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Antibacterial, anti-inflammatory and mutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock

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Abstract

Crude dichloromethane and 90% methanolic extracts of plants used traditionally in the treatment of wounds and retained placenta were screened for their antibacterial, anti-inflammatory (cyclooxygenase-1 and-2) activities and investigated for potential mutagenic effects. Antibacterial activity was evaluated using the micro-dilution assay. The bacterial strains used were *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). DCM extracts of *Dicerocaryum eriocarpum*, *Pterocarpus angolensis*, *Ricinus communis* and *Schkuhria pinnata* exhibited the highest antibacterial activity. In the anti-inflammatory assay, dichloromethane extracts of stems of *Cissus quadrangularis* and roots of *Jatropha zeyheri* showed selective inhibition of COX-2 (75% and 77%), while dichloromethane extracts of the shoots of *S. pinnata* showed selective inhibition of COX-1. None of the plant extracts were mutagenic in the *Salmonella typhimurium* Ames test (strain TA98). The results suggest that most plants used traditionally for treating wounds and retained placenta in animals are effective in combating infection and reduction of pain. Lack of mutagenicity suggests that these plants are probably safe for use.

Keywords: Antibacterial; Anti-inflammatory; Medicinal plants; Mutagenicity; Retained placenta; Wounds

1. Introduction

Ethnoveterinary medicine (EVM) is widely practiced by South African small scale farmers (Masika et al., 2000; Van der Merwe et al., 2001). People have used these remedies for generations and many reasons have been advanced as to why the practice continues. Farmers claim that medicinal plants are more efficacious than pharmaceuticals for chronic pathologies. They are reputed to have no side effects and no withdrawal periods for consumption of meat from treated animals are needed since the plants are thought to be non-toxic. In general, ethnoveterinary products are used after conventional pharmaceutical medicines, for chronic cases, proved ineffective.

Aligning indigenous names of diseases used by traditional farmers to modern veterinary practice is recognized as a difficult task (McCorkle and Mundy, 1992). Wounds are defined by farmers to include sores, warts and lumpy skin disease. Tsonga speaking people of South Africa invariably use the same name of 'matsuna' for wounds and lumpy skin disorders (Luseba and Van der Merwe, 2006). The Setswana term for sores and wounds (dintho) has a broad definition and includes inflamed skin lesions, abscesses and lesions of internal organs such as the lungs and liver (Van der Merwe et al., 2001). Farmers recognize tick bites as the biggest causes of wounds but thorns and other hard plant materials found in the Bushveld can contribute.

Farmers mostly use a range of plants (Table 1) as EVM remedies (Luseba and Van der Merwe, 2006). It is interesting that bitter (*Aloe marlothii*) and irritant (*Sarcostemma viminale*) plants are generally used in treatment of wounds. Van der Merwe et al. (2001) have also attributed the use of medicinal plants to the

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Table 1
Plants and parts administrated for treatment of wounds and retained placenta in animals in South Africa

