academicJournals

Vol. 12(30), pp. 4836-4840, 24 July, 2013
DOI: 10.5897/AJB2012.11917
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Antibacterial evaluation of *Anacardium occidentale* (Linn) (Anacardiaceae) in semiarid Brazil

Francianne Oliveira Santos[1]*, Elissandra Couras Angélico[1], José Galberto Martins da Costa[2], Fabíola F. G. Rodrigues[2], Onaldo Guedes Rodrigues[3] and Rosália Severo de Medeiros[3]

[1]Programa de Pós-graduação em Zootecnia, Universidade Federal de Campina Grande, Av. Universitária s/n, Bairro Santa Cecília, 58708-110 Patos-PB, Brasil.
[2]Departamento de Química Biológica, Laboratório de Pesquisa de Produtos Naturais, Universidade Regional do Cariri, Rua Cel. Antônio Luiz 1161, Pimenta, 63105-000 Crato-CE, Brasil.
[3]Laboratório de Multiusuários de Pesquisas Ambientais - LAMPA, Unidade Acadêmica de Ciências Biológicas, Universidade Federal de Campina Grande, Av. Universitária s/n, Bairro Santa Cecília, 58708-110 Patos-PB, Brasil.

Accepted 19 July, 2013

Microorganisms that cause losses are proving to be resistant to most known antibiotics, thereby encouraging the search for naturally occurring antibiotics. This study aimed to perform a phytochemical and bacterial study of ethanolic extracts of leaves and barks of *Anacardium occidentale* L. The samples were submitted for extraction using ethyl alcohol; the crude extract was used to perform phytochemical evaluation based on the identification of chemical constituents and to evaluate the antibacterial activity. The results of prospective chemical indicate the presence of tannins, phenols, alkaloids and catechins in the leaves and stem bark and compounds belonging to classes of flavonoids were found only in the leaves. Tests using the agar diffusion method for later determination of minimum inhibitory concentration (MIC) revealed that both parts of the plant have antibacterial activity, but the shell showed the largest zones of inhibition in most of the concentrations and strains. The MIC ranged from 512 to ≥ 1024 µg/ml, for the two parts of this species. The results indicate the need for further studies because this plant demonstrated considerable antibacterial therapeutic potential.

Key words: Antibacterial activity, phytochemicals, bacteria, minimum inhibitory concentration.

INTRODUCTION

The constant use of antibiotics has caused divers problems, among which are microbial resistance. This has led to the seeking of new antibiotics that are effective and opens paths for the evolution of research because development of any new antimicrobial is accompanied by the resistance of microorganisms (Silva et al., 2007). Brazil, for its vast and rich plant biodiversity, has great potential for discovery and development of new bioactive and natural molecules with antimicrobial effects. Many plants are used as medical therapy in several pathologies such as bacterial infections. The vegetation of the Caatinga has great potential botanists. Among the species of this biome, originally from tropical America, the cashew (*Anacardium occidentale* L.) cultivated species and more dispersed of the genus belongs to the Anacardiaceae family, which includes trees and shrubs of tropical and subtropical regions. Their uses include: for preparation of "cauim" or "mocororó" used for flour, the dry bagasse and almond roasted nut are used for feeding and preparing remedies and the decoction and infusion are used in popular medicine as hypoglycemic (Barbosa-Filho et al., 2005). Phytochemical study of medicinal

*Corresponding author. E-mail: francy_medvet@yahoo.com.br or galberto.martins@gmail.com.

plants constitutes a strategy alternative in seeking new therapeutic agents; both the bibliographical survey and popular knowledge serve as basis for identifying the pharmacological activity of medicinal plants. The importance of the demand for constituents of the secondary metabolism of plants has increased and stimulated the search for new compounds in plants with biological activities (Doss and Thangavel, 2011). In this way, several studies were based on traditional practices of popular medicine to make triage of antibacterial activity. Melo et al. (2006) evaluated the antibacterial activity *in vitro* of stem peels extract of *A. occidentale* L. against species of Streptococcus (*S. mitis*, *S. Mutans* and *S. sanguis)* present in the bacterial biofilm supra gingival, with diameters of the halos ranging from 11 to 19 mm (Bouzada et al., 2009). The extract of leaves of the cashew showed activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Salmonella enteric sorovar typhimurium* and *Klebsiela pneumonia*; their halos range from 10 to 23 mm, showing the great efficiency of the plant.

