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

Zubairu Aminu Yakubu 1, 2, *, Mukhtar Muhammad 1, Saidu Isah 2, Ibrahim Zulkiflu 1, Isah Sadiq 1, Garga M. Adamu 3 and Kebbi Haruna Sarkin 2

1 Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto State, Nigeria.
2 Department of Science Laboratory Technology, Umaru Ali Shinkafi Polytechnic, P.M.B. 2356, Sokoto, Nigeria.
3 Department of Tissue Culture and Plant Propagation, National Biotechnology Development Agency, P.M.B. 2140, Katsina State, Nigeria.

Publication history: Received on 30 August 2018; revised on 04 May 2019; accepted on 08 May 2019

Article DOI: https://doi.org/10.30574/gscbps.2019.7.2.0095

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, saponsins, 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

* Corresponding author
E-mail address: aminuyakubuzairu@gmail.com

Copyright © 2019 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0
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 cholecystitis, 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, Usman 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, Usman Danfodiyo University Sokoto, Sokoto State, Nigeria. The organisms were further authenticated via: Staphylococcus aureus using (BD BBL™Staphylolide™ 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 Usman 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 assembled, in which the soxhlet extractor chamber was inserted into pre weighed flask containing 250 cm³ 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 °C 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, Saponins 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, moreover 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].

Table 1. The presence of anthraquinones and volatile oil were completely absent in the extract. These bioactives component may be responsibele for the antibacterial activity of the extracts [26]. The present of alkaloids, flavonoids, tannins and saponins are in line with the findings of [27], who's also obtained the present of those compound in methanolic extracts of Canarium Schweinfurtiihi.

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 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

Acknowledgments

We also like to acknowledge the effort of Petroleum Technology Development Fund (PTDF) as well as Prof Aminu Muhammad Bayawa Faculty of Science, Department of Pure and Applied Chemistry Usmanu Danfodiyo University Sokoto, Nigeria, for accepting us and allowing us to conduct practical in Petroleum Research laboratory.

Disclosure of conflict of interest

The authors declare no conflict of interest.

References

[1] Sartoratto A, Machado ALM, Delarmelina C, Duarte MCT and Rehder VLG. (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of microbiology, 35, 275-280.

[2] Soberon M, Gomez I, Pardo-Lopez L, Munoz-Garay C, Fernandez LE, Perez C, Sanchez J and Bravo A. (2007). Role of receptor interaction in the mode of action of insecticidal Cry and Cyt toxins produced by Bacillus thuringiensis. Peptides 28, 169–173.

[3] Tchinda CF, Voukeng IK, Beng VP and Kuete V. (2016). Antibacterial activities of the methanol extracts of Albizia adiantifolia, Alchornea laxiflora, Lopertia ovatifolia and three other Cameroonian plants against multi-drug resistant gram-negative bacteria. Saudi journal of biological sciences, 650, 1-21.

[4] Ibrahim HA, Imam IA, Bello AM, Umar U, Muhammad S and Abdullahi SA. (2012). The Potential of Nigerian Medicinal Plants as Antimalarial Agent: A Review. International journal of science and technology, 2, 600-605.

[5] Atamgba AA, Margaret AA, Kayode D and Amonor JW. (2015). The biomedical significance of the phytochemical, proximate and mineral compositions of the leaf, stem bark and root of Jatropha curcas. Asian Pacific Journal of Tropical Biomedicine, 5, 650–657.

[6] De Souza TP, Gomez-Amoza JL, Martinez PR and Petrovick PR. (2009). Development of granules from Phyllanthus niruri spray-dried extract. Brazilian Journal of Pharmaceutical Sciences, 45, 669-675.

[7] Karuppiah P and Rajaram S. (2012). Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian pacific journal of tropical biomedicine, 2, 597-601.

[8] Singh N, Singh BK, Sinha N and Kumar B. (2008). A response to comment on the paper, growth and characterization of new non-linear optical thiourea L-alanine acetate single crystal. Journal of crystal growth, 310, 4487-4492.

[9] Gull I, Saeed M, Shaukat H, Aslam SM, Samra ZQ and Athar AM. (2012). Inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically important pathogenic bacteria. Annals of Clinical Microbiology and Antimicrobials, 11, 8.

[10] Alsaid B, Bessede T, Ferretti L, Pierre M, Bernabe J, Giuliano F, Karam I, Benoit G and Stéphane DS. (2010). effect of a local delivery of triiodothyronine (t3) within neuroregenerative guide on recovery of erectile function in a rat-model of cavernous nerve injury. The journal of sexual medicine, 7, 1798-1806.

