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Original Research Article

Isolation and Characterization of the Chemical constituents of Leaves of *Boerhavia coccinea*

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ABSRTACT

The aim of this study is the qualitative phytochemical evaluation, column chromatographic fractionation using different solvent systems, isolation and characterization, and TLC analysis of fractions of the whole plant of *B. coccinea*. The whole plant of *B. coccinea* was collected from Oria Abraka, Delta State, and authenticated in the Department of Botany, Delta State University, Abraka, Delta State, Nigeria. The collected sample was thoroughly washed under running tap water, air dried to a constant weight, and ground to powder using a mechanical blender. About 60 g of the ground powder was weighed using a weighing balance and extracted with redistilled n-hexane using Soxhlet apparatus. The extract was then redistilled to recover the solvent and gum was obtained. Qualitative phytochemical screening of the extract was carried out according to the standard analytical method, Column chromatographic fractionation of the extract was done using silica gel as stationary phase with different solvent system of n-hexane/chloroform and chloroform/methanol at 100%, 7:3, 1:1, and 3:7 ratios. Further TLC purification of separated fractions was carried out. The study revealed the presence of some secondary metabolites (alkaloids, steroids, saponins, flavonoids and tannins). Fraction A, B and C with weight 1.02 g, 1.04 g and 1.98 g had retention factors of 0.90, 0.86 and 0.79 respectively when analyzed using the TLC. Column chromatographic separation revealed the presence of Ar-OH (1), C-C (2), OH (2), R-C=N (1), C=C (1), Ar-C=C (1), R-O-R (1), and C-O-H (1) functional groups in all 3 fractions obtained.

Keywords: Chromatography, Ethnomedicine, Phytochemicals, Fractionation

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Introduction

Boerhavia coccinea (fam. Nyctaginaceae), commonly called scarlet spiderling or red spiderling, is reported to have many therapeutic benefits, including the management of hepatitis, pain, and inflammation, as well as oxidative stress as a result of its antioxidant activities. Its antimicrobial potential is employed in the treatment of urethritis, gastroenteritis, asthma, scabies, skin rashes, and oral candidiasis.[1] In ethnomedicine, *B. coccinea* is used in Abraka, Delta State, Nigeria, as anthelmintic for the treatment of veneral diseases, toothache, cough and measles.[2] The medicinal uses of *B. coccinea* is due to their phytochemical constituents, such as alkaloids, steroids, saponins, flavonoids, tannins, and terpenoids, which on isolation and characterization, may serve as precursors for the development of lead compounds, which may serve as templates for the discovery of new drugs.[3] *B. coccinea* is used to treat liver, pain, urinary and gastroenteric diseases, prolapsed uterus, asthma, scabies, skin rashes, small pox, oral candidiasis, aphthous ulcers, toothache and pneumonia.[4] The methodology employed in this study therefore involves preliminary qualitative investigation of secondary metabolites, fractionation and characterization of the leaves of *B. coccinea*.

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Further study may include structural elucidation of bioactive compounds. The aim of this study therefore is the qualitative phytochemical analysis of the methanolic extract of *B. coccinea* to justify its potential for ethnomedicinal uses, fractionation, isolation and characterization of the extract's using different solvent systems and the identification of the most suitable solvents for structural determination of bioactive compounds present in the plants.

Materials and Methods

Reagent specification

Methanol (99.85%, JHD, China), n-hexane (95%, Qualikems, India), silica gel (Chemical Laboratory CAS No. 112926-00-8), chloroform (99.0%, JHD, China), spray reagent, Vanillin, sulphuric acid (98%, Loba Chem).

Instrument/materials specification

Mechanical blender (QASA, Model: QBL-15L40), weighing balance (Shimadzu Coorporation, Model: TX4202L), column, micropipette, filter paper (Whatman 110mm), hot air dryer (Nickel Electro Ltd, Serial No. 80102), UV light (VIQUA, Canada, Model: 602806), hot air oven (Leader Model: GP/50/CLAD/250/HYD), rotary evaporator (Yangmai, China, Model: YMRE-2), beaker.

