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Is "Myrrh" Contaminated by Microbes and Heavy Metals? Analytical Study from Saudi Arabia

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Abstract

Background: There is a strong belief in myrrh's antibacterial and healing properties in Saudi culture. However, a number of negative outcomes such as surgical site infections have been recorded. The aim of this analysis was to determine the level of bacterial and heavy metal contamination in myrrh samples from Saudi markets.

There is a strong belief in myrrh's antibacterial and healing properties in Saudi culture. However, some negative outcomes such as surgical site infections have been recorded. This analysis aimed to determine the level of bacterial and heavy metal contamination in myrrh samples from Saudi markets.

Methods: This cross-sectional study was carried out at an academic centre in Riyadh, Saudi Arabia. We have collected seven samples of myrrh organic compound from native markets in numerous areas in Saudi Arabia (2 from Riyadh, 1 Hail, 1 Qassim, 1 Najran, 1 Dammam and 1 Jeddah). Bacterial counts were identified. All Samples were submitted and analyzed with ICP-MS (Agilent 7900).

Results: Four out of seven samples tested positive for bacterial contamination. The presence of trace amounts of heavy metals was within reasonable limits. The difference was 14%, 95% CI = -31.3315% to 52.3161%, Chi-squared 0.255.

Conclusion: The presence of viable bacteria noticed in many myrrh specimens represents a major health risk that could defeat the benefits of their usage. This raises the importance of reinforcement of proper environmental sanitation through appropriate legislation.

Keywords: Myrrh; Saudi Arabia; Herbal medicine; Commiphora; Bacterial contamination; Heavy metals

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Introduction

Myrrh is a natural resin extracted from a number of small tree species of the genus *Commiphora* [1]. While the Middle Eastern regions used myrrh ceremonially, the Far East was using it for healing purposes. *Commiphora myrrh* commonly named as myrrh is frequently used as an herbal remedy in Saudi culture as there's a strong belief of its antibacterial and healing effects. However, several adverse events have been noted after use of myrrh such as surgical site infection. This attracted our attention to analyze the myrrh obtainable in our markets in terms of their contamination with bacteria and heavy metal elements [2]. In Saudi Arabia there is a great believe among the public of the use of traditional medicine. Myrrh probably is the most commonly used via direct contact on wound or inhalation. It has been observed by several physicians, especially surgeons, the adverse effects on wounds like surgical site infection. The aim of this study was to assess the bacterial and heavy metal contamination in myrrh samples in Saudi Arabia. This analysis was the result of increasing popularity of myrrh, and the lack of quantitative and qualitative research on the composition of myrrh before being placed on the market [3].

Materials and Methods

The solvents, nitric acid, perchloric acid, hydrogen peroxide and other reagents used were

Table 1: The Instrumental for the ICP/MS based elemental analysis of myrrh extract.

Parameter	Value
Plasma mode	Low matrix
ORS mode	No gas and He
RF power	1550 W
Sampling depth	8 mm
Carrier gas	1.05 L/min
Ext 1 lens	0 V
Ext 2 lens	-140 V
He flows rate	4.3 mL/min
H2 flow rate	6 mL/min

HPLC grade. Arabian myrrh resin was collected randomly from local markets in various cities, Saudi Arabia (2 from Riyadh, 1 Hail, 1 Qassim, 1 Najran, 1Dammam and 1 Jeddah).

Sample preparation for ICP-MS analysis

One gram of dry myrrh sample and 50 ml of 20% Nitric Acid (HNO_3) were added to a flask. The mixture was heated to 70°C to 85°C for 48 h maintained at the same volume by adding 20% nitric acid. After the completion the mixture was filtered. The filtrate was collected in 100 ml volumetric flask and allowed to cool. After cooling, the volume was made up to 100 ml using deionized water and analyzed with ICP-MS. For the sample preparation, the glass-wares were washed with deionized water and rinsed three times with 20% nitric acid.

ICP-MS analysis

Agilent 7900 ICP-MS was utilized for the elemental analysis. The Instrumental setting details for the analysis done are given in Table 1.

Calibration of ICP/MS and internal standards

Instrument calibration was done by using solutions of Hg, As, Se, Al, Co, Na, Cd, Mg, Ni, Mn, Pb, Cu, Zn 1 ppb in 2% HNO_3. The tuning solution (Agilent) was used to optimize nebulizer gas flow, mass calibration, and resolution and Auto Lens calibration. A 20 ppb multi-element internal standard solution was used for all analyses. To prepare a 20-ppb internal standard solution, 1 ml of the 10-ppm stock (Agilent) was diluted into 500 ml 1% HNO_3.

