Original Article

EXTRACTION AND CHARACTERIZATION OF *SPERMACOCE HISPIDA* SEED OIL

*Rajkumar T[1], Sinha B N[2]

[1]Kottam Institute of Pharmacy, Mahabub Nagar, Andhra Pradesh, *India* - 509125.
[2]Birla Institute of technology, Mesra, Ranchi, India – 835 215.

Abstract

This paper carried out an experimental study, through extraction and characterization of crude Spermacoce hispida seed oil. Petroleum ether was used as solvent for the extraction process. The characterization analysis revealed that tested parameters, which include specific gravity, refractive index, acid value, saponification value and iodine value for crude oil produced, were within the American Society for Testing and Materials (ASTM) standard specifications. Characterization of the oils by standard techniques suggests that they contain high levels of saturated fatty acids, judging by their low iodine values. Hence they are unsuitable as alkyl resins for paint formulation but may, however, be used for soap production judging by their high saponification values. Spermacoce hispida showed the considerable reduction in Iodine value and increase in Peroxide value over a period of one month under storage conditions of light, darkness and refrigeration. In light, the Iodine Value (I.V) value dropped by 50% at the end of one month, while under the same conditions the Peroxide Value (P.V) increased by almost fivefold. Fewer profound changes in both I.V and P.V were observed for oil stored in darkness and under refrigeration. The observed profound changes were explained as arising from oxidative rancidity of the oils.

Keywords:- *Spermacoce hispida*, Seed oil, Iodine value, Saponification value, Peroxide value.

Introduction

There is a great demand for renewable sources of raw materials that have the nutritional and industrial potential. To meet the increasing demand for vegetable oils, improvements are being made with conventional crops as well as with selected plant species that have the ability to produce unique, desirable fats and oils. Seed oils are important sources of nutritional oils, industrial and pharmaceutical importance. The characteristics of oils from different sources depend mainly on their compositions and no oil from a single source can be suitable for all purposes. A lot of work has been carried out on analysis of seed oils by a number of workers, primarily because of extensive demands for oils both for human consumption and for industrial applications; consequently, there is an increasing need to search for oils from non-conventional sources to augment the available ones and also to meet specific applications [1].

The aim of presented investigation was to search for oils from non conventional sources, because of increasing needs for oil both for human consumption and industrial applications. Seed oils are known to deteriorate when processed inadequately with the principal decomposition reaction being oxidation.

*** *Author for Correspondence:***

T.Rajkumar , M.Pharm,
Kottam Institute of Pharmacy,
Eravally 'X' Roads, Mahabub nagar District,
Andhra Pradesh, India-509125.
Email: rajkutty1983@gmail.com

Oxidation of seed oil occurs through a free radical mechanism, initially characterized by the emergence of a sweetish and unpleasant odor which becomes progressively worse until it attains a characteristic smell of rancid fat. Heating is one of the most commonly used methods of food preparation in the home and industries and prolong use of oil for this purpose causes the change in its physical and chemical properties. Under the influence of temperature, fat and oils are susceptible to oxidation primarily leading to the formation of hydroperoxides. Due to their high reactivity, these hydroperoxides, especially at high temperatures rapidly react with secondary oxidative products. Examples: aldehydes, ketones, peroxides, hydrocarbons as well as cyclic compounds that exhibit very different possible toxic or carcinogenic properties.[2]

The products formed during this oxidative process can be determined by chemical analysis, one of the frequently used tests employed to predict the quality of seed oils is the determination of peroxide value and iodine value. A number of seed oils have been characterized but the vast majority had not been adequately evaluated.

Many of the species of Spermacoce are used traditionally for various diseases like an astringent, treating piles, diarrhea, gonorrhea, leprosy, leucoderma, erysipelas and abscesses. In traditional medicine, Spermacoce hispida is used to heal stomach ailments and also used as tonic, anti dandruff.

The flowers have been applied to boils, eruptions, swellings, also regarded as an emetic and as a remedy for coughs and malaria. The plant under study has been used since ages by folk because of its rich medicinal values. All the parts of the plant have an ethos medicinal importance.[3,4,5]

Until now a full characterization of the oil produced from the

seeds of Spermacoce hispida has not been reported. Additionally, the use of different methods of extraction and their effect on the composition and the characteristics of the oil has not been investigated.

Materials and Methods

The Seeds of Spermacoce hispida were collected and authenticated from the medicinal garden of Medicinal plants Revitalization and Rehabilitation Centre, Sevaiyur, Tamilnadu. Thirty kilograms of seeds were harvested, air-dried for one week, mixed well and divided into three individual portions of 10kgs each. The seeds were ground to powder using a grinder prior to oil extraction. All chemicals used in the study were analytical grade and used without further purification.

