Preliminary phytochemical screening of *Azadirachta indica* for identification of bioactive compounds

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Abstract

A crucial process that results in the isolation of new and novel compounds is phytochemical screening. Phytochemical screening is a key process before bulk extraction and isolation. The stem of *Azadirachta indica* has been chosen for phytochemical analysis in order to determine the various classes of secondary metabolites. The extracts indicated the presence of various classes of bioactive secondary metabolites including; terpenoids, flavonoids, and tannins. The bark displayed terpenoids and flavonoids. The important biological activities of *Azadirachta indica* is are due to the presence of these identified compounds. This work will direct the researcher to isolate new therapeutic agents from various parts of *Azadirachta indica* for new drug discovery.

Key words: *Azadirachta indica*, terpenoids, flavonoids, and tannins

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1. Introduction

We all recognize *Azadirachta indica* "neem." The indigenous to India and in nature occur in the tropical and subtropical regions throughout several nations. Azadirachta indica is widely used as medicine and is available all over the world. Numerous biologically active substances are isolated from the neem's chemical ingredient, which includes triterpenoids, alkaloids, flavonoids, carotenoids, phenolic compounds, ketones, and steroids [1]. Salannin, volatile oils, meliantriol, and Nimbin are other substances found in *Azadirachta indica* that have biological effects (Jacobson). The *Azadirachta indica* leaf has anti-inflammatory and antihyperglycemic

characteristics, making it useful for treating a variety of skin ailments like eczema, ringworm, and acne, as well as conditions that can lead to the development of gangrene. *Azadirachta indica* is thought to have been used to cleanse the blood, fight free radicals, and remove toxins from the body. *Azadirachta indica* also functions as an anticancer agent, has hepato-renal protecting characteristics, and has a hypolipidemic effect. The neem plant is huge, and Azadirachta indica has a semi-straight trunk and a height of around 25 meters. It is mostly recognized as a flowering plant and typically starts to accept fruit after 3-5 years. Within ten years, the tree begins to bear fruit [2]. Azadirachta indica trees have rough, grey bark. Azadirachta indica pinnet leaves size is 30 centimeters long, with serrated leaflets is10–12 that are each length is 7 centimeters and wide of each leaf is 2.5 centimeters. The neem trees thrive in the places with little rainfall, such as these [3]. Every portion of the chosen *Azadirachta indica* plant exhibited therapeutic qualities and is used as a medication to treat a variety of diseases and ailments. Smallpox and chicken pox are traditionally treated using the leaves paste to cure allergic skin reactions and as an antiviral [4].

Nimbin, which is largely responsible for the Neem oil's biological effects, has antipyretic, antihistamine, fungicidal, anti-inflammatory, and antiseptic characteristics. [5]. A potent acetylcholinesterase inhibitor called azadirachtin [6], and salannin are being tested for their ability to reduce NO generation. [7]. The plant neem all parts, including its flowers, leaves, roots, seeds, bark, fruits, and flowers, have been isolated in greater than 140 compounds, and these parts have been found to have interferon-inducing activity (bark), Antibacterial, antifungal, antiviral, and antiviral activity against skin illnesses activity (leaves), antimalarial, antipyretic and anti-inflammatory, immunomodulatory, antiulcer, antifungal, antiviral, and antiviral against skin maladies characteristics. [8, 9].

Azadirachta indica has long been a popular traditional anti-antimalarial remedy in the tropics [10, 11]. The extract of the leaf and seed have inhibitory action against both the sexual and asexual stages of the Plasmodium falciparum, according to various in vitro and in vivo investigations, [12][13]. Epoxyazadiradione, nimbolide, andgedunin in particular appear to be the primary metabolites in charge of an antimalarial action. [14, 15].

Numerous plants offer a variety of intriguing chemical classes, including terpenoids, alkaloids, polypeptides, tannins, quinones, flavonoids, and coumarins,. These chemical classes have a significant potential to prevent or treat fungal, protozoal, bacterial, and viral infections. Among these plants, *Azadirachta indica*, an endemic of India, was examined for its antihypertensive, anticancer, anti-inflammatory, antidiabetic, antihypertensive, and neuroprotective properties. It also grows in a number of Asian and African nations, as well as in America and Australia [5].

MATERIALS AND METHODS

2. Plant Material

Azadirachta indica parts such as stems were collected from the Maneriarea of district Swabi Pakistan's Khyber Pukhtun Khawa province in the month of March 2022. The plant samples were identified by Dr. Muhammad Ilyas Department of Botany in University of Swabi.

