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Phytochemical screening and antimicrobial activity of the leaf extract of *Vernonia amygdalina* (Bitter leaf)

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Abstract
This study was conducted to determine phytochemicals and antimicrobial activities of Vernonia amygdalina using standard methods. Results showed that V. amygdalina contains alkaloids, tannins, saponins, triterpenoids, steroids and cardiac glycosides that are responsible for its antibacterial activities. The minimum inhibitory concentration (MIC)results indicates that the aqueous and methanolic extracts of the plant leaves investigated exhibited antibacterial activity against Staphylococcus aureus, *Streptococcus pneumonia*, Escherichia coli, Pseudomonas aeruginosa and fungi candida albican, while the minimum bactericidal concentration (MBC) range between 100 – 400 mg/ml. Vernonia amygdalina leave extracts could therefore be a promising natural antibiotics with potential than conventionally antibiotics in controlling bacteria and fungi that can cause diseases.

Keywords: Bitter leaf, *Vernonia amygdalina*, *Streptococcus pneumonia*

Introduction

There is a growing concern in the public space to develop new drugs that will prevent or treat infectious diseases due to the increasing occurrence of antimicrobial drug resistance by most bacteria (Buchy *et al*., 2020; Taneja and Sharma, 2019; WHO, 2014) [26]. A search for alternative to the chemically laddened antibiotics has now shifted to plants considered to be medicinal (Andrew, 2021; Ali *et al*., 2020; Mordeniz, 2019; Umaru *et al*., 2019) [6, 5, 29]. Plants can synthesize and accumulate a great variety of phytochemicals in their cells including, tannins, cyanogenic, phenolic compounds, saponins, lignins, lignans, flavonoids, alkaloids and glycosides. These phytochemicals, which are secondary plant metabolites have been shown to protect against diseases (Andrew, 2021; Breslin, *et al*., 2017; Toyang and Verpoorte, 2013; Okwu, 2004) [6, 8, 27, 23]. They are the best candidates of antimicrobial agents since they have been used for centuries to combat infectious diseases without significant resistance development.

Vernonia amygdalina Del, 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 (Unegbu *et al*, 2020) [30]. *V. amygdalina* is well known as a medicinal plant that has been attributed to the prevention and treatment of various diseases such as diabetes, asthma, headache, skin infections such as ringworm, rashes and eczema, schistosomiasis, malaria, measles, diarrhea, tuberculosis, stomachache and intestine complaints, fevers, cough, induction of fertility in barren women and hyperlipidemia (Raimi *et al*., 2020) [24]. Due to this, its leaves are exported from several African countries and purchased in grocery stores aiming to serve African clients for about $1.50/225gm.

The roots of *V. amygdalina* have been used for gingivitis and toothache due to its proven antimicrobial activity (Ajibesin *et al.*, 2008; Mensah *et al.*, 2008; Gbolade, 2009). Studies have shown that V. amygdalina possesses antimicrobial, antidiebetic, anticancer, Antihelmitic, antimalarial and insecticidal properties, in addition to its immunomodulatory activities, anxiolytic, sedative and hypothermic effects (Andrew, 2021; Green *et al*., 2017; Oloruntobi *et al*., 2014) [6, 8, 22]. The bioactive components and antimicrobial effects of V. amygdalina have been widely studied using ethanol extracts and well documented (Andrew, 2021; Abideen *et al*., 2020; Raimi *et al*., 2020) [6, 1, 24]. Unfortunately, no substantial data is available on the phytochemical properties of *Vernonia amygdalina* (bitter leaf), which grow abundantly in Bauchi State and are used by local communities to treat several ailments. This study is therefore designed to determine the phyto-chemical profiles of the leaves aqueos and methanolic extracts of the plants and evaluate their antimicrobial effects. The study will provide baseline information on the plant extracts and its potential as alternative for antimicrobial drugs.

Materials and Methods

1. Plant Sample Collection and Preparation

Vernonia amygdalina (Bitter leaf) leaves were plucked from a farm within Rafin Zurfi, Bauchi State. The leaves were washed (to remove dirt), sliced and dried at room temperature for 7 days. They were afterwards pulverized to obtain finer ground powder, using an electric blender.

