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Insecticidal and Antifungal Activities of Crude Extracts and Pure Compounds from Rhizomes of *Curcuma longa* L. (Zingiberaceae)

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ABSTRACT

The insecticidal and antifungal activities of *Curcuma longa* L. rhizome extracts and two isolated compounds, namely, ar-turmerone and curcumin, were evaluated. Rhizomes of *C. longa* were extracted with n-hexane, methylene chloride, methanol and water, successively. Hexane, methylene chloride, and methanol extracts exhibited remarkable insecticidal activity against the larvae of *Culex pipiens*. The LC_{50} values were 5.28, 5.82, and 6.44 mg L^{-1}, respectively, after 48 h. In contrary, the extracts exhibited weak toxic effect on the third instar larvae of *Spodoptera littoralis*. The LC_{50} values of water, methylene chloride, and hexane extracts were 495.9, 565.7 and 709.7 mg L^{-1}, respectively after 48 hours. On the other hand, the extracts showed variable antifungal activity against plant pathogenic fungi, *Fusarium oxysporum*, *Pythium debaryanum*, *Phytophthora infestans*, *Fusarium solani* and *Alternaria alternata*. Methanol extract had the highest antifungal activity among the tested extracts with EC_{50} values of 159.8, 242.7, and 322.2 mg L^{-1} on *P. infestans*, *F. solani* and *A. alternata*, respectively. Two compounds, namely, ar-turmerone and curcumin were isolated from methylene chloride/methanol (1:1) extract of *C. longa* rhizomes and their chemical structures were identified by using spectroscopic analysis. Ar-Turmerone had moderate toxicity against *C. pipiens* larvae. The LC_{50} values were 158.5 and 117.6 mg L^{-1} after 24 and 48 hours, respectively. In addition, ar-turmerone showed moderate antifungal activity against *P. infestans* (EC_{50}= 588.9 mg L^{-1}) and weak activity against *F. solani* (EC_{50}= 820.6 mg L^{-1}). Curcumin caused 51.1 and 54.32% growth inhibition of *F. oxysporum* and *P. infestans* at 250 mg L^{-1}, respectively.

Keywords: Ar-Turmerone, Curcumin, Pest control, Turmeric.

INTRODUCTION

Higher plants are a rich source of natural compounds that can be used effectively in pest control. Insecticidal, herbicidal and fungicidal activities of many plants against several pests have been demonstrated (Isman, 2006; Dyan *et al.*, 2009; Boulenouar *et al.*, 2014; Kheradmand *et al.*, 2015). Public concern over use of synthetic pesticides is growing. This has led to the great growth in organic agriculture in which botanicals play an important role in pest control. Botanicals have certain advantages, such as rapid degradation, lack of persistence and bioaccumulation in the environment, and low mammalian toxicity (Cantrell *et al*., 2012).

Curcuma longa L., Zingiberaceae, develops to a height of 3 to 5 feet and it is widely cropped in India, China, and many countries with a tropical weather. Rhizome is the medicinal part of plant, which is boiled, cleaned, and dried, producing a yellow

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powder. Dried *C. longa* is the source of the spice turmeric, the ingredient that gives curry powder its characteristic yellow color. Turmeric is used extensively in foods for its flavor and color, as well as having a long tradition of use in the Chinese and Indian medicine. Current medicinal uses of turmeric include biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis (Akram *et al*., 2010).

The Egyptian cotton leafworm *Spodoptera littoralis* Boisd. is one of the most series agricultural lepidopterous pests of cultivated crops foremost in tropical and subtropical countries (Bakr *et al.*, 2013). *Culex pipiens* L. is a vector of *West Nile virus* and an important pest to humans, causing allergic responses that include local skin reaction and systemic reactions, such as angioedema, and urticaria (Cheng *et al.*, 2008). Plant pathogenic fungi cause significant pre-harvest and post-harvest loss in crop production. It is estimated that the world crop loss due to plant diseases may amount to 14%. Plant pathogenic fungi share 40-60% of total plant diseases loss (Mahy and van Regenmortel, 2009).

