



## ANTIMICROBIAL ACTIVITY OF *ADANSONIA DIGITATA* AND *COCHLOSPERMUM RELIGIOSUM* EXTRACTS AGAINST *E. COLI* AND *S.* *AUREUS* ISOLATES

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### Abstract:

The ethanolic and aq. Extracts of the *Adansonia digitata* and *Cochlospermum religiosum* were screened for the presence of possible anti-microbial activity using the well-agar plate diffusion method. They were tested against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* formium). The susceptibility of the microorganisms to the extracts of these plants compared on the basis of their zone of growth of inhibition. The antimicrobial activities of these plants were discussed according to their phytochemical components.

**Key words:** Antimicrobial activity, gram-positive, gram-negative, Medicinal plants, *Adansonia digitata*, *Cochlospermum religiosum*.

### Introduction:

In India *Adansonia digitata* L., family Bombacaceae, Vernacular name: *Baobab* (*Gorkhamali*) grows naturally in Mandhu region of Madhya Pradesh and it is cultivated in Uttar Pradesh, Tamil Nadu and Maharashtra (Nadkarni and Nadkarni, 2000). It is larger baobab trees height of 25 m. The stem having layers i.e. bark is greyish brown and normally smooth & leaves are hand-sized and divided into 5-7 finger-like leaflets. The large, pendulous flowers (up to 200 mm in diameter) are white and sweetly scented. The leaves are said to be rich in vitamin C, sugar, potassium tartrate and calcium. Hence freshly leaves are cooked or dried and crushed for later used by local people (Van wyk et al. 2005)

*Adansonia digitata* was found to be an excellent source of protein. Amino acid analyses revealed high glutamic and aspartic acid content and the sulfur containing amino acids as being the most limited amino acid (Osman, 2004). The presence of fatty acids (linoleic and linolene, vitamin E, carotenoid and minerals (iron, magnesium, calcium and zinc); they also mentioned that *Adasonia digitata* leaves were nutritionally superior to the fruit of the tree however the fruit contains useful quantity of potassium, phosphorus, zinc and acid. It is a good source of energy, protein and fat)

*Cochlospermum religiosum* Family *Cochlospermaceae* Vernacular name: Ganeri (Galgal) is a religious tree of 10 m height, thick bark with dark grey surface. Leaves simple, faint & flowers bright yellow. Fruit have a capsule, pear shaped, brown, 6 mm long, embedded in white cotton. *Cochlospermium religiosum* leaf was rich in phenols followed by alkaloids, flavonoids and tannins and barks of trees contain secondary metabolites. The

highest amount of plant lipid can as used as essential oils, spice oleoresins and natural food, colors etc. a number of data shows that the presence of phenolic in food is particularly important for the oxidative stability and antimicrobial productivity (Sasikala, 2013). It is traditionally used for the treatment of skin problems such as rashes on skin and chicken pox. The leaves are crushed and the extract is applied on skin before taking bath. It is also used in the treatment of herpes infection. The leaves are also consumed as medicine (Hullati and Rai, 2004). *C. religiosum* (Linn.) Alston. (Family *Cochlospermaceae*) is a small deciduous tree. The tree yields a gum which is known as katira. Traditionally it is used in treating cough, diarrhea, dysentery, pharyngitis, fistula, gonorrhoea, trachoma and syphilis. The dried leaf and flowers are used as stimulants, antipyretic, laxative and sedative (Kirtikar and Basu, 1975).

### Materials and Methods:

#### Collection and authentication of plant material

Fresh leaves, *Adansoni adigitata* (baobab) and *Cochlospermium religiosum* leaves were collected forest from Bhandara district in Maharashtra, India.

#### Preparation of plant extract

The plant material were washed well with water to separate the adhering soil material and dried in the shade. Dried leaves were comminuted to form coarse powder. Dried leaves were extracted with petroleum ether (60-80°C) for 24 h to remove fatty substances. Ethanolic extracts prepared by using maceration method.

#### Antibacterial Test microorganisms and growth media

*Staphylococcus aureus*, (gram-positive bacteria), *Escherichia coli* (gram-negative

bacteria) were chosen based on their clinical and pharmacological importance. All cultures were obtained from isolated in laboratory of Microbiology department, Sakoli. The bacterial cultures were incubated for 24 hours at 37°C on nutrient agar medium, following refrigeration storage at 4°C.

#### Preparation of inoculum

From a pure overnight culture the material is picked from at least 3-4 colonies, suspend totally in 4ml saline in tubes and mix. The inoculum were adjusted to 0.5 McFarland standard (approximately 10<sup>8</sup> colony forming unit (CFU) per milliliter by comparing turbidity) using a nephelometer (McFarland, 1987).

