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The Bioactive Compounds and Fatty Acid Profile of Bitter Apple Seed Oil Obtained in Hot, Arid Environments

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Abstract: Bitter apple or tumba (*Citrullus colocynthis* L.) is a prostrate annual herb belonging to the *Cucurbitaceae* family. It is highly tolerant against multiple abiotic stresses like drought, heat, and soil salinity and can easily grow on very marginal soil, even on sand dunes in hot, arid regions. Tumba fruit is a fleshy berry 5–10 cm in diameter and of a pale yellow color at ripening. The tumba fruit used in this research was harvested from the ICAR-CIAH, Bikaner research farm. The seeds were separated, and their oil was extracted to analyze its physical characteristics and composition (phytochemical compounds, fatty acid profile, etc.). The seeds of the tumba fruit contained 23–25% golden-yellow-colored oil with a specific gravity of 0.92 g/mL. The extracted oil contained appreciable amounts of phytochemical (bioactive) compounds like phenolics (5.39 mg GAE/100 g), flavonoids (938 mg catechin eq./100 g), carotenoids (79.5 mg/kg), oryzanol (0.066%), and lignans (0.012%), along with 70–122 mg AAE/100 g total antioxidant activity (depending on the determination method). The results of fatty acid profiling carried out by GC-MS/MS demonstrated that tumba seed oil contained about 70% unsaturated fatty acids with more than 51% polyunsaturated fatty acids. It mainly contained linoleic acid (C18:2n6; 50.3%), followed by oleic acid (C18:1n9; 18.0%), stearic acid (C18:0; 15.2%), and palmitic acid (C16:0; 12.4%). Therefore, this oil can be considered as a very good source of essential fatty acids like omega-6 fatty acid (linoleic acid), whereas it contains a lower concentration of omega-3 fatty acids (α-linolenic acid) and hydroxy polyunsaturated fatty acids. In addition, it also contains some odd chain fatty acids like pentadecanoic and heptadecanoic acid (C15:0 and C17:0, respectively), which have recently been demonstrated to be bioactive compounds in reducing the risk of cardiometabolic diseases. The results of this study suggest that tumba seed oil contains several health-promoting bioactive compounds with nutraceutical properties; hence, it can be an excellent dietary source.

Keywords: tumba; physico-chemicals; fatty acid profile; medicinal uses

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1. Introduction

In recent years, there has been an increasing interest in vegetable oils with functional properties. Vegetable oils are in great demand because they have diverse applications for ensuring safe food, for nutraceuticals and medicines, and in industry. India has a long history of cultivation and use of medicinal plants. However, speedy industrialization, urbanization, and overgrazing have caused the loss of medicinally and industrially important floras [1]. These floras may be an important source of functional food and nutraceutical compounds for the pharmaceutical industry.

The introduction and domestication of some economically important plants will not only help increase the vegetation cover in India's Thar Desert, but these actions may also

improve the socio-economic status of the people living in these areas. Plants from the *Cucurbitaceae* family, endemic to this region, are well adapted to the xeric conditions of the desert. Some of the naturally and commonly occurring plants of *Cucurbitaceae* family are tumba (*C. colocynthis)*, mateera or local watermelon (*Citrullus lanatus*), kachra or snap melon (*Cucumis melo* var. *utilissimus*), kachri (*Cucumis melo* var. *agrestis*), etc. [2]. Tumba is a trailing annual scabrid herb belonging to the *Cucurbitaceae* family and is known by different names in different regions, such as Hadla in Jordan, bitter cucumber or bitter apple in English-speaking countries, Abujahl watermelon or Kadu Hanzal in Persia [3]. In India, it is also known with different names such as Ghudmba in Punjab, Indark in Gujarat, Makal in Bengal and Kartama in southern India.

C. colocynthis (tumba or bitter apple) is a closely related species of cultivated watermelon (*Citrullus lanatus*) [4] grown and geographically distributed in various parts of the world, such as the deserts of the Middle East and in southern Europe and Africa [5–7]. This plant is highly tolerant to multiple abiotic stresses such as drought, heat, and soil salinity and can easily grow on very marginal soils, even on sand dunes in hot, arid regions. It grows profusely by producing multiple branches and, thanks to tendrils located at each node, spreads over sandy undulated plains and sand dunes and plays an important role in controlling soil erosion in desert areas. It is one of the most important biomass producers along with a naturally grown *Calligonum polygonoides* (phog, a shrub) under resource-limited environmental conditions in its habitat in India's Thar Desert [8]. The tumba plant has fast growing habits and starts flowering and fruiting at just 30 and 60 days, respectively, after sowing. Its creeping nature and the soil-binding properties of its roots are very helpful for preventing desertification through controlling and stabilizing sand dunes. All the organs of this plant, including the stem, leaves, fruits, seeds, and root are used as dried or fresh, either aqueous or oil extracts, and are reported to have anti-diabetic, anti-leprosy, anti-inflammatory, analgesic, vermifuge, hyperlipidemic laxative, hair-growth-promoting, antimicrobial, and antioxidant properties [4,9–14]. In spite of the several medicinal uses of tumba, some complications have also been reported from its direct use including diarrhea, colic, vomiting, nephrosis, hematochezia, and liver dysfunctions [15–17].

