

Study of Antibacterial Properties of *Azadirachta indica* and *Trachyspermum ammi* A Commonly Used Plant Material That Aids Digestion

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ABSTRACT

Azadirachta indica commonly called neem is a plant known for its medicinal value all over the world. In India it has been used as antibacterial, antifungal, anti-inflammatory agents in Ayurveda and unani medicine. Neem leaf extracts in specific are used to cure digestive problems and disturbances, intestinal parasites and also reduce discomfort. *Trachyspermum ammi* commonly known as ajwain is another plant material used to improve the digestion and the flavor of food. It is commonly added in those recipes which are difficult for digestion and lead to acidity and gas troubles. Aqueous extract of neem leaves and ajwain seeds were prepared to demonstrate their antimicrobial efficacy using pure culture of *E.coli*. *Escherichia coli* popularly abbreviated as *E.coli* is a common bacterial flora of human intestine, which helps in preventing the entry of pathogenic microorganisms. Thus it can serve as a better experimental model for bacteriological and anti-microbial studies. Growth curve was studied with the help of optical density with the help of Elico CL63 photometer. Growth curve and well diffusion were the methods used for the study. In comparative analysis of dry and fresh neem sterile aqueous extract it was found that fresh neem was more effective in inhibiting the growth of *E.coli*; whereas in further investigation with ajwain and fresh neem it was found that neem is more potent in inhibiting the *E.coli* growth.

Keywords : *Escherichia Coli*, *Trachyspermum Ammi*, *Azadirachta Indica*, Growth Inhibition.

I. INTRODUCTION

India is well known for its deep-rooted traditional medicinal knowledge and values. Ayurveda, Siddha, and Unani are some of the greatly known fields [1, 3-6, 8]. Before the development of science and technology the age old traditional methods were the only ones for the rescue of human race. Countries like India has rich array of herbal diversity. Many of them are widely used for various medicinal purposes [2]. Medicinal properties of these plants were explored and put to use under our indigenous field of medicine called Ayurveda in Vedic Period between 2500 and 500 BC in India [1]. Ayurveda is also called the “science of longevity”

because it offers a complete system to live a long healthy life. It offers programs to revitalize the body through proper diet and nourishment [1]. It not only includes treatment methods to cure commonly found diseases like cough, cold, fever, headache, but also the cancer, cardio vascular diseases, asthma, kidney stones, etc., which are considered to be deadly [3-6]. In comparison with the modern allopathic medicines they are highly cost effective, easily available and also efficient to cure the diseases with minimum side effects [1].

List of the herbs having medicinal properties grown in Asia would be enormous but incomplete without adding the two plants of immense importance as far

as their known medicinal properties are concern. Azadirachta indica and Trachyspermum ammi commonly called neem and amla are the plants known for their medicinal values easily availability and sustainability in tropical conditions.

Azadirachta indica commonly known as neem, nim tree or Indian lilac or Margosa, is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to the Indian subcontinent, i.e. India, Nepal, Pakistan, Bangladesh, Sri Lanka, and Maldives. It is typically grown in tropical and semi-tropical regions [7]. Neem trees also grow in islands located in the southern part of Iran. Its fruits and seeds are the source of neem oil. Neem products are believed by siddha and Ayurveda practioners to be antibacterial [10], antifungal [11], anti-inflammatory, anti-viral, anthelmintic, antidiabetic, and sedative [7]. Neem leaves are also proved to be effective in treating skin diseases and disorders [9].

Trachyspermum ammi also known as ajowan, ajwain, or carom is an annual herb in the family Apiaceae. Ajwain is a plant primarily found in countries like India Pakistan, south east and near east of Iran, where these seeds are predominantly used as a spice [17]. In Asian countries these seeds hold an important place in cuisines. They are better to taste, with a flavor similar to anise and oregano. Even a small amount can add great flavor to the dish. Ajwain is efficiently used in traditional Ayurvedic medicine primarily for stomach disorders such as indigestion, flatulence, and diarrhea. In Siddha medicine, it is used as a cleanser, detox, and antacid. The indigenous Indian system of

medicine uses ajwain as an antimicrobial, anti-hypersensitive, antispasmodic, bronchodialating and antilithiasis [19]. Ethanol and acetone extract of ajwain seeds possessed an antibacterial activity against two Gram negative food spoilage bacteria Pseudomonas aeruginosa and Escherichia coli [3, 18].

