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Proximate and phytochemical analyses on some indigenous plant seed-powders utilized in control of the domestic pest, *Blattella orientallis* L (Blattodea: Blattidae)

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Abstract
Compositional analyses were carried out on 3 indigenous plant seed powders that had shown some biocidal potential for control of *Blattella orientalis*. The proximate investigation revealed presence of moisture, crude protein, lipids, crude fiber, ash, and carbohydrate; while the phytochemical screening showed presence of flavonoid, tannins, saponin, alkaloid, terpenoids, and glycoside (important ingredients often associated with bioactivity in plants). The quantitative details indicated varying amounts in g/100g sample for certain constituents in the 3 plants (*P. guineense, A.melegueta and D. tripetala)* respectively as: flavonoid (6.08, 2.53, 4.05), tannins (3.85, 2.10, 2.10), alkaloids (3.47, 4.09, 3.17), glycosides (2.58, 1.95, 2.12), saponins (1.79, 2.43, 1.57) and terpenoids (1.28, 1.03, 0.79). The presence of these compounds in these plant powders, may therefore, have been responsible for the observed mortalities of the German cockroach, particularly with *P. guineense*, having shown marked and significantly high values throughout the investigation; and also demonstrated 60% effectiveness from the preliminary pilot studies, as against the 100% effectiveness recorded with the standard chemical insecticide, sniper. Further trials with *P. guineense* at higher concentration is recommended.

Keywords: Proximate, Phytochemical, Biocidal, Bioactivity, *Blattella*

Introduction

Through proximate analysis, basic composition and proportions of moisture, ash, protein, fat, fiber and carbohydrate etc., in samples and compounds are revealed (Leela et al 2016; Ganogpichayagrai & Suksaard, 2020); while phytochemical screening helps in unveiling the presence or absence of bioactive compounds responsible for pesticidal activities, such as alkaloids, flavonoids, saponins, tanins, phenolics and glycosides etc., (Ayodele et al 2015). Proximate and phytochemical analyses of plant seed powders may therefore, provide vital insights into a plant's physical, chemical and nutritional make-up and bioactivity. Such vital information is fundamental and crucial for determining products with pesticidal potentials, especially in our efforts towards development of novel, effective, and eco-friendly pest control solutions.

Over a thousand plant species are reported to possess pesticidal activities; out of which many have already been exploited as insect repellants, attractants, growth-regulators, behaviour-modifiers etc., (Verma et al 2018). The 3 indigenous plant seed powders used in this study, have shown promise as possessing both larvicidal and insecticidal potentials in controlling the notorious household cockroach, *Blattella* spp. and are thus being investigated for their proximate and phytochemical make-up.

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Alligator pepper (*Aframomum melegueta*), alias "grain of paradise" is a perennial plant endemic to western Africa whose seeds are used as spices and ingredient in ethno-medical remedies for snakebites, stomachaches and diarrhea. It is an antioxidant, and has both antibacterial and anti-inflammatory qualities that also fights cancers (Ilic et al., 2010). It is a member of the ginger family and recognized for health-improving effects (Kaefer & Milner, 2011); and also possesses aromatic ketones such as 6-paradol, 6-gingerol and 6-shogaol that are responsible for its peppery flavour (Sugita et al, 2013). **Pepper fruit (*Dennettia tripetala*)**, is similarly, a native, tropical fruit of West Africa; a spicy fruit (Ikpi & Nku, 2008), of the Annonaceae family, and a traditional cure for coughs, fevers, toothaches, diarrhea, diabetes and nausea in pregnant women (Ikpi & Nku, 2008). It is a tiny woody shrub and its bark has a distinctive scent. Their peppery flavour and insecticidal properties, most importantly, make them to be commonly employed to ward off insects, pests and weevils (Odeyemi et al., 2008). Presence of anti-oxidants, anti-allergens, terpenes, tannins, flavonoids alkaloids, antimicrobials and anti-inflammatory substances in pepper fruits, according to Akinbuluma et al., (2017) makes them useful in pharmaceuticals. **The Guinea cubeb (*Piper guineense*)** is also, native to West Africa and serve a wide variety of uses: medicinal, cosmetic and insecticidal (Anyanwu & Nwosu, 2014). Piperin molecules in the plant range between 5-8% and account for its spiciness. Beta-caryophyllene and flavonoids in the plant serve anti-inflammatory, anticancer, antiallergic and antiplatelet purposes (Pal & Verna, 2013); and also have preservative and antioxidant qualities (Pal & Verna, 2013).

