Phytochemical Composition of Aqueous and Ethanolic Leaf Extracts of *Piper guineense, Cassia alata, Tagetes erecta* and *Ocimum graticimum*

ABSTRACT

Phytochemicals are biologically active naturally occurring chemicals produced by plants to help them thrive or thwart competitors or pathogenic organisms. The phytochemical experiment revealed varying degrees of flavonoid, alkaloid, saponin, tanin and phenol as naturally occurring bioactive chemicals of water and ethanol leaf extracts of *Piper guineense, Cassia alata, Tagetes erecta* and *Ocimum graticimum. T. erecta* had the highest flavonoid, alkaloid, saponin and tanin content of 3.17%, 5.43%, 3.50% and 5.15% respectively in ethanol extract and this was significant ($P \leq 0.05$), and followed by *C. alata*, *O. graticimum*, and *P. guineense*. The highest phenol content of 3.50% was recorded in water extract of *C. alata* followed by water extract of *T. erecta, O. graticimum*, and ethanol extract of *P. guineense*. The exploitation of antimicrobial potentials of these phytochemicals in the control of plant diseases incited by pathogenic organisms is recommended.

INTRODUCTION

Plants are composed entirely of chemicals of various kinds [1] produced through primary or secondary metabolism for normal physiological functions and defense against competitors, pathogens, or predators [2,3]. Plant parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, contain natural chemical components that are bioactive that confer them with resistance against pathogens [4]. Thus, phytochemicals as biological active plant chemicals have protective or disease preventive properties [5]. Studies on the phytochemical compositions of some plants have been carried out and they were found to be rich in alkaloids, phenols, flavonoid, saponin and tannins [6]. [7] reported that the most important

classes of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Other classes include saponins, glucosides, anthraquinones, essential oils, steroids, and terpenes. Alkoloids are the largest group of secondary chemical constituents of plants which are readily soluble in organic solvents and slightly soluble in water except their salts [8,5]. Phenols occur as natural colour pigments in plants that are potentially toxic to the growth and development of pathogens [9] whereas Flavonoids are important group of the plant flora [8] whose activities include antioxidant property, protective effects and inhibition of the initiation, promotion and progression of tumors [10-12]. Saponins possess foaming characteristics due to the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part [5]. According to [11] saponins prevent disease invasion of plants by parasitic fungi thus, possessing antifungal properties [13]. Tanins which are widely distributed in plant flora are soluble in water and alcohol [5] and are used as antiseptic to inhibit the growth and development of pathogenic fungi [14,15]. These natural bioactive chemicals of plants have been reported to be responsible for the antimicrobial effects of plant extracts against pathogenic organisms [16-18]. The phytochemical composition of water and ethanol leaf extracts of *Ocimum gratissimum* (sweet basil), *Piper guineense* (black pepper), *Cassia alata* (Candle bush) and *Tagetes erecta* (African marigold) were evaluated and presented in this paper.

MATERIALS AND METHODS

Source of Plant Materials

The plant materials, *Ocimum gratissimum* leaf (Plate 1) and *Piper guineense* leaf (Plate 2) were obtained from market stalls in Umuahia, Abia State whereas *Cassia alata* leaf (Plate 3) and

Tagetes erecta leaf (Plate 4) were obtained from Umudike, Umuahia, Abia State. The materials were taken to the Department of Botany, Michael Okpara University of Agriculture, Umudike for identification before being taken to the laboratory for cleaning, drying and grinding into powder for further studies.

Plate 1: ***Ocimum gratissimum***

Plate 2: ***Piper guineense***

Plate 3: ***Cassia alata***

Plate 4: ***Tagetes erecta***

Extraction and Quantitative Phytochemical Screening of the Test Plant Materials

Fresh and healthy leaves of *O. gratissimum, P. guineense, C. alata* and *T. erecta* were thoroughly washed under running tap water and rinsed with sterile distilled water. The leaves were dried in an oven at 60°C for 24 hours and then ground into powder using a hand grinding machine. The powdered sample of each of the test plants was weighed out separately and stored in a storage bottle.

