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COMPARATIVE EVALUATION OF PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT, ANTI-INFLAMMATORY, AND ANTIDIABETIC ACTIVITIES OF *Curcuma amada* AND *Curcuma longa* ESSENTIAL OIL FROM UTTARAKHAND, INDIA

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ABSTRACT

The present research work aims to analyze the chemical, phytochemical composition and biological activities of *Curcuma amada* with *Curcuma longa* L. essential oil, the 2 variants collected from the diverse elevations and geographical variations of Kumaun and Garhwal regions of Uttarakhand, India. The analysis was done by using gas chromatography-mass spectrometry (GC/MS), UV-Visible Spectrophotometer, and other established biochemical methods. The results for essential oil yield varied from 0.212% - 0.396% and the major components identified were hedycaryol (8.7%), α -zingiberene (13.5%), α -santalene (13.5%) curlone (14%), eucalyptol (26.4%) and turmerone (44.3%). Further *in–vitro* antioxidant, biological activity was performed by DPPH free-radical scavenging, reducing power and metal chelation ability followed by anti-inflammatory, and anti-diabetic activities.The essential oil of *Curcuma longa* Garhwal species exhibited promising antioxidant activities and anti-diabetic activity (11.95 ± 0.11 μg/mL). A good anti-inflammatory activity was exhibited by Curcuma amada (7.46 ± 0.04 μg/mL) when compared with standard drugs. The relative difference in results can be influenced by the altitudinal variation, species, and testing methodology of the samples. The study strongly clarifies that essential oils from the local variant of Curcuma species possess promising antioxidant, anti-inflammatory, and anti-diabetic activity.

INTRODUCTION

Plants with medicinal properties avail the highest reputation in the indigenous systems of medicine all over the world. Despite the tremendous advancement in the field of synthetic drugs and antibiotics, plant with medicinal properties still considered as one of the major sources of drugs in modern as well as traditional systems of medicine (Bhutani and Gohil, 2010). The plants of the family Zingiberaceae are a rich repository of secondary metabolites and source of several natural immunomodulators and antioxidant properties providing the edge over synthetic antioxidants.The two rich reserve and valuable species of the Zingiberaceae family are *Curcuma amada* and *Curcuma longa*, combination of these two unique spices possess specific properties like antipyretic, aphrodisiac, diuretic, emollient, expectorant, laxative and provide cure to several diseases like biliousness, skin diseases, asthma, bronchitis, hiccough, fever, itching, and inflammation(Chowdhury *et al*., 2015).

Altitudinal variation has been demonstrated to have a major impact on the chemical composition of plant essential oils. Alterations in the aroma, flavour, and potential medicinal effects of the essential oils may occur from different altitudinal ranges favouring the synthesis of particular molecules. These variations are frequently studied by researchers and essential oil producers in order to better understand the variables impacting essential oil composition and identify the ideal environmental conditions for growing plants with desirable chemical profiles. The distribution of plants in different elevations and phytogeographical regions of Uttarakhand might considerably affect the contents and quality of essential oil which might be the possible reason for the diverse activities of essential oil (Akter *et al*., 2019; Mitra *et al*., 2020, Sontakke *et al*., 2019) .

Moreover, no significant comparative study has been reported earlier on the essential oil of diverse turmeric rhizomes growing at different elevations in Uttarakhand. In the view of aforesaid, the aim of the present study is on the systematic analysis of the chemical composition and varied biological activities of essential oils extracted from *Curcuma amada* and *Curcuma longa* L. rhizomes local cultivar collected from different

elevations of Uttarakhand.

MATERIALS AND METHODS

Plant material collection

The *Curcuma amada* rhizomes were collected in the month October-November from extension centers of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar *i.e.*, KrishiVigyan Kendra (KVK) Uttarakhand situated at 29.33ºN latitude and 79.43ºE longitude 1182 m above sea level and *Curcuma longa* rhizome samples were collected in same month from extension centers of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar *i.e.* KrishiVigyan Kendra (KVK) Uttarakhand of Kumaun region situated at the 29.33ºN latitude and 80.09º E longitude , 1615m above sea level and from Garhwal region situated at 30.57ºN latitude and 78.32ºE longitude, 909 m above the sea level .

Extraction of essential oil.

Fresh rhizomes of Curcuma were thoroughly washed, grated with peels, air-dried, and hydrodistilled by using the Clevenger apparatus. Further, any trace of water molecules was removed using anhydrous sodium sulphate and was further stored at 4ºC in amber vials for further phytochemical and biological activities. The yield of essential oil extracted from *Curcuma amada* rhizomes was 0.396% and the yield of essential oil of rhizomes of *Curcuma longa* L. from Kumaun region was 0.225% and from Garhwal region, it was 0.212% respectively (Table 1). The yield of essential oil shows a positive correlation between altitude and oil yield among *Curcuma longa* L. species.

