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Phytochemical characterization of some herbal concoctions made and sold in Lesotho that are claimed to treat COVID-19 and related respiratory ailments

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ABSTRACT

Introduction
Safety and efficacy of herbal products is a major health concern in countries with poor or no regulation regarding the ingredients, dosages, side effects, and contraindications of these traditional medicines. Herein we report the phytochemical characterization of some commercially available plant-derived concoctions in Lesotho, providing a possible scientific basis for the function and possible safety of such concoctions, based on the determined chemical compounds with the aid of documented phytochemical studies on medicinal plants.

Purpose
The purpose of this study was to use the documented phytochemical studies of medicinal plants in Lesotho and other parts of the world as a basis to study and characterize the commercially available traditional and herbal remedies that are claimed to fight respiratory ailments including those related to COVID-19 infection in Lesotho – Southern Africa.

Methods
Phytochemical screening was carried out on three herbal concoctions produced and sold in Lesotho using simple wet chemistry procedures. Solvent microextraction was carried out followed by gas chromatography and mass spectrometry for the purpose of qualitative analysis of the volatile organic compounds (VOCs).

Results
At least 5 major phytochemicals in each concoction were obtained with tannins and flavonoids quantified spectrophotometrically. One concoction (ROCK) had a total tannin content of about 75 µg/mL and a total flavonoid content of 300 µg/mL relative to the other plants averaging 20 and 50 µg/mL respectively. GC-MS analyses of the concoctions revealed the varying degrees of presence of VOCs with one showing hardly any peaks on the chromatogram indicating that either the concoction was not made from plants, or the VOCs had almost completely been lost during the processes of preparation. The other compounds detected, namely, benzoic acid (48% in ROCK), phthalic acid ester (detected in all concoctions), and glycerine, are consequent on processing indicating the importance of processing in the safety of processed plants as some of these are hazardous beyond certain thresholds.

Conclusion
The tested products show variable amounts of phytochemicals with ROCK showing more volatiles than the other two products. The detected phytochemicals indicate that the products are indeed plant derived while the VOCs profile indicates difference in treatment. The detection of phthalates suggests the importance of testing these products for the presence of unwanted chemicals.

INTRODUCTION

The COVID-19 pandemic, the outbreak of the dangerous and feared flu-like disease, whose fear travelled faster than the virus itself, caused global panic and mass hysteria. People were put under strict lockdown protocols to protect them from the fast-spreading coronavirus infection. Everything was put on hold; workplaces, schools, churches, and streets were empty, and most people lost their jobs, economies receded with prices for most goods, including foods, soaring. Hospitals where the pandemic reached early were overly populated and thousands of people died. There was a rapid change in life; cultural practices were suspended and any assemblies of people were forbidden, customs and norms of people were forced to change and people could not even bury their loved ones the way they desired.

During this ongoing pandemic, people took a variety of mitigation measures to fight and relieve the symptoms of this disease. In Africa, the medical measures included the use of steaming local herbs and drinking herbal remedies; as a result, the production and promotion of herbal concoctions produced locally that were claimed to fight respiratory ailments increased, with some outrightly claiming on local media that they fought and cured COVID-19. While some of the medicinal plants reported have been utilized for generations, there has never been such increased interest in the use of herbal products. The interest was not only limited to rural areas as it is commonly observed, but also urban areas (Matotoka & Masoko, 2018). Steaming of herbal concoctions became a common ritual whenever people went to public spaces.

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 themselves 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 capitalize on this upsurge by increasing the availability of herbal remedies to those who desire them (Ndhlala & Van Staden, 2012). 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 (Ndhlala & Van Staden, 2012).

The plant parts that are commonly used as ingredients in the preparation of herbal concoctions include leaves, stems, barks, roots, rhizomes, bulbs, and/or seeds. The complexity of the formulations is dependent on the severity of the illness (Matotoka & Masoko, 2018). Simple home remedies are usually prepared for common sicknesses such as cough and cold and many more. Information on herbal medicines is passed down family lines through oral tradition (Kose et al., 2015). This method of passing information increases the chances of wrong identification and misuse of some medicinal plants (Khan & Smillie, 2012). On the other hand, the ongoing use of these traditional herbal remedies and their fairly good performance intrigue scientists to carry out studies and research to fully document these remedies (Matotoka & Masoko, 2018). Several studies have also been done on the nature and composition of the medicinal herbs that are associated with the manufacture of herbal remedies and concoctions, to quantify and document the bioactive constituents of such herbal plants (Mugomeri et al., 2016). A few studies have documented information on medicinal plants in Lesotho (Moteetee & Van Wyk, 2011; Mugomeri et al., 2014; Kose et al., 2015; Masupha et al., 2012).

