DPPH Scavenging Ability, Ultraviolet-Visible and Infrared Spectral Analyses of the Oil Extract of *Diospyros Mespiliformis* (Jackal Berry)

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ABSTRACT

Oils in recent times have prompted numerous researches in the bid to find suitable, edible and healthy oils for consumption or for food supplements, which is the inspiration of this research. The oil was obtained by sohxlet exrtraction of the powder of *Diospyros mespiliformis* (Jackal berry) seeds using n-hexane as solvent. DPPH radicals scavenging assay (with ascorbic acid as the standard), as well as UV-Visible and infrared spectral analyses were performed on the oil. The percentage yield of the oil extracted was 6.90%. Percentage DPPH radical scavenging activity of the oil at lowest concentration (5 µg/ml) was 86.32±0.06, while it was 91.43±0.07 at the highest concentration of 500 µg/ml. The trend in percentage antioxidant activity with respect to concentration of both the sample and the control in this experiment differed by producing a zigzat curve. The oil absorbed at the UV-Vis region of 380-420nm (λ_{maxs} 390 nm & 417nm) and 640-700nm (λ_{max} 670nm). respectively. The FT-IR spectral analysis revealed characteristic vibrations corresponding to functional groups such as C=O stretches for esters (1735.04 cm^{-1}), C=C for alkenes bending stretch (980.64 cm^{-1}), C≡C stretches for alkynes (2291.72 cm^{-1}) and C-O alkyl stretching (1170.64 cm^{-1}). The oil from *Diospyros mespiliformis* seeds was successfully extracted which showed promise of being a good vegetable oil, but would rather be used as food supplement/natural antioxidant packaged in capsules because of it low yield, pending toxicity being carried out on the oil.

Keywords: *Diospyros mespiliformis*, DPPH, Scavenging, Oil, Antioxidant

INTRODUCTION

While wild Fruits (including berries) and seeds are not comparable to the cultivated crops in terms of nutrition, some have higher nutritional values [1]. Yet many wild fruits lack recognition as significant contributors to human diet in both the rural and urban areas. *Diospyros mespiliformis* Hochst. ex A.DC. (family *Ebenaceae*), commonly known as Jackal berry, or African ebony[2], is a deciduous tree that can grow up to 25 metres in height [3]. It is an Afro-tropical fruit tree whose seeds and pulp yield edible oil used traditionally for cooking, skin care, and wound management [4]. Beyond food use, the species is ethno botanically important for treating malaria, diabetes, fever, and inflammatory conditions [4]. In Nigeria, a leaf-infusion is taken as a mild laxative, and as a vermifuge [5, 6]. Hausa natives of Northern Nigeria chew the leaf, and fruit, or apply an infusion for gingivitis and toothache [6]. The liquid from the leaves boiled with 1-2 guinea grains is drunk in Ghana as a cure for whooping cough (Twi-nkonkon) which will be effected in 2-3 days [7].

These applications have stimulated phytochemical investigations that reveal a chemically rich matrix of naphthoquinones, triterpenoids, flavonoids, tannins, and tocopherols across different plant parts [4].

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Fig. 1: Picture of *D. mespiliformis (*jackal berry) unripe and ripped fruit

The fruit is known with different names in different cultures; it is called “Monkey guava”, “Persimmon” by English culture, popularly known as “Kanya” among the Hausa speaking people of Northern Nigeria [2], “Gyal” by the Bogghom people and “iSel” by the Tarok people, both of Plateau state, North-central communities of Nigeria.

Berry seed oils are utilized in food and cosmetic applications, because they contain polyunsaturated fatty acids [8] and other bioactive components such as antioxidants and vitamins and potentially are beneficial for human health.

The use of berry seed oils for the prevention of the skin lesions such as rash, gingivitis, and eczema has been patented [9].

Berries contain several essential micronutriends, dietary fibres and polyphenolic compounds such as ellagitannins, resveratrol, and anthocyannins [10]. While the fruit pulp of jackal berry is widely eaten, the seed are recognized as energy-dense by-product containing nutritious oil suitable for potential culinary, cosmetic and nutritional application [11].

While seeds and fruit oils are also rich in bioactive lipid, tocopherols, carotenoids, and phenolics constituents that may contribute to antioxidant activity. Oil extract provide a lipophilic matrix that can enhance the stability and bioavailability of certain antioxidant compounds. However, data on the antioxidant potential from *Diospyros mespiliformis* remain scarce compare to aqueous and alcoholic extracts. While polar extracts of *Diospyros mespiliformis* leaves, bark, roots, and fruits are well-studied for antioxidant activity, the lipophilic fraction remains comparatively underexplored.