Owing to the intense use of cashew in the Northeast for therapeutic, this study aimed to evaluate the antibacterial activity of ethanol extract of the leaves and stem peels of *A. occidentale* L. as alternative treatment for infections caused by bacterial agents.

MATERIALS AND METHODS

The experiment was conducted at the Research Laboratory Products Natural (RLPN) Regional University Cariri (URCA), Crato-CE. The leaves and stem peels of *A. occidentale* L., were collected in October 2011 at Forest Nursery at the Center for Rural Health and Technology, Federal University of Campina Grande (UFCG), in the morning. After collection, plants' parts were taken for botanical identification and preparation of voucher specimen, which was mounted and deposited in the Herbarium of UFCG, Patos-PB, under registry number 1522.

Obtaining the ethanol extract

To obtain the ethanol extracts of the stem peels and leaves of *A. occidentale* L. species, 310 and 193 g of respective parties were used, and then subjected to extraction with 95% ethanol at room temperature. After 48 h, the mixtures were filtered and distilled in a rotary evaporator at 65°C under reduced pressure and yielded crude extracts. These were heavy and stored at ambience temperature until the realization of the phytochemical analysis and antibacterial assays (Matos, 1997).

Phytochemical of prospective extracts

The identification of chemical classes present in extracts of *A. occidentale* L., according to Matos (1997)' method, is based on the observation of color change or formation of precipitate after the addition of specific reagents.

Antibacterial activity of stem peels and leaves of *Anacardium occidentale* L.

The species of bacteria used in microbiological tests were provided by the Laboratory of Microorganisms of Reference of the National Institute of Quality Control in Health (NIQCS), the Oswaldo Cruz Foundation (FIOCRUZ) and the Research Laboratory of Natural Products (RLNP) of Regional University Cariri/CE; they were three Gram-positives: *S. aureus* (ATCC12692), *S. aureus* MR (358), *B. cereus* (ATCC33018) and three Gram-negatives: *P. aeruginosa* (ATCC 15442), *K. pneumoniae* (ATCC 10031) and *Escherichia coli* (ATCC 25922). The lineages were grown in nutrient broth (BHI-Brain Heart Infusion - DIFCO) and incubated at 37°C for 24 h. The antibacterial activity was performed in duplicate, based on the technique of cavity gel by selecting the sensitive strains; then the Minimum Inhibitory Concentration (MIC) was determined, using orifices (6 mm in diameter) filled with 20 µl of the solution of the extract diluted in ethanol in the following concentrations: 10, 5, 2.5, 1.25, 0.6 and 0.3%. The plates were incubated in a bacteriological incubator at 37°C for 24 h reading of performance was done after 24 and 48 h.

The sensitivity of the sample was considered: halos ≥ 10 mm. The tests were accompanied by positive control with antibiotic chloramphenicol (30 mg) and negative control with ethanol (Romeiro, 2001).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was evaluated using the broth micro dilution methodology, based on document M7-A6 (CLSI, 2007) for bacteria. Previously, for the test, the bacterial lineages were activated in the Brain Hear Infusion Broth (BHI, 3.8%) for 24 h at 35 ± 2°C. After this, sub-culturing was the standardization of the inoculum, which consists of preparing a bacterial suspension in BHI; its turbidity tube was similar to the 0.5 Mc Farland scale (1×10^8 CFU/ml). Next, this suspension was diluted to 1×10^6 CFU/ml in BHI broth (10%), with 100 ml and then homogenized in the pits of a micro dilution plate supplemented with different concentrations of the natural product. This resulted in final inoculums of 5×10^5 CFU/ml (CLSI, 2007). The final concentrations of the extracts were 512, 256, 128, 64, 32, 16 and 08 g/ml. The tests were performed in duplicate and the plates were incubated at 35 ± 2°C for 24 h. 20 ml/pits of 0.01% resazurin were used. The negative control was performed with 100 ml BHI broth plus the standardized bacterial inoculum.

