Journal of Applied Pharmaceutical Science Vol. 3 (10), pp. 083-088, October, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.31014
ISSN 2231-3354 (cc) BY-NC-SA

Hyptis brevipes (Lamiaceae) Extracts Strongly Inhibit the Growth and Development of *Spodoptera littoralis* (Boisd.) Larvae (Lepidoptera: Noctuidae)

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ARTICLE INFO

Article history:
Received on: 10/06/2013
Revised on: 22/08/2013
Accepted on: 05/09/2013
Available online: 31/10/2013

Key words:
Hyptis brevipes, Lamiaceae, *Spodoptera littoralis*, growth disruption, flavonoids.

ABSTRACT

Hyptis brevipes (Lamiaceae) extracts are shown to exhibit strong insecticidal activity against the 3rd instar larva of the cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), inducing complete larval mortality due to the arrest and/or disruption of metamorphosis. This disruption induced a wide range of abnormalities. The LC_{50} value of the dichloromethane extract of *H. brevipes* was 3.0% (95% F.L. = 2.2% - 4.4%; slope = 3.185± 0.952) after three days of treatment. Two active compounds were isolated from the extract following bioassay-guided fractionation and were identified as 5-hydroxy-7,4'-dimethoxy-flavon-3-ol and 5-hydroxy-7-methoxy-2-(4`-methoxy-phenyl)-chromen-4-one based on spectroscopic data. This is the first report of these secondary metabolites in *H. brevipes*.

INTRODUCTION

Medicinal plants have been recognized as natural sources of remedies for the treatment of various diseases since antiquity, and they continue to play significant roles in primary health care around the world (Scorzoni *et al.,* 2007). Infectious diseases caused by bacteria, fungi, insects and arthropods are major threats to public health, despite the tremendous progress that has been made in the medicinal sciences (Cos *et al.,* 2006). Numerous synthetic acaricides and insecticides have been developed to address problems of these kinds caused by insects and mites but they are often expensive and some have negative effects on non-target organisms, including humans (Cetin *et al.,* 2010). As a result, there is a pressing need for readily-available, sustainable, cheap and environmentally friendly alternatives (Jaenson *et al.,* 2006). Moreover, the rising costs of treatments based on synthetic chemicals and the associated risks for humans have created interest in potentially more being natural products that can fulfil the same functions (Hostettmann, 1984). Natural products that are lethal to the intermediate hosts of insects are considered to be relatively safe and to possess fewer risks to the environment and health than synthetic insecticides (Su and Mulla, 1998; Panella *et al.,* 2005). We have previously tested the potential activity of plant extracts and the corresponding pure compounds using the larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Sakr and Abo-El-Mahasen, 2006). These larvae feed on a wide range of field crops, vegetables and ornamental plants, causing substantial economic damage (Hamshou *et al.,* 2010). We are therefore interested in identifying natural insecticides that are active against this species and could potentially be suitable for large scale applications.

The genus *Hyptis* comprises around 400 species, most of which are found in the Central States of Brazil. Its secondary metabolites are known for their medicinal utility and are used in folk medicine to treat conditions including skin diseases, gastric disorders and fever. Essential oils extracted from *H. savoeolens* and *H. spicigera* exhibit insecticidal and repellent activity against *Sitophilus granarius* adults (Conti *et al.,* 2011). In Indonesia, *H. brevipes* is used as a postpartum remedy in folk medicine (Roosita *et al.,* 2008).

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Moreover, both methylene chloride and methanol extracts of this species have antibacterial and antifungal activity (Goun *et al.*, 2003). However, the insecticidal activity of secondary metabolites from *H. brevipes* has not been investigated to date.

To address this deficiency, *H. brevipes* (Lamiaceae) was collected from Ecuador in the course of our ongoing chemical and pharmacological studies of medicinal plants (El-Seedi *et al.*, 2012a, 2012b). We therefore investigated the insecticidal activity of *H. brevipes* against 3rd instar *S. littoralis* (Boisd.) larvae (Lepidoptera: Noctuidae) with the aim of isolating, purifying and elucidating the structures of potential active compounds from the crude extract.

