International Journal of Medicine and Biomedical Research
Volume 2 Issue 2 May – August 2013
www.ijmbr.com


Original Article

# Quality and safety evaluation of a Ghanaian polyherbal product EAF-2011 for the management of superficial mycoses

Dickson R.A[1*], Mensah, M.L.K[2],Thomford K.P[3], Annan K[2], Mills-Robertson F.C[3],Appiah AA[3],Quarshie O[3], Antwi S[3], Kaminta S [3], Edoh D[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, [2]Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi,
[3]Centre for Scientific Research into Plant Medicine, Mampong-Akwapem, Ghana

* Corresponding author: radickson.pharm@knust.edu.gh, ritadickson2000@yahoo.co.uk

Received: 21.01.13; Accepted: 27.06.13

## ABSTRACT

**Background:** The widespread use of herbal medicines in recent years means that issues concerning their quality, safety and efficacy need to be answered. In the current study, the polyherbal formulation EAF-2011 used in the Centre for Scientific Research into Plant Medicine, Mampong-Akwapemfor the management of superficial mycoses was evaluated for its quality and safety. **Aim:** The study sought to evaluate the quality and safety of the product in the management of superficial mycoses. **Method:** The method of standardisation using the basic phytochemical screening and the thin layer chromatography procedures was used. The stability was also assessed using the thin layer chromatography, an antimicrobial assay and the physicochemical properties of the product. A safety evaluation of the product was also performed using a skin sensitisation and chronic toxicity test in male Sprague-Dawley rats. **Results:** The product demonstrated stability during the study period of one year as results obtained from the thin layer chromatography, physicochemical and biological assay did not indicate any significant change in characteristics. Alkaloids, phenols, flavonoids, sterols and triterpenes were detected in the product. Three spots obtained by the thin layer chromatography were shown to be ideal for use as analytical markers for the product. Haematological, biochemical, urine and histopathological data at the end of the study did not indicate any adverse effect from the herbal treatments. **Conclusion:** The study thus showed the ointment to be safe for the management of superficial mycoses.

**Key words:** Analytical markers, chronic toxicity, polyherbal formulation, safety, standardisation, superficial mycoses

## INTRODUCTION

Medicinal plants have become of interest in recent years due to their prospects in meeting the health needs of mankind.[1-4] The long history and widespread use of herbal medicines has always been seen as evidence to this fact.[] Recently however, these prospects have meant that herbal medicines and other natural products are being subjected to a lot of scrutiny.[6] The major issue of concern with these medicines have been with their quality, safety and efficacy.[7-8]

The evaluation of the quality of medicinal products is very essential as they are affected by factors that include seasonal changes, harvesting time, cultivation sites, post-harvesting processing, adulterants or substitutes of raw materials and procedures in extraction and preparation.[9] These factors are also known to also have implications on the safety and efficacy of resultant products.[9-10]

The availability of data in the area of quality, safety and efficacy is very scarce for majority of medicinal products in use.[9] A reason assigned for this limitation is the lack of adequate and accepted research methodologies for evaluating natural products.[11] However, steps have been taken by several agencies like the World Health Organisation (WHO) and the International Union of Pure and Applied Chemistry (IUPAC) aimed at addressing these challenges.[12-14] Significant among these interventions are the recommendations for the standardisation of all medicinal products.[14] Suggestions have been made for the preclinical screening of natural products for safety and efficacy especially when there is the absence of guidelines for carrying out actual clinical assessment.[12]

In the current study the polyherbal formulation labelled EAF-2011 from the Centre for Scientific Research into Plant Medicine, Mampong-Akwapem and used in the management of superficial fungal diseases was evaluated. The product formulated with five plants has no previous documentation on its quality and safety.

## METHODOLOGY

### Plant used in the study

The plants in the formulation were the dried leaves of *Alchorneacordifolia*(Schum. &Thonn.)Muell.Arg. (Euphorbiaceae), the dried leaves of *Psidiumguajava*(Linn) (Mrytaceae), the dried whole plant of *Tridaxprocumbens*(Linn) (Asteraceae), the dried stem bark of *Zanthoxylumzanthoxyloides*(Lam) (Rutaceae) and the dried fruit buds of *Eugenia caryophyllata*(Thumb) (Mrytaceae).

### Plant collection and authentication

The plants used were sourced from Mampong-Akwapem and its environs in November and December, 2010 except *Eugenia caryophyllata* which was purchased from some commercial collectors. The plants were authenticated by Mr. Ofori-Lartey, botanist at the Plant Development Department, Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akwapem. Voucher specimen number was allocated (Table 1.0) and the specimen was deposited at the herbarium of the CSRPM.

### Plant preparation

The various plant materials were air-dried in a cool, dry place under shade for 2 weeks at temperatures between 23ºC and 27ºC. The materials were pulverized using a hammer mill through 2mm screen. Each powdered plant material was extracted by macerating 1kg of the powdered plant material in 5 L of 70 % ($^{v}/_{v}$) ethanol for 3 days and then filtered. The ethanol was recovered using the rotary evaporator (Buchi™ R210) and the fluid extract evaporated on a water bath over 72hrs.

### Composition of the product

The individual herbal extracts were combined according to the established recipe used by the CSRPM in the formulation of the product.