The insecticidal activity of *C. longa* extracts against stored product insects (Matter *et al*., 2008; Abida *et al*., 2010) and Lepidopteran insects (Tavares *et al*., 2013) have been described. In addition, *C. longa* oil, extracts, and pure compounds have been shown to have insecticidal activity against mosquitoes, such as *Aedes aegyptii*, *A. albopictus* and *C. pipiens* (Zhu *et al*., 2008; Kalaivani *et al*., 2012; Sagnou *et al*., 2012). However, there were no reported studies on the insecticidal activity of *C. longa* extracts against *C. pipiens* and *S. littoralis*, except hexane extract against *C. pipiens* (Prak *et al.*, 2014). Therefore, the present study aimed to describe the insecticidal activity of hexane, methylene chloride, methanol, and water extracts of *C. longa* against *C. pipiens* and *S. littoralis*, and the antifungal activity of these extracts on five plant pathogenic fungi, namely, *Fusarium oxysporum*, *Pythium debaryanum*, *Phytophthora infestans*, *Fusarium solani*, and *Alternaria alternata*. Moreover, the isolation, identification and bioactivity of two active compounds [ar-turmerone (1) and curcumin (2)] were to be studied.

MATERIALS AND METHODS

Plant Materials

Rhizomes of *Curcuma longa* were purchased from Asala–mady Company, El-Mansheya, Alexandria, Egypt.

Culex pipiens

Culex pipiens L. (Diptera: Culicidae) colony maintained in the laboratory of Mosquito Bioassay, Department of Economic Entomology, for more than 10 years was used in this study. Mosquitoes were held at 27±1°C, 70±5% RH, and a photoperiod regime of 14:10 hours (light/dark). Adults were provided with a 10% sucrose solution as a food source. A pigeon was introduced twice per week for adult blood feeding. Larvae were reared in de-chlorinated water under the same temperature and light conditions and were fed daily with baby fish food.

Spodoptera littoralis

A laboratory strain of *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) was obtained from the Bioassay Laboratory, Faculty of Agriculture, Alexandria University. The colony was reared under laboratory conditions on castor bean leaves, *Ricinus communis* L. (Euphorbiaceae), at 26±2°C and 70±5% RH (El-Defrawi *et al*., 1964).

Fungi

Five phytopathogenic fungi species, *Fusarium oxysporum* (Schltdl.) isolated from *Zea mays* seeds, *Pythium debaryanum* (R. Hesse) isolated from *Cucumis sativus*, *Phytophthora infestans*

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(Mont. de Bary) isolated from *Solanum lycopersicum fruits*, *F. solani* (Mart. Sacc.) isolated from tubers of *Solanum tuberosum*, and *Alternaria alternata* (Fr. Keissl) isolated from *Phaesolus vulgaris* leaves were used in this study. The fungi were obtained from the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University. The fungi were maintained during the course of the experiments on Potato Dextrose Agar Medium (PDA: Potato 200, Dextrose 20, and Agar 15 g L^{-1} in distilled water) at 25 °C.

Bioassay of *C. longa* Extracts against *C. pipiens*

The World Health Organization (WHO) standard test method for mosquito larvae (Anonymous, 1996) was used to evaluate the insecticidal activities of *C. longa* crude extracts against the 4th instar larvae of mosquito (*C. pipiens*). The extracts were first prepared in acetone. Appropriate volumes of extracts stock solutions were added to 100 mL of distilled water containing 20 larvae in 200-mL glass cups. The extracts were tested at final concentrations of 1, 5, 10, 30, 50, 100, 250, 500 mg L^{-1}. The control was prepared with distilled water containing the same amount of acetone. Three replicates were used in each concentration and control. Malathion 95% (Kafr Elzayat Pesticides and Chemicals Co., Egypt) was used as a reference insecticide. The mortality percentages were recorded after 24 and 48 hours of treatment. Mortality data were subjected to Probit analysis to calculate the Lethal Concentration values (LC_{50}) of extracts and isolated compounds (Finney, 1971).

Bioassay of *C. longa* Extracts against *S. littoralis*

Insecticidal activity of *C. longa* extracts was tested by a residual film method (Ascher and Nissim, 1965) on the third instar larvae of *S. littoralis*. Leaf disks (1.8 cm diameter) of castor bean were immersed in acetone solutions of the crude extracts for 5 seconds. The leaf disks were left for 3 minutes to allow solvent evaporation. Then, five disks were placed in each Petri dish with 10 larvae of *S. littoralis*. The crude extracts were tested at concentrations of 5, 10, 50, 100, 500, 1,000 mg L^{-1}. Three replicates were carried out for each concentration and control (leaf disks were immersed in acetone only). The experiment was kept at room temperature (25°C). The mortality percentages were recorded after 24 and 48 hours and LC_{50} values were calculated as previously described.

