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PHYTOCHEMICAL ANALYSIS OF WILD SPIKENARD (*Hyptis suaveolens* L. Poit) WHOLE PLANT POWDER AS A BIOPESTICIDE FOR THE CONTROL OF STORAGE INSECT PESTS

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ABSTRACT
Phytochemical analysis of Wild Spikenard (Hyptis suaveolens L. Poit) whole plant powder as a biopesticide for the control of storage insect pests on stored produce was carried out in the Laboratory of Department of Chemistry, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria in 2015. This was to determine the chemical constituent of H. suaveolens whole powder and also the texture, colour and weight of the extract, organoleptic properties and the fluorescence under ordinary and ultra-violet light when subjected to various reagents such as acetone, petroleum, ether, ethanol, distilled water, methanol and chloroform. The study determined the phytochemicals present in Wild Spikenard (H. suaveolens) whole plant powder. Phytochemical analysis revealed the presence of constituents like phenols, tannins, flavonoids, glycosides, alkaloids, aldehydes, ketones, proteins and terpenoid. However, saponins and steroids were not present.

Key words: Phytochemical, Spikenard, Insect, Pest, Powder, Biopesticide.

INTRODUCTION

The extent of post-harvest crop losses and the need to shift emphasis from the excessive use of conventional synthetic insecticides for the control of *Caryedon serratus* on some host plants in store prompted this work. Moreover, the need to phase out ozone depleting pesticides has open a new window for searching alternative pesticides of plant origin to control insects and other pests of agricultural sector (Ahmedani *et al.,* 2007; Jada *et al*., 2013). Several methods of insect pests control have been employed by farmers and researchers have identified some efficient and effective control of the insect pests (Adebowale and Adedire, 2006). Some of these methods range from store hygiene, physical and cultural control methods and the use of inert materials. Chemical control appeared to be the most effective and efficient control method (Oaya *et al*., 2011) but it has tremendous adverse effect to man, the livestock and the environment (Adedire and Lajidire, 2000). Chemical related pesticides against insect pests are not only limited because of the health and environmental concern related to them but also due to their high costs and scarce availability in rural areas (Adda *et al*., 2011).

Against this background, a curious search for natural-product based agrochemicals which are biodegradable, eco-friendly, sustainable and save to humans and the environment was intensified (Jadhau and Jadhau, 2006). There is also the need for cheaper and safer alternative control practices. Over the years, farmers have learned to curtail insect population through the use of plant materials. The potential advantages of botanical insecticides over synthetic ones have been highlighted by Prakash *et al*. (2008); Oaya and Malgwi (2014). Many plants have been tested or identified as interesting botanical pesticides in Sub-Saharan Africa (Kossou *et al.,* 2001; Oparaeke *et al*., 2005; Sinzogan *et al*., 2006; Oparaeke *et al*., 2006; IITA, 2006; Okereke *et* *al*.,2007; Aboubakary *et al*., 2008; Adda *et al*., 2011; Oaya *et al*., 2012; Oaya *et al*., 2013 and Malgwi and Oaya, 2014) and are potentially useful in pest control programs taking into account both the needs of increased food and preserving the health of a growing population (Adda *et al*., 2011).

Hyptis suaveolens (L.) Poit, a potential anti-feedant plant product belongs to the family laminaceae (Raja *et al.,* 2005). Ethnobotanical studies conducted in Kenya on *Hyptis suaveolens* showed that, the plant can repel mosquitoes effectively when burned overnight in rooms (Abagli and Alavo, 2011). Duke (2007) also in his phytochemical and ethnobotanical database refers to the plant as insect repellant. *Hyptis suaveolens* is used for some ethnobotanical applications in rural communities (Koumauglo *et al*., 1993; Kossou *et al*., 2001; Edeoga *et al*., 2006 and Oaya *et al*., 2013) and the plant is readily available close to villages, along roadsides, on farm steads etc (Adda *et al*., 2011). It is established that, *Hyptis suaveolens* Poit contain some compounds that can control insects and nematodes (Oyedunmade, 1998; Musa *et al*., 2009). The leaves of *Hyptis suaveolens* (L) Poit have been utilized as a stimulant, carminative, sudoritic, galactogogue and as a cure for parasitic contageous disease (Siddiqui *et al.*, 2009). Crude leaf extract is also used as a relief to colic and stomachache. Leaf and twigs are considered to be antispasmodic and used in antisuporific baths and anti-inflammatory, anti-fertility agents (Ladan *et al.,* 2009).

