



THE ANTIMICROBIAL NATURE OF *AZADIRACHTA INDICA* (NEEM) IN VARIOUS POLAR AND AQUOEUS EXTRACTS AGAINST *ESCHERICHIA COLI*

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ABSTRACT

In the advent of development multiple drug resistant strains of microorganisms, the clinical and therapeutical fields have turned their interest to the traditional and ayurvedic form of cure and medicine. India, being a tropical wet and dry land, has a rich tradition and custom of the usage of medicinal and natural form of curing ailments and maintaining health. Given the rich history of the ayurvedic practices, the first literature mention of the use of medicinal plants is found in Rig-Veda literatures. One such widely used medicinal plant abundantly found in India is *Azadirachta indica*, commonly known as Neem. In this paper, the antimicrobial nature of this medicinal plant extract is tested, observed and recorded against a human pathogen *Escherichia coli*.

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INTRODUCTION

Looking into the pages of time and history, it is clearly seen that man has always depended upon nature for his existence, survival and nourishment. There is no doubt that nature is nourishment and nourishment has always been provided directly or indirectly by nature to us. Of all the food and fodder that we obtain from nature, the most important thing is the medicinal plant. Out of the many medicinal plants that occupy the wide expanses of the Earth's terrain, one of the frequently studied and over-used medicinal plants is *Azadirachta indica* that is neem [1] [2]. This paper is just a small effort in understanding the plant and one among the many properties that it exhibits.

Azadirachta indica, commonly known as the neem in Asian continents, is of Latin origin. In Persian language, Azadi means free and Dirachta means tree, in other words, the name literally means "The Free plant of India". In Sanskrit, neem is known as "sarvaroga nevarani" meaning, a cure to all diseases.

Classification and description of *Azadirachta indica*

The botanical Classification

The scientific classification of *Azadirachta indica* is given below

Azadirachta Indica:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rutales

Suborder: Rutinae

Family: Meliaceae

Subfamily: Melioideae

Tribe: Melieae

Genus: *Azadirachta*

Species: *Indica*

"A medicinal plant is that plant that has pharmacological activities to treat diseases as compared with an edible plant that is used in daily life as food", stated by Park and Pezzuto. A botanical cousin of mahogany tree, *Azadirachta indica* (neem) is a hardy evergreen tree found as common sight in arid and semi-arid regions of Asian continents. It is widely used in traditional practices and the use of neem has been recorded as early as in the ancient Indian scriptures "Rig Veda" [1][3].

Habitat and Description of *Azadirachta indica*

It is observed that the neem plants grows in tropical and sub-tropical region and are widely seen in Asian and African continents. These plants do not grow in cold climate and can survive even with meager rainfall and doesnot require any

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major maintenance attention.

The features and the description of this woody tree are dominant and are easily recognizable in one glance. The tree is a fast growing plant and bears physical appearance and aromatic nature that are unique only to neem plant. The tree grows to an average of 25-30 meters with a straight to semi straight trunk [4]. The tree is branched and the leaves are crowned and the tree is an evergreen tree capable of tolerating severe and long-lasting droughts and at extreme cases of drought, the tree losses nearly all its leaves.

The leaves that the tree bears are alternate and are arranged in the form of leaflets and the leaves have serrated edges. The coloration of the leaves vary from light green to very deep green and at diseased conditions, aged conditions and severe conditions, the leaves bear pale green to yellow color. The bark of the tree at adult age is very strong and the coloration of bark is grayish brown and dark brown with deep, rough and scaly in nature that is even evident in the young sapling [6]. The trees bear and blossoms at spring time and they bear small and fragrant white flowers. The fruits that are found in the tree are greenish yellow in coloration and bear olive-like appearance. The shade that the leaves provide is of godly status and the tree is known as life-giving tree [7]. Though the leaves may taste bitter, the air that is felt around the tree is sweet and the shade that is provided by this tree even in severe humid and hot season is compared to water that is found in midst of endless desert. It is said that the leaves of this tree detoxify the air and the roots of the tree are known for their anti-desertification properties and their ability to renew the polluted aspect of nature.

History

The history of neem ranges way back to about 4,500 years in the foundational scriptures of the ancient ayurvedic physicians like Charak Smahitha and Susruta Smahitha which formed the basis of the Indian system of natural treatment.

The medical practitioners for their healing abilities and preservation nature can trace the history of this tree way back to the ancient Harappa and Mohenjo-Daro civilization. The earliest indications that neem being in daily household for its medicinal values was as early as 5,000 years ago which was used on infants as well as adults to treat both internal and external ailments. Neem is also known as “Arista” in Sanskrit meaning perfect, complete and imperishable. In various literatures and scriptures, neem has been known by many names like “Nimba”, “Pinchumada”, etc. All these names are referred in respect to their medicinal or healing property, which has been described in detailed manner in various scriptures [1][7].

