Physicochemical Characterization and Antioxidant Activity of Essential Oils of Guggul (*Commiphora wightii*) Collected from Madhya Pradesh

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The present study reports physicochemical characterization and antioxidant activity of essential oils extracted from guggul (*Commiphora wightii*) exudates collected from different places in Madhya Pradesh, India. The guggul exudates were hydrodistilled for 3-4 h in Clevenger apparatus. The oil obtained was dried over anhydrous Na_2SO_4 and stored at 4° until testing. Before extraction of oils from the exudates, their % moisture and tristimulus values of the colors namely L (white-black), a (green-red) and b (blue-yellow) were determined. Physicochemical characterization of the extracted oils was carried out to determine their solubility, yield%, acid value (mg/KOH/g), saponification value (mg/KOH/g), ester value, iodine value (g/g), peroxide value (mEq/kg) and Fourier transformed infrared analyses. The storage-effect on the % moisture and tristimulus values of the colors of guggul exudates as also the % oil yield and physicochemical parameters of the essential oils extracted from them, were studied using three different packaging materials viz., local plastic, low density polyethylene (200 G) and high density polyethylene (200 G). The antioxidant potential of extracted oils was evaluated by free radical scavenging activity using 1,1-diphenyl-2-picryl hydrazyl assay.

Key words: Guggul, *Commiphora wightii*, essential oil, physicochemical characterization, antioxidant activity

Guggul, an oleo gum-resin, is plant exudates of *Commiphora wightii* (Arnott.) Bhand./*Commiphora mukul* (Hook, ex Stocks) Engl., belonging to family Burseraceae (Genus: *Commiphora*) and has been in use as medicine since *Vedic* period for treatment of a number of diseases such as atherosclerosis, hypercholesterolemia, rheumatism, obesity, respiratory diseases, liver disorders, digestive problems, menstrual irregularities. Guggul is a slow-growing, highly branched shrub or small tree, grows in the arid rocky tracts of Rajasthan, Gujarat, Madhya Pradesh and Karnataka States of India and in Hyderabad, Sind and Baluchistan provinces of Pakistan. Guggul comprises 61% resin, 29.3% gum, 0.6% volatile oils, 6.1% moisture, and 3.2%

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foreign matter[1]. In Madhya Pradesh, the tapping and collection of oleo gum-resin from erect type guggul plants starts with the onset of summer and the yield is about 200-800 g per plant.

Essential oils are secondary metabolite of plants whose constituents are basically a complex mixture of terpenic hydrocarbons and oxygenated derivatives such as aldehydes, ketones, epoxides, alcohols and esters. Monoterpenes, diterpenes, and even sesquiterpenes constitute the composition of many essential oils[2]. In oleo gum-resins, oils are bound with carbohydrates and are freed by distillation. The chief components of the essential oil from the resin of *Commiphora mukul* are myrcene, dimyrcene and polymyrcene, as reported by Bhati in 1950[3]. In 1982, Kakrani reported physicochemical properties of seed oil of *Commiphora mukul*[4], and in 1985 Kakrani *et al.* reported preliminary pharmacological activity of essential oil of *Commiphora mukul*[5]. Similarly, a number of researchers have reported variations in the chemical composition of essential oils of *Commiphora* species viz. *kua*[6], *guidoni*[7], and *myrrha*[8]. Singh *et al*. have reported the antioxidant activity of guggulsterone of guggul[9]. The chemical composition and characteristics of *Commiphora wightii* seed oil were reported by Patel *et al.* in 2009[10].

The present study reports physicochemical characterization and antioxidant activity of essential oils extracted from guggul (*Commiphora wightii*) exudates collected from different places in Madhya Pradesh, India. All the chemicals, reagents and solvents used for determination of physicochemical parameters of essential oils were of analytical grade and purchased from Rankem, New Delhi, India. DPPH was purchased from Sigma-Aldrich Chemical, St. Louis, MO, USA and ascorbic acid from Merck India Ltd., Mumbai, India.

