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Research Article

Quality characteristics, phytochemical analysis, and antioxidant of extract *Cuscuta reflexa* (Roxb.)

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Abstract

Cuscuta reflexa is an extensive leafless, parasitic climber that has been used since ancient times for various purposes and as a therapeutic plant in different areas of Pakistan. The phytochemical, antibacterial, antioxidant, and fatty acid investigations were carried out on the leaves, stems, and fruit extracts of *C. reflexa*. Preliminary phytochemical screening has shown the presence of various phytochemicals such as carbohydrates, protein, amino acids, alkaloids, flavonoids, phenols, glycosides, saponins, tannins, terpenoids, steroids, and phenolic compounds. The percentage variation of some components fluctuated like moisture (6.85-10.34%), ash (5.38-7.83%), acid insoluble ash (0.28-0.71%), water-soluble ash (0.78-0.96%), hexane extractives (1.23-1.91%), alcohol extractives (10.39-12.23%), water extractives (25.34-30.35%), loss on drying (1.16-1.47%) and crude fiber (15.04-18.26%) for leaves, stems, and fruits respectively. The level of free fatty acid was different as follows: leaves (2.37%), stems (2.16%), and fruits (2.94%); a high value of peroxide was observed in stems, followed by leaves and fruits. The iodine and saponification values in leaves, stems, and fruits were found in the range of 105-116 g I2/100 g, and 165-175 mg KOH/g. The antibacterial activity was carried out by the disc diffusion method against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria. It was found that all extracts of the selected plant were most active against *E. coli* with a zone of inhibition of 4, 4, and 6 at MIC 250 as compared to *S. aureus,* which showed a zone of inhibition of 3, 4, and 5 at MIC 250 µg/ml. The antioxidant potential of the leaves, stems, and fruit extracts, examined on the basis of their scavenging activity of free radicals, was found to be good. The result of the gas chromatography-mass spectroscopy (GC-MS) analysis reveals that linoleic acid (38.10–40.53%) is the most abundant fatty acid of *C. reflexa* and the smallest amount was found in tricosanoic acid (0.12-0.19%). In general, the isolated compounds were reported to possess anticarcinogenic, antitumor, antimicrobial, and anti-inflammatory properties. It was concluded that *C. reflexa* has a markedly therapeutic potential to heal wounds and may provide the pharmacological basis for

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its folk uses.

Keywords: antimicrobial activity; gas chromatography-mass spectroscopy; phytochemical analysis; radical scavenging activity

Introduction

Cuscuta reflexa Roxb. belongs to the Convolvulaceae and is a broad climber parasite. Generally, the species, recognized as Amberbel, has a place in the family Cuscutaceae. It is a tropical and subtropical spice that began as a parasite weed on other plants. It is more normally called "dodder" in English. Customary healers brought in the Urdu Akash bel. Interest in therapeutic plants is developing because of their expanding significance as restorative sources (Ashraf et al., 2020). *C. reflexa* is a perpetual parasitic spice tracked down everywhere, including Pakistan, Bangladesh, Nepal, and India (Anjum *et al.,* 2013). Cuscutaceae is a family of around 100–160 species. In Pakistan, *C. reflexa* is generally connected with parasitism in decorative plants, and its occurrence in therapeutic harvests is strange. This species, which originated in Pakistan, is common in the state's northern region (Pandit *et al.,* 2008; Bobbarala *et al.,* 2009).

As indicated by the examination, it is considered that the plant has extremely low degrees of photosynthesis and contains a small amount of chlorophyll. Be that as it may, different types of Cuscuta are completely reliant upon the host plants for sustenance. The stem is made of string-like fibers that are starting to develop and connect it to nearby host plants. Natural plants carry on with their whole existence. The ethnobotanical literature is replete with information on homegrown items used to treat chronic and severe ailments, including diarrhea, antispasmodics, constipation, and vomiting (Abu-Izneid *et al.,* 2021). connection to the ground. It has a long history of ethnomedicinal use. Additionally, homegrown cures are utilized in both developed and developing nations throughout the world, with a few restorative plants demonstrating new remedial sources (Saad *et al.,* 2005; Hamed *et al.,* 2019; Romeilah *et al.,* 2021). According to the World Health Organization (WHO), natural medicines are used by 90% of the population in agricultural countries (Muhammad *et al.,* 2020). The ethnobotanical literature is replete with information on homegrown items used to treat chronic and severe ailments such as diarrhea, antispasmodics, constipation, and vomiting (Abu-Izneid *et al.,* 2021).