MATERIAL AND METHODS

Tested plant

The *H. brevipes* plant used in this work was collected in July 2003 from Ecuador and identified by Dr. Felipe Ghia (Botany Department, Reversa Forestal Endesa, Provincia del Prichincha, Ecuador).

Instruments and materials

Melting points were determined using a digital melting apparatus (Model 1A 8103, Electrothermal Engineering Ltd., Southend-on-Sea, Essex, U.K.) and are uncorrected. UV spectra were recorded with a Perkin-Elmer Lambda 2 UV/vis spectrophotometer. IR spectra were recorded with a Nicolet MX-S spectrophotometer.

General ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 600 spectrometer at 600.1 and at 150.9 MHz, respectively, at 25 °C using CD_3OD as the solvent. All chemical shifts are expressed relative to TMS.

TLC was performed on percolated aluminum sheets [silica 60 F_{254}, 0.25 mm (Merck, Darmstadt, Germany)] and preparative TLC on pre-coated glass sheets [silica 60 F_{254}, 0.5 mm (Merck)], with detection under UV light (254 and 366 nm) and by spraying with vanillin-sulfuric acid followed by heating to 120 °C.

Accelerating gradient chromatography (AGC)

According to our established protocol, AGC was performed using variable-length medium pressure liquid chromatography (MPLC) glass columns (Baeckström Separo AB, Lidingö, Sweden) with inner diameters of 4.0, 2.5, and 1.5 cm, packed with silica gel 60, 40-63 µm (Merck), and an FMI Lab pump (model QD, Fluid Metering Inc., Oyster Bay, NY) delivering a flow rate of 15-18 ml/min. Fractions of 20 ml were collected manually. Analytes were eluted from the columns by continuous gradient elution running from CH_2Cl_2 through EtOAc, and then MeOH, to H_2O. The eluent mixtures were generated using a SEPARO constant-volume mixing chamber with an open reservoir. The mixing chamber initially contained 100 ml of CH_2Cl_2 and the reservoir contained the first of 15-20 premixed binary gradient mixtures of gradually increasing polarity, each of 15-30 ml, which were successively fed into the reservoir during the separation (El-Seedi, 2007).

Extraction and isolation of *H. brevipes*

The aerial parts of *H. brevipes* (1.4 kg) were extracted with CH_2Cl_2 at room temperature three times with occasional stirring and then filtered. The extracts were combined and evaporated in vacuo to give 50.7 g of crude material. The crude extract was then adsorbed onto silica gel (120 g) and chromatographed on a silica gel (280 g) column, eluting continuously with continuous hexane-CH_2Cl_2, CH_2Cl_2-MeOH, to MeOH gradients. The eluted tubes from AGC columns were evaluated by TLC to give 22 main fractions. Fraction 7 (3.3 g) was purified by repeated AGC eluting with $CHCl_3$-MeOH (21:1) to ultimately yield 15 mg of compound 1 and 10 mg of compound 2.

Screening experiment

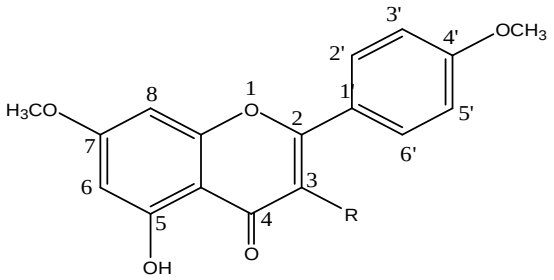
A portion of the crude dichloromethane extract was dissolved in a mixture of acetone and water (3:2 v/v) at a concentration of 5% (w/v) and tested against the 3rd larval instar of *S. littoralis*. Thirty pieces of castor bean leaves (with areas of 2 cm^2 each) were treated with 1 ml of plant extract. Leaves treated with solvent alone or water alone was used as positive and negative controls, respectively. The treated and control leaves were left to stand (at room temperature) to allow solvent evaporation and then offered to the tested larvae. These larvae (25 larvae per tested concentration and replicate) were fed on the treated leaves for three consecutive days, while control larvae were fed on leaves treated with the acetone: water mixture alone. Once the treated leaves had been consumed, the larvae were fed with fresh un-treated fresh castor bean leaves until pupation. All extract concentrations were tested in triplicate.

