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Antimicrobial, Antioxidant and Phytochemical Analysis of *Herniaria hirsuta*

Ghias Uddin[1], Jawad Ali*[2] and Sania Feroz[1]

[1]*Institute of Chemical Sciences, University of Peshawar, K.P.K Peshawar-25120, Pakistan*

[2] *Department of Chemistry, University of Swabi, Anbar, 23561, Khyber Pakhtunkhwa, Pakistan*

Abstract

This study examined to explore *Herniaria hirsuta* for the presence of secondary metabolites and pharmacological profile of crude extract. *H. hirsuta* was evaluated for phytochemical screening, antibacterial, antifungal and antioxidant activities. The phytochemical screening explored the presence of tannins, saponins, steroids, coumarins and betacyanins. Antimicrobial activities were performed against selected bacterial strain. The crude extract exhibited promising antibacterial effect, while low antifungal effect at 22mg/Ml. The crude extract and isolated fractions was also screen for free radical scavenging activity using DPPH the tested samples showed good anti-radical activity against Quercetin. In conclusion *H. hirsute* should be used as a good source of antimicrobial and antioxidant molecules.

Key words:*Herniariahirsuta*, Phytochemical screening, Antibacterial, Antifungal, Antioxidant activity.

**Corresponding author address:* Jawad Ali

Department of Chemistry, University of Swabi, Anbar, 23561, Khyber Pakhtunkhwa, Pakistan

E-mail:Jawad.ali719@gmail.com

1. Introduction

Herniaria hirsuta is a yearly, prostrate and spread herb of family Caryophyllaceae. Its stem and branches are 2- - 4 mm long with internodes and short hairs. Leaves are inverse and sessile, secured with solid short hairs. Blossoms are green and sessile, 1-1.5 mm long thickly secured with short, hardened, spreading hairs. Blossoms are pentamerous with 5 sepals, 5 petals and 3-5 stamens containing minute fibers and ovoid anthers. Its natural product is papillose close to the peak. Plant has minute, ovoid, tanish seeds. *H. hirsuta*is for the most part found in Belgium, France, Spain, Italy, Switzerland, Austria, Germany, Poland, Hungary, Yugoslavia, Greece, Turkey, Cyprus, Iran, Afghanistan, India and Pakistan particularly in Kashmir. *H. hirsuta* contains saponins, glycosides, herniarins and an alkaloids paronychin. It is utilized as grub for steers and camels. In Morocco, H. hirsuta is utilized for treatment of kidney stones [1].

Investigations on rats demonstrate that the concentrates of the plant coat the calcium oxalate precious crystals and avert them to store on kidney tissue [2] and keep renal epithelial cells from grip of these crystals [3]. The present examination manages the preliminary phytochemical screening, antimicrobial and antiradical action of *H. hirsuta.*

2. Materials and methods

2.1 Plant Material

The whole plant with stem, leaves and roots was collected form Takht Bhai, Mardan.The identification of plant was done by Ghulam Jelani, Department of Botany, University of Peshawar, Pakistan.

2.2 Extraction

The entire plant was absorbed methanol for 4 days. After 4 days, the methanolic extract was obtained by evaporating methanol by rotary evaporator. The obtained extract was concentrated and further used for activities.

2.3 Phytochemical Profiling

The crude concentrate was progressed to phytochemical tests to perceive bioactive secondary metabolites by utilizing standard techniques [4-10].

Alkaloids: 500 mg of crude extract was warmed with 2% H_2SO_4 for couple of minutes. The reaction mixtures were set to cool down and stained and added some drops of Dragendroff's reagent to each stained mixture. Orange red precipitate is the sign for the existence of alkaloids.

Tannins: 1000 mg of each extract was dissolved in water and heated on water bath and stained. Small amount of ferric chloride was added to each of the filtrate drop wise. A dark green solution shows the existence of tannins.

Anthraquinones: 500 mg of each extract was mixed with 10% HCl and boiled for few minutes on water bath. The reaction mixtures were allowed to cool down and stained. Equivalent quantity of $CHCl_3$ was added to each filterate. Small quantity of 10% ammonia was added to each mixture drop wise and heated. Appearance of rose pink color justifies the presence of anthraquinones.

Glycosides: Each extract was hydrolyzed with HCl and neutralized with NaOH solution. Fehling's solution A and B were added to each mixture drop wise. Appearance of red precipitate signifies the presence of glycosides.

Reducing Sugars: Each extract was dissolved in distilled water and shacked well and filtered. The filterates were boiled with few drops of Fehling's solution A and B for few minutes. Formation of orange red precipitates signifies the presence of reducing sugars.

