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Preliminary Phytochemical Screening, Antibacterial and Antifungal Activity of Artemisia Vulgaris

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Abstract

Artemisia vulgaris (L), commonly known as "mugwort", is a species of flowering plant in the daisy family Asteraceae, a species with great importance in the history of medicine and was called the "mother of herbs" in the Middle Ages. It is a common herbaceous plant that exhibits high morphological and phytochemical variability depending on the location where it occurs. This species is well known almost all over the world. It is a very important medicinal plant or herb which is used widely for the treatment of various ailments traditionally. The present study of leaves of Artemisia vulgaris was examined for their phytochemical screening, antibacterial and antifungal activity against different pathogenic micro-organisms Staphylococcus aureus, E. coli and Candida. Phytochemical analysis of the methanolic extracts showed the presence of various phytoconstituents like glycosides, flavonoids, saponins, proteins, triterpenoids, tannins and alkaloids. A total of 3 microorganisms were used to find out the antibacterial and antifungal activity of the plant extract. The methanol extract of plant showed strong antibacterial activity against Staphylococcus aureus and E. coli with zones of inhibition of 14 mm and 6 mm respectively. Similarly, the extract showed relatively weak antifungal activity against Candida with zones of inhibition of 12 mm. This finding concludes that the methanol extract has strong antibacterial activity and could be responsible for the presence of active compounds like flavonoids, tannins, saponins, alkaloids, and proteins. A further study should be done to investigate more active compounds of Artemisia vulgaris L, which can be beneficial for the pharmacology field.

Keywords: *A. vulgaris L., Extract, Traditional medicine, Antimicrobial and antioxidant activity, Phytochemicals*

Introduction

In Nepal, majority of peoples in the mountain region depend on traditional medicine for solving their health issues. Natural resources have been proved to be an important part of the primary health-care system of rural regions. Diverse geographical and climatic conditions offers huge possibilities of unique bioactive ingredient as sources of new natural products. Ethnological survey or study are still limited and poorly explored, which might helpful in different drug discovery process through the synthetic and semisynthetic route. In today's scenario over 60% of approved and proposed drug candidates are natural products or derived from them (Zhang and Demain, 2007; Cowan, 1999). Nepal harbors 3 percent of the world's bio-diversity, among the genetic resources, 6076 species of flowering plants have been identified for Nepal (Shrestha et al., 2000). Out of these, A. vulgaris L. locally known as "titepati" in Nepali, is a perennial herb of the Asteraceae family. It is a species with

great importance in the history of medicine and was called “mater herbarium” (the mother of herbs). It was used externally for treating wounds, against gout, and to remove leg fatigue, as well as in an attempt to treat fever. In addition, the plant gained popularity as a remedy for gastrointestinal ailments “resulting from cold” including stomach pain, diarrhea, and intestinal colic. Their pharmacological activity and potential applications make the Artemisia genus a current subject of interest (Abad et al., 2012; Kordali et al., 2005). According to literature survey, the Artemisia species have high levels of phenolic and flavonoids, which have powerful antioxidant and radical scavenging capabilities (Cha JD, Jung EK, Kil BS, 2007; Shi et al., 2010). An increasing number of research work and evaluation have been done to find antioxidative drugs, which not only extend the shelf life of food products but also contribute as radical scavengers in living organisms. Therefore, the present study was carried out on Artemisia vulgaris L. to contribute for the development of antibacterial as well as antifungal activities.

Materials and methods

Chemicals and reagents

Methanol, Distilled water, Conc H_2SO_4, Conc HNO_3, Dil. HCl, copper sulphate ($CuSO_4$), mercuric chloride ($HgCl_2$), potassium iodide (KI), Iodine (I_2), Ferric chloride ($FeCl_3$), Chloroform ($CHCl_3$), Glacial acetic acid (CH_3COOH), sodium hydroxide (NaOH), Fehling’s A solution, Fehling’s B solution, Benedict solution, MHA, saline, grease, etc. All reagents and solvents were of laboratory reagent grade.

Sample collection

Leaves of Artemisia vulgaris L. were manually collected from the Ratnanagar municipality during the month of July 2024 having the altitude ranging from 244 meters to 1945 meters above sea level. The taxonomic identification was carried out with the help of literature and comparing herbarium specimens deposited on TUCH.

Sample preparation

About 100 gm of fresh leaves of Artemisia vulgaris were properly rinse with tap water in order to remove unwanted dust particles from the surface of plant materials. After that it was allowed to shade dry for a 2 week at ambient room temperature with good air flow and and grounded to coarse size particles with the help of electric grinder. Thus obtained powder sample were kept in clean zipped plastic bag until further use.

