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Study on antioxidant activity and composition of *Olea cuspidata* seed pulp

Muhammad Umar Hayyat[1], Mariyam Mahmood[1], Amin Ul Haq Khan[1], Zafar Siddiq[2], Rashid Mahmood[1] and Farooq Ahmad[1*]

[1]Sustainable Development Study Centre GC University Lahore, Pakistan
[2]Department of Botany, GC University Lahore, Pakistan
****Corresponding author***

KEYWORDS

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optional value

A B S T R A C T

Olea cuspidata is a climax species of scrub dry sub-tropical broad leaved evergreen forest. The antioxidant activity, elemental constituents and oil contents were analyzed in seed pulp of *Olea cuspidata*, these are not already reported in literature moreover this study will help to provide an option to conserve this species. The plant extracts were made in butanol, ethyl acetate, chloroform and hexane. The antioxidant potential was determined by DPPH assay method. The highest inhibition of DPPH solution was observed in ethyl acetate extract after 25 minutes of reaction. The antioxidant activity in decreasing order in different solvents was: ethyl acetate > butanol > chloroform > hexane. The elemental composition was determined by CHNS analyzer and spectro photometric method and the elemental composition in the sample was the following: carbon (55.62%), hydrogen (8.568%), nitrogen (0.94%) sulphur (0.548%) and the total phosphorus was 38 ppm. The oil content was determined by Soxhlet extractor, which was 9.19%.

Introduction

Pakistan is an arid and semi-arid country. The total forest area is 4.2 million hectares, which accounts for 4.2% of total land area. Due to excessive grazing and cutting of trees the rate of deforestation is increasing in the country[1]. One of the important types of forest is scrub dry sub-tropical broad leaved forest. These are present on hills of Himalayas, Sulaiman, Kalachitta and Salt Range. The common species of these forests are *Olea cuspidata* and *Acacia modesta are* the climax species[2]. *Olea cuspidata* belongs to the family Oleacea and it is also considered as sub-species of Olea europea[3].

Olea cuspidata has certain economic and social uses. It is used for the restoration of disturbed lands. It is mostly used as fodder. Its wood is hard and flexible. The color of sapwood is white and of hardwood is light brown – black. Its wood is used for fuel purposes, construction and watershed management[2]. It also has great ecological importance. Wild animals and birds feed on its fruits. Due to excessive grazing and wood cutting the number of these plants is rapidly decreasing in Salt Range of Punjab, Pakistan.

Antioxidants are the compounds, which suppress the oxidation process. Free radicals are the reactive species and they are constantly produced in body as the by-products of different chemical reactions in the body. They cause oxidation of basic cell components such as proteins, carbohydrates and nucleic acids and disturb various metabolic processes[4]. This oxidative stress damages the different tissues of the body. Currently there is an increasing demand of natural antioxidants because they are not dangerous to use as compared to non-natural antioxidants[5, 6] . Plants are the major source of natural antioxidants. Antioxidants are very important from the health point of view. They reduce the chances of heart diseases and tumors[7]. They delay the ageing process and also used for the treatment of neurodegenerative diseases[8]. They are also play a vital role in food industry. They enhance the life of certain food products because they reduce the oxidation process[9].
The basic purpose of this work was to study the chemical characteristics of this plant such as antioxidant activity, oil content and composition of different elements and determine their concentrations in seed pulp of *Olea cuspidate* as any study on it was not reported. These constituents have nutritional value for the wildlife who feeds on this plant species. This study will provide options to conserve this species due to its antioxidant activity and other chemical constituents, which will enhance its ecological value as wildlife feed on its fruits and will also enhance its importance in pharmaceutical and food industry.

Material and Methods

Sample collection

The fruits of *Olea cuspidata* were collected from Soan Valley in Punjab, Pakistan. After collection the seed pulp of fresh fruits was removed.

