Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

ANTIBACTERIAL ACTIVITIES OF AQUEOUS AND ETHANOLIC EXTRACTS OF *TERMINALIA CATAPPA* LEAVES AND BARK AGAINST SOME PATHOGENIC BACTERIA

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Received: 29 Sep 2012, Revised and Accepted: 13 Nov 2012

ABSTRACT

The present investigation has been carried out to evaluate the antibacterial activities of aqueous and ethanolic extracts of *Terminalia catappa* leaves and bark against four bacterial pathogens namely *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Enterobacter aerogenes* respectively. Antibacterial activity was assessed by agar disc diffusion method. The activities of the extracts were measured by zone of inhibition and compared with a standard antibiotic Ciprofloxacin. Among the various concentrations (100,200,300 µg/ml), 300 µg/ml was found to be very effective.

Keywords: Antibacterial activity, Phytochemical analysis, *Terminalia catappa*

INTRODUCTION

Infectious diseases, also known as contagious diseases or transmissible diseases, comprise clinically evident illness resulting from the infection, presence and growth of pathogenic biological agents in an individual host organism. Infectious diseases are the worlds leading cause of premature death, killing almost 50,000 people everyday. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. How ever, the situation is alarmingly increasing in developing as well as developed countries due to indiscriminate use of antibiotics. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. While a spontaneous or induced genetic mutation in bacteria may confer resistance to antimicrobial drugs, genes that confer resistance can be transferred between bacteria in a horizontal fashion by conjugation, transduction, or transformation.

The drug resistant bacteria and fungal pathogens further complicate the treatment of infectious diseases in immune compromised AIDS and cancer patients. Multidrug resistant organisms are associated with nosocomial infections [1].

Thus infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide [2]. Until recently, research and development (R&D) efforts have provided new drugs in time to treat bacteria that became resistant to older antibiotics.

As bacterial antibiotic resistance continues to exhaust the supply of effective antibiotics, a global public health disaster appears likely. Substitutes from the nature to the antibiotics are becoming the prime need of the society in the present and in future.

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Traditional use of medicines is recognized as a way to learn about potential future medicines.

Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. By chance, some of these compounds, whilst being toxic to plant predators, turn out to have beneficial effects when used to treat human diseases. Such secondary metabolites are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen – substituted derivatives. At least 12,000 have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effects on the human body by binding to receptor molecules present in the body; such processes are identical to those already well understood for conventional drugs and as such herbal medicines do not differ greatly from conventional drugs in terms of how they work. Many of the herbs and spices used by humans to season food yield useful medicinal compounds [3].

The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural products chemists are combing the earth for phytochemicals and leads that could be developed for treatment of various diseases. In recent years, there has been a growing interest to evaluate plants possessing antimicrobial activity for various diseases. A number of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants.

Tropical almond (*Terminalia catappa*) is a large, spreading tree distributed throughout the tropics in coastal environments. The dried leaves are used for fish pathogen treatment, as an alternative to antibiotics. The leaves have antioxidant as well as anticlastogenic properties [4]. The various extracts of leaves and bark of *T. catappa* have been reported to be anticancer, anti-HIV reverse transcriptase [5] and hepato-protective [6] as well as anti-inflammatory [7], hepatitis [8] anti diabetic [9] and aphrodisiac [10].

The moderate consumption of the seed kernel is useful in the treatment of men with sexual dysfunctions, primarily from premature ejaculation. The ethanol extract of the leaves of *Terminalia catappa L.* (Combretaceae) inhibits osmotically-induced hemolysis of human erythrocytes in a dose-dependent manner. Punicalagin and punicalin, from the leaves are used to treat dermatitis and hepatitis as both have strong anti oxidative activity. In view of this, the present study was set up with the objective to assess the antibacterial activity of leaves and bark extracts of *Terminalia catappa* against some selected bacterial pathogens.

MATERIALS

Collection of plant material

Fresh Leaves and bark of *Terminalia catappa*, were collected from Pattukottai, Thanjavur Dt, Tamilnadu, India, which were carefully identified with the help of regional floras. The leaves and bark were washed thoroughly 2-3 times with running water and once with sterile distilled water, leaf and bark material were then air-dried on sterile blotter under shade. The plant was identified by Dr.S.John Britto, the Director, the Rapinet herbarium and centre for Molecular Systematics, St. Joseph's College, Thiruchirappalli and a voucher Specimen was deposited in the Rapinet herbarium of St. Joseph's College, Thiruchirappalli (Voucher No. P.N.001/2012).