Insect model

A susceptible strain of the cotton leaf worm *S. littoralis* was obtained from the Agricultural Research Center (Dokki, Giza, Egypt) in February 2006. Larvae were kept in the laboratory at 28 ± 2 °C and 65 % R.H. under a 14 h: 10 h light: dark cycle. The *Spodoptera* larvae were fed on castor bean leaves (*Ricinus communis*) until pupation. Pupae were separated in jars covered with muslin and tied around the neck with rubber bands until adult emergence. The adult stage was fed on 10% sucrose solution.

Larvicidal activity

Complete larval mortality was observed for the crude *H. brevipes* extract, whereas the mortality for negative control larvae was only 10%. Consequently, the activity of the extract against the 3rd larval instar of *S. littoralis* was tested at a range of different concentrations. In these experiments, the crude CH_2Cl_2 extract was redissolved in pure acetone when making up the solutions used to treat the leaves. LC_{50} values were calculated according to Finney (Finney, 1971) and reductions in adult emergence were calculated according to Khazanie (Khazanie, 1979).



1 R=OH
2 R=H

When, R= OH= 5-hydroxy-7,4'-dimethoxy-flavon-3-ol and 5-hydroxy-7-methoxy-2-(4`-methoxy-phenyl)-chromen-4-one, when R= H