2. Collection of test organisms and preparation of stock culture

Test organisms were received from Microbiology Laboratory of Abubakar Tafawa Balewa University, Bauchi State and reconfirmed by Gram staining and sub-culturing in appropriate selective media. The organisms include; *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.*

3. Extraction Procedure

The aqueous extract of *Vernonia amygdalina* leaves were separately homogenized with sterile distilled water at 1:8 w/v ratio in a pestle and mortar and filtered through muslin cloth. The filtrate thus obtained was further strained through Whattman No. 1 filter paper (Davis, 1956). The extraction was carried out at room temperature.

Methanolic extracts of the *Vernonia amygdalina* leaves were prepared by soaking 400g of the dry powdered plant leaves in 1000ml of absolute methanol at room temperature for 48hrs. The extract was thereafter filtered first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extract was then concentrated using a rotary evaporator with the water bath set at 40^0C to one-tenth its original volume and finally with a freeze drier.

The dried residue was then stored at 4ºC. Portions of the crude plant extract residue were weighed and dissolved in distilled water for experimental analysis.

Phytochemicals Screening

Tests were carried out on the aqueous extract to identify the phyto constituents using standard procedures as described by Harborne (1973) [17], Sofowara (1993) [25]; and Trease and Evans (1989) [28].

***Test for alkaloids*:** 200 mg of extract was mixed with 10 ml of methanol. To 2 ml of the filtrate was added 1% HCl and then steamed. To 1 ml of the filtrate was added 6 drops of Wagner's reagent. Brownish-red precipitate indicated the presence of alkaloids.

***Test for flavonoids*:** 5.0 cm^3 of the diluted ammonia solution was added to a portion of aqueous filtrate of plant extracts, this was followed by the addition of concentrated tetraoxosulphate (vi) acid. Appearance of yellow coloration showed the presence of flavonoids.

***Test for saponins*:** 2.0 g of the powdered samples were boiled in 20 ml of distilled water in a water bath and filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion showed the presence of saponins.

Test for tannins: 0.5 g of dried powdered samples were boiled in test tube containing 20 cm^3 of water. This was followed by filtration and addition of a few drops of 0.1% ferric chloride. Appearances of brownish green colour indicated the presence of tannins.

***Test for glycosides*:** 2.0 cm^3 of glacial acetic acid containing one drop of ferric chloride was added to 5 ml of the plant extracts. This will be under layer with 1.0 cm^3 of concentrated tetraoxosulphate (vi) acid. Formation of a brown ring at the interface indicated the presence of cardiac glycosides.

Test for terpenoids: 5.0 cm^3 of the plant extracts were mixed with 2.0 cm^3 of chloroform and 3ml of concentrated tetraoxosulphate (vi) acid. Formation of reddish-brown coloration at the interface indicated the presence of terpenoids.

***Test for steroids*:** 2.0 cm^3 of acetic anhydride was added to 0.5 ml of the methanolic extracts, followed by 2 cm^3 tetraoxosulphate (iv) acid. The color changed from violet to green, indicating the presence of steroids.

Antimicrobial Activity

Preparation of Antibacterial Medium

28.0g of the nutrient agar was weighed and transferred into a conical flask containing 100 $cm^3$of distilled water. The mixture was sterilized by steaming for 15 minutes. The medium was allowed to cool, transferred into a sterilized petri dish up to the mark, and allowed to solidify.

Test organisms

The antimicrobial activity of the crude extract was screened against gram-positive bacteria: *Staphylococcus aureus and streptococcus Pneumonia* and the gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa;* and a fungus, *candida albican*. These organisms were collected from the Microbiology Laboratory of Abubakar Tafawa Balewa University, Bauchi State. The bacteria were then sub-cultured into the newly prepared agar medium. *Staphylococcus aureus* were sub-cultured to Nutrient agar medium. *Escherichia coli* and *Pseudomonas aeruginosa* were sub-cultured to MacConkey agar medium. The petri dish was then incubated at 37ºC for 24 hours. After incubation, the growth was examined and the petri dish were wrapped with parafilm and store in refrigerator under 2-8ºC until further use.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extracts and was done by broth dilution method. Mueller Hinton broth was prepared, dispersed into test tubes and sterilized at 121°C for 15 minutes. The broth was then allowed to cool. Normal saline was prepared and 10 cm^3 was dispersed into a sterile test tube and the test organisms were inoculated and incubated at 39°C for 6 hours. Dilution of the test organisms was done in the normal saline until the turbidity marched that of the McFarland's standard scale by visual comparison (at a concentration of about 1.5 x 108 CFU/ cm^3). Two fold serial dilution of the extract in sterilized broth was made to obtain the concentrations of 5 mg/ cm^3, 2.5 mg/ cm^3, 1.25 mg/cm^3, 0.625 mg/cm^3 and 0.313 mg/cm^3. The initial concentration was obtained by dissolving 0.05 g of the crude extract in 10 ml of DMSO to obtain a concentration of 5 mg/cm^3 from which subsequent dilutions were made. Having obtained the different concentrations of the extracts in the sterile broth, they were observed for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was recorded to be the MIC.