Calibration of ICP/MS and quality assurance

The accuracy of the methods was checked by using both external assurance reference materials (CAB External Quality Assessment Scheme) and internal quality control samples the internal quality control samples. Specifically, following each calibration curve, one blank, one control samples at low level, and another control sample at high level were analyzed. After every 10 analyses it was analyzed one control sample, alternating between high and low levels. If any of the control sample values are outside the specified acceptance criteria (2 standard deviations from the mean based on 20 measurements), the instrument is recalibrated, and all samples analyzed since the last successful control sample analysis will be re-analyzed. To grantee more precision and accuracy, the full calibration curve was injected every 25 samples. The instrument platform should be cleaned daily and not to exceed 200 analyses between cleanings. The validation for the instrument and the analytical methods was done before starting the samples analysis. The accuracy and the precision were within the acceptable performance criteria (for the recovery was 100% ± 5% and % RSD was 0.25% to 7%). The Limit of Detection (LOD) was calculated as 3σ of the reagent blank.

Statistical analysis

Descriptive statistics for the study sample was presented in the form of frequencies and relative frequencies (percent) for categorical variables, while mean, standard deviation, were used for representing the numeric variables.

Comparison of the groups was done using Chi-square test or Fisher's exact test. IBM SPSS statistics software, version 26, was used for the analysis and p-value <0.05 was considered statistically significant.

Results

Table 1 showed the parameters and values of the Instrumental for the ICP/MS based elemental analysis of myrrh extract. Table 2 showed the level of trace elements in the seven myrrh samples and the inorganic constituents in myrrh resin (from *Commiphora myrrha*) estimated by ICP-MS. Table 3 showed the microbiology results of the seven samples.

Four out of seven samples (57%) test positive for infection and the presence of trace elements were within reasonable limits. The Difference was 14%, 95% CI = -31.3315% to 52.3161%, Chi-squared 0.255 and the p value was = 0.6137 which is insignificant. However when we repeat the same experiment on a big sample size of "700 samples" the results was totally different. The Difference was the same, 14%, 95% CI=8.7732% to 19.1187%, Chi-squared 27.420, the degree of freedom equal 1 and the significance level P<0.0001.

Discussion

The name myrrh is derived from the Arabic and Hebrew word "Morr", which means "bitter". Myrrh is a natural gum or resin extracted from a number of small, thorny tree species of the genus *Commiphora*. It is a reddish-brown mass, covered with a brownish yellow dust. It has a bitter and acidic taste and a balsamic odor; myrrh's characteristic odor is derived from "Furano -Ses- Quiterpenes". It is mixed with water to form an emulsion [4].

Myrrh is considered a popular herbal medicine in Saudi Arabia. It is perceived by many as being more effective than modern medicine. Traditionally, it is applied to surgical wounds, as there is a strong believe, coming from historical and to some extent evidence behind this, of its antibacterial and healing effect [5]. There are several reports coming from local data that embrace the use of myrrh as potential cause of Surgical Site Infection (SSI) [6].

We managed to culture bacillus species from 4 out of 7 (57.14%), despite the fact that all specimens were washed during preparation, this overpowers the argument that washing and heating myrrh will produce a safe disinfection effect. It is noteworthy that rinsing or boiling plant material may reduce soil-borne microorganisms [7] Herbal medication including myrrh is usually available through the same market [8]. Bakhotmah and Alzahrani [9] report topical use by (37.4%) in combination with honey in (12.1%) for diabetic foot. In addition, the high number of positive cultures indicating contamination is alarming, which might defeat the benefit of the usage and may explain the observed undesired effects on wounds e.g. infection. The contamination by microorganisms by myrrh could have been introduced during harvesting, handling, open-air drying, preserving, and manufacturing [10]. Despite the plenty of

Table 2: Inorganic constituents in myrrh resin (from *Commiphora myrrha*) estimated by ICP-MS. Level of trace elements in Myrrha samples.

