

## Ethnobotanical survey and comparative active ingredients in the leaves of two anti-measle botanicals in Osun state, Nigeria

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### Abstract

Medicinal plants have been used in treatment of human ailments and diseases before the advent of science. Paucity of knowledge of the active ingredients is a major setback in the practice of herbal medicine. This work compared the active ingredients in the leaves of two common anti-measle botanicals (*Calotropis procera*, *Vernonia amygdalina*) in Osun State, Nigeria. The ethnobotanical survey was carried out through visits to medicinal plant users such as native doctors, farmers, herbalists and the medicinal plant dealers within the State. 10g of sample of each medicinal plant measured into the amber bottle with the addition of 20ml methanol was subjected to High Performance Liquid Chromatography (HPLC); a method used to separate mixture of complex samples. Results of the phytochemical analysis revealed *Calotropis procera* having Tyranton as the highest phytochemical extract which possesses cytotoxic effects towards human viral infections. *Calotropis procera* and *Vernonia amygdalina* extracts are very useful in the utilization of anti-measle infection. These can be recommended to the medicinal plant users as being effective against the aforementioned ailment.

**Keywords:** *Calotropis procera*; HPLC; *Vernonia amygdalina*; Tyranton; Herbal medicine.

### 1. Introduction

Medicinal plants are widely used for pre and postnatal care in many parts of the world [1]; thus, various studies [2], [3], [4], [5], have documented many medicinal being used to treat obstetric and gynecological conditions including birth control, complications during pregnancy and child birth and problems associated with infertility. Indigenous people worldwide have used oral traditions and empirical means to compile detailed knowledge regarding the use of medicinal plants, and this information is disseminated from generation to generation [6].

Based on inherited knowledge and long-term usage for the treatment of various ailments over the centuries, medicinal plants are considered natural and therefore safer than conventional synthetic pharmaceuticals. There is, however, scarce scientific evidence supporting this belief [7]. Recent scientific evidence has revealed that many plants considered to be medicinal are potentially toxic, mutagenic and carcinogenic [8]. Poisoning by medicinal plants may be attributed to misidentification, incorrect preparation or inappropriate administration and dosage. Information from health centers and emergency rooms has reported many dangerous and lethal effects from the use of herbal products [9].

Many medicinal plants are also useful as raw materials for some industries, such as cosmetics, textile, biomass, food and confectionaries; thus, the pressure on the diversity of medicinal plants is extremely high. The overexploitation of medicinal plants is relatively higher in developing countries, where the majority of the population depend on medicinal

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plants for their primary healthcare services. Documenting the medicinal plants used in traditional maternal healthcare will therefore go a long way in providing baseline data that may aid the devising of conservation strategies, and the information gathered would be an invaluable source for pharmacological studies aimed at isolating additional compounds that could be useful for providing new drug leads. As a result, this study attempts to document the medicinal plants being used for traditional treatment of measles as well as highlighting their major phytochemical constituents, in Osun State, Nigeria.

Measles is a deadly viral disease that impacts vulnerable children around the world, many of who do not have access to healthcare. Measles is a viral illness acute in nature caused by a virus belonging to the family of paramyxovirus, genus Morbillivirus. The World Health Organization estimated that more than 20 million individuals are affected each year by the measles worldwide, out of which children are about 17 million [10]. Tragically it has been reported that more than 600 children die daily as a result of measles infection. This leaves measles as a leading cause of death among young children, especially in the developing countries of the world [11]. In 2006 alone an estimated 242,000 died from the disease, often from secondary complications related to pneumonia, diarrhea and encephalitis [12]. The measles disease begins with a fever that lasts for a couple of days, followed by a cough, runny nose, and conjunctivitis (pink eyes).

## 2. Materials and Methods

### 2.1. Ethnobotanical Survey

The potential medicinal plants were sourced for in different local government within Osun State by visiting the herbalists, farmers and other medicinal plant users and dealers. A number of medicinal plants collected were compared and contrasted; and four major ones were implicated to be responsible for the treatment of measles by all. Namely *Calotropis procera* and *Vernonia amygdalina*.

### 2.2. Methods of Extraction

10g of sample was measured into the amber bottle after which 20ml of methanol was added. Both were shaken vigorously for 30minutes the aqueous end was run off after shaking while the organic solvent end was collected into 25ml standard flask, made up to the moric and ready for the analysis.