The **primary objective** in this study was therefore, to investigate the proximate composition and the phytochemical profile of these 3 indigenous plant seed-powders found to possess some biocidal effects on the domestic pest, *Blattella orientalis*. The findings made here may hold answers for the sustainable management of this notorious domestic pest. According to Odeyemi et al 2008, "investigating plant materials for pest control properties may offer the opportunity to find alternative insecticides". Rahman et al (2007) similarly reported that botanical insecticides are a promising source of pest control; emphasizing furthermore, that the pool of plants with insecticide substances is considerably enormous (Rahman et al., 2007).

Materials and Methods

This work was conducted in the laboratory of the Biology Department, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rives State, Nigeria ((4.8068^0 N, 6.9343^0 E). Fresh seeds of the 3 plants namely, Alligator Pepper (*A. melegueta*), Pepper Fruit (*D. tripetala*), and Guinea Cubeb (*P. guineense*) were purchased from Igwuruta market in Ikwerre Local Government Area of Rivers State, Nigeria for this research project. All samples obtained were analyzed within 24 hrs. of purchase to prevent possible deteriorations. All the fresh fruits of Pepper Fruit (*D. tripetala*), and Guinea Cubeb (*P. guineense*) were thoroughly washed with distilled water and then oven dried, but the Alligator Pepper (*A. melegueta*) was purchased dry. 30 g of each seed was then ground into fine powder using an electrical blender (model: multifunctional BLG-412); then collected and stored in several air-tight plastic containers (10ml each); followed by proper labeling according to sample type. 15g of each finely ground, powder seeds were used for the phytochemical screening, with the remaining 15g for the proximate analysis work. All the investigations were replicated twice. Samples were analyzed following official methods of analyses recommended by the Association of Official and Analytical Chemicals (AOAC, 2005) for proximate composition of moisture, crude protein, ash, crude fat and total carbohydrate, respectively. Each investigation or replication was always preceded by thorough washing and rinsing of the electric grinder with sterile water. The phytochemical analysis for presence of alkaloids, phenols, saponins, flavonoids, glycosides etc., were done according to the methods described by Evans (1989) and Ajayi et al., (2014). Each analysis was duplicated (in other-words replicated twice). Results of sample means and standard errors were subjected to two-way analysis of variance (ANOVA) tests using the Statistical Package for Social Sciences (SPSS) and mean separations with LSD at ($P<0.05$) 5% level of significance.

Results

1) Determination of the proximate composition of Alligator pepper, Pepper fruit and Guinea cubeb seed powder.

The proximate composition results showed the 3 indigenous plants as containing the following substances, viz: carbohydrates, lipids, moisture, crude protein, crude fiber, and ash (as presented in a somewhat descending order of contents %) (Table 1). The carbohydrate content was predominant in all the plants (67.4%, 77.18%, 58.46%) i.e. for *P. guineense*, *A. melegueta*, and *D. tripetala* respectively; closely followed (in the same order) of the plant powders by lipids (4,82%, 6.95%, 10.16%), moisture (4.16%, 3.74%, 4.98%), crude protein (3.91%, 2.60%, 3.50%), fiber (2.78%, 5.13%, 2.12%) and ash (0.92%, 0.56%, 1.07%).

