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# Evaluation of Phytochemical and *In Vitro* Anti-Dermatophyte Activity of *Vernonia amygdalina* (Bitter Leaf) Locally Used in the Treatment of Ringworm Infection

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## ABSTRACT

Dermatophytoses is one of the most frequent human skin diseases of medical importance. Antimicrobial efficacy of *Vernonia amygdalina* leaves extracts on some human dermatophytes was studied among fifty (50) selected Almajiri school children with signs of ringworm, aged between 5 to 10 years and above in Bauchi metropolis. Aqueous and ethanolic extracts of the leaves were screened for alkaloids, anthraquinone, cardenolide, flavonoids, phenols, phlobatannins, saponins, steroids, tannins and terpenoids. Antifungal activity of the extracts was tested by agar well diffusion method and Minimum Inhibitory Concentration (MIC) determined. The leave extracts revealed the presence of all the phytochemicals with the exception of phenol. The disease is more common (56.5%) in children within 6 to 10 years. All the affected children had two or more spots on their scalp, indicating presence of *Tinea capitis* (scalp ringworm). Microsporum species was the most frequent (47.8%) dermatophyte isolated, followed by Trichophyton species (23.9%). The zones of inhibition exhibited by the extracts against the fungal isolates was found within the range of 10.20 to 22.50 mm and varies with the concentration of the extract. Highest MIC value of 65.10mg/ml was found against Epidermophyton and the least 57.50 mg/ml was obtained on Microsporum. These results revealed that the extracts had significant antimicrobial efficacy against the fungal isolates tested and can be a cheap source of bioactive materials for the production of anti-dermatophyte drugs.

**Keywords:** Dermatophytes, Ringworm, *Vernonia amygdalina*, Epidermophyton, Microsporum, Trichophyton

## INTRODUCTION

Dermatophytoses is a fungal infection widely distributed all over the world with various degrees. Many species of these fungi have been isolated from animals, but a few zoophilic are responsible for the majority of the cases [1]. The pathological importance of dermatophytes is associated with contagiousness among the subjects, high cost of treatment, difficulty of control and the public health outcomes [2].

Dermatophytes are the commonest fungal agents causing skin diseases. Ringworm is caused by mould fungi of the genera Microsporum, Trichophyton and Epidermophyton. The location involves are usually the surface of the body (*Tinea corporis*), the scalp (*Tinea capitis*), the foot and the nails (*Tinea unguium* or onychomycoses). The fungus settles on the skin, germinates and forms a mass of branching hyphae which grows out radially to produce circular lesion [3]. Scalp ringworm (*Tinea capitis*) is a superficial fungal infection of the scalp. It is most common in children 4-12 years of age, especially those of black decent [4] and involves red Itchy patches on the scalp leaving bald spots. It can be persistent and contagious, almost to the point of epidemic; however, it

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often disappears spontaneously.

Ringworm can pass from one person to another by direct skin to skin contact or by contact with contaminated items such as clothes, combs and bathrooms or pool surfaces. Ringworm can also be acquired from pets that carry the fungus. The first sign of ringworm of the scalp may be dandruff-like flakes appearing on the hair, round or bald patches. The skin may feel itchy, showing red and peeling. The rash may gradually spread all over a large area if prolonged or untreated. Once the hair is infected, it becomes brittle and breaks off near the root leading to bald spots. Dermatologic lesion is similar and suspected infection in human should be confirmed by culture to identify the source of dermatophyte infection [5]. Ringworm is very common among the less privileged school-age children especially the Almajiris due to overcrowding, lack of washing clothes, bathing, adequate and beddings. The Almajiri/Tsangaya system of education is a traditional qur'anic study where parent give out their sons usually at the age of five to seven and above years to an individual qur'anic scholar for learning the recitation and memorization of the holy Qur'an.

