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QUALITATIVE ANALYSIS OF MARKETED CASTOR OIL ACCORDING TO INDIAN PHARMACOPIEA AND FSSAI

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ABSTRACT

Castor oil is a fixed oil obtained from ***Ricinus Communis*** belonging to family **Euphorbiaceae**. The nutritional vegetable oils and fats are frequently adulterated with low grade oils and causes severe health effects. Ten samples of castor oil were procured locally and analyzed as per the guidelines of **Indian Pharmacopeia** and ***FSSAI***. Six unpacked samples of oils are observed to be adulterated with low grade oils as they possess high peroxide values, high acetyl values etc and concluded that these oils are unfit for consumption.

KEYWORDS: Castor oil, FSSAI, Indian Pharmacopeia, Qualitative analysis, Ricinoleic acid etc.

CASTOR OIL

Castor oil is the fixed oil obtained by cold expression from the seeds of

Ricinus communis Linn, (family – **Euphorbiaceae**). The plant is commonly called Palma Christi because of its large "hand shaped" leaves. Castor oil is a colourless to very pale yellow liquid with a distinct taste and odor. The castor seed contain ***ricin***, a toxic protein. Heating the oil during extraction process denatures and deactivates the protein.

Pharmaceutical, Therapeutic and other applications

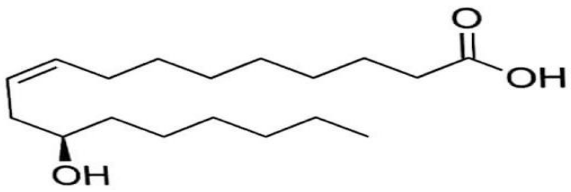
- Used to Emulsify and Solubilize oils and other water insoluble substances.
- Originally developed for use as Solubilizers and Emulsifiers.
- Has been used as a Stimulant Laxative to relieve Occasional Constipation.
- Castor oil can be used as Cathartic, Stimulant and Purgative.
- Hydrogenated castor oil is used as an Ointment Base.
- Sulphated castor oil is used as Water Absorbent Ointment and Cream.
- Castor oil and its derivatives are used in manufacturing of Pharmaceuticals, Perfumes etc.
- Castor oil Heals Inflamed Skin, Fights Signs of Ageing.
- Castor oil Reduces Acne, Moisturizes skin.
- Castor oil reduces Pigmentation, Boosts immunity.
- Castor oil reduces Joint pain / Arthritis.
- Castor oil or its derivatives is also used in production of Antifungal drugs, in Cancer Chemotherapy, Immune-suppressant drug, HIV Protease inhibitor, treatment for Skin Ulcers, a part from its historical usage as a laxative and to initiate labor pain in pregnant women.
- Castor oil, or a castor oil derivative such as **kolliphor EL** (polyehoxylated castor oil, a nonionic **surfactant**), is added to many drugs like **Miconazole** (an antifungal agent) and **Paclitaxel** (a mitotic inhibitor used in cancer chemotherapy) etc. (Kolliphor EL is used as an excipient to stabilize emulsions of non polar materials in water.)

Chemical Composition

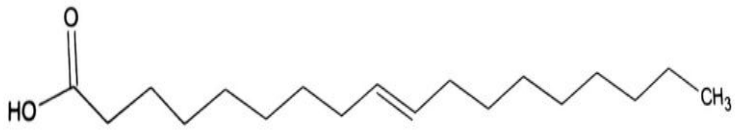
Castor oil is a well known source of ***ricinoleic acid***, a monounsaturated, 18 carbon fatty acid. Among fatty acids, ricinoleic acid is unusual in that it has hydroxyl functional group on 12^{th} carbon. This functional group causes ricinoleic acid to be more polar. The chemical reactivity of the alcohol group also allows chemical derivatization that is not possible with most other seed oils. Because of its ricinoleic acid content, castor oil is a valuable chemical in feed stocks, commanding a higher price than other seed oils.

Castor oil is composed of

1) Ricinoleic acid 85 – 95%
2) Oleic acid 2 – 6%
3) Linoleic acid 1 – 5%
4) Stearic acid 0.5 – 1%
5) Palmitic acid 0.5 – 1%



Structure of Ricinoleic acid



Structure of Oleic acid

Structure of Linoleic acid

Structure of Stearic acid

Structure of Palmitic acid

MONOGRAPH OF CASTOR OIL

Castor oil is the fixed oil obtained by cold expression from the seeds of **Ricinus Communis Linn** (family – **Euphorbiaceae**).

Description: A pale yellowish or almost colourless, transparent, viscous liquid; odour – slight or characteristic.

Tests: Light absorption: Absorbance of a 1% w/v solution in ethanol (95%) at the maximum at about 269nm, not more than 1.

Weight per ml: 0.945 – 0.965 gms.

Refractive index: 1.4758 – 1.4798.

Optical rotation: +3.5˚ to +6.0˚.

Peroxide value: Not More Than 5.

Acid value: Not More Than 2.

Acetyl value: Not Less Than 143.

Hydroxyl value: Not Less Than 150.

Saponification value: 176 – 187.

Iodine value: 82 to 90.

Foreign fatty substances

- A mixture of 2 ml of the substance under examination and 8ml of ethanol (95%) is clear.
- Shake 10ml with 20ml of light petroleum (60˚ - 80˚) and allow to separate, the volume of the lower layer is not less than 16ml.

Storage: store protected from light and moisture at a temperature not exceeding 15˚.

Labeling: The label states

1. The name and quantity of any added antioxidant;
2. Whether the contents are suitable for use in the manufacture of parenteral preparations. [7]

ANALYSIS OF OILS

In general oils can be identified and evaluated for their purity, quality and impurity if any by physical and also chemical constants, as recommended by **IP** or ***fssai*** standards.

Physical Constants: Refractive index, Viscosity, Specific gravity etc.

Refractive Index- The refractive index is defined as the ratio of the velocity of light in air to the velocity of light in the substance.

Significance: Refractive index of oils increases with the increase in unsaturation and also chain length of fatty acids. It is also valuable in identification of substances and detection of impurities.

Specific Gravity- The specific gravity is defined as the ratio of the weight of the liquid in air at the specified temperature to that of an equal volume of water at the same temperature.

Significance: It allows determination of fluid characteristics compared to that of a standard.

Chemical methods: Used to check for the type of fatty acid, extent of unsaturation and rancidity if any.

Iodine value- Iodine value is defined as the weight of iodine absorbed by 100 parts per weight of the given sample of oil. It gives the measure of extent of unsaturation in the oils. Oils which are unsaturated take up iodine to add up the bonds using iodine. The more the iodine value, the higher is unsaturation and thereby chances of rancidity.

Significance: It is a measure of degree of unsaturation present in fatty acids.

