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Estimation of nutritional, phytochemical and antioxidant activity of olive fruit (*Olea europaea* L.) grown in Bikaner, Rajasthan

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Abstract
Olive fruit (*Olea europaea* L.) a native of Mediterranean region is a famous edible oil tree crop used worldwide. Different standardized methods were used to remove the bitterness of olives. The physico chemical and proximate analysis of the fruit was determined using different standard analytical methods. In this review the dietetic quality of olive fruit (*Olea europaea* L.). described. This study emphasizes the analyzing the physic-chemical and nutritional value of the olive fruit. The result of the proximate composition showed that the fruit contain 86.16% moisture, 0.73% protein, 3.68% ash, 0.75% crude fiber, 7.22% fat, 76.74 Kcal/100 g carbohydrate. The total phenolic content of the fruit was 107.76 mg/100 g. Qualitative phytochemical testing of the studied plant extracts showed the presence of carbohydrate, glycoside, protein, phenolic compounds, flavonoids, steroid and terpenoids. The fruit of *Olea europaea* showed good physio-chemical properties and could be utilized successfully for human consumption and for commercial applications.

Keywords: *Olea europaea*, physic-chemical, nutritional, proximate composition, total phenolics, antioxidants, phytochemical

1. Introduction
Plant foods, such as fruits, vegetables, and whole grains contain many components that are beneficial to human health. Research supports that some of these foods, as part of an overall healthful diet, have the potential to delay the onset of many age-related diseases (Hasler, 2002) [13]. The health benefits of olive oil are unrivaled and research reveals more benefits nearly every day. In fact, we are only just beginning to understand the countless ways olive oil can improve our health and our lives. Olive oil benefits are so extensive that it is considered as functional food with components that contribute to its overall therapeutic qualities including a reduction of risk factors of coronary heart disease, the prevention of cancers, and alterations of immune and inflammatory responses. The olive tree (*Olea europaea* L.) a native of the Mediterranean region of Asia is now cultivated in many parts of the world for production of olive oil and table olives (Ghanbari *et al.*, 2012) [10]. The Olive fruit and its oils are in great demand due to its nutritional value (Mele *et al.*, 2019) [18]. The olive (*Olea europaea* L.) is a famous edible oil tree crop worldwide and has great commercial value due to its peculiar nutritional benefits (Conde *et al*., 2008) [8]. Due to the well-balanced oil composition (highly enriched in monounsaturated fatty acid) and rich minor components (such as polyphenols and phytosterols) in the fruits, olive trees are unique among oil plants (Sánchez and Harwood, 2002) [20]. Olive is as rich source of valuable nutrients and bioactives of medicinal and therapeutic interest. Olive fruit contains appreciable concentrations of multiple biological activities such as antioxidant, anticarcinogenic, anti-inflammatory, antimicrobial, antihypertensive, antidyslipidemic, cardiotonic, laxative and antiplatelet (Ghanbari *et al.*, 2012) [10].

2. Materials and Methods
2.1 Sample collection and preparation
Raw green olives were purchased from a farm at Momasar near Bikaner. Removing bitterness from green olive using alkaline is the most required method. Different standardized methods were used to remove the bitterness of olives.

Methods

2.1.1 Traditional treatment

Two kg of each olive variety were placed in plastic jars and pickling brine solution (12% Sodium chloride + 0.5% Citric acid) was added then the jars were closed tightly and left for 60 days until the end of the pickling process. (Ibrahim 2002; Ross *et al.*, 2002) [15-16].

2.1.2 Lye treatment

Olives of each variety (separately) were placed into tanks and soaked in a lye solution (1% w/v, sodium hydroxide) for about 8h for de-bittering. During this stage hydrolysis of oleuropein, which is labile under alkaline conditions, takes place. Lye is allowed to penetrate through three-quarters of the flesh, leaving a small volume around the stone unaffected. This part of the flesh provides the necessary sugars for subsequent fermentation and confers to the olives a slight bitter taste. Olives are washed with water twice in order to remove excess alkaline. Then, two kg of each olive variety were placed in plastic jars and pickling brine solution (12% sodium chloride + 0.5% citric acid) was added, then the jars were closed tightly and left for 60 days until the end of the pickling process. (Hurtado *et al.*, 2012) [14].