Sample Collection and Identification

The whole weed plant of *B. coccinea* was collected in Abraka, Delta State, Nigeria on the 10th of February, 2024 and authenticated in the Department of Botany, Delta State University, Abraka, Delta State, Nigeria with voucher number DELSUH302.

Sample Preparation and Extraction
The collected sample was thoroughly washed under running tap water to remove dust, soil particles and debris and air dried until a constant weight was observed. The dried sample was ground to uniform powder particle size using mechanical blender (QASA, Model: QBL-15L40). About 60 g of the powdered sample was weighed using a weighing balance and extracted with redistilled n-hexane using soxhlet apparatus. The extract was then redistilled to recover the solvent and a gum was obtained.

Phytochemical Screening
Qualitative phytochemical analysis of the extract was carried out for the presence of tannins, saponins, sterols, glycosides, alkaloids, phenols, flavonoids and anthraquinones according to the standard analytical methods described by Samell et al.[5]

Fractionation of Boerhavia coccinea Crude Extract using selected Solvent System in Column Chromatography
A medium sized column of diameter 4.0 cm and length 39.0 cm was used. The column was packed with 250 g of column silica gel of Merck 60:120 as the stationary phase. About 1 g of the gum like extract was weighed and dissolved in 10 mL of chloroform and placed on top of the packed column using a pipette and eluted with the following solvents 100% n-hexane, 7:3 n-hexane/chloroform, 1:1 n-hexane/chloroform, 3:7 n-hexane/chloroform, 100% chloroform, 7:3 chloroform/methanol, 1:1 chloroform/methanol, 3:7 chloroform/methanol, 100% methanol. As the mobile phase. Each of the eluted fractions was collected in 5 (10 mL) sterile sample containers. The eluents that gave the best separation was noted.

Analytical Thin Layer Chromatography and Bulking of Fractions
The 45 fractions obtained from the column chromatographic fractionation were bulked using TLC. The stationary phased used is precoated silica gel aluminum plate, n-hexane, chloroform and methanol as mobile phases with a 1.0 micropipette a spot of the sample was applied on the TLC plate 1 cm from the edge and air dried at room temperature. With the same pipette a smaller spot of the sample was applied to improve their separation. The plate was then developed in a glass chromatographic tank containing the solvents system lined with thick filter paper. The plant was then covered with a glass lid and the solvent was allowed to migrate until the solvent front was about 75% of the length of the plate. The plate was further dried by hot air dryer and viewed under UV light at 290, 271 and 261 nm to identify the fluorescent spot which was marked and then sprayed with spray reagent, 0.16 g vanillin in 30 mL concentrated H_2SO_4 for clear visibility of fluorescent spot, the plate was placed in a hot oven at 120°C for 3 seconds and the colour reaction recorded. The Rf was then calculated as follows[4]

$$Rf = \frac{\text{Distance travelled by the sample from the starting point}}{\text{Distance travelled by the solvent from the starting point to the solvent}} \ldots\ldots eqn\ 1$$

From the thin layer chromatography separation and their determined retention value, fractions with close TLC mobility profile and band were pooled and further analyzed using TLC. The bulked fractions placed in a beaker was then evaporated using a rotary evaporator (Yangmai, China, Model: YMRE-2) at 30°C, very low pressure and weighed with a weighing balance.

Infrared Analysis of B. coccinea
Infrared analysis of purified compounds of methanolic extract of *B. coccinea* obtained from TLC separated fractions was carried out using Fourier Transform Infrared Spectroscopy (FTIR).

Result and Discussion

The result of the phytochemical analysis and fractionation of the *Boerhavia coccinea* sample are presented in Tables 1 to 5 and figure 1 – 4.

