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Extraction and Characterization of Oil from *Trichilia Dregeana* (Luya) Seed Beans and Seed Shells

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ABSTRACT: The objective of this study was to extract oils from seeds and seed shells of *Trichilia dregeana* growing in Ethiopia. The oils were extracted using Soxhlet extraction technique and an organic solvent (n-hexane). The yields of the oils were in the range of 39.64-55.4%. The physicochemical properties of the oils such as density, acid value, saponification value and peroxide values were 0.8 ± 0.004 -0.84±0.014g/mL, 0.039±0.004 -5.667 mg KOH/g, 75.82±0.0 - 102.01±0.0 mg KOH/g and 0.01±0.01-0.0182±0.0, respectively. The results showed that all the observed physicochemical data of the oil products to be consistent with literature reported data for oils from seed crops and seeds of *Trichilia species.* GC-MS analyses also showed that all the oils contain palmitic acid (14-69%). Oleic acid and Linoleic acids were also found in different amounts. Elaidic acid was found (86%) only in one seed shell extract. Soaps were prepared using the extracted oils and alkali solution via saponification process. The properties of the soaps were found to be 9.10-9.81, 8-25.5% and 0.373-0.682 for pH, moisture contents and total alkali contents, respectively. These data and the data for foam and cleaning abilities of the prepared soaps were found to be in the acceptable limit. The results also suggested that *Trichilia dregeana* seeds and seed shells are rich in oils. High yields of oils from seeds and seed shells suggest their future potential in soap making and also as inputs for biofuel production. This could have significant contribution to economy of a given community where the plant grows in abundance.

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Trichilia dregeana is an ever green pant (tree) that belongs to the family of *Meliceae* (Grundy and Campbell, 1993**)**. It is a fast-growing plant and it provides shading to coffee and other crops. The plant is widely distributed in tropical African countries including Ethiopia (Gemechu and Getachew, 2020). Traditionally, oils extracted from seeds of *Trichilia* species are reported to be used in the production of natural soaps, candle making, hair oil, soaps, body ointment and lip balm and other cosmetics products (Jamieson, 1916; Komane *et al*., 2011). Reports revealed that the plant (its different morphological parts) have been traditionally used for treatment of several human illnesses (Lall and Kishore, 2014). For instance, bark decoctions are primarily used as enemas, (one teaspoon of bark, crushed, and mixed with one cup milk) to treat stomach disorders, kidney infections, backaches, and are also applied topically to treat scabies. The leaves are boiled, and the liquid is then used to bathe bruises. The seed oil of *Trichilia dregeana*, for instance, is known to possess antifungal and anti-inflammatory properties, wound healing properties, and it is also used to treat rheumatism (Coates-Palgrave, 1984). The oil is also one of the

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ingredients used in a leprosy remedy (Jamieson, 1916). The pulp left after crushing can be made into candles or used as fertilizer (Tredgold, 1986). Reports also revealed the promising potential of *Trichilia dregeana* and *Jatropha curcas* seed oils for the protection of stored maize against *Sitophilus zeamais* (Bizuneh, 2017). The bark has also been reported to play a role in lessening inflammation and suppressing pain (Jamieson, 1916; https://cjmgrowers.co.za/trichilia-dregeana-forest-natal-mahogany). Investigation of methanol extract of roots of the plant (*Trichilia dregeana*) has been reported to possess anti-Hepatitis C virus activities by inhibiting the entry step (Galani *et al*., 2015). Anti-inflammatory and anti-cholinesterase activities of compounds (e.g., cycloart-23-ene-3,25-diol) that were isolated from ethyl acetate extract of leaves of the plant on COX-1 and COX-2 enzymes have been reported (Eldeen *et al*., 2007). Isolation of compounds that belong to the class of limonoids have been reported from the seeds and stem, and found to show anti-fungal and anti-inflammatory properties (https://cjmgrowers.co.za/trichilia-dregeana-forest-natal-mahogany; Mulholland and Taylor, 1980). The use of aqueous extract of this plant was reported to be used in the treatment of gonorrhea and syphilis (Mohammadreza *et al*., 2019). In Ethiopia, the plant is used for treatment of different human illnesses toothache, wound, Trypanosomosis and also expelling intestinal parasite (Worku, 2019). There are no reports on the oil yields of *Trichilia dregeana* species growing in Ethiopia. The aim of the present study was, therefore, to determine the yields and properties of oils extracted from seeds and seed shells of *Trichilia dregeana* growing in Ethiopia. Soaps were also prepared from the extracted oils and their properties were determined.

