Effect of phytochemical and nutritional analysis of Cordia myxa and Cordia dichotoma from Dera Ismail Khan, Pakistan

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Abstract

Wild edibles fruits are nutritionally known as well-balanced food and have made major contribution to people's diet. The present study was designed to analyze the proximate, mineral, antioxidant, phytochemical, organoleptic and morphological examination of the selected wild edible fruit plants (WEFPs) (*Cordia myxa* and *Cordia dichotoma*) of Dera Ismail Khan. The selected WEFPs were examined for minerals by atomic absorption spectrophotometry, antioxidants by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and proximate including crude fats by soxhlet assembly, proteins by Kjeldahl method and ash content by muffle incineration, respectively. The proximates were determined using standard AOAC method. The results of proximate composition revealed that carbohydrate contents of *C. dichotoma* were 88.35% and *C. myxa* were 53.79%. In addition, copper levels in *C. myxa* and *C. dichotoma* were determined to be 525.00 and 477.70 mg/kg of dry weight, respectively. Following that, we found significant free radical scavenging activity of *C. myxa*

(IC_{50} 46.75 μg/mL) and the highest total phenolic content was reported in *C. myxa* (33.41mg GAE/g) and *C. dichotoma* (19.59 mg GAE/g), while overall total flavonoids were 0.463 mg QE/g in both fruits. These underutilized food sources with high levels of macronutrients, trace elements and antioxidants as well as potential health benefits, increase their relevance in both traditional and modern diets.

Keywords: *Cordia myxa*, *Cordia dichotom*a, DPPH assay, Phytochemical analysis, Nutritional analysis

Introduction

In developed countries, the food crisis occurred has emerged as a result of poverty, crop prices, joblessness, natural tragedy, limited agricultural fields and increase in population. As a result, people use wild edibles plants that are a good source of food and nutrients for their life activities [1,2]. During the lack of food or the unfavorable condition of cultivated food plants, the intake of wild edible fruits is an important component for human health [3]. These wild fruits and vegetables are rich in diet and provide vital nutrition exclusively to human beings with fibers, carbohydrates, proteins, and vitamins [4]. The edible plants have significant nutritional values that include some of the essential minerals such as calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), iron (Fe) and sodium (Na) *etc*.,[5].

Cordia dichotoma and *Cordia myxa* belong to the Boraginaceae family, locally referred to as "lasori" and "lasora", which are polygamodioecious in nature. These species of *C. dichotoma* produce jelly-like mass called *C. dichotoma* gum [6]. Its leaves are widely used as fodder for animals, fuel, pickles, and glue formations [7]. Pharmacological investigation of leaves and bark has been identified as antitumor, antioxidant, anti-inflammatory and analgesic activities [8]. Its fruits are used in paralytic folk medicine with various actions such as immunomodulator, anti-diabetic, hepatoprotective, and astringent [9]. It is used to treat eczema, anxiety and hypoglycemic symptoms [10]. The physiological characteristics of *C.*

myxa has shown that it is blackish in color on the ripening stage and the fruit of *C. myxa* has long been valued for its sticky mucilaginous pulp throughout its distribution area [11]. Pharmacological studies of *C. myxa* have been shown that antimicrobial, anti-inflammatory, antiparasitic, immunomodulator, gastrointestinal, cardiovascular and breast cancer cells can be treated with copper oxide nanoparticles synthesized from *C. myxa* [12]. Fruits are also used for cough, chest complaints, urinary tract, sore throat, lung, spleen diseases, astringent, diuretic, anthelminthic, and demulcent agent. Fruit tissue is also useful as an emollient for mature abscesses, rheumatic pain and anthelminthic. Bark powder is used for the treatment of skin diseases [13]. The present study, we have investigated the morphological, organoleptic, proximate, mineral, antioxidant and phytochemical analysis of *C. dichotma* and *C. myxa*.

Materials and Methods

2.1. Sample Collection and Preparation

Wild edible fruit samples were obtained from various localities Dera Ismail Khan, of District KPK, Pakistan. The collected plant specimens were identified and compared with available literature at Herbarium of Quaid-i-Azam University, Islamabad, Pakistan. These specimens have been deposited as voucher specimens in the herbarium of Pakistan (ISL) Quaid-i-Azam University. The fruit samples were dried in oven as well as under shade. The moisture free fruit was ground with pestle, mortal and sieved to achieve a particle size of 6-8 mm. Powder samples were packed in air-bound polythene bags to prevent moisture absorption.

