

# Antimicrobial Activity of Medicinal plants- *Azadirachta indica* A. Juss, *Allium cepa* L. and *Aloe vera* L.

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**Abstract:** The petroleum ether, methanol and aqueous extracts of the leaves of *Azadirachta indica* (Meliaceae), bulbs of *Allium cepa* (Liliaceae) and methanol extract of gel of *Aloe vera* (Liliaceae) were screened for their anti-microbial activity using the Cup plate agar diffusion method. They were tested against six bacteria; two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and four Gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and against two fungi (*Aspergillus niger* and *Candida albicans*). The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with selected antibiotics. The antimicrobial activities of these plants were discussed according to their phytochemical components. The methanol extract of *Azadirachta indica* exhibited pronounced activity against *Bacillus subtilis* (28 mm).

**Key words:** *Aloe vera*, *Azadirachta indica*, *Allium cepa*, *Candida albicans*, Antimicrobial activity.

## Introduction

Bacterial resistance to antibiotics is increasingly becoming a concern to public health. Currently used antibiotic agents are failing to bring an end to many bacterial infections due to super resistant strains. Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants [1]. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds [2].

*Aloe vera* L. has a long history of use as a therapeutic agent with many reported medicinal properties. *Aloe*

*vera* has been used to treat various skin conditions such as cuts, burns and eczema. Amongst its therapeutic properties, it has been shown to have anti-inflammatory activity [3], immunostimulatory activity [4], and cell growth stimulatory activity [5, 6]. Furthermore, activity against a variety of infectious agents has been attributed to *Aloe vera*; antibacterial [7], antiviral [8] and anti fungal [9] besides its skin soothing and cell protecting properties.

*Aloe vera* leaf gel can inhibit the growth of the two Gram-positive bacteria *Shigella flexneri* and *Streptococcus progenes* [7]. Specific plant compounds such as anthraquinones [10, 11] and dihydroxy -

anthraquinones [12], as well as saponins [13], have been proposed to have direct antimicrobial activity.

*Aloe vera* L is a succulent from the Aloe family (400 different species) with its origin in African continent. Its thick leaves contain the water supply for the plant to survive long periods of drought [14]. *Aloe* gel is perhaps the most widely recognized herbal remedy in the United State; it is used to relieve thermal burn, sunburn and promote wound healing [14]. In addition, research suggests that *Aloe* gel can help to stimulate the body's immune system [15]. The aloe leaf can be divided into two major parts, namely the outer green rind, including the vascular bundles, and the inner colorless parenchyma containing the aloe gel as shown in figure 1 [16]. The raw pulp of *A. vera* contains approximately 98.5% water, while the mucilage or gel consists of about 99.5% water [17]. The remaining 0.5–1% solid material consists of a range of compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids [18]. It has been hypothesized that this heterogenous composition of the *Aloe vera* pulp may contribute to the diverse pharmacological and therapeutic activities which have been observed for aloe gel products [19].

*Azadirachta indica* (Meliaceae) commonly known as neem is native of India and naturalized in most of tropical and subtropical countries is of great medicinal value and distributed widespread in the world. The Chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones, Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective [20]. Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin [21, 22].

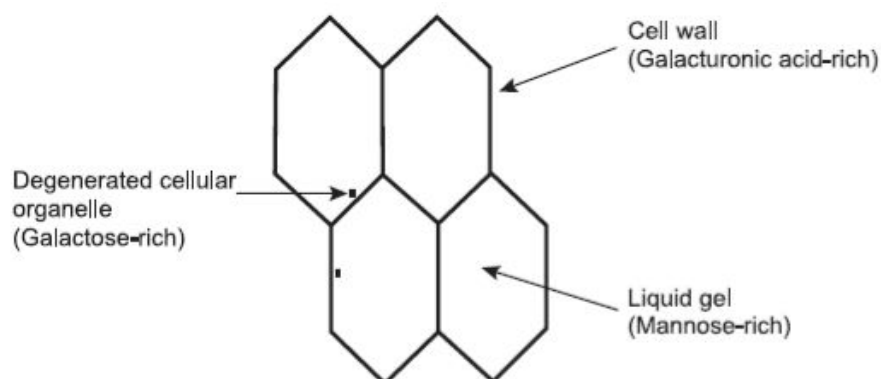
Neem leaf is effective in treating eczema, ringworm, acne, has anti-inflammatory, antiheperglycemic properties and it is used to heal chronic wounds, diabetic foot and gangrene developing conditions. It is believed to remove toxins from the body, neutralize free radicals and purify the blood. It is used as anti-cancer agent and it has hepato-renal protective activity and hypolipidemic effects [23].

