

IN VITRO ANTIMICROBIAL ACTIVITY OF VARIOUS PLANT LATEX AGAINST RESISTANT HUMAN PATHOGENS

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Received: 21 May 2011, Revised and Accepted: 30 June 2011

ABSTRACT

Medicinal plants are most important resource of potentially useful substances for the development of novel chemotherapeutic useful drugs. The present study was aimed to evaluate the antimicrobial effect of five different families of plant latex petroleum ether extract on some human pathogenic bacterial and fungal strains. Latex extract were screened *in vitro* against human pathogenic strains such as Gram positive; *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative; *Salmonella typhi*, *Klebsiella phenonemia* and two fungal strains; *aspergillus niger* and *candida albicans*. The inhibitory effect was assessed by agar well diffusion method. The minimum inhibitory concentrations (MIC) were also determined. The latex petroleum ether extract shows significant zone of inhibition compared with standard Ciprofloxacin and Fluconazole respectively.

Keywords: Plant latex, Antibacterial activity, Antifungal activity, MIC.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value^{1, 2}. Medicinal plants are the 'back bone' of traditional remedy³. In addition, the traditional medicine related to treatment of both human and animal mycoses with plant-derived preparations is considered a valuable knowledge for the discovery of new antimicrobial drugs⁴. Plants contain many biologically active molecules with different medicinal properties⁵. India is very rich in natural resources and the knowledge of traditional medicine and the use of plants as source of new drugs is an innate and very important component of healthcare system. However, very little information is available about many useful herbs as experimental data. Many plants of families *Cariaceae*, *Asclipidaceae*, *Moraceae*, *Euphorbiaceae* and *Apocynaceae* were growing widely throughout the tropical and subtropical regions of Karnataka. Some of plants from these families are popularly known to produces medicinally important latex. The latexes are source of various biologically active compounds, including glycosides, tannins and many proteins^{6, 7}.

Previous reports have shown that *Asclepias* latex, the protoplasmic content derived from the laticiferous cell which characterizes this genus, contains specialized substances including the poison, asclepiosin,⁸ proteolytic enzymes,⁹ cardiac glycosides toxic to birds¹⁰ and other substances poisonous to livestock.¹¹ These plants and their latex were extensively used as folk medicines,¹² including the treatment of various forms of cancer.¹³ In addition, the dilution of any remaining latex in the collected plant with the extracts from other cells and tissues, which form the bulk of the plant body, reduces the possibility of detecting antimicrobial substances present in the residual latex. The purpose of this investigation was to determine whether, latex exudates possessed antimicrobial properties which inhibited the growth of selected microorganisms. The present study was aimed to evaluate the antibacterial and antifungal activities of latex extract against human pathogenic strains by agar well diffusion method. Besides, the biological activity of the extract in terms of minimum inhibitory concentration (MIC) was determined.

MATERIALS AND METHODS

Sample Collection

The latex from various plants was collected from Kurudihally village, Chitradurga district, Karnataka, India. The plants used were *Carica Papaya* (*Cariaceae*), *Calatropes Procera* (*Asclipidaceae*), *Artocarpus heterophyllus* (*Moraceae*), *Jatropa carcass* (*Euphorbiaceae*) and *Thevetia peruviana* (*Apocynaceae*).

Extraction of antimicrobial compounds

Five ml of latex were collected separately from five different plants and extracted with petroleum ether was previously described;^{14, 15}

Experimental conditions

The experiments was carried out in a sterile aseptic condition at 25 ± 2 °C and equipments were sterilized using 5% formaldehyde and 90% ethanol, washed and dried before use. Bacterial strains such as, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella phenonemia* and two fungal strains; *Aspergillus niger* and *Candida albicans* were isolated from clinical specimens and stored at 4 °C.

Antimicrobial activity

The antimicrobial activity of petroleum ether extract latex was carried out using agar well diffusion method.¹⁶⁻¹⁸ The bacterial strains were collected from different infectious status of patients who had not administered any antibacterial drugs for at least two weeks with the suggestions of an authorized physician, in Kiran diagnostic health centre of Chitradurga, Karnataka state, India. Fungal strains were procured from the culture maintained at National College of Pharmacy Shimoga. The *invitro* antimicrobial activity was carried out against 24 h culture of four bacterial strains Gram positive *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative, *Salmonella typhi*, *Klebsiella phenonemia*. Two fungal strains were *Aspergillus niger* and *Candida albicans*. The petroleum ether extracts latexes were tested at 40 µg concentration against both bacterial and fungal strains. Petroleum ether was used as a vehicle. Ciprofloxacin (40 µg in 100µl) and Fluconazole (40 µg in 100µl) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 °C for antibacterial activity and 72 h at 25 °C for antifungal activity.