2.2. Morphological Study

The morphological features of fresh and preserved plant specimens were examined with the help of dissecting stereomicroscope [14]. The calculation of different parts of the plant was documented using the measuring scales and thread. Vegetative characteristics such as habit, leaf types (leaf shape, leaf apex, leaf margins, width and length of leaf), and reproductive structure (color, number of sepals, shape of petals, and types of inflorescence,

shape and length of fruit) were studied.

2.3. Organoleptic Assessments

Organoleptic evaluations of edible fruits including shape, color variation at the ripened and un-ripened stage, size, taste, length, width, and volume were conducted.

2.4. Nutritional evaluation

2.4.1. *Determination* of *Moisture Content*

Moisture contents of edible wild fruits were determined according to the standard procedure of [15] (AOAC 2000) with method No. 44-15A. The pre-weighed (4g) fresh fruit sample was heated in oven (Memmert, USA) at 70°C for 36 h until a constant weight of dry matter was obtained. The moisture content of fruit material was measured after drying.

$$\text{Percentage of moisture} = \frac{\text{Weight of fresh fruit} - \text{Weight of dry matter}}{\text{Weight of fresh fruit}} \times 100$$

2.4.2. *Determination of Ash Content*

Ash content of wild edible fruits was determined by incineration in muffle furnace according to the method [16]. Two grams of the dried fruit samples were weighed into the crucible and put in the muffle furnace model (Neycraft JFF 2000). Temperature of the muffle furnace was increased to 550°C before the white/gray ash was obtained. It was cooled at room temperature and measure in the desiccators. The percentage of ash content was calculated by the following formula:

$$\text{Percentage of ash content} = \frac{(\text{Weight of ash} + \text{crucible}) - \text{Weight of crucible}}{\text{Weight of fresh sample}} \times 100$$

2.4.3. *Determination of Crude Fats*

Crude fat contents of edible wild fruits were determined by the solvent extraction method as followed by [15]. Three to five grams of sample powder in thimble was placed into extraction chamber. Petroleum ether was used for extraction of crude lipid in Soxhlet extractor (model) for 4-6 hours at 60°C. The difference between the weight of thimble and

thimble contain final resulting material was expressed as percentage of crude fat content.

$$\text{Percentage of crude fat} = \frac{\text{Wt. of sample before extraction} - \text{Wt. of sample after extraction}}{\text{Wt. of sample used}} \times 100$$

2.4.4. *Determination of Crude Protein*

The crude protein content of wild edible fruits was determined according to previous method [17]. One gram of dried sample was digested with H_2SO_4 (98%) and digestion mixture ($FeSO_4$, $CuSO_4$, H_2SO_4, 1:2:10) at 360°C for 90 min. The digested solution was diluted up to 250 mL with distilled water. Approximately 10 mL of digested solution was distilled through a semi-digital distillation assembly. The ammonia released during this reaction was trapped in 4% boric acid solution. Finally, this solution containing ammonia was titrated against 0.1N Sulphuric acid (H_2SO_4), until the purple color appeared. Blank was carried out using methanol instead of sample. The crude protein percentage was calculated by the following formula:

$$\text{Percentage of crude protein} = \%\ \text{Nitrogen} \times 6.25$$

2.5. Mineral analysis

The quantity of macro and micro minerals was determined by atomic absorption spectrophotometry (Shimadzu AA-670). Samples were prepared by the acid of metal contents in wild edible fruits was done by the modified producer [18]. A cumulative amount of (15 mL) mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) was added with 2:1 in 1 g of edible fruit sample and solution was left overnight. It was heated for 10-15 minutes before the brown fumes of solutions were converted to white fumes. After cooling, this solution was filtered by Whatman filter paper no. 42 and diluted with double distilled water by raising the volume to 100 mL. It was transferred to the plastic bottles and kept at room temperature for metals detection. Detected metals included magnesium (Mg), iron (Fe), sodium (Na),

calcium(Ca), potassium (K), manganese (Mn), zinc (Zn), cobalt (Co), copper (Cu), chromium (Cr), strontium (Sr) and lead (Pb) by using the (AAS) atomic absorption spectrophotometer [19].The concentration of metal contents (mg/L) was converted into (mg/100 gm) by using the following formula:

$$\text{Mineral Concentration} = \text{Concentration of sample in mg} \times \text{Volume of sample}$$

