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(RESEARCH ARTICLE)

Antibacterial activity of methanolic extract of bitter leaf (*Vernonia amygdalina*) from various component fractions using column chromatography

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Abstract

This research work determined the antibacterial activities of various components at different ratios from column fractions of methanolic extract of bitter leaf (*Vernonia amygdalina*), meanwhile column chromatography been the best method of separating component nowadays. The column chromatography was perform using several type of solvent such as non-polar, semi-polar, polar solvent as well as their mixture in deference ratio. The phytochemical screening was carried out on the leaves extract of (*Vernonia amygdalina*) which revealed the presence of some active ingredients such as alkaloids, flavonoids, tannins, saponins, glycosides, cardiac glycoside and saponin glycosides in the extract, while anthraquinones and volatile oil were not identified. The research conclude that acetone- methanol 50:50 column fraction of methanolic extract of *Vernonia amygdalina* exhibits antibacterial activity against both gram positive and gram negative bacteria.

Keywords Antibacterial; Biter leaf; Column; *Escherichia coli*; *Salmonella typhi*; *Staphylococcus aureus*

1. Introduction

Numerous plant extracts and their essential oils have been initiated of having capability to control microorganisms i.e. both gram negative and gram-positive bacteria associated to skin, dental caries and food spoilage [1]. Plants are definitely the primary source of preparing remedies in the variety of alternative medicine. The search for plants with antibacterial activity has achieved by increasing impact nowadays due to the advent of antimicrobial drug resistance and frequently the occurrence of detrimental side effects of some antibiotics [2]. World Health Organization (WHO) reported, about 80% of the globe population relies on plants or its derivative products for the treatment of various sicknesses [3]. Medicinal plants, ever since ancestor's era, were employed in almost every society as a source of medicine. Traditional plants occupy considerable task in medical system in Nigeria and parts of the plant continue to be a major resource to resist serious diseases in the globe. Pharmacognostic researches of plants have been conducted to discover ideal drugs or outline for the development of latest therapeutic agents. Ever since many drugs, example, quinine and artemisinin were isolated from plants part and due to the improved resistance of many pathogens, example malaria parasites and bacteria, led to established drugs, search of the antioxidants within traditional plants is compulsory nowadays [4]. Medicinal plants have sustained to engage as well participate significant function in the improvement of innovative drugs and successful health care systems in many countries, developed and underdeveloped. In an analysis of plant contribution to drug expansion [5]. Atamgba *et al.,* reported that as minimum 119 chemical substances of plant origin can be considered as essential drugs that are used in one or more countries in

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the world [5]. Herbs and spices parts of plants from original or exotic origins are necessary parts of human diet as they develop taste, colour and aroma of foods as well as contributed in body imines system [6]. Besides they act as preservatives in many foods, they also contain minerals, antioxidants and antimicrobial properties which are essential to protect the body against diseases or serve as protecting agent that are capable to prevent harmful organisms from the body [7,8]. Herbs have been also used in human and veterinary medicine, herbs and spices are extremely essential and functional as beneficial agent against several pathological infections [9, 10]. The spices comprise an exclusive aroma and flavor which are consequential from compounds well-known as phytochemicals or secondary metabolites [11, 12]. The phytochemicals are antimicrobial substances present in the spices which are competent of attracting benefits and prevent harmful organisms; they also serve as photoprotectants and responds to environmental changes [12]. The habitual plant have been generally used and identified for their medicinal and aesthetic assessment ever since primevalera. About 60% of the globe populations entirely rely on traditional medicinal plants and their extracts for different healthcare requirements [13]. Bacterial infections are exerting difficulties on humans worldwide mainly because of the expansion of antibiotic resistance. In the previous 10 years, resistance in gram negative bacteria has been rising; gram negative bacteria quickly expand drug resistance, particularly in the occurrence of antibiotic collection pressure, increase in multidrug resistant (MDR) bacteria push the investigation of new ideal antibacterials to confront resistant phenotypes [14]. *Escherichia coli* (EHEC) are significant food borne threat in several countries around the world. Infection frequently causes (haemorrhagic diarrhoea*)* and irregularly to kidney failure and death while on the other hand, *Salmonella* is a bacterium which causes food borne sickness mainly from foods of animal origin all over the world [15]. Food borne diseases contains sequence of sickness and increasing community health difficulty globally, especially the gram positive bacteria *Staphylococcus aureus* (*S. aureus*) which is mostly accountable for positional active injury infectivity, toxic shock syndrome, endocarditis, osteomyelitis, and food poisoning. Moreover, *Listeria monocytogenes* (*L. monocytogenes*) is accountable for strict food borne sickness called listeriosis, which is well known among the rising zoonotic infections over the last two decades. The gram negative bacteria *Escherichia coli* (*E. coli*) is available in the human intestine and can cause urinary tract infection (UTI) and choleocystitis, or septicemia [16]. The causative bacteria usually comprise of the fecal flora, and the UTI incident is start, as soon as the urine flow in an human being is blocked by one or more reasons, for instance stinctures, calculi, tumours, prostatic hypertrophy, vesicourethral reflux, diabetes, anal disease, pregnancy, catheterization, several surgical procedure at the urinogenital region and cystoscopy. If the infections in patients are not treated, the microbes may acquire resistance to the functional antibiotics and a drug resistant cell continue to exist and predominates with related bacterial genetic make-up. In summary, there are many factors of antibiotic resistance in pathogenic bacteria and these conditions have become a clinical concern nowadays [17]. Microbial infections are well known to be of health concern due to increasing rate of antibiotic resistant bacteria. The U.S. Centers for Disease Control and Prevention, reported that about two million individuals are infected yearly with multi drug resistant bacteria, among those, 23,000 individuals die as a result of these pathogens. Anticipation and curing of these infections have led to significant consideration and presents a serious confronts to develop ideal antibiotics and/or antibacterial substances that are capable to kill or restrain bacterial growth [18]. Although, great numbers of plant derived antibiotics were identified, the scientific evaluations of plants derived antibiotics still remain an area of intensive investigation, this leads to our present study on antibacterial activity of methanolic extract of bitter leaf (*Vernonia amygdalina)* from various component fractions using column chromatography.

