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Research Article

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Assessment of the Phytochemical constituents and *in-vitro* antibacterial activity of *Vernonia amygdalina* extracts on someclinical isolates

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Abstract Antimicrobial resistance is on the rise and efforts are consequently being strengthened towards the search and improvement of sturdier antimicrobial agents with expectant safety and efficacy. This study was conducted to determine the phytochemical analysis and antibacterial activity of *Vernonia amygdalina* (bitter leaf) extracts obtained from a market in Sokoto state Nigeria on some clinical isolates. The efficiency of cold water, hot water and ethanol extracts on *Salmonella* Sp and *Escherichia coli* were evaluated by disc diffusion technique. Ethanolic extracts of *V. amygdalina* had higher inhibitory zones on the. Analysis of the phytonutrients revealed the presence of bioactive constituents. The tested bacterial isolates were susceptible to extract all extracts of the plant. This work confirmed that the leaves of *Vernonia amygdalina* contain bioactive compounds and its antimicrobial activity supports the traditional use of the extractant in diseases management.

Keywords Sokoto, *Vernonia amygdalina*, Antibacterial activity, Phytochemical analysis

Introduction

Active biomolecules naturally found in plants are phytochemicals. These secondary metabolites exert static and cidal activities against microorganisms. Examples of these bioactive constituents are Alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium [1]. A great number of such phytonutrients are used crudely in folk medicine worldwide [2].

The loss in efficiency of antimicrobial agents is on the rise due to the development of resistant strains and some of these agents have unwanted side effects [3]. Efforts are consequently strengthened towards the search and improvement of sturdier antimicrobial agents with expectant safety and efficacy [4].

Bitter leaf (*Vernonia amygdalina*) is the most studied plant of the *Vernonia*genusin the African continent. It belongs to the family *Compositae* with about 200 species. It is a tropical, drought tolerant shrub of about 2 to 5m with a petiolate leaf of about 6 mm in width and elliptic in shape [5]. The leaves are green with a characteristic whiff and bitter taste. Its bitter taste is due to bioactive components like alkaloids, Saponins, tannins and glycosides. It stimulates the digestive system as well as reduces fever [6].

Studies have shown that the nature and quantity of the phytochemicals differ according to the season and geographical location [7].Works have also revealed that bitter leaf extracts exert antibiotic action against gram negative bacteria [8] and [9].

This study seeks to investigate the phytochemical and in vitro antibacterial activity of water and ethanol extracts of *Vernonia amygdalina* obtained from a local market in Sokoto state Nigeria on clinical isolates of *Salmonella* sp and *E. coli*.

Materials and Methods

Collection and authentication of plant materials

The leaves were purchased locally from Sokoto state central market. The plant Species were identified locally as*chusar doki* (*shuwaka*) and scientifically by experts at the herbarium of botany unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto as *Vernonia amygdalina*.

Sample Extraction and Processing

Freshly acquired leaves of *V. amygdalina* were gathered and sorted to remove the dead ones. The desired ones were cleansed with tap water, rinsed with distilled water without squashing to remove debris and dust particles. The leaves were collected and air-dried and milled using a sterile electric blender to get a coarse powder. The powder (30g) were doused in 200 ml of distilled water (cold and hot) and methanol solvents to yield a concentration of 150mg/ml, the mixtures were allowed to stand for 24 hours after which muslin cloth was used to filter the plant residues and the filtrate thus obtained was further purified by filtration through Whatman filter paper No.42 (125mm).The filtered extracts were kept in air tight dark bottle at ambient temperature until required for phytochemical analysis. The extracts obtained were weighed after evaporation of the filtrates [10-12].

Phytochemical screening

The crude extracts obtained were subjected to phytochemical analysis to determine the presence of certain phytochemicals such as tannins, anthraquinones, alkaloids, glycosides, saponin glycosides, cardiac glycosides, saponins, flavonoids and steroids using standard protocols[17-18], Results were described qualitatively as Present, moderately present and not detected.