The MIC was defined as the lowest concentration that completely inhibited microbial growth in micro dilution pits as detected by the naked eye. The reading of the results for MIC determination was considered positive for the pits that remained with the blue color and negative for those with red color (Romeiro, 2001).

RESULTS AND DISCUSSION

After 48 h of extraction, the mixtures were filtered and distilled in a rotary evaporator. This yielded 8.7% for stem peels and 12.4% for leaves, with final mass of 27 and 24 g respectively.

In the ethanol extract of leaves of *A. occidentale* L. the presence of hydrolysable tannins, phenols, flavones, flavonols, xanthones, chalcones, auron, flavononois, catechins and alkaloids was identified. The extract from the stem peels showed flobabênicos tannins (condensed or catechists tannins), phenols, catechins and alkaloids (Table 1).

According to Gobbo and Lopes (2007), factors such as seasonality, circadian rhythm, age or development stage, temperature, water availability, UV radiation, mechanical

Table 1. Phytochemical screening of leaf and bark obtained from *Anacardium occidentale* L.

Phytochemical screening	Bark's stem	Leaf
Test of phenols and tannins	++	++
Test of anthocyanins, antocianidynes and flavonoids	---	--+
Test of leucoanthocyanidins, catechins and flavones	-+-	-++
Test for flavonols, flavanones, flavanonóis and xanthones	----	+-+++
Test alkaloids	+	+

+, Presence of compounds; - absence of compounds.

Table 2. Antibacterial activity of ethanol extract of stem peels and leaves of *Anacardium occidentale* L.

Plant part	Concentrations of the extracts in % (mg/ml)	Microorganism					
		P. aeruginosa	***S. aureus***	***S. aureus* M.R**	***E. coli***	***K. pneumoniae***	***B. cereus***
Peels	10	11.5±0.7*	23.5±2.12*	15.0±1.41*	9.0±0.0	9.5±0.7	21.0±0.0*
	5	9.5±0.7	15.0±1.41*	13.5±2.12*	8.0±0.0	10.0±0.0*	17.0±0.0*
	2.5	9.5±0.7	13.0±2.82*	12.0±1.41*	5.0±7.07	8.5±0.7	15.0±0.0*
	1.25	6.0±8.48	10.5±3.53*	10.0±0.0*	0.0±0.0	8.0±0.0	12.5±0.7*
	0.6	10.5±0.7*	5.0±7.07	9.5±0.7	0.0±0.0	4.0±5.65	11.5±0.7*
	0.3	5.0±7.07	0.0±0.0	7.5±0.7	0.0±0.0	3.5±4.94	10.0±1.41*
Leaves	10	10.0±0.0*	13.5±4.94*	10.0±2.82*	9.5±0.7	9.5±2.12	16.5±0.7*
	5	10.0±0.0*	8.0±1.41	6.0±8.48	9.5±0.7	9.0±1.41	10.0±1.41*
	2.5	9.0±0.0	7.5±0.7	0.0±0.0	7.5±0.7	4.5±6.36	11.0±1.41*
	1.25	8.0±0.0	7.5±0.7	0.0±0.0	7.5±0.7	4.5±6.36	9.0±0.0
	0.6	9.0±1.41	7.0±0.0	0.0±0.0	4.5±6.36	9.0±1.41	8.0±0.0
	0.3	0.0±0.0	8.0±1.41	0.0±0.0	3.5±4.94	3.5±4.94	0.0±0.0
Control (+)	Cloranfenicol	0.0±0.0	16.0±0.0*	21.0±0.0*	0.0±0.0	15.0±0.0*	23.0±0.0*
Control (-)	Ethanol	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Values are means of inhibition halos (mm diameter) (mean + standard deviation).

