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Phytochemical Analysis and Antibacterial Profile of Boerhavia Adscendens

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ABSTRACT

The phytochemical and antibacterial profiles of the ethanolic extract of B. adscendens was carried out using standard established procedures. The result of the qualitative phytochemical analysis revealed the presence of Alkaloid, Tannins, Glycosides, Steroids, Flavonoid and Phenol while Saponins, Terpenoid and Anthraquinone were not detected. The phytochemicals detected were quantitatively determined; Alkaloid content 0.91± 0.04mg/g, total phenolic 0.112±0.06 mg/g and total Tannins 0.87±0.03mg/g of extract. Antibacterial activity revealed the zone of inhibition of Klebsiella pnemoniae 7.13±0.34mm, Shigella dysentriae 9.2±1.13 mm, Proteus vulgaris 8.6±0.59 mm, E. coli 8.6±0.55 mm, Salmonella typhi 7.63±0.41 mm at 50 mg/mlwhile at 100 mg/ml Klebsiella pnemoniae 9.3±0.18, Shigella dysentriae 12.5±0.59 mm, Proteus vulgaris 10.6± 0.31 mm, E. coli 11.6± 0.26 mm, Salmonella typhi 13.9±0.60 mm. This study shows the ethanolic extract of B. adscendens demonstrated the presence of phytochemical compounds with antibacterial properties. The study therefore lays credence to the use of B. adscendens in traditional medicine practice.

Keywords: Phytochemical, antimicrobial, analysis, Boerhavia Adscendens

I. INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Sule et al., 2010). Countries in Africa, Asia and Latin America use traditional medicine to help meet some of their basic primary healthcare needs. A medicinal plant is any plant in which one or more of its organs contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. For instance, the people of South-eastern Nigeria and other West African countries utilize the plant Heinsia crinata for both food and for the treatment of ailments including typhoid fever, diarrhea, candidiasis e.t.c. (Andy et al., 2008).

In Africa today, up to 80 % of the population uses traditional medicine in primary healthcare (WHO, 2006). Many African plants are used in traditional medicine as antimicrobial agents but only few have been documented. In spite of vast improved healthcare system in the United States and Europe, millions of their citizens are turning back to traditional herbal medicines to prevent or treat many illnesses (WHO, 2006), so as to overcome drug resistance of many human pathogens to conventional antibiotics, suppress side effects like hyp

ersensitivity and Immune disorders. In Nigeria, traditional medical practitioners use a variety of herbal preparation to treat different microbial diseases, examples are pneumonia, meningitis etc (Sule et al., 2010). The plant kingdom has for long served as a prolific source of helpful drugs, food, additives, flavouring agents, colorants binders and lubricants etc. As a matter of fact, it was estimated that about 25 % of all prescribed medicines today are substances derived from plants (Sule et al., 2010), for the control of microbial infections and other diseases. Various synthetic drugs and chemical formulations have been used, but due to indiscriminate use, microbes have developed resistance against these synthetic drugs with broad-spectrum of activity. Consequently, there is a renewed impetus in the search for raw material resources containing useful compounds that are effective against drug resistant pathogens with minimal side effects when administrated to patients. The acceptance of traditional medicine as an alternative form of healthcare delivery compelled Researchers for further investigation of pharmacological properties of medicinal plants (WHO. 2006).

In recent years, research on medicinal plants had attracted a lot of attention globally, (Kumar et al., 2008) Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternative medicine systems for treatment of human diseases, like typhoid fever, dysentery and abdominal pains etc. (Sule et al., 2010). The chemical constituents in medicinal plant usually explain the rationale for the use of the plants in traditional medicine (Aliyu and Chedi, 2010). Phytochemical studies have also attracted the attention of plant scientist due to development of new and sophisticated techniques. These techniques played a significant role in the search of raw materials for pharmaceutical industries (Jiofac et al., 2009). Plants synthesize a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. With the development of natural product chemistry, the potential of chemotaxonomy is now becoming increasingly obvious (Jiofac et al., 2009).

II. METHODS AND MATERIAL

A. Apparatus and Reagents

Otary evaporator, Autoclaving machine, Incubator, UV Visible spectrophotometer, Refrigerator, weighing balance, Petri dishes, Pipettes of various sizes, 5ml multichannel pipette, Syringes of various sizes, Sterile universal bottles, Centrifuge tubes, Beakers of various sizes, Test tubes, Funnels, Whatman filter papers of various sizes; Mayer's reagent, Molish reagent, Wagner's reagent, Conc.H_2SO_4, Conc.HNO_3, HCl, NaOH, $FeCl_3$, Chloroform, Ethanol, Gallic acid.

