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PRELIMIARY PHYTOCHEMICAL ANALYSIS AND ANTIFUNGAL ACTIVITIES OF CRUDE EXTRACTS OF *ZALEYA PENTANDRA* AND *CORCHORUS DEPRESSUS* LINN.

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Abstract: *Zaleya pentandra* (*Zp*) and *Corchorus depressus* Linn. *(Cd)* have been considered as herbs with potential therapeutic benefits. *Zp* and *Cd* belong to the important family Aizoaceae and Tiliaceae, respectively.The extractions were carried out successively with methanol and dichloromethane at room temperature for 24 h. Preliminary phytochemical screening of *Zp* and *Cd* revealed the presence of steroids, alkaloids, saponins, and anthraquinones. The methanolic and dichloromethane extracts of selected plants were subjected to examination of antifungal activity by using agar tube dilution. The extracts were tested against different fungi such as *A. niger, A. flavus, F. solani, A. fumigatus* and *Mucor.* The dichloromethane extract of aerial parts of *Cd* showed high antifungal activity against *A. niger as* compared to all other tested extracts.

Keywords: *Zaleya pentandra*, *Corchorus depressus* Linn. Aizoaceae, Tiliaceae

The page of human history reveals that human beings irrespective of ethnicity or belonging to any civilization have been utilizing herbs as medicine for the purpose of healthcare (1). Medicinal plants are of great value in the mitigation and cure of diseases. Over the years, scientific research has advanced our knowledge of medicinal plants and new drugs (2).

In the Western world, as people are becoming aware of the potency and side effects of synthetic drugs, there is an increasing interest in plant-based medications. The future development of the pharmacognostic analysis of herbal drugs is largely dependent upon reliable methodologies for correct identification, standardization and quality assurance of herbal drugs (3).

In plants, defence system - natural active compounds perform a major role. These compounds are also known for the physiological action on human body. Primary metabolites in plants are: amino acids, sugars, proteins and chlorophyll. The secondary metabolites, like flavonoids, saponins, are very important therapeutic agents and are becoming very important part of the integrative health care system as alternative medicines. Infectious diseases - the main threats to the public health, are still due to the bacteria, viruses and fungi. In developing nations, this factor is prominent due to unavailability of the medicines. The second reason for the greater impact is drug resistance of microorganisms. Due to the factors mentioned there is the need for the discovery of new antimicrobial compounds (4).

Zaleya pentandra is widely distributed prostrate and branched herb. A genus of about 6 species found in Africa, Asia, Australia has only one specie, *Zp*, found in Pakistan. The plant has been used as astringent while locally used against malaria and snake bite. Previously, it was reported against influenza and phlegmatic cough (5). This plant has been reported to have antifungal, antiulcer, antisecretory, antivenom activity (6) and as cytoprotective (6, 7). Phytochemical evaluation of *Zp* showed the presence of phytosterolins (8). Another medicinal herb *Corchorus depressus* Linn. (*Cd*), commonly known as Boa-phalee, belongs to the family Tiliaceae. This family has 50 genera and 450 species which are distributed in tropical and temperate regions of mainly South Asia and South America. In Pakistan, about four genera and 24 species are found; *Cd* is included among cultivated species. *Cd*

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is used as a traditional medicine for the ailment of aches, dysentery, enteritis, fever and tumors (9). The genus Corchorus has been reported for anticancer and cardiac activity in Indian medicinal system. The infusion of leaves is a demulcent, laxative, carminative, stimulant, appetizer and tonic. *Cd* has been reported as analgesic and antipyretic (10). Literature survey of this plant revealed phytochemical constituents: triterpenoids, sterols (11) and flavonoids (12) reported from chloroform extract of *Cd. pentandraone,* a novel compound isolated from *Zp* (13). Plant is reported as antimalarial (14), antifungal and antibacterial (11, 15). Keeping in view above mentioned literature, it is obvious that both medicinal plants have great therapeutic potential and are available in ample amount by cultivation. The objective of this study was to validate the *Cd* and *Zp* plants against different strains of fungi by using different parts of plants in different types of solvents and preliminary phytochemical analysis of these emerging therapeutic agents .

MATERIALS AND METHODS

The plants *Cd* and *Zp* were collected from Peruwal (District Khanewal). The plants were identified by Prof. Dr. Altaf Ahmad Dasti, Plant Taxonomist, Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan, where their voucher specimens fl. p. 472/4 for *Cd* and fl. p. 235/5 for *Zp* were deposited.

