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Original Article

Analytical Evaluation of Processed and Unprocessed Castor Oil

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ABSTRACT

Background: Medicated oils and Clarified butter are often utilized in the Traditional System of Indian Medicine for internal and external use. To improve the durability and efficacy of therapeutic oil unique processing is advocated known as *Murchhana*. Specific ingredients are mentioned for each oil. The current work seeks to conduct a comparative analytical evaluation of processed and unprocessed castor oil. **Materials and Methods:** Both the oils were analyzed for rancidity, specific gravity at 25ºC, refractive index, iodine value, saponification value, acid value, peroxide value, free fatty acids, and HPTLC. **Results:** There was a significant difference between unprocessed and processed castor oil. Physicochemical and HPTLC analysis revealed significant differences in all samples, indicating that extra active chemicals were obtained in the processed oil. Saponification Value was increased in processed oil, however, iodine value, and acid value were found to decrease indicating the enhancement of the shelf life. Thus, utilizing adopting processing techniques the shelf life of the oil can be improved. **Conclusion:** Based on the results of analytical criteria, it implies the significance of processing the castor oil as mentioned in the Ayurvedic texts.

Keywords: Castor oil, HPTLC, Processing (Murchhana), Physicochemical analysis, Unprocessed.

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INTRODUCTION

The current trends for living a long and healthy life are entirely dependent on traditional medical systems, of which the traditional system of medicine is one of the most beneficial because it possesses several natural elements to eliminate the important causes of disease by reestablishing equilibrium and preventing further recurrence.[1] According to the World Health Organization, 80% of the world's population still relies on traditional or traditional ways of medicine for survival.[2]

In the Traditional system of medicine, formulations including fats and oils play a crucial role. Oil formulations made in Traditional systems of medicine pharmaceutics are widely used for both medical and cosmetic purposes.[3] Bhaishajya Kalpana is founded on the idea of modifying natural ingredients to enhance and maintain health as well as treat ailments.[4] It is a formulation in which there is little influence from pharmaceutical manufacturing. Medicated Lipid/Fatty formulation is noteworthy since it is a unique formulation that is effective both internally and externally. One of the most amazing techniques in traditional medicine and the pharmaceutical industry is the production of both fat-soluble and water-soluble extractives in an oil medium. Fixed oils, fatty acids (both saturated and unsaturated), free acids, and other components may be present in nonpolar crude oils used in the manufacturing of any medical oil. Oils in their crude form contain various undesirable constituents for therapeutic applications, as well as being more susceptible to rancidity factors. Even during the commercialization era, crude oils are found polluted in many places for financial gain, compromising the dignity of business and the health of the consumer.

In the 19th century, Bhaishajya Ratnavali, a book on the Traditional system of medicine pharmaceuticals, introduced the specific method of oil processing. It includes boiling the oil with decoction and a paste of a few herbs. Account of which is said to increase the High-Density Lipoprotein (HDL) and saponification value of medicated Clarified butter and oils in contrast to the unprocessed ones while decreasing the proportion of Low-Density Lipoprotein (LDL),[5,6] iodine value, acid value, peroxide value, and free fatty acids.[7] In light of this context, the current investigation compares the analytical findings of processed and unprocessed Castor Oil.

MATERIALS AND METHODS

Castor oil was obtained for the study from the local market by selecting a brand with FSSAI approval (Food Safety and Standards Authority of India). The required amount of material was drawn

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for the initial analytical study, and the remaining quantity was subjected to processing. The processing was carried out in the manner indicated in Text[8] (Table 1).

The Unprocessed Castor Oil was taken in a vessel. Heated over the mild fire until foam appear. The fire was lit off, then wait till it became stable. Individually, drugs were cleansed to remove foreign materials. Pulveriser was then used to powder the different medicines. All medications were sieved through mesh no. 80. Every medicine will be weighed separately. Herbal paste was made by combining medication powder with an appropriate amount of water. After that the Oil was placed over moderate fire, then the supernatant liquid portion in the curd and Sour gruel combination was poured into the oil, and Paste was added to the container and cooked with continuous stirring until oil desired result appeared. Then Oil was allowed to cool off on its own. After that, the Oil was filtered through doubled muslin cloth and kept in airtight glass bottles after cooling. Then Processed Erand Oil was obtained.

