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ANTIMICROBIAL ACTIVITY ASSAY OF *TABERNAEMONTANA CORONARIA*

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Abstract: The in vitro antimicrobial activity of *Tabernaemontana coronaria* leaf Fresh and Methanolic extracts has been investigated against *E. coli*, *K. pneumonia*, *S. typhimurium*, *S. flexneri* and *S. aureus.* The fresh and methanolic extract of *Tabernaemontana coronaria* showed activity against nearly all the test microorganisms. The inhibitory activities of methanolic extracts are highly significant in magnitude when compared with that of standard antibiotic used.

Keywords: *Tabernaemontana coronaria, E. coli*, *K. pneumonia*, *S. typhimurium*, *S. flexneri* and *S. aureus*

INTRODUCTION

Herbal remedies used in folk medicine provides an interesting and still largely unexplored source for the creation and development of potentially new drugs for the chemotherapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. Therefore it is of great interest to carry out a screening of these plants in order to validate their use in for medicine and to reveal the active principle by isolation and characterization of their constituents. The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of plants to the drug industry was possible, because of the large number of phytochemical and biological studies all over the world. Especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal remedies has risen in the last decades. Due to the plants continue to be a rich source of therapeutic agents. The antimicrobial properties of several plants had shown positive results[4,5,6,7,8,9,10].

Tabernaemontana coronaria (Apocynaceae) has a pan-tropical distribution. These plants are shrubs and small trees growing to 1-15 mm tall. Leaves are ever green, opposite 3-25cm long with milk sap. Hence it is one of the diverse plant genera commonly called milk wood[1].

The main constituents of *T. coronaria* are alkaloids, phenolic compounds, phytosterols and gums and mucilage present. A medicinal property of the T. coronaria is used to prevent eye injuries, anxiolytic and dementia[2].

MATERIALS AND METHODS

Chemicals used in the present study were Methanol 99% (Merck), Tetracycline (Merck), Nutrient agar medium (Hi-Media). Plant material leaves were collected from in and around Loni and confirmed their botanical details from P.V.P College, Botany department, then the leaves were wash under running tap water and surface sterilized by 0.1% $HgCl_2$ and again rinsed in distilled water to remove traces of $HgCl_2$ and leaves were dry in shade. Leaves were made into fine powder and stored in air-tight bottles for further use.

For fresh leaf extraction leaves were grind with motor and pestle that paste were taken into small vials or test tubes.

Extraction 10gm of dry powder was extracted in 100ml methanol by soxhlet apparatus. After 24hr to get the solvent extract the extract was kept at rotary vacuum evaporator to remove the solvent and finally get crude plant extract. Then it was stored in amber bottles at 4°C for further experimental use.

Bacterial cultures: Five strains of bacteria *E. coli* ATCC 25922, *Klebsiella pneumonia* ATCC 35657, *Salmonell typhimurium* MTCC 441, Shigella *flexneri* ATCC 29508 and *Staphylococcus aureus* ATCC 25923 obtained from the National chemical laboratory (NCL) PUNE, INDIA were maintained on Muller Hinton agar slants at 4°C.

Inoculums preparation: Bacterial strains were grown upto exponential phase in saline medium (0.85% NaCl) at 37°C for 24hrs and adjusted to final density of 10^4 CFU /ml to obtain a turbidity visually compare to 0.5 Mc Farland standards.

Antibacterial assay: The antimicrobial activities of fresh and methanolic extracts were assayed by agar well method (Rojas R et al., 2003). Tetracycline was used as standard and nutrient agar employed as medium. The *in vitro* screening of antimicrobial activity was carried out against *S. aureus* is Gram positive with Gram negative organisms *E. coli, S. typhi, Shigella* and *K. pneumonia*. The plates were inoculated with 18hrs culture of respective micro organisms. The wells were made aseptically with cork borer having 6mm diameter and 0.2ml of test solutions of each extracts as well as standard was added into the well using a micro pipette under aseptic conditions. The plates were kept in a refrigerator for 2hrs as period of pre-incubation diffusion followed by incubation at 37°±0.5° C, the zone of inhibition of microbial growth was measured after incubation for 18hrs. Each experiment was carried out in three replicates and the mean diameter of inhibition zone recorded.

RESULTS

In agar well diffusion the fresh and methanolic extracts of plant showed considerable activity against all bacteria (table.1). Comparatively fresh with methanolic extract showed good results all over. But methanolic is best activity than fresh. *S. aureus* is more resistant and *Shigella, K. pneumoniae* is sensitive in fresh and as well as methanolic extract.

Table I: Antimicrobial activity of for fresh and Methanolic extracts of *Tabernaemontana coronaria*.

Organism / Extract (100µl)	Zone of inhibition in mm				
	E. coli	S. flexneri	S. aureus	K. pneumonia	S. typhimurium
Fresh	15	10	16	10	14
Methanolic	17*	19*	20*	15*	21*
Positive control (Tetracycline)	16	14	15	13	16
Negative control (DMSO)	-	-	-	-	-

All Values are an average of 3 determinations *p<0.001 highly significant when compared with positive control

DISCUSSION

The activity of the plant against both Gram positive and Gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. *T. coronaria* demonstrates activity against the most prevalent Gram negative bacteria in eye infections *K. pneumoniae*.

Furthermore it may help to discover new chemical classes of antibiotics that could serve as selective agents for maintenance of animal or human health and provide biochemical tools for the study of infectious disease.

More over leaf methanolic extract of *T. coronaria* were active against *E. coli, K. pneumoniae*, *S. aureus, S. typhimurium* and detectable antibacterial activity is less in all organisms comparative to fresh extract with methanolic extract.

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