PHYTOCHEMICAL CHARACTERIZATION OF SELECTED HERBAL PRODUCTS IN LESOTHO

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ABSTRACT

Information on herbal products in Lesotho is lacking. The study characterised some herbal products found in Lesotho and compared herbalists' prescriptions with literature. FTIR spectroscopic scan of the herbal products was also done. Phytochemical screening of five powdered herbal products from the Lesotho Herbal Medicines Repository (LHMR) was done using qualitative and FTIR-spectroscopic standard methods. Phytochemical extraction of *Euclea coriacea*, *Hypoxis hemerocallidea, Xysmalobium undulatum*, *Senecio asperulus* and *Pelargonium sidoides* was done using water, methanol and acetone as solvents. *Euclea coriacea* was found to contain diterpenes and phytosterols while *Hypoxis hemerocallidea* had diterpenes, flavonoids and phytosterols. Phytosterols, flavonoids, glycosides were detected in *Senecio asperulus. Pelargonium sidoides* and *Xysmalobium undulatum* contained glycosides and phytosterols respectively. Although all the herbal plants analysed were found to have medicinal properties, local herbalists' prescriptions of *Euclea coriacea* and *Xysmalobium undulatum* did not concur with literature. Fourier Transform Infra-Red (FTIR) spectroscopy of herbal plants indicated specific spectra which can be used to identify herbal plant components.

INTRODUCTION

A large number of people in both developing and developed countries rely on herbal products for various ailments (Smith-Hall *et al.,* 2012; Sasidharan *et al.,* 2011). Most rural populations, especially in the developing world, depend on herbal products as their main source of primary health care. It was reported that trade in herbal products is a multi-million dollar business which is a major driver for rural economies (Katerere *et al.,* 2008). A survey in the USA found that people use herbal products because they are natural products, have fewer side effects, are less expensive and milder (Eloff *et al.*, 2011).

Use of herbal products needs to be regulated and coordinated for safety and sustainability (Ajazuddin & Shailendra, 2012). Quality control and stability testing is usually not done in most countries thereby rendering the herbal products unsafe for public use (WHO, 1996). Only 25 of 191 WHO member countries have policies and regulations on use of herbal products (Herman *et al.*, 2013). In Lesotho, the sale of traditional herbal products is not regulated. The use of herbal products by people in Lesotho including those with HIV is believed to be widespread, although insufficiently documented.

Ingredients, dosages, side effects and contraindications of herbal products sold in Lesotho are usually not listed or improperly labeled. In addition, most of the herbal products have multiple indications on the label. Herbal products can be toxic when inappropriately prepared and prescribed (Haneef *et al.*, 2013). Furthermore, excessive dosages, or prolonged use of some herbal products can have adverse effects (Phua *et al.*, 2009). For example, chronic use of aloe can cause loss of electrolytes (Saka *et al.*, 2012; Yonganandam *et al.*, 2010).

In some parts of the world, people mix herbal products with clinical drugs (Posadzki *et al.*, 2013, Savaliya *et al.*, 2010). Adulterated herbal products are potentially harmful due to adverse interactions with conventional drugs (Haneef *et al.*, 2013; Ernst, 2004). Adulteration of herbal products may have serious effects (Kozyrskyj, 1997). There is also a possibility of misidentification of herbal plants, potential contamination with other plant species products or other ingredients during processing (Khan & Smillie, 2012). Therefore, screening of herbal products for ingredients is important.

The storage conditions for herbal products are usually not specified. The conditions in which plant products are transported and stored may expose the products to bacterial and fungal contamination (Katerere *et al.*, 2008; Temu-Justin *et al.*, 1998). Contamination of herbal products poses health hazards to consumers of the herbal products.

Herbal products usually contain phytochemicals. Phytochemicals are non-nutritive, diversified plant chemicals that have disease preventive or curative properties. They are a lead used in developing new drug entities covering a wide range of therapeutic indications (Kar & Roy, 2012). Limited access to the expensive equipment required for the screening of phytochemicals could be one of the reasons why most countries do not regulate use of herbal products. Affordable, effective and rapid phytochemical screening methods that can detect clinical drugs in herbal products are therefore needed. There are newer analytical methods for phytochemical screening and detection of adulteration in herbal products (Haneef *et al.*, 2013). Fourier Transform Infra Red (FTIR) spectroscopy is one such method (Deisingh, 2005). FTIR analysis is rapid and needs no chemical treatment of samples prior to analysis. According to Sasidharan *et al.* (2011), FTIR spectra of pure plant compounds are unique and the spectra of unknown compounds are identifiable by referring to a library of known compounds.