The endpoint was discovered by locating a soundless burning of a piece of paper that had been made wet by some solid portion of the oil. Rancidity,[9] Specific gravity at 25°C,[10] Refractive index,[11] Iodine value,[12] Saponification value,[13] Unsaponifiable Value,[14] Acid value,[15] Peroxide value,[16] Free fatty acids[17] and HPTLC[18] were used to evaluate both unprocessed and processed oils.

Analytical Parameters of Castor Oil

Organoleptic Characters

Characteristics of Organoleptic.

The oil samples were visually examined.

Determination of Rancidity

1 mL of oil was mixed with 1 mL of concentrated HCl in a test tube. 1 mL of 1% solution of phloroglucinol in ether was added to it and mixed thoroughly with a fat-acid mixture. Color of *Taila* was observed and result was obtained.

Determination of Specific Gravity

Weighing an empty pycnometer that had been cleaned and dried. It was then weighed after being filled with oil. The procedure was repeated, but this time water was used in place of oil. By dividing the sample weight in g by the weight of the water, the specific gravity was obtained.

Determination of Refractive Index:

The refractive index was calculated using the Abbe's Refractometer laboratory model. Before switching between the prisms, the oil was sprayed on the dry prism surface. The sample was examined using the eyepiece. Using a lever, the black and white parts were adjusted to the cross wire. The scale reading was recorded.

Determination of Iodine Value

A dry iodine flask was used to collect an oil sample. To dissolve it, chloroform was used. The flask was maintained in the dark for 30 min after adding the iodine monochloride solution. It was treated with a potassium iodide and water solution. Then, using starch solution as the indication close to the endpoint, the solution was titrated against 0.1 N sodium thiosulphate. With the sample removed, the procedure was repeated.

$$\text{Iodine value} = 1.269 v/w$$

Where v=the difference in between the titrations and,

w=the weight of the sample in g.

Determination of Saponification Value

35-40 g potassium hydroxide was dissolved in 20 mL of water and diluted to 1 L with alcohol (95%), which was allowed to stand overnight before decanting the pure liquid. A precisely weighed 2gm sample was placed in a tarred 250 mL conical flask, and 25 mL of the alcoholic potassium hydroxide solution was added. It was then cooled after being refluxed over a water bath for one

Table 1: Herbal Ingredients Used for processing of castor oil.

Sl. No.	Scientific Name	Part to be used	Proportion
1.	*Rubia cordifolia* Linn.	Root	3 g
2.	*Gyperus rotindus* Linn.	Rhizomes	3 g
3.	*Coriandrum sativum* Linn.	Hole Plants	3 g
4.	*Terminalia chebula* Retz *Terminalia bellirica* Roxb *Emblica officinalis* Gaertn	Fruit Fruit Fruit	3 g *each*
5.	*Sesbania sesban* Merrill	Root	3 g
6.	Valeriana hardwickii wall	Root	3 g
7.	*Phoenix sylvestris* Roxb.	Flower	3 g
8.	*Ficus bengalensis* Linn.	Horn	3 g
9.	*Curcuma longa* Linn.	Tubber	3 g
10.	*Berberis aristata* DC.	Root, Tubber	3 g
11.	*Cinnamomum Verum* J.Presl	Bark	3 g
12.	*Pandanus odorotissimus* Linn.	Fruit	3 g
13.	*Zingiber officinale* Roscoe.	Rhizome	3 g
14.	*Ricinus communis* Linn.	Oil	768 mL
15.	Yogurt	Curd	1536 mL
16.	Sour gruel	Rice gruel	1536 mL

hour while periodically rotating the contents of the flask. 1 mL of phenolphthalein (1%) solution was added, and the surplus alkali was titrated with 0.5N HCl hydrochloric acid. The number of mL necessary (a) was recorded. The experiment was repeated with the same quantity of the same reagents but without the drug. The amount of mL necessary (b) was recorded. The saponification value was determined using the formula:

Value of saponification = (b-a) 28.05/ W

W=sample weight in g,

b=reading from a blank burette,

a=reading from a sample burette.

