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# Locational variations of physiochemical characteristics of selected medicinal plants

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The present study evaluates the seed oils of different medicinal plants for physiochemical characteristics and fatty acid composition. In this study, petroleum-ether (60–80°C) was used for the extraction of oil using Soxhlet technique. The yield of extractable seed oil were ranged from 6.4±0.1% to 42.5±0.4%. The peroxide value was ranged from 0.8±0.1 to 2.2±0.1 meq/kg. The mean iodine value of seed oil were lowest in case of *P. pinnata* and highest in *A. nilotica*. The saponification value of seed oil (184.2±1.0 to 219.4±0.7 mgKOH/g) and unsaponifiable matter was varied from (1.6±0.1–7.2±0.1%). The oleic acid and linoleic acid was detected as the major fatty acid in seed oil .

Keywords: Medicinal plants, physiochemical characteristics, oils, fatty acid.

## Introduction

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents[1]. Seed oils are mainly triacylglycerols which are the reaction product of glycerol and fatty acids. They are usually named by their biological sources (such as soybean oil, palm oils etc.) which have range of physical and chemical compositional parameters by which it can be recognized. They have different applications which are dependent on these physical and chemical compositional parameters[2]. These components determine the properties of the oil and vary from source to source and widely with plant variety and growing conditions. The utility of oils depend on their properties and these compositions[3]. Lack of information on the composition and utilization of the many and varied lesser known underutilized seed oils indigenous to the tropics are more of problem than the real shortage of oils[4].

## Results and discussion

*Percentage yield of seed oil:*

In this study, petroleum-ether (60–80°C) was used for the extraction of oil using Soxhlet technique. The yield of extractable seed oil were ranged from 6.4±0.1% to 42.5±0.4%. The high content of seed oil was found in *S. persica* (40.2±0.6% to 42.5±0.4%) followed by *P. pinnata* (39.2±0.7% to 40.3±0.4%) and *M. azedarach* (33.2±0.2% to 37.7±0.4%) in the locations Palwal and Hisar respectively (Fig. 1).

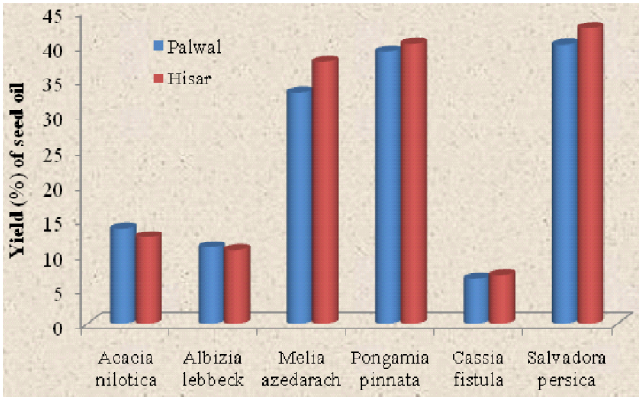


**Fig. 1.** Yield (%) of seed oils.

*Chemical characteristics of the oils:*

The extracted oil was analysed to determine some of the chemical characteristics (Tables 1 and 2) of two locations Palwal and Hisar respectively.

*Peroxide value:*

Peroxide value is measured in terms of milli equivalents of peroxide per 1000 g of the oil that oxidize potassium iodide to iodine. It gives initial evidence of rancidity in the oil. Natural oils and fats contain minute amounts of substances