2.1.3 Citric acid treatments

The concentration of citric acid was prepared as follow: 3% Citric acid concentration+12% Sodium chloride. The first part was soaked in citric acid solution (1% Citric acid + 12% NaCl) for 15 hr. followed by water soaking for 3hr. and repeated soaking in citric solution as well as soaking in water two times. Olive fruits were placed in plastic jars and pickling brine solution (12% Sodium chloride + 0.5% Citric acid) was added then the jars were closed tightly and left for 60 days until the end of the pickling process (Approximately two months).

2.1.4 Preparation of Methanolic Extracts

Approximately 400 g of each of the powdered plant materials was soaked in a liter of analytical grade methanol in a 2-liter capacity conical flask. The flasks containing each plant material were shaken regularly, corked, and left to stand for 48 hours at room temperature. In each case, the menstruum was separated by filtration through Whatsman filter paper No. 1. The filtrates were then concentrated using a rotary evaporator at 50 °C and later in a hot-air oven at 35 °C to dry completely. The concentrates were put in airtight containers and stored at 4 °C awaiting use in *in vitro* bioassay (Harborne, 1998) [12].

2.2 Nutritional Analysis

Proximate analysis was carried out according to the procedure of FSSAI Lab Manual- Fruit and Vegetables, (FSSAI, 2016) [17] to determine the moisture content, crude fiber and ash content, protein, crude fat, and estimation was done at CEG Test House and Research Centre Pvt. Ltd. (CEGTH), Jaipur, Rajasthan, India. Carbohydrate and energy was determined as per BIS specification (BIS, 2007) [6]. Total Phenolic content of the fruit was determined according to the standard analytical methods recommended by (API, 2008) [24].

2.3. Qualitative Phytochemical Screening.

Qualitative tests for various phytochemicals present in the methanolic extracts of *olive fruit* were carried out using standard phytochemical screening procedures given by (CCRAS, 2016) [9]. Visual examination of the appearance of colour or frothing was used as an indicator for the presence or absence of a given phytochemical group.

2.4. Antioxidant activity DPPH Assay and total flavonoids

2.4.1. Free radical scavenging activity

Free radical scavenging activity of different extracts of leaves and flowers of Ageratum conyzoides Linn. plant was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mm solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml). Here, only those extracts are used which are Solubilize in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS Shimadzu).15 Reference standard compound being used was ascorbic acid and experiment was done in triplicate.16 The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity.17 The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100. Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample (Shekhar and Anju, 2014) [21].

2.4.2 Total Flavonoids

The effects of (1) the herbal material: Solvent ratio (0.02, 0.03, 0.05, 0.07, and 0.08 g/mL), (2) stock solution volume (0.8, 2.3, 4.4, 6.5, and 8.0 mL) and (3) $AlCl_3$ volume (0.8, 1.0, 1.2, 1.4, and 1.6 mL) on the TFC were evaluated (Silva, *et al*., 2015) [22].

2.5 Value added product development of olive fruit

Different types of Olive toffees were developed and standardized using olive paste and olive powder along with addition of chocolates and walnuts. Toffee is a confection made by caramelizing sugar or molasses (creating inverted sugar) along with butter, and occasionally flour (en.wikipedia.org/wiki/Toffee).

3. Result and Discussion

Physico chemical analysis in Table 1 depicts that weight, length and diameter of raw olives was found to be 5.82 gm, 5.80 cm and 3.15 cm respectively

Table 1: Physico-chemical analysis of raw olives

Physico chemical analysis of raw olives			
Colour	Weight (gm)	Length (cm)	Diameter (cm)
Green	5.82±0.82	5.80±0.80	3.15±0.03

