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Pesticidal potential of the leaves of *Ocimum basilicum* linn. and *Hyptis spicigera* lam. on *Callosobruchus maculatus* F. (cowpea weevils) family lamiaceae

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ABSTRACT

A laboratory study was conducted on the pesticidal potentials of the aromatic plants Ocimum basilicum Linn and Hyptis spicigera Lamarck both of the family Lamiaceae against Callosobruchus maculatus (cowpea weevils), a pest of stored cowpeas. The essential oils produced by these plants which are a complex mixture of terpenes, sesquiterpenes, their oxygenated derivatives and other aromatic compounds were obtained by steam or hydro/hot-distillation method using a soxhlet extractor. 70g portions of Ocimum basilicum and Hyptis spicigera powdered leaves were separately extracted sequentially using the solvents; Hexane, ethylacetate, acetone, methanol and water in order of increasing polarities. Phytochemical screening of the both plant leaves showed the presence of alkaloids, cardiac glycosides, in all the extracts; flavournoids, tannins, steroids and carbohydrates in most of the extracts; while saponins and anthraquinones tested virtually absent. The extracts of both plants were tested for pesticidal activity against untreated cowpea seeds using cowpea weevils. Within the experimental time of 24-72hrs using 100mg and 200mg of 100mg/ml concentration, the solvent extracts generally demonstrated a knock down and mortality activity. Hexane extracts showed 80% mortality and 66.67% knockdown while acetone extracts showed 75% mortality and 60%knockdown. The effects were significant for both plants at P>0.05. Both plants exhibited an increase in effect with increasing concentration of the extracts.

Key words: *Ocimum basilicum*, *Hyptis spicigera*, pesticidal potential and Lamiaceae.

INTRODUCTION

Ocimum basilicum Linn *and Hyptis spicigera* Lamarck are both of the family Lamiaceae. Among all families of the plant kingdom, members of the Lamiaceae have been used for centuries in folk medicine. Lamiaceae or Labiatae, also known as the mint family, is a family of flowering plants and are aromatic herbs with environmental socio-economic value in flavouring, cosmetics, perfumery, infectionary and medicinal preparations. It had traditionally

been considered closely related to Verbenaceae [1] but in the 1990s, phylogenetic studies showed that many genera classified in Verbenaceae do not belong to Lamiaceae. Essential oils produced by these plants are a complex mixture of terpenes, sesquiterpenes, their oxygenated derivatives and other aromatic compounds. The actual chemical composition is a function of species, chemotype, climate, soil conditions and geographical location [2].

Ocimum basilicum, or Sweet Basil, is a culinary herb of major importance. Most culinary and ornamental basils are cultivars of the species *Ocimum basilicum*, but other species are also grown and there are many hybrids between species. Traditionally, it is a green plant, some varieties such as 'Purple Delight' are purple [3]. The common names include Basil, sweet basil, bush basil, wild basil, European basil, French basil, scent leave, garden basil and 'Dandoya kare' in Northern part of Nigeria.

Hyptis is a genus of flowering plant in the Lamiaceae family. These plants, known commonly as bushmints, are widespread in the tropics and warmer temperate regions of the Americas. There are 300 to 400 species, which may be annual or perennial [4]. It is called 'Bunsuru fadama' or 'Dai fadama' in Northern Nigeria.

MATERIALS AND METHODS

COLLECTION:

The leaves of the *Ocimum basilicum* Linn. and *Hyptis spicigera* Lam. were collected from near bushes in Jos North Local Government Area, Plateau State, Nigeria on the 21st of December 2010. The plants were identified in the field using the description and keys described by the 'Flora of West Tropical Africa [5] and the 'Woody plants of Ghana" [6]. The identities of the plants were authenticated at the Department of Horticulture and Landscape Technology, Federal College of Forestry, Jos, Plateau State, Nigeria.

PLANT PREPARATION:

The leaves of *Ocimum basilicum* and *Hyptis spicigera* were separately dried under shade for about 2 weeks after which they were powdered separately using mortar and pestle. The powdered drugs were sieved with a mesh of size 20 and stored in separate airtight containers until required for extraction.

EXTRACTION:

Pulverized sample (70g) of *Ocimum basilicum* was exhaustively extracted in a soxhlet extractor using hexane and labeled as hexane extract. The marc was again extracted using ethyl acetate and labeled as ethyl acetate extract. The same procedure was followed with methanol and water and labeled as methanol and water extracts respectively. This was also done for *Hyptis spicigera* pulverized sample [7].

