

Efficacy of leaf extracts of *Vernonia amygdalina* Del. from central Africa on atopic eczema

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Abstract

Vernonia amygdalina Del. is an edible plant which is a member of the Asteraceae family, genus *Vernonia*, growing in the tropical Africa, whose leaf extracts are used as a remedy for malaria, cough, gastrointestinal disorders and hepatitis, whereas both root and leaf extracts are used to treat fever, hiccups, kidney disease, diabetes and helminthiasis in African ethnomedicine. The plant extracts are reported to contain antioxidant flavonoid compounds (Luteolin, Luteolin 7-O-b-glucuroniside, Luteolin 7-O-b-glucoside) and saponins such as Vernoniosides A1, A2, A3, B1, B2, D3 and C. Their main active sesquiterpenes are Vernodalol, Vernodalol, Vernolepin and Vernomygdin and Vernolides. We report on the safety and efficacy of the water and alcoholic extracts of *Vernonia amygdalina* leaf in children with atopic eczema. In this preliminary clinical trial, 25 atopic eczema or atopic dermatitis (AD) school children (age: 5-17 y) who satisfied entry criteria and completed at least 2 weeks of treatment were considered in this study. Each patient was randomly assigned one of the following topical treatments: water (Vamex1) and ethanol extracts (Vamex2) of *V. amygdalina* leaf, dexamethasone or Vaseline (5-7 patients/group). Clinical assessment and the evaluation of disease severity were performed once a week using the Eczema Area Severity Index (EASI) scoring system. The treatment was administered topically twice daily; hematological assays and hepatic function assessments were performed.

Topical application of Vamex1, Vamex2, as well as dexamethasone, significantly reduced the disease severity (EASI score) (vs. Vaseline; $p < 0.05$); though dexamethasone showed a relatively better effect as compared to Vamex1 and Vamex2, the difference did not reach the significance level. In addition, topical Vamex1 and Vamex2 relieved itch within the second week of treatment (vs. Vaseline; $p < 0.05$). Results from the hematological and immunological assays showed a significant reduction in ESR in Vamex1, Vamex2 and dexamethasone-treated patients (vs. Vaseline; $p < 0.05$), whereas serum transaminases (AST, ALT) remained within normal ranges in all groups after a four-week treatment period. In addition, a decrease in total IgE was noted in dexamethasone and Vamex-treated patients, but not significantly. No side effects related to any of the treatments was noted. Results from this preliminary trial show that topical application of *Vernonia amygdalina* leaf extracts might be safer and has a potential to serve as natural alternative remedy for mild to moderate atopic dermatitis.

Key words : *Vernonia amygdalina* Del., atopic eczema, eczema area severity index, immunoglobulin E, transaminase, pruritus

Abbreviations

AD, atopic dermatitis; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BSA: body surface area; EASI: eczema area severity index; ESR: erythrocytes sedimentation rate; Vamex: *Vernonia amygdalina* extracts; WBC: white blood count.

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1. Introduction

Atopic dermatitis (AD) is a chronic, recurrent and relapsing inflammatory skin disease that has substantial impact on the quality of life (Drake *et al.*, 2001; Paller *et al.*, 2002), causing sleep disturbance, irritability (Bender *et al.*, 2003), changes in activity in children (Drake *et al.*, 2001). The hallmark symptom of AD is an intense pruritus (Schachner *et al.*, 2005) and, although it is a chronic condition, it resolves in about 60% of patients before adulthood (Catherine Mack Correa and Nebus, 2012).

AD is very common disease and affects males and females at about the same rate; though AD may occur at any age, it most often

begins in infancy and childhood (Bieber, 2010). It is estimated that 45%, 60% and 85% of children present with clinical symptoms by 6 months, 1 year, and 5 years of age, respectively (Bieber, 2010). We have demonstrated that both aqueous and alcoholic extracts of the plant leaf prevent and improve skin symptoms in a murine model of 2,4,6-trinitrochlorobenzene induced AD-like disease (Ngatu *et al.*, 2012).

