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Research article

Qualitative and quantitative assessment of cryptolepis-based herbal formulations within the Accra and Kumasi metropolis in Ghana

Adelaide Mensah, Noble Kuntworbe*, Raphael Johnson, Kwabena Ofori-Kwakye

Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical sciences, College of Health sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Key words: malaria, herbal formulations, *Cryptolepis sanguinolenta*, cryptolepine, HPLC.

***Corresponding Author: Noble Kuntworbe,** Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Abstract

Cryptolepis sanguinolenta of the family Asclepiadaceae contains the antimalarial alkaloid cryptolepine. Proliferation of *Cryptolepis*-based formulations in Ghana calls for pharmacovigilance to improve malaria treatment outcomes. The objective of this study is to qualitatively and quantitatively assess Cryptolepis-based formulations on the Ghanaian market. Fourteen registered brands of *Cryptolepis*-based aqueous formulations were purchased from pharmacies and herbal shops within the Accra and Kumasi metropolis of Ghana. The brands were coded A to N. Phytochemical, packaging and labeling assessments were performed. Microbial contaminations were assessed using the pour plate method. The cryptolepine content in each sample was determined using reverse phase HPLC and was compared using one way ANOVA followed by Bonferroni *post hoc* multiple comparison test. Samples were screened for possible antimalarial adulterants such as artemether, lumefantrine, artesunate and amodiaquine. None of the brands met the standard packaging and labeling requirements. Brands B, G and M passed the microbial contamination limit test whilst the rest failed. Glycosides, tannins, saponins, and sterols in addition to the alkaloids were detected. Cryptolepine was present in all brands except E and F. Batch 1 of brand M had the highest cryptolepine content (0.324 ± 0.043 mg/ml). There were significant ($P \leq 0.05$) variations in the cryptolepine content of the different batches of brands B, I, M and N. Artemether, lumefantrine and amodiaquine were not detected. Eleven of the fourteen brands gave positive test for artesunate. All the brands were defective in one or more of the basic requirements of pharmaceutical formulations. Eleven brands may have been adulterated with artesunate.

Introduction

The use of traditional medicines (TM)/herbal medicines (HM) is a common practice among different categories of people around the world [1, 2]. Herbal medicines are marketed in different dosage forms to manage, prevent and treat a wide range of diseases [1, 3] and is gradually becoming an integral part of some healthcare systems globally. However, there are challenges in the harmonization of regulatory standards for these medicines.

Cryptolepis sanguinolenta (Asclepiadaceae) is a climbing shrub that grows in the forest regions of Sub-Sahara Africa. Preparations from its roots have been used for decades by traditional healers for the treatment of various infectious diseases such as amoebiasis, enteric fever and malaria [4]. The plant has been scientifically investigated over the years to verify its folkloric uses as antimalarial, antihypertensive, hypoglycemic, antimicrobial, antifungal, and anticancer agent [5-8]. Results from these investigations have largely justified the continuous use of the plant especially for the management of malaria. Other studies have aimed at structural modifications [9-11] of the active component cryptolepine and using formulation techniques[12, 13] all in attempt to improve the efficacy and safety of the active component which is noted to be a DNA intercalator and topoisomerase II inhibitor [14-16]. Currently, there has been a continuous increase in the number of brands of cryptolepis-based herbal formulations on the Ghanaian market. Though these brands have been registered by the relevant regulatory authority, there is the need for vigilance to ascertain the quality of these brands. This is important because the claim that herbal formulations are harmless is no longer tenable as is evidenced by some key research findings such as adulteration with prescription drugs and other

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substances which make assessment of unwanted effects of such formulations difficult [17,18]. In addition, contamination with heavy metals have also been reported [19]. Studies have also shown that herbal formulations may contain microbial loads above permissible limits [20-22].

There are published evidence [23, 24] of the vulnerability of the drug market especially those in developing countries to the manufacture, distribution and sale of fake and substandard medicines. Investigations of the quality of herbal formulations elsewhere [20, 25] further stimulate research in this direction.

