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Chemical, antifungal, insecticidal and antioxidant studies on *Curcuma longa* essential oil and its oleoresin

Gurdip Singh[a]*, Sumitra Maurya[a], Cesar A. N. Catalan[b] and M.P. deLampasona[b]

ABSTRACT

GC and GC-MS analysis of essential oil of *Curcuma longa* showed the presence of 32 components accounting for the 83.0% of the total amount. ar-turmerone (49.14%) was found as a major component followed by α-turmerone (11.59), ar-curcumene (3.83), β-caryophyllene (2.79), (α-zingiberene (2.61), terpinolene (2.26). The analysis of oleoresin showed the presence of 27 components accounting for 71.41% of the total amount. A mixture (14.07%) of ar-turmerone (55%) and zingerone (45%) was found as a major component followed by β-sesquiphellanderene (9.29), ar-curcumene (9.04), linoleic acid (7.11), α-zingiberene (5.80), α-turmerone (5.14), β-caryophyllene (4.84), β-bisabolene (3.94), α-humulene (2.89), palmitic acid (2.64), α-atlantone (1.78). The essential oil possesses statistically significant antifungal as well as insecticidal character whereas, the oleoresin has been proved to be better antioxidant for rapeseed oil.

INTRODUCTION

Curcuma longa (Fam. Zingiberaceae), a perennial herb of the ginger family, is the major ingredient of curry powder and is also used in prepared mustard. It is extensively used in foods for both, colour and flavour. In Ayurvedic system of medicines it is used as an anti-inflammatory agent and in the treatment of numerous diseases (Leung, 1997). A perusal of literature (Ammon et al 1991; Chowdhury *et al* 2000; Lal *et al* 2000; Lutomski et al 1974; Singh et al 2002; Ravindranath, & Chandrasekhara 1980) reveals that the essential oil extracted from its rhizome possesses excellent antifungal, antibacterial, anti-inflammatory, anti-hepatotoxic and

*Corresponding author, E-mail: gsingh4u@ahoo.com
a Chemistry Department, D.D.U. Gorakhpur University, Gorakhpur-273009.
b Instituto de Quimica, Organica Universidad Nacional de Tucuman, Ayacucho 471, S.M. de Tucuman 4000, Argentina

anti-arthritic activities. Moreover, the antioxidant activity of curcumin, the major component of *C. longa*, is comparable to standard antioxidants, like vitamin C and E and butylated hydroxy anisole (BHA) & butylated hydroxy toluene (BHT). Because of its bright yellow colour and antioxidant properties against lipid peroxidation, curcurnin is used in lipid butter, margarine, cheese and other food products.

As a part of our on going research programme (Singh *et al* 2000; 2002; 2002 & 2004) on essential oils, the chemical, antifungal, insecticidal and antioxidant properties of *C. longa* oil and oleoresin have been undertaken. The present communication aims to compare the effect of *C. longa* essential oil and its oleoresin for antifungal activity against various food borne fungi, insecticidal behavior against *Tribolium castaneum* and antioxidant behavior for rapeseed oil.

MATERIALS AND METHODS

Plant material

Rhizomes of *C. longa* were purchased from the local market of Gorakhpur and voucher specimens were deposited at the Herbarium of the Faculty of Science, DDU Gorakhpur University, Gorakhpur.

Extraction of the oil

The thoroughly washed and crushed rhizomes of *C. longa* were hydrodistilled in a Clevenger type apparatus for 6h at 60 °C in accordance with European pharmacopoeia procedure (1983). Yellow colored oil (yield 2.2%) obtained was dried over anhydrous sodium sulphate to remove traces of moisture and stored in refrigerator in dark at 4°C until use.

Extraction of the oleoresin

After extraction of the essential oil, the crushed rhizomes were dried at 25°C. Oleoresin is obtained by extracting 20g of dried rhizomes with 900 mL of acetone for 6h in a Soxhlet apparatus. The extract was concentrated upto 20 mL. The remaining acetone was dried by placing the samples in a vacuum drier under reduced pressure. Dry extracts were stored in a freezer until use.