Family and botanical name	Common names	Indication	Plant part used	Application
Araliaceae *Cussonia spicata* Thunb. Dvd Merwe 63	Musenje: Tsonga; Musenzhe: Venda	Retained placenta	Bark	The bark is crushed and mixed with water
Asclepiadaceae *Sarcostemma viminale R. (Br.)* Dluseba 11/12/02	Neta: Tsonga; Mutungu: Venda	Wound	Aerial part	Crushed and applied directly on the wound. Very effective against maggots
Asphodelaceae *Aloe marlothii* Berger subsp. *marlothii* Kaalplaas 032	Mhangani: Tsonga; Tshikopa: Venda; Mokgopa: Setswana	NCD	Leaves	Leaves are crushed and the juice is applied on sores
Asteraceae *Schkuhria pinnata* (Lam.) Dvd Merwe 61	Santhloko lefero: Setswana;	Eye infections Pneumonia, diarrhoea Heartwater	Aerial parts	The shoots are crushed and mixed with water for dosing the animal for systemic ailments or applied directly on the eye
Euphorbiaceae *Jatropha zeyheri* Sond. Dluseba 10/11/02	Xidomeja/Mudomeja: Tsonga; mafuredonga: Venda	General ailments; Wounds	Roots	Together with *P. angolensis*, the most used plant. Fresh or dry roots are ground, soaked in water and dosed to animals
Euphorbiaceae *Ricinus communis* Linn. Dvd Merwe 29/01/02	Mokhura: Setswana	Wounds and sores	Leaves	Leaves are made into poultices and are widely applied to wounds, sores and boils
Fabaceae *Pterocarpus angolensis* DC. Dluseba 10/12/02	Vhangazi/Murhotso: Tsonga	General illness, thriftiness,	Bark	The bark is chopped, soaked in cold water, dosed once only with bottle or horn (approximately 750 ml) after the water has changed to a reddish colour or it is boiled for 30 to 60 min
Pedaliaceae *Dicerocaryum eriocarpum* (Decne.) Abels Dvd Merwe 101	Dinda/Dindza: Tsonga;	Dystocia, Retained placenta	Shoots	The shoots are crushed and mixed with water. It is applied topically as a lubricant during difficult calving and given orally (5 l) for retained placenta
Rhamnaceae *Ziziphus mucronata* Willd. *subsp. Mucronata* Dvd Merwe 100	Mutshetshete: Venda; Mokgalo: Setswana	Wounds and sores	Roots	Decoctions of roots are applied externally to boils, sores and glandular swelling
Vitaceae *Cissus quandrangularis* (Linn) Dluseba 10/12/02	Nyangala: Tsonga	Wounds; lactophore	Shoots	The shoots are crushed and applied directly on wounds

Source: (Van der Merwe et al., 2001; Luseba and Van der Merwe, 2006).

doctrine of 'signature', for example the use of *Pterocarpus angolensis* probably for its red colour, and *Dicerocaryum eriocarpum* which is soapy for a retained placenta.

Most EVM studies so far focused on capturing traditional knowledge and evaluating EVM remedies by empiric methods such as the veterinary consistency as stipulated by Kansonia and Ansay (1997) which relies on anecdotes provided by farmers. Otherwise, the scientific rationale behind the use of these remedies has been deducted in some cases from research in human traditional medicine. There is need for specific studies on animal pathologies and diseases. This study is a first attempt to evaluate the biological activities of the ten most used plants in North-West and Limpopo Provinces of South Africa as EVM remedies in treatment of wounds and retained placenta.

2. Materials and methods

2.1. Data collection

Rapid rural appraisal methods were used to collect the information on plants used in the treatment of wounds and retained placenta (Beebe, 1995) in two provinces of South Africa (Limpopo and North-West). Farmers were interviewed in groups using open-ended questions at the dipping tanks or individually thereafter. Information was also gathered from previous work done in Madikwe Reserve, North-West Province, South Africa (Van der Merwe et al., 2001). The veterinary consistency (Kansonia and Ansay, 1997) was used for empiric validation of the information. Plants were collected under guidance of farmers and only when the same plant was designated by at least two individuals for the same pathology was the information judged acceptable. Voucher specimens were submitted to the South African National Biodiversity Institute for identification and thereafter kept in the Herbarium at Onderstepoort Veterinary Institute. Table 1 lists the plants used in the treatment of wounds and retained placenta.

2.2. Plant processing and extraction

Plant materials (leaves, stems, bark or entire plant) were dried in the shade at room temperature before grinding to fine powders using a Buchi mixer (Labotec®). Approximately 5 g of ground material was extracted by sonication with dichloromethane (DCM) and 90% methanol (10 ml/g) (MeOH) respectively for 1 h. The extractants were filtered using Whatman No. 1 filter paper. All extracts were dried under reduced pressure at 40 °C.

