

Full Length Research Paper

A comparative study of antibacterial activities of wild and cultivated plants used in ethnoveterinary medicine

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Farmers generally collect fresh plant materials from the wild for ethnoveterinary uses. They are encouraged to harvest with caution and dry or cultivate important materials in order to protect the biodiversity. These recommendations are not validated scientifically. The microplate method for minimum inhibitory concentration (MIC) determination was used to compare wild with cultivated, and fresh with dry plant materials. The MIC values obtained ranged from 1.25 to 0.01 mg/ml. MIC values ≤ 0.3 mg/ml were considered as cut off point between effective and none effective inhibition. The multilevel linear models (hierarchical linear models), both unadjusted and adjusted models were employed. The plant (name) was considered as level-2 or higher level, while the actual observation was level-1 or lower level. The crude estimates of the odds ratio indicated that wild is significantly 0.57 times less likely than garden to yield MIC values of more than 0.3 mg/ml (p-value = 0.005). Also, fresh are about 4.195 times more likely than dry to yield MIC scores of more than 0.3 mg/ml (p-value < 0.001). Adjusting for conditions "dry and fresh", microbe and solvent; wild is significantly 0.52 times less likely than garden to yield MIC values of more than 0.3 mg/ml (p-value = 0.003). On the other hand, when adjusting for "wild or garden", type of solvents and type of microbes; fresh is significantly 4.202 times more likely than dry to yield MIC values of more than 0.3 mg/ml (p-value < 0.001). These results partially support farmers claiming that wild plant materials are more potent than the grown ones. On the contrary, the results are in favour of drying plant materials.

Key words: Antibacterial, medicinal plants, wild, cultivated, fresh, dry, odd ratio.

INTRODUCTION

Ethnoveterinary medicine (EVM) practices show some profound differences with the human traditional medicine. Farmers generally collect plants when needed. Some of the most prominent practices are the use of fresh materials in EVM; farmers only collect these remedies when the animal is sick, plants are generally collected from the wild and only few essential species are planted in the yard or kept in bundles of dry branches and roots. They claim that wild plants are more efficacious than cultivated ones because they are mature, contain more substances and

are easily accessible (Luseba and van der Merwe, 2006). The predominance of materials collected from the wild can also be attributed to the doctrine of signature. In China, the similarities in appearance of knotted wild roots to the human body symbolising the vitality and potency of the wild ginseng (Schippmann et al, 2002) explain why the grown ginseng which lacks this shape is unpopular. This is somehow supported by scientific evidence indicating that medicinal values of plants reside in their secondary metabolites produced in condition of stress and competition in the wild. Conversely, a study of essential oil composition of some wild and cultivated medicinal plants showed that the major constituents of two species out of the three studied was similar (Kizil, 2006).

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According to Lee (2004), cultivation enables both producers and buyers have more control over production and profits; cultivators can selectively breed for desired and consistent qualities and more potent products. The offer of raw materials with low risk of alteration and more concentrated returns are other advantages of cultivation. Since there is little reference to ancestral guidance in EVM, it can be argued that plants can be collected indiscriminately from the wild or homesteads without losing the perceived efficacy. We investigated the differences (if any) between plants harvested from the wild and those that are cultivated using the antibacterial activity as indicator of effectiveness in order to make valuable recommendations.

For the purpose of this study, plants considered are those growing easily and are highly exploited or are threatened by harvesting methods (e.g. whole plant) and can cure a wide range of ailments in both humans and animals. Five plant species, that is, *Aloe marlothii* A. Berger (Fam. Asphodelaceae), *Aloe ferox* Mill. (Fam. Asphodelaceae), *Asparagus falcatus* L. var. *falcatus* (Fam. Asparagaceae), *Eucomis autumnalis* (Mill) Chitt. (Fam. Hyacinthaceae) and *Hypoxis*.

hemerocallidae Fisch.Mey and Ave-Lall (Fam. Hypoxidaceae) were investigated.