Vernonia amygdalina (Figure 1A), a member of the Asteraceae family, genus *Vernonia*, is an African edible plant, used as vegetable or drink; leaf decoction is used as remedy for malaria, gastrointestinal disorders and hepatitis, whereas both root and leaf extracts are used to treat fever, hiccups, kidney disease, diabetes and helminthiasis in western and central African countries (Oboh and Masodje, 2009; Olorunfemi *et al.*, 2012) and as cough reliever in remote villages of the province of Bas-Congo, Democratic Republic of Congo. The plant is also reported to have a good nutritional value with its protein and mineral contents. For nutritional purpose, leaves are cut into small pieces and washed repeatedly until the bitter taste is removed, then boiled and cooked with fish or meat depending on local ethnic eating habits. However, a detailed chemical composition of lipid and sugar compounds of *V. amygdalina* from central Africa region has not been available in the literature.

The plant extracts are reported to contain antioxidant flavonoid compounds (Luteolin, Luteolin 7-O-b-glucoside, Luteolin 7-O-b-glucuronoside) and saponins such as Vernonioides A1, A2, A3, B1, B2, D3 and C. In addition, their main active sesquiterpenes are *Vernodaline*, *Vernodalol*, *Vernolepin* and *Vernomygdin* and *Vernolides* (Figure 1B) (Momoh *et al.*, 2012; Ifeoma and Chukwunonso, 2010).

Recent studies on the ethanolic and aqueous *Vernonia amygdalina* leaf extracts (Vamex) have shown anti-inflammatory (Ngatu *et al.*, 2012), antitumor (Izevbogie *et al.*, 2004) and antioxidant (Erasto *et al.*, 2007) activities. Moreover, the anti-inflammatory and antitumor properties of Vamex have been reported to be related to its ERK/MAPK pathways inhibitory effects, mainly attributed to their flavonoid and terpenoid compounds (Oyugi *et al.*, 2009; Ngatu *et al.*, 2011). We report on the safety and efficacy of *V. amygdalina* leaf extracts in patients with mild to moderate AD. In addition, the determination of lipid and carbohydrate compounds in the extracts is also provided.



Figure 1 A: *Vernonia amygdalina* Del. garden at Kinkole. Kinshasa, DR Congo (Photo Jules Mbala, RDC, 2012)

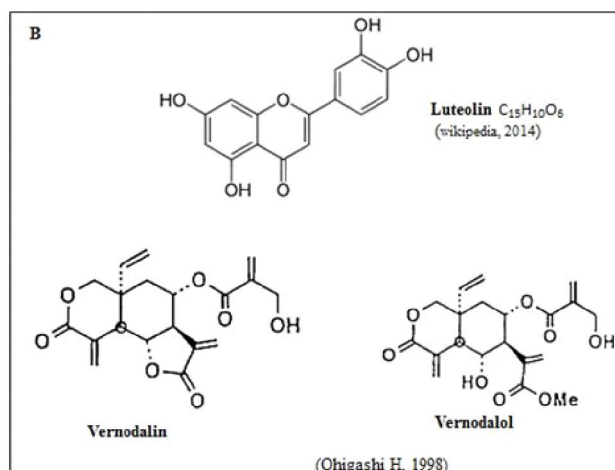


Figure 1B: Chemical structures of some its leaf extracts active compounds

2. Materials and Methods

2.1 Preliminary clinical trial

2.1.1 Study design and participants

This study was a multicenter randomized controlled preliminary trial carried out from November 2010 through March 2011. Patients were school children and staff who were enrolled in a dermatological screening and care conducted in five schools of the western province of Bas-Congo, Democratic Republic of Congo. Of the 2,728 children screened for skin disorders, there were 188 patients diagnosed with either atopic (AD) or allergic contact eczema (ACD). Data on fungal skin disorders have been reported elsewhere (Ngatu *et al.*, 2011). The present report comprises data from 25 out of 28 atopic dermatitis children who satisfied inclusion criteria, completed at least 21 days of treatment and were treated either with Vamex1 (6 patients), Vamex 2 (7 patients), *dexamethasone* (7 patients) or Vaseline (5 patients) as described in the study flow-diagram (Figure 2). Of the three patients who were lost to follow up, two from Vaseline group discontinued the treatment and the other one (from Vamex1 group) did not even show up at school, a week after starting treatment, for administrative reason.