The safety of herbal products is a major health concern in Lesotho. Ingredients, dosages, side effects, and contraindications of these traditional medicines sold in Lesotho are usually not listed, or improperly labelled and most products have multiple indications on the label (Mugomeri et al., 2014). Herbal products can pose a risk of toxicity when inappropriately used because of the wrong prescription and/or inappropriate labelling (Phua et al., 2009). The risk of toxicity associated with traditional herbal medicine is potentially high since herbalists do not necessarily screen for specific phytochemicals when identifying plants of medicinal value. Crude plant extracts which contain an arsenal of potentially harmful substances are usually used in high doses.

Phytochemicals are biologically active, plant-based secondary metabolites that aid plants in exerting a defense

response against pests and other predators. They are also described as the non-nutritive chemical compounds found in plant foods. The common phytochemicals of medicinal value include alkaloids, phenols, flavonoids, saponins, glycosides, tannins, terpenes, and amino acids (Sasidharan et al., 2011). However, the medicinal value of these phytochemicals depends on the specific nature of the molecules making up the phytochemical (Kar & Roy, 2012). Some phytochemicals have disease-preventive or curative properties, while others are toxic (Mugomeri et al., 2016).

The purpose of this study was to use the documented phytochemical studies of medicinal plants in Lesotho and other parts of the world as a basis to study and characterize the commercially available traditional and herbal remedies that are claimed to fight respiratory ailments including those related to COVID-19 infection in Lesotho – Southern Africa. This study thus provides the scientific basis for the function and potential safety, or lack of, of such concoctions based on the determined chemical compounds.

METHODS

Acquisition and storage of herbal concoctions

Three different concoctions were purchased from the individual distributors around the University campus in Roma, Maseru district of Lesotho. The different herbal remedies were defaced to hide their identities and labelled (ROCK, GRCK, and RRFK) for identification purposes. Thereafter, they were individually filtered using a Whatman No1 filter paper, and the filtrates were stored in properly labelled glass bottles at room temperature; the residue was discarded (De et al., 2010).

Phytochemical Screening

Different aliquots (exact volumes stated in each test) of the filtrates were then qualitatively tested for the presence of the following phytochemicals: flavonoids, alkaloids, terpenoids, tannins, steroids, and phenols.

Detection of flavonoids *(Alkaline reagent test)*

Aliquots of 0.2 g were treated with six drops of 2% sodium hydroxide solution. The formation of intense yellow colour, which developed into a colourless solution with the addition of dilute acid, gave an indication of the presence of *flavonoids* in the extracts (Adegoke et al., 2010).

Detection of alkaloids (Mayer's test)

Extracts (0.5 g) were dissolved in 5 mL of 1% dilute hydrochloric acid and filtered. Filtrate was treated with Mayer's reagent (Potassium mercuric iodide). The formation of a yellow-coloured precipitate gave a positive result for alkaloids in the extracts (Trease & Evans, 2002; Adegoke et al., 2010).

Detection of terpenoids (Salkowski's test)

To 0.1 g of the extracts, 0.5 mL of chloroform was added followed by 1 mL of concentrated sulphuric acid. The formation of a reddish-brown precipitate indicated the presence of terpenoids in the extracts (Trease & Evans, 2002).

Detection of tannins (Ferric chloride test)

A mass of 0.2 g of the extracts was mixed with an equal volume of distilled water in a test tube and three drops of dilute ferric chloride were added. The formation of brownish-blue or dark colour indicated the presence of tannins in the extracts (Adegoke et al., 2010).

Detection of steroids (Liebermann-Burchard's test)

Extracts (0.5g) were mixed with 2 mL of chloroform. 2 mL of concentrated sulphuric acid was then added to the mixture in a test tube. The appearance of red colour in the lower chloroform layer gave a positive result for steroids in the extracts (Adegoke et al., 2010).

