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Phytochemical evaluation of oil extract from three indigenous medicinal plants in South west Nigeria

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ABSTRACT

The study evaluated phytochemical components of plant oil extracted from three common indigenous plants; *Acalypha godseffiana*, *Annona muricata* and *Petiveria alliacea* spatially distributed in South west Nigeria. Oil extraction was performed in a standard laboratory according to Association of Official Analysts and Chemist (AOAC, 2003) procedures. Results from the assay shows that *A. godseffiana* oil extract possesses higher phytochemical contents; tannin (7.76mg/ml), flavonoids (14.3mg/ml), cardiac glycosides (20.9mg/ml) terpenoids (11.9 mg/ml) and alkaloids (24.38%) than the oil extract from the other medicinal plants. Furthermore, oil extract from *A. godseffiana* reflected higher cumulative phytochemical constituents. These phytochemicals were noted to act as insecticidal agents, thus essential in the management pest agricultural pest of economic importance. Rigorous biochemical characterization of the oil extracts is suggested for identification and isolation insecticidal compounds of interest.

INTRODUCTION

Plants are essential and vital to humankind existence, used in different ways and considered to be of nutritional, economic, medicinal, ecological and socio-cultural importance. Medicinal plants are of great importance to the health of individuals and communities in Nigeria (Edeoga et al., 2005). Many of these plants are indigenous to Nigeria, used as spices and food plants. Plants tissues contained several bioactive compounds and secondary metabolites which works on biological systems (plant, pests and microbes) in different manners. Nigeria is rich in diversity of plant genetic resources (PGR), many of which exist in wild forms in the plants' natural habitats and in diverse crop landraces/ecotypes/cultivars (NACGRAB, 2008).

These plant diversity include; *Acalypha godseffiana* (Muell Arg), *Annona muricata* (L.) and *Petiveria alliacea* (L.) that are spatially distributed in the various agro-ecologies of the country. *A. godseffiana* is a medicinal plant proven to have anti-malarial and anti-fungi efficacy, and used in the management of hypertension and diabetes (Ikewuchi et al., 2011). *A. muricata* (soursop) is a fruit bearing tree widely distributed throughout tropical and sub-tropical parts of the world. Its fruits are usually eaten raw, medicinal and have been reported as anticonvulsant and anticancer agent **(**Moghadamtousi et al., 2015). *P. alliaceae* is a perennial shrub in used in natural medicine and various preparations made from it are considered to have anti-inflammatory, anti-microbial, anti-spasmodic and diuretic (Kim et al*.*, 2006*).*

Plants are usually known to synthesize aromatic substances, which in many cases act as agents of plant defense against predation by microorganisms, insects, and herbivores (Nnama et al*.*, 2016). Currently, there has been renewed research interests in the use plant secondary metabolites as botanicals (plant insecticides) , and as a reliable alternative to synthetic pesticides for pest management in reaction to adverse effects of chemical pesticide on the environment ,pest resurgence, development of insecticidal resistance against pesticides by crop major pest and food contamination (Isman,2006 ; Ofuya,2015). The importance of plants as botanicals stemmed from the fact of their availability in nature, ease of use and processing, possession of low mammalian toxicity, and being both eco and environmental friendly. Secondary metabolites are the compounds that do not affect the normal growth and development of a plant but reduce the palatability of the plant tissues to crop pests (Howe and Jander, 2008). According to Aletor (1999) several plant chemical components are known to have insecticidal properties either as whole leaves, powders or water and or as oil extracts.

However, for rational and extensive economic utilisation of these plants as bio pesticides, it is imperative to validate their chemical composition. Thus, this study therefore investigated the phytochemical constituents of the oil extracts obtained from these important medicinal plants for their potential as promising pesticides of use in food and agriculture.

METHODOLOGY

Collection of the plant materials

The plants used in this study ; *A. muricata* and *P. alliacea* were collected from the Field Genebank of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan (07^o 23 11 3"N , 3^o 50 25 0"E). Fresh leaves of *A*. *godseffiana* were collected from Botanical Garden, Teaching and Research Farm of the Federal University of Technology, Akure (07^o18 24 4"N, 5^o 21 7 "E) .The identity of the plant materials was confirmed at the Herbarium of the NACGRAB.

