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DETERMINATION OF PHYTOCHEMICAL COMPOSITION OF HELLIOTROPIUM DASYCARPUM: ANTIBACTERIAL AND ANTIFUNGAL ASSAY

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Abstract:

The purpose of the current study is to determine the phytochemicals, antibacterial activity and antifungal activity of the plant Helliotropium dasycarpum obtained from Baluchistan province of Pakistan. Helliotropium dasycarpum which is an herb is situated in different regions of Baluchistan, Pakistan. Various solvents are applied for the extraction of the plant and its members in various studies, with Methanolic extract being the very popular solvent. When subjected to phytochemical test, it was revealed that the plant contains alkaloids, saponins, pyhtosteroles, phenols tannins, ditepenes and fixed oil. Plant extract also showed antibacterial activity against four species of bacteria namely E.coli, Klebsiella, Salmonella and Staphylococcus aureus. However, among these bacteria the maximum inhibition zone was that of Klebsiella pneumonia (21 mm zone of inhibition). The plant extract was also screened for antifungal activity using Aspergillus niger. The activity was checked using PDA (potatoes dextrose agar). After 24 hours, the drug showed significant inhibition zone as compared to control which contain PDB and Aspergillus niger. Furthermore the test sample was kept for further 15 days to check fungicidal effect. After a careful observation there was no fungal growth in the medium which reflects fungicidal effect.

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INTRODUCTION:

The use of plants for the treatment of diseases has taken its origin from beginning of the history of mankind. With the passage of time, every civilization gathered experiences and knowledge of plants usage for medicinal purposes. In this regard some well known medicinal systems were introduced like Western system of medicine was commenced in Egypt and Mesopotamia, while Unani and Ayurvedic systems were started in Western Asia. From the ancient times, people searched for drugs to find cure for diseases. Because of lack of sufficient knowledge about the disease or the plants, the plants use was instinctive [1].

From the ancient times, the people searched for drugs to find cure for diseases. In other words, the plants use were instinctive even the people were not aware of how plants can be used, and which part is used for the treatment purpose [2]. Within this context, phytochmicals are the substances obtained from plants, being an integral parts of the plants parts which are present in leaf, stem, roots and flowers. These have their specific function due to which it enables the plant to resist harm which may be temporary or continuous from the environment side [3]. That is the reason; ethno botany in Pakistan is gaining a great interest and is getting matured gradually with time [4].

Shifting out concerns to Heliotropium dasycarpum, the plant is a member of Boraginaceae family and can be commonly found in Iran, Afghanistan, Brazil and Turkmenistan. In Pakistan this species is found in Southern Punjab, Gilgit, Waziristan and Baluchistan. The plant is 40cm tall and perennial which is much branched. Currently, extracts of plants are taken by various methods and screened for various pharmacological activities including antifungal, antibacterial, analgesic antitumor antiviral. Therefore, the current study is aimed that with the help of this study the chemical constituents present in the plants will be identified and compared with other studies taken in other regions and also these phytochemical constituents will be screened for antifungal and antibacterial activities.

METHODOLOGY:

Identification and collection of the plant

Identification of plant was done by the help of an expert in botany. Visits were made to different regions in Baluchistan, including Ziarat, Chiltan Park, Hazarganji Park and Pishin. Most of the plants for the study were collected from Hazarganji Park.

Drying, crushing, extraction and filtration of the plant

Collected plants were dried on clear floor under shade for one month. The dried plants were crushed in powdered from and were put into a bag with well closed mouth. Methanol was used as extracting solvent; about five litters of methanol were added in a bucket of ten liter volume. It was kept in that bucket for about fifteen days with daily shaking.

Drying of the filtrate

The filtrate was dried by rotary drier first and then subjected to freeze drying, followed by grinding with the help of mortar and pestle. About 40 gram of crude extract was obtained in powdered form.

Performance of lab tests

By the help of chemical reagents and equipments, lab test were performed one by one in a sequence and the results were documented in the diary book with photographs.

Antibacterial assay

For antibacterial activity, the control applied in the study was DMSO, while the plant extract was presented in methanolic form. In the study of antibacterial assay agar well method was applied. Fresh culture were used which was of 24 hours duration and contained bacterial culture of all four bacteria. For this purpose, Petri dish (sterile) were taken and filled with MHA (mueller hinton agar), over the surface of these media bacterial lawns were positioned and wells of diameter 6mm were also bored in the media. An extract of 250 mg /ml was poured on each well, placed in an incubator at a temperature of $37C^0$ for 24 hours. The zone of inhibition for each bacterium was recorded with a scale in millimeter (Table 1). The zone of inhibition for bacterial species in sequence was 13 mm, 14 mm, 18 mm and 21 mm respectively.

Antifungal assay

For antifungal activity, potatoes-dextrose agar (PDA) was used. Fresh PDA (250ml) was first prepared in the lab aseptically, both control and test samples were prepared (100ml each) while remaining 50 ml was discarded. Only one species of fungi was used in the study and that was Niger (aspergillus Niger). Two flasks each containing 100 ml PDB, one of the flasks was added with plant extract along with PDB, while the second flask only contained the PDB, which was the control of the antifungal assay.

The preparation of the PDB and plant extract administration in the medium was carried aseptically using laminar hood flow. Eleven gram of the dried plant extract was added in 100 ml PDB (11%). When

both media were slightly solidified in the Petri dish, the fungus Aspergillus niger was taken using kocoborer and placed in the centre of both Petri dishes (control and test sample). Both Petri dishes were kept for few days, and checked after every two days.

RESULTS:

The plant extract was subjected in lab to various Phytochmical screening tests and the following results were recorded (Table 1).

