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Preliminary Phytochemical Screening and Antimicrobial Activity of *Hedera Helix* L.

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Abstract: *Hedera helix* L. is a plant of northern area of Pakistan which was screened for the natural products and antimicrobial activity. Preliminary phytochemical screening, using conventional natural products identification tests indicated the presence of different classes of secondary metabolites such as alkaloids, terpenoids, saponins and tannins. These secondary constituents vary in type in different parts of the plant and which also depends on the type of the solvent extraction. The chloroform and methanolic extracts revealed the presence of alkaloids, while terpenoids, saponins, tannins and reducing sugars were present in n-hexane, chloroform, ethyl acetate and methanolic extracts, respectively. The ethyl acetate and methanol extracts of *Hedera helix* is the most active, showing activity against three selected Gram positive and two Gram negative bacterial stains and thus displayed highest inhibitory zone of (18:0 mm) at the tested concentration (22 mg/ml) which could be attributed to the presence of these phytochemicals and signifies the potential of *Hedera helix* as a source of therapeutic agents. Infrared spectroscopic analysis of n-hexane, chloroform, ethyl acetate and methanol crude extract of *Hedera helix* indicated the presence of O-H, C=O, C-H, C=O, C=C, NH def, NO_2 and C-O-C bond stretching. The medicinal values of *Hedera helix* are due to the presence of detected metabolites.

Key words: Phytochemical screening · Antimicrobial activity · Infrared spectroscopic

INTRODUCTION

Plants are natural factories of producing valuable chemical constituents in the most efficient way and with precise selectivity. Since the middle of the 19th century, numerous bioactive constituents have been isolated and characterized. Many of these are beings used as the active ingredients of the modern medicine, or as the lead compounds for new drugs discovery. Several plants derived medicines, are rich in phenolic compounds [1] used in protection against coronary heart diseases and carcinogenesis [2]. *Hedera helix* is a member of Araliaceae family and using as a folk medicine for the treatment of hypertentation and stomach disorder by the presence of the bioactive constituents.

The fresh leaves and fruits of this plant are toxic and cause gastrointestinal irritation, dermatitis, bloody diarrhea and even death [3, 4]. The extracts of *Hedera helix* has also been shown to possess antibacterial [5], antihelmintic [6] leishmanicidic [7], *in vitro* antispasmodic [8], antifungal [9] and acute and chronic anti-inflammatory [10] activities. On the basis of these activities a great interest has been developed for phytochemical screening and antibacterial activity. The finding could be providing the scientific support for the traditional medicinal use and suggests that further studies should be conducted to isolate and characterize biologically active chemical constituents.

MATERIALS AND METHODS

Collection of Plant Material: *Hedera helix* whole plant was collected from District Swat, Khyber Pakhtunkhwa, Pakistan, during the month of February, 2009. The plant was identified taxonomically and authenticated at the herbarium (voucher specimen No. Rauf 634): Department of Botany, University of Peshawar, Peshawar, Pakistan.

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Extraction and Fractionation of Plant Material: The whole plant was dried at room temperature for 15 days. The dried plant material was milled and ground into fine powder which was successively extracted with methanol (5 days at room temperature). The extract was concentrated under reduced pressure at 40°C to give thick syrup that constituted the plant crude methanolic extract which was partitioned into n-hexane, chloroform and ethyl acetate to afford the corresponding fractions.

Microorganism Collection and Maintenance: Three strains of Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*) and two of Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia)* were used. These organisms were kept in Müller-Hinton agar in the refrigerator at 4°C, prior to subculture.

Antimicrobial Activity of the Various Extracts Against Selected Bacterial Species: The tests for susceptibility were done using modified agar well diffusion method to test the antibacterial activity of the extracts. The Müller-Hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 hours. 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. Wells were bored into the medium using 0.2 ml of the extracts (n-hexane, chloroform, ethyl acetate and methanol). Streptomycin (2 mg/ml) as the standard antimicrobial agent was used. Inoculation was done for 1 h to make possible the diffusion of the antimicrobial agent into the medium. The inoculation plates were incubated at 37°C for 24 h and the diameters of the zone of inhibition of microbial growth were measured in the plate in millimeters.

Phytochemical Screening: The chemical tests were performed on the hexane, chloroform, ethyl acetate and methanolic extracts of *Hedera helix* followed the standard procedures [11-13] for identification of the chemical constituents.

Alkaloids: 0.2 g of each of the fraction was warmed with 2% H_2SO_4 for two minutes. The reaction mixtures were filtered and added a few drops of Dragendroff's reagent to each filtrate. 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. A few drops of ferric chloride were added to each of the filtrates. A dark green solution indicates the presence of tannins.

Anthraquinones: 0.5 g of each extract was boiled with 10% HCl for few minutes on water bath. The reaction mixtures were filtered and allowed to cool. Equal volume of $CHCl_3$ was added to each filtrate. Few drops of 10% ammonia were added to each mixture and heated. Rose-pink color formation indicates the presence of anthraquinones.

Glycosides: Each extract 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 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: 0.2 g of each 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: 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCl were added. A yellow solution that turns colourless indicates the presence of flavonoids.

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

Steroids: 2 ml of acetic anhydride was added to the mixture of 0.5 g of each extract and H_2SO_4 (2 ml). The colour change from violet to blue or green in some samples indicates the presence of steroids.

Terpenoids: 0.2 g of the 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 indicates positive results for the presence of terpenoids.

Infrared Spectroscopic Analysis: Infrared spectroscopic of Shimadzu Corporation Japan; Model FTIR Prestige-21 was used. The dry crude powered extract was applied on the cell to obtain a thin film. The instrument was calibrated and recoded the spectrum.