2.1.2 Enrollment of patients and clinical evaluation

Patients aged 5 to 17 years (school children) with a diagnosis of mild to moderate atopic eczema, not being under a concurrent treatment and having 2% of affected body surface area (BSA) or more were considered. The severity of the disease was rated with the use of scores based on the Physician Assessment of Individual Sign (0, clear or absence; 1, mild; 2, moderate; 3, severe) for erythema, scaling/desquamation, skin thickness, induration, papulation and lichenification. The diagnosis of atopic dermatitis was made with the use of "Hanifin and Rajika criteria" (Hanifin and Rajka, 1980) and the treatment was administered topically.

To enter one of the treatment arms, each patient was instructed to randomly draw a card that had a number which allocate the participant to one of the treatment arms. Enrollment cards were prepared and numbered by a statistician who generated a series of

random numbers at the computer laboratory, College of Trade and Business (Institut Supérieur de Commerce, Matadi, Bas-Congo province, Democratic Republic of the Congo). Participants received medical care separately from non-participants at three medical centers, located in three different study sites. A weekly clinical evaluation was performed, using the EASI scoring system and a photo of affected body areas was taken for each patient on days 1, 10, 14, 21 and 28.

A list of treatment codes was handled by a local community health staff; the investigator and patients were blinded to treatments. Patients with two or more skin disease on the same body region, those with severe skin condition or history of hypersensitivity to one of the medications used in the study or under concurrent treatment were not enrolled in this trial and were taken care by hospital staff not involved in the study activities.

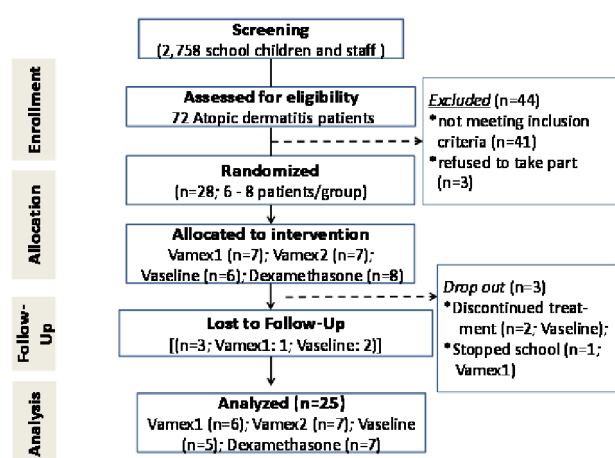


Figure 2: Study flow diagram

Legend: n, number of patients; Vamex1, water extracts of *Vernonia amygdalina* leaf; Vamex2: ethanolic extracts of *Vernonia amygdalina* leaf. The Figure 2 shows that, from a group of 72 eligible atopic eczema patients, 28 were enrolled satisfied inclusion criteria of which 25 completed at least 21 days of treatment and were treated either with Vamex1 (6 patients), Vamex 2 (7 patients), dexamethasone (7 patients) or Vaseline (5 patients).

2.1.3 Evaluation of primary and secondary efficacy outcomes and safety evaluation of Vamex1 and Vamex2

A clinical evaluation was performed on days 10, 14, 21 and 28. The primary efficacy end point was the treatment success rate, defined as the percentage of patients with Eczema Area Severity Index (EASI) score (Schachner *et al.*, 2005) equals zero and the severity of itch designated as “clear” on day 21. EASI is a validated composite score calculated from scores assigned to four of the individual signs of AD for erythema, induration/papulation, excoriation and lichenification combined with % BSA affected in head and neck, upper limbs, trunk and lower limbs. The secondary efficacy end point included the EASI score on day 28 of treatment.