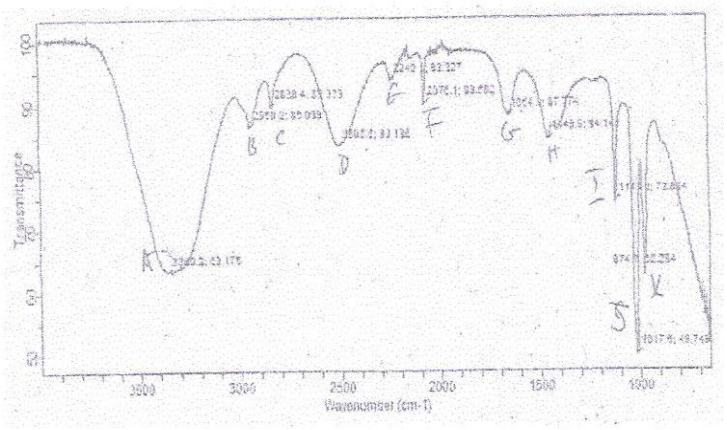

Figure 1: IR Spectrum of Fraction A

Phytochemical Determination
The result from Table 1 shows the presence of the following phytochemicals; alkaloids, steroids, saponins, flavonoids, tannins, with terpenoids and glycosides not detected. These secondary metabolites found maybe invariably responsible for the medicinal and therapeutic benefits of *B. coccinea*.
Alkaloids are naturally occurring organic compounds with nitrogen atoms known to have CNS effects such as sedation, reducing symptoms associated with stress and depression, it also has stimulant effect e.g. nicotine it also have antimicrobial, analgesic, cardiovascular benefit such as antiarrhythmia.[6]
Steroids found in extract of *B. coccinea* may be useful as an anti-inflammatory, antibacterial and immunosuppressant.[7] Saponins may have anti-lipophilic effects antibacterial and analgesic activities.[8] Among the array of secondary metabolites found in plant, flavonoids have the widest range of medicinal uses.[9] It has been reported that among its numerous medicinal uses flavonoids have anti-inflammatory, antioxidants, antibacterial, anti-allergy, it also has antiviral, cardio-protective and neuroprotective effect.[10] Tannins are reported to have bactericidal effects and is very popular in treatment of intestinal infection and intestinal bleeding.

Table 1: Phytochemical Constituents of *Boerhavia coccinea* Sample

Phytochemicals	Inference
Alkaloids	+
Steroids	+
Saponins	+
Flavonoids	+
Tannins	+
Terpenoids	-
Glycosides	-

Key: + = present, - = absent

Fraction of Bioactive Constituents
Medicinal plants possess a plethora of phytochemicals which may have many therapeutic values, interact negatively with useful secondary metabolites and may sometime be cytotoxic or poisonous which may undermine their medicinal uses and efficacy. These undesirable effects may be the result of the presence of some secondary metabolites with adverse physiological effects. This necessitates why fractionation of medicinal crude extract is mandatory to differentiate secondary metabolites with therapeutic potentials from those likely to cause adverse effect. This is therefore a critical step in isolating compounds with beneficial biological effects from plants. In this study 3 fractions as presented in Table 2, with likely useful biological or physiological effect are identified out of the many compounds found in *B. coccinea*.

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Infrared Analysis of Pure Compounds of B. coccinea

Infrared absorption characterization analysis was carried out from TLC separated fractions from methanolic extract of *B. coccinea* and the spectral obtained was recorded as in Table 4 above. The spectra shows the presence of functional group such as Aryl OH, C-C single bond, OH, R-C=N, C=C, Ar-C=C, Ar-O-H, R-O-R, C-O-H for compound A, B and C. these functional groups may confirm the presence of alkaloids, flavonoids, phenolics etc in the extract of *B. coccinea*

Compound A has the following function groups from the IR spectrum: O-H group which is hydrogen bonded showed a broad peak at 3337cm^{-1}(Table 3). The OH group appears to be hydrogen bonded due to intermolecular association. There is O-H stretch vibration at 2493cm^{-1} which belong to carboxylic acid group. This is supported by carbonyl stretch (C=O stretch) of a unsaturated at 1654cm^{-1} or bonded to an aromatic nucleus. There is also evidence of C-O stretch at 1015cm^{-1} of either an alcohol or of a carboxylic acid. There is C-H stretching vibration of an alkane or an alkyl at 2950cm^{-1} and 2850cm^{-1}. There is also C-H bend at 1443cm^{-1} of an alkane or alkyl. Finally, there is also, Ar-O-H stretching at 1215cm^{-1} of phenolic group and R-O-R' stretching at 1116cm^{-1} of an ether (Fig 1).