Bioassay of *C. longa* Extracts against Fungi

The antifungal activity of *C. longa* extracts was tested by using radial growth inhibition technique (Zambonelli *et al.*, 1996). Appropriate volumes of the stock solutions of the extracts in dimethyl sulfoxide (DMSO) were added to PDA medium immediately before it was poured into the Petri dishes (9.0 cm diameter) at 40-45ºC to obtain a series of concentrations (25, 50, 100, 200, 300, 400 and 500 mg L^{-1}). Each concentration was tested in triplicate. Parallel controls were maintained with DMSO mixed with PDA. Carbendazim (Kafr El-zayat Pesticides and Chemicals Co., Egypt) was used as a reference fungicide. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the center of Petri dishes. The treatments were incubated at 27ºC in the dark. The colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated from the following formula:

$$Mycelial\ growth\ inhibition = [(DC-DT)/DC] \times 100 \quad (1)$$

Where, DC and *DT* are average Diameters of fungal colony of the Control and Treatment, respectively. The concentration of the extract that inhibited the fungi mycelial growth by

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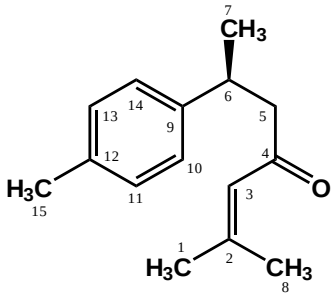
50% (EC_{50}) was determined by a linear regression method (Finney, 1971).

Isolation of Compounds from *C. longa* Rhizomes

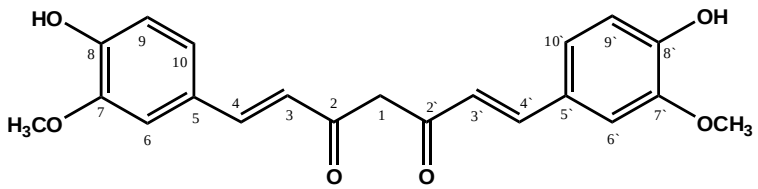
Rhizome powder (1.5 kg) was extracted with 4 liters of solvent mixture (methylene chloride-methanol, 1:1) for 14 days at room temperature. The extract was concentrated under reduced pressure to give 123.7 g of crude extract. The crude extract (15 g) was subjected to column chromatography packed with 500 g silica gel using ethyl acetate/hexane solvent system starting with 30%, followed by 40, 60, and 80% ethyl acetate/hexane and finally with ethyl acetate. Fifty fractions (each one 100 mL) were collected. Fractions 1 to 5 from the first solvent system (30% ethyl acetate/hexane) gave 2.5 g of oily compound (1) (Figure 1). Fractions 1 to 4 from the second solvent system (40% ethyl acetate/hexane) were combined to give 4 g crude extract that were chromatographed on 150 g silica gel column chromatography with methanol/methylene chloride solvent system. The solvent systems were 1, 2.5, and 5% and methanol (100%). Fourteen fractions of 50 mL were collected. Fractions 2 to 7 were combined to give 2 g. These 2 g were further purified on silica gel column chromatography using 1 and 2.5% methanol/methylene chloride, and methanol, respectively. The fractions collected from the first solvent system were further purified on column chromatography with acetone/hexane solvent system to give 50 fractions. The collected fractions from 50% acetone/hexane solvent system (920 mg) were chromatographed on silica gel with 1, 2.5, 5, and 10% methanol/methylene chloride followed by methanol. Fractions of 5% (390 mg) solvent system were repeatedly subjected to Preparative Thin Layer Chromatography (PTLC) with 10% methanol/methylene chloride to give 45 mg of compounds (2) as amorphous powder.

Insecticidal and Antifungal Activity of Ar-Turmerone (1) and Curcumin (2)

The insecticidal activity of ar-turmerone (1) was evaluated against the 4th instar larvae of *C. pipiens* at concentrations of 50, 100, 250, and



ar-Turmerone (1)



Curcumin (2)

Figure 1. Chemical structure of isolated compounds **(1)** and **(2)** from *Curcuma longa*.

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500 mg L^{-1}. This compound was tested for its fungicidal activity against fungi at concentrations of 5, 10, 25, 50, 100, 250, 500, 750, and 1,000 mg L^{-1}, as previously described. The antifungal activity of curcumin (2) was tested against *F. oxysporum* and *P. infestans* at 250 mg L^{-1} only, due to small amount isolated from this compound.

Statistical Analysis

The insect mortality of each concentration was calculated after 24 and 48 h of treatment as the mean of three replicates. The insect mortality and fungal growth inhibition percentages were subjected to probit analysis (Finney, 1971) to obtain the LC_{50} and EC_{50} values, using SPSS 12.0 (SPSS, Chicago, IL, USA). The values of LC_{50} and EC_{50} were considered significantly different if the 95% confidence limits did not overlap.

