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## Phytochemical analysis and Antibacterial properties of *Azadirachta indica* (Neem) leaves extract against *E.coli*

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**Abstract**

*Azadirachta indica* has great medicinal properties and distributed worldwide. The extract of *Azadirachta indica* show different properties like antibacterial, antifungal, antioxidant etc. In this work we prepared extract in different solvent i.e benzene, acetone, toluene, ethyl acetate, ethanol and butyl alcohol. Phytochemical analysis of plant extract also gave positive result for saponins, tannins, phenols, proteins, glycoside, terpenoids, carbohydrate, flavanoids, alkanoids. The aim of this study that screen out the active components and test the antibacterial activity of extract in different solvents as benzene, acetone, toluene, ethyl acetate, ethanol, butyl alcohol. The acetone extract showed the maximum bacterial growth inhibition 58.77% against *E. coli* strains. Therefore the *Azadirachta indica* leaf and other parts of this plant use for different purpose like antimicrobial, antioxidant in the form of powder, tablet and micro solution.

**Keywords:** phytochemical analysis, antibacterial, disc diffusion, *Azadirachta indica*

**Introduction**

*Azadirachta indica* we all commonly known as neem. It is native of India and naturally present in tropical and subtropical areas in different countries. *Azadirachta indica* has great medicinal value and distributed wide spread in the world. The Chemical constituents including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones contain many biologically active compound that can be extracted from neem. (Verkerk 1993) [18]. Other compounds present in *Azadirachta indica* have a biological activity are salannin, volatile oils, meliantriol and nimbin (Jacobson). The leaf of *Azadirachta indica* is effective in many skin problems as treating eczema, ringworm, acne, anti-inflammatory, antihyper glycaemic properties and it is also used to heal chronic wounds, diabetic food and gangrene developing conditions. It is believed that *Azadirachta indica* used to remove toxins from the body, neutralize free radicals and purify the blood. Another properties of *Azadirachta indica* is that it is used as anticancer agent and it has hepato-renal protective activity and hypolipidemic effects [Antimicrobial p1]. Neem is a large tree and the height of *Azadirachta indica* is approximately 25 meters with a semi-straight trunk. It is basically known a flowering plant and normally starts fruiting after 3–5 years. The tree becomes productive within 10 years (Maragathavalli 2012) [12]. The bark of *Azadirachta indica* tree is grey and rough. The length of *Azadirachta indica* pinnet leaves are up to 30 centimetres long and each leaf has 10–12 serrated leaf lets that are 7 centimetres long by 2.5 centimetres wide. The neem tree grows well in those countries where minimum rainfall occur (Mohashine 2009) [7]. All parts of the selected *Azadirachta indica* plant showed medicinal properties, are used as medicine for the treatment of many diseases and illnesses. Traditionally, the leaves and their paste are used for curing allergic skin reactions and antivirally treating smallpox and chicken pox (Khine 2013) [6]. Most countries as urban Nepalese, Indian and Bangladeshi used for cleaning their teeth with the twigs of *Azadirachta indica*. The juice from the leaves is used as a tonic to increase appetite and to remove intestinal worms (Kumar 2009) [1]. It is also used for its hypoglycaemic, hypolipidemic, hepatoprotective and hypotensive activities and to control fever (Chaturvedi 2011) [10]. Therapeutically, the leaf extract is used for its antimicrobial activity against dental pathogens (Khan 2010) [4]. In addition, in the Ayurvedic medicine system, the selected plant is used to treat malarial fever (Khan 2010) [4], (Gochukw 2009) [16]. Neem oil is very useful in the preparation of mosquito-repellent tablets and is now available in north-east India (Kumar 2009, Chaturvedi 2011, Khan 2010) [1, 10, 4]. There are also several medicinal uses of neem,

and its formulated products include treatments for cancer, skin diseases, digestive disorders and AIDS (Santhosh 2013)<sup>[17]</sup>. In Oman, it is used traditionally for the treatment of fever and diabetes. Several active chemical compounds are present in the selected plant, including glycosides, dihydrochalcone, coumarin, tannins, zadirachtin, nimbin, nimbidine, diterpenoids, triterpenoids, proteins, carbohydrates, sulphurous compounds, polyphenolics, among others (Mondali, 2009, Sudhir 2010)<sup>[9, 11]</sup>. of these, the most commonly active compounds found in neem are zadirachtin, nimbin and nimbidine (Mondali, 2009)<sup>[9]</sup>. The most active chemical compounds are slightly hydrophilic in nature; however, they are freely lipophilic and more soluble in organic solvents, such as alcohol, ketones and esters (Pankaj, 2011)<sup>[13]</sup>.

