WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 9, 324-330. Conference Article ISSN 2277– 7105

ANTIBACTERIAL POTENTIAL OF THE LEAF EXTRACTS OF AMPELOPTERIS PROLIFERA (RETZ.) COPEL - AN ETHNOMEDICINALLY IMPORTANT FERN IN ANGUL DISTRICT OF ODISHA (INDIA)

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Article Received on
19 March 2018,

Revised on 09 April 2018,
Accepted on 29 April 2018,

DOI: 10.20959/wjpr20189-12111

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ABSTRACT

The objective of the present investigation was to evaluate the antibacterial activity of ethanolic, methanolic and aqueous extracts of Ampelopteris prolifera (Retz.) Copel leaves. The antibacterial potential of the fern was tested against human pathogens such as Staphylococcus aureus (MTCC-1430), Vibrio choleriae (MTCC-3906), Pseudomonas aeruginosa (MTCC-1035), Shigella flexneri (MTCC-9543), Bacillus subtilis (MTCC-1305), Streptococcus mitis (MTCC-2897), Klebsiella pneumoniae (MTCC-109), Staphylococcus aureus (MTCC-1430), Salmonella paratyphi (MTCC-3220), Micrococcus luteus (MTCC-1809), Salmonella typhimurium (MTCC-98), Escherchia coli (MTCC-614), Bacillus circulans (MTCC-490) and Enterococcus faccalis (MTCC-459) by following agar well diffusion method. The result of The study revealed that ethanolic extract was highly effective against Streptococcus mitis and Klebsiella pneumoniae while Pseudomonas aeruginosa showed no response and Salmonella enterica ser typhi, Bacillus subtilis, Streptococcus mitis and Staphylococcus aureus responded moderately. The ethanolic extract had high inhibition against Streptococcus mitis (17.16 ± 0.20 mm) whereas Shigella flexneri showed no response and moderate effect against other test bacteria. Streptomycin was taken as the reference antibiotic.

KEYWORDS: *Ampelopteris prolifera*, Agar well diffusion, Antibacterial activity, Ethanol, Methanol, Streptomycin.

INTRODUCTION

Although the medicinal value of the ferns have been known to man for more than 2000 years, they have been found with very little application in modern chemotherapy as compared to the angiosperms. However, researches on the antimicrobial activity of this plant group are still in their infancy. The Greek botanist, Theophrastus (Ca. 372-287 B.C.), had referred to the medical value of ferns in one of his books. Although in India, total ferns' species were 1022, they have been recorded with respect to vast angiospermic diversity (15000 species), in that ferns' plants played a significant role on ethnomedicine. In ancient Indian medicine, several ferns were used, and in particular by Unani physicians in India and Western Asia. Reports showed that ferns were used by the people of India and in various other countries. Most of the diseases against which the Lycophytes are said to have curative properties, are caused by bacteria (gram-positive, gram-negative or acid-fast). The plant extract preparations were used successfully in the treatment of such diseases and are expected to possess antimicrobial properties.

MATERIALS AND METHODS

Collection and identification of plant material

The plant *Ampelopteris prolifera* (Retz.) Copel was collected from the "Angul reserve forest" area near Bhubaneswar, Odisha in the month of March, 2017. Identification of the voucher specimen was done by available literature.[12] The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India. The leaves were collected in bulk amount, washed in running tap water, dried under shade and made to coarse powdered form.

Processing of plant material and preparation of extract

The collected leaves were shade dried and ground to form coarse powder and had been successively extracted with the solvents such as ethanol, methanol and aqueous by Soxhlet apparatus,[13] and the extracts were recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.

Evaluation of the extracts for antibacterial activity

The in vitro antibacterial screening was carried out against selected four gram-positive, eight gram-negative and two gram-variable bacterial pathogens causing various diseases in human. The test bacterial pathogens were Staphylococcus aureus (MTCC-1430), Vibrio cholerae (MTCC-3906), Pseudomonas aeruginosa (MTCC-1035), Shigella flexneri (MTCC-95430),

Salmonella typhi (MTCC-733), Klebsiella pneumoniae (MTCC-109), Salmonella paratyphi (MTCC-3220), Bacillus subtilis (MTCC-1305), Micrococcus luteus (MTCC-1809), Salmonella typhimurium (MTCC-98), Escherichia coli (MTCC-614), Bacillus circulans (MTCC-490), Streptococcus mitis (MTCC-2897), Enterococcus faecalis (MTCC-459). These species were procured from Microbial Type Culture Collection Centre (MTCC) and Gene Bank, Chandigarh, India. The remaining bacterial species were procured from Post Graduate Department of Microbiology, OUAT, Bhubaneswar, Odisha. These organisms were identified by following standard microbial methods.[14] The antibacterial screening of the extracts was carried out by determining the zone of inhibition using agar well diffusion method[13] for bacteria.