Saponins: About1 ml of each extract was dissolved in 3 ml of distilled water and shacked well and heated till it boils. Frothing signifies the existence of saponins.

Flavonoids: 0.2 ml of each extract was dissolved in diluted NaOH and few drops of HCl were added. A yellow solution that becomes colorless implies the presence of flavonoids.

Phlobatanins: 0.5 ml of each extract was dissolved in distilled water and filtered. The filterate was boiled with 2% HCl solution. Red precipitate implies the presence of phlobatanins.

Steroids: To 1 ml of each extract, few drops of acetic acid were added. It was gently warmed and then cooled and then a drop of H_2SO_4 was added. The color changes to green which shows the presence of steroids.

Terpenoids: 0.2 ml of each extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface signifies positive results for the presence of Terpenoids.

Test for Caumarin: Exist 3 ml of 10% NaOH was added to 2ml of aqueous extract formation of yellow color indicates the presence of Caumarin.

Test for Emodins: Exact 2ml of NH4oH and 3ml of benzene was added to extract. Appearance of red color indicates the presence of emodins.

Test for Anthocyanin and Betacyanin: To 2ml of plant extract, 1ml of 2N NaOH was added and heated for 5 minutes at 100C. Formation of bluish green color indicates the presence of anthocyanin and formation of yellow color indicates the presence of betacyanin.

Test for Carbohydrates: Few drops of Molisch's reagent were added to each of the portion dissolved in distilled water; this was then followed by addition of 1 ml of conc.H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet color at the interphase of the two layers was a positive test.

Test for Monosaccharide's: About 0.5 g each fraction was dissolved in distilled water and filtered. 1 ml of the filterate was then mixed with 1ml of Barfoed's reagent in a test tube and then heated on a water bath for a period of 2 minutes. Reddish precipitate of cuprous oxide was considered as a positive test.

2.4 Anti-bacterial activity of extract

Three strains of Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis and Bacillus subtilis*) and one strain of gram negative bacteria (*Klebsiella pneumonia*) were used for antibacterial activity. These organisms were kept in Muller-Hinton agar in the refrigerator at 4ºC.

The tests for sensitivity were finished utilizing accustomed agar well dispersion strategy to tryout the antibacterial action of different portions. The Muller Hinton agar was the medium. The cultures were taken threefold at fostering temperature of 37ºC for 1 to 3 days. The chowder culture of the test species was set in a sanitized Petri-dish to which 20 ml of the disinfected liquid MHA was inserted. Wells were drilled into the medium utilizing 0.2 ml of the concentrates. The standard antimicrobial operator utilized was Streptomycin (2mg/ml) was utilized as standard antimicrobial mediator. Immunization was improved the situation 1 h to make conceivable the dispersion of the antimicrobial mediator into the medium. The immunization plates were hatched at 37ºC for 1 day and the breadth of the zone of restraint of bacterial development were measured in the plate in millimeters.

2.5 Antioxidant Activity By Using DPPH Radical Scavenging Assay

DPPH radical rummaging measure was utilized to perform cancer prevention agent movement by utilizing standard convention as talked about before [11-12]. The oxidation capacities of the said divisions and guidelines were measured from the dying of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) purple-hued solution in methanol. Compactly, a 1mM solution in methanol was set up by dissolving DPPH in it and 1ml of this solution was blended with 3ml of each division solution in methanol (containing 10-100µg) and clear (without sample). Every solution was kept in dull for 30 min, and after that absorbance was measured at 517 nm. Decrease in the DPPH solution absorbanceaffirms an expansion in the DPPH radical searching action. As percent radical rummaging exercises (%RSA) by utilizing DPPH, the searching of free radicals was figured as:

$$\%DPPH = (OD\ blank/control - OD\ sample) \times 100 / OD\ blank$$

Where OD blank indicates the absorbance of the blank solution and OD sample shows the absorbance of samples or standard sample.

