

Phytochemical analysis and antibacterial evaluation of ethanol stem bark extract of *Azadirachta indica* Grown in Dibila Kebelle, Tigray-Ethiopia

¹ Mengisteab Gebrehiwot Meressa

¹Head, department of chemistry

¹Department of Chemistry, College of Natural and Computational Science, Adigrat University P.O.Box 50, Adigrat, Ethiopia

Abstract-*Azadirachta indica* is member of the Meliaceae family and is popularly known as margosa tree. The aim of this study was to investigate the phytochemical composition and the antibacterial property of the medicinal plant, *Azadirachta indica*. For this purpose, the air dried powdered stem bark sample (400 g) of the plant was extracted by soaking in 1000ml of with ethanol for 72 hours and concentrated using rotary evaporator. The antibacterial activities of these extracts were investigated against gram positive bacteria (*S. b hemolytic* and *Staph. aureus*) and gram negative bacteria (*Salmonella typhi* and *Escherichia Coli*). The antibacterial activity was tested using Muller Hinton Agar medium by disc diffusion method and minimum inhibitory concentration assays. After incubation, zone of inhibition was measured in mm, a good inhibition (>9mm) was observed indicating the effective antibacterial activity of the bioactive compounds in the plant extract. This research work also demonstrated that the presence of Phytoconstituents such as Tannins, Saponins, Phlobatanins, Flavanoids, Cardiac glycosides and Alkaloids in the stem bark ethanol crude extract of *Azadirachta indica*.

Keywords-*Azadirachta indica*, Meliaceae, medicinal plant, Antibacterial, phytochemical, *Staphylococcus*, *Escherichia coli aureus*, Muller Hinton Agar medium.

I. INTRODUCTION

Since the prehistoric time, alleviation of diseases has been one of the primary concerns of mankind. Local practitioners have used indigenous plants and herbs showing definitive pharmacological actions such as purgative, emetics, central nervous system (CN) stimulants and depressants etc. for the treatment of a variety of ailments. In contrast to this, plants producing toxic effects were being used in the hunting or warfare, while plants derived products like opium, hashish has long been used as hallucinating agents [1].

Medicinal plants have been used for centuries as remedies for human diseases. They constitute an effective source of both traditional and modern medicine. The acceptance of traditional medicine as an alternative form of health care hassled researchers to further investigate antimicrobial activity of medicinal plants. Some countries in Africa, Asia, and Latin America use traditional medicine to help meet some of their primary health care needs [2]. The use of plant compounds to treat infections is an age-old practice in large parts of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases [3, 4]. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics [5, 6].

Studies revealed that herbal medicine represents one of the most important fields of traditional medicine. WHO recognized that medicinal plants have played an important role in the health care of about 80% of the world population in developing countries and depend largely on traditional medicine [7]. Herbal remedies are popular remedies for diseases used by a vast majority of the world's population. Recently, another report showed that out of all the plants that have proved useful for humanity, a few are distinguished by their astonishing versatility. Among these, Neem tree, commonly known as *Azadirachta indica*, is one of the most important ones found in arid and semi-arid regions of world. It has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine [8].

The tree is so much resourceful that almost all of its parts via leaves, flowers, seeds, roots and bark have been used in traditional medicine as household remedies against various human ailments in some form or another. From its roots to its spreading crown, the tree contains a plethora of important compounds useful for animals, people and plants. Neem tree's virtues are, to a large extent, attributable to its chemical constituents [9].

Several researchers revealed that neem (*Azadirachta indica*) is perhaps the most useful traditional medicinal plant in India. Almost all parts of the plant are endowed with medicinal properties and have been used as traditional medicine or household remedies against various human ailments, from antiquity. In this era, it is considered as valuable source of unique natural products for the development of medicines against various diseases only crude extracts of different parts of neem have been used as traditional medicine for treatment of various diseases (Aarati, *et al.*, 2011). Each part of the neem tree has some medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem [10].

The stem bark is reputed to treat amenorrhea, malarial fever, cutaneous diseases, general debility, and burning sensation near the heart, fatigue, thirst, bad taste in the mouth, cough, ulcers, blood complaints, leprosy and urinary discharge. The bark contains

sugiol, nimbiol, nimbosterol, deacetylnimbin, kulinone, methyl kulonate, sterols, polysaccharids, polydactyl derivatives and tricyclic diterpenoide [11].

Azadirachta indica is a member of the Mahogany family. It has similar properties to its close relative, *Melia Azederach*. The word *Azadirachta* is derived from the Persian *azadhirakt* (meaning 'noble tree'). The taxonomic positions of neem are Order: Rutales, Suborder: Rutineae, Family: Meliaceae, Subfamily: Melioideae, Tribe: Me1ieae, Genus: *Azadirachta*, and Species: *Azadirachta indica* [12].

