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An evaluation of relative stabilities of selected local herbal concoctions stored in *Calabash* fruit pods and clay pot containers

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ABSTRACT

Following long standing cultural beliefs, clay pots and dried fruit pods of Lagenaria siceraria (calabash) have been traditionally used for storing herbal preparations in most communities in Africa. Natural products are often prone to deterioration, especially during storage, leading to loss of active component, production of metabolites with no activity and, in extreme cases, production of toxic metabolites. The study of possible deterioration of stored herbal formulations has seen little attention in the scientific community. It is therefore imperative to evaluate the stability of herbal formulations, particularly during storage. In addressing the issue of stability of stored herbal products, one important consideration is to investigate possible interaction between formulations and the storage containers which may result in contamination or loss of activity. The present study compares relative stabilities of three local herbal concoctions in northern Ghana: Yoggu , Dirigu and Sankpannantoo stored in calabash and clay pots for 768 hours. The concoctions were screened for phytochemicals. Their UV absorbance, pH and thin layer chromatography were determined at various time intervals and the results were used to monitor the stabilities of the formulations in calabash and clay pots. These formulations were also stored in glass and used as control. The result showed varying degree of change under room temperature during a 768 hours monitoring period. Statistical analysis of the results using F-Test Two-Sample for Variances revealed that the stability of identical concoctions stored in calabash was statistically different from those stored in clay pots ($F > F_{crictical}$ and $P < 0.05$ for all measured parameters) but was comparable to those stored in glass container ($F < F_{crictical}$ and $P > 0.05$ for all measured parameters). This result thus supports the continued storage of herbal formulations in dried calabash fruit pods in many rural communities in African.

Keywords: Herbal storage, Natural products stability, *Calabash*, Clay pot, Phytochemistry.

INTRODUCTION

The curative natures of herb and plants have always shown the way to medicinal discoveries and the fact can hardly be denied that any effort to alienate man from nature will make his body system a play ground for diseases [1]. Medicinal plants have been part and parcel of the human society to combat diseases from the dawn of civilization [2]. They have been used in virtually all cultures as a source of medicine [3]. The widespread use of herbal remedies and health care preparations has been traced to the occurrence of natural products with medicinal properties.

Extracts from tissues of plant origin are prepared into herbal formulations in the treatment of a wide variety of diseases in the world over.

The importance of traditional medicine as a source of primary health care was first officially recognized by the world Health Organization (WHO) in the primary Health care declaration of Alma Ata (1978) and has been globally addressed since 1976 by the Traditional Medicine Programme of the WHO. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural product, being non-narcotic, having no side effects, easily available at affordable prices and sometimes the source of health care available to the poor [4].

In the rural communities of Africa, Asia and Latin America where the majority of the world's people live, the dependence on biomaterials can run to over 90 percent of human survival requirements [5].

Unlike in western medical treatment settings, herbal formulations are kept in dried matured *calabash* fruit pods and clay pot containers among most population of the third world. The formulations are usually stored between 1-3 weeks within which the formula is used or disposed off.

Because of the rising cost of conventional drugs coupled with inaccessibility to better health facilities, majority of the people in northern Ghana use plant-based traditional medicine for healthcare. In these areas, traditional herbal practitioners prepare a wide range of infusions and decoctions, majority of which are multi-component and store them in either *calabash* bottles or in clay pots without recourse to consideration of sterility and compatibility between the formulations and the container components. With the advancement of herbal drug treatments, it has now been observed that many of the constituents present in the drug may interact with each other, and with the components of the storage material raising serious concerns about the stability of such formulations. This is an important issue in the field of phytochemistry and natural medicines.

Calabash or gourds are dried mature fruits of *Lagenaria siceraria*. There are many sizes and several distinct shapes of *calabash* and each is put into special uses. The smaller ones are usually used as bottles while the larger ones can be cut open and used as dishes. Because of their woody nature, dried *calabash* fruit pods are susceptible to damage from insects, humidity, light, chemicals and dust. Reports have indicated the presence of triterpenoid cucurbitacins, saponins, essential fixed oils and vitamins in *calabash* [6].

