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Full Length Research Paper

Physicochemical, thin layer and gas-liquid chromatographic analysis of ungrafted desi mango flower oil and mineral estimation in its flowers

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In the present work, flower oil of a local cultivar of *Mangifera indica* L. called ungrafted desi mango was subjected to physicochemical analysis and thin layer (TLC) and gas-liquid chromatography (GLC). Oil was extracted from the flowers in hexane using Soxhlet apparatus and the yield was 1.63%. The saponification and iodine values were 180 and 75.23, respectively, while the percentage of unsaponifiable matter and free fatty acid content were 1.46 and 6.60%, respectively. The moisture content in the flowers was 0.15%. Hydrocarbons, wax ester, sterol ester, triglycerides, fatty acids, alcohols, sterols, diglycerides, monoglycerides, and phospholipids were identified in the oil with TLC by comparing their Rf values with standard compounds. Fatty acid composition of the oil was determined with GLC which showed the presence of C_{12} to C_{20} fatty acids in different amounts, $C_{18:0}$ being the highest (51.5%) and C_{12} and C_{14} very low. Macro and micro mineral elements were estimated in flowers of the mango plant using atomic absorption spectrophotometer. The amounts in ppm were sodium (693.68), potassium (629.25), iron (253.04), nickel (2.17), zinc (24.28), copper (23.24) and lead (9.31).

Key words: *Mangifera indica*, ungrafted desi mango, physicochemical, flower oil, mineral elements.

INTRODUCTION

Mangifera indica L. (family, Anacardiaceae) is a tropical tree considered to be indigenous to the Indo-Pakistan subcontinent and has been grown here for thousands of years (Thuma, 2001). The plant, with its numerous varieties, is known for its delicious fruit, which is produced on large scale in Asia, America and Africa (Ross, 1999; Rathore et al., 2007; Awan, 2008). The mango cultivation period in Pakistan is between December and February. The yellowish or reddish mango flowers are produced in the form of long inflorescences each consisting of nearly 2000 small flowers, and the mango fruit grows at the end of the long string like stem which is the former panicle (Litz, 2009; Anonymous, 2012). Most studies on *M. indica* have been focused on its fruit, mainly due its nutritional value, but also, for the medicinal reasons (Medlicott and Thompson, 1985; Bartley, 1988; Lasztity et al., 1988; El-Samahy et al., 2000; Ang and Ng, 2000; Shang et al., 2002; Pino et al., 2006; Quijano et al., 2007; Parmar and Anand, 2008; Pandit et al., 2009; Jha et al., 2009; Akhtar et al., 2010). Some studies have also been carried out on the other parts of the plant, such as leaves (Severi et al., 2009; Elzaawely and Twata, 2010), bark (Nong et al., 2005) and seed kernel (Ali et al., 2011).

However, very little attention has been given to mango flowers. Recently, volatile chemical components of mango flowers were studied using different techniques (Phutdhawong et al., 2007; Sandoval et al., 2007; Wang et al., 2010). To the best of our knowledge, no study has so far been reported on the physicochemical analysis of mango flower lipids, thus, the objective of the present study was to analyze the n-hexane extract of the flowers

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of ungrafted mango of Pakistan, which is locally known as desi mango.

The oil was subjected to physicochemical analysis, and its composition was determined with thin layer (TLC) and gas-liquid chromatography (GLC). Mineral elements were also estimated in the flowers.

MATERIALS AND METHODS

Collection of mango flowers and isolation of oil

For the present study, ungrafted or desi mango flowers were collected from a mango garden in Multan, Pakistan. For oil extraction, the flowers (fresh mass 533 g) were allowed to dry in shade for seven days. The dried flowers were ground to obtain a powder, which was heated for about 2 h in an oven at 105°C to remove moisture. The dried material (440 g) was stored in a desiccator. From the dried powder of the mango flowers, oil was extracted using Soxhlet apparatus. Distilled hexane was used as the solvent and the extraction was carried out for 3 h. The percent yield of the oil was calculated on the basis of the dried material. Whole flowers inflorescence was used for the estimation of nutrient elements.