Extraction

The extraction of the plant material was done by Soxhlet extraction method. 30 gm of the leaves sample was taken on 400 ml methanol. The round bottom flask was filled with methanol and adjusted to extractor. The solvent methanol was then heated at its boiling point 64.7°C. Green solution was obtained after 4 hours of extraction. To remove the solvent from the extract, the solution again went under the fitted rotary vacuum evaporator. The extract was poured into the round bottom flask, secured with keck clip. The rotation speed was adjusted at 155 rpm according to the size of the flask and volume of the sample. The rotation was started after adjusting the temp. to the boiling point of the solvent. Once all the solvent was removed, the rotation was stopped and then the extract was collected in a glass vial for further process.

Preliminary Phytochemical Screening

The detection of phytoconstituent present in the methanolic extract of A. vulgaris leaves was carried out by standard methods.

Test for Proteins (Xanthoproteic test):

In 2 ml of extract, 2ml of concentrated HNO_3 was added. The observation of orange color indicated the presence of proteins.

Test for Carbohydrates (Benedicts test):

In 2ml of extract, Benedicts reagent was added and boiled. Formation of orange red precipitate indicated the presence of carbohydrates.

Test for Alkaloids (Mayer's test):

In 2ml extract, drops of Mayer's reagent were slowly added by the side of the test tube, observation of a white or creamy precipitate indicated the presence of alkaloids.

Test for Flavonoids (alkaline reagent test):

In 2ml of extract, 2ml of 2% sodium hydroxide solution was added. The formation of yellow color precipitate indicated the presence of flavonoids.

Test for Triterpenoids (Salkowski test):

In 2ml extract, 1ml of chloroform was added followed by a few drops of concentrated H_2SO_4 on the side of the test tube and shaken well. The formation of yellow color at the lower layered indicated the presence of triterpenoids.

Test for Saponins (frothing test):

2ml of extract was diluted with 10ml of distilled water in a test tube and shaken for 5 mins. Formation of stable foam indicated the presence of saponins.

Test for tannins (ferric chloride test):

In 2ml of extract, few drops of 10% ferric chloride solution were added. The change of color into dark blue or green indicated the presence of tannins.

Test for Glycosides (Keller-Kilani test)

To 10ml of the extract, a mixture of 4ml of glacial acetic acid and 1 drop of 2% $FeCl_3$ was added followed by the addition of 1ml of concentrated H_2SO_4. Formation of brown ring between the layers indicated the presence of glycosides.

Antibacterial and Antifungal Susceptibility Test

Preparation of Bacterial and Fungal culture

The microbial strains employed were identified strains that were obtained from Central Department of Microbiology, T.U. The strains studied include three different types of microorganisms, one gram-positive (Staohylococcus aureus), one gram-negative (Escherichia coli) and a yeast (Candida albicans). They were taken on slants and later cultured on petri plates having nutrient agar. Before using the prepared bacteria and fungi, turbidity was maintained as McFarland standard by adding normal saline in the culture.

Plating and Inoculating Bacteria and Fungi and Extract

Using the agar-well diffusion method, the extract's antibacterial and antifungal activity was evaluated against harmful bacteria such as Staphylococcus aureus and E.

coli as well as harmful fungi such as Candida. Using sterilized cotton swabs and a broth culture containing the appropriate microorganisms, MHA plates were swabbed. Each petri plate had two 4mm well cut into it with a distance of 2-3 cm apart using a sterile corkborer. Separately, 30 µl of methanol extract of Artemisia vulgaris leaf material was added into a well and to compare the effectiveness of the extracts, 30 µl of solvent (methanol) was employed as a control and added into another well. The plates were then incubated for 24 hours at 37°C. Based on the measurement of the inhibition zone that formed around the well, the extract's antibacterial and antifungal activity was determined.

Results And Discussions

Phytochemical extraction

Methanolic extract of A shows the different phytochemical constituents presented in table 1. This qualitative estimation specifies leaves section is reservoirs of proteins, tannins, glycosides, saponins and triterpenoids etc.

