



Bioactive compounds in two brands of poly-herbal bitters sold in mile 3 area of port Harcourt city local government area, rivers state

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Abstract

Poly-herbal bitters are composed of numerous groups of chemical compounds extracted from herbs and roots and are characterized by their bitter taste. Currently, the use of poly-herbal bitters presumed to cure various types of diseases has been on the increase. This study, evaluates the bioactive compounds of two brands of poly-herbal bitters sold in Port-Harcourt using GC-MS analysis. The result revealed that the GC-MS spectral showed different peaks, identifying the presence of 17 bioactive compounds. The bitter composition of the prevailing compounds expressed are Pentadecanoic acid ($22 \pm 0.18\%$), Methyl stearate ($25 \pm 1.84\%$), Bis 2-ethylhexyl phthalate ($34 \pm 0.39\%$), Oleic acid ($15 \pm 0.30\%$), 9-Octadecanoic acid ($31 \pm 0.08\%$), Cyclodecane ($11 \pm 0.09\%$), diethyl phthalate ($16 \pm 0.14\%$), phthalic acid ($21 \pm 0.07\%$), Cyclotetradecane ($23 \pm 0.08\%$), and the (% Area) are 5.17, 5.68, 8.15, 1.20, 4.19, 0.90, 0.75, 1.94, 0.92, and 1.49 respectively. Only these 9 compounds identified have established and documented pharmacological use and supports the advertised usage of the formulations. Based on the upsurge in the quest to determine whether the consumption of poly-herbal remedy mixtures are safe, further research should be done with specific biomarkers.

Keywords: poly-herbal bitters, bioactive compounds, bitter composition, gas chromatography-mass spectrometry (GC-MS)

Introduction

Plants are of great value and significance. They are unequivocally necessary to humans, animals or largely, living organisms. There are compounds existing in plants, which possesses possible curative or remedial weapons against diseases or infections in addition to their nutritive and dietetic values. Plants with curative and therapeutic actions are referred to as medicinal plants and are the principal basis or source of vigorous and effective drugs from their raw form. Additionally, they also possess manifold physiological activities that could be essential or useful for the treatment of diverse ailments (Pieme *et al.*, 2006) [19].

Poly-herbal bitters are made of copious groups of chemical compounds, extracted from herbs and roots of plants and are characterized by their bitter taste. They help to increase the important energy centres of the body (Anionye, *et al.*, 2017) [4]. They are composed of different Plant constituents such with medicinal properties such as Vernonia amygdalina, Cajanus cajan, Zingiber officinale, allium sativum, Saccharum officinarum, Moringa oleifera, Magnifera indica, Citrus aurantifolia, Caramel. Poly-herbal bitters is a renowned and oldest form of health therapy known to mankind. It has been the major source and solution to the treatment of certain diseases or ailments. Poly-Herbal medicine resulting from plants are commonly denoted as Phyto-medicine. The usage of Poly-herbal medicines across all spectrum of the Nigerian society, is an alternative form of medical and clinical therapy resulting to their very high demand in many Nigerian homes (Sushruta *et al.*, 2016). In underdeveloped and developing societies, Poly-herbal bitters have been wholly used for the treatment of ailments and diseases, hence the need to identify the bioactive components of these products as well proper experimental assessment technique to certify and confirm the safety and

effectiveness of these Poly-herbal bitters (Ogbonnia *et al.*, 2018) [15]. The bioactive compounds in these poly-herbal bitters should exhibit certain therapeutic benefits such as anti-oxidant, anti-microbial, anti-cancer, anti-diabetic, and anti-inflammatory properties.

Bioactive compounds are biologically active compounds have the capacity and proficiency to interrelate with several compounds of living tissues, thus providing an extensive array of effects. They exist innately in different sources which includes aquatic, terrestrial, and microorganisms (especially bacteria, fungi and viruses). They can also be produced synthetically in laboratories (Abdelkarim *et al.*, 2014) [1]. Biologically active compounds comprises of chemical compounds that originates from natural plants and certain foods such as fruits, vegetables, nuts, oils and whole grains. Their actions or activities elicits certain functional benefits which involves promoting good health in the body such as the regulation of metabolic functions beyond the basic nutritional value. Most bioactive compounds elicit antioxidant, anti-carcinogenic, anti-inflammatory and antimicrobial properties. Also, many epidemiologic studies report that some of them have protective effects on cardiovascular diseases (Joanna, 2019) [12].

These compounds are experiencing a growing interest in wide range of applications such as geomedicine, plant science, modern pharmacology, agrochemicals, food industry, etc. In the pharmaceutical industry, natural products are the origin of all modern clinical drug development (Muhammad *et al.*, 2019) [14]. The National Agency for Food and Drug Administration and control (NAFDAC) which is a food and Drug Law Enforcement Agency in Nigeria is yet to provide a verified generated data in the public domain on the Scientific and Medical explanation of some Poly-herbal bitters in Nigeria based on its consumption. This has prompted frequently asked

questions and beliefs amongst people as to whether it is safe. Currently, the upsurge in its usage as maybe widely patronized and sighted in some areas, underscores research efforts to evaluate their bioactive components and definitions. The plant constituents present in medicinal plant products such as Poly-herbal bitters consists of multi-component mixtures which has triggered the quest to determine whether the consumption is a safe health practice and acclaimed to be beneficial to the health of the consumers.