RESULTS AND DISCUSSION

The weight percentage yield of n-hexane, chloroform, ethy lacetateand methanol fractions of *Hedera helix* whole plant are shown in Table 1. The ethyl acetate fraction contains a greater proportion by mass of the component compounds.

The preliminary phytochemical screening results of *Hedera helix* extracts showed the presence of bioactive secondary metabolites constituents such as alkaloids, anthraquinones, flavonoids, saponins, tannins and terpenoids (Table 2).

These components are well known to have curative activity against several human pathogens and therefore could suggest the use traditionally for the treatment of various diseases [14, 15]. The literature revealed that terpenoids showed different activities like antitumor and anticancer, anti-inflammatory, antiviral and antibacterial [16] while the chloroform extract was evidenced for the presence of alkaloids which showed central nervous system activities [17], antihelmintic activity [18], aphrodisiac [19], antibiotic activity [20], treatment of venereal diseases [21] and anti-malarial activity [22]. The antibacterial activities of the extracts could be as a result of alkaloids, anthraquinones, flavonoids, terpenoids, saponins and tannins.

The exploring of bioactive chemical components, the extracts were also subjected to antibacterial studies. The results of the antimicrobial determinations for all the extracts of *H. helix* against the five microorganisms were investigated by wells-diffusion assay. The antibacterial activity showed significant reduction in bacterial growth in the term of zone of inhibition, indicating that the plant exhibited antimicrobial activity against the selected microorganisms and the zone of inhibition was recorded and presented in the tabulation drawn (Table 3). The different extracts of the plant exhibited significant antibacterial activity against selected bacteria such as *E coli, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* and *Klebsiella pneumonia.*

The ethyl acetate extract exhibited the maximum inhibitory effect against *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* with a zone of inhibition ranging from 10-18 mm, showing its medicinal importance in the treatment of gastroenteritis and pneumonia. The hexane extract was also found active against three *Staphylococcus aureus, Klebsiella pneumonia* and *Bacillus subtilis* with a zone of inhibition ranging from 10-16 mm while the chloroform extract was active against *Bacillus subtilis* having a zone of inhibition equal to 16 mm.

Table 1: Total weight of the crude extracts and percentage yield of the crude extract of *Hedera helix*

Solvent	Weight of crude extract	Percentage yield
n-Hexane extract	0.98	14
Chloroform extract	1.55	22
Ethyl acetate extract	3	42
Methanol extract	7	1.4

Table 2: Phytochemical screening of n- hexane, chloroform, ethyl acetate and methanolic crude extracts of *Hedera helix*

Chemical components	N-Hexane (ext)	Chloroform (ext)	Ethyl acetate (ext)	Methanol (ext)
Alkaloids	-	+	-	+
Steroids	-	-	-	
Terpenoids	+	+	+	+
Flavonoids	-	-	-	-
Anthraquinones	-	-	-	-
Tannins	-	-	+	+
Phlobatanins	-	-	-	-
Saponins	-	+	-	+
Glycoside	-	-	-	-
Reducing sugars	-	-	+	+

Key= -absent, + = present, ext = extract

Table 3: Antimicrobial sensitivity activity of different extract of *Hedera helix*

Microorganism	Gram	Hexane	$CHCl_3$	EtOAc	MeOH
Escherichia coli	-	NA	NA	10	10
Staphylococcus aureus	+	10	NA	10	10
Klebsiella pneumonia	-	10	NA	NA	NA
Staphylococcus epidermidis	+	NA	NA	14	10
Bacillus subtilis	+	16	16	18	18

Key= NA (Not active)Well size: 4mm

Table 4: IR Spectroscopic data of different extract of *Hedera helix*

	Region (cm^{-1})			
Components	Hexane	Chloroform	Ethyl acetate	Methanol
OH	3369.64, 1238.30	3342.640, 1261.45	3360.00, 1255.66	3365.78, 1261.45
CH	2922.16	2927.94	2924.09	2926.01
C=O	1734.01, 1712.79	1693.50	1712.79	1693.50
C=C	_	1597.06	1604.77	1600.92
NH def	1558.48	1514.12	1516.05	1514.12
NO_2	1456.26, 1379.10	1456.26,1373.32	1456.26,1373.32	1456.26, 1373.32
C-O-C	1165.00	1193.94	1049.28	1157.29

The presence of the secondary metabolites functional groups in each extract was confirmed by IR spectroscopy analysis which gave results that suggested the presence of functional groups. The IR spectrums (Table 4) were exhibited strong absorption bands at 3369.64, 3342.640, 3360.00 and 3365.78 cm^{-1}, indicated O-H stretching, hydroxyl groups; 2922.16, 2927.94, 2924.09 and 2926.01 cm^{-1} indicated C-H stretching saturated; 1734.01, 1712.79, 1693.50, 1712.79 and 1693.50 indicated the presence of C=O stretching; 1597.06, 1604.77 and 1600.92 indicated the of C=C stretching; 1558.48, 1514.12, 1516.05 and 1514.12 indicated the stretching of NH; 1456.26,1379.10,1456.26,1373.32,1456.26,1373.32,1456.26 and 1373.32 indicated the presence of NO_2 stretching and 1165.00, 1193.94, 1049.28 and 1157.29 showed the presence of C-O-C bond stretching.

The present study clearly indicated that the *Hedera helix* extracts possess constituents with antibacterial properties and that can be further explored for the broad range of antibacterial activity. However, the present microbial study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. *Hedera helix* could serve as useful source of antimicrobial agents. Furthermore work will be needed bioassay directed isolation to isolate the pure antimicrobial chemical constituents and standardize new antibacterial drugs.

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