**Table 1.** Chemical characteristics of seed oils (Palwal)

| Parameters | *Acacia nilotica* | *Albizia lebbeck* | *Melia azedarach* | *Pongamia pinnata* | *Cassia fistula* | *Salvadora persica* |
|---|---|---|---|---|---|---|
| Yield of oil (%) | 13.6±0.3 | 10.9±0.4 | 33.2±0.2 | 39.2±0.7 | 6.4±0.1 | 40.2±0.6 |
| Peroxide value (meq/kg) | 1.4±0.1 | 0.9±0.1 | 2.2±0.1 | 2.0±0.1 | 1.1±0.4 | 0.8±0.1 |
| Iodine value (g/100 g) | 140.5±0.1 | 101.0±0.7 | 122.4±0.5 | 82.0±0.4 | 110.0±0.5 | 112.0±0.8 |
| Saponification value (mg KOH/g) | 219.4±0.7 | 204.7±1.0 | 184.7±0.6 | 184.2±1.0 | 199.0±1.3 | 190.0±1.4 |
| Unsaponifiable matter (%) | 6.1±0.2 | 3.7±0.2 | 2.2±0.0 | 1.9±0.3 | 4.9±0.3 | 6.9±0.6 |
| Free fatty acid (%) | 0.9±0.1 | 1.7±0.0 | 2.0±0.1 | 0.1±0.0 | 2.4±0.1 | 1.4±0.3 |

Values are mean of three replicates ± standard error.

**Table 2.** Chemical characteristics of seed oils (Hisar)

| Parameters | *Acacia nilotica* | *Albizia lebbeck* | *Melia azedarach* | *Pongamia pinnata* | *Cassia fistula* | *Salvadora persica* |
|---|---|---|---|---|---|---|
| Yield of oil (%) | 12.4±0.1 | 10.5±0.1 | 37.7±0.4 | 40.3±0.4 | 6.9±0.3 | 42.5±0.4 |
| Peroxide value (meq/kg) | 1.6±0.1 | 1.1±0.1 | 1.7±0.4 | 1.2±0.3 | 1.4±0.0 | 1.3±0.4 |
| Iodine value (g/100 g) | 144.7±0.3 | 103.0±0.5 | 124.6±0.7 | 85.0±0.2 | 130.0±0.6 | 108.0±0.6 |
| Saponification value (mg KOH/g) | 189.0±0.4 | 198.0±0.8 | 188.0±0.3 | 186.0±0.7 | 189.0±0.7 | 187.0±0.9 |
| Unsaponifiable matter (%) | 7.2±0.1 | 3.9±0.1 | 2.1±0.1 | 1.6±0.1 | 5.2±0.2 | 4.2±0.5 |
| Free fatty acid (%) | 0.7±0.1 | 1.4±0.1 | 2.7±0.1 | 0.8±0.3 | 1.8±0.3 | 1.2±0.0 |

Values are mean of three replicates ± standard error.

capable of inhibiting oxidation. Low peroxide values of the seed oil indicate that the seed may have low level of oxidative and lipolytic activities or contain high level of natural antioxidants like alpha tocopherol. The peroxide value was ranged from 0.8±0.1 to 2.2±0.1 meq/kg in all the oil from the seeds studied which is a good index for the stability of the oil and its susceptibility to rancidity during storage.

*Iodine value:*

It is the number of grams of iodine required to saturate 100 g oil. Iodine value indicates the presence of unsaturated fatty acid of oils but does not define the specific fatty acids. Iodine value analysis provides near theoretical values, except in the case of conjugated double bonds or when the double bond is near a carboxyl group.The iodine value ranged from 82.0±0.4 g/100 g to 144.7±0.3 g/100 g. The mean iodine value of seed oil were lowest in case of *P. pinnata* 85.0±0.2 g/100 g (Hisar) and 82.0±0.4 g/100 g (Palwal) and highest in *A. nilotica* 144.7±0.3 g/100 g (Hisar) and 140.5±0.1 g/100 g (Palwal). These values are favourably comparable with the reported value[5–8].

*Sponification value and unsponifiable matter:*

It is the number of milligram of potassium hydroxide required to saponify completely one gram of oil under specified condition. The saponification value of seed oil varied from 184.2±1.0 mg KOH/g to 219.4±0.7 mg KOH/g which was lowest in case of *P. pinnata* 184.2±1.0 mg KOH/g while highest in *A. nilotica* 219.4±0.7 mg KOH/g. Saponification value is a measure of the alkali reactive groups in fats and oils and is useful in predicting the type of glycerides in a sample. Glycerides containing short chain fatty acids have higher saponification value. The higher saponification value indicate the short alkyl chain in the fatty acids of triglycerides. Low saponification value also implies that average molecular weight of the fatty acids constituting the fats in the seed oils are greater than that for conventional oils.