2.6. Antioxidant activity

The antioxidant activity was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) DPPH free radical scavenging assay with some modifications [19]. A 20 µL of extract solution in DMSO was mixed with 180 µL of DPPH in methanol in 96-well plate. The plate was incubated in dark for 1 h, after which the absorbance of the solution was measured at 517 nm wavelength on microplate reader. Mixture of 180 µL of methanol and 20µL of DMSO was used as blank, while 20 µL of DMSO and 180µL of DPPH solution as positive control. Standard curve was prepared by ascorbic acid in methanol at various concentrations (12.33-1000 µg/mL) in serial diluation. Percentage inhibition was measured according to the following formula and IC_{50} value was calculated by table curve method.

$$\text{Scavenging activity (\%)} = \left(\frac{1 - \text{Absorbance of sample}}{\text{Absorbance of Control}}\right) \times 100$$

2.7. Phytochemical analysis

Total flavonoid content was determined by the aluminum chloride ($AlCl_3$) colorimetric method using quercetin as a standard [19]. The 20 µL of extracts was mixed with 10 µL of 1M Potassium acetate and 10 µL of 10% aluminum chloride then added 160 µL of distilled water in the 96-well plate. After mixing the solution plate was incubated for 30 min in a dark. Negative control was prepared using MeOH instead of extract. A calibration curve was prepared using 0.24-20 µg/mL concentration of Quercetin. The absorbance was measured at 450 nm using a micro plat reader.

Total phenolic content was determined by the Folin-Ciocalteu reagent method in 96 well-plate [20]. The 20 µL of extracts or standard solution was mixed with 90 µL of Folin-Ciocalteu reagent freshly diluted 1:10 with distilled water. After 5 minutes, solution was mixed with 90 µL of 6% sodium carbonate solution in each well and plate was incubated for 30 min in dark. A calibration curve of gallic acid (ranging from 0.24 to 20 µg/mL) was prepared and gallic acid was used as standard. Absorbance of all samples was measured at 630 nm using a microplate reader. Total phenolic content of wild edible fruit extracts was expressed as mg gallic acid equivalent (GAE)/g of dried extract.

3. RESULTS

3.1. Morphological Observation of Plants

C. myxa is a tall tree up to 2.5-6 m and the leaves are almost ovate to elliptical tri-nerved. The leaf margins are somewhat dentate to sinuate, lengths of the leaves range from 7.0-8.5 cm, width is 9.2-10.3 cm, the base of leaf is almost rounded and petiole is 2.3-4.6 cm long. Fruit is drupe with size of 20 mm, ovoid, apiculate, brownish-yellow, with base partially bounded by the distended, broadly cupular calyx (Fig. 1). *C. dichotoma* is up to 13.4-15.6 m in length, leaf is obovate with undulate margins and base is somewhat circular to oblique. The length of leaf is 5.6-5.9 cm, width 3.43.7 cm and leaf petiolate 2.3-3.6 cm. The inflorescence is cymes, ebracteate. In male flowers, sepals are lobed with campanulate petals, which are hairy from the inner side. Style is cleft and flower's color is yellowish red (Figure 1).

Figure.1. Morphology of *C. myxa* and *C. dichotoma*.

3.2. Organoleptic

The length of *C. myxa* is 1.6-2.2cm, width is 0.8-1.8cm and volume 2.2-3cm. Fruits are green in color during unripening stage and color became yellowish in ripening stage, sweet taste, odorless and circular at the

base. *C. dichotoma* fruits are small, green in color during unripening stage and color became yellowish in ripening stage, sweet taste, odorless and circular at the base. Fruit is 1-1.8 cm long, width 0.5-0.8 cm, volume 1-2.2 cm, color yellow, odorless, sweet and pointed at the base.

3.3. Proximate Analysis

The highest value of moisture contents noted was (2.7%), ash (5.89 %), fats (2.81%), fiber (23.43%), proteins (11.31%) was exhibited by *C. myxa* fruit, while prominent amount of carbohydrates (88.35%) and calorific value (382.51 kcal/g) were recorded *C. dichotoma* (Table 1).

