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Composition of Essential Oils in Turmeric Rhizome

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Abstract
Turmeric has been recognized as a pharmaceutical crop. It is valuable primarily for essential oil and curcumin content. Chemical composition of the essential oils obtained from the rhizome of turmeric was determined by GC/MS technique. More than 75 compounds were detected and 67 of them were identified. They accounted for 98.59% of essential oil. The essential oil contained 15 monoterpenes (5.58%), 43 sesquiterpenes (84.37%) and 10 non-terpenic components (8.64%). The major constituents were *ß*-turmeron, *a*-turmeron, *Epi-a*-patschutene, *ß*-sesquiphellandrene, 1,4-dimethyl-2-isobutylbenzene, (±)-dihydro-*ar*-turmerone, zingiberene, *E-a*-atlantone and (-)-caryophyllene oxide. Thin layer chromatographic finger printing and quantitative determination of phenolics in acetone extract of commercially available turmeric samples were carried out using Folin-Ciocalteu colorimetric method. Gallic acid was used as the standard for the estimation of phenolics. All the investigated turmeric extracts contained relatively high amount of phenolics.

Key words: *Curcuma longa*, essential oil, GC-MS, TLC fingerprint, total phenolics

Introduction
Turmeric is a rhizomatous herbaceous medicinal plant that contains 49 genera and 1400 species (Asghari *et al.* 2009). The medical property of it is very ancient and innumerable. It is used extensively in Ayurveda, Unani and Siddha medicine for various diseases. It is an important herb which is widely used worldwide as medicine, condiment, dyes and cosmetics. Turmeric is one of the major spice ingredients in the entire Indo-Nepalese kitchen and has a significant role in culinary culture. As a food additive, turmeric can improve the deliciousness, aesthetic appeal and shelf life of delicate product and also its powder is extensively used as a preservative and coloring agent.

Turmeric is one of the most extensively investigated plant species. There have been extensive *in vitro* and *in vivo* investigations carried out on turmeric extracts. It has shown various pharmacological activities like anti-oxidant (Jayaprakasha *et al.* 2006), antimicrobial (Negi *et al.* 1999), antifungal (Cho *et al.* 2006), hepatoprotective and cardioprotective (Rivera-Espinoza & Muriel 2009, Mohanty *et al*. 2004), cytotoxic, (Ramsewak *et al.* 2000) antitumour and anticarcinogenic (Kuo *et al.* 1996, Goel *et al.* 2001, Shao *et al.* 2002), chemoresistance and radioresistance (Bar-Sela *et al.* 2010), cholesterol, fatty acids and triglycerides lowering activities (Rukkumani *et al.* 2003) and blood glucose level suppressing activity (Nishiyama *et al.* 2005). The volatile oil shows anti-inflammatory (Chandra & Gupta1972), anti-proliferative and immunomodulatory activities (Yue *et al.* 2010) and anti-arthritic effects (Funk *et al.* 2010).

Turmeric has been recognized as a pharmaceutical crop and valued primarily for curcumin and essential oil content (Li *et al.* 2010). The contents of curcuminoids and essential oils in turmeric rhizomes vary often with genotypes, varieties, geographical locations, sources, cultivation conditions, environments, harvest methods and seasons, drying process, and storage conditions etc. (Rakhunde *et al.* 1998). The content of both curcuminoids and essential oils depends on the extraction methods. Thus, the commercial turmeric powder and products have significant variations in composition of bioactive compounds. The

curcuminoids, curcumin, demethoxycurcumin, and bis-demethoxycurcumin can be used as marker compounds for the quality control of turmeric. On the other hand, *ar*-turmerone, *a*-turmerone, and *ß*- turmerone can be used to control the product quality of turmeric oil and oleoresin products (Garg *et al.* 1999). Turmeric and turmeric products can also be authenticated simply by chromatographic techniques.