Chemicals

- ✓ Beef extract
- ✓ Peptone
- ✓ Sodium Chloride
- ✓ Agar

All the chemicals were purchased from Rankem, Chennai.

- ✓ Ciprofloxacin – 500 mg(cifran)

The drug obtained from the Ranbaxy Laboratories Ltd., 1208-1215 12th Floor, No. 6, Davika Tower, Nehru Place, New Delhi 110019.

Microorganisms

Microorganisms such as *Escherichia coli* (*E. coli* – MTCC 40), *Bacillus subtilis* (*B. subtilis* – MTCC441) *Staphylococcus aureus* (*S. aureus* – MTCC96), *Enterobacter aerogenes* (*E. aerogenes* – MTCC 111) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Sector 39-A, Chandigarh, India, were used as antibacterial test organisms. The bacteria were maintained on nutrient broth (NB).

METHODS

Extraction of plant Material:

Aqueous and ethanolic extracts of selected plant materials were prepared according to the methodology of Indian pharmacopoeia. The shade dried plant materials were subjected to pulverization to get coarse powder. The powdered materials were subjected to Soxhlet extraction separately and successively with ethanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40°-50°C). The aqueous and ethanolic extracts of leaves and bark were put in air tight containers separately and stored in a refrigerator till the time of use.

Phytochemical evaluation

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Anti bacterial activity of plant extracts:

The ethanolic and aqueous extracts of *T.catappa* leaves and bark were evaluated for anti bacterial activity by disc diffusion method. Different concentration s of the extracts (100, 200, 300 µg/ml) were prepared by reconstituting with ethanol and water respectively. The test microorganisms such as *E. coli, B. subtilis, S. aureus, E. aerogenes* were seeded into respective medium by spread plate method with 10µl (10^6 cells/ml) of 24h cultures of bacteria grown in nutrient broth. After solidification, the filter paper discs (5mm in diameter) impregnated with the extracts we87re placed on test organism-seeded plates. Ciprofloxacin (10µg/ml) was used as standard Antibiotic. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSION

In the recent years, research on medicinal plants has attracted a lot of attention globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids etc, which have been found *in vitro* to have antimicrobial properties [11,12].

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by the physicians; several are already being tested in humans. Scientists realize that the effective life span of any antibiotic is limited, so new sources especially plant sources are also being investigated. Second the public is becoming increasingly aware of the problems with the over prescription and misuse of traditional antibiotics. In addition many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over the counter from herbal suppliers and national food stores and the self medication with these substances is a common practice to certain extent.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [13]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [14,15,16]·[17,18,19]·[20]. Some of these observations have helped in identifying the active principle responsible for such activities and in developing drugs for the therapeutic use in human beings. However, not many reports are available on the exploitation of antibacterial property of plants for developing commercial formulations.

The efforts of scientists in establishing plants with promising antimicrobial property is yielding fruitful results as a number of plants with high antimicrobial property have been elucidated [21-26].

Considering the vast potentiality of plants as sources for antimicrobial drugs the current investigation was undertaken to screen the anti bacterial activity of *Terminalia catappa* against certain pathogenic bacteria.

Preliminary phytochemical analysis of the ethanolic and aqueous extracts of *Terminalia catappa* leaves revealed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Phenolic compounds, Triterpenoids, Phytosterols, fixed oil and fat, protein, carbohydrates and glycosides, Resins etc. Estimation of protein content revealed the presence of high protein content in leaves.(Tables 1,2,3)

Table 1: Qualitative phytochemical analysis of Ethanolic extracts of *Terminalia catappa* leaves and Bark

S. No.	Phytochemical constituents	Ethanolic Extract	
		Leaves	Bark
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Saponins	+	+
5	Phenolic compound	+	+
6	Triterpenoids	+	+
7	Phytosterol	+	+
8	Fixed oil and fat	+	+
9	Protein	+	+
10	Carbohydrate and Glycosides	+	+
11	Resins	+	+