#### Determination of minimum inhibitory concentration (MIC)

Micro-broth dilution method was used to determine the minimum inhibitory concentration (MIC) (Rattan A; 2000). Tubes containing 10ml Muller-Hinton broth with two fold dilutions of plant extracts are inoculated with 50µl inoculums suspension of the bacteria using a multichannel pipette and incubated at 37°C overnight for bacteria and 22°C overnight for fungi. For purity control the tube containing no antibiotic is inoculated with 10µl inoculum suspension of bacteria and put for incubation at 37°C overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. The lowest concentration inhibiting 99% growth of the organism is recorded as the MIC. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

#### Determination of zone of inhibition (ZOI)

Agar disk diffusion method was used to determine zone of inhibition. Mueller-Hinton agar plates (with 10cm diameter and uniform agar depth of approximately 4mm) are inoculated with 50µl inoculums suspension of the bacteria and antimicrobial disks (5 disks) are placed on the inoculated agar plate containing a known amount of a standardized antimicrobial agent. The plate is incubated for 18 to 24 hours at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. During incubation the antimicrobial agent diffuses into the agar and inhibits growth of the microorganism, producing a zone of inhibition around the disk. The diameter of this zone is measured and the results are interpreted as values <8 mm were considered as not active against microorganisms. To verify the purity agar plates are inoculated with 10µl inoculum

suspension of bacteria and put for incubation at 37 °C for 16 to 18 hours.

#### Test organism

*E. coli* was isolated from sewage water sample and *S. aureus* was isolated from the pus sample of patient from general hospital. These test organisms were subject to test against the different plant extracts. The bacterium was identified to Cheesbrough (1985).

#### Result:

##### Antibacterial Activity

**Minimum Inhibitory Concentration:** The Minimum Inhibitory Concentration (MIC) value of the extracts against both gram positive and gram negative bacteria shown in the following table. The MIC value was found in the range of the 62 to 200 µg/ml against the tested microorganisms. The MIC values against gram positive bacteria ranged from 100 to 200µg/ml and against gram negative bacteria from 62-200 µg/ml. Antibacterial potency of the plant extracts against these bacteria expressed as MIC indicated that the plant extract is more effective against gram negative at lower concentration than that against gram positive bacteria.

Antibacterial Activity			
Minimum Inhibitory Concentration in µg/ml			
S. N.	MIC in various Concentrations	<i>E. coli</i>	<i>S. aureus</i>
1	200 µg/ml	+	+
2	100 µg/ml	+	+
3	75 µg/ml	+	+
4	50 µg/ml	-	+
5	25 µg/ml	-	+

**Table 1: Minimum Inhibitory Concentration of ethanolic extracts *A. digitata* L. against gram positive-gram negative organisms.**

Antibacterial Activity			
Minimum Inhibitory Concentration in µg/ml			
S. N.	MIC in various Concentration	<i>E. coli</i>	<i>S. aureus</i>
1	200 µg/ml	+	+
2	100 µg/ml	+	+
3	75 µg/ml	+	+
4	50 µg/ml	+	+
5	25 µg/ml	+	+

**Table 2: Minimum Inhibitory Concentration of ethanolic extracts *C. religiosum* L. against gram positive- negative organisms**

Zone of inhibition studies of ethanolic extracts of *A. digitata* L, against bacterial strains reveals that the maximum zone of inhibition against the bacterial strain *E. coli* highest with

30 mm diameter of the zone of inhibition against with *S. aureus* at the concentration range of 200µg/ml.

Microorganisms	Zone of Inhibition in mm				
	Concentration of plant ( <i>Adansonia digitata</i> ) Methanolic extracts in µg/ml				
	25	50	75	100	200
<i>E. coli</i>	-	-	11	20	30
<i>S. Aureus</i>	12	14	16	18	20

Zone of inhibition studies of ethanolic extracts of *C. religiosum* L, against bacterial strains reveals that the maximum zone of inhibition against the bacterial strain *S. aureus* highest with 18 mm diameter of the zone of inhibition against with *E. coli* at the concentration range of 200µg/ml.

Microorganisms	Zone of Inhibition in mm				
	Concentration of plant ( <i>Cochlospermium religiosum</i> ) Methanolic extracts in µg/ml				
	25	50	75	100	200
<i>E. coli</i>	-	12	13	15	16
<i>S. Aureus</i>	13	14	15	17	18

#### Discussion:

The extract from *A. digitata*, others extract exhibited antibacterial activities against at least one of the tested bacteria. The differences in antibacterial activities were noted between the various extracts and could be related to the differences in their phytochemical composition. The alkaloids, phenols and polyphenols were detected in all extracts. The antibacterial activities of many molecules belonging to these classes of compounds were shown (Kuetze et al. 2009). Also associated the antibacterial activities of the medicinal plants to the presence of flavonoids, tannins and alkaloids. This could be explained by fact that the presence of the secondary metabolites in a plant depend on the environmental factors such as climate, chemical nature of the ground in which plant grow, the period of harvest, conditions of drying and extraction method.

Several former studies showed the presence of the terpenoids, phenolic and alkaloids in the extract of *A. digitata* (Wickens 1979; Chadare et al. 2009). The aqueous, ethanol, and petroleum-ether extracts of this plant have shown an antibacterial activity against *E. coli*. However, we observed a weak antibacterial activity of this plant. *Cochlospermium*

*religiosum* leaf was rich in phenols followed by alkaloids, flavonoids and tannins and barks of trees contain secondary metabolites. The highest amount of plant lipid can as used as essential oils, spice oleoresins and natural food, colors etc. It is traditionally used for the treatment of skin problems such as rashes on skin and chicken pox. The leaves are crushed and the extract is applied on skin before taking bath. It is also used in the treatment of herpes infection. The leaves are also consumed as medicine (Hullati and Rai, 2004).

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