The quality control and quality assurance of turmeric products still remains a challenge. Most of the turmeric powder available in the markets are adulterated. It is done by mixing with powders of certain other species of *Curcuma* (Remya *et al*. 2004). Usually, adulterated products have low content of curcuminoids and when *C. zedoaria* is a common adulterant, it has toxic effect (Latif *et al*. 1979). Thus, chemical analysis will provide useful information for any questionable samples of dried rhizomes, ground turmeric, turmeric oils or oleoresins, and curcumi- noids/curcumin. Chemical analysis becomes particularly necessary when exotic chemical adulterants such as Sudan dyes (Salmén *et al*. 1987, Di Anibal *et al*. 2009, Salmén *et al*. 1988) Metanil yellow, Orange II and lead chromate are present in turmeric powder which are detected by colorimetric, chromatogarphic or spectrophotometric techniques (Tripathi *et al.* 2004, 2007). The Thin Layer Chromatography (TLC) fingerprint with a visible pattern of bands provides fundamental data and is typically used to demonstrate the consistency and stability of herbal materials. It is a convenient method of determining the quality and possible adulteration of herbal products but may not be helpful in identifying adulterated products in the marketed samples of turmeric (Govindarajan *et al.*1980). However, chemical fingerprints appear to be the only approach to determine the product quality. High pressure liquid chromatography (Wichitnithad *et al*. 2009) or UV spectrophotometry (Di Anibal *et al.* 2009) have been used as powerful tools for identification of the quality of product in various studies.

Although turmeric is well investigated, the chemical constituents of the essential oil of *C. longa* of Nepalese origin have not previously been investigated and the total phenolic content is also not reported. This prompted us to carry out the detailed GC/MS analysis to access the quality of Nepalese turmeric. Thus, the present study has been conducted for the determination of chemical composition of the essential oil of Nepalese turmeric by GC-MS technique, determination of purity by simple TLC fingerprint and the total phenolic content in different turmeric extracts prepared from commercial samples as curcuminoids are the major phenolic compounds present in turmeric.

Methodology

Chromatographic materials and chemicals

Gallic acid and TLC foils (precoated) Silica gel 60 GF_{254}, 0.2 mm were purchased from Merck, Darmstadt, Germany. Folin Ciocalteu reagent was purchased from SD fine-chemicals. All other chemicals were of analytical grade.

Plant materials

Homemade turmeric powder was collected from Pokhara (PS). Other samples were purchased from Kalimati market (KS), Basantpur market (BS) and Ason market (AS). Similarly, Chandra (CS), Kiran (KS) and Surya brand (SS) turmeric powders were purchased from the local vendors.

Volatile oil extraction

The turmeric sample from Pokhara (PS, 50 g) was taken into a 500 ml round bottom flask. About 200 ml of distilled water was poured into the flask. The Clevenger apparatus was fitted on the mouth of the flask and it was heated to boil. The heating was continued for 4 hours. The oil was collected in the trap of the Clevenger apparatus. It was allowed to stand for sometime in order to separate the oil and water layer completely. The water was drawn off slowly and the oil was collected in a test tube and dried over Na_2SO_4 and stored at 4æ%C for further use.

Gas chromatography-mass spectroscopy

Analytical gas chromatography-mass spectrometry was recorded on GCMS QP 2010 plus Shimadzu, Japan fitted with a flame ionization detector using a capillary 30 m DB-1 column (J and W scientific, USA) with 0.25 mm internal diameter and 0.25 µm film thickness. The oven temperature program was maintained at 60^0C and gradually increases up to 250^0C at 7^0C/minute without hold time. The injection temperature was maintained at 280^0C. The carrier gas was Helium with a flow rate of 1 ml/min. MS was operated in the electron impact mode with an ionization energy of 70 eV connected to computer software GCMS real time solution and postrun analysis. Ion source temperature in MS was

maintained at 200^{0}C with maintaining interface temperature as 280^{0}C. The total flow was 22.8 mL/min and the column flow of 0.94 mL/min with the linear velocity 35.4 cm/sec. Total duration of the detection was 25 minute and was run in scan mode. The detected compounds were identified by processing the raw GC-MS data and comparing with WILEY 7, FFNSC 1.2, SZTERP library mass spectral database as well as by comparison of electron-impact-mass spectra with those of relevant reference materials and the literature.

Preparation of extracts for the determination of total phenolics

The sample from Pokhara (PS, 20 g) was extracted with methanol (200 ml) in a soxhlet extractor for 6 hours. The solvent was evaporated to get the crude methanolic extract. Again, another 20 g of PS was extracted first with acetone (200 ml) followed by methanol (200 ml) in a soxhlet extraction apparatus for 6 hours. The extracts were filtered and the solvent was evaporated to get the acetone and methanolic extracts. Similarly, the other turmeric samples, 20 g of each were extracted with acetone (200 ml) for 6 hours. The solvent was evaporated to get the acetone extracts.