2.1. Extraction

The plant materials were dried for 10 days at room temperature. *Azadirachta indica* dried plant material was ground into a fine powder. The components were pulverized, steeped in methanol for five days, and extracted until all plant materials were used up. The extracts were then concentrated using a rotary evaporator under decreased pressure at a temperature below 60 °C.

2.2. Phytochemical screening

Standard protocols were used in the chemical analyses to identify the components described by Sofowora in the n-hexane, chloroform, ethyl acetate, and methanolic extract of the stem of Azadirachta indica. [16], Trease [17], and Evans and Harborne [18].

2.3. Flavonoids

The presence of flavonoids was determined by dissolving 0.5 g of plant extract in a weak sodium hydroxide solution, followed by a few drops of hydrochloric acid solution. The yellowish solution subsequently turned colorless.

2.4. Steroids

0.3 g of each extract was combined with 2 ml of H_2SO_4; 2 ml of acetic anhydride, and 2 ml. Steroids were detected in certain samples when the color changed from violet to blue or green.

2.5. Tannins

Each extract was filtered after being boiled in a water bath after being diluted with water. A few drops of ferric chloride were added to the filtrate. A dark green solution indicates the presence of tannins.

2.6. Anthraquinones

A little amount of plant extract (0.5 g) and their minute fractions were boiled with 10 HCl for a brief length of time, the resultant liquid was filtered, cooled, and some CHCl3 was added to each filter along with a few drops of 10% ammonia, followed by heating. The rose-pink coloring indicates the presence of anthraquinones.

2.7. Saponins

After boiling plant extract (0.3 g) in distilled water (5 mL), a white foamy appearance implies the presence of saponins.

2.8. Phlobatanins:

Before filtering, the extract (0.5 g) was diluted in distilled water. A 2% HCl solution was used to boil the filtrate. Phlobatanins are visible as a red precipitate.

2.9. Terpenoids

Concentrated H SO (3 ml) was carefully added to 0.5 g of each extract in 2 ml of chloroform to create a layer. The interface was successful in producing a reddish-brown coloring to show that terpenoids were present.

3. Results

The result of the phytochemical screening test is given below (Table 1). Phytochemical screening is an important preliminary step for the isolation of active compounds. The methanol extract indicated the presence of flavonoids, steroids, tannins, anthraquinones, saponins, phlorotannins, phlobatanins, and terpenoids.

Table 1: Phytochemicals analysis of crude extracts of *Azadirachta indica* and the various fractions.

Phytochemicals	*n*-Hexane	Chlorofomm	Ethyl acetate	**Aqueous extract**
Flavonoids	-	-	+	+
Steroids	-	-	-	-
Tannins	+	-	+	+
Anthraquinones	-	-	-	-
Saponines	-	-	-	-
Phlobatanins	-	-	-	+
Terpenoids	-	-	-	-

4. Discussion

Phytochemicals are naturally occurring compounds present in various parts of plants. These are called primary and secondary metabolites. Flavonoids, tannins, and Phlobatanins were confirmed in the aqueous and ethyl acetate extracts of *Azadirachta indica.* The presence of tannins was also confirmed in *the n*-hexane extract. Plants are rich in various phytochemical constituents and these phytochemical constituents are so significance due to their multiple applications. They are used in folk medicines, modern medicines, synthetic drugs, and different food supplements [19]. Tannins have the characteristic feature of injury healing and flavonoids are potent antioxidants and free radical scavengers which inhibit the cells from being damaged. Flavonoids have also anticancer effects [19].

Tannins and flavonoids consist of a major class of significant polyphenolic compounds with vast biological properties including antioxidant, and anti-carcinogenic properties [20]. Polyphenolic compounds are the major class of phytochemicals that acts as primary reductant [21]. The different research reports reveal the antioxidant study of flavonoids [22]. The flavonoids show scavenging properties which prevent the formation of radicals and neutralized the already formed. The flavonoids, phenolic acid, and polyphenols are the phytochemicals that scavenged the free radical e.g. peroxide and lipid peroxyl that precede further the oxidative process which

led to degenerative disorders [22]. Tannins, alkaloids, terpenoids, and saponins are often screened in plants having medicinal values or used for pharmacological purposes. These natural products or secondary metabolites show antimicrobials, and antioxidant and antiviral activities [23]. The polar bioactive phytochemicals such as flavonoids and tannins are mostly screened in polar solvents and have shown anti-inflammatory and analgesic effects [24]. Flavonoids in the polar extract of methanol have been shown to obstruct the cyclooxygenase and 5-lipoxygenase routeways. This obstruction decrease annunciates of arachidonic acid [24]. The polar extracts have confirmed the presence of the phytochemical constituents of flavonoids and tannins and showed the power of anti-hyperglycemic and anti-inflammatory effects [25].

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