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The Usefulness of Common IVY (*Hedera helix* L.) Extracts Against Bacterial and Fungal Pathogens Found in Local Hospitals

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Abstract. The current study was carried out to highlights the antibacterial and antifungal activity of *Hedera helix* L. by using its different fractions. Both activities were carried out under agar well diffusion method, while for fractions obtaining the cold maceration method was followed which were tested against four fungal species including *Fusarium oxysporum, Aspergillus flavors, P. pallidum* and *Alternaria alternate* and four bacterial strains*, Staphylococcus aureus, Streptococcus mutans, Serratia marcescens* and *Methicillin resistance, Staphylococcus aureus.* The fractions which were extracted from the *Hedera helix* shown different zone of inhibition (ZOI) against both the fungal and bacterial strains. Chloroform and *n*-hexane extracted samples showed the highest antifungal activity against *Polyspondylium pallidum* with complete inhibition zone and the least activity was shown by an aqueous fraction. In the antibacterial activity, the crude methanolic extracts have shown the highest inhibition zone (46-57%) against the selected bacterial strains, while the lowest activity was reported from aqueous fractions (21-26%). The other fractions moderately inhibit the growth of the selected strains but were significant.

Keywords: *Hedera helix*, fungal activity, antibacterial activity, heavy metal analysis

Introduction

Hedera helix (ivy) is an evergreen woody plant belonging to the family Araliaceae. For the first time, it was reported from Europe. The plants have evergreen leaves. The younger leaves are palmate but when reaching to maturity they become rhomboid, cordate or ovate-lanceolate. The flowering condition starts from summer to late autumn and produces in umbel inflorescence. They are small with 3-5 cm in diameter and yellow or green. The fruits are berries and the ripening season is winter. Usually pollinated by birds and seeds germinate in the cluster or the dense form (Bottema, 2001). It is also growing as an ornamental plant in different countries of the world and also use as a medicinal plant against different diseases. In earlier times the plants were only used for energy purposes and shelters but later on people aware of its medicinal uses from headache to clinical problems by writings and other ways (Ali and Qaiser, 2009). After that such knowledge spread across the world. Pharmacologist of different areas starts their experiments on plants to know about the constituents and medicinal properties of plants. About seventy thousand (70,000) folk medicines were obtained from the plants (Marles and Farnsworth, 1995). In Pakistan, about 75% of people use plant medicines against different diseases (Hamayun, 2007; Hamayun *et al.*, 2006).

Plants consist of two types of metabolites primary metabolites and secondary metabolites. Primary metabolites are those which are necessary for the plants i.e. plant cannot survive without it, while secondary metabolites are those that are derived from the primary metabolites and required in small quantities by plants. Primary metabolites help in plant growth and development and secondary metabolites are used in the preparation of drugs that are active against different diseases. These metabolites are also used as agrochemicals and bio-pesticides. In recent reports, it was shown that about 12,000 secondary metabolites were reported from the plants and the drugs which are prepared from these metabolites or medicinal plants are called traditional drugs (Schultes, 1978). Since ancient times

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the leaf of *Hedera helix* has been used for medicinal purposes and pharmacologists also reported its therapeutic properties. Some of the metabolites which are reported from the selected plant are saponins, polyacetylenes, alkaloids, vitamins, sterol, flavonoids, phenolic compounds and amino acids, etc. Each one showed an important role and some of them such flavonoids are used for protection from a micro-organism (Sohn *et al.*, 2004). Others inhibit their growth e.g tannins etc. Initially, these metabolites were very active against micro-organisms but with time they showed resistance because of the failure of therapeutic properties and adaptation of micro-organisms (Rehman *et al.*, 2020b; Jansen, 1981).

