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Efficacy of Akanda (*Calotropis gigantea* L.) against some phytopathogenic bacteria

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Abstract: The antibacterial activities of ethyl alcohol, ethyl acetate, chloroform, acetone, n-hexane and dichloromethane extracts from the leaf, stem and root of akanda (*Calotropis gigantea* L.) were investigated against two phytopathogenic bacteria: *Xanthomonas oryzae pv. oryzae* and *Ralstonia solanacearum*, and a symbiotic bacterium *Rhizobium* sp. Leaf and root extracts of ethyl alcohol, ethyl acetate and chloroform; and stem extracts of ethyl alcohol and ethyl acetate showed antibacterial activities against all the test organisms. The maximum inhibition zone of all the test organisms were found with the leaf extracts followed by the root and the stem extracts. Among all the extracts, the maximum zone of inhibition of *X. oryzae pv. oryzae* have been observed with the leaf extracts of ethyl acetate followed by the stem and the root extracts of acetone, respectively. The maximum zone of inhibition of *R. solanacearum* and *Rhizobium* sp. was found with the ethyl alcohol leaf extract followed by the stem and the root extracts. The filter paper disc diffusion method and the broth serial dilution technique were applied to screen the antibacterial efficacies of the extracts and to determine the minimum inhibitory values, respectively. The minimum inhibitory concentration (MIC) of all the extracts ranged from 2.0 - 8.0 mg/mL. These results revealed that *C. gigantea* L. has antimicrobial activities against the three tested bacteria.
Key words: *Calotropis gigantea* L., antibacterial activity, *Xanthomonas oryzae* pv. *oryzae*, *Ralstonia solanacearum*, *Rhizobium* sp, MIC

Introduction

Plants are a rich source of different types of medicines. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological tests and a sustainable number of new antibiotics on the market are obtained from natural or semi-synthetic resources. It has been reported that between the years 1983 and 1994 the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria (Crag *et al.*, 1999). The uses of plant extracts and phytochemicals both with known antimicrobial properties are of great significance. In the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants (Nascimento *et al.*, 1990). Especially from the last decade, plants are used as a valuable source of natural products for maintaining human health with more intensive studies for natural therapies. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs (Santos *et al.*, 1995). Medicinal plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plants. These products are known by their active substances, for example, the phenolic compounds, which are a part of the essential oils (Jansen *et al.*, 1987) and tannin (Saxena *et al.*, 1994). Akanda (*Calotropis gigantea* L.) belonging to the family Asclepiadaceae grows widely throughout the Indian subcontinent and used as a traditional medicinal plant with its unique properties (Oudhia, 1999). Traditionally *C, gigantea* is used alone or with other medicinal plants to treat common human diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhoea (Das, 1996). According to Ayurveda, dried whole plant is a good tonic; expectorant, depurative and anthelmintic. Literally, it was also reported that alcoholic root extract of *C. gigantea* showed analgesic, anticonvulsant, anxiolytic and sedative effects in albino rats (Argal and Pathak, 2006). Extracts of different plant parts *viz*. root, stem, leaf and stem+leaf of *C. gigantea* L. affect germination and seedling vigor of many agricultural crops (Oudhia and Tripathi, 1999).

The purpose of the present study was aided to prepare different extracts from leaf, stem and root bark of *C. gigantea* L. to assess their antibacterial activities against two plant pathogenic bacteria namely *Xanthomonas oryzae* pv. *oryzae* and *Ralstonia solanacearum*, and a symbiotic bacterium, *Rhizobium* sp.

Materials and Methods

The experiment on antibacterial activities of Akanda (*Calotropis gigantea* L.) against three bacteria species was conducted at the laboratory, Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

Plant materials: The fresh leaves, stems and roots of *C. gigantea* L. were collected from the HSTU campus. At first, fresh specimens were washed thoroughly 2-3 times with running tap water followed by sterile water and shade-dried. After that the dried plant parts were made powder and used for extraction.

Test microorganisms: The test bacteria (*Xanthomonas oryzae* pv. *oryzae*, *Ralstonia solanacearum*) and a symbiotic bacterium (*Rhizobium* sp). were isolated from the bacterial leaf blight disease of rice, wilt of potato and root nodules of pulse crops. After isolation, the bacteria were cultured on nutrient agar medium and subsequently pure cultures of all bacteria were made at the laboratory.

