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Research Article

Development of a nutraceutical from natural products: A case study of a herbal-based low sodium table salt

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Background: *Cyperus papyrus* reed ash has been used traditionally as a salt substitute in Western Kenya. Previous work carried out at Kenya Medical Research Institute indicated that potassium salt substitution derived from local papyrus reed has a favourable potassium/sodium ratio that is suitable for use to regulate high blood pressure.

Objective: To develop and design a process flow for pilot scale production and to develop suitable analytical methods for quality assurance for a herbal based low sodium table salt.

Methodology: The plant material was collected from two study sites in Rift Valley then cleaned, chopped, dried, ashed and extracted at laboratory and optimized industrial scale to yield laboratory and pilot scale samples. Elemental analysis was determined using Atomic Absorption and Flame emission spectroscopy.

Results: The herbal salt yield for the laboratory scale processing was about 10% for both samples but 13% and 22%, respectively, using the optimized industrial procedure. Elemental analysis results indicate the presence of both essential and non-essential elements and heavy metal was within the World Health Organization acceptable limits. The potassium/sodium ratios obtained were between 3 and 11.

Discussion: The herbal salt has the capacity to preserve meat and is a source of other essential trace elements such as chromium, Zinc and manganese.

Key words: Nutraceuticals; process optimization; low sodium; herbal salt; elemental composition

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1. Introduction

About 80% of the local communities in sub-Saharan Africa rely on traditional medicine (WHO, 2002) that for the most part operates outside the public health matrix. Impediments to the integration of traditional medicine with conventional practice include the unavailability of safety, quality and efficacy data and value added herbal products (Chan, 1995). Therefore, promotion of the use of traditional medicine products requires evidence on standardization of the raw materials and finished products, clinical efficacy and safety data.

Cyperus papyrus reeds ash has been used traditionally as a salt substitute in Western Kenya (Kokwaro, 2009). Earlier studies carried out at Kenya Medical Research Institute (KEMRI) indicated that Potassium salt substitution derived from local papyrus reed has a favourable Potassium/ Sodium (K/Na) ratio that is suitable for use to regulate High Blood Pressure in hypertensive patients when used in place of table salt (Obel et al, 1985). A recent study of potassium salt substitution among Taiwanese veterans has further supported this finding (Chang et al, 2006). Part of the overall aim of this project was to establish a national development pipeline for the development of a nutraceutical from natural products. Thus, we report the findings of the process flow development and design for a pilot scale production and development of suitable analytical methods for quality assurance for a herbal based low sodium table salt which is being developed for the management of mild hypertension. Keeping in view the importance of the inorganic constituents of the herbal product, the elemental analysis was undertaken.

Although various preservatives are available today, salt is still widely used in preservation of cheese, dairy products, meat, pickles and sauces. The dual function of common salt is dehydrating ability and bacteriostatic effects. Hence, the food preservative potential of the herbal salt was determined. Food preservative should preserve the appearance, texture, flavor, edibility and nutritive value of the foods and also to prevent from food poisoning due to microbial action.