Clay is the raw material for production of earth. It has minute particles most of which are about 0.01mm in diameter. It is produced by the breakdown of felspathic rocks. The chief constituent of most clay is hydrated aluminum silicate called Kaolinite. Clay can absorb and desorb moisture as the humidity rises and falls. Excess moisture contributes to the growth of mold on the clay-pot. Mold grows best at high humidity and the presence of food source such as dust or oils. In traditional African settings, clay is usually molded into pots and heated to bake. These pots are then used as storage containers for water and herbal remedies.

Calabash and clay pots are less expensive but fragile. In certain traditions, it is believed that the potency of an herbal formulation is improved when stored in either a clay pot or *calabash*. However, contact between these containers and their contents can present a number of problems including leaching, sorption, permeation and chemical reactivity [7]. This interaction may render the herbal product toxic and possible loss of activity.

Previous studies on interaction between drugs and storage containers have largely been confined to conventional drugs in plastics. Incredibly, the use of *calabash* and clay pot containers in the herbalist treatment setting is increasing due to cultural beliefs and the advantages offered by their use.

Yoggu is an aqueous formulation prepared as a decoction by combining roots of *Securidaca longipedunculata*, bark of *Sclerocaryo birrea*, and whole *Hibiscus surattensis*. It is commonly used to treat anthrax in northern Ghana. *Dirigu* is an aqueous formulation from the combination of roots of *S. longipedunculata*, whole mistletoe on *Parkia biglobosa* , whole mistletoe on *Vitellaria paradoxa*, mistletoe on *Phragmipedium reticulatum* and seeds of *Cyperus articulatus*. It is used to treat migraine in northern Ghana. *Sankpannantoo* is an aqueous decoction of the bark of *Blighia sapida*. It is has been used in northern Ghana as an antibiotic. These concoctions are mostly stored in dried *calabash* fruit pods and clap pots and used until they are exhausted.

The purpose of the present study was to obtain data relating to physical and chemical stability of the three local herbal formulations: *Yoggu*, *Dirigu* and *Sankpannantoo* during storage in *calabash* and clay pots for 768 hours. This research may also reveal the extent of deterioration of these concoctions as a result of their possible interaction with *calabash* fruit pods or clay pots.

EXPERIMENTAL SECTION

Equipment, apparatus and reagents

Calabash bottles (medium size), Clay pot (medium size), pipette, syringe, beakers, tissue paper, aluminum foil, cotton, glass jars, assorted measuring cylinders, wash bottles, assorted beakers, assorted reagent bottles, electronic balance (sartorius), TLC glass plates, TLC developing tanks, pH-meter (crison), Shimadzu spectrophotometer (model UV-3000) and assorted glassware. All reagents used were of analytical grade.

Sample materials

Roots of *S. longepedunculata*, bark of *S. birrea*, whole part of *H. surattensis*, whole mistletoe on *P. biglobosa*, mistletoe on *P. reticulatum*, seeds of *C. articulatus*, whole mistletoe on *V. paradoxa* and bark of *B. sapida.*

Methods

Sample collection

Plant samples were taken based on their natural distribution. All samples were collected within a radius of 1.6 kilometers from the Tolon locality of the Northern Region of Ghana and identified by at the Department of Applied Biology of the University For Development Studies, Navrongo campus, Navrongo, Ghana.

Sample Preparation

Samples were thoroughly cleaned with distilled water to remove dirt and dust. The formulations were then prepared according to the herbalist recommended procedures. (Aqueous decoction).

Preparation of "Yoggu" formulation

Adequate quantities of roots of *S. longepedunculata,* bark of *S. birrea* and whole part of *H. surattensis* were put together in water and a decoction prepared at boiling temperature for 15 minutes.

Preparation of "Dirigu" formulation

Adequate quantities of roots of *S. longepedunculata*, whole mistletoe on *P. biglobosa*, mistletoe on *P. reticulatum*, seeds of *C. articulatus* and whole mistletoe on *V. paradoxa* were put together in about 1 litre of water and a decoction prepared at boiling point for 15 minutes.

Preparation of "Sankpannantoo" formulation

Adequate quantity of bark of *B. sapida* was put in distilled water a decoction prepared at boiling point for 15 minutes.

