Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Formulation and Antifungal Screening of *Vernonia amygdalina* Delile (Asteraceae) Leaf Extract Ointment

AREMU OLUSOLAISAAC [1]*, ASIRUIDAYATDAYO [2], FEMI-OYEWO NBANGNYONG [2]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin

[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus

ARTICLE DETAILS

Article history:
Received on 24 June 2017
Modified on 18 October 2017
Accepted on 25 October 2017

Keywords:
Vernonia amygdalina Leaf extract,
Ointment,
Inhibition,
Tricophyton tonsurans,
Tricophyton rubrum

ABSTRACT

The plant *Vernonia amygdalina* (Asteraceae) has been ethnomedicinally reported to have antifungal activities. Presently there is no known pharmaceutical dosage form that is available for use. This study therefore seeks to screen for the presence of secondary metabolites present in the plant and then evaluate the extract and ointment formulations against certain selected fungal organisms. The extract was obtained from pulverized dried leaves with ethanol 98%v/v at powdered leaves to solvent ratio 1:10. The plant extract was screened phytochemically using standard procedures. The extract was formulated into ointment at concentrations 0%w/w, 2.5%w/w, 5.0%w/w, 7.5%w/w and 10.0%w/w respectively. The ointment formulations were evaluated against *Tricophyton tonsurans* and *Tricophyton rubrum* as test organisms using Whitfield ointment as reference standard. The extraction gave a yield of 6.60%w/w. The phytochemical screening indicated presence of saponin, tannins, alkaloids, flavonoids and cardiac glycosides. The extract showed activity against *Tryhophyton tonsurans* with mean inhibition zones of 7.00± 0.01mm to 16.00±0.02mm and *Tricophyton rubrum* with mean inhibition zones of 2.00±0.01mm to 14.00±0.02mm for all the concentrations tested with the exception of 0%w/w, the negative control. Ointment formulations gave inhibition zone of 6.00±0.01mm to 12.00±0.02mm against *Tricophyton tonsurans* at all concentrations while it had inhibition zone of 4.00±0.01mm against *Tricophyton rubrum* at 7.5%w/w and 10.0%w/w concentrations respectively. This compares fairly well with Whitfield ointment with inhibition zone of 24.00 ±0.03mm and 37.50±0.03mm against *Tricophyton tonsurans* and *Tricophyton rubrum* respectively. The results of this work show that *Vernonia amygdalina* leaf extract ointment has potential in treating certain fungal infection of the skin.

INTRODUCTION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remained relevant in both developing and the developed nations of the world for various chemotherapeutic purposes. The use of plant derived natural compounds as part of herbal preparations for alternate source of medicament continues to play major roles in chemotherapy especially in third world countries [1]. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs.

Author for Correspondence:
Email: solabank@yahoo.com

The local use of natural plants as primary health remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa [2]. Many plants are consumed as food without in-depth knowledge of their exact chemical composition and contribution to health, although their utilization has passed through several ancestral generations who probably realized from experience that those plant food materials are beneficial [3]. The plant *Vernonia amygdalina*, commonly called bitter leaf, is a perennial shrub of 2-5m in height that grows throughout tropical Africa. It belongs to the family Asteraceae, has a rough bark with dense black straits, and elliptic leaves that are about 6 mm in length. The leaves are green and have a characteristic odor and bitter taste [4]. It does not produce flowers under normal circumstances, but studies found that some

white, fragrant and bee-infested flowers growing on copious corymbose panicles would be formed under drastic growth environment [5]. In many parts of West Africa, the plant has been domesticated [6]. It is known as 'Grawa' in Amharic, 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onugbu' in Igbo, 'Ityuna' in Tiv, 'Oriwo' in Edo and 'Chusar-doki' in Hausa [7]. *V.amygdalina* is drought tolerant (though it grows better in a humid environment). It thrives on a range of ecological zones and is used as a hedge plant in some communities [8]. *V.amygdalina* has been widely used in folk medicine as anti-malaria, purgative, anti-parasitic, treatment of eczema and for maintaining healthy blood glucose levels [9, 10]. The plant *V.amygdalina*is probably the most used medicinal plant in the genus *Vernonia* [11]. The observation that an apparently sick wild chimpanzee chewed *V. amygdalina* and seemed to return to normal activity after a while as reported by Huffman and Seifu [12] and Ohigashi *et al* [13] elicited the attention of the phytomedicine community such that dozens of studies have been done since then to test the efficacy of different extracts of the plant in managing a wide array of medical ailments. Igile *et al* [14] reported that in traditional medicine, practitioners use the plant as an anti-helminthic, anti-malarial, and as a laxative. Others use it as a digestive tonic, appetizer, febrifuge and for the topical treatment of wounds [15]. Dalziel[16] was about the first to report that the root and twig of the plant are used for the treatment of stomach and gastrointestinal problems by the Hausas of Northern Nigeria, while the decoction from the leaves is used in treating malaria fever in Guinea and cough in Ghana. In some parts of Nigeria, the stems are used as chew sticks for oral hygiene, and for the management of some dental problems.