The fruit of *C. colocynthis* is a large fleshy berry, globular in shape and smooth, like a gourd. It is 5–10 cm in diameter, pale yellow in color at ripening, and available during October and November. Tumba fruit has very high medicinal value and is used in indigenous medicine as a purgative agent. Tumba seed oil is used in the soap and candle industry in Rajasthan, India. It can also be used for oilseed feedstock and thus can replace lubricant or biodiesel to some extent [18]. The nutritional composition of tumba seeds of different regions of the world was reported to consist of 20–30% carbohydrate, 14–24% fat, 13–26% protein, and 2–4% ash [19,20]. The fatty acid composition of tumba seed oil differs widely according to its spatial distribution in various regions of the world; it varies between 55–74% for linoleic acid, 9–17% for oleic acid, 5.36–9.84% for stearic acid, and 8.35–11.70% for palmitic acid [19,21–27]. The present study aims to harness the tumba seed oil cultivated in the hot and arid region of Rajasthan, India, as a potential vegetable oil for culinary as well as nutraceutical applications through characterizing its bioactive compounds and fatty acid composition.

2. Materials and Methods

2.1. Experimental Site and Plant Material

Tumba fruit was collected during the Fall cropping season in 2019 from the research station of the ICAR-Central Institute for Arid Horticulture, Bikaner, India, located in the Thar Desert (28°06′0.21″ N; 73°21′22.17″ E; 224 m a.s.l.). The fruits were weighed and cut for seed extraction. The seeds were dried at room temperature and weighed.

2.2. Lipid Extraction

The recovered seeds were finely ground with an electric blender, and weighted samples were subjected to oil extraction through a Soxhlet apparatus (Lab C, The Laboratory

Glassware Co., Ambala Cantt, India) using petroleum ether as solvent at 50 °C [28]. After 3 h, the recovered hexane was evaporated and the extracted seed oil (pale yellow in color) was weighed to calculate the oil content.

2.3. Fatty Acid Profiling of Tumba Seed Oil Using GC-MS/MS

The extracted tumba seed oil was subjected to preparation of fatty acid methyl esters (FAMEs) using Boron-Trifluoride (BF_3) as per the AOAC Official Method [28]. The FAMEs were subjected to GC-MS/MS analysis using a Gas Chromatograph, consisting of an AOC-20i and interfaced to a QP 2010 Plus Mass Spectrometer (GC-2010 system, Shimadzu Corporation, Japan), equipped with a polar fused silica column, COL-ELITE-2560 (highly polar phase; biscyanopropylphenylpolysiloxane, 100 mt Length × 0.25 mm ID × 0.2 µmdf). The initial temperature of the oven was 100 °C (hold for 4 min); it was then increased to 240 °C (held for 15 min) with an injection temperature of 225 °C. The helium gas flow was 1.0 mL/min to a total of 65 min program.

The identification of FAMEs was accomplished by GC-MS mass spectrum explication and a comparison of retention times with mass spectra to those of commercial-standard fatty acid methyl esters mix, C8-C-24 (Supelco 37 Component FAME Mix, Sigma Aldrich). The amount of individual fatty acid in the tumba seed oil was determined with the help of standard curves adopted in the two methods, and the value was expressed as percentage of weight.

2.4. Nutraceutical Composition and Antioxidant Activity Determination of Tumba Seed Oil

2.4.1. Methanolic Extractives of Tumba Seed Oil

One gram of oil sample was extracted in triplicate with 10 mL of 80% aqueous methanol containing 0.1% HCl by shaking continuously for 1 h at room temperature. The extractives were stored under deep freezing conditions at −20 °C until further use.

2.4.2. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC of the methanolic extractives from the tumba seed oil was assessed according to the Folin–Ciocalteu reagent method described by Berwal et al. [29], and the values were expressed in gallic acid equivalents (GAE)/100 g of oil.

The TFC of the tumba seed oil was determined by the aluminum chloride–based colorimetric assay previously described [30]. One mL extractive was mixed with 0.3 mL each of sodium nitrite, aluminum chloride, and NaOH solution. After 15 min incubation at room temperature, absorbance was read with an UV-VIS Spectrophotometer (Shimadzu UV-2550, Shimadzu Corporation, Japan) at 510 nm against the blank. TFC was expressed as catechol equivalents/100 g of oil.

2.4.3. Estimation of Lignan, Oryzanol, and Carotenoid Content

The content of lignans was estimated using the method described by Bhatnagar et al. [31]. Briefly, 0.01 g oil samples were dissolved in a hexane + chloroform mixture (7:3, *v/v*) to reach a final volume of 10 mL. Then absorbance was read with a UV-VIS Spectrophotometer (Shimadzu UV-2550, Shimadzu Corporation, Tokyo, Japan) at 288 nm. The lignans content was intended by using a specific extinction coefficient ($E^{1\%}$/1 cm) for sesamoline, 231.1.

Total oryzanol content was estimated using the protocol described by Gopla Krishna et al. [32]. Briefly, 1 g of tumba seed oil was mixed with hexane up to a 10 mL final volume. The mixture absorbance was read at 314 nm against hexane (blank) with a UV-VIS Spectrophotometer (Shimadzu UV-2550, Shimadzu Corporation, Japan), and oryzanol content was calculated using a specific extinction coefficient ($E^{1\%}$/1 cm) for oryzanol, i.e., 358.9.

The carotenoid content of tumba seed oil was estimated using the method described by Kumar et al. [33]. One gram of oil sample was made up to 10 mL using hexane. This mixture was 10-times diluted before its absorbance was read with an UV-VIS Spectrophotometer

(Shimadzu UV-2550, Shimadzu Corporation, Tokyo, Japan) at 446 nm. The carotenoid content was calculated with the molar extinction coefficient of carotenoids, i.e., 383.