The ancient documents other than Charak and Susruta Smahitha, which celebrate and highlight the various known and unknown properties of neem in daily life, are Rig-Veda, Upavanavinod, Varahamihira, and Brihat Samhita. There are many descriptions of neem even in Persian and Unani literatures like ‘Shajar-e-Munarak’ and “Azad dirakht-I-Hind” [1] [2].

Many Hindu Mythologies have highlighted the neem in their central plot and their medicinal plots have been greatly celebrated as well. A popularly Hindu Mythology has that Once Indra (The head of Heaven) had sprinkled Amrita



Fig 1 An adult neem tree with the characteristic dense leaf crown



Fig 2 The hard scaly bark of the neem tree



Fig 3 The small fragrant white flowers of neem



Fig 4 A bunch of fruits of neem tree

(Celestial nectar) on to Earth and the places where the Amrita fell, Neem rose and hence the tree possessed all the healing and protective nature. It is said as well that planting neem tree "The Kalpa -Vriksha" will earn the planter a place in heaven [3]. The tree has been also associated with the God of Medicine 'Dhanvantri'. It is believed that neem has the ability to cleanse the surrounding air of harmful energy and particles thereby protecting the people who breathe in the air. It is scientifically proven that the air around neem has about 13% more oxygen in them compared to that of other trees.

As time advanced, this wonder tree due to its famed medicinal property, spread to the western countries. Neem's reputation in healing properties has spread to such an extent that it has been introduced to African and Latin Countries and have been

called as 'Mwarunaini', which literally means reliever of 40 human disorders. These countries conducted a series of detailed experiments to test and determine the factor in this tree, which contributes to its miraculous medicinal properties. After a lot of research funded by the developed countries, neem and its products were accepted in international grounds for their therapeutic values and for having no ill side effects on the user irrespective of their age [3].

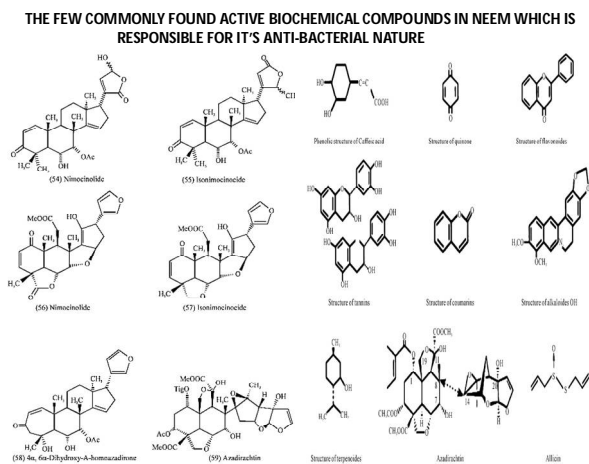
Now a days, the research on basis of the ancient Vedic and ayurvedic texts and scriptures are trying to unravel the unlimited bundle of treasure that this tree has to offer this society. It has now been cultivated in many countries to fully tap and discover their potential and to make them ready for commercial use.

Chemical Nature

It has been observed that the leaves of the neem plant are the storehouse of the organic compounds, having about 0.13% of essential oils attributing to the trademark neem smell to them. More than 140 active complex biochemical and phytochemical compounds have been found throughout the plant. Of all the compounds that are extracted from the plant, the compounds can be classified into two groups namely

Isoprenoids Compound: These compounds include compounds like Diiterpenoids, Triterpenoids, Vilasinin type of compounds, Limonoids and its derivatives, C - Secomeliacins, and various other type of compounds.

Non-Isoprenoids Compounds: These compounds include proteins, polysaccharides, sulphurous compounds, polyphenolic compound (flavonoids and their glycosides), aliphatic compounds and various other likewise compounds.



The above picture is the pictorial representation of the compounds, which are responsible for the anti-bacterial nature of the neem plant extract. The above compounds are the very few of the may actively found compounds in the neem plant that makes the plant very special and important to be entitled to preservation and protection [3].

As stated above, there are many biochemical compounds, which have been credited to the cause of the medicinal, and therapeutic properties exhibited by the plant. Few of the prominently observed, extracted and studied compounds are Nimbidin, Sodium nimbidate, Azadirachtin, Nimbin, Nimbolide, Gedunin, Mahmoodin, Gallic acid, (-)epicatechin, catechin, margolone, margolonone, isomargolonone, Cyclic trisulphide, Cyclic tetrasulphide, Polysaccharide G1A, G1B,

G2A, G3A, NB-2 peptidoglycan. Quercetin and β -sitosterol were first polyphenolic flavanoids which were extracted from fresh neem leaves which exhibited anti-fungal and anti-bacterial properties. Apart from the above stated compounds found in the leaves, active ingredients like nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, amino acids, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione and nimbiol. Along with the compounds in leaf, the seed and seed oil also hold a good amount of active biochemical compounds like nimbidin, gedunin and azadirachtin in them which exert the characteristic bitter taste, desired amount of medicinal and therapeutic properties [4] [14].