METHODS

Phytochemical screening:

The various extracts were subjected to phytochemical screening for the presence of chemical constituents such alkaloids, saponins, tannins, steroids, flavournoids, etc according to standard procedures [8].

Bioassay:

Bioassay method was used for the determination of the pesticidal effects and potency of the extracts. Adult cowpea weevils (*Callosobruchus maculatus*) were used for this experiment. Forty (40) cowpea seeds were placed in sterilized and dried beakers and100mg/ml of each extract was prepared, from which 2mls and 1ml were measured separately and mixed with the cowpea in the different beakers and labeled properly. This was allowed to dry for 24hrs. Ten (10) live and active weevils (*Callosobruchus maculatus*) were then selected and placed in the beakers. This was covered tightly with a muslin cloth and left for observation at a total time interval of 72hrs for number of death of weevils.

In the 2nd phase of the experiment, 1.0, 1.5 and 2.0g portions of crude dry powder samples were introduced into different 250ml beakers each containing 40 imperforated cowpea and 10 adult weevils (*Callosobruchus maculatus*). White muslin cloths were used to cover the mouth of the beakers to prevent the weevils from escaping out and to prevent suffocation and also to aid easy observation. The experiment set ups were allowed to stand for 72hrs also along side with a control set up containing no plant samples [7].

At 48 and 72 hrs, the number of live weevils were observed and recorded. The number of survivors was calculated by subtracting the mortals from the original number of life weevils introduced. A control experiment without the powder sample, but with other experimental conditions constant was also set-up in each case. This was conducted for both plants.

RESULTS

Table 1: Phytochemical screening

(a), *Ocimum basilicum*

Chemicals /extract	Alkaloid	Saponins	Tannin	Flavonoid	Carbo-hydrate	Steroids	Anthra-quinone	Cardiac glycoside
Hexane	+	-	-	+	+	+	-	+
Ethyl acetate	+	-	+	+	+	-	-	+
Acetone	+	-	+	+	+	+	-	+
Methanol	+	+	+	+	+	+	-	+
Water	+	+	+	+	+	-	-	+

(a) *Hyptis spicigera*

Chemicals /extract	Alkaloid	Saponins	Tannin	Flavonoid	Carbo-hydrate	Steroids	Anthra-quinone	Cardiac glycoside
Hexane	+	-	-	-	-	+	-	+
Ethyl acetate	+	-	+	+	+	-	-	+
Acetone	+	-	+	+	-	+	-	+
Methanol	+	-	+	+	+	+	-	+
Water	+	+	+	+	+	-	-	+

Key; (-) absent (+) = present

Table 2: Percent recovery of the cowpea weevils for crude dry powder

Crude plant	Solvent Percentage recovery (%)				
	Hexane	**Ethyl acetate**	**Acetone**	**Methanol**	**Water**
Ocimum basilicum	2.41	3.51	7.81	0.89	0.64
Hyptis spicigera	3.71	4.01	4.59	0.33	0.10

Table 3: Percent knockdown of the cowpea weevils for crude dry powder at different time interval

Extract	No. of survivors at 48hrs for			No. of survivors at 72hrs for			No. of survivors for Control at	
	1.0g	**1.5g**	**2.0g**	**1.0g**	**1.5g**	**2.0g**	**24hrs**	**48hrs**
Ocimum basilicum	10	7	6	9	6	5	6	6
%knockdown	0	17.65	25.0	5.26	25.0	33.3	25	25.0
Hyptis spicigera	10	6	9	10	7	9	9	8
%knockdown	0	25	5.26	0	17.6	5.26	5.26	11.1

Table 4(a-b): Bioassay of *Ocimum basilicum* solvent extracts at different concentrations

a) 200mg

Extract	Hexane	Ethyl acetate	Acetone	Methanol	Water
Average Total Mortality	8	5.5	3.5	4	4.5
%mortality	80	55	35	40	45
%knockdown	66.67	37.9	21.2	25	37.9
Control %knockdown	17.6	17.6	11.1	0	14.3

b) 100mg

Extract	Hexane	Ethylacetate	Acetone	Methanol	Water
Average Total Mortality	4	2.5	1	1.5	1.5
%mortality	40	25	10	15	15
%knockdown	25.0	14.3	5.26	8.1	8.1
Control %knockdown	17.6	17.6	11.1	0.0	14.3