2. Experimental

2.1. Sample collection and treatment

The sample of bitter leaf (*Vernonia amygdalina*) was collected from Yarkasuwan Mallan Bawa Mabera Area in Sokoto metropolis, Sokoto State, Nigeria. The sample was then authenticated at the Herbarium Laboratory of the Botany Unit of Biological Sciences Department, Usmanu Danfodiyo University Sokoto with Voucher number (UDUH/ANS/0161). The sample was shade dry for about 72 hours, then the sample were grinded to powdered formed using pestle and mortar. The powdered sample was weight (Wo) using electrical weighing balance in the laboratory.

2.2. Bacterial isolates

The bacterial isolates which comprised of *Escherichia coli* (*E. coli*), *Salmonella typhi* (*S.typhi*) and *Staphylococcus aureus* (*S. aureus*) were obtained from the stock culture collection of the Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. The organisms were further authenticated via: *Staphylococcus aureus* using (BD BBL ™Staphyloslide ™ Latex Test Kit), *Salmonella typhi* using (Widal slide agglutination test kit) and while *Escherichia coli* was authenticated using biochemical test.

2.3. Method of extraction

This was achieved by the use of soxhlet extractor in chemistry department laboratory at Usmanu Danfodiyo University Sokoto Nigeria.
Procedure: 100 g powdered sample of bitter leaf (*Vernonia amygdalina*) was divided into 4 portions, and then each portion was placed into a thimble. Each potion contain 25 g of the powder sample in the thimble, the thimble was inserted into the soxhlet extractor chamber, then the set up was assemble, in which the soxhlet extractor chamber was inserted into pre weighed flask containing 250 cm^3 methanol, the pre-weighed flask was heated for about 45 minute for each portion using heating mantle thermostat at 60 °C temperature with constant flow of water in the soxhlet condenser to regulate the temperature. After the extraction, the solvent was recovered and the remaining solvent was concentrated using water bath [19].

2.4. Column chromatographic separation method

2 g of crude extract was dissolved in 50 ml of methanol was applied on a column (5x40 cm) pack with sephadex LH-20 follow by addition n-hexane, used as first eluent, to allowed removing low molecular compound, also the isolation continuous for Sample B n-hexane/Acetone 50:50, Sample C Acetone 100, Sample D Acetone/ Methanol 50:50, Sample E Methanol/n-hexane 50:50 and Sample F Methanol 100 [19, 20].
Sample A n-hexane 100
Sample B n-hexane/Acetone 50:50
Sample C Acetone 100
Sample D Acetone/ Methanol 50:50
Sample E Methanol/n-hexane 50:50
Sample F Methanol 100

2.5. Antibacterial activity sensitivity tests

The antibacterial assays were carried out by standard disc diffusion method as described by Sivaperumal [9]. Whitman number one filter paper disc (5 mm diameter) was impregnated with the different fractions and then be placed on Mueller Hinton Agar (Himedia, Mumbai) which was previously been inoculated with the test organisms. Ciprofloxacin was used as control. All plates were incubated overnight at 37 ^oC for about 24 hours, the zones of inhibition was then recorded in millimeter.