The objectives of the current study are to carry out qualitative and quantitative assessment of available commercialized brands of *cryptolepis*-based aqueous formulations on the Ghanaian market specifically within the Accra and Kumasi Metropolis using physicochemical analysis and visual inspection.

The primary findings in this research have shown that there is the need for further improvement in the quality of *cryptolepis*-based formulations currently in circulation. The presence of artesunate in some brands raises doubt about their label claim of being herbal formulations. Also disturbing is the fact that some brands contain microbial contaminants beyond acceptable limits.

Experimental

Mannitol salt agar, nutrient agar, MacConkey agar, Sabouraud agar and bismuth sulphite agar were obtained from the chemical stores of the Department of Pharmaceutics, KNUST, Ghana and Department of Microbiology, Central university College, Ghana.

Dilute ammonia, chloroform, H_2SO_4, Dragendorffs reagent, Fehling's solution A and B, Lead acetate solution and all phytochemical screening reagents were obtained from the chemical store of the Department of Pharmacognosy, KNUST, Ghana.

Formic acid, methanol, acetonitrile, and diethylamine were donated by Ghana Standards Authority central store and were all HPLC grade. The Cryptolepine hydrochloride sample used in this study was isolated by Kuntworbe *et al.,* in a previous study [26]. *Cryptolepis sanguinolenta* root powder was prepared from root samples obtained from the Centre for Scientific Research into Plant Medicine, Mampong in the Eastern Region of Ghana. All other reagents were of analytical grade.

Sampling and Profiling of Cryptolepis-based formulations

The samples used in the study were purchased from wholesale and retail Herbal shops and pharmacies within the Accra and Kumasi Metropolises in the Greater Accra and Ashanti region respectively within a period of three months (from March to May, 2015). Five brands were obtained (purchased) from Accra and nine from Kumasi. For each brand, one to three different batches were obtained depending on availability. A total of 92 samples were used for the study. The samples were coded with specific reference letters A-N and profiled.

Packaging and Labelling assessment

The samples collected were assessed based on the following parameters; type of packaging, closure type, type of container, active ingredient(s), Food and Drugs Authority registration number, presence or otherwise of a dose measuring device such as a medicine spoon, a measuring cup or an oral syringe. The quality of print and information on the labels were assessed using the approach by Wang *et al.*, [27]. Basically the elements that were sought on the primary and/or secondary package included name of the product, quantity of medicine, batch identification number, storage instruction (s), expiry date, requirement for handling, manufacturing company, strength of active ingredient, instruction/direction for use and legibility of information.

Phytochemical screening

The powdered roots of *Cryptolepis sanguinolenta* as well as the samples were screened for the presence or otherwise of major phyto-constituents including alkaloids, tannins, glycosides, steroids and flavonoids using methods described elsewhere[28, 29]. Specific test for the presence or otherwise of the active compound cryptolepine was carried out by rendering 3 ml portions of each product distinctly alkaline (pH $\geq$10) with 2 ml of dilute ammonia, shaken with 5 ml of chloroform and observed visually. The presence of cryptolepine was characterized by deep purple colouration [26,] of the chloroform layer. The observations were made relative to a reference cryptolepine sample that was similarly treated.

Level of Microbial contamination

Different dilutions of the samples were prepared (1 in 10, 1 in 100, 1 in 1000, and 1 in 10,000). Using the pour plate method, 1 of each dilution was placed in 5 different petri dishes and 20 ml of nutrient agar, MacConkey agar, bismuth agar, mannitol salt agar and sabouraud agar were added to the samples respectively. The dishes were incubated at 37^{o}C for 48 hours (saboraud at 25^{o}C for 72 hours). The colony forming units were counted using a colony counter. Triplicate determinations were done for each the samples.

HPLC method development and validation

The HPLC method described here is based on an earlier work [26] with modification which is a reduction of the formic acid concentration to 0.66% instead of 1% in the previous method. The HPLC system and column used in the current study are also different.

