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Research Article

MINERAL PROFILE, ANTIOXIDANT AND ANTIMICROBIAL STUDIES OF *CORCHOROUS DEPRESSUS* LEAVES

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ABSTRACT

Corchorous depressus is a woody perennial plant, prostrate, the branches originated from a woody crown. In medical field*, Corchorous depressus* is used in general weakness, gonorrhea, diabetes, treachery troubles, improved sexual vigor have been reported. In the present work, the leaves of plant was extracted with methanol, ethanol, acetone, ether and n-hexane to determine the percentage yield, their antioxidant and antimicrobial activity and mineral profile. Total phenolic contents, total flavonoid contents, %age inhibition by linoleic acid system were determined. BHT was used as reference in DPPH and in linoleic acid system to check the scavenging activity of *Corchorous depressus*. The methanolic extract of leaves of plant was used to evaluate the antimicrobial activity against the pathogens *(E.coli*, *P.multocida*, *S. aureus*, *B. subtilis A. flavus*, *A. niger, R. solani,and F. solani)* by disc diffusion and MIC methods. For the evaluation of mineral profile of the leaves of plant, the extracts were analysed under the wet digestion method. By this method different metals like Pb, Zn Cu, Ni, Co and Fe are evaluated in different concentration.

Keywords: *Corchorous depressu,* Antioxidant Activity, Antimicrobial Activity, Heavy metals

INTRODUCTION

Plants are very important for life. About 80% of world population completely depends on plants for their health and solution of health related problems. [1] Vascular plant can produce a variety of organic molecules that are termed as secondary metabolites. These molecules protect plant from infections, wounding, UV radiation [2] Several Reports show that a variety of such plant's secondary metabolites have biological effects like antioxidant, antimicrobial and among them phenolic compounds form a major class of biological important compounds [3]. Plants including food and vegetable are rich source of phytochemicals like flavonoides, carotenoids and phenolic compounds [4]. Extraction isolation and identification of such health promoting plant constituents is the main target of the modern scientific investigation [5]. Plants including fruits and vegetables are the richest source of bioactive compounds [4]. It is an accepted fact that the human body is constantly at risk from being attacked by highly reactive free radical species. They are continuously synthesized in the body by the normal usage of oxygen as in aerobic respiration and immune functions of living cells [6]. Reactive oxygen species like superoxide and peroxyl radicals are produced under situation of oxidative stress. The efficient and reliable way to eliminate and diminish the effect of these free radical oxidative species is the antioxidant defense mechanisms [7]. The importance of the *Corchorous depressurs* can be demonstrated directly through modification of environmental conditions [8]. The alignment of species on an environmental gradient will evaluate which species will be able to utilize in the future [9]. The plants that are nearest to the gradient are the ones that are expected to compete more strongly.

An exhaustive literature survey on the secondary metabolites of *Corchorous* species has been carried out. Triterpenoids, Glycosides of Cardiac, phenolics,, sterols, ionones, carbohydrates and fatty acids have been reported from different species [10]. Much of the components have been present for the different biological characteristics e.g. digitalis glycosides like action, activity of anti-convulsive, action of anti-esterogenic, activity of anti-cancer and anti-pyretic activity etc. The reproductive potential of this plant was calculated as the product of average seed output and the fraction evaluated by the average germination [11]. Flowering Period of this plant is februrary to November. The whole plant is used against hepatitis i.e inflammation of liver, urine itching, prolong bleeding during menses and impotency in males. Leaves are used against retention in urine and heat stroke. The leaves of the plant are more effective as an emollient and a good cooling agent. Mucilage is used for the treatment of gonorrhea and used as a poultice for healing wounds. Decoction of seeds & leave's with milk and sugar is also a good tonic [12].

Nutrient content of *Corchorous* leaf (per 100g)

Energy	43-58kcal
Water	80-84g
Protein	4.5-5.6 g
Carbohydrates	7.6-12.4 g
Fats	1.0-1.3 g
Calcium	266-366 mg
Iron	7.2-7.7 mg
Potassium	440-444 mg
B-carotene (vitamin A)	6,410-7,850 mg
Thiamin (vitamin B)	130-150 mg
Riboflavin (vitamin B)	260-530 mg
Niacin (vitamin B)	1,100-1,200 mg

Antioxidants are such substances that have the ability to break free radical chain reactions. To eliminate the effects of oxidation, the use of synthetic antioxidants like butylatedhydrokytolune (BHT) and butylated hydroxyl anisole (BHA) in food have been restricted due to their suspected carcinogenic effect [13]. Several experiments have shown that the therapeutic effect of medicinal plants, fruits and vegetables is due to the antioxidant potential of their phytoconstituents. Thus the antioxidant potential of plant may be due to their phenolic components. Fruits and vegetables possess a number of antioxidants such as beta-carotene, terpenoids, ascorbic acid, alkaloids, carotene, polyphenoles such as flavonoids, flavones, glycosides, routines [14].