Most studies on *Diospyros. mespiliformis* (species) has focused on polar extract, methanolic fruit extracts from *Diospyros mespiliformis* inhibited 87.36% of DPPH radicals at 1 mg/mL, and root, fruit, and bark extracts showed IC_{50} values of 3.47 ± 0.05, 6.94 ± 0.49, and 7.82 ± 0.76 µg/mL, respectively, against DPPH [12]. Ethyl acetate fractions of leaves exhibited even stronger activity with IC_{50} = 1.08 ± 0.04 µg/mL, outperforming ascorbic acid at 5.08 ± 0.12 µg/mL [12]. Such potency has been linked to flavonoids like quercetin, luteolin, and their glycosides, naphthoquinones, and phenolic acids identified by UPLC-ESI-MS, GC-MS, and NMR [4, 13].

Seed and fruit oils of *Diospyros* species are emerging as sources of bioactive lipids. GC-MS analysis of *Diospyros mespiliformis* stem bark and fruit extracts has identified δ-tocopherol, β-sitosterol, lupeol, pentadecanoic acid, octadecanoic acid, and cis-vaccenic acid [4]. These constituents, tocopherols, phytosterols, unsaturated fatty acids, and phenolic derivatives are known contributors to DPPH radical scavenging and lipid peroxidation inhibition in plant oils. Indeed, crude ethanol extracts, ethyl acetate, and aqueous fractions of *Diospyros mespiliformis* stembark showed significant total antioxidant capacity, with the ethyl acetate fraction

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exhibiting the highest ferric thiocyanate inhibition and lowest malondialdehyde levels [14]. Despite this chemical promise, direct spectroscopic profiling of *Diospyros mespiliformis* oil extract remains undocumented.

There are documentation on antioxidant potential of polar extracts, the presence of tocopherols and phenolics in its lipid fraction of some parts of *Diospyros mespiliformis* [14, 15], but lack of dedicated spectral data for its oil, this study aims to: (1) evaluate the DPPH antioxidant scavenging ability of oil extract from *Diospyros mespiliformis* seed, and (2) characterize the extract using UV-visible and FT-IR spectroscopy to identify chromophores and functional groups. Establishing these parameters will bridge the analytical gap for jackal berry oil and provide a scientific basis for its potential development as a natural antioxidant in nutraceutical, cosmetic, and pharmaceutical applications.

MATERIALS AND METHODS

Sample Collection and Preparation

The sample material *Diospyros mespiliformis* (Jackal berry) was collected from Dangkang community of Kanke Local Government of Plateau State, Nigeria. While the fruits were collected as sample, the fruits, leaves and bark were also collected for identification. It was authenticated to be *Diospyros mespiliformis* Hochst. ex A.DC at the Herbarium of the Federal College of Forestry, Jos and assigned the voucher number FHN0504.

Sample Preparation:

The sample, seed of *D. mespiliformis* were gotten by breaking open the dried ripped fruit of the plant. It was washed thoroughly with clean water and then rinsed with distilled water. After washing, the seed was dried (spread on laboratory bench) with good ventilation and left to dry for two (2) weeks. The dried seed were pulverized into a coarse powder using mortar and pestle and then grinded with a laboratory mill grinder (ZX Laboratory, FW400A). The milled powder was sieved to a fine powder using a sieve with a sieve-size 2 mm in diameter and then kept in a black polythene bag for subsequent work.

Extraction:

The powdered sample of *D. mespiliformis* 100 g was measured using a weighing balance (Ohaus Pioneer PX224)), transferred to a Soxhlet extractor for extraction with 250 ml of n-hexane as solvent for extraction for about eight (8) hours. The oil obtained was then evaporated using a rotary evaporator operated at 60 ºC, then finally dried off at 50 ºC over a steam bath.

Percentage Oil Yield Determination:

The oil recovered from the extraction was weighed into a pre-weighted empty bottle and the weight of oil was determined by subtracting the weight of empty bottle from the weight of content (i.e bottle plus oil). Hence the percentage of oil yield was calculated using the formula:

$$\% \text{ oil Yield} = \frac{Weight\ of\ oil\ extracted}{Weight\ of\ Powdered\ sample}\ x\ 100$$

DPPH Radicals Scavenging Activity:

The antioxidant ability of the oil on DPPH radicals scavenging abilities was carried out using the method by Nimmyel *et al.,* 2024 [16].