The unsaponifiable matter was varied from 1.6±0.1% to 7.2±0.1% which is highest in *A. nilotica* 7.2±0.1% (Hisar) while lowest in case of *P. pinnata* 1.6±0.1% (Hisar). Lower the value of unsaponifiable matter in oil, higher the purity. Our result are in partial agreement with the previous findings by Tissouras *et al.*[9] that may be due to agroclimatic conditions.

*Free fatty acid:*

It is defined as the milligram of potassium hydroxide required to neutralize the free acids in one gram of the oil. It is an indicator of oil quality, the freshness of the oil. The concentration of free fatty acids in seed oil was ranged from

0.1±0.0% to 2.7±0.1% which is highest in *M. azedarach* 2.7±0.1% (Hisar) while lowest in case of *P. pinnata* 0.1±0.0% (Palwal). The findings are similar to earlier results[10].

*Fatty acids composition (%) of seed oils:*

The fatty acid profiles of seed oil are presented in Tables 3 and 4. In the present study of the seed oil, myristic acid was found 0.1±0.0% and 0.2±0.1% in *A. nilotica*, while it was highest in *S. persica* 54.0±0.3% and 55.5± 0.8% in both the locations. Palmitic acid was present in all the seed oil under study. It varied from 5.9±0.4% in *M. azedarach* to 20.2±0.4% in *S. persica* in the oil from Palwal location while it was in the range of 7.5±0.6% *M. azedarach* to 19.3±0.2 in *S. persica* in the oil from Hisar location. Stearic acid was also present in all the above oil studied, which ranged from 1.0±0.3% to 5.9±0.4% in both the locations. The other saturated fatty acids (arachidic acid and behanic acid) were present in low concentration. In *S. persica* oil the myristic acid and palmitic acid were more than 74% of the total oil in both the locations. Therefore the oil of *S. persica* will be very useful in the soap industry because of its high capacity for leather formation and its stability. Oleic acid ranged from 5.6± 0.5% (*S. persica*) to 58.7±0.7% (*P. pinnata*) and linoleic acid from 2.3±0.0% (*S. persica*) to 75.5±0.3% (*M. azedarach*) in the oil under study. The other unsaturated fatty acids linolenic acid and eicosenoic acids were present in low concentrations. Oils having oleic acid and linoleic acid are useful in health promoting effect. Therefore the oils of the above seeds having high amount of oleic acid and linoleic acid are useful for food and feed formulations. It was also observed that proportion of fatty acids were different among the oils of the seeds obtained from the two locations. The total unsaturated fatty acids in *A. nilotica* and *A. lebbeck* were higher in Hisar location which has hotter climate while it was found in lower concentration in the oil obtained from the seeds of

