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# Variation of Physiochemical Properties and Phytochemical Constituents of the Seed Oil of Selected Acacia Species Grown in Sudan

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## ABSTRACT

**Background and Objective:** *Acacia* genus (family *Fabaceae*) is of high importance in Sudan, it is used as an antibacterial, anti-inflammatory, antioxidant, antispasmodic, anti-arythmique and astringent. This study aims to present the physicochemical characteristics of the seed oil of eight Sudanese *Acacia* and to assess the secondary metabolites in the seed powder. **Materials and Methods:** Seeds of (*Acacia nilotica* ssp. *adansonia*, *A. mellifera*, *A. nilotica* ssp. *nilotica*, *A. polycantha* ssp. *campylacantha*, *A. oerfota*, *A. senegal* var. *senegal*, *A. sieberiana* var. *sieberiana* and *A. seyal* var. *seyal*) were collected, oil of the seeds extracted, physicochemical parameters (acid value, refractive index, specific gravity and saponification value) and phytochemical constituents (alkaloids, flavonoids, steroids, saponins and tannins) were calculated. **Results:** *Acacia* seed oil yield 12.51 to 4.09%. Specific gravity 0.60 to 0.90. The refractive index was similar. Acid value 27.040 to 6.564 mg $g^{-1}$. The Saponification value is 193.54 to 109.39. No saponification value in *A. sieberiana* var. *sieberiana*. The seed powders contain alkaloids, flavonoids, steroids, saponins and tannins. *A. senegal* was reported to have maximum chemical groups followed by A. *nilotica* ssp. *adansonia and A. sieberiana* var. *sieberiana*. **Conclusion:** The physical properties of *Acacia* oils indicated that the oils could be employed in the industry.

## KEYWORDS

*Acacia*, specific gravity, refractive index, acid value, saponification value, phytochemical screening



## INTRODUCTION

*Acacia* species are of high importance in Sudan because of their economic and medicinal uses and the gum produced by some species[1]. The genus *Acacia*, is a genus of the family *Fabaceae* comprising more than 1350 species of woody plants and shrubs in the arid and sub-arid portions of the world[2], The species are resistant to drought and salinity[3], tolerate extreme dryness and adapt well to degraded soils[4,5]. Acacia is used in traditional medicine for their antibacteria, anti-inflammatory, antioxidant, antispasmodic, anti-arythmique and astringent properties[6]. Their wood is hard and heavy. Vegetable oils are nutritionally

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important sources of natural antioxidants, there is a great increase in need due to the increase in the world population and industries needing vegetable oil as a major raw material. The situation of the seed oils can be known by the free fatty acids amount and acid value. Natural products are responsible for therapeutic effects and various plant constituents of the plant material may be detected by being subjected to preliminary phytochemical screening[7]. Flavonoids show antiallergic, anti-inflammatory and anticancer activities. Some flavonoids have also been reported to have the inhibition of giant cell formation in HIV-infected cell cultures[7]. Saponins for plants have long been employed for their detergent properties. In medicine, it is used in hyperglycaemia, antioxidant, anticancer, inflammation and weight loss[8]. Saponins have anti-carcinogen properties, immune saponins activity cholesterol-lowering activity and anti-fungal properties[9,10]. Tannins are known to be common in Mimosaceae and are known to exhibit antiviral, antibacterial and anti-tumor activity. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as diuretic, tannins are also saved of commercial tannic acids and tanning agents[7]. Several studies have shown that Acacia species are rich in secondary metabolites such as alkaloids, cyanogenic glycosides, cyclitols, gums, terpenes, flavonoids and condensed tannins[2].

This study aimed to present the physicochemical properties of the oil of selected eight Sudanese *Acacia* species and to assess the secondary metabolites of the methanolic extracts of the powder of the dried seeds, as the literature review revealed that no similar studies have been done for these species in Sudan.

## MATERIALS AND METHODS

**Study duration:** The study was carried out from July, 2020 to July, 2022.

**Plant materials:** Seeds of (*Acacia nilotica* ssp. *adansonia*, *A. mellifera*, *A. nilotica* ssp. *nilotica*, *A. polycantha* ssp. *campylacantha, A. oerfota*, *A. senegal* var. *senegal, A. sieberiana* var. *sieberiana* and *A. seyal* var. *seyal*) were selected for this study. The seeds of the studied plants were collected from different regions of Western Sudan in the year 2020. The studied seeds were identified and authenticated. Voucher specimens were kept at the Herbarium of Botany Department, Faculty of Science and Technology, Omdurman Islamic University, Sudan.