2.4.4. Total Antioxidant Activity

The total antioxidant assay by the phosphomolybdenum method was carried out as described by Prieto et al. [34]. A 0.3 mL aliquot of methanolic extractive was mixed with 28-mM sodium phosphate and 4-mM ammonium molybdate. The resultant mix was incubated for 90 min in a water bath set at 95 °C. Then the absorbance was read at 695 nm with an UV-VIS Spectrophotometer (Shimadzu UV-2550, Shimadzu Corporation, Tokyo, Japan). The total antioxidant activity was calculated with standard curves of ascorbic acid and expressed as mg ascorbic acid equivalents/100 g of oil.

2.5. Statistical Analysis

The physico-chemical parameters measured, including fatty acid analysis in tumba seed oil, were carried out in three replications. The mean values and standard deviations (SD) were calculated using MS-Office Excel.

3. Results and Discussion

3.1. The Fatty Acid Composition of Tumba Seed Oil

Seeds accounted for 2.75 ± 0.25% of total tumba fruit dry mass and contained about 24.75 ± 1.25% golden-yellow-colored oil with a 0.92 ± 0.01 g/mL specific gravity. The fatty acid composition of tumba seed oil from the hot, arid region of Rajasthan is reported in Table 1 and Figure 1. It is extremely rich in omega-6-polyunsaturated fatty acid (n6-PUFA), where linoleic acid (C18:2n6) was the most important n6-PUFA that accounted for 50.31% of the total fatty acids (Figure 1). The saturated fatty acids in the tumba seed oil contributed 30.38% of the total fatty acids and were mainly composed of palmitic acid (C16:0; 12.41%), stearic acid (C18:0; 15.15%), and arachidic acid (C20:0; 1.08%). The monounsaturated fatty acids (MUFAs) contributed about 18.83% of total fatty acids, with oleic acid (C18:1n9) being the major component (18.02%) followed by gadoleic acid (C20:1n9; 0.52%). Omega-3 fatty acids (n3-PUFA; i.e., α-linolenic acid) were also found at a minute level and accounted for about 0.50% in tumba seed oil.

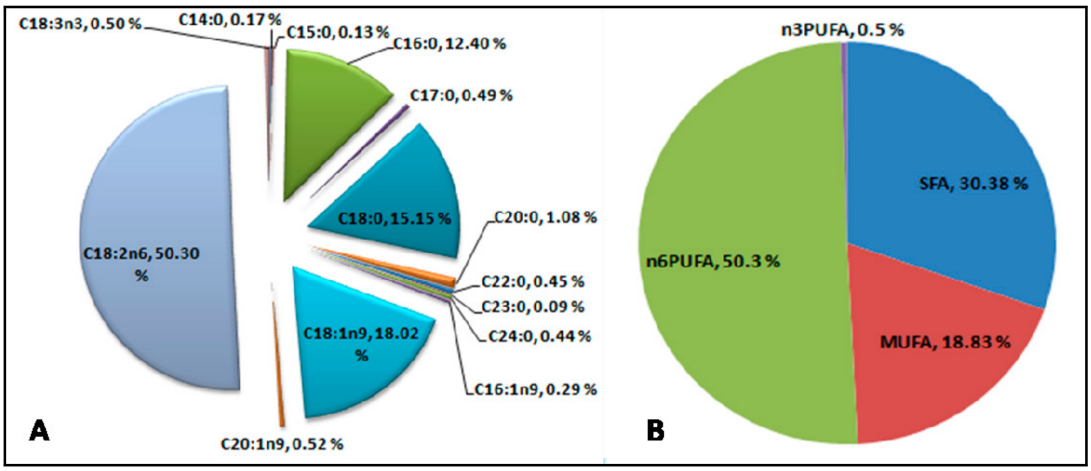

Figure 1. The fatty acid composition of tumba (*C. colocynthis*) seed oil obtained in the present study: (**A**) fatty acid profile and (**B**) fatty acid subclasses distribution in the tumba seed oil (SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; n6-PUFA: omega-6 polyunsaturated fatty acids; n3-PUFA: omega-3 polyunsaturated fatty acids). The values are presented as the weight percentage for individual fatty acids.

Table 1. The fatty acid composition of tumba (*Citrullus colocynthis* L.) seed oil obtained in the present study.

	Name of the Fatty Acid	Common Name	Formula	Peak Area (%)	Type of Fatty Acid
1	Tetradecanoate	Myristic acid	C14:0	0.17 ± 0.01	Saturated
2	Pentadecanoic acid	Pentadecylic acid	C15:0	0.13 ± 0.01	Saturated
3	Hexadecanoic acid	Palmitic acid	C16:0	12.41 ± 0.02	Saturated
4	9-Hexadecenoic acid, (Z)-	Palmitelaidic acid	C16:1n9	0.29 ± 0.01	MUFA
5	Heptadecanoic acid	Margaric acid	C17:0	0.49 ± 0.03	Saturated
6	Octadecanoic acid	Stearic acid	C18:0	15.15 ± 0.46	Saturated
7	9-Octadecenoic acid (Z)-	Oleic acid	C18:1n9	18.02 ± 0.36	MUFA
8	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	C18:2n6	50.31 ± 0.33	PUFA
9	9,12,15-Octadecatrienoic acid	α-Linolenic acid	C18:3n3	0.50 ± 0.05	PUFA
10	Eicosanoic acid	Arachidic acid	C20:0	1.08 ± 0.03	Saturated
11	11-Eicosenoic acid	Gadoleic acid	C20:1n9	0.52 ± 0.03	MUFA
12	Docosanoic acid	Behenic acid	C22:0	0.45 ± 0.02	Saturated
13	Tricosanoic acid	Tricosanoic acid	C23:0	0.09 ± 0.02	Saturated
14	Tetracosanoic acid	Lignoceric acid	C24:0	0.44 ± 0.04	Saturated