B. Plant Collection and Identification

The plant Boerhaavia adscendens herb was collected in Tsohuwar Kasuwa, Gombe, Gombe State Nigeria from Herbalists. The plant was identified by Botanist at Biological science department of Gombe State University. The plant was allowed to dry under the shade and grounded into coarse powder using grinding machine. The powdered plant material was kept in black polythene bags until required for use. The voucher specimen was deposited in the Herbarium of Biology Department of Gombe State University, Nigeria.

C. Extraction of Plant Sample

The coarse powder of Boerhaviaadscendens was extracted at room temperature with Ethanol for 5 days using maceration method. The extract was filtered using Whatman no 1 filter paper and then concentrated to dryness with rotary evaporator under reduced pressure.

D. Qualitative Phytochemical Analysis

Phytochemical Analysis: Phytochemical examinations was carried out on the extract using standard established methods as indicated below.

a. Detection of Alkaloids

2 ml of 1%HCl was added to 2ml of extract in a test tube and divided in to 2 equal portions:

a) 1ml of Mayer`s reagent was added to one portion and a creamy precipitate indicates the presence of alkaloids;
b) The second portion is treated with Wagner`s reagent, formation of brown precipitate indicates the presence of alkaloids.

b. Detection of Glycosides

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of glycosides.

c. Detection of Saponins

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

d. Detection of Phytosterols

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

e. Detection of Phenols

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

f. Detection of Tannins

Add 2ml of 5% $FeCl_3$ solution to 5ml of the ethanolic extract in a test tube. A greenish-black precipitate indicates the presence of tannin.

g. Detection of Flavonoids

a) Alkaline Reagent Test (Tiwari et al., 2011): Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Shinoda test (magnesium hydrochloride reduction test) (De et al., 2010). To the test Solution, add few fragments of magnesium ribbon and add concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

h. Detection of Terpenoids

5ml of the extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids (Sathyaprabha et al., 2011).

i. Detection of Steroids

2ml of acetic anhydride was added to 0.5 g ethanolic extract with 2 ml H_2S0_4. The colour changed from violet to blue or green indicating the presence of steroids (Sathyaprabha et al., 2011).

j. Detection of Anthraquinones

About 0.5g of extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool; equal volume of chloroform was added to the filtrates. Few drops of 10% NH_3 were added to the mixture and heated. Formation of rose-

pink colour indicates the presence of Anthraquinones (Sathyaprabha et al., 2011).

E. Quantitative Phytochemical Analysis

The amounts of Phytochemicals which are present in the Ethanol extracts of Boerhavia adscendens were determined and quantified by standard procedures.

a. Determination of Total Phenolic Content

100 µl (1mg/ml) of the plant sample was mixed with 1.5ml of folin-ciocalteu reagent (FCR); this was allowed to stand for 2minutes and then 1.2ml of (20%) Na_2CO_3 was added. This reaction mixture was left for 30minutes after which the absorbance was read at 765nm by using spectrophotometer (6405UV/Vis.). The total phenolic content(x) was calculated from the equation of calibration curve using gallic acid as standard. (Mdlolo,2009).

b. Determination of the Total Tannin content

Procedure: 5g of the finely ground sample was weighed and transferred into 250 mL conical flask and 50 mL of distilled water added and shook vigorously for an hour. The resulting solution was filtered into a volumetric flask and 5 mL of the filtrate pipetted out into a test tube. 0.1g of tannic acid was dissolved in 100 mL of water to form tannic acid solution. 5 mL of the tannic acid solution was pipetted out into another 50ml volumetric flask. A blank sample was also prepared using 5ml of distilled water. The three samples were incubated for 1.5 hours at 20 – 30^{0}C and the sample was then filled with distilled water up to mark of 50 mL of the volumetric flask. The absorbance of the three samples was measured at 760 nm using spectronic 21D. The values generated were used to calculate the tannin content (Igidi and Edene, 2014).

c. Determination of Alkaloid Content

Procedure : Applying alkaline precipitation gravimetric method, 5.0g of the sample was weighed out into a conical flask; 200ml of 10% acetic acid in ethanol was measured out and added into the conical flask containing the sample. The mixture was allowed to stand at room temperature for 4 hours before it was filtered through a filter paper. The filtrate was then reduced to a quarter of its original volume by evaporation over a steam bath. The alkaloid in the extract was precipitated by drop wise addition of concentrated NH_4OH until full turbidity was obtained. The precipitate was washed with 1% NH_4OH solution, dried in the oven at 1000c for an hour; it was cooled in a desiccator and reweighed. By difference, the weight of alkaloid was determined and expressed as a percentage of the sample analyzed (Harbone, 1973).