Seed index

Weight and volume occupied by 1000 seeds was determined.

Moisture and Volatilities

About 5 to 6g of seeds were accurately weighed in a periods and kept in a hot-air oven maintained at 110°C for 4 hrs. After cooling in a desiccator, the loss in weight was recorded in each case. This procedure was repeated until constant weight was obtained.

Operation of Soxhlet Extractor

300 ml of petroleum ether (60-800) was poured into a round bottom flask. 10 g of the sample was placed in the thimble and was inserted in the centre of the extractor. The Soxhlet was heated at 40-60°C. When the solvent was boiling the vapor rose through the vertical tube into the condenser at the top. The liquid condensate dripped into the filter paper thimble in the centre which contained the solid sample to be extracted. The extract seeped through the pores of the thimble and filled the siphon tube, where it flowed back down into the round bottom flask. This was allowed to continue for 30 min. It was then removed from the tube, dried in the oven, cooled in the desiccators and weighed again to determine the amount of oil extracted. Further extraction was carried out at 30 min intervals until the sample weight at further extraction Previous weight became equal. The experiment was repeated by placing 5 g of the sample into the thimble again. The weight of oil extracted was determined for each 30 min internal. At the end of the extraction, the resulting mixture containing the oil was heated to recover solvent from the oil.

Preliminary Phytochemical investigation

Phytochemical screening of Spermacoce Hispida was performed by standard procedures viz, Test for alkaloids, Test for reducing sugars (Fehling's test), Test for terpenoids (Salkowski test), Test for flavonoids, Test for saponins, Test for tannins [6].

Determination of the percentage of seed oil extracted

30g of the sample was placed in the thimble and about 150 ml of petroleum ether was poured into the round bottom flask. The apparatus was heated at 40-60% and allowed for 3h of continuous extraction using soxhlet apparatus. The experiment was repeated for different weights of the sample 35, 40 and 50g. At the end the solvent was distilled and the percentage of oil extracted was determined.

Determination of Viscosity

A clean, dried viscometer with a flow time above 200 seconds for the fluid to be tested was elected. The sample was filtered through a sintered glass (fine mesh screen) to eliminate dust and other solid material in the liquid sample The viscosity meter was charged with the sample by inverting the tube's thinner arm into the liquid sample and suction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed into a holder and inserted to a constant temperature bath set at 29°C and allowed approximately 10 minutes for the sample to come to the bath temperature at 29°C. The suction force was then applied to the thinner arm to draw the sample slightly above the upper timing mark The afflux time by timing the flow of the sample as it flows freely from the upper timing mark to the lower timing mark was recorded.

Determination of pH Value

2g of the sample was poured into a clean dry 25ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. It was then cooled in a cold-water bath to 25°C. The pH electrode was standardized with the buffer solution and the electrode immersed into the sample and the pH value was read to record.

Acid Value

Two grams of the pure oil were weighed accurately by transfer method into a 250 ml conical flask. Neutral ethanol (20 ml) was added by a pipette and the flask heated on a steam bath for 3-min then the flask was cooled and the contents titrated with 0.1N alcoholic potassium hydroxide solution using phenolphthalein as an indicator. A blank titration was also conducted side by side.

Unsaponifiable Matter

After the titration of the sample for saponification value was completed, the contents of the flask were made alkaline and extracted with light petroleum ether (60-80°C) and ether twice. The combined ethereal solution was washed thoroughly with distilled water, dried over sodium sulfate, solvent evaporated and the residue weighed. It was dissolved in neutral alcohol and the free acid titrated with 0.02N alcoholic potassium hydroxide solution using phenolphthalein as an indicator.

Determination of Saponification Value (S.V)

The indicator method was used as specified by ISO 3657 (1988). 2 g of the sample was weighed into a conical flask; 25 ml of 0.1N ethanolic potassium hydroxide was then added. The content which was constantly stirred was allowed to boil gently for 60 min. A reflux condenser was placed on the flask containing the mixture and a few drops of the phenolphthalein indicator was added to the warm

solution and then titrated with 0.5M HCl to the end point until the pink colour of the inculcator just disappeared. The same procedure was used for other samples and a blank. The expression for saponification value (SV) is given by:

$$S.V = 56.1\ N\ (V_o - V_i)/m$$

Where

V_o – The volume of the solution used for the blank Test

Vi – The volume of the solution used for determination

N – Actual normality of HCl used

m – Mass of the sample

Determination of Iodine Value (I.V)