**Table 3.** Fatty acid composition of oils (Palwal)

| Fatty acid | *Acacia nilotica* | *Albizia lebbeck* | *Melia azedarach* | *Pongamia pinnata* | *Cassia fistula* | *Salvadora persica* |
|---|---|---|---|---|---|---|
| Myristic acid ($C_{14:0}$) | 0.1±0.0 | – | – | – | – | 54.0±0.3 |
| Palmitic acid ($C_{16:0}$) | 13.7±0.4 | 13.5±0.3 | 5.9±0.4 | 9.2±0.6 | 18.5±0.3 | 20.2±0.4 |
| Palmitoleic acid ($C_{16:1}$) | – | 0.2±0.1 | – | – | – | – |
| Stearic acid ($C_{18:0}$) | 4.4±0.1 | 1.1±0.0 | 2.9±0.4 | 5.5±0.2 | 0.7±0.0 | 4.7±0.1 |
| Oleic acid ($C_{18:1}$) | 30.6±0.3 | 12.6±0.4 | 15.1±0.2 | 58.7±0.7 | 19.2±0.4 | 6.8±0.3 |
| Linoleic acid ($C_{18:2}$) | 40.5±0.4 | 62.7±0.8 | 75.5±0.3 | 16.8±0.1 | 54.3±0.7 | 3.4±0.3 |
| Linolenic acid ($C_{18:3}$) | 2.6±0.3 | 2.0±0.1 | – | – | 1.0±0.1 | 3.3±0.5 |
| Arachidic acid ($C_{20:0}$) | 0.1±0.1 | 0.9±0.1 | – | 2.4±0.4 | 0.3±0.1 | 2.1±0.1 |
| Eicosenoic acid ($C_{20:1}$) | – | – | – | – | 2.4±0.4 | 2.4±0.2 |
| Behanic acid ($C_{22:0}$) | 1.6±0.2 | 2.3±0.1 | – | 3.1±0.2 | 0.2±0.0 | 1.6±0.4 |

Values are mean of three replicates ± standard error.

**Table 4.** Fatty acid composition of oils (Hisar)

| Fatty acid | *Acacia nilotica* | *Albizia lebbeck* | *Melia azedarach* | *Pongamia pinnata* | *Cassia fistula* | *Salvadora persica* |
|---|---|---|---|---|---|---|
| Myristic acid ($C_{14:0}$) | 0.2±0.1 | – | – | – | – | 55.5±0.8 |
| Palmitic acid ($C_{16:0}$) | 13.4±0.2 | 14.3±0.4 | 7.5±0.6 | 9.1±0.3 | 18.3±0.4 | 19.3±0.2 |
| Palmitoleic acid ($C_{16:1}$) | – | 0.3±0.1 | – | – | – | – |
| Stearic acid ($C_{18:0}$) | 4.5±0.1 | 1.0±0.2 | 0.8±0.3 | 5.9±0.4 | 1.3±0.2 | 4.7±0.1 |
| Oleic acid ($C_{18:1}$) | 31.8±0.3 | 12.8±0.2 | 16.0±0.1 | 57.4±1.2 | 18.9±0.4 | 5.6±0.5 |
| Linoleic acid ($C_{18:2}$) | 39.6±0.2 | 64.8±0.5 | 74.0±0.6 | 17.6±0.1 | 53.6±0.7 | 2.3±0.0 |
| Linolenic acid ($C_{18:3}$) | 2.8±0.1 | 1.4±0.1 | – | – | 1.1±0.3 | 2.9±0.1 |
| Arachidic acid ($C_{20:0}$) | 0.1±0.0 | 0.6±0.0 | – | 2.4±0.1 | 0.1±0.1 | 3.1±0.4 |
| Eicosenoic acid ($C_{20:1}$) | – | – | – | – | 2.3±0.2 | 1.8±0.2 |
| Behanic acid ($C_{22:0}$) | 1.4±0.2 | 2.5±0.3 | – | 3.2±0.1 | 0.3±0.1 | 1.3±0.2 |

Values are mean of three replicates ± standard error.

Palwal location which has less harsh conditions than Hisar. The fatty acid profile of the seed oil generally exhibited dominant of two class MUFAs and PUFAs. MUFAs have been paid attention during past decades due to the beneficial effects on cardiovascular heart disease[11]. A MUFA rich-diet tends to decrease low density lipoprotein-cholesterol[12].

## Conclusion

The present study revealed that the yield of oil was found to be maximum in the seeds of *S. persica* and minimumin *C. fistula* as compared to other selected plants. In the seed oil of *S. persica,* myristic acid and palmitic acid together constitute more than 74% in both locations. In my study I have shown that different plants contain bioactive compounds at different concentration in different amount. These plants have different – 2 kinds of medicinal properties. As *Pongamia pinnata* seeds contains high amount of oil that's why it is used in biodiesel and energy sources. They all are good sources of protein, fibre and carbohydrate so they are used for feed purpose. These plants have good antioxidant properties so these are valuable sources of new drugs.This study highlights the need for further research on the isolation and characterization of the constituents from extracts in order to decode the specific phytochemical constituents responsible for the antioxidant activity of the plant.