Saponification value- Saponification value is the number of milligrams (mg) of potassium hydroxide (KOH) required to neutralize the free acids and to saponify the esters present in 1g of the substance.

Significance: It is a measure of average molecular weight of all the fatty acids present in the oil.

Unsaponifiable matter- The unsaponifiable matter is defined as the substances soluble in an oil which after saponification are insoluble in water but soluble in the solvent used for determination.

Significance: It is a measure of lipid fraction that cannot be transformed into soap.

Hydroxyl value: - Hydroxyl value is the number of milligrams of KOH required to neutralize the acetic acid which can combine by 1gm of oil by acetylation process.

Significance: Hydroxyl value is a measure of the content of free hydroxyl groups in a chemical substance.

Acetyl value - Acetyl value is defined as the number of milligrams (mg) of KOH required to neutralize the acetic acid obtained when 1gm of sample acetylates oil is saponified. Most oils have low acetyl value like 3 – 15 unlike castor oil which has a value of 150.

Significance: Acetyl value is a measure of the free hydroxyl groups in a substance (as of fat or oil) as determined by acetylation.

Acid value- Acid value is defined as the number of milligrams (mg) of KOH required to neutralize the free acids present in 1gm of fat. During rancidity, acid is released. The acid value indicates the extent of rancidity.

Significance: It is the measure of the amount of free fatty acids which have been liberated by hydrolysis from the glycerides due to action of moisture, temperature or lypolytic enzyme lipase.

MATERIALS AND METHODS

Qualitative Analysis Of Castor Oil

Castor oil of different brands were analyzed by conventional methods like titrimetric methods

Reagents and Chemicals

S.No	Name	Grade
1	Chloroform	HPLC
2	Iodine mono chloride	AR
3	Glacial acetic acid	AR
4	Petroleum ether	AR
5	Sodium thio sulphate	AR
6	Diethyl ether	AR

List of Instruments

S.No	Instrument name	Software	Model
1	Abbe refractometer	-	Tanco
2	Digital Balance	-	Contech CA-123
3	Ultra sonic bath/Sonicator	-	PCI Analytics 6.5 litres (capacity)
4	Hot air oven	-	Tempo Equipment Private Limited

Marketed Brands of Castor Oil with their Manufacturers

S.No	Samples	Manufacturers, Address	Batch / Lic No.	Date of Packing
1	Castor oil IP (Standard Sample)	Gandhi Herbal India Pvt. Ltd, Mhow-Neech Road, Guradiya Deda, Dist-MANDSAUR(M.P)	25B /4/ 2008	17-12-2016
2	Erandel tel (M1)	Status chemicals, Chandrayangutta, HYD.	05	1-12-2016
3	Sanjay brand (M2)	Sanjay marketed products, Esamia bazaar, HYD.	-	17-1-2017
4	Refined castor oil (M3)	Madina chemicals, Asif nagar, HYD.	C06	1-6-2016
5	Unpacked sample-1 (U1)	N. Balaiah oil merchant, Osmanganj, HYD.	-	3-1-2017
6	Unpacked sample-2 (U2)	Papalal oil merchant, Begum bazaar, HYD.	-	3-1-2017
7	Unpacked sample-3 (U3)	Bharani oil depot, Musheerabad, HYD.	-	3-1-2017
8	Unpacked sample-4 (U4)	Gulshan tea and oils, Tolichowki, HYD.	-	4-1-2017
9	Unpacked sample-5 (U5)	Sri Venkateshwara oil merchant, Secunderabad.	-	7-1-2017
10	Unpacked sample-6 (U6)	Krishna rao oil merchant, Rajendra nagar, HYD.	-	4-1-2017

Physical Analysis

- **Description**

Procedure- 5ml of oil was taken in clean 10 ml test tube and viewed under light for the appearance of its colour, odour and nature.

- **Solubility**

Procedure- Solubility of all the samples was observed in different solvents like alcohol, glacial acetic acid and chloroform.

1ml of oil was taken in a test tube and specified solvents like alcohol, glacial acetic acid and chloroform were taken respectively in different test tubes and their solubility were observed.

- **Weight per ml (or) Specific Gravity**

The specific gravity is the ratio of the weight of the liquid in air at specified temperature to that of an equal volume of water at the same temperature.

Procedure- A clean, dry pycnometer was selected and calibrated whose empty weight was noted (B). Then the weight of pycnometer filled with water (C) and oils (A) was noted separately which was measured at 30˚C.

The specific gravity was calculated using the formula:

$$\text{Specific Gravity} = \frac{A - B}{C - B}$$

Where A= weight of pycnometer + oil

B= weight of empty pycnometer

C= weight of pycnometer + water

Limits: 0.945 – 0.965 [18]

- **Refractive index**

The refractive index is defined as the ratio of the velocity of light in air to the velocity of light in the substance.

Principle

Refractive index is usually measured by Abbe refractometer. Abbe refractometer working principle is based on critical angle.

Procedure- A drop of each sample of oil was placed on the double prism by opening it with the help of a screw head. It was then allowed to stand for few minutes so that the temperature of the sample and instrument are the same and then the readings were noted for all the 10 samples.

Limits: 1.4758 – 1.4798.[19]

Chemical Analysis

- **Alkaline impurities**

Preparation of 0.01M HCl

0.85 ml of concentrated HCl was dissolved in little quantity of water and the volume was made up to 1000 ml with distilled water.

Procedure- Dissolve 1 g of oil in mixture of 1.5 ml of alcohol and 3 ml of toluene. Add 0.05 ml of 0.4 g/lit solution of Bromo phenol blue.

Limits: Not More Than 0.2 ml of 0.01M HCL is required to change the colour to yellow.

- **Peroxide value**

The peroxide value is the number of milli equivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.

Preparation of 2M HCl- 171 g of HCl was dissolved in little quantity of water and the volume was made up to 1000 ml with water.

Preparation of 0.01M $Na_2S_2O_3$- 248 g of Na2S2O3 was dissolved in little quantity of water and 2 g of sodium carbonate was added and the volume was made up to 1000 ml with distilled water.

Standardization of 0.01M $Na_2S_2O_3$ with potassium dichromate

4.9 g of potassium dichromate previously powdered and dried in a dessicator for 4 hrs was accurately weighed and dissolved in sufficient water to produce 1000 ml. From above solution, 20 ml of solution was pipette out and 1 g of the potassium iodide was added. Then 7 ml of 2M HCl and 250 ml of water was added and the resulting solution was titrated with 0.1M $Na_2S_2O_3$ until the colour changes from blue to light green using 3 ml of starch solution as indicator.