**Methods:** The oils from seeds were extracted by a Soxhlet extractor[11]. The solvent was evaporated under reduced pressure, using a rotary evaporator. The extracted oils were weighed and stored. The percentage yields of the oils were calculated. The physicochemical characteristics of the oils were studied. Analyses were done in triplicates.

### Physicochemical characters

**Specific gravity:** The specific gravity (density) was measured according to Chophi *et al.*[12] using a Pycnometer. The samples consisted of six units. The Pyknomter was first thoroughly cleaned, dried and weighed. Then it was fitted with distilled water and suspended in the thermostat at 20°C When the Pyknomter and content attained the temperature of the thermostat, the level of water was adjusted to the mark. It was then removed, carefully dried and weighed after attaining the temperature of the balance case. The Pyknomter was emptied, dried and filled with the oil. It was then placed in a thermostat and the oil level was similarly adjusted to the mark after the temperature of the thermostat was reached. After removal from the thermostat, its outside was carefully dried and weighed. The specific gravity of the oil, uncorrected for the buoyancy of air, can be calculated from the ratio of the weight of oil to that of water at the same temperature:

$$\text{Specific gravity} = \frac{\text{Weight of oil}}{\text{Weight of water}}$$

**Refractive index:** The refractive index of oil was measured according to AOAC[13] using a Zeiss Abbe refractometer. The refractive index of oil with reference to air is calculated as follows: The ratio of the sin of the angle of incidence to the sin of the angle of refraction of a beam of light passing from air into the oil.

**Acid value:** The determination of acid value was made titrametrically according to the purified diethyl ether (25 mL) was mixed with (25 mL) of 90% alcohol and the mixture was carefully neutralized with standard (N/10) alkali using phenolphthalein (1%) as an indicator. The exact weight of the oil was dissolved in the mixed neutralized solvent and titrated with (N/10) alcoholic potassium hydroxide. The acid value was calculated from the following relationship[13]:

$$\text{Acid value} = \frac{\text{Titre (in mLs of N / 10 KOH)} \times 5.61}{\text{Weight of sample}}$$

The acid value is a value equal to the number of milligrams of potassium hydroxide required to neutralize the free acid present in 1 g of the oil.

**Saponification value:** The determination of Saponification value was made titremetrically according to ACAO[13] Standard alcoholic potassium 0.5 N (25 mL), was added to an exact weight of the oil and the mixture was refluxed in a boiling water bath for one hour, with constant shaking. The excess hot alkali was then titrated with standard (N/2) HCL using phenolphthalein (1%) as an indicator. A blank titration was carried out and the saponification value was then calculated:

$$\text{Saponification value} = \frac{(b - a) \times 28.05}{\text{Weight of oil}}$$

Where:
a = Volume (mL) of hydrochloric acid used in the sample
b = Volume (mL) of hydrochloric acid used in the blank

**Preliminary phytochemical analysis:** As 10 g of the powder of the dry seeds of the studied plants were successively extracted with methanol (40-60°C) in the soxhlet apparatus. The different extracts were tested for alkaloids, flavonoids, steroids, saponins and tannins. The Phytochemical tests were performed according to Shaikh and Patil[14] Thusa and Mulmi[15] with minor modifications.

**Test for alkaloids:** Test solution taken with 2 mL HCl, an aqueous layer formed, decanted and one or two drops of Mayer's reagent added. White turbidity or precipitate indicated the presence of alkaloids.

**Test for flavonoids:** Magnesium and one or two drops of concentrated HCL were added to the test solution in alcohol and heat. The red or orange-red colour indicated a positive test for flavonoids.

**Test for steroids:** A few drops of $CHCl_3$, 3-4 drops of acetic anhydride and one drop of concentrated $HSO_4$ were added to the test solution. The purple colour changing to blue or green indicated the presence of steroids.

**Test for saponins:** Water was added to the test solution and shake, foamy lather indicated the presence of saponins.

**Test for tannins:** The $H_2O$ and lead acetate were added to the test solution. The presence of tannins was indicated by the appearance of a white precipitate.