TLC fingerprinting of different samples

The thin layer chromatographic (TLC) behaviour of acetone extract of six different turmeric samples (PS, KS, BS, AS, CS, KS and SS) was studied on precoated silica gel plate using dichloromethane-methanol (100:3) as the developing solvent.

Determination of total phenolic content in different extracts

Preparation of standard

The total phenolic content in turmeric extracts was determined by using Folin-Ciocalteu (FC) colourimetric method based on oxidation-reduction reaction (Waterhouse 2002). Various concentrations of gallic acid solutions in methanol (100, 75, 50, 25 and 10 µg/ml) were prepared. In a 20 ml test tube, 1 ml gallic acid of each concentration was added and to that 5 ml Folin-Ciocalteu reagent (10%) and 4 ml 7% Na_2CO_3 were added to get a total volume of 10 ml. The blue coloured mixture was shaken well and incubated for 30 minutes at 40 ^{0}C in a water bath. Then, the absorbance was measured at 760 nm against blank. All the experiments were carried out in triplicate. The average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

Preparation of sample

Various concentrations of the extracts (200, 100, 50 and 25 µg/ml) were prepared. Following the procedure described for standard, absorbance for each concentration of the extract was recorded. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram dry extract (mg/g). Total phenolic content in all samples were calculated using the formula: C = cV/m where, C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/ml, V = volume of extract in ml, m = mass of extract in gram.

Results and Discussion

Composition of essential oil

The essential oil obtained by the hydrodistillation of turmeric powder was orange coloured slightly viscous liquid with characteristic spicy odour. The yield was 3.0% on dry weight basis.

GC-MS analysis of turmeric essential oil showed 75 components and 67 of them were identified on the basis of retention time and comparing with mass spectral database of standard compounds. Relative amounts of detected compounds were calculated on the basis of GC peak areas. They accounted for 98.59% of the essential oil. The essential oil contained 15 monoterpenes (5.58%), 43 sesquiterpenes (84.37%) and 10 non-terpenic components (8.64%). The major constituents were *ß*-turmeron (17.74%), *a*-turmeron **(8.19%),** *Epi-a*-patschutene (7.19%), *ß*-sesquiphellandrene (4.99%), 1,4-dimethyl-2-isobutylbenzene (4.4%) and (±-)-dihydro-*ar*-turmerone (4.27%), zingiberene (4.03%), *E-a*-atlantone (3.06%), (-)-caryophyllene oxide (3.09%). The identified compounds and their percentages are listed in Table 1. It is known that turmerone is principle flavouring compound of turmeric. Turmerone and *ar*-turmerone together with 1,8-cineol imparts a camphory characteristic pungent smell. Among turmerons, *ar*-turmerone is a major bioactive compound of *C. longa*. It has been suggested that *ar*-turmerone inhibits microglia activation, a property that may be useful in treating neurodegenerative disease (Hucklenbroich *et al*. 2014).