Instrumentation

A Thermo Finnigan Spectra System HPLC system equipped with a quaternary gradient pump P400 SN112/12439-5,

vacuum membrane degasser SCM1000 SN112/202380, variable-loop Auto sampler AS300 with column oven and sample cooling system was used in this study. The chromatographic column used is a Microsorb™ S1 89-100-D5E61002 equipped with a column guard. Data acquisition analysis and reporting were performed by Chrom Quest (version 4.1) chromatographic software. A Thermo Finnigan dual wavelength UV/Vis programmable detector (UV 2000) was used to monitor the eluent.

The mobile phase consisted of Acetonitrile (phase A) and water containing 0.66% formic acid (phase B). The pH was adjusted with diethyl amine to 2.53 to produce peaks with excellent resolutions when an isocratic approach was used i.e. 30% of phase A and 70% of phase B.

Sample preparation and chromatographic run

The samples (4 ml) were rendered alkaline using dilute ammonia and extracted with 5 (5 ml) portions of chloroform using a separating funnel. The organic layer was evaporated to dryness. The residues were separately dissolved in 10 ml of mobile phase B, sonicated, filtered with syringe filters (0.4µM) and transferred into HPLC vials for analysis.

The detection and quantification of cryptolepine from the samples were achieved using an isocratic elution at a flow rate of 1.5 mL/min and sample injection volume of 10µL with UV detection at 280 nm.

The total chromatographic run time was 15 minutes followed by washing and re-equilibration. The column temperature was maintained at 25^{o}C.

The method was validated for linearity, precision, reproducibility and robustness in line with the earlier studies [26].

Determination of Adulterants

The presence of artemether, lumefantrine,artesunate and amodiaquine were investigated using HPLC methods [30, 31].

Results and Discussion

The increasing use of herbal products in the management of a number of health conditions requires continuous vigilance to ensure maintenance of efficacy, safety and the general quality of these products. This is particularly important on the evidence of continuous circulation of fake, substandard and falsified pharmaceutical formulations. Cryptolepis-based formulations are indicated for the treatment of malaria which is a deadly tropical disease and hence the quality of such formulations is important for malaria treatment outcomes.

In all, fourteen brands of commercialized herbal formulations containing *Cryptolepis sanguinolenta* were sampled from retail pharmacies in Accra and Kumasi which are two main cities in Ghana, West Africa. The samples were coded for referencing and also to conceal the identity of the brands as shown in Table 1.

Visual observations revealed that all the samples were packed in plastic amber-coloured medicine bottles with tamper evident seals which prevented leakages and contamination with environmental air.

One of the most important components of a pharmaceutical formulation is the label and the product information it carries. A complete label ensures correct usage and preservation of the product. Thorough assessment of the quality of the product labels and accompanying literature showed deficiencies in some of the brands (Table 2). These deficiencies included the absence of label strength and in some cases the quantity of product. Equally disturbing was the absence of manufacturing and expiry dates on the labels of some brands. The implication of a missing manufacturing and expiry dates is that such formulations may remain in circulation as long as stock last, putting patients in danger of consuming expired products. The absence of product strength and quantity makes it difficult for anyone to verify such products especially with respect to batch to batch consistency.

