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Antibacterial Activity of *Terminalia Chebula* Fruit by Agar well Diffusion Method

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Abstract: Antibacterial activity of ethanolic, acetonic and chloroform extract of the fruit of *Terminalia Chebula* were studied against *Escherichia* Coli and *Staphylococcus aureus* by agar well diffusion method. Results obtained showed that the growth of both coli and S. anreus were inhibited by all the three extracts of dried fruit of *Terminalia Chebula*. The antibacterial activity against selected strains depends on the type of solvent used for extraction and the bacterial stain tested. The results revealed that *Terminalia Chebula* can be exploited for new potent antibacterial agents.

1. INTRODUCTION

India has a rich heritage of traditional herbal medicine which formed the basis of health care since earliest days of mankind. A large number of herbs or medicinal plant parts are used in several formulations for the treatment of many diseases caused by microbes. Herbal medicine is still the main stay of about 75-80% of the whole population, mainly in developing countries. The World Health Organisation (WHO) estimated that almost 80% of the people worldwide rely on plant based medicines for their primary health care needs (Famsworth, 1985) and India happens to be the largest user of traditional medical cure, using 7000 plant species.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts (root, stem, leaf, flower, fruit, twigs etc.) extracts are used as raw drugs as they possess many medicinal properties. Some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use while many raw drugs are collected in larger quantities and traded to herbal

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industries as raw material (Uniyal et al., 2006). There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Parekh et al., 2005), but vast majority have not been adequately evaluated (Balandrin et al., 1985).

The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants for their potential antimicrobial activity (Ritch-Krc et al. 1996; Martins et al., 2001). Antibacterial properties of various plants parts have been well documented for some of the medicinal plants for the past two decades (Leven et al., 1979).

Virulent strains of *E.coli* can cause gastroenteritis, urinary tract infections and neonatal meningitis. *S. aureus* can cause a range of illness, from minor skin infections such as pimples, impetigo, boils (furuncles), cellulites, folliculitis, carbuncles, scalded skin syndrome, and abscesses, life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocardictic, toxic shock syndrome, bacteraemia and sepsis.

Terminalia Chebula is one of the traditional Ayurvedic medicines that have found to possess various qualities on curing different kind of diseases[9]. It is called the "King of medicines", in Tibet and is always listed top in ayurvedic material due to its extraordinary power of healing (Kirtikar and Basu, 1935). It is routinely used as traditional medicine by tribal of Tamil Nadu in India to cure several ailments such as fever, cough, diarrhea, skin diseases and wound infections (Dash, 1991; Bag et al., 2000). Very little studies have been done on the antibacterial activity of plant extracts of *Terminalia Chebula* (Chang at al., 2003). Keeping in view the importance of different types of infections caused by bacteria the present study was designed to find out the antibacterial potentiality of fruits of *Terminalia chebula* against selected stains of bacteria.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fruits of the *Terminalia chebula* plant was collected from the Bhadson area of district Patiala of Punjab (India). The plant was identified, confirmed and authenticated (Jain 1999).

2.2 Procurement of microorganisms

S. aureus and *E.coli* species were collected from Department of Biotechnology and the pure cultures of bacteria were maintained on nutrient agar slants for their vegetative growth. The cultures were maintained in incubator for use and regularly checked for contamination, and the periodic transfers were made aseptically.

2.3 Extract Preparation

Seed samples of *Terminalia chebula* was thoroughly washed and dried in hot air even at 100°c for 1 hr. The dried samples were then crushed in pestle and mortar into fine powder.

2.4 Extract Preparation

The extracts of seeds of *Terminalia Chebula* were prepared in ethanol , chloroform and acetone by following the methodology of Alam et al., 2010. All the dried powdered was extracted using soxhlet apparatus and the extraction was done for about 14 hrs in chloroform and for about 12 hrs in acetone and ethanol. Solvent was removed under reduced pressure or distilled about more than an house but less than two hours to get crude extract. The crude extract was further dried in vacuum desicator over anhydrous calcium chloride to get a dry solid for extract for further study.