RESULTS AND DISCUSSION

A preliminary screening experiment was used to evaluate the potential larvicidal activity of the *H. brevipes* extract used in this work. The CH_2Cl_2 extract induced 100% larval mortality when tested against 3rd instar *S. littoralis* larvae at a concentration of 5% using the feeding method (Table 1). Based on this result, the compounds within the extract were separated and characterized using AGC. This led to the isolation and identification of two flavonoid derivatives: 5-hydroxy-7,4'-dimethoxy-flavon-3-ol (1) and 5-hydroxy-7,4'-dimethoxy-flavone (2). 5-Hydroxy-7,4'-dimethoxy-flavon-3-ol (1) was obtained as amorphous solid whose HRFABMS (positive ion mode) mass spectrum contains a molecular ion peak at *m/z* 299 $[M+H]^+$ corresponding to the molecular formula $C_{17}H_{14}O_6$. Its structure was further confirmed by its ^1H NMR ($CDCl_3$) spectrum, in which *m*-coupling is apparent between the peaks at δ 6.48 ppm (1H, *d*, *J*=1.1 Hz, H-6), δ 6.65 ppm (1H, *d*, *J*=1.3 Hz, H-8), *p*-substitution is indicated by the peaks at δ 7.07 ppm (2H, *d*, *J*=5.9 Hz, H-3`, H-5') and δ 7.79 ppm (2H, *d*, *J*=6.2 Hz, H-2`, H-6`), and the presence of methoxy groups is indicated by peaks at δ 3.9 ppm (C-4`) and 4.1 ppm (C-7). Moreover, the ^{13}C NMR data for this species are consistent with those reported by Harborne and Mabry (1982). 5-Hydroxy-7,4'-dimethoxy-flavon (2) was obtained as amorphous solid whose HRFABMS (positive ion mode) mass spectrum contains a molecular ion peak at *m/z* 299 $[M+H]^+$ corresponding to the molecular formula $C_{17}H_{14}O_5$. Its ^1H NMR ($CDCl_3$) spectrum contains peaks corresponding to *m*-coupled aryl protons at δ 6.59 ppm (1H, *s*, H-3), δ 6.38 ppm (1H, *d*, *J*=1.8 Hz, H-6), and δ 6.49 ppm (1H, *d*, *J*=2.2 Hz, H-8); a *p*-substituted aryl ring with protons at δ 7.02 ppm (2H, *d*, *J*=5.6 Hz, H-3', H-5') and δ 7.85 ppm (2H, *d*, *J*=6.7 Hz, H-2`, H-6'); and methoxy groups at δ 3.9 ppm (C-4`), 4.1 ppm (C-7). The ^{13}C NMR data for this compound are consistent with those reported by Harborne and Mabry (1982). A literature search revealed that *Hyptis* species are known to produce a wide range of terpenoids, flavonoids and pyrones (Piozzi et al., 2009). It is well known that this genus produces secondary metabolites with diverse biological effects, including a novel trypsin inhibitor isolated from the seeds of *H. suaveolens* that exhibits potent and specific activity against specific trypsin-like enzymes from insects (Aguirre et al., 2004). Deng (2010) performed a cytotoxicity-guided fractionation of a *H. brevipes* extract using the MCF-7 human breast cancer cell line that led to the isolation of six new 5,6-dihydro-α-pyrone derivatives and one known analog, as well as six other known compounds. We also tested the insecticidal activity of the CH_2Cl_2 *H. brevipes* extract against 3rd instar *S. littoralis* larvae at different concentrations. The extract's LC_{50} value was 3.0 % (95% F.L. = 2.2 % - 4.4 %; slope = 3.185 ± 0.952) for three day treatments (Table 1). For all tested concentrations, non-zero mortality was observed within 24 h of the extract's application and mortality increased substantially over a three day period (Table 1). The extract severely inhibited the development of the treated larvae relative to controls in a dose-dependent fashion, ultimately causing death in all cases. Larvae treated with the extract had difficulty in shedding their cuticles and died during ecdysis (Fig. 1-5). Most larvae treated with the CH_2Cl_2 extract showed signs of incomplete moulting and became unable to feed normally. These larvae initiated apolysis but failed to achieve ecdysis, resulting in death. These results clearly indicate that the *H. brevipes* extract is toxic towards *S. littoralis* larvae. This activity may derive from the flavonoids, terpenoids and pyrones. Insect enzymes play important roles in the degradation and resistance to some insecticidal compounds. Sousa et al. (1993) reported that the gut enzymes of *S. littoralis* larvae (proteases) were able to digest the seed protein entrolobin before it could exert any toxic effect, whereas proteases from *Callosobruchus maculates* were unable to hydrolyse entrolobin. The glutathione S-transferase (GST) variant expressed by *Tenebrio molitor* offers passive protection against pyrethroid insecticides by sequestering them and thereby inhibiting their action (Kostaropoulos et al., 2001). Compounds in the ethanolic extract of *H. suaveolens* bind competitively to both the active and hydrophobic sites of the glutathione S-transferase of *C. Maculates* (Kolawole et al., 2011). It is possible that similar mechanisms are responsible for the toxicity of *H. brevipes* extract towards *S. littoralis* larvae. Numerous publications have described the deleterious effects of various plant extracts on insects. At a concentration of 9%, the CH_2Cl_2 extract of *Artemisia monosperma* CH_2Cl_2 decreased pupation and adult emergence in *S. littoralis* by 83.3% and 85.1%, respectively, when administered by feeding (Sakr and Abo-Elmahasen, 2006). Similarly, a methanolic extract of *Chrysanthemum macrotum* caused 100% larval mortality when administered to the 3rd larval instar of *S. littoralis*at 10 mg/g (Haouas et al., 2010). Moreover, the essential oil and ethanolic extracts of *H. suaveolens* have been shown to exhibit insecticidal activity against *Sitophilus granarius* (Conti et al., 2011) and *C. maculates* (Kolawole et al., 2011), respectively. The metamorphic changes induced by the *H. brevipes* extract as observed in this work may be due to the effects of the plant's secondary metabolites on the nervous system of the tested larvae. This would be consistent with the findings of authors such as Koolman *et al.* (1988), Younes et al. (1998), Shoukry et al. (2003), Sakr (2007) and Sahayaraj et al. (2011). The antifeeding activity observed in this work resembles that observed for the essential oil of *H. suaveolens* against *S. litura* (Raja et al., 2005), for the

Table. 1: Latent effects of the *H. brevipes* CH_2Cl_2 extract against the third larval instar of *S. littoralis* (feeding method) when applied over three days.