Table 1. Profile of samples for study

Sample Code	Batch Number	Manufacturing Date	Expiry Date
A1	DMP 006	06/ 2013	06/ 2015
A2	DMP OO3	-	-
B1	FM1/10/12	10/ 2012	10/ 2015
B2	FM2/02/13	02/ 2013	02/ 2015
C1	MM1/2014	01/ 2014	01/ 2016
C2	MM2/2014	05/ 2014	01/ 2016
D1	GM 002	11/ 2012	11/ 2014
D2	GM 001	06/ 2014	06/ 2015
E1	1073	07/ 2013	07/ 2015
E2	1074	10/ 2013	10/ 2015
F1	003-SF-12-CP	03/ 2014	03/ 2016
G1	2227635	12/ 2013	11/ 2014
G2	2276506	02/ 2014	01/ 2015
G3	2227667	08/ 2014	07/ 2016
H1	MR2/13	08/ 2013	08/ 2016
H2	MR3/14	03/ 2013	03/ 2016
I1	-	06/ 2013	06/ 2016
I2	-	06/ 2013	06/ 2016
I3	004	06/ 2014	06/ 2017
J1	EF 23-M06-13	06/ 2013	06/ 2015
K1	Mal50-	08/ 2013	08/ 2015
K2	Aug013wk3	05/ 2014	03/ 2017
K3	MLK50-May014wk4 Mal50-Jan13wk2	01/ 2013	01/ 2015
L	KKC 04	05/ 2013	05/ 2016
M1	23-06-14	06/ 2014	07/ 2016
M2	14-05-14	05/ 2015	07/ 2016
N1	00179	04/ 2014	04/ 2016
N2	00175	10/ 2013	10/ 2015
N3	00176	11/ 2013	11/ 2015

Note: 1, 2, 3 represent different batches of each brand.

Such omissions also raise suspicion of substandard spurious /falsely-labelled/falsified/counterfeit (SSFFC) medicines [32]. In addition, there were different batch numbers on the primary and secondary packages of brand A2. These inconsistencies are misleading and place the medications in an unsafe group as far as medication information is concerned. The inconsistency may be due to manufacturing errors such as a mixed up of labels and other packaging materials of different batches. Most of the brands did not have product leaflet and therefore no further information about those brands. Only two brands had dose measuring devices. Consumers would therefore have to rely on household cups and spoons which are usually inaccurate [32, 33] and could result in over and under dosing.

The samples for the study were of different colour shades (yellowish, yellowish-brown or brown).The mass to volume ratios (density) of the samples were between 1.16-1.68g/ml which points to the samples being aqueous formulations with relatively high solute concentrations. The samples were weakly acidic in nature with pH values in the range of 4.14-6.28 (Table 3). Within this pH range, the active ingredient cryptolepine will be largely ionized being a weak base. It has been reported that the ionized form of the compound is the most stable [34] and active [35]. Thus all the brands have the right pH for the stability and delivery of the active compound.

Phytochemical screening revealed the presence of tannins, glycosides, flavonoids, saponins, sterols and alkaloids in all the brands. Saponins and tannins have antimicrobial properties [6] hence depending on concentration; their presence in the samples could confer preservation on the preparations (Table 4).

Data on the microbial screening (Table 5) revealed unacceptable levels of microbial contamination (except brands B, G and M) with non-fastidious organisms, fungi, *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi.* Even though cryptolepine and the secondary metabolites from the phytochemical screening are known antimicrobial agents, their levels in the samples were either below their minimum inhibitory concentration or the samples were grossly contaminated (higher bio-burden) with the detected organisms. The presence of these microorganisms could potentially affect the health of consumers of these preparations.

The validated reverse-phase HPLC method for the detection and quantification of cryptolepine produced a correlation coefficient R^2 of 0.9996 when concentrations of pure cryptolepine of the range 0.0005 – 0.01%w/v were plotted against the corresponding peak areas. This high degree of correlation is in line with standard analytical method validation (ICH, 2005). The limit of detection LOD which is the amount of analyte that can be detected by the method but not necessarily quantified was 3.4221µg/mL, which was less than the lowest concentration for the calibration curve. Limit of Quantification LOQ which is the lowest quantifiable amount by the method was 10.37µg/mL which fell within the concentration range of the calibration curve details. These findings attest to the HPLC method being accurate. The capacity of the method to remain unaffected by intentional but small changes in chromatographic conditions was tested. Here, a deliberate alteration of pH and flow rate was done and their effect on retention time was noted. The relative standard deviation that was yielded at the end of the experiment fell below 2%, hence an indication that the method is robust and reliable during normal usage. The method was tested for its precision within the same day and different days to know whether laboratory variations will alter the method.