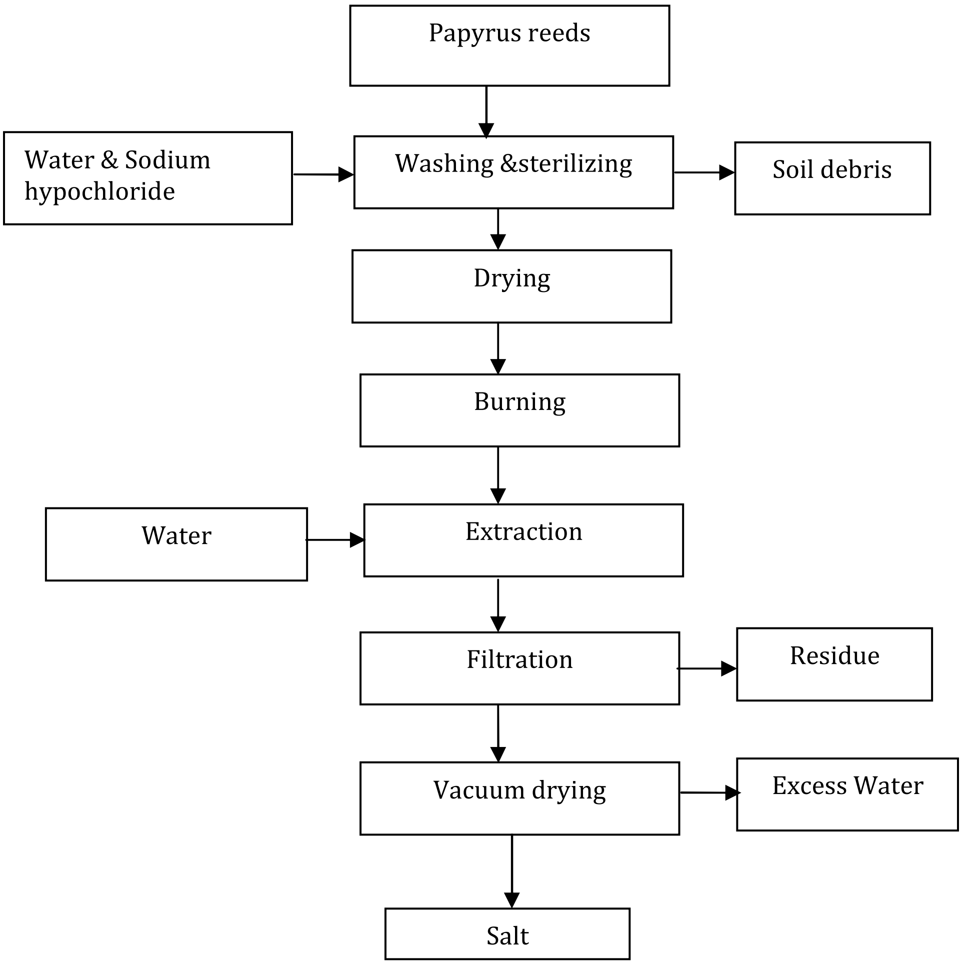

Figure 1: Pilot production flow chart for Herbal Salt

2. Methods

2.1 Plant materials

The *Cyperus papyrus* reeds, the raw materials for preparation of the herbal salt, were collected from two study sites of the Rift Valley Province in mid-September 2009. One area comprised the shores of Lake Naivasha while the second site was the swampy Mbaruk area near Nakuru town. Papyrus reeds, was identified by a KEMRI botanist and verified by a taxonomist at the East Africa Herbarium, National Museums of Kenya, Nairobi where voucher specimens were deposited. The Naivasha sample was assigned Mwitari-KEMRI/KIRDI/2009/1 while the Nakuru sample was assigned Mwitari-KEMRI/KIRDI/2009/2 as Voucher specimen numbers.

2.2 Plant collection and processing

The wet weights of the reeds collected from the two study sites were taken and the plant materials were then packed separately in gunny bags and transported to Kenya Industrial Research and Development Institute (KIRDI) South B Campus, Nairobi Kenya. The reeds were then cleaned, chopped into small pieces and air dried at room temperature (25 °C) under shade for a period of three weeks or until a constant weight was achieved.

2.3 Determination of Bulk Density of Papyrus reeds ash

The bulk density was determined using water displacement, where a container of known volume is packed with the ash of known weight and used to displace water, whose volume is recorded. The density determination formula is then employed to calculate the bulk density. Density=Mass/Volume.

2.4 Laboratory scale production and pilot scale process optimization

Aqueous hot infusions of each ash sample were prepared, in a ratio of 1:10 (ash material: distilled water), in a water bath at 60 °C for 1hr. The extracts that were obtained were filtered and then were lyophilized in a freeze-dryer to yield laboratory scale crude salt.

For pilot scale production, the plant materials were put through the process as indicated in **Figure 1** and once the ash was obtained, 5 samples of ash (material source – Lake Nakuru) each weighing 25 g, were extracted in varying amounts of water ranging from 200 ml to 400 ml. The 5th sample contained same amount of water as sample 4 but was heated at 70 °C for 1 hr. All the extracts were filtered and evaporated to dryness to yield a crude extract. Six kg of ash was extracted with 60 litres of soft water and filtered (ratio 1:10; material source – L. Nakuru). The filtrate was treated with 0.5 kg activated charcoal with heating at 80 °C for 2 hrs. The solution was then filtered and the water evaporated to dryness to yield the crude salt. The crude salt was re-dissolved in distilled water in the ratio of 1:10 and heated to boil, filtered and the water evaporated to dryness again to yield the pure salt. It was noted that the crude extract contained some water insoluble material that resisted dissolving in water even on prolonged heating. It was then filtered to yield the filtrate which was evaporated to yield purified herbal salt and water insoluble matter that was discarded.