Analysis of essential oils.

The GC–MS analysis of essential oils was carried out using GCMS- Shimadzu QP 2010 plus equipment with helium as carrier gas at the pressure of 69 kPa and the split ratio of 30.0. A DB-5 silica capillary column (30m x 0.25mm x 0.25um) was used for separating ions. The column flow rate was maintained at 1.21 mL/min during analysis and the total flow was 40.5 mL/min. The linear velocity and purge flow was maintained at 39.9 cm/sec and 3 mL/min, respectively. The carrier gas saver, splitter hold, and high-pressure injection were off, and the oven temperature was initially programmedat 50ºC RAMP@ 3ºC/min up to 210ºC (isotherm for 2 min) then at 6°C/min up to 280ºC (isotherm for 2 min), and finally held for 11 min. The compounds were identified by matching the mass spectra, Kovatt indices, and with the help of NIST-MS and FFNSC Wiley libraries and reported literature (Adams, 2007).

Compound identification

Identification of compounds by GC-MS was based on the comparison of their retention indices by comparison with their mass spectra with those of pure compounds compiled in computer libraries, NIST-MS and FFNSC Wiley libraries, and previously reported documents (Davies, 1990; Tonzibo *et al.*, 2013).

***In - vitro* Antioxidant activity**

DPPH (2, 2-diphenyl-2-picrylhydrazyl) radical scavenging activity

A fresh DPPH (2, 2-diphenyl-2-picrylhydrazyl) methanolic solution was used as test solution with varying concentrations from 5μg/mL - 25μg/mL (in the total volume of 0.1 mL) of the essential oil and further test was carried out as per the method given by Dhami *et al.*, 2019; Gairola *et al.*, 2021).

Reducing power activity

Different concentration (5μg/mL- 25μg/mL) of essential oil was taken to perform reducing power activity as per the method given by Amarowicz *et al.*, 2004. Gallic acid was used as the standard.

Metal complexation activity

Different volume of essential oil (5 μg/mL- 25μg/mL) was taken to perform metal-chelating activity using the method given by (Mau *et al.*, 2003, Gururani *et al.*, 2022)

***In-vitro* Anti-inflammatory activity**

Different volumes of the essential oil (5 μg/mL- 25 μg/mL) were added to a 2.8 mL freshly prepared phosphate buffer (pH 6.4) along with the aliquot of 0.2 mL egg albumin. Absorbance was measured at 660 nm using Thermo scientific Genesys 10S UV-VIS Spectrophotometer. Diclofenac was taken as a standard as used in procedure by Heendeniya *et al.*, 2018.

***In-vitro* Anti-diabetic activity**

The varying volume of turmeric essential oil (10 μg/mL - 50 μg/mL) was taken for anti-diabetic activity and same steps were followed as prescribed by Lekshmi *et al.*, 2012; Shai *et al.*, 2010).

Statistical analysis

All the experiments were carried out in three replicates and data on essential oil compositions and contents were subjected to analysis of variance (ANOVA)(Soni *et al.*,2019). The significant differences were tested by Duncan's multiple range tests at 5% level of significance (p 0.05). Correlation coefficients were performed to assay the differences among the essential oil components of each Curcuma species. Statistical analysis was performed using SPSS 13.

RESULTS AND DISCUSSION

The natural essential oil is enriched with antioxidant, anti-inflammatory, and anti-diabetic activity which provide advancement to deteriorate the effects of free radicals thereby reducing the prevalence of related diseases and helping in making a cost-effective potent drug with minimal toxicities to the physiological system, similar effect were found in volatile oils of *Curcuma amada* and *Curcuma longa* L. which were collected from varied altitudes and geographical locations of Uttarakhand and their yield ranges from 0.212% to 0.396% (v/w%) as shown in Table 1. A positive correlation of essential oil yield with the rise in altitude was observed in *Curcuma longa* L. species. The positive correlation of yield with altitude

Table 1: Essential oil yeilds of Curcuma species :-

Essential Oils of Curcuma species	Yeild
1.*Curcuma amada*	0.396%
2.*Curcuma longa* L. Kumaun	0.225%
3.*Curcuma longa* L.Garhwal	0.212%

Table 2: Comparative analysis tentatively identified compounds of turmeric essential oil of C. amada with C. longa from Uttarakhand

