromatography on a DB-5MS column capillary with column dimensions; 30 m × 0.25 mm × 0.25 µm. The inlet and detection temperatures were 250˚C and 320˚C. The equipment was run on split injection, with 20:1 split ratio, which utilized nitrogen as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 38 psi respectively. The oven temperature was run initially at 60˚C for 5 min. The first ramping was at 10˚C/min for 20 min and the second ramping at 15˚C/min for 4 min. The GC analysis of alkaloid extract was done in duplicate.

Extraction and Determination of Tannin Content; Extraction was done as outlined by Swain [15]. Exactly 0.2 g of the pulverized sample was measured into 50 ml borosilicate beaker. Then, 20 ml of 50 % methanol was added and covered with paraffin and placed in a water bath at 80 ^{o}C for 1 hour. The content was stirred with a glass rod to

prevent lumping. The extract was quantitatively filtered using a doubled layered Whatman No.1 filter paper into a 100 ml volumetric flask using 50 % methanol to rinse. This was concentrated to 2 ml in the borosilicate vial for the gas chromatographic analysis. One microliter (1 µ) was injected into the injection port of the gas chromatographic equipment. The extract obtained was subjected to gas chromatography on a HP-5 column with column dimensions; 30 m × 0.25 mm × 0.25 µm film. The inlet and detection temperatures were 250˚C and 320˚C. The equipment was run on split injection, with 20:1 split ratio, and utilised nitrogen as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 40 psi respectively. The oven temperature was run initially at 120˚C for 5 min and ramped at 10˚C for 20 min. The second ramping was at 15˚C/min for 5 min. The GC analysis of tannin extract was done in duplicate.

Statistical analyses

The duplicate data obtained for each isolate are presented in tables as mean±standard deviation and chromatographic profiles

RESULTS AND DISCUSSION

Phytochemicals are non-nutritive and protective chemicals that appear to act alone or in combination with vitamins and other nutrients in food to prevent or halt disease progression. In this study *C. dolichopentalum* leaves recorded high amounts of saponins, flavonoids, and tannins. Saponins showed high concentrations of tricogenin, sapogenin, tribuloin and saponine besides others (Figure 1; Table 1). Higher concentration of sapogenin was recorded in *C. dolichopentalum* compared to that reported in *Gongronema latifolium* (Benth) leaf [16]. Saponins protect DNA from damage by acting as antioxidant and anti-mutagenic agent. They can directly inhibit colon cancer. They posses antiviral effects and may be cardioprotective via their ability to lower cholesterol [17]. They are also responsible for many other important activities-Molluscidal, anthelmintic, antiulcerogenic, anticancer, antioxidant, immunomodulatory, anti-malarial, anti-bacterial, analgesic, anti-nociceptive, hepatoprotective [18].

Diosgenin one of the constituents of *C. dolichopentalum* is important in the control of metabolic diseases such as diabetes and obesity [19]. Several molecular candidates associated with fatty acid metabolism [20], inflammatory pathway [21], eicosanoid biosynthesis [22,23], cell proliferation and growth [24], apoptosis [22,23, and regulation of transcription [25] are affected (up or down-regulated) by diosgenin leading to tumour cell death.

Flavonoids such as apigenin, luteolin, kaempferol, isorhamnetin and quecertin were recorded in appreciable amount besides others (Figure 2; Table 2). Flavonoids exert a variety of biological effects [26] at cellular levels, as antioxidants, anti-inflammatory and anti-cancer agents. Concentrations of Apigenin, Kaempferol, and Isorhamnetin in *C. dolichopentalum* were higher than that reported in *Gongronema latifolium* (Benth) leaf [16]. Apigenin possess affinity for the opoid receptors, as a non-selective antagonist of all three opoid receptors [27]. Luteolin has antibacterial, anti-inflammatory, anti-mutagenic, antioxidant and immunomodulating activities [28]. Luteolin and apigenin activates the dopamine transporter [29].