Standard drugs used and preparation of doses for antibacterial assay

Streptomycin was used as Reference Antibiotics (RA). The stock solution of RA was prepared in sterile distilled water to give a concentration of 0.5 mg/ml.

Agar well diffusion assay

Agar well diffusion method,[12] was followed to determine the zone of inhibition of microbes in Nutrient Agar (NA, HiMedia Laboratories Ltd., Mumbai). Plates were swabbed (sterile cotton swabs) with 8 hr old broth culture of bacteria. Wells (8 mm diameter) were made in each of these plates using sterile cork borer. A stock solution of plant extracts was prepared at a concentration of 20 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 h. Control treatments comprising inoculums without plant extract were set up. The plates were incubated at 37 °C for 24 h for bacterial pathogens. Triplicates were maintained and the diameter of the zone of inhibition (mm) was measured and statistical analysis was carried out.

RESULTS AND DISCUSSION

Antibacterial screening

The result of antibacterial screening of the leaf extracts of Ampelopteris prolifera against the test organism revealed that the ethanolic extract was more effective in inhibiting the growth of test organisms than methanolic and aqueous extracts. In the present investigation ethanolic leaf extracts of the fern showed maximum inhibiting activity against Streptococcus mitis (17.16±0.20 mm) followed by Bacillus subtilis (17.33±2.05 mm), Micrococcus luteus (16±0.21), Salmonella typhi (15.66±1.69mm), Salmonella paratyphi (14.66±1.69 mm), Klebesiella pneumonia (14.33±1.24 mm), Bacillus circulans (14.66±1.69 mm), Vibrio

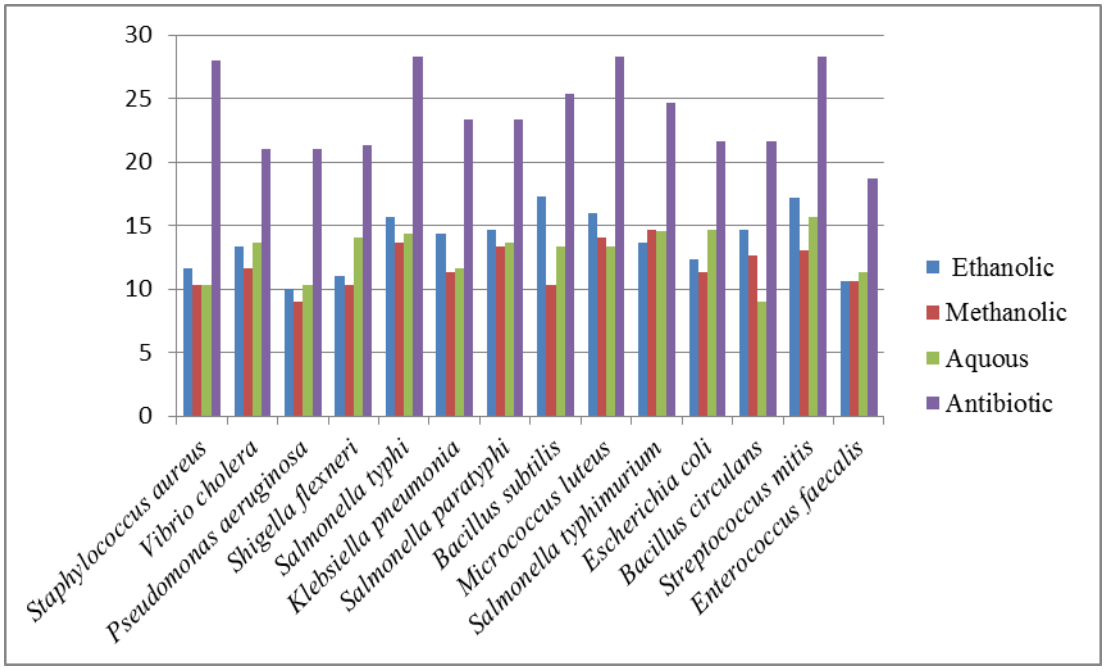
cholera (13.33±0.24 mm), Salmonella typhimurium (13.66±0.47 mm), Escherichia coli (12.33±1.24 mm), Enterococcus faccalis (10.66±1.24 mm), Shigella flexneri (11±1.24 mm), Staphylococcus aureus (11.66±1.63 mm) and Pseudomonas areuginosa (10±2.16 mm). The methanolic extract exhibited highest zone of inhibition against Salmonella typhimurium (14.66±1.24 mm) followed by Micrococcus luteus (14±0.81 mm), Salmonella typhi (13.66±0.69 mm), Salmonella paratyphi (13.33±1.24 mm), Streptococcus mitis (13±0.81 mm), Bacillus circulans (12.66±0.47 mm), Vibrio cholerae (11.66±0.94 mm), Escherichia coli (11.33±1.69 mm), Klebesiella pneumoniae (11.33±1.24 mm), Enterococcus faecalis (10.66±1.24), Bacillus subtilis (10.33±0.47 mm), Shigella flexneri (10.33±1.24 mm), Staphylococcus aureus (10.33±0.47) and least against Pseudomonas aeruginosa (9 mm).