## Experimental

*Seed material:*

The seeds of *Acacia nilotica, Albizia lebbeck, Melia azedarach, Pongamia pinnata, Cassia fistula* and *Salvadora persica* were collected from the forest area of CCS Haryana Agricultural University, Hisar and Palwal. After cleaning, the seeds were ground into fine powdered form.

*Chemicals:*

The commercially available chemicals from Qualigens, Merck and Ranbaxy, of highest purity, were used for various experimental procedures.

*Oil content:*

*Reagent:* Petroleum ether (60–80ºC).

*Methods:*

Dried and ground samples (100 g) of seed kernel were weighed in a thimble and placed in Soxhlet apparatus. Dried pre-weighed solvent flask containing petroleum ether and condenser were attached for each sample in three replicates. The heating rate was adjusted to give a condensation rate of 2–3 drops/s and extracted for 6 h. Removed the thimble and retained petroleum ether. The excess of petroleum ether was evaporated from the solvent flask on a hot water bath and dried the flasks in a dessicator and weighed.

*Calculation:*

$$\text{Oil content in sample (\% dry wt. basis)} = \frac{(b - a)\times 100}{\text{Wt. sample (g)}}$$

where $b$ = wt. of powdered seed before extraction and $a$ = wt. of powdered seed after extraction.

*Iodine value:*

*Reagents:*

Glacial acetic acid and chloroform.

Iodine monochloride 0.2 *N* (dissolved 16.2 g of iodine monochloride in enough glacial acetic acid to make 1 litre solution). Potassium iodide solution, 20% aq. standard 0.1 *N* sodium thiosulphate solution. Starch indicater 1% solution.

Five grams of oil sample was taken in a 500 ml glass stoppered conical flask containing 10 ml of $CHCl_3$. The flask was swirled until the sample entirely dissolved. Iodine monochloride (25 ml) was added to it and again swirled the mixture. The flask was allowed to stand in dark place for one hour. After that, 15 ml of KI solution and 100 ml of water were added. Flask was shaken vigorously and titrated with standard sodium thiosulphate solution. Two blanks were made in the same manner by omitting the sample. Blanks were also allowed to stand with test mixture for the same length of time.

*Calculation:*

$$\text{Iodine number (g/100 g)} = \frac{(B - S)(N)(12.7)}{W}$$

where $B$ = volume of standard sodium thiosulphate solution (ml) used for blank, $S$ = volume of standard sodium thiosulphate solution (ml) used for sample, $N$ = normality, and $W$ = weight of sample (g).

*Saponification value:*

*Material:*

Conical flask of 250–500 ml capacity fitted with water cooled reflux condenser. Alcoholic potassium hydroxide (28

g of KOH pellets dissolved in 20 ml of water and diluted to 1 liter with 95% v/v alcohol).

*Method:*

The oil (5 g) was taken into flask (*A*) and added 25 ml of 0.5 *N* alcoholic KOH solution. Reflux condenser was attached and heated on boiling water for 60 min. Flask was swirled frequently during heating. After that 2–3 drop of 1% phenolphthalein indicator was added and titrated with 0.5 *N* HCl. Operations were performed with blank (*B*) as well.

*Calculation:*

$$\text{Sponification value} = \frac{(B - A)\times 28.05}{\text{Wt. of oil (g)}}$$

where *A* = volume of 0.5 *N* HCl used for sample and *B* = volume of 0.5 *N* HCl used for blank.