2.6 Antifungal activity

The antifungal activity was screened out by utilizing Agar tube dilution technique. 20 mg of each extract was taken for tests in clean and disinfected vials. These weighed samples were then dissolved in 1ml sterile DMSO. They were then properly normalized. The growth media used for fungus in this bioassay was SDA (Sabourad dextrose agar). 9.75 g of the powdered SDA was taken and was dissolved in 150 ml distilled water. It was normalized. The media and micropipette tips were sanitized by Autoclave. The tubes were allowed to cool at 50^{o}C after autoclaving and test samples were loaded into the non solidified SDA in biological safety cabinet. Each sample was loaded in test tubes for the given fungal cultures and for negative control. Tubes were arranged with their corresponding samples in slanting positions on the table at room temperature for 24 hours to form slants. Next day slants were checked for their sterility and each sample tube was inoculated with 4mm diameter of fungus removed from seven day old cultures of fungus. The tubes were incubated in fungal incubators at 27^{o}C-29^{o}C for 3-7 days. The following formula is used to calculate inhibition of fungal growth,

Percentage inhibition =

$$100 - \text{Linear growth in test sample (mm)} / \text{Linear growth in control (mm)} \times 100$$

3. Results and discussion

Phytochemical screening conducted on the plant extract revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the plant extract revealed the presence of phytochemicals such as tannins, saponins, steroids, coumarins and betacyanins. (Table 1)

Antioxidant and antimicrobial properties of various extracts of many plants are of great interest in both fundamental science and alternative medicine, since their potential use as natural extracts has emerged from a growing tendency to replace synthetic antioxidants by natural ones. The present study confirmed the antimicrobial and antioxidant activities of methanolic extract of *H. hirsuta*. (Fig.1, Table 2 and Table 3 respectively)

Table 1: Phytochemical screening of methanolic crude extract of *H. hirsuta*

Alkaloids	-
Tannins	+
Anthraquinones	-
Glycosides	-
Reducing sugars	-
Saponins	+
Phlobatanins	-
Steroids	+
Terpenoids	-
Caumarin	+
Emodine	-
Anthocyanin	-
Betacyanin	+
Flavonoids	-

Table 2: Antibacterial activity of methanolic crude extract of *H. hirsuta*

Microorganism	Gram	Result	Streptomycin
Staphylococcus aureus	+	14	28
Staphylococcus epidermidis	+	16	28
Bacillus subtilis	+	14	28
Klebsiella pneumonia	-	14	28

Table 3: Antifungal activity of crude extract of *H. hirsuta*

Fungus	% Activity

Aspergillusflavus	90
Aspergillusniger	100
Alternariasolani	100
DMSO	100

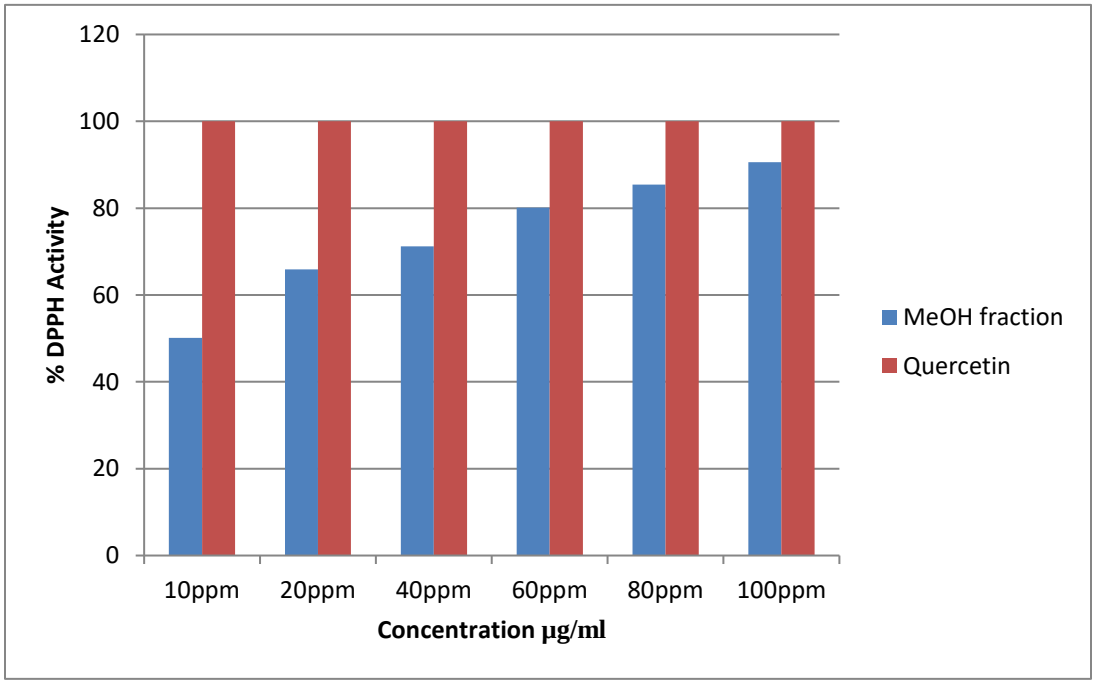

Fig. 1: Antioxidant activity of *H. hirsuta*methanolic extract

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