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PHARMACOGNOSTICAL STUDIES ON SEEDS OF JOJOBA (*SIMMONDSIA CHINENSIS)*

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ABSTRACT:

Jojoba was sourced from the seeds of *Simmondsia chinensis*, which was a woody, evergreen, desert shrub. Preliminary phytochemical studies indicated the presence of palmitic, palmitoleic, stearic, oleic, proteins, linoleic, linolenic, arachidic, eicosenoic, behenic, erucic as well as lignoceric and nervonic fatty acids. Also contains myristic acid that has an anti-inflammatory action. The seed oil(wax ester) was the fixed oil expressed as well as extracted from seeds of plant. Total acid value of jojoba found less than 1.0. Iodine values were in the range between 70 and 90. Saponification value was also constant and ranges from 85 - 100. High-Performance Liquid Chromatography (HPLC), Gas-Liquid Chromatography(GLC), Capillary GLC chromatographic methods were used in jojoba purity determination. Microwave-assisted extraction (MAE) technique was developed for the extraction of jojoba oil from seeds. Several influential parameters of the MAE procedure (ethanol concentration, solvent volume, microwave power and extraction time) were studied through single factor experiments and orthogonal experiment for the optimization of the extraction protocol. The optimal conditions of MAE were: ethanol concentration 60%, solvent volume 30 ml, microwave power 140 W and extraction time 20 min. In summation - jojoba's affinity to the skin makes it a superb moisturizing agent, reduces fine lines and increasing suppleness, with natural healing properties, which help with skin problem, including blackheads, pimples and acne. It furthermore helps to prevent water loss and dehydration. The seeds of jojoba was considered for this study with the aim of producing an anatomical description for diagnosis and for distinguishing it from its adulterants. The studies include physical and chemical properties associated with Natural Jojoba and new MAE procedure to increase the productivity of oil in comparison to conventional method.

KEY WORDS: *Microwave-assisted extraction (MAE), Simmondsia Chinensis, Palmitoleic.*

INTRODUCTION:

Jojoba is one of the finest cosmetic ingredients in the world. Its excellent inherent emolliency, moisturization and oxidative stability properties rank it as one of the top cosmetic lipid materials.

Jojoba (*Simmondisa chinensis*, family: Simmondsiaceae), the golden plant of Indian deserts is native to the Sonoran desert of Arizona, California and Maxico.Natural Jojoba is sourced from the seeds of *Simmondsia chinensis*, which is a woody, evergreen, desert shrub. Jojoba seeds are a dark, reddish-brown color and about 1.0 to 1.5 centimeters long. Approximately 50% of the weight of the seed is a mixture of long-chain liquid esters which is typically extracted by mechanical pressing[1]. Unlike most triglyceride seed and nut crude oils, Natural Jojoba is very low in or virtually devoid of tars, gums, free carboxylic acids, hydroperoxides, phosphatides, chlorophylls, color bodies and malodorous low molecular weight carbonyl compounds. Expelled, crude Jojoba is naturally of high quality and purity as it flows from the mechanical presses.

Jojoba liquid wax esters resist hydrolysis and oxidation for their intended use in non-occlusive moisture control and photo-protection on external surfaces of skin, hair, eyes and plant leaves.

GENERAL BOTANICAL CHARACTERISTICS:

Jojoba, [*Simmondsia chinensis*] is a new oil-producing industrial crop that has attracted much attention in recent years . Jojoba oil, commonly known as liquid wax, is colorless and odorless with unique physical and chemical properties. Unlike most other vegetable seed oils, which are triglycerides, jojoba oil is made of long-chain fatty acids and fatty alcohols with no side branching. This unique chemical configuration accords jojoba special characteristics unparalleled in the plant kingdom[2]. The most important uses are in the cosmetic industry, as a high-temperature high-pressure lubricant, and as a potential low-calorie edible oil. Other possible uses are as an anti-foaming agent in the fermentation industry and as a magnetic memory media lubricant. Jojoba oil can easily be hydrogenated into a soft wax and can be used in candle wax, various kinds of polishes, coating material for fruits and pills, and insulation for batteries and electrical wires[4].

