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Research Article

ANTIBACTERIAL ACTIVITY AGAINST BACTERIA ISOLATED FROM LEAVES OF TABERNAEMONTANA DIVARICATA

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A B S T R A C T

The ability of most infectious microbes evolving antimicrobial resistance necessitates discovery of novel antimicrobial agents from natural sources. For this study, a phytopathogen was isolated from infected leaf of *T. divericata* and identified as *Pseudomonas aeruginosa* with the standard methods. Antibacterial activity of standard drug i.e. ciprofloxacin and isolated compound from aqueous leaf extract of *T. divericata* was done against three phytopathogens i.e. *Pseudomonas aeruginosa*, *Escherichia coli and Staphylococcus aureus* in different dilutions (30µg/ml, 20 µg/ml, 10µg/ml) were studied. This study revealed that the isolated compound from aqueous extract of leaves of *T. divaricata* has capacity to act against phytopathogens. Isolated compound from aqueous extract of leaf showed resistant capacity against plant bacteria i.e. *P. aeruginosa*, *E. coli* and *S.aureus* through inhibition zone. Observation of results confirms that the 30 µg/ml concentration of isolated compound from aqueous extract of leaves of *T. divaricata* increased there is the possibility to observe more maximum inhibition against *E. coli* than *P. aeruginosa* and *S.aureus* bacterial species.

INTRODUCTION

Tabernaemontana divaricata is botanical name of crape jasmine which is an evergreen, well-branched shrub very common in India and all over world. *T. divaricata* possess a wide range of therapeutic activities including alexipharmic, emmenogogue, astringent, anticancer, hepatoprotective, aphrodisiac, digestible, purgative and antibacterial properties (Raj and Balasubramaniam, 2011).

In nature, host plant and pathogen are constantly changing with pathogens evolving new pathogenicity to overcome host defense systems and plants evolving to reduce pathogen attack. These coevolutionary interactions occur within ecological settings in which pathogen evolution and impact are tempered by environmental patchiness while host evolution is constrained by small population sizes and long generation times (Burdon and Thrall, 2009; Iranzo *et al*., 2015)

The *T. divaricata* leaves extracts (ethanol, petroleum ether, diethyl ether, methanol and aqueous) were found to possess maximum potency against infectious pathogens *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Enterococcus facealis*, *Staphylococcus pyogenes*, *Streptococcus agalactae*, *Salmonella typhi*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* (Ashikur *et al*., 2011). It is generally considered that compounds produced naturally rather than synthetically will be biodegraded more easily and therefore be more environmentally acceptable. Thus naturally antioxidants, antibacterian cytotoxic, antiviral, fungicidal agents and nutrients have gained popularity in recent years and their use and positive images among consumers are spreding (Pushpa *et al*., 2012).

In recent years, multiple drug resistance in both human and plant pathogenic microorganisms have been developed due to the indiscriminate use of commercially antimicrobial drugs commonly used in the treatment of infectious diseases (Davis, 1994). The ethanolic extract of flowers and stems showed inhibition against Bacillus subtilis, Escherichia coli and *Staphylococcus aureus*. The ethanolic extract of leaves showed inhibitory action against *Staphylococcus aureus* but no inhibition activity against *E. coli* and *B. subtilis* (Khan, 2011; Kalaimagal and Umamaheswari, 2014).

In order to find new therapeutic agents, plants that have antimicrobial activity have attracted attention now (Juliani and Simon, 2002; Falerio *et al*., 2003; Kalemba and Kunicka, 2003).

The ability of most infectious microbes evolving antimicrobial resistance necessitates discovery of novel antimicrobial agents from natural sources. The present study was focused on isolation of phytopathogen from infected leaf of *T. divericata* and its resistance against aqueous leaf extract with compare of standard drug. The subjected to isolated compound from

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aqueous extracts of *T. divaricata* leaf for antibacterial efficacy by agar well diffusion method and the minimum inhibitory concentration (MIC) testing were also studied.