stimulation and pathogenic attacks can influence the amount and nature of active constituents in the plant. Seasonal variations can change the content of virtually all classes of secondary metabolites such as essential oils, phenol acids, flavonoids, saponins, alkaloids, tannins, among others. There is also a correlation between solar radiation intensity and production of phenol compounds such as flavonoids, tannins and anthocyanins.

Table 2 shows the results obtained with the ethanol extracts of the stem peels and leaves of *A. occidentale* L. It demonstrated that there was some bacterial inhibition by verification of the training of halos around the pits where the solutions

Table 3. Values in minimal inhibitory concentration (µg/ml) (MIC) of ethanol extracts of leaves and stem peels of *Anacardium occidentale* L.

Microorganism	Leaves (µg/ml)	Peels (µg/ml)
P. aeruginosa	≥ 1024	512
S. aureus	≥ 1024	512
S. aureus M.R	≥ 1024	512
E. coli	512	512
K. pneumoniae	≥ 1024	≥ 1024
B. cereus	≥ 1024	≥ 1024

tions tested were deposited. It was considered as the concentration capable of developing halo of inhibition of bacterial growth ≥10 mm of diameter (Romeiro, 2001).

Moreover, it was observed that the Gram-negative bacteria, *P. aeruginosa* (ATCC 15442) and *K. pneumoniae* (ATCC 10031) were resistant to most of the concentrations of the extracts of peels and leaves; they were sensitive only to concentrations of 10 and 5% inhibition halos of 10 to 11.5 mm in diameter. As for *Escherichia coli* (ATCC25922), concentrations of the extracts of the two parts of the plant did not form inhibition halos ≥10mm in diameter; it is considered as the most resistant of all species tested.

In relation to Gram-positives bacteria: *S. aureus* (ATCC12692), *S. aureus* MR (358) and *B. cereus* (ATCC33018) were more sensitive to the concentrations of both extracts. The ethanol extract of stem bark showed the largest halos of inhibition for most concentrations compared to leaves; it got larger halo of 23.5 mm against the bacterium, *S. aureus* (ATCC12692) at a concentration of 10%. Of the microorganisms, *B. cereus* (ATCC33018) was considered as the most sensitive.

In dealing with controls, negative control showed no antibacterial activity in any lineage. This shows that ethanol did not interfere with the results of the extracts. While the positive control showed good results for Gram negatives and Gram positives, with 15.0mm to 23.0mm of inhibition halos.

The MIC ranged between 512 and ≥1024 µg/mL against the bacteria tested. MIC values were determined by visual reading after revealing with reassurance, which is an indicator of oxide reduction that has been used to evaluate the viability of bacterial cells. Table 3 represents the MIC's of the extract of stem peels and leaves of the cashew. The lowest concentration able to inhibit bacterial growth was 512 µg/mL in terms of *S. aureus* (ATCC12692), *S. aureus* MR (358), and *P. aeruginosa* (ATCC15442) for the bark extract and *E. coli* (ATCC25922) for both extracts. This showed more sensitive to this test compared to other lineages.

The greater resistance of Gram-negative organisms was expected, since they showed structural peculiarities in hindering the penetration of antibiotics such as the outer layer lipopolysaccharide, which determines surface properties (like permeability and susceptibility to antibiotics) (Yokota and Fujii, 2007). Gram-negative bacteria have a wall composed of several layers of peptidioglicanas, which differ in their chemical composition and consequently more complex than the wall of Gram-positives; although thicker ones show predominantly only one type of macro molecule. This shows the Gram-positive bacteria are more sensitive than the Gram-negative bacteria (Gonçalves and Gobbo, 2012).

