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### PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *TERMINALIA BELLIRICA* LEAF

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#### ABSTRACT

Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin. Plants are directly used as medicines by a majority of cultures around the world. The preliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Alkaloids, Carbohydrates and Flavonoids was done with Hydroalcoholic extract of *Terminalia bellirica* leaf. Antibacterial activities of *Terminalia bellirica* leaf extract against three pathogenic bacteria were investigated by the agar well diffusion method. Antibacterial activity data revealed that *Terminalia bellirica* leaf showed promising antibacterial activity especially towards Gram negative bacteria. The inhibitory zone of *E.coli* is  $16.3 \pm 1.02$ ,  $19.0 \pm 0.12$ ,  $22.6 \pm 1.08$  in *Pseudomonas aureus* inhibition of zone is  $12.2 \pm 0.14$ ,  $16.0 \pm 1.04$ ,  $23.4 \pm 0.06$  and for *Streptococcus pyrogens* the inhibition is  $18.3 \pm 1.04$ ,  $21.3 \pm 0.08$ ,  $24.0 \pm 1.02$  respectively. Specifically the zone of inhibition of *Streptococcus pyrogens* with 400  $\mu\text{g/ml}$  of *Terminalia bellirica* leaf extract is equal to that of standard.

#### KEY WORDS

*Terminalia bellirica* , *Pseudomonas aureus*, *Streptococcus pyrogens* and Hydroalcohol.

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#### INTRODUCTION

Plants have been used in treating human diseases for thousands of years. Some 60,000 years ago, it appears that Neanderthal man valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found. One of these allegedly ancient medicinal herbs, yarrow, is discussed in this work as a modern medicinal plant. Since prehistoric times, shamans or medicine men and women of Eurasia and the America acquired a tremendous knowledge of

medicinal plants. All of the native plant species discussed in detail in this work was used by native people in traditional medicine. The fact that hundreds of additional species were also used by First Nations Canadians suggests that many of these also have important pharmacological constituents that could be valuable in modern medicine <sup>1</sup>.

#### **Medicinal chemicals**

The medicinal qualities of plants are of course due to chemicals. Plants synthesize many compounds called primary metabolites that are critical to their existence. These include proteins, fats, and carbohydrates that serve a variety of purposes indispensable for sustenance and reproduction, not only for the plants themselves, but also for animals that feed on them. Plants also synthesize a dazzling array of additional components, called secondary metabolites, whose function has been debated. Many secondary metabolites are antibiotic in a broad sense, protecting the plants against fungi, bacteria, animals, and even other plants.

#### **Extinction of medicinal plant species**

Moreover 50 % of prescription drugs are derived from chemicals first identified in plants. As 2008 report from the Botanic Gardens Conservation International warned that cures for things such as cancer and HIV may become extinct before they are ever found. They identified 400 medicinal plants at risk of extinction from over collection and deforestation, threatening the discovery of future cures for disease. These included Yew trees (the bark is used for the cancer drug) Hoodia (from Namibia, a potential source of weight loss drugs) half of Magnolias (used as Chinese medicine for 5,000 years to fight cancer, dementia and heart disease) and Autumn crocus (for gout). Their report said that five billion people still rely on traditional plant-based medicine as their primary form of health care <sup>2</sup>.

#### **Natural antibiotic properties of plant secondary metabolites**

The plant chemicals are classified as primary or secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are often concentrated in seeds and

vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants for commercial use are high volume-low value bulk chemicals (e.g. vegetable oils, fatty oils, fatty acids, carbohydrates etc.). Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbiocides, pesticides and many pharmaceutical drugs. From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases <sup>3</sup>.

#### **Bacteria**

Bacteria are classified under a distinct kingdom because of its peculiar cellular and morphological characteristics that makes it different and distinct from all other kingdoms like fungi, animal and virus. Bacteria are microscopic, unicellular (single celled) ancient organisms that are responsible for a number of lethal diseases was shown in Table No.1.