In Pakistan, the plant is handled as different homegrown arrangements for the therapy of diarrhea, epilepsy, vomiting, paralysis, liver disorders, blood purification, joint pain, persistent chronic fever, jaundice, nausea, itching, lumbago, and muscle pain, among others. In such a manner, various natural items, like Majoon Ushbaare, Majoon Najah, Bukharin, and Aftimooni, are accessible on the Pakistani market and contain *C. reflexa* in different amounts, generally as a tonic. *C. reflexa* is known as a wonder plant, which explains why a large number of pharmacological studies have been conducted on it, utilizing various exploration techniques and investigations (Durdana *et al.,* 2010). These pharmacological examinations show the utilization of this plant as an antioxidant and antifungal (Parveen *et al.,* 2013), anti-inflammatory, lower blood pressure, antibacterial (Mala and Sofi, 2017), for the treatment of antihistamine, alopecia (Ambi *et al.,* 2017), anticonvulsant, anti-HIV, anticholinergic, antidiabetic (Mahmood *et al.,* 1997; Rahmatullah *et al.,* 2010), muscle relaxant, analgesic, antitumor, diuretic, CNS depression, and anthelmintic (Chatterjee *et al.,* 2011; Udavant *et al.,* 2012), It also has anti-inflammatory and anti-cancer activity (Suresh *et al.,* 2011). Various distributed articles have detailed the antidiarrheal employment of *C. reflexa* by various networks, albeit test proof is missing (Mukherjee *et al.,* 2006; Khan *et al.,* 2016; Sharma and Kapoor, 2014). In dairy cattle, *C. reflexa* stem juice is normally administered three times per day to treat diarrhoea (Gosh, 2008). The approved antihistamine, antifungal, anticholinergic, and antibacterial impacts of this plant propose further logical examination of its insect diarrheal impact. The objective of the present study was to assess the phytochemical study; antimicrobial also checks the antioxidant and fatty acid composition effects of the *C. reflexa* plant.

Materials and Methods

Plant material and chemicals

Different parts of the *C. reflexa* plant (i.e., leaves, stems, and fruits) were collected in March 2020 from the District of Sanghar (longitude: N 25.8577 and latitude: E 69.4785), Sindh, Pakistan. The plant was identified and authenticated at the Institute of Plant Sciences. A voucher specimen (12523) was deposited at the herbarium located at the same institute, the University of Sindh, Jamshoro. The plant samples were dried at room temperature under shade for a week and then crushed into powder for further analysis. All chemicals and reagents used in this research work were pure analytical grade.

Physicochemical evaluations

Physicochemical parameters like the level of total ash, water-soluble ash, loss on drying (LOD), water and alcohol soluble extractives acid insoluble ash, were estimated by hot extraction and cold maceration according to the method prescribed by WHO (2007); (Anonymous, 2002).

Phytochemical analysis

Carbohydrates, protein, amino acids, alkaloids, phlobatannins, flavonoids, phenols, glycosides, saponins, tannins, terpenoids, steroids, and phenolic compounds were tested using the reported method described by Edeoga *et al.* (2005), Siddiqui *et al.* (2009), and Kokate *et al.* (2001).

Determination of antimicrobial activities

The antibacterial activity and radical scavenging activity of *C. reflexa*, plants (leaves, stems, and fruits) were determined according to the method described by Rahman *et al.* (2017).

Gas chromatography-mass spectrometry (GC-MS)

C. reflexa Fatty acid methyl ester (FAME) of leaves, stems, and fruits was prepared according to the standard method (IUPAC 2.301). The FAME was investigated and recognized by GC-MS. The sample solution of (1.0 μl) methyl ester was infused on a capillary column, through an autosampler. Helium was utilized as a carrier gas with a (1.0 mLmin-1) flow rate; injector and detector temperatures were set in the scope of (150 °C and 270 °C), At first, the temperature of the oven was set at 150 °C for 2 min then the temperature was raised to 270 °C at the rate of 0 °C/min (4 min hold). The complete analysis time of sample running was 45 min. The mass spectrometer instrument was worked in the electron impact mode at 70 eV in the analyst range between50-550 m/z separately.

