



Phytochemical screening and pharmacological evaluation of herbal concoctions sold at Ga Maja Limpopo Province

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ABSTRACT

Informal street merchants and traditional health practitioners at Ga Maja (Limpopo Province) primarily offer consumers semi-processed herbal preparations that are indicated to have blood cleansing, detoxifying, antidiarrheal and pain relieving properties. The focus of this study was to evaluate the phytochemical composition of the concoctions and substantiate the pharmacological effects and safety indicated by the traders. Five herbal concoctions and plant material used in their preparation were purchased from five independent traders and a laboratory concoction was prepared according to the traders' instructions. Possible bacterial and fungal contaminants were isolated and identified using Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). Qualitative phytochemical analysis was determined using standard chemical tests and thin layer chromatography. Total polyphenol content was quantified. Antioxidant activity was quantified using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing power. Antimicrobial activities were determined using a broth micro-dilution assay and bioautography. Cell viability assay was used to determine the cytotoxicity of the concoctions. Pathogenic bacteria, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Escherichia coli* and *Klebsiella pneumoniae* were identified as bacterial contaminants. The commercial concoctions and the laboratory standard had similar phyto-constituents and phytochemical fingerprint profiles. The antimicrobial properties of the concoctions were a result of synergistic effects of the compounds because no single compound was observed to have antimicrobial activities on the bioautograms. The phenolic content, antioxidant and antimicrobial activities varied substantially amongst the concoctions. The lack of standardisation methods reduces the pharmacological potential of the products. This study concludes that while plants with biological activities were used by the traders to prepare the concoctions, the efficacious concentrations to produce a therapeutic response were not adequately measured and adhered to. Furthermore, although the concoctions did not exhibit cytotoxic effects, toxicities may arise from endotoxins produced by the microbial contaminants. Hygienic processing and packaging are essential to ensure that consumers receive quality products that are safe to consume.

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1. Background

Informal street merchants and traditional health practitioners primarily offer consumers semi-processed herbal preparations that are commonly prepared in small batches. In preparing the herbal concoctions, fresh or dry plant material can be used; the plant material can either be macerated in water for several days or generally boiled in hot water (Ndhala and Van Staden, 2012). In South Africa, herbal products that are sold by informal traders are usually indicated to be immune and energy boosters, blood cleansers, detoxifiers and aphrodisiacs (Ndhala et al., 2009).

Some of these formulations are unstable, vary in strength and generally have short shelf lives. The poor physical conditions employed in preparation, such as unsterile working environment and storage,

contribute to the short expiry period. Unfortunately, the production of such remedies leads to the depletion and wastage of plant material (Nwankwo et al., 2012).

The plant parts commonly used as ingredients in preparation of the herbal concoctions include leaves, stems, barks, roots, rhizomes, bulbs and/or seeds. The complexity of the formulations is dependent on the severity of the ailment. Simple home remedies can be prepared for trivial ailments such as diarrhoea, coughs, pains and gastrointestinal disorder. However, more elaborate procedures of preparations are required for life-threatening conditions (Cano and Volpato, 2004).

A single plant species can produce numerous bioactive compounds that are neither stringently required for metabolic processes nor do they form part of nutrition. The production of the compounds is subject to the interaction of the plant with the environment in which it is supposed to thrive (Okem et al., 2015). The biological activity of these compounds is attributed to their role in the plants' survival. Some of these compounds are synthesised to effectively shield the plant from

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bacterial, fungal, parasitic and viral attacks (Kennedy and Wightman, 2011). Therefore, in preparing herbal remedies by using various plant parts from different sources, one can realise the extent of the number of compounds which are present and the possibility for the constituents to chemically interact.

There has been increasing interest in the use of herbal products. This is witnessed by the frequent use of herbal products by people not only living in the rural locations, but also urban areas. This demonstrates that even though impoverished rural communities use herbal products due to a lack of healthcare infrastructure and the cost of modern pharmaceuticals (Marsland, 2007), people in urban locations use traditional medicine due to the lack of trust in the ability of western medicine to treat not only the diseases itself but rather also the mental aspect of ill-health.

The broad use of traditional herbal remedies has encouraged manufacturers, private traders and street merchants to capitalise on this upsurge by increasing the availability of herbal remedies to those who desire them (Ndhkala and Van Staden, 2012).

The allure in the use of traditional herbal medicines is the holistic approach which is used to treat an individual. The mode of treatment does not only involve the use of herbal remedies, but includes performing incarnations that are believed to give the remedy more strength.

Herbal concoctions here in this study were purchased from traders established at Ga Maja (Limpopo Province). The traders sell herbal concoctions indicated to have aphrodisiac, antidiarrheal, blood cleansing and pain relieving properties. The traders at these locations use 500 mL to 5 L recycled plastic bottles. The bottles are not labelled with product information and only the word of the trader has to be taken into account regarding the ingredients. This may compromise the products due to more prone possible microbial contamination, whilst the lack of labelling may permit adulteration regarding materials used.