Preparation of solvent extractions: The powdered plant materials (25 g) were extracted with ethyl alcohol, ethyl acetate, chloroform, n-hexane, acetone and dichloromethane solvents using a Soxhlet extractor for 48 hours and the extraction process was performed four times. The extracts were filtered through Whatman No. 1 filter paper. The filtrates were concentrated with a rotary evaporator under reduced pressure at 60^0C to have the crude extracts. After complete evaporation, each of those extracts were weighed and preserved at room temperature in airtight bottles until further use. One gram of each solvent residue was dissolved in 10.0 ml of respective solvents and used as the test extracts for antimicrobial activity assay.

Antibacterial activity assay: Antibacterial activity of the aqueous and solvent extracts was determined by the disc diffusion method on nutrient agar medium (Vander and Vlietnck, 1991). Sterile Whatman filter discs (6 mm

diameter) were made in the nutrient agar plate using sterile cork borer (5 mm). To study the antibacterial activities, standard kanamycin disc (30 µg/disc) and blank disc impregnated with the respective solvents were used as the positive and the negative controls, respectively. The antibacterial activities of ethyl alcohol, ethyl acetate, chloroform, n-hexane, acetone and dichloromethane were tested at concentrations of 30 µg/disc. The plates were incubated for 72 hours at 28^0C. At the end of the incubation period, the clear zone of inhibition around the disc was measured in millimeter (mm).

Determination of Minimum Inhibition Concentrations (MIC): The MIC values for the six solvents were determined by the serial tube dilution technique (Reiner, 1982) against *X. oryzae* pv. *oryzae*, *R. solanacearum* and *Rhizobium* sp.

Results

The results of the antibacterial activities of the leaf, stem and root extracts are presented in the Table 1 which revealed that all the six extracts exhibited positive response against the three test organisms.

Leaf extract: In case of *X. oryzae pv. oryzae* ethyl acetate extracts showed the highest zone of inhibition (5.0 mm) followed by chloroform (4.0 mm) and acetone (4.0 mm) and the lowest with ethyl alcohol and dichloromethane (3.0 mm) but n-hexane had no effect. Ethyl alcohol leaf extracts showed the highest inhibition zone (6.0 mm) against *R. solanacearum*, followed by n-hexane (4.5 mm) whereas the lowest one with chloroform (3.0 mm) and acetone extracts showed no activity. All the extracts had significant performance in terms of inhibition zone against the non-pathogenic bacterium where ethyl alcohol revealed the highest (8.0 mm) and dichloromethane the lowest (3.0 mm) inhibition zones. Thus, it can be concluded that *C. gigantea* leaf extracts was superior in inhibiting the growth of all the three organisms studied. The result revealed that non plant pathogenic bacteria were inhibited to the large extent compared to plant pathogenic bacteria. Between the plant pathogenic bacteria the inhibition zone of *R. solanacearum* was more than the *X. oryzae* pv. *oryzae* by leaf extracts of *C. gigantea.*

Stem extract: Various stem extracts of Akanda in six different solvents exhibited activity against the three test organisms studied (Table 1). The highest inhibition of *X. oryzae* pv. *oryzae* occurred by acetone extract (4.0 mm) followed by chloroform (3.0 mm) and two extracts dichloromethane and n-hexane had no activity. The maximum inhibition of *R. solanacearum* occurred by ethyl alcohol (5.0 mm) extracts and the minimum by ethyl acetate extracts (1.0 mm) whereas chloroform, n-hexane and acetone extract had no activity against *R. solanacearum.* In case of *Rhizobium* sp., the maximum inhibition (7.0 mm) was found in ethyl alcohol whereas chloroform had no effect. The growth of *Rhizobium* sp. was more inhibited than those of *X. oryzae* pv. *oryzae* and *R. solanacearum* when ethyl alcohol solvent extract was used. From the results it is concluded that ethyl alcohol stem extract showed better performance than that of other extracts.

Table 1. Antibacterial activity of Akanda (*Calotropis gigantea* L.) leaf, stem and root extracts

Source (Akanda)	Extracts	Zone of inhibition (mm)		
		Xanthomonas oryzae pv. *oryzae*	*Ralstonia solanacearum*	*Rhizobium sp.*
Leaf	Ethyl alcohol	3.0	6.0	8.0
	Ethyl acetate	5.0	4.0	6.0
	Chloroform	4.0	3.0	5.0
	n-Hexane	-	4.5	7.0
	Acetone	4.0	-	6.5
	Dichloromethane	3.0	4.0	3.0
Stem	Ethyl alcohol	0.5	5.0	7.0
	Ethyl acetate	1.0	1.0	1.0
	Chloroform	3.0	-	-
	n-Hexane	-	-	2.0
	Acetone	4.0	-	1.0
	Dichloromethane	-	4.0	1.0
Root	Ethyl alcohol	1.0	5.0	4.6
	Ethyl acetate	2.0	2.5	5.5
	Chloroform	2.5	2.0	2.0
	n-Hexane	-	2.0	4.0
	Acetone	2.6	-	5.0
	Dichloromethane	1.0	2.5	1.0

- indicates no inhibition.