2.5 Elemental analysis

The laboratory scale and the purified pilot scale herbal salt samples were sent to the Department of Mines and Geology for elemental analysis. The samples were digested with aqua regia and reduced with stanous chloride and the Mercury Atomic Absorption (AA) (AAS, SpactrAA-10, Varian) determined by cold vapour generation. Lithium, Calcium, Sodium and potassium were estimated using flame emission spectrophotometer after the samples were digested with aqua regia. For Arsenic, the samples were digested with aqua regia and reduced with sodium borohydride and AA determination by vapour generation (flame used for atomization). The other heavy metals including Zinc, Lead, Copper, Cadmium, Chromium and Manganese were determined using Atomic Absorption Spectroscopy after the samples were digested by aqua regia. Chlorides were determined by digesting the samples with nitric acid (trace metal grade) and titrated using 0.1N silver nitrate. The levels of heavy metal exposure was then calculated based on the safe limits that have been set by health authorities in terms of provisional tolerable weekly intake (PTWI) values /Acceptable Daily Intake (WHO, 2007; FAO/WHO, 1993). The estimation ash salt intake was determined according to the method of Garvey et al. (2001), where the estimated dose was taken as 1.0 g of material per day (mean adult body weight = 60 kg).

2.6 The salt preservative properties

Materials used included common salt, the herbal salt derived from papyrus reeds, glycerin, plastic sieve and meat. Meat was obtained from both a high-end butchery (Nakumatt supermarket) and from a butchery representative of the informal sector, characterized by less stringent hygiene and /quality assurance/ quality control standards in South C estate, Nairobi. Only fresh meat from the abattoir, as was verified by the owners of the butcheries, was included in the study. The meat was then chilled to temperatures ranging from 0 - 4 °C. All visible fat was trimmed from the meat and then washed in clean tap water of 4 °C. The meat was then cut into strips that were approximately 1 cm x 1 cm cross section with varying lengths. The strips were divided into two equal portions. One portion was dipped in 14% common salt solution while the other portion was dipped in 14% herbal salt solution. Both were left to soak for five minutes and then drained. The stripes were spread on trays that were greased using glycerin and dried in an electric drier for 48 hrs. The meat was weighed and then submitted to the lab for testing. The efficacy of this preservation system was assessed by parameters such as determination of bacterial count, isolation and characterization of microorganism, percentage weight loss during storage and determination of physical properties of treated meat.

3. Results

Process optimization

The wet weight for the reeds collected from L. Naivasha region was 171.0 kg while that collected from L. Nakuru region was 276 kg. The weight of the reeds on drying was 26.0 kg for the L. Naivasha and 44.0 kg for L. Nakuru samples. The percentage yield of the ash was 23.10% and 28.41% of the weight of dry reeds for L. Naivasha sample and L. Nakuru samples, respectively (**Table 1**). The ashes appeared blackish and fibrous despite the complete burning.

The percentage yield of the herbal salt obtained for the laboratory scale processing was 10.0 and 10.67 % for the sample collected from L. Nakuru and L. Naivasha, respectively as shown on Table 1. After process optimization, the percentage yield of the crude salt obtained was 13.61% and 21.91% of the ash weight for the Nakuru and Naivasha samples, respectively. These were higher compared with what was obtained using the laboratory scale extraction procedure with the yield from L. Naivasha being higher than L. Nakuru's.

Table 1: Reeds wet and dry weight and the percentage yield of ash, laboratory and pilot scale crude herbal salt

Study site	Wet reeds weight (kg)	Dry reeds weight (kg)	Weight of ash (kg)	Salt % yield (Lab scale)	Salt % yield (Pilot scale)
Naivasha (KTM-3)	171.0	26.0	6.0 (23.10)*	10.67	21.91
Nakuru (KTM-4)	276.0	44.0	12.5 (28.41)*	10.00	13.61

* *Ash percentage yield*

Table 2: Extraction process optimization (Material source - L. Nakuru)

Sample number	Ash weight taken (gm.)	Amount of water (ml.)	Weight of crude salt extract (gm.)	Crude salt % yield
1	25	200 (cold)	3.8	15.2
2	25	300 (cold)	2.9	11.6
3	25	400 (cold)	1.6	6.4
4	25	250 (cold)	2.2	8.8
5	25	250 (heated)	2.9	11.6

