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# Pharmacognostic standardization and insecticidal activity of the leaves of *Hyptis suaveolens* (L.) Poit (Lamiaceae)

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**Abstract**
Lamiaceae have traditionally been used in developing countries for their insecticidal and repellant properties against several insect species. *Hyptis suaveolens* has been reported to repel mosquitoes and other insects effectively when burnt overnight in rooms. This study was aimed at establishing the Pharmacognostic profile and insecticidal/repellant activity of the leaves of *H. suaveolens* (L.) Poit against maize/bean weevils. Evaluation of the fresh, powdered and anatomical sections of the leaves was carried out to determine the macromorphological, micromorphological, chemomicroscopic, numerical and phytochemical profile. Evaluation of the insecticidal activity involved the determination of antifeedant properties, repellant and insecticidal actions of the extract and its fractions against adult *Sitophilus zeamais* (maize weevil) and *Acanthscelides obtectus* (bean weevil). Macro and microscopic studies gave results that could serve as a basis for proper identification, collection and investigation of the leaves of *H. suaveolens*. Phytochemical screening revealed the presence of alkaloids, tannins, saponins and cardiac glycosides. The crude extract and fractions produced antifeedant, repellant and insecticidal activities to varying degrees against Adult *Sitophilus zeamais* (maize weevil) and *Acanthscelides obtectus* (bean weevil). *H. suaveolens* has the potential to act as a lead in the commercial production of insecticides of plant origin. This will go a long way in ameliorating the deleterious effects associated with synthetic chemicals.

*Keywords*: *H. suaveolens*; Pharmacognostic profile; Insecticidal activity; Phytochemical constituents; Weevils.

## INTRODUCTION

Concern about the deleterious effects associated with synthetic chemicals have revived interest to explore plants as a source of natural insecticides (Abagli and Alavo, 2011). More so, these plant products have been claimed to be more ecological friendly than synthetic chemicals such as temephos, fenthion, diflubenzuron and methoprene, used as both larvicides and insecticides. The insecticidal activity of ethanolic extracts of four tropical plants (*Vernonia amygalina, Sida acuta, Ocimum gratissimum* and *Telfaria occidentalis*) against bean weevils (*Acanthscelides obtectus*) has been established (Adeniyi *et al.*, 2010). The laboratory evaluation of four medicinal plants as protectants against the maize weevil, *Sitophilus zeamais* (Mots) has also been investigated (Arannilewa *et al.*, 2006). Insecticidal activity of powder and extracts of *Delonix regia* seed against maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) (Ajayi, 2013), evaluation of the powder of three medicinal botanicals in the control of maize weevil, *Sitophilus*

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*zeamais* Motschulsky (Omotoso, 2014) and insecticidal and antifeedant activities of medicinal plant extracts against *Attagenus unicolor* Japonicus (Coleoptera: Dermestidae) (Mi-Kyeong *et al.,* 2006) are other examples of insecticidal investigations of plant origin.

Phytochemical constituents such as alkaloids, tannins and glycosides from medicinal plants have been used extensively to control insect vectors due to their broad spectrum of activity, low mammalian toxicity and ability to degrade rapidly in the environment.

The mints, taxonomically known as Lamiaceae or Labiatae, are a family of flowering plants. They have traditionally been considered closely related to Verbenaceae. Lamiaceae is a large family of aromatic herbs and shrubs having flowers resembling the lips of a mouth and four-lobed ovaries yielding four one-seeded nutlets. It contains about 236 genera and 6,900 to 7,200 species (Catino *et al.*, 1992). Lamiaceae have traditionally been used in developing countries for their insecticidal and repellant properties against several insect species (Conti *et al.*, 2012).

The plant *Hyptis suaveolens* (L.) Poit commonly known as mosquito plant, Chinese mint, mintweed or bush tea is distributed throughout the tropics and subtropics. The leaves of *H. suaveolens* have been utilized as a stimulant, carminative and as a cure for cutaneous diseases. Crude leaf extract is also used as a relief to colic and stomachache. Leaves and twigs are considered to be antispasmodic and used in antirheumatic and anti-soporific baths (Mandal *et al.*, 2007).