Chemical investigation

GC: The oil and oleoresin were subjected to GC analysis by using a Hewlett Packard 5890 Series II gas chromatograph equipped with flame ionization detector (FID) and two HP fused silica column (column A and column B). The column A was an HP-5 (5% phenylmethylsilicone; length 30 m, inner diameter 0.32 mm and film thickness 0.25 mm). The injector and detector temperatures were maintained at 250 °C and 270 °C, respectively. Helium was used as carrier gas; flow rate 1.5 ml min^{-1}. The amount of sample injected was 0.1 mL (split mode). The oven temperature was programmed as follows: 60 °C (5 min), 60-140 °C (1°C min^{-1}), 140-270 °C (10 °C min^{-1}), 270 °C (5 min). Column B was an HP-innowax (length 30 m, inner diameter 0.53 mm and film thickness 1.0 mm). Injector and detector temperatures were 250 °C and the same amount of sample was injected in split mode; carrier gas helium, flow rate 1.0 mL min^{-1}. The oven temperature programmed was: 60°C (5 min), 60-140°C (1°C min^{-1}), 140-240°C (10°C min^{-1}), and 240°C (5 min).

GC-MS: The oil and oleoresin were subjected to GC-MS analysis using a Hewlett Packard HP 6890 series GC fitted with a Hewlett Packard mass detector (model 5973) and a HP-5MS column (length 30 m, i. d. 0.25 mm., film thickness 0.25 µm). The injector, GC-MS interphase, ion source and selective mass detector temperatures were maintained at 270, 280, 230 and 150°C respectively. Helium used as carrier gas with flow rate 1.0 mL

min^{-1}. Oven temperature, programmed for oil was as follows : 60°C (1 min), 60-185°C (1.5°C min^{-1}), 185°C (1 min), 185-275°C (9°C min^{-1}), 275°C (2 min) and that for the oleoresin was as follows: 70°C, 70-280°C (5°C min^{-1}), 280°C (1 min).

Identification of components

The chemical constituents have been identified by comparing their mass spectra with NBS 75 K library (Adams, 1995 & Hennerberg et al 1999) and by co-injection

Table 1. Chemical constituents of *Curcuma longa* essential oil

Compound	% MS	RI	Identification
toluene	0.06	---	--
α-pinene	0.04	0941	MS, RI, co-GC
myrcene	0.05	0993	MS, RI, co-GC
δ-2-carene	trace	1002	MS, RI, co-GC
α-phellandrene	0.13	1005	MS, RI
δ-3-carene	0.07	1013	MS, RI, co-GC
α-terpinene	0.11	1020	MS, RI
p-cymene	0.22	1026	MS, RI, co-GC
limonene	0.18	1031	MS, RI, co-GC
1,8-cineole	0.28	1032	MS, RI, co-GC
γ-terpinene	trace	1062	MS, RI, co-GC
terpinolene	2.26	1088	MS, RI, co-GC
epoxyterpinolene	0.04	1140	MS, RI
p-cymen-8-ol	0.26	1185	MS, RI
p-cymen-7-ol (cuminol)	0.09	1290	MS, RI, co-GC
β-caryophyllene	2.79	1420	MS, RI, co-GC
β-trans-bergamotene	trace	1437	MS, RI, co-GC
α-humulene	0.57	1455	MS, RI, co-GC
trans-b-farnesene	0.20	1459	MS, RI, co-GC
ar-curcumene	3.83	1482	MS, RI, co-GC
γ-curcumene	0.06	1485	MS, RI
α-zingiberene	2.61	1497	MS, RI, co-GC
β-bisabolene	0.83	1508	MS, RI, co-GC
β-curcumene	0.15	1517	MS, RI
β-sesquiphellandrene	3.69	1525	MS, RI
trans-γ-bisabolene	0.13	1533	MS, RI, co-GC
trans-nerolidol	0.54	1565	MS, RI, co-GC
caryophyllene oxide	0.71	1585	MS, RI, co-GC
zingiberenol	0.32	1623	MS, RI
ar-turmerone	49.14	1670	MS, RI, co-GC
β-turmerone	2.43	1674	MS, RI
α-turmerone	11.59	1703	MS, RI
Total	**83.00**		