RESULTS AND DISCUSSION

Insecticidal Activity of *C. longa* Extracts against *C. pipiens* Larvae

The toxicity of the four crude extracts of *C. longa* was evaluated against the fourth instar larvae of *C. pipiens*. Values of LC_{50}, 95% confidence limits and other parameters generated from regression lines are shown in Table 1. After 24 hours of treatment, the extracts of hexane, methylene chloride, and methanol exhibited remarkable insecticidal activity against the larvae of *C. pipiens* without significant differences among the extracts, as their 95% confidence limits were overlapped. The LC_{50} values of the hexane, methylene chloride, and methanol extracts were 12.87, 17.81, and 23.63 mg L^{-1}, respectively. Water extract revealed the lowest insecticidal activity among the tested extracts after 24 hours of treatment. The insecticidal activity of extracts was significantly increased after 48 hours of treatment. Hexane, methylene chloride, and methanol extracts showed potent insecticidal activity without significant differences among them, where their LC_{50} values were 5.28, 5.82, and 6.44 mg L^{-1}, respectively, while the water extract (LC_{50}= 17.40 mg L^{-1}) gave the least insecticidal activity. All tested *C. longa* extracts were less toxic to the larvae than a reference insecticide, Malathion.

The oil, oil fractions, hexane extract, and pure isolated compounds from *C. longa* have been described to possess insecticidal activity against mosquitoes, such as *Aedes*

Table 1. Comparative toxicity of *Curcuma longa* extracts against the fourth instar larvae of *Culex pipiens* after 24 and 48 hours of treatment.

Extracts	Exposure time (h)	LC_{50} [a] (mg L^{-1})	95% Confidence limits (mg L^{-1})		Slope ±*SE* [b]	Intercept ±*SE* [c]	(x^2) [d]
			Lower	Upper			
Hexane	24	12.87	7.92	19.56	1.35±0.08	-1.50±0.12	19.51
	48	5.28	1.73	10.65	1.07±0.08	-0.78±0.10	34.58
Methylene chloride	24	17.81	9.81	29.99	1.21±0.08	-1.51±0.12	26.49
	48	5.82	2.51	10.45	1.004±0.07	-0.77±0.10	21.62
Methanol	24	23.63	12.72	41.66	0.99±0.07	-1.36±0.11	23.11
	48	6.44	2.63	11.94	0.88±0.07	-0.71±0.10	19.83
Water	24	44.76	35.03	57.54	0.90±0.07	- 1.49±0.12	2.40
	48	17.40	12.94	22.88	0.80±0.06	-0.99±0.10	8.74
Malathion	24	2.2×10^{-3}	1.8×10^{-3}	2.7×10^{-3}	2.03±0.20	5.38±0.57	0.73
	48	1.6×10^{-3}	1.4×10^{-3}	1.9×10^{-3}	1.90±0.20	5.31±0.55	1.66

[a] The concentration causing 50% mortality. [b] Slope of the concentration-mortality regression line±Standard Error. [c] Intercept of the regression line±Standard Error. [d] *Chi* square value.

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aegyptii, *A. albopictus* and *C. pipiens* (Roth *et al*., 1998; Zhu *et al*., 2008; Kalaivani *et al*., 2012; Sagnou *et al*., 2012). In addition, the *C. longa* extracts were found to be toxic against stored product insects *Tribolium castaneum* (Abida *et al*., 2010) and *Rhyzopertha dominica* (Matter *et al*., 2008). The repellent activity of *C. longa* extracts on *T. castaneum*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *Sitophilus oryzae*, and *Corcyra cephalonica* was observed (Chander *et al*., 1992 and 2000).

Based on the LC_{50} values of the tested extracts on the larvae of *C. pipines*, the insecticidal activity of the tested extracts were more potent than those of *Acacia nilotica*, *Cassia senna*, *Calotropis procera*, *Ambrorsa maritima*, *Achillea santolina* and *Adhatoda vasica* extracts (Abdelgaleil, 2010; Zaitoun *et al*., 2012). On the other hand, the toxicity of tested extracts was similar to those of petroleum ether extracts of *Echinochloa stagninum* (Bream *et al*., 2010).