The method specified by ISO 3961 (1989) was used. 0.4g of the sample was weighed into a conical flask and 20ml of carbon tetra chloride was added to dissolve the oil. Then 25ml of Dam reagent was added to the flask using a safety pipette influenced chamber. A stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2h and 30min. At the end of this period, 20ml of 10% aqueous potassium iodide and 125ml of water were added using a measuring cylinder. The content was titrated with 0.1M sodium-thiosulphate solution until the yellow colour almost disappeared. A few drops of 1% starch indicator were added and the titration continued by adding thiosulphate drop-wise until blue coloration disappeared after vigorous shaking. The procedure was used for the blank test and for other samples. The iodine value (I.V) is given by the expression:

$$I.V = 12.69c\ (V_1 - V_2)\ m$$

Where

c – Concentration of sodium thiosulphate used,

V_1– Volume of sodium thiosulphate used for the blank

V_2– Volume of sodium thiosoulphate used for determination

m – Mass of the sample.

Determination of Peroxide Value (P.V)

To 1 g of the oil sample, 1 g of potassium iodide and 20 ml of the solvent mixture (glacial acetic acid/chloroform, 2/1 by volume) were added and the mixture was boiled for one minute. The hot solution was poured into a flask containing 20 ml of 5% potassium iodide. A few drops of starch solution were added to the mixture and the latter was titrated with 0.025 N sodium thiosulphate and the peroxide value was determined as follows:

$$P.V = \frac{S\ N\ 10^3}{W}$$

Where

S – Ml of $Na_2S_2O_3$

N – Normality of $Na_2S_2O_3$

W – Weight of oil sample (g)

Refractive Index

Refractive index was determined on Abbe's refractometer. The prisms were cleaned with xylene and dried. Place few drops of oil on the prism, close the prisms and allow to stand for 1-2 min, adjusted the instrument and light to obtain the most distinct reading and determine the refractive index. Refractive index of oil increases with the increase in unsaturation and also chain length of fatty acid.

Results

Table 1
Preliminary Phytochemical investigation

Extract	Alkaloids	Saponins	Tannins	Phenolics	Glycosides	Steroids	Essential oils	Carbohydrates	Flavonoids	Terpenoids
Petroleum ether extract	-	+	+	+	-	+	+	-	+	+

The Preliminary Phytochemical investigation conforms the presence of Saponins, Tannins, Phenolics, Steroids, Essential oils, Flavonoids & Terpenoids.

Table 2
Characterisation of *Spermacoce hispida* Seed

Sr. No.	Analytical parameter	Values
1	Weight of 1000 seeds	1g
2	Volume of 1000 seeds	7 mL
3	Oil content (% v/w)	43.6
4	Moisture and volatilities (% w/w)	5.8
5	Ash content (% w/w)	4.56
6	Colour	brownish black
7	Odour	Disagreeable
8	Taste	Bitter
9	Protein % w/w (on dry basis)	22.5

Table 3
Results of the analysis of *Spermacoce hispida* seed oil

Sr. No.	Parameter	Value
1	Seed index	1.540g
2	Moisture and volatalities	4.6
3	Content of seed oil (%)	43.60%
4	Viscosity	9.824
5	pH Value	7.5
6	Acid value (mg NaOH g-1of oil)	1.268
7	Saponification value (mg KOH g-1of oil)	194.82
8	Unsaponifiable matter (%)	1.5
9	Iodine value (I2g 100 g-1of oil)	65
10	Peroxide value (ml g-1of oil)	90
11	Refractive index (30-40°C)	1.428
12	Specific gravity	0.962

Table 4
Iodine and peroxide values of *Spermacoce hispida* during storage

Method of storage	Iodine value	Peroxide value
	Control	
Light	65	90
Dark	65	90
Refrigerated	65	90
	After 1 week	
Light	64.77	99.23
Dark	63.5	87.5
Refrigerated	62.23	87
	After 2 week	
Light	59.42	108
Dark	62.23	87.5
Refrigerated	50.9	86.3
	After 3 week	
Light	44.45	145
Dark	49.53	86
Refrigerated	51.44	85
	After 4 week	
Light	31.75	160
Dark	49	80
Refrigerated	46.99	84