Table 2: Column Chromatographic Fractions of *B. coccinea* and their Characteristics

Fraction	Weight (g)	Retention factor (Rf)	The sample colour @ 290, 271, 261nm	Spot number
Fraction A	1.02	0.90	Ash	1
Fraction B	1.04	0.86	Purple	1
Fraction C	1.98	0.79	Pink	1

Table 3: Infrared Spectra of TLC Separated Fractions of *B. coccinea* for Fraction A

Peak number	Peak position (cm^{-1}) and strength and shape	Functional group	Types of absorption
A	3337 strong/broad	OH or phenol group	O-H stretching vibration hydrogen bonded
B	2950 strong	C-C single bond	C-H stretching vibration of alkanes or alkyl group
C	2842 weak	C-C single bond	C-H stretching vibration of alkanes or alkyl group
D	2493 strong/broad	OH	O-H stretching vibration of alcohol
E	2234 weak-medium	R-C≡N	C≡N stretch
F	1654 broad	C=C	C=C stretch vibration of alkene
G	1443 broad	Ar-C=C aromatic carbon to carbon double bond	Ar-C=C stretching vibration
H	1215	Ar-O-H	C-O stretching vibration of an aromatic ring
I	1116 medium	R-O-R'	R-O-R' stretching of an ether
J	1015 stretching vibration	C-O-H	C-O stretching of an alcohol, ether or carboxylic acid

Compound B has the following functional groups from the IR spectrum: O-H group which is hydrogen bonded showed a broad peak at 3300cm^{-1} (Table 4). The OH group appears also to be hydrogen bonded. There is O-H stretch vibration at 2608cm^{-1} which shows carboxylic acid functional group. This is supported by alkene stretch (C=C stretch) at 1654cm^{-1} or bonded to an aromatic nucleus. At 1449cm^{-1} there appear to be an aromatic ring bonded to carbon to carbon double bond i.e. Ar-C=C.

There is also evidence of a stretch at 1017cm^{-1}. The spectral as well revealed that there are two types of C-H stretching vibration of an alkane or an alkyl at 2950cm^{-1} and 2836cm^{-1}. Finally, there is also R-O-R' stretching at 1113cm^{-1} of ether functional group, this is supported by the presence of C-O stretching vibration of 1017cm^{-1} (Fig 2).

The UV-visible spectrum also showed the presence of an aromatic ring. From the IR spectrum of sample three (3), the following functional groups were observed: O-H group which is hydrogen bonded at 3337cm^{-1} (Table 5). The OH group appears to be hydrogen bonded due to intermolecular association. There is O-H stretch vibration at 2508cm^{-1} which belong to either an ether or alcohol group. This is supported by carbonyl stretch (C=O stretching) at 1654cm^{-1} which appear to be bonded to an aromatic nucleus. There is C-H stretching vibration of an alkane or an alkyl at 2950cm^{-1} and 2840cm^{-1}. There is also C-H bend at 1450cm^{-1} of an alkane or alkyl. This revealed the presence of carbon to carbon single bonds (i.e. C-C-H). finally, there is R-O-R' stretching at 1118cm^{-1} of an ether and C-O stretching of either an ether or alcohol at 1015cm^{-1} (Fig 3).

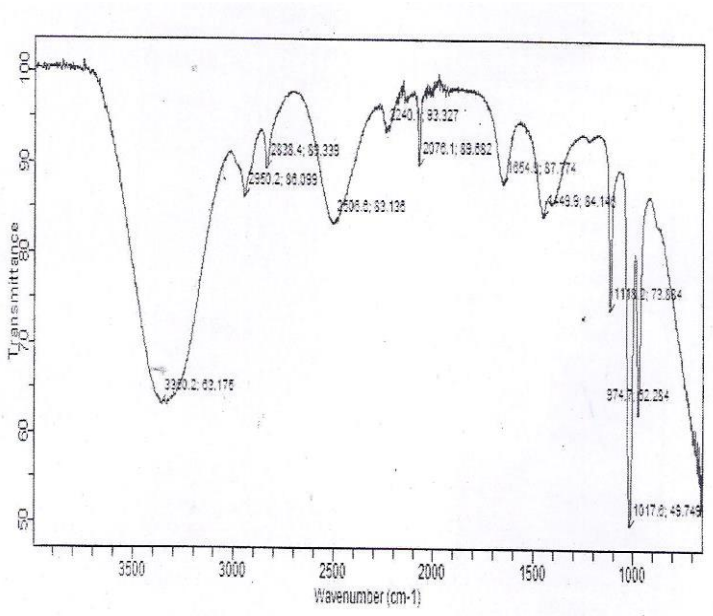

Figure 2: IR spectrum for Fraction B

Table 4: Infrared Spectra of TLC separated Fraction of *B. coccinea* for Fraction B

