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GC-FID phytochemical analysis of selected antidiabetic herbal preparations sold in Onitsha metropolis, Anambra state, Nigeria

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Abstract
Diabetes has become one of the world's most serious public health problems. The importance of plant extracts in diabetes management is widely acknowledged. This study was designed to determine the phytochemical compositions of the selected herbal preparations, which is used for treatment of Diabetes in Nigeria. Standard method was employed and gas chromatography-flame ionization detector (GC-FID) instrument used for the analysis and quantification of phytochemicals present in the samples. The analyses revealed the presence of 23 bioactive compounds and they include; Proanthocyanin, Phytate, Quinine, Naringin, Epihedrine, Anthocyanin, Lunamarin, Phenol, Spartein, Naringenin, Ribalinidine, Catechin, Resveratol, Flavonoid, Steriods, Kaempferol, Flavone, Sapogenin, Oxalate, Rutin, Epicatechin, Tannin and Flava 3 ol in different concentrations. However, tannin and flava 3 ol are absent in all except Blessed father which have oxalate, ribalinidine and phenol absent. Steroids and spartein are absent in colon cleanser, while epicatechin, rutin, oxalate, sapogenin, kaempferol and resveratrol are all absent in Ashietu Adams. Evaking herbal also does not contain epicathechin. The herbal preparations contained pharmacologically active compounds which support its traditional use for the treatment of Diabetes.

Keywords: GC-FID, phytochemicals, herbal preparations, diabetes, standardization, traditional medicine.

1. Introduction
It has been said that the prevalence of diabetes is increasing at an alarming rate and has become one of the world's most serious public health problems. Diabetes is also considered the most common endocrine disorder. Understanding and improving medication is vital for the management of diabetes and its complications [1]. Many of the currently available medicines for the management of diabetes have undesired side effects. Hence, interest in the scientific investigation to find alternative therapeutic strategies for the diabetes treatment has become inevitable [2]. Natural products are considered to be an effective alternative for the treatment and management of diabetes, because of higher efficacy and less to nil side effects [3]. World Health Organization (WHO) reports that traditional medicines play a major role in health maintenance, chronic disease prevention and treatment [4]. The importance of plant extracts in diabetes management is widely acknowledged and a large number of medicinal plants have been documented for their effective role in the diabetes management [5].
Herbal medicines include herbal extracts, herbal drug preparations and herbal drugs. Herbal preparations include comminuted or powdered materials or extracts, tinctures, and fatty oils herbal materials, which may be produced by extraction, fractionation, purification, concentration or other physical or biological processes [6]. The use of herbal medicine to treat and prevent diseases and ailments or to support health and healing, had long since been in use and presently gaining wide acceptance. According to WHO, 80% of the world's population are tending towards usage of herbal drugs for major health care [7]. Herbal preparations are used in traditional medicine as crude drugs in various dosage forms, as whole, crushed, powdered forms, decoctions, dried extracts, infusions, poultices and tinctures [8]. Many of these plants used in herbal preparations have been investigated in recent times and found to contain active substances that are medically useful, whereas many more are yet to be scientifically investigated. The World Health Organization (WHO) estimated that more than 4 billion inhabitants of the world rely mainly on traditional medicine for health care needs.

A major part of traditional. Medicine involves the use of plant extracts and their derived active principles [9]. In herbal medicine, the therapeutic effects varies as the phytochemical constituents varies [10]. So, there is an urgent need for the isolation and identification of bioactive compounds from the medicinal plants [11]. The presence of phytochemical compounds in medicinal plants has been reported [12]. Herbal preparations are greatly used in Nigeria for the treatment and management of diabetes and several other disease, and this may be attributed to the abundant phytochemicals present in these preparations.