Percentages are the mean of three runs, obtained from electronic integration measurements using selective mass detector

• The retention index was calculated for all volatile constituents using a homologous series of n-alkanes C_8-C_{18} with the oven temperature program, suggested by R. P. Adams (2001); Trace: <0.01%;

o Co-GC: co-injection with authentic sample;
MS: by computer matching with the WILEY 275 and National Institute of Standards Technology (NIST 3.0) MS libraries provided with the computer controlling GC-MS system and also by visual comparison with published spectra and available in our own files.

Table 2. Chemical constituents of *Curcuma longa* oleoresin

Compound	% MS	RI[a]	identification
ortho-guaiacol + p-cymenene	0.45	1090	MS, RI, co-GC
trans- anethole	trace	1284	MS, RI, co-GC
carvacrol	trace	1298	MS, RI, co-GC
4-vinyl-2-methoxy-phenol	0,88	1315	MS, RI
β-caryophyllene	4,84	1417	MS, RI, co-GC
α-*trans*-bergamotene	trace	1435	MS, RI, co-GC
α-humulene	2,89	1453	MS, RI, co-GC
ar-curcumene	9,04	1480	MS, RI, co-GC
α-zingiberene	5,80	1496	MS, RI, co-GC
β-bisabolene	3,94	1507	MS, RI, co-GC
β-sesquiphellandrene	9,29	1524	MS, RI, co-GC
ar-turmerone (55%) + zingerone, (45%)	14,07	1668	MS, RI, co-GC
β-turmerone	0,95	1673	MS, RI
α-turmerone	5,14	1702	MS, RI
trans-α-atlantone	1,78	1778	MS, RI
dehydrozingerone	0,56	---	MS
palmitic acid	2,64	---	MS, co-GC
methyl linoleate	0,16	---	MS, co-GC
2-nonadecanone	0,46	---	MS
linoleic acid	7,11	---	MS, co-GC
oleic acid	0,07	---	MS, co-GC
squalene	0,13	---	MS
α-tocopherol (vitamin E)	0,06	---	MS, co-GC
ergost-5-en-beta-ol	0,12	---	MS
stigmasterol	0,24	---	MS, co-GC
β-sitosterol	0,70	---	MS, co-GC
Total	**71,41**		

Percentages are the mean of three runs, obtained from electronic integration measurements using selective mass detector.

The retention index was calculated using a homologous series of n-alkanes C_6-C_{18} with the oven temperature program, suggested by R. P. Adams (2001);

o Co-GC: co-injection with authentic sample;

MS: by computer matching with the WILEY 275 and National Institute of Standards Technology (NIST 3.0) MS libraries provided with the computer controlling GC-MS system and also by visual comparison with published spectra and spectra available in our own files.

Trace: minor 0.03%

with available authentic samples. The results were reported in Tables 1 and 2, respectively for essential oil and oleoresin.

Antifungal investigation

The antifungal efficacy of the volatile oil and oleoresin for the pathogenic fungi *Aspergillus terrus* (AT) *Aspergillus niger* (AN), *Aspergillus flavus* (AF), *Trichothecium roseum* TR, *Fusarium monoliforme* (FM), *F. graminearum* (FG), *F. oxysporum* (FO), and *Curvularia palliscens* (CP) were confirmed using Inverted petriplate and food poison techniques (Rao et al 1994). The fungal strains were collected from MTCC, Chandigarh, India. Cultures were maintained on Czapek (DOX) agar media, adjusting pH 6.0-6.5 and slants were stored at 5°C. Each test was performed at three concentrations (2, 4 and 6 µL) and

replicated for three times. The results taken by inverted petriplate and food poison technique are given in Table 3 and 4, respectively.