The Medicinal and Therapeutic Uses of Neem Plant

The healing and various other medicinal properties of neem are attributed to the presence of biochemical and phytochemical compounds. These properties exhibited by the plant include anti-microbial, anti-inflammatory, anti-cancerous, anti-diabetic, immunomodulatory, anti-ulcer, anti-oxidant, antipyretic, antiarthritic, antitumour, anti-mutagenic, anti-carcinogenic, anti-malarial anti-dermal, oral hygiene maintenance and various others. It has been observed that the neem has about 35 biologically active compounds which are responsible for exhibiting the wide array of medicinal properties that contribute to the above stated and many more properties which are yet to be brought into lime light [5]. The main reason for the advancement in interest and research aspect of this nature's drugstore is the very minute possibilities of side effects and wide range of the advantageous effects of the plant and plant product. The nature of neem plant and her products have not recently come to light, but rather they have been celebrated by the beginning of time and many religious and spiritual scriptures like the Quran and Bible have shown their support and interest in the herb's amazing therapeutic properties. The main pioneer of this herb is the ayurvedic practices, Unani, Homeopathy [5]. Catching upon these ancient but effectively reliable practices, the modern medicine and pharmaceuticals have also started to use this plant product to cure age old ailments and newly arising disease against whom the new medicine no ground.

It is also found that apart from having high medicinal values, they play great beneficial roles in maintaining and nurturing both environment and the agricultural status of a particular place. The neem plant has high rate of photosynthesis compared to other terrestrial plants, thereby increasing the oxygen percentage of the place. With the ability to increase the fertility of the soil, the ability to reverse the erosion rate, the ability to prevent pests from attacking plants or crops and reducing the yield, the neem plant plays major role in improving both environmental and agricultural value of the place. The neem showed marked positive changes in both macroflora and micro flora surrounding it, which is a promising character, attributed in the current situation of natural resource erosion [6].

Not only is neem a positive entity in agricultural and natural environment aspect, it also plays a positive effect in removing the negative effects of man activity towards nature and her activities. It has also been observed that neem leaf powder exhibited biosorbant activity against Congo red dye from water.

Apart from the environmental and agricultural effects of neem and neem products, the neem is widely used in medicinal and clinical benefits of human as well our environment. Antibacterial, anti-cancerous, antifungal, antiviral, anticandidal, anti-inflammatory, antiplaque, anti-dermal effects are few of the very many behaviors exhibited by the neem and her products [7].

The Mode of the Action of the Active Compounds

The above stated points can be precisely stated that the active compounds in the neem plant and the products have clear stated therapeutic implications in disease prevention and curative properties. Although an extensive amount of research is conducted on the behavior and the explanation on the behavior of the neem plant on the preventive and the curative properties of diseases, the exact molecular explanation of the mode of action and mechanism of inhibition and control has not been correctly pointed out and explained precisely. An outline and an abstract idea of what might be the cause of the exertion of the therapeutic and medicinal properties have been stated below. The experiments and the research have pointed that the possible cause and explanation to the nature and the mode of the action is due to the rice source of antioxidants and other valuable compounds like Azadirachtin, nimbolin, nimbin, nimbidin, nimbidol, salannin and quercetin [8][9][10].

It is believed that the neem compounds show their antimicrobial properties via inhibitory effect on the microbial growth, by inhibiting the potential of breaking down the cell wall. The main compound in neem, Azadirachtin, a complex tetranortriterpenoid limonoid, is widely found in leaves and seeds, is the key compound which is responsible for exhibiting antifeedant and toxic effects in insects [11]. The ethanolic extracts of neem leaves have shown the maximum zone of inhibition in 100% concentration against clinical pathogens like *Staphylococcus aureus* and MRSA in an invitro assay. This result has a groundbreaking effect in the clinical field, as these two pathogens are known to be highly dangerous and fatal due to their inbuilt defense against the host. Apart from the above stated mode of action, the neem plants have various other methods to establish the medicinal properties to the host system and themselves as well. The azadirachtin and nimbolide, a rich source of antioxidants, are told to be the main reason for the free radical scavenging nature of the neem plant. The above stated two compounds shown concentration-dependent gradient to establish their antioxidant nature in the following order: nimbolide> azadirachtin> ascorbate [12]. The other main field where the neem products are showing their mind blowing effect is in the cancer and tumor management and preventive fields. This regulatory and preventive nature of neem towards cancerous and tumorous tissues is the modulation of various tumor suppressor genes like p53, pTEN, VEGF, NF- κ B, bcl2, bax and various genes which are directly or indirectly involved in the pathways of oncogenesis or angiogenesis. Further research has seen that the neem components also down regulate cell survival proteins and X-linked apoptotic proteins. The neem compounds have shown to actively contribute to various pathways and immune reactions like anti-inflammatory reactions by the regulation of proinflammatory enzyme activity like cyclooxygenase and lipoxygenase enzymes [13]. Along with the neem leaves and bark, the neem seeds and their oil have also shown inhibitory and toxic effects against

