International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 23-02-2022, Accepted: 11-03-2022, Published: 25-03-2022
Volume 7, Issue 3, 2022, Page No. 193-198

Qualitative analysis and *In vitro* antibacterial activity of *Azadirachta indica*

S Monisha, M Manju, S Mahalakshmi, Sathiya Savithri*
Department of Chemistry, Theivanai Ammal College for Women, Tamilnadu, India

Abstract
Neem is biologically called as Azadirachta Indica which is a traditional medicinal plant that grows in India, Nigeria and United States. The presence of phytochemical and biopesticide components was proven. The result revealed that saponins and terpenes were the most abundant, while tannins and glycosides were moderately abundant. Alkaloids, flavonoids and phenol were also present. The presence of these phytochemicals could account for neem's biological uses and antibacterial activity testing shows that it is more effective. Bacillus subtillis, staphylococcus aureus, Escherichia coli and pseudomonas aeruginosa are the names of four pathogens.

Keywords: neem, phytochemicals, antibacterial activity

Introduction

Through the augmentation of antioxidant activity, prevention of bacterial development [1], plant products or natural products play an essential role in biological activity and treatment [2]. Because of their low cost, the therapeutic role [3] of a variety of plants in disease management is still being vigorously reached. It is widely acknowledged that allopathic medications [4] are generated from natural resources, including medical plants, as is widely understood. In Ayurvedha, Unani, Homeopathy, and modern medicine, neem components are used to treat a variety of infections, metabolic, and malignant diseases [5].

There have been reports of antibacterial, antifungal, anti-inflammatory biological and Pharmacological activities. Recently, their anti-inflammatory [6], anti-arthritic [7], antipyretic [8], anti-gastric ulcer [9], antifungal, antibacterial and anti-tumour [10] activities have been demonstrated, and a study highlighted neem's many therapeutic roles. From the dawn of civilization, medicinal plants have been an integral part of human society in the fight against sickness. For more than 2000 years, A. Juss (syn. Melia azadirachta) has been known in India and its neighbouring countries as one of the most versatile medicinal plants with a wide range of biological activity [11].

The relevance of the neem tree has been recognized by the United States National Academy of science, which published a paper titled Neem a tree for solving world problems. According tomany scientific research, neem seeds contain chemical compounds that can control over 100 different insect and microbe species [12].

Immune modulatory [13], antiulcer, antimalarial, antifungal, antibacterial [14], antiviral, antioxidant [15], antimutagenic and anticarcinogenic activities have all been demonstrated for neem leaf and its constituents.

For generations, man has been aware of herbal treatments. Traditional medicine [16] practitioners have highlighted the medical usefulness of various indigenous plants for a variety of ailments. Secondary metabolites found in plants include tannins, terpenoids, alkaloids, flavonoids and other compounds that have been proven to therapeutic plant effects *In vitro* [17].

Plant Description

Fig 1: Structure of leaf and powder form of Azadirachta indica(neem).

Taxonomic position of Azadirachtaindica

- Order- Rutales
- Suborder- Rutinea
- Family-Meliaceae
- Subfamily- Melioideae
- Tribe- Melioideae
- Genus- Azadirachta
- Species- indica

The leaves, bark and seeds of this plant have yielded a large number of biologically active chemicals. Nimbidin, a bitter ingredient found in neem bark, has been reported to be antipyretic, non-irritant, and beneficial in skin conditions sucheczema, dermatitis, burn ulcers, herpes labialis, scabies and seborrheic dermatitis. Alkaloids, flavonoids, terpenoids, phenolic compounds, carotenoids, steroids and ketones are just a few of the biologically active compounds found in neem's chemical constituents.

Antibacterial and antifungal activity has been found in polyphenolic flavonoids isolated from fresh neem, Querestien, and sitosterol leaves [18]. Neem's antifungal, antibacterial, and anti-inflammatory properties, as well as its biological activity, have all been documented. Anti-inflammatory, antiarthritic, antipyretic, gastric ulcer, antifungal, antibacterial and antitumour activities [19] of A. indica phytochemicals have been confirmed by a number of researchers. Trichophyton, Epidermophyton, microsporum, Trichosporon and candida are among the fungus that extract from leaves, oil and seed kernels are effective against. Azadirachta indica has antibacterial action, as evidenced by these biological activities [20].