The phenolic components belong to a group of naturally occurring plant metabolites all of which have an aromatic ring with at least one hydroxyl group, i.e. a phenol. These are aromatic secondary plant metabolites present throughout the plant kingdom [15]. They are present both in edible and non-edible plants and have different biological effects mainly antioxidant potential. This property of phenolics is due to their redox properties. Redox potential means that they act as reducing agents, donators of hydrogen and singlet quencher of oxygen. Phenolic acids also have ability of matal chelation and they inhibit the oxidative degradation of lipids. They develop the quality as well as nutritional value of food. That's why they are necessary in food factories. Natural phenolic components are also attracting the interests of researchers, food manufacturers and consumers due to the useful food with specific health effects. Flavonoida are a group of polyphenolic compound having properties like free radical scavenging, anti-inflammatory action, hydrolytic oxidative enzymes etc [14]. In some research works that presented

here, there is variation in K, Na, Ca, P, Fe, and Zn contents in the different components of *Corchorus depressus* under the different environmental conditions of the Indian Thar desert. The principal component analysis (PCA) was performed to synthesize the relationship and future lines of study with the help of plant biomass and community dynamics (bottom-up factor), soil parameters (top-down factors) and plant metabolites. The K, Ca, and Fe were positively related to each other, but these metals prevent the concentration of steroidal sapogenin and phenol at significant levels. Changes in soil N and soil P however, favor these metabolites directly and alkaloid components indirectly [16].

MATERIALS AND METHODS

Collection of Samples

The plant part (leaves) of *Corchorous depressus* were selected for this research work. Plant was purchased from local market of Faisalabad city.

Drying and Grinding of Samples

The branches of whole plant were washed well under tap water first and then by using distilled water. After air drying, leaves were manually separated and then grinded into fine powder. The grinded material was stored in glass jars at room temperature.

Preparation of extracts

Five solvents methanol, ethanol, n-hexane, acetone and ether were used for extraction. 20g of ground sample were dissolved in 50 mL of each solvent in 250mL conical flasks and set on orbital shaker for extraction at the speed of 200 rpm at room temperature for 24 hours. After 24 hours, flasks were removed from orbital shaker and the contents were filtered using Whatman No. 1 filter paper. The resulting filtrates were concentrated under vacuum using rotary evaporator. The concentrated extracts were stored in sample voiles at 4^0C for further analysis and chemical tests.

Evaluation of Antioxidant Activity of plant leaves

For the determination of antioxidant potential of different extracts of *Corchorous depressus's* leaves, the following assays were performed.

Determination of Total Phenolics Contents (TPC)

Total Phenolic contents were determined using method as described [17].

Determination of Total Flavonoids Contents (TFC)

Amount of total flavonoids was assessed using method as described [18] with slight modifications.

DPPH Free Radical Scavenging Assay

Scavenging of *Corchorous depressus* extracts against DPPH radicals was assessed according to the method of (Iqbal *et al*., 2005) with some modifications. Three reading were recorded for each sample. According to following equation the inhibitory percentage of DPPH was calculated.

Scavenging Activity = (1 - absorbance of sample / absorbance of control) x 100

Determination of Antioxidant Activity in Linoleic System

Inhibition of linoleic acid peroxidation of *Corchorous depressus* extracts was measured using ammonium thoicyanate assay as described [19].

Antibacterial Assay of Plant Extract

Antibacterial activity was assessed using Disc Diffusion method as described [20].

Minimum inhibitory concentration (MIC)

96 well plate,100 µL sample, 50 µL medium (muller Hinton broth for fungal strain, Nutrient broth for bacterial strain), 10 µL of inoculums and 10 µL Resazurin indicator.

Procedure

Minimum inhibitory concentration (MIC) of plant extract was determined by a modified resazurin microliter-plates [21].

Determination of Mineral profile in plant

Wet digestion methods for elemental analysis in leaves of *Corchorous depressus* involve the chemical degradation of sample matrices in solution, usually with a combination of acids to increase solubility.

