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Phytochemical Screening and Chromatographic Purification of *Bauhunia semibifida* ROXB Hexane-Dichloromethane Leaf Extract

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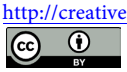

Abstract

Bauhunia semibifida is among the several plants that have been studied for their medicinal properties. Bauhunia is native to India. However, some species have been found in Africa. It has several important uses, such as anti-bacterial, anti-inflammatory and anti-cancerous. This study is aimed primarily at carrying out a preliminary phytochemical screening to ascertain the classes of bioactive compounds present in *Bauhunia semibifida* and ascertain the solvent system(s) for their separation. Several solvents were used for the extraction as well as in the chromatographic profiling of all the products of extraction. Thin layer chromatography was performed on silica gel-coated glass plates using several mobile phase protocols (Hexane: Ethylacetate, Dichloromethane (DCM): Methanol). Further purification of the extracts was done using classical column ($CcSiO_2$) and gel filtration chromatography. TLC profiling of DCM and Hexane extracts shows the same Rf values of less than 1 and are thus combined to give the Hexane/DCM extract, which gave two compounds following isocratic elution from column chromatography. Phytochemical screening of the extract showed the presence of Tannins, Cardiac glycosides, Alkaloids, Flavonoids and Phenolic compounds. Hexane/DCM extract thus contains phytocompounds which can further be studied.

Keywords

Bauhinia semibifida, Phytochemical Screening, Herbs, Medicinal Plants, Chromatography

1. Introduction

Nature is full of remedies that help in relieving various pathological disorders.

Herbs have constantly been used from prehistoric times for the cure of various disorders as it has been observed that natural therapy is most effective than synthetic ones [1].

Battling diseases have been an important aspect of interactions between human beings and the natural environment, and plants have forever been a catalyst for our healing. The plant contains a baffling diversity of secondary metabolites having most often very attractive bioactivities [2]. From natural sources, especially plants, many of which are well established in their use in traditional medicine, a significant number of effective drugs have been isolated [3]. Studies carried out on bacterial resistance have estimated that about 10 million people will die per year in 2050, costing about $100 trillion dollars per year. The World Health Organization (WHO) and some other groups around the world have, as a result, agreed on the urgency of developing an action plan that will address the issue globally and especially in the development of new drugs [4] [5].

Plants of medicinal value play a significant role in disease prevention, their promotion and use fit into all existing prevention strategies [6]. Information on herbs has thus been collected and a well-defined pharmacopeia developed [7]. Much of the pharmacopeia of scientific medicine was derived from the herbal lore of native peoples [8]. Medicinal plants are a source of several valuable drugs known as natural products. Only a handful of medicinal plants are cultivated, while most of them are still collected from the wild [9]. Information on plants is obtained through ethno-botanical surveys, which involve the study of plants as related to the culture of the people [10]. Phytochemicals, such as alkaloids, phenolics, terpenoids and tannins, have the potential to prevent diseases, act as anti-microbial, anti-inflammatory, anti-oxidant, anti-cancerous, detoxifying agent, immunity-potentiating agent and neuropharmacological agent [11]. Glycosides, polyphenols, etc. are among several secondary metabolites that plants accumulate, which are effective in the treatment of infectious diseases and have minimal side effects that are often associated with synthetic antimicrobials [12]. Nigeria is blessed with a vast floral environment with several data published on plants and their curative values and this has made available a wide range of information for scientific study and validation.

Classical column chromatography, gel filtration, and thin layer chromatography are some of the chromatographic techniques utilized in the separation, identification and estimation of different classes of bioactive compounds.

2. Materials and Methods

2.1. Chemicals and Reagents

Methanol (BDH, UK), Acetonitrile (Sigma-Aldrich Co.), N-hexane (Noah, USA), DCM (JHD, USA) Ethylacetate (Qualikems chemicals), Teteraoxosulphate (VI) acid (Loba Chemie PVT LTD, India), Ferric chloride, Normal phase silica gel (Loba Chemie PVT LTD India). All other chemicals were of analytical grade BDH Chemical Laboratory (England, UK).

2.2. Plant Collection

Bauhinia semibifida leaves were harvested at Akpueburu in Egbechukwu quarters Ezira, Orumba South Local Government Area, Anambra State, southeast Nigeria between the months of March and April. The harvested leaves were washed under running tap water to remove dirt and other debris. The leaves were spread out and allowed to dry at room temperature. The dried leaves were chopped into bits and pulverized using an electric blender to obtain a fine powder. This was then stored in a plastic container and covered tightly to prevent mold from growing until ready for use.

2.3. Extraction Protocol

2.7 kg of the pulverized leaf sample was loaded into 4000 ml round bottom flask and 2500 ml of methanol was added. This was placed on a heating mantle and allowed to reflux for 3 hours. The extract was filtered through a cotton wool into a conical flask at the end of the 3 hours. The procedure was repeated four more times to exhaustively extract from the sample. The filtrate was concentrated in vacuo using a rotary evaporator. The concentrated extract was placed in a water bath to fully remove the residual solvent.