#### **Botanic Description**

*Terminalia bellirica* is a large deciduous tree to 50 m tall and a diameter of 3 m with a rounded crown. The frequently buttressed bole at the base is branchless up to 20 m. The bark is bluish or ashy-grey covered with numerous fine longitudinal cracks, the inner bark yellowish. Leaves are large, glabrous, alternate, broadly elliptic to obovate-elliptical, 4-24 cm x 2-11 cm, base rounded to cuneate, rufous sericeous but soon glabrescent with 6-9 pairs of secondary veins. Secondary and tertiary venation prominent on both surfaces, clustered towards the ends of branchlets. Petiole 2.5-9 cm long <sup>4</sup>. Young leaves copper-red, soon becoming parrot green, then dark green. Flowers solitary, small, 3-15 cm long, greenish white, simple, axillary spikes; calyx tube densely sericeous or tomentulose, flowers appear along with new leaves and have a strong honey-like smell. Fruit sub-globular to broadly ellipsoid, 2-4 x 1.8-2.2 cm, densely velutinous or sericeous, light-yellow, obscurely 5-angled and minutely brown tomentosa. The generic name *Terminalia* comes from Latin word terminus or *terminalis*, and refers to the habit

of the leaves being crowded or borne on the tips of the shoots.

## MATERIALS AND METHODS

### Collection and authentication of *Terminalia bellirica* leaves

Leaves of *Terminalia bellirica* leaves were collected in Naidupet (Village), Nellore (District) and authenticated by a botanist Dr. N. Yasodamma, Sri Venkateswara University, Tirupati (Mandal), Chittoor (District). The leaves were dried in shade and made into coarse powder.

### Preparation of Hydroalcoholic extract of *Terminalia bellirica* leaves

The leaves were separated from plant and it was washed with absolute ethanol to avoid the microbial growth, the leaves were dried at open air under the shade, cut into small pieces and powdered mechanically, then 50 gm of powder *Terminalia bellirica* leaves was extracted with 250ml hydroalcohol (Water + Ethanol) 1:3 ratio in a soxhlet apparatus for 72 hrs. The extract obtained was concentrated by recovery of water and ethanol. The concentrated product was used as hydroalcoholic extract of leaves of *Terminalia bellirica*.

### Test for saponins

#### Foam test

Take 2ml of drug solution in a test tube, add small amount of water to it, shake well, then stable froth (foam) is formed<sup>5</sup>.

### Test for tannins

#### Ferric chloride test

A small amount of test solution is treated with ferric chloride solution, if blue colour appears then, hydrolysable tannins are present and if green colour appears then, condensed tannins are present.

### Test for amino acids

#### Millon's test

To the test solution, add 2ml of millon's reagent, white precipitate indicates presence of amino acid.

### Test for proteins

#### Warming test

The test solutions are taken in a test tube and heat it in boiling water bath then proteins get coagulated.

## Glycosides

### Legal test

The extract is dissolved in pyridine, then sodium nitroprusside solution is added to it and alkaline-pink or red colour is produced.

### Baljet test

To the section of sample, sodium picrate solution is added. It shows yellow to orange colour.

### Test for cardiac glycosides

#### Keddes test

Extract the drug with chloroform and evaporate to dryness. Add one drop of 90 % alcohol and 2 drops of 2 % 3, 5 Di nitro benzoic acid in 90 % alcohol. Make alkaline with 20 % sodium hydroxide solution, and then the purple colour is produced. The colour reaction with 3, 5 Di nitro benzoic acid depends on the presence of  $\alpha$ ,  $\beta$  unsaturated lactones in the aglycone.

#### Keller-kiliani test

Extract the drug with chloroform and evaporate it to dryness, then add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube, add carefully 0.5 ml of concentrated sulphuric acid through walls of the test tube. Acetic acid layer shows blue colour<sup>6</sup>.

### Test for alkaloids

#### Dragendorff's test

To 2-3 ml filtrate, add few drops of dragendorff's reagent. Orange brown precipitate is formed.

#### Mayer's test

2-3 ml filtrate with few drops of Mayer's reagent gives precipitate.

#### Hager's test

To 2-3ml filtrate, add few drops of Hager's reagent give yellow precipitate.

