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Original Research Article

Volatile Oil Constituents, Bioactivity and Formulations of Essential Oil from *Psidium guajava*

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ABSTRACT

With the availability of myriad of plants having insecticidal activity, the issue of toxicity while using synthetic compounds to eradicate pests would be resolved. Therefore, the search for and formulations of insecticidal plants have grown remarkably, in recent years. It is on this premise that this work sought to characterize, evaluate the bioactivity and formulations of essential oil of *Psidium guajava* (PG) for insecticidal activity. Volatile oil of from PG was characterized using Gas Chromatography-Mass Spectrometry to identify the compounds present. Filter paper and anti-feedant methods were used to test the oils for insecticidal activities against adult *Callosobruchus maculatus*. The releasing profiles of the volatile oil formulations were observed for 15 days at 3 days interval using bentonite and kaolin as carriers at different concentrations (10, 20 30 40 and 50 µl). Antimicrobial activities of the volatile oil were also carried out on some bacterial and fungus pathogens using standard methods. From the results, forty-nine (49) compounds were identified in PG volatile oil with α-Limonene (29.55 %), γ- cadinene (9.61 %), caryophyllene (9.44 %), nerolidol (7.19 %) and viridiflorol (4.29 %) as major components. After 6 hours and 24 hours of filter paper and anti-feedant test, the volatile oil toxicity results showed that the percentage mortality of *Callosobruchus maculatus* increased significantly ($p > 0.05$) with increase in concentration. Oil formulation results showed that the efficacies and the insecticidal activity decreased with time. Antimicrobial studies showed that the extracted oil inhibited the growth of tested organisms at different zones of inhibition.

Keywords: Volatile oil, *Psidium guajava*, Insecticidal activity, Formulations, Antimicrobial activity

Introduction

Pest/insect attack on agricultural crops affect food production and human health. Synthetic chemicals were used by farmers to control this pest attack and improve farm production in the past but have been reported to have debilitating side effects on the environment and on human and plant health due to accumulation. About 98% of pesticides used do not reach their targets but rather enter into groundwater, streams and wildlife. The early insecticide such as Dichlorodiphenyltrichloroethane killed fishes, eagles, birds and even people.[1] Moreover, the insecticides are very expensive, non-biodegradable and sometimes constitute health hazard to consumers.[2] It has been found that, indiscriminate use of these synthetic insecticides resulted in the development of resistance.[3-5]. To this end, it is necessary to substitute synthetic insecticides with plant components that are available, affordable, eco-friendly, biodegradable and less or non-toxic to animals while combating the activities of insects.

Natural insecticides have been used even in the past, an example is nicotine from tobacco, pyrethrum gotten from flower of *Chrysanthemum*. The need to revisit natural pesticides stem from the toxic effects of synthetic insecticides, as mentioned earlier.

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Volatile oils have been used by many cultures around the world for thousands of years due to its life enhancing benefits. It has various biological, medicinal and nutritional uses.[6] Volatile oils are embedded in plants, many of which are widely used for treating various ailments traditionally[7], accounting to about two-third (2/3) of the world's plant species having medicinal properties.[8] The medicinal properties displayed by these plants are not unconnected with the presence of active constituents present in them.[9] Due to the volatility and instability of volatile oils, microencapsulation can be used to protect unstable biodegradable essential oil.[10] Therefore, formulations that will reduce the releasing profile of the oil will be carried out using different diluents under tropical temperature. To the best of our knowledge and available literature, there is dearth of information on the formulation of *Psidium guajava* (PG) using both bentonite and kaolin.

This study, therefore, sought to investigate the chemical composition, antimicrobial properties, and formulations of volatile oil of PG using bentonite and kaolin, its insecticidal activity was also investigated.

Materials and Methods

Sample collection and identification

The aerial parts of the plant were freshly collected at the surrounding of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria around June, 2018 and authenticated at the Department of Plant Science and Biotechnology.

Extraction of Volatile oil by Hydro-distillation

Fresh aerial parts (500 g) of the plant was hydro-distilled for two hours using Clevenger apparatus. The oils were dried over anhydrous sodium sulphate, transferred into brown sample bottle and refrigerated at 4ºC prior analysis.

Identification of sample constituents
The analysis of the volatile oil constituents was carried out on a Hewlet Packard GC/MS system equipped with quartz capillary column; 30 m × 0.25 mn i.d and 0.25 mm film thickness was used. The carrier gas was helium (1ml/ min); oven temperature, 40 to 300ºC at the rate 4ºC/min; the injector port temperature was 240ºC. Different constituents were identified by comparing their retention time and mass spectra with those of the library[11-17].