The aquous extract exhibited highest zone of inhibition against Streptococcus mitis (15.66±1.24 mm) followed by Escherichia coli (14.66±0.81 mm), Salmonella typhimurium (14.54±0.69 mm), Salmonella typhi (14.33±1.24 mm), Shigella flexneri (14±0.81 mm), Vibrio cholerae (13.66±0.47 mm), Salmonella paratyphi (13.66±0.94 mm), Bacillus subtilis (13.33±1.69 mm), Micrococcus luteus (13.33±1.24 mm), Klebsiella pneumoniae (11.66±1.24), Enterococcus faecalis (11.33±0.47 mm), Staphylococcus aureus (10.33±1.24 mm), Pseudomonas aeruginosa (10.33±0.47) and least against Bacillus circulans (9 mm). The result of these three (ethaniloic, methanolic and aqueous) extracts were compared with reference antibiotic streptomycin and the zone of inhibition of streptomycin was found to range from 30.33±0.94 mm (Bacillus subtilis) to 16.66±3.09 mm (Enterococcus faecalis) (Table 1 and Fig. 1).

Table 1: *In vitro* antibacterial activity (zone of inhibition in mm) of different extracts of *Ampelopteris prolifera* (Retz) Copel.

Test organisms (Bacteria)	Zone of Inhibition (in mm)			
	Ethanolic extract (20 mg/ml)	Methanolic extract (20 mg/ml)	Aquous extract (20 mg/ml)	Antibiotic
Staphylococcus aureus	11.66±1.63	10.33±0.47	10.33±1.24	28±0.69
Vibrio cholerae	13.33±0.24	11.66±0.94	13.66±0.47	21±1.24
Pseudomonas aeruginosa	10±2.16	9±1.24	10.33±0.47	21±1.24
Shigella flexneri	11±1.24	10.33±1.24	14±0.81	21.33±1.69
Salmonella typhi	15.66±1.69	13.66±0.69	14.33±1.24	28.33±1.69
Klebsiella pneumoniae	14.33±1.24	11.33±1.24	11.66±1.24	23.33±0.81
Salmonella	14.66±1.69	13.33±1.24	13.66±0.94	23.33±0.81

paratyphi				
Bacillus subtilis	17.33±2.05	10.33±0.47	13.33±1.69	25.33±0.47
Micrococcus luteus	16±0.21	14±0.81	13.33±1.24	28.33±1.69
Salmonella typhimurium	13.66±0.47	14.66±1.24	14.54±0.69	24.66±0.47
Escherichia coli	12.33±1.24	11.33±1.69	14.66±0.81	21.66±0.81
Bacillus circulans	14.66±1.69	12.66±0.47	9±1.24	21.66±0.81
Streptococcus mitis	17.16±0.20	13±0.81	15.66±1.24	28.33±1.69
Enterococcus faecalis	10.66±1.24	10.66±1.24	11.33±0.47	18.66±0.20



The standard drug streptomycin was found effective at a much lower concentration than the leaf extracts. However, this plant is commonly used by the local people traditionally against a large number of infectious diseases. It is expected that this plant may have very less toxicity and one might conclude that the use of these plants would probably produce less side effects as compared with a conventional chemotherapeutic agent.

CONCLUSION

Although many works on antibacterial activities of the fern leaf extract has been extensively surveyed but its antimicrobial mechanisms have not been reported in great details. This study has shown that leaf extract of *Ampelopteris prolifera* (Retz.) Copel. possess rather a significant activity against different microorganisms, including human pathogens. These results confirm the potential use of *Ampelopteris prolifera* (Retz.) Copel leaf extract in the

food and pharmaceutical industries, which may be useful as an alternative antimicrobial agent in natural medicine for the treatment of numerous infectious diseases.

ACKNOWLEDGEMENT

The authors are thankful to the Head, Post Graduate Department of Botany, Utkal University, Bhubaneswar, Odisha (India) for providing necessary laboratory facilities to conduct the study. The financial assistance received from the University Grants Commission, Govt. of India, New Delhi in the form of BSR Fellowship to the first author is deeply acknowledged.

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