Materials and methods

Sample Collection

Poly-herbal bitters were purchased from a Poly-herbal bitter vendor in Port Harcourt, Rivers State. The Poly-herbal bitters were bought as liquid formulations and stored at room temperature throughout the period of the experiment.

Plant Composition

The plant composition of the two Poly-herbal mixture includes: Vernonia amygdalina, Allium sativum, Saccharum officinarum, Zingiber officinale, Cajanus cajan, Caramel, Moringa oleifera, Magnifera indica, Citrus aurantifolia.

Method

The milled sample was extracted in dichloromethane after soaking for 5days. 10ml of the sample was weighed into a well stopper bottle and 20mls of the organic solvent was added. The mixtures were vigorously agitated and were left to stand for 5days. The crude extract was collected by

filtering into a quartz beaker, the process was repeatedly carried out for two consecutive times. The combined aliquot collected was concentrated on a steam berth to about 5ml. this was purified by passing through a pasture pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air-dried to about 2ml for gas chromatographic analysis. The extract of the sample was subjected to GC-MS analysis, this group of powerful instruments interfaced helped to characterize the various compositions. The Gas Chromatographic Model: 7890A (GC) analysis was performed on an Agilent Technologies interfaced with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an ion-source temperature at 2500C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5 (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was at 600C held for 0.5 minute and ramped to 1400C at the rate of 40C/minutes holding for a minute, then ramped to 280 degrees while holding for 5 minutes at the rate of 80C/minutes. 1µl was auto injected.

Statistical analysis

The analysis was done in triplicates. Mean \pm standard deviations of triplicate determinations were used to analyze data. Data obtained was analyzed using One-way ANOVA (Analysis of variance) using SPSS 21 (IBM Statistical package of Biological and Social Sciences).

Results

Table 1: GC-MS Analysis of the Poly-herbal bitters

Chromatographic Peak	Chemical Name	Molecular formula	Molecular weight (g/mol)	Area%	Retention time
BITTERS A					
1.	Cyclododecane	C ₁₂ H ₂₄	168.32	0.91	8.60
2.	Cetene	C ₁₆ H ₃₂	224.42	0.75	11.06
3.	Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	222.24	1.94	11.16
4.	Phthalic acid	C ₈ H ₆ O ₄	166.14	0.92	14.12
5.	Phenyl-propyl ester	C ₁₁ H ₁₄ O ₂	190.24	1.22	15.14
BITTERS B					
6.	Pentadecanoic acid	C ₁₇ H ₃₄ O ₂	242.40	5.17	15.65
7.	Cyclotetradecane	C ₁₄ H ₈	196.37	1.49	16.28
8.	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5	5.68	16.55
9.	6-Chloropyridin-2-ol	C ₅ H ₄ CINO	129.54	0.79	17.98
10.	Oleic Acid	C ₁₈ H ₃₄ O ₂	282.47	0.99	19.16
11.	9-octadecanoicacid	C ₃₆ H ₇₀ O ₄	566.90	2.57	19.19
12.	Disparlure	C ₁₉ H ₃₈ O	282.50	7.84	19.17
13.	2-Methylene cyclohexanol	C ₇ H ₁₂ O	100.16	4.70	20.31
14.	Tricyclo-3-en	C ₁₂ H ₁₆ O	164.24	4.19	20.47
15.	1-Octen-3-yne	C ₈ H ₁₄ O	108.18	1.05	20.51
16.	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₄₈ O ₄	390.56	89.15	20.90
17.	Diisooctyl phthalate	C ₂₄ H ₄₈ O ₄	390.55	75.57	20.90

Table 1 above represents the chromatographic peak of the brands of poly-herbal bitters, showing the percentage area as well as the retention time.

Table 2: triplicate analysis of the brands of poly-herbal bitters

S/N	Chemical Name	Bitters Composition
1.	Cyclododecane	11 \pm 0.09
2.	Cetene	15 \pm 0.01
3.	Diethyl Phthalate	16 \pm 0.14
4.	Phthalic acid	21 \pm 0.07
5.	Phenyl-propyl ester	18 \pm 0.02
6.	Pentadecanoic acid	22 \pm 0.18
7.	Cyclotetradecane	23 \pm 0.08
8.	Methyl stearate	25 \pm 1.84

9.	6-Chloropyridin-2-ol	29±0.06
10.	Oleic Acid	27±0.10
11.	9-octadecanoic acid	29±0.08
12.	Disparlure	11±0.05
13.	2-Methylene cyclohexanol	33±0.11, 33±0.12
14.	Tricyclo-3-en	34±0.18
15.	1-Octen-3-yne	34±0.39
16.	Bis(2-ethylhexyl) phthalate	34±0.42
17.	Diisooctyl phthalate	34±0.44

Table 2 above shows the triplicate analysis of the two brands of bitters represented as Mean ± standard deviation.