Wet digestion of plant samples

Triplicate sets of 0.50 gm of each plant samples extract were weighed in separate beakers and treated with 10 mL conc. HNO3 to destroy the organic material, side by side 10 mL of mineral acid was also added in 100 mL beakers, which served as blanks for all procedure. For thermal agitation, the samples were placed on a hot plate and covered with crucible lids. The hot plate was set at 70 ±1 ˚C, which resulted in a slight boiling of the samples after heating for 2-3 hours, the temperature of hot plate, was then raised to 150±1 ˚C and then removed the crucible lids to evaporate the digestion mixture. Then 3 mL of conc. HNO3 and 5 mL of H_2O_2 were added. The H_2O_2 was added as decolorizing agent. All the digestion procedure was performed in the fume hood for the prevention of hazardous effects of HNO_3. Continued heating till complete decomposition of plant extract took place, and clear, transparent solution was obtained. The contents of beakers were cooled and then added deionized water. The solution was twice filtered through Whatman filter papers No. 42, and finally the volume was made up to 50 mL using deionized water**.**

Determination of minerals

Standards solutions of Na, K, Li, Zn, Pb, Ca, Mg, Cu, Mn and Cr, were prepared from stock standard solution (1000 mg L^{-1}), in 2 N HNO_3 and absorbance were noted for standard solution of each element. Na, K and Li were analyzed using flame photometeric analysis, while Zn, Pb, Ca, Mg, Cu, Mn, and Cr were determined using flame atomic absorption spectroscopy (FAAS). The calibration curves obtained for concentrations data were statistically analyzed using standard deviation. A blank reading was also taken and necessary corrections were made during the calculation of concentration of various elements [22].

Statistical Analysis

All the results and data obtained from this research work was analyzed by standard deviation and applying ANOVA [23].

Results and Discussion

Percentage Yield of Extracts

In the present research work, extraction of leaves of *Corchorous depressus* was done using five solvents ethanol, methanol, acetone, ether and n-hexane. Table 1 shows the percentage yield of all these extracts calculated on the basis of dry matter**.** From this table it is clear that mathanolic extract of leaves of *Corchorous depressus* gave the highest percentage yield that is 6.20%. Lowest yield was obtained from n-hexane extract of leaves of *Corchorous depressus* that is 3.06%.

Table 1: Percentage yield of extracts from leaves of *Corchorous depressus*

Extracts of leaves	%age yield
Methanol	6.20
Ethanol	6.05
Acetone	5.23
Ether	4.93
n-hexane	3.06

Antioxidant Assays

In the present research work, Antioxidant activity of leaves was assessed by adopting five assays namely Total Phenolic contents, Total Flavonoid Contents, Reducing power, DPPH radical scavenging activity and Inhibition of linoleic acid peroxidation assays.

Total Phenolic Contents

Amounts of total phenolic contents extracted from different extracts of *Corchorous depressus* are shown in table 2. This fig showed that TP varied greatly with varying solvent. This value varies from lowest amount 16.10 ± 0.06 to 41.05 ± 0.02 mg of Gallic acid equivalent per gram of dry matter. The highest amount of phenolic compounds was found in methanolic extract of leaves of *Corchorous depressus*.

Despite the higher polarity of ethanol solvent, the methanol extracts of leaves gave unexpected higher phenolic contents than those of other extracts. This may be attributed to different types of phenolic compound having different chemical compositions. On the other hand Ethanolic extracts had the higher TP than methanolic extracts. This might be due to the higher polar nature of ethanol and the polar nature of phenolic compounds found in leaves.

Table 2: Total Phenolic Contents (mg/100g) measured as GAE

Plant Part	Extracts	TPC
Leaves	Methanol	41.05 ± 0.02
Leaves	Ethanol	40.09 ± 0.07
Leaves	Acetone	29.16 ± 0.09
Leaves	Ether	21.24 ± 0.07
Leaves	n-hexane	16.10 ± 0.06

Total Flavonoids

The phenolic components of extracts from *Corchorous depressus* were measured and expressed as mg of catichin equivalent per gram of dry mass. Similar to phenolic compounds, flavonoid contents that are polyphenol group of compound vary greatly among different extracts. Table 3 showed the TF contents of extracts from *Corchorous depressus*. It was clear from the results that the highest TF were found in methanolic extract of leaves of *Corchorous depressus* i.e. 26.70 ± 0.07mg/g of CE and lowest TF contents was found in n-hexane extract leaves of *Corchorous depressus* i.e. 13.15 ± 0.08.

