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Proximate analysis and bioactive components of an aqueous polyherbal formulation used in traditional management of gastric ulcer

Blessing Amarachi Mba. [1, *], Chinwe Sylvester Alisi [1], Alloysius Chinedu Ene [1], Chidi Uzoma Igwe [1], Nnenna Chinenyenwa Osuji-Kalu [1] and Uloaku Cynthia Ogbuagu [2]

[1] *Department of Biochemistry, Faculty of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.*
[2] *Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Collage of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.*

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Abstract

This study evaluated the proximate and bioactive components of a trado-medical anti-ulcer polyherbal formulation consisting of *Chromolena odorata* leaf, honey from *Apies mellifera* and palm kernel oil from *Elaeis guineensis.* The polyherbal formulation was prepared using macerated aqueous extract of *C. odorata* leaf, honey and palm kernel oil combined in the ratio of 7:2:1. The bioactive component of the formulation was determined using gas chromatography mass spectrometry (GC-MS) while the proximate analysis was according to the methods of association of analytical chemists (AOAC). The result of the analysis showed that the polyherbal formulation had high moisture content (44.34%), carbohydrate (40.36%), protein (7.70%), and fat (4.85%). The bioactive compounds predominantly present in the polyherbal formulation were hexadecanoic acid, 6-octadienal, octadecenoic acid, vaccenic acid, oleic acid and octadecadienal. The presence of these bioactive compounds may be responsible for the therapeutic use of the formulation in traditional management of gastric ulcer. These bioactive compounds have been found to possess anti-inflammatory, antimicrobial, anticancer properties, cholesterol reducing property as well as several industrial applications. The presence of these bioactive compounds may be responsible for the therapeutic use of polyherbal formulation in the traditional management of gastric ulcers.

Keywords: Proximate; Bioactive Compounds; Gastric Ulcers; Polyherbal Formulation

1. Introduction

Traditional herbal medicine has been used across regions and cultures in human healing due to their therapeutic potentials as a result of the bioactive and phytochemicals of pharmacological importance they contain (Gupta et al., 2023). Polyherbal formulation involves using more than one herbs or plant extracts in medicinal preparation. These herbs of pharmaceutical importance are combined in specific ratio to prepare a formulation used for treatment of illnesses. This concept of polyherbalism has been developed in Ayurvedic and other traditional medicine systems for centuries and has faced severe setback in being integrated in modern medical practices due to lack of scientific and clinical data proving their efficacy and safety in human and animal use (Parasuraman, Thing & Dhanaraji, 2014). Polyherbalism offers great advantages due to their synergistic effects, reduced side effects due to greater therapeutic efficacy at lower dose, improving patient comfort and compliance by removing the need to take many single herbal formations at the same time (Lawal, Olajuyibe, Akinwuru & Olugbami, 2024).

Proximate analysis of food samples and plant extracts is used to quantify the major macronutrient components, which provide a basic understanding of their quality and nutritional value (Kotue et al., 2018). Plant foods are rich in nutrients

* Corresponding author: Mba Blessing A

that help to maintain the normal function of the body. These nutrients can be divided into two groups based on their requirements each day: which are macronutrients (proteins, carbohydrates, fats) and micronutrients (vitamins and minerals) (Ganogpichayagrai & Suksaard, 2020).

Medicinal plants are rich in bioactive compounds which are known to possess anticancer and antioxidant properties (Jasemi et al, 2024). These bioactive compounds especially the polyphenols are free radical scavengers and thus, can help in reducing oxidative stress (Karen, Patricia, Almeida & Milena, 2022).

2. Materials and Methods

2.1. Plant materials

The fresh leaves of the plant *Chromolaena odorata* (Awolowo leaf) was collected from the back of School of Agriculture and Agricultural Technology (SAAT) farm, Federal University of Technology, Owerri (FUTO), Imo State, Nigeria The plant was appropriately identified by a plant taxonomist Mr. Francis Iwunze of the Department of Forestry and Wild Life Technology, FUTO with voucher and herbarium number FUTO/FWT/HERB/2025/122.. Fresh local palm kernel oil from *Elaeis guineensis* was obtained from trusted vendor at Relief Market, Owerri Municipal Council, Imo State, and fresh a honey was sourced from a farm at Obinze in Owerri West Local Government Area, Imo State.

2.2. Formulation of the polyherbal drug

One thousand gramme (g) of fresh leaves of the plant *Chromolaena odorata* was thoroughly washed and squeezed to obtain an extract of 1300mlout of which 700ml of the extracts was collected and thoroughly mixed with 200ml of honey and 100ml of local fresh palm kernel oil to obtain 1000ml (1 litre) of the polyherbal formulation (in the ratio of 7:2:1) used in the study.

2.3. Proximate analysis

For the quantitative determination of proximate composition of the polyherbal formulation, moisture content was determined using hot air oven (Association of Official Analytical Chemists (AOAC) 2016), 2g of the formulation was incinerated at 105°C until constant weight was obtained. Crude protein content was determined according to Kjeldahl method (AOAC, 2016), total nitrogen was multiplied by a factor of 6.25. Total fat was determined according to the acid hydrolysis method (AOAC, 2016) using soxhlet extractor at 60°C, until constant weight. Crude fibre was determined using percentage weight loss after incineration (AOAC, 2016). A sample size of 2g was boiled for 30minutes with petroleum ether and acid. The solution filtered, washed and the final residue incinerated cooled and weighed. Ash content was determined using gravimetric method (AOAC, 2016) the polyherbal formulation was incinerated at 550°C for 3 hours, intermittently cooled and weighed until constant weight was obtained. Available carbohydrate was determined using the differential method by calculation.