[11] Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK and Siddique NA. (2013). A review on therapeutic potential of Nigella sativa: a miracle herb. Asian Pacific Journal of Tropical Biomedicine, 5, 337-52.

[12] Melvin EF, Heinecken C and Guy TJ. (2009). Optimizing tori line designs for pelagic tuna longline fisheries: South Africa. Report on work under special permit from the Republic of South Africa Department of Environmental Affairs and Tourism, Marine and Coastal Management Pelagic and High Seas Fishery Management Division. Washington sea grant.

[13] Fabiana AM, Kivia QDA, Juliana CFDS, Orlando RPA and Marilia OFG. (2015). Review article antioxidant therapy for treatment of inflammatory bowel disease: Does it Work? Redox Biology, 6, 617-639.

[14] Gurbani K, Rahul K, Shruti B, Archana S. (2016). A Review on Dietary antioxidants and their indispensable role in periodontal health. Journal of food and drug analysis, 1-8.
WHO (World Health Organization) Centre for Health Development (2002). Traditional Medicine: Planning for cost-effective traditional health services in the new century- a discussion paper. http://www.who.or.jp/tm/research

Xiao-Nan Y, Imran K and Sun CK. (2015). Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf essential oil of Forsythia koreana deciduous shrub. Asian pacific journal of tropical medicine, 8, 694–700.

Mishra MP, Sibanarayan R, Shasank SS, Goutam G, Debajyoti D and Rabindra NP. (2015). In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against uti causing mdr bacteria. Journal of King Saud University - Science, 1-12.

US Department of Health and Human Services. (2013). Centers for Disease Control and Prevention, Antibiotic Resistance Threats in the United States, US Centers for Disease Control and Prevention, 2013, http://www.cdc.gov/drugresistance/threat-report

Association of Official Analytical Chemists (2010). Official Methods of Analysis of AOAC International. 18th ed. Washington DC: Association of Official Analytical Chemists.

Magdalena K, Agnieszka K, Anna R and Ryszard A. (2007). Extraction and chromatographic separation of tannin fractions from tannin-rich material. Publish journal of food and nutrition sciences, 57, 471–474.

Warra AA, Umar RA, Sani I, Gafar MK, Nasiru A and Ado A. (2013). Preliminary phytochemical screening and physicochemical analysis of Gingerbread plum (Parinari macrophylla) seed oil. Journal of Pharmacognosy and Phytochemistry, 1(2), 20-25.

Warra AA, Wawata IG, Gunu SY and Atiku FA. (2011). Soap preparation from Soxhlet extracted Nigerian cotton seed oil. Advances applied science research, 2(5), 617-623.

CLSI, (2011). Clinical and Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing: twenty-first informational supplement, third 3rd ed. Clinical and Laboratory Standards Institute, Wayne, PA, USA (Document M200–S21).

Ahmed BA, Macalou S, Borrelli F, Capasso R, Fattorusso E, Taglialatela SO and Di-Pietro A. (2007). Nonprenylated rotenoids a new class of potent breast cancer resistance protein inhibitors. Journal of Medicinal Chemistry, 50, 1933-1938.

Kirbag S, Erecevit P, Zengin F and Guvenc AN. (2013). Antimicrobial Activities of Some Euphorbia Species. African Journal of Traditional, Complementary and Alternative Medicines, 5, 305-9.

Murugan R, Prabu J, Chandran V, Sajeesh T, Iniyavan M and Parmelazhagan T. (2015). Nutritional composition in vitro antioxidant and anti-diabetic potentials of Breynia Retusa (Dennst.) Alston. Food science and human wellness, 1-35.

Prakash D, Upadhyay G, Gupta C, Pushpangadan P and Singh KK. (2012). Antioxidant and free radical scavenging activities of some promising wild edible fruits. International Food Research Journal, 19, 1109-1116.

Saetae D and Suntornsuk W. (2010). Antifungal activities of ethanolic extract from Jatropha curcas seed cake. Journal of Microbiology and Biotechnology, 2, 319-24.

How to cite this article
Zubairu AY, Mukhtar M, Saidu I, Ibrahim Z, Isah S, Garga MA and Kebbi HS. (2019). Antibacterial activity of methanolic extract of bitter leaf (Vernonia amygdalina) from various component fractions using column chromatography. GSC Biological and Pharmaceutical Sciences, 7(2), 16-21.