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Full Length Research Paper

Antibacterial activity of the five South African *Erythroxylaceae* species

De Wet, H.

Department of Botany, University of Zululand, Private Bag X1001, KwaDlangezwa, 3886, South Africa.
E-mail*:* hdewet@pan.uzulu.ac.za. Tel: +27-35-9026108. Fax: +27-35-9026491.

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Until recently, no medicinal uses were recorded for the South African *Erythroxylaceae* species, although, this family is used world wide in traditional medicine. This study reveals for the first time that *Erythroxylum delagoense* and *Erythroxylum pictum* roots were used to treat dysentery and diarrhoea and that *Erythroxylum emarginatum* leaves decoction was used to treat asthma, kidney problems, arthritis, child bearing problems and influenza in South Africa. To validate some of the medicinal uses, antibacterial testing was done for the first time on all five South African species. Leaf and bark extracts of four of the five South African *Erythroxylaceae* species (*E. delagoense*, *E. emarginatum*, *E. pictum* and *Nectaropetalum capense*) showed some good antibacterial activities with MIC <1 mg/ml. *E. delagoense* showed good results against *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*; *E. emarginatum* against *Klebsiella pneumoniae*; *E. pictum* against *Bacillus subtilis* and *Klebsiella pneumonia*; *N. capense* against *Klebsiella pneumonia*.

Key words: Antimicrobial activity, *Erythroxylaceae*, medicinal uses, South Africa.

INTRODUCTION

The Erythroxylaceae family comprises four genera and 260 species. Three of the genera (Aneulophus, Nectaropetalum and Pinacopodium) are restricted to tropical Africa, while the fourth (Erythroxylum) occurs in the tropical part of Australia, Asia, Africa and America, with about 230 species. Erythroxylum rarely attains high densities where the genus occurs, but it is ecologically versatile. In South Africa, there are two genera and five species (Bredenkamp, 2000), which occur in the Northern Province, Mpumalanga, KwaZulu-Natal and the Eastern Cape (Table 1). The best known species in this family is *Erythroxylum coca* Lam. of which the leaves are traditionally chewed in the Andes region to relieve hunger and fatigue. It is also used by the Indians to treat gastro-intestinal ailments, motion sickness and as an antide-pressant, but it is best known for producing cocaine, which was the world's first commercial anesthetic (Van Wyk and Wink, 2004). The *Erythroxylaceae* species biological activities are ascribed to its rich diversity of alkaloids (Van Wyk and Wink, 2004). Some chemical compounds, which included alkaloids, were isolated in three of the South African species. *Erytrhoxylum delagoense* yielded *ent*-labda-8(17), 14-diene-3*β*,13R-diol, *ent*-dolabr-4(18)-en-15*S*,16-diol, *ent*-5α-dolabr-4(18)-en-15*S*,16-diol, *ent*-15*ξ*,16-dihydroxypict-4(18)-en-5-one (Ansell et al., 1993); *Erythroxylum pictum* yielded sisosterol and 29 diterpenes (Ansell et al., 1993) and *Erythroxylum emarginatum* yielded two tropane alkaloids, anhydroecgonine methyl ester *N*-oxide and anhydro-ecgonine methyl ester (Nishiyama, 2007). The presence of the alkaloids in the earlier mentioned species is a good indication that the South African species will most probably have some biological activities.

Although, this family is widely used for its medicinal properties, very little is known about the medicinal uses of the five South African *Erytrhoxylaceae* species. The aim of this study was to do an ethnobotanical survey on the uses of the South African species. When the survey revealed that one of the medicinal uses was the treatment of diarrhoea, antibacterial screening was thought to be suitable. The study was supported by results obtained from antimicrobial studies done on other *Erythroxylaceae* species which revealed good activities against various pathogens (Srinivasan et al., 2001; Gurib-Fakim et al., 2005; Mahomoodally et al., 2005; Rangasamy et al., 2007). A literature survey revealed that

Table 1. Localities and voucher specimens for the South African Erythroxylaceae species used for antibacterial screening.

Botanical name	Voucher specimen	Locality
Erythroxylum delagoense Schinz	SJ Siebert and AR Phamphe 2967 (ZULU)	Mhlangeni, Ithala Game Reserve, Lowsberg [2731AD]
E. emarginatum Thonn.	A Abbott, H de Wet and SJ Siebert 8557 (ZULU)	Port Edward, Umthamvuna Nature Reserve [3130AA]
E. pictum E.Mey ex Sond.	A Abbott, H de Wet and SJ Siebert 8558 (ZULU)	Port Edward, Umthamvuna Nature Reserve [3030CC]
Nectaropetalum capense (Bolus) Stapf & Boodle	A Abbott, H de Wet and SJ Siebert 8556 (ZULU)	Port Edward, Umthamvuna Nature Reserve [3130AA]
N. zuluense (Schonland) Corbishley	A Abbott, H de Wet and SJ Siebert 8555 (ZULU)	Port Edward, Umthamvuna Nature Reserve [3130AA]

no previous antibacterial screening has been carried out on any of the five South African Erythroxylaceae species.