Table 5(a-b) Bioassay result of *Hyptis spicigera* solvent extracts at different concentrations

a) 200mg

Extract	Hexane	Ethylacetate	Acetone	Methanol	Water
Average Total Mortality	4	6.5	7.5	6.5	1.5
%mortality	40	65	75	65	15
%knockdown	25.0	48.1	60.0	48.1s	8.1
Control %knockdown	17.65	14.3	8.1	11.1	2.6

(b) 100mg

Extract	Hexane	Ethyl acetate	Acetone	Methanol	Water
Average Total Mortality	2	3	3.5	3	0.5
%mortality	20.0	30.0	35	30	5.0
%knockdown	16.67	17.65	21.2	17.6	2.56
Control %knockdown	17.65	14.3	8.1	11.1	2.6

Table 6: Percentage mortality of extracts at different time intervals

Extract	Percent mortality at 24hr interval (%)			Percent mortality of Solvent controls		
	24hr	48hr	72hr	24hr	48hr	72hr
Ocimum basilicum						
Hexane	70	90	90	10	20	20
Ethylacetate	30	60	80	0	10	20
Acetone	10	30	50	0	20	30
Methanol	40	40	60	0	0	20
Water	30	40	80	20	30	30
Hyptis spicigera						
Hexane	10	40	40	0	20	20
Ethylacetate	40	60	80	0	20	40
Acetone	50	70	80	20	20	40
Methanol	10	10	20	0	20	20

Average No. of weevils = 10

DISCUSSION

The use of natural products from plant sources as drugs has persisted despite recent advances in synthetic organic chemistry due to the observation that they are generally safer and possess fewer side effects compared to their synthetic counterparts [9]. Similarly, natural insecticides may be less harmful to the environment compared to synthetic ones hence their use is encouraged. From the results of the phytochemical screening carried out, both plants were found to contain alkaloids, tannins, saponins and flavonoids among other constituents. The presence of alkaloids, saponins and flavonoids which have insecticidal properties could explain the use of these plants as insecticides in the northern part of Nigeria [10].

The pesticidal activity test results on the whole crude dried leave powdered samples of the two plants showed a minimal progressive increase in the percentage mortality and knockdown with increased storage time and concentration; (<50%) knockdown rates. This means that the two leaves have minimal pesticidal activity on cowpea weevils as against the potent effect claimed by the herbalists in the Upper Volta and in Northern Nigeria to protect cowpea against damage by weevils for a whole season [4].

The results of the bioassay for the two plants showed that they both have greater affinity for non- polar solvents (as seen with acetone) than the polar solvents (as seen with water). This could disprove the claim by the Hausas in the Northern Nigeria that the water macerate is the only effective extracts used to protect cowpea seeds against weevils [7].

Pesticidal potentials on cowpea weevils of both plants using percent mortality at different time intervals showed an increase in activity with increasing time with the highest effects seen with Hexane extract and least effect seen with acetone for *Ocimum basilicum* but for *Hyptis spicigera,* acetone gave the highest effects while water gave the least effects. The reason could be that the chemical constituents of both plants showed greater affinities for non-polar solvents than the polar solvents.

This can also be seen with the percent recovery of the plants extracts as it increases in the order- Water-methanol-Hexane -ethyl acetate- Acetone for *Ocimum basilicum* and Water-methanol-Hexane-ethyl acetate-Acetone respectively. From the foregoing, it can be said that the two plants contain more polar chemical constituents as compared to non-polar constituents.

Hexane extract showed the highest pesticidal activity in terms of percent mortality and percent knockdown values (80% and 66.67% respectively for 200mg extracts and 40% and 25% respectively for 100mg) extracts while acetone showed the least pesticidal activity (35% and 21.2% respectively for 200mg extracts and 10% and 5.26%respectively for 100mg extracts). The percent knockdown at the lower concentration (100%) was generally

low (<50%) while at higher concentration of 200mg, the percent knockdown was only >50% (high) for Hexane (66.67%).

CONCLUSION

From the analytical data obtained, it showed that pesticidal potential of *Ocimum basilicum* Linn. and *Hyptis spicigera* Lam. have a low knock down and mortality rate potency (within the experimental time limit used) against cowpea weevils (*Callosobruchus maculatus*). The potencies increase with time and concentrations of the plant extracts. Also, the chemical constituents that have greater affinities for non-polar solvent exhibited greater pesticidal activity for both plants.

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