S.No.	Compounds	Chemical Formula	KI Value	Curcuma Species		
				CLG	CLK	CA
1.	α-Thujene	$C_{10}H_{16}$	927	1.1	0.7	5.3
2.	Sabinene	$C_{10}H_{16}$	972	0.1	9	-
3.	p-Cymene	$C_{10}H_{14}$	1025	-	1.4	-
4.	Eucalyptol	$C_{10}H_{18}O$	1039	5.5	26.4	1.3
5.	Terpinolene	$C_{10}H_{16}$	1089	2.3	0.1	0.4
6.	Terpinen- 4 ol	$C_{10}H_{18}O$	1177	-	13.8	-
7.	α-Terpineol	$C_{10}H_{18}O$	1188	0.2	1.3	-
8.	Z-Caryophyllene	$C_{15}H_{24}$	1408	-	0.6	2.1
9.	α-Funebrene	$C_{15}H_{24}$	1413	-	-	5
10.	α-Santalene	$C_{15}H_{24}$	1417	13.5	0.2	-
11.	cisβ- Farnesene	$C_{15}H_{24}$	1442	-	1.6	-
12.	α-Humulene	$C_{15}H_{24}$	1454	7.4	1.5	0.3
13.	α-Curcumene	$C_{15}H_{22}$	1480	4.5	0.5	2.4
14.	Germacrene D 4-oL	$C_{15}H_{26}O$	1485	0.2	2.3	-
15.	α-Zingiberene	$C_{15}H_{24}$	1493	13.5	-	9.1
16.	β-Bisabolene	$C_{15}H_{24}$	1505	6.8	0.1	1
17.	β-Curcumene	$C_{15}H_{24}$	1515	-	-	4.5
18.	β-Sesquiphellandrene	$C_{15}H_{24}$	1522	8.5	-	6.3
19.	Hedycaryol	$C_{15}H_{26}O$	1546	-	8.7	-
20.	Humulene epoxide II	$C_{15}H_{24}O$	1608	-	1.2	0.2
21.	epi-γ-eudesmol	$C_{15}H_{26}O$	1632	-	1.3	-
22.	Unknown	-	1650	-	13.1	-
23.	α-Cadinol	$C_{15}H_{26}O$	1654	-	1.9	-
24.	ar-Tumerone	$C_{15}H_{22}O$	1670	10.7	-	44.3
25.	Germacron	$C_{15}H_{22}O$	1693	1.5	-	-
26.	Curlone	$C_{15}H_{22}O$	1697	3	-	14
27.	(E)-beta-Santalol	$C_{15}H_{24}O$	1739	1.5	0.1	1.7
Monoterpene hydrocarbons				3.5	11.2	5.7
Oxygenated monoterpenes				5.7	41.5	1.3
Sesquiterpene hydrocarbons				54.2	4.5	30.7
Oxygenated sesquiterpenes				16.9	28.6	60.2
Total				80.30%	85.80%	97.90%

Full form of abbreviations: -CLG: - *Curcuma longa* L. GarhwalCA: -Curcuma amada;CLK: -*Curcuma longa* L. Kumaun KI Value: - Kovatt indices t≤0.05 (t = trace)

Table 3: IC50 value of various antioxidant activities.

Samples	IC50 / RP 50 valuesof various antioxidant activities(μg/mL ± SD))		
	DPPH radical scavenging activity (μg/mL ± SD)	Metal chelating activity of Fe^{+2} (μg/mL ± SD)	Reducing power activity of Fe^{+3}(μg/mL ± SD)
CLG	9.34 ± .09	14.15 ± 0.05	15.44 ± 0.16
CA	11.8 ± .14	16.10 ± 0.07	15.64 ± 0.03
CLK	14.17 ± 0.03	14.57 ± 0.04	16.96 ± 0.07
A.A	11.72 ± 0.30	-	-
EDTA		13.77 ± 0.03	-
G.A	-	-	16.03 ± 0.30

Data analyzed with one way ANOVA at $p < 0.05$ followed by Dunkan test with replications.
IC50 :- 50% Inhibitory Concentration RP50:- 50% Reducing Power Capacity
CLG :-*Curcuma longa* L.GarhwalCA: -Curcuma amada
CLK : -*Curcuma longa* L.KumaunEDTA: - Ethylene DiamineTetraacetic Acid
A.A :- Ascorbic acid G.A:-Gallic Acid SD:-Standard deviation

might be due to variations in environmental conditions, humidity, vegetation, temperature, soil, and other edaphic factors.

