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Antibacterial Activity of a Leaf Extract from a Weed Plant *Tridax Procumbens*

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Abstract: Most of the plants are tributed as medicinal plants which are more useful to mankind. It is now believed that nature has given the cure for every disease in one way or another. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditionally, *Tridax procumbens* has been in use in India for wound healing and as an anticoagulant, antifungal, and insect repellent. The juice extracted from the leaves is directly applied on wounds. Its leaf extracts were used for infectious skin diseases in folk medicines. It is used in ayurvedic medicine for liver disorder, hepato protection, gastritis, and heart burn. *Tridax procumbens* is also used as treatment for boils, blisters, and cuts by local healers in parts of India. The leaves are antiseptic haemostatic and parasiticide. They are used as a treatment against bronchial catarrh, dysentery, diarrhea. The leaf powder, combined with *Cicer arietium* in a 2:1 ratio, is taken orally to treat diabetes. A fine paste of the leaf is applied externally to reduce swelling of hemorrhoids to stop bleeding. The leaf sap is applied topically to sores and ulcers. The leaf juice possess insecticidal and parasiticidal. The leaves are burnt, and the smoke act as a mosquito repellent activity. The leaves are used as a hair restorative and also a main ingredient in Bhringraj hair oil.

Keywords: *Tridax procumbens*, anti bacterial, anti fungal, wound healing property.

I. INTRODUCTION

Medicinal herbs have been discovered and used in traditional medicine practices since prehistoric times. Plant synthesis hundred of chemical compounds for functions including defense against insect, fungi, diseases, and herbivorous mammals. Numerous Phytochemical with anti microbial potential are established and many biological activities are identified. Medicinal plants are widely used in non industrialized society, mainly because they are readily available and cheaper than the modern medicine. The annual global export value of 50,000 to 70,000 types plants with suspected medicinal properties was estimated to be US \$2.2 billion in 2012 and in 2018, the potential global market of botanical extracts and medicines was estimated at several hundred billion dollars. The WHO coordinates a network to encourage safe and rational usage of medicinal plants. Most of the infectious wounds are caused by bacterial colonization, which is originating either from the normal flora on the skin, or bacteria from other parts of the body or from any other environment. The most common causative organism associated with wound infection include *Staphylococcus aureus*, *Streptococcus pyogenes*, Enterococci and *Pseudomonas aeruginosa*. In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects[1]. The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential source of new antimicrobial agents [2]. A number of chemical constituents were reported from the plant *Tridax procumbens* viz: alkaloids, flavonoids, carotenoids, β -sitosterol, n-hexane, fumaric acid, luteolin, quercetin, oxoester, lauric acid, myristic, palmitic, arachidic, linoleic acid and tannin etc[3].

II. MATERIALS AND METHOD

- 1) **Collection of Plants:** The weed plant *Tridax procumbens* linn (Asteraceae) was collected from the agricultural land, from a rural area of Avinashi, Tiruppur District, Tamil Nadu, in the month of January 2019. Fresh plants were collected in a sterile polyethylene bag, and the leaves were trimmed out for the ethanol extraction. Then the leaves were surface sterilized with sodium hypochlorite and rinsed thrice with distilled water. Collection of bacterial strains: Organism which were used in this research were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas sp*, *Proteus sp*, *Klebsiella sp*.
- 2) **Preparation of plant Extract:** Washed fresh leaves were strained away and dried under shade for one week. Then the dried leaves were finely powdered and sieved. Further extraction was made by Soxhlet apparatus 30 gm of powdered sample in a conical flask of 300 ml of 95% ethanol. Then the extract was collected and poured into a sterile petri plate and the solvent was allowed to evaporate. The sediments were scrapped off, weighed, and dissolved in DMSO.