1ml of 0.1M $Na_2S_2O_3 \cong 0.0049$g of $K_2Cr_2O_7$

Procedure- 5 g of substance under examination was weighed accurately and transferred to a 250 ml of glass stoppered conical flask, and then 30 ml mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform was added, swirled until sample got dissolved and 0.5 ml of saturated KI solution was added. Allowed to stand exactly for 1 min, with occasional shaking and 30 ml of water was added, and titrated gradually with continuous and vigorous shaking, with 0.01M $Na_2S_2O_3$ until the yellow colour almost disappears. 0.5 ml of starch solution was added, titration was continued by shaking vigorously until the blue colour just disappears (A). Blank titration omitting the substance under examination (B) was carried out.

Note: The volume of 0.01M $Na_2S_2O_3$ in the blank determination must not exceed 0.1 ml.

$$\textbf{Peroxide value} = \frac{10(a-b)}{W} X \frac{\text{actual molarity}}{\text{given molarity}}$$

Where, a = volume of 0.01M $Na_2S_2O_3$ consumed for disappearance of blue colour from sample solution.

b = volume of 0.01M $Na_2S_2O_3$ consumed for blank.

W = weight in grams of the substance, under examination.

Limits: NMT 5.0 [20]

- **Acid value**

The acid value is the number which expresses in milligrams, the amount of KOH necessary to neutralize the free acids present in 1g of substance.

Preparation of 0.1M KOH:- 5.611 g of KOH was dissolved in little amount of water and the volume was made up to 1000 ml of water.

Preparation of 0.1M oxalic acid-63 g of oxalic acid was dissolved in little quantity of water and the volume was made up to 1000 ml with distilled water.

Standardization of 0.1M KOH- 0.63 g of oxalic acid was weighed accurately in 100 ml volumetric flask and the volume was made up to 100 ml with distilled water. 10 ml of above solution was pipetted out into a conical flask and titrated against 0.1M KOH solution using phenolphthalein as indicator.

Procedure- 10 g of oil was weighed and mixed with 50 ml mixture of equal volumes of ethanol (95%) and ether, previously neutralized with 0.1M KOH to phenopthalein solution. 1 ml of phenopthalein solution was added and titrated with 0.1M KOH until the solution remained faintly pink for 30 sec.

$$\textbf{Acid value: } \frac{5.61n}{W} \text{ X } \frac{\text{actual molarity}}{\text{given molarity}}$$

Where, n = volume of 0.1M KOH consumed for sample.

W = weight in grams of the substance under examination.

Limits: NMT 2 [21]

- **Acetyl value**

Acetyl value is the number which expresses in milligrams the amount of KOH required to neutralize the acetic acid liberated by hydrolysis of 1g of acetylated substance.

Preparation of 0.5M alcoholic KOH- 30 g of KOH was dissolved in 20 ml of water and finally the volume was made up to 1000 ml with 95% ethanol.

Preparation of 0.5M sodium carbonate- 5.3 g of sodium carbonate was taken and dissolved in 100 ml of distilled water and the volume was made up with water.

Preparation of 0.5 N HCL- 42.5 ml of concentrated HCL was dissolved in little quantity of water and the volume was made up to 1000 ml with distilled water.

Standardization of 0.5M HCl- Weigh accurately 800 mg of anhydrous sodium carbonate, dissolve it in 100 ml of distilled water and then add 0.1 ml of methyl red solution. Add the acid slowly from the burette, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool and continue the titration. Heat again to boiling and titrate further as necessary until the faint pink colour is no longer affected by boiling.
1ml of 0.5 M HCL is equivalent to 0.02650 g of sodium carbonate.

Procedure- 10 g of oil was placed with 20 ml of acetic anhydride in a long necked Round Bottomed Flask of 200 ml attached to a reflux air condensor. Boiled gently for 2 hours, allowed to cool, poured into 600 ml of water, contained in a large beaker, 0.2 g of pumice powder was added and boiled for 30 min. Cooled, transferred to a separator and the lower layer was discarded. Acetylated product was washed with 3 quantities, each of 50 ml of warmed saturated solution of sodium chloride until washings are no longer acidic to litmus paper. Finally, shaked with 20 ml of warm water and the aqueous layer was removed as completely possible. Acetylated substance was poured into a small dish, 1 g of powdered anhydrous sodium sulphate was added and stirred thoroughly and then filtered through a dry pleated filter paper. Saponification value of acetylated substance was determined.

$$\textbf{Acetyl value} = \frac{\textbf{1335(b-a)}}{\textbf{(1335-a)}}$$

Where, a = saponification value of substance.
b = saponification value of acetylated substance.
Limits: NLT 143

- **Saponification value**

The saponification value is the number of milligrams of potassium hydroxide required to neutralize the free acids and to saponify the esters present in 1 g of the substance.

Principle

The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of excess potassium hydroxide with standard hydrochloric acid.

Preparation of 0.5M alcoholic KOH- 30 g of KOH was dissolved in 20 ml of water and finally the volume was made up to 1000 ml with 95% ethanol.

Preparation of 0.5M sodium carbonate- 5.3 g of sodium carbonate was taken and dissolved in 100 ml of distilled water and the volume was made up with water.

Preparation of 0.5 N HCL- 42.5 ml of concentrated HCL was dissolved in little quantity of water and the volume was made up to 1000 ml with distilled water.

Standardization of 0.5M HCl- Weigh accurately 800 mg of anhydrous sodium carbonate, dissolve it in 100 ml of distilled water and then add 0.1 ml of methyl red solution. Add the acid slowly from the burette, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool and continue the titration. Heat again to boiling and titrate further as necessary until the faint pink colour is no longer affected by boiling.
1ml of 0.5 M HCL is equivalent to 0.02650 g of sodium carbonate.

Procedure:- 2 g of oil under examination was weighed in a 200 ml flask of borosilicate glass fitted with a reflux condenser and 25 ml of 0.5 M ethanolic KOH was added. The flask was boiled under the reflux on a water bath for 30 min. 1 ml of phenolphthalein solution was added and titrated immediately with 0.5 M HCL (a ml). A blank titration was performed omitting the substance under examination. (b ml).
Saponification value was calculated from the following expression:

$$\textbf{Saponification value} = \frac{28.05\,(b-a)}{w} X \frac{\text{actual molarity}}{\text{given molarity}}$$

Where, w = weight in grams of the substance.

CALCULATIONS

$$\text{Saponification value} = \frac{28.05\,(b-a)}{w} X \frac{\text{actual molarity}}{\text{given molarity}}$$

Where, b = volume of 0.5M HCL consumed for blank
a = volume of 0.5M HCL consumed for substance under examination
w = weight in grams of substance under examination
Limits: 176 – 187.[22]

- **Unsaponifiable Matter**

The unsaponifiable matter consists of substances present in oils and fats which are not saponifiable by alkali hydroxides and are determined by extraction with an organic solvent of a solution of the saponified substance under examination.