Insecticidal activity

The oil and oleoresin were screened for their insecticidal activity against wheat insect *(Tribolium castaneum)* using 80 mm glass petriplates. The required dose of oil (0.5, 2, 3 and 6 µL per petriplate) was soaked in a piece of filter paper (10 mm dia.) and pasted on the inner surface of the cover of the petriplate. A group of ten insects along with 5 g of floor inside each petriplate. To get sufficient aeration inside the petriplates, a constant gap was maintained in between the pair of petriplates by inserting a small piece of filter paper. Similarly, a control experiment was also done by pasting blank filter paper disc (without additive) on the same surface of petriplate. In order to investigate the cidal nature of the oil and oleoresin, the insects revival was observed after transferring them to a fresh petriplate. Similarly, the insecticidal efficacy of the oil and oleoresin was also compared with two commercial synthetic insecticides (without dilution), namely endosulfan 35% (Thiodan) and chloropyriphos 20% (Primoban-20). The similar dose of synthetic insecticides was also undertaken and results are compared accounting the vapor action of essential oils and synthetic one.

Antioxidant activity

In order to assess the antioxidant activity (Andres *et al* 2000; Economou *et al 1991;* Yanishlieva *et al* 2002) of *C. longa* essential oil and its oleoresin, crude rapeseed oil, having initial peroxide value 2.2 meq kg^{-1} was taken for present investigation. This oil is most frequently used edible oil in central Europe and is unstable because of the presence of substantial amount of α-linoleic acid (8-12%). The present study was undertaken to examine the effect of oil and oleoresin on rapeseed oil peroxidation by three different experimental procedures.

Peroxide value (PV) method

For measuring the peroxide value a modified oven test (Frankel, 1998) was used. The antioxidant activities of volatile oil and oleoresin were compared with synthetic antioxidants, such as BHA and BHT. The calculated quantities of each (200 ppm) were added to 30g of rapeseed oil in open mouthed

Table 3: Investigation of antifungal activity of *Curcuma longa* essential oil and its oleoresin using inverted petriplate technique

S.No	Fungus	% mycelial zone inhibition at different doses of oil* (µl)					
		Essential oil			Oleoresin		
		2	4	6	2	4	6
1	AN	12.5	31.3	87.5	0	6.25	25.0
2	AF	12.5	18.7	50.0	12.5	18.7	25.0
3	AT	6.25	25.0	68.7	6.25	6.25	12.5
4	TR	12.5	25.0	50.0	0	5.0	12.5
5	FO	6.25	12.5	25.0	6.25	10.0	11.3
6	FG	6.25	25.0	50.0	12.5	15.0	25.0
7	FM	6.25	50.0	75.0	12.5	18.7	25.0
8	CP	12.5	18.7	37.5	18.7	25.0	50.0

*average of three replicates

of conjugated dienes by spectrophotometry is related to the content of polyunsaturated hydro-peroxides which are flavor precursors of oxidized lipids. After 6 days, the activity of oil started decreasing due to its volatile nature and becomes lesser than oleoresin and BHT. However, in general, the results obtained by spectrophotometric absorptions at 500nm (Fig-3) were closely related with PVs.

Some authors (Pokorny *et* al, 1997; Nguyen *et al* 1999) have reported the data concerning the improvement of oxidative stability of rapeseed oil by addition of various antioxidants and synergists.

Summarizing these results, it can be concluded that *Curcuma longa* essential oil, which is rich in ar-turmerone, possesses significant antifungal as well as insecticidal behavior. The oleoresin, which is a mixture of ar-turmerone (55%) and zingerone (45%), has been proved to be better antioxidant for rapeseed oil.

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