Detection of phenols (Ferric chloride test).

To 0.2g of the extracts, 2 mL of 5% aqueous ferric chloride was added. The formation of bluish colour gave a positive result for phenols in the extracts (Adegoke et al., 2010).

Quantitation of tannins and flavonoids

Determination of tannins

The tannins were determined by Folin-Ciocalteu method. About 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin Phenol reagent, 1 mL of 35% Na_2CO_3 solution and diluted to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80, and 100 µg/mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm with a UV/Visible spectrophotometer (Miean et al.,

2001; Marinova et al., 2005; Ael-M et al., 2012; Singh et al., 2012)

Determination of flavonoids

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture which consists of 1 mL of extract and 4 mL of distilled water was taken in a 10 mL volumetric flask. In the flask, 0.30 mL of 5 % sodium nitrite was treated and after 5 minutes, 0.3 mL of 10 % aluminium chloride was mixed. After 5 minutes, 2 mL of 1M sodium hydroxide was treated and diluted to 10 mL with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80, and 100 µg/mL) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with a UV/Visible spectrophotometer (Ghasemzadeh et al., 2010; Hajaji et al., 2010; Har et al., 2012).

Determination of total phenol content.

Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture which consists of 1 mL of concoction and 9 mL of distilled water was taken in a volumetric flask (25 mL). Folin-Ciocalteu phenol reagent (1 mL) was added to the mixture and shaken well. After 5 minutes, 10 mL of 7% Na_2CO_3 solution was added to the mixture. The volume was made up to 25 mL. A set of standard solutions of gallic acid (20, 40, 40, 60, 80, and 100 µg/mL) were prepared in the same manner as described earlier (Xu et al., 2008; Rasool et al., 2011; Stankovic et al., 2011). These were allowed to stay 90 minutes at room temperature. Thereafter, the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV)/visible spectrophotometer.

Determination of total alkaloid content

The determination of total alkaloids followed earlier reports as described elsewhere (Shamsa et al., 2008). The concoction (1mg) was dissolved in 1 mL of dimethyl sulphoxide to which 1 mL of 2 N HCl was added and shaken for homogeneity. The mixture was filtered, and the filtrate was transferred to a separating funnel, after which 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added. The mixture was extracted with 1-, 2-, 3-, and 4-mL portions of chloroform which were mixed in a 10-mL volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80, and 100 µg/mL) were prepared in the same manner. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with a UV/Visible spectrophotometer (Das et al., 2018).

GC-MS Analysis of the volatile organic compounds

Extraction of the volatile organic compounds was carried out by a miniaturized-liquid-liquid extraction (mLLE) where 0.50 mL of DCM was added to 1 mL of the infusion and extracted after separation of the two layers. A 10 µL Hamilton® syringe was used to draw 1µL aliquots of the organic sediment and injected that in the gas chromatograph (Shimadzu QP 2010 GC-MS [Kyoto, Japan]) with the injection port temperature set at 200 °C fitted with a 30 m × 0.25 mm × 0.25 µm Restek Rtx-5ms (5%phenyl- 95% dimethyl-polysiloxane) capillary column using a 1:10 split ratio. The optimized oven temperature programme began at 50 °C held for 4 minutes, then ramped to 200 °C at a rate of 10 °C/minute and held for 4 minutes. The UHP Helium (Afrox, Johannesburg, South Africa) was used as carrier gas pumped through the column at a constant flow rate of 1 mL/minute. The MS transfer line and the ion source temperatures were set at 250 and 200 °C, respectively with a scanning mode acquisition of mass spectra set in the mass range of 50 – 500 amu. The obtained compounds were compared against the mass spectral library embedded in the Shimadzu GC-MS

RESULTS AND DISCUSSION

Screening of different phytochemicals

Table 1 shows a summary of observations recorded during the screening of different phytochemicals where +/- indicates the presence/absence of a certain phytochemical denoted on the column heading.