Seven exudates of guggul were collected from different places i.e., Jabalpur, Morena, Neemuch and Bhind in Madhya Pradesh, India. Older exudates of 2010 were obtained from the firms/dealers, whereas freshly harvested (2011) were collected directly from the trees. The freshly harvested guggul exudates collected from Morena packed in local plastic bag was divided into three parts. The first part was processed immediately for extraction of essential oil. The second and the third part were processed after packaging them in low density polyethylene (LDPE, 200 gauge) and high density polyethylene (HDPE, 200 G), respectively, and storing at room temperature for three months to study the storage-effect on the % moisture and tristimulus values of the colors of guggul exudates as also the % oil yield and physicochemical parameters of the essential oils extracted from them. The essential oil was extracted from the crushed guggul exudates by hydrodistillation for 3-4 h in a Clevenger apparatus adopting standard procedure[6]. The oil obtained was dried over anhydrous Na_2SO_4 and stored at 4º until testing. Before extraction of essential oils from guggul exudates, their color parameters and % moisture were determined. The tristimulus values of the colors namely L (white-black), a (green-red) and b (blue-yellow) were measured using a Hunter, LabScan XE, USA, taking average of the three replicates. Percentage moisture was determined in an oven at 42º, adopting the standard procedure.

Physicochemical characterization of essential oils was carried out according to the method described by Association of Official Analytical Chemists[11] to determine their solubility, % yield, acid value (mg/KOH/g), saponification value (mg/KOH/g), ester value, peroxide value (mEq/kg) and iodine value (g/g).

The FT-IR spectra of essential oils were recorded in an IR spectrometer (ABB Bomem Inc., Canada; model: FTLA 2000-104) equipped with DTGS detector. A small quantity (2 µl) of the oil was deposited with the help of a Pasteur pipette between two well polished KBr disks, creating a thin film. The spectra were recorded from 4000 to 500 cm^{-1} with the number of scans being 20 at a resolution of 4 cm^{-1}.

The ability of the oils to scavenge the free radicals was estimated by *in vitro* method using a stable nitrogen centered radical viz. 1,1-diphenyl-2-picryl hydrazyl (DPPH)[12]. Briefly, 0.05 ml oil, dissolved in methanol, was added to methanol solution of DPPH (100 µM, 2.95 ml) at different concentration and the absorbance was recorded at 517 nm using a UV-180 spectrophotometer, Shimadzu, Kyoto, Japan. The same procedure was followed for all oil samples and ascorbic acid was used as positive control. The free radical scavenging activity was expressed as % inhibition, calculated using the formula: % Inhibition=[(absorbance of control–absorbance of control sample)/absorbance of control]×100.

Percent moisture and tristimulus values of the colors of guggul exudates are shown in Table 1. It has been observed that the freshly harvested guggul exudates had higher % moisture and the same decreased with the passage of time. Low % moisture in the guggul exudates is advantageous for their shelf-life, enabling preservation for a longer period. Measurement of tristimulus values of the colors (L, a and b) showed that the freshly harvested guggul exudates collected from Morena had the lowest L (white-black) value (13.82), whereas the older sample (2010) obtained from M/s Rudraksha Ayurvedic Pvt. Ltd., Jabalpur, India, had the highest L value (25.91). Table 2 shows the physicochemical characteristics of the essential oils extracted from the guggul exudates vis-a-vis the oil received, as gift, from M/s Rudraksha Ayurvedic Pvt. Ltd., Jabalpur (MP) and termed as 'standard oil' for comparison. The yield of the essential oils, obtained by hydrodistillation of the crushed exudates of guggul, varied from 0.34% to 0.86% and the color from yellowish brown to dark brown. Essential oils were soluble in alcohol, chloroform, carbon tetrachloride and hexane. Acid value (mg/KOH/g) is an important index of physicochemical properties, being indicative of age, quality, edibility and suitability of oils. The lowest acid value 5.24 (mg/KOH/g) was found in the essential oil obtained from freshly harvested guggul exudates collected from Morena and the highest 25.0 (mg/KOH/g) in the oil extracted from the exudates of guggul received from M/s Rudraksha Ayurvedic. The high acid value is indicative of oil becoming rancid due to storage of guggul exudates under improper conditions or adulteration there in. Saponification value (mg/KOH/g) gives an idea of the average molecular weight or the chain length of all the acids present. Higher the molecular weight, the lower the saponification value and being inversely related. The highest saponification value was found in the oil extracted from the exudates of guggul obtained from M/s Rudraksha Ayurvedic. The high saponification value of the oil is suggestive of the presence of high molecular weight fatty acids in it. Ester value is the difference of saponification value and acid value[13]. The peroxide value (mEq/kg) of an oil or fat is used as a measurement of the extent of rancidity reactions. Air, or specially oxygen in the air, can react with the oil and form various peroxide components which eventually affect odor, flavor and quality. Lower the peroxide value, the fresher the oil would be. In general, peroxide levels higher than 10.0 means less stable oil with a shorter shelf life[14]. Out of the seven essential oils obtained by hydrodistillation of the guggul exudates, the highest peroxide value was found in the oil extracted from the exudates of guggul obtained from M/s Rudraksha