Jojoba is a native, drought-resistant, evergreen shrub that may grow to 10 feet (3 m) or remain as a low mound 8 to 20 inches (20-50 cm) tall. The form varies in different environments; the more erect form is generally found on moist sites, whereas the semi-prostrate form is found on desert sites. Several stems arise from the root crown and branching is profuse. Younger stems are pubescent. In full light, lateral branching is prolific near the base. As the plant ages, the lower foliage is shade-pruned and a high canopy develops[5,6]. The bark is smooth.

Jojoba is usually dioecious (male and female flowers are borne on separate plants). Female flowers are small, pale green and commonly solitary or in clusters at the nodes. Male flowers are yellow, larger, and occur in clusters. Pollination occurs via wind or insect.

The leaves are thick and leathery, and are generally 0.8 to 1.6 inches (2-4 cm) long. They are vertically oriented on the plant to reduce exposure to the sun. Jojoba leaves may be shed during Severe drought ,but generally live two or three seasons depending on moisture and shade conditions. Jojoba is considered to be drought-resistant, and plants are physiologically active the entire year[10].

Jojoba fruits are dehiscent capsules that are generally one-seeded but may contain up to three acorn like seeds. The seeds are light brown to black and are large, generally 0.6 to 1.2 inches (1.5-3.0 cm) long. Approximately 50 percent of jojoba seed consists of lipids[11].

Jojoba may have several taproots that develop by forking below the root crown. The maximum depth of taproots is not known, but taproots have been observed at depths of 33 feet (10 m). Horizontal root growth does not occur except where subsurface strata prevent downward growth.

Shallow or subsurface feeder roots and true rhizomes are not developed[13].

Jojoba Seed (Nuts) An oblong to oval seed produced by the female jojoba plant weighing 0.2 - 1.5 grams each with a diameter of 3-15 mm and which at maturity is reddish brown with a wrinkled surface. Mature jojoba seeds contain approximately 3% moisture, 15% protein, 42% to 58% liquid wax esters, 5% to 7% simmondsin and the balance carbohydrate and fiber[12].

Jojoba Oil An array of natural golden liquid wax esters found in the seed of the jojoba plant. Although similar in appearance to other vegetable oils, the chemical composition of jojoba oil resembles that of outlawed sperm whale oil. Jojoba oil is composed principally of 40 and 42 carbon chain length esters, which are in turn composed of monounsaturated fatty acids and fatty alcohols of 16 to 24 carbon chain length, which make the oil susceptible to many different types of chemical manipulations. The extracted oil is relatively pure, non-toxic, biodegradable, and resistant to rancidity.

Jojoba oil is an unusually pure material made up of essentially all-wax esters with less than 1% triglyceride content. Since it contains Vitamin E (natural tocopherols) and has no polyunsaturated components, it is highly resistant to oxidation. It also contains small amounts of phospholipids that are themselves beneficial to the hair and skin, but that under certain conditions result in some turbidity in jojoba oil. Properly packaged, the oil can be stored indefinitely without degrading. Jojoba oil imparts unique and beneficial properties when used for a variety of industrial purposes. The oil has been proven to be an excellent lubricant for mechanical applications. At this time the principal use of jojoba oil is for its excellent cosmetic properties such as skin softening, skin penetration and emolliency.

Jojoba oil can be used as an alternate fuel oil. The viscosity index of jojoba oil is much higher than that of petroleum oil; therefore, it may be used as a high temperature, high pressure lubricant. Jojoba oil contained in seed is light yellow, unsaturated, of unusual stability, remarkably pure, and need not be refined for use as transformer oil or as a lubricant for high-speed machinery or machines operating at high temperatures.

Jojoba oil used for cooking, hair care, and for treatments of many medical problems such as poison ivy, sores, wounds, colds, cancer, and kidney malfunction.

Chromatographic Techniques of jojoba oil

High-Performance Liquid Chromatography (HPLC)

when used on jojoba oil, a complete separation of the homologous wax esters obtained.