Insect rearing and maintenance
Infested cowpea seeds was purchased from local market and used as the initial stock (*Callosobruchus maculatus*, F). New generation was reared from this stock at room temperature in the laboratory. New adults of *Callosobruchus maculatus* were subsequently sub-cultured on the same variety of cowpea over four generations before using for the experiment.

Antifeedant Test
Five concentrations of each oil (10, 20, 30, 40 and 50 µl) were dissolved separately in 500 µl acetone of analytical grade. Each concentrations of the oil was mixed with 5g of cowpea (50 ml glass jar) and stirred thoroughly using a glass rod for enough seeds coating with oil, until the acetone evaporates completely, as described by Ofuya and Lale.[18] Twenty *Callosobruchus maculatus* (3-5 days old, mixed sex) were put into each jar with replaced lid. Pure acetone (500 µl) was used to treat the control seeds while the second control was cowpea without treatment. The treatment and control were done three times. Mortality was observed after the introduction of the insects on the seeds at intervals of six and twenty-four hour. Insects not responding to touch were deemed dead.

Filter paper test
Bioassay on the toxicity of the volatile oil of PG leaves against adult *Callosobruchus maculatus* was done as described by Ukeh *et al*., 2012[19] in Pyrex glass Petri dishes (9cm diameter). Different doses of each volatile oil (10, 20, 30, 40 and 50 µl) were dissolved in 500 µl analytical graded acetone and put into the Petri dishes pre-lined with Whatman N° 1 filter paper. The filter paper was controlled using pure acetone. The solvent was allowed to escape, after which twenty mixed sex of *Callosobruchus maculatus* adults were put into each dish. The dishes were covered and left in the laboratory for 6 and 24 hours at surrounding temperature and relative humidity. All treatments were repeated three times for each dose of all volatile oils, and account of dead weevils was made at 6 h and 24 h interval.

Preparation of formulated volatile oil dusts
Three grams of each *bentonite* and *kaolin* (diluents) were weighed into separate wide-necked jars with lid. The volatile oil each of 10, 20, 30, 50 µl was dissolved separately in 500 µl analytical graded acetone. The mixture was added into each jar with the use of micro-pipette. The content of jar were mixed thoroughly and exposed to the air for the solvent to evaporate. 3 gram of phostoxin tablets (*Aluminum phosphide*) used as synthetic insecticide was obtained from an agrochemical store at Akure, Ondo State Nigeria was blended into powder.

Application of the formulated dust for insecticidal activity
Each formulated volatile oil and the synthetic insecticide (phostoxin) were exposed to the atmosphere throughout the duration of the study. From the exposed formulated volatile oil and phostoxin 0.5 gram each of the material were weighed into Petri dish at each time of exposure period and ten pairs of adult *Callosobruchus maculatus* were introduced into the Petri dish Olonisakin *et al*.[20,21]. The Petri- dish was covered and mortality of the *Callosobruchus maculatus* was observed for 24 hours after application, the same weight of pure bentonite and kaolin and the same number of weevils was used as control during each of time of exposure period. The treatment was carried out at three days interval for fifteen days.

Antimicrobial activity of volatile oils
Bacteria isolates used for the work were obtained from Lagos state university teaching hospital, Nigeria while the fungi isolates were obtained from the Department of Microbiology, AAUA. All the inoculums of various organisms used were reconstituted using aseptic condition for confirmation of pure isolates in the laboratory. With the aid of a sterile loop, the isolated colony of each pure culture was transferred into 5 ml of sterile nutrient broth and incubated for 24 hrs, after incubation, 0.1 ml of the isolated colony were transferred into 9ml of sterile distilled water contained in each test tube and mixed properly, it must be noted that the source of inoculums contains approximately 10^6 cfuml of bacteria suspension. Muller Hinton agar (oxoid) was prepared according manufacturers specification which is 38g per liter. The agar was sterilized using the autoclave atb121 for 15 minutes, after cooling to 42.
Inoculums were seeded into the sterile plates before the agar was poured and it was properly swirled in order for the inoculums to mix properly. Holes were bored with 6mm diameter size of cork borer. 0.5 ml of each volatile oil was introduced to the well and each well was labeled accordingly. Fungi plates were prepared also in which the inoculums was sporulated for 7 days before being standardized using the bacteria method and was tested with volatile oils. *Oflaxacin* tablets (200 mg) and *Mycoten* tablets were used for control experiment, as standard antibacterial and antifungal drugs respectively. The inoculated plates were left for an hour at room temperature for the extract to diffuse before the growth of the organism commence. The plates were incubated at 37ºC for 24 hours for bacteria while fungi were incubated for 7 days. Zones of inhibition were read and recorded using a metric ruler. The bacterial culture were maintained on nutrient broth while fungal cultures were maintained on sabourad liquid medium. The bacteria and fungi used include *Staphylococcus aureus, Bacillus sp, Streptococcus sp, E. coli, Klebsiella pneumonia* and four strains of fungi; *P. aeruginoses, A. niger, Fusarium sp, A. flavus.* The plate diffusion method of Willey *et al*.[22] was used for the antibiotic sensitivity test organism commence.