Principle

The unsaponifiable matter is defined as the substances soluble in an oil which after saponification are insoluble in water but soluble in the solvent used for the determination. It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments, vitamins and hydrocarbons as well as any foreign organic matter non volatile at 100˚C e.g. (mineral oil) which may be present. Light petroleum or diethyl ether is used as a solvent but in most cases results will differ according to the solvent selected and generally the use of diethyl ether will give a higher result.

Preparation of 3% w/v solution of KOH- 3 g of KOH was dissolved in little quantity of water and the volume was made up to 100 ml with distilled water.

Preparation of 0.1 M ethanolic KOH- 5.611 g of KOH was dissolved in little quantity of ethanol and the volume was made up to 1000 ml with ethanol.

Preparation of 0.1 M oxalic acid- 63 g of oxalic acid was dissolved in little quantity of water and the volume was made up to 1000 ml with distilled water.

Standardization of 0.1 M KOH- 0.63 g of oxalic acid was weighed accurately in 100 ml volumetric flask and the volume was made up to 100 ml with distilled water. 10 ml of above solution was pipetted out into a conical flask and titrated against 0.1 M KOH solution using phenolphthalein as indicator.

Procedure- 5 g of oil was accurately weighed into a 250 ml flask fitted with a reflux condenser. The solution of 2 g of KOH in 40 ml of ethanol (95%) was added and heated on a water bath for 1 hour with occasional shaking. Then the contents of the flask was transferred to a separating funnel with the aid of 100 ml of hot water and while the liquid is still warm, the contents are washed with 3 quantities each of 100 ml of peroxide-free ether. The ether extracts are combined in a second separating funnel containing 40 ml of water and allowed to separate in which the lower layer was rejected. Then the extract was further washed with 2

quantities each of 40 ml of water and with 3 quantities each of 40 ml of a 3% w/v solution of KOH, in which each treatment was being washed with 40 ml of water. Finally the extract was washed with each of 40 ml of water until the aqueous layer was not alkaline to phenolphthalein solution. The ether layer was transferred to a weighed flask, washing out the separating funnel with ether in which ether layer was distilled off and to the residue 6 ml of acetone was added. Then the solvent was completely removed with the aid of gentle current of air and dried at 100-105˚C for 30 minutes. The residue obtained was weighed and dissolved in 20 ml of ethanol (95%). The above solution was titrated with 0.1 M ethanolic KOH. If the volume of 0.1 M ethanolic KOH exceeds 0.2 ml, the amount weighed cannot be taken as the unsaponifiable matter and the test must be repeated.

Limits: NMT 0.8% [23]

- **Iodine value**

Preparation of iodine mono chloride solution-8 g of iodine trichloride was dissolved in 200 ml of glacial acetic acid and separately 9 g of iodine was dissolved in 300 ml of dichloromethane. The two solutions were mixed and diluted to 1000 ml with glacial acetic acid.

Preparation of potassium iodide (KI) solution- 166 g of potassium iodide was dissolved in little quantity of water and the volume was made up to 1000 ml with water.

Preparation of 2M HCl- 171 g of HCl was dissolved in little quantity of water and the volume was made up to 1000 ml with water.

Preparation of 0.1M sodium thio sulphate ($Na_2S_2O_3$)- 248 g of $Na_2S_2O_3$ dissolved in little quantity of water and 2 g of sodium carbonate was added and the volume was made up to 1000 ml with distilled water.

Standardization of 0.1M $Na_2S_2O_3$ with potassium dichromate

4.9 g of potassium dichromate previously powdered and dried in a dessicator for 4 hrs was accurately weighed and dissolved in sufficient water to produce 1000 ml. From above solution, 20 ml of solution was pipette out and 1 g of the potassium iodide was added. Then 7 ml of 2M HCl and 250 ml of water was added and the resulting solution was titrated with

0.1M $Na_2S_2O_3$ until the colour changes from blue to light green using 3 ml of starch solution as indicator.

1ml of 0.1M $Na_2S_2O_3$ ≅ 0.0049g of $K_2Cr_2O_7$

Procedure- 0.2 g of oil was placed in a dry 500 ml iodine flask, and 10 ml of carbon tetra chloride was added and dissolved.20 ml of Iodine mono chloride solution was added, stopper was inserted and allowed to stand in the dark at a temperature between 15˚ and 25˚ for 30 min.15 ml of potassium iodide solution was placed in the cup top, stopper was removed, the stopper and sides of flask was rinsed with 100 ml of water Shaken and titrated with 0.1M $Na_2S_2O_3$ using starch solution, added towards the end of titration, as indicator. The number of ml required (a) was noted, the operation was repeated without the substance under examination, and the number of ml required (b) was noted.

$$\textbf{Iodine value} = \frac{1.269(b-a)}{w}$$

Where, w = weight, in grams of substance.

a = No of ml of $Na_2S_2O_3$ required for Sample solution.

b = No of ml of $Na_2S_2O_3$ required for Blank.

Limits: 82 TO 90 [24]

- **Foreign Fatty Substances**

Procedure

10 ml of oil was shaked with 20 ml of light petroleum and allowed to separate.

Limits: Volume of lower layer is not less than (NLT) 16 ml.

- **Rancidity and Kries test**

Preparation of 0.1%w/v phloroglucinol solution

0.1g of phloroglucinol was dissolved in little quantity of ether and the volume of made up to 100ml with ether.

Procedure- 5 ml of oil was shaked vigorously with 5 ml of 0.1% phloroglucinol solution in Diethyl ether and 5 ml of conc. hydrochloric acid was added.

Appearance of pink to red colour indicates presence of rancidity.

- **Heavy metals**

The limit for heavy metals is indicated in the individual monographs in terms of ppm i.e. the parts of lead, pb, per million parts (by weight) of the substance under examination.

Preparation of lead nitrate stock solution- 0.1598 g of lead nitrate was dissolved in 100 ml of water to which has been added 1 ml of nitric acid and diluted to 1000 ml with water.

Preparation of lead standard solution- 10 ml of lead nitrate stock solution was diluted with little quantity of water and the volume was made up to 100 ml with water.

Preparation of lead standard solution (20 ppm pb)- 1 volume of lead standard solution was diluted with 5 volumes of water to get 20 ppm of lead standard solution.

Preparation of standard solution- 1 ml of lead standard solution was pipetted into 50 ml nessler cylinder and diluted up to 25 ml with water. The above solution was adjusted with either dilute acetic acid or dilute ammonia solution to pH between 3.0 and 4.0 and diluted up to 35 ml with water.

Preparation of test solution- 1 ml of substance under examination was dissolved in suitable solvent in 50 ml nessler cylinder and diluted up to 25 ml with water. The above solution was adjusted with either dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0 and diluted up to 35 ml with water.

Procedure- To each of the nessler cylinders containing the standard solution and test solution respectively, 10 ml of freshly prepared hydrogen sulphide solution was added and mixed well. Then the solutions were diluted up to 50 ml with water and allowed to stand for 5 min during which the colour produced was viewed against the white background. The colour produced in test solution should not be more intense than that produced with the standard solution.