Table 3: Extraction process optimization and herbal salt yield on purification

Material source	Weight of ash taken (g)	Weight of crude herbal salt obtained (g)	Percentage yield (%)	Weight of purified herbal salt obtained (g)	Percentage yield (%)	Insoluble matter in water (%)
L. Naivasha (KTM-3)	238	52.1567	**21.91**	9.550	**18.31**	3.61
L. Nakuru (KTM-4)	1,173.2	159.622	**13.61**	91.7667	**57.49**	6.09

Extraction process optimization

The least amount of water extracted the largest amount of crude salt (**Table 2**). Comparing samples no. 4 and 5, it is obvious that heating has positive effects of increasing extract yield. However, the heating need not reach boiling point. It was also noted that the first extraction contained some coloring matter which made the extract solution appear brownish. It was therefore necessary to decolorize the solution with activated charcoal to improve the color and product quality.

Further purification was required since the final product is intended for human consumption. The salt obtained using the optimized extraction method was further purified and pure herbal salt obtained. **Table 3** below shows the results of the yield of the pure salt in comparison to the crude salt. The purified salt percentage yield was 57.49% and 18.31% of the crude salt for L. Nakuru and L. Naivasha samples, respectively. Based on the process optimization outcome, the ratio of 1:10 of the ash to water quantities was taken as the ideal. Heating was found to increase the salt yield. The purified herbal salt was taken as the pilot scale herbal salt.

Bulk Density of Papyrus reeds ash

The papyrus reeds ash was found to have a bulk density of 0.1602 g/cc. This was used as a pointer of how much ash could be processed in a vessel of what capacity.

Elemental analysis and acid insoluble ash

Table 4 below shows the results for the elemental analysis and acid insoluble ash content. Elements in varying quantities were noted to be present in both laboratory and pilot scale samples. Lead, Copper, Cobalt and Boron were present in Naivasha sample (KTM-3) and Nakuru sample (KTM-4) laboratory scale but below detection limit (BDL) in the pilot scale samples. Chromium was found to be present in the Nakuru laboratory sample (KTM-4) but, absent in the pilot scale. KTM-4 laboratory scale was also found to contain very high levels of Manganese (Mn) (76.0 parts per million, ppm). The presence of heavy metals such as lead and cadmium were noted in both laboratory scale samples. KTM-3 was noted to contain higher lead content compared to KMT-4.

The K/Na ratio for KTM 3 was found to be 4.48 for laboratory scale and 3.34 for pilot scale while that of

KTM 4 was 10.41 and 8.54 for laboratory scale and pilot scale, respectively (**Table 4**). The percentage acid insoluble ash content for both laboratory scale and pilot scale were determined (**Table 4**). For KTM 3 the values were 5.14% w/w for laboratory scale and 1.97% w/w for pilot scale while for KTM 4 the values were 16.37% w/w and 2.36% w/w for the laboratory and pilot scale herbal salts, respectively.

Pilot extraction process: Material Balance

This was carried out from the dry reeds (22 kg) to the final product (material source – L. Nakuru). Six kg (27.3%) of ash was obtained from the 22 kg of dry reeds which yielded 1.05 kg (17.6%) of crude salt and on further purification, 0.96 kg of pure salt, 0.09 kg of water insoluble matter and 4.94 kg of residual ash were obtained. The percentage yield of the crude salt was 17.6%. This was higher than the 13.61 % obtained in the process optimization.

The salt preservative properties

The average weight loss was roughly the same in both of the samples. It was observed that the sample that was treated with the herbal salt retained the pink colour of meat for long and this was also evident in the final product. No Salmonella or any other pathogenic organisms were detected in both meat samples treated with both common salt and herbal salt. Also, the level of Coliforms were <10 in both test samples and the moisture content was 11.9% for the meat sample treated with herbal salt and 11.7% for the sample treated with common salt. The samples were also prepared in a safe and hygienic manner since the indicator organisms for contamination were not detected.

Table 4: Elemental and acid insoluble ash analysis for the laboratory and pilot scale herbal salt samples in ppm or percent ppm