+=Presence; -=Absence

Table 2: Estimation of protein content in the ethanolic and aqueous extracts Of Terminalia *catappa* leaves and bark

S. No.	Extract	Protein concentration mg/dL	
		Leaves	Bark
1	Ethanolic extract	60	54
2	Aqueous extract	80	75

Table 3: Qualitative phytochemical analysis of Aqueous Extracts of *Terminalia catappa* leaves and Bark

S. No.	Phytochemical constituents	Aqueous Extract	
		Leaves	Bark
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Saponins	+	+
5	Phenolic compound	+	+
6	Triterpenoids	+	+
7	Phytosterol	+	+
8	Fixed oil and fat	+	+
9	Protein	+	+
10	Carbohydrate and Glycosides	+	+
11	Resins	+	+

+=Presence; -=Absence

The anti bacterial activities of the ethanolic and aqueous extracts of leaf and bark of *Terminalia catappa* were evaluated by the disc diffusion method against four pathogenic organisms namely *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Enterobacter aerogenes*. Ethanolic and aqueous extracts of various concentrations (100, 200, 300 µg/ml) were found to be active against both gram positive and gram negative bacterial strains. Activity was more against the gram positive than gram negative bacterial strains. Bark extract was found to be equipotent as leaf extract in inhibiting the bacterial growth. The best antibacterial activity was shown by ethanolic extract than the aqueous extract. (Tables 4,5 and Figures 1a,1b,1c,2a and 2b)

Table 4: Antibacterial activity of ethanolic extracts of *Terminalia catappa* leaves and barks

S. No.	No of Microorganisms	Standard Antibiotic Ciprofloxacin (10µg/ml)	Zone of Inhibition					
			Diameter in mm Ethanolic Extract					
			Leaves µg/ml			Bark µg/ml		
			100	200	300	100	200	300
1	*E.coli*	12	7	9	13	7	10	12
2	*B.subtilis*	10	5	9	14	8	9	14
3	*S. aureus*	14	9	12	17	12	15	17
4	*E. aerogenes*	11	4	7	10	10	12	13

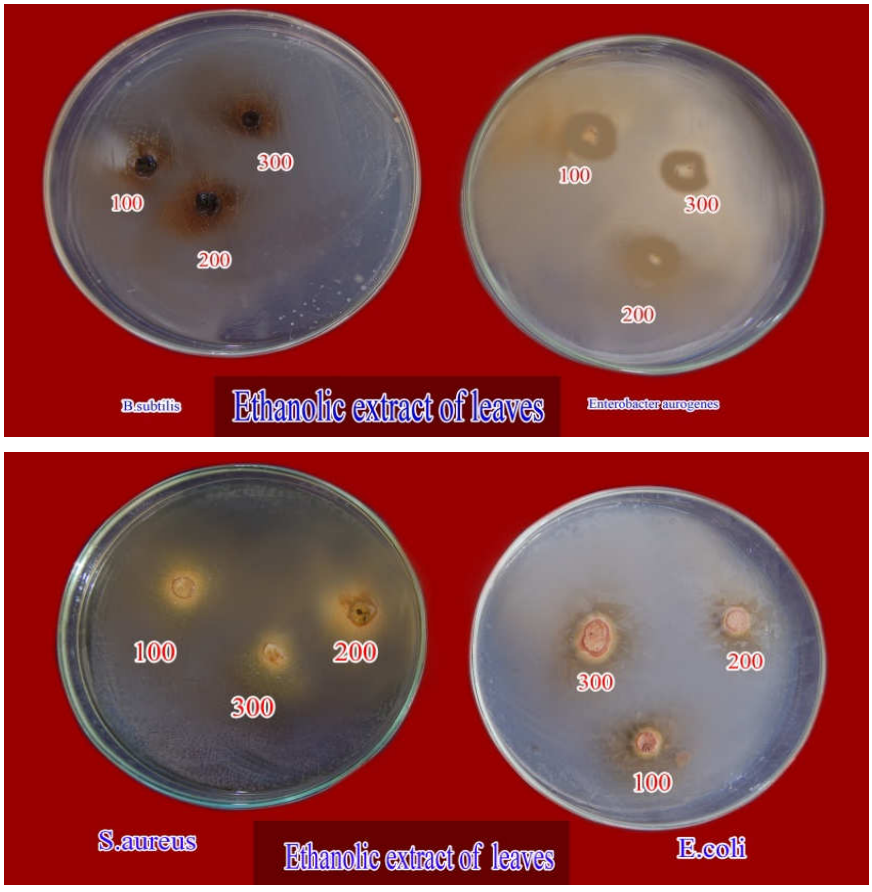

Fig. 1a: Antibacterial activity of ethanolic extracts of *Terminalia catappa* leaves