Statistical analysis

All of the analytical tests in the current study were replicated three times, and the results are presented as a mean value and standard deviation. ANOVA (one-way analysis of variance) was used to analyses the data, and Tukey's test of significant difference at p0.05 was used to distinguish between the means.

Results and Discussion

Table 1 illustrates the percentage of active chemical constituents in leaves, stems, and fruits was mentioned on an air-dried basis. The current values of *C. reflexa* for leaves, stems, and fruits were found to be at 8.23%, 10.34%, and 6.85%, respectively. A high level of moisture content was observed in stem extract as compared to leaves and fruit extract. The ash value determines the quality of the plant extract. The significance obtained for the leaves of *C. reflexa* is approximate; stems 7.83%, leaves 6.17%, and fruits 5.38%, respectively. The acid-insoluble ash was determined in the *C. reflexa* plant extract and the value was observed in the range of 0.28 to 0.71%, and the water-soluble ash was found in the range of 0.78-0.96% correspondingly. The extraction of any concentrates with explicit dissolvable yields an answer containing particular phyto-constituents. The association of these phyto-constituents in that particular dissolvable depends on the possibility of the concentrate and dissolvable used. The utilization of a solitary dissolvable can be a method for giving primer data on the nature of a specific medication test. The extractive value of the extract decides the quality just as the virtue of the plant material. The hexane, alcohol, and water-soluble extractive values of the leaves, stems, and fruits in the range were observed to be 1.23-1.91%, 10.39-12.23%, and 25.34-30.35%. The losses on drying of leaves, stems, and fruit extract were 1.23%, 1.47%, and 1.16%, respectively.

In the current investigation, the fiber content was found in leaves, stems, and fruit extracts to be around 18.26%, 16.14%, and 15.04%. The FFA level of *C. reflexa*, the extract, was observed at 2.37%, 2.16%, and 2.94% for leaves, stems, and fruits, respectively. The PV determination is most commonly used for the measurement of oxidative rancidity of oils and fats (Okechalu, 2011). In the present study, values of PV in the range of 5.25 to 7.21 meq O2/kg were observed. A high level of PV was noticed in stem extract, then leaves and fruits. To determine the quantity of unsaturation in fatty acids, we often use iodine numbers. The more C=C bonds are present in oil and fat, the higher the iodine number. In the current study, the iodine value was noticed in the range of 105.04 to 116.23 g I2/100g for leaves, stems, and fruits correspondingly. The level of SV was found in the range for leaves, stems, and fruits (165–175 mg KOH/g) respectively. According to the literature, oils with higher saponification values contain a higher proportion of lower fatty acids. Oils with low SV can be used for the production of soap, candles, and raw materials for lubricants (Agatemor, 2006; El-Beltagi *et al.,* 2019a). These results are in good agreement with the literature. The most valuable outcome obtained in the current study was relatively similar to the earlier reported study by Kumar *et al.* (2011).

Table 1. Summarized results of physiochemical properties of C. *reflexa*

Parameters	Leaves	Stems	Fruits
Moisture content (%)	8.23±1.34^{b}	10.34±2.35^{a}	6.85±0.75^{c}
Total Ash (%)	6.17±0.27^{b}	7.83±1.95^{a}	5.38±1.38^{c}
Acid insoluble ash (%)	0.71±0.18^{a}	0.35±0.21^{b}	0.28±0.05^{c}
Water soluble ash (%)	0.96±0.20^{a}	0.78±0.01^{c}	0.88±0.01^{b}
Hexane extractives (%)	1.67±0.01^{b}	1.23±0.65^{c}	1.91±0.45^{a}
Alcohol extractives (%)	10.39±1.24^{c}	12.23±2.83^{a}	11.26±1.75^{b}
Water extractives (%)	30.35±0.13^{a}	28.73±1.96^{b}	25.34±1.56^{c}
Loss on drying (%)	1.23±0.14^{b}	1.47±0.06^{a}	1.16±0.45^{c}
Crude fiber (%)	18.26±1.01^{a}	16.14±1.63^{b}	15.04±1.95^{c}
FFA (%)	2.37±0.12^{b}	2.16±0.56^{c}	2.94±1.44^{a}
PV (meqO_2/kg)	6.26±0.83^{b}	7.21±0.46^{a}	5.25±1.05^{c}
IV (gI_2/100g)	105.04±1.87^{c}	112.14±2.23^{b}	116.23±2.11^{a}
SV (mgKOH/g)	175.23±1.92^{a}	165.21±1.98^{c}	171.38±2.85^{b}

Each column contains the mean values of triplicate analyses with standard deviation (± SD). Means followed by different letters in the same column differ significantly according to Tukey's test at 0.05 p-level.