Table 1: Isolated and identified saponins in the leaves of *C. dolichopentalum* using gas chromatography

			mg/100g x10^{-2}	
Names of Identified Saponin	**1**	2	Mean	STDEV
Hispogenin	0.037225	0.031888	0.03	0.00
Solagenin	1.6927	1.4675	1.58	0.16
Diosgenin	3.16466	2.7297	2.95	0.31
Tigogenin	1.80633	1.5832	1.69	0.16
Neochlorogenin	9.49701	8.1961	8.85	0.92
Hecogenin	136.193	182.95	159.57	33.06
Sapogenin	6579.754	5727.77	6153.76	602.44
Tribuloin	29.0494	24.4102	26.73	3.28
Yanogenin	10.619	9.084	9.85	1.09
Conyzorgin	0.10844	0.22847	0.17	0.08
Saponine	2968.03	2569.18	2768.61	282.03
Total	9739.952	8527.631	9133.79	857.24

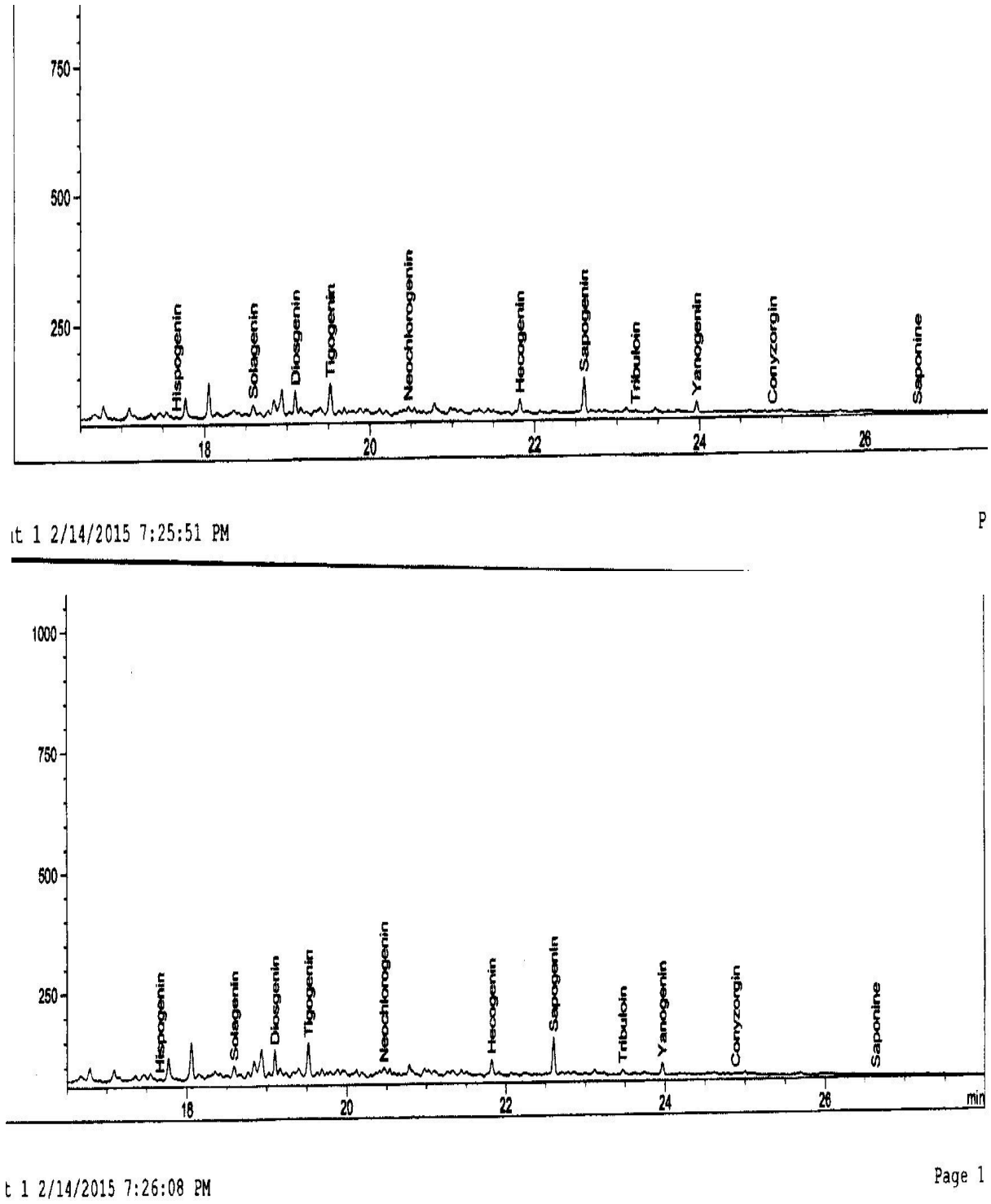
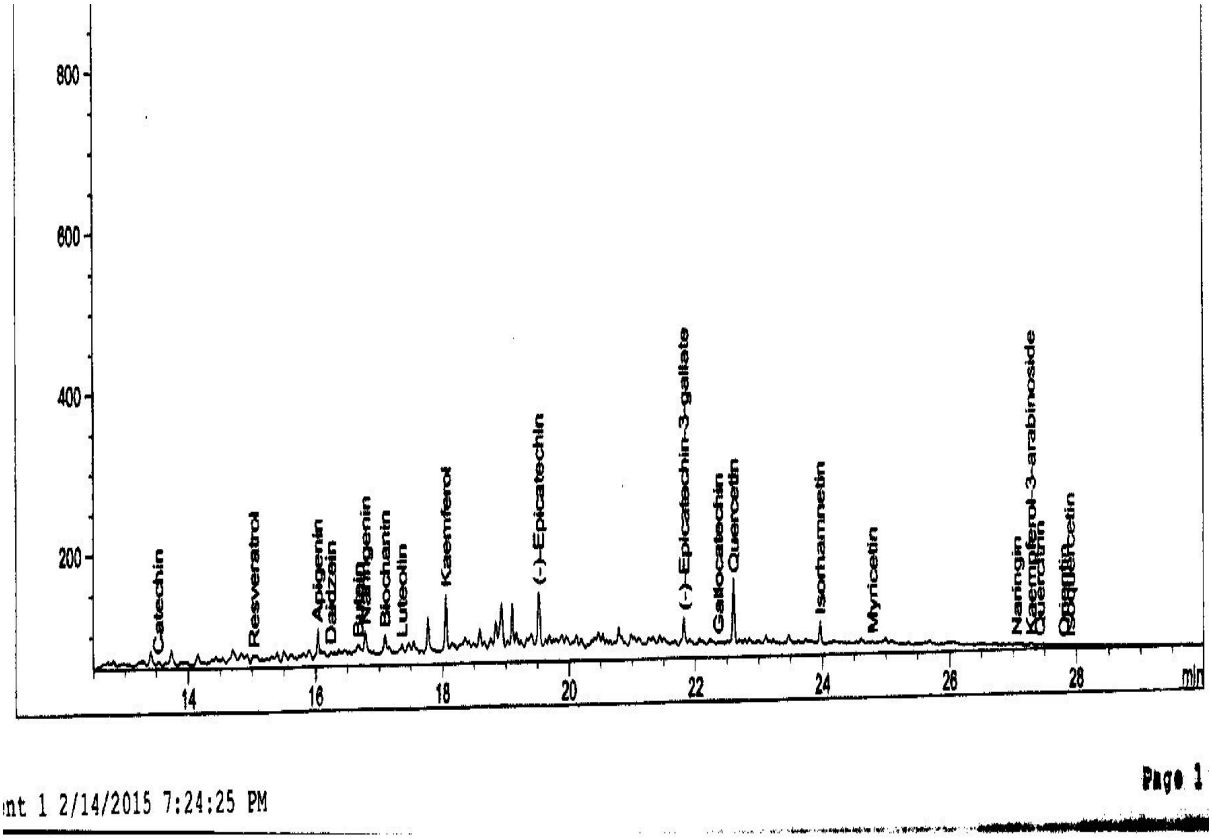


