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Extraction, Physicochemical properties, Phenol and Tocopherol of *Cassia Siamea Lam* Seed Oil

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***Abstract*—The objective of this study have determined the physicochemical properties, phenol, and tocopherol of *Cassia Siamea Lam* seed oil.*Cassia Siamea Lam* oil have done using the solvent extraction method. The oil was analyzed for the composition of tocopherol, phenols, and physicochemical properties (specific gravity, refractive index, iodine value, saponification value, unsaponifiable matter, acid value, and peroxide value). The physicochemical properties of the oil were analyzed by AOAC Official method (1999).The phenol was analyzed by spectrophotometer and tocopherol was analyzed by HPLC technique. The specific gravity obtained of*Cassia Siamea Lam*at 45^{0}C was 0.892, the peroxide value 4.09mEq/Kg and the refractive index was found 1.4642, the acid value of *Cassia Siamea Lam*oil obtained was 9.81mg KOH/g, the iodine value was found to be 108gm/g, saponification value of*Cassia Siamea Lam* oil was182 mg KOH/g, while the unsaponifiable matter of*Cassia Siamea Lam* oil was 0.7mg/KOH/g, phenols 538.4mg/100g and tocopherol 114%mg/100g. The studied properties of *Cassia Siamea Lam* oil were examined andfound that this oil may be useful for the domestic and industrial purposes (paints, cosmetics, soap, lubricants, and varnishes) and as synthetic drugs.**

***Index Terms*—Cassia Siamea Lam*, Oil, Extraction, Physicochemical properties, Phenol, Tocopherol.**

I. Introduction

Oils and fats from seeds constitute an important role in human nutrition as well as for several industrial purposes. The most common oil seeds are ground nut, soybean, cotton seed, sunflower seed, rapeseed, sesame seed, palm kernel seed, coconut seed, etc. [1]-[3].There is two types of oils 1- Edible oil, and 2- Industrial oils. Edible oils are used as food-grade oil that is most often encountered in odorless frying. Oil is low in unsaturated fats, but it is also an ingredient in many food products. Especially where healthy oil is required edible oils had made an important contribution to the diet in many countries, serving as a good source of protein, lipids and fatty acids for human nutrition including the repair of worn-out tissues. Now well formation as well as a useful source of energy [3]-[7].

There are many uses for industrial oil for example flaxseed oil, rich in the unsaturated fatty acid linoleic, is a drying oil and is used in protective coating (Paint, printing, Varnishes). Vegetable oils are used in putty, printing inks, erasers, coating or core oils, greases, plastics, etc. the oils have a very low degree of unsaturation and could be classified as non-drying oils, melon seed oil could be very good for soap-making oil seed meals from soybean, peanut, rapeseed, and flaxseed are rich in protein, when mixed with other ingredients (Cereal grains) and they provide nutritionally balanced feeds [8].The quality of the oil depends upon the physical and chemical parameters that are dependent on the sources of oil processing and storage conditions. Some physical parameters are moisture content, refractive index, viscosity, specific gravity, color, etc. Chemical parameters are saponification value, acid value, iodine value, ash content, and peroxide value which can be used to evaluate the purity and quality of the oil [9]. The protein quantity and quality, caloric values, and nutrient content of seed oils are good.

Phenolic compounds are as important as unsaturated lipids, carbonyl compounds and non-enzymatic browning in the development of adverse flavors and colors in food products the free phenolic acids are the particular concern because of enzymatic oxidant too-quinoas and subsequent binding to lysine and methionine in the protein [10].

Tocopherols are important biological antioxidants; Alpha tocopherol prevents oxidation of body lipids including polyunsaturated fatty acid and lipid components of cells. Tocopherol has been associated with the reduction of heart disease and the prevention of cancer. Tocopherols have widely been used for food, feed, pharmaceuticals cosmetics, and resins. In food, Tocopherol is used as antioxidants for frying oil, margarine, fried snacks, and so on[11].

Cassia Siamea Lam is a well-known Ayurvedic medicinal tree and its sub-family is Caesalpinioideae. *Cassia Siamea Lam* is an evergreen tree that grows almost 18 m long with up to 30cm straight trunk diameter and gray to light brown bark. Large branches of this tree up 20–30 cm in length and 13 cm in width have bright yellow flowers that are present terminally on the branch. *Cassia Siamea Lam* seeds are a maximum of 8mm long shiny dark brown in color and bean-shaped with a different area[12],[13].