*Allium cepa* (Liliaceae), commonly known as basal is distributed worldwide. The onion bulbs contains numerous organic sulfur compounds, including trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcysteine sulfoxide and cycloalliin; flavonoids; phenolic acids; sterols including cholesterol, stigma sterol, b-sitosterol; saponins; sugars and a trace of volatile oil composed mainly of sulfur compounds, including dipropyl disulfide [24, 25]. A fresh onion bulb contains fructans with a low degree of polymerization, and sulfur-containing compounds [26].

Onion is used to decrease cancer tumor initiation promote healing of stomach ulcers, inhibit the proliferation of cultured ovarian, breast and colon cancer cells; reduce the cholesterol, blood pressure and symptoms associated with diabetes mellitus, inhibit platelets aggregation (involved in thrombosis) and prevent inflammatory processes associated with asthma [27, 28]. Onion is used as antiseptic, antihelminthic, antispasmodic, carminative, diuretic, cholagogue, diaphoretic and expectorant. It is used also for coughs, the flu, parasites, wound, burns, dog bites, bee stings, ear aches, athletes' foot, warts, baldness, toothaches, intestinal infections, kidney infections, contaminated blood and heart failure. Raw onion can completely sterilize the mouth and throat [29].

In the present communication, an attempt has been made to explore antimicrobial principles, which involves an investigation on the efficacy of essential oils.

**Figure 1. Schematic representation of *A. vera* leaf pulp structure and its components [16].**



## **Materials and Methods**

### **Plant materials:**

The plants used in this study were *Azadirachta indica*, *Aloe vera* and *Allium cepa* collected locally from Dehradun.

### **Preparation of the crude extracts:**

The leaves of *Azadirachta indica* and bulbs of *Allium cepa* were air-dried, coarsely powdered and were then extracted. Hundred grams of each of the air-dried and coarsely powdered plant material was extracted for 2 hours with petroleum ether (60-80°C) in soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure using Rota-vapor. The extracted plant material was then air-dried, repacked in the soxhlet apparatus and extracted with methanol (98.8%) for 2 hours. The methanol extract was filtered and evaporated under reduced pressure using Rota-vapor. The extracts were dissolved in dimethyl-sulphoxide to make the final concentrations and refrigerated for further use.

Simultaneously, water extract was prepared by adding (10 ml) of boiled distilled water to 5 g of coarsely powdered plants leaves in a beaker on water bath with occasional stirring for 4 hours. The aqueous extract was then filtered and rewashed with small volume of boiled distilled water and added to the filtrate, which were then adjusted to (5 ml) volume and used immediately.

After cutting *Aloe vera* leaves and discarding green rind was discarded, the mucilaginous inner pulp was minced and thoroughly homogenised with a hand held blender. Each leaf produced approximately 120 ml of gel. The homogenised gel was lyophilised in vacuo at 22°C and the resultant lyophilised material was stored frozen until further extraction. 1 g of lyophilised *A. vera* gel was extracted extensively in 50 ml methanol for 2 hours at 22°C. The extract was filtered through filter paper (Whatman No. 54) under vacuum followed by drying by rotary evaporation in an Eppendorf concentrator. The resultant waxy red pellet was dissolved in 1 ml 20 % methanol giving a dark red extract. The extract was passed through 0.22 µm filter and stored at 4°C.

