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Evaluation of antimicrobial activity of root extract of *Asclepias curassavica*

Hemavani. C and B. Thippeswamy*

Department of P.G. Studies and Research in Microbiology, Bioscience complex, Kuvempu University, Jnana Sahyadri, Shankaraghatta-577 451, Shivamogga (Dist.). Karnataka. India.

Abstract

The plants belonging to the Asclepiadaceae family has very high medicinal property. *Asclepias curassavica* is one such plant which comes under Asclepiadaceae family. In the present study the effect of plant root extract of different solvents were screened against three bacteria and three fungi for their level of antimicrobial potential.To determine the compounds present that may produce an inhibitory effect on different classes of bacteria and fungi. Thin layer chromatography was used to assay for the compounds present and further these compounds were eluted through Column Chromatography.The crude extracts of petroleum ether, chloroform and methanol and two pure fractions obtained from methanol extract were tested for their antimicrobial property. The crude extract of chloroform was effective against *Pseudomonas solanacearum* and *Escherichia coli* than other extracts. The crude extract of methanol was effective against *Clavibacter michiganense* than other extracts. Compound 1 of methanol extract is effective than compound 2 against *Pseudomonas solanacearum* and compound 2 is effective than compound 1 against *Clavibacter michiganense* and *Escherichia coli.* The crude extract of chloroform was more effective against *Aspergillus niger* than *Helminthosporium oryzae* and *Fusarium oxysporum.* Whereas the petroleum ether and methanol extract has no antifungal property.

Keywords: *Asclepias curassavica,* antimicrobial activity, thin layer chromatography, Column chromatography.

INTRODUCTION

Medicinal plants are the plants whose extracts can be used directly or indirectly for the treatment of different ailments. Medicinal plants are an integral component of ethnoveterinary medicine. Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developed world.

An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies.

The plants belonging to the Asclepiadaceae family has very high medicinal property. *Asclepias curassavica* is one such plant which comes under Asclepiadaceae family.The plant is used medicinally in the tropics for the anodyne properties of its roots.

It has a white milky poisonous sap and grows to a height of about 3 feet with oblanceolate leaves and beautiful scarlet-orange flowers.The plant is used medicinally in the United states for the anodyne properties of its root and its rhizome and root have been employed successfully like those of *Asclepias tuberosa* both in powder and infusion, incases of asthma and typhus fever attended with catarrh, producing expectoration and relieving cough and pain .The decoction of the plant is used as an abortifacient.

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*Corresponding Author

B. Thippeswamy
Department of P.G. Studies and Research in Microbiology, Bioscience complex, Kuvempu University, Jnana Sahyadri, Shankaraghatta-577 451, Shivamogga (Dist.). Karnataka. India.

Email: thippeswamyb205@gmail.com

In Ayurvedic herbal medicine systems the plant is considered diaphoretic, anthelminthic, purgative and emetic. It is employed in India for stomach tumors, piles, gonorrhea, intestinal parasites, fever and warts.

A. curassavica is used in China to disperse fever (clears heat); improve blood circulation and to control bleeding. Entire plant is dried and decocted as a Cardiac tonic, also for tonsillitis, pneumonia, bronchitis, Urethritis, externally for wounds, other types of external and internal bleeding. Calotropin inhibits human nasopharyngeal tumors. It contains a mild cardiac glycoside (Cardiac steroids) cardenolide glycosides called alpha asclepiadin and beta asclepiadin, beta sitosterol; seed oil 53% linoleic acid but just 1% lenolenic acid (essential fatty acids).Some nicotine in sprouts as well as asclepiadin, Sitosteroid amyrin and tannins.

MATERIALS AND METHODS

Collection of plant material

Field survey was conducted during the month of January 2008. The plant was collected near Rangenahalli, Chikamagalore district. The plant was identified using the manuals and guides (Gamble, 1921; Guha Bakshi, 1999; Yoganarasimhan, 2005).

Preparation of plant extracts

The collected plant materials were brought to the laboratory. The plant materials were dried at room temperature; roots were separated and were powdered using a grinder. The powdered samples were stocked in sterilized bottles until when needed.