Analysis of phytochemicals by GC-FID is one of the modern techniques used to identify and isolate phytoconstituents. Phytochemical analysis of the selected herbal preparations will revealed its bioactive phytoconstituents, since no study on the GC-FID phytochemical analysis of liquid herbal predations used in Nigeria is contained in any literature. Therefore, the present study is aimed to study the GC-FID phytochemical analysis of selected herbal preparations.

2. Materials and Methods

2.1 Sample Collection

The study was carried out in the month of January, 2021. Five different herbal products termed as antidiabetic herbal preparations were purchased by popularity from different retail drug outlets in Onitsha metropolis. The samples were kept at room temperature of 25 ^{0}C and used within two weeks of procurement. The samples' codes, identities and other information are presented in the table below.

Table 1: Identity of studied Herbal Products

Product name	Dosage form	Manu-facture Date	Expiry Date	State of Manufacture	NAFDAC Reg. NO.	Contents	Therapeutic Claim
Evaking Herbal	Liquid	July, 2020	Agust, 2024	Anambra	A7-2235L	*Kudin, Aloe vera Allium satirum Juglans regia;*	Antidiabetic, Anti-inflammatory It can be used to reduce high blood pressure.
Blessed father herbal bitters	Liquid	April, 2018	April, 2024	Anambra	Nantmp 0996	*Fruits Herbs Roots*	Antidiabetic.
New hope herbal mixture	Liquid	July, 2019	July. 2022	Anambra	NUOMHP 11352	*Btter herbs*	Antidiabetic, Anti-inflammatory, Antibacterial, Antiviral, It is used for the treatment of stroke, fibroid and asthma.
Herbal colon cleanser	Liquid	May. 2019	May, 2022	Lagos	A7-1819L	*Aloe vera, Andrographis paniculata, Curcuma longa, Moringa oleifera,*	Antidiabetic, Anti-inflammatory, Antirheumatic, Improves libido, Supports immune system, Improves eye sight, Prevents pile, Removes toxins and Purifies blood.
Ashietu Adams Wasilat herbal formular	Liquid	Jan. 2018	Dec, 2021	Lagos	Nil	*Carpolobia lutea, Sphenocentrum jollyanum, Cnestis ferruginea, Lecaniodiscus laevis, Microdesmis kaeyaya,*	Antidiabetic.

Fig 1: Herbal products used for the study

2.2 GC-FID Identification and quantification of Phytochemical Constituents

For the GC-FID analysis, 1g of the herbal sample was weighed and transferred into a test tube. 15 ml of ethanol was added. The test tube was allowed to stand in a water bath at 60 ^{o}C for 60 minutes. Then the content of the test tube was carefully transferred into a separators funnel and the tube rinsed into the same funnel with 10ml of cold water, 10ml of hot water, 20ml of ethanol and 3ml of hexane. The extract in the test tube was washed three times with 10ml of 10% v/v ethanol solution. The extract solution was then dried with anhydrous sodium sulphate and the solvent was evaporated. A sample of the extract was then made soluble in 1000µl of hexane of which 200µl was transferred into a vial on the Gas Chromatography machine for phytochemical analysis.

The GC-FID phytochemical analysis was performed on a BUCK M910 Gas Chromatograph (GC) (BUCK Scientific, USA), equipped with a flame ionization detector (FID). A RESTEK 15 meter MXT-1 column (15m x 250µm x 0.15µm) was used. The injector temperature was 280 ^{o}C with splitless injection of 2 µl of sample and a linear velocity of 30cms^{-}1, Helium 5.0 Pas was the carrier gas with a flow rate of 40mlmin^{-}1. The oven operated initially at 200 ^{o}C, it was heated to 330 ^{o}C at a rate of 3 ^{o}C min^{-}1 and was kept at this temperature for 5min. The detector operated at a temperature of 320 ^{o}C.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals [11, 13, 14].