### **Preparation of the tested organisms:**

#### **A) Preparation of standard bacterial suspensions:**

The average number of viable, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* organisms per ml of the stock suspensions was determined by means of the surface viable counting technique [30]. About (10<sup>8</sup>-10<sup>9</sup>) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

#### **B) Preparation of standard fungal suspensions:**

The fungal cultures (*Aspergillus niger*, *Candida albicans*) were maintained on Sabouraud Dextrose Agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100 ml) of sterile normal saline and the suspension was maintained for further use.

### **Antimicrobial activity:**

#### **Testing for antibacterial activity:**

The cup-plate agar diffusion method was used [31] to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions of 10<sup>8</sup>-10<sup>9</sup> colony-forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates, 4 cups, 10 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1ml of each extracts using micropipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the growth inhibition zones were measured, averaged and the mean values were tabulated (Table 1).

#### **Testing for anti-fungal activity:**

The same method as for bacteria was followed. Instead of nutrient agar media, yeast and mould extract agar was used. The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for *Aspergillus niger*.

Table (1) Preliminary screening for antimicrobial activity of different plants against standard organisms:

Micro organisms	Mean Diameter Inhibition Zone(mm)						
	<i>Azadirachta indica</i>			<i>Allium cepa</i>			<i>Aloe vera</i>
	P.ether	Methanol	Water	P.ether	Methanol	Water	Methanol
<i>Bacillus Subtilis</i>	(-)	28	(-)	(-)	23	(-)	(-)
<i>Staph. Aureus</i>	(-)	18	(-)	(-)	(-)	(-)	(-)
<i>E. coli</i>	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>Proteus vulgaris</i>	(-)	18	(-)	(-)	20	(-)	(-)
<i>Pseudo aeruginosa</i>	(-)	14	(-)	(-)	23	(-)	(-)
<i>Salmonella Typhi</i>	(-)	20	(-)	(-)	(-)	(-)	(-)
<i>Aspergillus Niger</i>	(-)	(-)	(-)	(-)	(-)	(-)	12
<i>Candida albicans</i>	18	15	(-)	(-)	(-)	(-)	(-)

(-) : No Activity.

Table (2): Screening of antibacterial activity of Gentamicin and Tetracycline against standard organisms:

Drug	Conc. µg/ml	Mean diameter of growth inhibition zone in (mm).					
		<i>B.s.</i>	<i>S.a.</i>	<i>E.coli</i>	<i>Pr.v.</i>	<i>Ps.a.</i>	<i>Sa.t.</i>
Gentamicin	100	30	20	25	22	25	-
	40	30	18	24	20	20	-
	20	20	17	23	17	15	-
	10	20	14	20	15	14	-
Tetracycline	100	27	31	24	-	-	-
	40	25	30	25	-	-	-
	20	24	26	23	-	-	-
	10	20	22	20	-	-	-

*B.s:* *Bacillus subtilis**S.a:* *Staphylococcus aureus**E.coli:* *Escherichia coli**Pr.v:* *Proteus vulgaris**Ps.a:* *Pseudomonas aeruginosa**Sa.t:* *Salmonella typhi*

-: No inhibition zone

Table (3): Screening of antifungal activity of Nystatin against standard organisms:

Drug	Conc. µg/ml	Mean diameter of growth inhibition zone in (mm).	
		<i>A. niger</i>	<i>C. albicans</i>
Nystatin	50	17	28
	25	14	26
	12.5	-	23

*A. niger:* *Aspergillus niger**C. albicans:* *Candida albicans*

-: No inhibition zone

## **Results:**

The petroleum ether and aqueous extract of bulbs of *Allium cepa* was found to be inactive against all organisms tested. The methanol extract showed different antimicrobial activity toward test organisms. The methanol extract of bulbs of *Allium cepa* exhibited high activity against *Bacillus subtilis* (23mm), *Proteus vulgaris* (20mm), *Pseudomonas aeruginosa* (23mm) and inactive against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and against *Aspergillus niger* and *Candida albicans*. The methanol extract of the leaves of *Azadirachta indica* exhibited pronounced activity (28mm) against *Bacillus subtilis*, high activity (18mm) against the Gram-positive *Staphylococcus aureus* and the Gram-negative organisms *Proteus vulgaris* (18 mm) and *Salmonella typhi* (20 mm), low activity (14mm) against *Pseudomonas aeruginosa* and inactive against *Escherichia coli*. All extracts were inactive against *Aspergillus niger*. Both petroleum ether and methanol extracts of the leaves of *Azadirachta indica* showed high activity (15-18mm) against *Candida albicans*, while its aqueous extract was inactive.