This work considered not only the use of leaves in powder form but the whole *Hiptis* plant (leaves, seeds, stems and roots) ground into powder form. The knowledge of chemical constituent of the plant is desirable because such information aided in determining the quantity or the dosage of application which has been a major limitation to the use of bio-pesticides especially in the rural communities. This knowledge was also instrumental in evaluating the effect of *Hyptis suaveolens* L. Poit whole powder on the survival and reproductive potential of *Caryedon serratus* Olivier on stored groundnut and tamarind, in the search for alternative control method instead of the use of expensive, toxic, harmful and imported synthetic insecticides. This work was carried out to determine the phytochemical constituents of whole plant powder of *Hyptis suaveolens* (L.) Poit to be used as biopesticide for the control of storage insect pests such as *Caryedon serratus* Olivier *Callosobruchus maculatus* Fab., *Sitophilus zeamays* etc.

MATERIALS AND METHODS

Description of the Study Area

The phytochemical analysis of the plant material, *H. suaveolens* (whole powder) was carried out in the Laboratory of the Department of Chemistry, Modibbo Adama University of Technology, Yola, Adamawa State-Nigeria. The University is located in Sangere Village, Girei Local Government Area, within longitude 9° 14” North and Latitude 12° 13” East of the equator in the Northern Guinea Savannah agro- ecological zone of Nigeria (Adebayo, 1999).

Collection and Preparation of Plant Material, Wild Spikenard *(H. susveolens*)

The plant material used was the Wild Spikenard (*H. suaveolens*) and it belongs to the family Laminaceae. The plant material is readily available in Nigeria. It was collected from the vicinity of the main campus of Adamawa State College of Agriculture, Ganye, Adamawa State-Nigeria. The whole plant (leaves, seeds, stems, roots and flowers) was shade dried until all the water molecules evaporated and the plant organs well dried for grinding. The dried whole plant was mechanically powdered and sieved using 0.02mm mesh and stored in an air-tight container with proper labeling which was later used for further phytochemical analysis or qualitative test for the identification of various plant constituent and Laboratory test for insecticidal potentials of the whole powder. The shade dried Wild Spikenard (*H.*

suaveolens) whole and the prepared powder kept in a container are presented on plates I and II respectively.