MATERIALS AND METHODS

The five South African Erythroxylaceae species reported in Table 1 were collected during 2006. The specimens were identified by Dr. SJ Siebert at the University of Zululand, South Africa. Voucher specimens were deposited in the Herbarium of the Department of Botany, University of Zululand (ZULU).

Extractions

Acetone, methanol and hot water extracts were prepared from dried leaves and bark of the five species. The National Committee for Laboratory Standards (NCCLS) methodology and guidelines as well as Eloff's (1998) was used to determine the minimum inhibitory concentration (MIC). Bacterial cultures was sub-cultured from stock agar plates and grown in tryptone soya broth overnight. Sterile microtitre plates (96 U-Bottom, Nunc) were aseptically prepared by adding 100 µl distilled sterile water into each well. The plant extracts at starting stock concentrations of 64 mg/ml were transferred into the microtitre plate. Serial dilutions were performed. To each well was added 100 µl of an overnight culture diluted in fresh tryptone soya broth at a 1:100 ratio, yielding an approximate inoculums size of 1×10^6 colony forming units CFU/ml. The plates were then incubated for 24 h at 37°C. Neomycin (0.02 mg/ml) was used as a positive control, while acetone (64 mg/ml) and DMSO (64 mg/ml) were used as negative controls. The controls were included in each assay to confirm the antimicrobial susceptibility. After 24 h, 40 µl of 0.2 mg/ml of *p*-iodonitrotetrazolium (INT) (Sigma) violet was added into the plates. The plates were then kept for 6 h at ambient temperature before inspection for antibacterial activity. The INT was used as the bacterial growth inhibition indicator where by the pink purple or red colour represented bacterial growth, while no change or colourless appearance represented growth inhibition. The lowest concentration at which the plant extract inhibited bacterial growth after 6 h was considered as MIC value of the crude extracts. Tests were performed in duplicates.

The following ATCC (American Type Culture Collection) were used for the screening: *Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 12600, *Escherichia coli* ATCC 11775 and *Klebsiella pneumonia* ATCC 13883. *S. aureus* can cause diseases like otitis media and is also known to cause secondary infections in influenza patients. *K. pneumoniae* cause pneumonia especially in the very young and old. *E. coli* are responsible for an estimated one-half of all diarrhoea episodes in high risk regions (Nester et al., 2001).

RESULTS AND DISCUSSION

An ethnobotanical survey which was done in the KwaZulu-Natal Province on the five *Erythroxylaceae* species had revealed the following novel medicinal uses. *E. delagoense* and *E. pictum* roots are boiled and drank to treat dysentery and diarrhoea, while *E. emarginatum* leaves are boiled and drank to treat asthma, kidney problems, arthritis, child bearing problems and influenza. In a recent ethnobotanical study done by Corrigan et al. (2010), it was documented that the fruit of *E. delagoense* is used to treat throat and respiratory ailments in infants in the north eastern part of KwaZulul-Natal. The only other medicinal uses recorded for one of these species were in Kenya where the leaves and roots of *E. emarginatum* are used for pain relief and the leaves are also used to increase alertness and enhance sexual desire (Nishiyama et al., 2007).

Interpretation of the MIC value was as follows: Extracts having activities below 8 mg/ml were considered to have some antimicrobial activity (Fabry et al., 1998) and the MIC values of extracts below 1 mg/ml were considered noteworthy (Gibbons, 2004; Rios and Recio, 2005; Van Vuuren, 2008). Most of the five *Erythroxylaceae* species have antimicrobial activities against all four bacteria tested (Table 2). The medicinal use of *E. delagoense* and *E. pictum* roots for treating diarrhoea and dysentery has merit as almost all the chemical and water extracts of leaves and bark have a MIC of below 8 mg/ml for *E. coli*,

Table 2. Antimicrobial activity (MIC) of the five South African Erythroxylaceae species.

Pathogen	*B. subtilis* (mg/ml)			*E. coli* (mg/ml)			*K. pneumoniae* (mg/ml)			*S. aureus* (mg/ml)		
Species	**Acetone**	**Methanol**	**H_2O**	**Acetone**	**Methanol**	**H_2O**	**Acetone**	**Methanol**	**H_2O**	**Acetone**	**Methanol**	**H_2O**
E. delagoense												
Leaves	0.5	1.0	4.0	4.0	4.0	16.0	1.0	0.5	4.0	0.5	1.0	4.0
Bark	1.0	0.5	4.0	4.0	4.0	4.0	1.0	0.25	4.0	16.0	4.0	1.0
E. emarginatum												
Leaves	4.0	4.0	4.0	4.0	4.0	4.0	4.0	0.5	4.0	1.0	4.0	4.0
Bark	4.0	4.0	4.0	16.0	16.0	4.0	4.0	0.5	4.0	1.0	1.0	16.0
E. pictum												
Leaves	1.0	1.0	16.0	4.0	1.0	4.0	4.0	0.25	4.0	1.0	4.0	16.0
Bark	0.5	1.0	1.0	4.0	4.0	1.0	0.5	0.25	4.0	1.0	4.0	4.0
N. capense												
Leaves	4.0	1.0	1.0	16.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Bark	4.0	1.0	1.0	4.0	4.0	4.0	0.5	0.25	4.0	1.0	4.0	4.0
N. zuluense												
Leaves	4.0	1.0	4.0	16.0	16.0	4.0	4.0	0.25	4.0	1.0	4.0	16.0
Bark	4.0	4.0	4.0	16.0	16.0	4.0	4.0	1.0	4.0	1.0	4.0	4.0
Neomycin (mg/ml)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06