2.4. Partitioning of the Extract

The crude methanol extract was dissolved in 1.5 litre of water and transferred into a 5-litre separatory funnel. Equal volume of hexane was added to aqueous methanol extract, stirred and allowed to stand for two hours, after which the organic layer was recovered. This was repeated three more times for each of the solvents (hexane, dichloromethane and ethylacetate), after which the extracts were concentrated.

2.5. Phytochemical Screening of the Extracts

Preliminary phytochemical screening was carried out on the 3 extracts using standard methods to determine the secondary metabolites present in the leaf [13] [14] [15].

Test for tannins

Put about 2 ml portion of the extract in test tube and gently heat it for 2 min. Add 3 drops of Ferric chloride solution. An orange color indicates the presence of Tannin.

Test for flavonoids

Ferric chloride test: Little portion of the extract was dissolved in ethanol and boiled with a few drops of 10% ferric chloride solution. A violet colouration indicates the presence of a phenolic hydroxyl group [13].

Test for alkaloids

Dragendorff's, reagent was used to carry out the test. This was done by adding 1 ml of Dragendorff's to 2 ml of the extract warmed with 2% H_2SO_4. Formation of an orange-red precipitate indicates the presence of alkaloids [14].

Tests for glycosides

General test: A small quantity of each extract was boiled with 2 ml of 2.5 M

teteraoxosulphate (VI) acid. This was cooled and neutralized with 20% potassium hydroxide and then boiled again with 5 ml of a mixture of equal volume of Fehling's solution A and B. Formation of a brick-red precipitate shows the presence of glycosides [13].

Test for saponins

Frothing test: A small portion of the extracts were shaken with water in a test tube and then warmed in a water bath. Persistent frothing indicates the presence of saponin [15].

The result of the preliminary phytochemical screening is shown in Table 1.

2.6. TLC Profiling of the Extracts

Profiling was done using the following solvent systems; Hexane and ethylacetate in the ratio 10:1, 20:1, 5:1 and 3:1, as well as DCM and methanol in the ratio 100:1, 50:1 and 30:1, respectively. Hexane ethylacetate solvent system gave a better separation and thus was employed in classical column chromatography.

2.7. Chromatographic Purification

A classical column ($CcSiO_2$) as well as a gel filtration chromatographic technique was utilized in purifying the extracts. $CcSiO_2$ chromatography yielded several fractions, which were spotted on TLC plate dried and developed with a solvent (H1:E10). Similar fractions were pooled together to give a subfraction. DCM and hexane fractions show similar chromatographic patterns on TLC plate and thus were combined as one. DCM-Hexane fraction was subjected to further purification using sephadex LH_{-20}. Aliqot of 2 ml was taken and a total of 25 fractions were collected. The fractions were spotted on TLC plates and developed using DCM 10: MeOH 1. TLC plates were visualized using Uv lamps of 234nm and 365 nm. Developed plates were sprayed with ethanol in 5% sulphuric acid and heated over a hotplate. Fractions with the same Rf value were combined and re-spotted for a fresh TLC. This gave rise to two compounds that showed a single spot on TLC plate when developed with DCM 20: M3: H_2O 1 and DCM 10: M 1, respectively, with Rf values 0.42 and 0.61, respectively.

3. Results and Discussion

Table 1. Result of preliminary phytochemical screening of *Bauhinia semibifida* leaf extract.

Parameters	DCM	Hexane	Hexane
Tanins	+++	+++	+++
Saponins	–	–	–
Cardiac glycosides	+++	+++	+++
Alkaloids	+++	+++	+++
Flavonoids	–	+++	+++
Phenolic compounds	+++	+++	+++

Key: +: Present; –: Absent.

Pharmacological findings of pharmaceutical agents can be provided for chemical constituents using the qualitative phytochemical screening of plant extracts [16] [17]. In the present study, qualitative tests for all three extracts showed indications of the presence of metabolites. Alkaloids, phenolic compounds, Tannins, Cardiac glycosides and Flavonoids were found to be present in all except for DCM extract, where flavonoids were absent. Saponin was absent in all three extracts.

TLC profiling of the extracts gave results that lend credence to the number of phytochemicals present in the extracts. Phytochemicals provide different Rf values in different solvent systems. Thus, this variation in Rf values of the phytochemicals gives information that assists in understanding their polarity as well as helps in the selection of an appropriate solvent system for the separation of pure compounds by classical column chromatography and gel filtration. A blend of solvents with variable polarity in different ratios can be used for the separation of pure compounds from plant extract [16].

4. Conclusion

The findings from this study suggest that *Bauhunia semibifida* leaves are potential sources of secondary metabolites, which have great importance as therapeutic agents for many diseases. Further work aimed at characterizing the compound is ongoing for the DCM/hexane as well as the ethylacetate extracts.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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