Gas-Liquid Chromatography (GLC)

GLC provides more qualitative and quantitative information about the components in oils than any other single technique. Insofar as jojoba oil is concerned, the percentage of each wax ester homolog plus overall fatty acid and alcohol compositions easily obtained.

Capillary GLC is also the method of choice for analysis of the component alcohols and fatty acids of jojoba oil.

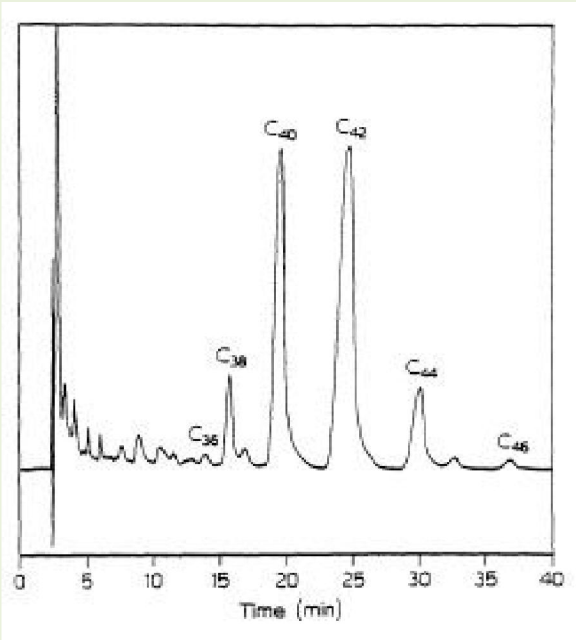

Fig.: High-performance liquid chromtography jojoba oil. Column: 250x4.5 mm ODS (octadecylailane) (Whatman). Solvent: Acetone/acetonitrile (3/1).

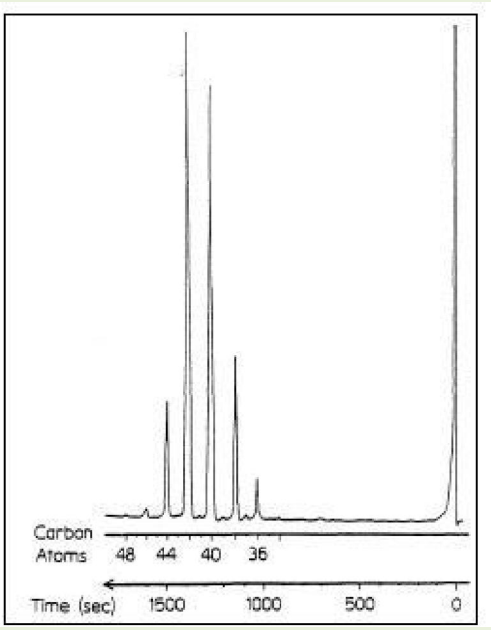

Fig: Gas-liquid chromatography of jojoba oil. Column: OV-1, 3mx 0.28 mm, with a 0.1 μm film thickness. Injector-325 C; detector-350 C; oven: 1 min at 190 C then programmed to 340 C at 5 C/min.

Conventional methods of jojoba oil extraction

Expeller Pressed Jojoba: Jojoba oil which has been removed from jojoba seed by passing the seed through mechanical presses known as "expellers". These machines "expel" the oil from jojoba seed through the application of pressure, and sometimes heat to facilitate release of the oil from the seed. In addition to jojoba oil, the processing of jojoba seed with expeller presses results in the formation of a jojoba "presscake". This presscake typically contains 12% - 18% residual jojoba oil, 5-8% moisture, 20-25% protein, 10-12% simmondsin and the balance carbohydrate and fiber.

Cold Pressed Jojoba: Jojoba oil that has been mechanically pressed from jojoba seed without the addition of heat to facilitate pressing. Cold pressed jojoba oil is typically light golden in color and has very little odour.