Peak number	Peak position (cm^{-1}) and strength and shape	Functional group	Types of absorption
A	3300 strong/broad	OH group	O-H stretching vibration hydrogen bonded
B	2950 strong	C-C single bond	C-H stretching vibration of alkanes or alkyl group
C	2836 weak	C-C single bond	C-H stretching vibration of alkanes or alkyl group
D	2608 strong/broad	OH	O-H stretching vibration of alcohol
E	2240 weak-medium	R-C≡N	C≡N stretch
F	1654 broad	C=C	C=C stretch vibration of alkene
G	1449 broad	Ar-C=C aromatic carbon to carbon double bond	Ar-C=C stretching vibration
H	1113 medium	R-O-R’	R-O-R’ stretching of an ether
I	1017 stretching vibration	C-O-H	C-O stretching of an alcohol, ether or carboxylic acid

Table 5: Infrared Spectra of TLC separated Fraction of *B. coccinea* for Fraction C

Peak number	Peak position (cm^{-1}) and strength and shape	Functional group	Types of absorption
A	3337 strong/broad	OH or phenol group	O-H stretching vibration hydrogen bonded
B	2950 strong	C-C single bond	C-H stretching vibration of alkanes or alkyl group
C	2840 weak	C-C single bond	C-H stretching vibration of alkanes or alkyl group
D	2508 strong/broad	OH	O-H stretching vibration of alcohol
E	2240 weak-medium	R-C≡N	C≡N stretch
F	1654 broad	C=C	C=C stretch vibration of alkene
G	1449 broad	Ar-C=C aromatic carbon to carbon double bond	Ar-C=C stretching vibration
I	1118 medium	R-O-R’	R-O-R’ stretching of an ether
J	1015 stretching vibration	C-O-H	C-O stretching of an alcohol, ether or carboxylic acid

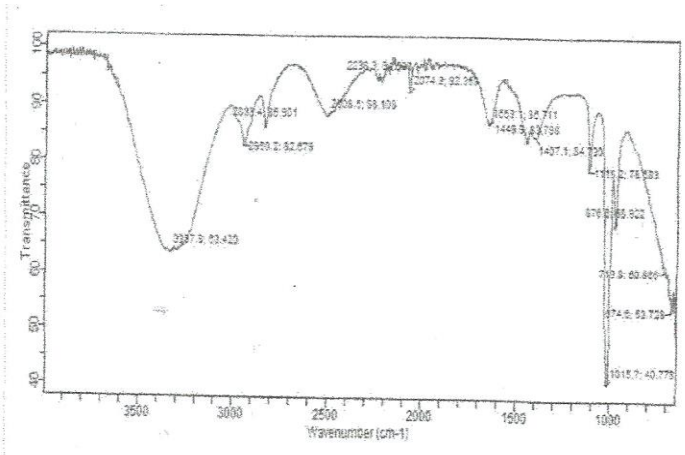

Figure 3: IR Spectrum for Fraction C

Conclusion

This study reveals the presence of phytochemicals; alkaloids, steroids, saponins, flavonoids, tannins in methanolic extract of *B. coccinea* which provides a basis for its therapeutic benefits and validates its ethnomedicinal uses in the treatment of peptic ulcer which may be the result of its antimicrobial effect. Infrared analysis of the studied sample shows promising peaks from the isolated fractions which have similar spectra and interpretation with similar functional group that may likely contain flavonoids, isoflavonoids and phenolic compounds. The spectra analysis of fractions will provide a lunch pad for further scientific purification or fractions, structural elucidation that may result in the development and design of lead compounds from the whole plant of *Boerhavia coccinea* with antimicrobial and anti-inflammatory activities.

Conflict of Interest

The authors has no conflict of interest.

Authors’ Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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