Different extractions of *Hedera helix* (polyacetylenes, falcarinol and falcarinol, etc) were found to act in the anti-fungal and anti-bacterial activity. It also showed good results in anti-inflammatory and chronic problems (Majester-Savornin *et al.*, 1991). The biological compounds through various modes of actions like destroying their cell wall or biding to its material inhibit the growth of microbes (Grayer and Harborne, 1994). During the absorption of soil materials plants also absorb heavy metals that are incorporated into the plant's bodies. Heavy metals are toxic but new reports show that huge consumption of heavy metals has a significant role in plants (Ur-Rehman *et al.*, 2019, Shamshad *et al.*, 2015, Tariq *et al.*, 2020). Then heavy metal may be of two types one shows a significant role in the plant and human body called fundamentals, the other are noxious heavy metals which disturb the processes of living bodies and cause diseases in them even if in a small amount (REHMAN). The selected plant species consist of both metabolites and some heavy metals which also shown a pharmacological effect. Although due to the presence of some heavy metals it produced ROS (reactive oxygen species) that are responsible for oxidative strains and these oxidative strains then cause problems like cancer in human beings and the natural process of the environment. In this regards the *Hedera helix* was selected to find its anti-bacterial, anti-fungal, and heavy metal analysis.

Material and Methods

Antibacterial activity. *Collection and processing of plant specimen*. Different areas of the Khyber Pakhtunkhwa were visited for the collection of the *Hedera helix* plant. The collected plants were washed and dried in the shadow followed by the oven drying method in the oven. The completely drying plants were powdered with the help of a grinder. Then the powder is stored at a cool place before extraction (Girish and Satish, 2008).

Preparation of extraction and fractions. The cold maceration method was used for the extraction of the active metabolites. One and a half Kg of the powdered plant material was dipped into two litter of ethanol and incubated for 5 days at 40 °C. The material filtered thrice and a clear filtrate was obtained. The filtrate was subjected to evapouration *via* a rotary evapourator at 40 °C. The obtained extract was dried and then dissolved in 100 mL distilled water. The solution was then fractionated by using different organic solvents including ethanol, *n*-hexane, chloroform, methanol and ethyl acetate by using a separating funnel. The entire fractions thus obtained were concentrated by rotary evapourator and designate for that solvent fraction (Portnoy and Magnuson, 1955).

Media preparation. The antimicrobial activity was assessed using the agar well diffusion method (Holder and Boyce, 1994). One liter of distilled water was to liquefy 25 g of Luria Both, PH of Miller powder was put at 7.0. The media put in the autoclave in a 250 mL flask. The selected four bacterial strains were introduced into the flask and kept overnight at 150RPM at 37 °C. After that agar converted into solid form and five holes were tunneled through a sterilized borer. The inoculums were introduced into the tunnels. The bacterial and fungal species were selected due to their frequent occurrence in local hospitals of Khyber Pakhtunkhwa (KPK) and also showing resistance to different drugs.

Test for bacterial strains. Among the four selected bacterial strains, three were gram-positive and one strain is gram-negative i.e MRSA (Methicillin resistance of *Staphylococcus aurous*), *Streptococcus mutans* and *Staphylococcus aureus*. And the gram-negative is *Serratia marcescens*.

Measurement of zones of inhibition. 75 µL of plant extract was introduced into the wells of Petteri dishes and incubated at 37 °C for 24 h. Two solutions *i.e* dimethyl sulfoxide 20 mg/mL was used as a negative control in which the extracts were dissolved and the Cefotaxime (the standard antibiotic) was used as a positive control. After the incubation period, the diameter of each transparent zone was measured. The experiment was repeated several times to record the standard data.

Test for fungal strains. The four fungal strains i.e *Fusarium oxysporum, Aspergillus flavors, Polyspondylium pallidum* and *Alternaria alternate* were selected for antifungal activity (Pfaller *et al.*, 1988).