Extraction of plant oils

Fresh leaves of *A. godseffiana* and *P. alliacea* were washed under running tap water to remove the surface pollutants. Mature fruits of *A. muricata* were cut longitudinally and the black seed removed with the aid of a sharp knife. The leaves were air dried at room temperature (27± 2°C) for 4-5 days. Dried seeds and leaves were milled separately into fine powder. Extraction of the oil from the plant materials was carried out following the procedures of Association of Official Analytical Chemist (AOAC, 2003). 500g sample of the powdered materials was macerated at room temperature (27 ± 2^oC) in 500ml of 99 % ethanol (BDH®) for 48hrs and then filtered with filter paper (Whatman, 9mm).The solvent was evaporated by using a rotary evaporator (Resona Technics®) .The resulting slurry was air dried to remove traces of the solvent .The oil obtained was kept in reagent bottles and stored in a deep freezer at 4^o C until needed.

Table 1: Profile of the medicinal plants evaluated

Scientific name	Family	Common name	Parts used
Acalypha godseffiana	Euphorbiaceae	copperleaf	leaves
Annona muricata	Annonaceae	soursop	seeds
Petiveria alliacea	Phytollaccaceae	garlic plant	leaves

Phytochemical analysis procedures

Plant oil extract was subjected to phytochemical screening using the method described by Evans (1996).The extracts were further screened for flavonoids, phenols, alkaloids, tannins, terpenoids and cardiac glycosides following the procedures of Harbone (1973) and Edeoga et al., (2005).

Qualitative bioassay

Alkaloids

0.5 g of oil extracts from *A.godseffiana*, *A.muricata* and *P.alliacea* was diluted with 10 ml of acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added to the mixture and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendoff's reagent to the other. The formation of a cream precipitate (Mayer's reagent) or reddish brown precipitate (Draggendoff's reagent) was regarded as positive for the presence of alkaloids.

Saponins

0.5 g of oil extract from *A. godseffiana*, *A.muricata* and *P.alliacea* was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An appearance of creamy mass of small bubbles indicated the presence of saponin.

Tannins

0.5 g of the oil extract from *A. godseffiana*, *A.muricata* and *P.alliacea* was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration indicating the presence of tannins.

Phlobatannins

0.5 g of plant oil extract from *A.godseffiana*, *A.muricata* and *P.alliacea* was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate indicated the presence of phlobatannins.

Flavonoids

0.5 g of oil extract from *A.godseffiana*, *A.muricata* and *P.alliacea* was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicated the presence of flavonoids.

Steroids

Two millimeter of acetic anhydride was added to 0.5 g of ethanol extract of each sample with 2 ml H_2SO4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski method)

0.5 g each of the oil extract from *A.godseffiana*, *A.muricata* and *P.alliacea* was added 2 ml of chloroform. 3ml concentrated H_2SO4 was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Cardiac glycosides (Keller-Killiani test)

0.5 g of the oil extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides.

Determination of Tannins: 0.2g of finely ground sample of the botanicals was weighed separately into a 50ml sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker and shaken for 2hours at 30^oC. Each solution was then centrifuge and the supernatant store in ice. 0.2ml of each solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5mg/ml of the stock and the solution made up to 1ml with distilled water. 0.5ml of Folin ciocateau reagent was added to both sample and standard followed by 2.5ml of 20% Na_2CO_3.The solution were then vortexed and allow to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a blank reagent concentration of the same solution from a standard tannic acid curve was prepared (Makkar and Goodchild, 1996).