Table 2. Minimum inhibitory concentration of six different solvent extracts of Akanda against antibacterial activities

Source (Akanda)	Extracts	MIC (mg/mL)		
		Xanthomonas oryzae pv. *oryzae*	*Ralstonia solanacearum*	*Rhizobium sp.*
Leaf	Ethyl alcohol	4.0	2.0	2.0
	Ethyl acetate	4.0	4.0	3.0
	Chloroform	3.0	3.0	4.0
	n-Hexane	5.0	4.0	2.0
	Acetone	3.0	6.0	3.0
	Dichloromethane	4.0	4.0	4.0
Stem	Ethyl alcohol	4.0	2.0	2.0
	Ethyl acetate	4.0	5.0	5.0
	Chloroform	3.0	5.0	4.0
	n-Hexane	4.0	4.0	8.0
	Acetone	2.0	4.0	5.0
	Dichloromethane	4.0	2.0	4.0
Root	Ethyl alcohol	3.0	2.0	3.0
	Ethyl acetate	3.0	3.0	2.0
	Chloroform	2.0	3.0	3.0
	n-Hexane	6.0	4.0	4.0
	Acetone	2.0	4.0	3.0
	Dichloromethane	4.0	3.0	4.0

Root extract: Root extracts of *C. gigantea* using different solvents showed different antibacterial activities (Table 1). The growth of *X. oryzae* pv. *oryzae* was inhibited by all the extracts except n-hexane but the maximum (2.6 mm) was found with acetone and the lowest with both ethyl alcohol and dichloromethane extracts (1.0 mm). The growth of *R. solanacearum* was inhibited by all the extracts none but acetone. The highest inhibition (5.0 mm) was observed in ethyl alcohol and the lowest (2mm) was in both chloroform and n-hexane. A considerable zone of inhibition (*Rhizobium* sp.) was found by all the root extracts. The maximum inhibition (5.5 mm) was found with acetone and the minimum (1.0 mm) with dichloromethane extracts. It is clear from the Table 1 that *Rhizobium* sp. inhibition was more followed by *R. solanacearum* and *X. oryzae* pv. *oryzae.*

Minimum inhibitory concentration (MIC) of solvent extracts: MIC of ethyl alcohol and dichloromethane extracts ranged from 2.0-4.0, ethyl acetate and chloroform 2.0-5.0, n-hexane 2.0-8.0, acetone 2.0-6.0 mg/mL (Table 2).

Discussion

Ethyl alcohol, ethyl acetate, chloroform, n-hexane, acetone, dichloromethane solvents and aqueous extracts of the leaf, stem and root were subjected to a preliminary test of antimicrobial activities against two phytopathogenic bacteria, *X. oryzae* pv. *oryzae* and *R. solanacearum* and a symbiotic bacterium, *Rhizobiaum* sp. It is clear from the present results that all the extracts exhibited pronounced activities against all the three tested bacteria. In vitro antibacterial activities against some pathogenic bacteria have been reported by Alam *et al.* (2008). The antibacterial activities of leaf extract were promising than the extracts of its root and stem. In case of leaf extracts, the growth of *X. oryzae* pv. *oryzae* is maximum inhibited by ethyl acetate, *R. solanacearum* by ethyl alcohol and *Rhizobium* sp. by ethyl alcohol too followed by n-hexane. Except n-hexane and dichloromethane, all other extracts had the inhibitory effects against all the bacteria whereas ethyl alcohol and ethyl acetate had the highest effects than other extracts. High activity of stem extracts against *X. oryzae* pv. *oryzae* was achieved in acetone followed by chloroform. Like leaf extracts, the maximum inhibition of *R. solanacearum* and *Rhizobium* sp. was found by ethyl alcohol extracts. The activity of root extracts revealed that *X. oryzae* pv. *oryzae* was maximum inhibited by chloroform, *R. solanacearum* by ethyl alcohol and *Rhizobium* sp. by ethyl acetate. Similar to leaf extracts of only ethyl alcohol, ethyl acetate and chloroform showed their antibacterial activities against all the test pathogens. This tends to show that the active ingredients of these plant parts are better extracted with the ethanol and chloroform solvent than all other solvents. The extracts may contain alkaloids, coumarins and tannins like methanol extracts (Okemo, 1996). Coumarins and tannins have antibacterial and antihelminthis properties (Hedberg *et al*., 1983). Eloff (1998) and Cowan (1999) also found that methanol was more efficient than acetone in extracting phytochemicals from plant materials. The absence of antibacterial activities of acetone, n-hexane and dichloromethane extracts indicates the insolubility of the active ingredients in these solvents. In general, the activities against test bacterial culture used have shown

