Butom (Pistacia palaestina, Boiss) fruit and its extracted oil functional characteristics

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Pistacia palaestina fruits "butom" and its extracted oil were characterized. The wild grown plant contains appreciable percentage of lipids (44.7%) and protein (6.25%). The weight of 1000 fruits was 56.15 grams, refractive index, specific gravity, saponifiables, unsaponifiables, and iodine number of extracted oil were 1.472, 0.914, 194 mg KOH/g oil, 2.3 g/kg oil, and 86.7 mg I_2/100g oil, respectively. Extracted *Pistacia palaestina* oil contains significant amounts of α-tocopherol (80 mg/kg), δ-tocopherol (20 mg/kg), and β- and γ-tocopherol (11 mg/kg). Diphenyl-1- picrryylhydazyl (DPPH) free radical scavenging activity expressed as IC_{50} µl/extract and IC_{50} µg phenol compounds were 25.1 and 45.5, respectively. These results were correlated to the phenolic compounds (1820 mg/100g) and flavonoids (525 mg/100g). Results of this study indicate that butom fruit contains greater amounts of nutrient elements compared to several nuts including almonds, hazelnut and pistachio and can be an important source of oil production in the food industry.

Keywords: *Pistacia palaestina*, antioxidants, bioactive compounds

INTRODUCTION

Pistacia palaestina plant, also known as "butom" is one of the most abundant species from the family *Anacardiaceae* that are wildly grows in Middle East. *Pistacia palaestina* plants from the family *Anacardiaceae* was reported to contain more than twenty species, but only five are most popular namely, *P. atlantica, P. vera, P. khinjuk, P. terebinthus* and *P. lentiscus* [1]. The plant is considered a deciduous tree that is between 3 and 5 m long, unisexual flowers with a fruits about 5mm in diameter, ovoid-globular, brownish and sometimes bluish color [2-3]. The ripe fruits of butom are used as a mix of aromatic food plants in the Middle East that called Zaatar. For instance, Flamini *et al*., (2004) [3] studied the composition of the essential oil of leaves, galls, and ripe and unripe fruits of Pistacia palaestina Boiss. The authors reported that ripe and unripe fruits of Pistacia palaestina Boiss were rich in monoterpenes and the main components in the leaves were alpha-pinene and myrcene (63.1% and 13.3%, respectively), while galls were rich in Alpha-pinene, sabinene, and limonene (49.4%, 22.8%, and 8.1%, respectively).

Furthermore, *Pistacia palaestina* is generally used as medicinal plant. More specifically, different parts of *Pistacia* species have been used in traditional medicine such as tonic, aphrodisiac, antihypertensive antiseptic, and management of dental, gastrointestinal, urinary tract, liver, and respiratory tract disorders [2-3]. The use of *Pistacia palaestina* extracts further demonstrated that the plant and its functional compounds would be a promising alternative or an adjuvant therapy for the treatment of several diseases and symptoms [4]

Various types of phytochemicals including phenolic compounds, terpenoids, fatty acids, and sterols have also been isolated and identified from different

parts of *Pistacia* species. For example, the composition of the essential oil of leaves, galls, and ripe and unripe fruits of Jordanian *Pistacia palaestina* showed that the oil is also rich in monoterpenes [5].
Characterisation of various medicinal plants and their constituents as a natural source of bioactive compounds is of great interest against several diseases based on their traditional claims [6]. Similar plants were studied in relation to the impact of soil and environment on its quality characteristic. In this regard, Gulsoy *et al*. (2011) [7] studied the indicatory species of turpentine tree *(Pistacia terebinthus* L. subsp. *palaestina* (Boiss)), in relation environmental factors. Cistus cretagus, Pinus brutia, and Quercus coccifera are the most important positive indicatory plants, according to the authors, whereas Cistus laurifolius, Pinus nigra, and Rosa canina are the most significant negative indicatory plants. Pulaj et al., [8] investigated the chemical composition and in vitro antibacterial activity of essential oils from Pistacia terebinthus collected from wild populations in Kosovo. Pistacia terebinthus was equal to or better than Tea Tree Oil control, according to the authors, suggesting that essential oils from this species may have the potential for development as an antibacterial agent.
There are limited studies regarding functional characteristics and bioactive properties of *Pistacia palaestina* fruits. Therefore, this study was carried out to investigate some physiochemical and antioxidant properties of *Pistacia palaestina* fruits "butom" and their extracted oil of this plant that were wildly grown and in Jordan.

