

# Phytochemical Analysis and *In vitro* Antioxidant Activity of Jojoba Oil

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## Abstract

### Aim

To evaluate the phytochemical constituents and *in vitro* antioxidant activity of jojoba oil.

### Objective

Jojoba oil is the liquid produced with the seed of the *Simmondsia chinensis*. Phytochemical constituents and *in vitro* antioxidant activity of jojoba oil is analysed.

### Background

The antioxidant defence system protects the cells against the free radicals. The antioxidant defence mechanism is affected by age, diet and health condition of individual. When formation of free radicals overtakes the antioxidant defence system, the free radicals start attacking the cell and resulting in several physiological disorders like Alzheimer's disease, cancer, diabetes, liver cirrhosis and rheumatism. Jojoba oil is found as an additive in many cosmetic products, especially those marketed as being made from natural.

### Reason

This research is done to analyse the phytochemical constituents and antioxidant activity of jojoba oil.

### Result.

Phytochemical tests and *in vitro* antioxidant assays were done and it was found out that jojoba oil contained antioxidant property.

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## INTRODUCTION

*Simmondsia chinensis* or simply Jojoba belongs to the family Simmondsiaceae, is mostly a woody, evergreen, perennial shrub that produce small seeds, which contains waxy liquid very similar to spermaceti<sup>[1,2]</sup>. Jojoba oil and wax is produced from its seeds. Jojoba is used to encourage weight loss, improve liver functions, elevate body immunity, also provides remedy for cancer and promotes hair growth<sup>[3]</sup>. Most of these uses were extensively studied and the plant wax and extracts showed promising activity as skin emollient<sup>[4,5]</sup>, anti-acne, anti psoriasis<sup>[6]</sup>, anti-inflammatory<sup>[7]</sup> and anti- hypercholesterolemia<sup>[8]</sup>. Jojoba contains 50% by weight oil which is more than soybeans and most oil seed crops. Jojoba oil is unique due to its unusual properties that differ from other oil seeds. The complete absence of glycerin makes it liquid wax and not fat. This jojoba liquid contains natural antioxidants and is used traditionally to treat sunburn, headache, chaffed skin, wounds and sore throat. In human studies, it was discovered that sulphurized jojoba oil can be used in the treatment of acne whereas unmodified jojoba wax can be used for treatment of psoriasis. The flavonoid profile of jojoba plant fruits may place this family among other families which are rich in flavones methyl ethers and flavonoid content which make the pericarp a valuable source for antioxidant and hepato-protective compounds<sup>[9]</sup>. Phytochemicals are basically plant chemicals, that are in active, non nutrient plant blend that are found in most plant

foods such as grains, vegetables and fruits. Phytochemicals prevent the substances we eat, drink and breathe from becoming carcinogenic, stimulate damaged cells to commit suicide before they can reproduce, regulate hormones, prevent DNA damage and also slows growth rate of cancer. Thereby reducing the risk of cancer and other major chronic diseases.<sup>[10]</sup>

Jojoba is powerful combination of antioxidant and moisturising properties. The human body has a strong antioxidant defence mechanism. This mechanism protects the body from free radicals. When activity of free radicals overtakes the antioxidant defence mechanism it results in major chronic diseases such as cancer, Alzheimers disease etc.

## MATERIALS AND METHODS

Jojoba oil used in the study was purchased from cyprus enterprises argumbakam, Chennai, India. The reagents required for the procedure was procured from Himedia.

## PHYTOCHEMICAL TESTS

The phytochemical tests were conducted according to Koleva et al<sup>[19]</sup>

### Test for carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish colour indicates the presence of carbohydrates.

**Test for tannins**

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

**Test for saponins**

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

**Test for flavonoids**

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow colour indicates the presence of flavonoids.

**Test for alkaloids**

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

**Test for quinones**

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red colour indicates presence of quinones.

**Test for glycosides**

To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicates presence of glycosides.

**Test for cardiac glycosides**

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

**Test for Terpenoids**

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicates presence of terpenoids.

**Test for phenols**

To 1ml of the extract, a few drops of Phenol Ciocalteu reagent was added followed by few drops of 15% Sodium carbonate solution. Formation of blue or green colour indicates presence of phenols.

**Test for coumarins**

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates presence of coumarins.

