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EVALUATION OF ESSENTIAL OIL COMPONENTS FROM THE FRUIT PEELINGS OF SINDHRI AND LANGRA VARIETIES OF MANGO (*MANGIFERA INDICA* L.)

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Abstract

The present study was carried out to evaluate essential oil contents found in the fruit peelings of the two varieties of Mango (*Mangifera indica* L.), belonging to family Anacardiaceae which is commonly called Cashew family. Genus *Mangifera* has about forty species in S.E. Asia and Indo Malaya region. Several Mango varieties are cultivated in many areas of Pakistan. For this study GC-MS was used for the characterization of the extracted essential oil. Two Mango varieties namely, Sindhri and Langra were selected from Mirpurkhas district. Essential oil was extracted from Mango peelings by hydro distillation method. The total 34 essential oil components ranging between 0.16-49.4% identified from the Sindhri and Langra Mango varieties. Bicyclo [4.1.0] hept-3-ene, 3, 7, 7-trimethyl-, (1S) was found abundant in both varieties with 49.46% and 47.93%, respectively. Yield of essential oil was found to be 3.25% in fresh Mango fruit peelings of Sindhri, whereas 1.04% was present in Langra variety. Result of present study indicated that peelings of Mango varieties could be used as a source of many useful components.

Key words: Anacardiaceae, *Mangifera indica* varieties, Sindhri, Langra, Essential oil.

Introduction

Mango, famously known as the "king of fruits" and occupied the second place as major fresh fruit in Pakistan. Mango is very popular fruit grown in tropical and sub-tropical environment and very rich source of various vitamins including A, B and C as well as it contains water, sugar, protein, iron, fats and fibers. It was originated in India more than 4000 years ago. Historically this fruit had gained great importance in India during the rule of Mughal Emperors. The famous king Akbar-e-Azam, who planted Lakh bag (one lac Mango trees), amateur, gardeners, nurserymen and farmers by means of selection and cloning**.** (Muhammed Usman *et al.*, 2001).Pakistan occupies the 5th place in Mango producing country and the 3rdlargest exporter of Mango in the world (Saeed Akhtar *et al*., 2010). Each cultivar has its own distinctive shape, size, color, aroma, flavor and pulp consistency (Ansari *et al*., 2004). Mangos produced in Sindh are mostly greenish yellow in color, very sweet in taste and with strong aroma. Most varieties of Mangos produced mainly in the different districts of Sindh province like Mirpurkhas, Hyderabad, Tndo Allahyar, Mitiari, Sanghar and Naushehro Feroze. Approximately 400,000 lac tons Mangos are produced in Sindh annually; about 40 percent of Mangos are exported to different countries. Main importers from Pakistan are Dubai, UK, and Saudi Arabia (Saeed Akhtar *et al*., 2010). Sindh covers 60467 hectares under Mango cultivation and the production was0.4 million tons in 2014.Mirpurkhas district has the highest Mango growing area with 12196 hectares under Mango cultivation**.** About 60 percent of the productions of Mango are consumed locally; Sindhri and Langra varieties of Mango are famous in Pakistan. Beside Sindhri and Langra other common commercial Mango varieties are grown in Sindh province are Gulab khasa, Dusehri, Saroli, Chaunsa, Neelum, Almas, Desi and Banganpali.

The essential oils are highly volatile components; found in leaves, flowers, pulp peeling, and in roots. Two varieties of Mango namely, Sindhri and Langra were selected for the extraction of essential oil from fruit peelings. Utilization of Mango peel for industrial purpose, (Rashad *et al*., 1990) must be encouraged more than before. Therefore, this research on essential oil components from fruit peeling eventually enhanced the chances to improve the aromatic quality of Sindh Mangos as well as increased the possible measures for utilization of Mango fruit peeling oil in industries. The volatile components of Mango have been extensively investigated (Eloisa Helena *et al*., 2000) and (Hong-Wu Wang, 2010). The aim of study was to separate, identify and quantify the essential oil components from the peeling of two different varieties of Mango and to compare them with each other. For the experiment hydro distillation method was used to isolate essential oil from samples. Gas chromatography and mass spectrometry GC-MS was used to separate determine and quantify the components **(**Laohaprasit *et al*., 2012).