The reduction ability of DPPH was determined by the decrease in its absorbance at 517 nm caused by antioxidants. DPPH (0.01 g) was measured and dissolved in 250 mL of ethanol in a volumetric flask which gives 0.004%. Ascorbic acid (0.05 g) was dissolved in 50 mL of ethanol to obtain 500 µg/mL of the standard. Using dilution formula ($C_1V_1 = C_2V_2$) other concentrations were prepared by serial dilution. To each concentration of freshly prepared *D. mespiliformis* fruit seed oil extract and ascorbic acid standard, 3 mL of

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DPPH reagent was added and allowed to stand for 30 minutes the absorbance was measured using UV-spectrophotometer at 517 nm.

The percentage scavenging activity on DPPH was calculated using the formula:

$$\% \text{ scavenging activity} = \frac{Ao-As}{Ao}\ x\ 100 \qquad [16]$$

Where, A_o is the absorbance of negative control and

A_s is the absorbance of the sample

Ultraviolet-visible absorption (UV):

The *D. mespiliformis* fruit seed oil was extract dissolved using the solvent of extraction and poured into a cuvette which was then analysed in uv-visible range between 200-780 nm using UV-visible spectrophotometer (UV-1800, Shimadzu) [17].

Infra-red Spectroscopy:

Under the influence of IR radiations the molecules shows various absorption spectrums selective to the functional group present in molecule. The spectrum of compound is unique features of molecular framework. The IR spectra of the fruit seed oil of *D. mespiliformis* oil was scanned on FT-IR spectrophotometer (Thermo Scientific Nicolet, iS20) over the frequency range from 4000-400 cm^{-1}[17].

RESULTS AND DISCUSSION

Results:

Percentage Yield:

The percentage yield of *D. mespiliformis* fruit seed oil was obtained to be 6.90% with physical characteristic color of canary yellow. This implies that it will require a lot more of the sample to produce a little quantity of the oil.

DPPH Radicals scavenging activity:

The percentage DPPH radical scavenging activities of the oil extract of *D. mespiliformis* fruit seed and ascorbic acid (positive control) is summarized in Table 1.

Table 1: Result of Percentage Scavenging on DPPH Radical

Concentration (µg/mL)	Scavenging Activity of DPPH (%)	
	***D. mespiliformis* oil Extract**	**Ascorbic Acid**
5	86.32±0.06^{b}	85.65±0.06^{b}
10	54.84±0.05^{d}	50.17±0.05^{e}
50	80.58±0.30^{c}	87.54±0.02^{a}
100	53.94±0.05^{e}	70.19±0.04^{d}
500	91.43±0.07^{a}	77.75±0.04^{c}

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Note: Values presented as Mean ± SD (n=3), Values in the same column having same letters are not significantly different ($p \leq 0.05$).

The results of the antioxidant analysis on the oil extract was compared with that of ascorbic acid (seen in Figure 2) where the least concentration of standard and extract (5 µg/mL), the percentage DPPH radical scavenging activities of ascorbic and *D. mespiliformis* seed oil were 85.65±0.06 and 86.32±0.06 respectively showing that the *D. mespiliformis* seed oil was more potent than the standard, ascorbic acid; while at the highest concentration (500 µg/mL), the corresponding activities were 77.75±0.04 and 91.43±0.07 respectively, also showing that the seed oil was more potent than the standard,.