### Standardisation of the product

The standardisation of the product involved two methods: the basic phytochemical screening and the thin layer chromatography. In the basic phytochemical screening, the ointment, the total crude extracts and the individual plant materials were each screened for the presence of alkaloids, saponins, flavonoids, sterols/triterpenes, anthracenosides and cyanogenic glycosides using methods described.[16-17]

The thin layer chromatography was also performed for the ointment, the total crude extracts and the plant materials according to the methods prescribed.[18-19] The total crude extract (TC) and the ointment were extracted in 70 % ($^{v}/_{v}$) of ethanol for three days. The aqueous fraction from this extraction was obtained by evaporating the ethanol using the rotary evaporator. This aqueous fraction was then extracted with ethyl acetate in a separating funnel (1:2) ($^{v}/_{v}$). The ethyl acetate fraction was spotted for the TLC on precoated silica gel 60 $F_{254}$ plates (Merck). Detection was done using

an Ultraviolet Lamp at 365nm and 254 nm and anis aldehyde detecting reagent at ambient temperature.

## Stability studies of the product

The stability of the product was tested by employing the methods of the thin layer chromatography, an antimicrobial assay and assessing the physical properties of the ointment. These studies were conducted at the baseline month 0, month 6, month 9 and month 12.[20]

## Thin layer chromatography

The thin layer chromatography was performed according to the guidelines described under the standardisation of the product.

### Antimicrobial assay

The agar well diffusion method was used for the antimicrobial assay as described in the B.P, (1988).An amount of 16g of the ointment was dissolved in 100mLof 20% dimethylsulphoxide (DMSO). Petri dishes of 100mm were filled with 25mLof sabouraud agar for the fungal organisms and 25mLof Mueller Hinton for the bacterial organisms. The plates were filled to a depth of 4mm and allowed to solidify. Each plate was then flooded with the pathogenic micro-organism and the plates were allowed drying at room temperature for one hour. A sterilised borer of an internal diameter of 4mm was used to bore holes in the media. The herbal products were dispensed into the bored holes. The filled petri dishes were kept in the refrigerator for 6 hrs to allow absorption of the extract into the media and then incubated at 27$^{o}$C for 72hrs for the fungal organisms and 38$^{o}$C for 24hrs for the bacterial organisms. DMSO was used as the negative control for all test organisms while fluconazole was used as the positive control for the fungal organisms and chloramphenicol for the bacterial organisms. After the incubation period, the diameter of each zone of inhibition was measured in millimetres with a metre rule. The measure of the zone of inhibition at each period of assay was compared and used as an indicator of the stability of the product.

### Organoleptics

The organoleptic features: colour, odour and consistencyof the product were observed over the duration of the stability studies. Observations were made by two independent investigators.

### pH

An amount of 1g of the ointment was dispersed in 100mL of distilled water by vigorous shaking and warming over a water bath to melt the ointment. The mixture was allowed to cool under room temperature to separate the insoluble wax from the aqueous phase. The insoluble wax was then decanted and the pH of the aqueous fraction determined using the digital pH meter (Cyberscan pH 310; EutechInst).[18]

## Preclinical chronic toxicity testing of the product

Skin sensitisation and chronic toxicity was conducted for the product to identify any possible toxic effects. All experiments were performed according to the protocol described by Nyarko *et al.* and conducted in accordance with accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC).[18]

### Animals

Male Sprague-Dawley rats (200-250g) were used for the study. Animals were fed on powdered feed from Ghana Agro Food Company (GAFCO) Tema, Ghana and also allowed free access to sterilized distilled water.

### Skin sensitization

The hair on the lateral portion of albino rats (about 9cm$^3$) was trimmed and shaved with a razor blade. The rats were divided into 5 groups (n=5). One group was injected intradermally with 0.1mL of 5% ($^{w}/_{v}$) of the highest dose of the ointment dissolved in glycerol. The control was also received an intradermal injection of 0.1Ml glycerol. The duration of the resultant swelling was observed, as well as any ulceration that appeared subsequently. The various dosage concentrations of the ointment were also applied topically to the three (3) groups of rats and the animals were observed daily for 6 months for any ulceration, irritation and/or inflammation. The last group did not receive any treatment and served as the control.

### Chronic toxicity

Four groups of five rats each were kept in three different metal cages. Group 1 was kept as control and they received sterilised

water and no treatment for six weeks. Groups 2, 3 and 4 were treated with 2% ($^{w}/_{w}$), 5 % ($^{w}/_{w}$) and 10 % ($^{w}/_{w}$) of the ointment respectively. Baseline readings for the liver, kidney and urine parameters were taken. These were then repeated at the 1st, 3rd and 6th months.

### Haematological, biochemical and urine analyses

Blood samples were obtained by tail bleeding (at baseline, 1st, 3rd and 6th month) into Eppendorf tubes without anticoagulant. Samples were centrifuged for 5 mins (Denley BS 400, England) and the serum stored at -40$^{o}$C for biochemical analysis. Blood samples were also collected for haematological analysis.

Haematological analysis was done using Haema-Screen 18 (Hospitex Diagnostics, Italy) according to the established protocol. Serum biochemical analysis was also done using protocols from Cypress Diagnostic Kits (Belgium) with a semi-automated blood chemistry analyser, photometer 4040. Urine samples were also collected and analysed for specific gravity, pH, glucose, bilirubin, ketones, proteins, urobilinogen, nitrates, blood and leucocytes using urine reagent strip UroColor™ 10 (Standard Diagnostic Inc., Korea).

### Histology

Two rats from each group were euthanized after 6 months of treatment. Biopsies were taken from the liver, spleen, kidneys and skin. The skin biopsy was obtained from the area where the ointment was applied. Specimen was preserved in 10% formaldehyde solution (formalin). Tissues were embedded in paraffin wax, sectioned into uniform thickness of 10 µm and stained with hematoxylin and eosin. Sectioned tissues were evaluated under a microscope for any morphological changes.