*Vernonia amygdalina* belongs to the plant family compositae. It is a small shrub that grows typically grows to a height of 2 to 5 m in tropical Africa. The leaves are petiolate, elliptical and up to 20 cm long and about 6 mm in diameter with rough bark. *Vernonia amygdalina* is commonly called bitter leaf in English because of its bitter taste. In Nigeria, the Hausa calls it *Shuwaka*, Igbo, *Onugbo* and Yoruba *Ewuro* [6]. The leaves are green with a characteristic odour and bitter taste. It does not produce seeds and has to be distributed or propagated through stem cutting [4]. It grows under a range of ecological zones in Africa with about 200 species and produces a lager mass of forage and it is drought tolerant. It is mainly used for human consumption and has to be washed to remove the bitter taste. Its bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides. It stimulates the digestive system as well as reduces fever [7]. Vernonia was named after a 17$^{th}$ Century English botanist and plant collector in North American, Vernon [8].

The plant curative and therapeutic properties raised over several others vegetables or culinary leaves people make use of in Nigeria [6]. Its goodness for a healthy body devoid of several diseases like diabetes and high blood cholesterol problem has also been substantiated by several research studies both within Nigeria and other African countries where the plant can be found [9]. The plant roots have been used for gingivitis and toothache due to its proven antimicrobial activity [10]. Many herbalists prescribe aqueous extracts for their patients as treatment for anaemia, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal track problems. *V. amygdalina* extracts have also been reported to help suppress, delay, or kill cancerous cells [11]. However, extract of bitter leaf had been reported to exert antibiotic action against drug resistant microorganisms and possess antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities [8]. The leaves and bark in local medicine are used as purgative, against menstrual pain and wound dressing [12].

Ringworm was observed to be common especially among less privileged children; suffering from poor parenting associated with personal hygiene and effective health care delivery. In essence, this work will create awareness on the usefulness and medicinal value of *V. amygdalina* as an alternative and affordable to synthetic therapeutic drug by testing it efficacy on ringworm cases among children. Most of the previous studies on the antimicrobial efficacy of *V. amygdalina* extracts [10,13,14] focused on bacterial infections. Hence the need for this study on dermatophytes. In Nigeria, the research for new and alternative drugs is on course, so the present study was designed to evaluate the phytochemical and *in vitro* anti-dermatophyte activity of Vernonia leaf extracts on fungal isolates from community cases of ringworm infections.

## MATERIALS AND METHODS

### Collection and preparation of Vernonia leaf samples

Fresh leaf samples of *V. amygdalina* were obtained from Fadama garden behind state secretariat, Bauchi. The plant was identified by a Botanist in the Department of Biological sciences, Abubakar Tafawa Balewa University (ATBU), Bauchi, Nigeria. The leaves were aseptically washed, air-dried and grinded into fine powder with pestle and mortar, and then finally stored in polythene bags until used for ethanolic and aqueous extractions.

### Preparation of cold ethanolic and aqueous extracts

20 g of the grounded powder of the leaves material was introduced into a conical flask and 200 ml of absolute ethanol and distilled water was then added, respectively. The extraction was carried out at room temperature for 24 h for the aqueous extract and 72 h for the ethanolic extract. The extract was decanted and filtered with a Whatman No. 1 filter paper (110 mm). The filtrate obtained was evaporated to dryness at 45°C, and the obtained residue was discarded as described by Newton et al. [15]. The extract stock solution was filter-sterilized, then stored in sterile capped tubes in refrigerator at 4°C before use.

### Phytochemical screening of the Vernonia leaves extracts

Phytochemical screening was done in order to detect the presence of following bioactive compounds: alkaloids, anthraquinone, cardenolide, flavonoids, phenols, phlobatannins, saponins, steroids, tannins and terpenoids

using the methods described by Wazis et al. [16] and Sofowora [17].

**Alkaloids:** A 3 mm of the ethanolic and aqueous extracts was stirred with 5 ml of 1% HCL on a steam bath for twenty minutes. The solution obtained was cooled and filtered and few drops of Mayer's reagent/picric acid were added to the filtrate. A cream precipitate indicated the presence of alkaloid.

**Anthraquinone:** A 0.5 g of the plant extract was mixed with 10 ml of aqueous $H_2SO4$ and then filtered while hot, the filtrate was shaked with 5 ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution was then added. The presence of violet or red coloration in the ammonical (lower) phase was taken as positive combined anthraquinone.