Table 3: Total Flavonoid Contents (mg/100g) measured as CE

Plant Part	Extracts	TFC
Leaves	Methanol	26.70 ± 0.07
Leaves	Ethanol	24.20 ± 0.06
Leaves	Acetone	23.16 ± 0.07
Leaves	Ether	20.09 ± 0.06
Leaves	n-hexane	13.15 ± 0.08

DPPH Scavenging Activity

DPPH is an organic free radical that is highly stable and gives intense violet colour. When it absorbs a proton from any hydrogen donating compound its colour faints due to loss of chromophore. DPPH radical scavenging activity enhances with the rate of hydroxylation of antioxidant compounds. Due to its sensitivity to many active species, DPPH is used to assess the antioxidant potential of plant extracts. In this research work extract of leaves of *Corchorous depressus* in ethanol, methanol, ether, acetone and n-hexane were employed to assess their antioxidant ability. In DPPH assay, BHT was used as standard. DPPH radical scavenging activity of various concentrations 20, 40, 60, 80, 100mg/mL) of these extract varies greatly. From these values inhibitory concentration IC_{50} (mg/mL) were calculated for all extracts. The half minimum inhibitory concentration IC_{50} (mg/mL) value is the minimum concentration of extract that inhibit 50% of absorbance. Higher the value of $IC_{50,}$ lower will be the DPPH radical scavenging activity of an extract. Table 4 shows the IC_{50} values of leaves of *Corchorous depressus* extracts and the standard BHT. These values vary from 11.8 ± 0.02 to 56.34 ± 0.06 (µg/mL). BHT exhibited the lowest value (76.45 ± 0.04 µg/mL) proving it a highly active free radical scavenger followed by methanol extract of leaves of *C.depressus* (56.34 ± 0.06 µg/mL), then ethanol extract of leaves (54.93 ± 0.06 µg/mL), acetone extract of leaves (42.01 ± 0.04 µg/mL), ether extract of leaves (24.23 ± 0.04 µg/mL) and n-hexane extract of leaves (11.8 ±0.02 µg/mL).

Table 4: DPPH Free Radical Scavenging Activity

Plant Part	Extracts	Scavenging Activity
Leaves	Methanol	56.34 ± 0.06
Leaves	Ethanol	54.93 ± 0.06
Leaves	Acetone	42.01 ± 0.04
Leaves	Ether	24.23 ± 0.03
Leaves	n-hexane	11.80 ± 0.02
Leaves	BHT	76.45 ± 0.04

Percentage inhibition of Peroxidation in Linoleic Acid system

The range of %age inhibition in linoleic acid system for leaves appeared in the range of 42.12% to 49.51%. In the present work, antioxidant activity of *Corchorous depressus* extracts was assessed in linoleic acid system by inhibition of lipid peroxidation adopting the ferric thiocyanate method. During linoleic peroxidation, peroxides are formed. These oxidizes ferrous ion into ferric ion which upon forming complex with SCN^- give absorbance. Higher the absorbance, higher will be the peroxide formation. Table 5 showed the result of extracts of leaves peroxidation in linoleic acid system.

BHT was also used as standard in this assay. Higher the absorbance, higher the peroxide formation and lower the antioxidant activity of the extract. In figure, value of BHT is higher even after 72 h incubation. Ethanol extract of leaves and methanol extract of leaves showed somewhat similar antioxidant potential. While all other extracts showed different absorbance. In the present research work, extracts of leaves of *corchorous depressus* showed antibacterial methanol extract inhibition of zone of 49.51 ± 0.08 mm. It is the maximum antibacterial effect shown by methanol extract of leaves.

Table 5: % age Inhibition of Peroxidation in linoleic Acid System

Plant Part	Extracts	% age Inhibition
Leaves	Methanol	49.51 ± 0.08
Leaves	Ethanol	49.06 ± 0.09
Leaves	Acetone	46.11 ± 0.08
Leaves	Ether	26.70 ± 0.08
Leaves	n-hexane	42.12 ± 0.07
Leaves	BHT	94.06 ± 0.89

Antimicrobial assay of Plant Extracts

Antibacterial activity of leaves of *Corchorous depressus* was assessed by adopting Disc Diffusion method. Refimpicine is used as standared that exhibit good antibacterial activity. In the present research work, extracts of leaves showed different antibacterial activity. The inhibition zone of *E. Coli, P. Multocida, S. Subtilus and S. Aureus* in methanol extract 17.23 ± 0.09mm, 18.23 ± 0.03mm, 16.63 ± 0.34mm and 12.84 ± 0.04mm. The inhibition zone of *A. Flavus, A. Niger, R. Solani, F. Solani* in methanol extract 0.187 ± 0.008mm, 0.194 ± 0.008mm,0.059 ± 0.005mm, 0.091 ± 0.007mm. The maximum inhibited zone was 22 ± 0.64mm by methanol extract of leaves of *Corchorous depressus*.