Parameter	Lab scale herbal salt samples		Pilot scale herbal salt samples	
	KTM 3	**KTM 4**	**KTM 3**	**KTM 4**
Copper (Cu)	4.00	6.43	BDL	BDL
Lead (Pb)	25.00	11.00	BDL	BDL
Manganese (Mn)	3.00	76.00	0.67	0.67
Zinc (Zn)	8.00	8.00	3.33	6.00
Cadmium (Cd)	3.00	4.00	BDL	BDL
Mercury (Hg)	ND	ND	BDL	BDL
Chromium (Cr)	BDL	4.00	BDL	BDL
Boron (B)	400.00	500.00	BDL	BDL
Lithium (Li)	5.00	2.00	2.67	2.67
Arsenic (As)	ND	ND	2.50	BDL
Aluminium (Al)	9.00	13.00	26.67	26.67
Cobalt (Co)	18.00	23.00	BDL	BDL
Iron (Fe)	26.00	26.00	24.66	25.33
Calcium (Ca)	663.00	347.00	1002.67	1002.67
Potassium (K)	27.33 %	24.78 %	12.53 %	26.73 %
Sodium (Na)	6.1 %	2.38 %	3.75 %	3.13 %
Chlorides (Cl^-)	7.50 %	10.50 %	ND	ND
Acid insoluble ash	5.14 % w/w	16.37 % w/w	1.97 % w/w	2.36 % w/w
K/Na ratio	4.48	10.41	3.34	8.54

ND: *Not determined;*

BDL: *Below Detection Limit. Selenium was not determined while Barium, Vanadium, Nickel and Molybdenium were BDL in both lab and pilot scale samples*.

4. Discussion

Cyperus papyrus (papyrus reeds) has been harvested and cultivated since ancient times for its wide array of uses ranging from the production of the first paper and boats in ancient Egypt's first dynasty (Bridget and Tait, 2000) to the manufacturing of ropes, baskets, house building material and furniture today (Terer et al, 2012; Terer, 2011; Gichuki et al, 2001; Muthuri and Kinyamario, 1989; Jones, 1983). *Cyperus papyrus* swamps are characteristic of many wetlands of tropical Africa including Kenya's fresh water lakes. However, like most wetland habitats worldwide, they are vulnerable to human pressure due to harvesting and reclamation for agriculture. Jones and Muthuri (1997) calculated the net primary production of a papyrus swamp at L. Naivasha in Kenya to be 6 kg dry weight per m^2 per year, which is among the highest recorded productivities for natural ecosystems. Based on this optimal regeneration rate we can therefore extrapolate that 6 kg/m^2/year would yield 0.304 g/m^2 of salt annually, from sustainably harvested papyrus reeds using the optimized extraction process we have describe in our methodology. This can be further extrapolated to give the equivalent of approximately 0.8 swamp acreage of sustainable papyrus swamp harvest from the lake per metric ton of salt production per year. These projections indicate that with the scale-up of papyrus derived herbal salt production, the establishment of papyrus cultivations to complement and/or replace sustainable harvesting will likely be required. *Cyperus papyrus* rice paddy cultivation techniques that have been developed for commercial production of papyrus paper can be adopted as alternative to sustainable harvesting (Atsushi et al, 1998). In addition, communities living around the swampy natural papyrus habitat would be educated on the importance of this valuable plant to discourage land reclamation and careless destruction of the reeds.

The extraction ratio of 1:10 of the ash to water with heating was found to be the optimum extraction conditions for the herbal salt. The K/Na ratio for the laboratory and pilot scale herbal salt samples ranged between 3.34 and 10.41. Minor variation in K/Na ratio between Lab and pilot scale samples were noted an indication that the pilot scale process did not affect the K/Na ratios. Previous studies have indicated K/Na ratio of between 2 and 10 for reeds harvested from Western Kenya region (Obel et al, 1985). Also unpublished data at KEMRI reported K/Na ratios of 2.85 and 3.19 for samples harvested from Ruiru in Central Province and along the shores of L. Victoria in Nyanza Province of Kenya, respectively.