Hot extraction method

About 100g of root powder was used in soxhlet apparatus

using different solvents like petroleum ether, chloroform and methanol for 24hrs (Balakrishnan *et al*, 2003). The solvent was removed and the extracts were concentrated and collected in separate bottles. The extracted crude residues were subjected to thin layer chromatography and column chromatography for the separation of different chemical compounds.

Thin layer chromatography

The crude residues obtained from Petroleum ether, chloroform and methanol were subjected to Thin-layer Chromatography to determine the compound present. TLC was carried out by using solvents such as petroleum ether, Ethyl acetate, Chloroform, Methanol. Various ratios among these solvents were used as mobile phase chloroform: methanol gave a good separation. Recording all the readings the crude extracts were subjected to column chromatography.

Column chromatography

Separation of compounds by column chromatography is one of the most widely used technique in biochemical work. Silica gel (120-160 mesh) was chosen as the stationary phase and solvents are taken as mobile phase. The gel was dried at 100^0C for 12 hrs to activate it. Then the column was filled with the activated silica gel using petroleum ether.

The crude residue from methanol extract was transferred on the bed of silica gel. At first the column was run by using petroleum ether to remove chlorophyll and some coloured pigments. Then, Chloroform and Methanol in the ratio 9:1 was used to remove unwanted compounds. Finally, chloroform and methanol in the ratio 8:2 and 7:3 was used to elute 2 fractions that were collected at an internal of 10ml each and were monitored by thin layer chromatography. These obtained fractions were evaporated to dryness and compounds were stored.

Antibacterial activity

The plant extracts were tested for antibacterial activity by the Agar well diffusion method (Varadarajan *et al,* 2007*)* using three bacterial strains, *Escherichia coli, Pseudomonas solanacearum* and *Clavibacter michiganense.* About 0.1ml of bacterial culture suspension of respective strains poured over the plates containing 10 ml of Nutrient Agar in sterile Petri dishes and spread using sterile L-shaped glass rod and wells were made at the centre with sterile stainless steel cork borer (d=4mm). About 50 mg/100ml of isolated fractions and crude extracts were loaded into corresponding wells. The standard antibiotic i.e. streptomycin was used (50mg/100ml of sterile water) in order to compare the result. The plates were incubated for 24 hrs at 37^0C and the diameter of the zone of complete inhibition of the bacteria was measured around each well and readings were recorded in millimeters.

Antifungal activity

The plant extracts were tested for antifungal activity by the Agar well diffusion method using three fungal strains, *Aspergillus niger*, *Fusarium oxysporum, Helminthosporium oryzae.* About 1ml of inoculum was added into 10 ml of Potato Dextrose Agar and Mixed well, and then poured into sterilized petriplate. After solidification at room temperature for a maximum of 20 minutes, wells were made in the agar with sterile stainless steel cork borer (d=4mm). Then 50mg/100 ml of isolated fractions and crude extracts were dissolved in respective solvents and were loaded into corresponding wells. Bavistin was used as standard fungicide (50mg/100ml of sterile water) in order to compare the result. The plates were incubated for 3-4 days at 12 hrs dark and 12 hrs light at room temperature. After incubation, the diameter of the zone of inhibition of the fungi was measured around each well and readings were recorded in millimeters.

RESULTS

The yield of crude extracts obtained from *Asclepias curassavica* using Soxhlet apparatus was recorded.

The crude extract of petroleum ether was in the form of green paste and yielded about 1.0g. The crude extract of Chloroform was in the form of brown paste and yielded about 1.5g and the crude extract of methanol was in the form of brown paste and yielded about 2.5g.

The crude extract of methanol when subjected to column chromatography using chloroform and methanol in the ratio 8:2 and 7:3 yielded 2 pure fractions of about 1.06 g and 1.08g respectively.