MATERIALS AND METHODS

PISTACIA PALAESTINA FRUITS COLLECTION

About 5 kg of fully maturated brownish-bluish *Pistacia palaestina* fruits were collected from Jarash forests, in the north of Jordan during 2019 season, which was during the last week of August, and transferred to the laboratory using polyethylene bags. To limit harvesting and maturation variability, only fully maturated brownish-bluish *Pistacia palaestina* fruits were collected from at least 350 trees. The fruits were cleaned and dusts, leaves, stems and immature fruits were removed. Fruits were then dried to a constant weight at room temperature, and then stored at refrigeration (5-7°C) using airtight polyethylene bags until subsequent analyses. Voucher specimens were kept at the Herbarium of the Department of Plant Production and Protection, Faculty of Agriculture, Al-Balqa Applied University, Jordan for maturation stage similarity and plant identification. Only fully mature plants were used in this study.

PISTACIA PALAESTINA OIL EXTRACTION

A 100 g of ground fruits were soaked in petroleum ether (40-60°C) for overnight and then filtered. The supernatant was collected, and the precipitated ground fruit were soaked again and the extraction was repeated. The combined supernatant solvents were evaporated at 35-40ºC under vacuum using a rotary evaporator. Two oils extractions samples were performed and the collected lipid fractions from the two runs were stored at -18°C until additional analyses.

PISTACIA PALAESTINA FRUITS AND OIL PHYSIOCHEMICAL ANALYSES

The weight of 1000 × 3 fruits of *Pistacia palaestina* was determined. Moisture, crude fat, crude protein, and ash contents of the fruit were determined according to the official methods of the Association of Official Analytical Chemists (AOAC 2000) [9]. Averages of three measurements were reported.
The refractive index of the oil was measured using Abbe digital reftractometer (Reichert, 1310499, USA) after calibration of the instrument at 20°C using distilled water (AOAC method no 921.08; [9]). Specific gravity of the extracted oil was estimated using a density bottle of 50 ml capacity at 30°C after calibration using water as described by AOAC method 920.212 [9]. Iodine value (mg I_2/100 g oil) was determined using Wijs solution according to the AOAC method no 920.159 [9]. Saponification value was determined according to AOAC method no 921.08, [9]. The unsaponifiable value was determined according to AOAC method 933.08 [9]. All measurements were carried out in triplicate and averages were reported.

METHANOLIC EXTRACT OF DEFATTED PISTACIA PALAESTINA FRUITS

Ten grams of the defatted ground fruits were suspended in 100 ml methanol with continuous shaking for two hours. The mixture was then filtered using Whatman No.1 filter paper and the solvent was evaporated using a rotary evaporator under vacuum after which the residue extract was weighed. The extracts were recovered using 50 ml methanol and analysed for its total phenolic compounds, total flavonoids and antioxidant activity using 2, 2-diphenyl-1-1-picrylhydrazyl (DPPH).

TOTAL POLYPHENOLS CONTENT (TPC)

Total polyphenols content in the methanolic extract of *Pistacia palaestina* fruits was performed in triplicate using Folin-Ciocalteu (FC reagent) colorimetric method, based on the reaction of the reagent with the functional hydroxyl groups of phenols [10]. Briefly, 1 ml of the extracted oil was placed into a 10 ml volumetric flask. Five millilitres of distilled water and 0.25 ml (2N) FC reagent (Sigma-Aldrich, USA) were added and vortexed for 3 minutes. Two ml of Na_2CO_3 (10%) were then added before the volume completed with distilled water to the mark, mixed and allowed to stand for 60 min. A calibration curve was prepared

using a pure phenolic compound (gallic acid) as a standard. The absorbance of the blue colour formed was measured at 725 nm in a Perkin Elmer, UV/Vis Spectrophotometer (Perkin Elmer Lambda, 25, 101 NB, USA) against a blank sample. The TPC was expressed as gallic acid equivalents (GAE) in mg/100g of defatted sample. Total polyphenols content was carried out in triplicate and averages were reported.

