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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF PHYLLANTHUS AMARUS PLANT EXTRACT AGAINST RESISTANT PATHOGENIC BACTERIAL STRAINS: AN ETHNOMEDICINAL PLANT

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ABSTRACT

Objective: To evaluate antimicrobial activity of Phyllanthus amarus plant extract against resistant pathogenic bacterial strains.
Methods: Aqueous and acetone extract of *Phyllanthus amarus (Family: Euphorbiaceae)* was studied against resistant pathogenic bacterial strains (*Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis, Salmonella typhi, Salmonella paratyphi B, Proteus vulgaris* and *Serratia marsescens)* by disc diffusion method comparable to that of a standard antibacterial Lomefloxacin.
Result: Aqueous extract showed 57% efficiency in inhibiting the pathogenic isolates while acetone extract showed 29% efficiency.
Conclusion: Phyllanthus amarus aqueous extract was found to be antimicrobially more effective than the acetone extract.

INTRODUCTION

Phyllanthus amarus is an erect annual herb cultivated throughout India. It has been found that the local tribes of Dhanbad region of India use the leaves of this plant as an antitussive and an astringent. The plant has been used by the local tribes of the Jharkhand state in India for treatment of jaundice, sores, fever, dysentery, and diarrheal infections (Artur, 2007). The plant was reported to have hepatoprotective activity (Selvamohan, 2012) Sane et al in 1997 reported the efficacy of the plant in inhibiting the hepatitis B virus. The leaves were found to possess antipyretic activity[4]. The present investigation was undertaken to evaluate the antibacterial efficacy of P. amarus on some drug resistant microbes and thereby justify the folklore claim of the plant in the traditional system of Indian medicine (Mohamed, 2010). The present investigation is aimed at studying the antimicrobial activity of aqueous and acetone extracts of *Phyllanthus amarus* against pathogens responsible for common infections of skin, respiratory, urinary and gastro-intestinal tracts. The plant exhibited significant antimicrobial potency, comparable to that of a standard antibiotic Lomefloxacin.

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MATERIAL AND METHODS

Plant material and extract

Phyllanthus amarus (Euphorbiaceae) were collected in April 2017 from the Dhanbad district of Jharkhand, India. The plant was then identified by Scientist-in-charge, Botanical Survey of India, Allahabad, U.P .The whole plant was then air-dried after washing and pulverized in a mechanical grinder. The dried powder was extracted with methanol in a Soxhlet apparatus (yield 2.4% w/w with respect to dried powdered extract).

Test Microorganism

Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis, Salmonella typhi, Salmonella paratyphi B, Proteus vulgaris and *Serratia marsescens.*were used in the present study. All these strains were found to show a high degree of resistance when tested against the standard antibacterial including Lomefloxacin.

Determination of antimicrobial activity

One loopful (loop diameter: 3 mm) of an overnight grown nutrient broth culture of the test organisms was added by checker board technique to the marked quadrant of the sterile 100 mm petridishes containing various concentrations of the aquas and acetone extract in nutrient agar (Table 1). The spot

inoculated plates were incubated at 37*C for 24 h to determine MIC of the extract against the microorganisms (Kavit, 2014 and Velraj, 2013). Antibacterial activity was then also tested by the disc diffusion method (Misbah, 2013).

Fig.1. Phyllanthus amarus

RESULTS

The aqueous extract was found effective in inhibiting *Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis, Salmonella typhi, Salmonella paratyphi B, Proteus vulgaris* and *Serratia marsescens*. The acetone extract was found effective in inhibiting *Enterococcus fecalis, Salmonella typhi, Salmonella paratyphi B* and *Proteus vulgaris* (Table-1). *Staphylococcus aureus* was found to be most sensitive to the aqueous extract. Comparative antimicrobial efficiencies of aqueous and acetone extracts against *Salmonella typhi* and *Enterococcus fecalis* were statistically highly significant, very highly significant and comparable against *Salmonella paratyphi B* and *Proteus vulgaris*. The aqueous extract showed 57% efficiency in inhibiting the pathogenic isolates while acetone extract showed 29% efficiency. Thus, the aqueous extract was found to be antimicrobially more effective than the acetone extract (Table 1).

Table 1. Zone of inhibition

S.No	Name of the Culture	Aqueous extract of *Phyllanthus amarus* Zone of inhibition (mm) ± S.E.M	Acetone extract of *Phyllanthus amarus* Zone of inhibition (mm) ± S.E.M	Standard Antibiotic Lomefloxacin (30 µg) Zone of inhibition (mm) ± S.E.M
1	*B.subtilis*	17.86 ± 1.53	-	Resistant
2	*S. aureus*	21.83 ± 1.56	-	20 ± 1.41
3	*E. fecalis*	19.5 ± 1.34	14.33 ± 1.63***	19.5 ± 1.04
4	*S. typhi*	19 ± 2.266	14.16 ± 1.72**	17.5 ± 1.04
5	*S. paratyphi B*	16 ± 1.27	13.83 ± 1.47	21.5 ± 1.04
6	*P. vulgaris*	15.5 ± 1.52	15 ± 1.41	28.66 ± 1.032
7	*S. marsescens*	19.66 ± 1.87	-	19.5 ± 1.04