- 3) *Preparation of Inoculums*: An inoculum of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas sp*, *Proteus sp*, *Klebsiella sp* was prepared by adding 1ml of collected strains in 6ml of Nutrient broth. Then the inoculums was incubated at 37°C for 3 hours.
- 4) *Anti-Bacterial Activity*: The antimicrobial activity of Tridax ethanolic leaf extract were tested in five different types of pathogenic bacteria which were cultured on agar plates supplemented with different concentrations of plant extract by Agar well diffusion method [4], and Disc diffusion method [5].
- 5) *Agar well Diffusion Method*: The plates were prepared using steaking method. About 15 ml of media was poured and the plates were allowed to solidify in refrigerator for about 2 hours. After that wells were bored on the solidified agar plates with the help of sterile cork borer. 50µl of drug extract and known antibiotics were poured into the 3 wells of diameter 7 mm. Then all the plates were allowed to stand at room temperature for 1 hour so that the drug diffuses in the agar. Then all the plates were incubated at 37°C for 24 hours. After completion of incubation period, the plates were observed for antimicrobial activity and the diameter of zone of inhibition of growth of microorganisms was measured.[5]
- 6) *Disk Diffusion Method*: The plates were prepared as per prior method using pour plate technique. The drug concentration for extract was 5mg and 10mg and standard antibiotic was used. 7 mm filter paper discs (whatman no. 3) were impregnated with 20 ml of each drug. The discs were allowed to remain at room temperature until complete diluents evaporation and kept under refrigeration until ready to be used. Disc loaded with drug extract and known antibiotic were placed on to the surface of agar. After completion of incubation period of 37°C for 24 hours , the plates were observed for antimicrobial activity and the diameter of zone of inhibition of growth of microorganisms was measured.
- 7) *Minimum Bactericidal Concentration [MBC]*: The MBC was defined as lowest concentration to kill any microbes. Dilution of the plant extract were prepared in a sterile nutrient broth to get a final concentration of 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and 10mg/ml. To each of the dilution a loop full of culture was inoculated and the inoculated tubes were incubated at 37°C for 24 hours. After incubation, a loop full from each tubes was inoculated on to nutrient agar plates. The plate without growth was recorded as MBC.

III. RESULT

- 1) *Anti-Bacterial activity of Tridax Procumbens Extract*: The antibacterial activity of Tridax procumbens extract was tested by Agar well diffusion method (table 3.1) Disc diffusion method (table 3.2) and MBC (table 3.3) was noted against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas sp*, *Proteus sp*, *Klebsiella sp*. The ethanol extract shows high significant activity against *Staphylococcus aureus*, *Pseudomonas sp*, *Klebsiella sp*.

Table No 3.1 Antibacterial activity of Ethanol extact by Agar Well diffusion Method

| Organism | Zone of inhibition (mm) | | Standard |
|------------------------------|-------------------------|------|--------------------------------|
| | 5mg | 10mg | |
| <i>Escherichia coli</i> | 5 | 10 | No zone in Kanamycin |
| <i>Staphylococcus aureus</i> | 12 | 14 | 5mm of zone in Kanamycin |
| <i>Pseudomonas sp</i> | 10 | 18 | No zone formation in Kanamycin |
| <i>Proteus sp</i> | 9.5 | 11 | 10 mm of zone in steptomycin |
| <i>Klebsiella sp</i> | 12.5 | 17.5 | 17 mm of zone in steptomycin |

Table No 3.2 Antibacterial activity of Ethanol extact by Disc diffusion Method

| Organism | Zone of inhibition (mm) | | Standard |
|------------------------------|-------------------------|--|----------------------------|
| | 5mg | | |
| <i>Escherichia coli</i> | 10 | | No zone in Amoxyclave |
| <i>Staphylococcus aureus</i> | 13 | | No zone in Amoxyclave |
| <i>Pseudomonas sp</i> | 14 | | 8.5 mm zone in Amoxyclave |
| <i>Proteus sp</i> | 11.5 | | 10 mm zone in streptomycin |
| <i>Klebsiella sp</i> | 13 | | 12 mm zone in streptomycin |

Table No 3.3 MBC of *Tridax procumbens* ethanol extract.

| Sl.No | Organism | Minimum bactericidal concentration (mg/ml) |
|-------|------------------------------|--|
| | | <i>Tridax procumbens</i> |
| 1 | <i>Escherichia coli</i> | 11 |
| 2 | <i>Staphylococcus aureus</i> | 5 |
| 3 | <i>Pseudomonas</i> sp | 6 |
| 4 | <i>Proteus</i> sp | 9 |
| 5 | <i>Klebsiella</i> sp | 10 |

- 2) *Minimum Bactericidal Concentration of Tridax Procumbens Extract*: Minimum bactericidal concentration is defined as the higher dilution or least concentration of the extract that kills the microorganism. After 24 hrs of incubation at 37°C the test tubes were observed for turbidity. Loopfull was plated on Nutrient agar plates. From the tubes, no turbidity was determined. The plate without growth was noted as the minimum bactericidal concentration of *Tridax procumbens* extract. Minimum bactericidal concentration of *Tridax procumbens* was 4mg/ml. The Minimum bactericidal concentration of *Tridax procumbens* ranged from 4 to 8 mg/ml. (Table 3.3)

IV. DISCUSSION

The leaves of *Tridax procumbens* Linn belonging to the Family Asteraceae has been investigated in a systematic way covering the phytochemical screening and the biological studies. The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts [6], [7]. It means that they are more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrades polyphenols in water extracts, whereas in methanol and ethanol they are inactive.

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