human carcinogenic pathway. The neem medicinal properties are not just restricted to molecular level, but as well to the hepatoprotective nature. This medicinal property is also attributed to azadirachtin and nimbolide compounds. Another therapeutic and well utilized property of neem is its wound healing property, which is achieved again by the up regulation of inflammatory response and neovascularization [14]. The neem root bark extract has also shown to exhibit antidiabetic properties in its ethanol and chloroform extract. This has been a good alternative source to manage and cure diabetes mellitus. The neem plant has also been exploited for its neuroprotective and growth promoting effects [15].

The research experiments have also established vaginal neem extract tablets when used by sexually active women based on invitro cases have shown effective results against *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Herpes simplex* [16].

Adverse Reactions and Toxicology

The clinical trials and the available research data provide evidence towards very few or no adverse reactions in adults with the normal usage dose. It has been observed among women who use vaginal neem extract tablets to shown transient genital itching or burning.

Among the neem plant products that used, the neem seeds have shown poisonous at large dosage level. The toxicity of neem products are characterized by metabolic acidosis, drowsiness, seizures, coma, and hepatoencephalopathy and, at lethal doses, the product can lead to death. It is clinically stated that the management of the poisoning is solely dependent of the manifestation of the symptoms; there is no clear antidote available for administration [17].

At clinical level assays, neem oil showed nonmutagenic characters in Ames mutagenetic assay. The biochemical nature of the main component of neem, Azadirachtin, is biodegradable, nonmutagenic and non-toxic to warm blooded marine animals, birds and even to fishes. The Environmental protection Agency has deemed the usage of neem product to be clearly safe in limited dose to nonfood crops in the form of pesticides [18][19].

Although the above stated facts are clearly on the advantage side of the neem, the neem oil has oral LD-50 value has been observed to be observed to differ in different animal models used in the clinical and research experiments. It was observed that the LD-50 value of neem oil in rats was 14ml/kg.

Diseases Affecting Neem Plant

The above stated points denote to the extreme levels of resistance and preventive measures against various clinical and phytopathogens. Although the features of the neem plant make it an extremely advantageous and a strong willed plant, the neem plant is bound to have diseases affecting them. The microorganisms those are capable of inflicting disease to the neem plant are narrowed to bacterial and fungal category [20]. Few of the commonly seen diseases that affect the neem plant are stated below;

- Damping off.
- Leaf web blight.
- *Colletotrichum* leaf spot and blight.
- *Alternaria* leaf spot and blight.

- *Pseudocercospora* leaf spot.
- Powdery mildew.

The disease description and the symptoms are discussed below;

Damping off: Of all the diseases that affect the plant, damping-off is the most prevalent and the most destructive disease that affects the plant and this disease are seen to cause the highest damage in the seedlings. This disease is grouped into two categories namely pre-emergence and post-emergency depending on the rate of the seed and seedling development course [21]. This disease is a fungal disease and various soil fungus which contribute to the development of this disease are *Pythium*, *Fusarium*, *Phytophthora* and *Rhizoctonia*; out of these soil fungus, *Fusarium* and *Rhizoctonia* contribute to an lesser extent compared to their two other counterparts. There are multiple parameters like high soil temperature, excessive soil moisture, high soil pH, high nitrogen content, low light intensity, clayey type soil. It is observed that such climatic and physiologic conditions are advantageous for the establishment and expression of the disease condition. The seedlings of the neem plant were prominently affected by *Fusarium oxysporum* in serious ratio and in high degree [22].

There are multiple practices, which are followed and employed in order to limit and prevent the establishment and spread of the disease condition and the causative organism. The prominently and most important practice is the proper checking and inspection of the seed and seedlings which are planted or shipped [22]. The proper usage of pesticides and fungicides are advised to be practiced and thereby limiting the strength of the disease. The prominent fungicides like Formalin and Bavistin are found to be effective in many cases. Along with the usage and application of fungicides, proper potting and planting techniques are to be followed for effective yield and healthy nature [23].