***Fssai* Protocol**

- **Test for presence of Sesame oil (Baudoin test)**

Procedure - 5 ml of oil was taken in 25 ml of measuring cylinder, 5 ml of concentrated HCL and 0.4 ml of furfural solution was added. Glass stopper was inserted and shaken for 2 min and allowed to separate. Development of pink or red colour in lower acid layer indicates

presence of sesame oil. Confirmed by adding 5 ml of water and shaked again, if the colour in acid layer persists, sesame oil is present, if colour disappears it is absent. [25]

- **Test for presence of Mineral oil (Holdes test)**

Procedure- 25 ml of alcoholic KOH was taken in conical flask and then 1 ml of oil sample to be tested was added. Boiled on a water bath until the solution became clear and no oil drops are found on sides of flask. Flask was taken out from water bath and the contents was transferred to a wide mouthed warm test tube and 25 ml of boiling distilled water was added along the sides of the test tube carefully. During addition, tube was shaken lightly from side to side.

Note: Turbidity indicates presence of mineral oil, the depth of turbidity depends on % of mineral oil present.[26]

- **Test for Presence of Cottonseed Oil (Halphen's Test)**

Principle

The development of red colour on heating the oil with a solution of sulphur in carbon disulphide indicates the presence of cottonseed oil. The test is also given by Hempseed oil, Kapok seed oil/ oil containing cyclopropenoid fatty acids (such as sterculic and malvalic acid). Hydrogenation and deodorization wholly or partially destroy the cromogens and react with diminished intensity. A positive reaction is not given by an oil heated to 250˚C or above.

Procedure- 5 ml of the oil was taken in a test tube and equal volume of the sulphur solution was added. The above solution was mixed thoroughly by shaking and heated gently on a water bath (70˚ to 80˚C) for a few min with occasional shaking until the carbon disulphide has boiled off and the sample stops foaming. The test tube was placed in an oil bath or a saturated brine-bath maintained at 110-115˚C for 2.5 hrs. A red colour at the end of this period indicates the presence of cottonseed oil.

RESULTS AND DISCUSSION

The results obtained by different analytical techniques were discussed in this chapter.

Qualitative analysis

Physical Analysis

Description

The oil samples were observed under light and found to be pale yellow and almost colourless, transparent, viscous liquid oily liquid, except **unpacked sample 3 (U3) which appeared as colourless turbid liquid.**

Solubility

Solubility of all the oil samples were observed and found to be **slightly soluble in light petroleum, miscible with methanol and very clearly soluble in glacial acetic acid and chloroform.**

Weight per ml or Specific Gravity

The results of Weight per ml for Standard, Marketed and Unpacked samples are given in Table.

Weight per ml or Specific Gravity

S.No	Samples	Weight per ml / Specific Gravity
1	Standard sample	0.946
2	Erandel tel (M1)	0.958
3	Sanjay brand (M2)	0.963
4	Refined castor oil (M3)	0.956
5	Unpacked sample-1 (U1)	0.953
6	Unpacked sample-2 (U2)	0.958
7	Unpacked sample-3 (U3)	0.967
8	Unpacked sample-4 (U4)	0.948
9	Unpacked sample-5 (U5)	0.959
10	Unpacked sample-6 (U6)	0.954

Specific Gravity bottles filled with oil samples

The specific gravity values for all the oil samples were found to be within the IP limits.

Refractive index

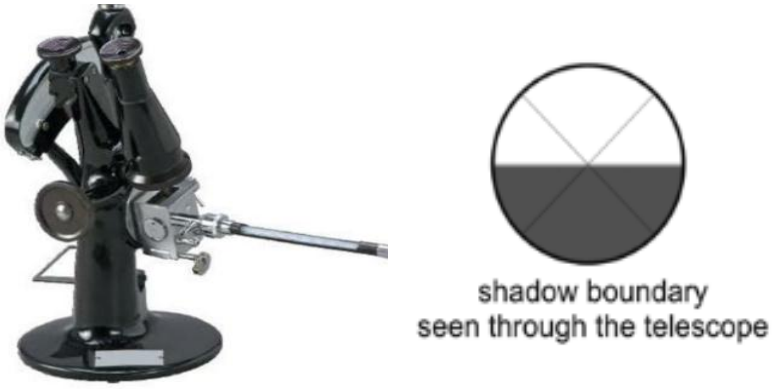


Diagram of Refractometer

The results of Refractive index values for Standard, Marketed and Unpacked samples are given in Table.

Refractive Index

S.No	Samples	Refractive index (1.4758-1.4798)
1	Standard sample	1.4752
2	Erandel tel (M1)	**1.4853**
3	Sanjay brand (M2)	1.4788
4	Refined castor oil (M3)	1.4732
5	Unpacked sample-1 (U1)	**1.4958**
6	Unpacked sample-2 (U2)	**1.4962**
7	Unpacked sample-3 (U3)	**1.4925**
8	Unpacked sample-4 (U4)	**1.4962**
9	Unpacked sample-5 (U5)	**1.4932**
10	Unpacked sample-6 (U6)	1.4759

The refractive index values for **erandel tel (M1)** and **all unpacked samples** of castor oil are not in limits when compared to IP limits. Refractive index of oils increases with increase in unsaturation and also chain length of fatty acids. The higher values, indicates, presence of more unsaturated fatty acids in Erandel tel and all the unpacked samples.

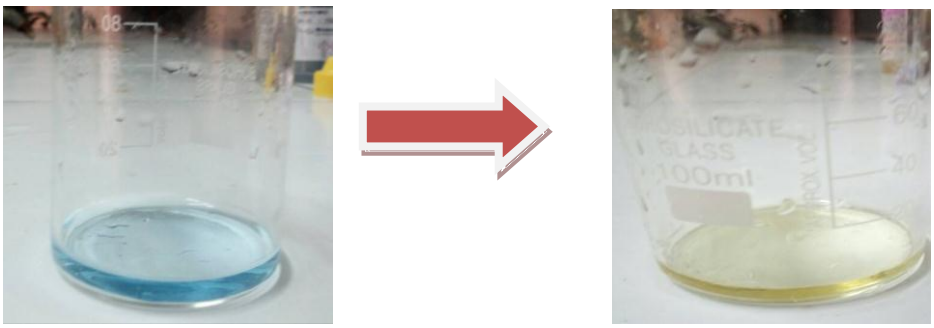
Chemical Analysis

Alkaline Impurities

The results of Alkaline Impurities for Standard, Marketed and Unpacked samples are given in Table.