Chromatographic identification through GC-MS were persuaded toward the identification of 16 and 20 compounds in volatile oils of *Curcuma longa* L. Garhwal and Kumaun region contributing 80.3% and 85.8 % of total volatile oil composition respectively and 15 compounds in Curcuma amada volatile oil constituting of about 97.9 % of total volatile composition reported in Table 2 along with their components isolated, chemical formula and KI values. The prominent compounds were ar-tumerone (44.3%), eucalyptol (26.4%), curlone (14%), α-zingiberene (13.5%), α-Santalene (13.5%), hedycaryol (8.7%), β-sesquiphellandrene (8.5%), α-humulene (7.4%), β-bisabolene (6.8%) and germacron (1.5%).The sample of Curcuma amada showed the significant amont of components such as Tumerone(44.3%),Curlone(14%),á-zinqiberene(9.1%).The samples of *Curcuma longa* L. from the Kumaun region showed a significant amount of eucalyptol (26.4%), terpinen-4-ol (13.8%), hedycaryol (8.7%), and other compounds identified in the sample were α-cadinol (1.9%),epi-γ- eudesmol (1.3%) with one unidentified compound constituting about 13% of total volatile oil composition whereas *Curcuma longa* L.fromGarhwal region consists of α-Santalene (13.5%), α-Zingiberene(13.5%), α-Humulene (7.4%), ar-tumerone (10.7%) in high amount when

compared with inter and intra species of Curcuma. Elevated tumerone content and increased oil yield might be due to the presence of ample organic phosphorous, carbon, nitrogen, and potassium elements in the Curcuma rhizomes oils (Kadam *et al*., 2020; Gounder and Lingamallu, 2012). The remarkable difference between the constituents of samples collected from different elevations may be due to several genetic, edaphic, and geographical factors.Earlier studies found that the production of certain compounds such as caryophyllene, *á*-curcumene, -bisabolene and β-curcumene found in *Curcuma rhizomes* can be robusted via the use of mycorrhizal fungi as a substitute for chemical fertilizers (De Ferrari *et al*., 2020) thereby enhancing the better antioxidant, anti-inflammatory, and anti-diabetic activities.

In – vitro Antioxidant Activities

DPPH (2, 2′-Diphenyl picrylhydrazyl) Free Radical Scavenging Activity: -

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a free radical that remains stable at room temperature and forms a diamagnetic molecule by accepting electron or free radical. The quenching of DPPH free radicals leads to the vanishing of deep violet-blue color to colorless or pale yellowish color which results in the reduction of absorbance (Avanço *et al*., 2017; Amarowicz *et al*., 2004). *Curcuma longa* L. from Garhwal shows better results as compared to *Curcuma amada* and *Curcuma longa* L(Kumaun).It might be due to the presence of α-zingiberene, eucalyptol, turmerone, ß-sesquiphellandrene, germacrone, and santalene as reported in previous studies (George *et al*., 2015). The DPPH activity is measured with the help of the IC50 value (Table 3). Previously, it has been documented that fresh rhizome of C. longa (mainly containing α-turmerone (42.6%), β-turmerone (16.0%) and ar-turmerone (12.9%)) exhibited dose-dependent DPPH radical activity with (IC50 10.03mg/mL) (Muchtaromah *et al*., 2017) C. longa rhizome essential oil exhibited strong antioxidant potential when combined with Z. officinale essential oil (IC50 3.75 µL/mL) than *C. longa* and Z. *officinale* oils alone (IC50 4.28 and7.19 µL /mL)(Prakash *et al*., 2012)..

Metal complexation activity of Fe^{+2}

The metal chelation activity is measured with the help of IC50 value which signifies the amount of total antioxidant required to chelate metal ion by 50%.(Ebrahimzadeh *et al*., 2008;Mau *et al*., 2003).The IC50 value of Curcuma essential oil ranges from (14.15 ± 0.05 to 16.10 ± 0.07 μg/mL) in the current study(Table 3). It has been reported that curcumin, one of the main constituents found in Curcuma species prevents the formation of Fe^{2+}-ferrozine complex by chelating the Fe^{2+} ions. The advancement of curcumin to bind with free Fe^{2+} ions is its active functional groups *i.e*. -OH and -OCH3 (Lindsay and Kerr 2014; Eruygur *et al*., 2019). Moreover, the essential oil is a complex mixture of terpenoids containing a number of double bonds and oxygen as a hetero atom which are rich electron sites and might be the possible region for chelation.(Gocer and Gulcin 2011)

Reducing power activity

The measure used for reducing power activity is the RP50 value and it is the value that total antioxidant required to reduce ferrous ions into ferric ions by 50%.(Gülçin, 2015).The RP50 value of Curcuma essential oil varies from 15.44 ± 016 to 16.96 ± 0.07 μL in the present study (Table 3). Studies have also confirmed constituents like *á*-caryophyllene, β-elemene, β-eudesmol, and germacrone possess good antioxidant activity (Zhang *et al*., 2017).