Insecticidal Activity of *C. longa* Extracts against *S. littoralis*

The results of insecticidal activity of *C. longa* different extracts on the third instar larvae of *S. littoralis* are given in Table 2. All extracts exhibited moderate toxic effect. After 24 hours of treatment, the tested extracts caused mortality less than 50% at the highest concentration (1000 mg L^{-1}). Therefore, LC_{50} values were not calculated. However, with increasing the time of treatment, there was increase in insect mortality, particularly in the case of water, methylene chloride, and hexane extracts, with LC_{50} values of 495.9, 565.7 and 709.7 mg L^{-1} after 48 hours, respectively.

Few researchers described the insecticidal activity of *C. longa* extracts and products against Lepidopteran insects (Lee *et al*., 2001a and b; Tavares *et al*., 2013). However, there are no reported studies on insecticidal activity of *C. longa* extracts against *S. littoralis*. Our results are supported by other studies in which the plant crude extracts showed insecticidal, anti-feedant and growth inhibitory effects against *S. littoralis* (Abdel-Rahman and Al-Mozini, 2007; Pavela, 2011; Barakat, 2011; El-Kholy *et al*., 2014).

Antifungal Activity of *C. longa* Extracts against Plant Pathogenic Fungi

Extracts of *C. longa* were examined for their antifungal activity against *F. solani*, *P. infestans*, *F. oxysporum*, *A. alternata*, and *P. debaryanum*. The results revealed that the extracts had variable antifungal activity as shown in Table 3. Hexane extract was effective only against *P. infestans* (EC_{50}= 287.2 mg L^{-1}). Likewise, methylene chloride was active only against *F. oxysporum* (EC_{50}= 395.5 mg L^{-1}). In addition, methanol extract was active against *P. infestans*, *F. solani*, and *A. alternata* with EC_{50} values of 159.8, 242.7, and 322.2 mg L^{-1}, respectively. Finally, water extract was effective only against *P. infestans* (EC_{50}= 295.8 mg L^{-1}). The *C. longa* extracts were less active than a reference fungicide, carbendazim.

Our results indicated that methanol extract of *C. longa* had the highest antifungal activity among the tested extracts against plant pathogenic fungi. These results are supported by the study of Ungphaiboon *et al.* (2005) who mentioned that the methanol extract of turmeric demonstrated antifungal activity against *Cryptococcus neoformans* and *Candida albicans* with *MIC* values of 128 and 256 µg mL^{-1}, respectively. Similarly, Aly and Gumgumjee (2011) stated that methanol extract of *C. longa* was more effective as compared to n-butanol extract against five fungal genera using agar well diffusion method, and the Minimum Inhibitory Concentrations (MIC) of the methanol extract ranged from 50 to175 µg mL^{-1}. The extracts of *C. longa* with ethanol, which has close polarity to methanol, showed antifungal activity against several fungi such as *Aspergillus flavus*, *A. parasiticus*, *F. moniliforme*, *Penicillium digitatum*, and *Trichophyton longifusus* (Khattak *et al*., 2005; Kumar *et al*., 2011). Not only polar extracts but also non-

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Table 2. Comparative residual toxicity of *Curcuma longa* extracts against the third instar larvae of *Spodoptera littoralis.*

Extracts	LC_{50}[a] (mg L^{-1})	95% Confidence limits (mg L^{-1})		Slope ±*SE*[b]	Intercept ±*SE*[c]	(x^2)[d]
		Lower	Upper			
Hexane	709.7	389.5	1722.7	0.52 ± 0.07	- 1.49 ± 0.15	2.01
Methylene chloride	565.7	313.5	1342.6	0.50 ± 0.07	- 1.38 ± 0.14	0.94
Methanol	> 1000	-	-	-	-	-
Water	495.9	292.5	1035.6	0.55 ± 0.07	- 1.49 ± 0.15	4.32
Chlorpyrifos	12.34	11.61	13.03	7.25±0.74	- 7.92±0.85	0.89

[a] The concentration causing 50% mortality. [b] Slope of the concentration-mortality regression line±Standard Error. [c] Intercept of the regression line±Standard Error.[d] *Chi* square value.

Table 3. Comparative antifungal activity of *Curcuma longa* extracts against plant pathogenic fungi[a].