### Statistical analysis

One-way analysis of variance (ANOVA) and independent sample *t*-test was conducted to determine statistical significance. Statistical tests were performed with Graphpad Prism software version 5.0 at an α-level of 5%.

## RESULTS

### Standardisation of the product

The results of the phytochemical screening showed the presence of alkaloids, phenols, sterols and triterpenes and flavonoids in the product. The results are compared to that of the individual plant extracts and the total crude extract in Table 2. No differences were observed between the plant extract, total crude extract and the ointment. The thin layer chromatograph provided three analytical markers for the product (Figure 1). The $R_f$ values of the spots were A (0.87), B (0.64) and C (0.57).

Table1: Voucher specimen number of plant materials used

| Plant Material | Voucher Specimen Number |
|---|---|
| *Alchorneacordifolia* | CSRPM 368 |
| *Psidiumguajava* | CSRPM 50 |
| *Tridaxprocumbens* | CSRPM 256 |
| *Zanthoxylumzanthoxyloides* | CSRPM 330 |
| *Eugenia caryophyllata* | CSRPM 001CM |

### Stability studies of the product

### Thin layer Chromatography

The chromatographic finger print obtained for the ointment and the total crude extract was repeated over a year period. The values obtained for each period of time were not different (Figure 2).The baseline and after one year of study for both test samples showed the ointment and the crude extracts to be stable as the spots obtained at the end of the study were not different from that obtained at the start of the study. Similar $R_f$ values were recorded A (0.87), B (0.64) and C (0.57).

### Antimicrobial assay

The antimicrobial assay of the ointment against three fungal pathogens: *Micosporumcanis, Trichophytonrubrum,* and *Candida albicans* and one bacterial pathogen (*Staphylococcus aureus*) produced similar results over the one year study period (Table 3). The differences observed in the readings were not significantly different at the start of the

study and after one year. The ointment also showed appreciable activity against the bacterial pathogen.

## Organoleptic characters and pH

The study of the organoleptic characters of the ointment at the baseline showed the ointment to be dark brown in colour, aromatic in odour, smooth with no lumps or grittiness in consistency. These same observations were made subsequently in the following period of study as shown in Table 4. A marginal change in pH values was recorded at the end of the study. The pH was recorded at the start of the study and during the study. This is also recorded in Table 4.

Table 2: Results of the phytochemical screening of the various samples

| Plant Extract | Alkaloids | Phenols | Sterol/ Triterpenes | Saponins | Flavonoids | Anthracenosides | Cyanogenetic Glycosides |
|---|---|---|---|---|---|---|---|
| *Alchorneacordifolia* | - | + | - | + | + | - | - |
| *Eugenia caryophyllata* | - | + | + | - | + | + | - |
| *Psidium. Guajava* | - | + | - | + | + | - | - |
| *Zanthoxylumzanthoxyloides* | + | + | + | - | + | - | - |
| *Tridaxprocumbens* | + | + | - | + | + | - | - |
| *Total Crude Extract* | + | + | + | - | + | - | - |
| *Ointment (*EAF-2011*)* | + | + | + | - | + | - | - |

Key: (-) Absent; (+) Present

Table 3: Zones of inhibition produced by the ointment (EAF-2011) over one year period

| Month | *Microsporum canis* | *Trichophyton rubrum* | *Candida albicans* | *Staphylococcus aureus* |
|---|---|---|---|---|
| 0 | 12.7 (0.22) | 13.3 (0.12) | 10.3(0.56) | 20.3(0.01) |
| 3 | 12.3 (0.31) | 13.0 (0.32) | 10.0(0.45) | 19.7(0.20) |
| 6 | 13.3 (0.25) | 14.0 (0.00) | 10.3(0.34) | 20.0(0.47) |
| 12 | 13.2 (0.88) | 13.8 (0.71) | 10.2(0.67) | 19.8(0.84) |

Results are Mean ±SD; n=3

Table 4: Organoleptic characters and pH of the ointment during the stability study

| Month | Colour | Odour | Consistency | pH |
|---|---|---|---|---|
| 0 | Dark Brown | Aromatic | No grittiness or lumps observed | 5.42 @ 29.2 $^0$C |
| 3 | Dark Brown | Aromatic | No grittiness or lumps observed | 5.29 @ 30.2 $^0$C |
| 6 | Dark Brown | Aromatic | No grittiness or lumps observed | 5.67 @ 30.4 $^0$C |
| 12 | Dark Brown | Aromatic | No grittiness or lumps observed | 5.71 @ 29.4 $^0$C |