Table 1:
Results from phytochemical screening of herbal concoctions

CONC	TANN	FLAV	PHEN	ALKA	TERP	STER
ROCK	+	+	+	+	+	-
GRCK	+	+	+	-	+	+
RRFK	+	+	+	-	+	+

Key:
CONC = Concoction, TANN = Tannins, FLAV = Flavonoids, PHEN = Phenols, ALKA = Alkaloids, TERP = Terpenoids, STER = Steroids

'+' indicates presence; '-' indicates absence

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The results in Table 1 show the presence of at least five phytochemicals in each concoction indicating the possibility that the concoctions were derived from plant materials. The quantification of the detected phytochemicals may be used to determine the identity of the plant materials used and the method of preparation. These data will also help in the formulation of the most effective concoction as the presence of each phytochemical may be manipulated to come up with the desired ratio of composition. That is, since the phytochemical profile is known, it will be easier to increase a certain phytochemical content by using its rich plant derivative, depending on the type and function of the desired concoction.

Quantitation of the tannins and flavonoids in the different concoctions

The abundances of tannins and flavonoids in three different herbal concoctions are shown in Table 2 following spectrophotometric determination.

Table 2:
Results obtained when quantifying the phytochemicals in herbal concoctions

Concoction	Tannins (µg/mL)	Flavonoids (µg/mL)
ROCK	75.1 (± nd)*	299.9 #
GRCK	15.1	43.5
RRFK	23.2	51.6
Linearity (R^2) [20 – 100 µg/mL]	0.9990	0.9978

Key:

± Indicate the standard deviation for three replicates (n = 3)

* The confidence interval was too small to include

Solution was diluted (1/10) and the concentration was calculated backward to the original solution.

The data collected in Table 2 shows that of the three concoctions analysed, ROCK has the highest quantity of tannins and flavonoids followed by RRFK and then GRCK with the lowest quantity. This indicates that ROCK may have been prepared using a method that is different from the one used in the preparation of RRFK and GRCK; because it has considerably higher quantities of the tannins (75 µg/mL) and flavonoids (300 µg/mL) than both RRFK and GRCK which have comparable quantities (ca. 15-23 and 43-52 µg/mL tannins and flavonoids respectively). Another reason for the observed difference in quantities of the phytochemicals may be the nature and type of the plant material used to prepare each concoction.

Analysis of the volatile organic compounds by GC MS

Figure 1 shows the comparison of the chromatograms of the three herbal infusions following a simple miniaturised liquid-liquid extraction of the concoctions using dichloromethane.

Figure 1:
The combined chromatograms of the organic extracts of the three herbal concoctions

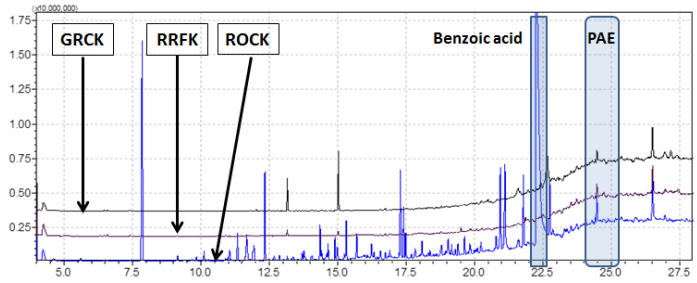

Table 3 shows the list of VOCs and their relative percentage abundance that were detected in three herbal concoctions as represented in the combined GCMS chromatograms in Figure 1. These compounds were picked by the Wiley Mass Spectral Library embedded in the GCMS Solution software that ran the GCMS. It must however be noted that the relative abundance is not a good measure of abundance as the relative is calculated from a total of 100% peak areas which standardise all the peaks to a sum of 100. The RRFK sample did not seem to have any significant peaks, yet the relative abundances of the detected peaks present a highly exaggerated picture owing to the high values. The other important aspect is that the similarities between the obtained spectra and that in the library were comparatively lower for RRFK owing to the lower intensity (lower signal/noise ratio) than in the other samples, particularly ROCK.