Storage of concoctions

For purposes of storage of samples of formulations under investigation, 200ml of equal concentrations (10mg/ml) of crude extracts from each preparation were put in *calabash* containers, clay pots and glass containers in triplicates. Each container was then wrapped with aluminum foil and kept on laboratory bench under room temperature.

Phytochemical screening of concoctions

Each decoction was screened for phytochemical components using standard methods reported by Sofowora [8] and Harbone [9] with some minor modification.

Monitoring and determination of parameters

The TLC, absorbance, and pH were recorded for the contents of each container at time intervals of 24, 48, 96, 192, 384, and 768 hours from the time of preparation of formulations.

Thin Layer Chromatography (TLC)

Preparation of plates

TLC plates were prepared by mixing the silica gel with small amounts of calcium sulphate (gypsum) and water. This mixture was spread as thick slurry on glass and the resultant plate is dried and activated by heating in an oven for thirty minutes at 110^0 C. The average thickness of the adsorbent layer was about 0.20mm.

Development of TLC plates

The TLC plates for all formulations were developed using aqueous ethanol (50% v/v) as mobile phase. Visible bands were marked under daylight and ultraviolet light (254 and 360 nm, Camac Universal UV lamp TL-600) before spraying with freshly prepared *p*-anisaldehyde (1 ml *p*-anisaldehyde, 18 ml ethanol, 1 ml sulphuric acid) or vanillin (0.1 g vanillin, 28 ml methanol, 1 ml sulphuric acid) spray reagents [10]. The plates were carefully heated at 105^0 C for optimal colour development. The number of spots were counted and recorded for each formulation at the respective time intervals.

Spectrophotometry

UV–visible absorption measurements of samples from formulations were carried out with a Shimadzu dual wavelength/double beam spectrophotometer (model UV-3000).

Prior to obtaining UV absorption spectra, solutions of the formulations were diluted to comply with limits for range of measurements by the instrument, using distilled water.

Cuvettes were filled with sample solution and inserted into the cuvette chamber. One was marked “B” for the blank and “S” for sample. The cuvettes were wiped with tissue before insertion into the cuvette chamber. Absorbance readings were then recorded for each sample at the appropriate time intervals.

Selection of wavelength for absorbance

Prior to running absorbance of samples, a calibration curve of absorbance versus wavelength was drawn for freshly prepared samples of the three formulations to obtain wavelength of maximum absorbance.

Sample	Yoggu	Dirigu	Sankpannantoo
λ_{max}	500nm	350nm	400nm

pH readings

The pH sensing electrode was calibrated with pH standards of pH = 4.01 and pH = 7.01 each time the pH of the sample solutions were to be taken. But once calibrated, the pH sensing Electrode could be used for a period of 24hrs. The pH values of the sample solutions were read as the pH reading stabilizes.

RESULTS AND DISCUSSION

Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries [11]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body [12]. Preliminary screening revealed the presence of saponins, flavonoids and phenolics in all the concoctions (Table 1). These phytochemicals are biologically active and may be responsible for the various biological activities demonstrated by the concoctions as evidenced in their use in folklore medical practices. Alkaloids and tannins were present only in *Dirigu* and *Sankpannantoo*. Flavotannins and anthraquinones were only present in *Yoggu* and *Dirigu* but absent in *Sankpannantoo*. *Yoggu* and *Sankpannantoo* tested positive for steroids. Cyanogenic glycosides and anthocyanins were only found to be present in *Yoggu* while cardiac glycosides and terpenes were only present in *Dirigu*.

pH values of contents of containers

Contents of all the containers under investigation showed variations in pH values during the 768 hour period (Table 2). The most significant variations in pH were observed in the contents of the clay pots. (pH range in clay pots: 7.10±0.057 – 3.82±0.101 for *Yoggu*, 5.50±0.000 – 1.47±0.101 for *Dirigu* and 8.10±0.057 – 11.23±0.202 for *Sankpannantoo*). The change in pH of the contents of the clay pot is an indication of possible changes in phytochemical composition arising from container-concoction interactions. There is also the possibility of the concoctions interacting with the surrounding because of the semi porous nature of the clay pot.