In Malawi and Uganda, *V.amygdalina* is used by traditional birth attendants to aid the expulsion of the placenta, after birth, aid post-partum uterine contraction, induce lactation and control post-partum hemorrhage [17, 18].

Ringworm is a common skin disorder otherwise known as 'tinea'. While there are multiple forms of ringworm, the most common affect the skin on the body (*Tineacorporis*), the scalp (*Tineacapitis)*, the feet (*Tineapedis* or athlete's foot) or the groin (*Tineacruris*).

Trichophyton rubrum is a fungus that is the most common cause of athlete's foot, jock itch and ringworm. An ointment is a homogenous viscous semi-solid preparation most commonly a greasy thick oil with a high viscosity that is intended for the application of active ingredients to the skin or mucous membranes. They are used as emollients for the application of active ingredients to the skin for protective, therapeutic or prophylactic purpose and where a degree of occlusion is desired. There is the need to channel various discoveries of traditional use of *V. amygdalina* plant parts into a delivery design that will be convenient for administration through oral or dermal route. There have been reported cases of bacteria and fungi resistance to some synthetic formulations some of which are even causing adverse skin reactions. In the present study, *V.amygdalina* leaf extract is being designed in a dermatological ointment formulation, evaluate its physico-chemical characteristics and then screen for antifungal activity against *Tineacapitis*.

MATERIALS AND METHODS

MATERIALS

Collection and preparation *V. amygdalina* leave extract

Fresh leaves of *V. amygdalina* were obtained from farms around Sagamu, Ogun state, Nigeria. The plant was identified and authenticated by taxonomist of the Forest Research Institute of Nigeria, Ibadan. The fresh leaves were sorted to remove the dead ones, washed without squeezing to remove debris and dust particles. Large quantities of the leaves were air-dried for about two weeks. The dried leaves were comminuted using blender model Mx-738 (Nakai, Japan) and stored in air tight containers. Ethanol 98%v/v was used as solvent for extraction at leaf weight: solvent of 1 to 10. The milled leaves of about 250µm size and 500g weight was macerated in the solvent for 10 days at ambient room temperature and filtered through a whatman filter paper (No.1) to remove the coarse leaf materials into pre-weighed sterile containers. The solvent was evaporated using rotary evaporator (Buchi Rotary evaporator, Germany).The extract was concentrated into mucilage form by removing any residual solvent using heating mantle. The extract was kept in air-tight containers and stored in a refrigerator.

Assessment of antifungal effect of *V. amygdalina* leaf extract

The plant, *V. amygdalina* leaf extract with Whitfield ointment as reference was tested against *Tricophyton tonsurans* and *Tricophyton rubrum* common organisms implicated in fungal

infections using agar pour plate method. Fresh isolates of *Trichophyton rubrum* and *Trichophyton tonsurans* obtained from Pharmaceutical microbiology laboratory, University of Ibadan, Ibadan, Nigeria were cultured on Sabouraud dextrose agar and incubated at 25°C for 10 days. Thereafter a loopful of the culture was transferred into 40% sucrose solution and then mixed thoroughly. One milliliter of each organism dispersed was added to 19ml of sterilized agar solution, mixed thoroughly and later poured into petri dishes for setting. The set plates were dried in hot air. After drying, 2 cups were bored using a sterile cork borer (5mm in diameter) on each plate ensuring that the cups were far apart as much as possible and not too close to the edge of the plate. Using sterile Pasteur pipettes, different concentrations of the extract (0.0%w/w, 2.5%, 5.0%, 7.5% and 10.0%) in replicate were introduced into the labeled cups and the plates were incubated at 25°C for 48hrs. At the end of incubation period, the plates were observed for clear zones of inhibition and recorded as mean ± SD.