Extraction

The shade-dried aerial parts and root part of *Cd* (1000 g) and aerial parts of *Zp* (1000 g) were subjected to extraction successively with dichloromethane and methanol (3 × 6 L), respectively, at room temperature with occasional shaking for 24 h. Extracts were concentrated by Rotavapor-R200 at 35°C. The methanolic and dichloromethane extracts of selected plants were collected in separate sample bottles labelled with different codes. The results are depicted in Table 1.

Antifungal assay

To study antifungal activity, we followed agar tube dilution method. It was according to the protocol of Duraipandiyan and Ignacimuthu (16) to determine this activity of plant extracts. In order to grow fungus we used potato dextrose agar (PDA -Merck) media and it was used for inoculum preparations. It was composed of agar 15 g/L, dextrose 20 g/L and potato infusion 4 g/L, approximately. For media preparation for fungus, we dissolved 9.75 g of potato dextrose agar (PDA) in 250 mL of distilled water and then autoclaved the PDA media. The samples (15 mg/mL) were prepared and got concentration (250 µL/mL) for antifungal assay. Nystatin 100,000 units/mL was used as positive control while DMSO was utilized as negative control.

Then, PDA media were dispensed as 4 mL volumes into autoclaved cotton plugged and screw capped test tubes and these were marked to 10 cm mark. These test tubes were cooled up to 50°C and these media were seeded with 67 µL of sample from stock solution of plant extracts with the help of pipette. In this way we got a final concentration of 250 µg/mL. Test tubes were then placed in slanting position and allowed to solidify at room temperature. A 4 mm part of fungus inoculum was subjected to inoculation in the tubes with solidified media and the test sample. Positive test tubes and negative test tubes having nystatin and DMSO, respectively, were inoculated with fungus. After that these test tubes were put in incubator at 30°C for 48 h. Then, linear growth of fungus was calculated in the slant. As far as growth inhibition was required, it was measured with reference to negative control of DMSO. The experiment was performed in triplicate for each fungus and sample. The percentage inhibi-

Table 1. Results of the extraction of the plants *Zaleya pentandra* and *Corchorus depressus*.

Plant name	Part used	Solvent	Weight of extract (g)	Abbreviation for the extracts
Corchorus depressus	Aerial parts (1000 g)	Dichloromethane	39.85	CDD
		Methanol	7.95	CDAM
Corchorus depressus	Roots (1000 g)	Dichloromethane	9.4	CDRD
		Methanol	17.84	CDRM
Zaleya pentandra	Aerial parts (1000 g)	Dichloromethane	33	ZPAD
		Methanol	35	ZPAM

tion of fungal growth was calculated by applying the formula given as follows:

$$\% \text{ inhibition of fungal growth} = \frac{10 - \text{Linear growth in test (cm)}}{\text{Linear growth in control (cm)}} \times 100$$

Detection of various classes of secondary metabolites

Phytochemical studies were carried out for the detection of alkaloids, glycosides and saponins in different parts of the plant *Cd* and Zp. These tests were performed as preliminary basis to identify various classes of metabolites.

Detection of alkaloids

Ten grams of the ground plant material was boiled with 10 mL of acidified water in test tube for 1 min, cooled, and then allowed the debris to settle. The supernatant liquid was filtered into another test tube, 1 mL of this filtrate was taken and 3 drops of Dragendorff reagent were added, there was no precipitate. The remainder of the filtrate was made alkaline by addition of dilute ammonia solution. Five milliliters of chloroform was added to the solution in separating funnel and two layers were observed. The lower chloroform layer was separated out into another test tube. Chloroform layer was extracted by addition of 10 mL of acetic acid and then chloroform was discarded. Then, the extracts were divided into three portions, to one portion few drops of Dragendorff reagent and to second few drops of Mayer's reagent were added. Turbidity or precipitate was compared with the third untreated control portion (16).