METHODOLOGY

Isolation of Pure Compound from Leaf of T. divericata

Leaves of *T. divaricata* were collected from local area of Bhopal, M.P., India and drying of fresh leaves was carried out in sun but under the shade. The dried plant material was grinded into fine powder and was extracted in water by meraceation method for 24 hrs after extraction the extract was concentrated by evaporation and stored as aqueous extract at 4°C for further study. 3 gm of aqueous extract of *T. divarivata* leaf was taken in beaker; to it silica used for column chromatography (60-120 mesh size) and sufficient amount of mobile phase i.e. Toluene: Ethyl acetate (8:2) was added. Fractions eluted out from column chromatography gave pure compound, which was used for antibacterial activity of phytopathogen.

Isolation of Phytopathogen from Infected Leaf of T. divericata

The infected leaf of *T. divaricata* was brought to the laboratory from infected plant and causative agent was isolated by the culture method (Aneja, 2003). Infected portions of leaves were cut into small size were thoroughly washed in sterile distilled water for three times and macerated in sterile water and kept for 10 mins to obtain a suspension. The infected leaves were taken and it is serially diluted with distilled water. The serial dilution 100 µL of sample was collected from dilution and were spread on nutrient agar plate. The pure colonies of bacteria were sub-cultured in nutrient agar slants, incubated at 37^{o}C to achieve vigorous growth and then preserved in 20% glycerol vials at -80^{o}C (Collins *et al*., 1989).

Antibacterial Activity against Phytopathogens

Isolated compound from aqueous leaf extracts of *T. divaricata* were tested for antibacterial activity by agar well diffusion method (Sofowora, 1993)against one isolated bacteria from infected leaf of *T. divericata* and other two available bacteria such as E. coli and S.aureus by using Muller Hinton agar media (MHA) and Sabouraud dextrose agar (SDA). Muller Hinton & Sabouraud dextrose agar plates were prepared by pouring 20 ml of molten media into sterile petriplates. Then to solidify the plates for 10 mins and 0.5% inoculums suspension of three bacterial species were swabbed uniformly on three different solidify agar plate to the individual plates using sterile cotton swabs.

For antibacterial activity, from the total dried 200 microgram of stock crude isolated compound from aqueous leaf extracts of *T. divaricata* was diluted to 10 µg/ml, 20 µg/ml and 30 µg/ml. Wells of 6 mm diameter were made on agar plates and then by using a micropipette 10 µl of standard drug ciprofloxacin and isolated compound from aqueous leaf extract was poured 10 µg/ml, 20 µg/ml and 30 µg/ml on to the each well of three different agar plates respectively. The plates were then incubated at 37ºC for 24 hrs (Baishya *et al*., 2018). At the end of the incubation period different level of zone of inhibition were measured by zone scale & in the control there was no zone of inhibition. Each experiment has three replicates and three determinations were conducted with means of variable and standard deviation was recorded.

RESULT & DISCUSSION

Isolated phytopathogen bacteria from infected leaf of *T. divericata identified as P. aeruginosa* with the standard method of test (Pacarynuk and Danyk 2004). Antibacterial activity of standard drug i.e. ciprofloxacin against three bacteria i.e. *P. aeruginosa, E. coli and S. aureus* in different dilutions such as 30µg/ml, 20 µg/ml, 10 µg/ml are depicted in Table 1. Photo plates of antibacterial activity of standard drug i.e. ciprofloxacin against three phytopathogens are showing in Figure 1.

Table 1 Antibacterial Activity of Standard Drug against Phytopathogens

S. No.	Name of drug	Phytopathogens	Zone of Inhibition (mm)		
			30µg/ml	20 µg/ml	10µg/ml
1.	Ciprofloxacin	*P. aeruginosa*	36±0.5	28±0.74	19±0.47
		E. coli	30±0.47	26±0.47	22±0.47
		S. aureus	34±0.47	29±0.47	25±0.47

(n=3; mean±SD)

Figure 1 Photo plates of antibacterial activity of standard drug against phytopathogens

Antibacterial activity of 30 µg/ml concentration of standard drug ciprofloxacin showed zone of inhibition against phytopathogens bacterial species i.e. 36±0.5 mm against *P. aeruginosa*, 30±0.47 mm against *E. coli* and 34±0.47 mm against *S. aureus*. 20 µg/ml concentration of ciprofloxacin showed zone of inhibition against phytopathogens bacterial species i.e. 28±0.74 mm against *P. aeruginosa*, 26±0.47 mm against *E. coli* and 29±0.47 mm against *S. aureus*. 10 µg/ml concentration of ciprofloxacin showed zone of inhibition against phytopathogens bacterial species i.e. 19±0.47 mm against *P.s aeruginosa*, 22±0.47 mm against *E. coli* and 25±0.47mm against *S. aureus*.