MATERIALS AND METHODS

Collection and preparation of plant materials: The Trichilia dregeana seed beans (coded 3028, 3029 and 3030) and seed shells (coded 3029SS and 3030SS) were obtained from Sheko forest of the Southwest Ethiopia's moist Afromontane forest. The sample coded 3029 was collected from Shimi village (7°04′59″ Latitude and 35°26′04″ Longitude; 1200m asl) and the seed samples coded 3028 was collected from East of Gizmeret village (7°06′34″ Latitude and 35°25′16″ Longitude; 1120m asl). The sample coded 3030 was collected from East of Bejika village (7°03′47″ Latitude and 35°27′26″ Longitude; 1260m asl). The samples were collected in November, 2019 and were stored in cold places till used for extraction. The seeds (3029 and 3030) were de-shelled. All the plant materials (seeds and shells) were then crushed into fine powders with the aid of coffee grinder electric machine (Figure 1).

Oil extraction: The fine powder of each plant material was subjected to Soxhlet extraction method using n-hexane (solvent) (Figure 2). The extraction was carried out following procedures reported in literatures (Warra *et al*., 2012; Suzana *et al*., 2003). It was done in portions and run for 8 hours. n-hexane was chosen as the best solvent for the Soxhlet extraction due to its relative low boiling temperature as compared to most organic solvents and also due to its easy disposability after use (Suzana *et al*., 2003). The extracts were then subjected to evaporation under reduced pressure using rotary evaporator to remove the solvent. The oils were then stored in refrigerator until used for furthers analyses.

Characterization of the extracted oils

Physicochemical property analyses of the oils: The physicochemical analyses carried out in the experiment were relative density, acid value, peroxide value and saponification value of the oils. All the experiments were carried out following standard procedures reported in literatures (Hautfenne, 1982). The above mentioned experiments were done in triplicates and the results are reported as mean value plus standard deviation (X±SD). The extraction and characterization of the extracted oils was carried out at The Department of Chemistry, Hawassa University, Ethiopia.

Trans-esterification of oils and GC-MS analyses of the oil samples: 200 mg of each of the oil sample was placed in the bottom of a screw-capped tube (Teflon-lined) and 2 ml of 2 N KOH (in methanol). Then the tubes were closed tightly and heated using water bath at 80°C for 1 hr. The mixture was allowed to cool to room temperature and then 5 ml n-hexane and 5 ml water were added into the cold mixture. The n-hexane layer was collected after a short centrifugation and was dried over anhydrous sodium and magnesium sulfate. The oils were first subjected to trans-esterification reactions with methanol in the presence of mineral acid as a catalyst. Then, the transesterified oils were subjected to GC-MS analyses. The GC-MS analyses of the oils were carried out using Agilent, GC-MS Machine (Single Quadrupole). The experiment was carried out at Adama Science and Technology University (ASTU). ASTU was chosen for the following two reasons. (i) It has GCMS machine of latest model and (ii) though there was no official MOU between Hawassa University and ASTU, there is good collaboration among the staffs of The Chemistry Departments of both Universities.