Table 3:
A list of different VOCs detected in three different herbal concoctions

Ret time/min	Name (as suggested by the GC-MS Wiley Library)	Relative abundances (%)		
		ROCK	GRCK	RRFK
7.851	2-Decenal, (E)-	10.44		
11.341	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	0.98		
11.667	Methyl formate	1.28		
11.942	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	0.59		
12.34	Cyclohexanone, 5-methyl-2-(1-methylethyl)-	3.28		

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13.16	1-Octanol		7.21	13.23
14.357	Pulegone	1.28		
14.901	3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-	0.79		
14.982	Borneol	0.63	12.6	7.47
15.313	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	1.48		
15.695	Cyclohexene, 1-(1,1-dimethylethoxy)-6-methyl-	0.92		
16.232	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl)-	0.59		
17.295	Phenylethyl Alcohol	3.43		
17.412	2-Oxabicyclo[2.2.2]octan-6-one, 1,3,3-trimethyl-	1.97		
17.49	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	0.99		
18.086	8-Oxabicyclo[5.1.0]oct-2-en-4-one, 3,6,6-trimethyl-	0.59		
19.042	1-Butanol, 2-amino-3-methyl-, (.+/-.)-	0.68		
19.4	1-Hydroxy-p-menth-3-one	0.52		
19.636	Sorbic Acid	0.7		3.64
20.789	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.58		
20.945	4-Hexadecanol	3.47		
21.113	Glycerine	4.58		
21.604	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester		5.2	
21.785	Ethyl hydrogen succinate	2.76		
21.961	Heptaethylene glycol monododecyl ether		1.63	
22.419	Benzoic acid	48.15		
22.656	6-Octadecenoic acid, (Z)-		22.18	
22.75	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	2.43		
24.473	Dibutyl phthalate	0.99	2.96	10.98
26.512	n-Hexadecanoic acid	2.29	11.73	27.91
26.969	Octaethylene glycol monododecyl ether		4.42	6.2
27.173	3-Ethoxy-4-methoxybenzaldehyde		4.36	5.35
27.404	Eicosanoic acid		2.23	9.68

NOTE: The relative abundances are calculated based on the total number of peaks identified (50) based on ROCK!

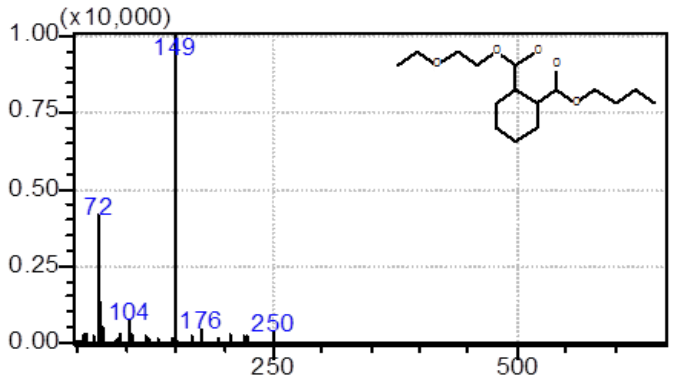
These GC-MS results support the classical analytical methods that showed that of all the three concoctions, ROCK has a relatively high concentration of phytochemicals. The results also showed that, although the GRCK and RRFK have different concentrations and quantities of phytochemicals (which are low), they are very much comparable in terms of composition. That is, it is likely that these were prepared using the same method. For example, they had very small amounts of volatiles and this usually happens when they are prepared by use of boiling water in an open system, which is a common practice. On the other hand, ROCK had very high amounts of volatiles and different organic compounds and this may have been achieved by the method of preparation which preserves the VOCs.

In addition to those organic molecules, the combined chromatograms in **Figure 1** showed that ROCK had high concentrations of the compound, benzoic acid (see the highlighted peak around 22.5 min) possibly derived from sodium benzoate preservative which may have been used as a preservative. ROCK has also shown the significant presence of benzoic acid possibly from the sodium benzoate preservative. Benzoic acid and its salts are well-known preservatives used in food, beverages, and pharmaceuticals at regulated dosage and it works by inhibiting the enzymes involved in the food spoilage processes (Jacob et al., 2016; Onwordi et al., 2017). Some people are allergic to benzoic acid; it may cause shortness of breath, immune depression, irritate the eyes, skin, lungs, and digestive tract, and hyperpnoea in experimental animals and humans. Benzoates, salts of benzoic acids, are also reported to react with ascorbic acid (Vitamin C) to form benzene, especially if they are stored for extended periods at high temperatures (dos Santos et al., 2015). Using a high dose of sodium benzoate causes the release of histamine and prostaglandin, ulcers, and gastric mucus secretion changes (Kreindler et al., 1980, Schaubschlager et al., 1991). Sodium benzoate also increases blood pressure, and this may damage the blood vessels (Shahmihammadi et al., 2016).

