

Original Research Article

COMPARATIVE ANALYSIS OF PHYTOCHEMICAL COMPOSITION OF FOUR SELECTED TROPICAL MEDICINAL PLANTS NAMELY: *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*.

ABSTRACT

Aim: The aim of the study was to carry out a comparative analysis of the phytochemical composition of the leaves of four selected tropical medicinal plants namely: *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*.

Methodology: The phytochemicals in the plant leaves were extracted by cold maceration in ethanol and subjected to both qualitative and quantitative analysis of the phytochemicals.

Results: The qualitative and quantitative analysis revealed the presence of the bioactive compounds alkaloids, Saponins, flavonoids, steroids, glycosides, terpenoids, polyphenols, specific cardiac glycosides, tannins, phytates and reducing compound in the leaves of each plant at varying quantities. Resins were only detected in *O.gratissimum*. From the quantitative analysis, *Gongronema latifolium* had the highest percentage content of alkaloids, glycosides, saponins, tannins and reducing sugars. *Ocimum gratissimum* had the highest flavonoid content. **Conclusion:** Taken together, *G. latifolium* on balance had a higher phytochemical content than the other three plants and thus should be more versatile in the treatment of a whole range of diseases. This was followed by *V. amygdalina*, *O. gratissimum* and *P. guineense* in that order. The fact that most of these phytochemicals have antioxidant activity may be responsible for their antidiabetic activities and treatment of other free radical prone diseases.

Key words: Phytochemical composition, Tropical medicinal plants, *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*.

1. INTRODUCTION

Plants have been used as medicines for the treatment of diseases in all cultures throughout history [1-3]. Plants are the medicine of choice for rural dwellers ostensibly because there are more accessible, cheaper and have a lower incidence of adverse effects after use compared to Orthodox medicine [4]. Plants synthesize hundreds of chemical compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified over the years making them most useful in the treatment of diseases and an important source of pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides some of which have antifungal, anti-inflammatory, fungistatic, molluscidal activity [5-6].

During the last few decades there has also been increasing acceptance and public interest in natural therapies in developed countries [7-10] because of the belief that it will result in a healthier life. Herbal medicines are also often viewed as a balanced and moderate approach to healing [2]. This has resulted in an increased interest in the study of medicinal plants and their traditional uses in different parts of the world.

Given the plethora of medicinal plants available it becomes difficult to identify which plant should be the best to be administered to ameliorate a given ailment. It is with a view to establishing the relative abundance of the phytochemicals in some commonly used medicinal plants in the southern region of Nigeria that the current study is being carried out. A comparative analysis of the phytochemical constituents of the four plants will provide a bio-rational basis for the choice of the plants for therapeutic remedy.

Ocimum gratissimum is an herbaceous perennial flowering plant which is woody at its base belonging to the family *Lamiaceae* (labiate). *Ocimum gratissimum* is commonly called African basil. The plant is pantropical and widely naturalized in many regions. It is widely distributed in tropical Africa and Asia, especially India. The plant is economically important for its essential oil and folklore medicinal use and insecticidal properties [11]. The essential oil of the plant has been widely used in food, cosmetic, pharmaceutical and soap industries [12,13].

Comment [W1]: Lamiaceae

Piper guineense commonly referred to as Ashanti pepper, West African pepper or African black pepper is a climbing perennial plant of the family *Piperaceae*. It is native to the tropical regions of Central and Western Africa and is widely distributed in countries like Nigeria, Cameroon, Ghana, Benin Republic and Guinea. *Piper guineense* is pharmacologically important for its medicinal, cosmetic and insecticidal uses [14]. It provides oil used as aromatics in the drink industry [15].

Gongronema latifolium (Family *Asclepiadaceae*) is a non-wood forest plant native to West Africa and is widely distributed elsewhere in tropical Africa and subtropical Asia. Commonly called "utazi," "aroeke" in the South Eastern and South Western parts of Nigeria respectively, it is, apart from its culinary uses, employed in traditional medicine [16]. The bark contains some quantity of latex and though it has been viewed with potential interest for its rubber, it has apparently never been exploited.

Vernonia amygdalina, Family *Asteraceae*, popularly called bitter leaf, is widely used in the West African sub-region for a number of medicinal purposes [17]. Traditional medicine practitioners use the plant as an anti-helminthic, anti-bacterial and as a laxative [18]. Others use it as a digestive tonic, appetizer and febrifuge for the topical treatment of wounds [19]. It

has been shown in our laboratory and elsewhere to have antidiabetic activity [20 and references therein].

2. MATERIALS AND METHOD

2.1 Materials

Mature leaf samples of *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina* were harvested from local farms in Cross River State, Nigeria.