Results and Discussions

Antifungal activity. The antifungal potential of *H. helix* was check and shown in Table 1. The *n*-hexane fraction was found to show a complete zone of inhibition against *P. pallidium* and 54% against *A. flavus*, 42% *A. alternate* while, 37% zone inhibition of *F. oxysporum*. Similarly, the chloroform fraction showed a complete zone of inhibition against *P. pallidium* and 42% of *A. flavus* and *F. oxysporum*. Like these two extracts, the crude methanolic extract inhibits the growth of *P. pallidum*, *A .alternate*, *F. oxusporum* and *A. flavus* by 56, 42, 37 and 54% respectively (Rehman *et al.*, 2020a). The aqueous fractions showed the highest inhibition against *A. flavus* (34%) followed by *A. alternate, F. oxysporum,* and *P. pallidum* as 12, 21 and 18%. The ethyl acetate fractions were most active against *A. flavus* (54%) followed by *A. alternate, F. oxysporum* and *P. pallidum.*

Anti-bacterial properties of *Hedera helix*. The same four extracts (chloroform, *n*-hexane, crude methanolic extracts and aqueous fractions) of *Hedera helix* which were tested against fungal species (Parvu *et al.*, 2015). their potential was also checked against the following four bacterial strains *i.e Staphylococcus aureus*, *Streptococcus mutans, Serratia marcescens* and Methicillin-resistance *Staphylococcus aureus* (MRSA). These species were selected from different hospitals of Khyber Pakhtunkhwa Pakistan based on their pathogenic and spreading nature to help our population and society. These four bacterial species cause various diseases in human beings so we selected them to knows the fighting potential of the extracts of research plants against them. The result of the anti-bacterial activity of *Hedera helix* is shown in Table 2. The growth of *Staphylococcus aureus*, *Streptococcus mutans, Serratia marcescens,* and Methicillin-resistance *Staphylococcus aureus* (MRSA) was inhibited by crude methanolic extracts by 50, 46, 57 and 18%, respectively. The *n*-hexane has shown a high zone of inhibition against *Serratia marcescens* (42%) followed by *Streptococcus mutans* (39%), *Staphylococcus aureus* (38%) and *Methicillin resistance Staphylococcus aureus* (MRSA) (10%), respectively. The chloroform fractions have shown 47, 46, 42 and 28% zone of inhibition against *Staphylococcus aureus*, *Streptococcus mutans, Serratia marcescens* and Methicillin-resistance *Staphylococcus* aureus (MRSA). The ethyl acetate fractions were most active against *Streptococcus mutans* followed by the other three species, *Serratia marcescens, Staphylococcus aureus* and Methicillin-resistance *Staphylococcus aureus.* The aqueous fractions inhibited the growth of tested bacterial species in the order of *Serratia marcescens* (33%), *Staphylococcus aureus* (26%), Methicillin-resistance *staphylococcus aureus* (24%) and *Streptococcus* mutans (21%). The pharmacologist throughout the world now concentrates on medicinal plants because their constituents are very active against the disease-causing organism (Wali *et al.*, 2019; Eloff and McGaw, 2006). About 75% of drugs are provided to the medical faculties are prepared from medicinal plants. These traditional drugs either shown no or negligible side effects.

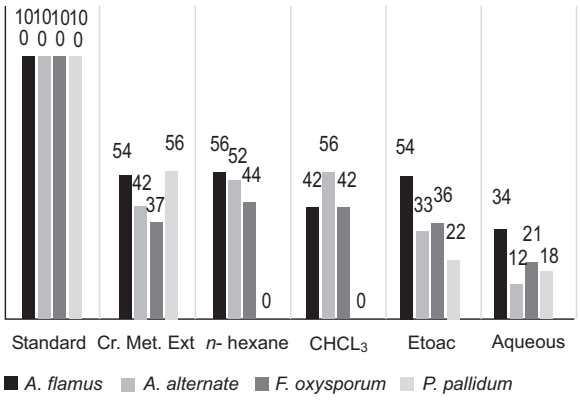

Fig. 1. Anti-fungal activity of *Hedera helix*.