### 2.3. Sample Preparation

The purified prescription was dissolved in 50% methanol/h<sub>2</sub>o and filtered through 0.22m nylon membrane microfilters (shimadzu- (Neramix). The chromatography analysis was achieved using a pumped pressure 15mpa High performance liquid chromatography with a UV detector (Diode array detector (DAD)). Reversed-phase separation was performed on HPLC column, Ubondapak C18, length 100mm and ID 4.6mm. Mobile phase comprise of methanol and water. The sample was injected (10l injection volume) onto the column and eluted at a fluid rate 0.08ml/mm (N) and 1ml/mm (A). The ultraviolet detection was set to 254nm.

### 2.4. Methodology of HPLC Analysis

High performance liquid chromatography (HPLC) is a method used to separate mixture of complex samples. HPLC is an active process in which the materials are pumped at high pressure through a separation column, which contain a stationary phase usually a chemical functional bead that separate the compound mixtures. Samples are introduced through the injector and carried via the noble phase across the stationary phase to affect the separation. After separation through the column, the samples are expressed for detector system that identify and qualify the individual compounds.

### 2.5. Preliminary Analysis

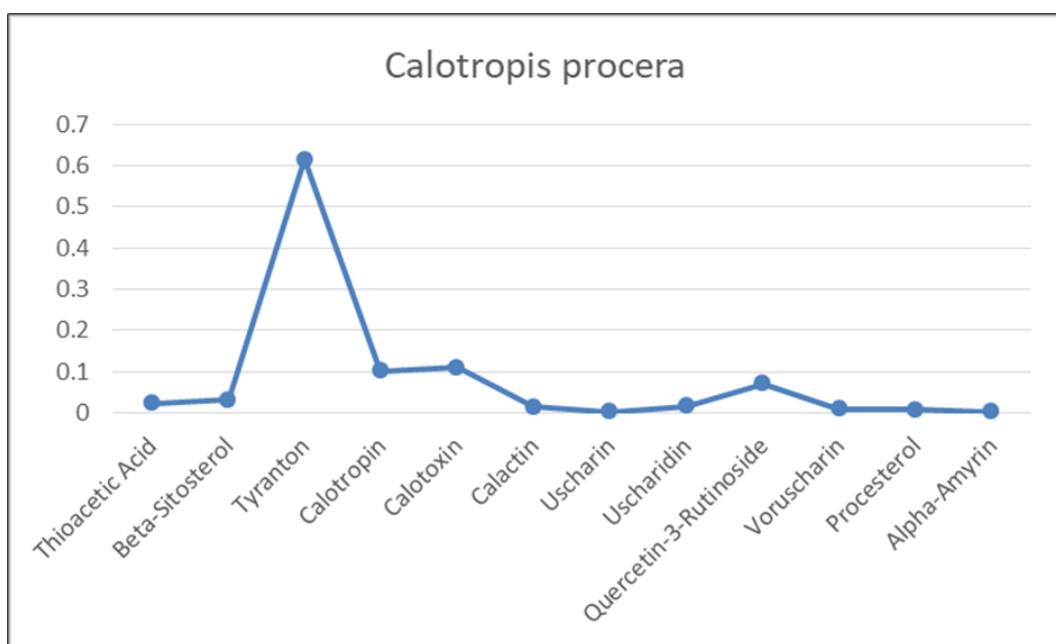
Standard form of analytes profile was injected into the HPLC and the grounded chromatograph with a given peak area and peak profile. These were used to create a window in the HPLC in preparation of the test samples analysis. Then, liquor of the extracted test sample was injected into the HPLC also, to obtain a corresponding peak area and peak problem in chromatogram. Then, the peak area of the sample is compared with that of the standard, relative to the concentration of the standard to obtain the concentration of the sample.