TOTAL FLAVONOIDS DETERMINATION

Total flavonoids content was determined according to the methods described by Ghazzawi and Al-Ismail [11] using aluminium trichloride (2% w/v). In summary, one ml of the extracted oil was added to 1 ml of aluminium trichloride (2% w/v). The absorbance was measured spectrophotometrically at 430 nm after 15 minutes of incubation, the results were expressed as mg quercetin equivalents per mg sample. Total flavonoids content was carried out in triplicate and averages were reported.

TOCOPHEROLS DETERMINATION

Tocopherols' analysis was carried out using a Knauer HPLC System (Germany) following the method of Gimeno et al. [12]. After diluting Pistacia palaestina fruits oil in hexane (1:10); an aliquot of 200 µl was transferred to a test tube containing 600 µl of methanol and 200 µl of the internal standard solution (300 mg/ml of δ-tocopherol acetate in ethanol). After that, the mixtures were mixed, centrifuged for 5 minutes at 3000 g, and filtered through a 0.45 mm pore size filter. For the HPLC analysis, fifty µl were directly injected into the Knaur HPLC system. The mobile phase was methanol-water (96:4, v/v) and the elution was performed at a flow rate of 2 ml/min. The analytical column was Venusil XBP, C18 (Agelant Technologies, USA), and was kept at 45°C. Detection was performed on 292 nm using Knauer UV detector (model Smartline 2500, Germany). All measurements were carried out in triplicate and averages were reported.

DIPHENYL-1- PICRRYYLHYDAZYL (DPPH) FREE RADICAL SCAVENGING ACTIVITY DETERMINATION

Free radicals scavenging activity of the extracted oil was determined as described by Al-Ismail et al. [13] using nine different aliquots of the methanolic solutions of the extract (2, 4, 6, 8, 10, 20, 30, 40, 50 µl) against 4 ml a blank of methanol containing 0.2 ml of DPPH solution. After incubation in the dark at ambient temperature for 45 minutes the absorbance was measured using spectrophotometer at 515 nm, and the radical scavenging activity of the sample was expressed as % inhibition according to the following formula:

$$\text{Inhibition \%} = [(\text{abs. control} - \text{abs. sample})/ \text{abs. control}] \times 100$$

IC_{50} (µl extract and µg phenol compounds) was calculated from their concentration-response curves.

FATTY ACIDS COMPOSITION

Fatty acid profile of the extracted oil was determined as described by Mezni et al. [14]. In brief, fifty mg of lipid extract was weighed, dissolved in 1 ml hexane (GC grade) and then vortexed for 1 min. A 200 µl of 2 M potassium hydroxide prepared in anhydrous methanol was then added and mixed for 30 seconds until the solution became clear. The prepared fatty acid methyl esters (FAMEs) were analysed using capillary GLC column (Restek, Rtx-225, USA, cross-bond 50%-cyanopropylmethyl 50%-phenylmethyl polysiloxane, 60 m, 0.25 mm/D, 0.25 µm df) immediately after esterification by injecting 1µl of the hexane layer through the injection port of the GLC (model GC-2010, Shimadzu. Inc., Koyoto, Japan). After setting the GLC conditions, the FAMEs were injected; the column oven temperature was set to 180°C for 10 minutes, then increased to 200°C (5°C/min) and held at 200°C for 5 minutes, before being raised to 210°C (3°C/min) and held at 210°C for 20 minutes. The injector was set to 250°C, the flame ionization detector to 260°C, the flow rate 1.2 ml/min N2, and the split ratio to 70. The FAMEs were identified using chromatogram of fatty acids standard.

STEROLS DETERMINATION

Phytosterols profile was analysed according to the method reported by Dabbour et al. [9]. Derivatization of the unsaponifiables, and gas chromatographic analysis of the sterols were carried out as follows.