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Table 1: Proximate composition of the Indigenous plant powders

	Plant samples		
Proximate composition	***Piper guineense***	***Aframomum melegueta***	***Dennettia tripetala***
Carbohydrates (%)	67.4 ± 4.7 f	77.18 ± 5.1 f	58.46 ± 4.4 d
Lipid (%)	4.82 ± 1.3 a	6.95 ± 1.5 a	10.16 ± 1.8 c
Moisture %	4.06 ± 1.2 a	3.74 ± 1.1 a	4.93 ± 1.3 a
Crude protein (%)	3.91 ± 1.1 b	2.60 ± 0.9 b	3.50 ± 1.1 b
Fiber (%)	2.78 ± 1.0 b	5.13 ± 1.3 a	2.12 ± 0.8 b
Ash (%)	0.92 ± 0.6 b	0.56 ± 0.4 b	1.07 ± 0.6 b

Different alphabets indicate significant differences ($p<0.05$) amongst different groups and exposure time
There were significant differences ($p<0.05$) amongst proximate composition of the different plant samples

2) The phytochemical composition of the 3 indigenous plant seed-powders

Results of the phytochemical screening for *P. guineense, A. melegueta* and *D. tripetala* is presented in Table 2 below (in a similar descending quantitative order) viz: flavonoids (6.08, 2.53, 4.05), tanins (3.85, 2.10, 2.10), alkaloids (3.47, 4.09, 3.17), glycosides (2.58, 1.95, 2.32), saponins (1.79, 2.43, 1.57) and terpenoids (1.28, 1.02, 0.79) respectively. *P. guineense* had significantly higher values for virtually all the components investigated.

Table 2: Quantitative phytochemical screening of the plant seed-powders

Phytochemicals	***P. guineense***	***A. melegueta***	***D. tripetala***
Flavoniods (%)	6.08 ± 1.7 a	2.53 ± 1.1 c	4.05 ± 1.4 ab
Tanins (%)	3.85 ± 1.4 a	2.10 ± 1.0 b	2.10 ± 1.0 c
Alkaloids (%)	3.47 ± 1.3 a	4.09 ± 1.4 a	3.17 ± 1.3 a
Glycoside (%)	2.58 ± 1.1 a	1.95 ± 1.0 ab	2.32 ± 1.1 a
Saponins (%)	1.79 ± 0.9 ab	2.43 ± 1.1 a	1.57 ± 0.9 ab
Terpenoids (%)	1.28 ± 0.8 b	1.02 ± 0.7 b	0.79 ± 0.6 b

Different alphabets indicate significant differences ($p<0.05$) amongst treatment groups and phytochemicals
There were significant differences ($P<0.05$) amongst plant samples and phytochemicals

Discussion

Information on a plant or a crop's proximate and phytochemical composition would reveal its status or suitability for use either as human food or livestock fodder or for insecticidal purposes. The results of the proximate investigation on the 3 indigenous plants, revealed the following (presented here in a somewhat descending quantitative order): carbohydrates (58.46-77.18%), lipids (4.82-10.10%), moisture (3.74-4.93%), crude protein (2.60-3.91%), fiber (2.12-5.13%) and ash (0.56-1.07%).

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The observed high carbohydrate content (58 – 77%) in these plants, may indicate their nutritional importance and may also serve as potential high energy sources for humans/livestock or pests etc when consumed as food. It may also perhaps account for the observed firm and rugged shape/structures of *A. melegueta* (alligator pepper) pods.

Similarly, the observed lipid contents (4.82 - 10.16%) in these plants, present them as being rich sources of rapid energy. Also, the fairly high percent crude protein contents for *P. guineense, A. melegueta* and *D. tripetala* (3.50%, 3.91% and 2.60%) respectively, may indicate the importance of these plants in body building and development: but the crude fiber contents (2.12 – 5.13%, particularly with *A. melegueta* (5.13%) may highlight usefulness in aiding gut movement and faecal elimination.

Although it may perhaps be proper/necessary to determine which of the 3 plants and/or seeds would be best for food, or livestock fodder; but it must however, be emphasized here, that certain plants are consumed by both man and livestock merely by their flavour and scent alone, regardless of their nutritional value.

