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ABSTRACT

Cordia obliqua is an underutilized forest tree having food, medicinal, and industrial applications. Its flower buds are in great demand and are consumed as a raw vegetable in different states of India, including Odisha. It is also used in traditional medicine to treat various diseases. However, there is a huge gap between conventional use (food and medicine) and scientific evidence. The elemental analysis (S, K, Mg, P, Mn, Ca, Fe, Zn, Ni, and Cu) of leaves and flower buds was performed by inductively coupled plasma optical emission spectrophotometry. The elements found in significant quantities are K (18740 mg/Kg), P (1530 mg/Kg), Fe (71.9 mg/Kg), and Cu (22.5 mg/Kg) in flower buds. In contrast, the major elements found in leaf samples are S (2280 mg/Kg), Mg (1400 mg/Kg), Mn (1700 mg/Kg), Ca (957 mg/Kg), Zn (58 mg/Kg), and Ni (23 mg/Kg). Nutritional analysis of flower buds revealed the presence of carbohydrates (38.7 mg/dry weight), proteins (89 mg/g dry weight), fiber (127 mg/g in dry weight), and Vitamin C (2.5 mg/g dry weight). In contrast, the leaves consist of carbohydrates (26.9 mg/g dry weight), proteins (30.4 mg/g dry weight), fiber (127 mg/g in dry weight), and Vitamin C (5.0 mg/g dry weight). The flower buds also have high antioxidant activity. Sufficient nutritional value was obtained from both the leaves and flower buds of *C. obliqua*, which could fulfill the required amount of diet supplements as per the suggestion of Nutrition Requirements and Recommended Dietary Allowance.

1. INTRODUCTION

Cordia obliqua Willd. is a medium-sized deciduous forest tree belonging to the family *Boraginaceae*, commonly known as Clammy cherry (in English), Lasora or Lessora (in Hindi), and Bahal (in Odia) [5,10]. *C. obliqua* is native to the mid-Himalayan region and distributed in different states of India, including Odisha [6]. Its plant parts have been used in the Indian traditional system of medicines, such as Ayurveda, Siddha, Unani, and Folk, for treating various diseases [27,10]. The bark, root, fruits, and seeds are used to cure dental disorders, including toothache [8]. The fruits are used to treat cough, chest pain, chronic fever, joint pain, and spleen disease [1,6,12]. Leaf decoction mixed with common salt is taken orally twice a day for 1 week to cure flu, cough, and cold fever [5]. In addition, this plant has several pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, analgesic,

antimalarial, hepatoprotective, hypotensive, respiratory stimulant, and diuretic [1,10,30]. These activities are due to the presence of various bioactive compounds, such as hesperetin-7-rhamnoside, hesperetin, lutea-20, 29-ene-3- α -D-maltoside, toxifolin-3, lutea-20 (29)-ene-3-O- α -L-rhamnopyranoside, and allantoin- β -sitosterol [4,30].

The raw fruits of this plant are used as vegetables and preparation of pickles [1,10]. Its flower buds are generally used as a raw vegetable in West Odisha, India. The fruits of this plant contain various nutritional components such as carbohydrates, protein, pectin, iron, magnesium, potassium, and phosphorus [1]. The mucilaginous substance of the ripened fruit is used as gum in the paper industry for pasting paper sheets and cardboard. In the pharmaceutical sector, mucilaginous substances from raw and ripened fruit are used as release matrix forming material for tablet formulation [1,19]. In summary, this plant has food, medicinal, pharmaceutical, and industrial applications. However, there are huge gaps between traditional uses of this plant as food, ethnomedicine, and its scientific research to validate the benefits or properties of this important plant. There is also no report on nutritional profiling and phytochemical analysis of leaves and flower buds of *C. obliqua*. Therefore, the present study was designed to profile different trace elements (such as sulfur, potassium, magnesium,

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phosphorus, manganese, calcium, iron, zinc, nickel, and copper), nutritional components (such as total carbohydrate, crude fiber, total protein, total free amino acid, ash, and Vitamin C), estimation of phytochemicals (total flavonoid, phenol, and tannin content), and antioxidant activity by 2-diphenyl-1-picrylhydrazyl (DPPH) assay and FRAP assay of both flower buds and leaf samples of *C. obliqua*. This study may help to explore the nutritional value of this forest plant.