**Steroids and phytosteroids**

To 1ml of plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

**Phlobatannins**

To 1ml of plant extract few drops of 2% HCL was added appearance of red colour precipitate indicates the presence of Phlobatannins.

**Anthraquinones**

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink colour precipitate indicates the presence of Anthraquinones.

**ANTIOXIDANT ASSAY**

**Nitric oxide radical inhibition assay**

Sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide; it interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess-Illsovoy reaction (Garrat, 1964). In the present investigation, Griess-Illsovoy reagent was modified using naphthylethylenediaminedihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and different concentration of oils (200–600 µg) or standard solution (0.5 ml) were incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylenediaminedihydrochloride (1%) was added, mixed and allowed to stand for 30 min. A pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank. Ascorbic acid was used as positive control. The scavenging activity was calculated using the formula.

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of Test}) / A \text{ of control} * 100$$

**DPPH Assay:**

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams et al. with slight modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of essential oil solution of varying concentrations (200, 400 and 600µg). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of Test}) / A \text{ of control} * 100$$

**ABTS Assay:**

ABTS radical scavenging activity was performed according to the protocol by Re R, Pellegrini N et al <sup>[12]</sup>. The stock solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution followed by incubation for 12 h at room temperature in the dark to yield a dark-colored solution containing ABTS•+ radicals. Free radical scavenging activity was assessed by mixing Different concentration of essential oil (200, 400 and 600 µg) with 3.0 ml of ABTS working standard. The decrease in absorbance was measured exactly 1 min after mixing the solution, the final absorbance was noted up to 6 min. Ascorbic acid was used as positive controls. The scavenging activity was estimated

based on the percentage of ABTS radicals scavenged by the following formula:

$$\% \text{ scavenging} = [(A_0 - A_s)/A_0] \times 100$$

Where A<sub>0</sub> is absorption of control, A<sub>s</sub> is absorption of tested extract solution

**RESULTS AND DISCUSSION**

The following results were obtained after performing the phytochemical tests ;

TABLE 1 :

S.No	Phytochemical Tests	Jojoba oil
1	Carbohydrates test	Weakly +
2	Tannins test	Weakly +
3	Saponins test	-
4	Flavonoids test	-
5	Alkaloid test	-
6	Quinones test	+
7	Glycosides test	-
8	Cardiac glycosides test	+

S.No	Phytochemical Tests	Jojoba oil
9	Terpenoids test	+
10	Phenols test	Weakly +
11	Coumarins test	-
12	Steroids & Phytosteroids	Steroids
13	Phlobatannins test	-
14	Anthraquinones test	-

+ Present  
- Absent

From the phytochemical tests done , Jojoba oil shows a strong presence of quinones , cardiac glycosides and terpenoids. Cardiac glycosides are a class of medicines used to treat cardiac anomalies like irregular heartbeats. Carbohydrates , tannins and phenols also show a weak presence. Quinones suggest anticancer potential of jojoba oil and phenols prove the strong antioxidant property of jojoba oil<sup>[23]</sup>. Tannins are also know for their antimutagenetic properties.<sup>[24]</sup>

**ANTIOXIDANT ASSAY**

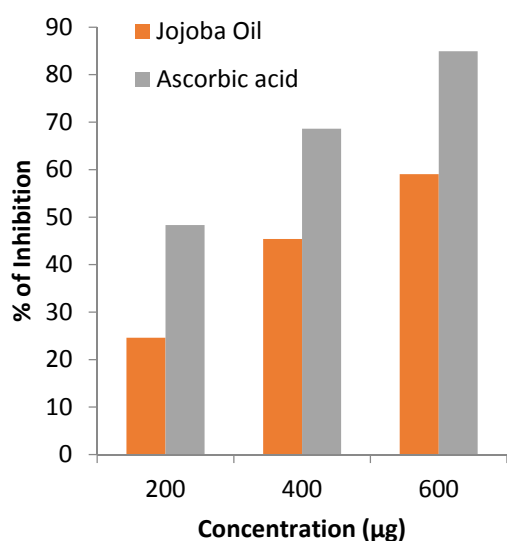
TABLE 2 : NITRIC OXIDE SCAVENGING ASSAY

Concentration (µg)	Control	Jojoba Oil	Ascorbic acid	% Jojoba Oil	% of Ascorbic acid
200	0.784	0.591	0.405	24.61734694	48.34183673
400	0.784	0.428	0.246	45.40816327	68.62244898
600	0.784	0.321	0.118	59.05612245	84.94897959