Materials and Methods

Sample collection: Fully ripped Mango fruits were collected from Mirpurkhas, Sindh, Pakistan. For the extraction of essential oil two Mango varieties (Sindhri and Langra) were selected for present study. Samples were washed with distilled water, peeled before analysis (Laohaprasit *et al*., 2012). Peelings were cut into fine pieces (Laohaprasit *et al*., 2012).

There are several extraction methods that can be used to isolate the volatile compounds the most popular being solvent extraction **(**Laohaprasit *et al*., 2012).Oil was extracted by hydro distillation method. Hexane (C_4H_{10}) is

a colorless non polar alkane with 68ºC boiling point was used as an extraction solvent. Hexane is a most efficient solvent because maximum number of essential oil components was extracted with larger concentrations. For oil extraction 250g of each variety of Mango fruit peelings was collected and oil was extracted by hydro distillation method. Assembly for the extraction of essential oil was run for4 hour. Fresh peels were taken in the round bottom flask measuring 1000 ml. Flask was filled with 400 ml of distilled water. The peelings were heated to boiling point. Another flask connected with condenser on the other side which contained small quantity of hexane for the extraction of oil. Traces of extracted essential oil travel with steam from round bottle flask through the condenser to the collecting flask. Steam condensed to water and oil was collected in an adjacent flask. After the extraction collected material was transferred into the separating funnel. Material was separated, when settled down. The extract was collected in a vial and weighed, water was eliminated.

The concentration of detected individual essential oil components were calculated on the basis of gas chromatographic area percentage, the sample weight and oil the weight (Hirotoshi Tamura *et al*., 2001). Yielded oil from Sindhri variety was 3.25% whereas as it was1.04% in Langra variety.

For getting the essential oil from 1kg fresh fruit peel of Mango varieties method was repeated several times. Care was taken while doing the procedure because essential oil is highly volatile and can be evaporating very fast. The essential oil samples extracted from Mango varieties were collected in vials and were covered with aluminum foil preserved at 4ºC for analysis (Laohaprasit *et al*., 2012). Three extractions of each sample were collected and essential oil was analyzed by GC-MS techniques for the separation and identification of the components from the samples of both varieties.

GC-MS analyses: The GC-MS analysis was carried out by using Agilent 6890 N gas chromatography and mass spectrometry. Combination of two powerful techniques coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent auto sampler 7683-B injector. For the separation of essential oil components from the mixture Gas chromatography was used and Mass spectrometry was used to identify and characterizes each component individually. GC is used to separate mixture into individual component using a temperature controlled capillarycolumn. MS is used to identify individual components from mass spectra by compared them with mass spectral data bases. A capillary column of HP-5MS (5% phenyl 1 methylsiloxane) 30mx 0.25mm i.d.; with 250 µm diameter and film thickness was 0.25 µm. The initial temperature of 100ºCwas hold for 1 min, increased to 200ºC at 5ºC/min. Split ratio was 5:1. Helium was used as carrier gas, with flow rate of 9.5 ml/min. The inlet/ injector and detector/transfer line temperatures were 260ºC and 270ºC respectively. A volume 1.0 µL sample was injected with syringe size 10.0 µL. Total sample run time was 31 minutes.

Identification of essential oils: The major volatile compounds found in the two varieties of Mango are monoterpene and sesquiterpene hydrocarbon (Pandit *et al*., 2009). The component of essential oils was identified through their retention indices (RI) with reference to the computer matching with MS-data library (Ghangro, 2013). The quantification of relative amount of the each individual essential oil component acquired was carried out by the area percentage method.

Relative percentage of each component was obtained by using following equation:

$$\text{Rel. \%} = \frac{\text{Conc. x 100}}{\text{Sum (all conc.)}}$$

where: Concentration is area percentage of individual essential oil component and sum is total of all area percentage.