The leaf extract is commonly used as an antibacterial agent. In addition, the neem has several applications, such as antiseptic, healing, anthelmintic; use in medicinal soaps, creams and toothpaste (Schmutterer, 2004; Mustafa, 2016)<sup>[14, 8]</sup>. Dental caries and oral health/dental health are multifactorial diseases related to diet, oral microbiota, hygiene, salivary characteristics, and are inseparable part of general health, which can lead to considerable pain and suffering (Chandra Shekar *et al.*, 2015; Sheiham, 2005)<sup>[3, 15]</sup>.

## Materials and Methods

### Collection of plant materials

The *Azadirachta indica* tree leaves were collected from Shobhit University campus, modipuram Meerut. The leaves were washed under distilled water to remove dust and other foreign particles. After washing these leaves were dried under shade area for the crushing.

### Preparation of leaves extract

- a) **Ethylacetate extract-** *Azadirachta indica* 50 gm leaves were converted into fine powder form using a pastal mortar and dip in 500 ml ethyl acetate solvent for overnight. After 24 hours the solvent was filtered by using Whatman filter paper. It was stored at 4 °C in black cap bottles for further uses.
- b) **Toluene extract-** *Azadirachta indica* 50 gm leaves were converted into fine powder form using a pastal mortar and dip in 500 ml toluene solvent for overnight. After 24 hours the solvent was filtered by using Whatman filter paper. It was stored at 4°C in black cap bottles for further uses.
- c) **Benzene extract-** *Azadirachta indica* 50 gm leaves were converted into fine powder form using a pastal mortar and dip in 500 ml benzene solvent for overnight. After 24 hours the solvent was filtered by using Whatman filter paper. It was stored at 4°C in black cap bottles for further uses.
- d) **Acetone extract-** *Azadirachta indica* 50 gm leaves were converted into fine powder form using a pastal mortar and dip in 500 ml acetone solvent for overnight. After 24 hours the solvent was filtered by using Whatman filter paper. It was stored at 4°C in black cap bottles for further uses.
- e) **Butyl alcohol extract-** *Azadirachta indica* 50 gm leaves were converted into fine powder form using a pastal mortar and dip in 500 ml butyl alcohol for overnight. After 24 hours the solvent was filtered by using Whatman filter paper. It was stored at 4°C in black cap bottles for further uses.

- f) **Ethanol extract-** *Azadirachta indica* 50 gm leaves were converted into fine powder form using a pastal mortar and dip in 500 ml ethanol solvent for overnight. After 24 hours the solvent was filtered by using Whatman filter paper. It was stored at 4 °C in black cap bottles for further uses.

### Phytochemical analysis of *Azadirachta indica* extract

- a) **Saponins** – In this process we take 2ml extract of each solvent, 6ml of distilled water were added in a test tube and mixed properly if appearance of bubbles or persistent foam indicate the presence of saponins
- b) **Tannins-** For this we take 2ml extract of each solvent, 10% of alcoholic ferric chloride was added, if formation of brownish blue or black color indicate the presence of tannins.
- c) **Phenols-** Take 2ml of extract of each solvent, 2ml of 5% aqueous ferric chloride were added. Formation of blue color indicates the presence of phenols in the sample.
- d) **Protein** - Take 2ml extract of each solvent, 1ml 40% of NaOH and few drops of 1% copper sulphate were added, formation of violet color indicate the presence of peptide linkage molecule in the sample.
- e) **Cardiac glycosides-** Take 1ml extract of each solvent, 0.5 ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added. Formation of brown ring at the interface indicate the presence of cardiac glycosides in the sample.
- f) **Terpenoids-** Take 1ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid. Formation of reddish brown precipitate indicates the presence of terpenoids in the sample.
- g) **Carbohydrates-** Take 1ml of extract of each solvent, add few drops of molish's reagent and then add 1ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet color indicates the presence of carbohydrates in the sample.
- h) **Flavonids-** Take 2 ml of each extract, add few drops of 20% sodium hydroxide. Formation of intense yellow color is observed. To this few drops of 70% dilute hydrochloric acid were added and yellow color was disappeared. Formation and disappearance of yellow color indicates the presence of flavonoids in the sample.
- i) **Alkaloids-** Take 1ml extract of each solvent add 1 ml of marquis reagent were added and mix properly, appearance of dark orange or purple color indicates the presence of alkaloid in the sample.