TABLE 3 : DPPH SCAVENGING ASSAY

Concentration (µg)	Control	% of Inhibition			
		Jojoba Oil	% of Inhibition	Ascorbic Acid	% of Inhibition
200	1.068	0.752	29.58801498	0.53	50.37453184
400	1.068	0.553	48.22097378	0.31	70.97378277
600	1.068	0.385	63.95131086	0.13	87.82771536

GRAPH 1 : NITRIC OXIDE SCAVENGING ASSAY



GRAPH 2 : DPPH SCAVENGING ASSAY

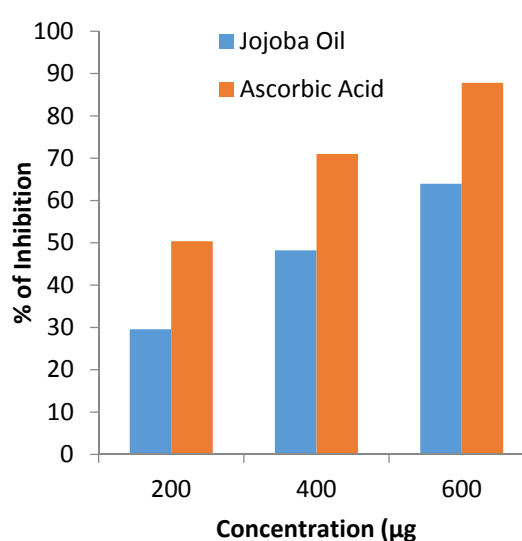
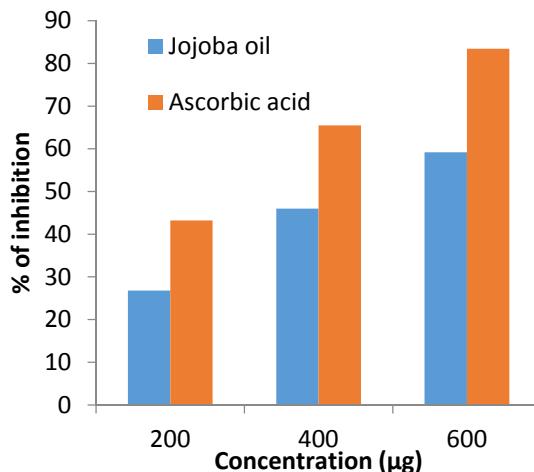


TABLE 4 : ABTS ASSAY

Concentration (µg)	Control	Jjoba oil	Ascorbic acid	% Jjoba Oil	% of Ascorbic acid
200	0.713	0.522	0.405	26.78821879	43.19775596
400	0.713	0.385	0.246	46.00280505	65.49789621
600	0.713	0.291	0.118	59.18653576	83.45021038

GRAPH 3 : ABTS ASSAY



Three tests were conducted to assess the in vitro antioxidant activity of jojoba oil. Nitric oxide scavenging assay clearly shows that there is a gradual increase in the percentage of inhibition of jojoba oil as the concentration increased. In DPPH scavenging assay, for a concentration of 200 µg, the percentage inhibition of jojoba oil was found to 29.58. In ABTS radical scavenging assay, the percentage of inhibition showed a similar increase with increase in concentration, 200 µg showed 26 % inhibition. All the tests were done against ascorbic acid as standard. The results show that jojoba oil possesses a strong in vitro antioxidant property.

**CONCLUSION**

The phytochemical screening indicated that the tested jojoba oil extract contains secondary metabolites like saponins, tannins, alkaloids, steroids and glycosides. Also, jojoba extracts exhibited antioxidant activity. The results from the phytochemical screening suggest that these extracts can have potential use as natural preservatives in food against the well-known agents of food-borne diseases and food spoilage. Antioxidant-based formulations can be used for prevention and treatment of many illnesses such as atherosclerosis, stroke, diabetes. The potential to use jojoba oil's antioxidant property for treatment of major chronic diseases will open new horizons in the pharmaceuticals industry and will be a dominant area of future research.

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