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Soxhlet extractor coupled with Liquid-liquid extraction: Setting up a pilot library of refined crude extracts from indigenous medicinal plants of Lesotho

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Abstract

Infections associated with multidrug resistant organisms result in increased morbidity and mortality in Lesotho and globally, thus, there is immediate need to discover new antibiotics against resistant bacteria through systematic examination of inexpensive local herbs. Here, we report for the first time, the well-defined and publicly available prototype library that consists of 20 different crude extracts derived from crude drug samples of medicinal plants of Lesotho used for treatment of infectious diseases. We also described the coupling of soxhlet extraction with liquid-liquid fractionation to give partially refined organic and aqueous extracts, and disclose that the extracts from the leaves and whole plant contained about twice as much organic soluble phytochemicals as aqueous soluble phytoconstituents. While the roots and bark contained about 2 and 5 times more of aqueous soluble than organic soluble phytochemicals respectively, especially when extracting with 90% ethanol. The library represents the potential phytochemicals in herbal prescriptions dispensed by traditional healers in Lesotho. The extracts are publicly available upon request through the corresponding author and efforts to expand the library are still continuing. This methodology could be followed to develop other libraries.

Keywords: Antibiotic resistance, soxhlet extraction, liquid-liquid extraction, crude drugs, prototype library

Introduction

The issue of antibiotic resistance is the universal concern, thus, there is immediate need to discover new antibiotics against resistant bacteria through systematic exploration of inexpensive local herbs [1]. According to Tim Cushnie *et al.*, [2] frequently encountered conventional antibiotics in clinical setting, such as β -lactams, aminoglycosides, tetracyclines and macrolides were discovered through systematic examination of traditional natural products and associated traditional knowledge. Even though the indigenous communities such as Basotho relied on traditional medicinal herbs for the treatment of infectious disease since time immemorial [3] new antibiotic discoveries from medicinal plants are especially rare possibly due to an over-reliance on synthetic chemical libraries. Well-defined and publicly-available libraries of crude extracts from crude drugs of plant origin are needed for the advancement of new antibiotic drug discovery research from traditional plants [4].

Plants are often described as the massive storage of pharmacologically active chemical compounds (soluble phytochemicals) that are obtained through the extraction process [5] Optimum extraction yields have been reported when extracting with aqueous organic solvents than their respective absolute organic solvent as well as by using a refluxing extraction technique [6]. Also, a more refined plant extraction procedure needs to be followed to produce relatively refined extract samples for stronger zones of inhibition [7] Our laboratory have been paying attention to developing a practical extraction technique that gives partially purified crude extracts from crude drugs. In this paper, we attempt to setup a pilot library composed of partially refined crude extracts from indigenous medicinal plants of Lesotho with antimicrobial properties [3]. We also described the coupling of soxhlet extractor with liquid-liquid extraction, the methodology used to generate the library. Finally, we compare the effects of using 90% acetone and 90% ethanol on extraction yields of individual crude drugs.

Materials and Methods

The study received the ethical approval from the National University of Lesotho (NUL), Research and Ethics Committee (ID08-2020). The solvents used were of analytical grade purchased from Prestige laboratory supplies (PTY) Ltd and used directly.