Escherichia coli being the commonly present bacterium in the human intestine, helps in preventing the entry of pathogenic microorganisms. E. coli are non-pathogenic in normal conditions, but if present in excess, can turn out to be a causative agent of various diseases like urinary tract infection, diarrhoea, vomiting etc. With increasing resistance of microorganisms to antibiotics, people have spun their way towards herbal medicines.

II. METHODS AND MATERIAL

Collection of Sample: Fresh Neem leaves were collected from Birla College campus and Ajwain seeds were procured from the local market.

Preparation of Extracts:

Aqueous soxhlet extract of neem:

The neem leaves were washed and divided in two parts. One part was used directly as fresh leaves to prepare the aqueous extract and the other part was kept at 40°C for drying. The aqueous extracts of fresh as well as dried neem leaves were prepared using the soxhlet apparatus. 25 Gms of fresh as well as dried neem leaves powder was used for preparing 100 ml of extract separately using distilled water. The extracts were further concentrated to 25 ml, autoclaved and stored in sterile condition at 4°C till further use.

Aqueous soxhlet extract of ajwain seeds: Sterile aqueous extract of ajwain seeds was prepared as explained above.

Crude extract preparation of fresh neem leaves and ajwain seeds:

Crude extract was prepared by boiling 25 Gms of sample (fresh neem leaves, ajwain seeds) in 50ml of distilled water and condensed to 25 ml by evaporation, cooled and preserved at 4°C till further use.

Preparation of bacterial culture:

Pure isolated colonies of the E.coli were obtained from the Department of Microbiology, Birla College Kalyan. E.coli was sub-cultured by growing the colonies on nutrient agar slants and Preserved in refrigerator.

Preparation of nutrient broth:

25 ml of sterile nutrient broth was prepared using distilled water, and stored at 4°C.

Preparation of nutrient agar plates:

20 ml of sterile nutrient agar was poured on sterile plates.

ANTIMICROBIAL ASSAY BY GROWTH CURVE METHOD:

A. Growth curve analysis using sterile aqueous extract:

24 ml of sterile nutrient broth was inoculated with 1ml of sterile fresh neem leaves extract and 1ml of

E.coli culture, and was incubated in Remi Orbital shaking incubator at 37°C for 24 hrs. The growth was analyzed at regular intervals of 1 hr. similarly growth analysis of E.coli was studied using ajwain extract. Parallel study was performed using 0.5ml of neem and ajwain extract separately. All the studies were performed in triplicates. Control was maintained similarly using sterile distilled water.

B. Growth curve analysis using crude extract:

24 ml of sterile autoclaved nutrient broth was inoculated 1ml of E.coli culture and 1ml of crude fresh neem leaves extract. Parallel study was also carried out using 0.5ml extract, and incubated in shaker incubator at 37°C for 3hrs. The growth was analysed at regular intervals of 10 min. Similar study was performed using 0.5ml and 1ml of ajwain extract individually. All the studies were performed in triplicates. Control was maintained similarly using sterile distilled water.

ANTIMICROBIAL ASSAY BY AGAR DITCH METHOD:

Sterile nutrient agar plates were used as medium for screening antibacterial activity. 100µl of E.coli culture was uniformly spread on sterile plates using a sterile spreader. Wells of 1cm were dug using a sterile cork borer in solidified pre inoculated agar medium. The plates were divided into 3 quadrants I, II, and III, each having 100µl of 1%, 10% and 100% individual crude extract of fresh neem leaves and ajwain seeds. The plates were incubated at 37°C for 8hrs and observed for clear inhibition zone formed around the wells.