Table 2. Information provided on primary (p) and secondary(s) labels of samples

Parameters	Samples													
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**
Name of Product	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Quantity of medicine (volume)	+	+	S only	+	+	+	+	+	+	+	+	+	+	+
Batch Number	p≠s	+	+	+	+	+	P only	+	+	+	+	+	+	+
Storage Instructions	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Expiry Date	+	+	+	+	+	+	P only	+	+	+	+	+	+	+
Manufacturer	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Strength of active ingredient(s)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Direction for use	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Active ingredients	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FDB registration number	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Legibility of information	+	+	+	+	poor	+	+	+	+	+	+	+	+	+

Note: p≠s, information on p is different from that on s; +, Parameter is present on sample; - Parameter is absent on sample.

Table 3. Density, pH and colour of samples

Samples	pH	Density (g/ml)	Colour
A1	4.71	1.44	Yellow
A2	4.74	1.46	Yellow
B1	4.14	1.38	yellowish brown
B2	4.15	1.29	yellowish brown
C1	5.76	1.46	Yellow
C2	5.76	1.47	Yellow
D1	4.45	1.41	yellowish brown
D2	4.63	1.40	yellowish brown
E1	5.83	1.50	Brown
E2	5.94	1.44	Brown
F	5.69	1.41	Brown
G1	5.18	1.38	yellowish brown
G2	5.19	1.38	yellowish brown
G3	5.18	1.39	yellowish brown
H1	4.23	1.17	Brown
H2	4.24	1.16	Brown
I1	6.27	1.37	Brown
I2	6.26	1.40	Brown
I3	6.28	1.36	Brown
J1	4.37	1.58	yellowish brown
K1	4.82	1.42	Brown
K2	4.82	1.45	Brown
K3	4.82	1.39	Brown
L	5.22	1.68	yellowish brown
M1	4.86	1.47	Yellow
M2	4.87	1.48	Yellow
N1	5.04	1.48	yellowish brown
N2	5.04	1.49	yellowish brown
N3	5.04	1.50	yellowish brown

Table 4. Phyto-constituents of the samples and the pure cryptolepine powder.

Phyto-chemicals	Samples														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Root powder
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Flavonoids	-	+	+	-	+	+	+	+	+	+	-	-	+	+	-
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cryptolepine	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Sterols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: +, indicates the presence of phytochemical; -, indicates the absence of the phytoconstituent

Table 5. Microbial load of the different batches of samples

Sample code	Colony forming units/mL				
	Non-fastidious Aerobic Organisms (x 100)	Fungi (x 100)	*Staphylococcus aureus* (x 100)	*Escherichia coli* (x 100)	*Salmonella typhi* (x 100)
A1	4500 ± 3.97	0 ± 0.00	300 ± 0.50	0 ± 0.00	0 ± 0.00
A2	25400 ± 42.35	0 ± 0.00	0 ± 0.00	2340000 ± 139.23	2300 ± 2.00
B1	92100 ± 13	1600 ± 4.63	0 ± 0.00	0 ± 0.00	0 ± 0.00
B2	11900 ± 5.17	1300 ± 2.67	0 ± 0.00	0 ± 0.00	0 ± 0.00
C1	43400 ± 7.40	24600 ± 4.00	28300 ± 4.50	0 ± 0.00	0 ± 0.00
C2	51500 ± 7.09	0 ± 0.00	0 ± 0.00	3200 ± 3.13	0 ± 0.00
D1	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
D2	14310 ± 376.56	4500 ± 5.84	0 ± 0.00	5400 ± 7.76	21500 ± 6.75
E1	1210000 ± 904.77	65700 ± 6.06	4200 ± 1.66	2310000 ± 4.50	6800 ± 3.38
E2	1340000 ± 573.97	15200 ± 4.64	143300 ± 4.09	8710000 ± 6.84	2000 ± 2.4
F	12900±43.59	28500 ± 5.89	10200 ± 2.47	23800 ± 4.36	4600 ± 1.79
G1	22100 ± 4.93	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
G2	14000 ± 3.80	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
H1	76000 ± 4.77	1700 ± 3.81	2300 ± 1.88	4000 ± 1.22	0 ± 0.00
H2	76000 ± 4.76	3600 ± 4.29	1300 ± 2.24	1300 ± 3.03	0 ± 0.00
I1	5500 ± 7.69	6100 ± 7.18	4700 ± 6.69	19100 ± 4.24	15700 ± 7.83
I2	10400 ± 318.00	9700 ± 2.13	8900 ± 4.92	21400 ± 5.38	0 ± 0.00
I3	11700 ± 6.04	9600 ± 5.39	3200 ± 2.50	1300 ± 3.77	200 ± 0.71
J	75200 ± 4.42	600 ± 1.58	7100 ± 2.30	0 ± 0.00	5100 ± 3.24
K1	92000 ± 5.34	4500 ± 6.65	8400 ± 2.26	42600 ± 5.10	10200 ± 4.00
K2	11600 ± 5	4800 ± 2.96	3500 ± 4.30	39400 ± 7.91	24700 ± 6.44
K3	12500 ± 6.84	136200 ± 6.04	18000 ± 3.43	25300 ± 3.81	0 ± 0.00
L	76600 ± 15.62	376 ± 5.94	63100 ± 7.33	0 ± 0.00	20800 ± 3.71
M1	46900 ± 4.87	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
M2	43400 ± 4.82	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
N1	56900 ± 3.24	5700 ± 2.70	0 ± 0.00	0 ± 0.00	0 ± 0.00
N2	80400 ± 5.25	1100 ± 2.39	1600 ± 3.71	43 ± 3.50	0 ± 0.00
N3	75200 ± 4.28	21400 ± 6.4	2100 ± 4.39	35 ± 3.21	0 ± 0.00