## RESULTS

**Percentage yield:** The yield of the seed oils extracted from eight *Acacia* species, oil yield percentage is relatively high in *Acacia mellifera* 12.51% and low oil in *Acacia sieberiana* var. *sieberiana* 4.09% as shown in Table 1.

**Physicochemical properties:** The physicochemical properties of the seed oil of the *Acacia* species studied are given in Table 1.

**Specific gravity:** Specific gravity ranged between (0.60 to 0.90) low for *Acacia nilotica* ssp. *nilotica* and *Acacia sieberiana* var. *sieberiana* and high in the *Acacia oerfota*.

**Refractive index (at 20°C):** The results show that the refractive index of the species studied were more or less similar.

**Acids value:** The acid value of *Acacia* species ranged between 27.040 mg $g^{-1}$ in *Acacia seyal* var. *seyal* acid values decreased gradually in other species studied, the lowest value obtained was 6.564 mg $g^{-1}$ in *Acacia olyacantha* ssp. *campylacantha*.

**Saponification value:** The saponification value of the studied *Acacia* species was found to be high ranging between (109.39 to 193.54 mg $g^{-1}$) the highest value appeared in *Acacia polyacantha* ssp. *campylacantha* and the lowest value observed in *Acacia nilotica* ssp. *adansonia*. However, the Saponification value is not detected in *Acacia sieberiana* var. *sieberiana*.

**Phytochemical screenings of the seed extract:** The present work of phytochemical screenings of the methanolic extracts of the dried seeds powder for Alkaloids, Flavonoids, Steroids, Saponins and Tannins. Table 2, showed that alkaloids were present in all *Acacia* studied. But flavonoids present in *Acacia polyacantha* ssp. *campylacantha, Acacia senegl and Acacia seyal* var. *seyal,* whereas, flavonoids were not detected in other *Acacia* species studied. The steroid compounds were detected through five *Acacia*

Table 1: Physicochemical properties of seed oil of *Acacia* species

| *Acacia* taxa | Yield (%) | Acid value (mg $g^{-1}$) | Refractive index (20°C) | Specific gravity | Saponification value (mg $g^{-1}$) |
|---|---|---|---|---|---|
| *Acacia mellifera* | 12.51±0.1817 | 08.262±0.0110 | 1.470±0.0962 | 0.80±0.0264 | 187.93±0.9904 |
| *Acacia nilotica* ssp. *adansonia* | 5.64±0.3153 | 08.206±0.1030 | 1.400±0.1045 | 0.70±0.0264 | 109.39±0.5553 |
| *Acacia nilotica* ssp. *nilotica* | 5.41±0.1006 | 13.362±0.093 | 1.410±0.0352 | 0.60±0.0173 | 140.25±0.1228 |
| *Acacia oerfota* | 5.68±0.1504 | 17.040±0.2649 | 1.430±0.1003 | 0.90±0.0416 | 182.54±0.9850 |
| *Acacia olyacantha* ssp. *campylacantha* | 3.93±0.0416 | 06.564±0.0381 | 1.420±0.0947 | 0.70±0.0321 | 193.54±0.5953 |
| *Acacia senegal* | 8.83±0.0519 | 11.530±0.4584 | 1.465±0.4532 | 0.70±0.0416 | 130.90±1.304 |
| *Acacia seyal* var. *seyal* | 7.78± 0.1504 | 27.040±0.0155 | 1.461±0.0717 | 0.81±0.0264 | 179.72±0.1250 |
| *Acacia sieberiana* var. *sieberiana* | 4.09±0.1106 | 18.820±0.0996 | 1.459±0.0665 | 0.60±0.0264 | 000.00±0.0000 |

Data are expressed in mean±standard error of the mean

Table 2: Phytochemical screenings of extract of eight *Acacia* taxa

| *Acacia* taxa | Alkaloids | Flavonoids | Steroids | Saponins | Tannins |
|---|---|---|---|---|---|
| *Acacia mellifera* | +ve | -ve | +ve | +ve | +ve |
| *Acacia nilotica* ssp. *adansonia* | +ve | -ve | +ve | +ve | +ve |
| *Acacia nilotica* ssp. *nilotica* | +ve | -ve | +ve | +ve | +ve |
| *Acacia oerfota* | +ve | -ve | -ve | +ve | -ve |
| *Acacia polyacantha* ssp. *campylacantha* | +ve | +ve | -ve | +ve | -ve |
| *Acacia senegal* | +ve | +ve | +ve | +ve | +ve |
| *Acacia seyal* var. *seyal* | +ve | +ve | -ve | +ve | +ve |
| *Acacia sieberiana* var. *sieberiana* | +ve | -ve | +ve | - ve | +ve |

