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ORIGINAL ARTICLE

Extraction and Characterization of oil from *Cannabis sativa* L.

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ABSTRACT
Extraction and characterization of Cannabis seeds was carried out for accessing its potential as an alternative biofuel crop. The extraction was carried out using absolute alcohol as solvent and the physiochemical characterization of oil was performed. The parameters studied in our study were pH value, color, specific gravity, oil percentage, peroxide value, iodine value and saponification value. The results when compared with available literature on oil extracted from Cannabis showed that the plant from India is a valuable non-conventional oil seed resource which could be exploited in future as an alternative to vegetable oil.
Keywords *: Cannabis, Hemp oil, Physiochemical properties, Oil quality, Bhaang*

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INTRODUCTION

Cannabis is a native of western and central Asia belongs to the family Cannabaceae and is represented by three members *C. sativa, C. indica, and C. ruderalis* [1]. The plant is cultivated worldwide and has the potential to be used for food, fibre and medicinal purpose [2]. However, the plant grows abundantly in the Himalayan region and has got its widespread presence ranging from Punjab, Bengal, Bihar and Deccan regions [3]. The oil from *Cannabis* contains d-9-tetrahydrocannabinol (THC) and polyunsaturated fatty acids and has been reported to be utilized for development of wide range of cosmetics, medicine, food material, narcotic drugs etc. [4-7]. Evaluation of *Cannabis* seeds elucidated its richness in vitamins (A, B and E), minerals, β- carotene and has been reported to have good nutritional and antioxidant properties which could also contribute towards the prevention of some chronic diseases [1-2, 8]. The *Cannabis* seed is reported to have a good combination of proteins (20-25 %), carbohydrates (20-30 %), oil (25-35 %), fibre (10-15 %) and minerals in appropriate quantities [1, 9]. The versatility of *Cannabis* seeds is expected to increase its demand for making useful products. There are reports on some lesser known non-conventional plants which may act as a good nutrient source for humans and cattles [10]. The increasing demands of vegetable oil has opened the eyes of various workers for exploring other less familiar plants which could be used as a substitute or complement the worldwide oil demand. Since the presence of *Cannabis* can be accessed in India on a wider scale, due to which considerable efforts may be put in for exploring the potentiality of the plant for human welfare. Moreover, a full characterization of oil from *Cannabis sativa* L. has yet not been reported from India. In the same context the present work focused on efficient extraction of oil and the chemical characterization of oil from the plants from Indian agro-ecological region.

MATERIALS AND METHODS

Procurement of seeds

The mature seeds of *Cannabis sativa* L. were collected from Pithoragarh, Uttarakhand, India. The seeds were then brought to Sharda University, Greater Noida, and India and then processed for oil extraction.

Cannabis Seed Processing

The cannabis seed was processed by three methods clearing, drying and grinding.

a) Clearing: Handpicking was done for separating foreign materials such as husk, stones, dry leaves, dirt etc.
b) Drying: The cleaned seeds were sun dried in open, until the casing splits and seeds were shed. The seeds were further dried in hot air oven at 60 ºC for 7 h for reducing its moisture content.
c) Grinding: Seeds were crushed using mortar and pestle for rupturing the cell wall and release *Cannabis* fats for extraction.

Screening of Solvents

The grinded seeds were added to various solvents used commonly by various workers viz. methanol, ethanol, isopropanol, water and glycerol. The solvent was screened for best and optimum results based upon their capacity to dissolve the grinded seeds.

Extraction of oil by Soxhlet apparatus

Absolute ethanol (400 ml) was poured into round bottom flask and sample (15 g) was inserted in the center of the extractor. Meanwhile, the heating mantle was set at 74 ºC and boiling of the solvent was observed due to which the vapor started to rise through the vertical tube and moved towards the condenser at the top. The liquid condensate dripped into the filter paper thimble in the center, which contained the solid sample to be extracted. The extract moved through the pores of the thimble and filled the siphon tube and flowed back towards the round bottom flask. The process was repeated for 40 consecutive cycles for the extraction of oil. The extracted oil was then removed from the tube, dried and cooled followed by weighing for determining the amount of oil extracted. At the end of the extraction, the resulting mixture containing the oil was purified by simple distillation to recover the solvent from the oil [11].