The seeds of *Cassia Siamea Lam* are used in intestinal worms or as an antidote for scorpion and snake bites[14]. However, there is a dearth of information on the physiochemical properties of *Cassia Siamea Lam* seed oil. The results determined the physicochemical properties of this seed oil and are expected to provide information on possible uses of *Cassia Siamea Lam* seed oils.

II. Materials and Method

A. Collection of plant material

Cassia Siamea Lam seeds were collected in Dhanipur Mandi Aligarh. Collected seeds were dried in direct sunlight for two days before being ground into a fine powder and

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stored in an airtight container for chemical analysis.

B. Oil extraction

The oil was extracted from *Cassia Siamea Lam* seed powder using a solvent extraction method with petroleum ether as a solvent, as described by [15]-[17]. 100 g powdered *Cassia Siamea Lam*seeds were packed in filter paper and inserted into the Soxhlet extractor, with petroleum ether as the extraction solvent. After 24 hours, the solvent was recovered using a water condenser, and the residual oil was transferred into a desiccator and stored in an airtight container until needed for analysis.

C. Physical properties[18]

was used to determine the percentage yield, color, moisture, and specific gravity, and the ash content was determined according to[19],[20], defined the refractive index of extracted oil.

D. Chemical properties

Acid value, Peroxide value, Iodine value, Saponification value, and Unsaponifiable matter, were determined by [21].

E. Determination of total phenols

Total polyphenol was extracted from an oil sample in accordance with[22]. 1 ml of the extracted sample in a 100 ml volumetric flask, diluted with water in separate Plastic tubes, 1 ml of Gallic acid standard Solution A, B, C, D, and E were placed. In each tube, add 5 ml of dilute Folin-Ciocalteu Phenol reagent and mix for 3 to 8 minutes. Following the addition of dilute Folin-Ciocalteu phenol reagent, 4 ml of a sodium carbonate solution was mixed into each tube and allowed to stand at room temperature for 60 minutes before being measured with a Spectrophotometer set at 765. The amount of polyphenol in the oil was calculated as mg of Gallic acid per kilogram of oil using external calibration curve (R^2=0.999) and calculated by these formulas and results were averaged.

Polyphenol (mg/Kg) = (Concentration(x)*dilution*0.988)/ (sample weight (g)).

F. Determination of Tocopherol acetate composition

Tocopherol acetate was analyzed using modified method following by [23]. Tocopherol acetate were evaluated using high performance liquid chromatography (HPLC) with capable of pressures up to 3000 psi with injector capable of 100 µL injections Operating conditions eluent flow rate 2.0 ±0.2 mL/min; temperature ambient Detector Capable of measuring absorbance at 280 nm, with sensitivity 0.02 AUFS. Precolumn set at 2 mm id × 2cm stainless steel, packed with 40µm pellicular reversed-phase C 18 and column set at 4.6 mm id × 25 cm stainless steel, packed with 5µm silica. The mobilephase consisted of hexane isopropyl(99.92+0.08, v/v), HPLC grate solvent. Degas 2-5 min under vacuum the injection volume 20 µL. The data wereintegrated and analyzed using the Chromatography Laboratory Automated Software system. Standard of and isomers of tocopherols were dissolved in hexane and usedfor identification and quantification of peaks. The amount of tocopherols in the oils was calculated as mg tocopherols per kg oilusing external calibration curves (R^2 =1), which were obtainedfor Tocopherol acetate standard. All chromatographic analysis wascarried out for three replications and the results were averaged and calculated by these formula.

Vitamin E mg/kg (A) = (PPM*Dil.)/ (wt.)*0.99

III. Result and Discussion

Table-I Physical properties of *Cassia Siamea Lam* seed Oil

Physical properties	Color	% Yield	Specific gravity at 45^{0}C	Refractive index 40^{0}C	pH Value
Cassia Siamea Lam seed Oil	Yellow	10	0.892	1.4642	4.20

Table-II Chemical properties of *Cassia Siamea Lam* seed oil

Chemical properties	*Cassia Siamea Lam* seed oil
Acid Value(mgKOH/g)	9.817
Peroxide Value (mg/kg)	4.09
Iodine ValueIodine/kg	102
Saponification Value (mgKOH/g)	154.83
Unsaponifiable Matter (g/kg)	4.714
Polyphenol(mg/100g)	538.4
Tocopherolacetate (mg/100g)	114.1

The physical and chemical properties of the oil are presented in the table-I and table-II. The oil of*Cassia Siamea Lam* was yellow in color and liquid at room temperature. The yield % of *Cassia Siamea Lam* oil is 10 shows in table-I.The specific gravity of oil depends on the type of oil and temperature. Different values of specific gravity may attribute to the differences in fatty acid composition, total solid content, and degree of unsaturation. The specific gravity of the oil increases as the degree of unsaturation increases[24]. The specific gravity of *Cassia Siamea Lam* oil was 0.892 if we compared with other oil so it was higher than the ground nut oil while lower than sesame oil.