Table 1. Phytochemical analysis of leaves. Key: (+): Present (−): Absent

SN	**Phytoconstituents**	**Reference**	**Result** (Methanol extract)
1	Proteins	Orange color appeared	+
2	Carbohydrates	Orange red ppt.	–
3	Alkaloids	White or creamy ppt.	–
4	Flavonoids	Yellow ppt.	–
5	Tannins	Change in color dark green	+
6	Glycosides	Brown rings	+
7	Saponins	Liquid foamed	+
8	Triterpenoids	Yellow rings on bottom	+

The phytochemicals in most of the plant extract had diverse biological properties, such as analgesic, anti- carcinogenic, anti-inflammatory and antioxidant activities and their presence also ensured the medicinal potential and their therapeutic activities. whereas according to the investigation (Awadhesh Kumar, 2020), phytochemical screening showed the presence of saponins, glycosides, flavonoids, proteins, triterpenoids in leaves extract. Monika and Kaur, 2016 have reasoned the phytoconstituents of methanol extracts of the plant are more effectively responsible for the antimicrobial activity when compared to other extracts.

Antibacterial Susceptibility Test

After 24 hours of incubation, the zone of inhibition (ZOI) was examined, and their values were tabulated (Table 2) below. It was found that methanolic extract seems to be effective against gram positive bacteria (S. aureus) with ZOI 14 mm, while least against gram negative one (E. coli) having 6 mm diameter. The results have evidenced that Artemisia vulgaris have potential antibacterial properties with gram positive bacteria (Staphylococcus Aureus) and fungi (Candida albicans).

Table 2: Antimicrobial activity of methanol extract of A. vulgaris leaves

SN	Organisms	Zone of Inhibition of methanol extract (in mm)
1	*Staphylococcus Aureus*	14
2	*E. coli*	6
3	*Candida*	12

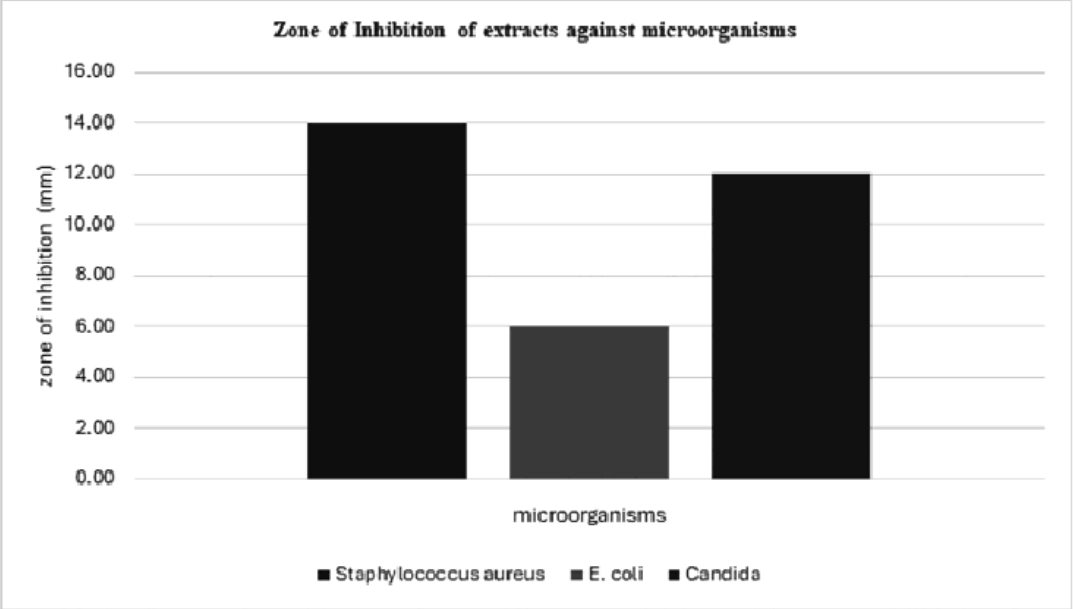

Figure 1: Zone of Inhibition of extract against different microorganisms

This suggests that the leaves of A. vulgaris L. have the potential to be utilized in developing various medicinal formulations aimed at preventing or delaying certain types of cellular damage due to their antioxidant properties. Additionally, the leaves of A. vulgaris L. could serve as a foundational material for creating antibacterial resistance drugs to treat several infections, owing to its antimicrobial activities.

Conclusions

The phytochemical analysis is very much important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants. Further, it provides the base for targeted isolation of compounds and to perform more precise investigations. The present study of screening of phytochemicals in methanol plants extract of Artemisia vulgaris leaves revealed the presence of bioactive compounds like proteins, tannins, glycosides, saponins and triterpenoids. This study suggested that the Artemisia

vulgaris extract possesses antibacterial as well as antifungal activity, which might be helpful in preventing or slowing the process of bacterial and fungal related disease. Thus, this plant can be used as a good therapeutic agent in the modern era and it can be used as home remedy to cure multiple diseases. Further research on this plant species is urgently required, along with immediate efforts toward its conservation and reforestation, which are essential in the current scenario.

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