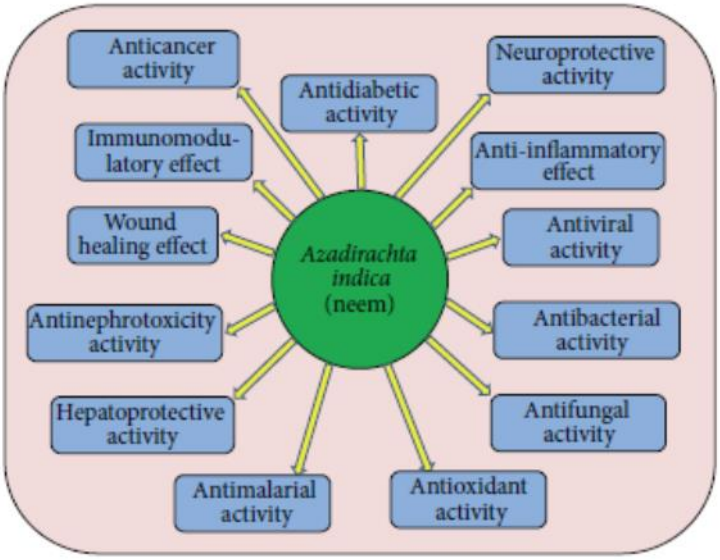

Fig 2: Pharmacological activities of Azadirachta indica in diseases management through the modulation of various activities.

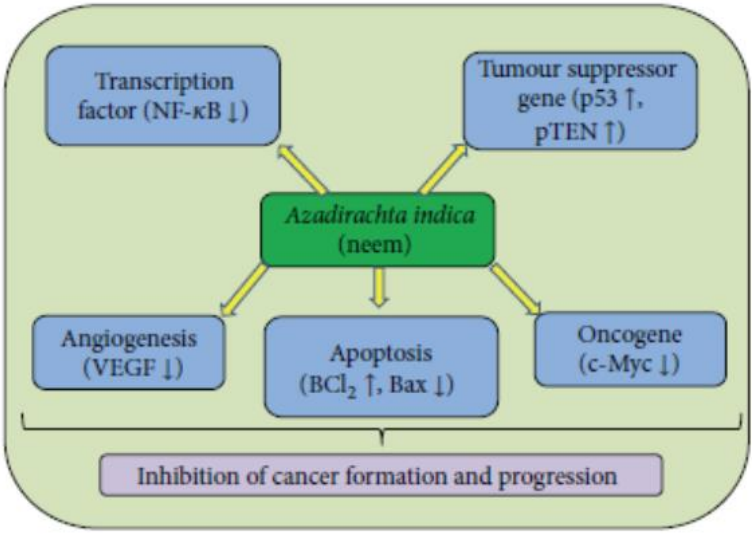

Fig 3: Antitumour activities of Azadirachtaindica through the modulation of various cell signalling pathways.

Materials and Methods

Experimental Section

Collection and Samples

The Azadirachta indica tree on campus provided the leaves for this project. It was checked to make sure the plant was healthy and free of disease. The leaves were properly cleaned and washed under running tap water to remove dust and other foreign particles, with some fresh leaves kept on hand.

Solvent Extract

Dried and fresh leaves were trampled into small bits, powdered and combined individually with ethanol, methanol, ether, and distilled water in a ratio of 1:10. The extractions were produced by grinding continuously using a motor and pestle and then filtering with whattman no.1 filter paper. The filtrates were then vacuum dried with a rotatory evaporator, and the concentrates were sored at 4 degree Celsius for further research.

Components of phytochemicals

Phytochemical studies solvents were carried out on the crude powder of leaves according to the procedures published by Trease and Evans for the identification of phytochemicals such as tannins, alkaloids, steroids, saponin, and flavonoids.

Result and Discussion

Phytochemical Analysis

Test For Carbohydrates

Add 1 ml of extract of each solvent, a few drops of Molish reagent and 1 ml of concentrated H_2SO_4 to the test tube's sidewalls. After that, the mixture was allowed to sit for 2 to 3 minutes. The presence of carbohydrates in the sample is shown by the formation of a red colour.