Ethanolic and aqueous extracts of the leaves of test plants were prepared by soaking 100g each of the dry powder of the test plant leaves separately in 1000 ml of absolute ethanol or sterile distilled water at room temperature (28^{o}C) for 48 hrs. The solution was then filtered through a Whatmann filter paper. The filtrates were thereafter concentrated using a rotary evaporator with

water bath set at 60°C. The concentrated crude extract was then stored at 4° C [19]. The ethanolic and aqueous extracts were subjected to various qualitative and quantitative phytochemical tests to determine the presence and quantity of active constituents present in the extracts using some standard procedures. The phytochemicals tested for were; alkaloids, flavonoids, phenol, tannins, and saponins. The methodology for the determination of the percentage composition of the phytochemicals in the test plant materials are as follows:

Determination of Percentage Tannin

The tannin content of the leaves of test plants was determined using the Folin Dennis spectrophotometric method of [9]. Each powered leaf sample of the test plants (2.0 g) was separately mixed with 50 ml of distilled water or ethanol and shaken for 30 minutes with a shaker. The mixture was filtered and the filtrate (5 ml) was separately measured into 50 ml volumetric flask and diluted with 3 ml of distilled water or ethanol. Similarly 5 ml of standard (tanuric acid solution) and 5 ml of each extracting solvent were added separately (control). One milligramme of Folin- Dennis reagent was added to each of the flask followed by 2.5 ml of saturated sodium carbonate solution. The content of each flask was made up to 50ml mark and left to stand for 90 minutes at room temperature (28°C). The absorbance of the developed colour was measured with spectrophotometer at 760 nm wave length with the reagent blank at zero. The process was repeated three times to get an average. The tannin content was calculated according to [9] as shown below:

$$\% \text{ tannin} = \frac{100}{W} \times \frac{AY}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times \frac{D}{1}$$

Where:

W = weight of sample analysed

AY = Absorbance of the standard solution

C = Concentration of standard in mg /ml.

VA = volume of filtrate analyzed

D = Dilution factor where applicable

AS = Absorbance of standard tannin solution

VF = volume of volumetric flask used

Determination of Percentage Alkaloid

The determination of the concentration of alkaloid in the leaves of the test plants was carried out using the alkaline precipitation gravimetric method of [9]. Five grams (5g) of the powdered sample of each plant material was separately soaked in 20 ml of sterile distilled water or 10% ethanolic acetic acid. The mixture was allowed to stand for 4 hrs at room temperature (28^{o}C). Thereafter, the mixture was filtered through Whatman filter paper (No. 42) and the filtrate concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added

in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60^{o}C for 30 minutes, cooled in a desiccator and reweighed. The process was repeated two times and the average was taken. The weight of alkaloid was determined as a percentage of weight of sample analyzed as shown below:

$$\% \text{ Alkaloid} = \frac{\text{Wt of Alkaloid ppt}}{\text{Weight of sample}} \times \frac{100}{1}$$

$$= \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where:

W_1 = weight of filter paper

W_2 = weight of sample + weight of filter paper

W_3 = weight of filter paper + alkaloid precipitate (ppt)

Determination of Percentage Phenol

The concentration of phenols in the leaves of test plants was determined using the folin- cio Caltean colorimetric method of [9]. Each of the powdered samples (0.2 g) was added into a test tube and 10ml of water or ethanol was separately added to it and shaken thoroughly. The mixture was left to stand for 15 minutes before being filtered using Whatman filter paper (No. 42). One milliliter (1 ml) of the extract filtrate was placed in a text-tube and I ml folin-cio Caltean reagent in 5ml of distilled water or ethanol was added and the colour was allowed to develop for 2 hours at room temperature. The absorbance of the developed colour was measured at 760 nm wave length. The process was repeated two more times and an average taken. The phenol content was calculated thus.

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{AY}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times \frac{D}{1}$$

Where,

W = weight of sample analysed

AY = Absorbance of the standard solution

C = Concentration of standard in mg /ml.

VA = volume of filtrate analysed

D = Dilution factor where applicable

AS = Absorbance of standard tannin solution

VF = Total filtrate volume.