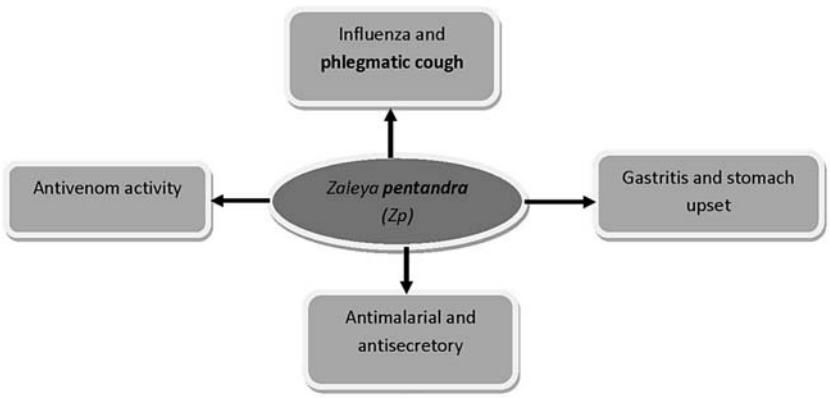

Figure 1. Reported pharmacological activities of *Zaleya pentandra*

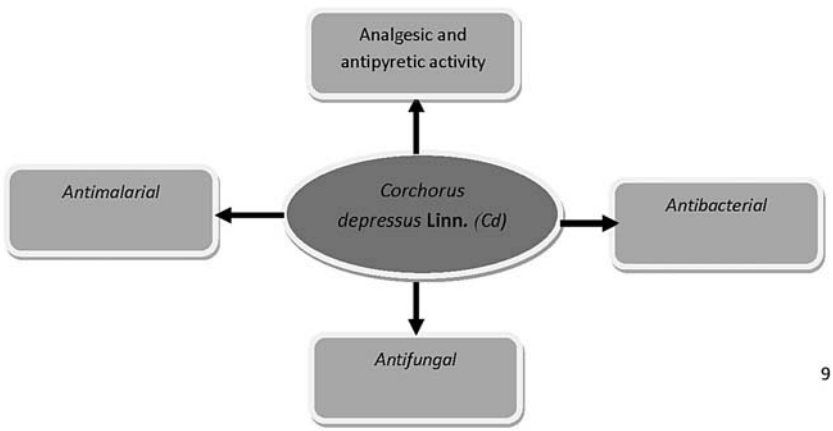

Figure 2. Reported pharmacological activities of *Corchorus depressus* Linn.

Detection of anthraquinone glycosides

Borntragers test

Two grams of powdered plant material were taken and extracted with 10 mL of hot water for 5 min, allowed to cool and filtered; the filtrate was extracted with 10 mL of carbon tetrachloride. Then, carbon tetrachloride layer was taken off, washed with 5 mL of water and then 5 mL dilute ammonia solution was added. No free anthraquinones were revealed as there was no appearance of pink to cherry red color in the ammoniacal layer.

Two grams of second sample of the plant material was extracted with 10 mL of ferric chloride solution and 5 mL of hydrochloric acid by heating it on water bath for 10 min and then filtered. The filtrate was cooled and treated as above (17).

Detection of cardioactive glycosides

Keller Kiliani test

One gram of ground plant material was taken in a test tube and 10 mL of 70% alcohol was added. It was then boiled for 2 min and filtered. The filtrate was diluted twice of its volume with water and then 1 mL of strong lead acetate solution was added. This treatment leads to the precipitation of chlorophyll and other pigments, which were then filtered off. The filtrate was extracted with an equal volume of chloroform. Chloroform layer was separated out and evaporated to dryness in a dish over a water bath. The residue was dissolved in 3 mL of 3.5% ferric chloride in glacial acetic acid and was transferred to test tube after leaving for 1 min. Sulfuric acid (1.5 mL) was then added, which formed a separate layer at the bottom. Cardioactive glycosides were revealed by the appearance of brown color at interface (due to deoxysugar) on standing and appearance of pale green color in the upper layer (due to the steroidal nucleus) (17).

Detection of saponins glycosides

For the detection of saponin, standard procedure for identification of plant constituents was adopted from previously reported methodology (18). Three grams of each extract was extracted with 300 mL of hot distilled water. After extraction, extracts were stored at 4^{o}C. Approximately 5 mL of each extract was diluted with equal volume of distilled water, put in a beaker and shaken vigorously for 2 min. For this test 0.5 g of powdered drug were taken in test tube and shaken with water. Persistent foam indicated the presence of saponins. Persistent foam formation, with the addition of olive oil and lasting for minimum 15 min confirmed the presence of saponins

RESULTS AND DISCUSSION

Medicinal plants have been studied extensively from pharmacological and pharmaceutical chemistry point of view to isolate and identify novel therapeutic compounds. As shown in Figures 1 and 2, substantial studies are required to endorse these medicinal plants as therapeutic remedy in different ailments. An effort was made to evaluate antifungal activities and preliminary phytochemical analysis of two medicinal plants of Pakistan *Cd* and *Zp*. Five strains of fungi *A. niger, A. flavus, F. solani, A. fumigates and Mucor* were selected to evaluate the antifungal activity of different extracts of these plants. Different parts of both plants were selected and different solvents were used to find out most active fraction of plants.