Results and Discussions

Fully ripened peeling of two varieties of *Mangifera indica* L., namely Sindhri and Langra were used for the extraction of essential oil. The similarities and variations in the concentrations of essential oil components of two varieties of Mangoes were compared. Total 34essential oil components with particular aromas were identified in Sindhri and Langra varieties (Figs. 1 and 2). Many monoterpene, sesquiterpene and alcohol give flavor and fragrance to different Mango varieties. Large number of alkanes was also identified as they gained great importance as many drugs, pesticides and other chemicals synthesized from it. Some volatile compounds are essential contributors to Mango flavor (Malundo *et al*., 1996). Caryophyllene has a spicy, terpenic and woody odor and taste is woody, pepper-like spicy with citrus background (Laohaprasit *et al*., 2012). 3-Carene gives Mango flavor and aroma (Laohaprasit *et al*., 2012). Borneol with camphor like odor used to make perfume it is a natural insect repellant.

Mango peelings are the waste product that can be used for oil extraction because it the most fragrant part of the Mango fruit, which contain volatile components. Results indicated that very valuable components are found to be present in the peel of both Mango varieties with larger concentration of essential oils i.e., 49.4%, 47.93% respectively.

During this study following 34 essential oil components were identified in the Sindhri and Langra Mango varieties (Table 1).

In current studies which comparing all essential oil components, 11 essential oil components were found to be common in both varieties with different concentrations. Bicyclo [4.1.0] hept-3-ene, 3, 7, 7-trimethyl-, (1S), was found abundant in both varieties. It was also observed in current studies that some particular compounds are responsible to give distinctive flavor and fragrance to different Mango varieties. 3-carene; borneol and caryophyllene give the particular light camphor type aromatic flavor to Sindhri Mango variety. While, 3-carene, caryophyllene attribute the particular turpentine flavor to Langra Mango varieties.