TABLE 1: PERCENT MOISTURE AND TRISTIMULUS VALUES OF THE COLORS OF GUGGUL EXUDATES

Sample-details	% moisture	Tristimulus values of the colors*		
		L	a	b
Guggul exudates (2010) from Jabalpur (MP)	1.83	25.91	5.44	12.72
Guggul exudates (2010) from Neemuch (MP)	1.90	20.88	5.42	12.80
Guggul exudates (2010) from Morena (MP)	1.97	22.33	4.35	12.58
Guggul exudates (2011) from Morena (MP) packed in local plastic bag	2.57	13.82	5.68	8.91
Guggul exudates (2011) from Morena (MP) packed in LDPE (200 gauge) and stored for a period of three months	2.30	17.58	6.19	10.77
Guggul exudates (2011) from Morena (MP) packed in HDPE (200 gauge) and stored for a period of three months	2.38	17.24	6.10	11.03
Guggul exudates (2011) from Bhind (MP)	2.22	18.26	4.83	11.21

*Average of three replicates, LDPE=low density polyethylene, HDPE=High density polyethylene

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF THE EXTRACTED ESSENTIAL OILS FROM GUGGUL EXUDATES

Parameters	Std. oil*	Oil 1	Oil 2	Oil 3	Oil 4	Oil 5	Oil 6	Oil 7
Yield%	-	0.34	0.60	0.60	0.86	0.65	0.70	0.57
Solubility	A, Cf, Ct, H	A, Cf, Ct, H	A, Cf, Ct, H	A, Cf, Ct, H	A, Cf, Ct, H	A, Cf, Ct, H	A, Cf, Ct, H	A, Cf, Ct, H
Acid value (mg/KOH/g)	6.0	25.0	7.43	5.85	5.24	5.96	5.32	5.28
Saponification value (mg/KOH/g)	117.93	258.89	83.98	148.23	60.89	78.15	60.81	69.54
Ester value	111.93	233.89	76.55	142.38	55.65	72.19	55.49	64.26
Peroxide value (mEq/kg)	5.64	8.74	6.86	6.08	4.38	6.44	5.88	5.06
Iodine value (g/g)	43.187	Rancid	90.34	90.57	41.02	84.64	42.08	42.14

*Oil received from M/s Rudraksha Ayurvedic Private Limited, Jabalpur (M.P.) as gift and termed as 'standard oil', A=Alcohol, Cf=Chloroform, Ct=Carbon tetrachloride, H=Hexane

Ayurvedic which also had highest acid value. Its iodine value could not be determined because of its rancidity. On the other hand, the low acid value and the low peroxide value of the remaining six essential oils are indicative of their resistance toward lipolytic hydrolysis and oxidative deterioration. Iodine value (g/g) indicates the number of double bonds present and, therefore, the degree of unsaturation. The higher the iodine value, the more the double bonds in the molecule as also the oil being more prone to rancidity. The iodine value of all the six essential oils, except oil No. 1, is below 100, which shows the presence of saturated fatty acids and places them in the category of non-drying oils[15].

Out of the two LDPE and HDPE packaging materials used for storage of freshly harvested guggul exudates over a period of 3 months, the % oil yield as also the physicochemical parameters were found to be better in the exudates packed in HDPE than LDPE because of the fact that HDPE has lower O_2/CO_2 permeability than LDPE at 25º[16]. Further, the high temperature during tapping and harvesting of guggul exudates has a definite effect on the % oil yield. The % yield of essential oils obtained from the freshly harvested guggul exudates collected from Bhind was 0.57%, where the temperature had been around 47.8º at the time of collection of the guggul exudates. Thus, the variations in % oil yield and physicochemical parameters of the extracted essential oils could be attributed to the quality of the exudates, their storage-period, the type of packaging materials used and the temperatures at the collection-points.