Solvent Extracted Jojoba: Oil Jojoba oil which has been chemically removed from jojoba presscake using chemical solvents such as hexane in an oil recovery process following, mechanical pressings of jojoba seed. Solvent extracted jojoba oil is typically dark in color, has a high level of odor and requires refining, bleaching and deodorization to prepare it for commercial use.

Simmondsin: A naturally occurring compound in the seed of the jojoba plant which has been found to suppress the appetites of animals (Dimethylsimmondsin) when incorporated in pet food formulas. Simmondsin and several of its analogs are present at 5% to 7% in jojoba seed and remain in the presscake after jojoba oil is removed from the seed[12].

Jojoba In Skin Care Cosmetics

Many of the most effective ingredients for skin care formulations are those with chemical composition and physical properties similar to the skin's own surface layers. Since jojoba is completely miscible with sebum, when it is applied to the skin, a very thin, non-greasy lipid layer of jojoba and sebum forms. This partially porous layer provides exceptional trans-epidermal respiration and moisture control. Unlike greasy occlusive materials such as petrolatum, mineral oils and some lanolin products, jojoba provides an absolutely non-tacky and non-greasy, dry emolliency[13].

At the same time jojoba significantly reduces trans-epidermal water loss without totally blocking transpiration of gases and water vapour. This function is enhanced by the kinking at jojoba's cis-configuration that helps avoid tight packing of hydrocarbon chains. Natural Jojoba serves as an excellent moisturizing agent with exceptional spread and lubricity, and leaves a rich velvety non-oily feel on the skin while retarding water loss and enhancing the flexibility and suppleness of the skin.

Pharmacodynamic studies of the penetrability of lipids have shown that there are six general factors that influence the rate of permeation into the stratum corneum:

- Viscosity: Low viscosity oils poses higher rates than high viscosity oils. Natural Jojoba has a low viscosity.
- Degree of Unsaturation: Unsaturated oils exhibit higher rates of permeation.

- Saponification Value: The lower saponification value, the higher the rate. Natural Jojoba has a low saponification number.
- Carbon Chain Length: The shorter the chain length, the higher the rate.
- Lecithin Content: The lesser the amount of lecithin in an oil, the greater the rate of penetration. Jojoba has no lecithin.
- Molecular Configuration: Straight chain and branched esters penetrate better than do triglyceride oils.

Natural Jojoba is comprised of monounsaturated fatty acids and monounsaturated fatty alcohols. It has a comparatively low saponification value and contains little to no lecithin.

Table-A: shows that jojoba compares extremely well to specialty oils used in cosmetics. The iodine value is a measure of unsaturation, specific gravity that indicates the heavy feel of an oil.

Table-A:-

OIL	IODINE VALUE	SAPONIFICATION VALUE	SPECIFIC GRAVITY (35^{O}C)
Jojoba	81-88	92-97	0.863
Almond	95-115	183-200	-
Castor	83-88	176-185	0.936
Olive Oil	79-88	190-195	0.912
Safflower	135-155	182-202	0.923
Sesame	103-116	188-195	0.918
Sunflower	120-140	185-195	-

The incorporation of jojoba into the oil phase of skin care formulations is a straight forward process. Natural Jojoba has a required hydrophilic/lipophilic balance number (HLB) of approximately 6. It is considered compatible with almost all anionic, cationic, amphoteric, and non-ionic cosmetic ingredients. Not only can multi-functional jojoba be considered as a replacement for mineral oil, triglycerides, lanolin, squalane and synthetic esters, but it can bring a whole new level of functionality to products.

Natural Jojoba's excellent moisture control and superb dry emolliency meet today's needs for simple, natural botanical ingredients that really work. Its safety is well-established by extensive testing and millions of uses without complications[10]. Its extraordinary oxidative stability can help formulators avoid the risk of rancidity. This characteristic also reduces the consumer's risk of damage from free radicals.

Physical and Chemical Properties of Natural Jojoba

Physical and chemical properties often define and distinguish chemical compounds and mixtures from one another. Based on these properties market specifications can be determined. Natural Jojoba is no different from other cosmetic ingredients in this aspect.