Element name (symbol) Element	Level of element (ppm of myrrh resin) sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	sample 7
Na Saudium 0.09	0.0912	0.0874	0.0951	0.1054	0.0847	0.0984	0.0913
Mg Magnesium 1.48	1.62	1.44	1.48	1.57	1.42	1.49	1.39
Al Aluminum 11.44	11.64	10.98	12.45	10.18	12.63	10.94	11.27
K Pottasium 0.86	0.863	0.828	0.871	0.837	0.836	0.911	0.934
Ca Calcium 171.08	183.14	164.26	170.21	175.31	160.24	167.14	177.28
Ti Thallium 1.28	1.227	1.142	1.347	1.335	1.254	1.298	1.411
Cr Chromium 11.49	11.57	11.52	12.41	12.05	10.26	10.93	11.71
Mn Manganese 0.61	0.663	0.618	0.527	0.704	0.614	0.602	0.557
Ni Nickel 0.29	0.302	0.281	0.274	0.326	0.294	0.307	0.284
Cu Copper 0.135	0.127	0.143	0.152	0.144	0.137	0.129	0.113
Zn Zinc 0.457	0.572	0.364	0.498	0.527	0.438	0.408	0.392
As Arsenic 0.82	0.881	0.904	0.853	0.749	0.806	0.774	0.827
Se Selenium 1.386	1.433	1.587	1.62	1.542	0.948	1.214	1.358
Cd Cadmium 0.019	0.0091	0.0072	0.0114	0.0083	0.0092	0.0105	0.079
Hg Mercury 0.02	0.0221	0.0273	0.0202	0.0176	0.0204	0.0195	0.0184

Table 3: Microbiology results.

Sample No. Element	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	sample 7
Type	Bacillus species	Bacillus species	No Growth	No Growth	Bacillus species	Bacillus species	No Growth
Gram	Positive	Positive			Negative	Positive	

bacteriostatic and wound healing effect in the literature about the beneficiary use of myrrh as there are many confounding factors, this is applicable for bacteria as well as heavy metal contamination. Heavy metals have a harmful effect on humans. There are international regulations on food quality that set the maximum permissible limit of toxic metals in human food [11]. Minerals are just as critical to maintaining optimal health. However, when taken in amounts over the recommended maximum allowable range, they can be toxic to health.

The present investigation found a total of elements in the myrrh resin (Table 3) selenium concentration were 1.386 ppm which is comparable to another research result [12]. Chromium, concentration average is 11.49 ppm. It is noteworthy that presence of the chromium in the myrrh resin can be viewed as a source of chromium supplement and be regarded as a medicinal asset along with its other therapeutic uses. In the present investigation zinc has been found to be present in myrrh resin in low amounts (0.457 ppm), which is slightly lower than the same study of 0.57 ppm [12]. Zinc is relatively nontoxic, particularly if taken orally; it may represent no harm if not small benefits [13].

Mercury level average in our specimens is 0.020 mg/kg it's within safe levels. Mercury occurs naturally in the environment; it is a toxic element and can pose a threat to human health and life. The maximum level of mercury content in dietary supplements is set out in the commission regulation that sets maximum levels for certain contaminants in foods. According to this regulation, the maximum level of mercury for dietary supplements is 0.1 mg/kg or 100 µg/kg.

In the present investigation three elements, besides essential ones, draw the attention for their considerable amount present in the myrrh resin. These include aluminum 11.441 ppm which is slightly higher than recommended dose, it may have toxic role if administered in higher doses. According to the agency for toxic substances and

disease registry (atsdr, 2008, atsdr, 2010) intermediate and chronic-duration Minimal Risk Level (MRL) for the aluminum is maximum allowed level of 0.2 ppm [14].

Arsenic which is a known toxic non-metal has been found in the myrrh extract [12]. However, the level of arsenic in the myrrh, when compared with the reference dose of arsenic for humans (US EPA), was found to be much lower than the expected toxic level [12].

The results of this study especially on the heavy bacterial contents of myrrh implicate an impending danger for consumers. Although we did not study the effect of presence of these bacteria on the rate of adverse wound event. However, we are assuming this is related to the presence of bacteria. Results of this study suggest that myrrh despite its natural benefits may pose potential health risks to patients and, through the presence of microbial contaminants and their products. The high bacterial contamination gave an indication of low environmental sanitation there was no expiry date on any of the containers tested and all were stored at room temperature [15].

Although this study showed heavy metal levels within the allowable limits, it is possible that some amounts can be taken up by the system and accumulates due to long term use, thus cause serious consequences. Even if these metals found in myrrh are less likely free to bind with molecules in our body and thus slower to be absorbed, the issue of safety and vigilance on its serious adverse effects is of concern [16]. The present work complements other myrrh associated investigations done in the past and provides additional data for future researches. Our study has its own limitations. Firstly, the study sample size was relatively small. We did not study the clinical effect of this contamination on patients.

Conclusion

Our experiments showed that the chance of contamination of local myrrh samples is high and may affect its bacteriostatic effects.

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