Blood samples were collected on days 1 and 21 of treatment; biological assays consisted of white blood cells (WBC), erythrocytes sedimentation rate (ESR), total serum immunoglobulin E (IgE), serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Lomo Medical, Kinshasa, Congo DR).

The safety of treatment was evaluated by considering major adverse effects and the serum levels of hepatic function parameters, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

2.2 Chemicals and reagents

2.2.1 Preparation of extracts

Extracts of *Vernonia amygdalina* leaf used in the main study were prepared at the department of Environmental Medicine, Kochi Medical School, Japan. *Vernonia amygdalina* leaves were harvested in a garden in Kinshasa, Democratic Republic of the Congo, sundried, crushed; they were then sorted and milled so as to get a coarse powder that was progressively used for the extraction. To prepare the extracts, a sample of powder (2 kg) was always divided into two separate equal samples; 1000 g of each sample they were separately mixed and macerated with hot distilled water (for water extracts or VAM1) and 95% ethanol (for EtOH extracts or VAM2) on an agitating mixer at room temperature (RT) for 24 h, as reported previously (Ngatu *et al.*, 2012). Later on, the mixtures were filtered. After solvent removal by rotary evaporation, 35.4 g (28%) and 30 g (%) of VAM1 and VAM2, respectively, were obtained. Each sample of solid residue was then mixed with distilled water at a concentration of 10% Vamex1 (w/v) and 10% Vamex2 (w/v) for the preliminary trial. The procedure was repeated each time there was a need of samples.

2.2.2 Other treatments

Apart from Vamex solutions, Vaseline (negative control) and 0.2% dexamethasone (positive control; Newpharma, Belgium) were purchased in Kinshasa, Democratic Republic of Congo. Drugs and Vamex solutions were always kept in the refrigerator at 4°C. They were put in similarly colored and codified vials. Patients with good response to the treatment could continue the same treatment at least until day 28; however, in case the patient's condition did not improve at all, another drug was prescribed but only after completing at least three weeks of treatment. Antihistamines were allowed only after day 21 of treatment. In case of occurrence of a major adverse effect, the medication had to be stopped.

2.2.3 Chemical analysis for determination main lipid and carbohydrate compounds

To determine lipid and sugar compounds in the extracts, gas chromatography mass spectrometry (GC-MS) were performed at Kaneko lab (JAIST, Japan) to identify the lipid and carbohydrate compounds in the water (Vamex 1) and alcoholic extracts of extracts of *V. amygdalina* leaf (Vamex2). The GC-MS measurement conditions were as follows: tetramethylsilylated samples of Vamex1 and Vamex2 were analyzed, using a gas chromatography-mass spectrometer (Crarus 500 Gas Chromatograph connected with Crarus 560 S mass spectrometer, Perkin Elmer Co. Ltd) equipped with a 15 m × 0.25 mm i.d. fused-silica capillary column (Elite-1, PerkinElmer); the operational conditions were as follows: GC-MS; the injector temperature was 200°C; and the flow rate of helium gas was 1.5 ml/min; the column temperature program was started at 140°C, increasing to 260°C at 8°C/min; the transfer line and the ion source temperatures were 200°C.

2.2.4 Ethical consideration

The study protocol was approved by the ethics committee of Kochi University Medical School, Japan (Ethics-committee No. 22-59).

On the Congolese side, an official written authorization to implement the study was obtained from the provincial health authority of Bas-Congo, Democratic Republic of Congo, where the study was conducted. Informed consent was obtained from parents for younger kids and from the patient in case of school staff. The research followed the guidelines of the declaration of Helsinki and Tokyo for humans.