FT-IR spectroscopy allows the qualitative determination of organic compounds as the characteristic vibrational mode of each molecular group causes the appearance of bands in the infrared spectrum at a specific frequency, which is further influenced by the surrounding functional groups[17]. Earlier researchers have used mid-infrared spectra for characterization of edible oils and fats[18-20]. The FT-IR spectra of three oil samples viz., oil No. 4, 'standard oil' and oil No. 6 are shown as fig. 1a-c, respectively. At higher frequencies i.e., 3700-3400 cm^{-1}, the broad band observed in the spectra of guggul oils can be related to the intermolecular hydrogen-bonded O–H stretching vibration of water (H–OH), hydroperoxides (ROOH) and their break-down products, namely alcohols (ROH). The 3025-2850 cm^{-1} region is known as the absorption zone of C–H stretching vibration of methylene and terminal methyl groups of fatty acid chains[18,20]. In conformity with this, the intensive band at 2926 cm^{-1} and 2924 cm^{-1} is present in all the three spectra. However, in FT-IR spectrum of oil no. 4, (fig. 1a), extracted from the freshly harvested guggul exudates from Morena, Madhya Pradesh, India, the intensive band at 2926 cm^{-1} is also accompanied by a $-CH_2$ group shoulder at 3069 cm^{-1}. In the centre of all the spectra, the bands at 1734 cm^{-1} and 1714 cm^{-1} are assigned to the C=O stretching absorption of the triglycerides ester linkages and similarly the bands at 1667, 1644 and 1635 cm^{-1} are assigned to C=C stretching vibrations. The bands at 1383, 1377 and 1350 cm^{-1} are associated with the symmetrical C–H bending vibrations of $-CH_3$ groups, while the bands at 1456, 1453 and 1445 cm^{-1} are associated with the asymmetrical bending vibrations which overlap the C–H bending vibrations of methylene groups. The bands at 1249, 1242 and 1239 cm^{-1} are associated with the stretching vibrations of C–O ester groups. The olefinic C–H out-of-plane bending vibration bands are obtained at 976, 950 and 887 cm^{-1}[21,22].

The antioxidant potential of four oil samples viz. 'standard oil', oil Nos. 1, 4 and 6 (Table 2) was determined by DPPH-free radical scavenging activity. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color typical of free DPPH radical decays, and the absorbance change at λ=517 nm is measured. The test provides information on the ability of a compound to donate a hydrogen atom[23]. Among all the four oils, the highest % inhibition was found in the 'standard oil' (89%) and

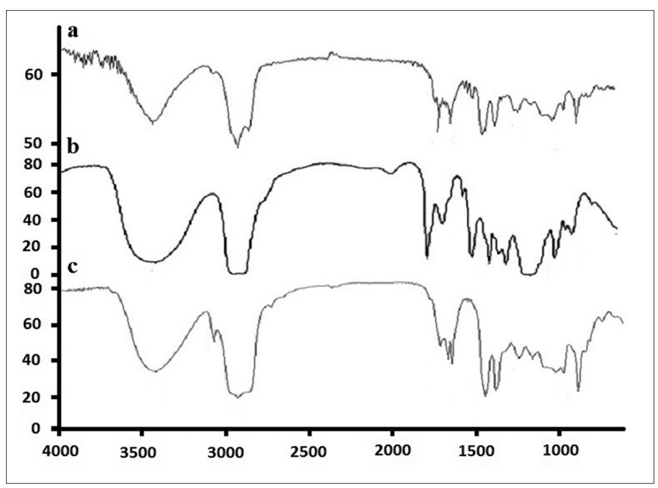

Fig. 1: FT-IR spectra of guggul oil samples.
FT-IR spectra of guggul oil (a) freshly collected exudates from Morena (MP); (b) M/s Rudraksha Ayurvedic Pvt. Ltd., Jabalpur (MP); (c) freshly collected exudates from Morena (MP) after packaging it in HDPE (200 G) and storing for a period of 3 months.

the lowest in the oil no. 1 (10.7%) as compared with the positive control (ascorbic acid) which showed 94% inhibition.

From the results of the present study, it may be concluded that the variations in % oil yield and physicochemical parameters of the extracted essential oils could be attributed to the quality of the exudates, their storage-period, the type of packaging materials used and the temperature at the collection-points of guggul exudates. The essential oils extracted from guggul exudates can be exploited as a source of antioxidant agent, besides their physicochemical parameters being comparable with the virgin oils.

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