*Saponification and removal of unsaponifiable material:*

Unsaponifiable material were determined as per the method (Ref. 13). Seed oil (5 g each) was refluxed separately with potassium hydroxide in 95% ethanol (2 *M*, 50 ml) for one hour. The mixture was allowed to cool, diluted with water (50 ml) and extracted with diethyl ether (3×50 ml). Sufficient quantities of water and ether were necessary for a good phase separation. The combined ether extracts were washed with distilled water (3×100 ml) and allowed to stand overnight. The ether was removed under vacuum at 40°C and the residue dried by evaporation with acetone to obtain the unsaponifiable matter.

*Peroxide value:*

It was estimated by Cox and Pearson[14] methods.

*Reagents:*

Acetic acid-chloroform mixture (3:1 v/v). Saturated KI solution. Standard 0.01 *N* sodium thiosulphatesolution and 1% starch indicator.

*Method:*

The oil (5 g) was taken into 500 ml conical flask. Acetic acid-$CHCl_3$ mixture (30 ml) was added to the flask. Saturated KI solution (0.5 ml) was added to it and allowed to stand for 1 min. About 450 ml of water was added to the flask and then titrated against standard 0.01 *N* sodium thiosulphate by using starch indicator to liberate all iodine free $CHCl_3$ layer until the blue colour just disappeared. Blank was titrated similarly in the absence of oil.

*Calculation:*

$$\text{Peroxide value} = \frac{A\times N\times 1000}{\text{Wt. of oil (g)}} \text{ meq/kg oil}$$

where *A* = ml $Na_2S_2O_3$ (Test-blank) and *N* = normality of $Na_2S_2O_3$ solution.

*Fatty acid spectrum:*

*Preparation of methyl esters:*

Methyl esters were prepared by the method of Luddy *et al.*[15] as described below.

*Reagents:*

(a) Sodium methoxide (0.5 *N*), (b) carbon disulphide and (c) activated charcoal.

*Method:*

A suitable amount of oil sample was taken in a test tube and 0.5 ml of 0.5 *N* sodium methoxide was added and covered with aluminium foil and then immersed in a water bath at 65°C to a depth of half inch and was shaken vigorously for 2–3 min. The mixture became homogenous indicating the complete esterification of the oil sample. The test tube was removed from the water bath and cooled to room temperature. One ml of carbon disulphide was added and shaken for 1–2 min. Separated the lower layer and approximately 100 mg of activated charcoal was added mixed uniformly and filtered through Whatman No. 1 filter paper. The filtrate constituted all the methyl esters of fatty acids.

*Fractination of methyl esters by GLC:*

Methyl esters of fatty acids were separated using Chemito 8610 HT Gas chromatograph equipped with FID and a BPX70, 0.25 ml fused silica column (SGE Pvt. Ltd., Ringwood, Victoria, Australia) was used. The carrier gas was hydrogen and injection was operated in the split mode, the split ratio being approximately 50:1. Injector and detector temperature were 270°C and 280°C respectively. The oven temperature was held at 70°C for 1 min and then programmed at 30°C/ min to 170°C followed by further programming at 30°C/ min to 200°C and held at this temperature for 6 min. Data was captured and analysed with, Chemito 5000 integrator (Tashniwal Instruments, India Ltd.)

*Free fatty acids:*

Free fatty acids were determined as per the method of *Rao et al.*[16].

*Reagents:*

(i) 1% phenolphthalein and (ii) 0. 1 *N* sodium hydroxide.

*Denatured alcohol:* Prepared by mixing 10 volume of ethyl alcohol with 1 volume of methyl alcohol and neutralized with 0.1 *N* sodium hydroxide using phenolphthalein as indicator.

*Method:*

Fifty ml of denatured alcohol was added to two gram of oil sample in a 250 ml conical flask. The flasks were swirled and few drops of phenolphthalein was added and the contents was titrated against 0.1 *N* sodium hydroxide till a permanent light pink colour appeared which persisted for at least 1 min. The percentage of free fatty acids was calculated by using the following formula:

Free fatty acids (in terms of oleic acid)

$$= \frac{100 \times 28.2 \times V}{\text{Wt. of oil} \times 10 \times 1000}$$

where $V$ = volume of 0.1 *N* sodium hydroxide used.

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