MATERIALS AND METHODS

Plant collection, processing and extraction

Samples of cultivated plants were collected from Jotello Soga Ethnoveterinary medicine garden at Onderstepoort Veterinary Institute (Pretoria, South Africa), a year and four months after the establishment of the garden. Seedlings of these plants were obtained from a nursery in Kwa-Zulu Natal near Durban (South Africa) and basic garden implementation and maintenance practices were applied. Precautions were taken not to use chemicals excessively besides one light application of N3P2K3^(R), a fertiliser, at planting time. Wild plants were collected from some rural areas or bought from the medicinal plant markets. Informal markets are supplied by plant gatherers that collect the plants from undisclosed locations (Street et al., 2009). In order to minimise differences in age and environmental factors, same species of wild plant samples were pooled. Besides the roots of *A. falcatus* which were dried directly under shade, succulent leaves of Aloes and bulbs of *H. hemerocallidae* and *E. autumnalis* were first shredded and thereafter freeze -dried and further ground and kept in brown bottles until used. Approximately 5 g of ground material was extracted by sonication with dichloromethane (DCM), an intermediate polarity solvent and 90% methanol (10 ml/g) (MeOH), a polar solvent, respectively for one hour. The extractants were filtered using Whatman No.1 filter paper. All extracts were dried under reduced pressure at 40 °C using a rotavapor (Labotec(R)).

Antibacterial activity

Antibacterial screening was carried out using the microplate method for minimum inhibitory concentration (MIC) determination (Eloff, 1998). Two millilitres cultures of four bacterial strains: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 21212) 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; the two extractants were used as negative controls. One hundred micro-litres of each bacterial culture were 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 (Eloff, 1998).

Thin layer chromatography (TLC) investigations were not conducted. The beneficial effect of medicinal plant materials results from the combination of secondary metabolites and not from a single compound (Das et al., 2010). Moreover, the identification of a single active chemical entity that is responsible for the antimicrobial activity of a plant is becoming more and more improbable. It is widely considered by most traditional medicine practitioners as a fruitless academic exercise. Research should focus on the investigation of a combination of compounds to achieve a greater efficacy (Van Vuuren, 2008).

Statistical analysis and data description

There were 48 extracts for each plant category per extractant (e.g. fresh *A. ferox*). The inhibitory effects of the extracts on test bacteria ranging from 1.25 to 0.01 ml were recorded. MIC value of 0.3 mg/ml, that is, two-fold dilution away from the 1.25 mg/ml starting point, was considered as a cut-off point between effective and none effective inhibition in this study as a novel technique. Therefore, inhibition values were grouped into those that were less or equal to 0.3, considered as effective and those that were greater than 0.3 were considered as non-effective.

The statistical software package used to analyze the data is Stata 11 (StataCorp, 2009). The analysis was performed at 95% confidence limit. The multilevel linear models, also known as hierarchical linear models, for binary outcomes for both unadjusted and adjusted models were employed. The plant (name) was considered level-2 or higher level, while the actual observation was level-1 or lower level (Hox, 1995).

RESULTS

The crude estimates and estimates from adjusted models are presented here-under. The adjustments were made for the two extractants and four test microbe strains were used. Table 1 presents the crude estimates or the results from unadjusted models. The crude estimates of the odds ratio (OR) indicates that the wild plant material is significantly 0.57 [95% Class interval (CI): 0.383 – 0.847] times less likely than the garden plant material to yield minimum inhibitory concentration (MIC) values of more than 0.3 mg/ml (p-value = 0.005). Also, fresh are about 4.195 (95% CI: 2.662 – 6.6122) more likely than dry to yield MIC scores of more than 0.3 mg/ml (p-value < 0.001).

Table 1. Crude estimate of the odds ratio.

MIC category	Odds ratio	Std. Err.	z	P> z	[95% Confidence interval]	
_lWorG_2	0.5698925	0.1152641	-2.78	0.005	0.3833826	0.8471365
_lCategory_2	4.195155	0.9738682	6.18	0.000	2.661639	6.612214

Std. Err. = standard error.

Table 2. Adjusting for conditions “dry and fresh” and “wild or garden”.

MIC category	Odds Ratio	Std. Err.	z	P> z	[95% Confidence interval]	
_lWorG_2	0.5468442	0.1135394	-2.91	0.004	0.3640253	0.8214775
_lCategory_2	3.791773	0.8552593	5.91	0.000	2.436959	5.899789

MIC = Minimum inhibitory concentration; Std. Err. = standard error.

Table 3. Adjusting for conditions “dry and fresh” and “wild or garden” and, type of microbes.

MIC category	Odds ratio	Std. Err.	z	P> z	[95% Confidence interval]	
_lWorG_2	0.530297	0.1143576	-2.94	0.003	0.3475034	0.8092435
_lCategory_2	4.065883	0.9640142	5.92	0.000	2.554679	6.471029
_lmicrobec~2	0.6862754	0.1934506	-1.34	0.182	0.3949641	1.192447
_lmicrobec~3	1.454235	0.434486	1.25	0.210	0.8096898	2.611864
_lmicrobec~4	2.012137	0.632242	2.23	0.026	1.086921	3.724921

MIC = Minimum inhibitory concentration; Std. Err. = standard error.