Table 6: Antifungal Activity by (ZID)

Plant Part	Extracts	A. Flavus	A. Niger	R. Solani	F. Solani
Leaves	Methanol	17.43 ± 0.11	18.63 ± 0.49	14.63 ± 0.011	11.61 ± 0.07
Leaves	Ethanol	15.66 ± 0.17	14.19 ± 0.37	13.99 ± 0.13	11.23 ± 0.06
Leaves	Acetone	15.23 ± 0.23	11.66 ± 0.19	11.02 ± 0.19	9.11 ± 0.07
Leaves	Ether	13.49 ± 0.19	10.23 ± 0.17	6.06 ± 0.14	6.84 ± 0.09
Leaves	n-hexane	9.26 ± 0.07	7.29 ± 0.21	5.16 ± 0.16	6.17 ± 0.06
Standard	Terbinafine	21.63 ± 0.07	19.51 ± 0.9	17.16 ± 0.09	17.20 ± 0.24

Table 7: Antibacterial Activity by (ZID)

Plant Part	Extracts	E. Coli	P. Multocida	S. Subtilus	S. Aureus
Leaves	Methanol	17.23 ± 0.09	18.23 ± 0.03	16.63 ± 0.34	12.84 ± 0.04
Leaves	Ethanol	14.21 ± 0.03	16.47 ± 0.05	15.94 ± 0.47	12.39 ± 0.04
Leaves	Acetone	9.29 ± 0.07	13.39 ± 0.11	15.24 ± 0.41	11.90 ± 0.05
Leaves	Ether	8.61 ± 0.07	11.24 ± 0.17	13.91 ± 0.43	10.47 ± 0.05
Leaves	n-hexane	7.69 ± 0.05	11.09 ± 0.19	1.24 ± 0.09	8.97 ± 0.03
Standard	Refimpicine	22.90 ± 0.66	24.19 ± 0.63	20.63 ± 0.62	21.62 ± 0.61

Table 8: Antifungal Activity by MIC (mg/mL)

Plant Part	Extracts	A. Flavus	A. Niger	R. Solani	F. Solani
Leaves	Methanol	0.187 ± 0.008	0.194 ± 0.008	0.059 ± 0.005	0.091 ± 0.007
Leaves	Ethanol	0.147 ± 0.008	0.187 ± 0.006	0.047 ± 0.005	0.087 ± 0.009
Leaves	Acetone	0.16 9± 0.006	0.141 ± 0.004	0.049 ± 0.007	0.063 ± 0.006
Leaves	Ether	0.16 ± 0.007	0.139 ± 0.005	0.044 ± 0.063	0.055 ± 0.009
Leaves	n-hexane	0.23 ± 0.003	0.67 ± 0.009	0.05 ± 0.001	0.24 ± 0.006
Standard	Terbinafine	0.212 ± 0.019	0.202 ± 0.017	0.196 ± 0.014	0.191 ± 0.018

Table 9: Antibacterial Activity by MIC (mg/mL)

Plant Part	Extracts	E. Coli	P. Multocida	S. Subtilus	S. Aureus
Leaves	Methanol	0.063 ± 0.01	0.047 ± 0.03	0.054 ± 0.019	0.059 ± 0.05
Leaves	Ethanol	0.061 ± 0.01	0.043 ± 0.01	0.049 ±0.018	0.056 ± 0.009
Leaves	Acetone	0.044± 0.03	0.039 ± 0.07	0.047 ± 0.07	0.053 ± 0.007
Leaves	Ether	0.036 ± 0.04	0.061 ± 0.01	0.026 ± 0.009	0.051 ± 0.003
Leaves	n-hexane	0.032 ± 0.01	0.037 ± 0.07	0.021 ± 0.07	0.033 ± 0.017
Standard	Refimpicine	0.88 ± 0.009	0.66 ± 0.017	0.61 ± 0.016	0.64 ± 0.009

Table 10: Metals Present in Leaves and their Concentration (ppm)

Metals	Concentration in ppm
Pb	0.01
Zn	1.5
Cu	0.055
Ni	0.005
Co	0.025
Fe	9.13

CONCLUSION

It is concluded from the result and discussion that *Corchorous depressus* exhibited considerable antioxidant and antimicrobial activity as all the tests including DPPH, total flavonoid, total phenolics, %age inhibition in linoleic acid system and reducing power analysis were performed. Different antioxidant parameters showed that plant possessed high antioxidant potential. Methanolic extracts of leaves of plant showed more effective antioxidant activity than ethanol extracts. Different extracts of leaves of plant also exhibited good antimicrobial activity. Different metals e.g. Pb, Zn, Cu, Ni, Co and Fe are also detected. Hence it is said that leaves of *Corchorous depressus* revealed substantial bioactivity.

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