#### Wagner's test

2-3 ml filtrate with few drops of Wagner's reagent gives reddish brown precipitate

### Test for carbohydrates

#### Molisch's test

The test is positive with soluble, as well as, insoluble carbohydrates. It consists of treating the compounds with  $\alpha$ - naphthol and concentrated sulphuric acid which gives purple colour ring at the junctions of two layer.

### Test for flavonoids

#### Shinoda test

To dry powder or extract, add 5 ml 95 % ethanol, few drops concentrated Hcl and 0.5 gm magnesium turnings then pink colour is observed<sup>7</sup>.

## RESULTS AND DISCUSSION

### Phytochemical investigation

The preliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the hydroalcoholic extract of *Terminalia bellirica* leaves according to the procedure. In the above chemical test the hydroalcoholic extract of *Terminalia bellirica* leaves showed positive results for Saponins, Tannins, Amino acids, Proteins, Alkaloids, Carbohydrates and Flavonoids. The results of preliminary test of the hydroalcoholic extract of leaves extract was shown in Table No.2.

### Microbial activity

*In vitro* antibacterial activity was examined for the extracts. Antibacterial activities of *Terminalia bellirica* leaf extract against three pathogenic bacteria (two negative and one positive) were investigated by the agar well diffusion method. The results were checked after the proper incubation. Antibacterial potential of extracts were assessed in terms of zone of inhibition of bacterial growth for 100, 200 and 400 µg/ml. The inhibitory zone of *E.coli* is 16.3±1.02, 19.0±0.12, 22.6±1.08 in *Pseudomonas aureus* inhibition of zone is 12.2±0.14, 16.0±1.04, 23.4±0.06 and for *Streptococcus pyrogens* the inhibition is 18.3±1.04, 21.3±0.08,

24.0±1.02 respectively. Specifically the zone of inhibition of *Streptococcus pyrogens* with 400 µg/ml of *Terminalia bellirica* leaf extract is equal to that of standard was shown in Table No.3 and Figure No.1.

## DISCUSSION

The microorganism used for this study was found to be susceptible to hydroalcoholic extract of *Terminalia bellirica*. It is suggesting that the antibacterial principle containing in the plant may be of broad spectrum. It was able to inhibit both gram positive and gram negative bacteria, which did not produce any antagonistic effect. The phytochemical screening of hydroalcoholic extract of *Terminalia bellirica* leaves showed positive results for Saponins, Tannins, Amino acids, Proteins, Alkaloids, Carbohydrates and Flavonoids. The antibacterial activity of plants may due to the presence of secondary metabolites such as tannins, alkaloids and flavonoids. Flavonoids are a major group of phenolic compounds reported for their antiviral<sup>8-12</sup>, antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties. Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties<sup>12-14</sup>. As a result of this the zone of inhibition of all microorganisms the hydroalcoholic extract of *Terminalia bellirica* shown more inhibitory zone against bacteria. The results show that increase in concentration of extract increased the zone of growth inhibition of some of the microorganisms.

Table No.1: Types of bacteria

S. No	Harmful bacteria	Beneficial bacteria
1	<i>Streptococcus Pyogenes</i>	<i>Lactobacillus acidophilus</i>
2	<i>Escherichia Coli</i>	<i>Bacillus subtilis</i>
3	<i>Vibrio Cholerae</i>	<i>Bifidobacterium animalis</i>
4	<i>Enteritis Salmonella</i>	<i>Streptococcus thermophilus</i>
5	<i>Salmonella Typhi</i>	<i>Lactobacillus reuteri</i>