Neem is a hardy, fast-growing evergreen tree with a straight trunk, long spreading branches and moderately thick, rough, longitudinally fissured bark. Mature trees attain a height of 7-15m. The tree starts producing the yellowish ellipsoidal drupes (fruits) in about 4 years, becomes fully productive in 10 years and may live more than 200 years. The leaves are compound; impair pinnate, comprising up to 15 leaflets arranged in alternate pairs with terminal leaflets. The leaflets are narrow, lanceolate, up to 6cm long. The flowers are abundant, sweet-smelling white particles in the leaf axils. Seed propagation in nurseries followed by direct planting in the field is accepted method to produce plantation stands. The one seed fruit is yellow when ripe and is about one inch long [13]

II. MATERIALS AND METHODS

Collection of Plant Material

The plant material used in this study was collected in September, 2016, from a small village called Debilla, in the north east of Tigray National Regional State (TNRS), 109km from Mekelle and 891 km northeast of Addis Ababa. The plant was taxonomically identified by Department of biology, College of natural and computational sciences, Addis Ababa University, and a voucher specimen was deposited at the National Herbarium (Ethiopia), Department of Biology, Addis Ababa University.

Preparation of Extraction

The stem bark part of the plant was manually separated, air dried, powdered, weighed and stored in air tight container. The air dried and powdered *azadirachita indica* stem bark (400 gm) was successively extracted with ethanol/ EtOH (1000 ml) for 72 hours. This was then filtered through Whatman No. 1 filter paper using a Speed vacuum pump, Buchner funnel and Buchner flask. The crude extract thus obtained was concentrated to a thick brownish yellow semi-solid mass using Rotary vacuum evaporator and kept in the refrigerator for further analysis.

Microbial strains

The bacterial strains *S. b* hemolytic, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* were procured from the quality control laboratory of Ethiopia's Food, Medicine and Health Care Administration and Authority, Addis Ababa-Ethiopia..

Phytochemical Analysis of Plant Extracts

The ethanolic extract of the plant was tested for the presence of various active principles using standard procedures [14, 15, 16, and 17].

Test for Tannins:

A 0.02g of the crude extract was boiled in 40 ml of water in a boiling tube. Few drops of 0.1 % of $FeCl_3$ were added. Formation of brownish green or a blue black coloration indicated the presence of tannins.

Test for Saponins:

A 0.04 g of the crude extract was boiled in 40 ml of distilled water in a water bath. Then it was mixed with 10 ml of distilled water and it was shaken well. Stable persistent froth indicated the presence of saponins.

Test for Phlobatanins:

A 0.04 g of the crude extract was boiled with 1 % aqueous hydrochloric acid. A deposition of a red precipitate indicated the presence of phlobatanins.

Test for Flavanoids:

A 0.04 g of the crude extract was dissolved in 4 ml of ethanol solvent. Con. HCl and Mg turnings were added. Formation of yellow colour indicated the presence of flavanoids.

Test for Steroids:

A 0.01 g of the crude extract was dissolved in 4 ml of ethanol solvent. 2 ml of acetic anhydride and 4 ml of con H_2SO_4 were added. A colour change from violet to blue or green indicated the presence of steroid.

Test for Cardiac glycosides:

A 0.02g of the crude extract was dissolved in 4 ml of ethanol and then 4 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underplayed with 2 ml of con. H_2SO_4 . Appearance of brown ring indicated the presence of the cardiac glycosides.

Test for Alkaloids:

A 0.02 g of crude extract was dissolved in ethanol and it was divided into two parts. Few drops of Mayer's reagent were added to one part. A creamy white precipitate indicated the presence of alkaloids. Few drops of Wagner's reagent were added to other part. A red-brown color precipitate indicated the presence of alkaloids.

Antibacterial Activity of the Crude Extracts

Following extraction, the crude extract was collected and tested by using disc diffusion and agar well diffusion methods against gram positive bacteria (*S. b* hemolytic and *Staph. aureus*) and gram negative bacteria (*Salmonella typhi*, and *Escherichia coli*).

The tested bacteria were prepared by mixing a few bacterial colonies (1ml) from exponential phase with 9 ml of sterile nutrient broth and the turbidity was adjusted with 0.5 McFarland standards which is equivalent to 106-108cfu/ml. The sterile swab was dipped into the properly adjusted inoculum and swabbed on the Mueller Hinton agar (MHA) plates. Sterile cork borer (6 mm diameter) was used to bore holes in the plate and 100 μ l of the crude extracts at a concentration of 10 mg/ml was carefully dispensed into bored holes in triplicate. Filter paper sterilized with methanol was used as negative control. The crude extracts

were allowed to diffuse for about 2 hrs before incubation and then incubated at 37°C for 24 hrs. After 24 hrs of incubation, the presence of a zone of inhibition around each well was recorded.