good activity when compared with standard antibiotics. High activity was found in all extracts against the *Rhizobium* sp. Sharif *et al*. (2006) reported that *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracted in chloroform were found to inhibit *E. coli* and *X. vasicatoria* at minimum inhibitory concentration (MIC) ranged between 0.25 -6.0 mg/mL. Sukanya *et al*. (2009) reported that *Chromolaena odorata* extracted in methanol, ethanol, ethyl acetate, chloroform, chloramphenicol and aqueous were found to inhibit *X. vesicatoria* and *R. solanacearum* at minimum inhibitory concentration (MIC) ranged between 2.0 - 9.0 mg/mL.

References

Alam, M.A., Habib, M.R., Nikkon, F., Rahman, M. and Karim, M.R. 2008. Antimicrobial activity of akanda (*Calotropis gigantea* L.) on some pathogenic bacteria. Bangladesh J. Sci. Ind. Res. 43(3): 397- 404.

Argal, A. and Pathak, A.K. 2006. CNS activity of *Calotropis gigantea* roots. J. Ethnopharmacology. 106(1): 142-145.

Cowan, M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-582.

Crag, G.M., Boyd, M.R.., Khanna, R., Kneller, R., Mays, T.D., Mazan, K.D., Newman, D.J. and Sausville, E.A. 1999. International collaboration in drug discovery and development: the NCI experience. Pure Appl. Chem. 71: 1619-1633.

Das, B.B. 1996. Rasraj Mahodadhi. Khemraj Shri Krishnadas Prakashan, Bombay.

Eloff, J.N. 1998. Which extractan should be used for screening and isolation of antimicrobial components from plants. J. Ethnopharm. 60: 1-8.

Hedberg, L., Hedberg, O., Madati. P., Mshigeni. K.E., Mshiu, E.N. and Samueisson, G. 1983. Inventory of plants used in traditional medicine in Tanzania. II Plants of the family dilleniaceae to opiliaceae. J. Ethnopharm. 9: 105-128.

Jansen, A.M., Cheffer, J.J.C. and Svendsen, A.B. 1987. Antimicrobial activity of essential oils; A 1976-1986 literature review. Aspects of test methods. Plant. Med. 40: 395-398.

Nascimento, S.C., Chiappeta, A. and Lima, R.M. 1990. Antimicrobial and cytotoxic activities in plants from pernambuco, Braz. Fitoter. 61: 353-355.

Okemo, P.O. 1996. Antimicrobial efficacy of selected medicinal plants used by Kenyan Herbal doctors. Ph.D thesis. Kenyatta University of Nairobi. pp. 173-190.

Oudhia, P. 1999. Int. Rice Res. Notes 24(1): 40.

Oudhia, P. and Tripathi, R.S. 1999. World Weeds 4: 109-119.

Reiner, R. 1982. Detection of antibiotic activity, *In*: *antibiotics-an introduction* (Roche Scientific Services, Swtzerland). pp. 21-27. microbial activity of selected Peruvian medicinal plants. J. Ethanopharm. 88: 199-204.

Santos, P.R.V., Oliveira, A.C.X. and Tomassini, T.C.B. 1995. Control microbiogicode products. Fitoterapicos. Rev. Farm. Bioquim. 31: 35-38.

Saxena, G., McCutcheon, A.R., Farmer, S., Towers, G.H.N and Hancock, R.E.W. 1994. Antimicrobial constituents of *Rhus glabra*. J. Ethanol. Pharm. 42: 95-99.

Sharif, N., Sudarshana, M.S., Umesha, S. and Hariprasad, P. 2006. Antimicrobial activity of *Rauvolfia tetraphylla* and *Phyusalis minima* leaf and callus extracts. Afr. J. Biotech. 5(10): 946-950.

Sukanya, S. L., Sudisha, J., Hariprasad, P., Niranjana, S.R., Prakash, H.S. and Fathima S. K. 2009. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. African J. of Biotech. 8(23): 6677-6682.

Vander, B.D.A. and Vlietnck. 1991. Screening methods for antibacterial and antiviral agents from higher plants. *In*: *Assay for Bioactivity*. (K. Hostiettman Academic Press, London). pp. 47-69.