DERIVATIZATION OF THE UNSAPONIFIABLES

The trimethylsilyl derivatives (TMS) of the unsaponifiable components were obtained according to the method reported by Dabbour et al. [10] , a portion of 500 µl of silylation reagent, consisting of a mixture of 9:3:1 (v/v/v) of pyridine (CBH, Nottingham, UK)/ hexamethyl disilazane (Janssen, Belgium)/trimethylchlorosilane (Fluka, Switzerland) was prepared and then added to the unsaponifiables. The vial was left for 40 min at room temperature before the pyridine was evaporated under a stream of extra pure nitrogen, and then 4 ml of hexane (GC grade, lab scan, Dublin) was added and shacked vigorously. The solution was transferred into a 5 ml screw capped test tube and centrifuged at 4000 rpm for 5 min (Hettich EBA-20, Germany). The hexane layer was analysed immediately by GLC.

GAS CHROMATOGRAPHIC ANALYSIS

The TMS derivatives of the unsaponifiables were analysed using Shimadzu gas chromatograph (model GC-2010, Shimadzu Inc., Koyoto, Japan) supplied with split-split injector port and flame ionization detector, an RTX-65 TG (Restek, USA) [10]. CP-SIL 8CB capillary column (30 m × 0.25 mm internal diameter, film thickness was 0.25 µm and the active ingredients were 35% diphenyl 65% dimethly polysiloxan) was used. The oven analysis temperature

was kept at 265°C and the holding time for analysis was set for 55 min. The injector temperature was set at 280°C and the detector temperature was kept at 290°C. The gas flow rate was 1.11 ml/min and split ratio was 1:30.

STATISTICAL ANALYSIS

Measurements were carried out in triplicate and differences between means as well as standard deviations were separated using least significant difference at probability level of 0.05 using SAS statistical analysis software version 9.1 [15].

RESULTS AND DISCUSSION

Physiochemical characteristics of *Pistacia palaestina* fruits and its extracted oil.

Table I presents the weight of 1000 fruits, moisture, oil content, crude protein contents ash of *Pistacia palaestina* fruits and some physicochemical properties and its extracted oil. Our results were comparable to that of several authors with minor differences. For example, the weight of 1000 fruits, moisture, and crude protein contents were lower than those reported for *Pistacia terebinthus* fruits by Özcan [5] while oil content like those obtained by Matthäus and Özcan [17]. Ash content of the *Pistacia palaestina* fruits was similar (P>0.05) to that of *Pistacia terebinthus* reported by Özcan [5].

These results were attributed to the variation of *Pistacia palaestina* fruits proximate composition Aydın and Özcan [18]. Furthermore, physiochemical characteristics of the *Pistacia palaestina* were comparable to that of *Pistacia terebinthus* L. fruits and oil. This might be attributed to that some alleles of *P. palaestina* and *P. terebinthus* had identical sequences. In fact, *Pistacia palaestina* is not well separated from *P. terebinthus* in either the plastid or nuclear DNA data sets [18].

The significant amount of extracted oil (i.e., 44.7%) of *Pistacia palaestina* fruits is more than other oily seeds such as soybean, corn, cottonseed and olive fruits and equivalent to sunflower, cola seed, safflower and palm which are industrially important [19]. This indicates that *Pistacia palaestina* fruits could be an important economical source of oil in the food industry.

The extracted oil was yellowish in colour with a refractive index and specific gravity of 1.472 and 0.914, respectively, which are lower than those values reported by Özcan [5] (i.e., 1.477 and 0.9742, respectively). However, refractive index, specific gravity, and saponification values of the extracted *Pistacia palaestina* oil within the acceptable values of most vegetable oils [20]. Gunstone [19] reported that the Refractive index (RI) and specific gravity of the oils were mostly affected by its fatty acid composition, the degree of unsaturation and the total solid contents. More specifically, the author reported an increase in RI with the increase unsaturated or long chain fatty acids in their triglycerides increases. The RI and specific gravity of our extracted *Pistacia palaestina* oil (i.e., 1.472 and 0.914, respectively) is within the level of the vegetable oils recommended by the FAO/WHO which is between 1.466-1.470 for RI and between 0.919-0.925 for specific gravity, respectively [21].

Saponification value of oils that measures the average chain length of the fatty acids, provides valuable information regarding oil quantity, glycerides type as well as the acids in each oil sample. Our results indicate that *Pistacia palaestina* oil has saponification value (194 mg KOH/g) within the recommended value (188-195 mg KOH/g) by the FAO/WHO [21].