Figure 1: Chromatograms of saponins identified and quantified from the leaves of *C. dolichopentalum*



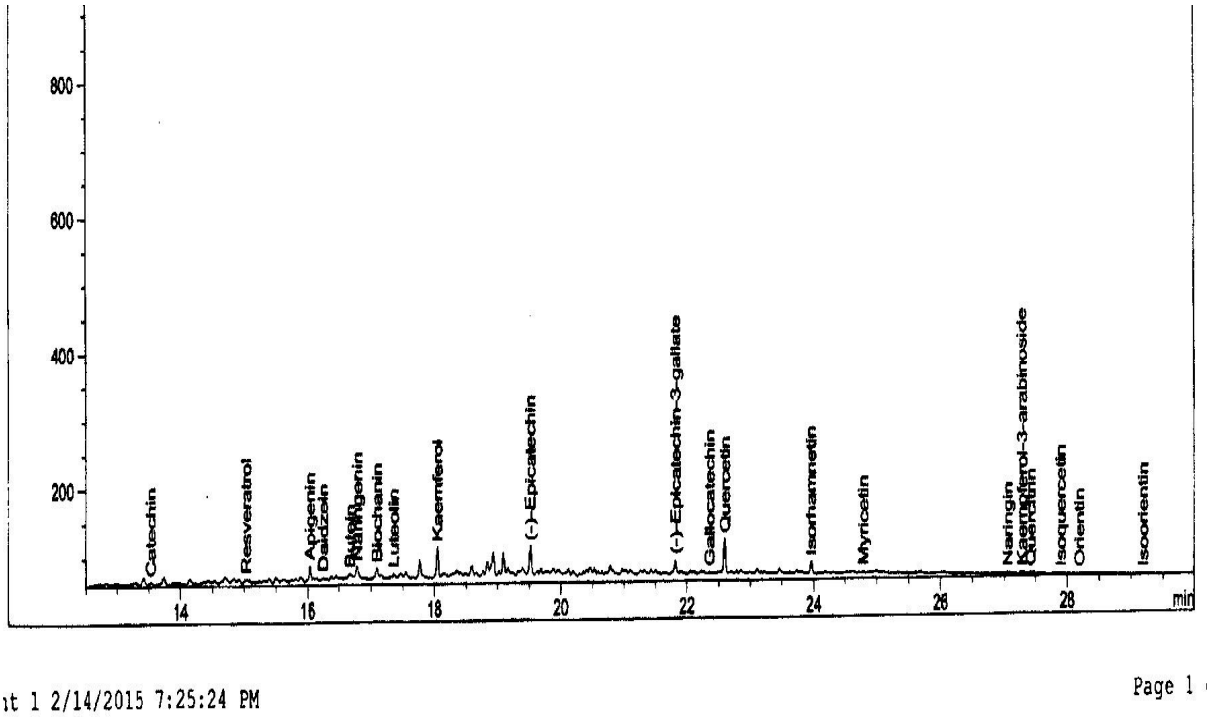


Figure 2: Chromatograms of flavonoids identified and quantified from the leaves of *C. dolichopentalum*

