Preliminary phytochemical screening and ethnomedicinal uses of *Phagnalon niveum*

Walimam Ulfat\(^a\), Nasratullah Mahboob\(^b\), Khalilullah Abed\(^c\), Azharulhaq Kamran\(^d\), Mohammad Azim Azimee\(^e\), Zardar Khan\(^f\)

\(^a\)Department of Chemistry, Faculty of Science, Nangarhar University, Nangarhar, Afghanistan  
\(^b\)Department of Chemistry, Faculty of Education, Samangan Higher Education Institute, Samangan, Afghanistan  
\(^c\)Biochemistry Department, Medical Faculty, Nangarhar University, Nangarhar, Afghanistan  

Corresponding author email id: walimamulfat@gmail.com

**Abstract:** Phytochemicals screening test is important for the isolation of new and novel compounds. The current results are directed to the detection of different classes of phytoconstituents responsible for the antioxidant properties of *Phagnalon niveum*. The crude methanolic extract of *Phagnalon niveum* showed the presence of Alkaloids, Terpenoids, Saponins, Tannins, Steroids and Glycosides, Carbohydrates, monosaccharides, combined reducing sugars and soluble starch. The extracts of *P. niveum* were screened for secondary metabolites by using methanol, crude extract. The methanolic extracts gave positive results for Alkaloids, Tannins, Flavonoids, Saponins, steroids, Reducing sugar, Terpenoid, Anthraquinone, Phlobatannin and Glycoside. This paper highlights the significance of the plant in traditional medicine and the presence of various phytochemicals in the plant. This study will serve as a benchmark for further detailed analysis of plant extract before drug development and its utilization in future.

**Keywords:** *Phagnalon niveum*, Phytochemicals, Preliminary screening, methanolic extract, antioxidant

1. Introduction

*Phagnalon niveum* Vahl, an herbaceous plant belongs to the family Polygonaceae, genus Phagnalon. This plant is used in traditional medicines. According to the local physician, it is used in the healing of wounds. Medicinal plants are good sources of therapeutic moieties, and provide safe, economical and effective remedies for different pathological conditions. The insecticides obtained from plant origin are comparatively more efficient, safer and possess negligible hazardous effects. Natural products are used widely for health care World Health Organization (WHO) reported that more than 80% of the population have used traditional medicines to face primary health care. For 30 years ago natural products were the main source for novel drugs, studies reported about 40% of all drugs is either natural products or derivatives of them\(^1\). Since thousands of years ago medicinal plants have been used for treating human and animal diseases. Hundreds of thousands of bio-organic compounds have been derived from plants and shown pharmacological properties. However, medicinal plants considering as the major source of novel drugs for curing hapless diseases \(^2\). For the existence of life on the earth, plants played a very important role from time immemorial. Human beings are directly relayed on plants to fulfil their daily life requirement. They are used as food, fuel, ornamentals, flavour and medicine. Higher plants are being employed to treat various infectious diseases throughout the world, they provide a lot of natural products including medicaments to fight against diseases\(^3\). To maintain the quality of the drug, there is a need to evaluate active constituents of raw material to ascertain their therapeutic effects. It enables the pharmacist to prescribe numerical values and ensure uniformity of standards. A medicinal plant can be defined as any part of the plant which contains substances that can be used for therapeutic purposes and its precursor for the synthesis of useful drugs. They contain nutrients and phytochemicals that can heal ailments of the body. Plant material that has cellular. The structure is referred to as an organized drug in pharmacy, whereas, the non-cellular structure is as an unorganized or a cellular drug\(^4\). Secondary metabolites are chemicals produced through secondary reactions resulting in primary carbohydrates, amino acids and lipids. Their direct function in plant metabolism is not been well recognized to date. However, their role in ecosystems. Particularly in plant-herbivore interaction, and chemotaxonomy\(^5\) has been well known. Plants that contain secondary metabolites such as alkaloids, saponins and tannins are generally avoided by grazing animals and leaf-feeding insects. Their presence in plants and intake at a high level reduces nutrient utilization, feed efficiency, animal productivity and in some cases may cause death\(^3\).

Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity\(^6\). The use of medicinal and aromatic plants for the treatment of diseases is as old as mankind. Medicinal plants receive attention from research centers because of their special importance in the safety of communities \(^7\). For over a decade, interest has been revived in the study and use of traditional medicine in different parts of the world. As a result, countries have sought cooperation in identifying and using safe positive components of traditional medicine in their national health
systems[8]. Since ancient times, people have been exploring nature, particularly plants, in search of new drugs, and this has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases[9]. Nearly 80% of the world’s population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. In India, almost 95% of the prescriptions have been reopened to be plant-based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha[10]. The most important phytochemicals extracted from medicinal plants include Alkaloids, Tannins, Flavonoids, Phenolic compounds and a lot of other chemicals. Mostly the phytochemicals show biological activities. Tannins have antimicrobial properties; Flavonoids have antibacterial, antifungal, anti-inflammatory, bactericidal and antimicrobial activities; Terpenes and Steroids are known to have bactericidal and antimicrobial properties against several pathogens. Phytochemicals are the sources of oils, tannins, gums and starting materials of the synthesis of complex substances and are important for the discovery of therapeutic agents and drugs. The discovery of antibiotics has decreased the spread and severity of a wide variety of diseases. However, and as a result of their uncontrolled use, the efficiency of many antibiotics is being threatened by the emergence of microbial resistance to existing chemotherapeutic agents. Bacteria and fungi are evolving numerous mechanisms to evade antimicrobial agents and the resistance to old and new antibiotics is rising in medical practice. Medicinal plants are the spine of traditional medicines. According to a report of the world health organization (WHO) almost 80% population of the developed countries, including about 49% (France), 70% (Canada) and 42% (United States of America) depends primarily on medicinal plants for the treatment of different diseases. Medicinal plants are considered as natural sources of new drugs, literature reports about 520 new drugs during 1983-1994 from natural sources[4]. Medicinal plants are considered an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the biological and phytochemical screening of plant extracts and/or extracts from traditional preparations used in popular medicine. Successful strategies for investigating these preparations involve the selection of test crude extracts based on a combination of ethnopharmacology and daily healer’s practices. Several traditional preparations from various medicinal plant species are used in Congolese traditional evaluating to justify their use[4]. Recently it has been observed that there is a steady increase in human infections, especially in tropical and subtropical developing countries [11]. This may be due to increases in the use of antibiotic drugs or an increase in resistance to these synthetic drugs [12]. Medicinal plants possess certain bio compounds, which are therapeutically important as precursors for yielding important drugs. The phytochemicals present in such plants produce certain physiological actions on the human body and relieve disease[4]. Phytochemicals are naturally found in plants. These are deriving colour, flavour and smell of plants. Besides, they are playing a key role in natural defense mechanisms in plants against diseases. They are well recognized as a therapeutic potential to human suffering and disease [13]. The most pronounced phytochemicals include Alkaloids, Tannins, Flavonoids, and Phenolic compounds, which possess bacteriostatic and bactericidal effects. Most of these bio compounds other than antimicrobials can also act as effective antioxidants [14].

However, intestinal amoebiasis is one of the current diseases in tropical regions causing diarrhoea. Traditional preparations from medicinal plants are still used with success for the treatment of amoebiasis. Extensive phytochemical studies of some plants that involved anti amoebic activity have resulted in the isolation and characterization of active constituents [15],[16]. Other investigations of purely natural products from different plant species allowed identification of some constituents with an interesting anti amoebic activity[17],[18]. Phagnalonniveum, an herbaceous plant belongs to the family Polygonaceae, genus Phagnalon. It is found in hilly areas. P. niveum has been reported for the treatment of cancer [19] The people of Bara Khyber agency KPK Pakistan have good knowledge about this plant for its medicinal use they used for healing of wounds. Based on this indigenous knowledge of Phagnalonniveum, the present study was carried out to analyze phytochemicals to seek scientific logic for their use in folk medicine. The extract of P. niveum has been found to have anti-ulcerogenic and cytoprotective properties applied on experimentally induced gastric lesions[3].

2. Material and methods:

2.1 Plant materials:

P. niveum was collected from the Bara Khyber agency KPK Pakistan in January 2017. The plant was identified by Sir Ghulam Jelani, Department of the Botany University of Peshawar where a voucher specimen of the plant had been deposited[18].

2.2 Preparation of crude extracts:

Shade dried plant of P. niveum was filled in the flask and extracted successively with methanol solvent in a soxhlet extractor for 50h. The solvent extract was concentrated under reduced pressure at 45°C using a rotary evaporator and suspended[20].
2.3 Phytochemical Profiling:
The phytochemical screening test is important for the isolation of new and novel compounds. The chemical tests were performed on the crude extract of plant *P. niveum* using the standard procedure to recognize the bioactive secondary metabolite[21].