Considering the wealth of constituents present in plants, the positive antibacterial activity of the extract of cashew stem peels can be due to the presence of compounds such as tannins and alkaloids previously found in the plant, which possibly has a higher concentration compared to the leaves. However, its potential is not due exclusively to a substance only, but a set of them.

These results confirm data from literature on the antibacterial activity of stem peels and leaves of cashew against a variety of microorganisms. In this study, the extract from the stem peels of *A. occidentale* was more effective in growth inhibition of the tested lineages compared to leaves and this experiment shows positive activity against Gram-positive lineages.

Conclusion

Based on these results, it is concluded that the dilutions of extracts from the leaves and stem peels showed antibacterial activity, but the peels showed the largest halos of inhibition and concentrations in most lineages; the Gram-positive bacterium, *B. cereus* was more sensitive to the concentrations of the two parts of the plant by diffusion technique. In relation to the method of micro dilution, the MIC varied between 512 and ≥1024 µg/mL in both parts of this species.

It is confirmed that with the therapeutic potential of *A. occidentale* L., it has great phytochemical potential. This proves the true biological value of this species; it has low cost and is easy to access by the population.

ACKNOWLEDGEMENTS

CAPESfor financial supportand the Oswaldo Cruz Foundation (FIOCRUZ) for grant bacteria standards.

REFERENCES

Barbosa-Filho JM, Vasconcelos THC, Alencar AA, Batista L, Oliveira RAG, Guedes DN, Falcão HS, Moura MD, Diniz MFFM, Modesto-Filho J (2005). Plants and their active constituents from South, Central, and North America with hypoglycemic activity. Bra. J. Pharmacol. 15(4):392-413.

Bouzada MLM, Fabri RL, Nogueira M, Konno TUP, Duarte G, Scio E.

(2009). Antibacterial, cytotoxic and phytochemical screening of some traditional medicinal plants in Brazil. Pharm. Biol. 47(1):44-52.
Clinical and Laboratory Standards Institute (CLSI)(2007). Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI document M100-S17 (ISBN 1-56238-625-5). Clinical and Laboratory Standards Institute, 940 We st Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.
Doss VA, Thangavel KP (2011). Antioxidant and antimicrobial activity using different extracts of *Anacardium occidentale* L. Int. J. Appl. Biol. Pharm. Technol. 2(3):436-443.
Gobbo-Neto L, Lopes NP (2007). Plantas Medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova* 30(2):374-381.
Gonçalves GMS, Gobbo J (2012). Antimicrobial Effect of *Anacardium Occidentale* Extract and Cosmetic Formulation Development. Braz. Arc. Biol. Technol. 55(6): 843-850.
Matos FJA (1997). *Introdução à fitoquímica experimental*. UFC Edições. pp. 44-46.
Melo AFM, Santos EJV, Souza LFC, Carvalho AAT, Pereira MSV, Higino JS (2006). Atividade antimicrobiana *in vitro* do extrato de *Anacardiumoccidentale*L. sobre espécies de *Streptococcus.* Braz. J. Pharmacol. 16(2):202-205.
Romeiro RS(2001). Métodos em bacteriologia de plantas (2001). Viçosa: Editora UFC.
Silva JG, Souza IA, Higino JS, Siqueira-Junior JP, PereiraJV, Pereira MSV (2007). Atividade antimicrobiana do extrato de *Anacardium occidentale* Linn. em amostras multirresistentes de *Staphylococcus aureus*. Braz. J. Pharmaco. 17(4):572-577.
Yokota S, Fujii N (2007). Contributions of the lipopolysaccharide outer core oligosaccharide region on the cell surface properties of *Pseudomonas aeruginosa*. Comp. Immunol. Microbiol. Infect. Dis. 30: 97-109.