Determination of Flavoniods

Flavonoid was determined using the method of [6]. The processed sample of test plant materials was weighed (5g) and boiled in 100 ml of 2M HCl solution under reflux for 40 minutes. It was allowed to cool and then filtered with a Whatman (No 42) filter paper. The filtrate was treated with equal volume of ethyl acetate (contained in the ethyl acetate portion) and was recovered by filtration using pre-weighed filter paper. The weight was obtained after drying in the oven at 60^{o}C and cooling in a desiccator. The process was repeated two more times to get an average. The quantity of flavonoid was determined as shown below:

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:

W_2 =weight of filter paper and flavonoid precipitate

W_1 =weight of filter paper alone

Determination of Saponin

The saponin content of the sample was determined by double extraction gravimetric method by [9]. Each of the powered sample (5g) of the test plant materials was mixed with 50 ml of distilled water or 20% ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55^{o} C. It was then filtered through Whatman filter paper (No. 42). The residue was extracted with 50 ml of 20% ethanol or distilled water and reduced to about 40 ml at 90^{o}C and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer became clear in colour. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60^{o} C in the oven and reweighed after cooling in a dessicator. The process was repeated two more times to get an average. The percentage Saponin content was determined as shown below:

$$\% \text{ Saponin} = \frac{\text{Wt of Saponin}}{\text{Weight of sample}} \times \frac{100}{1}$$

$$= \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where:

W_1 = weight of evaporating dish

W_2 =weight of dish + sample

W_3 = weight of dish + saponin

ANOVA

The experiments were laid out in completely randomized design (CRD) with three replicates. The data were analyzed using Analysis of Variance (AOVA) and means were separated using least significant difference (LSD) at 5% level of probability.

RESULTS

The quantitative phytochemical composition of water and ethanol extracts of the plant materials is shown in the Table 1. The result showed that with exception of phenol that recorded the highest value in water extract, the phytochemicals of the plant materials were more when ethanol was used as extracting solvent and this was significant ($P \leq 0.05$). *T. erecta* had the highest flavonoid, alkaloid, saponin and tanin contents in both ethanol and water extracts followed by *C. alata, O. graticimum* and *P. guineense.* The highest and lowest flavonoid content of 3.17 % and 1.07% were recorded in the ethanol extract of *T. erecta* and water extract of *P. guineense* respectively. The ethanol extract of *T. erecta* had the highest alkaloid content (5.43%) followed by *C. alata* (4.33%), *O. graticimum* (4.03%) and *P. guineense* (3.58%). The highest saponin and tannin contents of 3.50% and 5.15% respectively were recorded in *T. erecta* ethanol extract. The water extract of the plant materials recorded more phenol than ethanol extracts with highest phenol content of 3.50% in water extract of *C. alata*, followed by *T. erecta* (3.36%), *O. graticimum* (3.03%) and *P. guineense* which recorded the least value of 1.60% in water extract but highest value of 2.55% in ethanol extract. Generally, there were higher percentages of the phytochemicals in ethanol extracts than in water extracts except in phenol where the water

extract contained more than ethanol extract in all the test plants except *P. guineense* which recorded the highest percentage of phenol in ethanol extract.

Table 1: Phytochemical composition of aqueous and ethanol extracts of *O. graticimum, P. guineense, C. alata* and *T. erecta.*

Plant	Extracts and Phytochemical Composition (%)									
	Flavonoid		Alkaloid		Saponin		Tanin		Phenol	
	EE	WW	EE	WW	EE	WW	EE	WW	EE	WW
Piper guineense	2.07	1.07	3.58	2.17	1.82	0.83	1.88	1.06	2.55	1.60
Ocimum graticimum	2.50	1.55	4.03	3.10	2.10	1.07	3.98	1.63	0.54	3.03
Cassia alata	3.03	2.07	4.33	3.50	2.73	1.17	4.72	1.12	0.26	3.50
Tagetes erecta	3.17	2.58	5.43	4.07	3.50	1.50	5.15	2.48	0.23	3.36
LSD (5%)	**0.09**		**0.18**		**0.20**		**0.12**		**0.17**	