The reflexive index has performed the purity of the oil to follow hydrogenation and isomerization refractive index of oil increases with the increase in unsaturation and the chain length of fatty acids. The Refractive index value obtained for *Cassia Siamea Lam* oil 1.4642 was in close agreement with values reported for conventional oils from ground nut oil (1.450) and sesame oil (1.472). The high reflective index of these oils seems to confirm the high number of carbon atoms in their fatty acid [25].

The pH value is used to specify the acidity or basicity of an aqueous solution. In the case of fresh oil, the pH is 10, and pH 9 to 11 is appropriate. The pH value of *Cassia Siamea Lam* oil was obtained 4.2[26].They could also early polymerize during the deep frying of foods[27].

The acid value indicates the quality of fatty acids in oil; The Low acid value in oil indicates that the oil will be stable over a long period of time and protect against rancidity and peroxidation. High acid value in oil showed that the oil may not be suitable for use in cooking but be useful for the production of paint, soap, and shampoo. The acid value of*Cassia Siamea Lam* oil was 9.817mg KOH/g. This indicates that the high acid value in oil showed that the oil may not be suitable for use in cooking but be useful for the production of paints, liquid, soap, and shampoo[28],[29]. Unsaponified matter includes those substances frequently found dissolved in fats and oils which cannot be saponified by caustic alkali but are soluble in ordinary fat solvents[30].

The peroxide value of oil is commonly used to determine the rancidity of a sample containing fat or oil, so a high peroxide value of oil indicates a poor resistance to peroxidation during storage[31].General fresh oil has a

peroxide value of > 10milliequivalent/Kg. Range fresh oil has a peroxide value of 30-40milliequivalent/Kg range is generally associated with a rancid test. The peroxide value obtained for Cassia Siamea oil was 4.09 milliequivalent/Kg. While the peroxide value of groundnut oil is 1.15 milliequivalent/Kg and sesame oil 7.30milliequivalent/Kg respectively.

Iodine values of *Cassia Siamea Lam* oil was 103 g/100g .These result showed that Cassia siamea oil contained more unsaturated fatty acid than ground nut oil, and high iodine value is not without its disadvantages, for example, the oil will be more susceptible to oxidative deterioration, thereby making them difficult to store. The oil will not be suitable for use as biodiesel fuel because a high iodine value is an indication that the oil will be viscous and hence will have a low degree of atomization[32]. On the other hand, when unsaponifiable is too high (0>3%) the specific unsaponifable present do not provide significant benefits for soap making. Unsaponifiable matter obtained for *Cassia Siamea Lam*oil was significantly different from that of ground nut oil and coconut oil.

Saponification values are useful in determining the amount, type of glycerides, and mean weight of acids in the oil. The average chain length of the fatty acid that makes up the oil is measured by saponification[33],[34].The lower the saponification value, the higher the molecular weight of the fatty acids in the glycerides or the fewer the ester bonds[35].Saponification values of 154.83 mg KOH/g for Cassia Siamea oil have been reported. The saponification value of *Cassia Siamea Lam* seed oil indicated that the fatty acids in the oils contained a larger number of carbon atoms.

Unsaponifiable of*Cassia Siamea Lam* seed oil was 4.71g/kg .Unsaponifiable matter can be beneficial to soap formulas because they may have properties such as the iodine value is defining the unsaturation and identity characteristics of seed oil. If the Iodine value is high then it reduces heart diseases associated with cholesterol[36].Unsaponified matter includes those substances frequently found dissolved in fats and oils which cannot be saponified by caustic alkali but are soluble in ordinary fat solvents[37].

Phenolic compounds have been examined to contribute about 30% to oil stability[38]. Phenol has been shown to play important roles as an antioxidant and influence the flavor of oil. Table-III displays the concentration and absorbance of the A, B, C, D, and E solutions after spectroscopy analysis. Figure-I shows the graph between concentration and absorbance and Table-IVdisplays the total polyphenols calculation result. The total polyphenols content of the *Cassia siamea oil* was538.4mg/100g. This shows that *Cassia siamea oil* is more stable than groundnut oil and *Cassia siamea oil* will be a better source of dietary antioxidants compared to groundnut oil.