Table 4. Adjusting for conditions “dry and fresh” and “wild or garden” and, microbe and solvent.

MIC category	Odds ratio	Std. Err.	z	P> z	[95% Confidence interval]	
_lWorG_2	0.5216833	0.1130011	-3.00	0.003	0.3412159	0.797599
_lCategory_2	4.201871	1.000163	6.03	0.000	2.635318	6.699655
_lmicrobec~2	0.6786136	0.192562	-1.37	0.172	0.3891234	1.183471
_lmicrobec~3	1.468426	0.4400733	1.28	0.200	0.8161216	2.642099
_lmicrobec~4	2.045504	0.6435597	2.27	0.023	1.104063	3.789717
_lsolventc~2	2.315786	0.5092249	3.82	0.000	1.504961	3.563457

MIC = Minimum inhibitory concentration; Std. Err. = standard error.

Adjusting for conditions (dry and fresh); the wild is significantly 0.546 (95% CI: 0.364 – 0.821) times less likely than the garden forms to yield MIC values of more than 0.3 mg/ml (p-value = 0.004). Similarly, adjusting for the wild or garden, the fresh forms are significantly 3.79 (95%CI: 2.437 – 5.899) more likely than dry to yield MIC scores of more than 0.3 mg/ml (p-value < 0.001). Table 2 attempts to explicitly represent this information.

The results from further adjustment of covariants are given by Table 3. Adjusting for conditions (dry and fresh) and type of microbe; the wild form is significantly 0.530 (95% CI: 0.348 – 0.809) times less likely than the garden form to yield MIC values of more than 0.3 mg/ml or to produce non-effective inhibition values (p-value = 0.003). While adjusting for “wild or garden” and type of microbe,

fresh is significantly 4.06 (95% CI: 2.555 – 6.471) times more likely than dry to yield non-effective inhibition values (p-value < 0.001).

Adjusting for conditions “dry and fresh”, microbe and solvent (Table 4); wild is significantly 0.52 times less likely than Garden to yield MIC values of more than 0.3 mg/mL (p-value = 0.003). On the other hand, when adjusting for “wild or garden”, type of solvents and type of microbes; fresh is significantly 4.202 times more likely than dry to yield non-effective inhibition values (p-value < 0.001).

DISCUSSION

The results from the sample used in this study do support

partially, many reports from farmers who argue that wild plant materials are more potent than the grown ones (Luseba and van der Merwe, 2006). However, Kizil (2006) found similar major constituents of essential oil in both wild and cultivated *Thymbra* species. Moreover, the distribution of some essential oil constituents was not different between the two forms (wild versus cultivated). According to Aguilar-Stoen and Moe (2007), medicinal plants represent one of the most significant ways in which humans directly reap the benefits provided by diversity and further argue that a very low proportion is cultivated. It is therefore difficult to support medicinal plants cultivation blindly and set coercive measures against harvesting from the wild in order to ensure conservation and sustainable management.

It is generally accepted that harvesting from the wild as the main source of raw material is causing loss of genetic diversity and habitat destruction. Domestic cultivation is proposed as a viable alternative which offers the opportunity to overcome the problems that are inherent to herbal extracts. However, obstacles to bringing medicinal plants into successful commercial cultivation include the difficulty of predicting which extracts will remain marketable and the likely market preference for what is seen as naturally sourced extracts (Canter et al., 2005). Moreover, for pastoralists and small-scale farmers and livestock keepers, land issue and lack of stable homesteads might be a problem to ensure Good Agriculture Practices (GAP) which is the primary condition for ensuring quality in terms of safety and efficacy of medicinal plants.

Inappropriate methods of collection, processing and storage with undesirable contaminants in the products contribute negatively to the quality of traditional medicines. Moreover, natural products collected from the wild are not necessarily safe merely because they are natural (Street et al., 2008); the efficacy and safety testing of some of the study materials was undertaken by Luseba et al. (2007).

Research has also shown that cultivating and harvesting medicinal plants at different age will affect their chemical compound and bioactivities (Taylor and van Staden, 2001). In this study, plant collected from the garden might have reached their maturity after a year since they are annual crops; moreover, roots of *A. falcatus* and bulbs of *E. autumnalis* and *H. hemerocallidae* were used. Studies of anti-inflammatory activity of *E. autumnalis* showed that young plants had larger amount of COX-1 inhibition, particularly in leaves, whilst in older plants; activity was more prominent in the bulb (Taylor and van Staden, 2001).

