PHYTOCHEMICAL EVALUATION OF AQUEOUS AND ETHANOLIC EXTRACT OF NEEM LEAVES (Azadirachta indica)

Vibha Singh* and Divya Chauhan
Department of Food Science and Biotechnology, Jayoti Vidyapeeth Women’s University, Jaipur-303122, Rajasthan, India.

ARTICLE INFO
Article history
Received 10/12/2014
Available online 31/12/2014

Keywords
Azadirachtin, Phytochemical Screening, Precipitation, Phytoconstituents.

ABSTRACT
Phytochemicals are secondary metabolites, which are produced by medicinal plant. In Pharmacological studies, Azadirachta indica is considered as important medicinal plant. It is commonly known as Neem, belongs to family Meliaceae. This plant has main chemical constituent like Azadirachtin, Nimbin, Gedunin and Quercetin. It has been used for prevents illness and they were reported to have therapeutic values. The major aim of present study was to investigate the phytochemical screening of Aqueous and Ethanolic extracts of Azadirachta indica Leaves. Aqueous and Ethanolic extracts were prepared and subjected to phytochemical screening in which the secondary metabolites were confirmed based on tests of coloration and precipitation. The leaves have shown the presence of all the phytoconstituents like carbohydrate, alkaloids, glycosides, phenolic compounds and tannins, flavanoids etc. Among the both extracts used, the ethanolic extract of Azadirachta indica leaves was found to have accountable number of phytoconstituents.

Corresponding author
Vibha Singh
Department of Food Science and Biotechnology, Jayoti Vidyapeeth Women’s University, Jaipur-303122, Rajasthan, India. vibhapratapsingh16@gmail.com


Copyright © 2014 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. www.iajpr.com
INTRODUCTION

Medicinal Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources [1]. Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries [2]. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds [3].

Azadirachta indica, commonly known as Neem, belongs to family Meliaceae, and it is native of India and distributed wide spread in the world. Neem is called ‘aristha’ in Sanskrit a word that means ‘perfect, complete and imperishable’. Arishtha is the Sanskrit name of the Neem tree meaning ‘reliever of sicknesses’. It is a tall evergreen tree whose bark is hard rough and scaly. Its leaves are alternate, flowers are small and white in color. It reaches up to 15–20 m (about 50–65 feet) tall, and sometimes even to 35–40 m (115–131 feet) [4-6] [Fig. 1].

Fig.1: Azadirachta indica (Neem): a) Mother Plant; b) Flower; c) Leaves.

The Chemical constituents contain many biologically active compounds that can be extracted from Neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids, ketones, triterpenoids and limonoids: saladucin, valassin, meliacin, Nimbin Nimbicin, geducin and Azadirachtin etc [7-9]. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective [10]. Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin [11, 4]. The isolation of nimbin was reported as first bitter compounds isolated from neem oil, more than 135 compounds have been isolated from different parts of neem [12]. Azadirachtin, a major compound of the neem has potent anti- fedent, growth and reproductive regulating properties. Likewise, nimbin, a limonoid from neem, is also involved in improving pesticide properties [13].

Neem leaf is effective in treating eczema, ringworm, acne, anti-inflammatory, anti-heperglycemic properties and it is used to heal chronic wounds, diabetic food and gangrene developing conditions. It is believed to remove toxins from the body, neutralize free radicals and purify the blood. It is used as anticancer agent and it has hepato-renal protective activity and hypolipidemic effects [14].

Neem extract has been reported to have anti-fungal, antibacterial, anti-protozoal, antidiabetic and antiviral activity. The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children [15-16]. It is also considered as a natural insecti- cide/pesticide plant and the quality of pesticide and pharmacological products depend upon the contents of azadirachtin and nimbin in the plant [13].
Accordingly, all parts of this plant are useful and have been used in treatment of diseases ranging from teeth decay, ulcers, swollen liver, malaria, dysentery, diarrhea etc.\textsuperscript{[17-21]} They possess astringent, purgative anti-inflammatory, moderate anti-tumor and bactericidal effects\textsuperscript{[22-24]}.

The present study was carried out to find the solvent system among the aqueous and ethanolic solvents used and also to find the maximum number of phytoconstituents in Azadirachta indica leaves.

**MATERIALS AND METHODS**

**Collection and Identification**

Fresh leaves of Neem (Azadirachta indica) collected from Herbal garden of Jayoti Vidyapeeth Women’s University, Jaipur, Rajasthan and taxonomic identification of the plant from Department of Botany University of Rajasthan [RUBL 211373].

**Preparation of Powder**

Fresh plant leaves were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

**Extraction of Plant Material**

**Aqueous extraction**

20 gm powder of Azadirachta indica leaves was taken and extracted with sufficient quantity of distilled water using Soxhlet apparatus for 24 hrs at 60-80°C. The extracts were filtered in each step, concentrated, and the solvent was removed by rotary evaporator. The extracts were dried in incubator (45°C). The extract was concentrated and stored in refrigerator (4°C) for further analysis\textsuperscript{[25]}.

**Ethanol extraction**

20 gm powder of Azadirachta indica leaves was taken and extracted with sufficient quantity of ethanol using Soxhlet apparatus for 24 hrs at 60-80°C. The extracts were filtered in each step, concentrated, and the solvent was removed by rotary evaporator. The extracts were dried in incubator (45°C). The extract was concentrated and stored in refrigerator (4°C) for further analysis\textsuperscript{[25]}.