Data Analysis
The study was carried out in a complete randomized block design with each treatment replicated four times. Data collected include the number of cowpea bruchid mortality in each treatment. Percentage data were transformed and entire data were subjected to a one – way analysis of variance (Duncan multiple range) and differences between means were determined using the least significant differences (LSD) ($p \leq 0.05$) statistic.

Results and Discussion

The GC/MS and chemotype results of the volatile oil of PG are presented in Tables 1 and 2, respectively. Furthermore, the result in Table 3 shows the percentage mortality of *Callosobruchus maculatus* of volatile oil of PG application using filter paper method at different concentrations for 6 hours and 24 hours. The result of the acute toxicity of the volatile oil of PG against *Callosobruchus maculatus* using Anti-feedant method at different concentrations at 6 hrs and 24 hrs are also shown in Table 4. Table 5 shows the result of percentage mortality of *Callosobruchus maculatus* after application of volatile oil formulations using *bentonite*. Table 6 shows the result of volatile oil formulations using *kaolin*. Also, Table 7 shows the result of zones of inhibition of volatile oil against some bacteria organisms. The result of zones of inhibition of the volatile oil against some fungi organisms is shown in Table 8.

Components, sources and Chemotypes of the plants
A yield of 0.9 ml per kilogram weight was observed on the colourless volatile oil from PG leaves. PG, a *myteace* is been used locally for the treatment of gastroenteritis, dysentery, diarrhea etc.
From table 1, a total of 49 compounds were identified, accounting for 88.73 % of the oil composition. The major component of the volatile oil were *α*–Limonene (29.55 %), *γ*- Cadinene (9.61 %), Caryophyllene (9.44 %), Nerolidol (7.19 %), Viridiflorol (4.29 %), *α*-Bisabolol (2.97 %), Caryophyllene oxide (2.83 %), *δ*–Cadinene (2.65 %), Cedr-8-ene (2.19 %), *α*- Humulene (2.16 %). It is graphically represented in Figure 1. From the percentage chemical composition, it is limonene rich chemotype; this is in agreement with Ogunwande *et al*.[11] from Nigeria.

The main chemical class of the volatile oil was monoterpenes (32.47 %) followed by sesquiterpenoid (30.11 %), sesquiterpenes (22.30 %), monoterpenoids (2.31 %) *and* hydrocarbons (1.54 %) which represent 88.73 % of the oil composition, the earlier investigation of volatile oil from the leaves of PG from Kathmandu Nepal has major percentage composition of E-Nerolidol (35.6 %) and E-Caryophyllene (15.8 %) with lower concentration of (2Z-6E) Farnesol (6.7 %) and Ledol (5.3 %)[24]. According to Khadiri *et al*.[13] viridiflorol (36.4 %) and trans-caryophyllene (5.9%*)* were dominant in volatile oil from Tunisia. According to Arain *et al*.[14], PG leaves volatile oil from Mirpurkhas Sindh province in Pakistan were found to contain major compound -*β*-Caryophyllene (20.34 %), Globulol (8.2 %), *trans*-Nerolidol (7.72 %), Aromadendrene (4.34 %), Limonene (3.09 %) and *δ*–Cadinene (2.52 %*).* Similarly, Ogunwande *et al.*[11] reported that PG leaves volatile oil from Nigeria contained high concentration of Limonene (42.1 %) and *β*–Caryophyllene (21.3 %). The oil of PG was constituted of *α*-pinene (23.9 %), 1,8-cineole (21.4 %) and *β*-bisabolol (9.2 %) as reported by DaSilva *et al*.[15] Chen *et al*.[16] reported that the major constituents identified in the volatile oil of PG from Taiwan were: *β*-caryophyllene (27.7 %), *α*-pinene (14.7 %) and 1,8-cineole (12.4 %).