The result of the present study will not only aid in establishing the Pharmacognostic standardization of *H. suaveolens,* but will also be useful in promoting research aimed at the development of new agents from medicinal plants for insect control.

## EXPERIMENTAL

**Preparation of plant material.** The leaves of *Hyptis suaveolens* (L.) Poit (Lamiaceae) were collected a residential area of Ewbuomore quarters, Benin City, Edo State, Nigeria. The plants were authenticated by Mr. Sunday Nweke, the plant curator at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City where voucher specimens were deposited. The fresh leaves were air-dried for 72 h and powdered using an electric mill.

**Extraction and partitioning.** The dried leaves of *Hyptis suaveolens* (L.) Poit (Lamiaceae) (1.36 kg) were extracted using maceration method with MeOH (3 X 2L). Evaporating the solvent using rotary evaporator at 40 $^{o}$C yielded an extract which was subsequently re-suspended in water and successively partitioned into petroleum ether (3 X 2L), Chloroform (3 X 2L) and n-BuOH (3 X 2L). The fractions were concentrated *in vacuo* and used for insecticidal experiments.

**Insect culture.** Adult *Sitophilus zeamais* (maize weevil) and *Acanthscelides obtectus* (Bean weevil) used for the study were obtained from naturally infested maize and beans respectively in Uselu market, Benin City, Nigeria. From these stocks, pure cultures were raised to obtain new generations in the laboratory following standard procedure (Asawalam, 2006). Freshly emerged adults were used for the experiments.

**Macroscopy.** The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste (Wallis, 1985 and Evans, 2006).

**Microscopy.** The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound

microscope. The presence/absence of the following was observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed (AP, 1986).

**Chemomicroscopic examination.** Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques (Evans, 2006).

**Phytochemical investigation.** Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins (phenazone; iron complex; formaldehyde and modified iron complex tests were carried out on the aqueous extract to detect the presence of hydrolysable, condensed and pseudo tannins), cardiac glycosides (Keller-Killiani and Kedde tests were carried out on the methanolic extract to detect the presence of a deoxy sugar, whose natural occurrence is to date, known only in association with cardiac glycosides and to indicate the presence of a lactone ring on the cardenolides respectively), alkaloids (Mayer's, Dragendorff's, Wagner's and 1% picric acid reagents to detect the presence of alkaloidal salts and bases), saponins glycosides (frothing of the aqueous extract when shaken and haemolysis test on blood agar plates were carried out to indicate and confirm the presence of saponins), anthracene derivatives (Borntrager's test for combined and free anthraquinones, where aglycones were extracted using chloroform and shaken with dilute ammonia) and cyanogenetic glycosides (sodium picrate paper test were used to test for the presence of hydrocyanic acid in the sample. Conversion to sodium isopurpurate indicates the presence of cyanogenetic glycosides) (Evans, 2006; Brain and Turner, 1975; Ciulei, 1981 and Harborne, 1992).

**Quantitative investigation.** Quantitative investigations to determine moisture content, total ash, acid–insoluble ash, water–soluble ash, alcohol (90 % ethanol) and water soluble extractive values were carried out using standard procedures (AP, 1986 and BP, 1980).

**Moisture content.** The powdered drug (2.0 g) was weighed into a clean crucible of known weight. After oven drying at 105 $^{o}$C for 5 hours and cooled, the crucible was weighed again to determine weight loss in the powdered drug. The average percentage weight loss, with reference to the air dried powdered drug was determined for thirty replicates.

**Total ash determination.** The crucibles were washed thoroughly, dried in hot oven at 100 $^{o}$C, cooled in desiccators and weighed. A 2.0 g portion of each of the samples were weighed into the crucible and put in the furnace. Heating was started gradually until temperature of 600 $^{o}$C was reached. This temperature was maintained for 6 hours. The crucible was then put inside the desiccators and cooled. After cooling the sample was reweighed and the percentage ash calculated. Fifteen replicates were determined.

$$\% \text{ Ash} = \frac{W-Z \times 100}{N}$$

where W = weight of the crucible and ash; Z = weight of empty crucible; N = Weight of Sample.