**Cardenolide:** A 0.5 g of the plant extract was dissolved in 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was then underlayed with 1ml of concentrated tetraoxosulphate (VI) acid. Appearance of a brown at the interphase showed the presence of digitoxose sugar characteristic of cardenolide.

**Flavonoids:** A volume of 3 mm of the ethanolic and aqueous extract was added to a volume of 1 ml of 10% sodium hydroxide. A yellow coloration indicated the presence of flavonoids.

**Glycosides:** A 2 ml of chloroform was added to a volume of 3 ml of the ethanolic and aqueous extract. Dilute sulphuric acid was carefully added to form a lower layer. A reddish brown colour at interface indicated the presence of a steroidal ring.

**Phenolics:** Two drops of 5% ferric chloride were added to 5 ml of the ethanolic and aqueous extracts in a test tube. A greenish precipitate was observed as positive for phenolics.

**Phlobatannins:** A 1% hydrochloric acid was added to a volume of 1 ml of the ethanolic and aqueous extracts. A red precipitate was regarded as the presence of phlobatannins.

**Saponins:** 2 ml of the aqueous and ethanolic extracts in a test tube was shaken for 2 min. Frothing which persisted on shaking was taken as evidence for the presence of saponins.

**Steroids:** To a volume of 1 ml of the extracts, five drops of concentrated tetra-oxoosulphate VI acid ($H_2SO_4$) was added. Red coloration indicated the presence of steroids.

**Tannins:** A volume of 1 ml of freshly prepared 10% potassium hydroxide was added to a volume of 1 ml of the ethanolic extracts and aqueous extracts. The presence of a dirty white precipitate was considered as indication of tannins.

**Terpenoids:** A 10 ml of extracts was mixed with 2 ml Chloroform and 3 ml of concentrated $H_2SO_4$ was carefully added to form a layer. A reddish brown coloration of the interface formed indicating the presence of terpenoids.

**Sample collection**

The specimens were collected from different parts of the body or scalp of 50 randomly selected school-age children in Almajiri houses (Tsangaya) within Yelwa and Gwallameji area of Bauchi metropolis. A new surgical blade was used for each individual child. The specimens were collected by gently scraping affected spots into clean sheets of paper which were then transferred into sterile containers that had been properly labelled with respect to each individual's data; these were brought to the laboratory for inoculation. Informed consent of the child and their teachers was obtained before the sample collection. The participation was open and voluntary.

**Microscopic identification**

A drop of potassium hydroxide solution was placed on a clean sterile glass slide and small clean pieces of the specimen was transferred to the drop of potassium hydroxide and covered with a cover slip. The preparation was then examined using 10x and 40x objectives with the condenser iris diaphragm closed sufficiently to give good contrast for the presence of branching hyphae and rounded anthrospores.

**Culture methods**

The specimen was inoculated onto Sabouraud dextrose agar (SDA) media (Oxoid, UK) and incubated at room temperature for four days, after which it was sub-cultured. The isolates were finally stored on SDA slants in the refrigerator at 4°C prior to use, as described by Chander [18].

**Screening for antifungal activity of the extracts**

The ethanolic extract of the Vernonia leaves was applied on the fungal isolates Epidermophyton, Trichophyton and Microsporum species using agar diffusion as described by Newton et al. [15]. The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) (Oxoid, UK) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. 100 µl of the standardized fungal spore suspension was evenly spread on SDA media. Wells were then bored into the agar media using a sterile 6 mm cork borer and then carefully filled with the extracts. The plates were allowed to stand on the laboratory bench for 1 hour to allow for proper diffusion of the extract into the media. Dimethyl Sulfoxide (DMSO) was used as a negative control and Griseofulvin was used as a positive control. The plates

were incubated at 25℃ for 96 h and later examined for zones of inhibition.

**Determination of minimum inhibitory concentration (MIC)**

The MIC of the aqueous extract of Vernonia leaves was estimated using the methods of Rebell and Taplin [19]. Two-fold dilutions of the extract was prepared and 2 ml aliquots of different concentrations of the solution were added to 18 ml of pre-sterilized molten SDA for fungi at 40℃ to give final concentration solutions of 10 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to air-dry under laminar flow, then inoculated with the old fungal cultures. The plates were later incubated at 25℃ for 3 days and later observed for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test isolates.

