



3. A Study on the Antibacterial Activities and Medical Properties of Water Chestnut

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ABSTRACT

Fruits and vegetables are generally known as very good and abundant sources of several vitamins and minerals, not only that but also they are very effective in increasing immunity power against several microbial infections.

In the matter of discussion of preventing bacterial infections the underwater vegetables are not staying back. One of the very effective and used under water vegetable is Water Chestnut. Water chestnut is not a nut at all, but it is a very useful aquatic vegetable that grows under Water in the mud. Eleocharis dulcis, belongs to family Cyperaceae, commonly called Chinese water chestnut. It was stem-like, its green leaves grow to about 1.5m.

The small, round forms have crisp. White stuff may be eaten fresh, boiled grilled and also used as pickled. Water Chestnuts are popular dishes in china. It also used for making many cake or delicious food. It is grown in many countries like Asia (china, India & Japan etc.), Africa, Australia, and Island. Water chestnut used in agricultural department. It also plays important role in medical Field to development medical science.

Its peels extracts, seed used for product many type of medicine. It showed a high-level antibacterial activity against some bacteria like Bacillus subtilis, Escherichia coli, Salmonella typhi etc. In medicinal field this plant use to cured many type of diseases like inflammation, urinary, discharges, fractures, leprosy, astringents, bad teeth and malaria.

KEYWORDS

Water Chestnut, Microbiology, Pharmaceutical Sciences, Biology.

Introduction:

Water chestnut is not a nut at all, but it is a very useful aquatic vegetable that grows under Water in the mud. *Eleocharis dulcis*, belongs to family Cyperaceae, commonly called Chinese water chestnut. It was stem-like, it's green leaves grow to about 1.5m.

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In medicinal field this plant use to cured many type of diseases like inflammation, urinary, discharges, fractures, leprosy, astringents, bad teeth and malaria.



Fig.1: Water Chestnut

Taxonomy classification of Water Chestnut

- Kingdom: Plantae
- Clade: Tracheophytes
- Clade: Angiosperms
- Clade: Monocots
- Clade: Commelinids
- Order: Poales
- Family: Cyperaceae
- Genus: *Eleocharis*
- Species: *E. dulcis*

Materials and Methods

Collection of Plant

The plant water chestnut was taken for study on its antimicrobial activity and antibacterial significance of water chestnut collected from Champadanga, Hooghly, and West Bengal.

Preparation of Plant Extract

At first we washed the plant peels with distilled water and kept them for dry under shade, and grind them with the help of mixer grinder to a dust powder. And keep it in an air tight container at the room temperature in dark until used.

The powdered samples are extracted by the following methods.

- **Ether Extraction** To prepare the Ether extraction first, 5mg of dried of water chestnuts peel extract powder mixed with 25ml ether to each solution was stored at 4°C after collecting in sterile test tubes until use.
- **Hexane Extraction** About 5 gm of dried Water Chestnut peel extract powder taken, then dispersed in 25 ml of benzene solution and shaken in a rotary shaker for 10 minutes. Then closed with paper and tightened with a band few holes were made in the paper to facilitate air circulation and room temperature maintenance for 5 dates.
- **Benzene Extraction** About 5 gm of dried Water Chestnut peel extract powder taken, then dispersed in 25 ml of benzene solution and shaken in a rotary shaker for 10 minutes. Then closed with paper and tightened with a band. Few holes were made in the paper to facilitate air circulation and room temperature maintenance for 5 dates.
- **Chloroform Extraction** For making Chloroform extraction 5mg of dried of water chestnut peel extract powder mixed with 25ml Chloroform. Each solution was stored at 4°C after collecting in sterile test tubes until use.
- **Ethanol Extraction** First, 5mg of dried of water chestnut peel extract powder mixed with 25ml Ethyl Acetate. Each solution was stored at 4°C after collecting in sterile test tubes until use.
- **Ethanol Extraction** For preparing the Ethanol extraction, 5mg of dried of water chestnut peel extract powder mixed with 25ml ethanol. Each solution was stored at 4°C after collecting in sterile test tubes until use.
- **Medium** In this we use Mueller Hinton agar (MHA) is used as base medium for the screening of antibacterial activity and Mueller Hinton broth (MHB) is used for the preparation of inoculums.