Results

The GC-FID analysis revealed the presence of 23 bioactive compounds in the herbal samples and they include; Proanthocyanin, Phytate, Quinine, Naringin, Epihedrine, Anthocyanin, Lunamarin, Phenol, Spartein, Naringenin, Ribalinidine, Catechin, Resveratol, Flavonoid, Steriods, Kaempferol, Flavone, Sapogenin, Oxalate, Rutin, Epicatechin, Tannin and Flava 3 ol in different concentrations. However, tannin and flava 3 ol are absent in all except Blessed father which have oxalate, ribalinidine and phenol absent. Steroids and spartein are absent in colon cleanser, while epicatechin, rutin, oxalate, sapogenin, kaempferol and resveratrol are all absent in Ashietu Adams. Evaking herbal also does not contain epicathechin. (fig 2,3,4,5,6 and table 2)

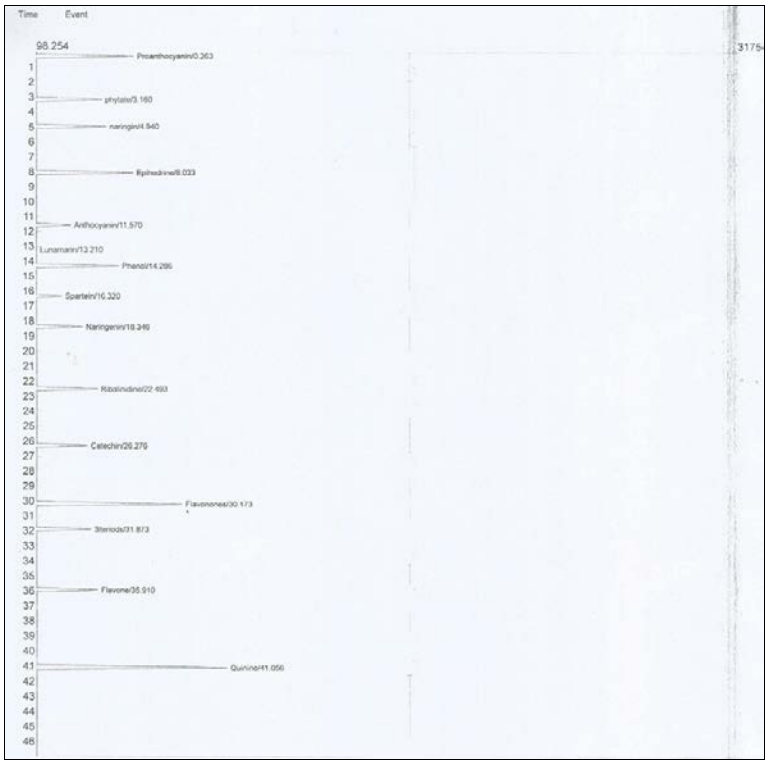

Fig 2: Chromatogram showing the phytochemical constituents of Ashietu Adams wasilat herbal preparation