Determination of Unsaponifiable Value

Unsaponifiable matter is defined as substances in oils or fats that can be extracted with ether but cannot be saponified by alkali hydroxides. The oil was saponified with 2M ethanolic potassium hydroxide. The contents of the flask were washed with water and extracted with ether. The ether layer was washed with water continually and treated with a potassium hydroxide solution. The ether coating was then rinsed with water to remove any alkali. After the ether had been distilled away, acetone was added. Acetone was removed by vacuum drying. After drying to a constant weight at temperatures ranging from 100°C to 105°C, the residue was weighed at room temperature.

The percentage of unsaponifiable materials=100a/w

Where, a=residue weight and,

w=sample weight.

Determination of Acid Value

10g of sample was properly weighed into a 250 mL flask, to which a mixture of 25 mL ether and 25 mL alcohol (95%) was added, which was then neutralized by the addition of 1 mL of phenolphthalein and gently heated in a water bath. The solution was then titrated with 0.1N potassium hydroxide (5.611 g KOH dissolved in water to contain 1000 mL) and agitated constantly until it turned pink. The number of mL necessary was recorded. The acid value was determined using the following formula:

$$Acid\ value\ = \frac{a \times\ 0.00561\ \times 1000}{w}$$

a=No. of mL of 0.1 N KOH,

W=Wt. of the sample in g.

Determination of Peroxide Value

In a clean 250 mL conical flask with a stopper, a 5 g sample was inserted. It was mixed with 2 parts chloroform and 3 parts glacial acetic acid. After whirling the flask for 1 min to dissolve the substance, 0.5 mL of saturated KI solution was added and allowed to stand. With repeated shaking, 30 mL of distilled water was added and gradually titrated with 0.01M sodium thiosulphate with continuous and vigorous shaking until the yellow tint was practically gone. The titration was then repeated with 0.5 mL of the starch solution, vigorously shaking until the blue hue was gone (a mL). A blank titration (b mL) was done without the medication. In the blank determination, the volume of 0.01 M sodium thiosulphate must not exceed

The peroxide value was calculated using the following formula

The peroxide value is equal to 10 x a-b/ w.

Where, a=the amount of NaOH needed to neutralize the material.

b=the amount of NaOH necessary for the blank;

w=the weight of the substance in g.

Determination of Free Fatty Acid

The free fatty acid content of the oil is determined by titration against KOH in the presence of a phenolphthalein indicator. The acid number is the amount of KOH needed to neutralize one gram of free fatty acids. The free fatty acid concentration, on the other hand, is expressed in oleic acid equivalents.

Formula: Free Fatty Acid=Oil Acid Value/2

Phytochemical Screening

Standard technique was used to perform a qualitative assessment of the extracts (Water soluble and Alcohol Soluble) for the presence of phytoconstituents such as carbohydrates, alkaloids, flavonoids, glycosides, tannins, and proteins, among others.[19-21] The formulations' aqueous and alcoholic extracts were made and subjected to preliminary phytochemical screening.[22]

Chromatographic Study

Alcohol, methanol, n-hexane, chloroform, and acetic acid were chemicals utilized in HPTLC and were acquired from Merck (Mumbai, India). On CAMAG equipment with a UV-vis detector (SPD-10A VP), binary pumps (LC-10AT VP), and system controller (SCL-10A VP) with linomat injector (20 L) that is controlled by win cat software, HPTLC analysis was carried out. Along with them, a pH meter (Mettler-Toledo FiveEasy-A211), an ultrasonicator (Labman®), and an analytical balance (Mettler-Toledo ME-205) were employed throughout the HPTLC study.

RESULTS

Castor oil's color changed from yellowish brown to darkish red and unprocessed Castor Oil color yellowish brown (Figure 1). After processing, samples of oil produced a distinctly pleasant odor that was similar to the aroma of the herbal substances used

in the process. The consistency of both oils has altered from clear translucent to dark semi-transparent (Table 2).

All oil samples tested in the study were free of rancidity. After processing, physicochemical parameters such as Specific gravity at 25°C, Refractive index, and Saponification value increased in processed Castor oil. After processing, parameters such as iodine value, acid value, peroxide value, and free fatty acids are found to have decreased values (Table 2). HPTLC study revealed that treated oil contained more fluorescent bands than unprocessed oil (Table 3; Figures 2 and 3). These findings point to a change in the composition of oil after processing, which may be responsible for the improved stability and efficacy of processed oil. The preliminary phytochemical screening tests reveal the presence of a variety of bioactive secondary metabolites, which may be responsible for the therapeutic properties of the compounds. The methods for conducting preliminary qualitative phytochemical testing on plant extracts are described in the Tables 4 and 5.