The results obtained in our study are consistent with previous reports describing the fatty acid composition of *C. colocynthis* seed oils obtained in different regions of the world, viz. India, Malaysia, Israel, Jordan, and Algeria, but the absolute values of the single fatty acids varied to some extent (Table 2) [19,21–27]. It was observed that palmitic, stearic, oleic, and linoleic acids are the major fatty acids in tumba seed oil, as they contributed more than 95 % of the total fatty acids. Among these, linoleic acid was the most prevalent fatty acid as its content ranged between 50.31 and 74.77%. The highest content of linoleic acid was previously reported in Jordan seed oil (74.77%), and the lowest content was reported in the current study (50.31%). Likewise, other major fatty acids also varied: palmitic acid ranged between 8.35 (Jordan) [26] and 12.41% (current study), stearic acid between 5.35 (Jordan) [26] and 15.15% (current study), and oleic acid between 9.04 (Jordan) [19,21–27] and 18.02% (current study) (Table 2). The fatty acid composition and unsaturated fatty acid content found in the present study were similar to those of major vegetable oils previously studied [35–37]. Orsavova et al. [37] studied the fatty acid profile of 14 major vegetable oils—safflower, grape, milk thistle, hemp, sunflower, wheat germ, pumpkin seed, sesame, rice bran, almond, rapeseed, peanut, olive, and coconut oil—and reported that palmitic, oleic and linoleic acids are major contributing fatty acids in these oils with 4.6–20%, 6.2–71.1% and 1.6–79%, respectively. Interestingly, in the present study palmitic, stearic, oleic, and linoleic acids are the major contributors, with approximately 70% unsaturated fatty acids.

In addition to the aforementioned fatty acids, some odd chain saturated fatty acids (OCFAs) were also detected in tumba seed oil at a minute level (about 0.71% of the total fatty acids). The main contributors were pentadecylic acid (C15:0; 0.13%), margaric acid (C17:0, 0.49%), and tricosanoic acid (C23:0; 0.09%) (Table 1). These results diverged from previous studies that reported only heptadecanoic acid (C17:0; 0.075–0.08%) [20] for this group of compounds. This group of fatty acids, specifically pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0), is essential for human health. The higher dietary intake of these OCFAs is linked with reduced risks of cardiovascular disease, adiposity, chronic inflammation, type-2 diabetes, metabolic syndrome, nonalcoholic steatohepatitis (NASH), chronic obstructive pulmonary disease, pancreatic cancer, and other conditions [38–42].

Table 2. Comparison of the major fatty acid composition (by weight %) of tumba (*Citrullus colocynthis* L.) seed obtained in the present study with that obtained in other countries and regions of the world.

Fatty Acids	India [a]	India [b]	India [c]	India [d]	Malaysia [e]	Israel [f]	Jordan [g]	Algeria [h]	Current Study
					wt %				
Palmitic acid (C16:0)	9.38	10.43	11.70	10.30	10.48	10.10	8.35	10.22	12.41
Stearic acid (C18:0)	7.34	9.84	9.70	8.00	9.72	6.70	5.36	8.98	15.15
Oleic acid (C18:1)	17.04	15.90	11.40	24.50	17.95	13.10	9.04	9.36	18.02
Linoleic acid (C18:2)	61.05	62.81	66.10	55.90	61.41	70.10	74.77	68.49	50.31

[a] Ashish et al. [21]; [b] Gurudeeban et al. [22]; [c] Kulkarni et al. [23]; [d] Kamalakar et al. [19]; [e] Solomon et al. [24]; [f] Zohara et al. [25]; [g] Al-Hwaiti et al. [26]; [h] Bireche et al. [27].

3.2. Nutraceutical Composition and Antioxidant Activity of Tumba Seed Oil

The nutraceutical composition and antioxidant activity (total phenolic, total flavonoids, oryzanol, lignans, carotenoids, and total antioxidant activity) of the extracted tumba seed oil are reported in Table 3. Nutraceuticals are natural bioactive compounds produced by plants as their secondary metabolites, which include phenols, flavonoids, terpenes, pigments, lignans, oryzanol, etc. [43]. All these compounds exert strong antioxidant activity.

Table 3. The bioactive compound content and antioxidant activity of the Tumba (*Citrullus colocynthis* L.) seed oil obtained in the present study.

Parameters	Content
Total phenolic content (mg/100 g of oil gallic acid Eq.)	5.39 ± 0.73
Total flavonoids content (mg/100 g of oil catechin Eq.)	938.0 ± 18.0
Oryzanol (%)	0.066 ± 0.003
Lignans (%)	0.012 ±0.002
Carotenoids (mg/kg)	79.5 ± 16.1
Total antioxidant activity by phosphomolybdate method (mg/100 g oil ascorbic acid Eq.)	70.83 ± 2.37

All the values are mean ± SD of three replicates.