Soap preparation: Attempts were also made to prepare laundry soaps by treating the extracted oils with NaOH (alkaline solution) using a process known as **s**aponification reaction. The objective was to assess the suitability (potentials) of the extracted oils for preparation of soaps by the local community where the seeds are available in abundance. The soaps were prepared following standard procedures and some of their important physicochemical properties (moisture content, pH, Free Alkali Content, foam ability, and cleaning abilities and solubility) were also determined. Moreover, the data were compared with the data obtained from commercial laundry soap products (**Solar** and **S-55**) purchased from local market in Hawassa city.

Fig 1. The fine powders of seeds and seed shells of *Trichilia dregeana* used in the study (**Photo**: Phillipo T., April, 2021).

Fig 2. Soxhlet extraction of oils from *Trichilia dregeana* seed beans and their shells (**Photo**: Phillipo T., April, 2021).

RESULTS AND DISCUSSION

Oil content the seeds and shells: Determination of oil content in seeds is very important because it helps to predict the potential of a particular seed as source of oil and/or biofuels (Haq *et al*., 2018). The amount of oil extracted by maceration and Soxhlet extraction from *Trichilia dregeana* seed beans and seed shells are given below (Table 1). High oil content in seeds implies that processing such seeds for oil production would be economically viable. The yield (percent yield) of oil extracted from *Trichilia dregeana* seed beans and seed shells (Figure 3) were found to be in the ranges of 39.63-55.5% (Table 1). The data are consistent with literature reports that showed the oil yield of seed of *Trichilia* species to be in the range of 55 - 65% (Grundy, 1993). The findings of the present study also revealed that the seed shells have relatively lower oil yield than the seeds. The oil yields are generally higher than literature reported oil yields of some seeds such as Jatropha seeds extracted by Soxhlet extraction method (42.19%) (Teklit and Afework, 2015). Reports from FAO stated that seeds with oil yield greater than 17% are considered as oil seeds (CODEX Alimentarius Commission, 1969). These relatively high percentage oil yields of the seed and seed shell of the *Trichilia dregeana*, indicated that this plant could be used for economically viable soap making purpose.

Figure 3. The oils extracted from seeds of *Trichilia dregeana* (Photo Fillipo T. April, 2021)

Table 1. Oil yields of the seeds and seed shells of *Trichilia dregeana*

S.No.	Plant material	Mass used (g)	Yield of oil (g)	Percent yield of oil (%)
1	Seed (code 3028)	300	147	49
2	Seed (code 3029)	310	154.2	49.74
3	Seed (code 3030)	360	200.9	55.4
4	Seed shell (code 3029SS)	142.3	58.2	40.89
5	Seed shell (code 3030SS)	168	66.6	39.64

*Relative density***:** The relative densities of oils obtained from seeds and seed shells were also found to be comparable to each other. Moreover, the values were found to be lower than the density of water (1 g/cm^3) (Table 2). This can be considered as a good advantage that qualifies the oil for soap production due to high tendency of the oil soap to easily emulsify and transport through aqueous solution. Moreover, the relative densities of the oils are comparable to relative density of palm oil (0.904g/ml) and reported relative densities of most vegetable oils **(**0.86 - 0.90 g/ml)(Sarjadi, 2019). Acid value: Acid value (AV) refers the proportion of free fatty acids present in oil or fat products (Sarjadi, 2019). It may also be defined as the number of milligrams of caustic potash (KOH solution) required in neutralizing the acid in 1 g of oil/fat sample. The AVs determined in the present study were found to be in the range of 4.039 ± 0.004 mg KOH/g to 4.667 ± 0.001 mg KOH/g. The values from oils of from the seeds and seed shells were also comparable to each other (Table 2). Reports revealed that low AV of an oil product indicates that the oil will be stable over a long period of time and stable against rancidity so that it will be suitable for soap making (Ibeto, 2012). The AVs of the oils in the present study were also found to be to comparable to the reported AV of ground nut (4 mgKOH/g) recommended by a Codex Alimentarius commission for using such an oil for making good quality soap (FAO, 2015).