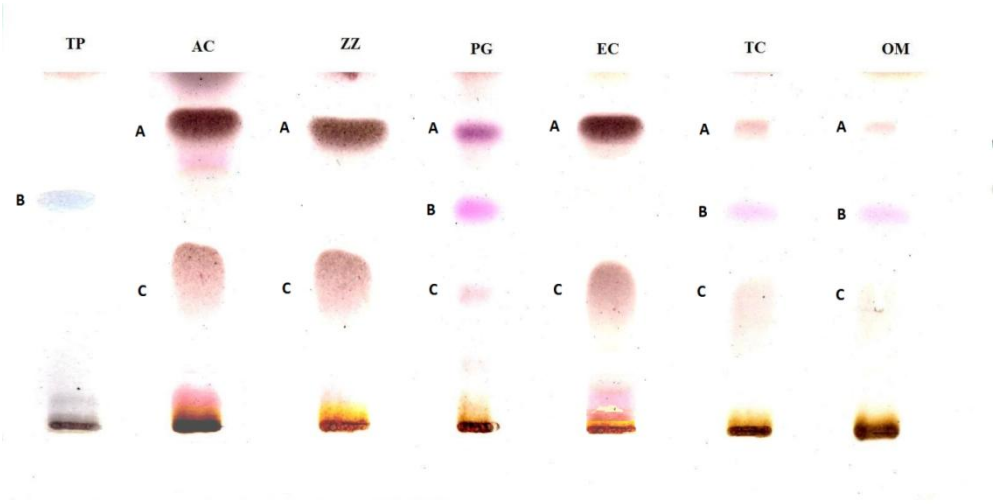


Figure 1: Chromatographic fingerprint obtained for the individual plant extracts. *Tridaxprocumbens (TP), Alchorneacordifolia (AC), Zanthoxylumzanthoxyloides (ZZ), Psidiumguajava (PG), Eugenia caryophyllata (EG), Total crude extract (TC) and the ointment (OM)*. Solvent system: ethyl acetate: petroleum ether (1:4). The $R_f$ values obtained for the spots were A: 0.87, B: 0.64, C: 0.57.

Table 5: Effect of ointment on haematological parameters at termination

| Haematology | Treatment | | | |
|---|---|---|---|---|
| | Control | 2% | 5% | 10% |
| NEU($mm^3$) | 13.37 ±0.98 | 14.57 ± 2.41 | 18.03 ± 0.69 | 28.53 ± 11.79 |
| LYM($mm^3$) | 81.93 ±0.81 | 56.37 ± 20.34 | 56.27 ± 19.62 | 66.90 ± 11.36 |
| MON($mm^3$) | 3.93 ± 0.29 | 29.67 ± 23.97 | 27.83 ± 22.54 | 3.40 ± 1.32 |
| EOS($mm^3$) | 0.30 ± 0.00 | 2.13 ± 1.83 | 3.00 ± 2.65 | 0.70 ± 0.15 |
| BAS($mm^3$) | 0.47 ± 0.07 | 0.47 ± 0.03 | 0.47 ± 0.07 | 0.47 ± 0.07 |
| RBC($mm^3$) | 8.68 ± 0.16 | 5.82 ± 2.71 | 5.86 ± 2.78 | 8.01 ± 0.64 |
| HB(g/dl) | 16.50 ±0.23 | 13.58 ± 2.62 | 14.01 ± 2.79 | 15.10 ±1.30 |
| HCT(%) | 48.70 ±0.45 | 36.90 ± 10.10 | 37.67 ± 10.94 | 44.00 ± 4.11 |
| MCV($\mu m^3$) | 56.00 ±1.00 | 53.10 ± 1.99 | 52.77 ± 3.23 | 54.67 ± 0.88 |
| MCH(pg) | 19.03 ±0.33 | 32.30 ± 13.35 | 31.30 ± 11.85 | 18.87 ± 0.09 |
| MCHC(g/dl) | 33.90 ±0.20 | 29.63 ± 4.82 | 29.30 ± 5.30 | 34.43 ± 0.32 |
| RDW(%) | 10.83 ±0.58 | 10.87 ± 0.15 | 18.47 ± 7.82 | 10.80 ± 0.46 |
| PLT($mm^3$) | 614.00±128.58 | 587.67 ±219.85 | 772.00 ± 47.32 | 696.67 ± 44.64 |
| MPV($\mu m^3$) | 5.80 ± 0.12 | 6.27 ± 0.48 | 5.87 ± 0.07 | 5.97 ± 0.26 |
| WBC($mm^3$) | 12.00 ± 0.56 | 11.50 ± 1.93 | 11.43 ± 0.81 | 11.80 ± 0.90 |

Results are Mean ± S.E.M; n=5

Key: HB - Haemoglobin, HCT - Haematocrit, BAS - Basophils, LYM - Lymphocytes,MCHC - Mean Corpsular Haemoglobin Concentration, MCH - Mean Corpsular Haemoglobin, MCV - Mean Corpsular Volume, MPV - Mean Platelet Volume, MON – Monocytes, NEU - Neutrophils, PLT - Platelets, RBC - Red Blood Cells, RDW - Red Blood Cell Distribution Width, WBC - White Blood Cells. No significant difference was observed were observed between the test and control for all parameters in all the treatment groups ($p$<0.05). Implying the ointment has no effect on the blood cells.

Table 6: Effect of the ointment on the liver and kidneys after treatment

| | | | Treatment | |
|---|---|---|---|---|
| | Control | 2% | 5% | 10% |
| Kidney Function | | | | |
| UREA(mmol/l) | 3.43 ± 0.30 | 3.13 ± 0.30 | 2.93 ± 0.07 | 2.90 ± 0.10 |
| CREATININE (mmol/l) | 36.07 ± 3.33 | 25.07 ± 2.93 | 44.75 ± 6.93 | 28.33 ± 3.31 |
| Liver Function | | | | |
| ALBUMIN(g/L) | 30.85 ± 2.08 | 32.35 ± 1.01 | 32.82 ± 1.39 | 32.70 ± 0.87 |
| ALT(u/L) | 104.67±0.91 | 98.67 ± 3.72 | 99.67 ±13.17 | 87.10 ± 8.98 |
| AST(u/L) | 139.45 ±8.51 | 170.14 ± 11.08 | 132.07 ± 28.09 | 147.43±25.87 |
| GGT(u/L) | 1.20 ± 0.23 | 1.10 ± 0.15 | 1.43 ± 0.35 | 2.60 ± 0.62 |
| ALP(u/L) | 4.67 ± 0.88 | 4.67 ± 0.33 | 3.33 ± 0.88 | 4.00 ± 0.58 |

Results are Mean of ±S.E.M; n= 5

Key: ALT – Alanine Transaminase, AST – Aspartate Transaminase, GGT – Gamma GlutamylTransferase, ALP – Alkaline Phosphatatse. No significant difference was observed ($p$<0.05) were observed between the test and control animals for all parameters in all the treatment groups. Implying the ointment has no harmful effect on the liver and kidneys.