TABLE-B: lists typical values for many of the physical and chemical properties associated with Natural Jojoba.

Table-B:- Properties of Jojoba Oil

PARAMETER	TEST RESULT	STANDARD (IJEC)
Specific gravity, at 25 °C	0.863	0.86-0.87
Iodine value, g/100g	82	80-85
Saponification value, mg KOH/g	92	88-96
Acid value, mg KOH/g	<2	<2
Acetyl value	2	-

MATERIALS AND METHODS:

Plant material

Simmondsia chinensis was collected from the local market of Jodhpur, Rajasthan and authenticated by Botanist from *Lachoo Memorial College of Science & Technology, Jodhpur.* The voucher specimen was kept in the college museum.

Microwave-assisted extraction (MAE)

MAE experiments were performed with microwave oven (CATA-2R Scientific Microwave System, MG- 605AP, 700 W, 230 V, 50 Hz) along with a glass tube inside to prevent bumping, Clevenger Apparatus. A 20 ml ethanol solution was added to 1.0 g of slightly crushed jojoba seeds placed in an inner vessel. The extraction was carried out with different extraction conditions. Microwave-assisted extraction (MAE) technique was developed for the extraction of jojoba oil from seeds.

RESULTS AND DISCUSSION:

The factors concerning MAE include ethanol concentration, solvent volume, microwave power and extraction time (Table-I & Table-II). The influence of each factor was studied by single factor experiments.

Table-I: The factors and levels for the orthogonal design

S. NO.	SOLVENT STRENGTH (ALCOHOL) % (A)	SOLVENT VOLUME (ML) (B)	MICROWAVE POWER (W) (C)	EXTRACTION TIME (MIN.) (D)
1.	40	20	140	5
2.	50	30	210	10
3.	60	40	350	20

Table-II: The result of orthogonal experiment (factorial design)

S.NO.	A	B	C	B	EXTRACTION YIELD % YIELD
1.	1	1	1	1	14.66
2.	1	2	2	2	13.66

3.	1	3	3	3	8.66
4.	2	1	2	3	9.00
5.	2	2	3	1	11.33
6.	2	3	1	2	12.33
7.	3	1	3	2	10.66
8.	3	2	1	3	18.66
9.	3	3	2	1	16.33

Effect of solvent strength (alcohol) on extraction yield

The selection of the most suitable solvent for extracting the analytes of interest from the sample matrix is a fundamental step in developing any extraction method. Methanol has a relatively higher dissipation factor, which means that it could absorb much of the microwave energy and transform it into heat better than other solvents. However, methanol was not tested in this study, because it is highly toxic and is not practical for use in food and pharmaceutical processing. For this reason, mixtures of ethanol–water were tested under the same conditions. The results were summarized in Table-(A). It can be observed that the extraction yield of jojoba oil were greatly influenced by the ethanol concentration. The extraction yield of jojoba oil increased sharply with the increase of ethanol concentration up to 60 %. When ethanol concentration increased from 40 to 60 %, however, extraction yield increased. From these results, it is clear that the addition of some amount of water enhances the extraction efficiency. One possible reason for the increased efficiency with a presence of some water might be due to the increase in swelling of plant material by water, which increased the contact surface area between the plant matrix and the solvent[7].

Table- (A) Effect of solvent strength (alcohol) on extraction yield

S. NO.	SOLVENT STRENGTH (ALCOHOL) %	SOLVENT VOLUME (ML)	MICROWAVE POWER (W)	EXTRACTION TIME (MIN.)	EXTRACTION YIELD (%)
1.	40	30	140	20	13.00
2.	50	30	140	20	14.00
3.	60	30	140	20	18.66

Effect of solvent volume (ml) on extraction yield

Generally in conventional extraction techniques, a higher volume of solvent will increase the recovery, but in MAE, a higher solvent volume may give lower recoveries. To investigate the influence of solvent volume on extraction yield of jojoba oil, experiments were performed by increasing the extractant solvent volume from 20 to 40 ml under the experimental conditions described above. It is seen in Table- (B) that the extraction yield of jojoba oil increased with the increase of solvent volume and reached its maximum at 30 ml/g. It decreased as solvent volume was above 30 ml/g. This was probably due to the larger volume of 60% ethanol causing excessive swelling of the material by water and absorbing the effective constituent. Therefore, a volume of 30 ml was enough for extraction[8].