2. MATERIALS AND METHODS

2.1. Collection and Processing of the Samples

The leaves and flower buds of *C. obliqua* were collected from Sambalpur University campus, Burla, Sambalpur, Odisha, India, 2019. The plant species was identified and authenticated by Dr. Pratap Chandra Panda, Principal Scientist, Plant Taxonomy and Conservation Division, Regional Plant Resource Center (RPRC), Bhubaneswar, Odisha, and the voucher specimen was deposited in the Herbarium of RPRC, Bhubaneswar, Odisha. The samples were washed with tap water thoroughly, rinsed with distilled water, and dried under shade at room temperature to get a constant weight of the sample. Then, dried samples were ground to make powder and kept in an airtight container for future use.

2.2. Elemental Analysis

2.2.1. Sample preparation

About 0.2 g each of the leaves and flower buds powder samples of *C. obliqua* were taken for digestion in an advanced microwave digestion system (Milestone Ethos Easy, Italy). Microwave digestion was carried out with a mixture of concentrated acid (6 ml nitric acid and 2 ml hydrogen peroxide). The microwave-digested sample solution was collected by rinsing with double-distilled water in the digestion vessels and filtered using a glass filter. The final volume of the filtrate was made to 100 ml and used for elemental analysis.

2.2.2. Element analysis by inductively coupled plasma optical emission spectrophotometry (ICP-OES)

The concentration of trace elements such as sulfur (S), potassium (K), magnesium (Mg), phosphorus (P), manganese (Mn), calcium (Ca), iron (Fe), zinc (Zn), nickel (Ni), and copper (Cu) of both flower buds and leaf samples of *C. obliqua* was analyzed by ICP-OES (PerkinElmer Avio™, USA) at Central Instrumentation Facility, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha. This instrument was equipped with Syngistix™ software to measure the elemental concentrations. About 1 ml of each sample was loaded separately to estimate the concentration of elements. The instrument conditions were set as gas flow 8 l/min, auxiliary gas flow 0.7 l/min, RF power 1500 watts, and replicates-3 peristaltic pump flow rate 1.3 ml/min.

2.3. Nutritional Analysis

Nutritional contents such as total carbohydrate, crude fiber, total protein, total free amino acid, ash, and Vitamin C of both the flower buds and leaves powder of *C. obliqua* were estimated as per the standard procedure described by Sadasivam and Manickam (1992) [22] with minor modification. Nutritional analysis was carried out using spectrophotometry method (Shimadzu, Mumbai, India). The data were expressed in mg/g dry weight.

2.4. Phytochemical Analysis

2.4.1. Preparation of extracts

Ten grams of flower buds and leaves powder of *C. obliqua* were taken in 200 ml of methanol and solvent extraction was performed

by a hydrodistillation method using Soxhlet apparatus (Borosil, India) for 24 h. The extract solutions were filtered through Whatman No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. The concentrated filtered samples were dried in a hot air oven at 40°C until a constant weight was achieved and stored in a refrigerator for phytochemical and antioxidant analysis.

2.4.2. Estimation of total flavonoid, phenol and tannin content

For the estimation of polyphenols in flower buds and leaf samples, 2 mg of dried methanol extract was taken separately and dissolved in 2 ml methanol. The methanol extract was analyzed for total flavonoids and phenol content by the method described by Panigrahy *et al.* (2017) [18]. Total tannin content was estimated by the protocol described by Behera *et al.* (2018) [2] with slight modification. The flavonoid content was expressed as mg/g dry weight of the flower buds and leaf samples. Similarly, the total phenol contents and total tannin contents were estimated based on the gallic acid equivalent standard curve and tannic acid equivalent, respectively. The data were expressed in mg standard equivalent weight/g of the dry weight of flower buds and leaf samples.