Preparation of Extract Concentration

Four concentrations were made 25, 50, 100, 200mg/ml from each of the four extract Ethanol, Benzene extract, Chloroform, Ethyl acetate and Ether. In every case 1gm of extract was mixed with 5ml DMSO to prepare 200mg/ml stock concentration. Other concentrations were made by adding extra DMSO with stock in other test tube.

Determination of Antibacterial Activity

Bacterial Stains

We take bacteria such as *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* bacteria were selected for antibacterial activity test. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Preparation of Bacterial Inoculum

Bacterial inoculums were prepared by 0.1 ml of cultures was transferred to the agar plates and incubated at 37°C for 4-5 hours.

Agar Diffusion Methods

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plant. This phenomenon forms the basis of the agar diffusion assay that is used to determine the susceptibility or resistance of a bacterial strain to an antibacterial agent. Disc diffusion method was carried on Mueller Hinton agar plates.

Petri plates were prepared by 20ml of Mueller Hinton agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1ml of inoculums was poured and spread and allow to dry. After the cork borer agar well made on agar plate and poured different concentration of extract into the well. The Penicillin is used as positive control and DMSO was used as negative or blind control. The plates were incubated at 37°C for 1 day.

The zone of inhibition were observed and measured. The agar plates were incubated at 4°C for 1 hour and were then incubated also at 37°C. After 24 to 48 hours of incubation, the antibacterial activity was evaluated by measuring the width of zone of inhibition of growth against the indicator organisms in comparison to a control of reference standards. The tests were study in triplicate.

Phytochemical Analysis

Phytochemical analysis of all the evaporated solvent ex-tracts was conducted following the procedure of Indian Pharmacopoeia (1985).

- **Test For Alkaloids** For testing of alkaloids first we take 200 mg plant material in 10 ml methanol, (filtered) and a 2ml filtrate + 1% HCl + steam mixed and then 1 ml filtrate + 6 drops of Mayer's reagents or Wagner's reagent added then the creamish precipitate/brownish-red precipitate/orange precipitate found that indicated the presence of respective alkaloids.
- **Test For Tannins** For testing of tannins 200 mg plant material in 10 ml distilled water added (filtered), a 2 ml filtrate + 2 ml FeCl₃ mixed then blue-black precipitate that indicated found the presence of tannins.

- **Test For Flavonoids** For testing of flavonoids 200 mg plant material in 10 ml ethanol (filtered), a 2 ml filtrate + conc. HCl + magnesium mixed and then ribbon pink-tomato red color indicated the presence of flavonoids.
- **Test For Steroids** For testing of steroids we follow the Liebermann - Burchard reaction and there we take 200 mg plant material in 10 ml chloroform, (filtered), a 2 ml filtrate + 2 ml acetic anhydride + conc. H₂SO₄ was added then the founded Blue-green ring indicated the presence of steroids.
- **Test For Phenols** For testing of phenols first 1ml of each solvent extracts dissolved in alcohol or water was separately treated with a few ml of neutral ferric chloride solution. The change in color indicated the presence of phenols.

Table 1: Phytochemical Analysis

No	Phytochemical	Method	Result
1	Tannin	Ferric chloride test	-
2	Flavonoid	Alkaline Reagent test	+
3	Phenol	Ferric chloride test	-
4	Carbohydrate	Molisch's test	+
5	Protein	Biuret test	+
6	Sapronin	Foam test	-
7	Starch	Iodine Test	+++

Result

Antibacterial activity of water chestnut peel extract against bacterial culture of Escherichia coli, Bacillus subtilis, salmonella typhi etc. It help in to examine inhibition zone. Antibacterial activity of water chestnuts peels extract against many type potential of many extract. The extract of water chestnut showed antibacterial efficiency against many organisms like Bacillus subtilis, Escherichia coli, and Salmonella typhi. Pore efficiency was found against the Staphylococcus aureus.

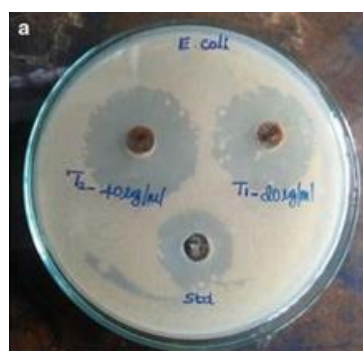


Fig.2: Escherichia coli.