Saponification value: Saponification value (SV) gives information concerning the characteristics of the fatty acids of the fat or oil. It is also considered as a measure of the average molecular weight (or chain length) of all the fatty acids present (Hautfenne, 1982). In the present study, the SVs of the extracted oils were found to be in the ranges of 75.82± 0.01 to 102.01 ± 0.00 mg KOH/g) (Table 2). The values were also comparable for the oils obtained from the seeds and seed shells of *Trichilia dregeana*. It has been reported that oils with high SV are better for soap making (FAO, 2015). Though the observed values are lower than that of palm oil (199.1mgKOH/g), they are higher than the oil obtained from cassia siamea (56.10 mgKOH/g) and beeswax (93 mgKOH/g)(Oshinowo, 1987) which are commonly used for soap making. This indicates that oils from seeds and seed shells of *Trichilia dregeana* can be used for soap production since their SVs fall within the acceptable ranges reported in literature (Oshinowo, 1987).

Peroxide value: The peroxide values (PVs) of the oils obtained from seeds and seed shells of were found to be comparable to each other and also are in the range of 0.0100 ± 0.01 to 0.0182 ± 0.01 meq O_2/Kg (Table 2). According to Epka and Epke (Mabrouk, 2005), high PV is associated with high rancidity rate. Thus, the observed low PVs for the oil investigated in the present study indicated that the oils were less liable to rancidity, and therefore, could be used for soap production without any risk of getting rancid as a soap or biofuels prepared from oils with low PV have longer shelf-life.

Table 2. The relative density, acid values, saponification values and peroxide values of the extracted oils

S.No.	Oil source	Relative density	Acid value (mg KOH/g)	Saponification value (mg KOH/g)	Peroxide value (meq O_2/Kg)
1	Seed (code 3028)	0.80± 0.014	4.039 ± 0.004	90.32 ± 0.00	0.0125± 0.00
2	Seed (code 3029)	0.86± 0.014	4.33 ± 0.0014	83.78 ± 0.00	0.0121 ± 0.00
3	Seed (code 3030)	0.84± 0.014	4.52 ± 0.001	102.01 ± 0.00	0.0100 ± 0.01
4	Seed shell (code 3029SS)	0.83± 0.001	4.071 ± 0.001	75.82± 0.01	0.0110 ± 0.01
5	Seed shell (code 3030SS)	0.84± 0.000	4.667 ± 0.001	86.58± 0.00	0.0182 ± 0.01

GC-MS analyses of oils

The extracted oils were subjected to transesterifiction using methanol as an alcohol to prepare ester samples for GC-MS analysis. The aim was to identify the fatty acid compositions of the oils. The results indicated that the oil composition were almost similar in the three seed samples and two seed shells (Table 3). Moreover, the data are comparable with literature reports that stated the seed oils of *Trichilia dregeana* seed beans gave palmitic acid, oleic acids and Elaidic acid (Table 3). The seeds coded as 3028 and 3030 were found to contain palmitic acid and oleic acids in comparable amounts. The oil yields were 69% (palmitic acid) 31% (oleic acid) for seed coded 3028 whereas these acids were found to be 57.37% (palmitic acid) and 42.63% (oleic acid) in a seed coded 3030. The variation could be due to difference plant origin (Section 2.1). The seed shell coded 3030SS contain the same acids but with higher percent of oleic acid (80.28%) and lower percentage of palmitic acid (19.72%) (Table 3). The seed coded 3029 and its shell (Code 3029SS) contain two fatty acids namely palmitic acid, linoleic acid and Elaidic acid (or *trans* form of oleic acid). In both cases, the percent yield of Elaidic acid > Linoleic acid > Palmitic acid (Table 3). These data are consistent with literature reports that stated the presence of palmitic acid (34%), oleic acid (51%), and linoleic acid (11%) (Jamieson, 1916). Other reports also revealed that palm oil that is commonly used for soap making contains approximately 44% palmitic acid, 5% stearic acid, 40% oleic acid and 10% linoleic acid. These fatty acids provide soap a conditioning and stable lather (plamitic acid) and conditioning and moisturizing properties (Oleic acid and linoleic acid) (Noh *et al*., 2002). The present finding also showed the presence of these important fatty acids in the seeds and seed shells of *Trichilia dregeana.* Therefore, *Trichilia dregeana* seed beans and seed shells obtained from Sheko forest, Southwest Ethiopia, give high yield and essential acid compositions to be used for soap making.