### Antimicrobial property

The antibacterial property were carried out by disc diffusion method. The plant extracts were prepared by taking 1:10 ratio in 6 different organic solvents as benzene, toluene, acetone, water, ethyl acetate, ethanol and butyl alcohol for 24 hours. *E. coli* Suspensions were prepared in nutrient both by adding one loopful culture and incubate for 24 to 48 hours at 37°C. For this experiment nutrient agar media were prepared and autoclave at 121 °C for 15 psi. After autoclaving, poured media was poured in petriplates and allowed to solidify. Sterilized sterile disc of 6 mm diameter (Hi Media) were dip in to the extract for 1min and then aseptically inoculated on the agar surface of the nutrient agar medium plates in which 1 ml suspension were spread using spreader. Inoculated

petriplates were incubated at 37 °C and the observations were (which were mean value of five replicates in each case) recorded after 24 hr. Percentage of bacterial growth Inhibition (BGI%) was calculated as per formula.

$$BGI (\%) = \frac{dc - dt}{dc} \times 100$$

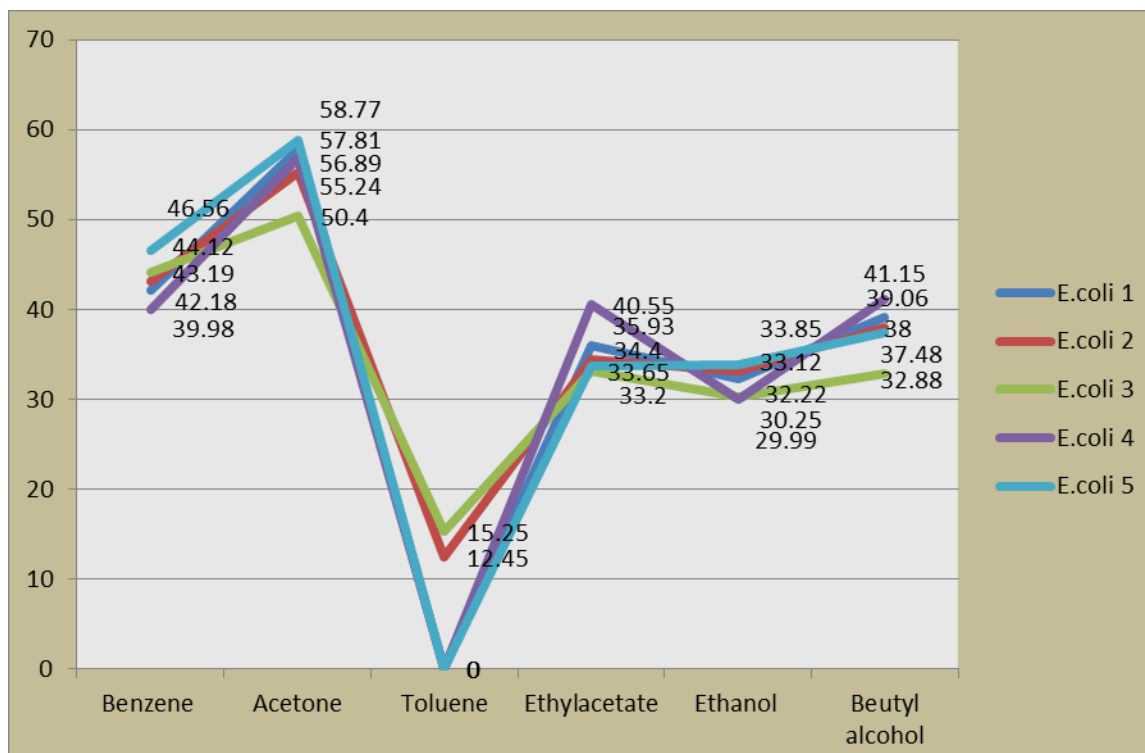
Where,  
 DC = growth diameter in control  
 DT = growth diameter in treatment