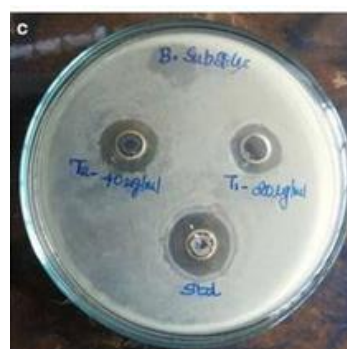


Fig.3: Bacillus subtilis

Table-2: The antimicrobial activity of Chloroform extract against different bacteria

Concentration (mg/ml)	Zone of inhibition (mm)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhi</i>
200 mg/ml	29.0±2	25.2±1	26.3±1	31.5±2
100 mg/ml	24.7±2	24.2±2	26.7±2	26.1±1
50mg/ml	22.7±1	20.5±1	21.9±1	21.9±2
25mg/ml	18.2±2	18.7±2	17.5±2	18.6±1

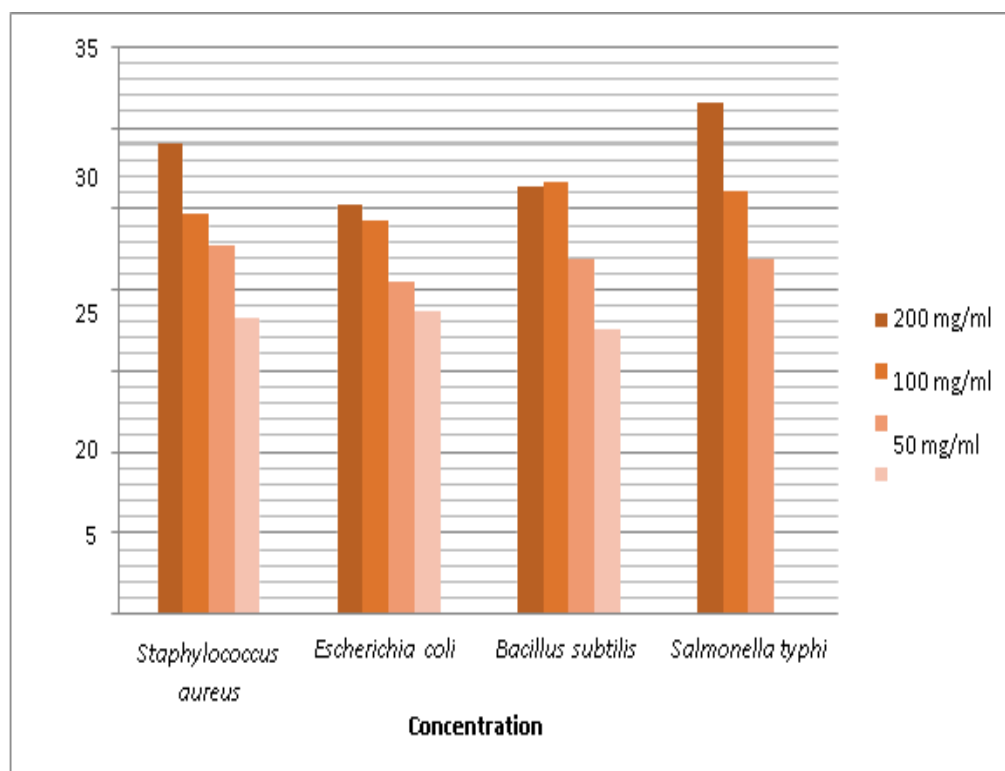


Fig 4: The antimicrobial activity of chloroform extracts against different bacteria

Table-3: The antimicrobial activity of Ethanol extract against different bacteria

Concentration (mg/ml)	Zone of inhibition (mm)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhi</i>
200 mg/ml	22.1±2	26.1±1	26.3±1	30.5±2
100 mg/ml	24.6±2	24.2±2	25.7±2	26.1±1
50 mg/ml	18.9±1	21.1±1	23.4±2	30.1±2
25 mg/ml	17.2±2	22.9±2	17.5±2	19.1±1