III. RESULTS AND DISCUSSION

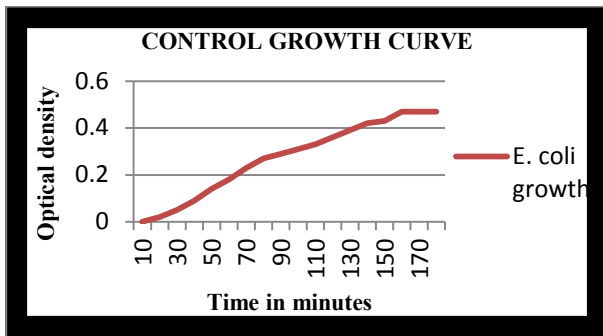


Fig.1

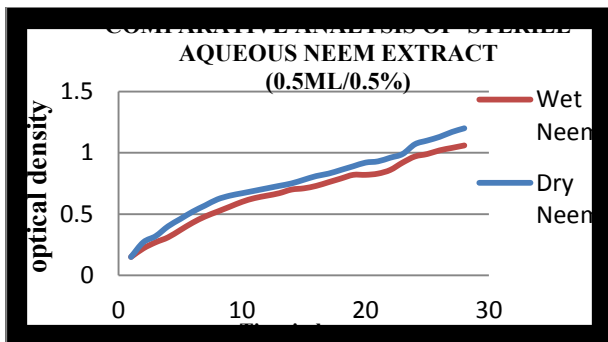


Fig.2

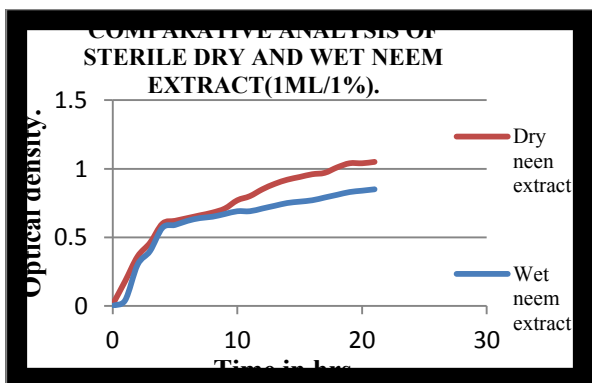


Fig.3

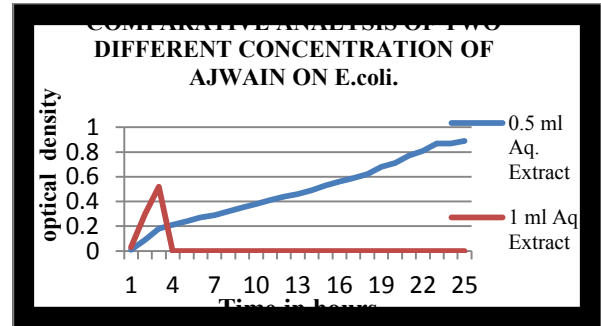


Fig.4

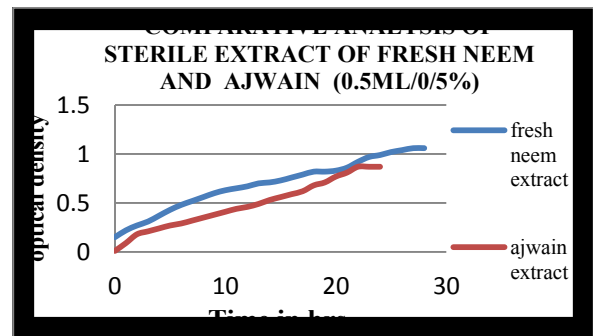


Fig.5

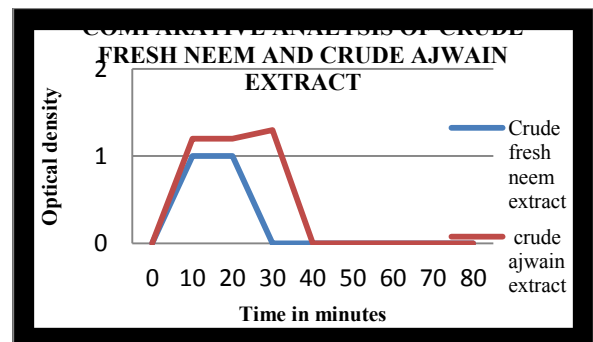


Fig.6.



Fig.7 Control inhibition zone on agar plate

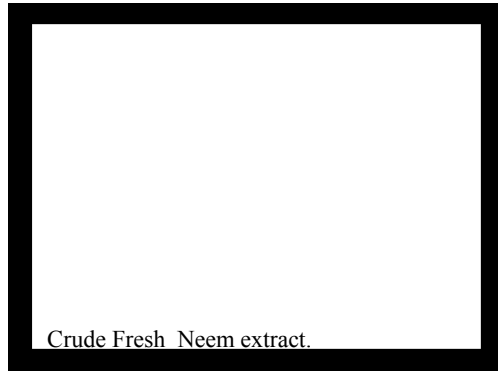


Fig.8. Inhibition zone of crude fresh neem extract at different concentrations.

