PHYTOCHEMICAL SCREENING OF METHANOL EXTRACTS OF Prosopis africana SEEDS, Ocimum gratissimum AND Vernonia amygdalina LEAVES

^{1*}M. N. Ugwu, ¹A. A. Asuk, K. Dasofunjo, ¹A. I. Okafor, ¹U. P. Ujong

¹Department of Medical Biochemistry, Cross River University of Technology, Faculty of Basic Medical Sciences, Okuku Campus, Nigeria

Correspondence e-mail: ugwumelvin@crutech.edu.ng, melvincrux@yahoo.com; Tel: +2348038728570

ABSTRACT

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. In this study qualitative and quantitative phytochemical analysis of *Prosopis* africana seeds, Ocimum gratissimum (scent leaf) and Vernonia amygdalina (bitter leaf) extracts was carried out. The analysis revealed that the extracts contain bioactive compounds which include: alkaloids, saponins, tannins, phlobatannins, cardiac glycosides, flavonoids, sugars, carbohydrates, terpenes and anthraquinones, steroids, oxalates, phenols, phytates, anthraquinones, glycosides, and terpenoids. In the quantitative analysis of Prosopis africana it was found that tannins were the highest, followed by carbohydrates while saponins was the lowest. Ocimum gratissimum revealed that phytates was found to be highest, followed by saponins while anthraquinones was the lowest. In Vernonia amygdalina, the most abundant phytochemical was flavonoids followed by alkaloids while terpenoids was the lowest. The availability of these bioactive compounds in Prosopis africana seeds, Ocimum gratissimum and Vernonia amygdalina as revealed in this study suggests strongly the possibility of using these plants in managing different diseases. Based on the finding, it can be concluded that the application of P. africana seeds, O. gratissimum and V amygdalina leaves in ethno medicine is justified.

Keywords: Phytochemicals, Prosopis africana, Ocimum gratissimum, Vernonia amygdalina and food condiment

1.0 INTRODUCTION

The trees of Prosopis africana are common in the Middle belt and Northern parts of Nigeria and are referred to as "Kiriya" and "Okpehe" in Hausa Idoma/Tiv and languages in Nigeria respectively (Ajiboye, 2009). In many areas where the trees are grown or available, the fermented seeds of P. africana are used as a food condiment; its young leaves and shoot are fodder that is highly sought after towards the end of the dry season. Prosopis africana yields a gum, tannin or dyestuff, the back and roots contain 14-16% tannin and a colouring matter that gives a reddish tint to leather, pounded dry fruits are suitable as a fish poison (Ajiboye, 2009). Some studies have established that *Prosopis africana* has

several therapeutic values (Yarkwan, 2020; Yanda *et al.*, 2022)

Ocimum gratissimum is an erect aromatic perennial and branched plant, one metre high or less. It is a plant belonging to the Lamiacea family. It is a small shrub with many branches, commonly found in many gardens around village huts in Nigeria and planted for its medicinal uses. Ocimum gratissimum is grown for the essential oil in its leaves and stems while eugenol and to a lesser extent, thymol extracted from the oil are substitutes for clove oil and thyme oil. The essential oil possesses antibacterial properties and is also an important insect repellent so also are the leaves when left dry and burnt. They are primarily used as

vegetables (Sulistiarini, 1999), as spice because of its aromatic nature to spice various kinds of soup and other delicious meals like porridge. The whole plant has many applications in traditional medicine especially in Africa and India (Ugwu et al., 2011 & 2013; Oladosu-Ajayi et al., 2017; Rizwan & Amah, 2021). The applications include the treatment of ringworms, gout and fungal infections, malaria, catarrh, aches, colon pain. The juice obtained by squeezing its leaf can be used to cure several stomach related illnesses like cholera, diarrhea, dysentery, vomiting and convulsion (Sulistiarini, 1999).

Vernonia amygdalina is commonly called bitter leaf (English), Onugbu (Igbo); Etidot (Efik, Ijaw and Ibibio), Ewuro (Yoruba), Oriwo (Edo) and Chusa-doki (Hausa) languages (Momoh et al., 2015). In many parts of Nigeria, the plant has been domesticated and used in the treatment of various infection and diseases. The leaves are extremely bitter because of its composition (Lasekan et al., 1998), which gives the plant its therapeutic capacity (Okoh et al., 1995; Oloya et al., 2022). Vernonia amygdalina was reported to have a broad spectrum of medicinal relevance (Ugwu et al., 2018a; Asuk and Ugwu, 2018; Emiri & Enaregha, 2020; Ugbogu et al., 2021).

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remained relevant in both developing and the developed nations of the world for various chemotherapeutic purposes. Plants have the ability to synthesise a wide variety of chemical compounds such as resins, alkaloids, glycosides, saponins, and essential oils (Kwee & Niemeyer, 2011). Many of these phytochemicals have beneficial effects in human health and may be used to effectively treat human disease (Lai & Roy, 2004; Ugwu et al., 2018b).

phytochemical investigations The and elemental analysis of some plant extracts indicate the presence of pharmacologically useful classes of compounds such as saponins, alkaloids, flavonoids, cardiac glycosides, terpenes, steroids, tannins and carbohydrates (Sanni et al., 2008). Tannins were reported to possess physiological astringent properties, which hasten wound healing and ameliorate inflamed mucus membrane (Gupta, 1994). It also has haemostatic properties (Awosika, 1991). Saponin present in plants is cardiotonic in nature (Trease and Evans, 1989).