+: Present and –:Absent

species analyzed, but the steroids were absent in *A. oerfota* and *A. polyacantha* ssp. *campylacantha* and *A. seyal* var. *seyal*. Saponins were found in all *Acacia* species studied except *A. sieberiana* var. *sieberiana*. From the present work, tannins are not found in *A. oerfota* and *A. polyacantha* ssp. *campylacantha*, but present in all other *Acacia* species studied.

## DISCUSSION

The physiochemical properties of *Acacia* oils studied showed that saponification values were high. The phytochemical examination of the seeds of the eight studied *Acacia* species afforded different secondary metabolites such as alkaloids, flavonoids, steroids, saponins and tannins, alkaloids were present in all *Acacia* studied. The extracts of *A. polyacantha* ssp. *campylacantha, A. senegal* and *A. seyal* var. *seyal*, gave positive results for saponins and flavonoids. Steroids and tritepenes are found in *A. mellifera* and *A. nilotica* ssp. *adansonia and A. sieberiana* var. *sieberiana*. High saponification values indicate that the fatty acids present in the oils of *Acacia* have a high number of carbon atoms[16]. The number of carbon atoms in *Acacia* oils is high, after hydrogenation, which will help in using it as a substitute for some traditional oils in making soap[17]. *Acacia* species are rich in secondary metabolites such as alkaloids, cyanogenic glycosides, cyclitols, gums, terpenes, flavonoids and condensed tannins[2]. Phytochemical screening is usually carried out to screen for and characterize constituents available in a given plant sample. Secondary metabolites are generally used for the protection of the plant against herbivores and have biological activities for animals. Oil from *Acacia* seeds can be proposed as a potential source of oil with economic benefit to populations in developing countries. It was interesting to bring attention that *in vivo* tests are needed to confirm the beneficial quality of *Acacia* seed oil[18]. Phytochemical study of *Acacia nilotica* fruits has shown the presence of tannins, flavonoids and saponosides[19]. Secondary metabolites like protein, phenolic, flavonoid and carotenoid contents are extracted from *Acacia* seeds and can be used as food ingredients, without detrimental affecting the efficacy of functional ingredients and the food's palatability[20].

This study showed that the seed oils of the eight studied *Acacia* species differ in their physical and chemical properties and indicated that the oil of *Acacia* seeds can be used in the industry, the high saponification values of these oils will help in using them as substitutes for some traditional oils in making soap. The physical properties of *Acacia* oils from this study indicated that the oils could be used as lubrication oil at low temperatures and employed in studies relating to optics. *A. senegal* was found to have maximum studied chemical groups, followed by *A. nilotica* ssp. *adansonia* and *A. sieberiana* var. *sieberiana* so it is recommended that it can be utilized as medicinal ingredients, in cosmetics, pesticides and other industries. Deep phytochemical studies of these oils are needed because some chemical constituents of these oils may be poisonous.

## CONCLUSION

The seed oils of the eight studied *Acacia* species differ in their physical and chemical properties, physiochemical properties of *Acacia* oils indicated that the oils could be employed in studies relating to optics and used as lubrication oil at low temperatures, the *Acacia* seed oil properties showed that the oil can be used in industries like soap making. The phytochemical examination of the seeds of the eight studied *Acacia* species afforded different secondary metabolites such as alkaloids, flavonoids, steroids, saponins and tannins. In this investigation, *A. senegal* was reported to have the maximum studied chemical groups. Followed by *nilotica* ssp. *adansonia and A. sieberiana* var. *sieberiana*.

## SIGNIFICANCE STATEMENT

This study aimed to present the physicochemical properties and to assess the secondary metabolites of the methanolic extracts of the powder of the dried seeds of selected eight *Acacia* species grown in Central Sudan. The results of the studied characters and parameters obtained for the *Acacia* seed oils indicated

that the oils of these plants possess different physical and chemical properties among the different species. These oils possess characteristics to enable them to be used in the industry and help in development and economics. Further studies are needed for these oils and oils from other related species to provide maximum benefits from these valuable oils.

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