Leaf web blight: The predominant causative agent of this disease in neem plant is *Rhizoctonia solani*. The establishment of the disease symptoms is after regular monsoon rains. The symptoms of this disease are the physical appearance of greyish brown blotches. Along with this physical manifestation, the fungal activity and size on the infected plant; especially the leaf and leaf blade. With progression of disease, the disease also affects the adjacent plant parts as well. The leaf, which is infected displays a spider web like fungal hyphae thereby, the name leaf webs blight. It is observed that the disease spreads via physical contact [24].

Like the previously stated control measures, the disease is either controlled by proper inspection of seeds and seedling along with proper potting and planting techniques [25]. The use of fungicides like various concentrations of Bavistin was found to be effective in multiple cases of application.

***Colletotrichum* Leaf spot and Blight**

The causative agent of this disease is *Colletotrichum gloesporioides*. The disease is found to be at the maximum capacity at the onset of September and the first week of October. The most avidly seen symptoms are the leaf spots that cover the leaf surface and are observed to have the capacity to increase the affecting area as the disease advances.

The leaf which is affected is observed to hold brightly appearing blighted area which eventually leads to the defoliation of the leaf [27][28]. The seedlings, which bear the infection, are observed to be the holders of premature defoliations. To control the infection, the weekly application of Blixton fungicides is effective in multiple cases.

Alternaria Leaf spot and Blight

This disease is caused by another member of the fungus family namely *Alternaria alternata*. This fungus is known for its destructive nature and its season of infection is in the late growing season spanning from the last week of October to early November. The heavily infected leaves bear pale complexion and they have low amount of soluble sugar in them.

The control measures of this disease are very similar to that of the previously mentioned disease. The use of Blitox fungicide at 0.2% at a time interval of fortnight is effective in controlling the disease [29].

Powdery Mildew

This is a fungal disease, which is caused by a fungal species known as *Oidium azadirachtae*. The physical manifestation of the disease is seen in the infected leaves in the form of white patches [26]. These patches, by nature, are coalesced. On the spread of the infection, the entire leaf surface along with the lamina bears greyish appearance. On the extreme instance of the disease, the infected leaf and leaflets wilt and defoliate prematurely [31].

The preventive measures for the disease are the proper quarantining of the influx and marketing/ planting of the plants which originate from potential target area of the causative pathogen. The use of fungicidal spray like Bavistin in the form of foliar spray at a very low concentration was found to be very effective in many cases [32].

It was observed that most of the diseases that affect this incredible plant are targeted to the foliar area and at the seedling stage. Many of the other diseases that affect the plant are the bacterial leaf spot, which is caused by the bacterial specimen *Xanthomonas azadirachtii*, and *Pseudomonas viticola*; the seedling wilt disease caused by *Fusarium solani* [35].

In this particular review point, the neem plant samples are observed in the antimicrobial aspect. Any medicinal plant in clinical aspect is observed in the parameter of the degree of microbial resistance [33]. The resistance exhibited the plant against various clinical and phytopathogens are due to the various bioactive chemical compounds, which may be of primary nature or even secondary metabolites in nature. The effectiveness of such compounds are tested by extracting them from their natural sources like the leaves, bark, stem, fruit, seeds or even the rhizosphere by using various polar and non-polar solvent [34]. The phytochemical extraction is done but a simple method or soaking and filtration method or by using Soxhlet method, to obtain a paste of the plant extract. The plant extract obtained is then made into different concentrations and these are further used to test for the degree of the plant compounds in different concentrations against the few selected microorganisms. The degree of the plant extract in terms of the anti-microbial parameter can be done using either slip disc method or agar well diffusion

method and calculated in terms zone of inhibition in mm (millimeter) [35].

It is also observed that the plant extract not only shows the normal microorganism but also against Multiple Drug Resistant Microorganisms, which are the currently arising threat to human and our counterpart organisms [36].

Thereby, the study of neem in its anti-microbial parameter is of great significance and advantage for medicinal, clinical and pharmaceutical fields. What further makes neem an ideal alternative source of medicinal and clinical cure is that, in India, it is not that much felled due to its major role in religious and cultural believes, thereby ensuring that the compound obtained from neem and its extraction doesn't affect the environmental and cultural believes[37].

All the above mentioned points and the effects given by the neem plant gives us enough reasons to conclude that neem is

"The golden or the wonder tree of india".

MATERIALS AND METHODS

Plant material: 400 grams of fresh and healthy neem leaves was collected from a healthy fully grown neem tree near Srirampura area, Mysore, Karnataka.

The fresh leaves were then washed and completely dried using a tissue paper. The leaves were then shade dried for about 7 days with occasional change in the sheets on which they were dried. Upon complete drying, the leaves were then powder to obtain fine powder. This powder was then weighed and used for the compound extraction.