In- vitro anti-inflammatory activity

One of the proclaimed causes of inflammation is denaturation of proteins. Protein loses its tertiary structure due to breaking of several bonds causing protein denaturation. Numerous inflammatory mediators are synthesized and secreted in physiological system like interferons, TNF-α, prostaglandins, COX-2 (cyclooxygenase) and leukotrienes causing inflammation, pain, swelling and fever (Makabe *et al*., 2006). The present investigation shows that the anti -inflammatory capacity of essential oils was done on the basis of dose dependent manner (5-25 μg/mL of oil). IB50 is the 50% inhibition of denaturation of protein (Leelaprakash, and Dass 2011). The IB50 value of essential oil ranges from 7.46 ± 0.04 to 12.47 ± 0.12 μg/mL in the present study. As reported earlier this activity might be due to the presence of α-curcumene, α-santalol, α-thujene, curlone, hedycarol, terpineol, terpinolene and turmerone in the essential oils (Ibáñez and Blázquez 2021; Ibrahim *et al*., 2023; Raina *et al*., 2005). IB50 of anti-inflammatory activity of essential oils is shown in Table 4. Turmeric essential oil components have shown remarkable anti-inflammatory activity by inhibiting iNOShaving IB503.2 μg/mL (Hong *et al*., 2002).Essential oils from C. elataoil (54.64% inhibition), C. nankunshanensis (55.23% inhibition), C. sichuanensis (68.43% inhibition), thereby inhibiting IKK, COX2, NF-κB and TNF-α (Xiang *et al*., 2018). Several reports sggest that compounds like ar-tumerone, and germacrone are responsible for anti-inflammatory activity and helps in revention and attenuation of several inflammatory diseases (Chen *et al*., 2018; Yang *et al*., 2020).

In- vitro anti-diabetic activity

The essential oil of turmeric rhizome is used to check the anti-diabetic activity in dose dependent manner (10 μg/mL-50 μg/mL) in this activity (Lekshmi *et al*., 2012). The essential oils showed significant in-vitroantidiabetic activity as compared to Acarbose (16.92 ± 0.42) and IA50 was taken as measure to quantify the anti-diabetic activity by seeing % of *á*-amylase inhibition i.e. inhibition is positively correlated with the anti-diabetic activity of essential oil which might be attributed to the presence of high amount of tumerone, curlone, α-curcumene and α-thujeneTable 5. The IA50 value of Curcuma essential oil varies from 11.95 ± 0.11 to 23.06 ± 0.08 μg/mL

Table4: IB50 of anti-inflammatory activity of essential oils.

S.No.	Sample Name	Mean IB50 values with SD (μg/mL ± SD)
1	CLG	8.87 ± 0.08
2	CA	7.46 ± 0.04
3	CLK	12.47 ± 0.12
4	Diclofenac sodium (DC)	8.21 ± 0.08

Data analyzed with one way ANOVA at $p < 0.05$ followed by Dunkan test with replications.
IB50:- 50% Inhibiting denaturing of protein
CLG:-*Curcuma longa* L. Garhwal CA:-Curcuma amada
CLK: -*Curcuma longa* L. Kumaun DC: - Diclofenac Sodium

Table5 : IA50 of anti-diabetic activity of diverse turmeric rhizome essential oils.

S.No.	Sample Name	Mean IA50 values with SD (μg/mL $\pm$ SD)
1	CLG	11.95 ± 0.11
2	CA	15.16 ± 0.27
3	CLK	23.06 ± 0.08
4	Acarbose	16.92 ± 0.42

Data analyzed with one way ANOVA at $p < 0.05$ followed by Dunkan test with replications.
IA50 :- 50% anti-diabetic activity ; CLG:-*Curcuma longa* L. Garhwal; CA:-Curcuma amada CLK: -*Curcuma longa* L. Kumaun

in the present study. Earlier studies suggest that turmeric volatile oil from dried rhizome can inhibit glycosidase enzyme with IA50 Value of 0.38µg/mL, fresh rhizome have IA50 value of 1.32µg/mL. Previous studies with turmeric spent oleoresin possess IA50 value of 0.16 µg/mL. The ethyl acetate extract of C. caesia, C. longa and C. aromatica % inhibition ranges from 97.72, 96.66 and 95.37 (Dosoky, *et al*., 2019).

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Declarations of competing interest

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