**Acid–insoluble ash determination.** The total ash was treated with 25 ml dilute hydrochloric acid in a crucible, boiled gently for 5 min while covered with a watch glass and filtered through ashless filter paper (Whatman No.1) of known weight. The crucible was washed with hot water and the washings passed through the filter paper. This was continued until the filtrate became neutral to litmus paper. The paper with the insoluble matter was dried to a constant weight at 105 $^{o}$C. The

weight of the insoluble matter was determined by subtracting the weight of the filter paper from the dry weight of the filter paper containing the insoluble ash. The percentage of the acid-insoluble ash with reference to the air-dried material was calculated. Fifteen replicates were determined.

**Water–soluble ash determination.** The water-soluble ash was determined by adding 25 ml of water to the ash. After boiling gently for 5 min, the content of the crucible was filtered through previously weighed dried ashless filter paper. After washing the residue with hot water, the filter paper was dried in an oven at 105 $^{o}$C until a constant weight was obtained. The weight of the residue was obtained by subtracting the weight of the dry filter paper from the weight of the residue and the filter paper. The weight of the water soluble ash was then obtained by subtracting the weight of the insoluble ash (i.e. the residue) from the weight of the total ash. The percentage of water soluble ash with reference to the air-dried powdered material was then determined. Fifteen replicates were determined.

**Alcohol soluble extractive value.** Powdered leaf drug (5.0 g) was weighed into a 250 ml stopper conical flask. Ethanol 90 % (100 ml) was added to the conical flask and stoppered. The flask was shaken in a mechanical shaker for 6 hours and then allowed to stand for 18 hours. The extract was filtered by suction filtration using a Buckner funnel. The weight of a heated cooled flat bottom porcelain crucible was accurately determined. The filtrate was poured into weighed crucible and evaporated to dryness at 100 $^{o}$C. The residue was dried to constant weight and the final weight noted. The weight of the residue obtained from the extract was determined by subtracting the constant weight of crucible from the residue. The alcohol extractive was then calculated with reference to the initial weight of the powdered drug and expressed as percentage. Fifteen replicates were determined.

**Water soluble extractive value.** The above experiment was repeated using water.

**Insecticidal evaluation.** The determinations of antifeedant and repellency activities (Khani *et al.*, 2011) and insecticidal activity (Arannilewa *et al.*, 2006) were carried out as follows:

**Determination of anti-feedant properties**. Bean weevils (20) were put into each of six (6) conical flasks containing a cube of sugar. Each cube was treated with 0.3 µg/mL of the crude extract, fractions (Pet ether, chloroform, N-butanol and aqueous fractions) and distilled water respectively. The flasks were covered with a stopper and the movement of the insects noted for 5mins at every hour for the first 10 hours and at the end of 24th hour. The experiment was repeated using maize weevils.

**Determination of repellant action.** Bean weevils (20) were put into each of six (6) conical flasks. With the aid of a micro syringe, 0.3 µg/mL of the crude extract and fractions (Pet ether, chloroform, N-butanol and aqueous fractions) dissolved in DMSO were put at the bottom of the flasks and DMSO (diluent) served as the control. The flasks were covered with a stopper and the movement of the insects noted for 5mins at every hour for the first 10 hours and at the end of 24th hour. The experiment was repeated using maize weevils.

**Determination of insecticidal action.** Bean weevils (20) were put into each of five (6) conical flasks. With the aid of a micro syringe, graded concentrations of the crude extract (0.1, 0.2, 0.3 µg/mL) and 0.3 µg/mL fractions (Pet ether, chloroform, N-butanol and aqueous fractions) dissolved in DMSO were sprayed into the flasks respectively. DMSO served as the control. The time of injection was noted and the conical flaks were covered to prevent the weevils from escaping.