Alkaline Impurities

S.No	Samples	Limits-Volume in ml (NMT 0.2 ml)
1	Standard sample	0.13
2	Erandel tel (M1)	0.15
3	Sanjay brand (M2)	**0.22**
4	Refined castor oil (M3)	0.20
5	Unpacked sample-1 (U1)	**0.24**
6	Unpacked sample-2 (U2)	**0.21**
7	Unpacked sample-3 (U3)	0.10
8	Unpacked sample-4 (U4)	0.05
9	Unpacked sample-5 (U5)	0.10
10	Unpacked sample-6 (U6)	0.16



End Point of Alkaline Impurities Colour Change

The alkaline impurities test depends upon the volume of 0.01M HCl consumed by the oil. As it indicates the presence of impurities, the volume 0.01M HCl consumed was more for **sanjay brand, unpacked samples 1 and 2** when compared to standard sample.

Peroxide value

The results of Peroxide value for Standard, Marketed and Unpacked samples are given in Table.

Peroxide value

S.No	Samples	Volume	Weight	Peroxide value (NMT 5)
1	Standard sample	2.0 ml	5.040 g	3.76
2	Erandel tel (M1)	1.9 ml	5.130 g	3.50
3	Sanjay brand (M2)	1.0 ml	5.130 g	1.75
4	Refined castor oil (M3)	1.6 ml	5.030 g	2.98
5	Unpacked sample-1 (U1)	2.4 ml	5.170 g	4.44
6	Unpacked sample-2 (U2)	2.0 ml	5.230 g	3.63
7	Unpacked sample-3 (U3)	1.5 ml	5.520 g	2.53
8	Unpacked sample-4 (U4)	6.4 ml	5.020 g	**12.54**
9	Unpacked sample-5 (U5)	2.9 ml	5.020 g	**5.57**
10	Unpacked sample-6 (U6)	1.8 ml	5.020 g	3.26

End Point of Peroxide value colour change

The peroxide values for **unpacked samples 4 and 5** were more when compared to IP limits. The increase in Peroxide value for unpacked samples may be due to increase in oxidation of oil during storage. As the extent of oxidation was more, the volume of 0.01M $Na_2S_2O_3$ consumed by unpacked samples was more.

Acid value

The results of Acid value for Standard, Marketed and Unpacked samples are given in Table.

Acid value

S.No	Samples	Weight of samples taken (w)	Volume of 0.1M KOH consumed (n)	Acid value (NMT 2)
1	Standard sample	10.050 g	3.7 ml	1.854
2	Erandel tel (M1)	10.020 g	4.0 ml	**2.01**
3	Sanjay brand (M2)	10.030 g	3.1 ml	1.56
4	Refined castor oil (M3)	10.048 g	4.3 ml	**2.1**
5	Unpacked sample-1 (U1)	10.010 g	6.5 ml	**3.27**
6	Unpacked sample-2 (U2)	10.010 g	3.5 ml	1.76
7	Unpacked sample-3 (U3)	10.050 g	3.1 ml	1.73
8	Unpacked sample-4 (U4)	10.000 g	5.6 ml	**2.82**
9	Unpacked sample-5 (U5)	10.040 g	3.9 ml	1.96
10	Unpacked sample-6 (U6)	10.000 g	2.5 ml	1.4

End Point of Acid value

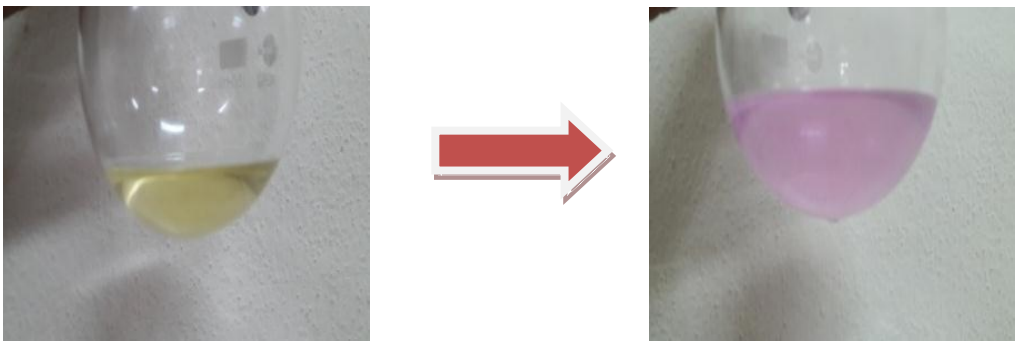
The acid values of **refined castor oil (M3) and unpacked samples 1 and 4** were more when compared to Standard sample. Acid value for refined castor oil (M3) and unpacked samples (1 and 4) increased, may be due to the increase in free fatty acids resulted by oxidation, improper storage of oils or due to adulteration with other oils. As the free fatty acids content is more, the volume of 0.1M KOH consumed by marketed and unpacked samples increases.

Acetyl value

The results of Acetyl values for Standard, Marketed and Unpacked samples are given in the Table.

Acetyl value

S.No	Samples	Saponification value (a)	Saponification value (b)	Acetyl value (NLT 143)
1	Standard sample	168.2	306.3	158
2	Erandel tel (M1)	201.7	309.7	**127.7**
3	Sanjay brand (M2)	189.8	310.9	**141.17**
4	Refined castor oil (M3)	151.3	306.3	174.8
5	Unpacked sample-1 (U1)	190.8	297.7	**124.7**
6	Unpacked sample-2 (U2)	175.5	293.5	**135.8**
7	Unpacked sample-3 (U3)	175.7	297.6	**140.3**
8	Unpacked sample-4 (U4)	168.06	293.6	143.6
9	Unpacked sample-5 (U5)	199.05	289.3	**106.06**
10	Unpacked sample-6 (U6)	185.8	308.9	143



End Point of Acetyl value colour change

Acetyl value is the measure of hydroxyl acids in lipids. **Erandel tel (M1), sanjay brand (M2) and unpacked samples (1, 2, 3 and 5)** are out of the limits.

Unsaponifiable Matter

The results of Unsaponifiable matter for Standard, Marketed and Unpacked samples are given in Table.