Table 1. Proximate composition of *C. myxa and C. dichotoma*

Proximates	***C. myxa***	***C. dichotoma***
Moisture	2.7%	1.4%
Ash	5.89%	3.76%
Crude fats	2.81%	2.23%
Fiber	23.43%	20%
Crude Proteins	11.31%	2.26%
Carbohydrates	53.79%	88.35%
Calorific value	285.69 kcal/g	382.51 kcal/g

3.4. Mineral analysis

The high amount of Cu (538.00±10.55 mg/Kg), Cd (125.33±4.03 mg/Kg), and (Zn (532.33±5.47 mg/Kg) was observed in *C. myxa* compared to *C. dichatom.* The overall metal concentration in *C. myxa* is in the following order Cu>Zn>Cd>Cr>Mg>K>Na>Pb>Fe>Ni>K>Co (Table 2). However, elevated levels of Cr (146.15±4.89 mg/Kg), Fe (5.52±1.81 mg/Kg), and Ni (2.27±0.96 mg/Kg) were found in *C. dichatom* compare to *C. myxa*. The overall ranking of metals concentration is as follow Cd>Cu>Zn>Cr>Na>Mg>Fe>K>Pb>Ni>Co>Mn (Table 2).

Table 2. Metals concentration in selected edible fruits

Metals	***C. myxa***	***C. dichatoma***
Cu	538.00±10.55	481.67±3.72
Cd	125.33±4.03	489.73±7.01
Zn	532.33±5.47	359.23±12.85
Mg	34.85±4.04	9.97±1.91
Fe	2.45±1.01	5.52±1.81
Cr	101.47±24.11	146.15±4.89
Na	10.98±2.14	6.60±1.26
Ni	0.56±0.53	2.27±0.96
Mn	0.30±0.16	0.07±0.02
K	22.93±1.34	5.31±1.48
Co	0.15±0.09	0.12±0.07

Pb	10.37±0.90	4.63±2.28

3.5 Free Radical Scavenging Activity

DPPH assay was used to investigate the free radical scavenging activity of *C. myxa* and *C. dichatoma.* Our results showed that there was a significant inhibition of 80.22% by *C. myxa* with an IC_{50} value of 46.75 µg/mL. However, *C. dichatoma* also exhibited 64.55% inhibition and IC_{50} value 209.2 µg/mL in dose-dependent manner (Table 3).

Table 3. Percentage inhibition and IC_{50} values of selected fruits

Fruits	Concentration (µg/mL)	Percentage Inhibition	IC_{50} µg/mL	R^2
C. myxa	1000.00	80.22	46.75	0.9682
	333.33	77.33		
	111.00	66.0		
	37.00	48.22		
	12.33	27.33		
C. dichatoma	1000.00	64.55	209.2	0.9315
	333.33	55.22		
	111.00	46.77		
	37.00	35.11		
	12.33	12.66		
Ascorbic Acids	1000.00	95.62	6.29	0.9864
	333.33	88.63		
	111.00	85.32		
	37.00	75.63		
	12.33	58.42		

3.6. Phytochemical Analysis

In this study, we have found the highest total phenolic content in *C. myxa* compared to *C. dichatoma* with standard curve of gallic acid (R2= 0.9861). Result of total phenolic content in *C. myxa* was recorded as 33.41 mg GAE/g, while *C. dichatoma* fruit contain 19.59 mg GAE/g (Table 4). Next, we evaluated total flavonoid content with the calibration curve of Quercetin (R2= 0.9953). Our findings indicated that *C. myxa* and *C. dichatoma* fruits contain flavonoid contents 0.463 mg QE/g (Table 4).

Table 4. Total Phenolic and flavonoid contents *C. myxa* and *C. dichatoma*

Fruits	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
C. myxa	33.41	0.463
C. dichatoma	19.59	0.463

4. DISCUSSION

The Arecaceae family has 1914 genera and 2,500 species which are distributed worldwide, particularly in Central America, South America and Southeast Asia. *C. myxa* and

C. dichotoma belongs to the family Boraginaceae which has 100 genera and 2000 species that exist in temperate, especially Mediterranean, and tropical regions. In Pakistan the family is represented by 32 genera and 135 species [21]. Medicinally the *C. myxa* is used as an expectorant and effective in treating the diseases of the lungs. In raw form, it contains gum which can be used beneficially in gonorrhea. while, *C. dichotoma* also has a lot of medicinal value as the leaf decoction with traditional salt used to cure cold and cough; it is also used in ulcers and headaches, although the entire plant is useful in snake bites [22].