Test organisms

Pure Isolates of *Salmonella* Sp., and *E. coli was* obtained from Microbiology Department, Usmanu Danfodiyo University teaching hospital Sokoto, Nigeria. The organisms were stored at 4°C on Nutrient Agar Slant and subcultures were made and preserved until used.

Inoculum preparation

A loopful of isolated colonies were inoculated into 4 ml of peptone water, incubated aerobically at 37 °C for 24 hours. The bacterial cell suspension was then adjusted with peptone water so as to obtain turbidity similar to that of 0.5 McFarland standard made by mixing 0.05ml of 1.75% (w/v) barium chloride dehydrate ($BaCl_2$. $2H_2O$) with 9.95 ml of 1% (v/v) tetraoxosulphate (vi) acid (H_2SO_4). This turbidity is equivalent toapproximately10^8 colony forming units per ml (CFU/ml).

Determination of Minimum Inhibitory Concentration (MIC) of the extracts on the test organisms

A Two-fold serial dilution of the test extracts (150 mg/ml) was carried out by transferring 5 ml of the plant extract into 5 ml of sterile nutrient broth to obtain dilutions of 75 mg/ml, 37.5 mg/ml, 18.25mg/ml, and 9.125 mg/ml [13-14]. The respective concentrations were inoculated with 0.1 ml of the bacterial inoculum and incubated at 37 °C for 24 hours. The lowest concentration of the extract with visible turbidity was taken as the Minimum Inhibitory Concentration (MIC).

Preparation of discs

A 6mm paper discs will be prepared using a paper puncture, placed on a Petri dish transferred to a hot air oven and sterilized at 195 degrees for 10 minutes. Twenty microliters of the concentrations 75 mg/ml, 37.5 mg/ml, 18.25mg/ml, and 9.125 mg/ml was transferred into different group of sterilized discs in already appropriately labeled Petri dishes and dried at 35 degrees in a dryer [15-16].

Antibacterial susceptibility test:

In-vitro antibacterial activity was carried out by Kirby Bauer diffusion method. Muller Hinton Agar (MHA) plates were prepared by pouring 20 ml of melted media into sterile Petri dishes. The plates were then allowed to solidify for 5 minutes and 100 microliters of inoculum suspension was dispensed aseptically on the already prepared MHA using a mono channel pipette. After which a sterile swab stick was used to make a bacterial lawn. The inoculum was allowed to dry for 5 minutes. The prepared discs were placed on the surface of medium and allowed to diffuse for 5 minutes. The plates were kept for incubation at 37 ^oC for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Statistical analysis

Analysis of data was done using GraphPad prism (version 7.0, GraphPad software Inc. 1992-2016). The result was presented in tables and charts. Analysis of variance (ANOVA) was used to compare the activity of the extracts.

