Original Article

Isolation and Characterization of Oil Obtained from *Leucaena leucocephala* (Lam.) De Wit

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ABSTRACT

In the present study, we have collected the leucocephala seeds which are seasonal and having good amount of oil content. The oil seeds can be used as the one of the adulterant for the coffee. The whole plant having importance for human being, it could be as the food or furniture. The immature seeds, pods and flower buds are used as the salad while the gum obtained from this plant can be used for the confectionary, pharmaceutics, and cosmetics industry. In some of the region, it is considered as the recalcitrant and attracts rodents. The plant is well-known in traditional medicine and seeds are the rich source of several necessary metabolites such as proteins, tannins, carotene, leucenol, and flavonol glycosides and having therapeutic potential like anti-inflammatory and antidiabetic activity. Hence, in our present investigation, we focus for the collection of seeds, and extraction of oil and its characterizations. The dried pods and seeds were collected from Waghi Village, near Nanded, Maharashtra. The obtained extract were further characterized for the confirmation of oil content by measuring lipid content, specific gravity, density, protein, and glucose content, and further analysis with thin layer chromatography. The obtained extract is also subjected to the anti-microbial activity.

Introduction

White Popinac or white lead tree is typically tropical plant species, grows in warm conditions and this plant scientifically known by the name *Leucaena leucocephala* (Lam.) de Wit belongs to Fabaceae family of plants. The plant members of Fabaceae are supposed to nodule forming; hence, also termed as the Leguminosae, the plants of this family can be easily identified by some key markers such as these plants frequently forms symbiotic relationship with nitrogen fixing bacteria, leaves are compounds, and alternatively arranged, margin is smooth, flower are bisexual, the fruits are legume or loment types. Cultivation of this plants has been done since long time for the health and other necessary benefits. White Popinac is small shrubby in size, supposed to be fast growing with higher photosynthetic rate, responsible for massive biomass production which accounts for around 30 tons dry matter $ha^{-1}.yr^{-1}$, on average 3-15

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meter tall and the maximum height riches to 20 m, numerous branches, young branches covers with smooth, grey to brown, rusty orange-brown vertical fissures barks. This plant could be better solution for the deforestation and lesser plantations [1]. The plant is short lived (average 20-40 years) evergreen in nature and mature and immature pods can be at same time on the plant. The plant is resistant to several extreme conditions such as pH (upto 4.1), drought and salinity. The plants have higher resistance to biotic and abiotic stresses, which shows the greater production of secondary metabolites necessary for it [2]. The medicinal plants have special place in all Indian therapeutic system like Unani-Tabbi, Ayurved, Siddha, Homeopathy, *etc*. [3] and White Popinac have versatile medicinal properties and treated as the miracle tree for food, forage and timber [4]. This plant has potential use in folk medicine to increase the milk production and weight gain in ruminants [4] and have potential as the insect repellant. The major secondary metabolites and necessary phytochemicals obtained from this plants can be used as the antidiabetic [5, 6], anticancer, antimetastatic, antibacterial [7], and antihelminthic. The notable medicinal use of this plant as the contraception and abortifacient potential and also helps to reduce stomachache.

This plant species have great potential to sustain in dry conditions and commonly having versatile applications as per the different sites of global locations [8]. It is to be used as the salad or along with other food materials in countries like Indonesia, India, and Thailand. Young plant parts, leaves, and shoots are majorly used to prepare several food dishes while dry seeds were used as the alternative to coffee powder [9], hence also known by the name bush coffee. The countries like Mexico and Central America, where immature seeds and pods are used as the food. The gums secreted by this plant are used as the important ingredient in Confectionary, Cosmetics, and Pharmaceutics.

In some regions, this plant is also supposed to weed and can easily survive and spread in any season, in each season, this plant spreads thousands of seeds that travels long ways and attracts rodents. The invasion of White papinoc observed roadsides, wastelands, cultivated lands, riverbanks, and forest edges which prevent survival of herbs, shrubs, and trees and this plant influence the particular habitat in patterns of vegetation, plant succession, and community assembly [10].

The seeds are good source of oil and it can be used for the production of biodiesel, and also it is rich source of lipid, carbohydrate, and protein [11]. The oil obtained from this plant can be used for bio-membrane modeling against xenobiotic compounds [12] and anti-corrosive for the mild steel and copper. Plants have multi-facet application like prevention of soil erosion, fuel wood, timber, fodder, green manure, etc. The major amount of oils are coming from the plants in the form of margarine, salad oil, and specially derived products which is necessary component of food processing and preparations. In the present investigation, we have collected the seeds and extracted the oil content in it. The oil obtained from this plant with therapeutic potential. Therefore, we conducted chemical characterization and bioactivity of the obtained oil product.