Preparation of *V. amygdalina* leaf extracts ointment

Simple ointment BPC was used as ointment base. Appropriate amount of cetostearyl alcohol, hard paraffin, wool fat and white soft paraffin were weighed into a clean evaporating dish and placed in a water bath to melt and stirred together until cold. Vernonia leaf extract was incorporated into the base at the molten stage to get the desired test concentrations of (0%, 2.5%, 5.0%, 7.5% and 10% w/w). Twenty-five gram (25g) of each sample contained 25.00g, 24.37g, 23.75g, 23.12g and 22.50g of the base respectively. The details of ingredients used in the formulation *V.amygdalina* leaf extract ointment are shown in Table 1.

Table 1: *V.amygdalina* leaf extract ointment formulations

Sample	Extract(g)	Base(g)	Concentration(%w/w)
1	-	25.0	0
2	0.63	24.40	2.5
3	1.25	23.80	5.0
4	1.90	23.10	7.5
5	2.50	22.50	10.0

Assessment of Antifungal Effect of *V. Amygdalina* Leaf Extract Ointment

Different concentrations of *V.amygdalina* leaf extract ointment formulations were evaluated against *Tricophyton tonsurans* and *Tricophyton rubrum* in duplicate using pour plate method as in the case of pure extract. The leaf extract was replaced with ointment formulations.

RESULTS

Percentage yield of the extract was 6.64%w/w. The outcome of the phytochemical screening is presented in Table 2. The results of the diameter of inhibition zones of the leaf extract of *V.amygdalina* against *Triochophyton tonsurans* and *Tricophyton rubrum*is shown in Table 3 and that of ointment formulation is presented in Table 4.

The result of the screened extract revealing the presence of secondary metabolites is presented in Table 2. The presence of secondary metabolites in most plants is always responsible for their medicinal activities in the crude form.

Table 2: Results of the phytochemical screening of *V.amygdalina* leaf extract**.**

QUALITATIVE TEST	RESULT
SAPONIN	+
TANNINS	+
CYANOGENETIC GLYCOSIDES	-
FLAVONIODS	+
ALKALOIDS	+
CARDIAC GLYCOSIDES	+
COMBINED ANTHRAQUINONES	-
FREE ANTHRAQUINONES	-

KEY**:** Present + ; Absent–

There is need for preliminary antifungal screening of the extract before formulation engineering in order to determine the extent of influence of the formulation vehicle on the activity of the extract. The outcome of this anti-infective evaluation is presented in Table 3.

The result of the antifungal effect of the ointment formulation of the extract is presented in Table 4.

DISCUSSIONS

There have been rampant occurrences of fungal infections of the skin especially among the school children in the third world countries. This probably is as a result poor hygiene condition, low immunity as a result of poor nutrition and so on. Many synthetic agents have been associated with one form of drawback or the other necessitating search for alternatives [19]. In the present study further attempt has been made to

Table 3: Assessment of the Antifungal properties of *Vernonia amygdalina* leaf extract showing inhibition zone in (mm) at various concentrations

Sample	Organism					
	Trichophyton rubrum			***Trichophyton tonsurans***		
	Value 1 (mm)	**Value 2 (mm)**	**Mean**	**Value 1**	**Value 2**	**Mean**
2.5%	2.00	2.00	2mm±0.01	6.5mm	7.5mm	7mm±0.01
5.0%	6.00	8.00	7mm±0.01	9mm	11mm	10mm±0.02
7.5%	12.00	12.00	12mm±0.02	12mm	12mm	12mm±0.02
10%	14.00	14.00	14mm±0.02	16mm	16mm	16mm±0.02
Withfield ointment	33.00	42.00	37.5mm±0.03	23mm	25mm	24mm±0.03

Table 4: Assessment of the antifungal properties of *Vernonia amygdalina* leaf extract ointment showing inhibition zone in (mm) at various concentrations

Sample	Organism					
	Trichophyton rubrum			***Trichophyton tonsurans***		
	Value 1	**Value 2**	**Mean**	**Value 1**	**Value 2**	**Mean**
2.5%	_	_	_	5.5mm	6.5mm	6mm±0.01
5.0%	_	_	_	8mm	8mm	8mm±0.01
7.5%	4mm	4mm	4mm±0.01	9mm	9mm	9mm±0.02
10%	4mm	4mm	4mm±0.01	11mm	13mm	12mm±0.03
Withfield ointment	33mm	42mm	37.5mm±0.03	23mm	25mm	24mm±0.03