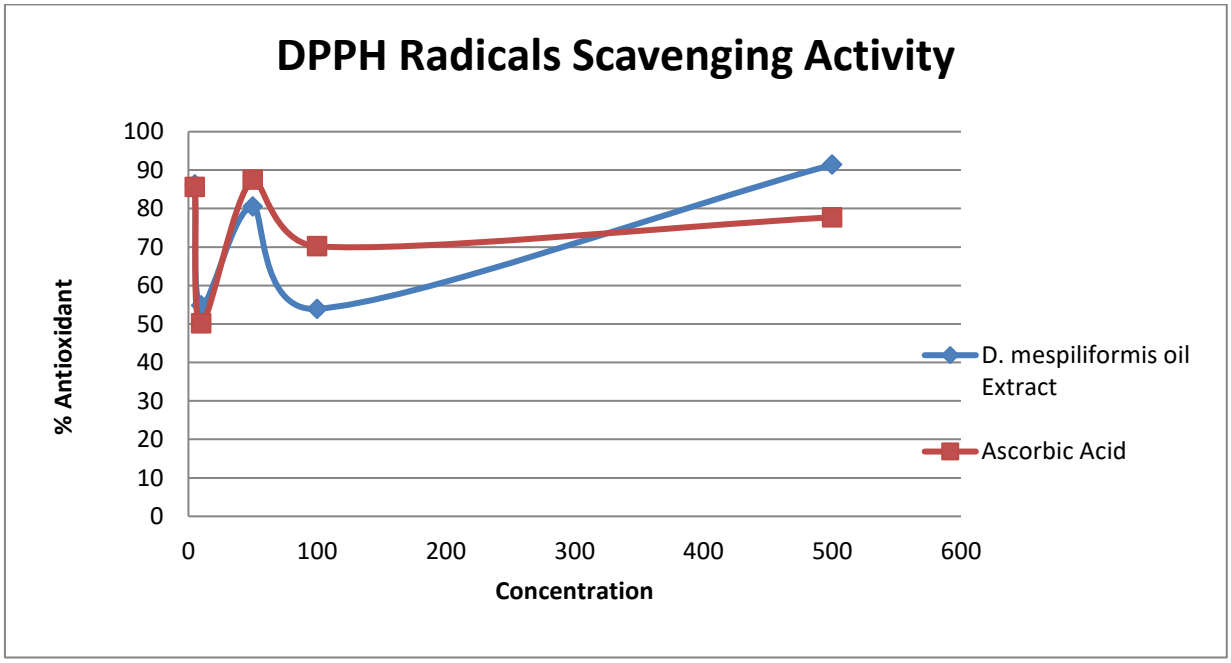

Fig. 2: Graph of Concentration against percentage DPPH radicals scavenging activity

UV-Visible Spectrum of the *D. mespiliformis* oil extract is shown in Figure 3. The UV spectrum showed absorption maxima at 380-420nm (λ_{maxs} 390 nm & 417nm) and 640-700nm (λ_{max} 670nm).