Fungi	Extracts	EC_{50}[b] (mg L^{-1})	95% Confidence limits (mg L^{-1})		Slope ±*SE*[c]	Intercept ±*SE*[d]	(x^2)[e]
			Upper	Lower			
F. oxysporum	Methylene chloride	395.5	252.5	752.9	0.70±0.08	- 1.83±0.16	6.19
P. infestans	Hexane	287.2	193.9	629.4	1.08±0.10	- 2.64±0.19	10.53
	Methanol	159.8	95.7	332.6	1.21±0.09	- 2.66±0.19	17.52
	Water	295.8	181.2	636.5	1.19±0.10	- 2.94±0.21	11.71
A. alternata	Methanol	322.2	210.1	659.9	1.83±0.16	- 4.59±0.37	18.09
F. solani	Methanol	242.7	182.8	345.7	1.01±0.09	- 2.41±0.18	0.59
	Water	443.4	319.7	684.4	1.08±0.10	- 2.85± 0.22	1.80
F. oxysporum	Carbendazim	37.98	27.73	55.59	0.94±0.096	- 1.48±0.14	1.46
P. debaryanum		13.63	10.61	17.53	1.15±0.098	- 1.30±0.13	5.07
P. infestans		4.91	2.35	8.61	1.19±0.10	- 0.82 ±0.11	5.93
F. solani		13.46	10.77	16.85	1.33±0.10	- 1.50±0.13	4.88

[a] EC_{50} of other extracts are more than 500 mg L^{-1}. [b] The concentration causing 50% inhibition. [c] Slope of the concentration-inhibition regression line±Standard Error. [d] Intercept of the regression line±Standard Error. [e] *Chi* square value.

polar extracts of *C. longa* (hexane, ethyl acetate, ether, and chloroform extracts) were reported to inhibit the growth of fungal strains of *Rhizoctonia solani*, *P. infestans*, *Erysiphe graminis*, *Helminthosporium* sp., *Pyricularia oryzae*, *Sclerotium oryzae,* and *Sclerotium rolfsii* (Kim *et al*., 2003; Alonso, 2004; Kumar *et al*., 2011).

Isolation and Structure Elucidation of Ar-Turmerone (1) and Curcumin (2) from *C. longa*

After extraction with methylene chloride/hexane (1:1), the *C. longa* rhizome extract was subjected to repetitive chromatographic separation using SiO_2 with different solvent systems. Further purification on TLC led to isolation of the two compounds, **(1)** and **(2).** The chemical structures of these compounds were determined based on their spectral data of ^{1}H NMR, IR and UV (Table 4).

Compound **(1)** was obtained as yellow oil. Its molecular weight (216.32) and molecular formula ($C_{15}H_{20}O$) were obtained from GC/MS analysis. The IR spectrum showed absorptions due to carbon-carbon double bonds (2,926 cm^{-1}), carbonyl (1,683 and 1,618 cm^{-1}) and benzene ring (817 and 753 cm^{-1}). Analysis of the ^{1}H NMR spectra

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Table 4. Spectroscopic data of ar-turmerone **(1)** and curcumin **(2).**

Ar-Turmerone **(1)**		Curcumin **(2)**	
UV			
λ_{max}= 237 nm, ε= 4000		λ_{max}= 207 nm; ε= 50000 λ_{max}= 245 nm; ε= 35000	
IR			
ν_{max} cm^{-1}: 3433, 2926, 2355, 1683, 1618, 1447, 1378, 817 and 753		ν_{max} cm^{-1}: 3350-3550, 2356, 1633, 1515, 1443, 1273, 1168, 1138 and 983	
^{1}HNMR			
Atom No.	Chemical shift (δ, ppm)	Atom No	Chemical shift (δ, ppm)
	2.08 (d, 1.3 Hz, 3H)	1	4.88 (s, 2H)
2	-	2, 2`	-
3	6.0 (br s, 1H)	3, 3`	6.60 (d, 15.8 Hz, 2H)
4	-	4, 4`	7.65 (d, 15.8 Hz, 2H)
5a	2.60 (dd, 15.0, 8.1 Hz, 1H)	5, 5`	-
5b	2.69 (dd, 15.0, 8.1 Hz, 1H)	6, 6`	6.79 (br s, 2H)
6	3.26 (ddq, 10.0, 6.9, 6.3 Hz, 1H)	7, 7`	-
7	1.23 (d, 5.0 Hz, 3H)	8, 8`	-
8	1.86 (d, 1.3 Hz, 3H)	9, 9`	6.81 (d, 8.0 Hz, 2H)
9	-	10, 10`	7.45 (d, 8.0 Hz, 2H)
10	7.04-7.14 (m, 1H)	OCH_3	3.28 (s, 3H)
11	7.04-7.14 (m, 1H)	OCH_3	3.33 (s, 3H)
12	-	-	-
13	7.04-7.14 (m, 1H)	-	-
14	7.04-7.14 (m, 1H)	-	-
15	2.28 (s, 3H)	-	-