Table 7: Effect of the ointment on urine parameters at termination of treatment

| Urine Parameter | Treatment | | | |
|---|---|---|---|---|
| | Control | 10% | 2% | 5% |
| Urobilinogen | - | - | - | - |
| Glucose | - | - | - | - |
| Ketones | - | - | - | - |
| S. G | 1.024 | 1.022 | 1.023 | 1.012 |
| Blood | - | - | - | - |
| pH | 7.0 | 7.2 | 7.3 | 7.3 |
| Proteins | ++ | + | ++ | + |
| Nitrites | - | - | - | - |

Key: (-): negative; (+): present in moderate quantities; (++) present in large quantities. There was no significant difference between the controls and test meaning there is no adverse effects on the kidneys.

Table 8: Post treatment effect of EAF-2011 on the organ weights (weight to body ratio) of rats

| Organ | Control | 2 % | 5 % | 10 % |
|---|---|---|---|---|
| Kidney | 2.29 ± 0.16 | 2.24 ± 0.57 | 2.16 ± 0.24 | 2.21± 0.84 |
| Spleen | 0.71± 0.64 | 0.71 ± 0.15 | 0.63 ± 0.11 | 0.65 ± 0.18 |
| Liver | 12.61 ± 1.71 | 12.19 ± 2.90 | 10.50 ± 0.85 | 11.29 ± 2.82 |

Results are Mean ± SEM; n=5

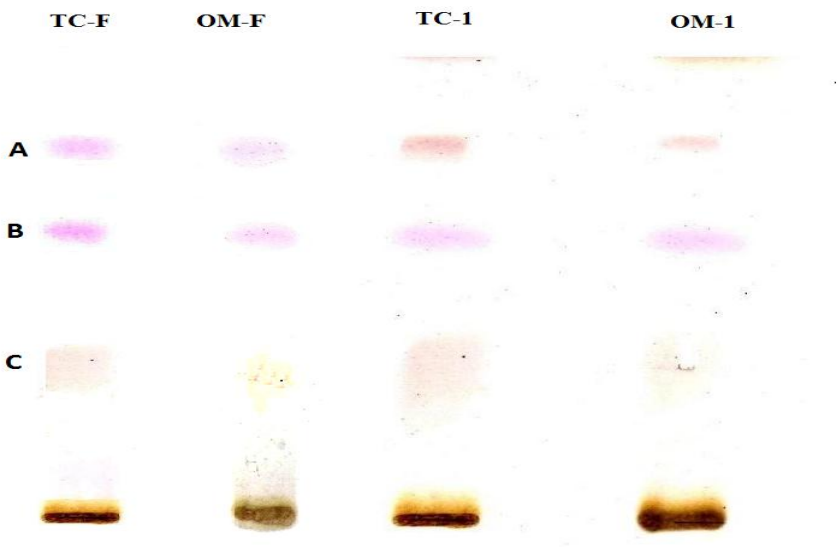


Figure 2: The chromatograph of the ointment and total crude extract at the baseline and the end of the study.Solvent system: Ethyl acetate: Petroleum ether (1:4).The $R_f$ values obtained for the spots were A: 0.87, B: 0.64, C: 0.57.

Key: Ointment at the start (OM-1), Ointment after a year (OM-F), Total crude extract at the start (TC-1) and Total crude extract after a year (TC-F).

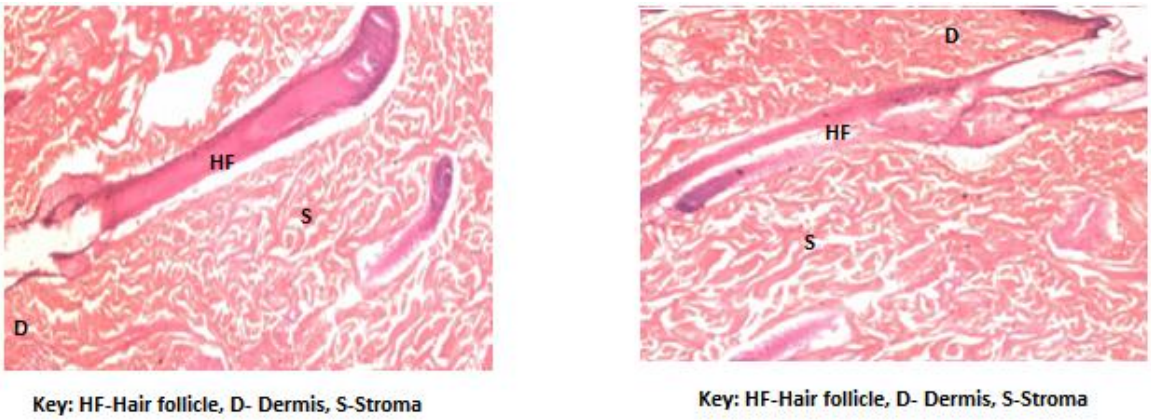


Figure 3: A photomicrograph comparing the longitudinal section of the skin from the animals treated with the 10 %EAF-2011 (Left) and the control (Right). Magnification x 400

