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Phytochemical Screening and Biological Activities of Different Parts of *Centaurea montana*

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Abstract

The crude extracts and its derived fractions {n-hexane, chloroform, ethyl acetate, n-butanol and residual aqueous fraction} of rhizome, leaves and flowers of medicinal plant *Centaurea* were subjected to microbicidy against *Vibrio cholerae* (ATTC9459), *Salmonella typhe* (ATTC700931), *Acinitobacter baumanni* (ATTC17978), *Shigella dysenteriae* (ATTC13313), *Bacillus anthrax* (ATTC14578), *Moraxella lacunata* (ATTC17967), *Penicillium chrysogenum* (ATTC28089), *Candida albicans (ATTC2876), Aspergillus fumigatus (ATTC3626)* and evaluation of chemical profile. The results revealed that *n* - hexane fraction obtained from rhizome of the plant showed outstanding (87.40% inhibition) antibacterial activity against *Moraxella lacunata* followed by crude extract of rhizome against *Acinitobacter baumanni* showing 82.50% inhibition. The crude extract and its derived fractions obtained from leaves of the plant showed moderate inhibition against all tested bacterial strains. The crude extracts/fractions obtained from the flowers of the plant were found inactive against all tested bacterial and fungal strains. Surprisingly none of the crude extracts/ fractions of the plant showed antifungal activity against the three tested fungal pathogens. Preliminary phytochemical screening of plant showed that rhizome and leaves are the rich source of fatty acids, flavonoids, alkaloids and glycoside.

Keywords

Medicinal Plants, *Centaurea montana,* Asteraceae, Antibacterial Activity, Antifungal Activity, Phytochemical Screening

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1. Introduction

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. These phytochemicals also defend plants against the attack from predators such as insects, fungi and herbivorous mammals. The medicinal properties of plants have been explored in the light of up-to-date scientific expansion all over the world, due to their exciting pharmacological activities and little toxicity [Sharma *et al*., 1992; Vaquero *et al*., 2010]. Medical plants are extensively used in the cure of different diseases. Plant extracts and their diverse formulations in the healing and reduction of several diseases in folk remedy have been dated back to the primeval times. Further, some natural products also exist in vegetables, fruits and beverages [Ozturk and Ercisli 2006]. Natural products from medicinal plants are known to be chemically balanced, effective and least injurious with none or much

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reduced side effects as compared to synthetic medicines. As described of World Health Organization (WHO), in subcontinent, plant producing medicines have been used widely since long time [Shinwari *et al*., 2006]. According to assessment accomplished by WHO, traditional healers treat 65% patients in Srilanka, 60% in Indonesia, 75% in Nepal, 85% in Mayanmer, 80% in India, and 90% in Bangladesh. In Pakistan, 60% of the people, particularly in villages are receiving health care by traditional practitioner [Ahmed *et al*., 2004]. Ethnobotany is recognized as an effective way to discover future medicines.

The genus *Centaurea* is a member of family Asteraceae with 350 to 600 species. *Centaurea montana* (white perennial cornflower) is a deciduous, rhizomatous, mat-forming plant growing up to height of 40cm. The plant is found in sandy rocky soil. Flowering season is from June to August (Nasir 1990). The plant is medicinally used as astringent, purgative, ophthalmic, emmenagogue, tonic, diuretic and antitussive (Stuart). Locally the plant is used for the washing of eyes.

2. Materials and Methods

2.1. Plant Collection

The plant was collected in 2014 from District Karak Khyber Pakhtunkhwa, Pakistan. The plant was botanically identified by the Curator, Department of Botany, Kohat University of Science and Technology with the help of available literature. A voucher specimen (accession #1302) was deposited at the herbarium of the department.