Plate I:
Shade dried *H. suaveolens* L. Poit whole plant

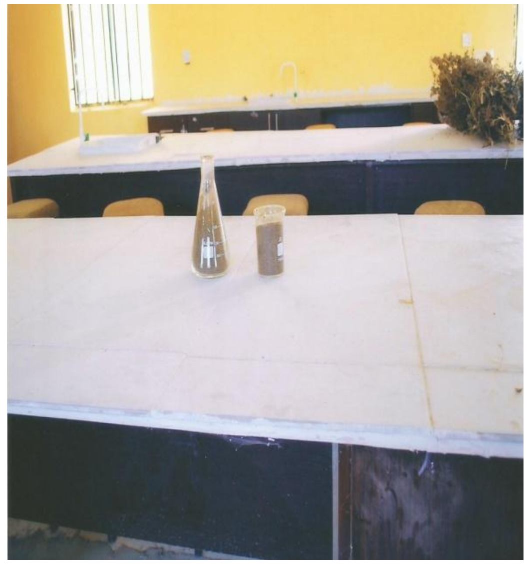

Plate II:
H. suaveolens L. Poit whole plant powder

Phytochemical Analysis of Wild Spikenad (*H. suaveolens*) Whole Powder

Phytochemical analysis of whole plant powder of Wild Spikenard, *H. suaveolens* was carried out in the Laboratory. Whole plant powder of *H. suaveolens* with different chemical reagents such as acetone, petroleum ether, ethanol, methanol, chloroform and distilled water were subjected to various qualitative tests to determine the presence or absence of phytochemical constituents like glycosides, tannins, phytosterols, steroids, alkaloids, saponins, flavonoids, ketones in *H. suaveolens* whole plant powder. The phytochemical constituents were detected by the usual prescribed method as reported by Reddy *et al*. (1999) and Okwu (2004). Fluorescence analysis was also carried out for the whole powder and for extract using standard procedures as reported by Harbone (1998). The methods are described below:

Preparation of Plant Extracts

Five gram (5g) of dried powdered plant material, *H. suaveolens* was taken in a beaker and 200 ml of distilled water was added. The mixture was heated on a hot plate with continuous stirring at 30-40^{o}C for 20 minutes. Then the water extract was filtered through filter paper and the filtrate was used for phytochemical analysis. The water extract was kept in a refrigerator when not in use (Yadav and Agarwala, 2011).

Preparation of Solvent Extract

Crude plant extract was prepared by Soxhlet extraction method as reported by Yadav and Agarwala (2011). About 20g of powdered plant material, *H. suaveolens* was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvents used were: methanol, petroleum ether, chloroform, distilled water, acetone and ethanol. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker kept on hot plate and heated at 30-40^{o}C till all the solvent gor evaporated. Dried extract was kept in refrigerator at 4^{o}C for future use.

Qualitative Phytochemical Analysis

The extract was tested for the presence of bioactive compounds by using the following standard methods as suggested by Harbone (1998) and Sofawora (1993).

Test for Proteins

Crude extract of the plant material, *H. suaveolens* whole plant power mixed with 2ml of the reagents, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Test for Phenols and Tannins

Crude extract of the plant material, *H. suaveolens* whole plant powder was mixed with 2ml of the reagents solution. A dark blue-green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoids

Crude extract of the plant material, *H. suaveolens* whole plant powder was mixed with 2ml of th reagents. Pink scarlet color appared after few minutes which indicated the presence of flavonoids.

Test for Alkaloids

Crude extract of the plant material, *H. suaveolens* whole plant powder was mixed with 2ml of the reagents. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of alkaloids.

Test for Glycosides

Crude extract of the plant material, *H. suaveolens* whole plant powder was mixed with 2ml of the reagents in a test tube. The mixture was cooled in ice. A color change from violet to blue to green indicated the presence of steroidal nucleus, which is a glycone portion of glycoside.

Test for Terpenoids

Crude extract of the plant material, *H. suaveolens* whole plant powder was dissolved on 2ml of the reagents and allowed to evaporate to dryness. A grayish colour indicated the presence of terpenoids.

Test for Aldehydes

Crude extract of 2ml of the plant material, *H. suaveolens* whole plant powder was mixed with the reagents in a tilted test tube. A silver mirror on the surface of the test tube is a positive test for aldehydes.

Test for Ketones

Crude extract of 2ml of the plant material, *H. suaveolens* whole plant powder was mixed with the reagents. The test tube was stopped and shaken vigorously. A positive test resulted from a brown colour of the mixture disappearing and a yellow iodo-form solid precipitating out of the solution indicating the presence of ketones.

The phytochemical analysis was instrumental for determining the dosage of application or the quantity of plant material whole powder used for the control of the groundnut bruchid, *C. serratus* on shelled groundnut seeds and tamarind whole pods in the store.