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The analysis of bioactive components of the polyherbal formulation was performed on a BUCK M910 Gas chromatography using Agilent Technologies GC systems with a 7000C GC/MS Triple Quad model (Sparkman, Penton, & Kitson, 2011) equipped with HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. Relative quantity of the chemical compounds present in each of the polyherbal formulation and synthesized silver nanoparticles were expressed as percentage based on peak area produced in the chromatogram.

3. Results

Table 1 Proximate composition of the aqueous polyherbal formulation

Parameter	Composition (%)
Moisture	44.34
Ash	1.90
Crude fibre	0.85
Fat	4.85
Crude Protein	7.70
Carbohydrate	40.36

Table 2 Volatile organic compounds found in the polyherbal formulaion

Peak No	Retention time	Area (%)	Library ID/Compound Name	Molecular Formula	Molecular weight (g/mol)
1	7.081	2.43	10- Undecen-4-one, 2,2,6,6-tetramethyl-	$C_{15}H_{28}O$	224.38
2	7.691	4.23	2,6-Octadienal, 3,7-dimethyl-, (E)	$C_{10}H_{16}O$	152.23
3	16.993	16.45	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5
4	17.641	25.52	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
5	18.808	18.1	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5
6	18.974	2.82	Fumaric acid, pent-4-en-2-yl tridecyl Ester	$C_{22}H_{38}O_4$	366.5
7	19.05	4.01	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298.5
8	19.448	15.23	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5
9	19.647	8.04	Oleic Acid	$C_{18}H_{34}O_2$	282.5
10	19.699	2.43	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280.5
11	19.799	0.59	1H-Indene, 2-butyl-5-hexyloctahydro-9,17-Octadecadienal, (Z)- $C_{18}H_{32}O$ 264.4	$C_{19}H_{36}$	264.5

Table 2: showing the result of GC-MS analysis of aqueous polyherbal formulation. The mass spectra peaks of these compounds were matched with blue-prints in the National Institute of Standard and Technology (NIST) spectral database.

4. Discussion

Proximate analysis was carried out on the polyherbal formulation to determine its nutritive composition. From the result, moisture had the highest value while crude fiber had the lowest value. High moisture content of plant extract or food samples has been implicated in early spoilage of food due to increased microbial activities.

According to Chukwu and Abdullahi (2025), the most important single factor that encourages mould growth on food samples is increased moisture content during storage. Low moisture content prevents microbial growth and prolongs the shelf-life of food sample (Shukla, Vats & Shukla, 2015). Crude fibre had the lowest composition in the polyherbal formulation. Crude fibre is nutritionally beneficial because it aids in food digestion and absorption of trace element in the gut (Adamu, Paul, Ndukwe & Gimba, 2018). Foods low in dietry fibre can lead digestive issues, constipation and negatively impact the gut health microbiome (Mathers, 2023). The result also showed that the polyherbal formulationn can be utilized as a good source of protein and carbohydrates. Proteins, which contain amino acids can be used for the synthesis of numerous other proteins needed for the functioning of cells, to repair worn out tissues as well as energy source within the cell (Essiett & Ukpong, 2014). High content of carbohydrate observed in the formulation could serve as an energy source which is needed for the metabolic activities within the cells.

The proximate analysis also revealed that the formulation can be a good source of crude fat and minerals. The ash constitutes the residue remaining after all the moisture as well as the organic materials have been burnt. Ash content is generally taken as a measure of mineral composition of a food or feed sample, which is essential in estimating the nutritional value of such sample (Ismali, 2017). The fat content of the formulation if further analyzed could be a source of free fatty acid and other fat soluble vitamins as well as a source of energy.

Furthermore, analysis on the bioactive components of the polyherbal formulation showed that methyl ester cis -13-octa decenoic acid was one of the volatile organic compounds found in the polyherbal formulation. It is a fatty acid methyl ester derived from oleic acid. Methyl ester cis-13-octa decenoic acid has found use in synthetic industries as a lubricant, intermediate in detergent production, emulsifier and a preservative (Pichat, 2013). It is also used in the agricultural industries as an oil carrier as it is biodegradable and less toxic to crops. It is also used in cosmetic industries as a skin moisturizer, preventing water loss and maintaining hydration (Hayes, 2017).

Cis vaccenic acid was another volatile organic compound found in the polyherbal formulation. It is a naturally occurring trans fatty acid and also an omega-7 fatty acid. It is mostly found in human milk, fats of ruminants and in some dairy products like butter, yogurt (Precht & Molkentin, 1999). A study showed that obese rats fed with a diet enriched with cis-vaccenic acid for 16 weeks had a significant reduction in total cholesterol, LDL-cholesterol and triacylglyceride level (Gebauer et al., 2015).

N- Hexadecanoic acid also known at palmitic acid was another fatty acid found in the polyherbal formulation. It has been shown to exhibit anti-inflammatory properties by competitively inhibiting the activity of the enzyme phospholipase A (2) (Aparna et al. 2012). Hence, it accounts for the use of oils rich in n-hexadecanoic acid in the traditional medicine system for the treatment of rheumaotoid symptoms.

5. Conclusion

The Polyherbal formulation was found to possess significant moisture, carbohydrates, protein and fat, making it an alternative source of these nutrients for humans and animals. Also, it was found to contain important bioactive compounds which have application in health sector and industries. These bioactive compounds of pharmacological and industrial importance when harnessed could be utilized for the management of some diseases as well as other industrial applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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