Minimum Bactericidal / Fungicidal Concentrations

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were evaluated by plating the bacterial suspensions from individual well at the beginning and at the end of the experiments on Mueller Hinton agar medium for estimation of MBC. The culture from MIC well was taken and streaked on the surface of fresh Mueller Hinton agar in a 90-mm plate with division and incubated at 37°C for 24 hours (for the bacteria) and 30°C for 1 – 7 days (for the fungi) after which the plates of the medium were observed for colony growth. The MBC/MFC values were the plates with lowest concentrations of the extract without colony growth.

Results and Discussion

The results of the percentage crude yield extract and the phytochemical screening of *Vernonia amygdalina* leaves

extracts showed presence of some phyto compounds both for methanol and aqueous extracts (Tables 1 and 2).

Table 1: Physical Properties and Percentage Recovery of Leaf Extract of *Vernonia amygdalin*

Physical Parameters	Extracts	
	Methanol Extracts	Aqueous Extracts
Colour	Green	Brown
Texture	Crystalline	Rough
Percentage recovery (%)	5.0	5.5

From Table 1, the color of the methanol and aqueous of the Leaf Extract of *Vernonia amygdalin* were green and brown respectively. The methanol extract has a crystalline texture while the aqueous extract has a rough texture. The aqueous extracts of the leaf used yielded the highest extracts (5.5 %), followed by the water extracts of the same part of the plant (5.0 %). The result of the phytochemical screening of the aqueous extract of vernonia amygdalina in Table 2 shows the presence of saponins, cardiac glycosides, alkaloids and tannins while methanolic extract shows the presence of terpenoids, tannins, saponins and alkaloids. The aqueous extract of the V. *amygdalina* in this study is similar to those reported in Raimi *et al* (2020) [24] and Unegbu *et al*., (2020) [30] except for flavonoid. Abideen *et al.* (2020) [1] also reported that *vernonia amygdalina* ethanolic extract showed the presence of tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids, teriterpenoids and absence of anthraquinones. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals and humans (Omoregie *et al.*, 2011) [21].

Table 2: Results of Phytochemicals Screening of *Vernonia amygdalina*

Sites	Aqueous extract	Methanolic extract
Alkaloids	+	+
Flavonoids	−	−
Saponins	++	+++
Tannins	+	+
Glycosides	+	−−
Terpinoids	−	+

Key: + = Present, − = absent.

Tannins are useful for treatment of inflamed or ulcerated tissues and in the prevention of cancer (Adegboye *et al.*, 2008). Most alkaloids are used by plant as defense against herbivore and attacks by microbial pathogens and invertebrate pests, all these actions were as a result of a strong bitter taste and its toxic nature. They have also been reported to reduce headache and fever due to their analgesic and antibacterial properties (Raimi *et al*., 2020) [24]. Saponin is a natural product that form pores in cell membrane bilayers as a result of complexation with cholesterol, hence, they are useful cholesterol lowering agent as well as anti-cholesterol agents. Cardiac glycosides are essential class of naturally occurring drugs that exhibit activity which helps in the treatment of congestive heart failure (Francis *et al.*, 2002) [16]. The presence of alkaloids, steroids, glycosides, alkaloids, tannins, saponins, and terpenoids in the extracts of *V. amygdalina* may explain the reason for its antimicrobial actions (Nenaah, 2013; Al-Harbi *et al.*, 2017; Jin *et al.*, 2017) [13, 4, 10].