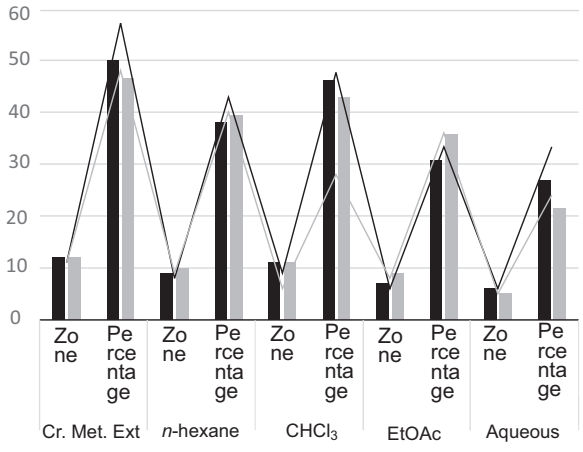

Fig. 2. Anti-bacterial activity of *Hedera helix*.

Table 1. Antifunal potential of *Hedera helix*

Anova: two-factors						
Summary	Count	Sum	Average	Variance		
A.flavus	6	340	56.66667	524.2667		
A. alternate	6	295	49.16667	866.5667		
n-hexane	4	152	38	666.6667		
$CHCl_3$	4	140	35	588		
EtOAc	4	145	36.25	176.25		
Aqueous						
Anova						
Source of variation	SS	MS	F	P-value		
Rows	1810.125	3	603.375	3.020777	0.062656	3.287382
F. oxysporum	6	280	46.66667	747.8667		
P. pallidum	6	196	32.66667	1508.267		
Standard	4	400	100	0		
Cr. Met. Ext	4	189	47.25	84.91667		
	4	85	21.25	86.25		
		df				*F. crit*
Columns	15238.71	5	3047.742	15.25842	2.02E-05	2.901295
Error	2996.125	15	199.7417			
Total	**20044.96**	**23**				

Table 2. Antibacterial properties of *Hedera helix*

Anova: two-factor without replication						
Summary	Count	Sum	Average	Variance		
S. aureus	6	70	11.66667	47.86667		
S. mutans	6	74	12.33333	57.46667		
S. marcescens	6	60	10	27.6		
MRSA	6	63	10.5	48.3		
Standard	4	96	24	8.666667		
Cr. Met. Ext	4	46	11.5	0.333333		
n- *hexane*	4	36	9	0.666667		
$CHCl_3$	4	37	9.25	5.583333		
EtOAc	4	30	7.5	1.666667		
Aqueous	4	22	5.5	0.333333		
Anova						
Source of variation	SS	df	MS	F	P-value	F crit
Rows	20.45833	3	6.819444	3.268975	0.050775	3.287382
Columns	874.875	5	174.975	83.87617	2.02E-10	2.901295
Error	31.29167	15	2.086111			
Total	**926.625**	**23**				

Conclusion

The outcome of our experiment strongly supports that *Hedera Helix has* significant antibacterial and antifungal activities, so the plant is an effective antibacterial and antifungal agent.

Conflict of Interest. The authors declare no conflict of interest.

References

Ali, H., Qaiser, M. 2009. The ethno-botany of Chitral valley, Pakistan with particular reference to medicinal plants. *Pakistan Journal of Botany*, **41:** 2009-2041.

Bottema, S. 2001. A note on the pollen representation of ivy (*Hedera helix* L.). *Review of Palaeobotany and Palynology*, **117:** 159-166.

Eloff, J.N., Mcgaw, L.J. 2006. Plant extracts used to manage bacterial, fungal and parasitic infections in southern Africa. In: *Modern Phytomedicine: Turning Medicinal Plants into Drugs*, Ahmad, I., Aqil, F., Owais, M. (eds.), Wiley, pp. 97-119.

Girish, H., Satish, S. 2008. Antibacterial activity of important medicinal plants on human pathogenic bacteria-a comparative analysis. *World Applied Sciences Journal*, **5:** 267-271.

Grayer, R.J., Harborne, J.B. 1994. A survey of antifungal compounds from higher plants, 1982-1993. *Phytochemistry*, **37:** 19-42.