2.3. Antibacterial activity

Antibacterial screening was carried out using the microplate method for minimum inhibitory concentration (MIC) determination (Eloff, 1998). Two milliliters of cultures of three bacterial strains: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853)

were prepared and placed in a water bath overnight at 37 °C. The overnight cultures were diluted with sterile MH broth (1 ml bacteria/50 ml MH). The DCM and MeOH extract residues were redissolved in acetone to a final concentration of 20 mg/ml. For each of the three bacteria used, 100 µl of each of the plant extracts tested was two-fold serially diluted with 100 µl sterile distilled water in a sterile 96-well micro-plate. A similar two-fold serial dilution of neomycin (Sigma) (0.1 mg/ml) was used as a positive control against each bacterium. One hundred microliters of each bacterial culture was added to each well. The plates (two replicates and two repeats) were covered and incubated overnight at 37 °C. To indicate bacterial growth, 50 µl of 0.2 mg/ml *p*-iodonitrotetrazolium violet (INT) (Sigma Chemical Company, St Louis, MO) was added to each well and the plates incubated at 37 °C for 30 min. Bacterial growth inhibition was indicated by a reduction in the red colour whereas clear wells indicated a lethal effect.

2.4. Anti-inflammatory activity

Anti-inflammatory activity was determined using the cyclooxygenase-1 and 2 assays as described by Zschocke and Van Staden (2000). The COX-1 (isolated from ram seminal vesicles) and COX-2 (human recombinant) (Sigma-Aldrich) enzymes were activated with co-factor solution and pre-incubated on ice for 5 min. Sixty microliters of this enzyme/co-factor solution was added to 20 µl of test solution (2.5 µl of the compound ethanolic solutions + 17.5 µl water) and pre-incubated for 5 min at room temperature. Twenty microliters of [1-^{14}C]arachidonic acid was added to the tested samples and incubated at 37 °C for 10 min. After incubation, the reaction was terminated by adding 10 µl 2 N HCl. Four controls were run. Two were background in which the enzyme was inactivated with HCl before the addition of [1-^{14}C]arachidonic acid, and two were solvent blanks. Indomethacin was included as a standard. Percentage inhibition of the tested compound was calculated by comparing the amount of radioactivity present in the sample to that in the blank solvent.

2.5. Mutagenicity test

Mutagenicity was tested using the *Salmonella* microsome assay based on the plate-incorporation procedure with *Salmonella typhimurium* tester strain TA98. The assay was performed according to Maron and Ames (1983). Stock (100 µl) bacteria in 20 ml Oxoid nutrient broth No. 2 were incubated for 16 h at 37 °C. The bacterial cultures (100 µl) were added to 100 µl of plant extract in 500 µl phosphate buffer and 2 ml of agar containing biotin–histidine (0.5 mM). The mixture was poured on a minimal agar plate and incubated at 37 °C for 48 h. Samples were tested in triplicate with two replicates. Three dilutions (50, 500, 5000 µg) were used per sample. 4-Nitoquinoline-*N*-oxide (4NQO) was used as a positive control.

3. Results and discussion

Wounds of several months duration with deep structure alterations are most frequently polymicrobial, involving numerous microorganisms that are potentially pathogenic (Bowler et al., 2001). This might be the case in EVM because farmers, most frequently, treat old wounds. The results of the general screening for antibacterial activity (MIC values) as shown in Table 2 suggest that five plants are effective in treatment of wounds because they are effective against at least *S. aureus* and *E. coli*. *S. aureus* and coagulase-negative staphylococci are the predominant organisms isolated from different types of wounds (Howell-Jones et al., 2005). The most effective plants were *Cissus quandrangularis*, *J. zeyheri*, *P. angolensis*, *R. communis* and *S. pinnata*. Of the 20 plant extracts screened, nine (6 DCM and 3 MeOH) showed activity against *S. aureus* while seven inhibited *E. coli* growth. None of the plant extracts was effective against *P. aeruginosa*. The results for anti-inflammatory activity of the different plant extracts are given in Table 3. DCM extracts of *C. quandrangularis*, *P. angolensis* and *S. pinnata* showed high inhibitory activity against COX-1 (>60%). On the other hand, extracts of *C. quandrangularis* (DCM and 90% MeOH), MeOH extracts of *D. eriocarpum*, DCM extracts of *J. zeyheri* and *P. angolensis* showed high inhibitory activity against COX-2 (>70%). COX-1 is the constitutive form which has a clear physiological function whereas COX-2 is induced by mediatory inflammation (Botting, 2006). However, it is worth mentioning that prolonged use of plant extracts that showed selectivity to either COX enzymes may have detrimental effects. Prolonged use of COX-1 selective inhibitors causes severe damage to the gastrointestinal tract (Méric et al., 2006), while COX-2 selective inhibitors which cause less or no gastrointestinal damage result in cardiovascular adverse effects (Modica et al., 2005).