Values are mean ± S.E.M. (n=3)

Table 2. MIC values of aqueous and acetone extracts of *Phyllanthus amarus* on test organisms

MIC in mg/ml						
B.subtilis	*S. aureus*	*E. fecalis*	*S. typhi*	*S. paratyphi B*	*P. vulgaris*	*S. marsescens*
20.3	22.2	20.1	24.0	16.8	12.2	19.9

Table 3. Relative Percentage inhibition of Phyllanthus amarus compare to standard

S.No	Name of the Culture	Relative percentage (%) inhibition of aqueous extrac	Relative percentage (%) inhibition of Acetone extract
1	*B.subtilis (B.s.)*	100	0
2	*S. aureus (S.a.)*	105	0
3	*E. fecalis (E.f.)*	100	73
4	*S. typhi (S.t.)*	108	80
5	*S. paratyphi B (SpB)*	76	64
6	*P. vulgaris (P.v.)*	53	53
7	*S. marsescens (S.m.)*	100	0

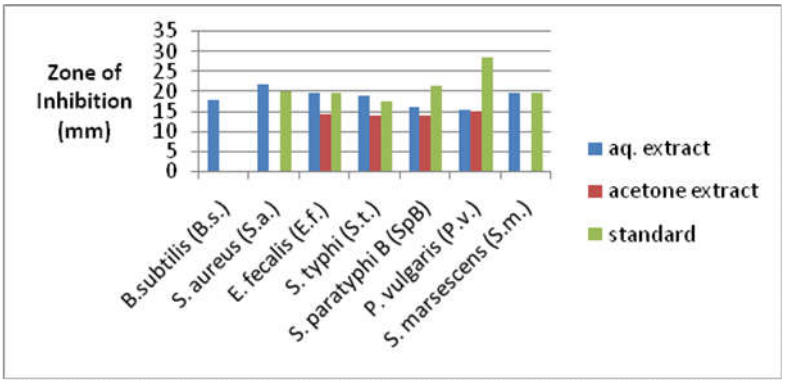

Figure 2. Antimicrobial activity of *Phyllanthus amarus*

Antimicrobial activity of most commonly used standard antibiotic Lomefloxacin (30 mcg) was studied against all the pathogenic isolates. *Bacillus subtilis* was found to be resistant to Lomefloxacin while susceptible to the aqueous extract. All other pathogenic isolates were found to show comparable susceptibility towards the aqueous and acetone extracts of *Phyllanthus amarus* and Lomefloxacin.

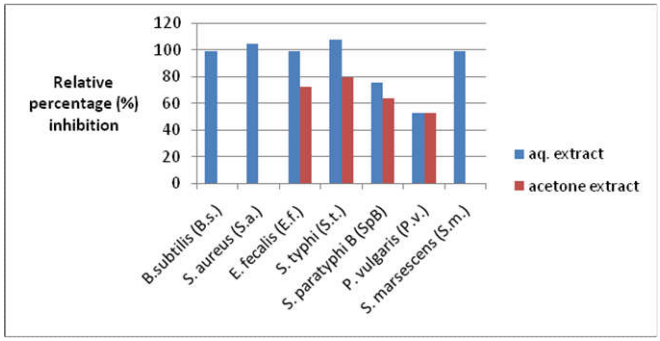

Figure 3. Relative Percentage inhibition

DISCUSSION

Observed values for the antimicrobial aqueous plant extracts were more effective against pathogenic bacteria *Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis, Salmonella typhi, Salmonella paratyphi B, Proteus vulgaris* and *Serratia marsescens*. This then suggest that, they are promising antimicrobial agents which agreed with the suggestion of Takazawa et al. (2007), that there is a need to employ broad range of solvents in the extraction of possible phytochemicals from medicinal plants. It is not common to have commercial antibiotics with antibacterial effectiveness, this report open up need to concentrate on the possibility of searching for active ingredients in these plants that can be deployed into world's chemotherapeutic usage.

Conclusion

In conclusion, antimicrobial activity of Lomefloxacin was observed to be comparable to that of the aqueous extract of *Phyllanthus amarus* with reference to the zones of inhibitions shown by *S. aureus*, *E. fecalis*, *S. typhi* and *S. marsescens* while that of the acetone extract of *Phyllanthus amarus* with reference to the zones of inhibitions shown by *E. fecalis* and *S. typhi.* aqueous extract showed 57% efficiency compare to acetone extract efficiency. Thus, the aqueous extract was found to be antimicrobially more effective than the acetone extract.

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Authors Contribution

All the authors have contributed in various degrees to conception and design, acquisition of data, analysis, interpretation of data or writing present article.

Conflicts of Interests

All the author(s): Manish K Pathak, U.K Singh, Gourav Upadhyay declare that there is no conflict of interest regarding the publication of this paper.

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