Table 2 showed that the chemotype of volatile oil from the aerial part of PG from different region. The chemotype were determined by selecting the constituents with greater concentrations from different region. According to Prabodh *et al.*[12], the cluster analysis of the PG leaf oil has revealed at least nine chemotype. Thus, Nigeria, Ecuador, Philippines and present study have the same chemotype (Limonene rich)[16,22]. Viridiflorol, *trans*-Caryophyllene were found to be in greater concentration in the volatile oil from leaves of PG growing in Tunisia[13]. Also, from Costa Rica *Hexenal/Benzaldehyde and Cineole*.[23] According to Dasilva *et al*.[15] and Santos *et al*.[24], pinene and cineole are most prominent in Brazil and China. From Cuba and Nepal, the major compounds are Nerolidol/Caryophyllene[12,25]. It is obvious that there are variations in the chemical compositions of PG leaf essential oils from different regions and there does not seem to be a correlation with geographical location.[12] It is evident that the composition of any plant's essential oil is influenced by several factors, such as geographical location, seasonal variation, soil type and experimental conditions.[26]

Table 1: Chemical composition of the volatile oil from the leaves of *psidium guajava*

S/N	Compounds	Retention time	Concentration %
1	*α*-pinene	6.624	0.42
2	*α*-thujene	6.950	0.06
3	Pthalaldehyde	7.500	0.20
4	Benzaldehyde	7.565	0.51
5	*β*-myrcene	8.316	1.69
6	*α*-limonene	10.017	29.55
7	*β*-ocimene	10.250	0.25
8	*γ*-terpinene	10.606	0.13
9	Terpinoleon	11.502	0.05
10	Linalol	12.057	0.11
11	Verbenol	12.803	0.07
12	methylcyclohexane	13.079	0.16
13	Mentha-2,8 dien-1-ol	13.314	0.03
14	Terpinen-4-ol	14.813	0.13
15	Butanoic acid-3-hexeny	15.000	0.03
16	Carveol	15.122	0.45
17	*α*-Terpineol	15.387	0.94
18	Verbenone	15.962	0.03
19	Thujen-3-ol	16.557	0.44
20	D-carvone	17.019	0.08
21	Menthan-1,8-dien-7-	17.392	0.02
22	Carveol acetate	19.113	0.03
23	*α*-Terpinene	19.918	0.09
24	*α*-cubebene	20.410	0.02
25	Nerol acetate	20.846	0.10
26	*α* – muurolene	21.113	0.02
27	Copaene	21.377	1.24
28	*α* –longipinene	22.319	0.20
29	Caryophyllene	22.940	9.44
30	D-limonene	23.177	0.23
31	*α*-Humulene	23.917	2.16
32	*β*-Himachalene	24.143	0.29

33	γ-muurolene	24.490	1.08
34	α -Guaiene	24.905	0.26
35	2-Norpinene	25.127	0.32
36	Aristolene	25.229	0.59
37	Cedr-8-ene	25.603	2.19
38	∂- cadinene	25.878	2.65
39	Napthalene	26.295	1.35
40	Ledane	26.903	0.81
41	Nerolidol	27.342	7.19
42	Viridiflorol	27.823	4.29
43	Caryophyllene oxide	28.570	2.83
44	γ-cadinene	29.601	9.61
45	Ledol	29.975	3.22
46	bisabolol	30.865	2.97
47	Ascabiol	32.875	0.04
48	Linoleic	41.389	0.20
49	Methyl linoleate	45.171	0.03

Table 2: Chemotype of the volatile oil of aerial part of *psidium guajava* from different regions.

Chemotype	**Regions**	**References**
Hexenal/benzaldehyde and cineole	Costa rica	Cole *et al*., 2007[23]
Viridiflorol rich	Tunisia	Khadiri *et al.*, 2014[13]
Bisabolene/sesquiphellandrene	Arizona	Shrestha *et a*l., 2015[27]
Pinene/cineole	Brazil/China	Dasilva *et al.*, 2003[15]
Nerolidol/Caryophyllene	Cuba/Nepal	Prabodh *et al.*, 2015[12]
Limonene–rich	Nigeria/Ecuador/Phillipines	Ogunwande et al., 2003[11]
Salinene/Caryophyllene	Australia	Sagrego *et al*., 1994[28]
Humulene/Caryophyllene	Brazil/Argentina	Siani *et al.*, 2013[29]
Caryophyllene/cineole	Taiwan/Egypt/Tahiti	El-ahmady *et* al., 2013[30]
β-caryophyllene rich	Pakistan	Arain *et al*., 2018[14]
Limonene/Cadinene/Caryophyllene	Nigeria	Present study