Phytochemical analysis

The phytochemical screening of the hexane, methanol, ethyl acetate, acetone, and water extracts was analyzed. It has been observed that chemically therapeutic compounds like flavonoids, saponins, glycosides, tannins, terpenoids, steroids, and phenolic compounds are available in satisfactory sums in the fruits, leaves, and stems of *C. reflexa* (Table 2).

Table 2. Phytochemical screening (leaves, stems, and fruits) extracts of C. *reflexa* plant

Compound	Hexane	Methanol	Ethyl Acetate	Acetone	Water
			Leaves Extract		
Carbohydrates	+	+	+	+	+
Proteins	+	-	-	-	-
Amino acids	-	-	-	-	-
Alkaloids	-	-	-	-	-
Phlobatannins	-	-	+	-	+
Flavonoids	+	+	+	+	+
Phenols	-	-	-	+	+
Glycosides	+	-	+	-	-
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
			Stems Extract		
Carbohydrates	+	+	+	+	+
Proteins	+	+	-	-	-
Amino acids	-	-	-	-	-
Alkaloids	-	-	-	-	-
Phlobatannins	-	-	+	+	-
Flavonoids	+	+	+	+	+
Phenols	+	-	+	+	+
Glycosides	+	-	+	-	+
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
			Fruits Extract		
Carbohydrates	+	+	+	+	+
Proteins	+	+	-	+	-
Amino acids	-	-	-	-	-
Alkaloids	-	-	-	-	-
Phlobatannins	+	-	+	-	+
Flavonoids	+	+	+	+	+
Phenols	+	+	+	+	+
Glycosides	-	+	-	+	-
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+

However, the amino acid and alkaloid show no activity against all five extracts, although protein shows positive in leaves, stems, and fruits against hexane extract, methanol positive in the stems, and fruit extract, respectively. While negative activity is shown against leaf extract, acetone shows a positive against fruit extract, whereas leaves and stems extract show no activity. Water extract shows negative activity against leaves, stems, and fruit extracts. In the same way, phlobatannins are present in ethyl acetate, water extract, and negative against hexane, methanol, and acetone for leaf extract. Leaves, stems, and fruit extracts were all negative for acetone and hexane extracts, respectively.

The phytochemical screening of the extracts revealed the presence of flavonoids, saponins, tannins, terpenoids, steroids, and phenolics. It is evident from the literature that the therapeutic potency of any important component may be increased by the presence of carbohydrates (Orhan *et al.,* 2007; Ahmad *et al.,* 2016; El-Beltagi *et al.,* 2022). It was also found that tannins show anti-diarrheal activity, and these constituents may precipitate proteins on the enterocytes which reduces peristaltic movement and intestinal secretion. In addition to that, saponins have hypoglycemic, cardiotonic, and expectorant activity (Muthee *et al.,* 2016). Furthermore, glycosides exhibit antiseptic, diuretic, and laxative properties, as well as flavonoids, which have significant antimicrobial, anti-diabetic, antioxidant, hypoglycemic, anti-inflammatory, anti-tumour (Rubini *et al.,* 2012), and free radical-scavenging activities (Sofy *et al.,* 2021). The current experiment revealed that the hexane, methanol, ethyl acetate, acetone, and water extract of *C. reflexa* may have anti-inflammatory, antioxidant agents. The presence of flavonoids and tannins may exhibit free radical-scavenging and anti-diarrheal activity, respectively. In addition to that, the plant sample may also show cytotoxicity and insecticidal activity due to the presence of terpenoids (Yadav *et al.,* 2011; Mohamed and Abd–El Hameed, 2014).