### 3. Results

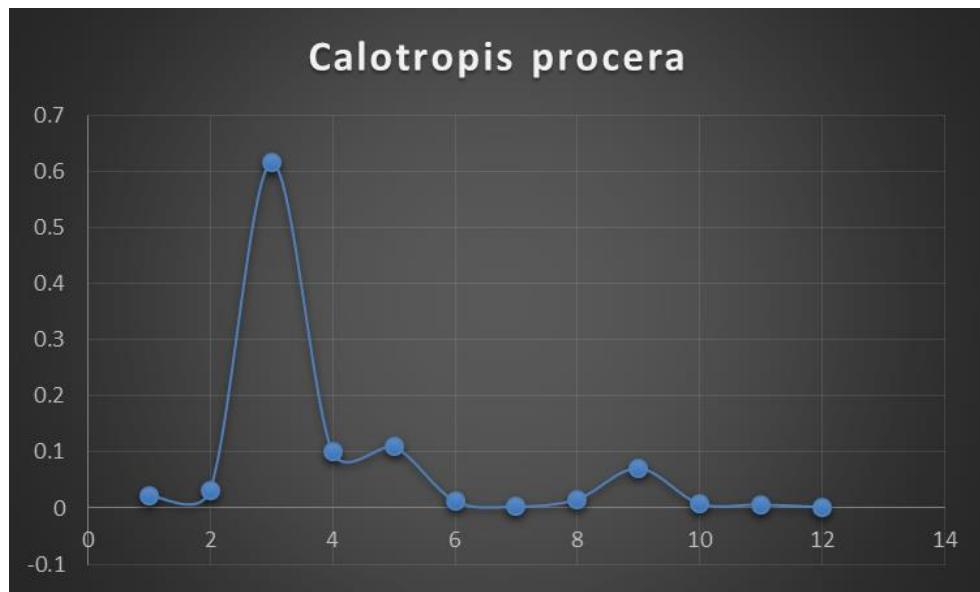
Table 1 presents the phytochemical analysis of *Calotropis procera*. In this table, phytochemical analysis of *Calotropis procera* shows that Tyranton has the highest phytochemical extract, which is flammable and incompatible with strong oxidizing agents, amines, ammonia, strong acids, strong bases, alkalines, aluminium (13).

**Table 1** Phytochemical Analysis of *Calotropis procera*

<i>Calotropis procera</i>	Areas	Concentration mol/litre	Percentage Composition
Thioacetic Acid	337.0240	0.02260	2.26
Beta-Sitosterol	471.1030	0.03159	3.15
Tyranton	9170.3010	0.61508	61.50
Calotropin	1503.0130	0.10081	10.08
Calotoxin	1636.5080	0.10976	10.97
Calactin	198.1040	0.01328	1.32
Uscharin	42.8000	0.00287	0.28
Uscharidin	239.5975	0.01607	1.60
Quercetin-3-Rutinoside	1061.3910	0.07119	7.11
Voruscharin	120.8390	0.00810	0.81
Procesterol	98.2620	0.00659	0.65
Alpha-Amyrin	30.0280	0.00201	0.20
	14908.9705	0.9999	89.85%



**Figure 1a** Line chart of phytochemical Analysis of *Calotropis procera* (conc mol/litre on Y axis, Active Substance on X axis)