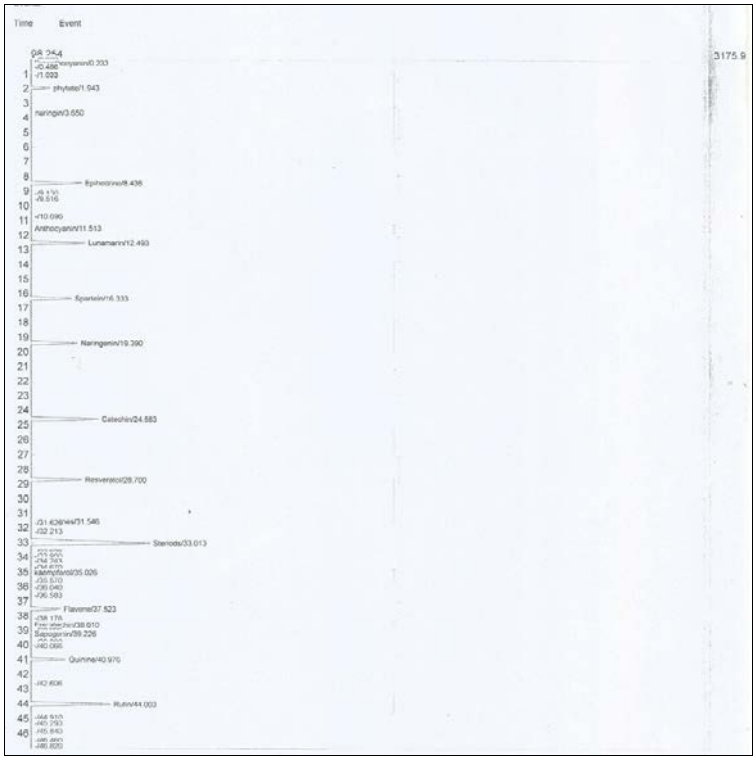

Fig 3: Chromatogram showing the phytochemical constituents of Blessed father herbal preparation

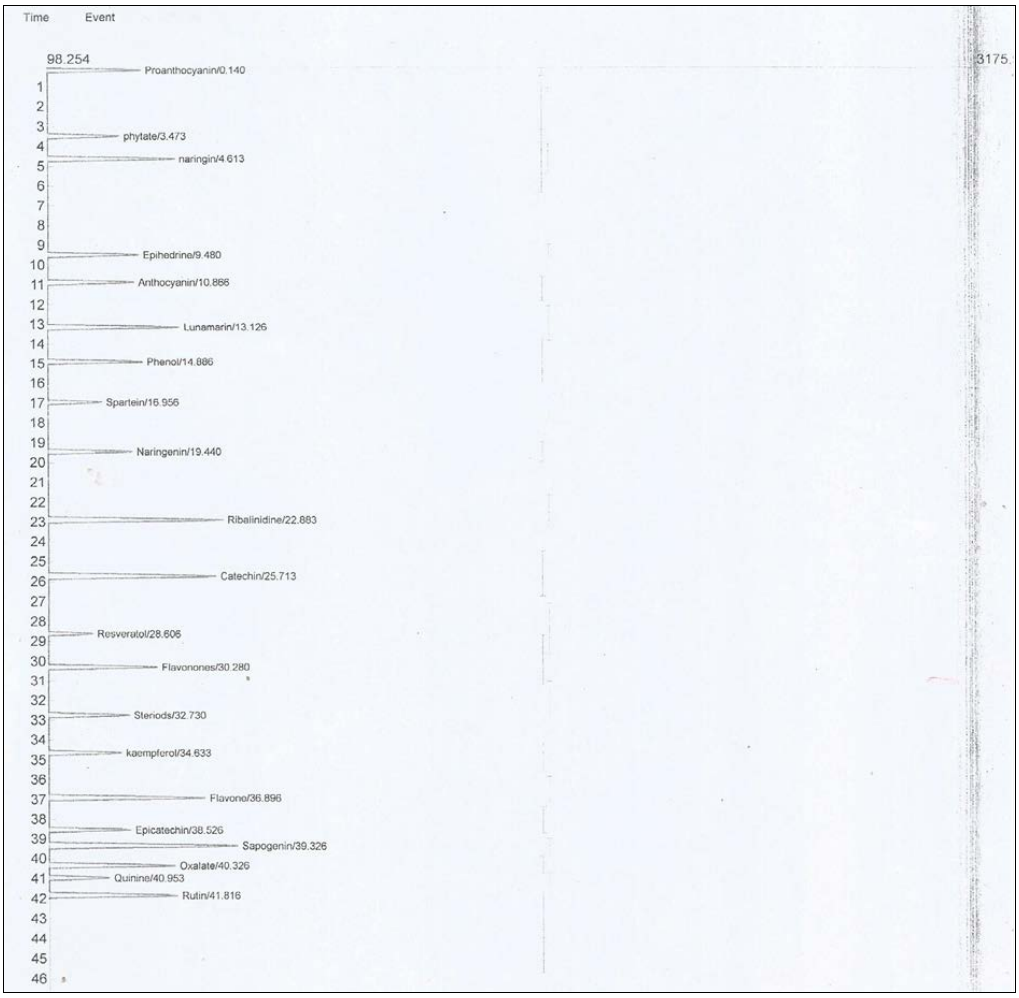

Fig 4: Chromatogram showing the phytochemical constituents of Biotrend colon cleanser herbal preparation