Test microorganism: Human pathogenic bacteria, *Escherichia coli*, a Gram negative rod aerophilic bacterium was collected from JSS Medical College, Mysore, India (Anil Kumar Sir). The plates obtained were cultured on Nutrient Agar Medium[22]. This parent culture was sub cultured in NA medium for 48 hrs. to obtain stable strains. This test microorganism was then used on the plant extract to test the degree of antimicrobial character exhibited by the extract.

Preparation of plant extract: About 45 g of shade dried powdered neem leaf was taken and divided into 3 groups of each 15g of leaf powder. Each group was then subjected to polar and aqueous extract. The leaf powder was soaked in 50 ml of the respective solvents like ethanol, methanol and aqueous extract [26]. The soaked plant extracts were then incubated at room temperature for two days. The obtained concentrated yield is of crude nature and this is further used to test the anti-microbial nature of the neem leaf extract against the test microorganisms [38].

Determination of anti-microbial nature of the plant extract

The anti-microbial nature of the plant extract was tested by slip disc method. In this method, each Petri plate, which was swabbed with the test microorganism, was placed with Whatmann no.1 filter paper discs of different concentrations. Now these plates are placed in the incubator for 48 hr. The zone of inhibition (in mm diameter) was measured at regular time intervals of 0 hr., 12 hr., 24 hr. and 48 hr. Control was simultaneously taken and the results were tabulated and inferred. The comparative potent of the anti-microbial nature of the different solvent extracts of the leaves were then evaluated by comparing the ZOI exhibited [30][34].

RESULTS AND DISCUSSION

The anti-microbial characteristic of the solvent extracts of powdered neem leaves against the test microorganism *E.coli* was evaluated by the ZOI given in the below tabular column. The above tabular column which shows the ZOI (in mm diameter) exhibited by various solvent extracts against the test microorganism shows that at T24, ethanolic extracts shows the maximum zone of inhibition at 300mg/ml concentration. But at T48, both ethanolic and methanolic extracts show maximum ZOI at 300mg/ml.

It has also been observed that the control without any plant extract in them also show inhibitory action against the test microorganism due to the self-instilled anti-microbial nature in the solvent. This proves that the plant extract along with the plant extract shows anti-microbial character.

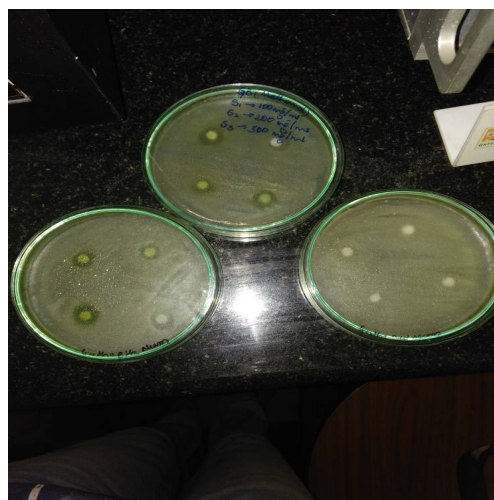
CONCLUSION

Even before the discovery of analgesic drugs, Neem and its products have been used as home remedy against army of disorders that trouble human and other animals. It has been observed that neem products have the ability to protect not only animals but also the fellow plant beings from both natural and man introduced dangers. The effect of neem in controlling almost all the fields of human life has made it such a plant of dominating effects that the researchers are bent on discovering more about the plant's miraculous effects and make it available for the world. True to its name, there is no part of the plant that has been proved to have no medicinal effect. So more, the rhizosphere of the roots of this plant have also been proved to show beneficial effects and thereby they stand tall and proud among the plants which are beneficial to Earth [39].

| Sl.no | Solvent Used | Solvent Conc | Micro Organism | Zone of Inhibition Exhibited | | | |
|-------|-----------------|--------------|----------------|------------------------------|-------|-------|-------|
| | | | | T0 | T12 | T24 | T48 |
| 1 | Ethanol | 0mg/ml | <i>E.coli</i> | 0 mm | 0.4mm | 0.6mm | 1mm |
| | | 100mg/ml | <i>E.coli</i> | 0mm | 1.2mm | 1.4mm | 1.5mm |
| | | 200mg/ml | <i>E.coli</i> | 0mm | 1.5mm | 1.6mm | 1.2mm |
| | | 300mg/ml | <i>E.coli</i> | 0mm | 1.7mm | 1.9mm | 1.7mm |
| 2 | Methanol | 0mg/ml | <i>E.coli</i> | 0mm | 0.3mm | 0.5mm | 1.2mm |
| | | 100mg/ml | <i>E.coli</i> | 0mm | 1.6mm | 1.7mm | 1.5mm |
| | | 200mg/ml | <i>E.coli</i> | 0mm | 1.3mm | 1.5mm | 1.3mm |
| | | 300mg/ml | <i>E.coli</i> | 0mm | 1.9mm | 1.6mm | 1.7mm |
| 3 | Distilled Water | 0mg/ml | <i>E.coli</i> | 0mm | * | 0.4mm | 0.7mm |
| | | 100mg/ml | <i>E.coli</i> | 0mm | * | 0.9mm | 1.2mm |
| | | 200mg/ml | <i>E.coli</i> | 0mm | * | 1mm | 1.3mm |
| | | 300mg/ml | <i>E.coli</i> | 0mm | * | 1.1mm | 1.5mm |