The proper labelling of herbal products is of utmost importance. Labels on herbal products provide the consumer with information about its contents, that is; the list and quantity of the active ingredients, the mode and frequency of administration and potential side effects. Moreover, the labels should include details about the expiry date, any additives such as preservatives and appropriate methods for storing and maintaining the product.

The degree of chemical purity of phyto-medicines can be assessed using an array of analytical methods. In order to analyse the phyto-chemical profile of a complex mixture, techniques such a thin layer chromatography (TLC) become useful and powerful. It is time efficient and a quick resolution towards challenges involved with being able to discriminate and develop fingerprints for major chemical classes that are present in mixtures (Zeng et al., 2008).

In order for the herbal concoctions to be reputable, maintain quality, reliability and be marketable, their efficacy and safety status must meet quality health standards. However, investigating herbal products is accompanied by the challenge that some herbalists, traditional healers

and/or traders are reluctant to divulge the ingredients and formulae to some of their products. This underscores research efforts aimed at standardising and providing evidence-based pharmacological effects of the remedies (Matotoka and Masoko, 2017).

This study was undertaken to evaluate and compare the phytochemical profiles of different commercial concoctions as well as to substantiate the pharmacological effects indicated by the traders. Safety regarding the consumption on these concoctions was evaluated by assessing microbial contamination and cytotoxic effects.

2. Methods and materials

2.1. Sample procurement and processing

Five herbal concoctions were randomly purchased from five independent traders from Ga Maja; this included the plant material used in their preparation. Table 1 shows the list of the collected plant material. The tables also include the vernacular names of the plants and the concoction samples in which they are added as ingredients. The voucher specimens for the collected plant species were deposited in the Larry Leach herbarium of the University of Limpopo. The plant material was left to dry at room temperature in a well ventilated room away from sunlight. The corms were cut into smaller pieces to increase the surface area of the parts to allow for a quicker drying period. The dried material was ground to fine powder using a commercial blender and stored in airtight glass containers. The samples were stored at room temperature in the dark.

2.2. Isolation and identification of microbial contaminants from concoctions

The traders prepared the herbal concoctions by boiling the plant material (Table 1) in water and packaged them in recycled plastic bottles. The five products were cultured on nutrient agar (NA) (Fluka, Switzerland) and Potato dextrose agar (PDA) (Fluka, Switzerland) plates immediately on the day of collection, using the spread-plate technique. Under aseptic conditions, 100 µL of each concoction were spread over the surface of separate and labelled plates using a sterile bent glass rod. The PDA plates were incubated at 25 °C for 48 h and the NA plates at 37 °C for 24 h. Viable colonies were purified using light microscopy and Gram staining procedures. The identification of the pure cultures of bacteria was carried out using Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). This technology is rapid and primarily relies on the detection of a great range of proteins which enables it to differentiate and classify even closely related organisms (Biswas and Rolain, 2013).

2.3. Sample preparation

In preparation, the traders rely on visual observation to try and keep the amount of plant material (leaves and roots) added constant.

Table 1
Plant material used by traders to prepare herbal concoctions.

Plant material	Voucher number	Vernacular name	HC1	HC2	HC3	HC4	HC5
<i>Kirkia wilmsii</i> (leaves)	SS 94	<i>Legaba/modumela</i>	+	+	+	+	—
<i>Kirkia wilmsii</i> (corm)	SS 94	<i>Legaba/modumela</i>	+	+	+	+	+
<i>Kirkia wilmsii</i> (twigs)	SS 94	<i>Legaba/modumela</i>	—	—	—	+	+
<i>Hypoxis hemerocallidea</i> (corm)	SS 115	<i>Monna maledu</i>	+	+	+	+	+
<i>Monsonia angustifolia</i> (leaves)	121393	<i>Tee ya thaba</i>	+	+	+	+	+
<i>Drimia elata</i> (corm)	S 18	<i>Sekanama</i>	+	+	+	—	—
<i>Sarcostemma viminalae</i>	121404	<i>Moema</i>	—	—	+	+	+
<i>Vahlia capensis</i>	121394	<i>Makgonatsohle</i>	+	+	+	+	+
"Tshikwana/Morotwa tshwene" (powder) ^a			—	+	+	+	+

Key: (+): Included, (—): Not included, HC1-sample 1; HC2- sample 2; HC3-Sample 3; HC4-Sample 4; HC5-Sample5; LC- Lab Concoction.

^a To the best of our knowledge, *Tshikwana/Morotwa tshwene* is a powdered plant mixture consisting *D. elata*, *S. viminalae*, *V. capensis* and roots of unspecified plant species. The traditional healers were reluctant to divulge all the components of this mixture citing that secrecy contributed efficacy of these powders because intervention bytheir ancestral gods enhanced the therapeutic action of the treatment.

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