Results

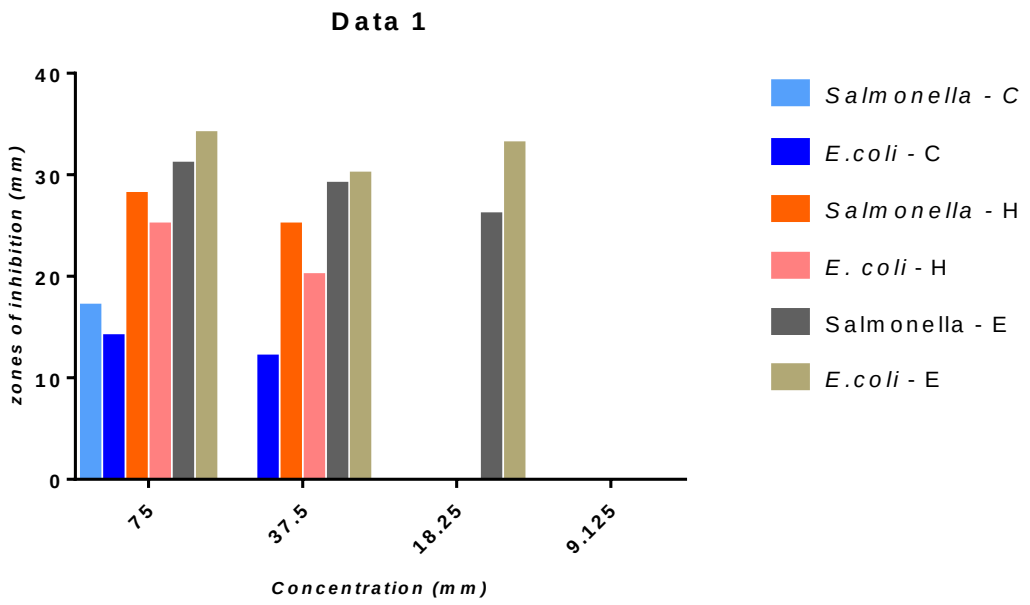
Table 1 shows that the phytonutrients alkaloids, cardiac glycoside, flavonoids, Saponin glycoside, Saponins, steroids, tannins and glycosides were present in extracts of *Vernonia amygdalina* leaves. Conversely, Cardiacglycosides and steroids were not revealed in hot water extracts. In addition, anthraquinones was not detected in all of the extracts. In table 2 the ethanol leaf extracts presented minimum inhibitory concentration (MIC) against *Salmonella* sp and *E. coli* at 18.25 mg/ml while the hot water extract showed MIC against the test isolates at 37.5 mg/ml.The cold-water extract conversely showed MIC against *Salmonella* sp. and *E. coli* at concentrations of 75 mg/ml and 37.5 mg/ml respectively. Antibacterial profile of *Vernonia amygdalina* revealed that the ethanolic extracts of had higher inhibitory zones than the water extracts (Figure 1). All extracts (ethanol, Hot water and cold water) inhibited the growth of test organisms. Ethanolic extract of had a greater zone of inhibition (34 mm) at 75mg/ml against *E. coli* and lowest (26 mm) at 18.25 mg/ml. The hot and cold-water extracts both had their highest inhibition on *Salmonella sp*. (28 mm and 17 mm respectively) at 75mg/ml and their lowest zone of inhibition (20 mm and 12 mm respectively) at 37.5mg/ml. All the extracts were active at 75mg/ml concentration but no activity was observed at 9.125 mg/ml.

Table 1: Phytochemical analysis of *Vernonia amygdalina*

Phytochemicals	**Extracts of *Vernonia amygdalina***		
	Ethanol	**Hot water**	**Cold water**
Alkaloids	Moderately Present	Present	Present
Anthraquinone	Not detected	Not detected	Not detected
Cardiac glycosides	Present	Not detected	Moderately present
Flavonoids	Present	Present	Present
Saponin glycoside	Present	Present	Present
Saponins	Present	Present	Present
Steroids	Moderately present	Not detected	Present
Tannins	Present	Present	Present
Glycosides	Extremelypresent	Present	Moderately present

Table 2: Minimum inhibitory concentration of *Vernonia amygdalina*

Extracts	**Concentration (mg/ml)**	**Organisms**	
		***Salmomella* sp.**	***E. coli***
Ethanol	75	++	++
	37.5	++	++
	18.25	++	++
	9.125	-	-
Hot water	75	++	++
	37.5	++	++
	18.25	-	-
	9.125	-	-
Cold water	75	++	++
	37.5	-	++
	18.25	-	-
	9.125	-	-



Key; C= Coldwater extract; H = Hot water extract; E= Ethanol extract

Figure 1: Antibacterial activity of Vernonia amygdalina

Discussion

Phytochemicals like Alkaloids, flavonoids, Saponins and tannins found in this study (all extracts) has been soundly documented for their anti-microbial activity. This may explain the robust bioactivity of all extracts against the test isolates. These phytochemicals exert their activity by forming complex with bacterial cell wall by binding to adhesions (e.g. flavonoids), enzyme inhibition, substrate deprivation, membrane disruption, metal ion complexation (e.g. Tannins) and by intercalation into cell wall and DNA of parasites (Alkaloids). This outcome is consistent with the results of a study by [6].