This image shows the zone of inhibition taken at 0 hour for the neem extract using various solvents



This image shows the zone of inhibition taken at 48 hours for the neem extracts using various solvents.

It can be concluded from the above experiment and studies that, *Azadirachta indica* shows anti-microbial character against *E.coli*. The results observed have shown that the use of medicinal plants like neem in traditional methods can control the growth and spread of infectious disease without any harmful side effects in the body [40].

The plant is easily available and its usage has been described in our Vedic scriptures and thereby is a cheaper substitute for the conventional drugs. Isolating and purifying the active compound and subjecting to animal models can further take this ahead.

Like priorly said, Nature has all for the needs of man, but not for the greed of him. Thought nature has such miraculous medicinal plants, the products and protection they provide us is to be repaid by protecting and saving these plants from the greed and injudicious use. With the knowledge of age-old science supported by the modern scientific techniques, the replenishing of this lost ocean of miraculous compounds is possible.

References

1. PIER (n.d.), Weed risk assessment for *Azadirachta indica* (neem), www.hear.org/pier/wra/pacific/azadirachta_indica_htmlwra.htm
2. http://agritech.tnau.ac.in/forestry/forest_disease_neem.html.
3. Friend, J (1999), *Neem in Australia: history and overview*, pp. 3-10, RP Singh & RC Saxena (eds), *Azadirachta indica* A. Juss: International Neem Conference, Science Publishers Inc., Enfield, United States.