Table 1. Constituents of turmeric essential oil

Name	GC %	Name	GC %	Name	GC %
1,8-cineole	0.10	(*E*)-*β*-farnesene	1.04	1,7,7-trimethylbicyclo[2.2.1]hept-2-yl-3-methyl-2-butenoate	1.40
a-*?*-dimethylstyrene	0.08	*β*-cedrene	0.38	*β*- tumerone	17.74
Linalool	0.15	*a*-humulene	0.81	Epi-*a*-patschulene	3.52
1,8-menthadien-4-ol	0.15	*a*-copaene	0.14	*a*-tumerone	8.19
?-cymen-8-ol	0.83	1-(1,5-dimethyl-4-hexenyl)-4-methyl- Benzene	3.80	Spathulenol	0.42
Azulene	0.16	*β*-cedrene	0.40	Neocurdione	0.83
a-terpineol	0.08	Zingiberene	4.03	Curcumenol	1.51
4-(1-methylethyl)-benzaldehyde	0.86	Dihydroarylcurcumen	0.89	(6*R*,1'*R*)-6-(1',5'-dimethylhex-4'-enyl)-3-methylcyclohex-2-enone	1.79
2-caren-10-al	0.24	*β*-bisabolene	2.30	(-)-neocloven-(II)	0.70
4-vinylguaiacol	0.11	3,5,7,7-tetramethylcycloocta-2,4-dien-1-one	0.27	1-[1-bromo-2-ethyl-3-methylcycloprop-1-yl]cyclopent-2-en-1-ol	0.67
2-cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)	0.19	*β*-sesquiphellandrene	4.99	Artemisia ketone	0.64
d-elemene	0.18	*a*-patchoulene	0.81	Atlantone	3.06
3-allyl-6-methoxyphenol	0.26	Sesquisabinene hydrate	0.26	*β*-ionol	0.22
Aristolen	0.11	*β*-cedrene	1.14	Atlantone	0.11
4-methyl-4-phenyl-2-pentanone	0.24	Nerolidol *E*-farnesol	1.48	Allyl ionone	0.14
a-cedrol	0.48	1-(1,2,3-trimethyl-cyclopent-2-enyl-ethanone.	1.14	Artemisia ketone	0.70
β-elemene	0.81	4,5,9,10-dehydro-isolongifolene	0.31	Ledenoxide-(I)	0.38
7-epi-sesquithujene	0.47	*ar*-tumerone	0.83	2,6,10-trimethylundecan-(5Z)-2,5,9-trien-4-one	0.07
β-patchoulene	0.88	2,3-dibromo-8-phenyl-*p*-menthane	2.39	2-methyl-1-phenyl-3-(?-tolyl)-1,3-propandiol	0.13
(-)-aristolene	0.28	(-)-caryophyllene oxide	3.09	*?* -elemene	1.14
trans-caryophyllene	1.95	1,4-dimethyl-2-(2-methylpropyl)-Benzene,	4.40	Guaia-3,9-dien	0.17
(±)-dihydro-*ar*-turmerone	4.27	Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-7-(4-methyl-3-pentenyl)	3.52	*β*-humulen	0.09
Epi-*a*-patschulene	3.67			Total	98.59

Many reports about the chemical constituents of *C. longa* essential oil are available. In the rhizome oil of *C. longa* from Brazil, the main components were *ar*-turmerone (33.2%), *a*-turmerone (23.5%) and *β*-turmerone (22.7%) (Braga *et al*. 2003). In Bangladesh origin, *ar*-turmerone (27.78%), tumerone (17.16%) and culone (13.82%) were the main constituents (Chowdhury *et al*. 2008). In lower Himalayan region of Northern India, *a*-turmerone (44.1%), *β*-turmerone (18.5%) and *ar*-turmerone (5.4%) were the main constituents (Raina *et al*. 2005). Similarly, *ar*-turmerone (45.8%) and Zerumbone (3.5%) were the major component collected from Malaysia (Jantan *et al.*

1999). In contrast, bisabolene (13.9%), *trans*-ocimene (9.8%), myrcene (7.6%), 1,8-cineole (6.9%), thujene (6.7%) and thymol (6.4%) were the major component collected from Nigeria (Usman *et al*. 2009). The main constituent from Sichuan Province, China were *ar*-turmerone (49.04%), humulene oxide (16.59%) and *β*-Selinene (10.18%) (Tsai *et al*. 2011). In the oil sample from Kerela, the major components were *ar*-turmerone (31.1%), turmerone (10.00%), curlone (10.6%) and *ar*-curcumene (6.3%) (Leela *et al*. 2002). In the samples from Iran, -turmerone (68.9%) and -turmerone (20.9%) were reported as the major component (Asghari *et al*. 2009). However, in our turmeric α sample, *β*-turmeron (17.74 %) was found to be the major constituent.

Extractive values of different samples of turmeric in different solvents

For the determination of total phenolics in turmeric extracts, different extracts were prepared. The yield of the extract varied depending on the solvent and turmeric samples used. The results are summarized in table 2.

Table 2. Extractive values from 20 g of different turmeric samples and total phenolic content

Sample	†Methanol extract (g)	Total phenolic (mg/g) GAE	Acetone extract (g)	Total phenolic mg/g GAE	‡Methanol extract (g)	Total phenolic (mg/g) GAE
PS	2.98	475.00 ±1.15	2.46	360.00±0.00	0.48	147. 00 ±1.29
BS	-	-	2.69	347.00±1.50	-	-
AS	-	-	1.93	319.00±1.00	-	-
CS	-	-	1.63	293.00±1.00	-	-
SS	-	-	2.16	356.00±3.86	-	-
KS	-	-	1.98	343.00±1.63	-	-

†Direct MeOH extract of PS, ‡MeOH extract obtained after extraction of PS with acetone