Materials and Methods

Collection of Sample

The dried pods and seeds were collected from the village Waghi, near Nanded, Maharashtra. The seed sample was further dried in the sunlight for 10 to 12 days. The dried seeds were distributed in two parts, half of the seeds were kept soaked in water for a week and remaining portion of the seeds were soaked in boiling water for 2-3 hours.

Oil Extraction

The crude extracts obtained in normal water and boiling water [13] were thoroughly mixed with organic solvent such as petroleum ether.

After proper mixing, with the help of separating funnel upper organic layer were separated and subjected to distillation for evaporation of excess solvent present in it. The obtained crude extract further collected in amber colored screw cap bottle and stored in cooling condition till further use.

Detection and Characterization of Oil

The crude oil obtained through the extraction were further analyze by measuring gravity and density of the crude extract [14].The obtained crude extract was further analyzed through thin layer chromatography [15], saponification [16], and estimation of oil content in it [17].

Evaluation of Microbial Contaminations

The obtained seed oil allowed to exposed microbial contaminants present in the laboratory. The obtained microbes were detected by microscopic method with the identification of morphological key markers [18].

Results and Discussion

The collected seeds were sundried divided into two equal halves. Half amount of seeds around 50 gm were soaked in distilled water for few days and remaining half amount of seeds were boiled in water for 3-4 hours. The extract obtained from soaked and boiled seeds appears to be brownish in color because of its seed coat color. The oil content present in the seed sample were found to be floating on the water and later on separation extracted oil were done with the ether extraction using separating funnel. Seeds have medicinal values, and it also contains very good amount of natural oil in it [8]. The oil components were further has been confirmed with the help of thin layer chromatography.

The oil produced by the seeds were gradually accumulated at the upper surface of boiling water or normal water (Figure 1). The oil collected at the surface of boiling water were further mixed with petroleum ether and separated with separating funnel (Figure 2). The oil extract were further concentrated by the removal of petroleum ether by keeping the temperature of heating mantle at 45 ◦C around the boiling point of petroleum ether. The distillation helps to remove extra solvent present in the extract. The obtained extract were further characterized by measuring lipid content, specific gravity, density, protein, and glucose content. The compounds present in the ether extract were further analyzed and confirmed through the thin layer chromatography. The obtained extract is also subjected to the anti-microbial activity. In an anti-microbial assay, we found that instead of killing or inhibiting the growth of microbes, in the presence of extracted oil, confluent microbial growth were observed in comparison to control. There may be some nutrient component present in the extract responsible for enhanced microbial growth.

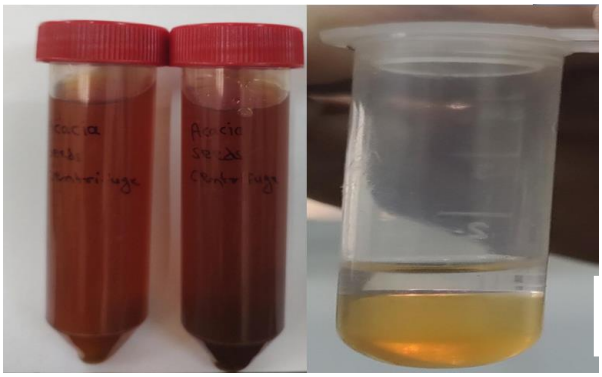

Figure 1 Extraction of oil from plant seeds and further separation with separating funnel

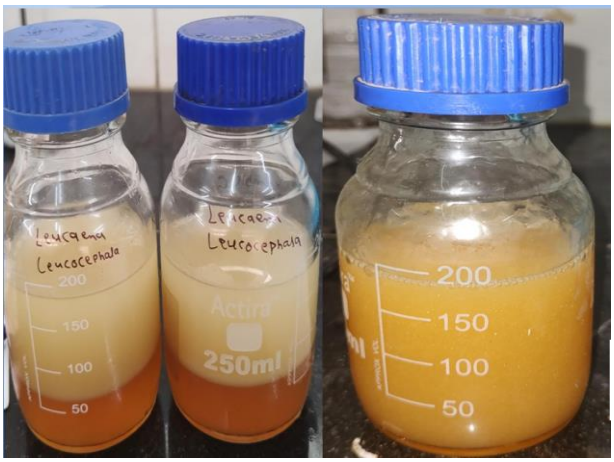

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Figure 2 Determination of lipid content of oil by Saponification method