2.2. Extract Preparation

The fresh plant parts roots (4.5 kg), leaves (4 kg), and flowers (2.5 kg) were collected and shade dried which were later coarsely powdered in a Willy Mill to 60-mesh size and used for solvent extraction. For sample preparation dried powdered samples were extracted thrice with methanol at room temperature for 21 days and concentrated using a rotary evaporator under reduced pressure to yield the crude extracts. The residue (crude extract) was suspended in distal water and partitioned successively with n-hexane, chloroform, ethyl acetate, n-butanol and soluble residual aqueous fraction yielding respective fractions.

2.3. Microbiological Assay

For antimicrobial activities agar diffusion method was used as described by Shinwari *et al.,* (2013) and Khan *et al.,* (2011). In this method, the bacterial inoculums was uniformly spread using sterile cotton swab on a sterile Petri plate MH agar (0.5 MF). Wells were made in petriplates (90 mm in diameter), the required concentration (20 gm/ml) of stock solution of each extract/fraction were poured in these wells (6 mm diameter holes cut in the agar gel, 20 mm apart from one another) and after incubation of 24 hours, the inhibition zones were observed around the wells which were measured and compared with the zones made around the standard antibiotic (Chloramphenicol 20 mg/mL) used. The concentration of media used was kept 9.5g/250mL.

2.4. Phytochemical Screening

Phytochemical screening of crude extracts/fractions of different parts of our research plant was carried out for the presence of fats, alkaloids, cardiac glycosides, flavonoids, saponins, Terpenes and phenolic compounds as per established protocol (Prabhu, 2009).

3. Results

The crude extract and its derived fractions obtained from the root of the plant were subjected to antibacterial and antifungal activities. The results revealed that *n*-hexane fraction of the root showed outstanding inhibition that is 87.40% against *M. lacunata* followed by 82.50% inhibitions by crude extract against *A. baumanni.* The crude extract also showed good activities against *V. cholerae* (78.35% inhibition) and *M. lacunata* (71.50% inhibition). None of the fraction obtained from root of the plant showed activities against the last three fungal pathogens (Table 1). It is been observed that extract and its derived fractions obtained from the leaves of the plant showed moderate activities (60% inhibition) against all tested bacterial pathogens while found inactive against last three fungal pathogens (Table 2). The extract/fraction obtained from the flowers of plant was found inactive against all pathogens.

Present study explored the naturally occurring substances such as fatty acids, alkaloids, flavonoids, Phenolic compound, saponins, tannins and glycosides in various extracts/fractions of plant samples (Table 3 and Table 4). Methanol extracts/fractions of the tested plant samples showed maximum number of plant constituents.

4. Discussions

Hence synthetic drugs are quite expensive and have associated side effects therefore the development of new effective and safe products for the treatment of different diseases caused by human pathogens is highly desirable [Victor *et al.,* 2004]. The plant under investigation showed significant biological activities which support the traditional use of the plant to treat various diseases. Therefore this plant species could be an excellent natural source for the treatment of diseases and might be potential targets for the activity

guided isolation of its active constituents.

Antibacterial activities of our selected plants may be, because of the presence of the fatty acid esters in the extract/fractions. Preethi *et al.,* (2010) suggested that plants having fatty acid esters in their extract are more potent antimicrobial agents. Similar type of finding is illustrated by Gohar *et al.,* (2010) in characterization of marine antibacterial agents, where they isolated hexadecanoic acid ethyl ester and other components from marine bacteria and found out their antibacterial potential against different pathogens. Although they observed antibacterial activity of isolated compound but at the same time reported that activity of crude ethanol extract was more than isolated compounds.

Chemical substances of plants are the real cause of the medicinal value of plants and they produce their effects by interacting with human physiology [Hill, 1952]. Biological activities of plant extracts/fractions are due to the presence of major phytocompounds, including terphinoids, fatty acids, carotenes, phenolics, alkaloids, glycosides, flavonoids, tannins [Aqil *et al*., 2006].