**Table 1:** Phytochemical analysis of *Azadirachta indica* leaves extract

Test Extract	Saponin	Tannins	Phenol	Protein	Glycoside	Terpenoids	Carbohydrate	Flavanoids	Alkaloids
Benzene	+	-	-	+	-	-	-	+	+
Ethanol	-	+	+	-	+	+	+	+	-
Toluene	+	-	-	+	+	+	-	+	+
Beutyl alcohol	+	+	+	+	+	+	+	+	+
Ethyl acetate	+	+	-	+	+	+	+	+	+
Acetone	+	+	+	-	+	+	+	-	-

**Table 2:** antibacterial activity of *Azadirachta indica* leaves extract against *E. coli*strains

Plant extract	Percentage of bacterial growth inhibition (BGI %)					
	Benzene	Acetone	Toluene	Ethyl acetate	Ethanol	Beutyl alcohol
<i>E. coli</i> -1	42.18	57.81	-	35.93	32.22	39.06
<i>E. coli</i> -2	43.19	55.24	12.45	34.40	33.12	38.00
<i>E. coli</i> -3	44.12	50.40	15.25	33.20	30.25	32.88
<i>E. coli</i> -4	39.98	56.89	-	40.55	29.99	41.15
<i>E. coli</i> -5	46.56	58.77	-	33.65	33.85	37.48



**Fig 1:** Graphical representation of antimicrobial activity

**Result and Discussion**

The Phytochemical test was done to find out the presence of active chemical constituents such as saponins, tannins, phenols, proteins, glycoside, terpenoids, carbohydrate, flavanoids, alkanoids. The phytochemical analysis of extracts using benzene, acetone, toluene, ethyl acetate, ethanol, beutyl alcohol. Showed in table No.1. The saponins present in all *Azadirachta indica* leaves extract (benzene, toluene, beutyl alcohol, ethylacetate, and acetone) except in ethanol solvent. Tanins found in all extract except in benzene. Phenol was to be present in all extract except benzene and ethyl acetate. Presence of protein was observed in all extract, except ethanol and acetone. Glycoside found in all extract except ethanol.

Presence of Terpenoids was found to be positive, except benzene. Presence of carbohydrate was observed in all extract, except in benzene and toluene. Flavanoid present in all extract except in acetone extract. Alkaloid was observed to be present in all extract, except ethanol and acetone.

The results of antibacterial activity of *Azadirachta indica* leaves determined by disc diffusion method showed in table no.2. All the extract of *Azadirachta indica* leaves showed the antibacterial activity against the *E. coli*strains. The benzene extract of *Azadirachta indica* leaves showed maximum growth inhibition 46.56% against *E. coli*5 and also showed minimum growth inhibition 39.98% against *E. coli*4. The acetone extracts of *Azadirachta indica* leaves showed

maximum growth inhibition 58.77% against *E. coli*5 and also showed minimum growth inhibition 50.40% against *E. coli*3. The toluene extract of *Azadirachta indica* leaves showed maximum growth inhibitions 15.25% against *E. coli*3 and also showed minimum growth inhibition 0% against *E. coli*1, 3, 5. The ethyl acetate extracts of *Azadirachta indica* leaves showed maximum growth inhibition 40.55% against *E. coli*4 and also showed minimum growth inhibition 33.20% against *E. coli*3. The ethanol extracts of *Azadirachta indica* leaves showed maximum growth inhibition 33.85% against *E. coli*5 and also showed minimum growth inhibition 29.99% against *E. coli*4. The butyl alcohol extracts of *Azadirachta indica* leaves showed maximum growth inhibition 41.15% against *E. coli*4 and also showed minimum growth inhibition 32.88% against *E. coli*3.

### Conclusion

We concluded from this study that each extract of *Azadirachta indica* leaves contains many chemical components as saponins, tannins, protein, carbohydrate, alkaloid and phenols which justified that plant has biological activities as-antibacterial activity. The plant could be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made via a simple process of maceration or infusion. Development of modern non-toxic drugs from neem has earlier been suggested (Verkerk 1993) [18]. These positive result encourage us the continuation of the present research study.

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