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Full Length Research Paper

Biological screening of ethyl acetate extract of *Hedera nepalensis* stem

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***Hedera nepalensis* is commonly used in folk medicines for the treatment of cough and other human ailments; therefore it was selected for phytochemical and antimicrobial screening. Its phytochemical screening showed the presence of different classes of secondary metabolites. The ethyl acetate extract of its stem showed the presence of steroids, terpenoids, saponins and flavonoids. Terpenoids and flavonoids were present in chloroform and ethyl acetate fractions, steroids were present in chloroform and ethyl acetate crude extract. Ethyl acetate and chloroform fractions were found most active. Among the organisms tested, *Escherichia coli* was the most sensitive to the plant extract as it displayed the greatest inhibitory zone (20:0 mm).**

Key words: Phytochemical screening, *Hedera nepalensis*, antibacterial activity.

INTRODUCTION

Plants are the major source of bioactive phytochemicals that can be used as medicine. The history of medicinal plants is as old as human civilization itself. Plants provide about 40% of medicine in the form of neutraceuticals and herbal drugs. Drug discovery and phytochemicals continue to be the source of medicinal plants research (Balick et al., 1996; Shulz et al., 2001; Skidmore and Roth, 2004). The genus *Hedera* belongs to family Araliaceae habitated mainly in Europe, North Africa, Asia, China, Japan and Afghanistan. One species of the genus *Hedera* is present in Hazara division of Pakistan. It has been considered one of the most useful plant containing saponins for the treatment of cough and other human ailments.

The literature survey revealed that saponins from the leaves of *Hedera helix* have spasmolytic, sedative, anti-fungal, anthelmintic, molluscicidal, antileishmanial, and antimutagenic activities (Timon et al., 1980; Julien et al., 1985). Aqueous and methanolic extracts of *H. helix* were found to reduce blood glucose level in rabbits to a significant level (Ibrar et al., 2003), and the leaves of *H. helix* also exhibited cytotoxicity (Ibrar et al., 2001). The fresh leaves and fruits of *H. helix* are toxic and cause gastrointestinal irritation, dermatitis, diarrheal bleeding and even death (Ghias et al., 2011). Despite its multipurpose traditional uses, very little data exist on its phytochemical constituents and antibacterial activity. Hence, the current study has been designed to evaluate its antibacterial potential and phytochemical constituents.

MATERIALS AND METHODS

Collection of plant material

The stem of *Hedera nepalensis* was collected from Bara Gali Khyber Pakhtunkhwa province of Pakistan in the month of February 2009. The plant was identified by Abdul Majid Taxonomist Department of Botany, Hazara University, Khyber Pakhtunkhwa, Pakistan, and a voucher specimen HU/hn/012 was deposited at the herbarium of Hazara University.

Extraction and fractionation

Shade dried powdered stem bark was soaked in ethyl acetate for 5

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Table 1. Phytochemical screening of chloroform, ethyl acetate and ethyl acetate crude extracts of *Hedera nepalensis.*

Chemical component	$CHCl_3$ fraction	EtOAc fraction	EtOAc crude extract
Alkaloids	-	-	-
Steroids	+	+	-
Terpenoids	+	+	+
Flavonoids	+	+	+
Anthraquinones	-	-	-
Tannins	-	+	+
Phlobatannins	-	-	-
Saponins	-	+	+
Glycoside	-	-	-
Reducing sugars	-	-	-

Table 2. Antimicrobial sensitivity testing chloroform, ethyl acetate and crud extract of *Hedera nepalensis.*

Microorganism	Gram	$CHCl_3$ fraction	EtOAc fraction	EtOAc crude extract	Std(Streptomycin)
Escherichia coli	-	0	20	20	30
Staphylococcus aureus	+	11	0	0	32
Klebsiella pneumoniae	-	16	12	14	32
Staphylococcus epidermidis	+	0	0	0	30
Bacillus subtilis	+	13	16	16	30

Std = Standard; – = absent; + = present; NA = not active; ext- extract. Well size: 6 mm.

days in closed container. The extract was concentrated under vacuum at 40°C. The crude extract was suspended in water and successively partitioned with chloroform, ethyl acetate to their corresponding fractions.

Microbial culture preparation

The microbes used in this study were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus epidermidis* and *Bacillus subtilis.* They were collected from stock culture in the Phytopharmaceutical and Nutraceutical Research laboratory, Institute of chemical Sciences, University of Peshawar, Pakistan. These organisms were placed in Muller-Hinton agar in the refrigerator at 4°C prior to subculture.

Antimicrobial activity of the various extracts against selected bacterial species

The activity was done using modified agar-well diffusion method to test the antibacterial properties of the extracts. The muller-hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 h. The broth culture (0.6 ml) of the test organism was placed in a sterile Petri-dish to which 20 ml of the sterile molten MHA was added. Holes were bored in to the medium using 0.2 ml of the extract. Streptomycin was used as standard antimicrobial agent at a concentration of 2 mg/ml. Inoculation was done for 1 h to make possible the diffusion of the antimicrobial agent into the medium. Incubation was done at 37°C for 24 h and the diameters of the zone of inhibition of microbial growth were measured in millimeters. The results are summarized in Table 2.

Phytochemical screening

The chemical tests were performed on the chloroform fraction, ethyl acetate fraction and crude ethyl acetate extract of *H. nepalensis* following the standard procedures (Sofowora, 1993; Trease and Evans., 1989; Herborne, 1973) for the identification of chemical constituents. The results are summarized in Table 1.