WEFPs are very important to overcome nutritional and revenue insecurity. To achieve these goals, it should be focused on the appropriate species of WEPF. Plants are very rich sources of proteins, carbohydrates and their importance to human health are well known. These wild edible plants fruit are good source of physiochemical which are very beneficial for human health [23]. In the present study edible wild fruits were analyzed for their proximate composition in order to assess their nutritional potential. The recorded data revealed that the carbohydrates in *C. dichatoma* (88.35%) were followed by *C. myxa* (53.79%). Similar values of carbohydrates have been reported by [24,25,26]. The proteins are very affective for human health. The percentage of the crude proteins presented in *C. dichatoma* (2.26%) and *C. dichatoma* (20%). These values were comparable with previous reports for *cordial* [24, 27]. Similarly, maximum crude fiber content was found in *C. myxa* (23.43%). Study reported that 25.7 % fiber in *C. myxa* is comparable to the present finding [25]. Maximum amount of extracted crude fats were found in the fruit of *C. dichatoma* (2.2%). Previous studies reported the association values of fats for *C. dichatoma* [28,29]. The ash content of *C. dichatoma* and *C. myxa* are also in agreement with the values reported previously. The calorific value of *C. myxa* was highest which indicated a good source of dietary caloric due to high lipid contents. The energy values recorded for *C. dichatoma* were 382.51 kcal/100g. Thus, the studied edibles are promising source of energy for indigenous

communities.

The present study depicts higher metals concentration in *C. dichatoma* as compared to the *C. myxa*. Valavi and co-workers found a high amount of metals such as K, Ca, Mg, Na, Fe, Zn, Cu, and Mn [30]. Our results indicated a higher amount of Na, Ca, Fe, K and Zn in the *C. myxa* fruits which are compared with the reported amounts. Wild fruits are considered to be a good source of mineral nutrients such as vitamins, carbohydrates and proteins *etc.*, and a possible supplement of food in the areas which facing problem of food crisis [31]. Different metals ions play a key role in the determination of nutritional value of fruits such as potassium, calcium, and magnesium. Among the calcium play a vital role in the storage quality of fruits [31]. The significance of mineral nutrients to human body fitness is well known. Balance amount of such nutrients in human diet is good for healthy body [33]. In plants the percentage of these metal ions mainly depend on the availability of the ions in the soil fertility [34]. The fruit of *C. myxa* is the good source of Na and K. Calcium helps in bone formation and blood coagulation and sodium is the main inorganic cation of extracellular tissue fluids, potassium functions principally as the cation of the cell and also in nerve and muscle excitability. Iron is important as a constituent of haem, an essential part of haemochromagens important in respiration [35, 36]. However, all the essential metals except non-essential metals play a vital role in plants and humans. They are required in normal quantities for functioning of vicious body activities. The non-essential metals *viz.* Pb, Cd, Cr and Ni are toxic to plant even at low concentration and destabilize various functions of the plants. Consumption of such contaminated plant with these toxic metals will cause health risks to humans. The percentage inhibition of methanolic fruit extract of *C. dichotoma* was 64.55 % and IC_{50} value 4.183 µg/mL. In previous study of DPPH inhibition potential of *C. dichotoma* fruit showed 19.46% and IC_{50} value as 57.22 µg/mL. Thus, a higher value of inhibition in the present study was probably due to climatic changes and variation in

collection of fruits time. The quantitative phytochemicals like flavonoids (0.463 mg/g) and phenolics (19.59 mg/g) of *C. dichotoma* fruit were deliberately investigated. In addition, flavonoid and phenolic contents in *C. myxa* fruit were 0.463 mg/g and 33.42 mg/g respectively. According to Afzal *et al*., DPPH scavenging activity of *C. myxa* was 80.22 % with IC_{50} 0.934µg/g. The phenolics and percent inhibition values were 373.91 mg/199g and 2.05 % for the fruits *C. myxa*. Literature review on flavonoids of *C. myxa* was not found. Therefore, phenolic content of *C. myxa* is closely in agreement with previous reports [38].

5. CONCLUSION

The present study was carried out to evaluate the mineral composition, antioxidant, phytochemical, organoleptic and morphological analysis of the selected wild edible fruit plants. The results of present study indicated that *C. myxa* fruit has the highest energy value compared to *C. dichotoma* fruit. Copper was found to be the leading nutrient in the mineral composition of twelve selected metals. The study has shown that *C. myxa* has the highest concentration of antioxidant and phytochemical contents. It is concluded that the fruits of Cordia species are a rich source of antioxidant activity and phytochemicals for biological activities. Therefore, the presence of these compounds is biologically important and contributes to their nutritive value and can be potential sources of useful foods.

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Conflicts of Interests

Authors declare no competing financial interests.

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