Table- (B) Effect of solvent volume (ml) on extraction yield

S. NO.	SOLVENT STRENGTH (ALCOHOL) %	SOLVENT VOLUME (ML)	MICROWAVE POWER (W)	EXTRACTION TIME (MIN.)	EXTRACTION YIELD (%)
1.	60	20	140	20	9.68
2.	60	30	140	20	18.66
3.	60	40	140	20	15.00

Effect of microwave power (w) on extraction yield

Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipole rotation of molecules and migration of dissolved ions[9]. Microwave irradiation energy can enhance the penetration of the solvent into the matrix and deliver efficiently to materials through molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted. In order to evaluate the effect of microwave power MAE, the different microwave powers were controlled for 20 min, e.g., 140 210, and 350 W. The results are shown in Table- (C). The experimental results demonstrate that the extraction yield of jojoba oil increase with the reduction of the microwave power.

Table- (C) Effect of microwave power (w) on extraction yield

S. NO.	SOLVENT STRENGTH (ALCOHOL) %	SOLVENT VOLUME (ML)	MICROWAVE POWER (W)	EXTRACTION TIME (MIN.)	EXTRACTION YIELD (%)
1.	60	30	140	20	18.66
2.	60	30	210	20	13.00
3.	60	30	350	20	12.11

Effect of extraction time (min.) on extraction yield

Extraction time is a factor that must be studied to increase the effectiveness of extraction of analytes employing MAE. Studies were carried out at different times, e.g., 5, 10 and 20 min. The results are shown in Table- (D). The experimental results demonstrates that the extraction yield of jojoba oil with the increase of the extraction time from 5 to 20 min Therefore, 20 min was considered as the appropriate extraction time.

Table- (D) Effect of extraction time (min.) on extraction yield

S. NO.	SOLVENT STRENGTH (ALCOHOL) %	SOLVENT VOLUME (ML)	MICROWAVE POWER (W)	EXTRACTION TIME (MIN.)	EXTRACTION YIELD (%)
1.	60	30	140	5	11.46
2.	60	30	140	10	12.31
3.	60	30	140	20	18.66

Optimization of MAE conditions of jojoba oil

Since various parameters potentially affect the MAE process, the optimization of the experimental conditions represents a critical step in the development of a MAE method. In this work, four parameters were evaluated: (A) ethanol concentration, (B) solvent volume, (C) microwave power, (D) extraction time. On the basis of the effects of single factor in above chapters, for each variable, three levels were set to detect the most suitable extraction conditions as described in Table-I. In general, a full evaluation of the effect of four parameters at four levels on the yield would require 81(34) experiments. In order to reduce the number of experiments , an L9 (34) orthogonal design graph was used (Table-II). In this way, only 9 experiments were necessary[3]. As seen from Table II, we can find that the influence to the mean extraction yields of jojoba oil decreases in the order: C > A > B> D according to the R values. Microwave power was found to be the most important determinant of extraction yield of jojoba oil. The best combination shown was A3 B2 C1 D3 which is in specific, ethanol concentration was 60%, solvent volume was 30 ml, microwave power was 140 W and extraction time was 20 min. That was also the optimal extraction condition, while extraction yield of jojoba oil was 18.66 %.

CONCLUSION:

Microwave-assisted extraction was determined to be an effective method for extracting jojoba oil from *Simmondsia chinensis*. Extraction yield of jojoba oil were affected by ethanol concentration, solvent volume, microwave power and extraction time. The optimal condition through single factor experiments and orthogonal experiment was determined as followings: ethanol concentration 60%, solvent volume 30 ml, microwave power 140 W and extraction time 20 min. This showed great potential for industrial application in the near future.

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