Insecticidal activity of the volatile oils
Filter paper method
The result in Table 3 and the showed the percentage mortality of *Callosobruchus maculatus* of essential oil application using filter paper test PG volatile oil at different concentrations using µl/9mml filter paper for 6 hrs and 24 hrs. At 6 hours, the percentage mortality of *Callosobruchus maculatus* increased significantly ($p > 0.05$) in 50 µl (50.00 ± 0.00^e) when compared with the control groups A and B (0.00±0.00). The percentage mortality of *Callosobruchus maculatus* increased significantly with increased in concentration of the oil. At 24 hours, the percentage mortality increased significantly ($p > 0.05$) in 50 µl (100.00 ± 0.00^e) when compared with the control group A and B (0.00±0.00). The results also depicted increase in percentage mortality with increase in concentrations. The percentage mortality increased significantly ($p > 0.05$) with increase in concentration and increase in time for 24 hrs when compared with 6hrs for the volatile oils. It is graphically represented in Figures 2a and b.

Anti-feedant method
At 6 hours, the percentage mortality of *C. maculatus* increased significantly ($p > 0.05$) in 50 µl concentration (50.00±0.00) when compared with other lower concentrations. The percentage mortality of *C. maculatus* increased significantly with increased in concentration of the oil. In 24 hours, the percentage mortality increased significantly ($P>0.05$) in 50 µl (100.00 ± 0.00^e) when compared with the control group A and B (0.00±0.00). The results also depicted increase in percentage mortality with increase in concentrations and time. It is graphically represented in Figures 3a and b.
From the results, it can be deduced that the higher the concentrations of the essential oil with increase in time of exposure of the weevils, the higher the rate of mortality. There is no record of mortality of *Callosobruchus maculatus* for the filter paper lazed with acetone and the beans coated with acetone. In comparing the filter paper and anti-feedant method, it was observed that the mortality rate of *Callosobruchus maculatus* is faster and more in antifeedant over time than filter paper method, the reason may be due to direct feeding on the treated beans with essential oils unlike the filter paper method where some of the weevils tends to move to the upper part of the petri dishes, away from the filter paper lazed with essential oils, this means that the essential oils have repellent activity, as such that increase in time of exposure of the weevils affect the respiratory system of the weevils, thus, brings about the mortality of the weevils.

Table 3: Percentage mortality of *Callosobruchus maculatus* of essential oils application (filter paper method)

Plant Spp	Conc.	6hrs	24hrs	Control A	Control B
P. guajava	10ul	18.33 ± 2.88[a]	21.67 ± 5.77[a]	0.00 ± 0.00	0.00 ± 0.00
	20ul	26.67 ± 2.88[b]	36.67 ± 2.88[b]	0.00 ± 0.00	0.00 ± 0.00
	30ul	36.67 ± 2.88[c]	63.33 ± 2.88[c]	0.00 ± 0.00	0.00 ± 0.00
	40ul	43.33 ± 2.88[d]	75.00 ± 5.00[d]	0.00 ± 0.00	0.00 ± 0.00
	50ul	50.00 ± 0.00[e]	100.00 ± 0.00[e]	0.00 ± 0.00	0.00 ± 0.00

All the values are reported as Mean ± SD. Values carrying different notations down a column are statistically different at p<0.05
Control A = filter paper with acetone; Control B= filter paper without acaetone

Table 4: Percentage mortality of *Callosobruchus maculatus* of essential oils application (Anti-feedant method)

Plant Spp	Conc.	6hrs	24hrs	Control A	Control B
P. guajava	10	30.00 ± 0.00[a]	51.67 ± 2.88[a]	0.00 ± 0.00	0.00 ± 0.00
	20	31.67 ± 2.88[a]	65.00 ± 5.00[b]	0.00 ± 0.00	0.00 ± 0.00
	30	40.00 ± 0.00[b]	78.33 ± 2.88[c]	0.00 ± 0.00	0.00 ± 0.00
	40	46.67 ± 2.88[c]	90.00 ± 5.00[d]	0.00 ± 0.00	0.00 ± 0.00
	50	50.00 ± 0.00[d]	100.00 ± 0.00[e]	0.00 ± 0.00	0.00 ± 0.00

All the values are reported as Mean ± SD. Values carrying different notations down a column are statistically different at p<0.05
Control A = Infested beans with acetone; Control B= Infested beans without acetone