indicated that (1) contains four tertiary methyls [δ 1.23 (d, J= 5.0 Hz), 1.86 (d, J= 1.3 Hz), 2.08 (d, J= 1.3 Hz), 2.28 (s)]. The presence of a characteristic low-field H-3 proton at δ 6.00 (brs) indicated the presence C2-C3 double bond. This bond was further confirmed by low-field proton signals of two methyls at δ 2.08 (d) and 1.86 (d). Three up-field proton signals at δ 2.60 (dd, 15.0, 8.1 Hz, 1H), 2.69 (dd, 15.0, 8.1 Hz, 1H) and 3.26 (ddq, 10.0, 6.9, 6.3 Hz, 1H) indicated the presence of one methylene (C5) and one methane (C6). Finally, the low-field proton signals at δ 7.04-7.14 (m, 4H) indicated the presence of aromatic ring (C9-C15). From these data, it was concluded that compound (1) was ar-turmerone that belongs to sesquiterpenes.

Compound **(2)** was obtained as yellowish amorphous powder. It was shown to have the molecular formula $C_{21}H_{20}O_6$ from GC/MS analysis. The strong UV absorption at λ_{max} 207 nm (ε= 50,000) and 245 nm (ε= 35,000) indicated the presence of conjugation carbon system in the molecule. The IR absorption at 3,550–3,350, 2,356, 1,633, and 983 cm^{-1} showed the presence of hydroxyl groups,

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carbon–carbon double bonds, carbonyl groups, and aromatic rings. The ^{1}H NMR spectrum showed the presence of two methoxy groups at δ 3.28 (s, 3H) and 3.33 (s, 3H). The low-field proton signal at δ 4.88 (s, 2H, H1) suggested the presence of methylene group adjacent to two carbonyl groups. In addition, the ^{1}H NMR spectrum showed proton signal at δ 6.60 (d, 15.8 Hz, 2H, H3 and H3`) and this signal is coupled with the signal at δ 7.65 (d, 15.8 Hz, 2H, H4 and H4`). The low-field of later signal confirmed the location of this signal at β position of carbonyl group. The presence of two aromatic rings was suggested from the three proton signals at δ 6.79 (br s, 2H, H6 and H6`), 6.81 (d, 8.0 Hz, 2H, H9 and H9`) and 7.45 (d, 8.0 Hz, 2H, H10 and H10`). Based on these spectral data, compound (2) was assigned to be curcumin. This compound belongs to polyphenolics and/or curcuminoids. The obtained spectral data of the isolated compounds were in agreement with those reported in the literature (Tavares *et al*., 2013, Verma, 2014, Nabati *et al*., 2014).

Biological Activity of Ar-Turmerone (1) and Curcumin (2)

The insecticidal activity of ar-turmerone **(**1) on the fourth instar larvae of *C. pipiens* is given in Table 5. The results showed that ar-turmerone (1) had pronounced toxicity. The LC_{50} values were 158.5 and 117.6 mg L^{-1} after 24 and 48 hours, respectively. On the other hand, ar-turmerone (1) showed moderate antifungal activity against *P. infestans* (EC_{50}= 588.9 mg L^{-1}) and weak activity against *F. solani* (EC_{50}= 820.6 mg L^{-1}). This compound was not effective against *F. oxysporum* and *A. solani* as EC_{50} values were higher than 1000 mg L^{-1}. In addition, curcumin (2) was tested against *F. oxysporum* and *P. infestans* at 250 mg L^{-1} only, due to small amount isolated from this compound. Curcumin (2) showed strong antifungal activity with radial growth inhibition of 51.1 and 54.32 % on *F. oxysporum* and *P. infestans*, respectively.

The results of the present study revealed that ar-turmerone (1) had insecticidal activity against *C. pipiens* larvae. These results are supported by Roth *et al*. (1998) who stated that ar-turmerone displayed insecticidal activity against mosquitoes with LC_{99} of 50 µg mL^{-1} on *Aedes aegypti* larvae. In addition, ar-turmerone was reported to possess insecticidal activity against *Plutella xylostella*, *Spodoptera litura*, *Sitophilus zeamais*, and *Spodoptera frugiperda* (Lee *et al.,* 2001b; Tavares *et al*., 2013). The repellent effect of ar-turmerone on *T. castaneum* was also described by Su *et al*. (1982).

On the other hand, our results indicated that curcumin (2) possessed antifungal activity

Table 5. Toxicity of ar-turmerone **(1)** against the fourth instar larvae of *Culex pipiens* after 24 and 48 hours of treatment.