Phenolics constitute one of the most widely and ubiquitously distributed plant secondary metabolite groups, with more than 8000 known phenolic compounds, which have been reported to exhibit various biological functions including antimicrobial, antioxidant, and antidiabetic ones [44]. A number of studies demonstrated that phenolic content in plants is directly linked with their antioxidant potential due to their redox properties, which make them strong reducing agents, hydrogen donors, and quenchers of singlet oxygen species [44,45]. Phenolic compounds in seed oil are a relevant oil quality index because these compounds protect lipids from peroxidation through scavenging free radicals. TPC in tumba seed oil was found to be 5.39 mg gallic acid eq./100 g of oil (Table 3), which is comparable to the TPC content of other vegetable oils like coconut, ground nut, rice bran, and sunflower oil, which were reported to contain 3.09, 1.8, 0.89, and 0.49 mg per 100 g gallic acid eq., respectively [46]. Other vegetable oils that have even higher content of TPC are *Basella rubra* (32.99 mg), white mustard (150 mg), coriander seeds (20 mg), and caraway seeds (78 mg) [34,38–41,47]. The differences in TPC composition of these oils may be linked to the crop species as well as to the extraction, processing, and refining conditions. Flavonoids are an abundant sub-group of plant phenolics that include more than 4000 natural compounds [48]. The TFC of tumba seed oil (Table 3) was higher (938 mg of catechin equivalent/100 g) than that of other vegetable oils. Kumar et al. [33] also reported very high TFC content (557.88 mg/100 g) in *B. rubra* seed oils. Xuan et al. [49] studied the TFC content of 14 different vegetable oils and reported contents ranging between 3 mg/100 g (safflower oil) and 34 mg/100 g (Inca Inchi oil). TFC is reported to exhibit a high antioxidant potential and to improve the oil's shelf life. The higher level of TFC in tumba seed oil makes it a good source of dietary antioxidants and improves its shelf life.

Oryzanol is one of the most important phytochemicals in rice bran oil and exhibits important biological activities. Tumba seed oil also contains oryzanol (0.066%), nearly

similar to that of *B. rubra* seed oil (0.01% oryzanol content) [33]. The highest oryzanol content is in rice bran oil, which varied from 26.7 to 61.6 mg/100 g (i.e., 0.027 to 0.06%) in different rice varieties [32,50]. It has also been demonstrated that rice bran extract–enriched (1000 ppm) soybean oil is less prone to oxidative degradation during frying than oil enriched with millet and barley bran extracts [51]. This study gives a clue about the effectiveness of oryzanol against the oxidative degradation of oil during frying. Rice bran oil is the richest source of oryzanol, and the value of oryzanol content obtained in tumba seed oil in this study is similar to that of rice bran oil. Therefore, tumba seed oil, due to its high oryzanol content, can be considered as less prone to oxidative degradation during frying and can be used for deep frying.

Lignans are a group of natural compounds derived from the oxidative coupling of β-hydroxyphenylpropane, which includes sesamin, sesamolin, sesaminol, and sesamolinol. These compounds have distinctive bioactivity and physiological and nutritional properties [31,52]. Sesamin has a typical lignan structure of β-β′ (8–8′) linked to the product of two coniferyl alcohol radicals, while sesamolin has a unique structure made up of one acetal oxygen bridge in a sesamin-type structure. Both sesamin and sesamolin are characteristic lignans of sesame seeds [31]. The lignans content of tumba seed oil is reported in Table 3. The lignans content of tumba seed oil (0.012 %) was similar to that of *B. rubra* seed oils (0.02%) [33], and among vegetable oils sesame is the richest source of lignans, containing 0.26 to 1.16 % lignans (1.08%) [52,53].

Carotenoids are a group of more than 750 types of yellow, orange, and red pigments synthesized by plants, algae, and photosynthetic bacteria. These fat-soluble phytochemicals have also gained substantial popularity for food because of their bioactivity as provitamin A and antioxidant potential [54]. The most important dietary carotenoids are the following: α-carotene, β-carotene, zeaxanthin, β-cryptoxanthin, lutein, and lycopene [55]. In our study, we found that the total carotenoid content in tumba seed oil was about 79.5 mg/kg total carotenoids (Table 3) and that they play an appreciable part in the overall antioxidant capacity of the samples. These pigments were also well represented in palm oil (53.5 mg/100 g) and *B. rubra* seed oils (30.5 mg/100 g) [33,44,56,57].

The richness of the natural antioxidants in vegetable oils delays lipid peroxidation and contributes to consumer acceptance of the food products made from them by improving their shelf life [33]. The antioxidants of oils play a key role in preserving their nutritive value and quality by scavenging the free radicals and thus protecting them from lipid peroxidation [57]. The tumba seed oil contained an appreciable amount of antioxidant compounds (about 70.83 mg ascorbic acid eq./100 g; Table 3). High antioxidant activities (evaluated with the phosphomolybdenum assay as in the present study) were previously observed also for *B. rubra* seed oils [33]. Thanks to its appreciable antioxidant activity, the use of tumba seed oil helps increase the quality and shelf life of food products.

4. Conclusions

Plant-based products, containing phytochemical-rich oils, can be useful for the preparation of food and for other uses, suggesting new topics for research and development. In recent times, plant-based food products are gaining popularity among the urban population due to their important functional and nutraceutical properties and the safety of their consumption. Tumba seed oil obtained from the hot region of Rajasthan (India) is mainly constituted of unsaturated fatty acids (about 70%) similarly to other major vegetable oils. Its major part is represented by polyunsaturated fatty acids along with some important odd chain fatty acids which have recently been suggested as essential for human health. It also contained an appreciable amount of natural bioactive compounds with strong antioxidant potential (phenolics, flavonoids, oryzanols, lignana, carotenoids, etc.), which make it more stable against oxidative degradation during frying as well as a very good source of antioxidants with nutraceutical and pharmaceutical applications. Consequently, tumba seed oil, with its good fatty acid profile and good antioxidant activity, has the potential to be a choice for vegetable oil for culinary purposes with a large number of health-promoting properties.

However, before tumba seed oil can be suggested as a vegetable oil for culinary purposes, more research is required to better evaluate its anti-nutritional factors and subsequent effects on human health.