Antibacterial activity

The antibacterial activity was screened by the agar well diffusion method against three bacterial strains *Pseudomonas solanacearum, Escherichia coli* and *Clavibacter michiganense.* The inhibition zone of chloroform (26mm) recorded was more than petroleum ether (25mm) and methanol (22mm) but was less than streptomycin (35mm) against *Pseudomonas solanacearum*. The inhibition zone of methanol (28mm) was more than chloroform (27mm), Petroleum ether (20mm) and streptomycin (15mm) against *Clavibacter michiganense.* The inhibition zone of chloroform (30mm) was more than methanol (22mm) and petroleum ether(15mm) but less than streptomycin (40mm) against *Escherichia coli* (Fig 1,2,3,6,7). The inhibition zone of compound I (12mm) was less than streptomycin (35mm) whereas compound II and control did not show any inhibition zone against *Pseudomonas solanacearum*. The inhibition zone of compound II (13mm) recorded was more than streptomycin (5mm) whereas compound I and control did not show any inhibition zone against *Clavibacter michiganense*. The inhibition zone of compound II (21mm) was more than compound I (6mm) but less than streptomycin (40mm) against *Escherichia coli*. There was no inhibition zone in control. (Fig 4,5,6,7)

Antifungal activity

Antifungal activity was evaluated using agar well diffusion method against *Fusarium oxysporum, Helminthosporium oryzae and Aspergillus niger.* The chloroform extract showed inhibition zone of 13mm ,19mm and 13mm against *Helminthosporium oryzae, Aspergillus niger* and *Fusarium oxysporum* respectively, whereas petroleum ether extract and methanol extract did not show any inhibition zone. Bavistin showed inhibition zone against *Aspergillus niger (16mm)* and *Fusarium oxysporum (13mm)* but there was no inhibition zone against *Helminthosporium oryzae.(fig 8,9,10,13,14).* The compounds obtained from the methanol extract

did not show any inhibition zone against *Fusarium oxysporum, Aspergillus niger and Helminthosporium oryzae.* But Bavistin showed inhibition zone of 16 mm and 13mm against *Aspergillus niger* and *Fusarium oxysporum* respectively.(fig 11,12,13,14)

Table 1. Growth inhibition zone (mm) of crude extracts and methanol extracts against different bacteria in agar diffusion method

Extracts	Growth inhibition zone (in mm)		
	Clavibacter michiganense	***Pseudomonas solanacearum***	***Escherichia coli***
Petroleum ether extract (Crude)	20	25	15
Methanol extract (Crude)	28	22	22
Chloroform extract (Crude)	27	26	30
Methanol extract I	0	12	6
Methanol extract II	13	0	21
Streptomycin (standard)	15	35	40

Table 2. Growth inhibition zone (mm) of crude extracts and methanol extracts against different fungi in agar diffusion method

Extracts	Growth inhibition zone (in mm)		
	Aspergillus niger	***Helminthosporium oryzae***	***Fusarium oxysprorum***
Petroleum ether extract (Crude)	0	0	0
Methanol extract (Crude)	0	0	0
Chloroform extract (Crude)	19	13	13
Methanol extract I	0	0	0
Methanol extract II	0	0	0

DISCUSSION

There is no reported antimicrobial activity of *Asclepias curassavica,* but it is found that the latex of this plant can inhibit the growth of *Candida albicans* (Moulin - Traffort *et al., 1990).* In the present work chloroform extract showed maximum antimicrobial activity. Raja *et al.* (2005) have screened the extract of the aerial part of *Asclepias curassavica* for both invitro and invivo antioxidant activity. Different concentrations of different extracts (Chloroform, ethyl acetate, Methanol and Hydro alcohol) of *Asclepias curassavica* were investigated for invitro antioxidant activity using the thiocyanate method. The hydro alcoholic extract was administered to rats. The hydro alcoholic extract of *Asclepias curassavica* had significant antioxidant activity, which might be helpful in preventing various oxidative stress related diseases. Koike *et al*. (1980) have shown the cytotoxic activity *Asclepias albicans*. Kubmarawa *et al*. (2007) have screened Ethanolic extracts of plants from Nigeria for their antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Averineni Ravi Kumar *et al*. (2007) have conducted the phytochemical screening of selected medicinal plants of Asclepiadaceae family.

CONCLUSION

Asclepias curassavica is known for its medicinal property .It has a good antimicrobial property. The root extracts of *Asclepias curassavica* can be used as broad spectrum antibiotic. It has the capacity to control the plant pathogens as well as human pathogens. It is ecofriendly. The plant should be conserved for future use.