Test For Phenol

Combine 2 ml of each solvent extract with 5% aqueous Ferric chloride. A blue colour indicates the presence of phenol in the sample.

Test For Alkaloids

Azadirachta indica extract was evaporated to dryness, then heated in a boiling water bath with 3% Hydrochloric acid. After cooling the mixture was then treated with few drops Meyers reagent. After that, the sample was examined for yellow precipitation.

Test For Glycoside

In a test tube, combine 3 ml test solution and 5 ml glacial acetic acid and 2 drops of 6% Ferric chloride. The test tube sides were used to include 0.5 ml of concentrated sulphuric acid. The presence of glycosides was shown by the formation of blue colour in the acetic acid layer.

Test Forflavonoids

Using 5 ml of extract solution, 2 ml of 60% methanol solution was added. The solution was warmed before the magnesium was added. 6-8 drops of concentrated Hydrochloric acid were added to this solution, the presence of flavonoids was detected by the presence of orange colour.

Test For Saponins

In order to test for saponins, 3 ml of extract was vigorously shaken with 6 ml of distilled water in a test tube and heated gently. The presence of saponins were accepted as evidenced by the formation of stable foam.

Test For Protein

Using 3 ml of each solvent extract, 2ml of 50% Sodium Hydroxide, and a few drops of 3% copper sulphate was added. A violet hue was formed, indicating the presence of peptide linkage molecules in the sample.