Unsaponifiable matter

S No	Samples	Percentage obtained (NMT 0.8%)
1	Standard sample	0.65
2	Erandel tel (M1)	**0.9**
3	Sanjay brand (M2)	0.8
4	Refined castor oil (M3)	**0.92**
5	Unpacked sample-1 (U1)	**1.2**
6	Unpacked sample-2 (U2)	**1.4**
7	Unpacked sample-3 (U3)	**1.2**
8	Unpacked sample-4 (U4)	**1.3**
9	Unpacked sample-5 (U5)	**1.15**
10	Unpacked sample-6 (U6)	**0.9**

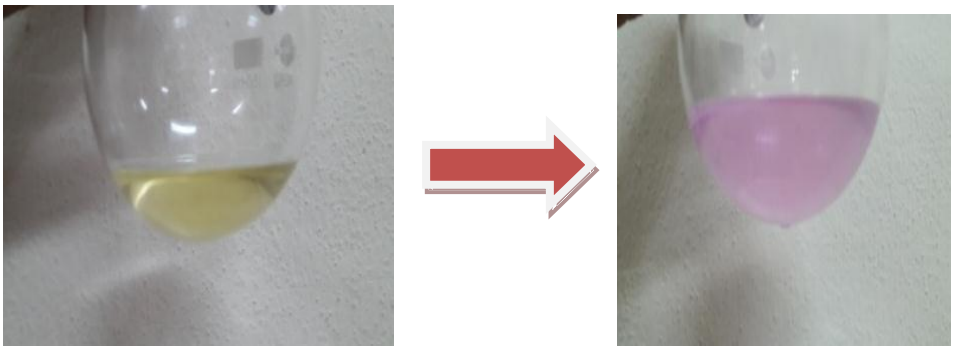
The unsaponifiable matter for erandel tel (M1), refined castor oil (M3) and all unpacked samples were deviated from IP limits indicating that presence of unsaponifiables in the samples.

Saponification value

The results of Saponification value for Standard, Marketed and Unpacked samples are given in Table.

Saponification value

S.No	Samples	Weight of samples taken (w)	Volume of 0.5M HCL consumed (a)	Saponification value (176-187)
1	Standard sample	2.010 g	10.6 ml	181.8
2	Erandel tel (M1)	2.030 g	9.0 ml	**201.7**
3	Sanjay brand (M2)	2.100 g	9.4 ml	**189.8**
4	Refined castor oil (M3)	2.070 g	12.5 ml	**151.3**
5	Unpacked sample-1 (U1)	2.060 g	9.6 ml	**190.8**
6	Unpacked sample-2 (U2)	2.020 g	11.0 ml	**175.5**
7	Unpacked sample-3 (U3)	2.010 g	11.0 ml	**175.5**
8	Unpacked sample-4 (U4)	2.110 g	11.9 ml	**168.06**
9	Unpacked sample-5 (U5)	2.030 g	9.2 ml	**199.05**
10	Unpacked sample-6 (U6)	2.100 g	9.7 ml	185.8



End Point of Saponification value

The saponification value of **Erandel tel, sanjay brand, unpacked samples 1 and 5** were more and remaining all the samples are less when compared to Standard sample. As the fatty acids content present in all marketed and loose samples was more and less, the volume of 0.5M HCl required to neutralize the free fatty acids and saponify the esters was more and less when compared to pure oil.

Iodine value

The results of Iodine value for Standard, Marketed and Unpacked samples are given in Table.

Iodine value

S.No	Samples	Volume of 0.1M $Na_2S_2O_3$ consumed	Iodine value (82-90)
1	Standard sample	20.0 ml	86.5
2	Erandel tel (M1)	27.4 ml	**45.2**
3	Sanjay brand (M2)	21.2 ml	**78.8**
4	Refined castor oil (M3)	8.9 ml	**128.3**
5	Unpacked sample-1 (U1)	9.6 ml	**155.7**
6	Unpacked sample-2 (U2)	7.0 ml	**140.4**
7	Unpacked sample-3 (U3)	7.5 ml	**131.6**
8	Unpacked sample-4 (U4)	9.8 ml	**147.3**
9	Unpacked sample-5 (U5)	10.2 ml	**139.8**
10	Unpacked sample-6 (U6)	13.7 ml	**95.5**

End Point of Iodine value

The iodine values for **refined castor oil (M3) and unpacked samples (1, 2, 3, 4, 5 and 6) were more, and for Erandel tel and sanjay brand were less** when compared to standard sample. There was an increase in Iodine value for refined castor oil and unpacked samples (1, 2, 3, 4 and 5), due to increase in unsaturation of free fatty acids. As the extent of unsaturation was more, the volume of 0.01M $Na_2S_2O_3$ consumed by samples was more.

Foreign Fatty Substances

The results of Foreign Fatty Substances values for Standard, Marketed and Unpacked samples are given in the Table.

Foreign Fatty Substances

S.No	Samples	Volume of lower layer
1	Standard sample	16.2 ml
2	Erandel tel (M1)	10 ml
3	Sanjay brand (M2)	11.2 ml
4	Refined castor oil (M3)	10 ml
5	Unpacked sample-1 (U1)	10 ml
6	Unpacked sample-2 (U2)	9.6 ml
7	Unpacked sample-3 (U3)	12 ml
8	Unpacked sample-4 (U4)	13.6 ml
9	Unpacked sample-5 (U5)	12 ml
10	Unpacked sample-6 (U6)	15.6 ml

The volume was found less in all the samples **except Standard sample (Castor oil IP)**

Rancidity and Kries test

Colour change in Rancidity

Colour was produced in two samples like **Heated pure extract (M4) and Marketed sample 1 (Refined castor oil)** when compared to standard sample. As rancidity indicates the chemical decomposition of oils and fats, the samples might be decomposed.

Heavy metals

All samples were tested for the presence of heavy metals by comparing the intensity of colour produced by the standard solution to that of the test solutions. As the colour produced by the test solutions were less intense than that of standard solution, all the samples were found to be free from heavy metals.

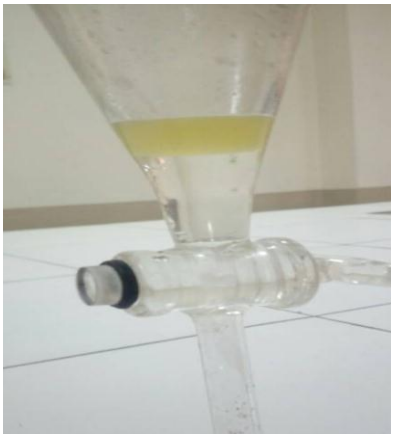
Test for presence of Sesame oil (Baudoin test)

The results of Standard, Marketed and Unpacked samples are given in the Table.

Test for presence of Sesame oil

S.No	Samples	Colour Produced
1	Standard sample	No colour
2	Erandel tel (M1)	No colour
3	Sanjay brand (M2)	**Light pink was produced**
4	Refined castor oil (M3)	No colour
5	Unpacked sample-1 (U1)	**Dark pink was produced**
6	Unpacked sample-2 (U2)	**Dark pink was produced**
7	Unpacked sample-3 (U3)	No colour
8	Unpacked sample-4 (U4)	**Light pink was produced**
9	Unpacked sample-5 (U5)	**Dark pink was produced**
10	Unpacked sample-6 (U6)	No colour

Presence of sesame oil



Absence of sesame oil

Colour produced due to presence of sesame oil

The development of pink colour with furfural solution in the presence of HCl indicates the presence of sesame oil. The colour is produced on account of reaction with sesamolin present in sesame oil. As development of pink colour was observed in **sanjay brand and unpacked samples (U1, U2, U4 and U5)**, it indicates the presence of sesame oil.