The detection of lipid content in the ether extract was done through the esterifications, during the esterification, we observed the formations of some unknown compounds and the probability of this compound could be Fatty Acid Methyl Esters (FAME) [19] (Figure 3). The estimation of lipid content in the extracted oil were found around 4.88 %, similarly in vegetable oil blend, other researchers found the oil content in vegetable oil blend around oleic acid (C18:1n-9; 63.3%), linoleic acid (C18:2n-6; 4.7%), and linolenic acid (C18:3n-6; 5.1%) [20] Major components of the natural oils are terpenoids and phenolic compounds [21].The natural and essential oil obtained from the plants or plants parts are basically composed of several chemical compounds or secondary metabolites such as terpenoids, shikimates, polyketides, and alkaloids which are produced through the shikimate pathways. These compounds have potential role in the food, cosmetic, and pharmaceutical industries (Figure 4) [6].

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Figure 3 Detection and separation of some unknown esterified product after esterification

The analysis of crude oil extract was done through the thin layer chromatography, in the chromatogram we observed uniform banding pattern in all samples of the crude oil (Figure 5). The samples seems to have pure oil components in the plant seeds with Rf values of 0.24, 0.45, 0.54, and 0.72. In the characterization of oil yielding seed like soybean, it was observed the oil components were observed at their respective Rf value such as Phospholipids at 0.01 ± 0.002, Monoacylglycerides at 0.04 ± 0.013,

Triacylglycerides at 0.20 ± 0.034, Tocopherols at 0.66 ± 0.105, and Fatty acid esters at 0.95 ± 0.14 [22]. The characterization of secondary metabolites in the *Asplenium indicum* also have done through the thin layer chromatography [23]. In India, we have around sixteen edible oil yielding plants and numerous plants known for the extraction of medicinal oil. The oil can be extracted from different plant parts such as root and shoot (leaves, fruits, seeds, bark, wood, flowers, and buds). The most of secondary metabolites are excluded from essential oils of the plant such as cellulose, glycerides, starches, sugars, tannins, salts, and minerals. The yield of the oil from the plant sources is ranges from 0.05-18.0%.

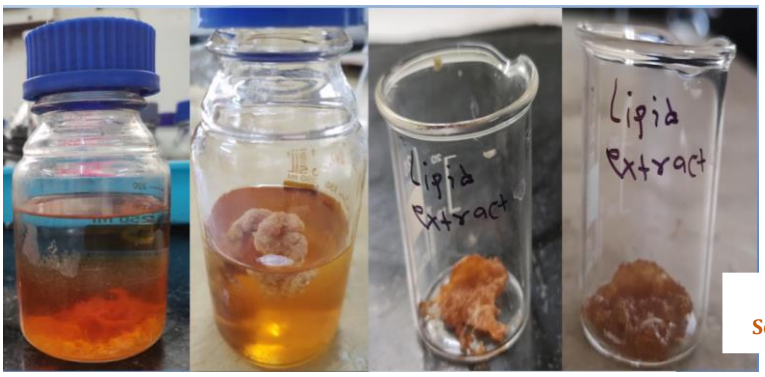

Figure 4 Isolation and estimation of lipid content in oil extracts

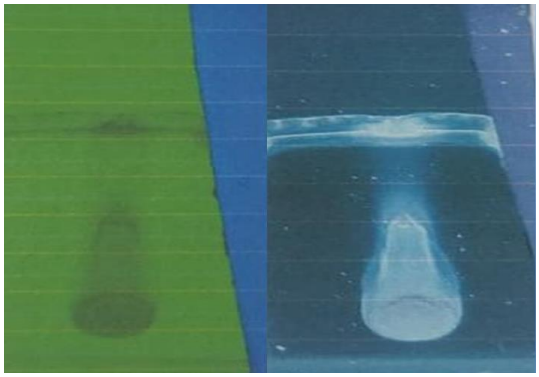

Figure 5 Analysis of crude oil extract by thin layer chromatography

The difference in the density of the crude oil extracts were observes in comparison to the water sample (Figure 6). The 5 ml sample shows the density of crude oil extract was 4.6 gm/cm^3. The natural density of vegetable or plants oils comes in the range of 700-950 kg/m^3. Hence, during the oil extraction we find oil floating over the water. The freezing of such oils at lower temperature can indicates the presence of polyunsaturated fatty acids in it. Similar results we obtained during the experiments we kept soaking the seeds for the several days at low temperature, the components present in the mixture are frozen over there. The density and viscosity of the sample plays an important role in the efficiency, lubrications, and functioning.