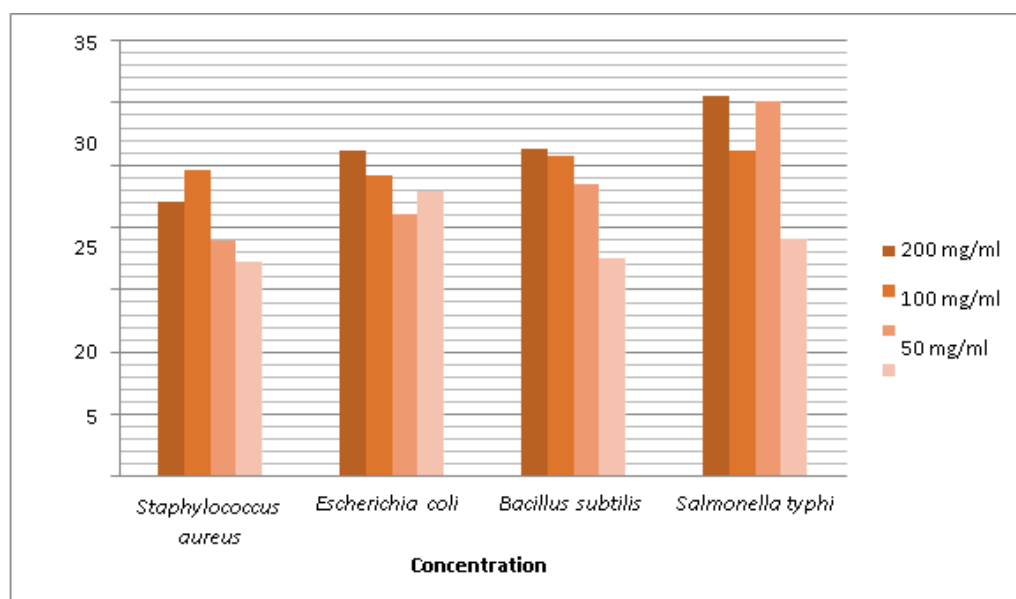


Fig 5: The antimicrobial activity of Ethanol extract against different bacteria

Table-4: The antimicrobial activity of Ethanol extract against different bacteria

Concentration (mg/ml)	Zone of inhibition (mm)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhi</i>
200mg/ml	22.1±2	26.1±1	26.3±1	30.5±2
100mg/ml	24.6±2	24.2±2	25.7±2	26.1±1
50mg/ml	18.9±1	21.1±1	23.4±2	30.1±2
25mg/ml	17.2±2	22.9±2	17.5±2	19.1±1