Concentration(%)	% Larval mortality					*%Pupation	*% Adult emergence	
	1st day	2nd day	3rd day	4th day	Total		Total	+Reduction (%)
5	50	50	80	100	100	0	0	100
4	40	40	60	100	100	0	0	100
3	10	30	50	70	100	0	0	100
2	10	30	40	60	100	0	0	100
0	10	10	10	10	10	90	90	0

LC_{50} (%)	95% confidence intervals (%)		Slope ±SE
3.0 %	**Lower**	**Upper**	**3.185±0.952**
	2.2	**4.4**	

*Relative to the number of larvae.

+ Reduction %= (C-T/C) X 100 [Khazanie, 1979]; where C = number of emerged adults in control and T = number of emerged adults after treatment.

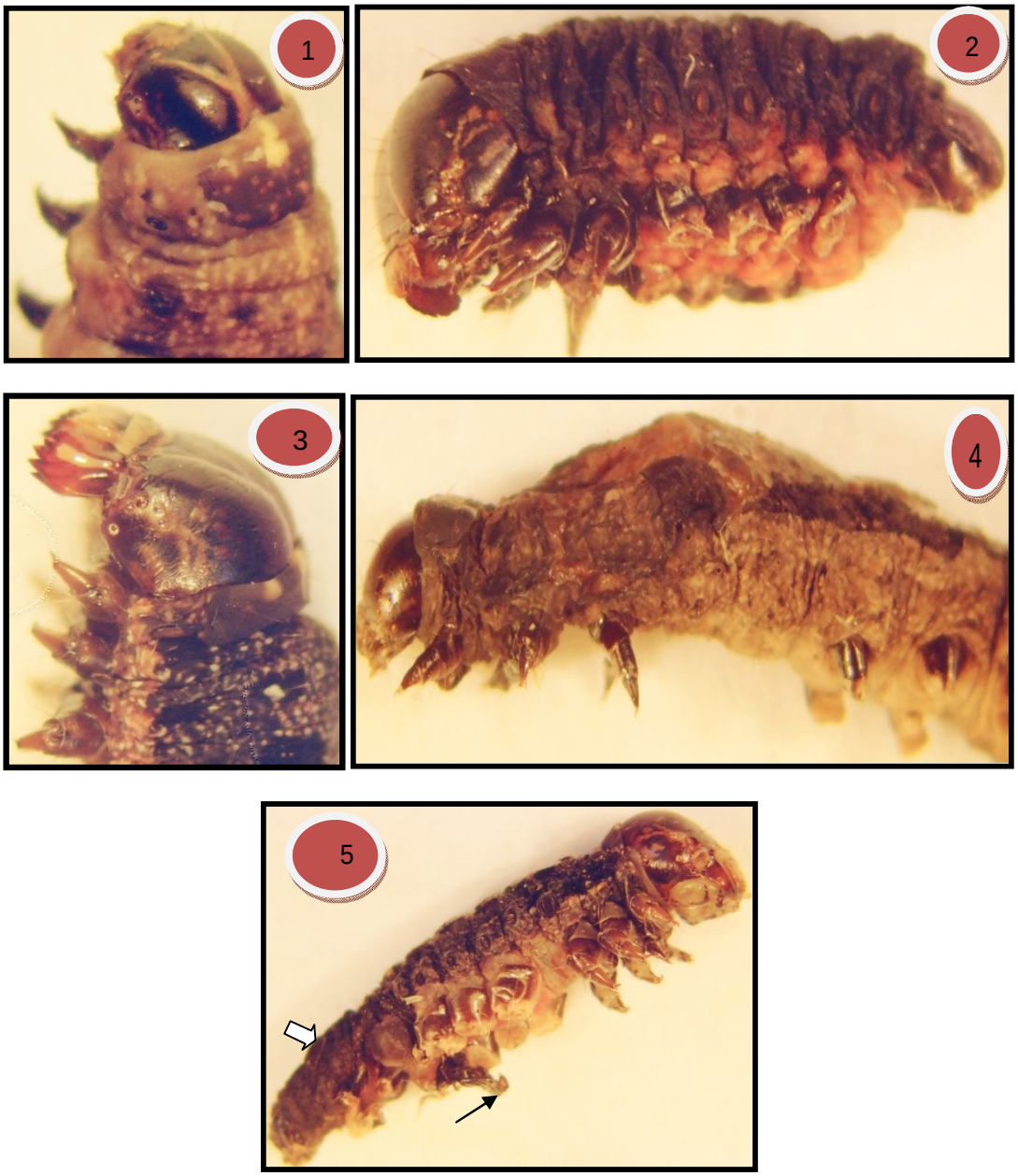


Fig. 1-5: Morphological changes induced by the crude *H. brevipes* extract. 1- Dorsal view of the head and thorax of a control *S. littoralis* larva showing normal mouth parts. 2- Lateral view of a treated larva that completely failed to shed its old cuticle, resulting in shrinkage and finally death. 3- Lateral view of the non-functional mouth parts in the ecdysis-inhibited treated larva. 4-5: Lateral view of treated larvae, showing the partial moulting that preceded death in some cases. 4- Moulting occurred only on the dorsal side of this treated larva. 5- A larva with a partially-shed old cuticle and a deformed head and abdominal legs (indicated by the dark arrow) while other body parts remain encased in the old cuticle (white arrow).

furocoumarin and rutamine compounds isolated from ethanol extracts of *Ruta chalepensis* against *S. littoralis* (Megally et al., 2009) and the ethyl acetate extract of *Pergularia daemia* against *Helicoverpa armigera* and *S. litura* (Pavunrai et al., 2011).