except the water extract for *E. delagoense* leaves (16 mg/ml). The treatment of respiratory ailments with *E. delagoense* and *E. emarginatum* roots can be validated with some of the excellent MIC values (<1 mg/ml) against *K. pneumoniae* and *S. aureus* as shown in Table 2.

The results of this study compared well with three antimicrobial studies done on four *Erythroxylum* species found in Mauritius (Gurib-Fakim et al., 2005; Mahomoodally et al., 2005; Rangasamy et al., 2007). Methanol plant extracts of *Erythroxylum hypericifolium* Lam., *Erythroxylum laurifolium* Lam., *Erythroxylum macrocarpum* O.E. Schulz and *Erythroxylum sideroxyloides* Lam., showed activity against *S. aureus* (MIC between 1 and 8 mg/ml) and *E. macrocarpum* showed activity against *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Rangasmy et al. (2007) reported that a methanol leaf extract of *E. laurifolium* has a MIC value of 1 mg/ml against *B. subtilis*, which are the same for four of the leaves extract of the South African species tested against *B. subtilis* (Table 2). *E. emarginatum* leaves have a higher MIC value of 4 mg/ml against *B. subtilis*. Another study on Indian medicinal plants showed positive antimicrobial activity against *E. coli* for an aqueous leaf extract of *Erythroxylum monogynum* Roxb. In the present study, only *E. delagoense* leaf water extract showed no activity against *E. coli*.

Although, the ethnobotanical information in our survey indicated that the people preferred the use of roots for the treatment of various ailments, the

results show that the bark and the leaves can be active. This is also confirmed by the study done on the *Erythroxylum* species in Mauritius, where the plant leaves and stem are used to treat fever, kidneys stones and are used as a diuretic. For conservation purposes, it is much better to use the leaves and stems. Although, there are no records of any medicinal uses of *Nectaropetalum capense* and *Nectaropetalum zuluense*, they both have some excellent MIC values against the four micro-organisms tested.

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REFERENCES

Ansell SM, Pegel KH, Taylor DAH (1993). Diterpenes from the timber of *Erythroxylum pictum*. Phytochemistry, 32: 945-953.

Bredenkamp CL (2000). Erythroxylaceae. In Leistner OA (ed.). Seed plants of southern Africa: families and genera. Strelitzia 10, National Botanical Institute, Pretoria, South Africa, p. 248.

Corrigan BM, Van Wyk B-E, Geldenhuys CJ, Jardine JM (2010). Ethnobotanical plant uses in the KwaNibela Peninsula, St Lucia, South Africa, S. Afr. J. Bot., 77: 346-359.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimum inhibition concentration of plant extracts for bacteria. Planta Medica, 64:711-713.

Gurib-Fakim A, Subratty H, Narod F, Govinden-Soulange J, Mahomoodally F (2005). Biological activity from indigenous medicinal plants of Mauritius. Pure Äppl. Chem., 77:41-51.

Mahomoodally MF, Gurib-Fakim A, Subratty AH (2005). Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. Pharmaceut. Biol. 43:237-242.

Nester EW, Anderson DG, Roberts CE, Pearsall NN, Nester MT (2001). Microbiology: A Human Perspective, third ed. McGraw-Hill, New York.

Nishiyama Y, Moriyasu M, Ichimaru MS, Iwasa K, Kato A, Juma FD, Mathenge SG, Chalo Mutiso PB (2007). Tropane alkaloids from *Erythroxylum emarginatum*. J. Nat. Med., 61: 56-58.

Rangasamy O, Raoelison G, Rakotoniriana FE, Cheuk K, Urverg-Ratsimamanga S, Quetin-Leclercq J, Gurib-Fakim A, Subratty AH (2007). Screening for anti-infective properties of several medicinal plants of the Mauritians flora. J. Ethnopharmacol., 109: 331-337.

Srinivasan D, Nathan S, Suresh T, Perumalsamy PL (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharmacol., 74:217-220.

Van Vuuren SF (2008). Antimicrobial activity of South African medicinal plants. J. Ethnopharmacol., 119: 462-472.

Van Wyk B-E, Wink M (2004). Medicinal Plants of the World. Briza, Pretoria, South Africa, p. 137.