F. Agar Disc Difussion Method for the Antibacterial Activity

The antibacterial activity of Boerhaavia adscendens was evaluated by agar disc diffusion method and performed in accordance with the guidelines of National Committee for Clinical Laboratory Standard. A18/24-hour old culture of E. coli, S. aureus and P. aeruginosa were mixed with sterile physiological saline (0.85%) and the turbidity was adjusted to the standard inoculums of 0.5 McFarland standard. Petri dishes containing Mueller Hinton agar were used to inoculate the bacterial suspension. Filter paper disc (Whatman no. 1. Diameter 6mm) was impregnated with the extract solution (50mg/ml) and placed on the inoculated agar petri dishes and incubated for 24hours at 37^{0}C. A paper disc of Erythromycin (0.128mg/ml) was used as control. The inhibition zone diameter was measured in millimeters.

a. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of the extracts were determined using the tube dilution method (Baker and Silverton, 1993). Dilution of the plant extracts were incorporated in nutrient broth in 1:1 ratio initial rough estimates of the MIC values of the plant extracts against the test organisms were estimated to determine the range of MIC values.

Consequently, the following concentrations were prepared for each extract, using the dilution formula: 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78mg/ml. in addition, 0.1ml of standard suspension of the test organisms was added to each tube. The tubes were incubated at 37ºC for 24 hours. A tube containing extract and growth (turbid solution) or absence of growth (clear solution) at the end of incubation period was recorded. The lowest concentration of the extracts showing no growth was regarded as the minimum inhibitory concentration (MIC).

b. Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration, (MBC) were determined by sub culturing the last test dilution that showed visible growth (turbidity) and all others in which there was no growth on fresh extract solid medium and incubated for 24 hours. The highest dilution in that showed no single bacterial colony was taken as the minimum bactericidal concentration (MBC) as reported by (Baker and Silverton, 1993)

III.RESULTS AND DISCUSSION

A. Qualitative Phytochemical Screening

Various phytochemical compounds were screened qualitatively in the ethanolic extract of B.adscendens (Table 1)

Table 1 : Result of the Qualitative Phytochemical Screening of the Ethanolic Extract of B. adscendens (Babban Juji)

Phytochemical constituents	Status
Alkaloid	+
Flavonoids	+
Tannins	+
Saponins	-
Terpenoid	-
Glycosides	+
Anthraquinone	-
Steroids	-
Phenols	+

Key: + (plus) indicates present and –(negative) indicate not detected.

The ethanol extract of B. adscendens was screened for the presence of secondary metabolites. The result of qualitative phytochemical screening showed the presence of alkaloid, flavonoid, tannins, phenol and glycoside. Saponin, steroids, anthraquinone and terpenoidswere not detected in the ethanol extract. This also correspond to the work of Abdul (2018). He detected the presence of phenols, flavonoid, alkaloid, tannins and saponins in the crude extract of B. procumbens. Some of the phytoconstituents detected in B. adscendens were also reported to exist in other species of B. adscendensand endows the plant with several pharmacological propertiessuch as anti-inflammatory, anti-oxidant, antiviral, analgesic and antiangiogenic properties (Sarin et al., 2014). Phenolic constituents are the major plant metabolites responsible for free radical scavenging ability. Flavonoidshad shown considerable chelating properties and to suppresses carcinogenesis in various animal models (Manthey et al., 2001). Phenolic and flavonoid metabolites possess diverse biological activities such as anti-carcinogenic, anti-atherosclerotic, anti-inflammatory and radical scavenging properties. Flavonoids, alkaloids, glycosides and steroids had shown anti-stress activity (Desai et al., 2009).

B. Quantitative Phytochemical Analysis

The total phenol, total alkaloid and total tannin compound in the crude extract of B. adscendens are presented in table 2, figure 1 and 2.

Table 2 : Result for qualitative phytochemical analysis of the ethanol extract of B.adscendens (Babban juji)

S/N	Phytochemical constitute	Quantity (amount)
1	Total phenol compound	0.112±0.06mg/g
2	Total alkaloid	0.91±0.04mg/g
3	Total tannin	0.87±0.03mg/g

The phytoconstituents detected in B. adscendens were quatitative determined yielding alkaloid content as 0.91±0.04mg/g total phenolic as 0.112±0.06 mg/g and total tannins as 0.87±0.03mg/g. B. adscendens contain significant amount of alkaloid, flavonoid and polyphenols. Natural antioxidants mainly come from plants in the form of phenolic compound such as flavonoids and phenolic acid (Ali et al., 2008). Abdul (2018) found out that the total phenolic compound in B. procumbenmethanolic extract was 82.80 ± 0.08 mg/g which is much greater than the one obtained in B. adscendens.