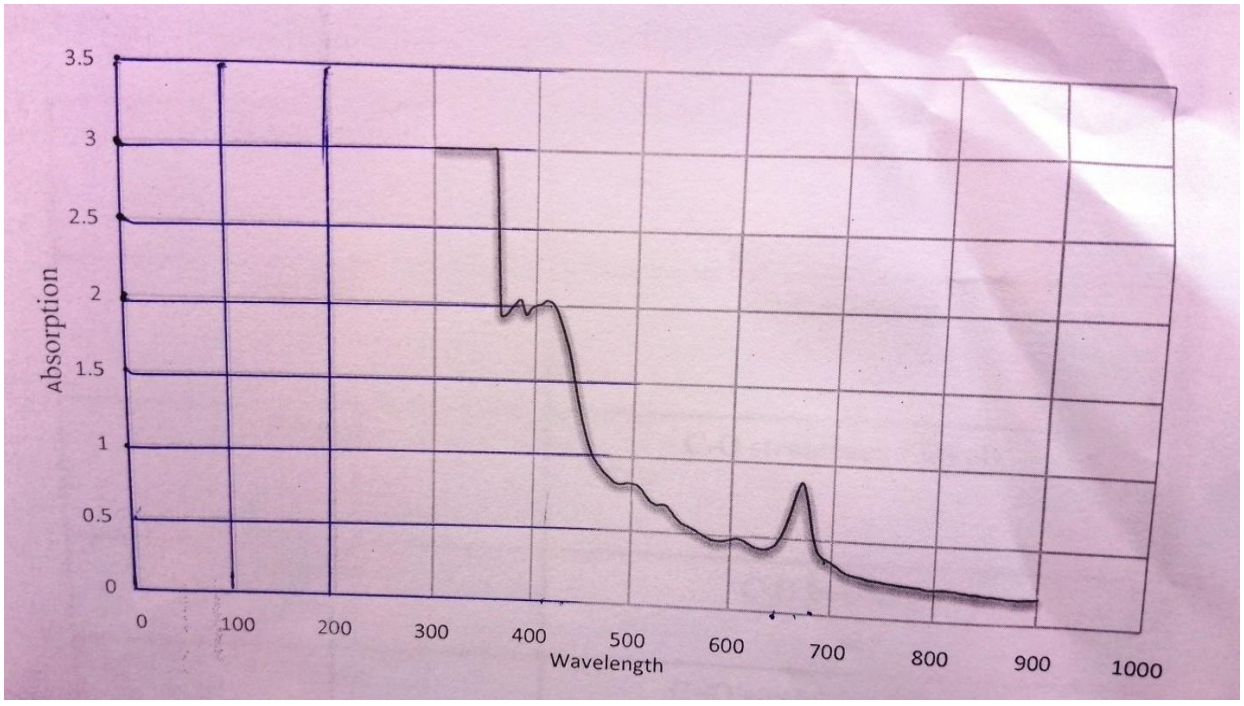

Fig. 3: UV-Visible Spectrum of *D. mespiliformis* Fruit seed oil extract

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IR spectra of the *D. mespiliformis* oil extract are shown in Figure 4. The infrared region approximately 4000 - 400 cm^{-1} was used to study the different modes of vibration spectrum.

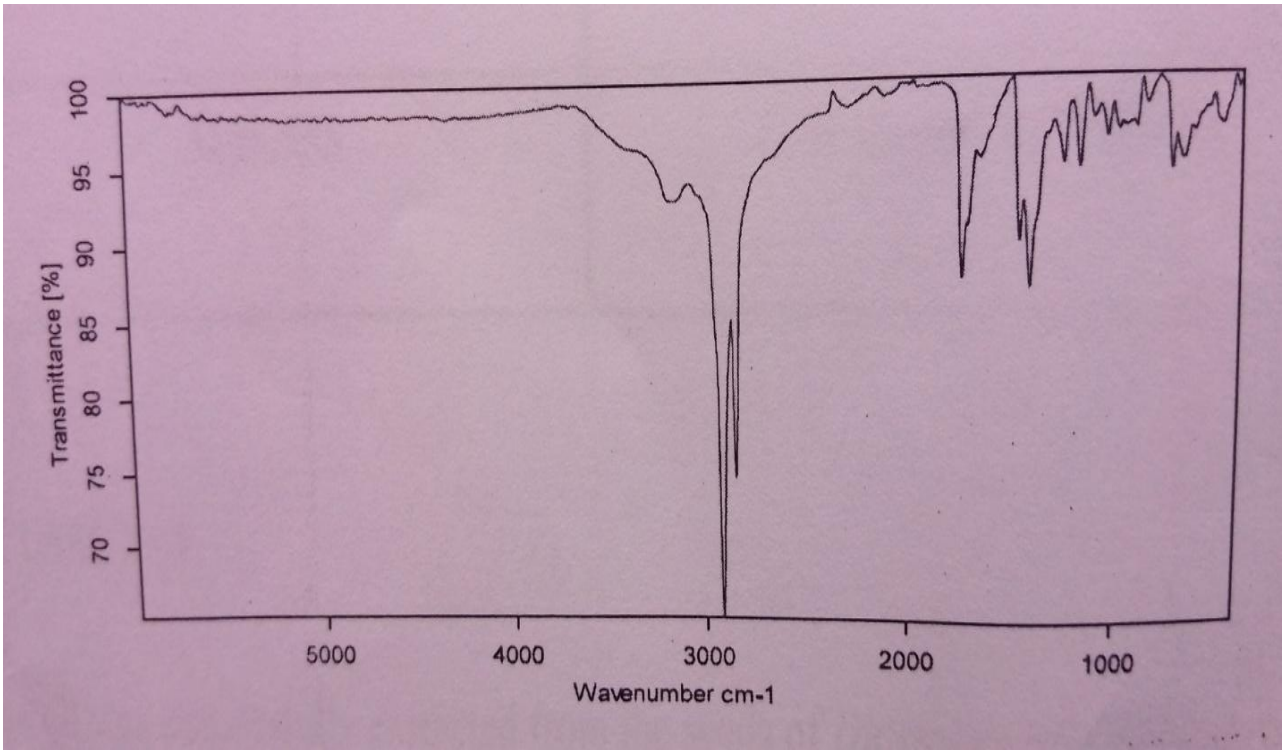

Fig. 4: FT-IR Spectrum of *D. mespiliformis* Fruit seed oil extract

Table 2: FT-IR important bands and their possible assignments

S/n	Spectrum band (cm^{-1})	Assignments
1.	980.64	C=C bending*
2.	1170.64	C-O stretching (Alkyl)
3.	1460.46	C-H bending
4.	1600-1450	C = C Aromatic
5.	1735.04	C=O stretching (Esters)
6.	2291.72	C≡C stretching
7.	2850.36	C-H stretching (saturated)
8.	2918.21	$-CH_2$ and $–CH_3$