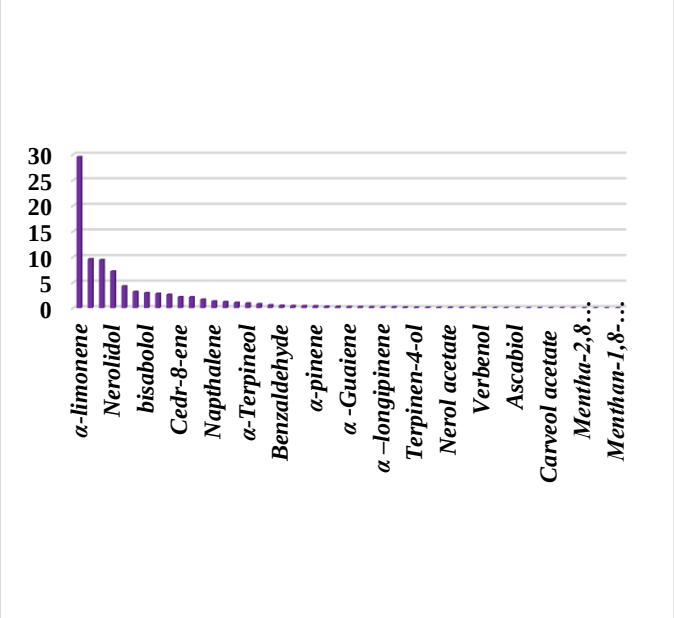


Figure 1: Chemical composition (%) of the volatile oil from the leaves of *Psidium*

Volatile oils formulations

Formulation using bentonite

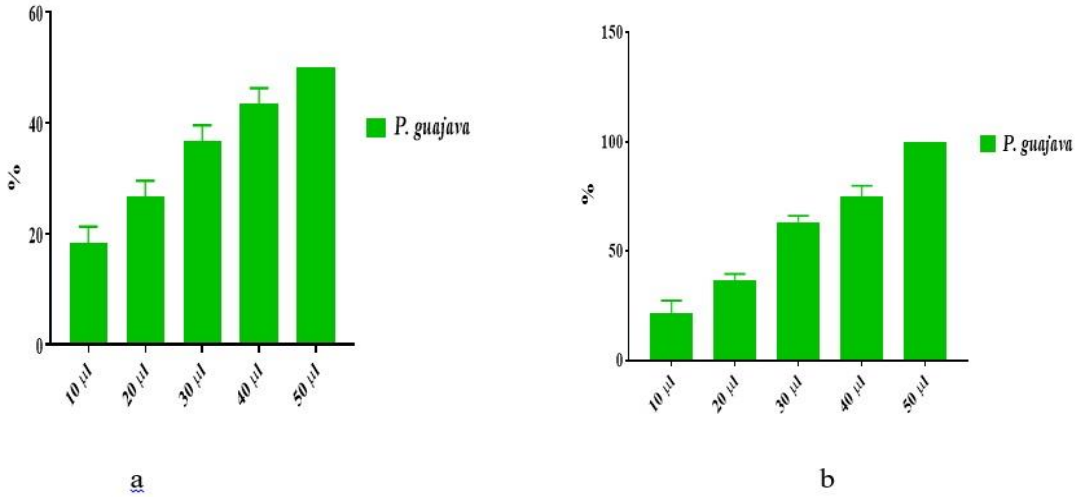
The result in Table 5 showed the percentage mortality of *Callosobruchus maculatus* with essential oils formulated with bentonite. The results show no significant difference ($p \geq 0.05$) in the percentage mortality rate of *Callosobruchus maculatus* between the activity of the formulated essential oil and phostoxin at different concentrations in Day 0. In Day 3, the mortality rate of *Callosobruchus maculatus* decreased significantly ($p < 0.05$) in groups treated with lower concentration (10 and 20 µl) of formulated volatile oils when compared with the groups treated with high concentrations of formulated volatile oil and phostoxin. In Day 6, the biological activities of formulated essential oils and phostoxin have reduced due to increase in the time of exposure which led to decrease in their efficacies. However, the mortality rate of *Callosobruchus maculatus* decreased significantly ($p < 0.05$) in groups treated with lower concentration (10 and 20 and 30 µl) of formulated volatile oil when compared with the groups treated with high concentrations of formulated volatile oil.

In Day 9, *phostoxin* has completely lost its efficacy on exposure, however, formulated essential oils still show some efficacy with significant increase ($p > 0.05$) in percentage mortality of *Callosobruchus maculatus* when compared the groups treated with lower concentrations(10, 20 and 30 µl) with (40 and 50 µl). In days 12 and 15, phostoxin and lower concentrated formulated volatile oils (10, 20 and 30 µl) have lost their efficacies, thereby showed significant decrease ($p < 0.05$) in mortality rate of *Callosobruchus maculatus* when compared with (40 and 50 µl). The control has no significant effect on the *Callosobruchus maculatus*, the formulated essential oils with bentonite has retention value and more efficacies than phostoxin.