Table 3. The fatty acid composition of the extracted oil samples (GC-MS data).

S.No.	Oils obtained from	Fatty acid	Percentage value
1	Seed (code 3028S)	Palmitic acid, methyl ester	69.020
		Oleic acid, methyl ester	30.980
2	Seed (code 3029S)	Palmitic acid, methyl ester	15.280
		Linoleic acid, methyl ester	32.202
		Elaidic acid, methyl ester	49.011
3	Seed (code 3030S)	Palmitic acid, methyl ester	57.37
		Oleic acid, methyl ester	42.63
4	Seed shell (code 3029SS)	Palmitic acid, methyl ester	14.49839
		Linoleic acid, methyl ester	19.11281
		Elaidic acid, methyl ester	66.3888
5	Seed shell (code 3030SS)	Palmitic acid, methyl ester	19.71682
		Oleic acid, methyl ester	80.28318

Soap preparation and characterization of its physicochemical properties: pH determination: The ability of a cleaning chemical agent to neutralize fatty substances depends on the alkalinity of a particular soap product. A soap product with very high pH value has the greatest cleansing ability but has the great damage on cotton fabrics. In the present study, the pH values of the prepared soap materials (Figure 4) were found to be in the range of 9.10±0.00 to 9.81±0.00 (Table 3). The values for samples without additives (WOA) and with additives (WA) were not different. They are almost comparable to each other. Though the pH values were slightly lower than that of the reference or commercial soap products (Solar and S-55), they are in the acceptable pH values for laundry soaps as recommended by National Agency for Food and Drug Administration and control (NAFDAC) (Umar, 2002).

Fig 4. The prepared soap products from oils obtained from seeds and seed shells of *Trichilia dregeana* (Photo: Fillipo T. April 2021).

Moisture content: Moisture content (MC) is a parameter that is used in assessing the shelf-life of a soap product (Hautfenne, 1982). The MCs of the soaps prepared from the extracted oils were found to be in the range of 4 to 25% (Table 3). The soaps were prepared with and without additives. For most of the soaps, the MC values were found to be in the range of the recommended percentage (10-15%) (Mabrouk, 2005) and were also comparable with that of commercial soaps used in the study (Table 3). Low MC values of soap products are indicative of good foaming and cleansing properties of a given soap product. Since the MC values determined for all the analyzed soap samples (Table 3) in the present study were within the range of the EAS (https://www.rsb.gov.rw/fileadmin/user_upload/files/pdf/new_stds/Covid-19_stds/RS_EAS_186_2013_Toilet_soap_Specification.pdf) and ISO standard (less than 30%), production of laundry soap from the oils of *Trichilia dregeana* seed and seed shells would result in high quality soap products that meet international standards (https://www.iso.org/standard/4880.html).

Total alkali content: The total alkali content (TAC) is the sum of free caustic alkali and the free carbonated alkali contents expressed as a percentage by mass either as NaOH for sodium soaps or KOH for potassium soaps (Hautfenne, 1982). The TACs of the soaps prepared from the extracted oils were found to be in the range of 0.372-0.620, and these values are slightly higher than the commercial soap products used in the study (Table 3). These values are lower than the specification set by ISO of which specifies that soaps must have TAC of less than 5% (https://www.complianceonline.com/analysis-of-soaps-determinationof-total-alkali-content-and-total-fatty-matter-content-standards-809484-prdp). The values were also lower than the maximum TAC value of 2% specified by Thus, the obtained lower TAC values in this study could indicate that the prepared soap materials have acceptable qualities (https://www.complianceonline.com/analysis-of-soaps-determinationof-total-alkali-content-and-total-fatty-matter-content-standards-809484-prdp).