2.0 MATERIALS AND METHODS

2.1 Plant Materials

2.1.1 Collection of *Prosopis africana* seeds and preparation

Prosopis africana seeds were purchased from Ishibori market in Ogoja Local Government of Cross river State, Nigeria. The seeds (500g) were sorted, cleaned and boiled for 5h using a gas cooker and allowed to cool to room temperature. The boiling helps to soften the hulls for easy removal and separation of the cotyledons. After it was dehulled, the dehulled and boiled seeds were washed again with clean water. The processing of dehulled seeds was done by hand squeezing the seeds and washing with clean water. The wet dehulled seeds were kept in a large polythene sack to exclude air and were fermented for three days according to the method described by (Achi, 1992). The fermentation was done at room temperature for 72h. The fermented seeds were then sun-dried to a constant weight and milled using hammer mill to produce Prosopis seed flour (Yusuf et al., 2008). The flour was kept in a refrigerator at 4°C prior to use.

2.1.2 Collection of *Ocimum gratissimum* and *Vernonia amygdalina* leaves

Fresh leaves of *Ocimum gratissimum* and *Vernonia amygdalina* were harvested from a garden in Okuku in Yala, Local Government

Area of Cross River State, South-South, Nigeria. The plants were identified at the herbarium unit of the Department of Biological Sciences, University of Calabar, Calabar, Nigeria.

2.1.2.1. Preparation of Methanol Extract of Ocimum gratissimum and Vernonia amygdalina

The fresh leaves of *Ocimum gratissimum* and *Vernonia amygdalina* were washed clean. The leaves were air-dried for 30 days at room temperature. The air-dried samples were ground to fine powder using a blender. 500 g of each sample of the powdered material were soaked in 1200 ml of distilled water for 72 h. These were filtered and were freeze-dried to obtain the extract powder. The extracts were kept in the freezer at 4^{0} C and used for the studies.

2.2. Chemicals

All chemicals and reagents used were of analytical grade, purchased from Sigma Chemical Company, USA and used without further purification.

2.3. Phytochemical Screening

2.3.1. Qualitative Phytochemical Analysis Phytochemical screening of the extract was carried out to identify the constituents, using phytochemical standard methods as described by Trease and Evans (1989; 2002) as well as Sofowora (1993). The screening involves detection of alkaloids, flavonoids, terpenoids. steroids. phenols, saponins, tannins. anthraquinones, cardiac and glycosides.

2.3.2. Quantitative Phytochemical Analysis

Different methods were used in evaluating the quantity of phytochemical constituents of the plant materials used. Spectrophotometric method was used to determine terpenoids, tannins and steroids as described by Ali and Ibrahim (2019). Folin-Ciocalteu procedure was used to determine phenol content. Flavonoids, alkaloids and saponins were determined by the methods described by Adeniyi *et al.* (2009).

3.0 RESULTS

Table 1 shows the qualitative and quantitative phytochemical composition of the extract of P. africana seeds. Qualitative analysis indicates that some bioactive compound such as alkaloids, saponins, tannins, phlobatannins, cardiac glycosides, flavonoids, sugars, carbohydrates, terpenes and anthraquinones are present. While the phytochemical quantitative composition indicate that bioactive compounds such as tanins were found to be highest, followed by carbohydrates while saponins was the lowest.

Table 2 also shows the qualitative and quantitative phytochemical composition of methanol extract Ocimum the of gratissimum leaves. Qualitative analysis indicates following bioactive that the compounds present, flavonoids, are alkaloids. steroids, saponins, oxalates, phenols, phytates, anthraquinones, tannins and cyanogenic glycosides. The quantitative phytochemical composition indicate that phytates were found to be highest, followed by saponins while anthraquinones was the lowest.

Table 3 contains the qualitative and quantitative phytochemical composition of the methanol extract of Vernonia *amygdalina* leaves. Qualitative analysis shows that the following phytochemicals are present. alkaloids. saponins, tannins, phlobatannins, glycosides, flavonoids, terpenoids. steroids. phenols and Quantitative determination indicates that flavonoids were found to be highest, followed by alkaloids while terpenoids was the lowest.

Table 1: The Phytochemical Constituents of Extract of Prosopis africana seed				
Phytochemical	Qualitative analysis	Quantitative analysis		
Alkaloids	Present	2.12±0.28		
Saponins	Present	1.50±0.31		
Tannins	Present	4.84±1.25		
Phlobatannins	Present	1.59±0.23		
Cardiac Glycosides	Present	2.01±0.33		
Flavonoids	Present	1.52±0.20		
Sugars	Present	2.11±0.27		
Carbohydrates	Present	4.52±1.35		
Terpenes	Present	1.67±0.41		
Anthraquinones	Present	2.15±0.22		

All experiments were done in triplicate and data are presented as mean ± standard deviation (SD).