After twenty-four hours, the number of dead weevils was counted and the rate of kill determined. The experiment was repeated using maize weevils.

## RESULTS

**Macroscopic description of the leaves of *H. suaveolens.*** The leaves were simple leaf with a cordate shape and subcordate base. They were dark green in the upper surface and light green in the lower surface. Both the upper and lower surfaces were pubescent with serrated margins and reticulate venation. The apex was acute and the petiole long, slender and hairy. Average leaf size was 3.45 cm $\pm$ 0.4 (length) and 2.53 cm $\pm$ 0.2 (breadth). The fresh leaf had a bitter taste and a characteristic odour.

**Microscopic description of the leaves of *H. suaveolens.*** Micromorphological features revealed that anticlinal walls are wavy. Stomata were present in both upper and lower epidermi, though the number is higher on the lower surface. The stoma was surrounded by two subsidiary cells whose common wall was at right angle to the long axis of the guard cells (Diacytic arrangement). There were numerous uniseriate multicellular covering trichomes present on both surfaces. Glandular Trichomes with bicellular heads were also present. A transverse section of the leaf across the mid–rib shows an upper and lower epidermi consisting of cells of same sizes. There are uniseriate covering trichomes on both surfaces. It has a bifacial surface i.e. there are two different surfaces with different identities, hence dorsoventral. The mesophyll consists of a palisade and the spongy mesophyll, embedding a crystal sheath. There are cluster crystals of calcium oxalate in the spongy mesophyll. The mid–rib bundle is surrounded by a zone of collenchymas on both surfaces. The phloem vessels embed the xylem vessels. Chemomicroscopic examination of the leaves revealed the presence of starch, mucilage, calcium oxalate crystals and cellulose.

**Numerical data of the leaves of *H. suaveolens.*** The moisture content of *H. suaveolens* which fell within the Pharmacopoeia limit, the ash values as well as the amounts of constituents which were extractable by methanol and water under specified conditions are presented in Table 1.

**Table 1:** Numerical data of leaves of *H. suaveolens*

| Parameter | Mean $\pm$ SEM (% w/w) |
|---|---|
| Moisture content* | 12.11 ± 0.47 |
| Total ash* | 9.52 ± 0.26 |
| Acid – insoluble ash† | 1.74 ± 0.21 |
| Water – soluble ash† | 4.72 ± 0.91 |
| Alcohol – soluble extractive* | 11.45 ± 0.82 |
| Water – soluble extractive* | 7.44 ± 0.06 |

*n = 30 † n = 15

**Table 2.** Insecticidal activity of the crude extract of *H. suaveolens*

| Sample/ system | Insect | Concentration (µg/mL) | Mortality after 24 h | Mortality rate after 24 h (%) |
|---|---|---|---|---|
| Extract | Bean weevil | 0.1 | 05 ± 0.32 | 25 |
| Extract | Bean weevil | 0.2 | 18 ± 0.71 | 90 |
| Extract | Bean weevil | 0.3 | 18 ± 0.71 | 90 |
| Extract | Maize weevil | 0.1 | 03 ± 0.44 | 15 |
| Extract | Maize weevil | 0.2 | 08 ± 0.02 | 40 |
| Extract | Maize weevil | 0.3 | 10 ± 0.96 | 50 |
| DMSO | Both weevils | 0.2 | 0 | 0 |

*Number of weevils/experiment = 20

**Table 3.** Insecticidal activity of the fractions of *H. suaveolens* extract against Bean and maize weevils

| Solvent system | Insect | Concentration (µg/mL) | Mortality after 24 h | Mortality rate after 24 h (%) |
|---|---|---|---|---|
| Petroleum ether | Bean weevil | 0.3 | 05 ± 0.32 | 25 |
| Chloroform | Bean weevil | 0.3 | 10 ± 0.71 | 50 |
| Aqueous | Bean weevil | 0.3 | 15 ± 0.94 | 75 |
| *n*-Butanol | Bean weevil | 0.3 | 16 ± 0.84 | 80 |
| Petroleum ether | Maize weevil | 0.3 | 02 ± 0.04 | 10 |
| Chloroform | Maize weevil | 0.3 | 06 ± 0.56 | 30 |
| Aqueous | Maize weevil | 0.3 | 08 ± 0.27 | 40 |
| *n*-Butanol | Maize weevil | 0.3 | 08 ± 0.08 | 40 |
| DMSO | Both weevils | 0.3 | 0 | 0 |