**Phytochemical Studies**\textsuperscript{[26]}

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, phenolic compound, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure:

**Test for Alkaloids**

For detection of alkaloids, 500 mg of extract was dissolved with 20 ml of HCl (1%) than filter and following test was performed:

- 2 ml of filtrate, 1 ml of Mayer’s reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.
- 2 ml filtrate; 2 ml of Wagner’s reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.
- 2 ml of filtrate, 1-2 ml of Hager’s reagent were added. A prominent yellow precipitate indicated the test as positive.

**Test for Carbohydrates**

Crude extract (400 mg) was dissolved in 20 ml of distilled water and filtered. The filtrate was subjected to following test:

- To 2 ml of filtrate, molish reagent was added slowly drop wise along the side of test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.
- 1 ml of filtrate was boiled on water bath with 1 ml of each Fehling solution A and B. A red precipitate indicated the presence of sugar.
- To 1 ml of filtrate, 1 ml of Barfoed’s reagent was added and heated on boiling water bath for 2 min. Red precipitate indicated the presence of sugar.
- To 1 ml of filtrate, 1 ml of Benedict’s reagent was added. The mixture was heated in boiling water bath for 2 min. A characteristic colored precipitate indicated the presence of sugar.
**Test for glycosides**

For detection of glycoside, 500 mg of extract was dissolved with 20 ml of concentrated HCl than filter and following test was performed:

- To 2 ml of filtrate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonium solution was added to it. Pink color indicated the presence of glycosides.
- 2 ml of filtrate was mixed with each of the 2 ml of chloroform and 2 ml of acetic acid. The mixture was cooled on ice and carefully concentrated H$_2$SO$_4$ was added. A color change from violet to blue to green indicated the presence of steroidal nucleus.

Extract was mixed with 1ml of glacial acetic acid containing 2 drops of 2% solution of FeCl$_3$. The mixture was then poured into another test tube containing 1ml of concentrated H$_2$SO$_4$. A brown ring at the interphase indicated the presence of glycosides.

**Test for Proteins**

For detection of proteins, 100 mg of extract was dissolved with 10 ml of distilled water then filtered through Whatman’s filter paper and the following test will be performed:

- 2 ml of filtrate, 1-2ml of million’s reagent was added. A white precipitate indicated the presence of proteins.
- 2 ml of filtrate was treated with 0.5 ml of 2% CuSO$_4$ solution, then 1 ml of 95% Ethanol was added, followed by 1 potassium pellet, pink color in the ethanolic layer indicated the presence of proteins.

**Test for Phenolic compound and Tannins**

2 ml of filtrate, 1-2 drops of 5% FeCl$_3$ solution was added. A dark green color indicated the presence of phenolic compound.

2ml of filtrate, 0.5 ml of lead acetate solution was added. A bulky white precipitate indicated the presence of Phenolic compounds.

**Test for Flavanoids**

Extract were treated with 3 ml of 2% NaOH solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid (H$_2$SO$_4$), indicates the presence of flavanoids.

The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

**Saponins**

The extracts (aqueous and ethanolic) were diluted with 20 ml of distilled water separately and further shaken for 15 mins in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicated the presence of saponins.

**Fixed oils & Fats**

Small quantity of extracts was pressed between two filter papers separately. An oily stain on filter paper indicated the presence of fixed oil.

**RESULT AND DISCUSSION**

The secondary metabolites contribute much in the direction of the biological activities of medicinal plants such as hypoglycemic, anti-diabetic, antioxidant, anti-inflammatory, anticancer agent, anti-carcinogenic, anti-malarial, anti-cholinergic, antileprosy activities, antimicrobial activity etc [14-16]. Several therapeutic effects of Azadirachta indica was established in Indian system of medicine. Various phytochemicals that are present in leaves of Azadirachta indica are responsible for this therapeutic effect. Presences of phytochemicals were analyzed by the qualitative tests which are shown in table 1.

**Table No. 1: Phytochemical study of Neem (Azadirachta indica) leaves:**

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fixed oils and fats</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

“Each test done in triploids”, “+”presence, “-” absence
In table 1, Ethanolic extract of A. indica recorded the presence of glycoside, flavonoids, proteins, carbohydrates, phenolic compounds, tannins and saponins. Aqueous extract was found to have maximum number of phytoconstituents except saponins. Aqueous extract also shows the presence of alkaloids.

The phytoconstituents are well known for its curative activity against several human problems such as ulcers, swollen liver, malaria, dysentery, diarrhea etc. [17-21]. A variety of herbs and herbal extracts contain different phytochemicals with biological action that can be of valuable therapeutic index. Much of the protective effect of herbal plants has been attributed by phytochemicals, which are the non-nutrient compounds [27].

Existing literatures signify that medicinal plants are the backbone of traditional medicine. Alkaloids, flavonoids, glycosides and phenols have been reported to exert multiple biological effects like anti-inflammatory, anti allergic, antioxidant, anti-diabetic, anti-viral and anti cancer activities, anti-leprosy activities, antimicrobial activity etc. [14-16].

CONCLUSION

This study provides evidences that both leave extract of A. indica found to have phytoconstituents and support that the ethanolic extract of A. indica leaves have more number of phytoconstituents when compared to aqueous leave extract of this plant. A. indica leaves showed abundant amount of phytochemicals as secondary metabolites which participate in various pathophysiological conditions of different diseases. Intense study in this plant will help to identify the active principle and this can be used as clues for developing new drugs which may be used in the pharmaceutical industries for modern drug discoveries.

REFERENCE