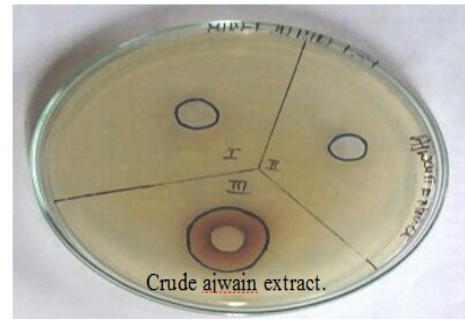


Fig.9 Inhibition zone of crude ajwain extract at different concentrations

Table 1: showing the inhibition zone of crude extract of fresh neem leaves and ajwain seeds at three different concentrations

	Crude extract of fresh neem leaves.			Crude extract of ajwain seeds.			Control
Concentration.	1%	10%	100%	1%	10%	100%	100µl E.coli.
Inhibition zone.(cm)	1.2cm	1.4cm	3.4cm	1.2cm	1.4cm	3cm	0 cm

Fig. 1 represents the growth curve of control sample which was not exposed to any herbal extract while the Fig. 2 represents the growth curve of E. coli where the sterile aqueous extract of fresh neem leaves was incorporated with the culture medium at the concentration of 0.5 ml/25 ml of nutrient broth and dry neem leaf sterile aqueous extract at the same concentration. It was found that sterile aqueous extract of fresh neem leaves was more effective in inhibiting the growth of E.coli. Studies carried out using 1ml of sterile aqueous extract of fresh neem and dry neem leaves / 25ml of nutrient broth also shows similar results indicating that fresh neem leaves extract is more effective than dry neem leaves, as seen in Fig 3. Fig 4 represents that

in comparative analysis of sterile aqueous extract of ajwain seeds, 1ml extract /25 ml of nutrient broth is more effective than 0.5ml of extract. From Fig 5 it is evident that sterile aqueous extract of ajwain seed is effective than neem leaves extract. On similar lines on comparing fig 3 and fig .4, it is observed that ajwain seed extract is much effective in inhibiting the bacterial growth in less time in comparison to fresh neem leaves extract at the concentration of 0.5ml as well as 1ml /25ml NB. Fig.6 indicates the potential growth inhibition obtained using crude extract of fresh neem leaves than in comparison to crude extract of ajwain seed. The agar ditch method primarily focus on the inhibition zones formed using three different

concentrations of crude extracts of fresh neem leaves and ajwain seeds respectively. As seen in fig .7 no inhibition zone is seen in control agar ditch filled with sterile distilled water. Whereas the inhibition zones formed around the neem leaves extract and ajwain seed extract increases with an increase in concentration used (fig 8, 9, table 1).

Margosa that is *Azadirachta Indica* is well known plant for the wide range of medicinal uses it has. It is possible that the medicinal properties of the plant are due to the different active components present in the plant. The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7- benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol. Quercetin and β - sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties and seeds hold valuable constituents including gedunin and azadirachtin^[21]. In past many workers have assessed the antimicrobial properties of this plant using various extraction solvents such as ethanol, methanol etc^[20,21,23]. As stated above in this investigation water was used as solvent when compared with the earlier literature where methanolic samples were used^[20]. It was found that results obtained using aqueous crude extract were less effective than methanolic extract^[20].

Ajwain also known as *Trachyspermum ammi* is a commonly used across India in various Indian cuisines. It is used to add flavor as well to help in digestion of food. Apart from these values it is also a

fundamental remedy on acidity problems, stomach ache, and other problems related to indigestion. Ajwain seeds contain several phytochemicals such as, tannins, glycosides, saponins, flavones and mineral matter like calcium, phosphorus, iron and nicotinic acid. Essential oil present in the ajwain contains the major constituent thymol. The remainder non-thymol fractions called thymene contains p-cymene, β -pinene, limonene with γ -pinenes and β -pinene. Alcoholic extract contains highly hygroscopic saponin. It has been reported that minute amount of camphene, myrcene and D3-carene is also present. A yellow crystalline flavone and steroid like substance have been isolated from the fruit of ajwain and it also contains a glucoside 6-O- β - glucopyranosyloxythymol. The major constituents of *T. ammi* are carvone, limonene and dillapiole^[24]. Ajwain was studied in the past and was reported that it has antimicrobial properties and a great pharmacological use^[24]. In the current investigation it was found that ajwain seeds aqueous soxhlet extract shows better antimicrobial activity than neem in growth curve assay.

IV. CONCLUSION

The growth curve experimental set up for 24hrs proved that ajwain seeds are more effective in inhibiting the growth of gram negative bacteria *E.coli*. Whereas the agar ditch method indicated that the crude extract of neem leaves was more effective than the ajwain extract in inhibiting the *E.coli* growth.

V. REFERENCES

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