*Number of weevils/experiment = 20

**Phytochemical screening.** Phytochemical screening of the leaves of *H. suaveolens* for secondary plant metabolites revealed the presence of alkaloids, tannins, saponins and cardiac glycosides.

**Antifeedant activity.** Within the 24-hour observation period, there was evidence of antifeedant activity as both the bean and maize weevils did not feed on the sugar source where the extracts and fractions were present, but fed on the sugar source with distilled water.

**Repellant activity.** Within the 24-hour observation period, there was evidence of repellant action as both the bean and maize weevils moved from the bottom of the flasks where the extracts and fractions were present, but remained at the bottom of flask in the case where DMSO was present.

**Insecticidal activity.** The crude extract (table 2) of *H. suaveolens* as well as the fractions (table 3) showed different rates of insecticidal activity.

## DISCUSSION

The macro- and micro-morphological parameters described in this study could therefore, serve as a basis of proper identification, collection and investigation of the leaves of *H. suaveolens*, in view of the fact that they are closely related to species of the Verbenaceae family. Also, there were no significant differences in the microscopy of the Indian specie of *H. suaveolens* (Jelani and Prabhakar, 1991) when compared to the Nigerian specie that we reported on, with regards to the nature and types of Stomata arrangement and Trichomes.

The moisture content of *H. suaveolens* obtained in the determination of quantitative standards met the pharmacopoeial limits of water content for vegetable drugs, which is between 8 – 14 % (AP, 1986). *H. suaveolens* can be conveniently stored at room temperature without the deterioration of their active constituents. The total ash value is of importance as it indicates to some extent the amount of care taken in the preparation of the drug (Evans, 2006). The total ash usually consists of carbonates, phosphates, silicates and silica. When the total ash was treated with dilute hydrochloric acid, the percentage of acid – insoluble ash was determined. This usually consists mainly of silica, as most of the natural ash is soluble leaving the silica as acid – insoluble ash which represents most of the ash from the contaminating soil (Evans, 2006). A high acid–insoluble ash in drugs such as senna, cloves, valerian and tragacanth indicates contamination with earthly material. Senna leaf, which may be used directly as the powdered drug, is required to have a low acid–insoluble ash (2.5 %) while hyoscyamus, which unavoidably attracts grit onto its sticky trichomes is allowed a higher value (12. 0 %). *H. suaveolens* which had a total ash value of 9.52 ± 0.26 and an acid–insoluble ash value

of 1. 74 ± 0. 21 % may therefore be used directly as a powdered drug.

The development of safe and cost effective insecticides using indigenous plants is being investigated by researchers. However, most research on plant products that have been said to be protective against insect damage have centered on volatile oils. Though the insecticidal activities of the volatile oils of *H. suaveolens* has been determined (Conti *et al.,* 2011), Gomez-Peralta *et al.,* 2009 had investigated the effect of the leaves on the survival of *Sitophilus zeamais* in maize and *Zabrotes subfasciatus* in bean seeds, with the powered leaves having only effects on the mortality of *S. zeamays*. From the results of this study, the leaf extract of *H. suaveolens* has shown to possess insecticidal, antifeedant activities and repellant action, due mainly to the presence of chemical constituents in the plants. The reported sensitivity of the weevils demonstrated in this study may largely be due to synergistic effect of the secondary plant metabolites present in *H. suaveolens.*

**Conclusion.** The results obtained from this study showed that *H. suaveolens* possesses unique Pharmacognostic parameters that can aid its standardization and quality control. The plant has the potential to act as a lead in the commercial production of insecticides of plant origin.

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