2.4 Preparation of samples for testing
One hundred milligrams of dried extract were separately dissolved in 10 ml distilled water to obtain stock solutions of 10 mg/ml. These stock solutions were diluted two-fold with a culture medium to obtain a series of concentrations of the test sample. Metronidazole from the Laboratoires Analyze et de Controlee des Medicaments et des Diner’s Aliment Aires(LACOMEDA) of the ICS (University of Peshawar) was used as an antiamoebic reference product. Ten milligrams of metronidazole was dissolved in 2ml MeOH and treated in the same manner as described for extracts[18].

**Test for Alkaloids:** About 0.2g of each of the fractions was warm with 2% H2SO4 for two minutes. The reaction mixture was filtered and added a few drops of dragendorff’s reagent was to each filtrate. Orange-red precipitate indicates the presence of alkaloids moiety.

**Test for Tannins:** A small quantity of each extract was mixed with water and heated on a water bath and filtered. A few drops of ferric chloride were added to each filtrate. A dark green solution indicates the presence of tannins.

**Test for Anthraquinone:** About 0.5g of each extract was boiled with 10% HCl for a few minutes on a water bath. The reaction mixture was filtered and allows to cool. An equal volume of CHCl3 was added to each filtrate. Few3 drops of 10% ammonia were added to each mixture and heated. Rose-pink colour formation indicates the presence of anthraquinone.

**Test for Glycosides:** Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of feelings solution A and B were added to each mixture. The formation of a red precipitate indicates the presence of glycosides.

**Test for Reducing Sugar:** Each extract was shaken with an orange-red precipitate indicating the presence of reducing sugars.

**Test for Saponins:** 0.2g of each extract was shaken with 5ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins test for flavonoids: About 0.2g of each extract was dissolved in dilute NaOH and a few drops of HCl were added. A yellow solution that turns colourless indicates the presence of flavonoids.

**Test for Phlobatanins:** About 0.5g 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.

**Test for Steroids:** Exact 2ml of acetic anhydride was added to the mixture of 0.5 g of each extract and H2SO4 (2ml). The colour change from violet to blue or treen in some samples indicates the presence of steroids.

**Test for Terpenoids:** About 0.2g of each extract was mixed with 2ml of chloroform and concentrated H2SO4 (3ml). was carefully added to form a layer. The formation of a reddish-brown colouration at the interface indicates positive results for the presence of terpenoids.

**Test for Cardiac Glycoside:** To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added. Then few drops of concentrated H2SO4 were added. The presence of greenish-blue colour indicates the presence of cardiac glycosides.

**Test for Emodins:** Exact 2ml of NH4OH and 3ml of benzene was added to extract. The appearance of red colour indicates the presence of emodins.

**Test for Caumarine:** Exist 3ml of 10% NaOH was added to 2ml of aqueous extract formation of yellow color indicates the presence of coumarin.

**Test for Anthocyanin and Betacyanin:** To 2ml of plant extract, 1ml of 2N NaOH was added and heated for 5 minutes at 100˚C. The formation of bluish-green colour indicates the presence of anthocyanin and the formation of yellow colour indicates the presence of betacyanin[20]

3. Result and discussion:

Phytochemical screening on the crude methanol extracts of *P. niveum* was done. *P. niveum* was collected from the Bara Khyber agency KPK Pakistan. The plant was identified by Sir Ghulam Jelani of Department of botany University of Peshawar, Pakistan. 1Kg shade dried plant of *P. niveum* was filled in the flask and extracted
successively with methanol solvent in soxhlet extractor for 30h. All these solvent extracts were evaporated and concentrated under reduced pressure at 55˚C with the help of a rotary evaporator, obtained 70g plant extract.

Phytochemical screening tests which we have done on p. niveum are listed below in the table.

<table>
<thead>
<tr>
<th>Chemical compounds tests</th>
<th>Crude methanol Extract</th>
<th>Chemical compounds tests</th>
<th>Crude methanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>Soluble starch</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>Emodins</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
<td>Anthocyanine</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>Cardiac Glycoside</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>Anthraquinone</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Caumarine</td>
<td>+</td>
<td>Phlobatanins</td>
<td>-</td>
</tr>
<tr>
<td>Beta Cyanine</td>
<td>+</td>
<td>The Alkaloids present in P. niveum extract</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>Key= - = absent</td>
<td></td>
</tr>
<tr>
<td>Free Reducing Sugar</td>
<td>+</td>
<td>+=Present</td>
<td></td>
</tr>
<tr>
<td>Combined Reducing sugar</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Conclusion

It is concluded that P. niveum are the rich sources of antioxidant molecules and these plants are recommended to use as antioxidant for the management of various ailments.

References