Test for presence of Mineral oil (Holdes test)

The presence of mineral oil is indicated by the development of turbidity when hot distilled water is added to a freshly made alcoholic KOH solution of the soap formed by the oil. As no turbidity was observed, all the samples were found to be free from the presence of mineral oil.

Test for Presence of Cottonseed Oil (Halphen's Test)

The presence of cottonseed oil is indicated by the development of red colour on heating the oil with solution of sulphur in carbon disulphide. All samples showed the development of red colour indicating the presence of cottonseed oil.

CONCLUSION

The quality of marketed castor oil was evaluated as per **IP** and ***fssai*** guidelines. The results revealed that some of the oils were unfit for consumption as they possess high acid value, high peroxide value, alkaline impurities, high iodine value. Some of the oils were also found to be mixed with low grade of sesame oils or oil may be oxidized to liberate more number of free fatty acids. It is strongly suggested that the government should take a stringent measures to prevent the adulteration of oils and to safeguard the health of human beings. A proper mechanism should be adopted to control the sale of unpacked oils. It is suggested that the nutritional vegetable oils and fats should follow the conditions as per Prevention of Food Adulteration Act.

1. The oils should be double refined.
2. The manufactured oils should be stored in good sanitary conditions to prevent rancidity and oxidation of oils.
3. All the fixed oils should be packed and labelled as per *fssai* guidelines with all details of manufacturing, composition, date of manufacture, date of expiry, storage conditions etc.
4. The manufacturers should adopt a proper mechanism to discard the expired oils.

The qualitative and quantitative determination of fixed oils should be carried out regularly at every required levels to prevent the adulteration and safeguard the human health. Increasing the awareness and educating people is the best solution to prevent the adulteration.

REFERENCES

1. Kokate CK, Purohit AP, Gokhale SB Pharmacognosy, I: 10.1- 10.6, 11.12.
2. Chemistry of fixed oils. [Online]. Available: https://theodora.com/encyclopedia/o/oils.html
3. Uses of Fixed Oils. [Online]. Available: http://www.thepharmacistpharma.blogspot.in
4. India's First F&B News Website, A Saffron Media Website, Food Beverage News e-paper, Saffron Media Private LTD. [Online]. Available: http://www.fnbnews.com
5. Pharmaceutical Applications of Castor oil. [Online]. Available: (http://en.m.wikipedia.org)

6. Kunal Shah, 'Castor Crop Production, Imports & Exports', Castor Seed Market Analysis & Outlook 2017 by NIRMAL BANG (a relationship beyond broking), 2017; 2-5.
7. Indian Pharmacopoeia volume III. Indian Pharmacopoeia Commission Ghaziabad, 2014; 96-97, 100, 102, 109-110.
8. Chatwal GR, Anand SK Instrumental Methods of Chemical Analysis. 5th edition. Himalya Publishing House, 2002; 2.149-2.184.
9. Skoog W Principles of Instrumental Analysis, 5th edition. Thomson Asia Pvt Ltd., 1992; 710-716.
10. Yusuf AK, Mamza PAP, Ahmed AS, Agunwa U Extraction and Characterization of Castor seed oil from wild Ricinus Communis Linn, International Journal of Science, Environment and Technology, 2015; 4(5): 1392-1404.
11. Ravichandran S 'Food adulteration has taken away the joy of life', International Journal of MediPharm Research, 2015; 1(3): 150-154.
12. Marc Plante, Chris Crafts, Bruce Bailey, and Ian Acworth Characterization of Castor oil by HPLC and Charged Aerosol Detection, 2011. Available: http://www.dionex.com
13. Braz Chem J 'Determination of biodiesel adulteration with raw Vegetable oil from ATR-FTIR data using chemometric tools', Journal of Brazilian Chemical Society, 2011; 22(7): 1229-1235.
14. Abitogun A, Alademeyin O, Oloye D 'Extraction and Characterization of Castor Seed Oil', The Internet Journal of Nutrition and Wellness, 2008; 8(2): 1-5.
15. Akpan UG, Jimoh A, Mohammed AD Extraction, Characterization and Modification of Castor Seed Oil. Department of Chemical Engineering, Federal University of Technology, Minna P.M.B. 65, Niger State, Nigeria, 2006.
16. Babu S, Sudershan RV, Sharma RK, Ramesh V. Bhat 'A simple and rapid polarimetric method for quantitative determination of castor oil', Journal of the American Oil Chemists' Society, 1996; 73(3): 397-398.
17. Indian Pharmacopoeia vol I. Indian Pharmacopoeia Commission, Ghaziabad, 2014; 3201.
18. A.O.A.C 17th edition, 2000, Official method 920.212 Specific gravity (Apparent) of Oils, Pycnometer method / I.S.I Handbook of Food analysis (Part XIII), 1984; 72.
19. A.O.A.C (Association Of Analytical Chemists) 17th edition, 2000, Official method 921.08 – Index of refraction of oils and fats / I.S.I Handbook of Food analysis (Part XIII) 1984; 70.
20. A.O.A.C 17th edition, 2000, Official method 965.33 Peroxide Value in Oils and Fats / Pearsons Composition and Analysis of Foods 9th edition, 641.

21. I.S.I (Indian Standards Institute) Handbook of Food analysis (Part XIII) 1984, page 67 / IUPAC 2. 201(1979) / I.S: 548 (Part I) – 1964, Methods of Sampling and Test for Oils and Fats/ ISO 660:1996 Determination of acid value and acidity.
22. A.O.A.C 17th edition, 2000, Official method 920.160 Saponification number of oils and fats / IUPAC 2. 202 / I.S.I Handbook of Food analysis (Part XIII), 1984; 78.
23. I.S.I (Indian Standards Institute) Handbook of Food analysis (Part XIII), 1984; 67. /A.O.A.C 17th edn, 2000, Official method 933.08, Residue (unsaponifiable) of oils and fats.
24. A.O.A.C 17th edition, 2000, Official method 920. 159 – Iodine absorption number of oils and fats / I.S.I Handbook of Food analysis (Part XIII), 1984; 76.
25. I.S.I Handbook of Food analysis (Part XIII), 1984; 86/ A.O.A.C 17th edition, 2000, Official method 893.01 – Oil (sesame) in Oils and Fats Modified Villavecchia Test.
26. I.S.I Handbook of Food analysis (Part XIII), 1984; 86/ A.O.A.C 17th edition, 2000, Official method 945.102 – Oil (mineral) in fats – Qualitative Test).
27. I.S.I Handbook of Food analysis (Part XIII), 1984; 86/ A.O.A.C 17th edition, 2000, Official method 197.02 – Oil (cottonseed) in Oils and Fats / F.A.O. Manuals of Food Quality Control, 14/8 271.