Data are means of three replicates in two separate experiments

WW = water extract, EE = ethanol extract

DISCUSSION

The screening of *O. graticimum, P. guineense, C. alata* and *T. erecta* for phytochemicals confirmed that the leaves of the test plant materials contain Saponin, flavonoid, Alkaloid, Tannis, and phenol which agreed with the work of [20] who reported the presence of bioactive secondary metabolites like alkaloids, tannins, saponins, flavonoids, and phenols in plants with antimicrobial

properties. There were differences in the phytochemical compositions in the water and ethanol extract of the test plants. The ethanolic extracts were found to contain more phytochemicals than water extracts except phenol across all the test plant materials. The difference may be due to the extracting solvents used or the solubility of the bioactive chemicals in the extracting solvent with higher solubility of the phytochemicals in ethanol than water as extracting solvent [21.22].

Phytochemicals are employed by plants to protect themselves against pathogens (Bacteria, Fungi or Protozoa) or insects and different mechanisms of action have been suggested against pathogenic organisms, such as interference with the phospholipid bilayer of the cell membrane, damage of the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material [5]. In general, the mechanism of action of phytochemicals is channeled towards inhibition of the growth of microorganisms, interfering with some metabolic processes or may modulate gene expression and signal transduction pathways [4,23-25], disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents [26]. Thus, phytochemicals may either be used as chemo-therapeutic or chemo-preventive agents. Chemo-prevention referring to the use of phytochemicals to inhibit, deter or retard growth of an organism and chemo-therapeutic referring to the use of the bioactive chemicals to exterminate an organism after it has established [8,4]. The Phytochemicals of the test plant materials are therefore suitable for exploitation as potent pesticides and possible substitute for synthetic pesticides in the control plant diseases and reduce food losses arising from diseases caused by pathogenic organisms.

CONCLUSION

The aqueous and ethanol leaf extracts of *O. graticimum, P. guineense, C. alata* and *T. erecta* contained varying degrees of saponin, flavonoid, alkaloid, tannis, and phenol. The ethanolic extracts recorded more phytochemicals than water extracts except phenol which was more in water extracts across all the test plant materials. These natural bioactive chemicals of test plant materials could be exploited for their antimicrobial effects against pathogenic organisms.

REFERENCES

1. Breslin, Andrew (2017) "The Chemical Composition of Green Plants". *Sciencing, Leaf Group Ltd.* UK, p. 76
2. Molyneux, RJ; Lee, ST; Gardner, DR; Panter, KE; James, LF (2007). "Phytochemicals: the good, the bad and the ugly?". Phytochemistry. **68**: 22–24.
3. Sangoyomi T.E., Asiedu R., and Ekpo E.J.A., (2010). Effects of ten plant extracts on mycelial growth and conidial production of four fungi associated with yam tuber rot, *AJRTC*, 8 (1): 24-30.
4. Doughari J. H., Human I. S., Bennade S., and Ndakidemi P. A., (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal Plants Research*, 3(11): 839-848
5. Doughari, James Hamuel (2012). Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, Dr Venketeshwer Rao (Ed.), InTech
6. Omodamiro O.D. and Ekeleme C.M., (2013). Comparative study of in vitro antioxidant and antimicrobical activities of *Piper guineense, Curmuma longa, Gongronema latifolium, Allium sativum, Ocimum gratissimum*. *World J. Med. Med. Sci*, 1 (4): 51-69
7. Okwu D.E., (2001). "Evaluation of the Chemical Composition of Indigenous Spices and Flavouring Agents", *Global J. Pure Appl. Sci.*, 7 (3): 455-459
8. Rufai Y., Isah Y. and Isyaka M.S., (2016). Comparative Phyto-Constituents Analysis from the Root Bark and Root Core Extractives of *Cassia ferruginea* (Schrad D. C) Plant. Sch *J Agric Vet Sci*, 3 (4): 275-283
9. Osuagwu G. G. E. and Eme C. F. (2013). The Phytochemical Composition and Antimicrobial Activity of *Dialium Guineense*, *Vitex Doniana* and *Dennettia Tripetala* Leaves. *Asian J of Natural & Applied Sci*, 2 (3): 69-81
10. Kim S.Y., Kim J.H., Kim S.K., Ohandy M.J. and Jung M.Y., (1994). Antioxidant activities of selected oriental herb extracts. *J. Am Oil Chem Soc*., 71: 633-640