This study aimed at characterising some herbal products sold in Lesotho using qualitative analysis and comparing herbalists' prescriptions with literature. FTIR spectroscopic scan for the herbal products was also done.

METHODOLOGY

Study design

The study involved qualitative and FTIR-spectroscopic screening of five dried powdered herbal products from the Lesotho Herbal Medicines Repository (LHMR) in the Department of Pharmacy at the National University of Lesotho (NUL). The herbs, their indications and plant parts used, according to herbalists, are shown in Table 1.

Table 1: Names and parts of herbs used in the study and indications given by herbalists

Scientific name	Local name	Parts	Indication
Euclea coriacea (Ebenaceae)	*Leleme la khomo*	Roots, leaves and stems	Gonorrhea
Hypoxis hemerocallidea (Hypoxidaceae)	*Moli* (common name- African potato)	Roots	Poor immunity (boosts immune system), chronic wounds
Xysmalobium undulatum (Asclepiadoideae)	*Hloenya*	Roots	High blood pressure
Senecio asperulus (Apocynaceae, subfamily- Asteraceae)	*Moferefere*	Roots and leaves	Back pain, swollen feet
Pelargonium sidoides (Geraniaceae)	*Pitsa ea litsolo*	Roots	Severe diarrhea

Extraction and screening of phytochemicals

Phytochemical extraction of the herbs was done using water, methanol and acetone as solvents. The method of extraction used was plant tissue homogenization. Portions of each sample (0.5g) were dissolved in 5ml of methanol and acetone separately. For water extraction, the samples (0.5g) were heated in 10ml of water but not allowed to boil. All the extracts were shaken vigorously for 5 minutes and left for 24 hours, after which the extracts were centrifuged for clarification. Then supernatants were separated, bottled (see Figure 1) and stored at -22°C before screening for phytochemicals. Phytochemical screening was done as per the standard methods of the tests shown in Table 2 (Tiwari *et al.*, 2011).

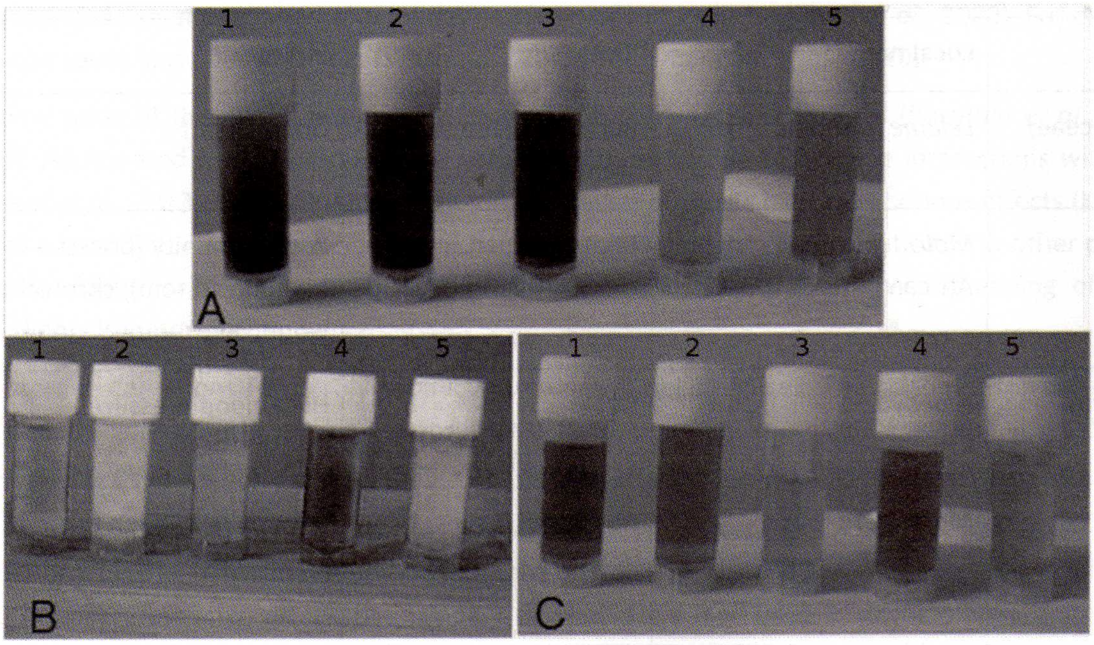


Figure 1: Water (A), methanol (B) and acetone (C) extracts of plant samples

1 = *Euclea coriacea*, 2 = *Hypoxis hemerocallidea*, 3 = *Xysmalobium undulatum*, 4 = *Senecio asperulus and* 5 = *Pelargonium sidoides*