Determination of antibacterial activities

Significant antimicrobial effects expressed as MIC of *C. reflexa*, plant extract against test microorganisms is given in Table 3. The methanolic extract of leaves, stems, and fruits exhibited a maximum zone of inhibition of *E. coli* by about 14, 6, 4, 0 mm, 12, 7, 4, 0 mm, and 11, 7, 6, 0 mm, respectively. However, *S. aureus* showed an inhibition zone about 10, 5, 3, 0 mm, 9, 6, 4, 0 mm, and 11, 6, 5, 0 mm at different concentrations (1000, 500, 250, and 10 μg/ml), respectively. Compared to that, the maximum These discoveries demonstrate that the plant extricates tried in this investigation could be utilized as normal additive specialists in food to wipe out or control the development of deterioration and pathogenic microorganisms. found to be in leave extract. This investigation upholds past discoveries in the literature that antimicrobial activity has an immediate connection to expanding the centralization of the concentrate (%) (Bhalodia and Shukla, 2011). It has been reported already that the concentrates from a few plants like cumin, oregano, sage, cinnamon, and different spices possessed significant ($P<0.05$) antifungal and antibacterial activities against a wide scope of food waste microbes (Gram-positive and Gram-negative), such as yeast and form (Nassan *et al.,* 2015; Liu *et al.,* 2017). These findings show that the plant extracts tested in this study could be used as typical food additive specialists to eliminate or control the growth of pathogenic and deteriorating microorganisms (Aly *et al.,* 2017).

Table 3. Inhibition zones (mm) for antibacterial activities of methanol extracts of leaves, stems, and fruits

Microorganism strains		Concentration in μg/mL					MIC Value (μg/mL)
		1000	500	250	10	Control	
Leaves	*Escherichia coli*	14	6	4	0	-	250
	Staphylococcus aureus	10	5	3	0	-	250
Stems	*Escherichia coli*	12	7	4	0	-	250
	Staphylococcus aureus	9	6	4	0	-	250
Fruits	*Escherichia coli*	11	7	6	0	-	250
	Staphylococcus aureus	11	6	5	0	-	250

Radical scavenging activity (RSA) assay

In the current examination, DPPH is utilized to assess the antioxidant potential of *C. reflexa* plants (leaves, stems, and fruits). The DPPH radical has been widely utilized as a stable free radical to determine the decreasing substances or antioxidant activity of plant extracts (Öztürk *et al.,* 2007; Ashry *et al.,* 2018; Ghonaim *et al.,* 2021). Plant extracts contain polyphenols that can give electrons or hydrogen atoms and catch free radicals (Wong *et al.,* 2006; Stoilova *et al.,* 2007; Naeem *et al.,* 2020; Ahmad *et al.,* 2021a, b). Hence, the purple-concealed DPPH was reduced to a yellow-shaded complex. The DPPH test is used as the main method to evaluate in vitro cancer prevention agents of plants (El-Beltagi *et al.,* 2019 b, c). Table 4 represents the n-hexane, ethanolic and methanolic, extracts of *C. reflexa*, plant (leaves, stems, and fruits) at various concentrations (5-100 µg/ml) and reference compound (gallic acid) respectively. The highest DPPH inhibition (74.9%) was found by fruit extract (methanol), whereas the lowest was observed (54.45%) for leaves (n-hexane) extract, respectively. The lower IC50 value would represent a higher antioxidant potential. The highest IC50 value of 129.4 µg/mL was found for ethanol extract, whereas the lowest IC50 value of 106.7 µg/mL was found for methanol extract. DPPH is a nitrogen-centered stable violet-colored free radical that is converted to yellow due to reduction by hydrogen or the electron-donating ability of antioxidants present in tested extracts. The selected plant parts in this study were capable of scavenging DPPH free radicals. The current studies were similar to the results reported by Sati *et al.* (2013), Guntupalli *et al.* (2012), and Chandran *et al.* (2013), addressing that plants' optional metabolites hold a solid anti-oxidative property. In the current study, the *C. reflexa* plant has significant antibacterial and antioxidant properties and, accordingly, could be valuable as helpful specialists in forestalling and decreasing the advancement of maturing that is associated with degenerative infections.