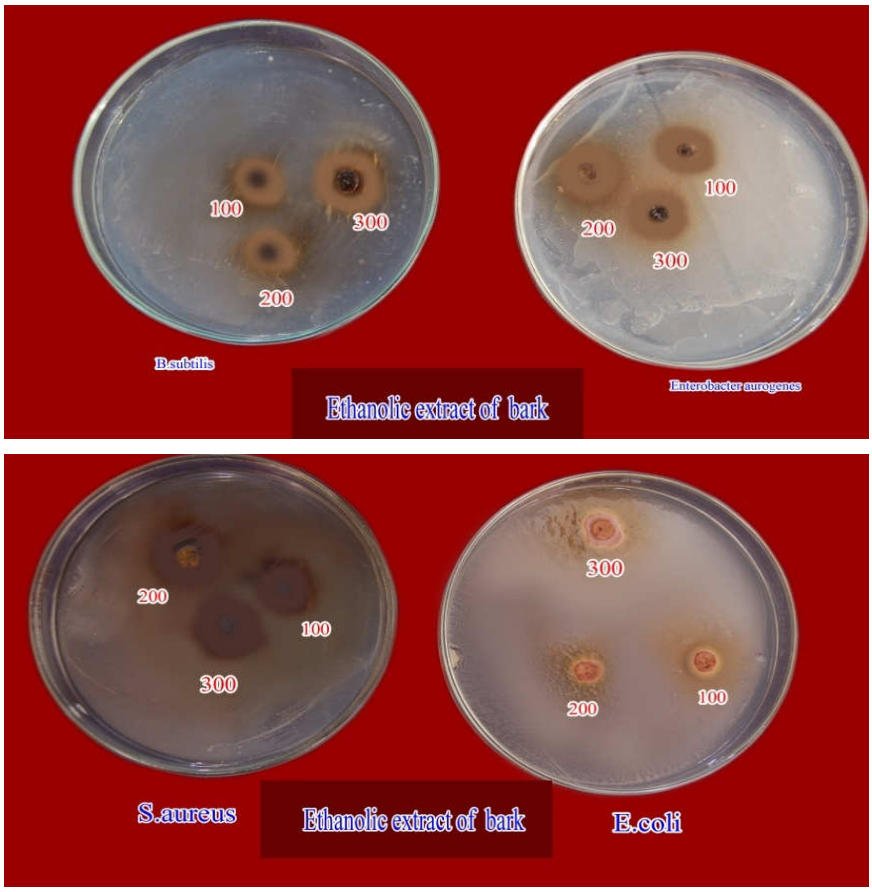

Fig. 1b: Antibacterial activity of ethanolic extracts of *Terminalia catappa* bark