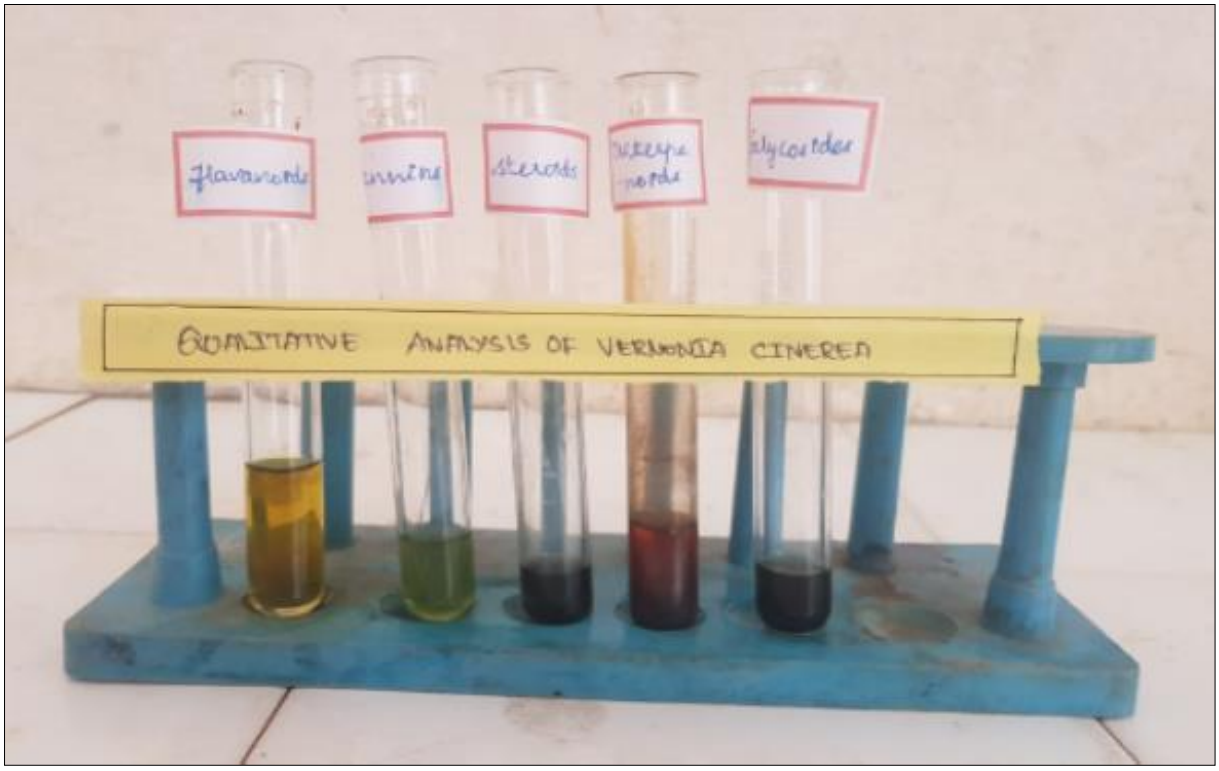

Fig 4: Qualitative analysis of Azadirachta indica

Test For Tannins

To a 3 ml of each solvent, 15% alcoholic ferric chloride was added. The production of brownish blue colour shows the presence of tannins.

Test For Terpenoids

Mix 2 ml of each solvent extract with 1 ml of chloroform and a few drops of concentrated sulphuric acid was added to make a reddish brown precipitate, which shows the presence of terpenoids.

***In vitro* antibacterial activity**

Biological methods were used to study the antibacterial activity of Azadirachta indica in vitro against twogram positive bacteria, B. subtilis and S. aureus as well as two negative bacteria, E. coli and P. aeruginosa. Azadirachta indica was examined for antibacterial activity at various concentrations, including 25,50,75 and 100 µg/ml. when compared to the conventional antibiotic streptomycin, all of the Azadirachta indica increase as the concentration of plant rises in the current investigation. Due to a higher rate of diffusion of Azadirachta indica in agar medium at a higherconcentrations, the mechanism of antibacterial action involves disruption of the membrane with a high rate of multiplication of surface oxygen species, causing pathogen death. The Azadirachta indica ruptures the cell's outer walls and inner walls, causing disarray and cell membrane leaking. The production of reactive oxygen species may be responsible for antibacterial activity, the ROS toxicity to bacteria is attributed to their high reactivity and oxidising properties.

Table 1: Zone of Inhibition of the Azadirachta indica

S. No	Positive and negative Pathogen	Zone of inhibition (diameter in mm) Azadirachtaindica				Typical (Streptomycin)
		25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	
1	Bacillus Subtilis	9	11	12	13	29
2	Staphylococcus aureus	14	15	17	19	20
3	Escherichia coli,	12	14	20	26	29
4	Pseudomonas aeruginosa,	13	17	20	26	29
5	Control (DMSO)	NI	NI	NI	NI	NI

NI:No Inhibition

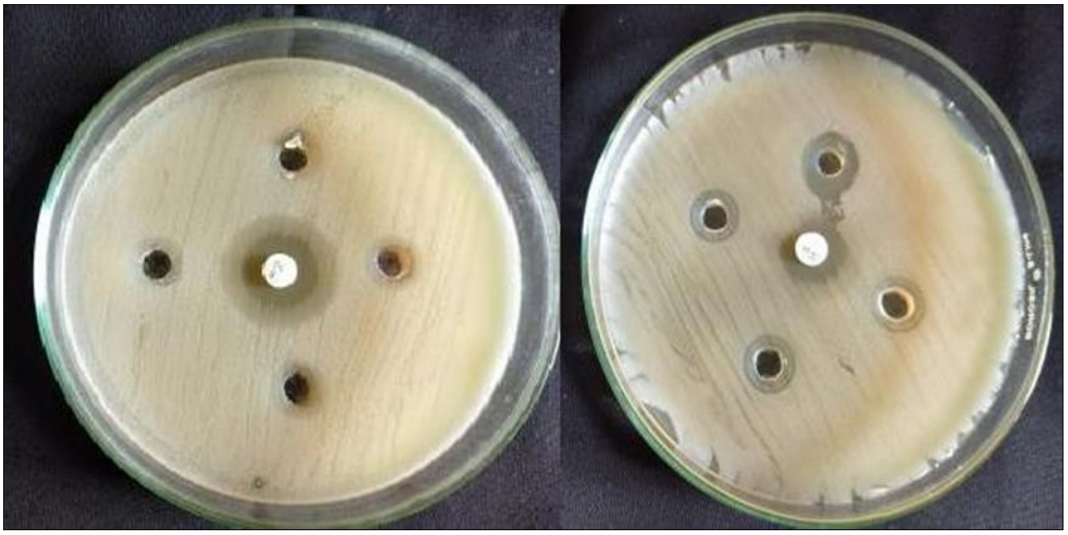

Fig 2: Antibacterial activity of the Azadirachta indica against positive pathogen Bacillus Subtilis and Staphylococcus aureus.