Note: Bold figures indicate samples that failed a particular organism's range of values. Reference number of acceptable microorganism for the samples are as follows; Non-fastidious Aerobic Organisms less than (100,000)10^ 5, Fungi less than (10,000)10^4, *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* should be completely absent

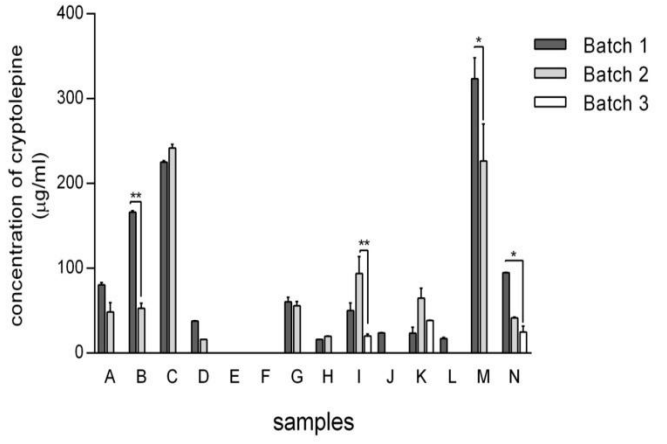


Figure 1: Concentrations of cryptolepine within the various batches of the brands.
Data is expressed as mean±SEM (n=3) *p<0.05, * * p<0.01(One-way ANOVA followed by Bonferroni p*ost hoc* test).

The relative standard deviations for this work fell below 2% indicating that the method is precise. The validated HPLC method was used to quantify the amount of cryptolepine in the samples. This was achieved by using the calibration curve equation. Cryptolepine was detected in all samples with the exception of E and F. No detectable amount of cryptolepine was found in E and F and this was forecasted by the initial determination of cryptolepine in the samples during the phytochemical screening. This is intriguing in the sense that the samples were clearly stated to contain the plant *Cryptolepis sanguinolenta,* the main source of cryptolepine but not even an amount as low as 3.4221µg/mL was detected. Reasons that can account for this finding are that;

The samples could have been wrongly labelled to contain the plant.

The plant could be present as stated but the source, plant part, time and method for collection of the plant could have influenced the content of cryptolepine.

Possible interaction of other constituents of the formulation with the cryptolepine in the samples.

Microbial degradation of the cryptolepine present since samples showed considerable high bio-burden.