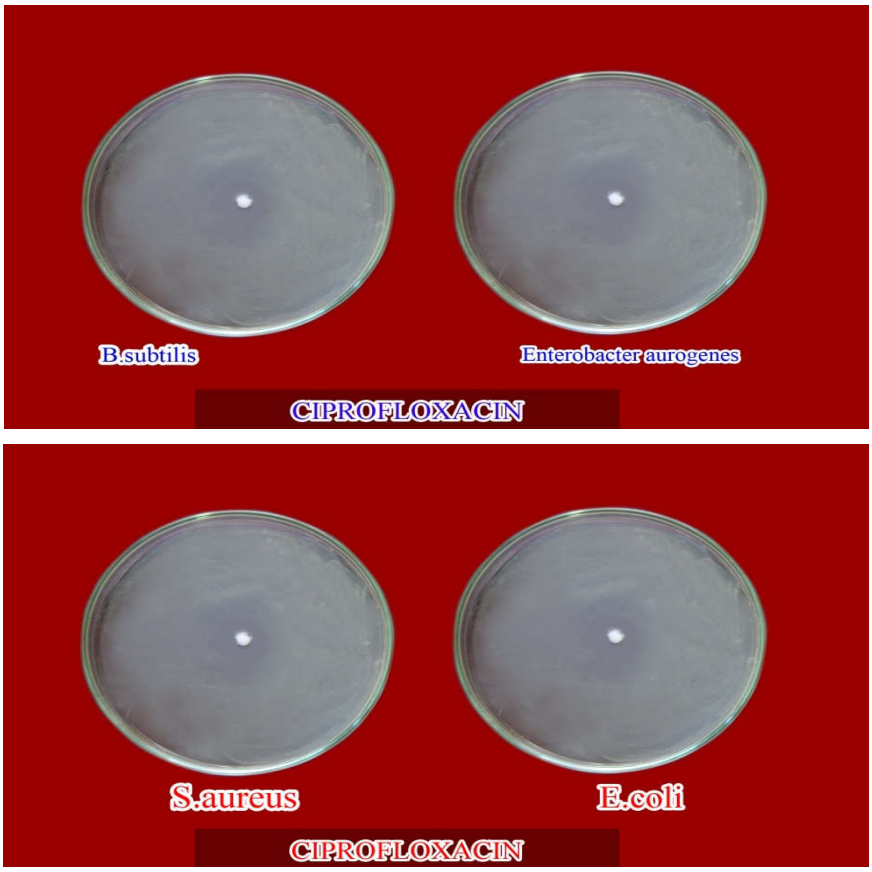

Fig. 1c: Standard Antibiotic –Ciprofloxacin

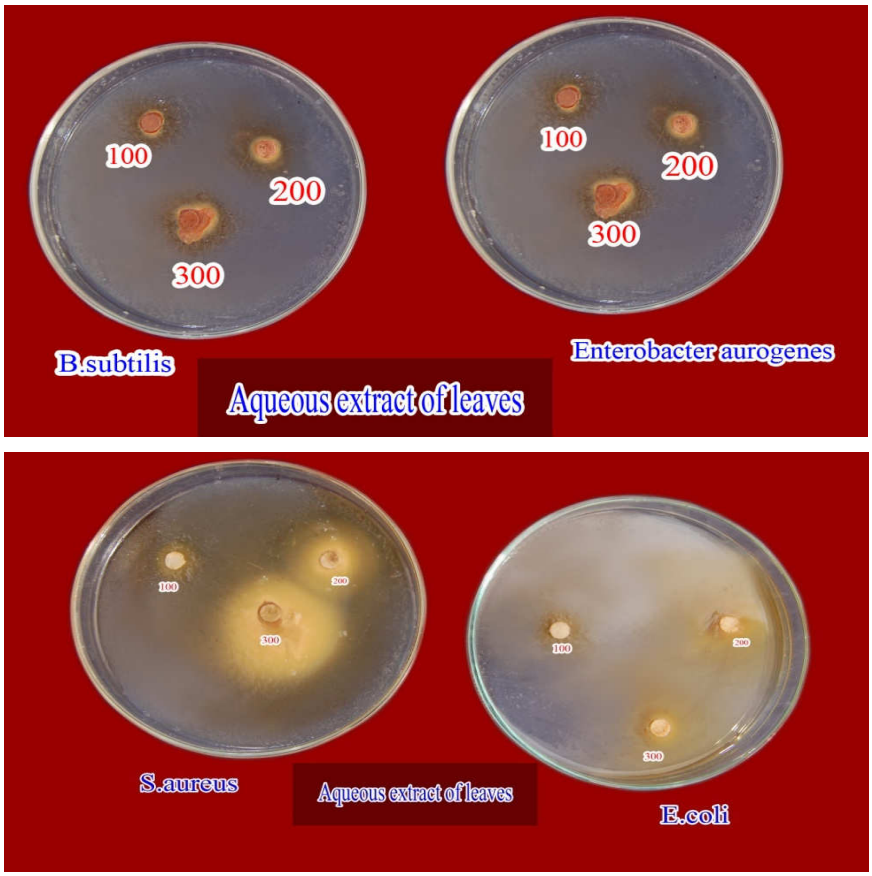

Fig. 2a: Antibacterial activity of Aqueous extracts of *Terminalia catappa* leaves