2.2 Methods

2.2.1 Extraction Procedure

Fresh leaves of each plant were washed and air dried at room temperature (25°C) for two weeks. The dried leaves were pulverized using a mechanical grinder. A weighed quantity, 200g, of each plant material was extracted by cold maceration in absolute ethanol for 48 hours. The extracts were first filtered with a white muslin cloth after which the filtrates were re-filtered with Whatman no.1 filter paper. The resulting ethanol leaf extracts were concentrated in vacuum using a rotary evaporator (at temperatures between 40°C and 45°C to avoid denaturation of the active ingredients) to obtain a semi-solid mass. Weighed quantities of each extract was dissolved in 5% Tween 80 solution for use in the biochemical assays.

Comment [W2]: Mention how the aqueous extract was prepared.

2.2.2 Phytochemical Analysis

The fresh leaves of each plant were subjected to phytochemical analysis according to established standard methods. Preliminary qualitative phytochemical analysis to detect the presence of secondary metabolites such as Alkaloids, Glycoside, Saponins, Tannins, Flavonoids, Reducing Compounds, Terpenoids, Steroids, Polyphenols, Anthraquinones, Resin, Phlobatannins, Phytate, Specific Cardiac Glycosides and Oxalate was carried out according to methods outlined by Harborne [21] and Trease and Evans [22].

Quantitative determination of reducing compounds in the plant extracts was carried out using Clegg Anthrone Colorimetric Method. Saponin content was determined using the method of Birk *et al.* [23] as modified by Hudson and El Difrawi [24]. Alkaloids and flavonoids were determined using the gravimetric method of Harborne [25]. Tannin was determined by the photometric method using Follin Dennis spectrophotometric method [26].

3. RESULTS & DISCUSSIONS

3.1 Phytochemical screening

The result of the qualitative screening of the Plant leaf extracts is shown in Table 1 while the quantitative screening of the plant leaf extracts is shown in Figure 1.

Qualitative phytochemical screening revealed the presence of the bioactive compounds alkaloids, glycosides, terpenoids, polyphenols, specific cardiac glycosides and reducing compound in the leaves of each plant at varying quantities. Saponins, flavonoids, steroids and phytate were present in most of the plants, while resins were only detected in *O.gratissimum*. Similar reports had been documented for *O.gratissimum* [11], *V. amygdalina* [27] and *G. latifolium* [28-30]. Specific cardiac Glycosides appear to be the predominant glycosides present in the leaves of *P.guineense*, *G.latifolium*, and *V. amygdalina* while glycosides were detected at low intensity in *P.guineense* and high intensities in *O.gratissimum*, *G.latifolium* and *V. amygdalina*.

The medical properties of these component phyto compounds have been well documented [31-33]. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains have been reported [34].

Table 4.1: Qualitative analysis of phytochemical components in ethanol and aqueous extracts of *O. gratissimum*, *P. guineense*, *G. latifolium* and *V. amygladina*

| Constituents | <i>O. gratissimum</i> | | <i>P. guineense</i> | | <i>G. latifolium</i> | | <i>V. amygladina</i> | |
|----------------------------|-----------------------|---------|---------------------|---------|----------------------|---------|----------------------|---------|
| | Ethanol | aqueous | Ethanol | aqueous | Ethanol | aqueous | Ethanol | aqueous |
| Alkaloids | + | + | ++ | + | ++ | ++ | ++ | + |
| Saponins | + | + | ++ | + | +++ | ++ | +++ | + |
| Flavonoids | +++ | + | + | + | + | + | ++ | + |
| Phytates | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Glycosides | +++ | ++ | + | ++ | +++ | ++ | +++ | ++ |
| Specific cardiac glycoside | ++ | + | +++ | +++ | +++ | +++ | ++ | ++ |
| Terpenoids | +++ | ++ | +++ | +++ | ++ | ++ | + | + |
| Steroids | ND | + | + | + | + | + | +++ | +++ |
| Polyphenols | ++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Reducing compound | + | + | ND | + | +++ | + | +++ | +++ |
| Tannins | ++ | + | +++ | + | +++ | + | +++ | ++ |
| Phlobatannins | ND | ND | ND | ND | ND | ND | ND | ND |
| Anthraquinones | ND | ND | ND | ND | ND | ND | ND | ND |
| Resins | + | ND | ND | ND | ND | ND | ND | ND |
| oxalate | ND | ND | ND | ND | ND | ND | ND | ND |

Comment [W3]: In the qualitative analysis, the aqueous extracts contain alkaloids, terpenoids and steroids. But these compounds are water insoluble compounds. In case of *O. Gratissimum*, steroids are not present in ethanol extract but present in aqueous extract. How it is possible?