RESULTS

Phytochemical Analysis of Wild Spikenard (*Hyptis suaveolens*)

The phytochemical analysis of Wild Spikenard (*H. suaveolens*) whole powder extract was carried out in the Laboratory of the Department of Chemistry, Modibbo Adama University of Technology, Yola in 2015. This was to determine the chemical constituent of *H. suaveolens* whole powder and also the texture, colour and weight of the extract, organoleptic properties and the fluorescence under ordinary and ultra-violet light when subjected to various reagents such as acetone, petroleum, ether, ethanol, distilled water, methanol and chloroform. The results are presented in Tables 1, 2, 3, and 4 respectively.

Texture, colour and weight of extract obtained from Wild Spikenard (*H. suaveolens*) whole powder

The result of texture, colour and weight of extract obtained from *H. suaveolens* whole plant powder is presented in Table 1. The result showed the texture, colour and the weight of extracts of plant material in various solvents. *H. suaveolens* whole plant powder exhibited various textures, colours and weights when tested or treated with some reagent. Sticky texture and dark colour were observed when the whole plant extract was treated with acetone, petroleum ether, ethanol and distilled water while the *H. suaveolens* whole plant extract treated with methanol and chloroform also gave sticky texture but dark brown colour. The highest weight in grams of the plant material whole extract was observed in distilled treated extract (0.95) followed by petroleum ether treated extract (0.70) and the least was seen in methanol treated extract (0.20) as presented in Table 1.

Table 1: Texture, Colour and Weight of Solvent Soluble Extracts Obtained from Wild Spikenard *H. suaveolens* Plant Extract

Reagents	**Texture**	**Colour**	**Weight (g)**
Acetone	Sticky	Dark Green	0.60
Petroleum Ether	Sticky	Dark Green	0.70
Ethanol	Sticky	Dark Green	0.25
Distilled water	Sticky	Dark Green	0.95
Methanol	Sticky	Dark Brown	0.20
Chloroform	Sticky	Dark Brown	0.60

Flourescence analysis of Wild Spikenard, *H. suaveolens* whole powder under ordinary and ulta-violet light.

H. suaveolens whole plant powder was treated with various chemical reagents and had exhibited various colours under ordinary and ultra-violet light. When the whole powder was analyzed alone, the colour was brown black under the ordinary light and dull brown under the ultra-violet light. The colour was greenish brown and dark brown respectively in an aqueous solution under ordinary and ultra-violet light. Moreover, the reagent aqueous residue gave dark brown colour under ordinary and ultra-violet light respectively. Consequently, the reagent alcoholic extract gave dark brown and brown black colour under ordinary and ultra-violet light. Similarly, alcoholic residue gave brown black and dark brown colours under ordinary and ultra-violet light. Also, chloroform extract of whole plant powder of *H. suaveolens* produces dark brown colours under day light or ordinary and ultra-violet light. Brown black and greenish brown colours were observed in *H. suaveolens* whole powder treated with chloroform residue as presented in Table 2.

Phytochemical constituent of Wild Spikenard (*H. suaveolens*) whole powder.

Phytochemical characteristics of whole plant powder of Wild Spikenard (*H. suaveolens*) were tested and it is presented in Table 3. The result revealed the presence of medicinally active compounds in *H. suaveolens* whole powder extracts. From the table, it could be deduced that, alkaloid, tannins, flavonols, terpenolds, phenols, proteins, glycosides, ketones and aldehydes were present when *H. saveolens* whole powder extract was subjected to various reagents such as petroleum ether, ethanol, methanol, chloroform and distilled water except in acetone. However, saponins and steroids were not present in the plant material whole powder extract treated with various reagents.

The results as presented in Table 3 showed that, tannins were present in all the powder treated with various reagents except in acetone solution. Similarly, aldehydes, ketones, terpenoids and phenols were also present in *H. suaveolens* whole plant powder treated with various chemical reagents except in acetone solution and distilled water. Moreover, alkaloids, flavonols, glycosides and proteins were absent in four of the *H. suaveolens* whole powder treated reagents (acetone, petroleum ether, chloroform and distilled water) but were present in the remaining as shown in Table 3.