The use of herbal medicinal products is not generally expected to contribute significantly to the exposure of the population to heavy metal contaminants. However, it should be understood that the heavy metal content of herbal medicines adds to the burden originating from food (WHO, 2007) and several studies have revealed that herbal remedies contained high levels of heavy metals sufficient to cause adverse health effect when regularly taken as recommended (Garvey et al, 2001; Koh and Woo, 2000; Ernst, 1998; Schicher, 1983). Thus it is critical that heavy metal contamination is minimized. This study revealed that both KTM-3 and KTM-4 laboratory scale samples contained lead, cadmium and copper but in levels that are within the Food and Agricultural Organization/ World Health Organization (FAO/WHO) provisional tolerable intake (FAO/WHO, 1993) but these elements were below detection limit in the pilot scale samples. However, Arsenic levels was not determined in laboratory scale samples but was present in KTM-3 pilot scale sample in quantities below permissible limits 15 µg/kg bw/week (FAO/WHO, 1993). Clinical features in arsenic poisoning include intense thirst, abdominal pain and vomiting. Severe gastroenteritis and circulatory collapse may occur. Nephrotoxicity, aminoaciduria, glycosuria and tubular necrosis are some of the manifestations of cadmium poisoning and it has been noted to occur in levels even below the PTWI (7 µg/kg bw/week) suggesting the current PTWI is not sufficiently restrictive to protect the general population (Satarug and Moore, 2004). Haemolytic anaemia, blue line on gums, lead encephalopathy, peripheral neuritis, constipation, colic, fits, chronic nephritis and hypertension are some of the lead poisoning clinical symptoms. The calculated week intake for lead was 2.9 µg/kg bw/week for the L. Naivasha and 1.3 µg/kg bw/week for L. Nakuru Laboratory scale samples while the FAO/WHO provisional tolerable intake is 25µg/kg bw/week (FAO/WHO, 1993). Copper is relevant to humans because it is both essential and toxic depending on the dose and duration of exposure. Acute copper poisoning results in gastrointestinal disturbances such as salivation, epigastric pain, nausea, vomiting and diarrhoea, all of which are probably due to the irritant effect of copper on the mucosa (Williams, 1982), whereas chronic effects from long-term overexposure results in liver damage (Bremmer, 1998). The maximum permissible level of copper is 12,000 µg/day (NRC, 1989) whereas the estimated levels were below 10 µg/day for both laboratory scale herbal samples.

Presence of essential micronutrients such as Zinc (Zn), chromium (Cr) and manganese (Mn) were noted in both samples. The quantities of manganese were about 3 µg/ gram of the laboratory salt for KTM-3 and 76 µg/ gram for KTM-4. Hence, this herbal product could be a minor source of Mn since a typical supplemental intake of Mn ranges from 2 - 5 mg/ day (NRC, 1989). Manganese has been found to have antioxidant activity, important for proper food digestion and for normal bone structure. The quantity of Mn in the pilot scale samples was low at 0.67 ppm which could be as a result of elimination during the purification stage. Low levels of Zn of 8 µg per gram for the two laboratory salt samples and 3 and 6 µg per gram for pilot scale. The Zinc daily recommended intake is between 10 and 20 mg/ day (NRC, 1989) therefore; this herbal product might not be a significant source of Zn. Chromium was noted only in the KTM 4 laboratory scale sample but below detection limit in the others. Chromium is known to enhance the action of insulin, a hormone critical to the metabolism and storage of carbohydrate, fat, and protein in the body (Mertz, 1998) and it also known to have beneficial effects in impaired glucose tolerance, intestinal diabetes, type - 2 diabetes and lowering of blood lipid (Anderson, 1998). In 1989, the National Academy of Sciences established an "estimated safe and adequate daily dietary intake" range for chromium as 50 to 200 µg/ day (NRC, 1989).

Salt has been used as a natural food preservative since ancient times, especially for meat. The phenomenon behind adding salt as a preservative is that it dehydrates microbes through the process of osmosis. Thus, it inhibits the bacteria that cause food spoilage and protects food from yeasts and molds. Hence, the preservative potential of the herbal salt was also determined and the meat sample that was treated with the herbal salt was found to lose its water more slowly as compared with the meat that was treated with common table salt. Also, the herbal salt treated meat sample retained a more uniform structure as compared with the common salt treated meat sample that collapsed as soon as it started losing its water. Both herbal salt and common table salt were found to inhibit the growth of spoilage and pathogen micro-organisms. The herbal salt was found to preserve meat just as well as normal table salt does.

5. Conclusion

Over one-third of the population in developing countries lack access to essential medicines and the provision of safe and effective traditional medicines therapies could be a critical tool to increasing access to health care. However, issues of safety especially heavy metal contamination should be noted critically since medicinal plants may contain heavy metals when grown in seriously polluted soil (Schicher, 1983). In this study, both KMT-3 and KTM-4 laboratory processed samples were within the recommended limits for the heavy metal contamination. More importantly, the scaled-up production and purification processes described in the methodology section above, advantageously reduced the heavy metal contamination to below detectable levels whilst not altering the Na/K ratio when compared to laboratory processed samples. This product can be a source of other essential micronutrients such as chromium, manganese, Zinc among others and macronutrients like calcium and potassium. It can also be concluded that the herbal salt have the capacity to preserve meat and for management of hypertension due to its favourable K/Na ratios.

Conflict of Interest declaration

The authors declare no conflict of interest

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