Oil Yield Determination (Percentage)

The oil obtained was transferred into a measuring cylinder and placed over water bath for 30 minutes at 74 ºC for complete evaporation of the residual solvent. The final volume of the oil was measured and expressed as oil content (%). The oil content was calculated using the formulae Oil content = ((weight of the oil)/(weight of the sample)) x 100 % [12].

Determination of Acid Value (AV)

Diethyl ether (25 ml) and ethanol (25 ml) were mixed and added with oil (10 g) followed by addition of phenolphthalein (few drops). The mixture obtained was titrated against 0.1 M NaOH with consistent shaking. At the end of the reaction dark pink colour was observed and the volume of 0.1 M NaOH (V_0) was noted [13]. The free Fatty Acid (FFA) was calculated using formula

$$FFA=\frac{Vo}{Wo} \times 2.82 \times 100$$

where 100 ml of 0.l M NaOH = 2.83 g of Oleic acid, W_0 = Sample weight , then $A.V=FFA \times 2$

Determination of Saponification Value

Sample (2 g) was weighed and 0.1 N (25 ml) ethanolic potassium hydroxide was added to it. The content was constantly stirred and allowed to boil for 1 h. A reflux condenser was placed on the flask containing the mixture and few drops of phenolphthalein indicator was added to the warm solution and titrated against 0.5 M HCl to the end point until the pink colour disappeared. The procedure was similarly repeated for blank. The saponification value (S.V.) was calculated by: S.V = [56.1 N (V_0-V_1)]/M, where V_0 = the volume of the solution used for blank test; V_I = the volume of the solution used for determination; N = Actual normality of the HCl used; M = Mass of the sample [13].

Determination of Iodine Value

Sample (0.4 g) was weighed and carbon tetra chloride (20 ml) was added for dissolving the oil. Dam's reagent (25 ml) was added to the prepared mixture using a safety pipette in fume chamber. The content were vigorously swirled and was then placed in dark for 150 min for incubating the sample. This was followed by addition of aqueous potassium iodide (20 ml, 10%) and water (125 ml) and the resulting mixture was titrated with 0.1 M sodium-thiosulphate till the yellow colour disappeared. It was followed by addition of few drops of 1 % starch indicator and the titration was continued again by addition of thiosulphate (drop wise) until blue color completely disappeared. The procedure was repeated for blank test as well. The iodine value (I.V) was calculated using a formula: I.V = [12.69 C (V_1-V_2)]/M, where C = Concentration of sodium thiosulphate used, V_1 = Volume of sodium thiosulphate used for blank, V_2 = Volume of sodium thiosulphate used for determination, M = Mass of the sample [13].

Determination of Specific Gravity

A clean and dry density bottle of 25 ml capacity was weighed (W_0) and filled with the oil. Further the stopper was inserted and reweighed (W_1). The oil was decanted and the bottle was washed and dried. The bottle was filled with water and then weighed again (W_2). The specific gravity (Sp.gr) was calculated using the formula: Sp.gr = $(W_1-W_0)/(W_2-W_0)$ [13].

Determination of pH Value

Sample (2 g) was poured into a clean dry beaker and hot distilled water (13 ml) was added to the sample with stirring. The mixture cooled in a circulating water bath at 25° C. This followed by pH measurement.

Determination of Peroxide Value

Potassium iodide (1 g) and solvent mixture (glacial acetic acid: chloroform; 20 ml; 2:1 v/v) were added to the oil sample (1 g) and the resulting mixture was boiled. The hot solution was then poured into a flask containing KIO_3 (20 ml, 5%) solution. Starch solution (few drops) were added to the mixture and then titrated with 0.025 M $Na_2S_2O_3$ solution. The final peroxide value was calculated by the formula:P.V $=\frac{S}{W} \times N \times 1000$; Where S $=Na_2S_2O_3$ solution in ml, N= normality of the solution and W= weight of oil sample in g [13].