Discussion

The peak results obtained from the analysis of the Poly-herbal bitters is summarized in Table 1 above. Peak 1 occurred at m/z 8.60 and is identified as Cyclododecane. Cetene was identified as peak 2 at m/z 11.06. Peak 3 appeared at m/z 11.16 identified as Diethyl Phthalate, Peak 4 appeared at m/z 314.12 identified as Phthalic acid. Peak 5 occurred at m/z 15.14 and the compound identified is Phenyl-propyl ester. Pentadecanoic acid was identified at a peak 6 at m/z 15.65. Cyclopentadecanone was identified at m/z 16.28 at chromatographic peak 7. Methyl stearate was identified at m/z 16.55 at chromatographic peak 8. Peak 9 appeared at m/z 17.98, identified as 6-Chloropyridin-2-ol. Peak 10 appeared at m/z 19.16, identified as Oleic Acid. 9-octadecanoic acid was identified at m/z 19.19 at peak 11. Disparlure was identified at m/z 19.17 at peak 12. The bioactive compound 2-Methylene cyclohexanol was also identified at peak 13 with m/z 20.31. The bioactive compound Tricyclo-3-en appeared at m/z 20.47 at peak 14. 1-Octen-3-yne was identified as peak 15 at m/z 20.51. Bis (2-ethylhexyl) phthalate appeared at m/z 20.90 at peak 16, and the bioactive compound Diisooctyl phthalate was identified at m/z 20.90 at peak 17.

From this result, 17 different bioactive compounds were identified but only 9 compounds identified have established and documented pharmacological use and supports the advertised usage of the formulations. These compounds are Pentadecanoic acid, Methyl stearate, Bis 2-ethylhexyl phthalate, Oleic acid, 9-Octadecanoic acid, Cyclodecane, diethyl phthalate, phthalic acid and Cyclotetradecane. These bioactive compounds identified possesses certain health benefits besides their nutritional benefits. For instance, Oleic Acid replaces saturated fats in the diet, and is capable of improving heart conditions by lowering cholesterol and reducing inflammation. 9-Octadecanoic Acid possesses hypocholesterolaemia properties, Phenyl-propyl ester and 2-methylene cyclohexanol, have an antioxidant, cancer preventive, hypercholesterolemic and antiarthritic properties. Cyclotetradecane possesses an antimicrobial activity, good antiepileptic activity, and anti-inflammatory properties. Cyclododecane has an antioxidant, cancer preventive and antiarthritic properties. Pentadecanoic acid belongs to the long chain fatty acid and possesses an antifungal and antibacterial activity. It also have an anti-inflammatory property and in the prevention of Alzheimer's disease, a progressive disease that destroys or affects memory and other important mental functions. Ojekale *et al.*, (2019)^[17], identified a total of 31 bioactive compounds in three different herbal bitter drinks and only 11 compounds out of the total number of identified compounds were of documented pharmacological importance which includes Hexadecanoic acid, Octadecanoic acid, Phthalic acid, Decanoic acid, Mono (2-ethylhexyl) phthalate, etc.

Also, James-Okoro *et al.*, (2012), on their assessment on the bioactive component in a herbal bitters, revealed the presence of some bioactive components found in it such as D-allose, 1, 6-anhydro-beta-Dglucufuranose, 5-hydroxymethylfurfural and Z-6-Pentadecen-1-ol acetate.

The different herbal mixtures analyzed are composed of different Plant constituents with medicinal properties such as Vernonia amygdalina, Cajanus cajan, Zingiber officinale, allium sativum, Saccharum officinarum, Moringa oleifera, Magnifera indica, Citrus aurantifolia, Caramel. According to the research of Muhammad *et al.*, to determine the bioactive constituents and antibacterial activity of Vernonia amygdalina leaf extracts against some bacteria associated with gastroenteritis, they discovered that the leaves of Vernonia amygdalina possesses antimicrobial potential against some bacterial isolates associated with gastroenteritis. Vernonia amygdalina whose common name is bitter leaf, has several health benefits such as anti-cancer, anti-bacterial, anti-malarial and anti-parasitic agent. In traditional medical practices, its roots and leaves are used to treat fever, hiccups, joint pain, diabetes, etc. According to the research of Pal *et al.*, (2011), Cajanus cajan leaves (which is a major component in the three poly-herbal mixtures analyzed in this research), significantly increased the fasting blood glucose levels of wistar rats.

Conclusion

This study justifies the consumption of the brands of poly-herbal mixtures assayed for the bioactive components present in them. That is to say, that the assessment of the herbal mixtures indicates that it possesses some vital bioactive components that are health promoting and can be used as alternatives to synthetic drugs. However, further investigation or research should be conducted to determine the effects of these poly-herbal bitters in selected or specific biomarkers on experimental animals.

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