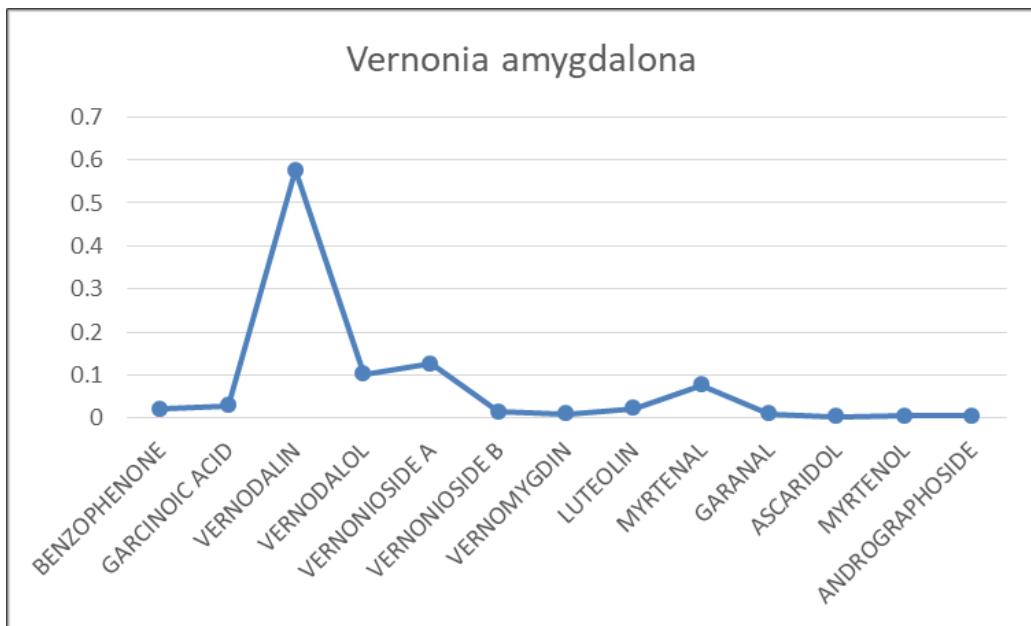


**Figure 1b** Line chart of phytochemical Analysis of *Calotropis procera* (conc mol/litre axis, Active Substance on X axis)

**Table 2** Phytochemical Analysis of *Vernonia amygdalina*

<i>Vernonia amygdalina</i>	Areas	Concentration mol/litre	Percentage Composition
Benzophenone	343.8435	0.02123	2.12
Garcinoic Acid	480.9895	0.02971	2.97
Vernodalin	9318.2950	0.57557	57.55
Vernodalol	1644.2785	0.10156	10.15
Vernonioside A	2044.0075	0.12625	12.65
Vernonioside B	241.1560	0.01489	1.48
Vernomygdin	146.9230	0.00907	0.90
Luteolin	357.3940	0.02207	2.20
Myrtenal	1238.5030	0.07650	7.65
Garanal	150.3320	0.00928	0.92
Ascaridol	65.1735	0.00402	0.40
Myrtenol	76.2240	0.00470	0.47
Andrographoside	82.3300	0.00508	0.50
	16189.4495	0.9999	99.96%

Table 2 presents the phytochemical Analysis of *Vernonia amygdalina* in the table phytochemical analysis shows that Vernodalin, Vernodalol and Vernonioside have highest phytochemical constituent in the plant which suggest the extracts of the plant possess cytotoxic effects towards human carcinoma cells of the nasopharyn [14].



**Figure 2a** Line chart of phytochemical Analysis of *Vernonia amygdalina* (conc mol/litre axis, Active Substance on X axis)



**Figure 2b** Line chart of phytochemical Analysis of *Vernonia amygdalina* (conc mol/litre)

#### 4. Discussion

*Calotropis procera* is a multipurpose plant, which can be utilized for medicine, fodder, and fuel purposes, timber and fiber production, phytoremediation, and synthesis of nanoparticles. It has been widely used in traditional medicinal systems across North Africa, Middle East Asia, and South-East Asia. At present, it is being extensively explored for its potential pharmacological applications [15]. In this study the phytochemical analysis of *Calotropis procera* shows that Tyranton has the highest phytochemical extract, which is flammable and Incompatible with strong oxidizing agents, amines, ammonia, strong acids, strong bases, alkalies, aluminium [16].

*Vernonia amygdalina* is a medicinal phytochemical that inhibit, reverse or retard diseases caused by oxidative and inflammatory processes [17]

## 5. Conclusion and Recommendation

In conclusion, phytochemical analysis through HPLC revealed that tyranton, vernodalin, methyl-gallate and chlorogenic acid were extracts with the highest concentrations in *Calotropis procera* and *Vernonia amygdalina*, respectively. This suggests their inclusion in the herbal and orthodox preparations of anti-measles medicines

There is still the need for researchers to explore this vital information through scientific validation to the claim of the indigenous people. Also screening, isolation and characterization of active constituents of the plants would give leads in the production of novel drug and there is a need for validation, standardization of phytomedicines and traditional medical practices so that this sector can be accorded to rightful place in the health care system. Government should provide a global forum for growers, traders, manufacturer of herbal medicine and professionals in the field of traditional and alternative therapies to share knowledge, experiences and ideas. Scientists, therefore should rise to get the knowledge from the elders and encourage the herb sellers to register with the government agency and make them available so that herbal medicine could easily be accessible and cheap for the less privileged. Further research should be carried out to confirm the efficacy of some of the most commonly used anti-viral herbs against bacterial infections.

## Compliance with ethical standards

### Disclosure of conflict of interest

No conflict of interest to disclosed.

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