4. W. Shah, N. Rane, M.B. Kekare, V. Vaidya, Estimation of two bioactive compounds from *Azadirachta indica* a. juss leaves using HPLC, *International Journal of Pharmaceutical Bioscience* 2(2010) 185-192.
5. Phytochemical analysis and antibacterial activities of *Azadirachta indica* A Juss, *International Journal of Research in Plant Sciences* 2 (2012) 50-55.
6. R. Subapriya, S. Nagini, Medicinal properties of Neem leaves: A Review Source, *Current Medicinal Chemistry - Anti-Cancer Agents*, 5 (2005) 149-156.
7. Phytochemical analysis and antibacterial activities of *Azadirachta indica* A Juss, *International Journal of Research in Plant Sciences* 2 (2012) 50-55.
8. Abdul Wadood *, Mehreen Ghufraan , Syed Babar Jamal , Muhammad Naeem , Ajmal Khan , Rukhsana Ghaffar and Asnad, Phytochemical analysis of the medicinal plants occurring in local area of Mordan, *Biochemical and Analytical Biochemistry open access Journal* (2013) 1-4.
9. The neem tree; produced by HDRA - the organic organization (1- 20).
10. 'Neem: A Tree for solving Global problems' (1992) National research Council, National Academy of Sciences.
11. Yogisha S, Raveesha KA (2009) In-vitro antibacterial effect of selected medicinal plant extracts. *J Nat Prod* 2: 64-69.
12. Buwa, L.V., Staden, J.V., (2006): Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *Journal of Ethno pharmacology*, 103: 139-142.
13. Heisey, R.M., Gorham, B.K., (1992): Antimicrobial effects of plant extracts on *Staphylococcus aureus*, *Candida albicans*, *Trichophyton rubrum* and other microorganisms. *Letters in Applied Microbiology*, 14: 136-139.
14. Naqvi, S.A.H., Khan, M.S.Y., Vohora, S.B., 1991. Anti-bacterial, anti-fungal and anthelmintic investigations on Indian medicinal plants. *Fitoterapia* 62, 221-228.
15. Leven, M., Vanden Berghe, D.A., Mertens, F., Vlietinck, A., Lammens, E., 1979. Screening of higher plants for biological activities. 1. Antimicrobial activity. *Planta Medica* 36, 311-321.
16. Hailu T, Endris M, Kaleab A, Tsige GM (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *J. Ethnopharm.* 100:168-175
17. Rios JL, Recio MC (2005). Medicinal plants and antimicrobial activity. *J. Ethnopharm.* 100:80-84
18. Rios JL, Recio MC, Villar A (1988). Screening methods for natural products with antimicrobial activity. A review of the Literature. *J. Ethnopharm.* 23:127-149.
19. Thomson WAR. (ed). *Medicines from the Earth*. McGraw-Hill Book Co, Maidenhead, United Kingdom, 1978.
20. Tewari DN. Monograph on Neem (*Azadirachta indica* A. Juss.). Indian Council of Forestry Research and Education (ICFRE), International Book Distributors, Dehra Dun, 1992.
21. Gajendrasinh PR, Bhavika MK, Rohit S, Hetal A, Prajapati PK. In vitro Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (*Azadirachta indica* L.) and Tulsi (*Ocimum sanctum* L.). *IJPBA* 2012; 3(3):582-586
22. Mamman, PH, Mshelia WP, Susbatrus, SC, Sambo KW. Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli*, *salmonella* spp and *staphylococcus aureus*. *Int. J Med. Med. Sci.* 2013; 5(1):14-18.
23. Ranjit R. Raut1* , Ajit R. Sawant2 and Bhagyashree B. Jamge3; Antimicrobial activity of *Azadirachta indica* (Neem) against Pathogenic Microorganisms, *Journal of Academia and Industrial Research (JAIR)* Volume 3, Issue 7 December 2014 (327-340).
24. Maragatharavlli, S., Brinda, S., Kaviyaarshi, N.S. and Gangwar, S.R. 2012. Antimicrobial activity in leaf extract of neem (*Azadirachta indica* Linn.). *Int. J. Sci. Nature.* 3(1): 110-113.
25. Mehrotra, S., Srivastava, A.K. and Nandi, S.P. 2010. Comparative antimicrobial activities of Neem, Amla, Aloe, Assam tea and Clove extracts against *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J. Med. Pl. Res.* 4: 2473-2478
26. Koonna, S.J. and Budida, S. 2011. Antimicrobial potential of the extracts of the leaves of *Azadirachta indica* Linn. *Nat. Sci. Biol.* 3(1): 65-69.
27. Ganguli, S. 2002. Neem: A therapeutic for all seasons. *Curr. Sci.* 82: 1304
28. Bandyopadhyay, U., Biswas, K., Chatterjee, R., Bandyopadhyay, D., Chattopadhyay, I., Ganguly, C.K., Chakraborty, T., Bhattacharya, K. and Banerjee, R.K. 2002. Gastroprotective effect of neem (*Azadirachta indica*) bark extract: Possible involvement of H⁺-K⁺-ATPase inhibition and scavenging of hydroxyl radical. *Life Sci.* 71: 2845-2865
29. Srivastava A Shukla Kumar YN (2000) Recent development in plant derived antimicrobial constituents A Review. *J Med Arom Pl. Sci.* 20: 717-72.
30. Saradhajyothi Koonna, Subbarao Budida (2011) Antimicrobial potential of the extracts of the leave of *Azadirachta indica*, *Linn. Nat Sci Biol*, 3(1) 65-69.
31. Almas, K. Ansal lafi, T.R. (1995) The natural toothbrush. *World health Forum* 16: 206-210.
32. Faiza Aslam, Khalil.Ur. Rehman, Mohammad Asghar and Muhammed Sarwar (2009) Antibacterial activity of various Phytoconstituents of Neem. *Pak. J.Agric. Sci.*, Vol. 46(3), 456-463.
33. Bauer, A.W., W.M.M. Kirby and T. Sherris. 1996. Antibiotic susceptibility testing by standard single disc method. *American Journal of Clinical Pathology.* 45:493
34. Fabry, W., P.P. Okemo and R. Ansorg, 1998. Antibacterial Activity of East African Medicinal plants. *Journal of Ethnopharmacology.* 60: 79-84.
35. Kausik, B., I. Chattopadhyey, R.K. Benerjee and U. Bandyopdyey. 2002. Biological activities and medicinal properties of neem. *Current Science.* 82(11): 1336-1344.
36. Khan, M., Wassilew, S.W., *Natural Pesticides from the Neem Tree and Other Tropical Plants*, GTZ, Eschborn, Germany, 645-650 (1987).

37. Chattopadhyay, R.R., Chattopadhyay, R.N., Maitra, S.N., Possible mechanism of anti-inflammatory activity of Azadirachta indica leaf extract, *Indian J. Pharm.*, 25: 99-100 (1993).
38. Gurib-Fakim A. Medicinal plants: Tradition of yesterday and drugs of tomorrow. Review article. *Mol. Aspects Med*, 2006; 27(1): 1-93.
39. Eisner, T., 1989. Prospecting for nature's chemical riches. *Issues Sci. Technol.* 6 (2), 31-34.
40. Mehrotra, S., Srivastava, A.K. and Nandi, S.P. 2010. Comparative antimicrobial activities of Neem, Amla, Aloe, Assam tea and Clove extracts against *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J. Med. Pl. Res.* 4: 2473-2478

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