Minimum Inhibitory Concentrations (MIC)

The MIC of all petroleum ether extract latex was determined by a micro dilution method.^{17, 19} The respective clinical strains were plated separately on the agar containing medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The latexes at different concentrations viz. (10, 20, 30, 40 and 50 µg) was dissolved in (25, 50, 75, 100 and 125 µL) of Petroleum ether respectively and loaded into corresponding wells in the plates. The standard drug Ciprofloxacin (40 µg in 100µl) and Fluconazole (40 µg in 100µl) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 °C for antibacterial activity and 72 h at 25 °C for antifungal activity. The results of these experiments are expressed

as mean \pm SE of three replicates in each test. The data's were evaluated by one-way ANOVA followed by Turkey's pair-wise comparison test and results were considered significant when $P < 0.05$.

RESULT AND DISCUSSION

Searching of compounds with antimicrobial properties has generally targeted to the plants with a history of ethno botanical uses,^{20,21} while a few studies have targeted randomly collected plants.²² The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial

infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.^{23, 24} Present study targeted to search the antibacterial activity of randomly selected plants latexes. As latexes are known to have a defensive purpose in plant, they may contain strong antimicrobial activity and plants may provide the good source of antimicrobial compounds. The antibacterial activity of Petroleum ether of different plant latexes namely; *Carica Papaya*, *Calatropes Procera*, *Artocarpus heterophyllus* fruits, *Jatropha carcass*, *Thevetia peruviana* against different human pathogenic bacterial and fungal strains of Table 1.

Table 1: Antibacterial activity of petroleum ether extract latexes against infectious strains

Compound	Streptococcus haemolyticus	B.subtilis	S.typhi	Klebsiella Pneumoniae	A. niger	C. albicans
	Antibacterial strains			Antifungal Strains		
Carica Papaya	19.00 \pm 1.15	18.33 \pm 1.20	18.67 \pm 1.86	17.00 \pm 1.00	17.33 \pm 1.45	17.00 \pm 1.53
Calatropes Procera	20.33 \pm 0.33	20.33 \pm 0.33	18.33 \pm 2.19	20.33 \pm 0.33	20.67 \pm 0.33	18.00 \pm 1.53
Artocarpus heterophyllus	19.33 \pm 0.67	19.00 \pm 1.00	17.67 \pm 1.86	19.33 \pm 1.20	20.00 \pm 0.58	16.33 \pm 1.33
<i>Jatropha carcass</i>	18.33 \pm 0.88	18.67 \pm 0.88	19.67 \pm 0.33	19.67 \pm 1.45	19.00 \pm 1.15	18.67 \pm 2.03
<i>Thevetia peruviana</i>	20.00 \pm 1.53	20.33 \pm 0.88	20.67 \pm 0.67	18.33 \pm 1.86	20.00 \pm 1.53	21.00 \pm 1.00
Ciprofloxacin	21.00 \pm 0.58	22.00 \pm 0.31	21.00 \pm 0.26	21.33 \pm 0.88	-	-
Fluconazole	-	-	-	-	21.67 \pm 0.33	21.33 \pm 0.67
Control	0.22 \pm 0.01	0.20 \pm 0.01	0.20 \pm 0.02	0.21 \pm 0.01	0.22 \pm 0.01	0.20 \pm 0.01

Note: The value of each constituents consisted of Mean \pm SE of 3 replicates; Value is significantly different when $p < 0.05$; Standard drug used: ciprofloxacin. (40 μ g in 100 μ L); Petroleum ether extract latexes: (40 μ g in 100 μ L); Control: Petroleum ether

The minimum inhibitory concentrations (MIC) were also determined in which *C. Papaya*, *A. heterophyllus*, *J. carcass* showed MIC at 20 μ g whereas, *T. peruviana*, *C. procera* showed at 30 μ g concentration for both bacterial and fungal strains. Plants and plant products are being used as a source of medicine since long; the medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antimicrobial activities, no side effects and economic viability.^{25,26,27} Traditional Chinese drugs (Kampo drugs) are used for treatment and prevention of many diseases in China and Japan and are formulated from several crude drugs composed of dried plants or insects.²⁸

Moreover, only glycosides and saponins extracted from *F. carica* leaves using alcohol as solvent had biological effects but they had no effects on *C. albicans*, *S. aureus* and *E. coli*,²⁹ compared to our study latex extracts are more active than leaf extracts on human pathogenic bacteria and fungi. However, further research is needed to identify the active agents responsible for the antibacterial and antifungal activities of petroleum ether extract latex. The next work will be carried out on ethyl acetate fraction (Antiviral activity of poxvirus and cytotoxic and anti-inflammatory reactions).

ACKNOWLEDGEMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors" the authors are thanks the authorities of Kuvempu university for providing necessary facility and to carry out the present work.

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