DISCUSSION

Every oil processing stage that involves the addition of pharmacologically active herbal constituents, such as heating, trituration, and mixing, can change the pharmacological properties of the processed oil.[23,24] It is one method of infusing new phytochemical constituents into oil to improve its characteristics and stability. Two procedures are used in the Traditional system of medicine pharmaceutical to prepare medicinal oils. They are made by either using unprocessed oil or oil that has been processed in a certain way.[25,26]

The former method is commonly used in the pharmaceutical sector, whereas the latter is used by the Traditional system of medicine physicians. Oil Processed is a specific oil processing method that incorporates adding a decoction and paste of selected herbs to oil and heating it until the water content is completely vaporized.[27] Processed oil is presently used as a foundation for the manufacture of medicinal oils.[28] According to the industry, such processing seems time and expense-demanding and is not encouraged until the benefits of such processing are established.

This study is an attempt to examine and analyze the changes that occur in oil as a result of the processed process. Any possibility of substituting Processed Castor Oil for unprocessed Castor oil in formulations is also explored here. A comparison of the analytical profiles of Unprocessed Castor oil with processed Castor oil revealed that oil processing improves stability. Specific gravity, refractive index, and saponification values of treated oil were discovered to be higher. Nonetheless, the iodine, acid, and peroxide readings were reduced. These modifications indicate that the oil is more stable and hence has a longer shelf life.[29] Reduced free fatty acid concentration leads to a higher content of low molecular weight fatty acids, which are favorable to the body and have superior bioavailability on both external and internal administration.[30]

Figure 1: Images of a pharmaceutical preparation of Castor Oil Processed.

Table 2: Organoleptic and Physico-chemical observation of Unprocessed and Processed Oil Samples.

Sl. No.	Parameters	Unprocessed Castor Oil	Processed Castor Oil
1.	Color	Pale Yellow	Brownish red
2.	Odor	Characteristic	Aromatic of Sour gruel
3.	Consistency	Oily more viscous	Oily more viscous (++)
4.	Appearance	Clear and Transparent	Dark brownish red color liquid
5.	Rancidity	Absent	Absent
6.	Specific gravity at 25°C	0.642	0.842
7.	Refractive index	1.4301	1.4370
8.	Iodine value	95.91	83.85
9.	Saponification value	179.87	192.63
10.	Unsaponifiable value	0.06%	0.03%
11.	Acid value	0.94	0.66
12.	Peroxide value	12.67	10.92
13.	Free Fatty Acid	0.47	0.33
14.	Total Fatty Matter	2.81	2.74
15.	Viscosity	281.40	309.40
16.	pH	7.94	6.20

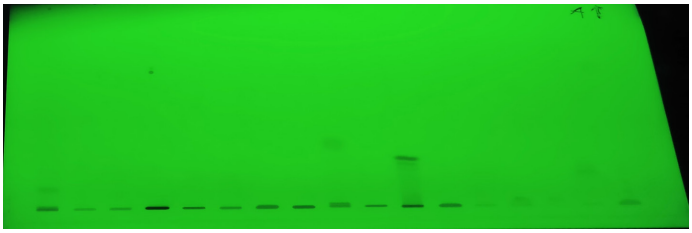

Figure 2: HPTLC analysis at 254nm.

After processing, Castor Oil has a darkish-red color and a distinct odor. The specific gravity increased after the processes, i.e., In PCO (0.842), which may be attributed to the addition of some active bio-constituents from the herbs employed in the Castor oil Processes. When compared to PCO samples, the specific gravity of UPCO (0.642) was found to be lower. It can be assumed that the treatment of the oil causes more active principles to dissolve in the oil, resulting in higher therapeutic efficacy than unprocessed samples. The PCO had a higher RI (1.4370) than the UPCO (1.4301). The increased value of RI in the processed sample was discovered to be greater, which might be attributed to coloring and additional phyto-constituents. The amount of free fatty acid present in oil and fat is indicated by the acid value. A high acid value in the oil could result in early rancidity. The acid value of PCO (0.66) was lower than that of UPCO (0.94).