3.1 Analysis of Olive Fruit

Olive fruit were physiochemically, nutritionally analyzed following table 2 depicts the detailed analysis of olive fruit. The moisture content of the olive fruit was 86.16% which was higher than that reported by (Cheng 2017) [7], where the moisture content of the fruit varied from content varied from 55.22 ±0.81% to 77.01 ±1.05%. The fat content was estimated

to be 7.22 percent. (Boskou *et al.*, 2015) [3] reported the lipid content of the fruit (up to 30% in ripe olives, i.e., up to 300 g·Kg^{-1}). The ripe olive fruit contained 92% of triacylglycerols (TAGs) in the lipid pool as reported by (Bianchi *et al.*, 1994) [2]. As such, lipids are the most abundant chemical components in this foodstuff, also being important for their nutritional value. The protein content of 0.73 percent obtained in our sample was lower than 1.6 percent obtained by (Boskou *et al*., 1996) [4]. Crude fiber of the fruit was estimated as per the procedures maintained by FSSAI Lab Manual and contributed to 0.75 percent which was lower than values obtained by (Gulfraz *et al.*, 2009) [11] for different varieties of wild olive fruit which ranged from 2.6 ±0.4 to 6.5 ±0.3%, while the ash content was 3.68% which was higher than the values 1.7 ±0.2 to 2.1 ±0.4% as obtained by (Gulfraz *et al.*, 2009) [11]. The ash content of the fruit sample was 3.68%. The ash content of eight olive cultivars grown in Saudi Arabia was reported by (Al-Ruqaie *et al*., 2016) [1] as values ranging from (1.38-5.22%). Carbohydrate content is 2.21g/100 g which is lower than the value (6.3g/100 g) reported by (USDA, 2019). The energy value (76.74Kcal/100 g) was also lower than the value (115 Kcal/100 g) reported by (USDA, 2019).

Phenolic compounds, includes phenolic acids, phenolic alcohols and flavonoids, are one of the most important minor components and directly affect the fruit quality (Brahmi *et al.*, 2013) [5]. In this study, olive manifests a total phenolic content of 107.26 mg/100 g. Among the minor components of the fruit, the phenolic ones are relevant for the health effects. In particular, epidemiological studies indicate that dietary consumption of phenol enriched Extra Virgin Olive Oil has a cardio protective effect in Mediterranean populations (Romani *et al.*, 2019) [19].

Table 2: Nutritional analysis of raw olives

S. No	Test parameters	Methods of test	Test results	Unit
1	Moisture	FSSAI Lab Manual- Fruit and Vegetables, 2016	86.16	%
2	Fat	CEGTH/STP/C/203	7.22	%
3	Protein	CEGTH/STP/C/139	0.73	%
4	Crude Fiber	FSSAI Lab Manual- Fruit and Vegetables, 2016	0.75	%
5	Ash COntent	FSSAI Lab Manual- Fruit and Vegetables, 2016	3.68	%
6	Carbohydrate	IS 1656-2007, RA 2012	2.21	g/100 g
7	Energy	IS 14433-2007, RA 2012	76.74	Kcal/100 g
8	Total Phenolics	API, Part-I, Volume-VI, 2008	107.26	Mg/100 g

Qualitative Phytochemical Screening. Qualitative phytochemical testing of the studied plant extracts showed the presence of carbohydrate, glycoside, protein, phenolic compounds, flavonoids, steroid, terpenoids. However the sample of the given fruit lacked tannin, Alkaloids, starch and saponins (Table 3).

Table 3: Qualitative Phytochemical composition of olive fruit

Test parameters	Methods for testing	Test result
Carbohydrate Test (Fehling solution)	Guide for ASU drugs, CCRAS, 2010	+
Glycoside (Borntrager's Test)	Guide for ASU drugs, CCRAS, 2010	+
Protein Test (Biuret Test)	Guide for ASU drugs, CCRAS, 2010	+
Tannin (Ferric chloride Test)	Guide for ASU drugs, CCRAS, 2010	-
Phenolic Compound Test	Guide for ASU drugs, CCRAS, 2010	+
Alkaloids Test (Mayer Reagent)	Guide for ASU drugs, CCRAS, 2010	-
Starch (Iodine Test)	Guide for ASU drugs, CCRAS, 2010	-
Flavonoid (Shinoda Test)	Guide for ASU drugs, CCRAS, 2010	+
Steroid (Salkowaski Test)	Guide for ASU drugs, CCRAS, 2010	+
Terpenoids Test	Guide for ASU drugs, CCRAS, 2010	+
Saponins (Foam Test)	Guide for ASU drugs, CCRAS, 2010	-