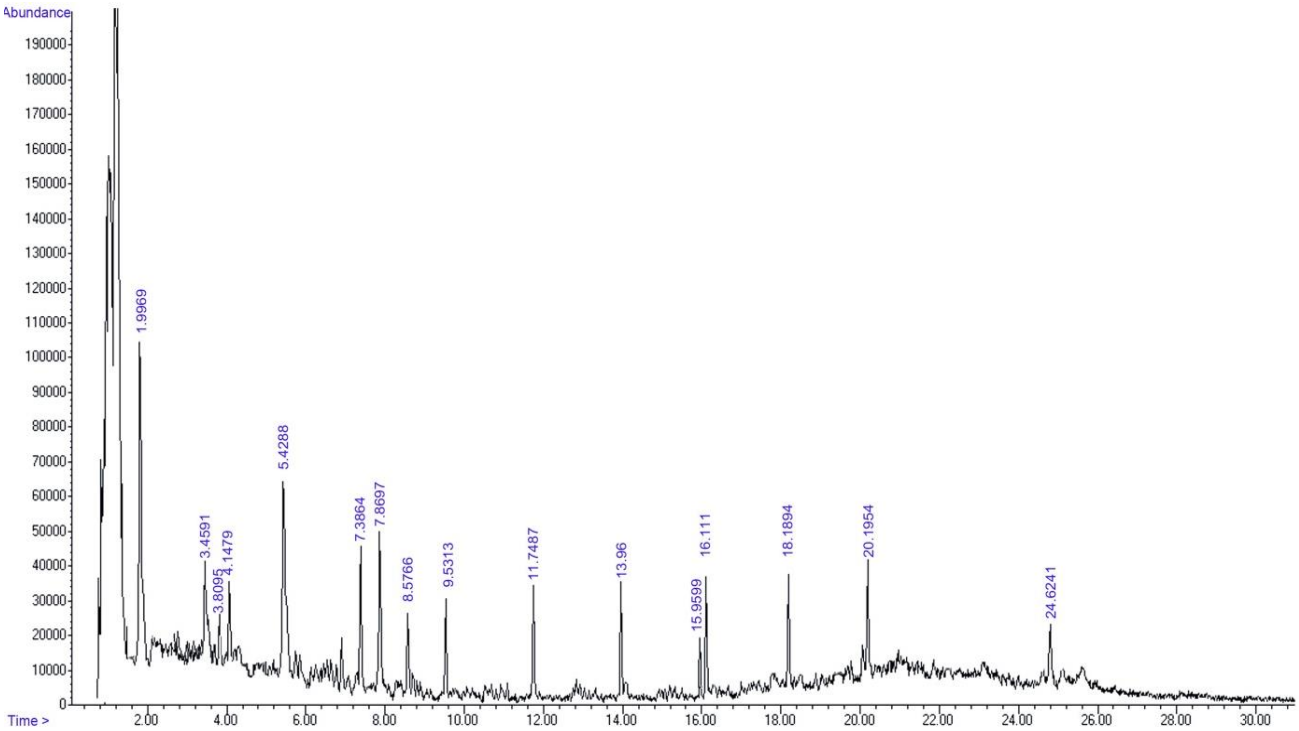

Fig. 1. GC-MS chromatogram of the hydro distilled sample of Sindhri variety of Mango.

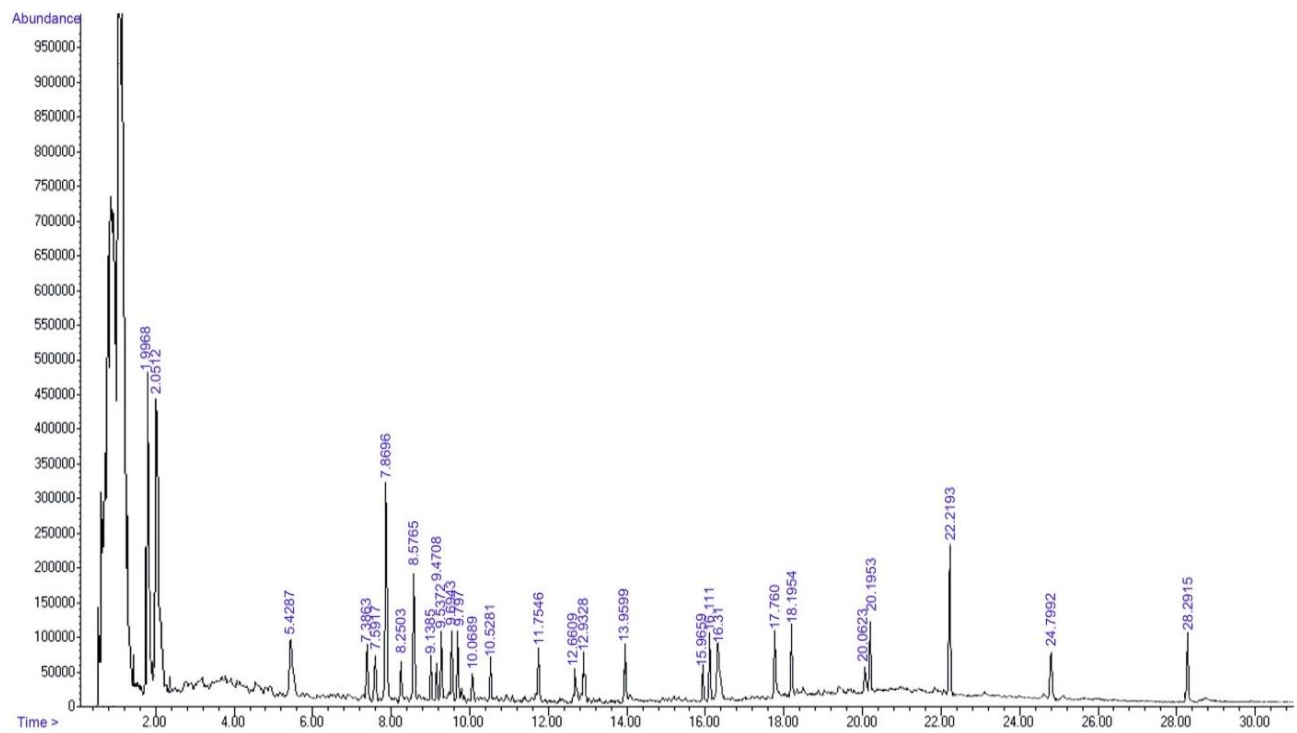

Fig. 2. GC-MS Chromatogram of the Hydro distilled sample of Langra variety of Mango.