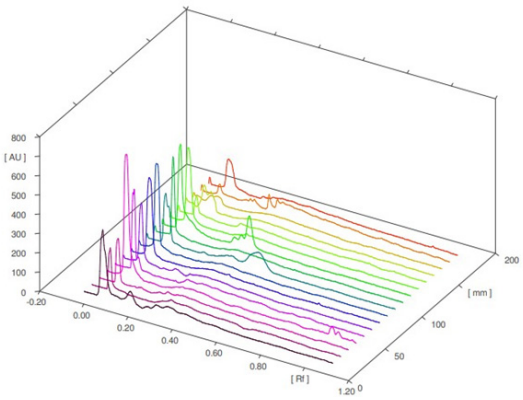

Figure 3: HPTLC analysis tracks at 254 wavelength.

The saponification value in PCO was 192.63, and in UPCO it was 179.87. The saponification value indicates the molecular weight of fats/oils. Because the saponification number and molecular weight of oil are inversely proportional, high saponification values indicate that the fat is composed of low molecular fatty acids and vice versa.[31]

Increased saponification value improves oil stability. The un-saponified matter discovered in PCO (0.03%) was lower than in UPCO (0.06%), confirming the refining process: the lower the un-saponified matter, the more refined the oil. The iodine value of oil shows its degree of unsaturation. The higher the degree of unsaturation, the greater the risk of the oil becoming rancid owing to air oxidation.[32] The iodine value measured in PCO was 83.85, whereas in UPCO it was 95.91; the lower iodine value in PCO indicates that the oil has a longer shelf life. The amount of peroxide is measured in mg per kilogram of fat. Aldehydes, ketones, and other byproducts are quickly converted from peroxides (O_2), which are intermediate results of fat oxidation. The most well-known test for evaluating the stability of oils is peroxide value analysis.[33] It was discovered that PCO had a peroxide value of 10.92 and UPCO had a value of 12.67. As Manjishtha, Haridra, and Vatankura contain antioxidant characteristics in their pharmacology, the lowered peroxide value in the processed sample may have been caused by antioxidant compounds.[34] This implies an increase in PCO stability.

The Processed process involves the incorporation of both fat-soluble (from herbal paste) and water-soluble (from Sour gruel and the supernatant liquid portion in the curd), Phyto-constitutes into the oil, resulting in a homogeneous solution and enhancing the oil's quality.[35]

HPTLC examination of processed oil samples clearly shows that more Phyto-constitutes were impregnated by the herbs used in processing. Because the herbs used include pharmacologically active chemicals, it is reasonable to conclude that the processed oil may have a few additional therapeutic properties not found in unprocessed oil. Because oil processing alters its medicinal potential, it is a recommended approach for developing more effective medicated oils. All evaluated analytical parameters in

Table 3: R_f values of oil samples observed by HPTLC samples.

Solvent system-n-Hexane: Chloroform: acetic acid (17:3:4)			
Visualization	**Samples**	**No. of Spots**	**R_f values**
254 nm	*Rubia cordifolia* Linn.	6	0.04, 0.16, 0.24, 0.28, 0.33, 0.40
	Gyperus rotindus Linn.	5	0.04, 0.31, 0.38, 0.59, 0.70
	Coriandrum sativum Linn.	4	0.04, 0.14, 0.23, 0.26
	Combination of *Terminalia chebula* Retz. *Terminalia bellirica* Roxb. *Emblica officinalis* Gaertn.	6	0.04, 0.29, 0.38, 0.80, 0.97, 1.00
	Sesbania sesban Merrill.	4	0.04, 0.28, 0.61, 0.75
	Valeriana hardwickii wall.	4	0.04, 0.19, 0.26, 0.33
	Phoenix sylvestris Roxb.	3	0.04, 0.33, 0.40
	Ficus bengalensis Linn.	3	0.03, 0.33, 0.38
	Curcuma longa Linn.	3	0.04, 0.34, 0.46
	Berberis aristata DC.	3	0.04, 0.33, 0.43
	Cinnamomum Verum J.Presl.	4	0.04, 0.28, 0.31, 0.35
	Pandanus odorotissimus Linn.	2	0.04, 0.30
	Zingiber officinale Roscoe.	3	0.04, 0.24, 0.82
	Yogurt	5	-0.04, -0.01, 0.04, 0.07, 0.30
	Sour gruel	7	0.02, 0.38, 0.45, 0.53, 0.65, 0.83, 0.99
	Unprocessed Castor Oil	8	0.04, 0.16, 0.18, 0.19, 0.26, 0.30, 0.40, 0.77
	Processed Castor Oil	4	0.04, 0.20, 0.25, 0.41

Table 4: Phytochemical Evaluation of Drugs of Processed Castor Oil in Aqueous Extract.