Another notable potential additive is the glycerol (glycerine) observed at 21.11 min. Glycerine is a known additive to hydrophilic plant extracts to improve stability, act as a humectant and reduce water activity thus affording a preservative effect to the concoctions (Padmawar & Bhadoriya, 2018). Because of its sweet taste, glycerine can also be added to the herbal extracts to mask their mostly bitter taste. This is another factor that indicates further that this concoction was prepared differently from the other two.

The results further showed the presence of a phthalic acid ester (highlighted at 24.5 min), potentially bis-2ethylhexyl-phthalate which is a well-documented plasticizer, possibly leaching from the plastic bottle making the consumption of these concoctions somewhat unsafe for continuous use since phthalic acid esters (PAEs) are endocrine-disrupting chemicals that are harmful to the reproductive, neurological, and developmental systems of human beings from multiple exposure pathways (Wang et al., 2021). This compound has been reported locally in solid waste leachates suggesting considerable use (George et al., 2019). While the relative abundance value of this compound in **Table 3** is small in ROCK, the reader is referred to the relative intensity of the peak in **Figure 1**. It should be borne in mind that relative abundance compares the abundance of each peak relative to all the other peaks. Therefore, it can be argued that the level of PAE in ROCK seems to be slightly more than in the other concoctions much as it reads 0.99% (cf. 3% for GRCK) in the table due to the presence of many other VOCs obtained in ROCK. PAEs are unmistakable with their dominant *m/z* 149 peaks (George et al., 2017; George et al., 2019). **Figure 2** shows the obtained mass spectrum of the peak 24.5 min with the suggested chemical structure from the mass spectral library embedded in the GC-MS Software used in obtaining and analysing the data. It should be noted though that the suggested compound in the spectrum is not necessarily correct, but it was picked for presentation purposes.

Figure 2:
The mass spectra of the peak obtained at 24.5 min with the library-suggested chemical structure



CONCLUSION

All the herbal concoctions tested positive for the presence of at least four phytochemicals (flavonoids, tannins, phenolics, and terpenes) although they were in relatively high concentration in ROCK (1.50 mg/mL tannins and 5.98 mg/mL flavonoids) and very low concentrations (undetectable) in RRFK and GRCK as determined using the test-tube chemistry. The analysis of VOCs revealed the presence of harmful xenobiotic substances in the concoctions, namely benzoic acid which may cause allergic reactions in high amounts, and phthalic acid esters that are classified as endocrine disrupting chemicals from preservation processes and packaging respectively. It is therefore wise for the producers of the herbal concoctions to replace the plastic containers with glass containers to evade this plasticiser leachate. It would be equally important to indicate the safety precautions and list of contents on the product to protect the lives and well-being of the customers.

Given the relatively low levels of the VOCs in the other concoctions, this could indicate the preparation method such as either excess drying in direct sunlight or too hot environments, or excess boiling leading to a significant loss of the desired VOCs. This also suggests that if the activity against respiratory ailments is related to the VOCs as claimed in literature, then the effectiveness of these concoctions is likely to be reduced. However, their activity could still be due to the phenolic (non-VOCs) as such no conclusive statement could be made regarding potency. It is therefore recommended that standard methods should be developed and adopted for the preparation of these concoctions to minimise the risk of losing the plant's identity (characteristic organic compounds of the plant) for better efficacy of the plant-derived medicines.

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Ethical Approval: Nil

Conflicts of Interest: None declared.

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REFERENCES

Adegoke, A. A., IberI, P. A., Akinpelu, D. A., Aiyegoro, O. A., & Mboto, C. I. (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products, 3* (3), 6–12.