Results of present study clearly indicated that essential oil extracted from peelings of different Mango varieties is a good source of many valuable components. Many monoterpene, sesquiterpene, alkane, alcohol, ether and antioxidant are found to be present in the fruit peelings of Mango varieties. Economically they are very important and can be used in different industries like perfumes, cosmetic and food. Medicinally they are useful for the prevention of different cancers. Another benefit of research is that some of essential oil components can be used as insecticide, because of their insect repellant property. Essential oil components of both varieties with their percentage were compared (Table 2.). Some essential oil components were available in both varieties while others were not. Difference in presence and percentage of essential oil components could be due to ripening of fruit peel at different time and weather conditions.

Table 1. Essential oil components identified in the Sindhri and Langra Mango varieties.

S.No.	Components
1.	Bicyclo [4.1.0] hept-3-ene,3,7,7- trimethyl-, (1S)-
2.	3-Carene
3.	Bicyclo [2, 2, 1] heptan-2-ol, 1, 7,7- trimethyl-[1S- endo) BorneolTln3.
4.	Dodecane
5.	2-Cyclohexane-1-ol,2-methyl-5-(1-methylethenyl)-, cis Carveol
6.	Tridecane
7.	Tetradecane
8.	1,4-Methanoazulene,decahydro-4,8,8-trimethyl-9-methylene-,[1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]-Longifolene.
9.	Caryophyllene
10.	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1.alpha.,4.alpha.,7.alpha.)]-alpha-Guaiene
11.	1,4,7,- Cycloundecatriene,1,5,9,9-tetramethyl-, Z, Z, Z-
12.	Cyclohexene,6-ethenyl-6-methyl-1-(1-methylethyl)- 3- (1-methylethylidene)-, (S)- α-Elemene
13.	1H- Cyclopropa [a] naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-,[1aS-(1a.alpha.3a.alpha. γ-Maaliene
14.	Pentadecane
15.	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1.alpha.,7.alpha.,8a.beta.)]-alpha-Bulnesene-Guaiene, δ-Guaijene
16.	Phenol, 2,4-bis (1,1-dimethylethyl)-
17.	Naphthalene,1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1S-(1.alpha.,4a.beta.,8a.alpha.)]-alpha-cadinene
18.	Naphthalene, 1 , 6, 7-trimethyl-
19.	Hexadecane
20.	Bicyclo [4.4.0] dec-1-ene,2-isopropyl-5-methyl-9- methylene-
21.	.alpha.-Cadinol
22.	Heptadecane
23.	1-Nonadecane
24.	Carbonic acid, heptadecyl propyl ester
25.	Octadecane
26.	Hexadecane,2,6,10,14-tetramethyl-Phytane
27.	Methyl 6,8-dodecadienyl ether
28.	Nonadecane
29.	Cycloeicosane
30.	Eicosane
31.	Heneicosane
32.	17-Pentatriacontene
33.	Docosane
34.	Tetratriacontane

Table 2. Comparison of concentration of essential oil components between Sindhri and Langra variety of Mango (Sindhri = V1, Langra=V2).