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References

1. Berwal, M.K.; Haldhar, S.M.; Ram, C.; Shil, S.; Kumar, R.; Gora, J.S.; Singh, D.; Samadia, D.K.; Kumar, M.; Mekhemar, M. *Calligonum polygonoides* L. as Novel Source of Bioactive Compounds in Hot Arid Regions: Evaluation of Phytochemical Composition and Antioxidant Activity. *Plants* **2021**, *10*, 1156. [CrossRef] [PubMed]
2. Kumar, P.; Khapte, P.S.; Meghwal, P.R. Genetic Diversity of Vegetables in Arid Region. In *Horticulture Based Integrated Farming System*; Shukla, A.K., Gupta, D.K., Jangid, B.L., Keerthika, A., Noor Mohammad, M.B., Mehta, R.S., Eds.; New India Publishing Agency: New Delhi, India, 2021; pp. 35–41.
3. Javadzadeh, H.R.; Davoudi, A.; Davoudi, F.; Valizadegan, G.; Goodarzi, H.; Mahmoodi, S.; Ghane, M.R.; Faraji, M. *Citrullus colocynthis* as the Cause of Acute Rectorrhagia. *Case Rep. Emerg. Med.* **2013**, *2013*, 652192. [CrossRef] [PubMed]
4. Bikdeloo, M.; Colla, G.; Rouphael, Y.; Hassandokht, M.R.; Soltani, F.; Salehi, R.; Kumar, P.; Cardarelli, M. Morphological and Physio-Biochemical Responses of Watermelon Grafted onto Rootstocks of Wild Watermelon [*Citrullus colocynthis* (L.) Schrad] and Commercial Interspecific Cucurbita Hybrid to Drought Stress. *Horticulturae* **2021**, *7*, 359. [CrossRef]
5. Dane, F.; Liu, J.; Zhang, C. Phylogeography of the bitter apple, *Citrullus colocynthis*. *Genet. Resour. Crop Evol.* **2006**, *54*, 327–336. [CrossRef]
6. Hassanane, M.S.; EL-Fiky, S.; Abd EL-Bbaset, S.A. A genotoxic study of the *Citrullus colocynthis* extract. *Bull. Natl. Res. Cent.* **2001**, *26*, 223–235.
7. Rahimi, R.; Amin, G.; Ardekani, M.R. A review on *Citrullus colocynthis* Schrad.: From traditional Iranian medicine to modern phytotherapy. *J. Altern. Complement. Med.* **2012**, *18*, 551–554. [CrossRef]
8. Berwal, M.K.; Haldhar, S.M.; Ram, C.; Saroj, P.L. Determination of total phenolic & flavonoids and antioxidant activity in *Calligonum polygonoides* L. from Thar Desert. *J. Environ. Biol.* **2021**, *42*, 1347–1354. [CrossRef]
9. Rani, R.; Dushyant, S.; Monika, C.; Jaya, P.Y. Antibacterial activity of twenty different endophytic fungi isolated from *Calotropis procera* and time kill assay. *Clin. Microbiol.* **2017**, *6*, 3. [CrossRef]
10. Abdel-Hassan, I.A.; Mohammeda, S.T. The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. *J. Ethnopharmacol.* **2000**, *71*, 325–330. [CrossRef]
11. Ardekani, M.R.S.; Rahimi, R.; Javadi, B.; Abdi, L.; Khanavi, M. Relationship between temperaments of medicinal plants and their major chemical compounds. *J. Tradit. Chin. Med.* **2011**, *31*, 27–31. [CrossRef]
12. Huseini, H.F.; Darvishzadeh, F.; Heshmat, R.; Jafariazar, Z.; Raza, M.; Larijani, B. The clinical investigation of *Citrullus colocynthis* (L.) schrad fruit in treatment of type II diabetic patients: A randomized, double blind, placebo-controlled clinical trial. *Phytother. Res.* **2009**, *23*, 1186–1189. [CrossRef]
13. Rahbar, A.R.; Nabipour, I. The hypolipidemic effect of *Citrullus colocynthis* on patients with hyperlipidemia. *Pak. J. Biol. Sci.* **2010**, *13*, 1202–1207. [CrossRef] [PubMed]
14. Marzouk, B.; Marzouk, Z.; Haloui, E.; Fenina, N.; Bouraoui, A.; Aouni, M. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. *J. Ethnopharmacol.* **2010**, *128*, 15–19. [CrossRef]
15. Tannin-Spitz, T.; Bergman, M.; Grossman, S. Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. *Biochem. Biophys. Res. Commun.* **2007**, *364*, 181–186. [CrossRef] [PubMed]
16. Nmila, R.; Gross, R.; Rchid, H.; Roye, M.; Manteghetti, M.; Petit, P.; Tijane, M.; Ribes, G.; Sauvaire, Y. Insulinotropic effect of *Citrullus colocynthis* fruit extracts. *Planta Med.* **2000**, *66*, 418–423. [CrossRef] [PubMed]
17. Dehghani, F.; Panjehshahin, M.R. The toxic effect of alcoholic extract of *Citrullus colocynthis* on rat liver. *Iran. J. Pharm. Ther.* **2006**, *5*, 117–119.
18. Jouad, H.; Haloui, M.; Rhiouani, H.; El Hilaly, J.; Eddouks, M. Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez-Boulemane). *J. Ethnopharmacol.* **2001**, *77*, 175–182. [CrossRef]
19. Kamalakar, K.; Sai-Manoj, G.N.V.T.; Prasad, R.B.N.; Karuna, M.S.L. Thumba (*Citrullus colocynthis* L.) seed oil: A potential bio-lubricant base-stock. *Grasas Aceites* **2015**, *66*, e055. [CrossRef]