Table 2:	The	Phytochemical	Constituents	of	Methanol	Extract	of	Ocimum	gratissimum
Leaves									

Phytochemical	Qualitative analysis	Quantitative analysis
Flavonoids	Present	1.53±0.25
Alkaloids	Present	1.10±0.31
Steroids	Present	0.51±0.19
Saponins	Present	4.34±0.42
Oxalates	Present	0.59±0.33
Phenols	Present	0.91±0.08
Phytates	Present	6.14±0.43
Anthraquinones	Present	0.28 ± 0.07
Tannins	Present	3.11±0.53
Cyanogenic glycoside	Present	2.45±0.31

All experiments were done in triplicate and data are presented as mean ± standard deviation (SD).

Table 3: The Phytochemical Constituents of Methanol Extract of Vernonia amygdalina

 Leaves

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Phytochemical	Qualitative analysis	Quantitative analysis
Alkaloids	Present	5.30±0.31
Saponins	Present	3.50±0.36
Tannins	Present	2.06±0.05
Phlobatannins	Present	4.13±0.13
Glycosides	Present	2.50±0.50
Flavonoids	Present	$10.51 \pm .80$
Steroids	Present	5.25±0.36
Phenols	Present	4.40±0.18
Terpenoids	Present	2.03±0.06

All experiments were done in triplicate and data are presented as mean ± standard deviation (SD).

4.0 DISCUSSION

The current study was conducted to assess the phytochemical constituents of *Prosopis africana* seed, *Ocimum gratissimum* and *Vernonia amygdalina*. Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection provided by diets from some plants (Arts & Hollman, 2005). In this study the phytochemical screening of fermented Prosopis africana seeds contain alkaloids, saponins, tannins, phlobatannins, cardiac glycosides, flavonoids, sugars, carbohydrates, terpenes and anthraquinones. phytochemical Ocimum gratissimum following analysis indicates that the

bioactive compounds are present, flavonoids, alkaloids, steroids, saponins, oxalates, phenols, phytates, anthraquinones, tannins and cyanogenic glycosides. While the screening of the leaves extract of Vernonia amygdalina revealed the presence of various concentrations of secondary metabolites such as flavonoids, alkaloids, steroids. saponins, oxalates. phenols, phytates, anthraquinones, tannins and cyanogenic glycosides.

Some of these naturally occurring phytochemicals are anti-ulcerative agents, anti-hepatic agents, anti-carcinogenic and some possess other beneficial properties, while some prevent oxidation by free radicals and therefore known as chemopreventers. Antioxidant activity is one of the predominant mechanisms of their protective action which is because of their capacity to scavenge free radicals (Del Rio, 1997). All phytochemicals have these various pharmacological and therapeutic properties which are very essential in maintenance of good health.

In Nigeria, fermented Prosopis africana seeds are popularly used as food seasoning. It is evident that fermented food condiments are good sources of nutrients and could be used to produce complementary food supplements (Achi, 2005). Ocimum gratissimum is a plant distinguished for its therapeutic value (Ugwu et al., 2011), and generally, the plants of genus Ocimum are rich in antioxidant compounds such as phenolic and are much valuable for their curative potentials (Marjakahkonen et al., 1999; Ugwu et al., 2013). Vernonia amygdalina is a valuable medicinal plant that is widespread in West Africa and flavour, and can be used as an active anticancer, antibacterial, antimalarial and antiparastic agent.

Alkaloids have pharmacological applications as anesthetics and central nervous system stimulants. Flavonoids are important group of polyphenols widely distributed among the plant flora (Kar, 2007). The consumption of foods containing flavonoids has been linked to numerous health benefits. Tannins are astringent, bitter plant polyphenols that both binds and precipitate proteins and various other organic compounds including amino acids and alkaloid (Petridis, 2010). Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumour activity, and a wide range of anti-infective actions, have been assigned to tannins (Haslam, 1996).

Steroids are triterpenes with cyclopentane hydrophenanthrene ring system (Harborne, 1998). In all eukaryotes, sterols form integral components of the membrane lipid bilayer where they play an important role in the regulation of membrane fluidity and permeability (Corey et al., 1993). Saponins are also necessary for activity of cardiac glycosides (Kar, 2007). They possess a bitter and acrid taste, besides causing irritation to mucous membranes. Saponins are also important therapeutically as they are shown to have hypolipidemic and anticancer activity (Sarker & Nahar, 2007). They have also been shown to possess beneficial effects such as cholesterol lowering properties and structure dependent exhibit biological activity (Harborne, 1998). Terpenes are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins (Firn, 2010).

The presence of wealth of phytochemicals indentified from this study can justify the use of *Prosopis africana* seeds, *Ocimum gratissimum* and *Vernonia amygdalina* leaf in traditional medicine as a curative agent and can explain the long-established use of these plants. As result, the extracts can be used to develop new herbal formulation for treating several diseases.

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