Fig 5: Chromatogram showing the phytochemical constituents of Evaking herbal preparation

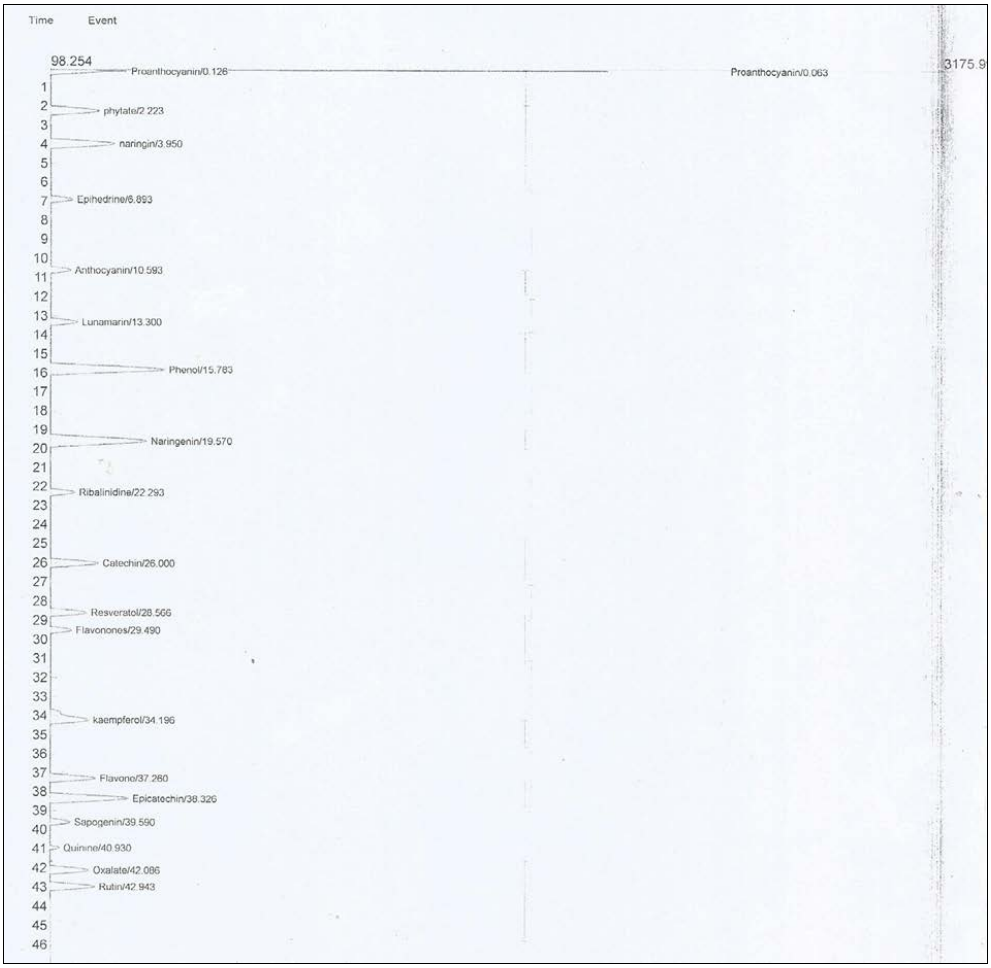

Fig 6: Chromatogram showing the phytochemical constituents of New hope herbal preparation