Table 4. Radical scavenging activity (RSA) assay of C. *reflexa* plant (leaves, stems, and fruits)

Solvents	Concentration (μg/mL)	% Inhibition (mean ± SD)		
		Leaves	Stems	Fruits
n-Hexane	5	9.92 ± 0.07^{b}	8.34 ± 0.08^{c}	14.16 ± 0.08^{a}
	10	12.63 ± 0.08^{c}	13.43 ± 0.10^{b}	17.93 ± 0.10^{a}
	15	17.34 ± 0.15^{c}	19.32 ± 0.17^{b}	21.58 ± 0.17^{a}
	20	20.36 ± 0.19^{b}	19.23 ± 0.23^{c}	26.09 ± 0.24^{a}
	25	22.35 ± 0.25^{b}	21.47 ± 0.31^{c}	28.02 ± 0.29^{a}
	50	34.54 ± 0.29^{b}	32.67 ± 0.37^{c}	40.47 ± 0.32^{a}
	75	45.45 ± 0.41^{c}	46.24 ± 0.41^{b}	52.34 ± 0.42^{a}
	100	54.45 ± 0.50^{c}	58.34 ± 0.56^{b}	63.02 ± 0.56^{a}
	IC_{50} (μg/mL)	128.1	117.9	126.9
Methanol	5	17.67 ± 0.08^{b}	18.01 ± 0.11^{a}	16.1 ± 0.09^{c}
	10	20.67 ± 0.11^{b}	21.34 ± 0.14^{a}	20.4 ± 0.11^{c}
	15	23.09 ± 0.17^{c}	25.56 ± 0.18^{a}	24.67 ± 0.18^{b}
	20	29.23 ± 0.23^{c}	30.87 ± 0.26^{a}	30.34 ± 0.25^{b}
	25	33.02 ± 0.25^{c}	34.23 ± 0.29^{a}	33.67 ± 0.33^{b}
	50	43.45 ± 0.34^{c}	46.12 ± 0.34^{b}	48.78 ± 0.38^{a}
	75	57.03 ± 0.44^{c}	58.9 ± 0.41^{b}	63.89 ± 0.46^{a}
	100	68.62 ± 0.57^{c}	71.44 ± 0.61^{b}	74.9 ± 0.66^{a}
	IC_{50} (μg/mL)	125.5	123.2	106.7
Ethanol	5	10.01 ± 0.08^{c}	12.67 ± 0.08^{b}	15.1 ± 0.11^{a}
	10	13.34 ± 0.11^{c}	15.67 ± 0.11^{b}	18.4 ± 0.16^{a}
	15	18.56 ± 0.16^{c}	22.09 ± 0.17^{b}	24.67 ± 0.19^{a}
	20	20.87 ± 0.19^{c}	25.23 ± 0.21^{b}	28.34 ± 0.26^{a}
	25	23.23 ± 0.23^{c}	28.19 ± 0.25^{b}	31.67 ± 0.28^{a}

	50	33.12±0.29[c]	38.45±0.33[b]	41.78±0.33[a]
	75	45.9±0.35[c]	49.03±0.41[b]	54.89±0.42[a]
	100	55.44±0.44[c]	66.62±0.52[b]	68.9±0.53[a]
	IC_{50} (μg/mL)	129.4	118.5	121.2

The values are the mean of triplicate analysis with standard deviation. Means followed by different letters in the same column differ significantly according to Tukey's test at 0.05 p-level.

Fatty acid composition

Identification and quantification of the constituents present in the experimental plant extract were performed by the GC-MS method. A total of 13 compounds were identified from the plant (leaves, stems, and fruits) of *C. reflexa* by GC-MS analysis, which represents the medicinal quality of the plant sample, and the different analyzed compounds of *C. reflexa* are shown in Table 5. Among them, the most abundant fatty acids in the *C. reflexa* were linoleic acid (38.10-40.53%), followed by oleic acid (23.94-28.03%), palmitic acid (13.81-14.60%), stearic acid (9.88-12.08%), arachidic acid (2.09-3.56%), behenic acid (2.75-3.26%), lignoceric acid (2.22-2.59%), and the smallest amount was found to be in the range of myristic acid (0.19-0.23%), margaric acid (0.18-0.23%), gondoic acid (0.45-0.50%), tricosanoic acid (0.12-0.19%), pentacosanic acid (0.39-0.47%), and cerotic acid (0.58-0.66%) respectively. Afrin *et al.* (2019) described 21 saturated/unsaturated fatty acids from the *C. reflexa* plant. Tetrapentacontane (29.98%) was found to be the most abundant fatty acid in *C. reflexa*, followed by Tris (2,4-di-tert-butyl phenyl) phosphate (20.87%) and Tris (2,4-di-tert-butyl) phosphate (13.40%). There were also smaller amounts of 2,6-di-tert-butyl-4-hylidenecyclohexa-2,5-dien-1-one (0.51%), 6-ethyl-2-methyl Octane (0.56%), and 1-Nonadecene (0.62%) respectively. In another study, according to Rai et al. (2016), nearly 12 compounds were identified in the *C. reflexa* plant samples. The most plentiful fatty acid was found to be (1,4 Bis4' 5' Bis (Trimethylfluromethyl) 1', 3'-Dithiac, 28.40%), and the smallest amount was found in (TMS-8,11-Di–OHTetrahydrocannabinol, 5.66%), respectively. Higher dietary intakes of saturated fatty acids are associated with lower risks of pancreatic cancer, type 2 diabetes, chronic obstructive pulmonary disease, adiposity, chronic inflammation, cardiovascular disease, and other diseases (Berwal *et al.,* 2022).