		Phytoconstituent								
Sl. No.	**Drugs**	**Alkaloid**	**Amino acids**	**Carbohydrates**	**Glycosides**	**Flavonoids**	**Tannins**	**Steroids**	**Proteins**	**Saponins**
1.	*Rubia cordifolia* Linn.	+	+	+	+	-	+	-	-	+
2.	*Gyperus rotindus* Linn.	+	-	+	+	-	+	+	-	-
3.	*Coriandrum sativum* Linn.	+	+	+	-	+	+	-	+	+
4.	Combination of *Terminalia chebula* Retz. *Terminalia bellirica* Roxb. *Emblica officinalis* Gaertn.	+	-	+	-	+	+	+	-	+
5.	*Sesbania sesban* Merrill.	+	-	+	-	+	+	+	+	-
6.	*Valeriana hardwickii* wall.	+	-	+	+	-	+	+	+	+
7.	*Curcuma longa* Linn.	+	+	+	-	+	-	-	-	+
8.	*Berberis arista* DC.	+	+	+	+	+	-	-	+	+

		Phytoconstituent								
Sl. No.	Drugs	Alkaloid	Amino acids	Carbohydrates	Glycosides	Flavonoids	Tannins	Steroids	Proteins	Saponins
9.	*Cinnamomum Verum* J.Presl.	-	-	-	+	-	-	-	-	-
10.	*Zingiber officinale* Roscoe.	-	-	+	+	+	-	-	-	-

Table 5: Phytochemical Evaluation of Drugs of Processed Castor Oil in Alcohol Extract.

		Phytoconstituent								
Sl. No.	Drugs	Alkaloid	Amino acids	Carbohydrates	Glycosides	Flavonoids	Tannins	Steroids	Proteins	Saponins
1.	*Rubia cordifolia* Linn.	+	+	-	-	-	+	-	-	-
2.	*Gyperus rotindus* Linn.	+	-	+	+	+	-	+	-	-
3.	*Coriandrum sativum* Linn.	+	+	-	-	+	+	-	+	+
4.	Combination of *Terminalia chebula* Retz. *Terminalia bellirica* Roxb. *Emblica officinalis* Gaertn.	+	-	-	-	+	+	+	+	+
5.	*Sesbania sesban* Merrill.	+	-	+	-	+	+	+	+	+
6.	*Valeriana hardwickii* wall.	+	-	+	+	-	+	+	+	+
7.	*Curcuma longa* Linn.	+	+	+	+	-	-	+	+	+
8.	*Berberis aristata* DC.	+	+	+	+	+	-	-	+	-
9.	*Cinnamomum Verum* J.Presl.	-	-	-	+	-	-	-	-	-
10.	*Zingiber officinale* Roscoe.	-	-	+	+	+	-	+	-	-

unprocessed and processed Castor oil are shown to be better than in unprocessed Castor oil.

CONCLUSION

The higher the saponification and acid levels, the higher the rancidity factor and the lower the self-life and therapeutic value. As a result, decreasing these levels will increase the benefit and acceptance of medicinal oil preparations. The herbal substances employed in the processed process may be important in lowering the refractive index, saponification value, and acid value of crude oil. They may also create an increase in therapeutic benefits by adding many water-soluble and fat-soluble extractives to the initial oil, which has a positive effect on the human system. Heating or boiling the oil is also an auxiliary step that may result in a decrease in rancidity factors since heating causes the evaporation of any moisture contents. Ultimately, the processed procedure reduces the degree of saturation of oils and increases the degree of unsaturation, which is favorable to human health. As a result, medicinal oil should be made using processed oil as the foundation, rather than Unprocessed oil. The traditional system of medicine oil processing (Processed) has a positive impact on increasing the stability of processed oil and is also beneficial in initiating new therapeutic qualities generated from the components used in the processing. Further experimental

and clinical research is needed to corroborate the conclusions of this study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UPCO: Unprocessed Castor Oil; **PCO:** Processed Castor Oil; **HPTLC:** High-Performance Thin Layer Chromatography.

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