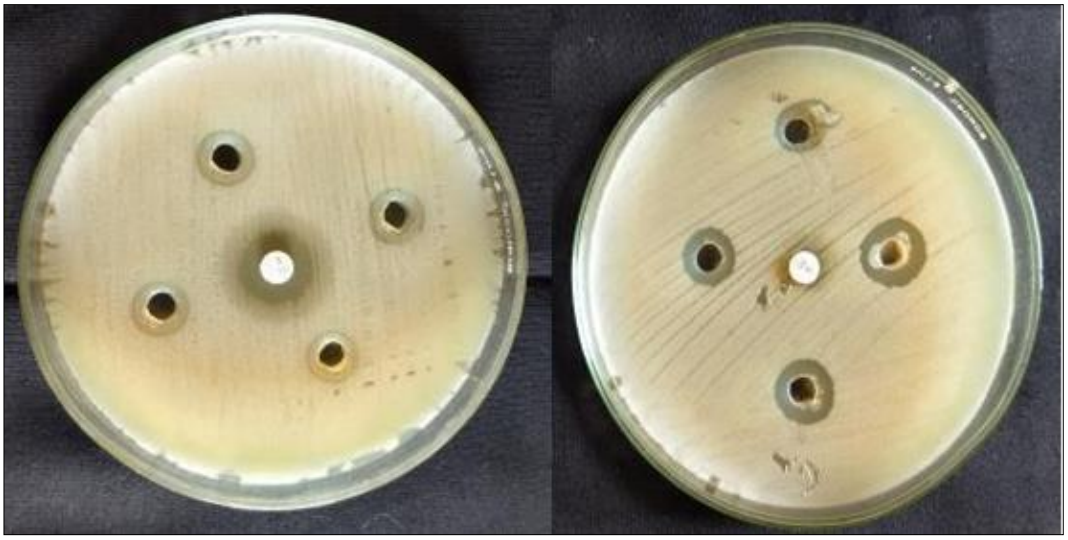

Fig 3: Antibacterial Activity of Azadirachta indica compared with standard.