Halliwell, B. 2002. Effect of diet on cancer development: is oxidative DNA damage a biomarker? *Free Radical Biology and Medicine*, **32:** 968-974.

Hamayun, M. 2007. Traditional uses of some medicinal plants of Swat valley, Pakistan. *Indian Journal of Traditional Knowledge*, **6:** 836-841.

Hamayun, M., Khan, S.A., Sohn, E.Y., Lee, I.-J. 2006. Folk medicinal knowledge and conservation status of some economically valued medicinal plants of district Swat, Pakistan. *Lyonia*, **11:** 101-113.

Holder, I., Boyce, S. 1994. Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. *Burns*, **20:** 426-429.

Jansen, P.C.M. 1981. *Spices, Condiments and Medicinal Plants in Ethiopia, their Taxonomy and Agricultural Significance*, Pudoc-ISBN 97890 22007679-327.

Majester-Savornin, B., Elias, R., Diaz-Lanza, A., Balansard, G., Gasquet, M., Delmas, F. 1991. Saponins of the ivy plant, *Hedera helix*, and their leishmanicidic activity. *Planta Medica*, **57:** 260-262.

Marles, R.J., Farnsworth, N.R. 1995. Antidiabetic plants and their active constituents. *Phytomedicine*, **2:** 137-189.

Parvu, M., Vlase, L., Parvu, A.E., Rosca-Casian, O., Gheldiu, A.-M., Parvu, O. 2015. Phenolic compounds and antifungal activity of *Hedera helix* L. (ivy) flowers and fruits. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **43:** 53-58.

Pfaller, M.A., Burmeister, L., Bartlett, M., Rinaldi, M. 1988. Multicenter evaluation of four methods of yeast inoculum preparation. *Journal of Clinical Microbiology*, **26:** 1437-1441.

Portnoy, J., Magnuson, H.J. 1955. Immunologie studies with fractions of virulent treponema pallidum. I. Preparation of an antigen by desoxycholate extraction and its use in complement fixation. *Journal of Immunology*, **75:** 348-355.

Rehman, K.U., Hamayun, M., Ahmad, G., Khan, S., Muhammad, S., Gul, R., Yaseen, T. 2020a. Antibacterial and antifungal prospective of cypress vine (*Ipomoea quamoclit* linn). *Pure and Applied Biology (PAB)*, **9:** 464-470.

Rehman, K.U., Hamayun, M., Khan, S.A., Khan, S.S., Wali, S. 2020b. Competence of benzoil tree (*Moringa oleifera* L.) as antibacterial and anti-fungal agent. *Advancements in Life Sciences*, **7:** 135-139.

Schultes, R.E. 1978. The kingdom of plants. *Medicines from the Earth*, McGraw Hill Book Co., 208 p., New Yark, USA.

Shamshad, I., Khan, S., Waqas, M., Ahmad, N., -Ur-Rehman, K., Khan, K. 2015. Removal and bioaccumulation of heavy metals from aqueous solutions using freshwater algae. *Water Science and Technology*, **71:** 38-44.

Tariq, F., Asim, M., Ahmad, I., Wali, S., Rehman, K.U., Bibi, H. 2020. Spectrometric analysis of heavy metal in *Parthenium hysterophorus* medicinal plant collected from district Karak and Peshawar. *Pure and Applied Biology (PAB)*, **9:** 1376-1384.

Ur-Rehman, K., Hamayun, M., Khan, S.A., Iqbal, A., Hussain, A. 2019. Heavy metal analysis of locally available anticancer medicinal plants. *Biosciences Biotechnology Research Asia*, **16:** 105-111.

Wali, S., Ur-Rehman, K., Ullah, B., Yaseen, T., Ahmad, G. 2019. Efficiency of common water hyacinth (*Eichhornia crassipes*) in controlling growth of fungal and bacterial clinical strains. *Pure and Applied Biology (PAB)*, **8:** 2178-2186.