Antibacterial activity of isolated compound from aqueous leaf extract of *T. divericata* against three phytopathogens bacterial species i.e. *P. aeruginosa*, *E. coli* and *S. aureus* in different dilutions such as 30µg/ml,20 µg/ml,10µg/ml are depicted in Table 2. Photoplates of antibacterial activity of isolated compound from aqueous leaf extract of *T. divericata* against three phytopathogens bacterial species are showing in Figure 2.

Table 2 Antibacterial Activity of isolated compound from aqueous Leaf Extract of *T. divericata* against Phytopathogens bacterial species

S. No.	Phytopathogens	Zone of Inhibition (mm)		
		30 µg/ml	20 µg/ml	10 µg/ml
1.	*P. aeruginosa*	14±0.74	11±0.47	7±0.57
2.	*E. coli*	25±0.47	12±0.47	8±0.47
3.	*S. aureus*	34±0	14±0.47	9±0.47

(n=3; mean±SD)

The antibacterial activity of 30 µg/ml concentration of isolated compound from aqueous extract of leaves of *T. divaricata* showed zone of inhibition against phytopathogens bacterial

species i.e. 14±0.74 mm against *P. aeruginosa*, 25±0.47 mm against *E. coli* and 34±0 mm against *S. aureus*.

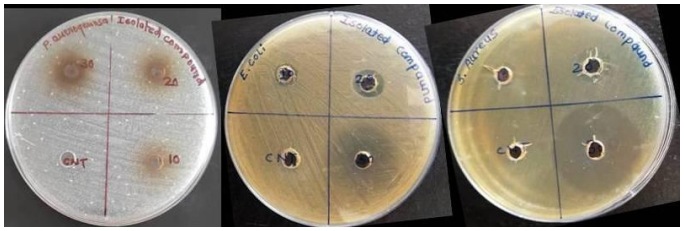

Figure 2 Photoplates of antibacterial activity of isolated compound from aqueous leaf extract of *T. divericata* against phytopathogens bacterial species

20 µg/ml concentration of isolated compound from aqueous extract of leaves of *T. divaricata* showed zone of inhibition against phytopathogens bacterial species i.e. 11±0.47 mm against *P. aeruginosa*, 12±0.47 mm against *E. coli* and 14±0.47 mm against *S. aureus*. 10 µg/ml concentration of isolated compound from aqueous extract of leaves of *T. divaricata* showed zone of inhibition against phytopathogens bacterial species i.e. 7±0.57 mm against *P. aeruginosa*, 8±0.47 mm against *E.coli* and 9±0.47 mm against *S. aureus*.

The maximum zone of inhibition (ZOI) 36±0.5 mm against *P.aeruginosa* was observed in 30 µg/ml concentration of standard drug ciprofloxacin, where as the maximum ZOI, 25±0.47 mm against *E. coli* was observed in 30 µg/ml concentration of isolated compound from aqueous extract of leaves of *T. divaricata*.

CONCLUSION

The present study concluded that the isolated compound from aqueous extract of leaves of *T.divaricata* had inhibitory action against *P. aeruginosa E. coli* and *S. aureus*. This study revealed that the isolated compound from aqueous extract of leaves of *T. divaricata* has capacity to act against phytopathogens bacterial species. Isolated compound from aqueous extract of leaf showed resistant capacity against *P. aeruginosa*, *E. coli* and *S.aureus* through inhibition zone. Observation of results confirms that the 30 µg/ml concentration of isolated compound from aqueous extract of leaves of *T. divaricata* increased there is the possibility to observe more maximum inhibition against *E. coli* than *P. aeruginosa* and *S.aureus* bacterial species.

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