FTIR-spectroscopic of screening herbal products

Small amounts (500 mg) of powdered herbal products were crushed until fine powders were obtained using a mortar and a pestle and were sieved with 0.25 mm pore sized sieves. A small portion of each powdered sample (20 mg) and 100 mg Potassium Bromide (KBr) were thoroughly mixed using a mortar and a pestle and loaded on the FTIR spectrometer sample holders. FTIR spectroscopic transmittance spectra were obtained in the region 4500 – 450 cm-1 at resolution 4 cm-1 with 10 scans using Shimadzu IR-Prestige-21 Fourier spectrometer.

PHYTOCHEMICAL CHARACTERIZATION

Table 2: Methods used to screen for phytochemicals

Phytochemical test	Phytochemical tested for	Method
Wagner's test	Alkaloids	The extracts were dissolved individually in dilute hydrochloric acid and filtered, and then the filtrates were treated with wagner's reagent; iodine in potassium iodide.
Borntrager's test	Glycosides	The extracts were hydrolysed with diluted hydrolysed hydrochloric acid and treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution.
Froth test	Saponins	The extracts were treated with 5ml of distilled and shaken in a graduated cylinder for 15 minutes.
Salkowski's test	Phytosterols	The extracts were treated with chloroform and filtered. The filtrates were then treated with 3 drops of concentrated sulphuric acid, shaken and allowed to stand.
Ferric chloride test	phenols	Extracts were treated with 3 drops of ferric chloride solution.
Gelatin test	tannins	The extracts were treated with 1% of gelatin solution containing sodium chloride.
Alkaline reagent and lead acetate tests	flavonoids	The extracts were treated with few 3 drops of sodium hydroxide solution. The extracts were also treated with few drops of lead acetate solution.
Ninhydrin test	proteins and aminoacids	0.25% w/v ninhydrin reagent was added to the extracts and boiled for 3 minutes.
Copper acetate test	diterpenes	The extracts were dissolved in water and treated with 3 drops of copper acetate solution.

RESULTS

All screened samples tested negative for alkaloids, phenols, tannins, proteins and saponnins. Phytochemicals that tested positive in at least one of the extracts were flavonoids, glycosides, phytosterols and diterpenes (Table 3).

PHYTOCHEMICAL CHARACTERIZATION

Depending on the solvent used, different plant extracts yielded different phytochemicals. Four of the five plants were found to contain phytosterols. Some plants were found to possess up to three phytochemicals. *Euclea coriacea* was found to contain diterpenes and phytosterols while *Hypoxis hemerocallidea* had diterpenes, flavonoids and phytosterols. Phytosterols, flavonoids, glycosides were detected in *Senecio asperulus. Pelargonium sidoides* and *Xysmalobium undulatum* contained glycosides and phytosterols respectively.

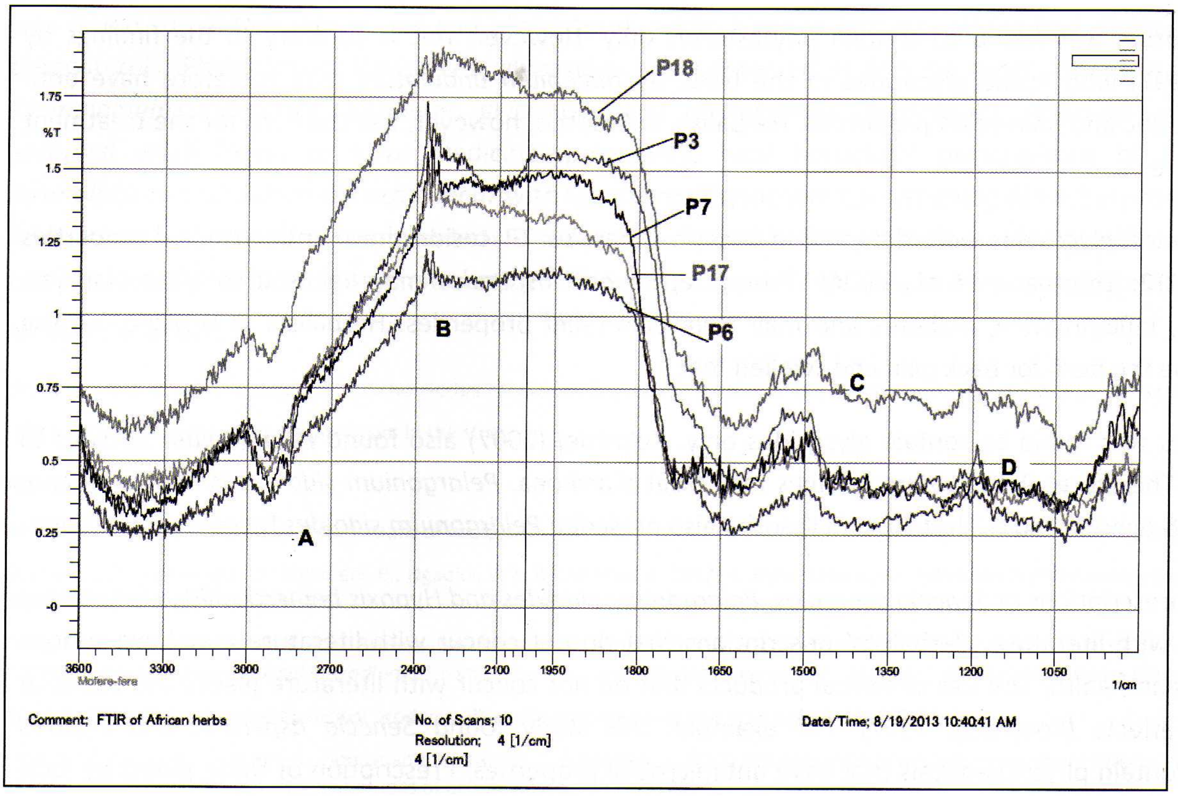
Table 3: Results of phytochemical screening of the herbal products