Table 2: Result for GC-FID phytochemical screening of the herbal preparations

Component	EVA King Herbal ug/ml	Blessed Father ug/ml	Ashietu Adam Wasilat Herbal ug/ml	Colon Cleanser ug/ml	New Hope ug/ml
Proanthocyanin	7.0247	1.0172	10.9479	1.3389	7.0015
Phytate	8.3784	1.3226	3.1894	6.6623	2.7631
Quinine	7.174	2.9208	12.5508	3.7247	3.9165
Naringin	18.6135	0.8332	5.0752	8.354	6.5009
Epihedrine	9.7857	4.0699	7.4345	5.1934	5.628
Anthocyanin	16.4953	0.0275	2.6049	3.6459	3.9369
Lunamarin	6.3892	1.6393	0.0218	5.0246	6.6349
Phenol	6.1025	0	6.9607	15.7124	6.1475
Spartein	6.2639	1.6393	1.4555	0	1.857
Naringenin	16.4176	4.2287	4.7955	16.2224	5.89
Ribalinidine	22.2503	0	10.5165	11.0384	19.1304
Catechin	14.2346	6.2198	5.616	9.6098	11.2001
Resveratol	19.775	5.9971	0	9.9288	5.0698
Flavonoid	5.7046	0.0552	9.4157	4.6473	5.7765
Steriods	20.9727	13.8596	6.0872	0	6.4897
Kaempferol	7.8183	0.0274	0	11.834	6.3007
Flavone	6.2785	1.1901	4.0446	5.965	6.7076
Sapogenin	16.7554	0.0361	0	7.1513	14.3314
Oxalate	5.0359	0	0	8.5254	8.8936
Rutin	9.4653	4.565	0	5.8442	5.2759
Epicatechin	0	0.0328	0	12.9727	6.0703
Tannin	0	0.0337	0	0	0
Flava 3 ol	0	5.5853	0	0	0

4. Discussion

It has been reported that plants used for medicinal purposes are very rich in variety of bioactive compounds and the presence of these phytoconstituents in herbal preparations gives it the essential therapeutic effects [11, 15].

The use of herbal remedies for diabetes has been in place over time, these phytoconstituents present may delay the development of diabetic complications and may regulate the metabolic abnormalities through a variety of mechanisms. However most of these phytochemicals have been reported as potent hypoglycemic agents [16].

The phytochemical results of this current study shown in table 2 revealed the presence of seven groups of these bioactive substances which are alkaloids (ribalinidine, lunamarin, spartein, epihedrine and quinine), flavonoids (proanthocyanin, anthocyanin, naringin, naringenin, flava 3 ol, flavone,

Flavonoid, rutin, kaempferol, epicathechin and cathechin), tannins, saponins (sapogenin), phenolics (phenol and resveratrol), steroids and anti-nutrient (oxalate and phytate). These groups and specific phytochemicals have been reported to possess various medicinal and therapeutic potentials.

Flavonoids are well known for their valuable health benefits especially with their attributable anti-oxidative, anti-mutagenic, anti-inflammatory, anti-carcinogenic, antipyretic, hypoglycemic, antifungal, antibacterial, anti-tumour and wound healing properties [14] it also has enzyme modulatory functions [15, 16]. Proanthocyanin and anthocyanin serve as stress protectants and health-promoting components because of their potent antioxidant activity [17]. Anthocyanins have been reported to play a beneficial role in visual acuity, treatment of cancer, heart disease, age-related neurodegenerative disorders and in angiogenesis [18]. Naringenin is beneficial in the management of cancer, cardiovascular diseases and osteoporosis [19, 20], other beneficial properties of naringenin include its ability to reduce oxidative stress [21], anti-inflammatory [22], anti-diabetic [23], anti-hyperlipidaemia [24], antioxidant [25] and antidepressant properties [26]. Flavones are known to possess anti-microbial and anti-fungal activities while Flavonoid are known to possess antioxidant, antihyperlipidemic and anti-inflammatory properties [27]. Rutin is an antioxidant, cytoprotective, vasoprotective, cardio protective, neuroprotective and anti-carcinogenic [28, 29], with antimicrobial activity [30]. Kaempferol Just like other flavonoids, possess anti-diabetic, anti-cancer, anti-inflammatory activities [31]. It has also been implicated with anti-microbial activity [32, 33]. Cathechin possesses enormous health benefits such as anti-obesity, anticancer, hepatoprotective, and antidiabetic and neuro-protective effects while epicatechins are known to possess cardio-protective, antioxidant, anti-diabetic and anti-cancer activities [14].