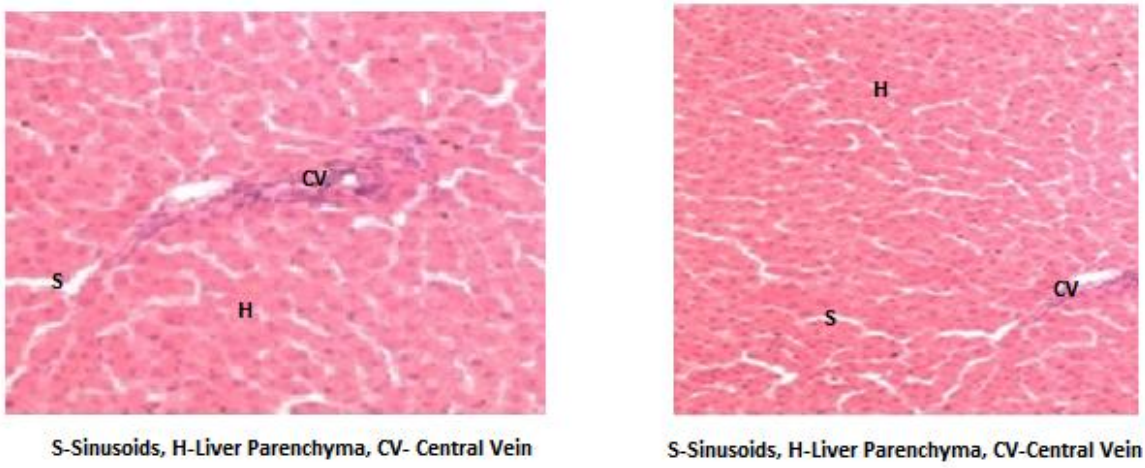


Figure 4: A photomicrograph of the transverse section of the liver from the animals treated with the 10 %EAF-2011 (Left) and the control (Right). Magnification x400

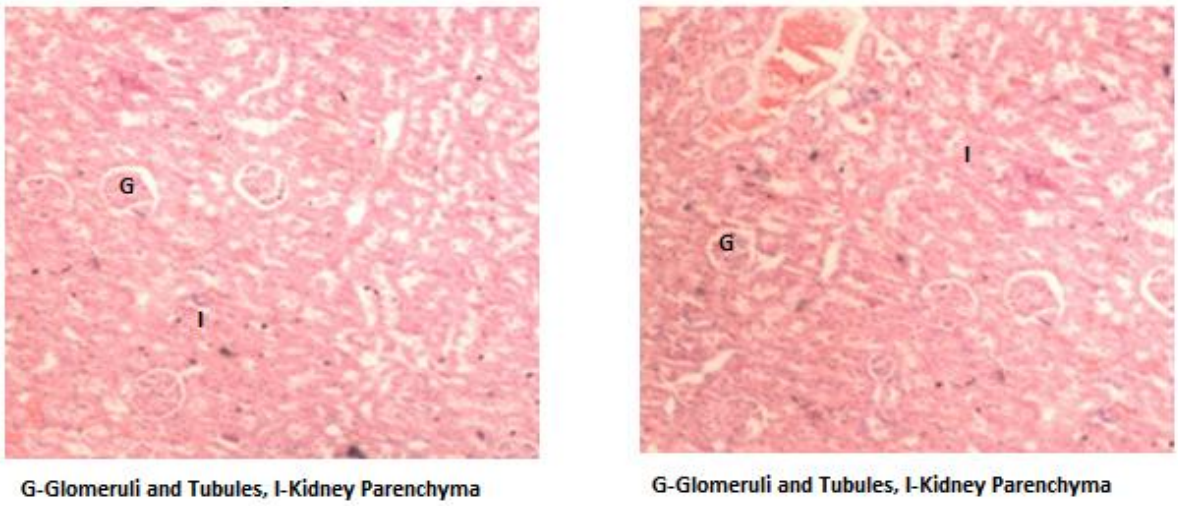


Figure 5: A photomicrograph of the transverse section of the kidney from the Sprague-Dawley rats treated with the 10 %EAF-2011 (Left) and the control (Right). Magnification x400

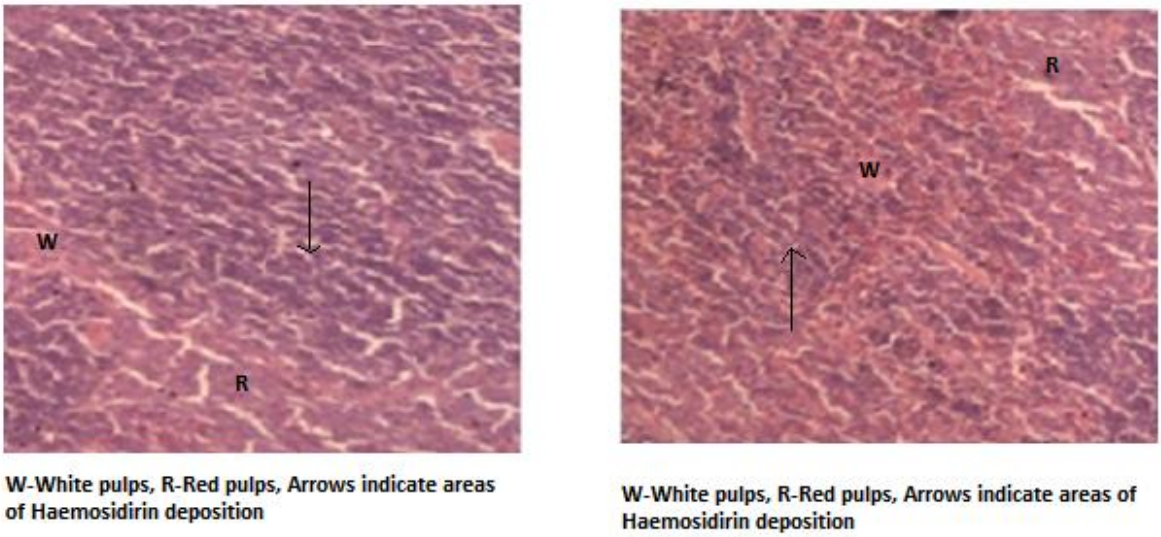


Figure 6: A photomicrograph comparing the spleen from Sprague-Dawley rats treated with the 10 % EAF-2011 (Left) and the control (Right). Magnification x 400