Table 1: Phytochemical constituents in formulations

Phytochemical	Formulations		
	Yoggu	***Dirigu***	***Sankpannantoo***
Alkaloids	-	+	++
Saponins	+++	++	++
Flavonoids	++	+	+
Anthocyanins	+	-	-
Tannins	-	+	++
Phenolics	+	+	+
Cardiac glycosides	-	+	-
Terpenes	-	++	-
Flavotanins	+	+	-
Anthraquinones	+	+	-
Cyanogenic glycosides	+	-	-
Steroids	+	-	++

+ = present ++ = moderately present +++ = abundantly present - = absent

Although there were variations in pH values recorded for the *calabash* contents during the 768 hour period , the variations in pH values were statistically not different from identical contents of the glass ($P > 0.05$ and $F < F_{crictical}$). The result therefore suggests that there is little interaction between the contents and *calabash* or the surrounding. The variations in pH values recorded for the *calabash* contents may be the result of herb-herb interaction rather than herb-container interaction. The use of *calabash* pods to store concoctions is thus supported by these results.

Table 2: pH of contents of containers

	Calabash container			**Clay pot**			**Glass**		
Time/hrs	Yoggu	Dirigu	Sankpannantoo	Yoggu	Dirigu	Sankpannantoo	Yoggu	Dirigu	Sakpannantoo
0	7.53±0.003	5.48±0.016	7.96±0.090	7.10±0.057	5.50±0.000	8.10±0.057	7.53±0.000	5.50±0.000	8.00±0.001
24	7.53±0.014	5.06±0.066	8.57±0.280	6.76±0.145	4.33±0.166	8.67±0.120	7.52±0.001	5.50±0.001	8.10±0.001
48	7.45±0.050	4.68±0.158	9.00±0.350	6.13±0.067	3.13±0.088	9.46±0.067	7.52±0.000	5.45±0.002	8.15±0.002
96	7.43±0.110	4.41±0.110	9.30±0.250	5.33±0.060	2.67±0.145	9.76±0.145	7.51±0.000	5.45±0.000	8.50±0.001
192	7.35±0.076	4.03±0.033	9.60±0.250	5.07±0.060	2.05±0.073	10.26±0.185	7.51±0.000	5.45±0.003	8.50±0.000
384	7.28±0.016	4.22±0.116	10.05±0.280	4.53±0.088	1.67±0.176	10.73±0.120	7.51±0.001	5.40±0.000	8.80±0.001
768	7.10±0.058	3.93±0.066	10.50±0.260	3.82±0.101	1.47±0.101	11.23±0.202	7.50±0.001	5.00±0.001	9.20±0.000

Values are expressed as means±SEM and analyzed by F-Test Two-Sample for Variances. (α = 0.05)

Absorbance values of contents of containers

Absorbances of contents of containers were also recorded throughout the period of investigation (Table 3). Absorbance values for all contents showed some variation during the period. Once again, the contents of the clay pots recorded the most significant variations in absorbance. (Abs. range in clay pot: 0.55±0.000 – 0.91±0.015 for *Yoggu*, 0.77±0.005 – 0.44±0.017 for *Dirigu*, and 0.33±0.003 – 0.78±0.005 for *Sankpannantoo*). The absorbance range recorded for the contents of *calabash* were 0.57±0.014 – 0.37± 0.006 for *Yoggu*, 0.77±0.003 – 0.62±0.005 for *Dirigu* and 0.32±0.006 – 0.15±0.006 for *Sankpannantoo*. When compared with those of the glass contents, it was found that the variations in absorbance of identical contents of the *calabash* were not statistically different. ($F < F_{crictical}$ and $P > 0.005$). Since glass has little interaction with its contents, it can be concluded that *calabash* container has little interaction with its contents. Thus dried *calabash* fruit pods may be as good as glass in the storage of the concoctions for the 768 hour period.