RESULTS

Screening of solvents for oil extraction

Various solvents like ethanol, glycerol, iso-propanol, methanol and water were used to access the best solvent which could be used for oil extraction. It was observed that out of all these solvents used ethanol resulted into a clear solution when the crushed seeds were dissolved into it (**Table 1**).

Accessment of physical properties of oil

The color of the extracted oil appeared to be brownish green and the calculated pH value was 6.3 for the extracted oil.

Oil Yield Determination (Percentage)

The average oil content of 29.87 % was obtained from the experimental set up.

Assessment of physio-chemical properties of extracted oil

Acid value, Iodine value, peroxide value, saponification value and specific gravity of the extracted oil were calculated and represented in **Table 2.**

Table 1. Solvents used for initial screening for best dissolution of crushed seed material

S.No.	Solvent	Observation
1	Ethanol	Non toxic, clearly dissolved and supernatant was clear layer on centrifugation.
2	Glycerol	Seeds were not dissolved completely.
3	Isopropanol	Seeds showed some impurities on centrifuging also.
4	Methanol	Toxic, clearly dissolved on centrifuging.
5	Water	Dissolving of seeds was not proper.

Table 2. Physio-chemical Properties of the extracted *Cannabis* oil

Property	Values
Acid Value [mg NaOH/g of Oil]	1.128 ± 0.2
Iodine Value [g I_2/100g of Oil]	155.7 ± 0. 3
Peroxide Value[meq/kg of Oil]	37.5 ± 0.1
Saponification Value [mg KOH/g of Oil]	192 ± 0.5
Specific gravity	0.95

DISCUSSION

Hempseed has always been regarded as a food material and consumed either as food or for therapeutic preparation [4, 14]. The oil extracted from other species of *Cannabis* has been regarded as balanced oil with respect to PUFA, linoleic and linolenic acid contents [4]. The average oil content of the extracted oil was 29.87% which is quite similar to that of previous results from worldwide hemp oil researcher which emphasized the general range of about 30-32 % [5, 15]. The variation in oil yield on the basis of geographical locations might be an indicator of implications of agro-climatic conditions prevailing over the region. The color of the oil obtained was slight brownish green and was different from the reported non-conventional vegetable oils [16-17]. and may be an implication of the steps involved in refining of the oil. The iodine value was 155.7 which is comparable to previous report of [1] where a broad range of 154.00–165.00 g of iodine/100 g of oil was observed. The saponification value was 192 mg KOH/ g of oil

in our case which is more than the highest value reported for hempoil in previous reports of [1]. The higher saponification value obtained in our case is also an indicative of better oil content in studied species and is supported by the studies on oil extracted from olive, sunflower and cotton seeds [18]. The results obtained for peroxide value and the specific gravity are also comparable under the general regulation of the previous reports [19]. The primary objective of the present study was to extract and investigate the physiochemical characteristics of *Cannabis sativa* L. collected from its native region in India. The study becomes important considering the report of [20] which has deciphered the variations in tocopherol content and FA profile of oil from *Cannabis* collected from different locations of Russia. Thus, it can be concluded that the properties of *Cannabis* varies depending upon the harvesting time, climatic conditions and geographical locations. Similarily, the possibility of *Cannabis* seed having different/contrasting properties growing in different geography cannot be ruled out. India primarily being agriculture based country, should have the capability of fulfilling the necessity of oil only from its own self. The comprehensive analysis of the oil extracted in our study represents the potential of these seeds to combat the shortage of oil demand in the country.

CONCLUSION

The analytical findings observed in our study are in close relations to that of other literature reports and can accelerate the ongoing research for this immense valuable oilseeds plant. The extraction contributes to the scientific knowledge by deciphering a new solvent which could be used for extraction of *Cannabis* oil and put forward the ignored potentials to be used as a bio-fuel.

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AUTHOR'S CONTRIBUTION

BRM collected the seeds from Uttarakhand, India ; RP and MK extracted the oil; BRM, RJ and MK characterized the extracted oil, VBS planned the work, inferred the results and drafted the manuscript.

CONFLICT OF INTEREST

Authors declare none

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