explore possible use of an herbal formulation of leaf extract of *V. amugdalina* in the treatment of dermatophytosis. Leaves have been chosen because ethnomedicinally, it is this part of the plant that have been used often in the treatment of various ailments. Also it is thought that the highest concentrations of bioactive compounds are concentrated in the leaves of this particular plant. The yield of the extract was found to be 6.6%w/w. This appears low to support any possible commercialization but because it is easily grown and is commonly available especially in the tropics, the idea of herbal formulation of the extract may still be visible. Beyond this, isolation, purification and characterization of the bioactive compounds present in the leaves may pave way for synthetic alternatives that can serve as source of active constituent. The result of the phytochemical screening shows the presence of saponins, tannins, flavonoids, alkaloids and cardiac glycosides as secondary metabolites in the leaf extract of V.*amygdalina*. Phytoconstituents present in plants namely flavonoid, alkaloids, tannins and triterpenoids are producing exciting opportunities for the expansion of modern chemotherapies against wide range of microorganisms. Tannins especially has been found to form irreversible complex with proline rich protein resulting in the inhibition of cell wall synthesis thereby leading to the death of the organisms. In another study, Aremu and Adekoya [20], have been able to establish anti-infective effect of these similar constituents. The activity is also thought to be a product of synergistic effect of the bioactive molecules present in the plant [21].

The antifungal activity of the Vernonia leaf extract against test organisms is as shown in Table 3. The extract showed activity against *Tricophyton rubrum* and Tryhophyton tonsurans in the order of 10.00>7.50>5.00>2.50%w/w for both organisms. However it was observed that the leaf extract exerted higher inhibition zones against *Tricophyton tonsurans* across the concentrations range studied and therefore higher antimicrobial activity against *Tricophyton tonsurans*. The difference in the antifungal activity between these two organisms may be attributable to the difference in their cellular structure. It is suspected that cellular wall structure of *Tricophyton tonsurans* is more susceptible to the lethal effect of bioactive compounds present in the leaf extract such as it was found in earlier work by Aremu and

Adekoya [20], where this same organism demonstrated a higher susceptibility than *Epidermophyton floccosum* using *Acalypha wikesiana* leaf extract. The reference compound (Whitfield ointment) exhibited higher antifungal activity 37.50±0.02mm mean inhibition zone for *Tricophyton rubrum* and 25.00±0.01mm mean inhibition zone for *Tricophyton tonsurans* respectively. This clearly indicates a better efficacy than the leaf extract. This may possibly be due to mechanism of action of the synthetic agent present in the reference compound on one hand and it could also be as result of its better potency. Generally it has been found out that antimicrobials that bind to cell wall of microbes often show higher death ability of the organisms than those that bind to the protein. Ointment base, a semisolid was chosen as the vehicle for the formulation of the extract because of its occlusive property and gradual diffusion of medicament from the base. These attributes are essential in the treatment of dermatophytosis. The antifungal activity of *V.amygdalina* leaf extract ointment formulations is as presented in Table 4. It was observed that there was inhibition against *Trychophyton tonsurans* at concentrations of 2.50 to 10.00%w/wand *Tricophyton rubrum* at concentrations 5.00% and 10.00%w/w. Ointment formulations had lesser activity as compared with extract and reference sample, Whitfield ointment that showed a remarkable activity against test organisms with inhibition zones 24.00±0.02mm and 37.50±0.02mm for *Trichophyton tonsurans* and *Tricophyton rubrum* respectively. The rate of release of the extract from the base which is a function of time, concentration, diffusion co-efficient and temperature of storage may account for reduced or absence of inhibition. The minimum inhibition concentration becomes a critical factor at the level of formulations as compared with crude extract since other factors as highlighted above come into play for the availability of the bioactive metabolites. Further increase in the concentrations of the extract in the formulations may lead to increase in better availability and higher inhibition zones that may compare favorably or even perform better than the reference sample.

CONCLUSION

The plant *V.amygdalina* leaf extract ointment has shown promising antifungal property against common dermatophytes often implicated in dermatophytosis from the foregoing results especially at higher concentrations. Therefore the herbal formulation of the leaf extract may be useful in the treatment of certain skin infections where *Tricophyton tonsurans* and *Tricophyton rubrum* have been suspected to be the causative organisms.

ACKNOWLEDGEMENT

The authors are grateful for the assistance rendered by the technical staff of the department of Pharmaceutics and Pharmaceutical Technology, Olabisi Onabanjo University, Sagamu campus during the course of this study.

REFERENCES

[1] Joy PP, Thomas J, Matthew S, Skaria BP. Medicinal Plants. Kerala Agricultural University, Kerala, India. 1998. pp 3-8.