Iodine value of *Pistacia palaestina* oil was 86.7 mg I_2/100g oil which lies in the range of olive oil (Jordanian Standard and Metrology, b 2012) and slightly lower than that of *Pistacia terebinthus* oil (89 mg I_2/100g) [5]. Iodine value provides a measurement of the degree of the oils and fats unsaturation, it increases as the double bonds increase [22]. Duel [23] proposed that an iodine value above 100 means oil is to be classified as drying oil while below 100 is non-drying oil. Based on this assumption, *Pistacia palaestina* oil might be classified as non-drying oil.

Unsaponifiable matter content of this oil was 23 g/kg, which is greater than that of *Pistacia terebinthus* oil and most of other vegetable oils, but it lies within the range of unsaponifiable matter of palm kernel oil [5, 20].

Tocopherol contents of the *Pistacia palaestina* oil reveal that α-tocopherols, δ-tocopherol, and β- and γ-tocopherol were 80.3, 20.0, and 11.1 mg/kg oil, respectively. Total tocopherols content of this oil was lower than that of the most vegetable oils except babasuu and palm kernel oil, but higher than that of coconut oil [20]. More specifically α-tocopherols in *Pistacia palaestina* oil is twice the amount found in

Table I - Physiochemical characteristics of the *Pistacia palaestina* fruits and its extracted crude oil.

Property	Value
Weight of 1000 fruits (gm)	56.15 ± 1.20
Moisture%	4.52 ± 0.05
Oil %	44.70 ± 0.60
Protein%	6.25 ± 0.35
Ash%	3.00 ± 0.66
Refractive index (n^D 40°C)	1.47 ± 0.00
Specific gravity	0.91 ± 0.03
Idodine value (mg I_2/100 g oil)	86.70 ± 1.24
Saponification value (mg KOH/g oil)	194.20 ± 1.00
Unsaponifiable (g/kg oil)	23.30 ± 2.80
α-tocopherol (mg/kg oil)	80.30 ± 2.00
β- and γ-tocopherol (mg/kg oil)	11.10 ± 0.50
δ-tocopherol (mg/kg oil)	20.00 ± 0.70

Data were expressed as mean±SD (n=3)

Almond and Hazelnut [24]. δ-tocopherol was significantly greater than that reported for Almond, Pistachio, Hazelnut and Cashew. This implies that the oil of these fruits is expected to have low resistance to oxidation in the absence of phenolic and tocopherol compounds [16].

TOTAL PHENOLS, FLAVONOIDS AND ANTIOXIDANT ACTIVITY

Total phenolic compounds (TPC) of the methanolic extract of *Pistacia palaestina* was 2440 mg/100g sample, while flavonoids content was 850 mg/100g sample (Tab. II). Diets rich in polyphenol and flavonoids have many health benefits and currently they are of great interest due to their antioxidant and anti-carcinogenic effects. These bioactive compounds also act as free radical scavengers, reducing agents, and quencher of singlet oxygen formation [25]. DPPH was therefore, measured as one of the main antioxidant parameters expressed as IC_{50} µl/ml extract IC_{50} µg phenol/ml compounds were 181.8 and 24.7, respectively. These free radicals scavenging capacities could be attributed to the phenolic and tocopherol compounds of *Pistacia palaestina* fruits (Tab. I and II).

FATTY ACIDS PROFILE OF THE EXTRACTED OIL

Fatty acid composition of *Pistacia palaestina* oil is presented in Table III. Results indicated that *Pistacia palaestina* oil is highly composed of unsaturated fatty acids (i.e., 77% content) of which monounsaturated fatty acids represent 53% and about 24% for polyunsaturated fatty acids. Saturated fatty acids in the *Pistacia palaestina* oil were the lowest representing only 22.43%. Oleic acid has the highest concentration in the amount of 50%. However, this level is still below the range of oleic acids in olive oil [26].

The levels of *Pistacia terebinthus* fatty acids were comparable to those for *Pistacia lentiscus* seeds [5, 14, 16]. More specifically, Palmitic acid (C16:0) represents 23.52% (i.e., that represents twice the amount found in Cashew and Pistachio, four times that found in Almont and five time that in Hazelnut), Linoleic acid (C18:2) accounts for 23% 9 (i.e., results like Almund, Cashew, a slightly lower than Pistachio and a twice as Hazelnut [24]. Palmitoleic, stearic and linolenic acids represent minor amounts of the total fatty acids as found in Almond, Pistachio, Hazelnut and Cashew [24]. Our results further indicated that fatty acids of *Pistacia palaestina* oil were like those illustrated by Matthäus and Özcan [16] for *Pistacia terebinthus*.