Raw olive fruits taste bitter and are not edible without debittering. Common debittering process promotes the loss of olive antioxidant activity and the magnitude of loss depends on the debittering reagent used (Sueishi and Nii, 2020) [23]. Table 4 indicates the Antioxidant activity and total flavonoid content of the olive fruit where, the methanol extract of olive fruit showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. The absorbance at 517 nm by UV visible spectrophotometer were found to be as 0.14 and 0.0989 for standard ascorbic acid and the Free radical scavenging assay (IC50) for Std. Gallic Acid obtained was as 0.0021 mg/ml. The total flavonoids (Equivalent to Quercitin content was estimated at 90.77 mg/100 g.

Table 4: Antioxidant Activity of olive fruit

Antioxidant Activity (DPPH Assay)	Method of Testing	Test results	Unit
Free radical Scavenging Assay (IC50)- For Methanolic Extract	American Journal of Ethnomedicene, 2012, vol.1, No.4, 244-249	0.14	Mg/ml
Free radical Scavenging Assay (IC50)- For Std. Gallic Acid		0.0021	Mg/ml
Total Flavonoids (Equivalent to Quercrtin content)	Pharmacogn Mag. 2015 Jan – Mar; 11(41:96-101)	90.77	Mg/100 g

Four different types of toffees prepared using olive fruit powder along with the control. Samples were found to be "liked slightly" to "liked very much" in the terms of sensory attributes. The overall acceptability scores of sample I (toffee with olive paste), sample II (toffee with olive powder), sample III (toffee with choco) and sample IV (toffee with walnut) recorded to be 8.84, 8.7, 09.0 and 8.8 respectively. Control sample prepared with choco observed highest (9.0) for overall acceptability. However, the difference in the score found to be significant at 1% level of significant.

Table 5: Organoleptic acceptability of Toffees based on olive fruit

Toffee samples	Values[1] of sensory characteristics on 9 point hedonic rating scale (mean±SD)[2]					
	Colour	Appearance	Flavor	Texture	Taste	Overall Acceptability
T with olive paste	8.80±0.8	8.80±0.35	8.82±0.20	8.80±0.35	9.00±0.00	8.84±0.35
T with olive powder	8.65±0.33	8.65±0.33	8.80±0.26	8.65±0.25	8.80±0.35	8.70±0.24
T with choco	9.00±0.00	8.80±0.26	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00
T with walnut	8.80±0.26	8.65±0.28	8.85±0.26	8.82±0.24	8.80±0.26	8.80±0.26
SEm	0.140	0.145	0.099	0.119	0.099	0.126
F value[3]	2.360 *	1.096	1.714	3.202 **	2.204 *	0.661
CD	0.369	NS	NS	0.374	0.310	NS

[1]Toffees Values are mean (n=10) ±SD scores, [2]Values with different superscrits are significantly different (p<0.05), [3]p<0.05 with superscripts *significantly different at 95%. (One way ANOVA: Post hoc method (tuckey's)

4. Conclusions and future perspective

Olea europaea fruit and oil consumption has been recognized as a key factor supporting the beneficial effects of the "Mediterranean diet" owing to the well-balanced oil composition (highly enriched in monounsaturated fatty acid) and rich minor components (such as polyphenols and phytosterols) in the fruits. Ripe olives contain high levels of bitter phenolic compounds that make the fruit inedible so the fruit must undergo some form of processing, fermentation, or curing to reduce the concentration of these bitter phenolic compounds. From the obtained results, it was concluded that the methanolic extracts of olive fruit (*Olea europaea* L) have appreciable antioxidant capacity and antioxidant-associated phytochemicals. The nutritional content of the fruit makes it unique among oil plants that can be successfully utilized as a potential source for many medicinal and therapeutic activities. Further, toxicity studies on the extracts of olive fruit (*Olea europaea* L) should be performed to determine their safety.

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6. Conflicts of Interest

The authors declare that they have no conflicts of interest.

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