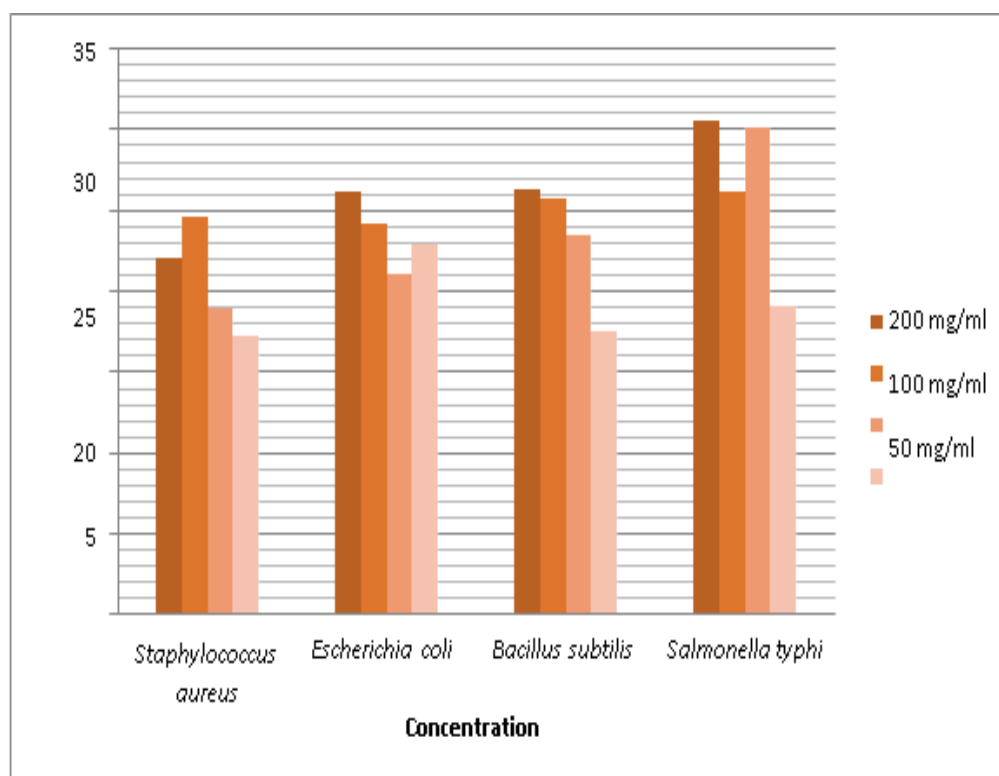


Fig 6: The antimicrobial activity of Ethanol extract against different bacteria.

Table-5: The antimicrobial activity of Benzene extract against different bacteria

Concentration (mg/ml)	Zone of inhibition (mm)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhi</i>
200mg/ml	26.9±2	27.4±1	25.8±1	28.5±2
100mg/ml	19.4±2	23.4±2	26.1±2	30.1±1
50mg/ml	22.4±1	22.9±1	24.3±2	31.2±2
25mg/ml	17.5±2	18.1±2	15.9±2	18.3±1

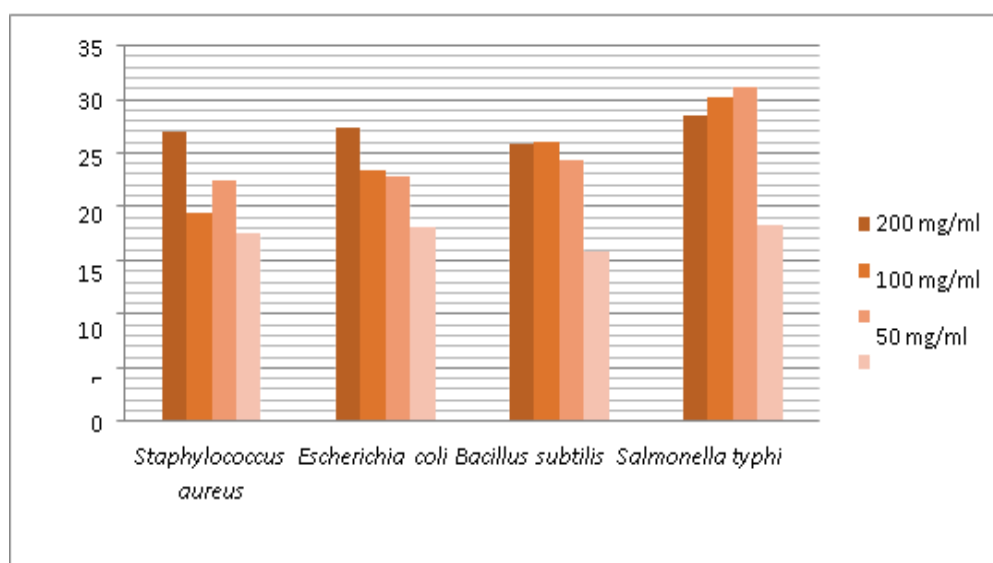


Fig 7: The antimicrobial activity of Benzene extract against different bacteria

Discussion

The methanol extract of water chestnut fruit was found as very important antibacterial activity that inhibits both Gram positive and Gram negative bacteria. It helps to increase of antibacterial resistance of various microorganisms.

From result it seemed that water chestnut showed low antibacterial activity on staphylococcus aureus then other bacteria. Water chestnut showed high efficiency against *Bacillus subtilis*, *salmonella typhi* and *Escherichia coli*. Water chestnut play very important role in medical field to produce various type of medicines. It's effective process help to control infection of many diseases.

Conclusion

In this paper we observed that phytochemical property of fruit to analyze their quality for their use. Hexane, Benzene, Chloroform, Ethanol extract physicochemical test showed positive result. It can be conclude that water chestnut showed antibacterial activity against many bacteria.

It helps to control infection of many diseases. This plant contains medicinally important compound that use in medical field for the treatment of various diseases. This plant helps in development medical field.

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