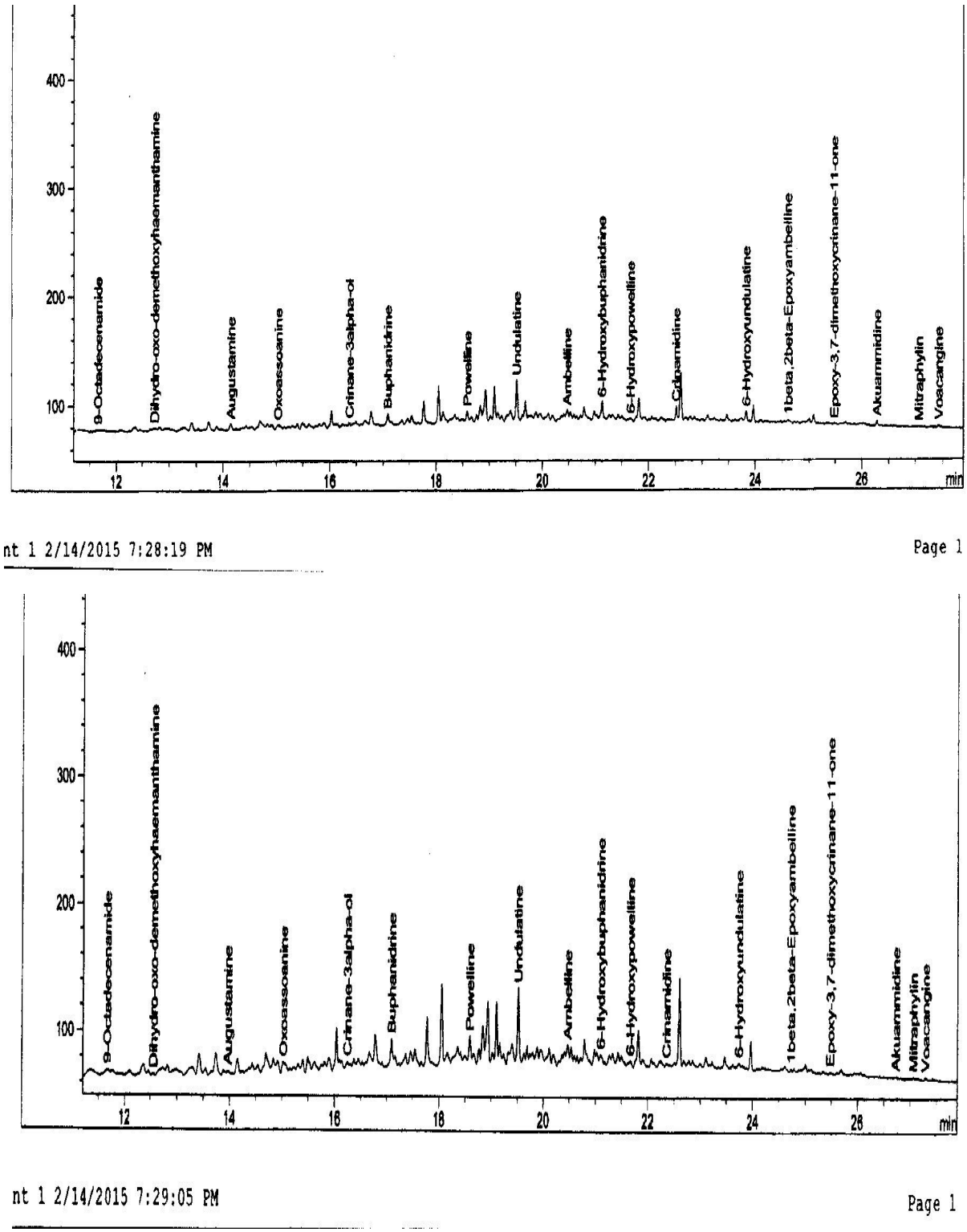


Figure 3: Chromatograms of alkaloids identified and quantified from the leaves of *C. dolichopentalum*

Table 2: Isolated and identified flavonoids in leaves of *C. dolichopentalum* using gas chromatography

			mg/100g x10^{-4}	
Names of Identified Flavonoids	**1**	2	Mean	STDEV
Catechin	0.02181	0.03576	0.03	0.01
Resvaratrol	1.0884	1.7968	1.44	0.5
Apigenin	33586	34067	33826.5	340.12
Daidzein	1.29822	2.1132	1.71	0.58
Butein	2.0742	3.3609	2.72	0.91
Naringenin	6.1529	9.9984	8.08	2.72
Biochanin	3.7986	6.2431	5.02	1.73
Luteolin	26502	22136	24319	3087.23
Kaempferol	308798	283032	295915	18219.3
(-)-Epicatechin	5.9374	9.6932	7.82	2.66
(-)-Epicatechin-3-gallate	0.1043	0.10726	0.11	0
Gallocatechin	1.4664	2.399	1.93	0.66
Quercetin	62359	51328	56843.5	7800.09
Isorhamnetin	55433	44075	49754	8031.32
Myricetin	835.16	43.291	439.23	559.94
Naringin	0.7983	1.2046	1	0.29
Kaempferol-3-arabinoside	0.58587	0.92309	0.75	0.24
Quercitrin	0.9921	0.45662	0.72	0.38
Isoquercetin	310.06	488.97	399.52	126.51
Orientin	0.48125	0.72309	0.6	0.17
Isoorientin	8.713	432.18	220.45	299.44
Total	487857	435642	461749	36921.8

Table 3: Isolated and identified alkaloids in the leaves of *C. dolichopentalum* using gas chromatography

			mg/100g x10^{-3}	
Names of Identified Alkaloids	**1**	2	Mean	STDEV
9. Octadecenamide	3.0514	4.9179	3.985	1.32
Dihydro-oxo-demethoxyhaemanthamine	3.7166	2.1746	2.946	1.09
Augustamine	0.79432	0.81176	0.803	0.012
Oxoassoanine	6.4266	7.4229	6.925	0.704
Crinane-3alpha-ol	13.579	16.804	15.192	2.28
Buphanidrine	29.116	56.339	42.728	19.25
Powelline	6.07674	10.415	8.246	3.068
Undulatine	5.3104	7.9602	6.635	1.874
Ambelline	6.61421	9.4801	8.047	2.026
6-hydroxybuphanidrine	3.9351	4.0891	4.012	0.109
hydroxypowelline	7.8645	10.47	9.167	1.842
Crinamidine	31.567	38.955	35.261	5.224
6-hydroxyundulantine	9.4319	18.182	13.807	6.187
1 beta, 2 beta-Epoxyambelline	9.2786	3.7761	6.527	3.891
Epoxy-3, 7 dimethoxycrinane-11-one	9.27721	11.933	10.605	1.878
Akuamidine	42.399	40.505	41.452	1.339
Mitrsaphylin	4.4844	4.6347	4.56	0.106
Voacangine	23.948	18.6013	21.275	3.781
Total	216.87	267.47	242.17	35.78