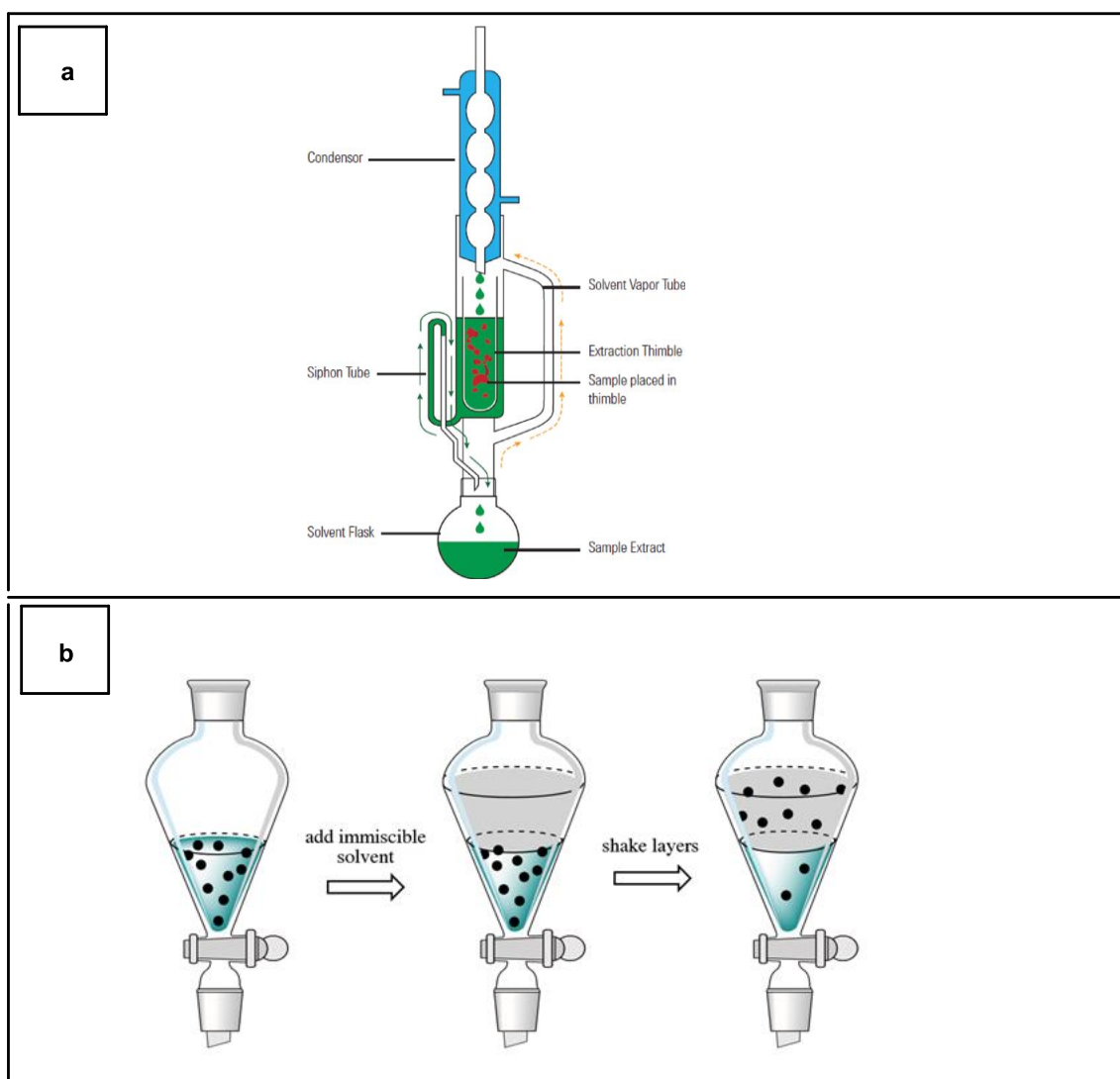
Plant materials

Medicinal plant parts collected from different areas in Lesotho (between 15 January 2020 and 20 March 2020) were authenticated by the expert botanist from NUL biology department and the voucher specimen were deposited in NUL Pharmacy department. The samples were dried, reduced to powder of 1.0 mm maximum particle size and packaged according to the procedure published by Mugomeri *et al.* [3].

Extracts preparation

Soxhlet extraction was performed using operation procedure reported by Raynie in 2019 (figure 1(a)) [6]. Each 50 g of powdered plant sample was extracted using 500 ml of solvent systems; acetone/water (9:1 v/v) and ethanol /water (9:1 v/v), in Soxhlet extraction apparatus Ace Glass Incorporated,

Vineland, NJ. After 24 hours the organic solvent was allowed to evaporate and leave the aqueous layer residuals. The liquid-liquid extraction by vigorous agitation of ethyl acetate to aqueous layer mixture (2:1 v/v) was performed according to the reported procedure (figure 1(b)) [8]. Each aqueous layer residue was extracted three (3) times with ethyl acetate using separatory funnel. Combined organic layer was washed first with deionized water and then with saturated sodium chloride solution, dried over sodium sulphate, filtered through a Whatman No.1 filter paper and concentrated under reduced pressure at 45 °C using 0.25-2 l rotary evaporator ROVA-100 from mrc Laboratory equipment manufacturer Beijing, CHINA. The residual organic solvent was further removed *in vacuo* to give the crude organic extract. Combined water layer was filtered through a Whatman No.1 filter paper and then evaporated over a water bath to remove water. The extract was air dried thoroughly to remove traces of water to produce crude aqueous extract. The procedure was repeated three (3) times for each plant sample to extract each sample in triplicates and values were expressed in mean \pm standard deviation.



Source: after Raynie [6] and Nichols [8]

Fig 1: Diagram of a Soxhlet extractor (a) and schematic of Liquid-liquid extraction (b)

Setting up pilot library

The crude extracts were weight, stored in air-tight 20 ml scintillation vials and refrigerated at below 4 °C. The vials

were labeled using designated sample codes [3] as code-Ace-org or code-Ace-aq to denominate acetone organic or acetone aqueous extracts, and as code-EtOH-org or code-EtOH-aq to

denominate ethanol organic or ethanol aqueous extracts. The information regarding the samples that included masses as well as plants medical uses, conservation status, geographical distribution and known phytochemical components [3] were captured in an excel sheet. The extracts that were used for preliminary antimicrobial assay were reconstituted in dimethyl sulfoxide (DMSO) before the assay, which was performed using the agar well diffusion method against *S. aureus* [9].

Results and Discussions

The extraction yields data summarized in table 1 were recorded for crude drug samples from five plant species harvested from different locations in Lesotho. The relative ratios of organic to water extract yields of different crude drugs were summarized in figure 2. The preliminary antimicrobial assay was performed using the agar well diffusion method against *S. aureus* and confirmed the antimicrobial activities [10-13].