20. Mariod, A.A.; Mirghani, M.E.S.; Hussein, I. Citrullus colocynthis Colocynth, Bitter Apple, Bitter Gourd. In *Unconventional Oilseeds and Oil Sources*; Mariod, A.A., Mirghani, M.E.S., Hussein, I., Eds.; Academic Press: Cambridge, MA, USA, 2017; pp. 99–105. [CrossRef]
21. Ashish, K.; Naveen, K.; Hasan, M.M.; Rajeev, C.; Arshad, N.S.; Zahid, A.K. Production of biodiesel from thumba oil: Optimization of process parameters. *Iran. J. Energy Environ.* **2010**, *1*, 352–358.
22. Gurudeeban, S.; Satyavani, K.; Ramanathan, T. Bitter apple (*Citrullus colocynthis*): An overview of chemical composition and biomedical potentials. *Asian J. Plant Sci.* **2010**, *9*, 394–401. [CrossRef]
23. Kulkarni, A.S.; Khotpal, R.R.; Karadbhajane, V.Y.; More, V.I. Physico-chemical Composition and lipid classes of *Aegle marmelos* (Bael) and *Citrullus colocynthis* (Tumba) Seed Oils. *J. Chem. Pharm. Res.* **2012**, *4*, 1486–1488.
24. Solomon, G.; Luqman, C.A.; Nor, M.A. Investigating "Egusi" (*Citrullus colocynthis* L.) Seed Oil as Potential Biodiesel Feedstock. *Energies* **2010**, *3*, 607–618. [CrossRef]
25. Yaniv, Z.; Shabelsky, E.; Schafferman, D. Perspectives on new crops and new uses. In *Colocynth: Potential Arid Land Oilseed from an Ancient Cucurbit*; Janick, J., Ed.; ASHS Press: Alexandria, VA, USA, 1999; pp. 257–261.
26. Al-Hwaiti, M.S.; Alsbou, E.M.; Abu Sheikha, G.; Bakchiche, B.; Pham, T.H.; Thomas, R.H.; Bardaweel, S.K. Evaluation of the anticancer activity and fatty acids composition of "Handal" (*Citrullus colocynthis* L.) seed oil, a desert plant from south Jordan. *Food Sci. Nutr.* **2021**, *9*, 282–289. [CrossRef]
27. Bireche, M.; Gherib, A.; Bakchiche, B.; Berrabah, M.; Maatallah, M. Positional distribution of fatty acids in the triglycerides of *Citrullus colocynthis* seed oil growing in Algeria. *J. Mater. Environ. Sci.* **2017**, *8*, 622–627.
28. AOCS. *Official Methods and Recommended Practices of the American Oil Chemist's Society*; AOCS: Champaign, IL, USA, 2003.
29. Berwal, M.K.; Haldhar, S.M.; Ram, C.; Shil, S.; Gora, J.S. Effect of extraction solvent on total phenolics, flavonoids and antioxidant capacity of flower bud and foliage of *Calligonum polygonoides* L. *Indian J. Agric. Biochem.* **2021**, *34*, 61–67. [CrossRef]
30. Medini, F.; Fellah, H.; Ksouri, R.; Abdelly, C. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. *J. Taibah Univ. Sci.* **2014**, *8*, 216–224. [CrossRef]
31. Bhatnagar, A.S.; Hemavathy, J.; Gopala Krishna, A.G. Development of a rapid method for determination of lignans content in sesame oil. *Food Sci. Technol.* **2015**, *52*, 521–527. [CrossRef]
32. Gopala Krishna, A.G.; Hemakumar, K.H.; Khatoon, S. Study on the composition of rice bran oil and its higher free fatty acids value. *J. Am. Oil Chem. Soc.* **2006**, *83*, 117–120. [CrossRef]
33. Kumar, S.S.; Manasa, V.; Tumaney, A.W.; Bettadaiah, B.K.; Chaudari, S.R.; Giridhar, P. Chemical composition, nutraceuticals characterization, NMR confirmation of squalene and antioxidant activities of *Basella rubra* L. seed oil. *RSC Adv.* **2020**, *10*, 31863–31873. [CrossRef]
34. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin, E. *Anal. Biochem.* **1999**, *269*, 337–341. [CrossRef]
35. Kostik, V.; Memeti, S.; Bauer, B. Fatty Acid Composition of Edible Oils and Fats. *J. Hyg. Eng. Design* **2013**, *4*, 112–116.
36. Zambiazi, R.C.; Przybylski, R.; Zambiazi, M.W.; Mendonca, C.B. Fatty Acid Composition of Vegetable Oils and Fats. *Bol. Cent. Pesqui. Processamento Aliment.* **2007**, *25*, 111–120.
37. Orsavova, J.; Misurcova, L.; Ambrozova, J.V.; Vicha, R.; Mlcek, J. Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of the Cardiovascular Mortality on Dietary Intake of Fatty Acids. *Int. J. Mol. Sci.* **2015**, *16*, 12871–12890. [CrossRef] [PubMed]
38. Venn-Watson, S.; Lumpkin, R.; Dennis, E.A. Efficacy of dietary odd-chain saturated fatty acid pentadecanoic acid parallels broad associated health benefits in humans: Could it be essential? *Sci. Rep.* **2020**, *10*, 8161. [CrossRef] [PubMed]
39. Jimenez-Cepeda, A.; Davila-Said, G.; Orea-Tejeda, A.; González-Islas, D.; Elizondo-Montes, M.; Pérez-Cortes, G.; Keirns-Davies, C.; Castillo-Aguilar, L.F.; Verdeja-Vendrell, L.; Peláez-Hernández, V.; et al. Dietary intake of fatty acids and its relationship with FEV_1/FVC in patients with chronic obstructive pulmonary disease. *Clin. Nutr. ESPEN* **2019**, *29*, 92–96. [CrossRef]
40. Huang, L.; Lin, J.S.; Aris, I.M.; Yang, G.; Chen, W.Q.; Li, L.J. Circulating Saturated Fatty Acids and Incident Type 2 Diabetes: A Systematic Review and Meta-Analysis. *Nutrients* **2019**, *11*, 998. [CrossRef] [PubMed]
41. Aglago, E.K.; Biessy, C.; Torres-Mejia, G.; Angeles-Llerenas, A.; Gunter, M.J.; Romieu, I.; Chajes, V. Association between serum phospholipid fatty acid levels and adiposity in Mexican women. *J. Lipid Res.* **2017**, *58*, 1462–1470. [CrossRef]
42. Kurotani, K.; Sato, M.; Yasuda, K.; Kashima, K.; Tanaka, S.; Hayashi, T.; Shirouchi, B.; Akter, S.; Kashino, I.; Hayabuchi, H.; et al. Even- and odd-chain saturated fatty acids in serum phospholipids are differentially associated with adipokines. *PLoS ONE* **2017**, *12*, e0178192. [CrossRef]
43. Bravo, L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317–333. [CrossRef]
44. Thusoo, S.; Gupta, S.; Sudan, R.; Kour, J.; Bhagat, S.; Hussain, R.; Bhagat, M. Antioxidant Activity of Essential Oil and Extracts of Valeriana jatamansi Roots. *BioMed Res. Int.* **2014**, *2014*, 614187. [CrossRef]
45. Chang, S.T.; Wu, J.H.; Wang, S.Y.; Kang, P.L.; Yang, N.S.; Shyur, L.F. Antioxidant activity of extracts from *Acacia confusa* Bark and Heartwood. *J. Agric. Food Chem.* **2001**, *49*, 3420–3424. [CrossRef] [PubMed]
46. Janu, C.; Kumar, D.S.; Reshma, M.V.; Jayamurthy, P.; Sundaresan, A.; Nisha, P. Comparative Study on the Total Phenolic Content and Radical Scavenging Activity of Common Edible Vegetable Oils. *J. Food Biochem.* **2014**, *38*, 38–49. [CrossRef]