The crude oil obtained from seeds soaking in normal water labeled as sample 1, while sample 2 and sample 3 were obtained from boiling water. In one of the experiment, the sample viscosity was qualitatively done with the help of glass balls, we allowed to sink the glass ball through the test tube filled with sample. The ball present in sample takes more time in

comparison to water and acetone. We have three samples of extract sample 1 shows 2.5 sec, sample 2 shows 3.2 sec, sample 3 shows 5.1 sec, water shows 1.8 sec, and acetone shows 2.3 sec. Among this value, we can easily predict that the sample 3 of crude oil extract is more viscous in comparison water and acetone and other crude samples. The solvent viscosity is directly depending on the temperature, we found that the natural oil obtained in this experiment having higher viscosity in comparison to water and alcohol. Viscosity is the important parameter for the process development of fatty acids in the industry or we can say it is important screening parameter for the elution of fatty acids from the columns. The viscosity of the liquid also helps in the designing heat transfer equipments.

Figure 6 The difference in the density of the crude oil extracts in comparison to the water sample

During our investigation, we observed several contaminations of the microbes on our crude oil extracts [24]. Dominant contaminants are *Candida* and *Aspergillus* in our samples (Figure 7). The fungal contaminations such as yeast and mould is prevalent in vegetable edible oil [25], similar contamination is also observed in the case of oil yielding seeds [26].The major prevalence of microbes was observed in stored products of refinery in which significant were detected [27]. These sort of contaminations are not only observed in extracted oil, but also in dried leaves which are used for the tea bagging. The microbes having potential to produce lipase are the most prominent microbes that can be observed on oil obtained from plants or natural resources [28].

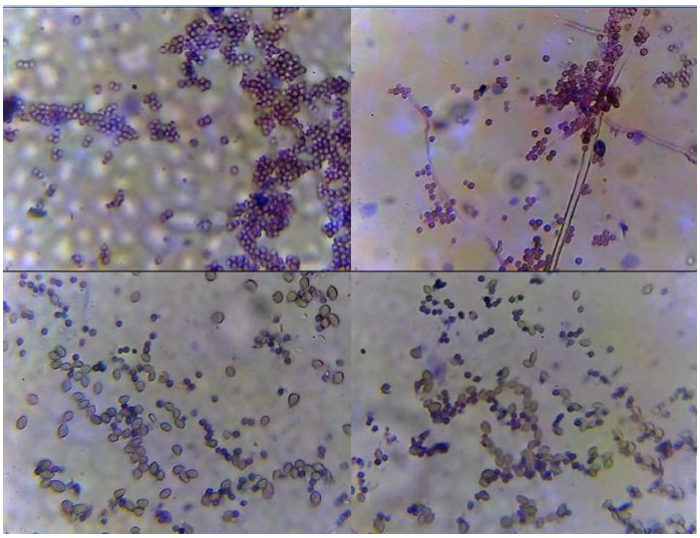

Figure 7 Microscopic images of contaminated microbes in crude oil extracts

Conclusion

The plant of white Popinac or leucocephala has versatile characteristics, hence it has wide application in medicinal, cosmetics, pharmaceutics, and confectionary to timber, almost all field. The plant is fast growing having higher photosynthetic rate; this plant can be can alternative to increasing deforestation and increasing global warming. Along rapid growth plant also provides timber for the furniture industry. From roots to shoots, each and every part of the plant has special use in human life such as food and medicine. The plant thought to be miracle timber tree as it is rapidly producing greater biomass; in folk medicine, it is to be used for the ruminants to increase the milk production and weight gain and have therapeutic value such as anthelmintic, antidiabetic, and also it is used to manage stomachache, contraception, and abortifacient. The major secondary metabolites present in this plant are alkaloid, cardiac glycosides, tannins, flavonoids, saponins, and Glycosides. In this investigation, we found that the plants taken into consideration are highly oil secreting with potential amount of nutrient content. The obtained oil has good amount of lipid content around 4.88% and fatty acids. In thin layer chromatography, we detected the presence of numerous fatty acids by comparing Rf values such as Phospholipids at 0.01 ± 0.002, Monoacylglycerides at 0.04 ± 0.013, Triacylglycerides at 0.20 ± 0.034, Tocopherols at 0.66 ± 0.105, and Fatty acid esters at 0.95 ± 0.14. Also, we observed that the oil sample obtained from boiling water with higher viscosity in comparison to normal water sample.

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Consent for Publications

All authors read and approved the final manuscript for publication.

Availability of data and material

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate

No actual animal studies were performed in the present investigations.

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