Afify, A. E. M. M., El-Beltagi, H. S., Abd El-Salam, S. M., & Omran, A. A. (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. *Asian Pacific Journal of Tropical Biomedicine, 2*(3), 203-9. https://doi.org/10.1016/S2221-1691(12)60042-2

Dos Santos, V. P. S., Salgado, A. M., Torres, A. G., & Pereira, K. S. (2015). Benzene as a chemical hazard
in processed foods. *International Journal of Food Science*. https://doi.org/10.1155/2015/545640

Das, K., Dang, R., Sivaraman, G., & Ellath, R. P. (2018). Phytochemical screening for various secondary metabolites, antioxidant, and anthelmintic activity of *Coscinium fenestratum* fruit pulp: A new biosource for novel drug discovery. *Turkish Journal of Pharmaceutical Sciences*, *15*(2), 156-1645. DOI: 10.4274/tjps.54376

George, M. J., Tagwa, O. R., & Sichilongo, K. F. (2017). Extraction and pre-concentration of phthalic acid esters from aqueous solutions using agitation-assisted dispersed binary solvents microextraction. *South African Journal of Chemistry*, *70*, 100-107. http://dx.doi.org/10.17159/0379-4350/2017/v70a14

George, M. J., Sichilongo, K. F., Ramabulana, T., Madala, N. E., & Dubery, I. A. (2019). Comparison of Soxhlet and reflux techniques for extraction and characterisation of potential endocrine-disrupting compounds from solid waste dumpsite soil. *Environmental Monitoring and Assessment*, *191,* 1-11. https://doi.org/10.1007/s10661-019-7294-6

George, M. J., Madala, N. E., & Dubery, I. A. (2019). Extraction of phthalic acid esters from soil samples using aqueous room temperature sonication coupled to bubble-in-drop single-drop microextraction. *International Journal of Environmental Analytical Chemistry*, *99*(12), 1198-1210. https://doi.org/10.1080/03067319.2019.1618458

Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules 15*, 4324-4333. https://doi.org/10.3390/molecules15064324

Hajaji, H. E., Lachkar, N., Alaoui, K., Cherrah, Y., Farah, A., Ennabili, A., El Bali, B., & Lachkar, M. (2010). Antioxidant properties and total phenolic content of three varieties of Carob tree leaves from Morocco. *Records of Natural Products, 4*(4), 193-204.

Har, L. W., & Ismail, I. S. (2012). Antioxidant activity, total phenolics and total flavonoids of *Syzygium polyanthum* (Wight) Walp leaves. *International Journal of Medicinal and Aromatic Plants, 2(*2), 219-228. https://www.cabdirect.org/cabdirect/abstract/20123308669

Jacob, S. E., Hill, H., Lucero, H., & Nedorost S. (2016). Benzoate allergy in children - From foods to personal hygiene products. *Pediatric Dermatology, 33*(2), 213–215. https://doi.org/10.1111/pde.12759

Kar, S. & Roy, K. (2012). QSAR of phytochemicals for the design of better drugs. *Expert Opinion on Drug Discovery,* 7, 877-902. https://doi.org/10.1517/17460441.2012.716420

Kose L.S., Moteetee A., van Vuuren, S. (2015). Ethnobotanical survey of medicines used in the Maseru district of Lesotho. *Journal of Ethnopharmacology* 170: 184-200. https://doi.org/10.1016/j.jep.2015.04.047

Khan, I.A. & Smillie, T. (2012). Implementing a "quality by design" approach to assure the safety and integrity of botanical dietary supplements. *Journal of Natural Products,* 75(9), 1665-1673. https://doi.org/10.1021/np300434j

Kreindler, J.J., Slutsky, J., Haddad, Z.H. (1980). The effect of food colors and sodium benzoate on rat peritoneal mast cells. *Ann Allergy.* 44(2):76–81.