The validation of the use of plants in traditional medicine based on the results with organic solvents might be considered irrelevant because traditionally, water instead is used as a solvent for most of the preparations (Elgorashi and Van Staden, 2004). However, in the case of wound treatment in livestock, this dilution factor could be considered insignificant because the whole plant material is processed and applied as such locally giving the opportunity for the substances to be released. It is also not known what changes occur when the mixtures, given orally, are digested by the animal in

Table 2
Antibacterial activity (MIC) of plant extracts (mg/ml) used in South African ethnoveterinary medicine as determined using the micro-dilution assay

Plant species	Part tested	DCM extracts			90% MeOH extracts		
		S.a.	E.c.	P.a.	S.a	E.c.	P.a.
A. marlothii	Leaves	0.31*	1.25	1.25	1.25	1.25	1.25
C. spicata	Bark	1.25	1.25	1.25	1.25	1.25	1.25
C. quandrangularis	Stem	0.63*	2.5	2.5	0.31*	1.25	2.5
D. eriocarpum	Shoots	0.63*	2.5	2.5	0.31*	1.25	1.25
J. zeyheri	Root	0.63*	1.25	2.5	2.5	0.63*	2.5
P. angolensis	Bark	0.31*	0.63*	2.5	2.5	0.63*	2.5
R. communis	Leaves/Stem	0.16*	0.24*	1.25	1.25	0.4*	0.78
S. pinnata	Shoots	0.31*	0.63*	1.25	0.31*	0.31*	1.25
S. viminale	Stem	1.25	1.25	1.25	1.25	1.25	1.25
Z. mucronata	Bark	2.5	2.5	2.5	2.5	2.5	1.25

S.a. = *Staphylococcus aureus*; E.c. = *Escherichia coli*; P.a. = *Pseudomonas aeruginosa*. Values with astersisks (*) are considered as active. The MIC values (µg/ml) for Neomycin® (positive control) were: *E. coli* = 2.44; *P. aeruginosa* = 0.78; *S. aureus* = 0.305.

Table 3
Inhibition (%) of prostaglandin synthesis by extracts (250 μg/ml) obtained from plants used in South African ethnoveterinary medicine as determined by the cyclooxygenase (COX-1 and -2) assays

Plant species	Part used	COX-1		COX-2	
		DCM	Methanol	DCM	Methanol
A. marlothii	Leaves	42.3±2.5	21.8±0.9	8.3±2.5	0
C. spicata	Root	35.2±5.3	28.4±5.7	2.1±1.2	19.2±4.3
C. quandrangulata	Stem	62.3±1.5	23±5.8	75.8±4.1*	70.3±3.9*
D. eriocarpum	Shoots	35.5±0.4	33.5±5.5	16.7±2.5	77±3.2*
J. zeyheri	Root	43.0±5.3	17.3±0.8	77±2.8*	25±1.4
P. angolensis	Bark	87.5±2.3*	59.3±0.6	83±1.4*	19.5±2.1
R. communis	Leaves/Stem	36.1±5.9	36.2±1.7	14.7±0.7	9.1±2.4
S. pinnata	Shoots	93.4±4.5*	9.8±2.8	54.7±2.5*	0
S. viminale	Stem	44.7±5.7	31.3±4.2	18.7±1.1	25.5±5.6
Z. mucronata	Bark	43.1±0.8	68.5±7.8	44.5±4.1	66.2±4.6*

Values with asterisks (*) are considered as active. The percentage prostaglandin synthesis inhibition by indomethacin® (positive control) in COX-1 was 71.8–79.8% and in COX-2 54–70%.