2.2.5 Statistical analysis

Data are expressed as percentages of total EASI score and severity of itch, while biological parameters are expressed as means of data from representative patients. Unpaired t and Chi-square tests were used to compare treatment groups for parameters related to the disease severity and values of biological parameters, respectively. All statistical analyses were performed with the use of Stata package version 10 (STATA Inc, Chicago, USA). The significance level of the difference between treatment groups was set at $p < 0.05$.

3. Results

3.1 Topical Vamex reduces itch and the severity of disease in AD patients

Data of patients' self-reported pruritus showed a significant decrease in the severity of this symptom within the second week of treatment in patients treated with Vamex1 ($p=0.045$), Vamex2 ($p=0.033$) and Dexamethasone ($p=0.047$), as compared to Vaseline group, whereas no significant difference was found between Vamex1 and Vamex2 ($p=0.446$), Dexamethasone and Vamex1 ($p=0.443$), also between Dexamethasone and Vamex2 ($p=0.392$) (Figure 3). Furthermore, a marked reduction of the disease severity (total EASI score) was noted in Dexamethasone ($p=0.034$), Vamex2 ($p=0.047$) and Vamex1 ($p=0.035$)-treated patients' groups when compared with the Vaseline-treated group. Though a relatively better effect of Dexamethasone was noted as compared to Vamex1 ($p=0.077$) and Vamex2 ($p=0.0934$), the difference did not reach the significance level (Figure 4).

The proportion of patients clinically cured (EASI score = 0) after 3 weeks of treatment was 71% (5/7) for dexamethasone, 69% for Vamex1 (4/6), 71% for Vamex2 (5/7) and 0% (0/5) for Vaseline group, whereas the proportion of those with total relief of itch in treatment groups was 100% (7/7), 100% (6/6), 100% (7/7) and 20% (1/5), respectively.

Trend in total severity of itch (n= 5 - 7 per group)

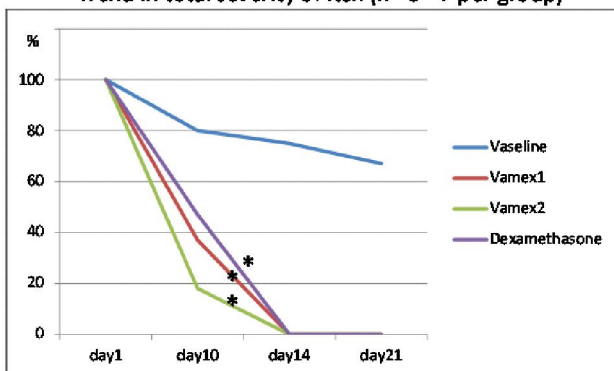


Figure 3: Trend of total severity score (%) for itch in each treatment group

Legend: n, number of patients; Vamex1, water extracts of *Vernonia amygdalina* leaf; Vamex2: ethanolic extracts of *Vernonia amygdalina* leaf. The Figure 3 shows a significant reduction of severity of itch in patients treated with Vamex2, Vamex1 and dexamethasone (vs. Vaseline; $p < 0.05$). No statistically significant difference was noted when comparing Vamex1, Vamex2 and Dexamethasone groups ($p > 0.05$).

Total EASI score (n= 5 - 7 per group)

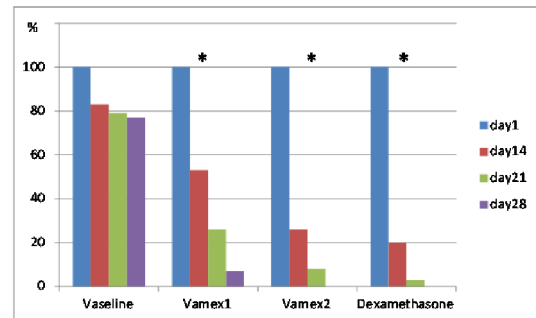


Figure 4: Distribution of the eczema area severity index (EASI) score according to treatment groups

Legend: n, number of patients; Vamex1, water extracts of *Vernonia amygdalina* leaf; Vamex2: ethanolic extracts of *Vernonia amygdalina* leaf. The Figure 4 shows a significant decrease of the severity (EASI) score in Dexamethasone, Vamex1 and Vamex2 groups after 4 weeks of treatment (vs. Vaseline group; $p < 0.05$). Though Dexamethasone group had lower EASI score than Vamex1 and Vamex2 groups, the difference was not reach statistically significant ($p > 0.05$).