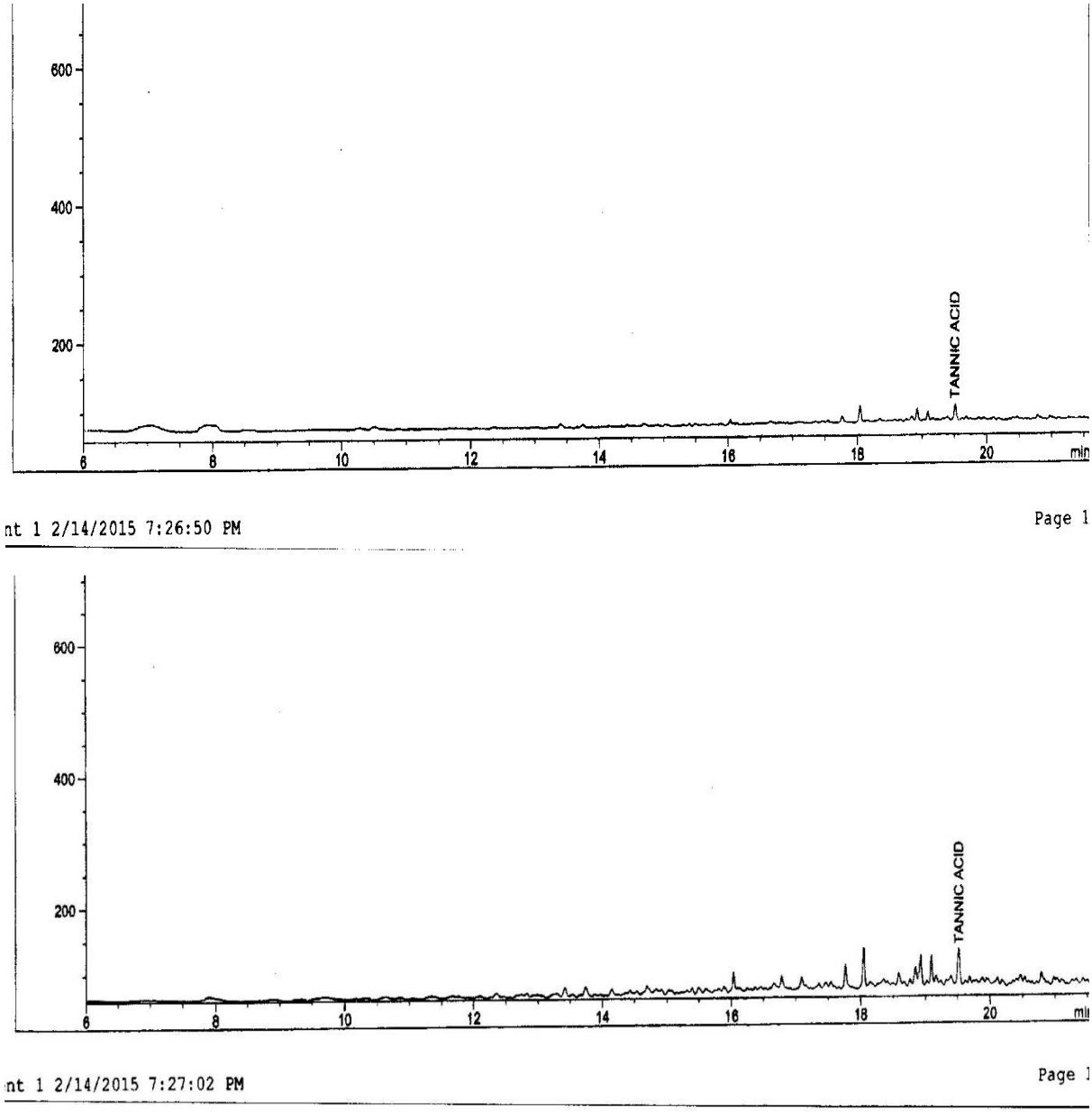


Figure 4: Chromatograms of tannins identified and quantified from the leaves of *C. dolichopentalum*