Tannins are used as astringent as well as a diuretic. They have also been used in the treatment of diarrhoea, gastrointestinal ulcers and tumours [34, 35]. They also possess antibacterial, antioxidants, antimicrobial, anti-inflammatory, antitumor, antivirus, antidiarrheal, antihaemorrhoid, and antimalarial activities [11].

The pharmacological effects ascribed to saponins include ability to heal wounds and inflamed mucous membranes [36]. It also has anti-hyper cholesterol and haemolytic effects [37, 38] it possesses immunomodulatory, anti-inflammatory, anti-fungal, antiviral, antibacterial, hypercholesterolaemic and anti-carcinogenic properties [39, 40], as well as hypoglycemic activity [11, 41].

Steriods are Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antiasthma, Haepato-protective and also hypoglycemic [42].

Phenols have diverse physiological functions, including anti-inflammatory, antioxidant and antimalarial activities [43, 44, 45].

Alkaloids possess a wide range of pharmacological activities and have been used as a component of many herbal remedies [46]. They have anticancer, psychedelics and antimalarial properties (quinine) [47], analgesic, antispasmodic and bactericidal [36, 48], also antioxidant and stimulating activities [49]. Spartein, lunamarine and ribalinidine amongst other alkaloids have been shown to possess potent antimalarial, anti-inflammatory, antimicrobial and antiprotozoal properties [50]. Lunamarin and ribalinidine have been reported to have radical scavenging function [51]. Also, lunamarin possess anticancer, immunomodulatory, anti-estrogenic, anti-amoebic properties [52, 53, 54].

Phytates and oxalates are some of the well-known anti-nutrients found to be contained in the herbal preparations studied, Anti-nutrients are mostly of organic or synthetic structure and are highly reactive, hence capable of toxic effects.They are thought of as not so beneficial, though, some also have valued health benefits. Phytate has been shown to exhibit anti-inflammatory and cholesterol lowering effects [55]. It also act as an antioxidant and metal chelator [56, 57, 58]. anti-nutrients, on the other hand, interfere with the absorption of minerals. Their interference with nutrient absorption has been known to cause headaches, rashes, nausea, bloating and nutritional deficiencies [59].

This result therefore show that the samples studied possess abundant phytochemicals which have medicinal uses and therapeutic properties. This further stresses the reason why one herbal product can be effective and can be used in the treatment and management of variety of sicknesses. The GC-FID elucidated bioactive compounds present in these herbal prepations studied justifies the use of their use in traditional medicine for treatment and management of diabetes and other ailments.

Conclusion

This study shows that the antidiabetic herbal preparations revealed 23 bioactive compounds in the herbal samples and they include; Proanthocyanin, Phytate, Quinine, Naringin, Epihedrine, Anthocyanin, Lunamarin, Phenol, Spartein, Naringenin, Ribalinidine, Catechin, Resveratol, Flavonoid, Steriods, Kaempferol, Flavone, Sapogenin, Oxalate, Rutin, Epicatechin, Tannin and Flava 3 ol in different concentrations. These abundant very essential phytochemicals have useful phyto-medicinal and nutraceutical benefits to human health and hence their use in management of diabetes and other ailments. However, further studies into the isolation and identification of the individual bioactive compounds responsible for its therapeutic activity and the elucidation of their mechanism(s) of action is needed.

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