CONCLUSION

The extracts of *H. brevipes* (Lamiaceae) displayed insecticidal activity against the 3rd instar larva of the cotton leaf worm *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae). *H. brevipes* hasnot been investigated chemically or pharmacologically before. The CH_2Cl_2 extract showed activity with LC_{50} 3.0% (95% F.L. = 2.2% - 4.4%; slope =3.185± 0.952) after three days of treatment. Bio-assay guided isolation strategy leads to identification of two bioactive flavonoids named; 5-hydroxy-7,4'-dimethoxy-flavon-3-ol and 5-hydroxy-7-methoxy-2-(4`-methoxy-phenyl)-chromen-4-one.

ACKNOWLEDGMENTS

We are grateful for financial support in the form of a Grant in Aid for Scientific Research [2007-6738] awarded to H.R.E., A.-K.B.-K. and U.G. under the auspices of the Swedish Research Links program operated by the Swedish International Development Agency – Middle East and North Africa [SIDA-MENA]. H.R.E. thanks the Chemistry Department of El-Menoufia University for granting him leave to visit Uppsala University on several occasions. U.G. is supported by the Swedish Foundation for Strategic Research and the Swedish Research Council.

REFERENCES

Aguirre C, Valdes-Rodriguez S, Mendoza-Hernandez G, Rojo-Dominguez A, Blanco-Labra A. A novel 8.7 kDa protease inhibitor from chan seeds (*Hyptis suaveolens* L.) inhibits proteases from the larger grain borer *Prostephanus truncatus* (Coleoptera: Bostrichidae). Comp Biochem Physiol Part B, 2004; 138:81-89.