TESTS	SAMPLES	WATER EXTRACT	METHANOL EXTRACT	ACETONE EXTRACT	INTERPRETATION
GLYCOSIDES Borntrager's test	*Euclea coriacea*	Negative	Negative	Negative	Glycosides present in *Senecio asperulus* and *Pelargonium sidoides*
	Hypoxis hemerocallidea	Negative	Negative	Negative	
	Xysmalobium undulatum	Negative	Negative	Negative	
	Senecio asperulus	Positive	Negative	Negative	
	Pelargonium sidoides	Positive	Negative	Negative	
PHYTOSTEROLS Salkowski's test	*Euclea coriacea*	-	Negative	Positive	Phytosterols present *Euclea coriacea*, *Hypoxis hemerocallidea*, *Xysmalobium undulatum* and *Senecio asperulus*.
	Hypoxis hemerocallidea	-	Negative	Positive	
	Xysmalobium undulatum	-	Positive	Negative	
	Senecio asperulus	-	Negative	Positive	
	Pelargonium sidoides	-	Negative	Negative	
FLAVONOIDS Alkaline test	*Euclea coriacea*	-	Negative	Negative	Flavonoids resent in *Hypoxis hemerocallidea*, *Senecio asperulus*.
	Hypoxis hemerocallidea	-	Negative	Positive	
	Xysmalobium undulatum	-	Negative	Negative	
	Senecio asperulus	-	Positive	Positive	
	Pelargonium sidoides	-	Negative	Negative	
DITERPENES Copper acetate test	*Euclea coriacea*	Positive	Negative	-	Diterpenes present *Euclea coriacea*, *Hypoxis hemerocallidea*
	Hypoxis hemerocallidea	Positive	Positive	-	
	Xysmalobium undulatum	Negative	Negative	-	
	Senecio asperulus	Negative	Negative	-	
	Pelargonium sidoides	Negative	Negative	-	

A dash (-) indicates that the phytochemical was not tested for because the phytochemical is insoluble in the extract.

PHYTOCHEMICAL CHARACTERIZATION

FTIR spectra for the plant samples in the region 3600 – 900 cm-1 at resolution 4 cm-1 with 10 scans are presented in Figure 2. The spectra obtained showed similar trends for all plant samples with peaks indicated by A – D.



Fugure 2: Characteristic FTIR Spectra from the powdered herbal products

P03 = *Euclea coriacea;* P06 = *Hypoxis hemerocallidea;* P07 = *Xysmalobium undulatum;* P17 = *Senecio asperulus;* P18 = *Pelargonium sidoides*

DISCUSSION

The findings of this study confirmed that the herbs contained phytochemicals. All the herbal plants screened contained at least one phytochemical, which implies that these plants may have medicinal properties.