Alkaloids

Briefly, 0.2 g of each fraction and crude was warmed with 2% H_2SO_4 for 2 min. The reaction mixtures were filtered and then a few drops of Dragendroff's reagent were added to each filtrated fraction. An orange red precipitate indicates the presence of alkaloids moiety.

Tannins

A small quantity of each extract was mixed with water and heated on water bath and filtered. Next, a few drops of ferric chloride was added to each of the filtrated fraction. A dark green color indicates the presence of tannins.

Anthraquinones

In brief, 0.5 g of each fraction and crude extract was boiled with 10% HCl for few minutes in a water bath. The reaction mixtures were filtered and allowed to cool. Equal volume of $CHCl_3$ was added to each filtrated fraction, and then few drops of 10% ammonia was added to each mixture and heated. Rose-pink color

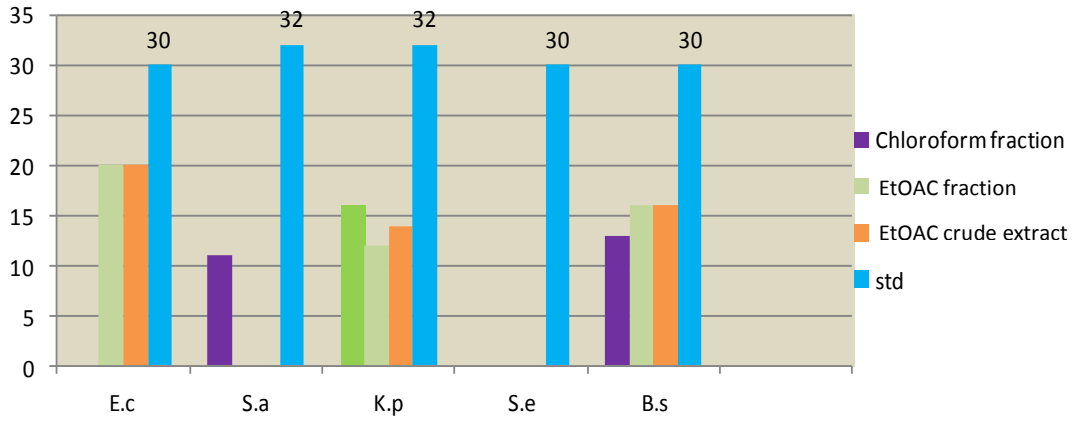

Figure 1. Comparison of antibacterial activity of various fraction and standard antimicrobial agent (Streptomycin). E.c: *Escherichia coli*; S.a: *Staphylococcus aureus*; K.p: *Klebsiella pneumonia*e; S.e: *Staphylococcus epidermidis* and B.s: *Bacillus subtilis.* Ext: Extract; std: Standard.

formation indicates the presence of anthraquinones.

Glycosides

Each fraction was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added to each mixture. Formation of red precipitate indicates the presence of glycosides.

Reducing sugars

Each fraction and extract was shaken with distilled water and filtered. The filtrates were boiled with few drops of Fehling's solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

Saponins

Briefly, 0.2 g of each fraction and crude extract was shaken with 5 ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Flavonoids

In brief, 0.2 g of each fraction and extract was dissolved in diluted NaOH, and then few drops of HCl were added. A yellow solution that turns colourless indicates the presence of flavonoids.

Phlobatannins

In brief, 0.5 g of fraction and crude extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatannins.

Steroids

Briefly, 2 ml of acetic anhydride was added to the mixture of 0.5 g of each fraction followed by adding H_2SO_4 (2 ml). The colour change from violet to blue or green in some samples indicates the presence of steroids.

Terpenoids

Briefly, 0.2 g of the each fraction was mixed with 2 ml of chloroform followed by careful addition of concentrated H_2SO_4 (3 ml). The formation of a reddish brown coloration at the interface indicates positive results for terpenoids.

RESULTS AND DISCUSSION

Pakistan is a rich source of medicinal plants majority of which are unexplored for their potential. Currently many studies have been carried out to authenticate tagged medicinal activities of these plants (Nisar et al., 2010a; b; c; 2011; Qayum et al., 2012; Rizwan et al., 2012; Zia-Ul-Haq et al., 2011). Hence, the current study has been designed for evaluating the antibacterial activity of *H. nepalensis.*

Phytochemical screening is an important step that leads to the isolation of important bioactive phytochemicals. The current results revealed that the ethyl acetate crude extract of *H. nepalensis* showed more varieties of the polar phytoconstituents such as steroids, terpenoids, flavonoids, tannins, saponins. The antiviral/ antibacterial activity of the plant is due to the presence of terpenoids (Shashi et al., 1997). In our biological study, the zones of inhibition against different bacterial species were between 10 and 20 mm (Figure 1 and Table 2). The pharmacological activity of the plant can be confirmed from the antimicrobial activity of various fractions obtained. Ethyl acetate fraction of the title plant possessed activity against *E. coli, K. pneumoniae and B. subtilis*, with a zone of inhibition ranging from 16 – 20 mm, hence suggesting its possible efficiency in the treatment of gastroenteritis and pneumonia. The ethyl acetate crude extract of the selected plant was also found to be active

against *E. coli, K. pneumoniae and B. subtilis* with a zone of inhibition ranging from 14 – 18 mm. The chloroform fraction showed activity against *S. aureus, K. pneumoniae and B. subtilis* with an inhibition ranging from 11 – 16 mm.

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