III. RESULT AND DISCUSSION

Phytochemical Analysis of Plant Extracts

Table 1 presents the result of the phytochemical screening of the stem bark of *Azadirachta indica*. It indicated that phytochemical screening of the ethanolic extract of *A. indica* revealed the presence of Tannins, Saponins, Phlobatanins, Flavanoids, Cardiac glycosides and Alkaloids, but we could not found positive result for steroids. Therefore; this plant could serve as a good source for Tannins, Saponins, Phlobatanins, Flavanoids, Cardiac glycosides and Alkaloids.

Table 1. Phytochemical screening of the stem bark of *Azadirachta indica*

S.No	Test for	Ethanol extract
1	Tannins	+
2	Saponins	+
3	Phlobatanins	+
4	Flavanoids	+
5	Steroids	-
6	Cardiac glycosides	+
7	Alkaloids	+

Key: (+) = Presence; (-) = Absence.

Antibacterial Activity the Crude Extract Against

The results in Table 2 and fig.1 indicated that the antibacterial activity of Ethanol extract of the stem bark of *Azadirachta indica* showed maximum zone of inhibition (20mm) against *Salmonella typhi*, followed by *Escherichia Coli* (16mm), *Staphylococcus aureus* (10mm) and *S. b hemolytic* (9mm).

Table 2. Antimicrobial activity of stem bark extract of *Azadirachta indica*.

S.No	Test organism	Average inhibition zone(mm)
1	<i>Escherichia coli</i>	16
2	<i>Salmonella typhi</i>	20
3	<i>S. b hemolytic</i> ,	9
4	<i>Staphylococcus aureus</i>	10

Figure 1 showed that the ethanol extract of the stem bark of *Azadirachta indica* had maximum zone of inhibition against *Salmonella typhi* and minimum zone of inhibition against *S. b hemolytic*.

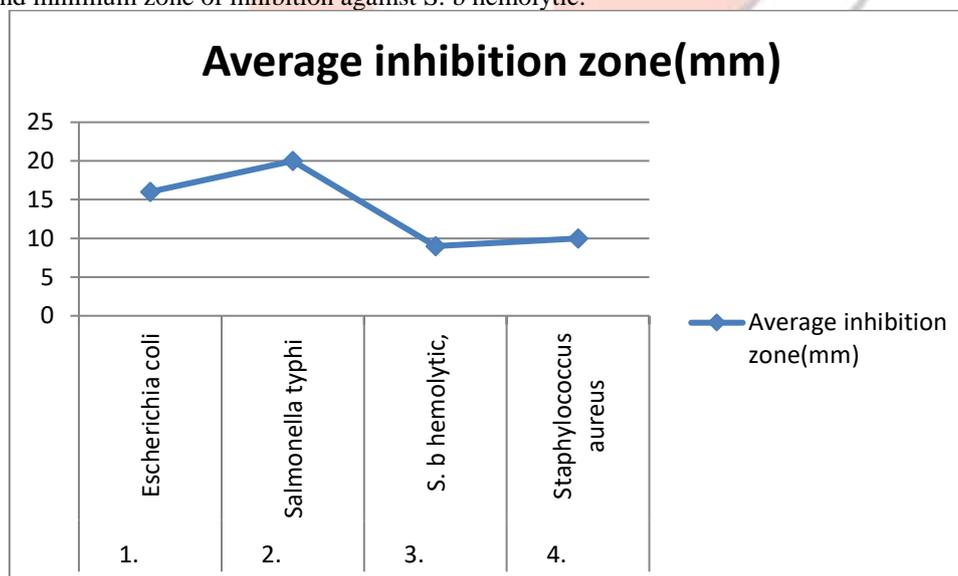


Figure 1 . Antimicrobial activity of stem bark extract of *Azadirachta indica*.

IV. CONCLUSION

This work was intended to study the phytochemical analysis and antibacterial activity of the stem bark of neem. Results from the qualitative analysis showed that Tannins, Saponins, Phlobatanins, Flavanoids, Cardiac glycosides and Alkaloids were found in the stem bark of *Azadirachta indica*. The analyses of the results from antibacterial tests confirmed the presence of active compounds which were extracted by ethanol that has a wide range of zone of inhabitation against the tested bacterial strains.

Since the results obtained from the qualitative analysis on the phytoconstituents of the plant part have clearly shown that the crude extract of the stem bark of neem contains Tannins, Saponins, Phlobatanins, Flavanoids, Cardiac glycosides and Alkaloids. Therefore; the researcher has recommended that these extracts should be used in the production of drugs in the area of modern pharmaceutical industries and as a source of pesticide, insecticide, and herbicide in the agricultural area so as to improve the yield

of crops and reduce pests, weeds and other organism which are competing with man specially in developing countries like Ethiopia.

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