treatment of retained placenta. We suspect that the active ingredients are then released and the materials become effective. Some plants have obvious properties that are expected to stimulate healing or combat infections. For instance, *C. quandrangularis* has a high content of vitamin C, carotene A, calcium and anabolic steroidal substances (Deka et al., 1994; Demling, 2000). *J. zeyheri* is one of the most used and stored plants by the Tsonga speaking people (Luseba and Van der Merwe, 2006). It contains a diterpenoid that showed antibacterial activity against *Streptococcus pyogenes* and some fungi (Dekker et al., 1987). *P. angolensis* compares well with *J. zeyheri* in Tsonga Traditional Medicine with regard to frequency of use. *P. angolensis* is well known for its tannin content. This may explain its antibacterial activity. *S. pinnata* was shown to exhibit good antibacterial activity on many other bacteria including *Cornebacterium*, *Diphthericeae*, *Neisseria*, *Streptococcus* and *Streptobacillus* spp. (Hussein and Deeni, 1991). *R. communis* proved to be the most potent plant with the best MIC values. Leaves, roots and seed oil of this plant have been studied extensively and compounds isolated comprise tannins, flavonoids and indole-3-acetic acid. Flavonoids are reported to have anti-inflammatory activity (Ilavarasan et al., 2006). This explains why Duke (1983) reported its indication in more than 80 ailments of which the majority are of an inflammatory nature. Water extracts were not evaluated because from our experience, their antibacterial activities are most of the time not detectable. For instance, 23 out 27 water plant extracts were not active for plant used for medicinal purposes (infections) (Rabe and Van Staden, 1997).

The concept of inflammation might not be distinct from that of infection among local farmers. It was shown that farmers do not use different plants for different degrees of inflammation or stages of infection (Van der Merwe et al., 2001). Surprisingly, in this study, the same plants showed simultaneously good antibacterial and anti-inflammatory activities. As is the case for antibacterial activity, the anti-inflammatory activity of some plant extracts can be explained by the chemical constituents of the plant. For instance, for *S. pinnata*, this can be attributed to procyanidin. Procyanidin has an antioxidant effect and has been used in inflammatory diseases and wound healing (Grim et al., 2004). The peroxynitrite scavenging properties of procyanidins condense tannin oligomers and can protect endothelial cells from damage (Stoclet et al., 2004). The anabolic steroids found in *C. quandrangularis* might also improve the healing process indirectly. They are thought to counteract the catabolic effects of some substances on wound healing (Ovington, 1998). The

Table 4
Number of His^+ revertants in *Salmonella typhimurium* strain TA98 produced by crude plant extracts

Plant species	Plant part	Number of colonies					
		DCM extracts (μg/ml)			90% Methanol extracts (μg/ml)		
		5000	500	50	5000	500	50
A. marlothii	Leaves	24±2.1	23.6±3.1	32.3±6.1	29.6±1.2	36.3±3.7	37±1.7
C. spicata	Root	31.3±3.2	32±5.3	31.3±6.4	36.3±4.9	37±3.6	23±1.4
C. quandrangulata	Stem	23.3±7.3	27.0±4.3	24.0±7.0	30.6±2.5	28.3±4.5	36.3±3.5
D. eriocarpum	Whole plant	48±3	29.3±5.7	35±5.3	36.3±6.4	26.6±2.8	27.6±4.7
P. mixta	Stem	38.3±6.6	31.3±2.3	33±1.4	30.3±3.2	29±6.9	27±3
J. zeyheri	Root	24±1.7	25.6±1.2	28.6±2.1	25±3	28±5	26.6±2.1
P. angolensis	Bark	20±7.8	25.3±3.2	27.3±4.5	22.3±2.5	25.6±4.0	24±3.6
R. communis	Leaves/Stem	33±7	38.6±3.7	35±2	35.6±6.8	36.1±1.4	38.3±2.1
S. pinnata	Aerial parts	38.5±7.7	33±4.3	32±5.3	32±1	32±3	30±5
S. birrea	Bark	27.5±3.5	31.6±7.7	30.0±2.1	34.6±7.5	33.6±2.8	32±2.1
S. viminale	Stem	30.3±0.7	30.3±1.4	32±3.6	34.6±6.4	28±6	28.3±2.3
Z. mucronata	Bark	27.5±2.1	37.6±2.8	33.6±2.3	32.3±4.9	37.6±1.2	37.6±6