Table 3: Absorbance of contents of containers

	Calabash container (10mg/ml)			**Clay pot (10mg/ml)**			**Glass (10mg/ml)**		
Time/hrs	Yoggu	Dirigu	Sankpannantoo	Yoggu	Dirigu	Sankpannantoo	Yoggu	Dirigu	Sakpannantoo
0	0.57±0.014	0.77±0.003	0.32±0.006	0.55±0.000	0.77±0.005	0.33±0.003	0.55±0.001	0.76±0.000	0.32±0.000
24	0.53±0.025	0.76±0.003	0.30±0.003	0.59±0.008	0.69±0.006	0.37±0.008	0.55±0.000	0.76±0.000	0.31±0.001
48	0.50±0.027	0.73±0.008	0.28±0.003	0.68±0.005	0.63±0.012	0.41±0.020	0.51±0.000	0.74±0.012	0.31±0.011
96	0.48±0.041	0.71±0.008	0.26±0.003	0.72±0.024	0.58±0.013	0.48±0.012	0.49±0.002	0.73±0.012	0.30±0.012
192	0.44±0.033	0.69±0.005	0.24±0.013	0.79±0.016	0.53±0.020	0.58±0.006	0.47±0.012	0.73±0.001	0.28±0.002
384	0.41±0.006	0.65±0.011	0.21±0.008	0.85±0.011	0.49±0.017	0.66±0.015	0.47±0.002	0.66±0.001	0.27±0.002
768	0.37±0.006	0.62±0.005	0.15±0.006	0.91±0.015	0.44±0.017	0.78±0.005	0.44±0.011	0.64±0.000	0.20±0.000

Values are expressed as means±SEM and analyzed by F-Test Two-Sample for Variances. (α = 0.05)

Table 4: Thin layer chromatography of contents of containers

	Calabash			Clay pot			Glass		
	Yoggu	Dirigu	Sankpannantoo	Yoggu	Dirigu	Sankpannantoo	Yoggu	Dirigu	Sankpannantoo
Time/hrs	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots
0	5	4	3	5	4	3	5	4	3
24	5	4	3	6	6	5	5	4	4
48	5	4	4	6	9	7	5	4	4
96	6	4	5	6	9	8	5	4	4
192	6	5	4	8	9	8	6	3	4
384	7	5	5	8	12	9	6	4	3
768	7	5	3	9	11	9	6	4	3

Values are analyzed by F-Test Two-Sample for Variances. ($\alpha = 0.05$)

Thin layer chromatography values of contents of containers

TLC was used to monitor possible changes in the number of components in each extract. The number of TLC spots for *calabash* contents ranged between 5 and 7 for *Yoggu*, 4 and 5 for *Dirigu* and 3 and 5 for *Sankpannantoo* during the 768 hour experimental period. The number of spots recorded for the clay pot contents ranged between 5 and 9 for *Yoggu*, 4 and 11 for *Dirigu* and 3 and 9 for *Sankpannantoo*. Contents that show greater changes in the number of TLC spots are regarded as unstable. The TLC values obtained for *calabash* and clay pot contents were compared with those of the glass. The values obtained for the *calabash* contents were statistically different from those of the contents of the clay pot ($F > F_{crictical}$ and $p < 0.05$). These values were however comparable to those of the glass contents ($F < F_{crictical}$ and $P > 0.05$).

CONCLUSION

Phytochemicals are a heterogeneous group of compounds found in all plants [13-14]. They constitute an important component of human nutrition. Medicinal plants are rich in phytochemicals and antioxidants and have been reported in several literatures to protect against some degenerative diseases that affect humans. Concoctions prepared from medicinal plants for the purposes of treating various ailments are often stored in containers prior to use and also during usage. These concoctions are often prone to various chemical and physical changes during storage. The changes may arise from interactions between the concoctions and their respective containers or interactions with the environment. *Yoggu*, *Dirigu* and *Sankpannantoo* are three local herbal concoctions used in the northern parts of Ghana in the treatment of various diseases. These concoctions are often stored in dried *calabash* fruit pods and clay pots during usage. In the present study, we investigated the stabilities of these concoctions during storage by measuring their pH, absorbance and TLC for 768 hours. Results revealed that the stability of formulations stored in *calabash* fruit pods was statistically different from those stored in clay pots ($F > F_{crictical}$ and $p < 0.05$ for all measured parameters) but was comparable to those stored in glass container ($F < F_{crictical}$ and $P > 0.05$ for all measured parameters). This result thus supports the continued storage of herbal formulations in dried *calabash* fruit pods in many rural communities in African. It can be concluded that dried *calabash* fruit pods are as good containers as glass in the storage of herbal concoctions.

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