Table 3 and 4 show the antibacterial activity of methanolic and aqueous extracts against *Staphylococcus aureus*, *streptococcus Pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa* and the fungus *Candida albican*. The result in Table 3 shows that the higher the concentrations of the extracts, the higher the zones of inhibition. The test indicates that all the test organisms were effectively inhibited by V. amydalina extracts and as such the leaves of *V. amygdalina* possess antimicrobial potential against the studied isolates. Methanol and aqueous extracts showed more activity against the bacterial isolates. This may be due to the higher volatility of the methanol which tends to extracts more active compounds from the samples than water. The results in this study agree with those of Ogundare (2011) [20] and Muhammad *et al.* (2019) [19], who found that alkanol extract of *V. amygdalina* leaf is effective against *S. aureus* and *Shigella*. The results also justifies the findings of Zubairu *et al.* (2019) [32] on the effectiveness of *V. amygdalina* leaf extract against *E. coli, S. aureus* and *S. typhi*. The inhibition results in this study is similar to other work by Ibekwe *et al.* (2001) [9]; Adetunji *et al.* (2013) [2]; Adetutu *et al.* (2011) [3]; Koduru *et al.*, (2006) [11], and Moreno *et al.* (2006) [12] which showed that the ethanolic and acetone extracts possesses more antimicrobial activity than aqueous extract. While the results obtained here for the effect of the aqueous extracts of the study plant on *S. aureus* and *E.coli* compare favorably with those reported in Unegbu *et al*., (2020) [30], the zone of inhibition for these organisms for methanol extract in the present study were higher than those produced by the ethanolic extracts.

Table 3: Zone of Inhibition (mm) against test bacteria and fungi isolate

Extract	Conc. in Mg/ml	*S. Aureus*	*S. Pneumoniae*	*P. aeruginosa*	*E. coli*	*C. albican*
Methanol	400	21.00	14.00	09.00	23.00	12.00
	200	17.00	12.00	07.00	18.00	10.00
	100	10.00	10.00	05.00	14.00	00.00
	50	07.00	06.00	00.00	10.00	00.00
Aqueous	400	16.00	11.00	10.50	17.00	08.00
	200	12.00	10.00	08.00	15.00	06.00
	100	08.00	04.00	06.00	10.00	00.00
	50	04.00	04.00	00.00	05.00	00.00

(Gram-positive bacteria: *Staphylococcus aureus, streptococcus Pneumonia*. Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*; Fungi: *candida albican*

With the exception of *S. aureus* in Table 4, the minimum inhibitory concentrations of the aqueous and methanolic extracts were similar, ranging from 100 – 200 mg/ml. The minimum bactericidal concentration (200 – 400) of both extracts were also found to be similar except for *S. aureus* and *E.coli*

Table 4: Minimum inhibitory concentration (MIC) (mg/ml) and minimum bactericidal concentration (MBC) of the extracts.

Organism/ Extract	*S. Aureus* MIC MBC	*S. Pneumoniae* MIC MBC	*P. aeruginosa* MIC MBC	*E. coli* MIC MBC	*C. albican* MIC MBC
Methanol	100	200	200	100	200
	400	400	400	200	400
Aqueous	100	200	200	100	200
	200	400	400	400	400

Conclusion and Recommendations

The methanol and aqueous extracts of *V. amygdalina leaves* possess antibacterial properties against *Staphylococcus*

aureus, Streptococcus Pneumonia, Escherichia coli, Pseudomonas aeruginosa; and antifungi property against *candida albican*. Knowledge of the phytochemical constituents of *V. amygdalina* leaves is useful in evaluating the medicinal values of the leaves. Flavonoids and tannins have antimicrobial and antioxidant properties. Alkaloids have pronounced physiological effects particularly on the nervous system. These plant extracts could be a promising natural antibiotics with potential applications in controlling bacteria and fungi that can cause diseases. The activity of the extracts against the isolates is due to present of bioactive compounds in the extracts such as alkaloids, saponin, tannin and flavonoid. The isolation and purification of the phyto compounds from this plant leaves is therefore recommended in order to develop new herbal drug formulations for the prevention and cure for infections. The determination of the respective antimicrobial potencies and toxicological evaluation for the formulation of antibiotics should be the future direction for investigation.

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