REFERENCES

[1] Averineni Ravikumar, K.M. Subbu Rathinam and G.Prabakar, 2007, Phytochemical Screening of selected medicinal plant of Asclepiadaceae family. *Asian journal of Microbiology, Biotechnology and Environmental Science,* Vol.9, 177-180.

[2] Anwar M.N, Jaripa Begum, Shoma Dutta,S. Khan, Md. Yusuf and J.U. Chowdhury, 2007, Screening of thirty eight medicinal plants of Bangladesh for antibacterial activities. *Asian Journal of Microbiology, Biotechnology, and Environmental Science,* Vol. 9, 627-632.

[3] Balakrishnan B.R., B. Sangameswaran, B.Arul and V.H. Bhaskar, 2003, Antibacterial Activity of Aerial Part Extracts of *Achyranthes bidentata* Blume. *Indian Journal of Pharmaceutical Science*,186-187

[4] Bohra N.K. and D.K. Purohit, 2002, Effect of some aqueous plant extracts on toxigenic strain of *Aspergillus flavus. Advances in Plant Sciences*, 15: 103-106.

[5] Dushyent Gehlot and A.Bohra, 1998, Antimicrobial Activity of Various plant part extracts of *Aerva Persica*. *Advances in plant science, 11:* 109-111.

[6] Dushyent Gehlot and A. Bohra, 2002, Anti-Salmonellae activity of stem extracts from arid zone plants. *Advances in plant Sciences*, 15:25-27.

[7] Gamble.J.S,1921,Flora of the presidency of Madras, Adlard and son, limited, 833.

[8] Guha bakshi. D.N., P.Sensarma, D.C.Pal ,1999, A lexicon of Medicinal plants in India, Naya prakash, Calcutta, 585.

[9] Kubmarawa D, G.A. Ajoku, N.M.Enwerem and D.A.Okorie, 2007, Preliminary Phytochemical and Antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology,* Vol. 6:1690-1696.

[10] Leena Parihar, Pradeep Parihar and Achaleshwar Bohra, 2007, Antimicrobial Activity of root extracts of some species plant. *Advances in plant sciences*, 20:379-381.

[11] Moulin–Traffort.J, R.Giordani and P.Regli, 1990, Antifungal action of latex saps from *Lactuca sativa* and *Asclepias*

curassavica.Mycoses, 33:383-392.

[12] Parekh J., S.Chanda, 2007, Invitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogen from Enterobacteriaceae. *African Journal of Microbiology Research*, Vol. 1:92-99.

[13] Prabha purohit and A. Bohra, 2002, Antifungal activity of various Spice plants against Phytopathogenic *Fungi. Advances in plant sciences,* 15: 615-517.

[14] Srinivasan K., D. Natarajan, C. Mohanasundari, G.Perumal, M.Abdul Nazar Dheen, Anbu Ganapathi, and T. Rajarajan, 2005, Antifungal properties of three medicinal plant extracts against *Cercospora arachidicola*. *Advances in plant sciences* 18: 45-47.

[15] Sumathi and Pushpa, 2007, Evaluation of antibacterial activity of some Indian Medicinal Plants. *Asian Journal of Microbiology, Biotechnology and Environmental Science*, Vol. 9:201-205.

[16] Venkatesan S and R. Ravi, 2003, Antifungal Activity of *Eclipta alba*. *Indian Journal of Pharmaceutical Science*, 97-98.

[17] Vimala Y., P.Suneetha and K.M. Elizabeth, 2007, Antimicrobial Activity of Ethanol and Chloroform Extracts of *Decalepis hamiltoni* on pathogenic microorganisms and microbial isolates of spoiled vegetables. *Asian journal of microbiology, Biotechnology, and environmental science*, vol 9: 79-81.

[18] Varadarajan M., V.Guruchandran, S.M. Nagarajan, E. Natarajan, R. Punitha, C. Geetha and S. Ramya, 2007, Screening for the evaluation of antibacterial activity of *Solanum nigrum.* Asian *Journal of Microbiology, Biotechnology, Environmental Science,* Vol. 9: 79-81.

[19] Yoganarasimhan.S.N, 2005, Flora of Chikmagalur District, Karnataka, India, International book distributors, India: 211.