### Preclinical chronic toxicity tests of the product

### Skin sensitivity testing

The skin sensitivity test using the male Sprague- Dawley rats showed no dermal irritation in the form of ulcerations was observed during the 6 months of treatment. Subcutaneous papule that was formed after intradermal injection of the ointment dissolved in glycerol resolved without any ulceration after 72hrs of observation as in the control treatment.

### Chronic toxicity studies

The results from the chronic toxicity study indicated that the ointment did not cause any significant change in the haematological, biochemical and urine parameters after 6 months of administration. The results of the haematological, biochemical and urine parameters are shown in Table 5, 6 and 7 respectively.

### Histology

The gross features of the organs isolated after termination were detected to have no abnormalities. No significant differences were observed in the organ weights between the control and the herbal treatments (Table 8). Microscopic examination of the tissues did not indicate any histopathological changes to the tissues sampled. The skin epidermis, dermis and adnexal tissues were regular in pattern. The spleen had regular red and white pulps with some haemosiderin deposition which was considered as physiological. In the kidneys the glomeruli, tubules and interstitium were normal. There was no indication of an inflammation or necrosis. The liver's portal tracts, hepatocytes and sinusoids were normal with the absence of inflammation or fibrosis. There was also no hepatocyte degeneration. These features were identified in the Whitfield, 2 %, 5 % and 10 % herbal treatment. Samples micrographs from histopathology are shown in Figures 3-6.

## DISCUSSION

The quality evaluation of medicinal products has become a key component in the assessment of herbal medicines. The value of these studies for natural products cannot be overemphasised as the variability in medically active constituents in these products has implications on the therapeutic effects produced by them.[21] Standardisation of all natural remedies has been touted as a solution to this challenge.[21]

The basic phytochemical screening of the product EAF-2011 indicated the presence of secondary metabolites like the alkaloids, phenols, flavonoids, sterol and triterpenesin the product. The results apart from providing a standard, also corroborates the indication given to the product as some compounds belonging to this class of secondary metabolites detected have shown some antifungal

activity. Ursolic acid a triterpene found in *Psidiumguajava* has been reported for its antifungal activity against C*andida albicans.*[20] Quercetin and kaempferolall flavonoids previously identified in *Eugenia caryophyllata, Psidiumguajava* and *Tridaxprocumbens*have also showed some antifungal activity.[23] Alkaloids like the berberine and furoquinoline have been reported to be active against*Trichophyton,Epidermophyton, Micosporum* and *Candida* species isolated from some individuals with dermatophytosi.[24-25] These two alkaloids are noted to be present in *Zanthoxylumzanthoxyloides*one of the plants in the product. The activity of the product can hence be attributed to the presence of these secondary metabolites.

Despite the importance of the basic phytochemical screening in standardisation, the use of multiple methods in this process provides a more reliable guarantee about the quality of products.[26] A confirmation to this statement has been made by some authors who have suggested methods of standardisation that have been classified under the broad categories of physical, botanical, chemical and biological standardisation.[26-27] These suggestions make the results of the thin layer chromatography, antimicrobial assay and the physicochemical properties employed during the stability study also relevant for the process of standardisation.[27] Also, the markers obtained by the thin layer chromatography; shown to be mostly phenolic compounds by their colour, can be considered as analytical markers for the product based on their stability and adequate detection with little interference (Figures 1and 2).[28] The markers from the individual plant can be used for monitoring the quality of plant materials collected and during the manufacturing process as they were shown on the chromatograph to contribute to the markers in the final product.[28]

With the safety of herbal medicinal products considered as relevant as any other procedures in the drug evaluation process, it is important that the product did not induce any change in the haematological, biochemical and urine parameters which by extension means that the product was not likely to have any harmful effect on the human subjects (Tables 6 to 8).[14] The skin sensitivity ruled out any irritations and immune response that may be elicited on the administration of the product a finding that was further confirmed on the histopathological examination of the sampled skin tissue by the absence of immuno-modulatory structures like the mast cells.[29] These results are important despite the product having some history of use in humans because the reliance on data from folkloric knowledge for safety has its own limitation such as the inability to detect toxic effects that develop in long term usage and those that occur as a result of hypersensitivity to the products.[30]

## CONCLUSION

The results from the study showed the product, EAF 2011, to be of good quality (stable) over the study period as well as safe for use under the specified conditions of the experiment.Despite the fact that only one mammalian specie (rats) was used in the study for the safety, the long history of use enables a less stringent assessment to be made on the product. Also, data obtained from the studyprovides a monograph for the product to guarantee quality in subsequent products.