On the other hand, two chemical constituents namely saponins and steroids were absent in all the *H. suaveolens* whole plant powder treated reagents as shown in Table 3 below.

Table 2: Fluorescence Analysis of Wild Spikenard, *H. suaveolens* Whole Powder under Ordinary and Ultra-violet Light.

Sample	**Ordinary Light (Day Light)**	**Ultra-Violet Light**
Dry Powder	Brown Black	Dull Brown
Aqueous	Gold Brown	Dark Brown
Aqueous Residue	Dull Brown	Dark Brown
Alcoholic Extract	Dark Brown	Brown Black
Alcoholic Residue	Brown Black	Dark Brown
Chloroform Extract	Dark Brown	Dark Brown
Chloroform Residue	Brown Black	Greenish Brown

Table 3: Phytochemical Analysis of Solvent Extracts from Wild Spikenard, *H. suaveolens* Whole Plant Powder.

Phyt. Constituent	**Acetone**	**Petroleum Ether**	**Ethanol**	**Distilled Water**	**Methanol**	**Chloroform**
Alkaloids	-ve	+ve	+ve	-ve	+ve	-v
Tannins	-ve	+ve	+ve	+ve	+ve	+v
Saponins	-ve	-ve	-ve	-ve	-ve	-v
Flavonols	-ve	-ve	+ve	-ve	+ve	+v
Aldehydes	-ve	+ve	+ve	+ve	+ve	+v
Ketones	-ve	+ve	+ve	+ve	+ve	+v
Steroids	-ve	-ve	-ve	-ve	-ve	-v
Terpenoids	-ve	-ve	+ve	+ve	+ve	+v
Glycosides	-ve	-ve	+ve	-ve	+ve	+v
Phenols	-ve	+ve	+ve	-ve	+ve	+v
Proteins	-ve	-ve	+ve	+ve	+ve	+v

+ve: Constituent Present; -ve: Constituent Absent.

DISCUSSION

Phytochemical Constituent of Wild Spikenard, *H. suaveolens* Whole Powder

Plants have been a major source of pesticides and cure for human diseases since time immemorial. It is no wonder that the world's one-forth population that is 2 billion people are dependent on traditional and plant based pesticides and medicines. Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants growing wild in different parts of the world. Although hundreds of plant species have been tested for chemical properties; the vast majority have not been adequately evaluated

(Balandrin *et al.,* 1985 and Niranjan *et al.,* 2011).

Phytochemical analysis conducted on the extract of whole powder of Wild Spikenard, *H. suaveolens* using reagents such as acetone, petroleum ether, ethanol, distilled water, methanol and chloroform revealed the presence of chemical constituents which are known to exhibit or demonstrate insecticidal as well as pesticidal properties (Sofowora, 1993). The chemical analysis of Wild Spikenard, *H. suaveolens* whole powder showed the presence of phytochemicals such as phenols, tannins, flavonoids, glycosides, proteins, terpenoids, alkaloids, aldehydes and ketones.

Phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (^{-}OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol also called carbolic acid C_6H_5OH. Phenol was found in petroleum ether, ethanol, methanol and chloroform when *H. suaveolens* powder was subjected to phytochemical analysis. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule (Khoddami, 2013: Amorati and Valgimigli, 2012; Robbins and Rebecca, 2003). The phenolic compounds are among the largest and most ubiquitous group of plant metabolites. They possess biological properties such as anticarcinogen inhibitory effects as well as cell proliferation activities (Yadav and Agarwala, 2011). Several studies have agreed and described the antioxidant and insecticidal properties of plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Han *et al.,* 2007).