Chemicals and apparatus

All the chemicals used were of analytical grade. Refractive index of the oil was determined with Abbe Refractometer. Schimadzu Ge equipped with flame ionization detector (FID) and a glass column PEG (3 m × 3 mm I. D.) was used for gas chromatography.

Physicochemical analysis

Free fatty acid value

Free fatty acid content in the oil was determined by titrating the oil with aqueous potassium hydroxide (KOH). To 2 g of oil in a 250-ml conical flask, 50 ml of solvent mixture of isopropyl alcohol : toluene (1:1) was added and mixed thoroughly. The mixture was heated to boiling and was titrated with 0.1 N KOH solutions using phenolphthalein as indicator.

Iodine value

Iodine value is the measure of the unsaturation of oils and is expressed as the number of grams of iodine absorbed by 100 g of an oil. After dissolving the flower oil (0.1158 g) in 15 ml of CCl_4, 25 ml of Wijs' reagent (0.1 mol/L iodine monochloride solution in acetic acid) was added and the content was mixed well by swirling. Then, the stoppered flask was kept in dark for 30 min to complete the reaction of iodine with unsaturated oil molecules. Then, 20 ml of 10% KI solution and 100 ml of distilled water was added, and the mixture was titrated with 0.1 N standard sodium thiosulfate solution using starch solution as indicator. The blank sample was titrated in the same way. The difference between the blank and the sample showed the amount of iodine absorbed by the oil.

Saponification value

Saponification value is the number of milligrams of KOH required to hydrolyze (saponify) 1 g of oil. In a 250-ml flask, 5.0 g of oil was mixed with 50 ml of 0.5 N alcoholic KOH. For hydrolysis to occur, the content was refluxed on a water bath for about 30 min till a clear solution was obtained. This was titrated with 0.5 N HCl solution using phenolphthalein as indicator to estimate the amount of unreacted alkali. The same procedure was repeated with blank that comprised of 50 ml of 0.5 N alcoholic KOH and no oil.

Unsaponifiable matter

Essential oils and other substances which are not hydrolyzed are termed unsaponifiable matter. The mango flower oil (0.5 g) was refluxed with 25 ml of 0.5 N alcoholic KOH for 2 h. This hydrolyzed the saponifiable matter. The unsaponifiable matter from this was extracted with diethyl ether using a separatory funnel. After removing moisture from the ether layer with anhydrous sodium sulfate, diethyl ether was removed under reduced pressure and the unsaponifiable matter was weighed.

Unsaponifiable matter (%) = [Mass of unsaponifiable matter/Mass of sample] × 100

Identification of lipid components with TLC

The mango flower oil, dissolved in hexane-acetic acid (80:20), was subjected to thin layer chromatography on plates (20 × 20 cm) having 0.25 mm thick silica gel layer, air dried and activated in an oven at 105°C for 1 h. The solvent system was: hexane-diethyl ether-acetic acid (78:20:2), and 2,7-dichlorofluorescein was used as locating reagent to get purple yellow coloured bands under ultraviolet light at λ 366 nm (Bello-Perez et al., 2005).

Determination of lipid composition with GLC

The mango flower oil was treated with a mixture of methanol and boron trifluoride (BF_3) to convert the free fatty acids present in the oil into their methyl esters (Morrison and Smith, 1964). The methyl esters were then subjected to gas liquid chromatography. Nitrogen gas was used as carrier gas. The temperature of detector and injector was maintained at 250 and 230°C, respectively. The fatty acids were identified by comparing their retention times with those of the standard methyl esters chromatographed under the same condition of temperature.

Moisture content

The moisture content of the flowers was determined following a reported method (Ashraf et al., 2011). Briefly, the dried sample (3 g) was first incinerated at low temperature and then kept at 550°C in a Muffle furnace until a light gray ash residue was obtained.