Table II - Total phenols, flavonoids and antioxidant activity of *Pistacia palaestina* fruits

Property	Value
Total phenol compounds (mg/100g defatted sample)	2440.0 ± 66.4
Flavonoids (mg/100g defatted sample)	850.0 ± 22.1
DPPH (IC_{50} µl extract/ml)	181.8 ± 13.2
DPPH (IC_{50} µg PC /ml)	24.7 ± 2.1

Data were expressed as mean±SD (n=3)
DPPH represent the Diphenyl-1-picrryylhydazyl free radical scavenging activity
IC_{50} represent the concentration of extract (mg/ml) needed to scavenge 50% of the DPPH radicals (mg/ml)]

Table III - Fatty acid composition of *Pistacia palaestina* oil

Property	Percentage
C14:0	0.12 ± 0.02
C16:0	20.15 ± 0.10
C16:1	1.85 ± 0.07
C17:0	0.05 ± 0.01
C17:1	0.46 ± 0.09
C18:0	2.00 ± 0.3.00
C18:1	50.00 ± 0.70
C18:2	23.00 ± 0.30
C18:3	0.55 ± 0.02
C20:0	0.11 ± 0.07
C20:1	0.17 ± 0.01
∑SFA	22.43 ± 0.50
∑MUFA	53.08 ± 0.90
∑PUFA	23.95 ± 0.40

Data were expressed as mean±SD (n=3)
SFA represent the saturated fatty acids
MUFA represent the monounsaturated fatty acids
PUFA represent the poly unsaturated fatty acids

STEROLS CONTENT OF THE EXTRACTED OIL

Eleven phytosterols were detected in the extracted oil of *Pistacia palaestina* fruits with total amount of 1838 mg/kg oil, where β-sitosterol is the abundant sterols representing 85.56% of the total sterols followed by campesterol (i.e., 4.47% mg/kg oil) and then Δ-5-avenasterol (i.e., 3.68%) (Tab. IV).

These results coincide with the sterols content of *Pistacia palaestina* grow wild in Turkey [16]. Results indicated that sterols of *Pistacia palaestina* oil were comparable to those of olive oil with respect to campesterol, stigmasterol and β-sitosterol and that the summation of β-sitosterol and other sterols (Sistanol, Δ-5-avenasterol, Δ-5-24-sigmastadienol and Δ-7-stigmastenol) were like those set for olive oil (i.e., less than 93%) [26].

CONCLUSIONS

Characterisation of *Pistacia palaestina* "butom" fruits and their extracted oil indicated that these fruits are rich source of lipids, protein, total minerals, unsaturated fatty acids, and sterols. Moreover, appreciable amounts of phenolic compounds, flavonoids, and tocopherols were found, and as the result, high free radicals scavenging capacity was detected. The nutritional and antioxidant results of the butom fruits provided potential human health values of these

Table IV - Sterols contents of the *Pistacia palaestina* oil (mg/kg oil)

Sterol Type	Value (mg/kg oil)	Value (percent of sterol from total sterols)
Cholesterol	9.9 ±1.4	0.54
Brasicasterol	6.7 ± 1.6	0.36
Campesterol	82.2 ± 2.7	4.47
Stigmasterol	44.5 ± 0.5	2.42
Δ-7-campesterol	5.3 ± 0.7	0.29
Clerosterol	20.7 ± 1.0	1.13
β-sitosterol	1572.7 ± 30.0	85.60
Sistanol	12.0 ± 1.6	0.65
Δ-5-avenasterol	67.7 ± 5.9	3.68
Δ-5-24-sigmastadienol	11.0 ± 1.2	0.60
Δ-7-stigmastenol	6.0 ± 0.2	0.65
Total sterols (mg/kg oil)	1838.0 ± 53.0	100.00

Data were expressed as mean±SD (n=3)

edible fruits. Results of this research highlighted the need for further investigation of using butom fruit as a potential therapeutic plant as well as a source for oil production in the food industry.

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