Bioactive substances possess anti- disease potential particularly minimizes the risk of oxidative injuries [Etuk *et al*., 2009]. In literature, many medicinal plants indicated their strength through antimicrobial, cytotoxic and antioxidant behavior that was endorsed with high concentration of flavonoids and alkaloids [Miller and Rice-Evans, 1997; Sharififar *et al*., 2009]. A combination of chemical screening with biological screening is the fastest way to explore new lead compounds from plants. For many plants there is no relevant literature available so, biological activity must be evaluated using more direct methods such as pharmacological testing or screening. Generally, extracts must be active in at least one of the bioassays adopted in the screening to be used for further studies.

5. Conclusion

The present study supports the tested medicinal plants as big source of bioactive chemicals specifically with reference to phenolic compounds, fatty acids, alkaloids, flavonoids, saponins and glycosides that ought to be isolated and monitored for biological activities as reported in traditional and therapeutic utilization. Summing preliminary screening tests, it is believable that detection of bioactive secondary metabolites may lead to the drug discovery and development.

Table 1. Bacterial Inhibition (in Percentage) of Crude extract/fractions of *Centaurea* Root.

Pathogens	Crude extract	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Aqueous	Antibiotic drug
V. cholerae	78.35	30.40	43.10	54.20	30.40	15.10	94.40
S. typhe	35.40	20.50	45.30	43.10	30.10	13.50	92.40
A. baumanni	82.50	57.30	48.20	43.40	36.20	18.40	89.50
S. dysenteriae	60.20	56.30	45.50	50.10	32.40	20.50	93.40
B. anthrax	60.20	60.50	43.50	55.30	32.40	19.30	94.50
M. lacunata	71.50	87.40	60.10	62.40	34.50	39.50	93.30
P. chrysogenum	Nil	Nil	Nil	Nil	Nil	Nil	88.50
C. albicans	Nil	Nil	Nil	Nil	Nil	Nil	90.40
A. fumigatus	Nil	Nil	Nil	Nil	Nil	Nil	88.50

Table 2. Bacterial Inhibition (in Percentage) of Crude extract/fractions of *Centaurea* Leaves

Pathogens	Crude extract	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Aqueous	Antibiotic drug
V. cholerae	60.30	39.20	47.40	38.10	33.60	19.30	95.10
S. typhe	55.20	40.40	42.50	42.50	36.20	18.50	93.20
A. baumanni	55.50	50.20	41.50	45.10	34.50	16.50	91.10
S. dysenteriae	61.20	51.20	50.30	53.40	37.20	19.50	92.50
B. anthrax	57.50	54.40	49.40	59.20	36.30	22.50	93.40
M. lacunata	58.30	60.30	61.30	60.50	37.30	31.20	92.50
P. chrysogenum	Nil	Nil	Nil	Nil	Nil	Nil	89.10
C. albicans	Nil	Nil	Nil	Nil	Nil	Nil	89.40
A. fumigatus	Nil	Nil	Nil	Nil	Nil	Nil	90.10

Table 3. Preliminary Phytochemical Profile of *Centaurea* Root.

S. No	Phytochemical Tests	Methanol	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Water fraction
1	Phenolic compound	+	+	+	+	-	-
2	Terpenes	+	+	+	+	+	-
3	Flavonoids	+	+	+	+	-	-
4	Alkaloid	+	+	+	+	+	+
5	Saponins	+	+	-	-	+	+
6	Glycosides	+	+	+	+	+	+
7	Fats	+	+	+	+	+	-

+ = Present - = Absent

Table 4. Preliminary Phytochemical Profile of *Centaurea* Leaves.

S. No	Phytochemical Tests	Methanol	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Water fraction
1	Phenolic compound	+	+	+	-	-	-
2	Terpenes	+	+	+	+	+	-
3	Flavonoids	+	+	+	-	-	-
4	Alkaloid	+	+	+	+	-	-
5	Saponins	+	+	-	-	-	-
6	Glycosides	+	+	+	+	+	-
7	Fats	+	+	+	+	-	-

+ = Present - = Absent

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