These results are also in favour of drying plant materials as fresh materials had MIC values higher than 0.3 mg/ml cut-off point. Drying plants can also be encouraged on the basis that having the remedies on hand when needed can help in controlling indiscriminate harvest brought about when hastened to treat the animal. Moreover, it can be speculated that succulent plants

might act better when dried. This is obviously the consequence of concentration or accumulation of substances in dried materials. As water is the fundamental requirement in microbial growth (Cooke and Whips, 1993), the dried plant materials in either powdery or chopped form are better protected from microorganism infestations. Fungi do also grow easily on succulent plant materials. From the Ayurvedic point of view (Dick, 2002), drying plant materials affect potency. There are numerous hydrophilic (alkaloids, glycosides and tannins) and lipophilic (e.g. volatile oils, resins and balsams) substances in the herb. These substances migrate together and active principles, partially precipitated, and segregated enzymes commingle causing reactions such as hydrolysis, oxidation and polymerization during drying and storage. Further, endogenous enzymes act to digest the plant material, as part of natural biodegradation.

Luseba and Van der Merwe (2006) noted that traditional healers and knowledgeable farmers dry essential plant remedies in bundles. It has been also recommended to process these materials in powder for better dosage determination and manufacturing of easier delivery forms such as capsules. This has been recognised as a major limitation in traditional medicine processing (Hillenbrand, 2006). Extraction of fleshy bulbs is more complicated; mucilage present in fresh materials might be a problem in extraction of succulent materials such as fleshy bulbs of *H. hemerocallidae* and *E. autumnalis*. However, freezing by inducing cellular membrane damage allows more intra-cellular components to be extracted. This is important for industry purpose where maximal extraction is important (Naidoo et al., 2004).

Substitution of plant parts that are more destructive to biodiversity like corms is sometimes advocated for conservation purpose as it was demonstrated with *Prunus africana*. However, Katerere and Eloff (2008) demonstrated clear chemical and bioactive differences between aerial and underground parts of *Hypoxis* spp. implying that it is not always possible to substitute one part with the other. Cultivation of *H. hemerocallidae* like other endangered plant species is therefore considered as imperative.

Furthermore, it seems that recommendations to grow medicinal plants should be made in consideration of some limitations such as uncertainty of the market demand and specific characteristics, restrictions for sale and purchase compared with cash produces and supply growing which is sometimes faster than demand (Tsutsui and Saiprasert, 1996). Field-grown plants are naturally inhabited by a variety of bacteria, insects and fungi which can affect the level of metabolites (Murch et al., 2000). Growing conditions and management practices for a given crop, that is, photoperiod, intensity, and spectrum of the available light during a cropping season have also been shown to influence the synthesis of secondary metabolites. Warm weather conditions increases the synthesis of secondary metabolites but rainy weather can inhibit alkaloid production in many species (Murch et al.,

2000). Conversely, according to Aguilar-Stoen and Moe (2007), medicinal plant species are widely distributed across the globe; these plant species thriving in disturbed and secondary growth habitats where they are in high competition and adapted to increased herbivory, produce more secondary metabolites as defence mechanisms and therefore are more potent as medicines. However, what is not known is the amount of stress that might also be present in plants grown in an artificial environment and among different or strange plant populations constituted by the garden landscape.

Conclusions

According to Street et al. (2009), many South African medicinal plants have been screened for biological activity with biological activity of crude plant extracts being considered sufficient to validate the uses in traditional medicine. This study goes a step further in validating the two main categories, that is, "wild and grown" and "fresh or dry" medicinal plants based on their biological activity. Wild and dry medicinal plants had better biological activities; however, cultivating and drying medicinal plants might be a more suitable option for prompt response to treatment of livestock diseases by small-scale farmers and biodiversity conservation.

However, cultivation cannot be forced on communities; there are limitations which are inherent with the farming system involved, in this case, the small scale system where farmers, pastoralists and livestock keepers might not even have appropriate land for sustainable cultivation of essential materials. Cultivation has significance on the conservation of endangered plants because by growing and processing plant materials, farmers will avoid uncontrolled harvest of precious biomes. Unfortunately, cultivation is still limited to few plant species; harvesting from the wild, in time and drying can be an appropriate recommendation.

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