2.5. Antioxidant Activity

2.5.1. DPPH free radical scavenging assay

Antioxidant activity of methanolic extracts of flower buds and leaf samples of *C. obliqua* was estimated by scavenging DPPH free radical following the procedure reported by Panigrahy *et al.* (2017) [18]. Briefly, a solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of methanol extract containing 0.02–0.1 mg/ml of the sample. The reaction mixture was incubated in the dark condition at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using BHT as a reference. The following equation calculated the ability to scavenge DPPH radical.

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / (\text{Abs}_{\text{control}})] \times 100}$$

Where, $\text{Abs}_{\text{control}}$ is the absorbance of DPPH free radical + methanol and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical + sample extract or standard.

2.5.2. Ferric reducing antioxidant power (FRAP) assay

Like DPPH assay, the ferric reducing ability of plasma (FRAP) assay was conducted using the method described by Wong *et al.* (2014) [29]. To the 200 µl of the extract, 3.0 ml of FRAP reagent was mixed and incubated in a water bath at 37°C for 30 min. The increase in absorbance was measured at 593 nm. The percentage of antioxidant activity was calculated using the following formula.

$$\text{Antioxidant activity (\%)} = \frac{[(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}) / (\text{Abs}_{\text{sample}})] \times 100}$$

2.6. Data Analysis

All the experiments were repeated 3 times. Data were represented in mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Element Analysis

The presence of elements in food sources is essential for growth, body maintenance, enzymatic reaction, and hormonal activities [3]. *C. obliqua* flower buds and leaves are commonly used as raw vegetables. However, there is a lack of study on their elemental analysis. We have performed

the elemental analysis (S, K, Mg, P, Mn, Ca, Fe, Zn, Ni, and Cu) of leaves and flower buds by ICP-OES. The major elements present are S, K, Mg, P, Mn, and Ca in both leaves and flower buds [Table 1]. The elements found in significant quantities are K (18740 mg/Kg), P (1530 mg/Kg), Fe (71.9 mg/Kg), and Cu (22.5 mg/Kg) in flower buds compared to leaf samples. In contrast, the major elements found in leaf samples are S (2280 mg/Kg), Mg (1400 mg/Kg), Mn (1700 mg/Kg), Ca (957 mg/Kg), Zn (58 mg/Kg), and Ni (23 mg/Kg) compared to flower buds [Table 1]. Previously, several elements such as iron, magnesium, potassium, and phosphorus were detected in *C. obliqua* fruits [1]. As per the Indian Council of Medical Research (ICMR) suggestion, about 3750–3225 mg of K, 340–310 mg of Mg, 17–21 mg of Fe, and 12–10 mg of Zn are essential per day for Indian men and women, respectively, while 600 mg of P, 4 mg of Mn, 600 mg of Ca, and 1.7 mg of Cu are required for both Indian men and women. More nutrient elements are needed for pregnant or lactating women than others [Table 1]. All the above elements are essential in the human body's growth and development [20]. Sulfur is an essential element required for the synthesis of cysteine, methionine, and glutathione. Glutathione is a potent antioxidant that protects the cell from free radicals and protects the body from allergies, osteoarthritis, and muscle soreness [15]. Potassium is accumulated inside the cell through Na⁺/K⁺ pump, which helps in the regulation of insulin secretion, muscle contraction, and activation of the enzyme [23]. Decreasing the level of K in the blood showed several symptoms such as vomiting, muscle weakness, cardiac arrhythmia, and a decrease in reflex response [25]. Phosphorus plays a critical role to keep bones and teeth healthy [17]. Manganese is mainly required for the human body to regulate sugar levels in the blood, metabolism of carbohydrates and fat, helps in calcium absorption, and bone formation, etc. [20]. Calcium plays a crucial role in cell signaling, cell growth, blood coagulation, heartbeat, muscle contraction, and different enzyme activity [11]. Iron is also essential to hemoglobin and myoglobin; it helps transport oxygen, acts as a cofactor for some enzymes, and maintains the body's immune system [14,20,25]. Zinc is required to smoothly carry out specific biological and physiological processes of the human body, including immune function, cell proliferation, and free radical scavenging activities [9]. Nickel is an essential trace element that helps in iron absorption, glucose, and adrenaline metabolism [23]. Copper helps

in ferrous ion oxidation, neurotransmitter synthesis, skin and hair pigmentation, and connective tissue stabilization [16].