Table 3. The pH, moisture contents and free alkali contents of soap samples prepared from the oils extracted from seeds and seed shells of *Trichilia dregeana*

S/No	Soap sample form oil	pH value	Moisture content (%)	Total alkali content (TAC)
1	Seed (3028)_WOA	9.63±0.01	20.5	0.620
2	Seed (3029)_WOA	9.50±0.00	18	0.434
3	Seed (3030)_WOA	9.50±0.00	11	0.682
4	Seed shell (3029)_WOA	9.47±0.01	13	0.558
5	Seed shell (3030)_WOA	9.81±0.00	4	0.434
6	Seed (3028)_WA	9.40±0.00	23	0.372
7	Seed (3029)_WA	9.10±0.00	12	0.682
8	Seed (3030)_WA	9.8±0.00	25.5	0.558
9	Seed shell (3029)_WA	9.23±0.01	22	0.434
10	Seed shell (3030)_WA	9.67±0.01	8	0.434
11	Solar soap (CS)	10.41±0.00	5.5	0.310
12	S-55	10.91±0.01	4.5	0.168

☛ *WOA: soaps prepared without additives; WA: soaps prepared without additives*

Foam ability of analyzed soaps: Lathering power or foam ability refers to the amount of foam that soap forms. Although foam generation has little to do with cleansing ability (Hautfenne, 1982), it is of interesting importance to the consumer. It is, therefore, considered as important parameter in evaluating soaps and detergents. The foam heights (in cm) of the soaps (with and without additives) were determined in this study. The results were found to be in the range of 3.8±0.00 to 4.86±0.014 cm. The data were also comparable to each other and but slightly lower than the value for the commercial soaps used in the study (Table 4).

Solubility and cleansing ability of soaps: The solubility of any soap gives an indication on the ease with which the soap will dissolve in water under washing conditions. The amount of cloudy film formed gives an indication on the number of micelles (soap molecules) formed by the soap in water (Mabrouk, 2005). The cloudier the soapy water becomes the more micelles are formed. The number of micelles formed can be attributed to the cleaning action of a particular soap. The data revealed that all the prepared soaps were found to show moderately to highly soluble (Table 4). The cleaning abilities of the prepared the prepared soaps (Table 4) also revealed that the extracted oils have good potential/suitability to prepare laundry soaps.

Table 4. The foam height, solubility in water and cleaning abilities of the soap products

S/No	Soap sample form oil	Foam height (cm)	Solubility in water	Cleaning ability
1	Seed (3028)_WOA	3.8±0.00	Highly soluble	Very good
2	Seed (3029)_WOA	3.96±0.24	Highly soluble	Very good
3	Seed (3030)_WOA	4.33±0.01	Highly soluble	Very good
4	Seed shell (3029)_WOA	4.86±0.014	Moderately soluble	Good
5	Seed shell (3030)_WOA	3.9±0.00	Highly soluble	Very good
6	Seed (3028)_WA	4.76±0.014	Highly soluble	Good
7	Seed (3029)_WA	4.37±0.01	Highly soluble	Good
8	Seed (3030)_WA	4.53±0.01	Soluble	Good
9	Seed shell (3029)_WA	5.3±0.00	Soluble	Good
10	Seed shell (3030)_WA	4.7±0.00	Moderately soluble	Good
11	Solar soap (CS)	6.56±0.014	Highly soluble	Very good
12	S-55	5.7±0.00	Highly soluble	Very good

Conclusions: The physicochemical properties of the extracted oils were found to be consistent with those values reported for oils used for soap making (e.g, palm oil). The soaps prepared from these oils also showed acceptable properties of laundry soaps. All the findings suggest that the seeds and seed shells of *Trichilia dregeana* that grow abundantly in Sheko area, Southwestern Ethiopia, suggest huge potential of the plant to be used as oil source for preparation of soaps and other cosmetic products.

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