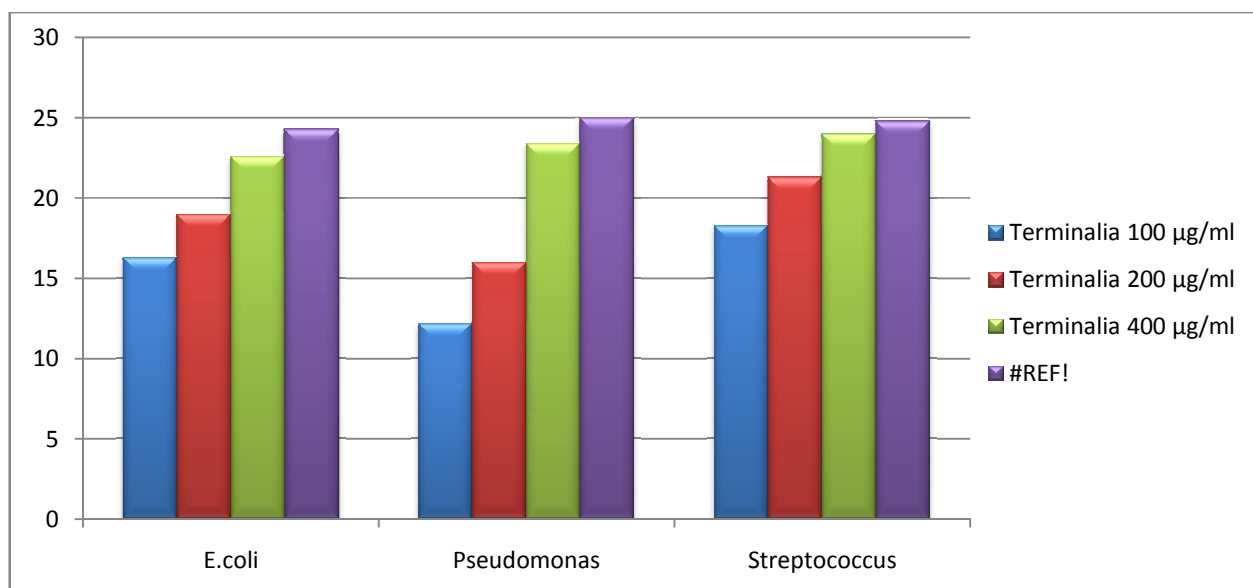
**Table No.2: Phytochemical screening results of *Terminalia bellirica* leaves**

S. NO	PHYTOCONSTITUENT	RESULT
1.	Saponins	+
2.	Tannins	+
3.	Amino acids	+
4.	Proteins	+
5.	Glycosides	—
6.	Cardiac glycosides	—
7.	Alkaloids	+
8.	Carbohydrates	+
9.	Flavonoids	+

PRESENT = (+), ABSENT = (-)

**Table No.3: Antimicrobial Screening of Hydroalcoholic Extract of *Terminalia bellirica* Leaves**

Micro Organisms	<i>Terminalia bellirica</i> - Zone of Inhibition		
	Concentration in µg/ml - Hydroalcoholic Extract		
	100 µg/ml	200 µg/ml	400 µg/ml
<i>Escherichia coli</i>	16.3±1.02	19.0±0.12	22.6±1.08
<i>Pseudomonas aureus</i>	12.2±0.14	16.0±1.04	23.4±0.06
<i>Streptococcus pyrogens</i>	18.3±1.04	21.3±0.08	24.0±1.02
Standard (10 µg/ml)	24.3±0.08	25.0±0.18	24.8±1.04
Control	----	----	----



**Figure No.1: Antimicrobial Screening of Hydroalcoholic Extract of *Terminalia bellirica* Leaves**

## SUMMARY AND CONCLUSION

Antibacterial activity was carried out by cup-plate agar diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* was compared with ciprofloxacin as standard. Antibacterial activity data revealed that *Terminalia bellirica* leaf showed promising antibacterial activity especially towards Gram negative bacteria. This is very promising because it is reported that plant extracts are more active against Gram positive bacteria and the search is always going on for plants extracts which are able to inhibit the dangerous Gram negative bacteria. Studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

Further large scale, well designed clinical trials are required to prove more conclusive proof of their efficacy, the problem of microbial resistance is growing and the outlook for use antimicrobial drugs in future is still uncertain. Action must be taken to reduce this problem to control the use of antibiotic and to develop research to better understand the genetic mechanisms of resistance and to continue

studies to develop new drugs from natural plant sources such as *Terminalia bellirica*.

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