[2] Bibitha B, Jisha VK, Salitha CV, Mohan S, Valsa AK. Antibacterial activity of different plant extracts. Short communication. Indian J Microbiol 2002;42:361-363

[3] Ghani A, Abdulrahamam EM, Onaolapo JA. ("Chemical and Microbiological Evaluation of some Nigerian traditional preparations" Reporter October 7, 1989, Kaduna.

[4] Sayed MD, Zaki AY, El-Merzabani MM, Doss SL. Preliminary phytochemical examination and tumor inhibitory effect of *Vernonia amygdalina* Del. cultivated in Egypt. Egyptian J. Pharm. Sci. 1982; 20: 229-239.

[5] Kayode J. Eco-physiological and conservation studies on *Vernonia amygdalina* in Ekiti State, Nigeria. Pak. J. Sci. Ind. Res. 2004; 47:227-234

[6] Igile GO, Oleszek W, Jurzysta M, Burda S, Fanfunso M, Fasanmade AA. Flavonoids from *Vernonia amygdalina*and their antioxidant activities. J. Agric. Food Chem. 1994; 42: 2445-2448.

[7] Egedigwe CA. Effect of dietary incorporation of *Vernonia amygdalina*and *Vernonia colorata* on blood lipid profile and relative organ weights in albino rats. MSc., Dissertation, Dept. Biochem., MOUAU, Nigeria 2010.

[8] Bonsi MLK, Osuji PO, Tuah AK, Umunna MN. *Vernonia amygdalina*as supplement of teff straw (Eragrostistef) fed to Ethiopian Menz sheep. Agroforestry Syst. 1995; 31: 229-244.

[9] Nwanjo HU, Nwokoro EA. Antidiabetic and biochemical effects of aqueous extract of *Vernonia amygdalina* leaf in normoglycaemic and diabetic rats. J. Innov. Life Sci. 2004; 7: 6-10.

[10] Nwanjo HU. Efficacy of aqueous leaf extract of *Vernonia amygdalina*on plasma lipoprotein and oxidative status in diabetic rat models. Nig. J. Physiol. Sci.2005; 20: 39-42.

[11] Erasto P, Grierson DS, Afolayan AJ Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. J. Ethnopharmacol. 2006; 106: 117-120

[12] Huffman MA, Seifu M. Observations on the illness and composition of a possibly medicinal plant *Vernonia amygdalina*(Del.) by a wild chimpanzee in the Mohale Mountains National Park, Tanzania. Primates 1989; 30: 51-63.

[13] Ohigashi H, Jisaka M, Takagaki T, Nozaki H, Tada T, Huffman MA, Nishida T, Kaji M, Koshimizu K. Bitter Principle and a Related Steroid Glucoside from Vernonia-Amygdalina a Possible Medicinal Plant for Wild Chimpanzees. Agric. Biol. Chem. 1991; 55: 1201-1205.

[14] Igile GO, Pleszek W, Jurzysta M, Aquino R, De Tommasi N, Pizza C. Vernoniosides D and E, two novel saponins from *Vernonia amygdalina*. J. Nat. Prod. 1995; 58: 1438-1443

[15] Iwu MM. Introduction: Therapeutic agents from ethnomedicine. In: Iwu, M.M., Wootton, J.C editors. Advances in Phytomedicine, Volume 1: Ethnomedicine and drug discovery, Elsevier Science B.V., Amsterdam, 2002; pg 12.

[16] Dalziel JM. The useful plants of west tropical Africa. Crown Agents for the Colonies, London, 1937; pp. 421-422.

[17] Bullough HW, Leary WP. Herbal medicines used by traditional birth attendants in Malawi. Trop. Geograph. Med. 1982; 34: 81-85.

[18] Kamatenesi-Mugisha M, Oryem-Origa H, Makawiti OO. Ethnopharmacological screening of *Vernonia amygdalina* and *Cleome gynandra* traditionally used in childbirth in Western Uganda. Proc 11th NAPRECA Symposium, Antanarivo, Madagascar, 2005; 9- 12: 110-115.

[19] Aremu OI, Ikuyajesin T. Formulation and Evaluation of the antifungal activity of Neem seed oil ointment against *Tineacaptis.* West African Journal of Pharmacy 2016; 27(1): 126-134.

[20] Aremu OI, Adekoya A. Phytochemical and antimicrobial screening of Carica papaya.Nigerian Journal of Pharmaceutical and Applied Science Research 2016; 5(2):18-24.

[21] Hodek P, Trefil P, Stiborova M. Flavonoids – Potent and versatile biologically active compounds interacting with cytochrome P450. Chemico-biol. Int. 2002; 139(1): 1-21.