Reference: [18, *19]

DISCUSSION

The percentage extraction of *Diospyros mespiliformis* seed oil was obtained to be 6.90%. This value is quite small and implies that it would require a lot more of the sample to produce a little quantity of the oil. [18] opined that the percentage extraction of jackal berry seed is notably low ranging between 0.70% and 5.31% by dry weight. A study on Nigerian varieties shows an oil yield of 4.72% [20], showing that the percentage obtained from this present research (6.90%) is higher than those documented. The reason for this low yield is

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that unlike other traditional oil seeds like groundnuts, the seed mass of jackal berry consist largely of other organic compounds and phytochemicals like alkaloids, saponins, sterols and tannins rather than lipids [21].

DPPH is a stable nitrogen-centered free radical which produces violet/purple color in ethanol solution and fades to shades of yellow color in the presence of antioxidants. One peculiarity of this method is that it allows for testing of both lipophilic and hydrophilic compounds. The purple color of the DPPH solution was reduced to a yellow color to a colorless in a concentration dependant manner [22].

The DPPH radical scavenging activity of *D. mespiliformis* fruit seed oil and ascorbic acid (as control) is presented in table 1 as percentage antioxidant activity. The relationship between the percentage (%) antioxidant with respect to their changing concentrations is shown in Figure 2.

At the least concentration of standard and extract (5 µg/mL), the percentage DPPH radical scavenging activities of ascorbic and *D. mespiliformis* seed oil were 85.65±0.06 and 86.32±0.06 respectively; while at the highest concentration (500 µg/mL), the corresponding activities were 77.75±0.04 and 91.43±0.07 respectively. All these revealed that the *D. mespiliformis* seed oil was more potent than the standard, ascorbic acid. The trend in percentage antioxidant activity with respect to concentration of both the sample and the control in this experiment differ by producing a zigzat curve (Figure 2). The inhibition was high at minimum concentration (5 µg/ml), then suddenly drops at concentration of 5 µg/ml, increase again when the concentration was raised to 50 µg/mL. At 100 µg/mL, the percentage inhibition activity decreases to a point before finally increasing with increasing concentration to a final 500 µg/mL.

[14] reported inhibition percentage of 43% to 48% at maximum concentration of 1 mg/mL unrefined polar extract of the leave of *D. mespiliformis.* A dose dependant DPPH radical reducing power and anion radical effect on peroxide anion using a colorimetric method with increasing concentration on the leave methanolic extract was also reported [24]. It can be seen here that the oil of jackal berry has a significant antioxidant activity that goes beyond the ascorbic acid (positive control) at both minimum and maximum concentration and activity is concentration dependant.

The UV region has a range of 100-400 nm and for the visible region the range is from 400-700 nm. From the UV-Visible spectrum, the *D. mespiliformis* seed oil extract absorbed at between 380-420 nm and 640-700 nm with maximum adsorption at 420 and 670 nm. Visible region (400 – 750) nm captures the characteristic natural color profile of the oil. Peaks around 430 – 470 nm caused by carotenoids (like lutein and β-carotene) which is canary yellow [23].

From Table, the FT-IR spectral analyses showed functional groups present in the oil as; carbonyl functional groups, C=O stretches for esters, C=C for alkenes bending stretch, saturated and unsaturated carbon-hydrogen stretch, C≡C stretches for alkynes and C-O alkyl stretching [18, 19]. Something of worth noting in the infrared spectrum is the conspicuous absence of OH peak present in phenolics which are the most abundant known naturally occurring antioxidant compounds [24, 25]. Absence of OH peak could be attributed reasons including: poor phasing or baseline, calibration issues in instrumentation; humidity/wet sample which could lead to too much broadening and overstretching of the peak [26]; Strong intramolecular hydrogen bonding which can shift and broaden the OH making it almost invisible; presence of phenolic OH which can be very broad and blend with the baseline; etc [27, 28, 29].

CONCLUSION

Quality assessment of functional oils requires instrumental fingerprinting; UV-visible spectroscopy detects conjugated chromophores, FT-IR identifies key functional groups and bioactive activity like antioxidant activities. These data provide a foundation for evaluating jackal berry oil as a natural antioxidant for food preservation and nutraceutical applications.

The oil from *Diospyros mespiliformis* seed was successfully extracted which show promise of been a good vegetable oil but would rather be used as food supplement/natural antioxidant packaged in capsules because of it low yield but pending it toxicity test which is being carried out.

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