## RESULTS

**Bioactive constituents of *Vernonia amygdalina* leave extracts**

Phytochemical screening of the Vernonia leaves extracts in this study **(Table 1)** revealed the presence of alkaloids, anthraquinone, cardenolide, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids, with the exception of phenols, in both the aqueous and ethanolic extracts.

**Table 1.** Phytochemical components of *Vernonia amygdalina* leave extracts.

| Phytochemicals | Vernonia leaves extracts and test | |
|---|---|---|
| | Aqueous | Ethanolic |
| Alkaloids | + | + |
| Anthraquinone | + | + |
| Cardenolide | + | + |
| Flavonoid | + | + |
| Glycosides | + | + |
| Phenolics | - | - |
| Phlobatannins | + | + |
| Saponins | + | + |
| Steroids | + | + |
| Tannins | + | + |
| Terpenoids | + | + |

*Key: + = Present; - = Absent*

**Children affected with dermatophytoses**

These studies observe the prevalence of dermatophytes among the Almajiri children in relation to their age **(Table 2)**. It was found that the disease is most prevalent (56.5%) in children within 6 to 10 years, followed by those above. However, some of the children were found as young as 4 to 5 years and uncircumcised. All the affected children have two or more spots on their scalp, indicating presence of *Tinea capitis* (scalp ringworm).

**Table 2.** Distribution of dermatophyte isolates according to age range of the children.

| Age (years) | No. of specimens collected | No. of positive isolate | Percentage (%) |
|---|---|---|---|
| <5 | 04 | 04 | 8.7 |
| 6-10 | 29 | 26 | 56.5 |
| >10 | 17 | 16 | 34.8 |
| **Total** | **50** | **46** | **100** |

This study also found that Microsporum species was the most frequent (47.8%) dermatophyte isolated, followed by Trichophyton species (23.9%) **(Table 3)**.

**Table 3.** Distribution of dermatophytes according to frequency of isolates.

| Dermatophyte species | No. of positive isolate | Percentage positive (%) |
|---|---|---|
| Epidermophyton | 09 | 19.6 |
| Microsporum | 22 | 47.8 |
| Trichophyton | 11 | 23.9 |
| Mixed growth | 04 | 8.7 |
| **Total** | 46 | **100** |

**Antidermatophyte activity of *V. amygdalina* leaves extracts**

Antifungal efficacy of the Vernonia leaves extracts was tested against the isolates of Epidermophyton Microsporum and Trichophyton species. The average of the zones of inhibition for each extract was then calculated **(Table 4)**. The zones of inhibition exhibited by the extracts against the fungal isolates was found within the range of 10.20 to 22.50 mm and varies with the concentration of the extract. The diameter zone of inhibition decrease with decrease in the concentration in both the extracts, where the highest zones correspond to highest concentration.

**Table 4.** Anti-dermatophyte activity of aqueous and ethanolic extracts of *V. amygdalina* leaves.

| Concentration (mg/ml) | Dermatophyte isolates and extracts zones of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Epidermophyton | | Microsporum | | Trichophyton | |
| | AE | EE | AE | EE | AE | EE |
| 250 | 20.50 | 22.50 | 19.20 | 21.50 | 18.10 | 19.10 |
| 200 | 18.20 | 19.20 | 17.30 | 18.60 | 16.50 | 18.50 |
| 150 | 17.50 | 18.40 | 16.40 | 17.40 | 15.00 | 18.20 |
| 100 | 16.40 | 17.20 | 15.60 | 16.80 | 14.50 | 17.50 |
| 50 | 13.50 | 14.50 | 11.50 | 12.50 | 10.20 | 11.40 |

*Key: AE: Aqueous Extracts; EE: Ethanolic Extracts*

The minimum inhibitory concentrations (MIC) of the ethanolic extracts of *Vernonia amygdalina* (mg/ml) was also analyzed in this study **(Table 5)**. The highest MIC value of 65.10 mg/ml was found ($P<0.05$) against Epidermophyton and least MIC value of 57.50 mg/ml was obtained against Microsporum.

