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Silica Cotton Ball (SCB) Technique: A cost Effective Procedure for Microbiology, Plant Pathology and Applied Pharmacology

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For the authenticity of a new Rapid Antimicrobial Susceptibility Test (RAST) technique, antibacterial activity of *Phyllanthus fraternus* Webster., available in Tripura, was evaluated by a modified technique and Disc-Diffusion (DD) method. Leaf extract was prepared in ethanol solvent to be evaluated against – *Xanthomonas oryzae* pv. *oryzae, Xanthomonas campestris* pv. *campestris* and *Ralstonia soloanacearum.* Compared to Streptomycin, the leaf extract exhibited *in vitro* high antibacterial activity by the Silica Cotton ball technique which proves more economical than the conventional disc diffusion technique.

Key words: Phytopathogenic bacteria, Silica-Cotton Ball disc (SCB), Disc-Diffusion (DD) method.

Techniques are to be utilized and modified time to time for the better enrichment of the cost effective procedures in the laboratory from the economical point of view. An attempt was tried for the search of a rapid and modified antimicrobial susceptibility test which will be generally carried out on the microbiology and plant pathology laboratories by the students and researchers. The researchers of the Indian laboratories consider conventional Disc-Diffusion method as a rapid and sensitive test. But there are some problems raised when a new researcher decides to prepare it manually. It is costly, having less diffusion rate, less available, having difficulty in changing the concentration, uni- dimensional and having chances for mechanical injury. To overcome these problems, a modified Silica-Cotton Ball (SCB) technique was introduced for the first time. Basically, all the routine tests of the allelopathology, toxicology, applied pharmacology, microbiology and plant-pathology can be experimented by this newly evolved technique and for comparison of control tests, again can be experimented with the conventional Disc-Diffusion (DD) method also.

MATERIAL AND METHODS

To test the authenticity of SCB technique, culture of *Xanthomonas oryzae* pv. *oryzae, Xanthomonas campestris* pv. *campestris* and *Ralstonia soloanacearum* were prepared by sample collected from rice, mustard and brinjal plants respectively and they were cultivated *in vitro* as per general procedure. *X. oryzae* pv. *oryzae* (Xoo) causes bacterial leaf blight of rice and is a Gram negative bacteria belonging to the family

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Pseudomonadaceae. *Xanthomonas campestris* pv. *campestris* (Xcc), also a Gram negetive aerobic rod shaped bacteria and is the causal agent of black rot, which affects crucifers such as *Brassica* and *Arabidopsis*. Whereas, *Ralstonia soloanacearum*(RS) is a Gram negative soil bacteria causes wilt of potato, tomato and egg plants. Since plants have co-evolved with pathogens, they understandably have also developed the chemical protection pathways against the parasitic organisms. Therefore, it is reasonable to expect a variety of plant compounds with specific as well as general antimicrobial activity and antibiotic potential (Wade, 1997). Based on some preliminary findings and hypothesizing that *Phyllanthus* leaves must possess some protective mechanism against microbial attack, the present study was undertaken to screen out for antibacterial activity of the leaves. *Phyllanthus fraternus* Webster syn. *Phyllanthus niruri* is a medicinal plant commonly found in Tripura (Deb, 1981) and grows wildly. Literature is available for its medicinal value and active biochemical constituents for this species (Santos *et al*., 2000; Sailaja and Setty, 2006; *http://www.pharmainfo.net*, 2008).

SCB technique employing the antibacterial agents from the plant sources should be interpreted on the basis of presence or absence of a definite zone of inhibition and should be considered as only qualitative until and unless the time interpretative zones have been established. Thus, this modified technique has been evaluated for susceptibility test for *P. fraternus* against *X. oryzae* pv. *oryzae, Xanthomonas campestris* and *Ralstonia soloanacearum* respectively. The SCB technique was applied for ethanolic plant extracts from leaves of *P. fraternus* in accordance with the inhibition of bacterial culture growth. The technique utilized was with little modifications from Bauer *et al.*(1966) and ASM(1999) using nutrient agar as a constituent of inoculation media. The *in vitro* anti-bacterial activity was evaluated by disc diffusion (DD) method (Pankaj *et al*.,1993; Nene and Thapliyal,1979) and Silica-Cotton (SC) technique for comparative analysis. In DD method, the extracts were reconstituted in dimethyl sulfoxide (DMSO) and 30ìg applied on Whatman no.4 paper disc (diameter 6 mm) by micropipette. In SC technique, the dilute hot nutrient agar was poured in Petri plates until it became cool and semisolid (gel), 1g of silica powder mixed with 0.5ml distilled water was poured on the marginal area of Petri plate to prevent contamination through air. The bacterial ooze was spread by autoclaved cotton swab moistened with bacterial solution. Later, a small cotton ball mixed with silica was placed on the bacterial swab containing nutrient agar. The silica-cotton ball was autoclaved and impregnated with 30ìg plant extract for testing. Streptomycin (10ìg/disc) was used as standard for reference purpose (Bauer *et al.,* 1966; ASM,1999; NCCLS,2003).Paper discs (1 mm thickness) were prepared with the help of a cork borer (6 mm diameter).The results are shown on the following Table 1.

RESULTS AND CONCLUSION

Thus it is concluded that the ethanolic extract of leaf of *Phyllanthus* showed maximum inhibitory effect against *Xanthomonas oryzae* pv.

Table 1. Susceptibility test procedure for antibacterial activity of leaf extracts of *Phyllanthus fraternus* on ethanol solvent against three phytopathogens at a single concentration (30µg/disc)

Test pathogen	Inhibition zone (mm) in Disc Diffusion method(DD)	Inhibition zone (mm) in Silica Cotton method(SC)	Inhibition zone (mm) by Streptomycin disc
Xanthomonas oryzae pv. *oryzae*	25±0.054*	26±0.009	30±0.043
Xanthomonas campestris pv. *campestris*	20±0.018	22±0.011	31±0.022
Ralstonia soloanacearum	22±0.028	25±0.079	30±0.079
Critical difference($p<0.05$)	5.752	3.791	5.002

* Disc diameter = 6mm. All the readings are based on triplicates ± standard error of mean.

oryzae than other two bacterial strains. Although the ethanol extract showed moderate to high inhibitory effect to various extent against the other two test bacterial strains and their inhibitory effect was found significant for all the pathogenic strains at 5% level of significance. Further, the present study proves the higher efficiency of the SCB techniques than the conventional methods as applied within the laboratory. Due to scarcity of literature about such SCB technique in world, it is duly said that it is a new method and this modified technique may be recommended for first time for the research area of Microbiology and Plant Pathology.

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