11. Okwu, D.E., (2004). Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. *Journal of Sustainable African Environment*, 6: 30-37.
12. Kar A., (2007). Pharmaocgnosy and Pharmacobiotechnology *(Revised-Expanded Second Edition)*. New Age International Limted Publishres, New Delhi. p. 332-600
13. Osuagwu, G. G. E. and Ihenwosu, A. O. (2014) Phtochemical composition and antimicrobial activity of the leaves of *Alchornea cordifolia* (Schum and Thonn), *Sanseviera liberica* (Gerand Labr) and *Uvaria chamae* (P. Beauv), AJPCT, 2 (1): 1-12
14. Burkill I.H., (1995). The Useful Plants of West Tropical Africa (Vol 3 Families J-L). Royal Botanical Garden, Kew, p. 605.
15. Okigbo R.N., Opara P.U. and Anuagasi, C.L., (2015). Efficacy of extracts of water yam (*Dioscorea alata*) and aerial yam (*Dioscorea bulbifera*) peels in the control of white yam (*Dioscorea rotundata*) rot. *Journal of Agricultural Technology*, 11 (8): 1823-1842
16. Abo, K.A., Ogunleye, V.O., and Ashidi, J.S., (1991). Antimicrobial Poteintial of *Spondias mombin, Croton zambesicus* and *Zygotritonia crocea*. *J Pharm Res*, 5 (13): 494-497.
17. Liu R.H., (2004). Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. *Journal of Nutrition*. 134 (12): 3479-3485
18. Nweze E.L., Okafor J.L. and Njoku O., (2004). Antimicrobial Activities of Methanolic extracts of *Trume guineesis* (Scchumn and Thorn) and *Morinda lucinda* used in Nigerian Herbal Medicinal practice. *J. Biol.Res. Biotech*., 2 (1): 34-46.
19. Usunobun, U., Okolie, N. P., Anyanwu, O. G., Adegbegi, A.J. and Egharevba, M. E. (2015). Phytochemical screening and proximate composition of *Annona Muricata* Leaves. *European Journal of Botany Plaint Science and Phytology*, 2 (1): 18-28.
20. El-Mahmood A.M., Doughari J.H., and Ladan N., (2008). Antimicrobial screening of stem bark extracts of *Vittellaria paradixa* against some enteric pathogenic microorganisms. Afr J Pharm Sci., 2: 89–94.
21. Amadioha, A. C. (2003) Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. Acta Phytopathologica, 38: 259 – 265.
22. Amadioha A. C., Uzoanya, O. and Opara, J. O. (2012) Control of postharvest micronbial deterioration of tomato (*Lycopeersicon esculentum* Mill) using plant leaf extracts. J Sust Agric and Environ, 13 (1):91 -98.
23. Kris-Etherton P.M., Hecker K.D., Bonanome A., Coval S.M., Binkoski A.E., Hilpert K.F., Griel A.E., Etherton T.D., (2002). Bioactive Compounds in Foods: Their Role in the Prevention of Cardiovascular Disease and Cancer. *Am. J. Med*., 113: 71-88
24. Manson M.M., (2003). Cancer prevention: the potential for diet to modulate molecular signalling. Trends Mol. Med., 9: 11-18.
25. Surh Y.J., (2003). Cancer Chemoprevention with Dietary Phytochemicals. Nat. Rev. Cancer, 3 (10): 768-780
26. Kotzekidou P., Giannakidis P., and Boulamatsis A., (2008). Antimicrobial Activity of Some Plant Extracts and Essential Oils against Food-borne Pathogens *in vitro* and on the fate of Inoculated Pathogens in Chocolate. LWT, 41 : 119-127

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