3.2 Hematological, immunological parameters (ESR, IgE, transaminases, eosinophils) and adverse effects

Results from the hematological and immunological assays showed a significant reduction of ESR at day28 of treatment in Dexamethasone, Vamex1 and Vamex2-treated representative patients (vs. Vaseline; $p < 0.05$) (Figure 5). The mean serum levels of ALT and AST remained within the normal ranges all representative of different treatment groups ($p > 0.05$) (not shown), whereas a relatively reduced serum total IgE was found in Dexamethasone, Vamex1, Vamex2 groups (vs. Vaseline group; $p > 0.05$) (Figure 6) and an elevated proportion of eosinophils in Vaseline patients but not significantly ($p > 0.05$) (not shown). In total, two patients had complaints consisting of worsening of itch in the Vaseline group (including one drop out case), whereas no adverse effect was noted in dexamethasone and Vamex-treated groups.

Erythrocytes sedimentation rate (ESR) (mean +/-SD)

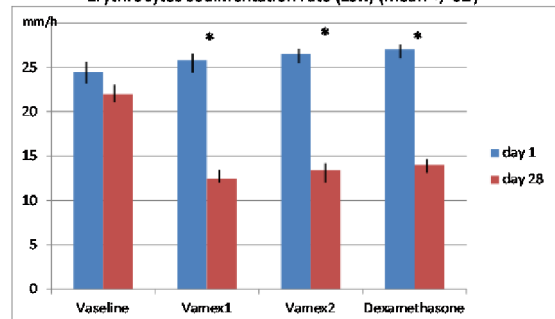


Figure 5: Distribution of erythrocytes sedimentation rate (ESR) according to treatment groups

Legend: ESR, erythrocytes sedimentation rate; SD, standard deviation; n, number of patients; Vamex1, water extracts of *Vernonia amygdalina* leaf; Vamex2: ethanolic extracts of *Vernonia amygdalina* leaf. The Figure 5 shows a markedly lower ESR in dexamethasone, Vamex1 and Vamex2-treated patients (vs. Vaseline group; $p < 0.05$). No statistical difference was observed between Dexamethasone and vamex1 groups, and also between dexamethasone and Vamex2 groups ($p > 0.05$).

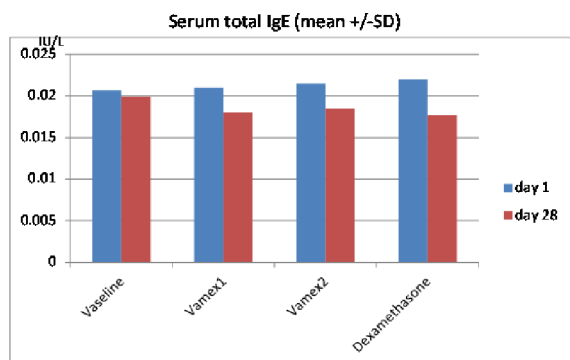


Figure 6: Distribution of total serum IgE levels according to treatment groups

Legend: IgE, immunoglobulin E; SD, standard deviation; n, number of patients; Vamex1, water extracts of *Vernonia amygdalina* leaf; Vamex2: ethanolic extracts of *Vernonia amygdalina* leaf. The Figure 6 shows relatively lower total IgE levels in dexamethasone, Vamex 1 and Vamex2-treated patients as compared with Vaseline group; but not significantly.