## REFERENCES

1. Ameh SJ, Obodozie OO, Inyang SU, Abubakar SM, Garba M. Current phytotherapy. A perspective on the science and regulation of herbal medicine. J Med Plants Res 2010;4:72-81.
2. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 2001;109:69-75.
3. Ajayi A.F, Akhigbe R.E, Adewumi O.M, Olaleye S.B. Haematological evaluation of *Cryptolepis sanguinolenta* stem ethanolic extract in rats. Int J Med Biomed Res 2012;1:56-61
4. Ige S.F, Akhigbe R.E, Olaleye S.B, Adeyemi J.W. Gastroprotective of the methanolic extract of *Garcinia kola* in rats. Int J Med Biomed Res 2012;1:172-178
5. Thorne S, Paterson B, Russell C, Schultz A. Complementary/alternative medicine in chronic illness as Informed self-care decision making. Int J Nurs Stud 2002;39:671-83.
6. Talalay P, Talalay P. The importance of using scientific principles in the development

of medicinal agents from plants. Acad Med 2001;76:238-47.
7. Ahmad I, Aqil F, Owais M. Turning medicinal plants into drugs. Mod Phytomed 2006;2006:67-72.
8. De Smet PAGM. Health risks of herbal remedies: an update. Clin Pharmacol Ther 2004;76:1-17.
9. Songlin L, Quanbin H, Chunfeng Q, Jingzheng S, Chuen L, Cheng HX. Chemical markers for the quality control of herbal medicines: An overview. Chin Med 2008;3:7-23.
10. Brijesh S, Poonam GD, Tannaz JB. Standardization of medicinal plant preparations. In: Arvind AN, editor. Approaches towards evaluation of medicinal plants prior to clinical trials; 6th November; Pune, India: The Foundation for Medical Research; 2006. p. 102-18.
11. Yi-Zeng L, Peishan X, Kelvin C. Quality control of herbal medicines. J Chromatography B 2004;812:53-70.
12. WHO. General guidelines for methodologies on research and evaluation of traditional medicine: World Health Organisation 2000 Contract No.: WHO/EDM/TRM/2000.1.
13. WHO. WHO guidelines for assessing quality of Herbal Medicines with reference to contaminants and residues. Geneva. Geneva: World Health Organisation 2007.
14. Moshihuzzaman M, Choudary MI. Protocols on safety, efficacy, standardization and documentation of herbal medicine. Pure Appl Chem 2008;80:2195-230.
15. Marriott JF, Wilson KA, Langley CA, Belcher D, editors. Pharmaceutical compounding and dispensing: Royal Pharmaceutical Society of Great Britain 2006.
16. Sofowora A. Medicinal plants and traditional medicine in Africa. 2 ed: Spectrum Books Limited, Ibadan;1993.
17. Evans WC. Trease and Evans Pharmacognosy. 15 ed. Edinburgh,: W.B. Saunders;2002.
18. Nyarko KA, Asiedu-Gyekye JI, Sittie AA. A manual of harmonised procedures for assessing the safety, efficacy and quality of plant medicines in Ghana: Ghana National Drugs Programme;2005.
19. Furniss BS, Hannaford AJ, Smith WGP, Tatchel RA. Textbook of Practical Organic Chemistry. 5ed. Essex: Addison Wesley Longman; 1989.
20. WHO. Stability testing of active pharmaceutical ingredients and pharmaceutical products: World Health Organisation2007 Contract No.: Rev.1.
21. Thakur AK, Prasad NA, Laddha KS. Stability testing of herbal products.: The Pharma Review. KONGPOSH Publications;2008.
22. Shai LJ, McGaw LJ, Aderogba MA, Mdee LK, Eloff JN. Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm. leaves. J Ethnopharmacol 2008;119:238-44.
23. Jindal A, Kumar P. Antimicrobial flavonoids from *Tridax procumbens* Taylor and Francis Online; 2011 [cited 2012 18th June]; Available from: http://www.tandfonline.com/doi/abs/10.1080/14786419.2011.617746.
24. Zhao W, Wolfender J-L, Hostettmann K, Xu R, Qin G. Antifungal Alkaloids and Limonoid derivatives from *Dictamnus Dasycarpus*. Phytochemistry 1998;47:7-11.
25. Volleková A, Kostálová D, Kettmann V, Tóth J. Antifungal activity of *Mahonia aquifolium* extract and its major protoberberine alkaloids. Phytother Res 2003;17:834-7.
26. Choudhary N, Sekhon SB. An overview of advances in the standardization of herbal drugs. J Pharm Educ Res 2011;2:55.
27. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines - A review. IJBC 2012;4:101-112.
28. Duron RR, Almaguer LC, Garza-Juarez ADJ, Salazar-Cavaroz MD, Waksman-De-Torres N. Development and validation of thin-layer chromatographic methods for quality control of medicinal products. Acta Chromatogr 2009;21:203-15.
29. Bala S, Weaver J, Hastings KL. Clinical relevance of preclinical testing for allergic side effects. Toxicology 2000;209:195-200.
30. Remirez DC. Update in pre-clinical regulatory requirements for phytomedicines in Latin America. J Compl Int Med 2006;3:Article 3. Available at http://www.bepress.com/jcim/vol3/iss1/3,2006. Accessed on 06 fev.2008

**doi:** http://dx.doi.org/10.14194/ijmbr.222

**How to cite this article:** Dickson R.A, Mensah, M.L.K,Thomford K.P, Annan K, Mills-Robertson F.C,Appiah AA, Quarshie O, Antwi S, Kaminta S, Edoh D. Quality and safety evaluation of a Ghanaian polyherbal product EAF-2011 for the management of superficial mycoses. Int J Med Biomed Res 2013;2(2):91-102

**Conflict of Interest:** None declared