The methanol extract of *Azadirachta indica* leaves was found as effective as 100µg/ml Gentamicin against *Bacillus subtilis* and 20µg/ml Tetracycline against both *Staphylococcus aureus* and *Proteus vulgaris* and 10µg/ml Gentamycin against *Pseudomonas aeruginosa*. The bulbs methanol extract of *Allium cepa* at 100mg/ml concentration was found to be effective similar to that of 20µg/ml Tetracycline against *Bacillus subtilis*, 40µg/ml Gentamicin against *Proteus vulgaris* and 100µg/ml Gentamicin against *Pseudomonas aeruginosa* (Table 2). Table 3 showed the antifungal activity of Nystatin against *C. albicans* and *A. niger*. The *Aloe vera* extract showed activity against only *A. niger*.

## **Discussion:**

The petroleum ether, methanol and aqueous extracts of the leaves of *Azadirachta indica* and bulbs of *Allium cepa* were subjected to a preliminary screening for antimicrobial activity against six standard bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and two fungi (*Aspergillus niger* and *Candida albicans*). It is clear from table 1, that both petroleum ether and aqueous extracts of the leaves of *Azadirachta indica* and bulbs of *Allium cepa* showed no activity against all organisms tested, unlike its methanol extracts.

The methanol extract of *Azadirachta indica* exhibited pronounced activity against *Bacillus subtilis* (28 mm), high activity against the Gram-positive organism *Staphylococcus aureus* (18mm), the Gram-negative bacteria *Proteus vulgaris* (18 mm) and *Salmonella typhi* (20 mm), low activity against *Pseudomonas aeruginosa* (14 mm) and inactive against *Escherichia coli*. These might be due to presence of triterpenoids, phenolic compounds, Carotenoids, steroids, valavainoids, ketones and tetratriterpenoids Azadirachtin [32, 33]. All extracts were inactive against *Aspergillus niger*. The petroleum ether and methanolic extracts of *Azadirachta indica* exhibited high activity against *Candida albicans* (15-18mm); while its aqueous extract was inactive against *Candida albicans*.

The methanolic extract of bulbs of *Allium cepa* showed pronounced activity (23mm) against *Bacillus subtilis* and *Pseudomonas aeruginosa*, high activity (20mm) against *Proteus vulgaris*, while inactive against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The onion bulbs contains numerous organic sulfur compounds, including trans-S-(1- propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcysteine sulfoxide and cycloalliin; flavonoids; phenolic acids; sterols including cholesterol, stigma sterol, b-sitosterol; saponins; sugars and a trace of volatile oil composed mainly of sulfur compounds. Although *Allium*, *Azadirachta* and *Aloe* extracts did not show any activity against *Staphylococcus aureus* [34, 35].

Extract of bulb of *Allium cepa* was inactive against both *Aspergillus niger* and *Candida albicans*. *Aspergillus niger* was significantly inhibited at high concentration of *Allium* extract [35]. *Allium cepa* was found to be active against *Candida albicans* [36]. Onion extracts have both antibacterial and antifungal properties [29].

The fungi tested in the present study were shown to have limited susceptibility to *Aloe vera* gel and extracted fractions. *A. niger* growth was inhibited by the extract. This is an important result as this strain of *A. niger* was resistant to all other antimicrobial agents tested except ciprofloxacin. The findings of this study have established the susceptibilities of a broad range of bacteria to fractions isolated from *Aloe vera* inner leaf gel. Gram-negative bacilli were found to be particularly susceptible to *Aloe vera* gel components. Of the bacterial classes tested, only the Gram-positive cocci bacteria were resistant to the *Aloe vera* components.

The identification of natural antimicrobial compounds and the future development of these compounds through structure/activity studies

provide a promising avenue of research for novel antimicrobials.

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