The TLC fingerprinting of acetone extracts

The TLC fingerprinting of acetone extract of six different samples were studied on precoated silica gel plate using dichloromethane-methanol (100:3) as the developing solvent. The three curcuminoids, curcumin (R_f 0.56), demethoxycurcumin (R_f 0.36) and bis-demethoxycurcumin (R_f 0.22) were clearly separated in all the samples. The base line of the PS seems nearly colourless. But in other five commercial samples, the yellow colour persisted at the base line. This indicated that these samples were not seem to be pure but to some extent adulterated. However, no further investigation was carried out to identify the type of adulterants in commercial samples. The chromatogram has been shown in Figure 1.

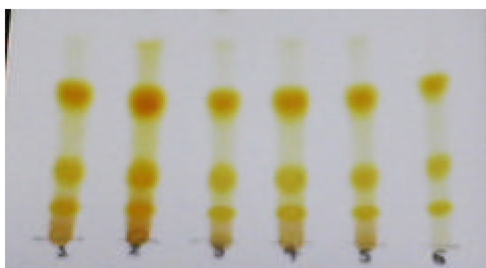

Fig. 1. TLC fingerprint of acetone extracts 1=BS, 2=AS, 3=CS, 4=SS, 5=KS, 6=PS

Total phenolic content in different plant extracts

The total phenolic content in turmeric extracts was determined by using Folin-Ciocalteu colourimetric method. Gallic acid was used as a standard compound. The absorbance values obtained at different concentrations of gallic acid was used for the construction of calibration curve (Fig 2).

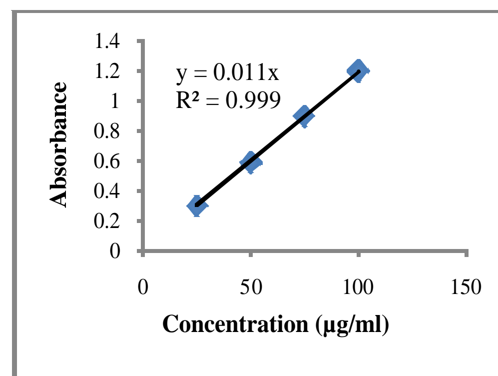

Fig. 2. Calibration curve for standard gallic acid

FC method is based on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybedic/phosphotungstic acid complexes to form blue coloured complexes, $(PMoW_{11}O_{40})^{-4}$ that

are determined spectrophotometrically at 760 nm. Total phenolic content of the extracts was calculated from the regression equation of calibration curve (y = 0.011x, R^2 = 0.999) and expressed as mg gallic acid equivalents (GAE) per gram of extract (mg/g). The results are presented in Table 2.

The results of the investigation indicated that the total phenolic content directly depends on the type of solvent used for the extraction process. Total phenolic content in methanol extract of PS was found to be the highest (475.00 ±1.15 mg/GAE). In acetone extract, total phenolic content was also very high (360.00±0.00mg/GAE). However, total phenolic content in methanol extract after extraction with acetone was found to be relatively low (147± 1.29 mg/GAE). It was reported that among different extraction solvents, ethanol extraction gives the highest yield of curcuminoids (Brag *et al*, 2003). Again in another study it was reported that acetone extract gave the optimum amount of individual curcuminoids than methanol extract (Revathy *et al*. 2011). In our study, we have prepared both methanol and acetone extract of PS for the estimation total phenolic content. Although total phenolic content in methanol extract is very high, the TLC fingerprinting of acetone extract indicated that curcuminoids were easily extracted with acetone. This was supported by the fact that the TLC examination of methanol extract obtained after extraction with acetone did not indicated the presence of three curcmminoids. This could be the reason for the relatively low amount of phenolics in methanol extract obtained after extraction with acetone. Hence acetone extract could be good source for the isolation of individual curcuminoids and we have prepared only acetone extracts for other commercial samples. Among acetone extracts, the highest amount of phenolic was detected in PS and the lowest amount of phenolic was detected in CS. There is not much variation observed in the commercial samples in terms of phenolic content. Therefore, the quality of commercial samples were not assured in terms of phenolic content.

Conclusion

The results of this investigation indicated that the chemical constituent of turmeric oil of Nepalese origin is different from that collected from other geographical regions of the world. The total phenolic content directly depends on the type of solvent used for the extraction process.

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