47. Kozlowska, M.; Gruczynska, E.; Scibisz, I.; Rudzinska, M. Fatty acids and sterols composition, and antioxidant activity of oils extracted from plant seeds. *Food Chem.* **2016**, *213*, 450–456. [CrossRef] [PubMed]
48. Harborne, J.B.; Baxter, H.; Moss, G.P. *Phytochemical Dictionary: Handbook of Bioactive Compounds from Plants*, 2nd ed.; Taylor & Francis: London, UK, 1999.
49. Xuan, T.D.; Gangqiang, G.; Minh, T.N.; Quy, T.N.; Khanh, T.D. An Overview of Chemical Profiles, Antioxidant and Antimicrobial Activities of Commercial Vegetable Edible Oils Marketed in Japan. *Foods* **2018**, *7*, 21. [CrossRef] [PubMed]
50. Kim, H.W.; Kim, J.B.; Cho, S.M.; Cho, I.K.; Li, O.X.; Jang, H.H.; Lee, S.H.; Lee, Y.M.; Hwang, K.A. Characterization and quantification of γ-oryzanol in grains of 16 Korean rice varieties. *Int. J. Food. Sci. Nutr.* **2015**, *66*, 166–174. [CrossRef] [PubMed]
51. Ajala, A.W.; Ghavami, A. Evaluation of the effectiveness of cereal bran extract for sunflower oil stability during frying. *Int. J. Food Stud.* **2020**, *9*, SI52–SI61. [CrossRef]
52. Moazzami, A.A.; Kamal-Eldin, A. Sesame seed is a rich source of dietary lignans. *J. Am. Oil Chem. Soc.* **2006**, *83*, 719–723. [CrossRef]
53. Reshma, M.V.; Balachandran, C.; Arumughan, C.; Sunderasan, A.; Sukumaran, D.; Thomas, S.; Saritha, S.S. Extraction, separation and characterisation of sesame oil lignan for nutraceutical applications. *Food Chem.* **2010**, *120*, 1041–1046. [CrossRef]
54. Ribayamercado, J.D.; Solon, S.F.; Tang, G.; Cabal-Borza, M.; Perfecto, S.C.; Russel, R.M. Bionconversion of plant carotenoids to vit-A in Filipino school-aged children varies inversely with vit-A status. *Am. J. Clin. Nutr.* **2000**, *72*, 455–465. [CrossRef]
55. Wang, X.D. Carotenoids. In *Modern Nutrition in Health and Disease*, 11th ed.; Ross, C.A., Caballero, B., Cousins, R.J., Tucker, K.L., Ziegler, T.R., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2014; pp. 427–439.
56. Chandrasekaram, K.; Ng, M.H.; Choo, Y.M.; Chuah, C.H. Effect of storage temperature on the stability of phytonutrients in palm concentrates. *Am. J. Appl. Sci.* **2009**, *6*, 529–533. [CrossRef]
57. Hemalatha, S.; Ghafoorunissa. Sesame lignans enhance the thermal stability of edible vegetable oils. *Food Chem.* **2007**, *105*, 1076–1085. [CrossRef]