Determination of Total flavonoid: The total flavonoid content of the extract was determined using a colourimeter assay procedure of Bao (2005). 0.2ml of the oil extract was added to 0.3ml of 5% $NaNO_3$ at zero time. After 5min, 0.6ml of 10% $AlCl_3$ was added and after 6min, 2ml of 1M NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

Determination of Saponin: The spectrophotometric method of Brunner (1984) was adopted for the saponin determination. 2g of the finely grinded sample of the botanicals was weighed into a 250ml beaker and 100ml of isobutyl alcohol or (But-2-ol) added. The mixture was shaked with mechanical shaker for 5hours to ensure

uniform mixing. The mixture was filtered with Whatman filter paper (No 1) into 100ml beaker containing 20ml of 40% saturated solution of magnesium carbonate ($MgC0_3$). The mixture was further filtered through Whatman filter paper (No 1) to obtain a clean colourless solution. 1ml of the colourless solution was taken into 50ml volumetric flask using pipette, 2ml of 5% Iron (III) Chloride ($FeCl_3$) solution added and made up to the mark with distill water. It was allow to be standing for 30min for the colour to develop. The absorbance was observed against the blank at 380nm.

Determination of Cardiac glycosides: 10ml the oil extract of *A.godseffiana*, *A. muricata* and *P. alliacea* was pipetted into a 50ml conical flask. 50ml chloroform was added and shaken on vortex mixer for 1hour. The mixture was filtered into 100ml conical flask. 10ml of pyridine and 2ml of 29% of sodium nitroprusside were added and shaken thoroughly for 10min. 3ml of 20% NaOH was added to develop a brownish yellow colour. A concentration which range from 0 – 50mg/ml. Glycosides standard (Digitoxin)were prepared from stock solution and the absorbance read at 510nm.

Determination of terpenoid: 0.5g of finely grounded sample of the botanicals was weighed into a 50ml conical flask. 20ml of chloroform: methanol, 2:1 was added, the mixture was shaken thoroughly and allowed to stand for 15min at room temp. The suspension was centrifuge at 3000rpm the supernatant discarded and the precipitate re-washed with 20ml chloroform: methanol 2:1 and then re-centrifuge. Again, the precipitate was dissolve in 40ml of 10% sodium dodecyl sulphate (SDS) solution. 1ml of 0.01M ferric chloride was added and allowed to stand for 30min before taken the absorbance at 510nm. The standard terpenoid (alpa terpineol) concentration ranging from 0-5mg/ml was read from the stock solution (Sofowora, 1993).

Determination of Steroid: Quantitative determination of steroid was assessed by weighing a 5g of the finely powdered sample of the botanicals into 100m conical flask and 50ml of pyridine was added to it, and shake for 30mins at room temperature. 3ml of 250mg/ml metallic copper powder or Copper (1) Oxide was added and allow to incubate for 1hr in the dark and the absorbance was measure at 350nm against reagent blank Sofowora (1993).

Data Analysis

Data collected on the percentage composition and concentration of the respective phytochemicals obtained from the oil extracts and their respective standard error values was analyzed by one – way analysis of variance (ANOVA). Pertinent means were separated using Turkey post hoc test at 0.05 level of probability.

RESULT

Results from the phytochemical evaluation of the plant oil extracts revealed the presence of major plant phytochemicals; saponnin, tannin, flavonoids, cardiac glycosides, terpenoids and alkaloids (Table 2). The phytochemicals identified were in varying concentration with the respective plants. Fig 1. shows that *A. godseffiana* oil extract recorded highest concentration of the all these phytochemicals ; tannin (7.76mg/ml), flavonoid (14.3mg/ml) , cardiac glycosides (20.9mg/ml) terpenoid (11.9 mg/ml) and alkaloid(24.38%) , but however yielded lower concentration of saponin (1.91mg/ml). Similarly, of three of oil extracts assessed, *A. muricata* oil yielded higher concentration of saponnin (21.00 mg/ml) while *A. godseffiana* recorded the highest cumulative amount of the major phytochemicals (Fig. 1).