Determination of mineral elements with atomic absorption spectrophotometer

Mineral elements present in the flowers of ungrafted desi mango were determined on atomic absorption spectrophotometer using a reported method (Ahmed et al., 2011) with slight modification. Concentrated sulfuric acid (two drops) was added to the plant sample in a China crucible and the content was heated on a burner for 10 min. It was then strongly heated in Muffle furnace at 700°C. The ash obtained was dissolved in distilled water in a 50-ml measuring cylinder and the volume was made up to the mark. Standard solutions of different elements were used for calibration of the instrument.

Table 1. Physicochemical evaluation of flower oil of ungrafted desi cultivar of mango (*M. indica* L.).

S/N	Physicochemical parameter	Value
1	Oil yield	1.63%
2	Moisture content	0.15%
3	Refractive index (40°C)	1.47
4	Saponification value	180
5	Iodine value	75.23
6	Unsaponifiable matter	1.45 %

Table 2. TLC of flower oil of ungrafted desi mango (*M. indica* L.), the standard Rf values and identified oil components.

S/N	Standards used	Rf value	Oil component
1	Octadecane	0.83	Hydrocarbon
2	Bees wax (WE)	0.74	Wax ester (WE)
3	Cholesterol octadecanoate (SE)	0.66	Sterol ester (SE)
4	Tristerin (TG)	0.56	Triglycerides (TG)
5	Stearic acid (FA)	0.49	Fatty acids (FA)
6	Cetyl alcohol (AL)	0.30	Alcohols (AL)
7	Cholesterol (ST)	0.17	Sterols (ST)
8	Disterin (DG)	0.18	Diglycerides (DG)
9	Monopalmitein (MG)	0.15	Monoglycerides (MG)
10	Lecithin (PL)	0.07	Phospholipids (PL)

Solvent system: n-hexane/diethyl ether/acetic acid (80/20/2); adsorbent: Silica gel (Merck Reference No. 7739 HF_{254}).

RESULTS AND DISCUSSION

The oil was extracted in n-hexane from the flowers of ungrafted desi mango (*M. indica* L.) collected from Multan, Pakistan, and subjected to analysis of a number of physico-chemical characteristics. The refractive index, iodine value, free fatty acid value, saponification value, and unsaponifiable matter were evaluated using standard methods. The chemical composition of the oil was determined by TLC and GLC. Moisture content and mineral elements present in the flowers were also estimated.

Free fatty acids

The percentage of free (or non-esterified) fatty acids in the oil extracted from ungrafted mango flowers was found to be 6.6%. Although, fatty acids are a good source of energy, recent inconclusive studies have shown that they are somehow linked with obesity and diabetes (Charlotte Grayson, 2011). Thus, it is always desirable to know the percentage of free fatty acids in a fat or oil before consumption.

Unsaponifiable matter

The oil extracted from the flowers of ungrafted desi Mango contained 1.41% unsaponifiable matter. The unsaponifiable matter in flower oil contains compounds that are not hydrolyzed or neutralized with alkalis. TLC (Table 2) showed the presence of alcohols, hydrocarbons and sterols in the flower oil. Previous studies on the volatile components from the mango flower showed the presence of a number of terpenoids which are part of the unsaponifiable matter of the flowers (Sandoval et al., 2007; Wang et al., 2008).

The oil extracted from the kernel of mango (Ali et al., 2007) had a lower value of unsaponifiable matter. This showed that the flower oil had higher quantity of unsaponifiable matter than the oil extracted from the kernel of mango fruit.

Saponification value

The saponification value of the flower oil of ungrafted desi mango was determined using standard method and the result is given in Table 1. Most common vegetable oils

Table 3. Fatty acids C_{12} to C_{20} composition analysis of the flower oil of ungrafted desi mango (*M. indica* L.) by GLC.

S/N	Fatty acid	Composition (%)
1	$C_{12:0}$	Traces
2	$C_{14:0}$	Traces
3	$C_{16:0}$	7.84
4	$C_{16:1}$	3.15
5	$C_{18:0}$	51.5
6	$C_{18:1}$	28.98
7	$C_{18:3}$	-
8	$C_{18:2}$	7.91
9	$C_{20:0}$	0.18

Table 4. Mineral elements in flower of ungrafted desi mango (M. *indica* L.).