Marinova, D., Ribarova, F., Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy,* 40 (3): 255-260.

https://www.researchgate.net/publication/258769164

Marsland, R. (2007). The modern traditional healer: locating 'hybridity' in modern traditional medicine, southern Tanzania. *Journal of Southern African Studies* 33, 751–765. https://doi.org/10.1080/03057070701646845

Masupha, P., Thamae, L., Phaqane, M. (2012). Policy implications for intellectual property systems for traditional healers in Lesotho. *African Technology Policy Studies Network: Special Paper Series*. https://africaportal.org/wp-content/uploads/2023/05/pb371.pdf

Matotoka, M.M., Masoko P. (2018). Phytochemical screening and pharmacological evaluation of herbal concoctions sold at Ga Maja Limpopo Province. *South African Journal of Botany* 117, 1–10. https://doi.org/10.1016/j.sajb.2018.04.013

Miean, K. H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry*. *49* (6), 3106-12. https://doi.org/10.1021/jf000892m

Moteetee, A., & Van Wyk, B. E. (2011). The medical ethnobotany of Lesotho: A review. *Bothalia*, 41, 209-228. https://doi.org/10.4102/abc.v41i1.52

Mugomeri, E., Chatanga, P., Hlapisi, S., & Rahlao, L. (2014). Phytochemical characterization of selected herbal products in Lesotho. *Lesotho Medical Association Journal 12,* 38-47. https://www.leboha.org.ls/journals/LMJ_January_2014.pdf#page=39

Mugomeri E., Chatanga P., Ralitlatla T.,Makara M., & Tarirai C. (2016). Ethnobotanical study and conservation status of local medical plants: towards repository and monograph of herbal medicines in Lesotho. *African Journal of Traditional, Complementary and Alternative Medicines,* 13(1): 143-156. DOI: 10.4314/ajtcam.v13i1.20

Ndhlala, A. R., & Van Staden, J., (2012). Smokescreens and mirrors in safety and quality of herbal medicines: a case of commercialized herbal preparations. *South African Journal of Botany* 82, 4-10. https://doi.org/10.1016/j.sajb.2012.06.008

Onwordi, C.T., Olanrewaju, A.J., Wusu, A.D., Oguntade, B.K. (2017). Levels of benzoic acid, sulphur (IV) oxide and sorbic acid in carbonated drinks sold in Lagos, Nigeria. *American Journal of Food Science and Technology,* 5(2):38-44.

Padmawar, A., & Bhadoriya, U. (2018). Glycol and glycerine: Pivotal role in herbal industry as solvent/co-solvent. *World Journal of Pharmaceutical and Medical Research*, *4*(5), 153-155.

Rasool, N., Rızwan, K., Zubaır, M., Naveed, K. U. R, Imran, I., & Ahmed, V. U. (2011). Antioxidant potential of different extracts and fractions of *Catharanthus roseus* shoots. *International Journal of Phytomedicine 1*(3), 8-114.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & YogA, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, *8,* 1-10. https://doi.org/10.4314/ajtcam.v8i1.60483

Schaubschlager, W., Becker, W-M., Schade, U., Zabel, P., & Schlaak M (1991). Release of mediators from human gastric mucosa and blood in adverse reactions to benzoate. *International Archives of Allergy and Immunology, 96*(2), 97–101. https://doi.org/10.1159/000235478

Shahmihammadi, M., Javadi, M., & Nassiri-Asl, M. (2016). An overview on the effects of sodium benzoate as a preservative in food products. *Biotechnology and Health Sciences. 3*(3). http://dx.doi.org/10.17795/bhs-35084

Shamsa, F., Monsef, H., Ghamooshi, R., & Verdian-rizi, M. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *The Thai Journal of Pharmaceutical Sciences, 32*, 17-20. https://digital.car.chula.ac.th/tjps/vol32/iss1/4

Singh, R., Verma, P. K., & Singh, G. (2012). Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. *Journal of Complementary Medicine Research, 1* (2), 101-104. http://dx.doi.org/10.5455/jice.20120525014326

Stankovic, M. S. (2011). Total phenolic content, flavonoid concentration, and antioxidant activity of *Marrubium peregrinum* extracts. *Kragujevac Journal of Science, 33,* 63-72.

Trease, G. E., & Evans, W. C., (2002). *Pharmacognosy*, *9*th Ed. Saunders Elsevier, pp. 214–393. http://sutlib2.sut.ac.th/sut_contents/H74301.pdf

Wang, Y., & Qian, H. (2021). Phthalates and their impacts on human health. *Healthcare*, 9,
603. https://doi.org/10.3390/healthcare9050603

Xu, B. J., & Chang, S. K. (2008). Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods. *Journal of Food Science* 73(2), H19-27. https://doi.org/10.1111/j.1750-3841.2007.00625.x