3.3 Lipid and carbohydrate compounds in *V. amygdalina* leaf extracts

GC-MS analysis of Vamex1 (water extracts) and Vamex2 (alcoholic extracts) revealed the lipid content of the extracts with a predominance of inositols (87%) followed by threitol (12.2%) in Vamex 1, and aliphatic acids (including octadecanoic acid, 36.1%, and hexadecanoic acid, 25.1%) in Vamex2 (Supplementary Figure1 and Table1). Other lipids and carbohydrates found in Vamex 2 were Inositol (12%), threitol (10.8%) and ribitol (7.9%) (Table1 and Figure.7).

The Figure shows two peaks with higher intensity that correspond to octadecanoic and hexadecanoic acids for the ethanolic extracts, while mainly peaks corresponding to inositols were found in the water extracts.

4. Discussion

The present preliminary trial, which followed an experimental study on a murine model of hapten-induced AD-like disease (Ngatu *et al.*, 2012), consisted mainly on evaluating the antiallergic efficacy and safety of topical application of aqueous and ethanolic extracts of *Vernonia amygdalina* leaf, an African edible plant used in ethnomedicine, in AD patients. We have previously reported the first case of a chronic and recalcitrant eczematous disorder in an African boy that was successfully treated with the ethanolic extracts of the plant leaf (Ngatu *et al.*, 2012).

The present preliminary trial showed that extracts from *Vernonia amygdalina* leaf were relatively as effective as dexamethasone in alleviating AD skin symptoms. Regarding the anti-itch activity, treatment with Vamex relieved pruritus in almost all patients within the second week of treatment; however, no statistically significant difference was noted when compared to dexamethasone. This finding confirms the anti-itch effect of *Vernonia amygdalina* leaf extracts we have previously reported (Ngatu *et al.*, 2012).

Those clinical outcomes from topical Vamex may be due, at least, to the decrease of serum immunoglobulin E (IgE) observed in Vamex-treated patients, which is considered as a major allergic inflammation marker that contribute to the induction of itch and aggravation of AD in the majority of patients. Previous works have suggested that the severity of AD correlates with the serum levels of IgE. The high affinity receptor for IgE (FcεRI) expressed on mast cells binds IgE, leading to mast cell activation and secretion of inflammatory mediators, including histamine; and histamine is known to induce itch and scratching which worsens skin lesions (Liu *et al.*, 2011; Metzger, 1992; Kawakami *et al.*, 2009).

In this study, treatment with Vamex1 and Vamex2 did not induce adverse effects in patients, as none of them has reported any discomfort related to the treatment. It is important to mention that the plant is often used in the country as an ethnomedical remedy for other illnesses by some inhabitants. This is the first trial that shows the antiallergic effects of *Vernonia amygdalina* extracts in a sample of human subjects. There are only two previously reported human studies, one related to antimalarial effects of the plant and, the antidiabetic activity for the other (Challand and Willcox, 2009; Okolie *et al.*, 2008).

This study has a limitation. Though extracts from *Vernonia amygdalina* leaf showed promising therapeutic benefits, the small sample size (when considering patients who fulfilled inclusion criteria and completed the treatment) might not definitely confirm their effectiveness. Another clinical trial with a greater number of patients should be envisaged.

5. Conclusion

This preliminary trial showed that topical application of polyphenol and terpenoid-rich *Vernonia amygdalina* leaf extracts might be safer in central Africans and has a potential to serve as alternative remedy against mild to moderate AD.