Cetin H, Cilek JE, Oz E, Aydin L, Deveci O, Yanikoglu A. Acaricidal activity of *Satureja thymbra* L. essential oil and its major components, carvacrol and gamma-terpinene against adult *Hyalomma marginatum* (Acari: Ixodidae). Vet Parasitol, 2010; 170:287-290.

Conti B, Canale A, Cioni PL, Flamini G, Rifici A. *Hyptis suaveolens* and *Hyptis spicigera* (Lamiaceae) essential oils: Qualitative analysis, contact toxicity and repellent activity against *Sitophilus granarius* (L.) (Coleoptera: Dryophthoridae). J Pest Sci, 2011; 84:219-228.

Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. J Ethnopharmacol, 2006; 106:290-302.

Deng Y. Bioactive constituents of two medicinal plants from Indonesia. Ph.D. Thesis in Pharmacy ,Ohio State University ,USA. 2010.

El-Seedi HR, El-Shabasy RM, Sakr H, Zayed M, El-Said A, Helmy KMH. Gaara AHM, Turki Z, Azeem M, Ahmed MA, Boulos L, Borg-Karlson A-K, Göransson U. Anti-schistosomiasis triterpene glycoside from the Egyptian medicinal plant *Asparagus stipularis*. Braz J Pharmacogn, 2012a; 22:314-318.

El-Seedi HR, Khalil NS, Azeem M, Taher E, Göransson U, Pålsson K, Borg-Karlson AK. Chemical composition and repellency of essential oils from some Egyptian plants against *Ixodes ricinus* (L.) nymphs (Acari: Ixodidae). J Med Entomol, 2012b; 49:1067-1075.

El-Seedi HR. Antimicrobial arylcoumarins from *Asphodelus microcarpus*. J Nat Prod, 2007; 70:118-120.

Finney DJ. 1971. Probit Analysis 3rd ed. Cambridge University Press: New York.

Goun E, Cunningham G, Chu D, Nguyen C, Miles D. Antibacterial and antifungal activity of Indonesian ethnomedical plants. Fitotrapia, 2003; 74:592-596.

Hamshou M, Van-Mamme JM, Smagghe G. Entomotoxic effects of fungal lectin from *Rhizoctonia solani* towards *Spodoptera littoralis*. Fungal Biol, 2010; 114:34-40.

Haouas D, Flamini G, Halima-Kamel MB, Hamouda MHB. Feeding perturbation and toxic activity of five *Chrysanthemum* species crude extracts against *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Crop Protec, 2010; 29:992-997.

Harborne JB, Mabry TJ. 1982. The flavonoids advances in research p. 101. Great Britain at the University of Cambridge.

Hostettmann K. On the use of plants and plant-derived compounds for the control of schistosomiasis. Naturwissenschaften, 1984; 71:247-251.

Jaenson TGT, Garboui S, Pålsson K. Repellency of oils of lemon eucalyptus, geranium, and lavender and the mosquito repellent Mygg A natural to *Ixodes ricinus (*Acari: ixodidae) in the laboratory and field. J Med Entomol, 2006; 43:731-736.

Khazanie R. 1979. Elementary statistics. Good year publishing Co.: California, USA, P. 488.

Kolawole AO, Okonji RE, Ajele JO. *Tithnia diversifolia*, *Cyperus rotundus* and *Hyptis suaveloensis* ethanol extracts combinatorially and competitively inhibit affinity purified cowpea storage bruchid (*Callosobrochus maculatus*) glutathione S-transferase. Arthropod-Plant Interact, 2011; 5:175-184.

Koolman J, Bidmon HJ, Lehmann M, Kauser G. 1988. In: Endocrinological frontier in physiological insect ecology (Sehnah F; ZabA; Denlinger DL eds), 1: 55.Wroclaw Technical Univ. Press, Wroclaw.

Kostaropoulos I, Papadopoulos AI, Mexataxakis A, Boukouvala E, Papadopoulou-Mourkidou E. Glutathione S-transferase in the defence against pyrethroids in insects. Insect Biochem. Mol Biol, 2001; 31:313-319.