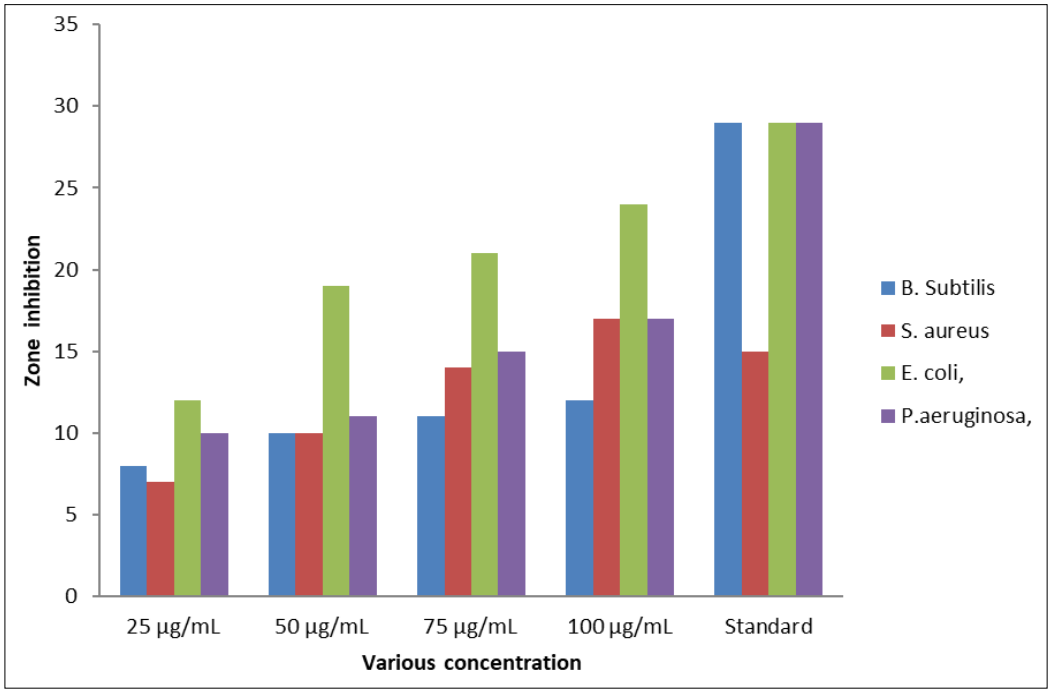

Fig 4: Antibacterial Activity of Azadirachta indica compared with standard.

Conclusion

Pseudomonas aeruginosa and Escherichia coli were used to access the antibacterial activity of the ethanolic extract of Azadirachta indica. The findings revealed that the microorganism had powerful antibiotic properties. Antibacterial activity was observed to be higher in Azadirachta indica extract.

References

1. A Zong, Cao H, Wang F. Anticancer polysaccharides from natural resoures: a review of recent research," *Carbohydrate polymers*,2021:90:1395-1410.
2. Efferth TE. Koch complex interactions between phytochemicals. The Multi Target Therapeutic concept of phytotherapy," current Drugs Targets,2011:12:122-132.
3. Brahmachari G. Neem-an omnipotent plant: *a retrospection*",2004:5(4):408-421.
4. Ketkar AY, Ketkar CM. various uses of neem products," in The Neem Tree, H.schmutterer, J*ohn Wiley and Sons Weinheim*, Germany, 2004, 518-525.
5. Singh N, Sastry MS. Antimicrobial activity of essential oil," Indian Journal of pharmacology,1997:13:102-106.
6. Paul R, Prasad M, Sah NK. "Anticancer biology of Azadirachta indica L(neem): a mini review," Cancer Biology and Therapy,2011:12:6:467-476.
7. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. "Biological activities and medicinal properties of neem (Azadirachta indica), "Current science,2002:82:11:1336-1345.
8. Girish K, Neem SB. "A green treasure," Electronic Journal of Biology,2008:4:102-111.
9. Ali A. Textbook of pharmacognosy, publication and Information Directorate, New Delhi, India, 1993.
10. Siddiqui A, M Ali. Practical pharmaceutical chemistry 1st ed, *CBS publisheds and distributors*, New Delhi, 1997, 126-131.
11. Trease GE, Evans WC. pharmacognosy. (Christiane [10]). London: 13 edn. Bailliere Tindall,1989:176-180.
12. Anonymous, The India pharmocopoeia. Goverment of India New Delhi. *Ministry of Health and family welfare*, 1996.
13. EskilHulkin, Thin layer chromatography of plant extracts, *act a chemical Scandinavia*,20, 1588-1592, 1966.
14. Bishnu Joshi, Phytochemical extraction and antimicrobial properties of different medicinal plants journal of microbiology ana Antimicrobial,2011:39:1-7.
15. Kubmarawa ME, Khan A, M Punahans Hassan. Phytochemical screening and antibacterial activity of extracts from paekiaclappererotonianakeay against human pathogenic bacteria. Journal of medicinal plants research,2008:2:352-355.
16. Kraus WR. Cramer and G. Sawizhil. Tetranotripenoids from seeds of *Azadirachta indica phytochemistry*,1981:20:117-120.
17. Zaika LL. Spices and herbs: their antibacterial activity and its determination *J. Food Saf.,* 23, 97-118,1988.
18. Cristiane, P. Victorio Flavonoid extraction from Alpinia zerumbet (Pers) Exl.Quim., *Sao Paulo*,2009:34, 19-2.
19. Hossain MA, Shah MD, Sakari M. Gaschromatograpy- mass spectrometry analysis of various organic extracts of Merremiborneenis from sabah, Asian Pacific *Journal of Tropical Medicine,* 4, 8, 637-641, 2011.
20. Mordue (Luntz) AJ, Nisbet AJ. Azadirachta from the neem tree Azadirachta indica: its action against insects, *Anaisdasociedade Entomologica do Brasil,2000:*29:4:615-632.