Table 1: Phytochemical tests of Helliotropium Dasycarpum

S. NO	Name of phytochemicals For detection	Present +ve	Absent –ve	Any other comments
1.	Alkaloids' detection Wagner's test	Positive		Alkaloids' present in extract
2.	Glycosides detection Legal s test		Negative	Glycoside absent in the plant extract.
3.	Saponin detection Froth test Foam test	 Positive Positive		Saponin is present in the extract
4.	Phytosterol Salkowski test	Positive		Phytosterol present in the extract
5.	Phenol detection Ferric chloride test	Positive		Phenol present in plant extract
6.	Tannins Gelatin test			Tannins present in plant extract
7.	Protein and amino acids Xanthoprotic test		Negative	Proteins and amino acids absent in the plant extract
8.	Diterpenes Copper acetate test	 Positive		Diterpenes present in the extract
9.	Gums and mucilage		Negative	Gums and mucilage absent
10.	Fixed oil Spot test	Positive		Fixed oil present in the extract

Detection of alkaloids: The extract was dissolved in dilute hydrochloric acid and then filtered. Filtrate was treated with Wagner's reagent (solution of Iodine in Potassium Iodide). Brown/reddish precipitate indicated the presence of alkaloids.

Detection of carbohydrates: The extract were dissolved individually in 5 ml distilled water and filtered. The Molisch's Test was used where filtrate was treated with 2 to 3 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of Carbohydrates; this test comes out to be negative.

For the Benedict test, the filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars, this test also came out to be negative showing the absence of carbohydrates. The filtrate was hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Detection of glycosides: The extracts were hydrolyzed with dilute HCl, and then subjected to Test for glycosides. The extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicated the presence of cardiac glycosides and this test came out to be negative.

Detection of saponins: Froth Test: The extract was first diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm thick layer of foam indicated the presence of Saponins. This test came out to be positive.

Detection of phytosterols: Salkowski's Test: The extract was treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicated the

presence of triterpenes. This test came out positive.

Detection of phenols: The extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols. The test showed the presence of phenols.

Detection of tannins: Gelatin Test: The extract was added with 1% gelatin solution containing sodium chloride. Formation of white precipitate indicated the presence of tannins.

Detection of proteins and aminoacids: The extract was treated with few drops of concentrated nitric acid. Yellow color indicated the presence of proteins.

Antibacterial Assay

For antibacterial activity four species of bacteria were used. In this study of antibacterial assay agar well method was applied. Fresh culture were used which was of 24 hours duration and contained bacterial culture. The zone of inhibition for bacterial species in sequence was 13mm, 14mm, 18mm and 21 mm respectively (Table 2).

Table 2: Antibacterial activity of the Helliotropium Dasycarpum

Bacterial Species	Extract Applied	Control	Zone Of Inhibition (Mm)	Remarks/Comments
E.coli	Methanolic	DMSO	13	antibacterial activity is seen
Salmonella	Methanolic	DMSO	14	antibacterial activity is seen
S. aureus	Methanolic	DMSO	18	antibacterial activity is seen
Klebsiella	Methanolic	DMSO	21	antibacterial activity is seen

NOTE. DMSO stands for dimethyl sulfoxide

Antifungal Assay

For antifungal activity potatoes-dextrose agar (PDA) was used. The results are mentioned in the Table 3.

Table 3: Antifungal activity of Helliotropium Dasycarpum

Name of fungus	medium used	control	test	inhibition zone after four days in control	inhibition zone after four days in test sample
Aspergillus niger	PDB	PDB + niger	PDB + niger + plant extract	No inhibition zone with clear fungal growth	Clear inhibition zone with no fungal growth

NOTE (PDB = potatoes -dextrose agar)

DISCUSSION:

Quinones are detected in plant H. ovalifolium but this phytochemical property was not frequently found in most members of the heliotropium [5]. Interestingly alklaloid were present in all but were absent in H. strigosum. It is also negative for glycosides, but positive for saponins and tannins [6]. In the current study, the phytochemical constituents and test reagents applied showed that plant contained alkaloids, tannins, terpenoides, saponins flavonides but is negative for glycosides. Furthermore test for carbohydrates has also been carried out which came out to be negative.

Several pharmacological tests were performed on the plant H. dasycarpum and its members, their results revealed different activities including: antibacterial, antifungal, anticataract action, antihyperglycemic, wound healing activity, neurotoxic activity, hyperlipidimic and antioxidants activities. An interesting result came into observations that the current study on the plant H. dassycarpum has shown both antibacterial and antifungal activity but in another study taken by Ghaffari et all 2013. There was no antibacterial activity shown by the same plant however very slight antifungal activity has been shown by the Methanolic extract. This difference may be due to geographical distribution. It must be noted here that bacterial species used by Ghaffari et al were different but two bacteria were similar to the bacteria used in this study (Staphylococcus aureus and E.coli). Regarding antifungal activity, various members have shown slight antifungal activity but the methanolic extract of the plant H. dasycarpum has shown activity against microsporus cavis, and no activity expressed by dichloromethane fraction [1]. But in this study the plant extract which belongs to Baluchistan has shown antifungal activity against the single used fungal species Aspergellus niger.

CONCLUSION:

H. dasycarpum which is scattered in various regions of Baluchistan has been collected and its photochemical constituents are determined. It

contains alkaloids, Saponins, tannins terpenoides and flavonides. Antibacterial activity shown by the plant was different from plant of the other location. The plant has shown significant activity against Aspergilus Niger. This study recommends the isolation of active ingredients from the extract of the plant, which may be useful as an important antibacterial agent.

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