Table 4: Isolated and identified tannin in the leaves of *C. dolichopentalum* using gas chromatography

			mg/100g	
Name of Tannin	**1**	2	Mean	STDEV
Tannin acid	55.72	47.32	51.52	5.95
Total	55.72	47.32	51.52	5.95

Isorhamnetin may alternate diabetes complications, such as diabetic cataract, lipid peroxidation and high blood glucose levels [30]. Kaempferol has strong antioxidant and anti-inflammatory properties [31]. It has antibacterial, anti-cancer, anti-fungal, antioxidant, hypocholesterolemic, cardio-protective, hepatoprotective, hypoglycemic, hypotensive and immunomodulatory activities [31,32]. Kaempferol is a possible agent for cancer treatment [33,34,35], this is because kaempferol reduces the resistance of cancer cells to Vinblastine and paclitaxel- anticancer drugs [36]. Quercetin is a strong antioxidant and may induce insulin secretion by activation of L-type calcium channels in the pancreatic β-cells [37]. Catechins have anti-carcinogenic, antimicrobial, antioxidant, and hypocholesterolemic activities [38,39]. Myricetin found in *C. dolichopentalum* exhibits antibacterial, anti-gonadotropic, antioxidant [28]. Biochanin A, one of the bioactive compounds found in *C. dolichopentalum* has been reported to have anticancer, anti-inflammatory, antimicrobial, antioxidant, immunomodulatory and hepatoprotective [40] activities. Naringenin has antiatherogenic, anticancer, anti-fibrogenic, anti-inflammatory, anti-mutagenic,

antioxidant, hepatoprotective, hypocholesterolemic [41], anti-ulcer and cardioprotective [42] properties. Diadzein have antithrombotic, hypocholesterolemic, hypotensive [43], anticancer and estrogen like properties [28].

Alkaloids are the bioactive constituent of some important compounds in medicine due to their physiological effects [44,45,46,47]. Present in *C. dolichopentalum* leaves are 18 alkaloids (Figure 3; Table 3), with buphanidrine recording the highest concentration. The concentrations of 9- Octadecinamide, Dihydroxyhaemanthamine, Crinane - 3-alpha-ol, Buphanidrine, hydroxypowelline, Crinamidine, 1beta-2beta-epoxyambelline, Hydroxyundulantine, Akuamidine and Voacangine in *C. dolichopentalum* were higher than those reported for *Gongronema latifolium* (Benth) leaf, while Augustamine, Oxoassonine, Powelline and Undulantine were reported less [16].

Crinamidine, 6- hydroxyl powelline, 6-hydroxyl undulantine, and Buphanidrine possess antiproliferative potencies, and antitumour activity in cancer cells. Octadecenamide is an amide with antimicrobial effect [48]. Akuamidine has hypotensive, anti-plasmodial, anti-depressant, skeletal muscle relaxant and local analgesic activities [49,50]. Its local analgesic activity is about 3 times as potent as cocaine. It acts selectively as a sympatholytic, unaccompanied by para-sympatholytic effects. It inhibits the irritability of the sympathetic nervous system and opposes akuamidine [51]. Voacangine exhibits cardiovascular toning, central nervous system depressant, anti-convulsive, anti-pyretic, analgesic, local anaesthetic and anti-leishmanial activities, potentiation of barbiturates hypnotic and anti-cholinesterase effects [52,53].

The constituent of Tannin is shown in Figure 4 and Table 4. The leaves of *C. dolichopentalum* indicated lower total tannin content than *Tridax procumbens* Linn Leaves [54]. Tannins have antioxidant, antimicrobial [28], anticancer [55] activities. Tannic acid has antihypertensive, anti-diarrheal, anti-cancer, anti-asthmatic, cardioprotective, anti-diabetic, anti-cataractogenic, tumour inhibition, anti-inflammatory, anti-adipogenic [55] and hepatoprotective [56] activities. Reported to tar the outermost layer of the mucosa [57] and as a result decrease its permeability by xenobiotics, tannins offer resistant to chemical and mechanical injury or irritation. Due to the ability of tannins to complex with macromolecules such as proteins, tannins are used as astringent or antidote for various poisons and as a tropical haemostatic.

CONCLUSION

This screening shows the enormous bioactive content of *C. dolichopentalum.* These natural bioactive compounds in *C. dolichopentalum* leaves may serve as replacement to synthetic agents. This is as a result of increasing usage limitations of synthetic therapeutic agents due to side effects and subsequent resistances.

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