S. No.	Formula	RT	V1 Rel. % age SD	V2 Rel. % age SD
1.	$C_{10}H_{16}$	1.9968	49.4 ± 4.1	47.93 ± 4.3
2.	$C_{10}H_{16}$	2.0512	ND	27.20 ± 2.4
3.	$C_{10}H_{18}O$	3.4591	11.06 ± 1.0	ND
4.	$C_{12}H_{26}$	3.8095	7.24 ± 0.6	ND
5.	$C_{10}H_{16}O$	4.1479	3.14 ± 0.02	ND
6.	$C_{13}H_{28}$	5.4287	8.78 ± 0.7	1.11 ± 0.08
7.	$C_{14}H_{30}$	7.3863	2.79 ± 0.04	1.06 ± 0.05
8.	$C_{15}H_{24}$	7.5917	ND	0.46 ± 0.02
9.	$C_{15}H_{24}$	7.8696	4.05 ± 0.3	4.88 ± 0.3
10.	$C_{15}H_{24}$	8.2503	ND	0.57 ± 0.03
11.	$C_{15}H_{24}$	8.5765	1.70 ± 0.02	2.81 ± 0.1
12.	$C_{15}H_{24}$	9.1385	ND	0.38 ± 0.01
13.	$C_{15}H_{24}$	9.4708	ND	0.38 ± 0.01
14.	$C_{15}H_{24}$	9.5372	1.44 ± 0.09	1.20 ± 0.09
15.	$C_{15}H_{24}$	9.6943	ND	1.01 ± 0.08
16.	$C_{14}H_{22}O$	9.797	ND	0.25 ± 0.01
17.	$C_{15}H_{24}$	10.0689	ND	0.57 ± 0.02
18.	$C_{13}H_{14}$	10.5281	ND	0.28 ± 0.01
19.	$C_{16}H_{34}$	11.7546	1.73 ± 0.09	0.88 ± 0.03
20.	$C_{15}H_{24}$	12.6609	ND	0.28 ± 0.01
21.	$C_{15}H_{26}O$	12.9328	ND	0.38 ± 0.02
22.	$C_{17}H_{36}$	13.9599	1.66 ± 0.06	0.93 ± 0.06
23.	$C_{19}H_{38}$	15.9599	0.24 ± 0.01	ND
24.	$C_{21}H_{42}O_3$	15.9659	ND	0.16 ± 0.01
25.	$C_{18}H_{38}$	16.1109	0.93 ± 0.04	1.73 ± 0.06
26.	$C_{20}H_{42}$	16.3103	ND	0.34 ± 0.01
27.	$C_{13}H_{24}O$	17.7664	ND	1.29 ± 0.06
28.	$C_{19}H_{40}$	18.1954	1.91 ± 0.02	1.02 ± 0.08
29.	$C_{20}H_{40}$	20.0623	ND	0.48 ± 0.03
30.	$C_{20}H_{42}$	20.1953	2.28 ± 0.1	0.93 ± 0.08
31.	$C_{21}H_{44}$	22.2193	ND	0.78 ± 0.05
32.	$C_{35}H_{72}$	24.6241	0.71 ± 0.04	ND
33.	$C_{22}H_{46}$	24.7992	ND	0.69 ± 0.03
34.	$C_{34}H_{70}$	28.2915	ND	0.53 ± 0.04

Abbreviations used in Table – 2; RT- Retention time; V1- Variety 1 (Sindhri Mango); V2-Variety 2 (Langra Mango); Rel. % age-relative percentage (n=3); SD-Standard deviation; ND-Not detected

Conclusions

It was observed in current studies that Sindhri and Langra Mango varieties found in Mirpurkhas region showed considerable variation in the color and in the percentage of yielded oil. 3.25% of essential oil with yellow golden color was extracted from 1kg fresh Mango peelings of Sindhri variety, whereas 1.04% pista green/yellowish green color was extracted from Langra variety. Comparatively greater amount of essential oil was present in Sindhri (3.25) as compare to Langra variety (1.04).Total34 essential oil components with characteristic aromas were identified in Sindhri and Langra varieties. Bicyclo [4.1.0] hept-3-ene, 3, 7, 7-trimethyl-, (1S) - was found as major component found present in both varieties. Both varieties also found to share eleven other essential oil components with different concentrations. Bicyclo [4.1.0] hept-3-ene, 3,7,7-trimethyl-, (1S)-, BorneolTln3, Tridecane, Dodecane, Carveol, cis and caryophyllene were found in higher concentrations in Sindhri variety. While Bicyclo [4.1.0] hept-3-ene, 3,7,7-trimethyl-, (1S)-, 3-carene and caryophyllene were found in Langra variety.

Results of present analysis indicated that GC-MS has provided excellent separation of essential oil in both Mango varieties. Moreover, essential oil present in the peelings can be used as an important taxonomic tool for their identification.

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