Determination of antioxidant activity

Antioxidant activity the sample extract was made with for different solvents. For that purpose 10 g of seed pulp sample of *Olea cuspidata* was dipped in 200 ml. methanol (100%) for 48 hours. The residue was again dipped in methanol to ensure maximum extraction. The methanol was evaporated from sample to get crude residue. The crude residue was suspended in 200 ml. distilled water. The aqueous solution was separated with n-hexane, ethyl acetate, chloroform and butanol. 25 ml of each reagent was used for each extraction. The organic layers were separated and stored at 4 ^{0}C till their analysis[10]. 3 ml of DPPH solution was taken in a test tube and 600 µl butanol extract was added in it. Similarly the samples were prepared for the other three extracts; ethyl acetate, chloroform and hexane and the sample absorbance was noted at 517 nm. For blank, DPPH solution was used. The readings were taken with the interval of 5 minutes. The inhibition of DPPH was calculated, by the following formula:% Inhibition of DPPH=DPPH $_{t=t}$ /DPPH $_{t=0}$ × 100

CHNS, Oil and Phosphorus

The concentration of C, H, N, and S in seed pulp of *Olea cuspidata* was determined by CHNS analyzer (Vario Micro). In the analyzer the helium gas was passed through the sample to remove atmospheric nitrogen and the catalytic combustion of sample was taken place at 1200 ^{0}C. The components of gas mixture were separated and detected by thermal conductivity detector. The C/H and C/N ratios were calculated from the concentrations of these elements[11]. Oil content was determined by Soxhlet Extractor and hexane was used as solvent[12]. The total phosphorus was determined by using spectrophotometer (SpectroScan80D).

Results and Discussion

Determination of antioxidant activity

The antioxidant activity of seed pulp of *Olea cuspidata* was determined by DPPH (2, 2-Diphenyl-1-picryl hydrazyl) radical scavenging activity. The antioxidant activity of the seed pulp sample in different solvents was calculated (Table 1). The % inhibition of DPPH in different solvents in decreasing order was:

Ethyl acetate > butanol > chloroform > hexane

The lower percent of inhibition shows the higher antioxidant activity e.g. if a material shows 14 % inhibition of DPPH solution after 20 or 25 minutes of reaction it means it reacts with the 86 % of DPPH and only 14 % DPPH remains unreacted. The seed pulp showed different antioxidant activity in different solvents, which showed that the solvent has effect on inhibition of DPPH (Figure 1). The solvent play a role in the reaction between concentration of antioxidants in a sample and % inhibition of DPPH. It affects the kinetics of reaction [13, 14, 15]. The antioxidant activity of a material is affected by the type and polarity of the solvent, which is used for extraction [16, 17].

The polarity index of Ethyl acetate is 4.4, chloroform is 4.1, butanol is 4 and of hexane is zero[18]. But their influence mechanism on antioxidant activity of a material is not found in literature. The antioxidant activity also depends upon those compounds, which show the antioxidant activity. These compounds are polar in nature and they inhibit the different % of DPPH in different solvent extracts[19]. Different types of plants show different type of antioxidant potential. The seed pulp of *Olea cuspidata* showed 85.60 % antioxidant activity in ethyl acetae extract, which is higher as compared to the antioxidant activity of different plants such as *Cassia occidentalis*, *Clitoria tematea* and *Anisomeles malabarica* (Table 2).

Elemental analysis

Elemental analysis is done to find out the percent composition of different elements in a compound. The elements which were found in the analysis were carbon, hydrogen, nitrogen and sulphur. Nitrogen and sulphure are macronutrients and are very important for the growth of plants. The presence of different elements in a plant material is not only useful for plants but also the source of essential elements for those who consume it. The higher percent of carbon and hydrogen indicates that plant is a source of carbohydrates and hydrocarbons. The higher percent of nitrogen and sulphur indicates that plant is also a source of proteins and vitamins, which are very important for health[20]. The results show that the necessary percent of elements is present in *Olea cuspidata*.

The composition of carbon is 55.62 % and of hydrogen is 8.568 % in seed pulp (Table 3) which is higher as compared to the necessary elemental composition which should not be less than 45 % and 6 % respectively[21]. The higher percent of carbon and hydrogen means the higher amount of carbohydrates, which provide energy to the consumers. So according to results, the seed pulp is a good source of carbohydrates. The values of nitrogen and sulphur are the 0.94 % and 0.548 % respectively, which are within limits as compare to the necessary elemental composition which should be 1% for nitrogen and <1 % for sulphur. The nitrogen is a structural component of proteins and sulphur is also present in proteins and vitamins.