Formulation using kaolin

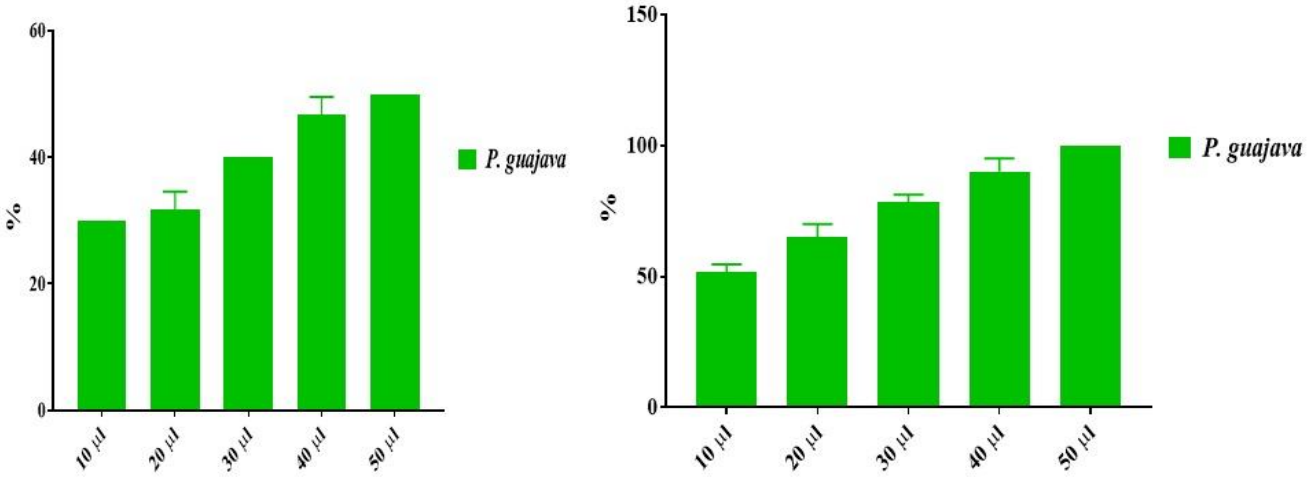
The results in Table 6 showed the percentage mortality rate of *Callosobruchus maculatus* using formulated volatile with kaolin. The control showed efficacy of insecticidal activity from the record of mortality rate of *Callosobruchus maculatus* throughout the fifteen days of experiment. In Day 0 and Day 3, there is no significant difference ($p \geq 0.05$) in the percentage mortality rate of *Callosobruchus maculatus* of the formulated essential oil and phostoxin at different concentrations. In Day 6, the biological activity of formulated essential oil and phostoxin has reduced due to increase in the time of exposure which has led to decrease in their efficacy. However, there is significant increase in percentage rate of mortality of *Callosobruchus maculatus* ($p > 0.05$) with increase in concentration from 10, 20, 30, 40 and 50 µl. In Day 9, 12 and 15, phostoxin has completely lost its efficacy with exposure, however, formulated essential oils still show some efficacies with significant increase ($p > 0.05$) in percentage mortality of *Callosobruchus maculatus* from (10, 20 30, 40 and 50 µl).

In contrast, kaolin (control) showed some insecticidal activity throughout the experimental days (Table 6) while bentonite has no efficacy. In day 0 the insecticidal activity shown by the formulated essential oils using kaolin and bentonite shows no significant difference ($p \geq 0.05$) in percentage mortality rate *of Callosobruchus maculatus.* However, formulated volatile oil with kaolin showed significant increase $p > 0.05$ in percentage mortality rate of *Callosobruchus maculatus* in Day 3, 6, 9, 12 and 15 when compared with formulated essential oil using bentonite. Conclusively, kaolin has more potential of reducing volatility of essential oils; it also contributed to the insecticidal activity of essential oils.



a b

Figure 2: Percentage mortality of *Callosobruchus maculatus* of essential oils application for (a) 6 hrs and (b) 24 hrs (filter paper method)



a b

Figure 3: Percentage mortality of *Callosobruchus maculatus* of essential oils application for (a) 6 hrs and (b) 24 hrs (anti-feedant method)