Table 2: Extraction yield (mean \pm SD% w/w) for samples of each medicinal plant material extracted individually in triplicates

Botanical name & family	Vernacular name (Sesotho)	Plant parts used	Solvent (90%)	Extraction yield (mean \pm SD% w/w)		
				Organic extract	Water extract	Total extraction
<i>Leucosida sericae</i> Eckl. & Zeyh. (Rosaceae)	Cheche	Leaves	Acetone	9.83 \pm 0.39	4.17 \pm 0.47	14.00 \pm 0.61
			Ethanol	10.84 \pm 0.19	5.94 \pm 0.26	16.78 \pm 0.32
<i>Gazania krebsiana</i> Less. (Asteraceae)	Tsikitlana	Whole plant	Acetone	7.21 \pm 0.76	3.55 \pm 0.16	10.76 \pm 0.78
			Ethanol	8.17 \pm 0.36	4.88 \pm 0.14	13.05 \pm 0.39
<i>Ledebouria marginata</i> (Baker) Jessop (Asparagaceae)	Bokhoe	Root bulbs	Acetone	2.48 \pm 0.33	3.84 \pm 0.52	6.32 \pm 0.62
			Ethanol	3.97 \pm 0.21	7.58 \pm 0.44	11.55 \pm 0.49
<i>Hermannia depressa</i> N.E. Br. (Malvaceae)	Seletjane	Tubular roots	Acetone	3.25 \pm 0.58	5.31 \pm 0.33	8.56 \pm 0.67
			Ethanol	5.67 \pm 0.37	8.09 \pm 0.61	13.76 \pm 0.71
<i>Xysmalobium undulatum</i> (L.) W.T.Aiton (Apocynaceae)	Pohots'ehla	Bark	Acetone	5.22 \pm 0.18	12.60 \pm 0.53	17.82 \pm 0.56
			Ethanol	3.73 \pm 0.38	19.08 \pm 0.44	22.81 \pm 0.58

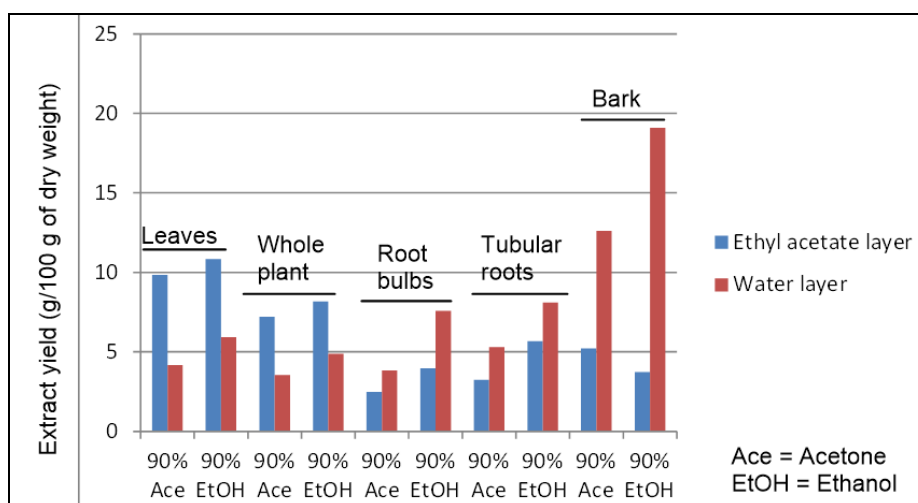


Fig 2: Relative ratios of organic to water extract yields of different crude drugs

Our results suggest that the extracts from the leaves and whole plant contained about twice as much organic soluble phytochemicals as aqueous soluble ones. While the roots and bark contained about 2 and 5 times more of aqueous soluble than organic soluble phytochemicals respectively. It is evident that 90% aqueous ethanol is the best extraction solvent than 90% acetone. These findings are in agreement with those reported by Sultana *et al.*, [14] who reported good extract yields from the bark of different plants when extracting with 80% ethanol. Moreover, Yaqoob *et al.*, [15] reported the phytochemical screening of leaves, fruits, and shoots of *Ferula jaeschkeana* Vatke that revealed presence of more number of phytochemicals in shoots than leaves and fruits, especially with ethanolic extracts. We suspect that the bark contains the highest extractable phytochemicals followed by leaves, and then roots [14, 15]. This library represents the potential phytochemicals in herbal prescriptions dispensed by traditional healers in Lesotho and serves for primary access for screening against various target pathogenic microbes, as well as for isolation of active compounds toward new antibiotic drug discovery. The extracts are publicly available upon request through the corresponding author and the efforts to expand the library are continuing in our laboratory.

Extraction procedure followed in this study could be used to develop other libraries.

Conclusion

This study describes the natural product extraction procedure that employs soxhlet extractor coupled with liquid-liquid extraction to organise the prototype library of medicinal plant crude extracts for future screening against various target pathogenic microbes, as well as for isolation of active components toward new antibiotic drug discovery. The library is publicly available for access to both local and international researchers, and the extraction procedure followed in this study was more refined to produce relatively clean extracts samples and could be used to develop other libraries.

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