Table.1 % inhibition of DPPH in different solvent extracts

Time (minutes)	% inhibition of DPPH in different solvents							
	Butanol		Ethyl acetate		Hexane		Chloroform	
	X	SD	X	SD	X	SD	X	SD
0	100		100		100		100	
5	80.4	±0.52	72.83	±0.76	84	±1.00	75.06	±0.97
10	67.93	±0.90	60.63	±0.55	69.23	±0.75	62.80	±0.72
15	54.80	±0.72	53.16	±1.04	63.83	±0.76	50.53	±0.50
20	44.46	±0.50	40.33	±0.76	54.10	±0.17	26.66	±1.52
25	19.70	±0.60	14.40	±0.52	24.96	±0.90	17.06	±1.00

X= Mean Value
SD= Standard deviation

Table.2 Antioxidant activity of different plants

Plants	Antioxidant activity	Source
Anisomeles malabarica	80.12 %	Sini 2010[27]
Clitoria tematea	82.87 %	Sini 2010[27]
Cassia occidentalis	84.23 %	Sini 2010[27]
Olea cuspidata	85.60 %	Mahmood 2012[28]
Capparis zeylanica	87.12 %	Sini 2010[27]
Plumbago zeylanica	88.45 %	Sini 2010[27]

Table.3 Oil content and elemental composition of seed pulp of *Olea cuspidate*

Elements	**Percentage composition**
Carbon	55.62
Hydrogen	8.568
Sulphur	0.548
Nitrogen	0.94
Oil contents	9.19 %
Total phosphorus	38 ppm

Elemental ratios

The organic materials are made up of carbohydrates, proteins and lipids. Carbon, hydrogen and nitrogen are the major components of these compounds. The ratios between different elements are very important parameters of an organic material. The carbon to nitrogen (6.5) and carbon to hydrogen (59.2) ratios of the seed pulp sample (Table 3). The high carbon to nitrogen ratio of an organic material shows it is carbonaceous and has less nitrogen content. If the content of nitrogen is higher in plants then carbon to nitrogen ratio becomes low. This low ratio enhances the vegetative growth[22]. The high carbon to nitrogen ratio also increases the strength of the cell wall[23]. The seed pulp has higher C/N ratio as compare to the straw and Alfalfa Hay which shows that it has carbonaceous nature and low nitrogen content. The carbon

to hydrogen ratio of an organic material shows that it is a source of hydrocarbons. Hydrocarbons are oily in nature[24]. The carbon to hydrogen ratio of seed pulp sample shows that it contains hydrocarbons.

Determination of Oil content

Olive oil is a rich source of fatty acids and α-tocopherol content, which are very important from nutritional point of view. They maintain the level of cholesterol in blood and also minimize the risk of breast cancer[25]. The 9.19% of oil content was extracted from seed pulp of *Olea cuspidate* (Table 3), which showed that it is a good source of oil. The color of the oil was greenish yellow and had characteristic smell like oil of *Olea europea.*

Determination of total phosphorus by spectrophotometric method

Phosphorus is an important element and is a macronutrient and it is necessary for the proper growth of plants. Phosphorus made upto 0.2 % of total dry weight of plant. It is a key component of nucleic acid, phospholipids and ATP in a cell. It controls the metabolic processes of a cell. It increases the growth of seeds and flowers. The total phosphorus in seed pulp of *Olea cuspidata* was 38 ppm (Table 3). For the proper growth of plants the concentration of phosphorus in soil should not be less than 20 ppm[26]. This amount of phosphorus present in the seed pulp of *Olea cuspidata* enhances the fertility of soil when seeds are decomposed.

Conclusions

In present study the seed pulp of *Olea cuspidata* showed the higher antioxidant activity as compared to the different plants such as *Cassia occidentalis, Clitoria tematea* and *Anisomeles malabarica.* It is a potential source of natural antioxidants. Along with antioxidant potential, the seed pulp is also the source of oil content. The study also reveals that *Olea cuspidata* has showed necessary composition of macro elements, which are carbon, hydrogen, nitrogen, phosphorus and sulphur. The antioxidant potential and other chemical constituents can enhance its ecological and pharmaceutical importance along with its other uses like wood and grazing purposes. The antioxidants can also be used in cosmetics industry after refining as they are antiaging.

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