The shade-dried aerial parts and root part of *Cd* and aerial parts of *Zp* were subjected to extraction successively with dichloromethane and methanol at room temperature with occasional shaking for 24 h. The extracts were concentrated under reduced pressure. Then, they were collected in sample bottles labelled with different codes.

Phytochemical screening of the selected plants revealed the presence of alkaloids, steroids, anthraquinones and saponins (Table 2). The methanolic and dichloromethane extracts of selected plants were subjected to estimation of antifungal

Table 2. Results of phytochemical screening of *Corchorus depressus* and *Zaleya pentandra*.

Name of plant	Alkaloids	Anthraquinones	Cardiac glycosides	Saponins
Corchorus depressus	+	-	+	+
Corchorus depressus (root part)	+	-	+	+
Zaleya pentandra	+	-	+	+

+ = present; - = absent

Table 3. Results of antifungal activities of dichloromethane and methanol extracts of *Corchorus depressus* and *Zaleya pentandra* (Growth inhibition of fungal strains after 48 h).

Plant extract	Percentage inhibition of fungal growth of				
	A. niger	*A. flavus*	*F. solani*	*A. fumigates*	*Mucor*
ZPAD	89.70	0	49.07	0	0
ZPAM	47.02	0	34.13	6.48	88.47
CDRD	43.39	87.82	64.88	19.70	49.13
CDRM	87.31	77.59	55.31	12.33	55.07
CDAM	61.20	89.24	24.78	6.94	77.70
CDAD	90.08	51.24	29.86	3.32	0
P Control	117.45	113.88	102.68	109.42	106.44
N Control	27.61	21.70	12.35	19.82	14.64

P Control = Positive control (standard); N Control = Negative control (standard).

Table 4. Results of antifungal activities of dichloromethane and methanol extracts of *Corchorus depressus* and *Zaleya pentandra* (Growth inhibition of fungal strains after 72 h).

Plant extract	Percentage inhibition of fungal growth of				
	A. niger	*A. flavus*	*F. solani*	*A. fumigates*	*Mucor*
ZPD	59.01	0	27.58	0	0
ZPAM	28.96	0	1.26	0	64.45
CDRD	35.53	67.28	30.98	0	24.43
CDRM	59.12	51.31	18.46	0	22.83
CDAM	52.63	65.49	0	0	55.29
CDAD	73.04	27.70	0	0	0
P Control	102.16	96.74	89.03	97.15	96.39
N Control	14.62	5.26	0	9.63	5.88

P Control = Positive control (standard); N Control = Negative control (standard).

activity by using agar tube dilution. The antifungal activity of the plant extracts were determined against the five different fungi named above (Tables 3, 4).The dichloromethane extract of *Zp* and methanolic extract of *Cd* of root part and dichloromethane extract of *Cd* of aerial part actively inhibited the growth of fungus *A. niger* and the maximum growth was 3.15 cm with these extracts. *A. flavus* showed maximum growth inhibition with dichloromethane and methanolic extract of root and aerial parts of *Cd*. The growth with methanolic extract of root part of *Cd* was 3.63 cm. In the presence of plant extract, dichloromethane extract of root part of *Cd* against *F. solani* showed little growth (4.22 cm.) The growth of *Mucor* with methanolic extract of *Zp* and methanolic extract of *Cd* weres 2.3 cm and 3.2 cm, respectively. The plant extracts also showed growth inhibition after 72 h of incubation .The dichloromethane extract of *Zp*, methanolic root part and dichloromethane extract of *Cd* showed 80% or more growth inhibition against *A. niger.* It has been reported that *A. niger* is the cause of pulmonary infection (19).

Similarly, methanolic extract of aerial part and dichloromethane extract of root part of *Cd* showed more than 80% growth inhibition against *A. flavus*. It has been reported that *A. flavus* cause alatoxicosis (20). The methanolic extract of *Zp* showed more than 80% growth inhibition against *Mucor* after 48 h. This extract showed more than 55% growth inhibition after 72 h. Interestingly, in the case of *A. fumigatus,* no significant growth inhibition was observed. The methanolic extract of root parts showed 55% and dichloromethane extract of *Cd* showed 64% growth inhibition respectively, against fungus *F. solani*.

CONCLUSION

Among all the solvents and plant parts, methanolic extract of aerial part of *Zp* showed the highest percentage of growth inhibition against all strains of fungi except *A. flavus,* whereas methanolic extract of aerial parts of *Cd* showed greater percentage of growth inhibition against all strains of fungi. Both plants have been proved as potential antifungal agents.

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