Concentration (mg L^{-1})	Mortality (%)	
	24 h	48 h
0	0	0
50	20.00	20
100	30.00	40
250	46.67	73.3
500	100.00	100.00
LC_{50} (mg L^{-1})[a]	158.8	117.6
95% Confidence limits (mg L^{-1})	-	(31.63 – 300.5)
Slope±SE^b	2.24±0.21	2.65±0.49
Intercept±SE^c	–4.94±0.45	–5.50±0.49
$(x^2)^d$	38.6	9.92

[a] The concentration causing 50% mortality. [b] Slope of the concentration-mortality regression line±Standard Error. [c] Intercept of the regression line±Standard Error. [d] *Chi* square value.

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against *P. infestans* and *F. solani* at concentration of 250 mg L^{-1}. These data are in accordance with other studies in which curcumin at 500 mg L^{-1} showed antifungal activity against *R. solani*, *Puccinia recondita*, and *P. infestans* (Kim *et al.*, 2003). Curcumin and turmeric oil exert antifungal effect against *F. solani* and *Helminthosporium oryzae* (Chowdhury *et al.*, 2008).

In summary, the results of the present study indicated that the *C. longa* extracts and ar-turmerone (1) possessed pronounced insecticidal activity against *C. pipiens* and moderate insecticidal activity against *S. littoralis*. In addition, methanol and water extracts of *C. longa*, and curcumin (2) had significant antifungal activity against plant pathogenic fungi. It can be concluded from these studies that *C. longa* extracts and the isolated compounds might be considered as key products for developing safe alternatives for managing *C. pipiens* and plant pathogenic fungi.

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فعالیت های حشره کشی و قارچ کشی عصاره های خام و مواد خالص ریزوم *Curcuma longa* L. (Zingiberaceae)

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چکیده

در این پژوهش، فعالیت های حشره کشی و قارچ کشی عصاره های خام ریزوم *Curcuma longa*و دو ماده خالص جداشده به نام ar-turmerone و curcumin ارزیابی شد. ریزوم های *C. longa* به ترتیب با n-hexane، کلرید متیلن، متانول، و آب عصاره گیری شد. عصاره های هگزین،کلرید متیلن، و متانول فعالیت حشره کشی قابل ملاحظه ای بر علیه لارو *Culex pipiens* نشان دادند. ارزش عددی LC_{50} بعد از ۴۸ ساعت به ترتیب برابر ۵/۲۸ ، ۵/۸۲، و ۶/۴۴ میلی گرم د رلیتر بود. بر خلاف این اثر، عصاره های مزبور اثر سمی کمی روی سن سوم لارو *Spodoptera littoralis* داشتند. همچنین، LC_{50} عصاره های آب، کلرید متیلن و هگزین، بعد از ۴۸ ساعت به ترتیب برابر ۴۹۵/۹، ۵۶۵/۷، و ۷۰۹/۷ میلی گرم درلیتر بود. از سوی دیگر، فعالیت های قارچ کشی این عصاره ها روی قارچ

های بیمارگر گیاهی شامل *Fusarium oxysporum*، *Pythium debaryanum*، *Fusarium solani*، و *Alternaria alternata* متغییر بود. در میان عصاره های مطالعه شده، عصاره متانول بیشترین اثر قارچ کشی را بر علیه *P. infestans*، *F. solani*، و *A. alternata* با EC_{50} برابر ۱۵۹/۸، ۲۴۲/۷، و ۳۲۲/۲ داشت. دو ماده به نام ar-turmerone و curcumin از عصاره گیری با کلرید متیلن/متانول (۱:۱) از ریزوم *C. longa* جدا سازی شده و ساختمان شیمیایی آنها به روش تجزیه اسپکترواسکوپی شناسایی شد. ar-turmerone سمیت متوسطی بر علیه لارو *C. pipiens* داشت. مقدار LC_{50} آن بعد از ۲۴ و ۴۸ ساعت برابر ۱۵۸/۵ و ۱۱۷/۶ میلی گرم د رلیتر بود. افزون بر این، ar-turmerone فعالیت قارچ کشی متوسطی بر علیه *P. infestans* (EC_{50} = 588.9 میلی گرم د رلیتر) و فعالیت ضعیف بر علیه *F. solani* (EC_{50} = 820.6 mg L^{-1}). نشان داد. نیز، Curcumin در غلظت ۲۵۰ میلی گرم در لیتر باعث جلوگیری از رشد *F. oxysporum* و *P. infestans* به ترتیب برابر با ۵۱/۱٪ و ۵۴/۳۲٪ شد.

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