Megally NY, Eman AM, Swelam ES. Furocoumarin and quinolone alkaloid with larvicidal and antifeedant activities isolated from *Ruta chalepensis* leaves. J Nat Prod, 2009; 2:10-22.

Panella NA, Dolan MC, Karchesy JJ, Xiong Y, Peralta-Cruz J, Khasawneh M, Montenieri JA, Maupin GO. Use of novel compounds for pest control: insecticidal and acaricidal activity of essential oil components from heartwood of Alaska yellow cedar. J Med Entomol, 2005; 42:352-358.

Pavunrai M, Muthu C, Ignacimuthu S, Janarthanan S, Duraipandiyan V, Raja N, Vimalraj S. Antifeedant activity of a novel 6-(4,7-hydroxy-heptyl) quinone from the leaves of the milkweed *Pergularia daemia* on the cotton bollworm *Helicoverpa armigera* (Hub.) and the tobacco armyworm *Spodoptera litura* (Fab.). Phytoparasitica. 2011; 39:145-150.

Piozzi F, Bruno M, Rosselli S, Maggio A. The diterpenoids from the genus *Hyptis* (Lamiaceae). *Heterocycles*, 2009; 78:1413-1426.

Raja N, Jeyasankar A, Venkatesan SJ, Ignacimuthu S. Efficacy of *Hyptis suaveolens* against lepidopteran pests. Cur Sci, 2005; 88:220-222.

Roosita K, Kusharto CM, Sekiyama M, Fachrurozi Y, Ohtsuka R. Medicinal plants used by villagers of a Sundanese community in West Java, Indonesia. J Ethnopharmacol, 2008; 115:72-81.

Sahayaraj K, Kombiah P, Siva KD. Evaluation of insecticidal activity of fermented plant products on *Spodoptera litura* (Fab.). Indian J Agr Res, 2011; 45:77-82.

Sakr HH, Abo-El-Mahasen M. Biological and biochemical studies on *Spodoptera littoralis* (Biosduval) (Lepidoptera: Noctuidae) treated with *Artemisia monosperma* (Compositae) extracts. Proc 4th Int Con Biol Sci (Zool), 2006; 359-366.

Sakr HH. Toxicity of *Streptomyces lavendulae* (Streptomycetaceae) culture filtrate to *Spodoptera littoralis* (Boisd.) larvae (Lepidoptera: Noctuidae). J Exp Biol Zool, 2007; 3:197-202.

Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VS, Gianinni MJSM. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida sp* and *Cryptococcus sp*. Braz J Microbiol, 2007; 38:391-397.

Shoukry I, Abdel Fatah A, Hussein KT, Khater KS. Toxicological evaluation of some botanical oils on biological aspects in the Indian meal moth *Polodia interpunctella* HB (Lepidoptera: Pyralidae). Egyptian J Biology, 2003; 5:155-163.

Sousa MV, Morhy L, Richardson M, Hilder VA, Gatehouse AMR. Effects of the cytolytic seed protein enterolobin on coleopteran and lepidopteran insect larvae. Entomol Exp Appl, 1993; 69:231-238.

Su T, Mulla MS. Ovicidal activity of neem products *(azadirachtin)* against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J Am Mosq Control Assoc, 1998; 14:204-209.

Younes MWF, Ryan MF, Asser AA, Sakr HH. Effects of ethanolic extracts of various plant species on the growth and development of *Tribolium castaneum* (Herbst) larvae (Coleoptera: Tenebrionidae). J Egypt Ger Soc Zool, 1998; 26:135-149.

How to cite this article:

Hanem H. Sakr, Shimaa H. Roshdy and Hesham R. El-Seedi. *Hyptis brevipes* (Lamiaceae) Extracts Strongly Inhibit the Growth and Development of *Spodoptera littoralis* (Boisd.) Larvae (Lepidoptera: Noctuidae). J App Pharm Sci, 2013; 3 (10): 083-088.