**Table 5.** Minimum inhibitory concentrations (MIC) of aqueous and ethanolic extracts of *V. amygdalina* leaves.

| Dermatophyte isolates | Minimum Inhibitory Concentrations (mg/ml) values | |
|---|---|---|
| | Aqueous extracts | Ethanolic extracts |
| Epidermophyton | 58.60 | 65.10 |
| Microsporum | 49.40 | 57.50 |
| Trichophyton | 55.80 | 58.20 |
| Mixed growth | 56.10 | 62.30 |

## DISCUSSION

Phytochemical substances have profound antimicrobial activity on various infectious agents through different mode of actions. The metabolites are found to be biologically active and play vital roles in the therapeutic activity of medicinal plants with specific action on human

body. Previous studies by Imaga and Bamigbetan [7] on *Vernonia amygdalina* leaves extracts revealed also confirmed the presence of these compounds. However, phenols was found in Vernonia leaves in this study, the analysis of ethanolic extract of the Vernonia leaves by Alara et al. [2] revealed the presence of these compound but with absence of anthraquinone. Among these phytochemicals, alkaloids are having useful effect on humans, as it serves as a component of powerful pain relievers [10]. The present study observed the efficacy Vernonia leaves extracts against fungi.

Dermatophytoses is a fungal infection commonly affecting school age children especially the less privileged. Scalp ringworm is highly contagious especially among children [3]. The Almajiri children in northern Nigeria suffered from total negligence due to poor parental background, non-chalant attitudes and religious misconception of some parents. The children are left without proper daycare needs such as beddings, bathing and washing, due to their large number under a single Qur'anic scholar and the children had to cater for all the daily needs by roaming about the streets, begging for food and money. They sometimes scout for junks along waterways and refuse dumps to sell. These ill-health conditions exposed them to some fungal pathogens, including the dermatophytes. *Microsporum canis* was found to be the most common fungal agent associated with dermatophytoses and accounting for up to 70% of the infection in a similar study by Bokhari [1].

The rise in the prevalence of side effects of many synthetic antimicrobial agents and emergence of multidrug resistant fungi encouraged research for plant-based drugs of therapeutic potentials. *Vernonia amygdalina* was reported to have such potential of high medicinal value [2]. In this study, aqueous extracts of this plant showed high antifungal activity against the isolates of Epidermophyton, Microsporum and Trichophyton species at 58.60, 49.40 and 55.80 mg/ml concentrations respectively. While for ethanolic extracts had the highest respective concentrations and activity ($P<0.05$) as 65.10, 57.50 and 58.20 against the isolates. The ethanolic extracts of the leaves show more antifungal properties than the aqueous extracts ($P<0.05$), which may due to the solvents used. Organic extract was found to be more active than water extract due to the better solubility of the active components in organic solvents [5].

The extracts however, were active against fungi of medical importance. The presence of alkaloids, anthraquinone, cardenolide, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids in the extracts of *V. amygdalina* in this study may explain the reason for its antifungal activities as the antimicrobial properties of most of these phytochemicals have been previously reported [5,6].

## CONCLUSION

This study showed that the extracts from these leaves revealed significant antifungal activities ($P<0.05$) on all the fungal isolates tested and might be source of active ingredients for the synthesis of antibiotics. The phytochemical components are quite promising and have strongly indicated the anti-dermatophyte efficacy of the plant leaves. As the findings of this study compared favorably with previous studies on fungal infections, the plant holds great promise for use as antimicrobial agent. The efficacy gives impetus to the use of these plants in meeting health care needs of infected children. Further studies are required to characterize the Vernonia leaves active components by molecular techniques of these plants. Cytotoxicity levels should be evaluated using laboratory animals so that these extracts can be formulated into tablets and creams that can be used to treat dermatophytoses and other related fungal infections. Other methods of extraction should be tried to determine the best method for optimal yield of the bioactive constituents.

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