Keys:

+ = Slightly Present
 ++ = Medium Presence
 +++ = Heavy Presence
 ND = Not Detected

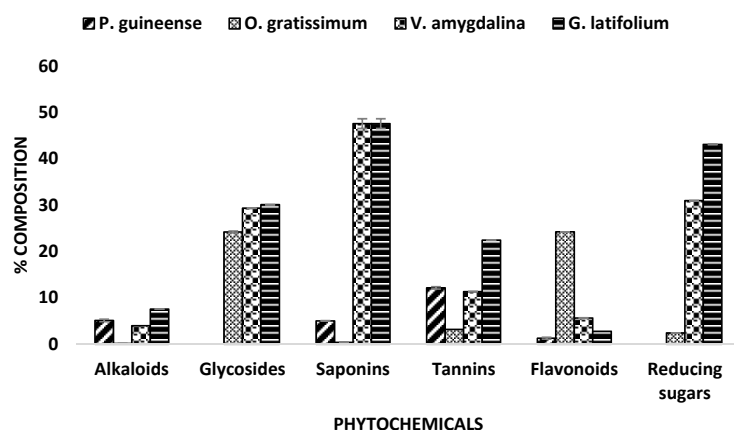


Fig 1: Quantitative Analysis of Phytochemical composition in *P.guineense*, *O.gratissimum*, and *V. amygdalina* and *G.latifolium* Values are expressed as mean + SEM.

The quantitative results, just like the qualitative results, also revealed the presence of the phytochemicals in varying amounts. Following is the presentation and discussion of the quantitative results.

3.1.1 Alkaloids: The results showed *G. latifolium* to have the highest alkaloid content followed by *P. guineense* and *V. amygdalina* with *O. gratissimum* having a negligible content. *G. latifolium* will thus be the plant of choice for alkaloid sensitive ailments. Alkaloids are a large and complex group of cyclic compounds that contain N. They almost uniformly evoke a bitter taste [35]. A whole range of pharmacological activities have been ascribed to Alkaloids. This includes antimalarial, anticancer [36], cholinomimetic [37], vasodilatory, antiarrhythmic [38], antibacterial [39], antihyperglycemic [40], psychotropic and stimulant activities [41]. Others include antitussive, hallucinogenic, uterotonic, emesis, antipyretic, antihypertensive and antitumor activities [42]. It has also been used as aphrodisiacs, in vasoconstriction and as inhibitor of acetylcholinesterase [42]. On the down side, Alkaloids can be toxic [43] and has indeed been used as poisons.

3.1.2 Glycosides: A Glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. *G. latifolium* again had the highest glycoside content followed closely by *V. amygdalina* and *O. gratissimum*. The glycoside content was too low to be quantified in *P. guineense*. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides which can be activated by enzyme hydrolysis [44]. This hydrolysis causes the sugar part to be broken off, making the other part available for use. Many such plant glycosides are used as medications (e.g. saponins). In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body.

Prominent among the glycosides are the cardiac glycosides (here the aglycone part is a steroidal nucleus) whose most important use is its effects in treatment of cardiac failure [45]. This it does by inhibiting Na⁺, K⁺-ATPase, and consequently increase the force of myocardial contraction [45,46]. Some cardiac glycosides have shown promise for their antitumor activity [47]. In addition, it has been reported that some cardiac glycosides display an inhibitory activity against rhinovirus [48]. The presence of specific cardiac glycosides at relatively high quantities in *P. guineense* and *G. latifolium* (Table 1) indicates the potentials of the plants in the management and treatment of cardiac arrhythmias, atrial fibrillation, atrial flutter, congestive heart failures and dropsy.

3.1.3. Saponins: *G. latifolium* and *V. amygdalina* had the highest saponin content followed, but not closely, by *P. guineense*. For *G. latifolium* and *V. amygdalina* saponins constituted the major phytochemical. *O. gratissimum* had minimal amount of saponins. Saponins are a special group of glycosides with foaming characteristics and a bitter taste. They consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have a wide a range of medicinal and non-medicinal uses. Saponins have been shown to have antimicrobial activities [21,49] and may contribute to the antimicrobial activities exhibited by *G. latifolium* and *O. gratissimum* [50-52]. Their antimicrobial action is thought to be due to their ability to emulsify lipid [49]. Once in direct contact with microbes, saponins causes cell rupture and eventual death of the microorganism. Saponins have also been shown to have pronounced haemolytic properties when given intravenously [53,54] by a mechanism similar to that of microbial rupture; the interaction of saponins with membrane-bound sterols, causes an increase in the permeability of the plasma membrane, bringing about the destruction of the cell. However, when administered enterally, saponins are harmless to man and other animal as it is rapidly hydrolysed by digestive enzymes, releasing the aglycones or sapogenins in free form as well as the sugar moieties. The aglycones, which are easily absorbed from the GIT, are equally capable of inhibiting the growth and development of pathogens [21]. It is these aglycones that actually exhibit the biological activities attributed to saponins. Saponins have also been used as expectorant and for their corticoid and anti-inflammatory effects [55]. Saponins are also natural ruminal antiprotozoal agents [55]. The presence of steroids indicates the cholesterol lowering potential of the plants which may likely be of great benefit in the prevention and treatment of atherosclerosis and heart disease as well as diabetes. As a matter of fact all four plants have been shown to have antidiabetic activities [56-59].