Table-III .Concentration and Absorbance table

Sol.	Aliquot (ml)	Volume (ml)	Std. Conc. (x) (ppm)	Absorbance (Y)
A	0.2	25	8.368	0.119
B	0.4	25	16.736	0.225
C	0.8	25	33.472	0.417
D	1.2	25	50.208	0.621

Fig.-I.UV-Spectrophotometer Graph on Concentration and absorbance

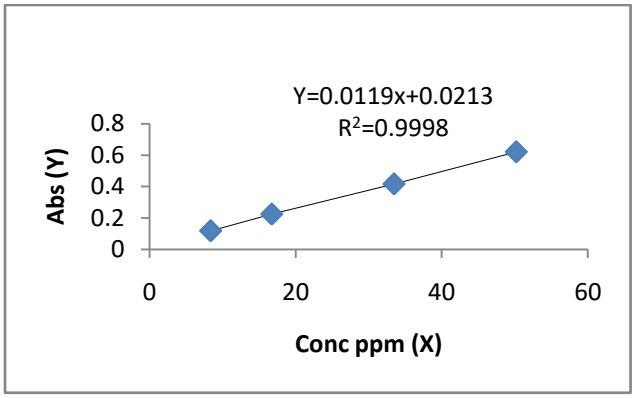

Table-IV. Calculation result of polyphenols

Sample wt.(g)	Dilution (ml)	Abs.	Conc.(mg. kg^{-1})	Polyphenols $m.kg^{-1}$	Polyphenols $mg.kg^{-1}$
0.5240	50	0.089	5.6891	536.3	
1.0501	50	0.158	11.4874	540.4	538.4

Tocopherols are important antioxidants .α Tocopherols prevent the oxidation of body lipids including polyunsaturated fatty acid and lipid components of cells and organelle membranes. Tocopherol has been associated with the reduction of heart disease[38].Table-V display theArea and ppm of the A, B, C, and D solutions after HPLC analysis. Figure-II shows the graph between Area and ppm and Table-VI displays the Tocopherol calculation result. Total Tocopherol values in the oil sample are present in table-II. The Tocopherol of*Cassia Siamea Lam*oil was114.1 mg/100g significantly. This implies that *Cassia Siamea Lam*oil will have a longer shelf-life compared to groundnut and sesame oil. They will also be better dietary sources of this impotent vitamin.

Table-V. Area and ppm table

Sol.	Ppm	Area	Rf
A	242.5	1993338	0.000122
B	606.3	4880570	0.000124
C	1212.6	9603876	0.000126
D	2425.2	18908286	0.000128

Fig-II. HPLC Graph on Area and ppm

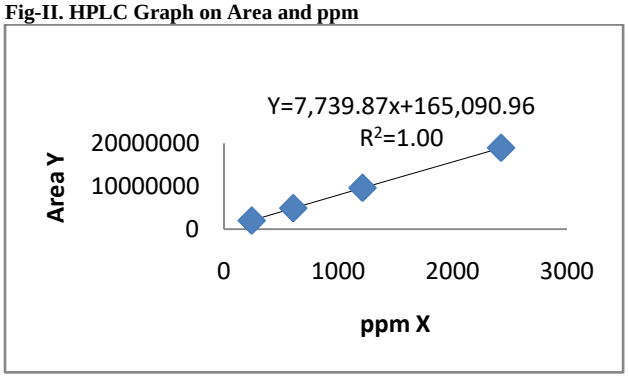

Table-VI.Calculation table of Tocopherol

Sample area	Ppm	Wt. (g)	Dil. (ml)	Tocopherol (mg/kg)	Tocopherol Avg ($mg.kg^{-1}$)	Tocopherol ($mg.100g^{-1}$)
4643920	578.67	1.0143	2	1136.4589	1140.8	114.08
2763281	335.69	1.1679	4	1145.1198		

IV. CONCLUSION

Present work will be helpful to collect the information for *Cassia Siamea Lam* and explore the plant seed oil for many opportunities. The important test of physicochemical properties of *Cassia Siamea Lam* seed oil revealed that the oil is edible as well as no-edible. The oil extract from the seed of *Cassia Siamea Lam* is a good source of phenols and tocopherol so it could be seen as a potential source of drug. The studied properties of *Cassia siamea* oil were examined that this oil may be useful for the domestic and industrial purposes (paints, cosmetics, soap, lubricants, and varnishes) and synthetic drugs.

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