From this study, it was observed that bioactive components are abundant in these leaves and that the ethanol extracts exhibited higher inhibitory activity on the test organisms. The ethanolic extract of *Vernonia amygdalina* presented the lowest MIC (18.25mg/ml) and had the most bioactivity against the test isolates (*salmonella* sp. and *E coli*) compared to the aqueous extracts (hot and cold water). This agrees with the work of [9]. This observed difference can be attributed to the presence of higher amounts of bioactive constituents as compared to aqueous extracts. Additionally, ethanol finds it easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material due to its polarity [19]. Also, nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds that are most often obtained through ethanol extraction processes [20].

Statistical analysis (Two-way ANOVA with multiple comparison) revealed that there is a significant difference (P=0.0123) between the activity of ethanolic and aqueous (hot and cold) extract. Phenolic compounds like flavonoids, tannins realized in this study, are produced in plants fairly as a result of physiological and ecological pressures like pathogenic microbes, insect attack and radiation [21]. Therefore, an explanation for the relatively reduced activity of aqueous extract in this study can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in ethanol they are inactive [22]. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol [22].

There was no statistical significance between the hot and cold-water extracts (P=0.1548) in this study. However, the relatively higher activity of hot water extracts as compared to cold water extract observed may be because of some basic oil materials that could have melted in hot water extracts which have the ability to probe the cell wall of Gram negative isolates (*Salmonella sp* and *E. coli*) and can interfere with the workings of cytoplasmic membrane. The presence of the more active substances in hot water extracts than cold water that can break the surface of the outer membrane of the bacteria-rich multiple liposaccharide molecules may have correspondingly contributed to its higher activity. This is consistent with the results obtained in previous studies. The findings of this study concur with that of several researchers that demonstrated that *V. amygdalina* extracts have an antibacterial activity against several species of bacteria [23].

Conclusion

The traditional use of both water and ethanol extracts of these plants was offered a logical explanation in this study. Our findings also corroborate the traditional claim that these medicinal plants are better extracted in ethanol. The results obtained also further assist in the accumulation of epidemiological information on the vulnerability of *Salmonella sp and E. coli* within the community to the extracts.