Comment [W4]: Ruminal or Luminal?

3.1.4 Tannin composition was more in *G. latifolium* followed by *P. guineense*, *V. amygdalina* and to a lesser extent *O. gratissimum*. Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that have been reported to be responsible for decreases in feed intake, and protein digestibility [reviewed in 60]. On the upside many tannin components have been suggested to be anti-carcinogenic and have also been shown to reduce the mutagenic activity of a number of mutagens [60]. These potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. As metal ion chelators, tannins exert their biological antioxidant activity by preventing the availability of ion for the production of free radicals such as hydroxyl radical via the Fenton and Haber-Weiss reactions [61]. Tannins have indeed been reported to inhibit the generation of superoxide radicals. Tannins have also been shown to have considerable antimicrobial activity. The growth of many fungi, yeasts, bacteria, and viruses have been shown to be inhibited by tannins [34,60,62]. They have been demonstrated to be toxic to rumen microorganisms, like *Streptococcus boris*, *Butyrivibrio fibrosolvens*, *Fibrobacter Succinogens* etc. [62]. However, tannins have little or no systemic effects as they are poorly absorbed in the gastro-intestinal tract (GIT), due to their very strong ability to complex or chelate macromolecules and metal ions [61]. As protein chelators, tannins are employed as astringent both in the GIT (as purgatives) and on skin abrasions (as clothing agents). Their presence enhances the treatment of burns and wounds by providing a protective coat under which the regeneration of tissues takes place [63]. Tannins are probably responsible for the use of *V. amygdalina* as astringent [64]. However, as a result of their chelating activity, consumption of tannin rich foods may deplete enzyme cofactor and precipitate deficiency diseases such as anemia [65]. Other physiological effects that have been reported to be exerted by tannins include acceleration of blood clotting, reduction of blood pressure, decrease of serum lipid level, production of liver necrosis, and modulation of immune responses.

3.1.5 Flavonoids: The Plant with the highest flavonoid composition by far was *O. gratissimum*. Flavonoids and glycosides were the major phytochemicals in *O. gratissimum*. This was followed by *V. amygdalina*, *G. latifolium* and *P. guineense* in that order. *O. gratissimum* will thus be a plant of choice in the treatment of flavonoid sensitive diseases. Flavonoids are phytochemicals with variable phenolic structures. Flavonoids are well established antioxidants [66,67]. They have been reported to possess antioxidant activity approximately 50 times that of vitamin C and E [68 and references therein]. Available reports also show that flavonoids possess anti-allergic [69], anti-inflammatory [66,69], anti-microbial [70-73], anti-cancer [66] and anti-diarrheal activities [74]. Flavonoids have also been shown to inhibit topoisomerase enzymes [75,76] and to induce DNA mutations in the mixed-lineage leukaemia (*MLL*) gene in *in vitro* studies [77]. Its anti-inflammatory properties makes it a candidate for the treatment of cancer [78], cardiovascular disorders [79], diabetes mellitus [80] and celiac disease [81] where Inflammation has been implicated as a possible origin of the diseases. The various mechanisms that have been proposed for the anti-inflammatory activities of flavonoids include its ability to inhibit reactive oxygen or nitrogen compounds and to inhibit the pro-inflammatory activity of enzymes involved in free radical production such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase [82,83] and to modify intracellular signaling pathways in immune cells [82] or in brain cells after a stroke [84].

3.1.6 Reducing sugars: The compositions of reducing sugars was in the same order as that for the glycosides ie *G. latifolium*, *V. amygdalina* and to a lesser extent *O. gratissimum*. As in the case of glycosides, reducing sugars were too low to be quantified in *P. guineense*.

4. CONCLUSION Taken together, *G. latifolium*, on balance had a higher phytochemical content than the other three plants and thus should be more versatile in the treatment of a whole range of diseases. This is followed by *V. amygdalina*, *O. gratissimum* and *P. guineense* in that order. *O. gratissimum* however had the highest flavonoid composition. The fact that all these phytochemicals have antioxidant activity may be responsible for their antidiabetic activities and treatment of other free radical prone diseases.

In summary, this work shows the relative abundance of phytochemicals in the four medicinal plants namely *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina* employed in ethno pharmacology in the West African sub region and elsewhere. Both the qualitative and quantitative results revealed the presence of a number of phytochemicals in varying amounts. The results provide a bio rational basis for which of the plants, or combination of plants, should best be administered to ameliorate a given ailment.

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