Acknowledgment

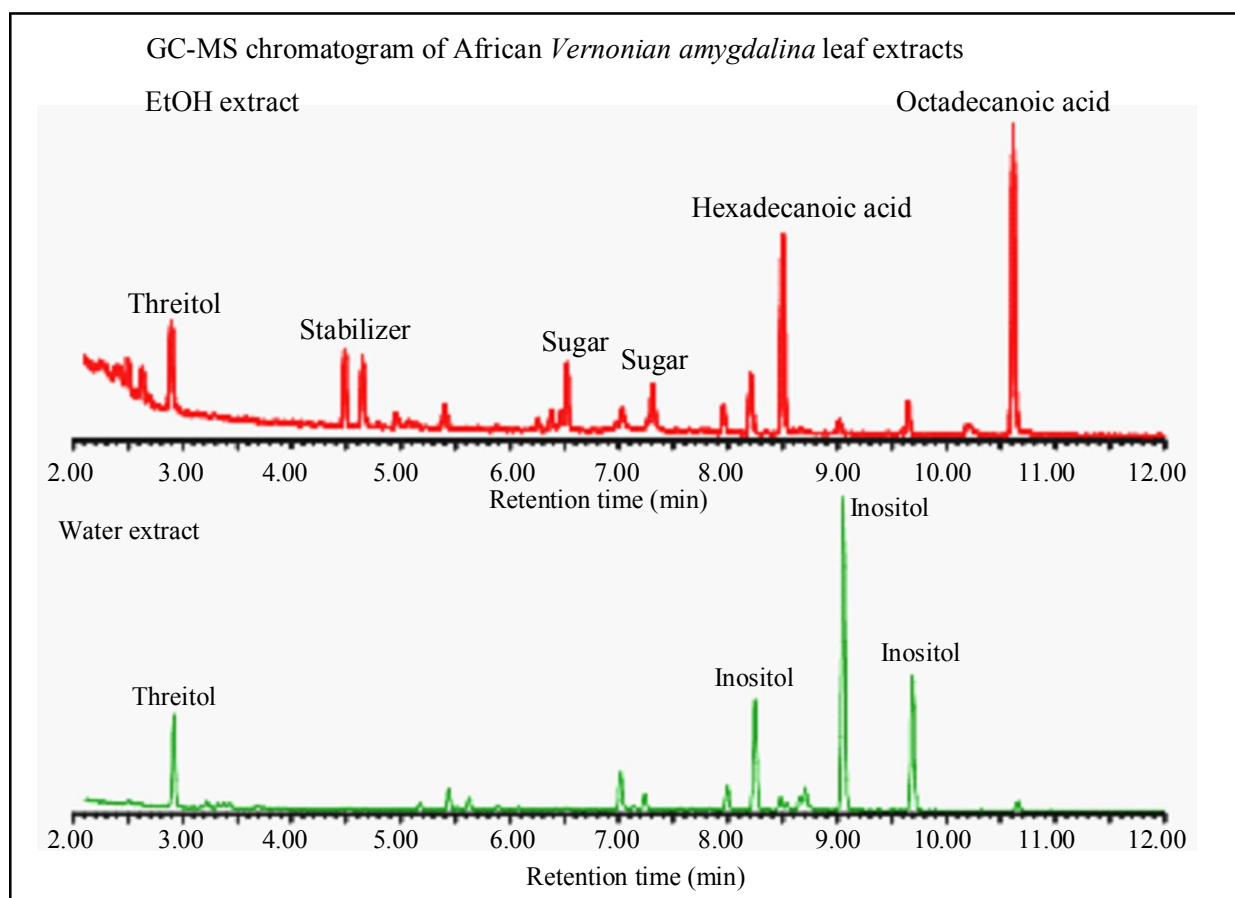
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Conflict of interest

The authors actually declare no conflict of interest related to this study and would, at least, mention that the product investigated in this study (Vamex) was subject of a patent of invention in 2012.

Table 1: Lipid and carbohydrate compounds in African *Vernonia amygdalina* leaf extracts

EtOH extract			Water extract		
Components	Intensity	Composition %	Components	Intensity	Composition %
Threitol	1399928	10.8334124	Threitol	6648015	1229722139
Plastic stabilizer	1063881	8232895987	Inositol	9607736	1777199009
Ribitol	1027199	7949030507	Inositol	26182582	4843145022
Inositol	986383	7633173862	Inositol	11622782	214993383
Hexadecanoic acid	3250241	2515215149			
Inositol	528816	4092268895			
Octadecanoic acid	4665870	3610706686			
Total	12922318	100	total	54061115	100

**Figure 7:** GC-MS chromatogram of African *Vernonia amygdalina* leaf extracts

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