Table 5. Fatty acid composition of extract C. *reflexa* plant

S.NO	Compounds (%)	Leaves (%)	Stems (%)	Fruits (%)
1	Myristic acid	0.19±0.02[c]	0.23±0.03[a]	0.20±0.02[b]
2	Palmitic acid	14.60±0.53[a]	13.81±0.41[c]	14.01±0.48[b]
3	Margaric acid	0.18±0.02[c]	0.20±0.02[b]	0.23±0.03[a]
4	Oleic acid	23.94±0.48[c]	28.03±0.58[a]	26.02±0.54[b]
5	Linoleic acid	40.53±0.74[a]	38.31±0.68[b]	38.10±0.57[c]
6	Stearic acid	10.35±0.46[b]	9.88±0.39[c]	12.08±0.42[a]
7	Gondoic acid	0.49±0.03[b]	0.45±0.03[c]	0.50±0.04[a]
8	Arachidic acid	3.56±0.21[a]	2.36±0.19[b]	2.09±0.16[c]
9	Behenic acid	2.75±0.18[c]	2.94±0.19[b]	3.26±0.20[a]
10	Tricosanoic acid	0.12±0.01[c]	0.15±0.02[b]	0.19±0.02[a]
11	Lignoceric acid	2.30±0.18[b]	2.59±0.19[a]	2.22±0.17[c]
12	Pentacosanic acid	0.42±0.03[b]	0.39 ±0.03[c]	0.47±0.04[a]
13	Cerotic acid	0.58±0.03[c]	0.66 ±0.04[a]	0.62±0.03[b]

The values are the mean of triplicate analysis with standard deviation. Means followed by different letters in the same column differ significantly according to Tukey's test at 0.05 p-level.

Conclusions

The present study provides an overview of the presence of a large number of secondary metabolites in the whole plant of *C. reflexa* (leaves, stems, and fruits) and indicates the presence of different classes of compounds that may have pharmacological importance. This entire plant is remedially helpful in the weakness of the stomach, liver, and spleen. This medication is likewise utilized for melancholia, paralysis, facial palsy, insanity, epilepsy, arthritis, worm infestation, numbness, and jaundice. Phyto-synthetic techniques can also be used to validate and improve the principles for crude medications. To guarantee the reproducible nature of herbal products, appropriate control of beginning material is of the utmost fundamental. Accordingly, as of late, there has been an accent on the standardization of medicinal plants of therapeutic potential.

Authors' Contributions

Conceptualization: ARS, AB, AH, SM, SA, TK, HIM, and AEL; Data curation; ARS, AB, AH, SM, SA, and TK, Formal analysis; ARS, AB, AH, SM, SA, and TK, Funding acquisition; ARS, AB, AH, SM, SA, TK, HIM, and AEL, Investigation; ARS, AB, AH, SM, SA, and TK, Methodology; ARS, AB, AH, SM, SA, and TK, Project administration; HIM, Resources; ARS, AB, AH, SM, SA, TK, HIM, and AEL, Software; ARS, AB, AH, SM, SA, TK, HIM, and AEL; Supervision; HIM Validation; Visualization; ARS, AB, AH, SM, SA, TK, HIM, and AEL; Writing - original draft; ARS, AB, AH, SM, SA, TK, HIM, and AEL; Writing - review and editing; HIM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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