Euclea coriacea was found to contain diterpenes and phytosterols. Diterpenes are known to have anti-cancer effects in human cells (Li *et al.*, 2013; Fronza *et al.*, 2012; Pesic *et al.*, 2011). Phytosterols have been demonstrated to have anti-inflamatory (Garcia *et al.*, 1999), antidiabetic (Tanaka *et al.*, 2006) and pain-relief (antinociceptive) (Santos *et al.*, 2011) effects. Therefore, *Euclea coriacea* may have anti-inflamatory, antidiabetic and pain-relief effects. However, herbalists in Lesotho prescribe *Euclea coriacea* for gonorrhea treatment.

Hypoxis hemerocallidea was found to contain diterpenes, flavonoids and phytosterols. Flavonoids have antimicrobial (Dzoyem *et al.*, 2013, Leite *et al.*, 2012, Li *et al.*, 2012) and antidiarrhoeal (Yao *et al.*, 2011) effects. *Hypoxis hemerocallidea* may be prescribed for these conditions. Ojewole (2006) found *Hypoxis hemerocallidea* to have pain-relief, anti-inflammatory and antidiabetic properties. Nonetheless, local herbalists prescribe *Hypoxis hemerocallidea* for poor immunity and chronic wounds.

Xysmalobium undulatum was found to contain phytosterols only. However, this is contrary to the findings by (Ghorbani *et al.*, 1997) who found glycosides in the herb. *Xysmalobium undulatum* may therefore have anti-inflamatory, antidiabetic and pain-relief properties. Herbalists in Lesotho, however, use the herb for the treatment of high blood pressure.

Phytosterols, flavonoids, glycosides were detected in *Senecio asperulus.* Glycosides have antidiarrhoeal properties (Schmiedl *et al.*, 2012; Thurmann *et al.*, 2004). Therefore, *Senecio asperulus* may be used to treat diarrhea, microbial infections, inflammation, diabetes and may have pain-relief properties. Herbalists in Lesotho, on the other hand, prescribe the herb for back pain and swollen feet.

Pelargonium sidoides was found to contain glycosides only. Kolodziej (2007) also found *Pelargonium sidoides* to possess glycosides. Therefore, *Pelargonium sidoides* may treat diarrhoea. *Pelargonium sidoides* is known to cure diarrhoea (Noldner & Schotz, 2007). Herbalists in Lesotho also prescribe *Pelargonium sidoides* for severe diarrhoea.

Overall, herbalists' prescriptions of *Senecio asperulus, Pelargonium sidoides and Hypoxis hemerocallidea* for various ailments concurred with literature. Herbalists' priscriptions that do not concur with literature may have serious implications on human health. The use of herbal products that do not concur with literature places the public at risk of unwanted effects (Kozyrskyj, 1997). For example, this study found *Senecio asperulus* and *Hypoxis hemerocallidea* to contain phytochemicals that have antimicrobial properties. Prescription of these plants by local herbalists may result in the development of antimicrobial resistance in the recipients. To improve concurrence between herbalists' prescriptions and literature, there is need for screening for phytochemicals in more herbs used by herbalists in Lesotho.

The FTIR spectra for the plants indicated that herbal plants have specific spectra which can be used to identify herbal plants. FTIR spectroscopic herbal spectra have been used to identify herbal chemical components using software that enable clustering and discriminative analysis of the phytochemicals (Tong *et al.*, 2011; Suo *et al.*, 2010). Tong *et al.* (2011) proposed application of FTIR spectroscopy for the identification of adulteration in herbal products. Since some people mix herbal products with clinical drugs (Posadzki *et al.*, 2013), adulterated herbal products can therefore be detected using FTIR spectroscopy. The current study did not compare herbal FTIR spectra with reference library compounds. FTIR spectroscopy needs to be coupled with library software to identify chemical components (Suo *et al.*, 2010). Although FTIR spectroscopic analysis in this study could not identify the herbal components including the phytochemicals present in the samples, the spectra suggest absence of adulteration.

CONCLUSION

All herbal products analysed in the current study were found to possess at least one phytochemical. *Euclea coriacea* was found to contain diterpenes and phytosterols while *Hypoxis hemerocallidea* had diterpenes, flavonoids and phytosterols. Phytosterols, flavonoids, glycosides were detected in *Senecio asperulus. Pelargonium sidoides* and *Xysmalobium undulatum* contained glycosides and phytosterols respectively. Although all the herbal plants analysed were found to have medicinal properties, local herbalists' prescriptions of *Euclea coriacea* and *Xysmalobium undulatum* did not concur with literature. Phytochemical screening of herbal products in Lesotho on a wider scale is therefore recommended. FTIR spectroscopy of herbal plants indicated specific spectra which can be used to identify herbal plant components. FTIR spectroscopy may be used to detect adulterated herbal products.

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