2.5 Phytochemical Analysis

The crude Extracts were analyzed for the presence of alkaloids, terpenoids, carbohydrates, saponins, tannins and flavonoids (Oloyede, 2009).

2.6 Culture of Test Microbes

For the cultivation of bacteria, Nutrient Agar Medium (Beef extract – 1.0 g, Yeast extract – 2.0 g, Peptone - 5.0 g, NaCl – 5.0g, Agar – 15.0 g, distilled water 1L) were prepared and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar rest plates were prepared by pouring approximately 15 ml of Nutrient Agar medium into the Petri dish under aseptic conditions.

2.7 Agar Well Diffusion Method

The ethanol, chloroform and acetonic extracts of seeds of *Terminalia chebula* were tested by Agar Well Diffusion method (Alam et al., 2010) (4 mm) holes were punched aseptically in nutrient agar plate by using a sterilized cork borer. The cotton swabs were dipped into the broth culture of the test organisms and were gently squeezed against the inside of the tube to remove excess fluid. *E.coli* and *S.aureus* were swabbed on Agar plates. Swabbing was done in outside diameter of the plates. The plates were allowed to dry for about 5 minutes. Then the extracts of *Terminalia chebula* for four different concentrations (25%, 50%, 75% and 100%) were added into wells of Petri plates. Pure solvents were used as control whereas amoxicillin was used as reference for bacterial species. The plates were incubated at 37°C for 24 hrs. The zones of inhibition were measured in millimeters (mm), using Vernier caliper. The Zone size was recorded and all the cultures were discarded by autoclaving.

Table 1: The observations of the Phytochemical tests.

Test	Chloroform	Acetone	Ethanol
Alkaloids wanger's reagent (Reddish brown color)	+	+	+
Terpenoids (Reddish brown color at interface)	+	+	+
Carbohydrates Molish reagent (Reddish violet color)	+	+	+
Saponins Foam test (Presence of foam at surface)	+	–	+
Tannin Ferric chloride test (dark green color)	+	+	+
Flavonoids (color changes from yellow to colorless)	+	+	+

– absent; + present

Table 2: The zones of inhibition with different extract of dried fruit powder.

Conc. of extract	Test organism	Zone of ethanolic extract	Chloroform extract	Acetonic extract
25%	S. aureus	8 mm	4 mm	4 mm
	E. Coli	10 mm	5 mm	10 mm
50%	S. aureus	12 mm	7 mm	10 mm
	E. Coli	14 mm	7 mm	14 mm
75%	S. aureus	17 mm	9 mm	14 mm
	E. Coli	16 mm	10 mm	16 mm
100%	S. aureus	19 mm	12 mm	17 mm
	E. Coli	18 mm	15 mm	19 mm
Control	S. aureus	nil	nil	nil
	E. Coli	nil	nil	nil
Standard	S. aureus	24 mm	24 mm	24 mm
Amoxicillin	E. coli	24 mm	22 mm	24 mm

3. RESULTS AND DISCUSSION

The extracts were tested for alkaloids, terpenoids, carbohydrates, saponins and tannins, and results are as reported in table 1 and the results of zones of inhibition are reported in table 2.

The zones of inhibition of solvent control were nil and of standard (amoxicillin) the zone of inhibition for *S. aureus* and *E. Coli* were 24 mm and 22 mm. The maximum activity was observed at 100% concentration of different extracts of dried fruit powder. Thus, the growth of both *S. aureus* and *E. Coli* were inhibited by all the three extracts of *Terminalia chebula* fruit.

CONCLUSION

The study has revealed the presence of many secondary metabolites in the extract of *Terminalia chebula*. It has further confirmed that the plant extracts could be used for the treatment of various infections. The extracts of *Terminalia chebula* have potent antibacterial activity when compared with conventionally used drugs and is almost equipotent to the standard (Amoxicillin) antibacterial drug. The results lend credence to the folkloric use of this plant in treating bacterial infection and show that *Terminalia chebula* can be exploited for new potent antibacterial agents.

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