Organisms that synthesize phenolic compounds do so in response to ecological and physiological pressures such as pathogen and insect attack, Ultra Violet radiation and wounding (Khoddami, 2013) and they can function as antioxidants (Klepacka, 2011). As they are present in food consumed in human diets and in plants used in traditional medicine of several cultures, their role in human health and disease is a subject of research (Mishra and Tiwari, 2011). According to them, some phenols are germicidal and are used in formulating disinfectants and others possess estrogenic or endocrine disrupting activity.

Tannin was also present in *H. suaveolens* whole powder extract when it was subjected to various chemical processes. It was found in reagents such as petroleum ether, ethanol, distilled water, methanol and chloroform. Haslam (2007) reported that, tannin (or tannoid) is an astringent, polyphenolic biomolecule that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. Tannin binds to proline rich protein and also interferes with protein synthesis (Yadav and Agarwala, 2011). The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation and perhaps also as pesticides and in plant growth regulation (McGee, 2004). Flavonoids in the same vane were present and are portrayed as hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to show pesticidal activities and influence against wide array of microorganisms in-vitro. This is in agreement with Marjorie (1996) who suggested that, the activity of flavonoid is probably due to their ability to complex with extracellular and soluble proteins and also complex with bacterial cell wall. Yadav and Agarwala (2011) also reported that, flavonoids are effective antioxidant and pesticides that show strong anticancer activities.

The *H. suaveolens* whole powder extracts also contain alkaloids. Alkaloid was found in reagents such as petroleum ether, ethanol and methanol. They have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity, which is the state of being toxic to cells. This is in consonant with Okwu (2001) who reported that, alkaloids contain some analgesic antispasmodic and antibacterial properties. Andreas (2009), in his findings found out that, *a*lkaloids are any of the large class of organic, nitrogen-containing ring compounds of vegetable origin and sometimes synthesized, some of which are liquid but most of which are solid. According to him, they have a bitter taste, that are usually water-insoluble and alcohol-soluble, that combine with acids without the loss of a water molecule to form water-soluble hydrochlorides, hydrobromides, or the like. They usually exhibit pharmacological action as nicotine, morphine, or quinine. György *et al.* (2002) reported that, prior to the development of a wide range of relatively low-toxic synthetic pesticides, some alkaloids such as salts of nicotine and anabasine were used as insecticides. Their use was limited by their high toxicity to humans.

Glycoside was also present in *H. suaveolens* whole powder extract treated with some reagents. Glycoside was present in methanol, ethanol and chroroform. The chemical constituent is known to lower the blood pressure according to reports by Nyarko and Addy (1990). Glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms (Brito-Arias, 2007). Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis (Brito-Arias, 2007) which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications and pesticides. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body (Lindhorst, 2007).

Aldehydes were present in petroleum ether, ethanol, distilled water, and chloroform. Proteins were found in methanol, distilled water, methanol and chloroform. Ketones were also found in petroleum ether, ethanol and distilled water. Terpenolds were present in ethanol, distilled water, methanol and chloroform. Flavonoids were also found in reagents such as ethanol, methanol and chloroform. These chemical properties were present in considerable proportion or amount. This agrees with Elamathi *et al.* (2012) who reported that although some therapeutic benefits can be traced to specific plant compounds, many herps contain dozens of active chemical constituents that together combine to give the plant its therapeutic value.

CONCLUSION

The excessive damage caused by storage insect pests such as groundnut bruchid, *C. serratus, C. maculates, Sitophilus zeamays* etc to stored products, the need for empirical knowledge of the chemical constituents of the plant material, Wild Spikenard (*H. suaveolens*) whole powder, the risk and the hazards associated with continues use of synthetic insecticides, the need for a shift of emphasis to the use of insecticides of plant origin that are safe, biodegradable, eco-friendly, sustainable, economically and viable, prompted this work. The results of this work revealed that the plant material, Wild Spikenard (*H. suaveolens*) whole plant powder contain certain phytochemicals namely; tannins, phenols, flavonoids, aldehydes, alkaloids, glycosides, terpenoids, proteins and ketones which might have accounted for its reported insecticidal potentials.

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