2.6. Phytochemical screening test

The crude sample of methanolic extract was subjected to phytochemical test for the presence of Alkaloids, Anthraquinones, Cardial glycosides, Flavonoids, Saponiins glycosides, Saponin glycosides, Tannin and Volatile oil using Standard Method reported by Treas and Evans; Harborne adopted by [21].

3. Results and discussion

Table 1 Results of Qualitative Analysis of Phytochemicals Test

Test	Extract Leaves
Alkaloids	+
Anthraquinones	-
Cardial glycosides	+
Flavonoids	+
Glycosides	+
Saponin glycosides	+
Saponins	+
Tannins	+
Volatile oil	-

(-): Absent; (+): present.

The results of phytochemicals analysis of methanolic extract of bitter leaf (*Vernonia amygdalina*) leaves reveals the presence of alkaloids, flavonoids, tannins, saponins, glycosides, cardiac glycoside and saponin glycosides, while anthraquinones and volatile oil were absent. Alkaloids was found to be present, the present of alkaloids in the crude methanolic extract as seen in this present study may be due to the pore formation in the cell wall and the leakage of cytoplasmic constituents by the active component [22]. Flavonoids were identified in the crude methanolic extract, it has been reported that flavonoids are important group of polyphenols are known to have antimicrobial and anti-inflammatory properties [22]. Tannins were found positive in the crude extract, more over tannins play important role as potent antioxidants, besides, it plays a role in curing diarrhea infection and as antihemorrhagic agent [23]. Cardiac glycoside, glycosides, saponins and saponin glycosides were also identified in this study, those compounds has been reported to be among the bioactive component that are responsible for activity against gram negative and gram positive bacteria [24, 25]. Phytochemical screening of the plants extracts in table 1. Report that secondary metabolites such as saponins, tannins, flavonoids and alkaloids were found to be present in leaf of *Vernonia amygdalina*. However anthraquinones and volatile oil were completely absent in the extract. These bioactives component (ingredients) may be responsible for the antibacterial activity of the extracts [26]. The present of alkaloids, flavonoids, tanins and saponins are in line with the findings of [27], who's also obtained the present of those compound in methanolic extracts of *Canarium Schweinfurtihii***.**

Table 2 Results for Antibacterial Activity

Sample	**Solvent**	**Ratio**	**zone of inhibition in (mm)**		
			S. aureus	*E. coli*	*S. typhi*
Sample A	n-hexane	100	-	-	-
Sample B	n-hexane/Acetone	50:50	-	-	-
Sample C	Acetone	100	-	-	-
Sample D	Acetone/ Methanol	50:50	20	24	23
Sample E	Methanol/n-hexane	50:50	-	-	-
Sample F	Methanol	100	-	-	-
Control Drug	Ciprofloxacin		32	31	30

Keys: *E. coli* =*Escherichia coli*, *S. typhi* = *Salmonella typhi*, *S. aureus* = *Staphylococcus aureus*
(-) = Absent, (+) = present.

Table 2: Showed that the test fractions detects selective antibacterial activities, the best activity was recorded with acetone-methanol fractions (50:50) with zone of inhibition (24 mm) against *Escherichia coli* (*E. coli*), followed by (23 mm) against *Salmonella typhi* (*S. typhi*) and lastly (20 mm) against *Staphylococcus aureus* (*S. aureus*) respectively. Moreover, the other fractions n-hexane (100%), n-hexane/Acetone (50:50), acetone (100%), methanol/n-hexane (50:50) and methanol (100%) shows no zone of inhibition against all the bacterial isolates. This indicates that's the active components of methanolic extract of *Vernonia amygdalina* lies in the various component fractions of *Vernonia amygdalina* extract. It was observed in this study that acetone-methanol column fraction from methanolic extract *Vernonia amygdalina* has broad spectrum of activity against gram positive and gram negative bacteria. This is in line with findings of [28].

4. Conclusion and recommendation

From this study, it can be deduce that acetone- methanol 50:50 column fraction of methanolic extract of *Vernonia amygdalina* exhibits antibacterial activity against both gram positive and gram negative bacteria. Moreover, the extract can be used to develop new herbal antibacterial formulation in the ethanomedicinal practice. Further research will be required to be carried out to explore the medicinal important of the leaves of *Vernonia amygdalina* plant.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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