Table 2: Phytochemical screening of *Acalypa godseffiana*, *Annona muricat*a and *Petiveria alliaceae* oil extracts

Phytochemicals	*Acalypa godseffiana*	*Annona muricata*	*Petiveria alliacea*
Saponin	+	+	+
Tannin	+	+	+
Phlobatannin	-	-	-
Flavonoid	+	+	+
Steroid	-	-	-
Terpenoid	+	+	+
Alkaloid	+	+	-
Anthraquinone	-	-	-
Cardiac Glycosides			
Legal test	+	+	+
Keller kiliani test	+	+	+
Salkwoski test	+	+	+
Lieberman test	-	-	-

Note: + detected, – absence

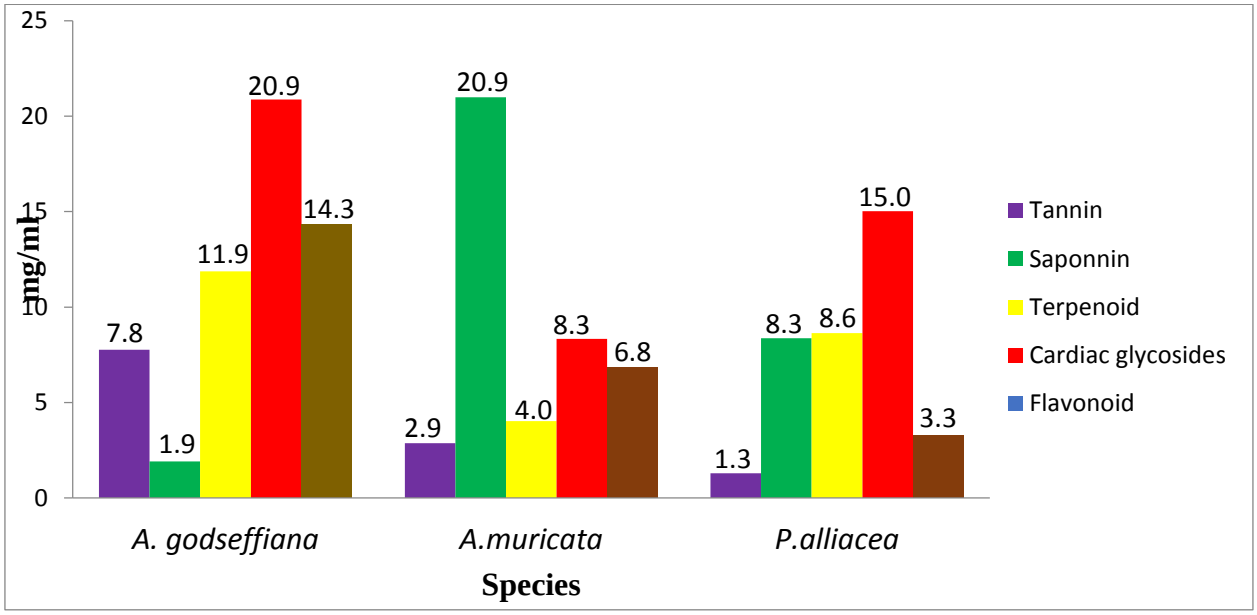

Figure 1; Comparison of phytochemical constituents of oil extract of *A. godseffiana, A.muricata* and *P.alliacea*

Table 3 shows the effect of species on the phytochemical components of the oil extracts obtained from the three plants. There was significant differences ($p < 0.05$) in the tannin, saponnin, terpenoid, cardiac glycosides, flavonoid and alkaloid contents of the three oil extracts. *A. godseffina* oil extract had the highest tannin (7.76mg/ml), terpenoid (11.87mg/ml), cardiac glycosides (20.88mg/ml), flavonoid (14.35mg/ml) and alkaloid (24.38mg/ml) content which was significantly different from other oil extracts. However, *A. muricata* oil extract presented the highest saponnin (21.00mg/ml) content which differs significantly from other oil extracts evaluated. Furthermore, the highest percentage of alkaloid glycoside was yielded by *A.godseffiana* (24.38%) oil followed by *A. muricata* (10.81%) oil extract (Table 3).