C. Antibacterial Activity

Crude extract B.adscendens were screened for antibacterial activity (table 3) the crude extract showed different zones of inhibition on clinical isolate. All values are expressed as mean ± standard deviation. (n=3)

Table 3: Result of the Antibacterial Activity of the Ethanolic Extract of Boerhavia adscendens

Micro-organism	Inhibition zone (mm) 50 mg/mL Extract	Inhibition zone (mm) 100 mg/mL Extract	Inhibition zone (mm) Erythromycin
Escherichia coli	8.6±0.55	11.6±0.26	17.9±2.05
Proteus vulgaris	8.67±0.59	10.65±0.68	26.57±1.52
Shigella dysentriae	9.2±0.60	12.5±0.70	24.3±1.65
Klebsiella pnemoniae	7.13±0.34	9.33±0.18	36.47±3.30
Salmonella typhi	7.63±0.26	13.97±0.60	34.47±2.45

The antibacterial activity showed that the ethanol extract at 100 mg/ml exhibited highest activity of 13.97± 0.60 mm on S. typhi while E. coli and P. vulgaris exhibit activity in the range of 10 – 12 mm. K. pneumonia has the least activity at 9.33 ± 0.18 mm, while E. coli has a highest value of both the MIC (6.25) and MBC (12.25). This shows that E. coliis the most resistant pathogen among the isolates under test. The in-vitro antibacterial test showed broad spectrum of activity against the bacterial strains studied.The result obtained is in line with the studies conducted by Agrawal et al., (2003), who investigated the ethyl acetate extracts of aerial parts and roots of B. diffusa against M. gypseum, M. fulvum and M. canis using broth dilution method. Rahman et al., (2014) screened the crude methanolic extract of B. repens against S. aureus, B. subtilis, B. cereus, E. coli, V. cholera, S. typhimurium as well as fungal strains such as C. albicans and A. brasiliensis using agar disc diffusion method. The extract showed mild activity against S. aureus (12.17mm) while moderate activity was observed against S. typhimurium (17.12 mm) and C. albicans (16.77mm). Sheila et al., (2013) also evaluated methanolic extract of leaf and root of B. coccinea against B. anthracis, S. pyogens, V. cholera, S. dysentriae, C. albicans and C. neoformans. Moderate inhibitory activity was found against C. neoformans (MIC 0.625 mg/ml) and C. albicans (MIC 1.250 mg/ml) by leaf and root extracts,respectively. Both extract, exhibited weak activity against B. anthracis whereas, S. pyogens, V. cholera, S.dysenteriae were resistant at the maximum concentration of 5mg/ml. Gautam et al., (2016) tested the n-hexane, ethyl acetate (EtOAc) crude extracts of B. diffusa against S. aureus, B. cereus, S. typhi and E. coli. EtOAc extract showed no activity against E. coli whereas S. aureus and S. typhi were resistant against crude hexane extract. Baskaran et al., (2011) observed the crude extracts of B. diffusa against S. aureus, B. cereus, M. luteus (Gram positive bacteria), E. coli, P. aeruginosa, K. pneumonia (Gram-negative bacteria) and fungal strains like A. flavus, A. Niger, and C. albicans.

The ethanol extract showed activity against S. aureus (11 mm) and E. coli (9mm).The crude extract of B.adscendens studied may be used for treatment of

infections causes by the pathogens tested. This also contains the relevance of B. adscendens in traditional medicine.

3.1.4 MIC and MBC of B. adscendens On Some Selected Clinical Isolates

Table 4 : Result of MIC and MBC for the Test of Organism to the Boerhavia adscendens Extract.

Organism	MIC (mg/mL)	MBC (mg/mL)
E.coli	6.25	12.25
S. dysentriae	3.12	6.25
K. pnemoniae	3.12	6.25
P. vulgaris	0.78	1.56
S. typhi	1.56	3.12

IV.CONCLUSION

The phytochemical analysis of B. adscendens revealed the presence of secondary metabolites which are known to be active components responsible for observed therapeutic effects in medicinal plants (Sarin et al., 2014). From this study,B. adscendens is active against E.coli, Salmonella typhi and Shigella dysenteriae. This implies that B. adscendens can be used to treat infectious diseases caused by these microorganisms. However, the observed antibacterial activity cannot be attributed to a particular chemical compound present in the plant extract.

Conflict of interest

No conflict of interest declared

V. REFERENCES

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