3.2. Nutritional Analysis

Nutrition such as carbohydrates, protein, amino acid, fiber, ash, and Vitamin C is most important for the growth and development of an organism [7]. Due to the deficiency of these nutrients, different types of diseases occur. Carbohydrates' primary role is to provide energy to the body for growth and function. About 38.7 and 26.9 mg/g of carbohydrate were found in flower buds and leaves of *C. obliqua*, respectively. Proteins are the primary food supplements required by the body and should be in an adequate quantity in the diet. Dietary proteins are essential for the body to develop antibodies, enzymes, hormones, and tissue repair [13]. In this study, the protein content of 89 and 30.4 mg/g dry weight was found in *C. obliqua* flower bud and leaf, respectively [Table 2]. According to the ICMR, about 60 g and 55 g of proteins are required daily for Indian men and women. Approximately 78 g of protein is needed per day for pregnant women. Therefore, *C. obliqua* flower bud could fulfill the daily required amount of protein for men, women, as well as pregnant women. Similarly, the fiber content of 114 and 127 mg/g in dry weight was found in flower buds and leaves, respectively. It is one of the most essential components in food, which helps in the proper function of the elementary canal and also helps in cleansing the digestive tract [21]. According to the ICMR, about 40 mg of Vitamin C is required for both Indian men and women. Whereas, this plant contains 2.5 mg/g and 5.0 mg/g of Vitamin C in flower buds and leaves, respectively [Table 2]. Ash is the inorganic residue of food after the destruction of organic matter by heating. Inorganic components in food represent minerals such as Na, K, Ca, Mg, Mn, P, Fe, Zn, and Cu. About 43 and 41 mg/g of ash contents were found from *C. obliqua* leaves and flower buds, respectively [Table 2].

3.3. Phytochemical Analysis

Secondary metabolites such as phenolics, flavonoids, and tannins are the principal constituents of medicinal plants responsible for pharmacological activity [28]. The ethnomedicinal study has reported several therapeutic and pharmacological values for *C. obliqua* [1]. The above phytochemicals were found to be in higher quantity in

Table 1: Elemental profiling of *C. obliqua* by ICP-OES technique.

Elements (mg/Kg)	Flower bud	Leaf	US-EPA RfD ¹ (mg Kg/bw/day)	FSSAI-DRA ² (mg/day)
S	1340±11.0	2280±9.7	Not assessed	Not assessed
K	18740±19.0	9695±13.3	Not assessed	For men – 3750 mg; for women – 3225 mg
Mg	1254±7.5	1400±9.2	Not assessed	For men – 340 mg; for women – 310 mg
P	1530±10.2	1450±8.7	0.00002	For men and women – 600 mg; for pregnant and lactating women – 1200 mg
Mn	1650±7.6	1700±11.2	0.14	For both men and women – 4 mg
Ca	569±4.3	957±4.0	Not assessed	For men and women – 600 mg; for pregnant or lactating (0–12 months) women – 1200 mg
Fe	71.9±3.3	42.1±2.7	0.7	For men – 17 mg; for women – 21 mg; for pregnant women – 35 mg; for lactating women (0–12 months) – 21 mg
Zn	32±1.6	58±2.3	0.3	For men – 12 mg; for women – 10 mg; for pregnant/lactating women (0–12 months) – 12 mg
Ni	15±1.0	23±2.1	0.02	Not assessed
Cu	22.5±1.4	18.5±1.7	0.04	For both men and women – 1.7 mg

FSSAI: Food Safety and Standards Authority of India, RDA: Recommended Dietary Allowance, bw: Body weight, RfD: Reference dose, mg/Kg: Milligram per kilogram, mg/day: Milligram per day, ¹reference dose (RfD) provided by the US-Environmental Protection Agency (US-EPA), ²Indian Council of Medical Research (ICMR) (Nutrition Requirements and RDA for Indians – A report of the expert group of the ICMR, 2020).