Figure 1
Iodine value versus storage time

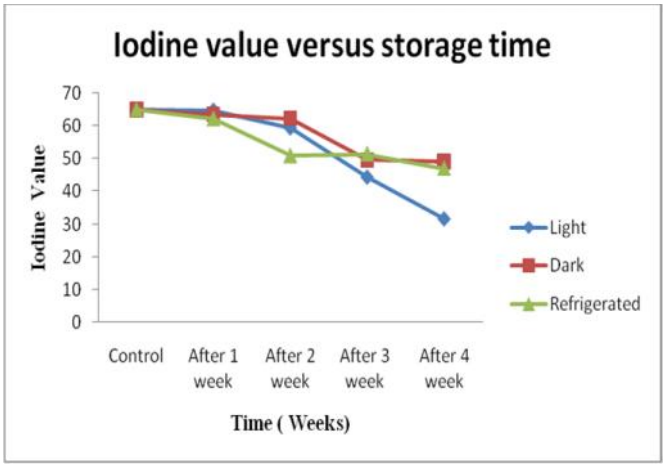
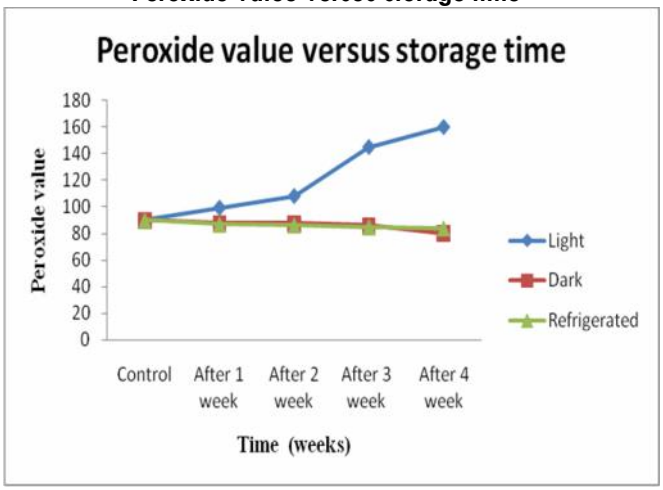


Figure 2
Peroxide value versus storage time



Conclusion

The percentage oil content of castor seed was found to be 43.60% of the total weight of 1.540g. As such a satisfactory result cannot be achieved by the solvent extraction process using laboratory Soxhlet apparatus. The seed oil produced in this research work was analyzed for specific gravity, Viscosity at 28°C, refractive index at 28°C, acid value, saponification value and iodine value. Their respective values are 0.9620, 9.824 St, 1.428, 1.268, 194.82 (mg KOH/g oil) and 65 (mg Na2SO3/g oil) for crude oil (Table: 2&3).Most of the values comply with the standard specified by ASTM (D960-52, 1952). The oil is of good quality and could be recommended suitable for industrial usage. Figure 1&2 is a plot of the iodine value and peroxide value versus time of storage of oil of Spermacoce hispida. In the first two weeks of storage, the I.V dropped much more rapidly for the refrigerated oil, but at the end of the fourth week the I.V was much lower for the oil exposed to light. The overall results shown in table 4 indicate considerable loss of instauration of oil by photo-catalyzed reactions, suggesting that the oil is better stored in the dark. Psychotropic organisms secreting oxidative enzymes can grow at low temperatures (even at 5°C), which may account for drop in I.V in the cold. The increase in the corresponding peroxide values (P.V) is much greater for the oil which was stored in the light. This suggests a high level of photo-catalyzed oxidation of the oil. The values of I.V and P.V for both oils stored in the darkened refrigerated do not differ significantly.

Reference

1. Mohammed. R.F, Jorf-Thomas.M, Determination of the lipid classes and fatty acid profile of Niger seed (Guizotia abyssinica Cass), Phytochem.Anal.14, 2003, 366-370.
2. Oderinde R.A, Ajayi I.A, Adewuyi A, Characterization of seed and seed oil of hura crepitans and the kinetics of degradation of the oil during heating, EJEAF Che., 8 (3), 2009, 201-208.
3. *Spermacoce* species.http://en.wikipedia.org/wiki/ *Spermacoce*.
4. Orwa., *Spermacoce Hispida* botanical information. Agroforestry Database, 4.0, 2009, 2.
5. Woongchon M, Ghee T.T, Geoffrey A.C, John M.P, Biological activity of novel macrocyclic alkaloids (budmunchiamines) from *Albizia amara* detected on the basis of interaction with DNA, J. Nat. Prod., 54 (6), 1991, 1531-42.
6. Khandelwal K.R, Practical Pharmacognosy –Techniques and experiments, Nirali Prakasam, 17th Ed. 2007, 149-61.
7. Akpan U. G, Jimoh A, Mohammed A. D., Extraction, Characterization and Modification of Castor Seed Oil, Leonardo Journal of Sciences, 8, 2006, 43-52.
8. Kyari M.Z, Extraction and Characterization of Seed Oils, Int. Agrophysics, 22, 2008, 139-142.