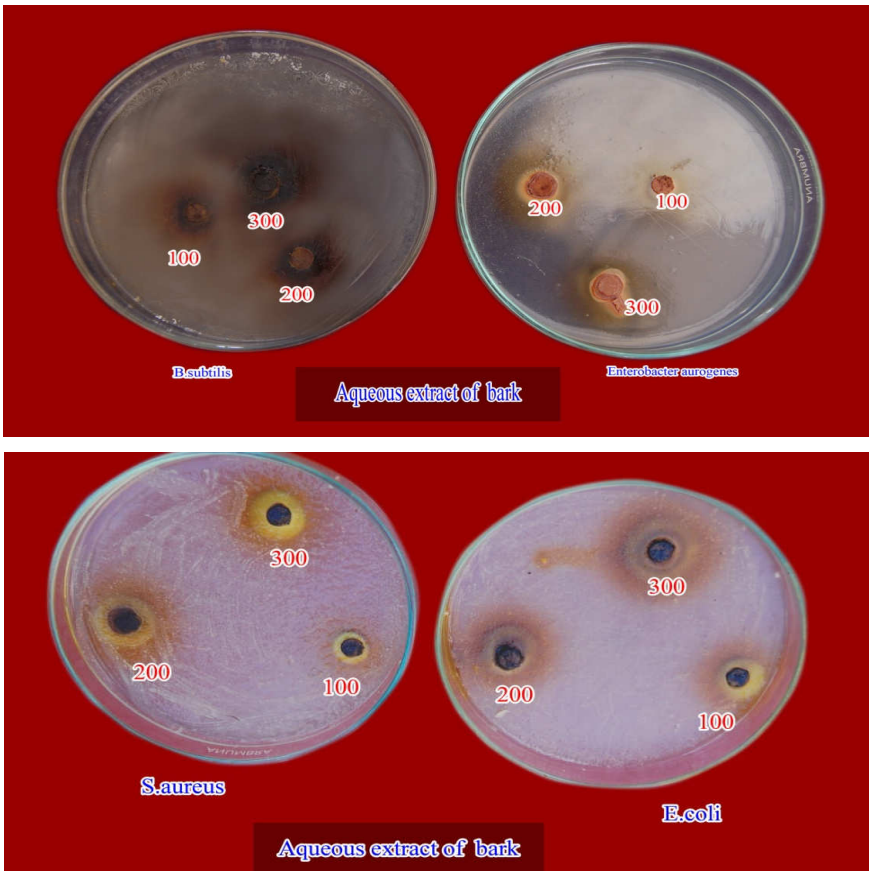

Fig. 2b: Antibacterial activity of Aqueous extracts of *Terminalia catappa* bark

Table 5: Antibacterial activity of Aqueous extracts of *Terminalia catappa* leaves and Bark

S. No.	No. of Microorganisms	Zone of Inhibition Diameter in mm Aqueous Extract					
		Leaves concentration in µg/ml			Bark concentration in µg/ml		
		100	200	300	100	200	300
1	*E.coli*	6	8	8	4	6	9
2	*B. subtilis*	3	5	9	6	7	10
3	*S. aureus*	4	10	12	8	10	12
4	*E. aerogenes*	3	6	8	5	6	8

Similar results were also shown by previous studies [27,28].The Gram positive bacteria were more susceptible than Gram negative bacteria. This is in agreement with previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria [29,30,31]. These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure [32].

The most striking feature of the present findings is that the plant extracts showed moderate to good antibacterial activity when compared to that of the standard antibiotic Ciprofloxacin (10 µg/ml). The need of the hour is to find new antimicrobial because the microorganisms are getting resistant to the existing antibiotics [33,34]. The persistent increase in multi drug resistant strains compels the search for more potent new antibiotics. Thus there is a need for a continuous search for new effective and affordable antimicrobial drugs. The results of present study signify the potentiality of *Terminalia catappa* leaf as a source of therapeutic agent which may provide leads in the ongoing search for antimicrobial botanicals.

The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. So it can be recommended as a plant of phyto pharmaceutical importance.

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