Antimicrobial activity of Volatile Oils of Psidium Guajava

The susceptibility of each organism against the volatile oil was evaluated and presented (Table 7 and 8). A total of six bacterial and three fungal organisms were tested with the volatile oil. *Oflaxacin* was used as the antibacterial control and it inhibited the growth of all the organisms as follows: *B. cereus* (28 mm), *S. aureus* (22 mm), *E. coli* (21 mm), *K. pneumonia* (21 mm), *S. pneumonia* (20 mm), *P. aeruginoses* (10 mm). The volatile oil inhibited the growth of all the organisms. PG volatile oil inhibited the growth of *S. aureus* (23 mm), *B. cereus* (20 mm), *S. pneumonia* (20 mm), *E. coli* (29 mm), *K. pneumonia* (24 mm), *P. aeruginoses* (14 mm). Sanches *et al.*[31] findings showed that aqueous extract of PG was effective against *staphylococcus and Bacillus*. Also, Nascimento *et al.*[32] conducted a study that PG extract was able to inhibit *Staphylococcus* and *Bacillus* and no effect on the *Escherichia coli.*

In Table 8, mycoten was used as the antifungal standard control and it inhibited the growth of all the organisms as follows; *A. niger* (28 mm), *Fusarium sp* (26 mm), *A. flavus* (23 mm). The volatile oil of PG inhibited the growth of all the fungi organisms It has been reported by Rathish *et al*.[33] that acetone, methanol and hexane extracts of PG effectively inhibited *Aspergillus species*. Priya[34] revealed that volatile oil of PG showed moderate inhibitory activity against *A. Niger.*

Conclusion

The major constituents of the volatile oil of *Psidium guajava* (PG) is *α*-limonene. The chemical composition of PG varies across the regions considered, which might be as a result of genetic factor, soil factor, chemotype, time of harvest and method of preserving the oil. In this study, volatile oil from PG was found effective against *Callosobruchus maculatus*. The anti-feedant test showed that the treatment of cowpea seeds after infestation adequately enhanced the mortality of *Callosobruchus maculatus.* The volatile oil was found to inhibit some pathogenic organisms. The present results showed that the volatile oil from PG has the potential to be used in pathogen control and as non-toxic insecticide.

Conflict of Interest

The authors declare no conflict of interest.

Table 5: Percentage mortality of *Callosobruchus maculatus* after application of essential oil formulations using bentonite

	Control	Phostoxin	**10**	**20**	**30**	**40**	**50**
Day 0	0.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Day 3	0.00 ± 0.00	100.00 ± 0.00	80.00 ± 0.00	85.00 ± 1.57	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Day 6	0.00 ± 0.00	10.00 ± 0.00	40.00 ± 1.44	50.00 ± 0.00	65.00 ± 1.44	75.00 ± 1.67	80.00 ± 0.00
Day 9	0.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.00	35.00 ± 1.67	50.00 ± 0.00	65.00 ± 1.44	80.00 ± 0.00
Day 12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.00	30.00 ± 0.00	45.00 ± 0.78	55.00 ± 1.67
Day 15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	.00 ± 0.00	20.00 ± 0.00	25.00 ± 1.00	40.00 ± 0.00

Table 6: Percentage mortality of *Callosobruchus maculatus* after application of volatile oil formulations using *Kaolin*

Days	**Control**	Phostoxin	**10ul**	**20ul**	**30ul**	**40ul**	**50ul**
Day 0	10.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Day 3	10.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Day 6	10.00 ± 0.00	10.00 ± 0.00	50.00 ± 0.00	60.00 ± 0.00	70.00 ± 0.00	80.00 ± 0.00	90.00 ± 0.00
Day 9	10.00 ± 0.00	0.00 ± 0.00	40.00 ± 0.00	50.00 ± 0.00	60.00 ± 0.00	65.00 ± 0.00	90.00 ± 0.00
Day 12	10.00 ± 0.00	0.00 ± 0.00	15.00 ± 1.22	20.00 ± 0.00	30.00 ± 0.00	40.00 ± 0.00	60.00 ± 0.00
Day 15	10.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00	15.00 ± 1.33	25.00 ± 1.33	35.00 ± 2.44	50.00 ± 0.00

Table 7: Zones of inhibition (mm) by volatile oil of PG against some bacteria organisms

Names of Organisms	**Zones of Inhibition (mm)**	**Oflaxacin (control)**
Staphylococcus aureus	23	22
Bacillus sp	20	28
Streptococcus sp	20	19
Escherichia coli	29	21
Klebsiella pneumonia	24	21
Pseudomonas aeruginoses	14	10

Table 8: Zones of inhibition (mm) by volatile oils against some fungal organisms

Names of Organisms	**Zones of Inhibition (mm)**	**Mycoten (control)**
Aspergillus niger	22	28
Fusarium sp	22	26
Aspergillus flavus	18	23

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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