Furthermore, and even more importantly, certain plants are also, globally known to possess some active natural ingredients for protection against insect pest infestations (Epidi et al., 2008; Udo 2008). In fact, Ukoroije and Bobmanuel (2019); Ukoroije and Otayor (2020) even reported that certain plant powders are effective against insecticide-resistant bugs. Harnessing this knowledge of plants biochemical or phytochemical make-up would therefore, be crucial and essential for novel, and more strategic and sustainable insect pest control efforts.

Phytochemicals are naturally occurring compounds in plants, contributing to their colours, flavour, smell, and also to their natural defense mechanisms against pest and disease infestations (Eleazu et al., 2012). High resistances to insect pests and disease attacks are often due to high levels of these natural self-defense compounds (phytochemicals) in plants (Peter, 1991; Eleazu et al 2012).

The results of the phytochemical screening of *P. guineense, A. melegueta* and *D. tripetala* revealed the presence of the following (presented here in somewhat decreasing quantitative order, for the plants respectively) as: flavonoid (4.05, 2.53, 6.08), terpenoids (0.79, 1.02, 1.28), alkanoid (3.17. 4.09. .3.47), saponins (1.57, 2.43, 1.79), tannins (2.10, 2.10, 3.85) and glycoside (2.32, 1.95, 2.58).

Terpenoids, steroids, phenols, flavonoids, tannins, alkaloids, and cyanogenic glycosides, according to Okafor et al., 2001, all have toxic effects on insect pests. This is consistent with the findings of Salunke et al. (2005), who found that phytochemicals influence the midgut epithelium first, then the gastric caeca and malpighian tubules of insects, resulting in antifeedant qualities and eventual insect mortality. Consequently, their presence in these 3 indigenous plants under study, serve as additional evidence that these plants may be potential bio-insecticides; hence the observed biocidal effects against the cockroach (*Blattella orientalis*).

The observed result is also similar to that obtained by Doherty et at. (2010) on *Aframomum melenguta*. which equally contained tannins, alkaloids, phenols, and flavonoids. Dike and Nnamdi (2012), in like manner also reported that *A. melegueta* contained saponnins, tannins, alkaloids, and flavonoids.

P. guineense powders had stronger activity than those of the other two plants, which could be attributed to higher flavonoid concentration. Flavonoids have been discovered to cause death in insects via altering moulting. The majority of flavonoids tested, either act as anti-estrogens or inhibit the production and activity of the cytochrome P450 isozyme (Salunke et at., 2005). They disrupt enzymatic systems and cell membranes to kill insects (Verma et al., 2018)

Terpenoids are known to disrupt cuticle integrity and then affects nervous and respiratory systems (Verma et al., 2018). Alkaloids, on the other hand, according to Eleazu et at. (2012), acts as a neurotoxin. It disrupts the nervous system of insects to cause death (Verma et al., 2018). Tannins on their part, interferes with digestive processes of insects (Verma et al., 2018).

Conclusion

Several plant species are now globally known to possess biocidal capacities for effective control and management of pest and disease infestations. Proximate and phytochemical screenings therefore, become expedient for rapid detection of plants' chemical constituents. This study on *P. guineense* powder has confirmed it as having a rich chemical composition and with significantly high phytochemical levels, and consequentially a potential biopesticide. More detailed and follow-up studies therefore, are hereby proposed to establish its pesticidal status, for possible and immediate exploitation in novel, sustainable and eco-friendly control trials.

Recommendations

Phytochemicals (aka botanical insecticides) have several tremendous and diverse benefits/advantages over conventional insecticides; being locally and readily available, relatively cheap, and with little/no toxicities or residue issues to man, natural enemies and environment; no pest resistance nor resurgence issues etc... *P. guineense* (or Guinea cubeb) powders closely fits this botanical insecticide description above; and is consequently, recommended here for more in-depth and specific studies, particularly:

- to ascertain the exact chemical substances (phytochemicals) involved in its pesticidal activity
- to determine the toxicity levels of the active ingredient(s)
- to establish if observed toxicity levels are below dosages that are deleterious to humans and animals

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