The concentration of cryptolepine in the samples ranged from 0.016±0.001 – 0.324±0.043mg/ml with significant batch variations within a brand as well as variations among the brands ($p<0.05$) as shown in Figure 1. This is an evidence of the little or no consensus among manufactures on the collection of plant, standard procedure and storage conditions.

The concentrations exceeds the IC_{50} values established for anti-malarial properties which are 0.134 ± 0.037µg/mL [7] and 0.033± 0.0001µg/mL [6]. The variations in the concentrations of cryptolepine poses a threat to the consumption of such products because different amounts of the active alkaloids are consumed with each batch and this could lead to an over or under dose of the medication. A serious effect will be seen when these concentrations get to values close to lethal doses that have been established already in other researches.

These concentrations when extrapolated can be used to determine the amount of crude root powder needed to yield a particular amount of cryptolepine needed for further analysis and serves as a milestone in standardizing herbal formulations containing Cryptolepine.

An acute toxicity study by Tay *et al.,* [36] established that two of the brands under study in this work had LD_{50} of 300 mg/kg body weight. The brand with highest content of cryptolepine per dose was 9.705mg (30 ml) which will achieve a maximum daily concentration of 29.115 mg (3 times daily dosing as stated by manufactures). This is therefore too low to cause any acute toxicity in consumers.

The cytotoxicity of cryptolepine was pegged at 100 mg/ml on Chinese hamster lung fibroblast cell line V79 which are mammalian cells [37]. This value compared to concentrations of cryptolepine in the samples confirms that the samples are safe as far as cytotoxicity is concerned.

It is tempting to adulterate herbal formulations with orthodox medicines known to be potent in the treatment of the indication for that particular herbal formulation. Usually the adulterants are relatively cheaper, readily available in desired quantities and have the tendency to alleviate ailment within a shorter time frame. In view of this, the presence of artemisinin-based combination drugs such as artemether, lumefantrine, artesunate and amodiaquine were investigated in the samples. These four were chosen because they are composites of malaria chemotherapy in Ghana. They are readily available over-the-counter medicines that can be found in the country. The samples for the study did not contain artemether, lumefantrine and amodiaquine. However, all the samples with the exception of samples B, M and N contained artesunate (Table 6).

This finding adds up to the above that the safety of these herbal medications is questionable. Artesunate which is highly water soluble was likely to have been deliberately added to the samples to enhance the anti-malarial activity of the medication. It could potentiate the effect of the cryptolepine present, or in the case of samples E and F are the actual source of anti-malarial agent in the sample. If so, then the assiduous attempt made by the World Health Organization to rule out monotherapy in the treatment of malarial has come to no avail. This could result in more complex anti-malarial resistant strains of the human Plasmodium species.

Table 6. Identification of adulteration of samples with synthetic antimalarial drugs

	Adulterants			
Sample	**Artemether**	**Lumifantrine**	**Amodiaquine**	**Artesunate**
A	-	-	-	+
B	-	-	-	-
C	-	-	-	+
D	-	-	-	+
E	-	-	-	+
F	-	-	-	+
G	-	-	-	+
H	-	-	-	+
I	-	-	-	+
J	-	-	-	+
K	-	-	-	+
L	-	-	-	+
M	-	-	-	-
N	-	-	-	-

Conclusion

This study was set out to assess the safety and quality of cryptolepis-containing herbal formulations on the Ghanaian market. The findings of the study can conclude that the fourteen (14) brands of herbal formulations studied did not all meet the standard quality assessment parameters. Investigations on the presence of adulterants revealed that about 79 % of the samples have been adulterated with artesunate. A similar trend was indicated in the level of microbial bio-burden. Only about 21% of the study samples could be passed for microbial safety.

This study developed a simple, robust, specific and precise HPLC protocol for identification and quantification of the cryptolepine alkaloid in herbal formulations. Two (2) of the fourteen (14) samples under study revealed no detectable amounts of the cryptolepine and hence raised suspicion on their quality. Quantitatively, there was batch-to-batch variation in cryptolepine content of the brands under study.

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