Table 3: Variability in the phytochemical constituents in the plant oil extracts

Species	Tannin (mg/ml)	Saponnnin (mg/ml)	Terpenoids (mg/ml)	Cardiac glycosides (mg/ml)	Flavonoids (mg/ml)	Alkaloid (%)
A.godseffiana	7.76±0.02a	1.91±0.05c	11.87±0.01a	20.88±0.05a	14.35±0.03a	24.38±0.03a
A.muricata	2.87±0.04b	21.00±0.37a	4.03±0.04c	8.34±0.04c	6.83±0.03b	10.81±0.01b
P.alliacea	1.30±0.00c	8.36±0.32b	8.63±0.05b	15.03±0.02b	3.26±0.09c	0.00±0.00c

Values are mean ± standard error of three replicates. Values followed by the same letters within the same column are not significantly ($p > 0.05$) different from each other using Tukey post hoc test

DISCUSSION

Plants are rich sources of bioactive chemicals and thereby may be an alternative source of insect control agents. Evaluation of the oil extracts obtained from the biopesticide plants *A.godseffiana, A. muricata and P.alliacea* yielded important phytochemicals; namely tannin, saponnin, terpenoid, cardiac glycosides, flavonoid and alkaloid in varying concentration, and are of economic and pesticidal importance. Adekunle and Adekunle, (2009) reported that several medicinal plants are rich in secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids. *A. godseffiana* oil extract yielded higher concentration of tannin (7.76mg/ml), terpenoid (11.87mg/ml), cardiac glycosides (20.88 mg/ml), flavonoid (14.35mg/ ml) and alkaloid (24.38 %), but with lower concentration of saponnin (1.91mg/ml). Similar results were reported by Iniagbe *et al.*, (2009) and Ikewuchi *et al*., (2010) for *A.hispida and A. wilkessianna* oil extract respectively. However, this is contrast to the findings of Oni et al., (2018) which reported flavonoid as the higher constituents of *A. godseffiana* oil. On the other hand, higher concentration of saponnin (21.00 mg/ml) was found in oil extract of *A. muricata*. Saponins have been noted to possess insect repellent or deterrent activity and provoke insect moulting defects or cause cellular toxicity in insect pest (Singh and Kaur, 2018). Additionally, the oil extracts

elucidated from the insecticidal plants yielded high terpernoid content (11.88mg/ml, 4.09 mg/ml and 8.7mg/ml) respectively. Terpenoids are known to be neurotoxin on insect and have been reported to be either insecticidal, repellent and /or possess antifeedant properties (Ukeh, 2008). Kouninki *et al*., (2007) reported the toxicity of some terpenoids of essential oil of *Xylopia aethiopica* against *Sitophilus zeamais*. Flavonoids are a major class of plant secondary metabolites and constituting 5- 10% of known secondary products in plants .In the chemical industry, flavonoids are used in the manufacture of insecticides through the isoflavonoid and rotenone (Harborne, 1967) and present in higher concentration (14. 35mg/ml) in *A.godseffiana* oil extract. The isolated flavonoid from *Ricinus communis* aqueous leaf extract have been shown to have insecticidal, ovicidal and oviposition deterrent potentials against *Callosobruchus chinensis* in stored pulses (Upasani *et al.*,2003). Furthermore, *A. godseffiana* oil extracts possesses higher alkaloids (26.3%) content, which had been earlier reported to play an important role as insecticidal (Rattan, 2010).

CONCLUSION

Plants are integral part of our biodiversity with nutritional, medicinal, economic and ecosystem attributes. Current pest management procedures calls for utilisation of alternative innovative plant products that are user friendly, affordable, sustainable and resilient as alternative to chemical pesticides. This study had clearly shown oil extracts of *A. godseffiana*, *A. muricata* and *P.alliaceae* as potential sources of biorotational products which can be exploited for the management of insect pest in agricultural practices. In view of the economic and environmental potentials of these plants, concerted efforts should be made to develop protocols for their vegetative propagation and ex-situ conservation to prevent erosion of the gene pool as well as promotion of their utilisation for food and agriculture. Further characterization of these plant oil extracts is recommended for identification and isolation for compounds of insecticidal interests.

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