References

1. Muttalib, L.Y. and Naqishbandi, A.M., (2017). Antibacterial and Phytochemical Study of Iraqi Salvia Officinalis Leave Extracts. *Iraqi Journal of Pharmaceutical Sciences*, *21*(1), 93-97.
2. Ramdane, F., Essid, R., Mkadmini, K., Hammami, M., Fares, N., Mahammed, M.H., El Ouassis, D., Tabbene, O., Limam, F. and Hadj, M.D.O., (2017). Phytochemical composition and biological activities of Asteriscus graveolens (Forssk) extracts. *Process Biochemistry*, *56*, 186-192.
3. Granados-Chinchilla, F. and Rodríguez, C., (2017). Tetracyclines in Food and Feeding stuffs: From Regulation to Analytical Methods, Bacterial Resistance, and Environmental and Health Implications. *Journal of analytical methods in chemistry*, https://doi.org/10.1155/2017/1315497.
4. Hussain, Z., Thu, H.E., Amjad, M.W., Ahmed, T.A. and Khan, S., (2017). Exploring recent developments to improve antioxidant, anti-inflammatory and antimicrobial efficacy of curcumin: A review of new trends and future perspectives. *Materials Science and Engineering: C*, 77, 1316-1326.
5. Oshim, I.O., Desmond, C.O., Nwobu, R.A.U., Ezugwu, U.M. and Urama, E.U., (2016). Kinetics of Minimum Inhibitory Concentration, Minimum Bactericidal Concentration and Minimum Fungicidal Concentration of Vernonia amygdalina (Bitter leaf) on Microorganisms Isolated from Wound Infections. *International Journal of Surgical Research*, *5*(1), 8-14.
6. Usunobun, U. and Ngozi, O., (2016). Phytochemical analysis and proximate composition of Vernonia amygdalina. *International Journal of Scientific World*, *4*(1), 11-14.
7. Kumar, S., Yadav, A., Yadav, M. and Yadav, J.P., (2017). Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm. f. *BMC research notes*, *10*(1), 60.
8. Walugembe, J., Iramiot, J.S. and Katuura, E., (2016). Indigenous knowledge and antibacterial activity of selected herbs used locally to treat common cold in Central Uganda. *Journal of Medicinal Plants Research*, *10*(31), 520-528.
9. Anyanwu, M.U. and Okoye, R.C., (2017). Antimicrobial activity of Nigerian medicinal plants. *Journal of intercultural ethnopharmacology*, *6*(2), 240.
10. Okigbo, R.N. and Nnadiri, P.C., (2017). Effect of Three Tropical African Plants on some Fungal Rot of Stored Cocoyam (*Colocasia Esculenta* L). *Technology*, *13*(2), 183-203.
11. Junaid, S.A., Olabode, A.O., Onwuliri, F.C., Okwori, A.E.J. and Agina, S.E., (2006). The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *African journal of Biotechnology*, *5*(22), 2315-2321.
12. Harborne, J.B., (1984). Methods of plant analysis. In *Phytochemical methods* (pp. 1-36). Springer

Netherlands.

13. Alade, P.I. and Irobi, O.N., (1993). Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. *Journal of Ethnopharmacology*, *39*(3), 171-174.
14. Nafeesh Ahmed, K.L. & Mathur, A., (2017). Study of the Antimicrobial Profile and Phytochemical Composition of Solvent Extracts of Leaves and Female Cones of *Cycas revoluta*. International Journal of Current Microbiology and Applied Sciences, 6(4), 2514–2522.
15. Rios, J.L., Recio, M.C. and Villar, A., (1988). Screening methods for natural products with antimicrobial activity: a review of the literature. *Journal of ethnopharmacology*, *23*(2-3), 127-149.
16. Paterson, (1999). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. . J. B. Harborne. 15 x 23.4 cm, 302 pp. London: Chapman & Hall, 1988. Price f79. ISBN 0-412-57260 (hardback). Plant Pathology, 48(1), 146–146.
17. Evans, W.C. & Evans, D., (2009). Biological and geographical sources of drugs. Trease and Evans' Pharmacognosy, 15–17.
18. Vijayan, C.H.I.T.H.R.A., Adersh, M., Reji, S.R. and Nair, G.M., (2013). Screening biological activities of Orthosiphon aristatus. *Int J Pharm Pharm Sci*, *5*(4), 594-600.
19. Gurjar, M.S., Ali, S., Akhtar, M. and Singh, K.S., (2012). Efficacy of plant extracts in plant disease management. *Agricultural Sciences*, *3*(3), 425.
20. Kennedy, D.O. and Wightman, E.L., (2011). Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition: An International Review Journal*, *2*(1), 32-50.
21. Gouzi, H., Depagne, C. and Coradin, T., (2011). Kinetics and thermodynamics of the thermal inactivation of polyphenol oxidase in an aqueous extract from Agaricus bisporus. *Journal of agricultural and food chemistry*, *60*(1), 500-506.
22. Lapornik, B., Prošek, M. and Wondra, A.G., (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of food engineering*, *71*(2), 214-222.
23. Anibijuwon, I.I., Oladejo, B.O., Adetitun, D.O. and Kolawole, O.M., (2012). Antimicrobial activities of Vernonia amygdalina against oral microbes. *Global journal of Pharmacology*, *6*(3), 178-185.