tannin-containing multi-component fractions of plant extracts were reported to have very high and significant anti-inflammatory activity (Osadebe and Okoye, 2003) suggesting that plants such as *P. angolensis* and *J. zeyheri* have the same effect due to their tannin content and flavanoids.

Chronic and non-healing wounds are frequently hypoxic as a consequence of poor blood perfusion (Bowler et al., 2001). In general wound management, it is usually recommended to surgically remove all foreign bodies and aerate the site. We suspect that by using irritating plants as EVM remedies, this function is achieved and the positive results obtained in this study support this assertion.

With reference to retained placenta in particular, it is common knowledge in veterinary medicine that the placental membranes are normally released between 2 and 6 h post-partum. Retained placenta is defined as retention of foetal membranes for longer than 12 h post-partum. However, in untreated animals, retained placenta would be released between 2 and 10 days (Eilen and Hopkins, 1993). Farmers have reportedly used medicinal plants during this period. In this case, it is difficult to conclude that plants are used for their known biological properties. However, it was noted that some plants used in the treatment of retained placenta have both mechanical and biological properties. Previous research (Van der Merwe et al., 2001) attributes this to the Doctrine of Signatures. The Doctrine of Signatures is a metaphysical method of discovering pharmaceutical value. It proposes the idea that God gave everything in nature its unique healing powers that can be discovered in its appearance. The soapy aspect of *D. eriocarpum* may have a soothing and cooling effect on inflamed skin and mucosa lesions as noted for *A. marlothii* by Van Wyk et al. (1997). The scientific validation could also be based on plant constituents. Tannin-containing plants such as *P. angolensis* and *S. pinnata* are probably effective where protection of underlying tissues by skin or mucosa is compromised as seen in the case of wounds and retained placenta. Tannin–protein complexation limits fluid loss and forms a physical barrier to further tissue insult. They have also a vasoconstructive effect on small blood vessels that limits bleeding and oozing of fluids through damaged skin or mucous membranes (Bruneton, 1995). We can also speculate that the well-known astringent property of tannins has a physiological effect such as a contraction of the uterus which might facilitate the removal of retained placenta. According to Manspeaker (2007), by shrinking of the small blood vessels, the capillary pressure is lessened and separation of foetal membranes occurs.

The Ames test which was used in this study to detect genetic damage induced directly or indirectly using *S. typhimurium* (TA98) is based on the number of His^+ revertants in *S. typhimurium* strain TA98 produced by crude plant extracts. An extract was considered mutagenic if the number of revertants per plate (mean of 5 or 3 replicate plates as indicated above) was at least double that of the spontaneous revertant frequency. None of the plant extracts had a value approaching the value indicating genotoxicity. It should be noted, however, leaf extracts of *Ziziphus mucronata* showed genotoxic effects in human peripheral blood lymphocytes using the micronucleus and the alkaline comet assays for both the DCM and MeOH extracts. Furthermore, MeOH extracts from the roots of *R. communis* were genotoxic in the micronucleus test (Taylor et al., 2003). These plants are perhaps more dangerous considering that they are used for extended periods of time and may cause long-term damage to the animals. Although the results were negative, the mutagenicity data are reported in Table 4 for reference purpose.

4. Conclusion

As far as we can ascertain, this work is the first to report on the antibacterial and anti-inflammatory activities of ethnoveterinary remedies used in the treatment of wounds and retained placenta. The results indicate that most plants used traditionally for treating these pathologies might be effective although organic solvents were used rather than the water decoctions or infusions used by farmers. Furthermore, lack of mutagenicity suggests that most of these plants are probably safe though other tests need to be conducted for further safety testing.

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