Table 2: Nutritional analysis of *C. obliqua* bud and leaf sample.

Nutrition (mg/g in DW)	Flower bud	Leaf	FSSAI-DRA ² (g or mg/d)
Carbohydrate	38.7±1.2	26.9±1.8	Not assessed
Protein	89.0±3.9	30.4±1.7	For men – 60 g/d; for women – 55 g/d; for pregnant women – 78 g/d
Free amino acid	0.9±0.0	4.1±0.2	Not assessed
Crude fiber	114.0±1.6	127.0±2.3	Not assessed
Ash	41.0±0.5	43.0±1.4	Not assessed
Vitamin C	2.5±0.0	5.0±0.0	For men and women – 40 mg/d; for pregnant and lactating women – 60–80 mg/d

FSSAI: Food Safety and Standards Authority of India, RDA: Recommended Dietary Allowance, g/day: Gram per day, mg/day: Milligram per day, ²Indian Council of Medical Research (ICMR) (Nutrition Requirements and RDA for Indians – A report of the expert group of the ICMR, 2020).

Table 3: Phytochemical content and antioxidant activity of *C. obliqua* flower bud and leaf sample.

Phytochemicals	Flower bud	Leaf
Tannins (mg TAE/g DW)	65.7±2.3	98.3±2.0
Phenol (mg GAE/g DW)	50.7±1.7	149.0±2.5
Flavonoids (mg/g DW)	14.9±0.2	71.3±0.7
DPPH (%)	72.6±0.4	65.2±0.3
FRAP (mg TE/g DW)	40.6±1.3	40.8±0.5

Tannins, phenol, flavonoid, and FRAP expressed as mg tannic acid equivalent/100 g, mg gallic acid equivalent/100 g, mg quercetin equivalent/100 g, and mg Trolox equivalent/100 g, respectively.

leaf samples than in flower buds [Table 3]. Flavonoids are a group of polyphenolic compounds responsible for free radical scavenging inhibition of hydrolytic and oxidative enzymes, as well as anti-inflammatory action [26]. These are vital in combating the free radicals which damage human cells. Numerous epidemiological studies confirm a significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk. These phenolic compounds possess a wide spectrum of biochemical activities, such as antioxidant, antimutagenic, and anticarcinogenic, as well as the ability to modify gene expression [24].

Furthermore, the antioxidant activity of leaf and flower buds was also estimated by DPPH and FRAP assay. The result showed that flower bud samples have higher antioxidant activity than leaf samples [Table 3]. A relatively higher absorbance value indicated more reduction of ferric ions to ferrous ions. Samples having a higher reducing power had a higher absorbance value at 700 nm. Highest antioxidant activity was found in *C. obliqua* leaves (40.8 mg/g) and flower buds (40.6 mg/g). The high FRAP reduction value indicates its suitable scavenging property.

4. CONCLUSION

C. obliqua is an important, underutilized forest plant having numerous medicinal, nutritional, and industrial applications. Its flower bud is used as a raw vegetable mainly because of its high nutritional value. It also contains various elements such as, S, K, Mg, P, Mn, Ca, Fe, Zn, Ni, and Cu. Besides, high amounts of protein, Vitamin C, phenols, and flavonoids were found in the flower buds. The antioxidant activity was also found to be very significant in the flower bud. All the data

taken together revealed that the flower buds and leaf of *C. obliqua* could fulfill the demand of nutrients required per day as per the recommendation of the Indian Council of Medical Research for Indian and cope with the nation's food security.

5. AUTHORS' CONTRIBUTIONS

PKN designed the experiment. SB and MN conducted the elemental analysis. MN, SI, and SKB performed nutritional and phytochemical analyses. SB and SKB analysed the data and prepared the first draft of the manuscript. PKN edited the final manuscript. Finally, all authors approved the manuscript for publication.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated or analysed during this study are included in this manuscript.

10. ACKNOWLEDGEMENTS

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