S/N	Metal	Amount (ppm)
1	Sodium	693.68
2	Potassium	629.25
3	Iron	253.04
4	Nickel	2.17
5	Zinc	24.28
6	Copper	23.24
7	Lead	9.31

have saponification value of 190 to 195. Values higher than 195 indicate a higher quantity of fatty acids with chain length shorter than 18 carbons, such as that found in butter fat, palm kernel and coconut oils. Our saponification value was low (180) which indicated the presence of large amount of unsaponifiable matter and/or the presence of fatty acids with chains longer than 18 carbons, the first possibility being supported by the TLC and GLC analysis.

Iodine value

The iodine value of oil is a measure of its unsaturation, that is, how much unsaturated fatty acid content is present in the given oil. Our value (75.23) was higher than that reported by Ali et al. (2007) from mango kernel oil suggesting that mango flowers oil contained higher concentration of unsaturated oils than its kernel oil (Bello-Perez et al., 2005).

Refractive index

Refractive index of oil sample was found to be 1.47 at 40°C, which was similar to that found by Wang et al. (2008) for Chinese mango flower oil. The refractive index which increases with the increase in unsaturation and the length of carbon chain, can give an idea of the fatty acid content of the oil (Ali et al., 2007).

Analysis of oil composition with TLC

The results of thin layer chromatography experiment are as shown in Table 2. Different standards including octadecane (HCN), bees wax (WE), cholesterol octadecanoate (SE), tristerin (TG), stearic acid (FA), cetyl alcohol (AL), cholesterol (ST), disterin (DG), monopalmitein (MG), and lecithin (PL) were used to compare the Rf values with the compounds from the mango flowers oil. The coloring reagent 2,7-dichlorofluorescein was used to see the spots under ultraviolet (UV) light at λ 356 nm. The mango flowers oil, thus, contained a number of saponifiable and unsaponifiable components, including hydrocarbons, wax ester (WE), sterol esters (SE), triglycerides (TG), fatty acids (FA), alcohols (AL), sterols (ST), diglycerides (DG), monoglycerides (MG), and phospholipids (PL).

Identification of oil components with GLC

The fatty acids present in the mango flower oil were analyzed by GLC as their methyl esters and the results are displayed in Table 3. Mango flower oil contained a number of fatty acids which included C_{12} to C_{20} saturated, monounsaturated and polyunsaturated fatty acids. The present research work can be compared with previous work on desi mango kernel oil by Ali et al. (2007). The amount of fatty acids with chain length C_{12}, C_{14}, C_{16}, and C_{20} was lower in flower oil than that found in the kernel oil, while the percentages of $C_{16.1}$, $C_{18.0}$, $C_{18.1}$ and $C_{18.2}$ were higher than those of the kernel oil. In the flower oil, $C_{18:0}$ was 51%, while $C_{18:1}$ was 29%. Other acids were in lower quantities.

Moisture content

Moisture content in the flower of desi mango was determined and the results are as shown in Table 1. A number of workers studied the moisture content in different varieties of mango (Jha et al., 2009; Peter et al., 2006; Nzikou et al., 2010; Bello-Perez et al., 2005). These findings show variation depending on the climate and weather conditions.

Mineral element analysis

The flowers of ungrafted desi mango variety were subjected to the estimation of mineral elements, sodium, potassium, iron, copper, zinc and lead, using atomic absorption spectrophotometer and the results are as shown in Table 4. The table shows that the flowers of desi mango are good source of some of the mineral elements. They contained good amounts of potassium, iron and zinc. The quantity of sodium is slightly higher than that of potassium. Although, flowers are non-edible

part of mango tree, they can be used as source of some of the nutrient elements. For instance, they contained notable amount of iron (253.04 ppm) which is an essential nutrient and part of hemoglobin.

Conclusion

The oil extracted form ungrafted desi mango flowers was studied for various physicochemical parameters. Sodium, potassium, iron, copper, zinc, nickel and lead were estimated in the whole flowers. The chemical composition of the flower oil was studied with TLC and GLC, and a number of constituents were identified. The oil was a mixture of saturated and unsaturated components, and contained a good quantity of unsaponifiable matter.

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