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PHYTOCHEMICAL EVALUATION OF LEAVES OF FIVE PLANT SPECIES FROM SOUTH EAST ZONE OF NIGERIA.

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Abstract

The phytochemical evaluation of leaf extracts of five medicinal plant species: *Boerhavia diffusa, Cissus araliodes, Commelina benghalensis, Leucas martinicensis, and Senna occidentalis* from Southeast zone of Nigeria, was conducted using standard procedures of Harborne.The results revealed that the plants are rich in important bioactive compounds occurring in varying concentrations as: alkaloids (2.42%,3.31%,1.82%,1.21%, and 2.23%);tannins (1.24%,10.20%, 17.78%,1.45%, and 2.04%); flavonoids (1.80%, 11.13%, 6.50%, 7.46%, and 1.67%); saponins (10.73%, 4.70%, 2.90%, 8.94%, and 13.48%); phenols (4.12mg/100g, 13.12mg/100g, 5.14mg/100g, 3.21mg/100g, and 4.38mg/100g); terpenoids (4.28%,15.16%,2.68%,4.85%,and 8.39%), and cardiac glycosides (0.13mg/l, 0.28mg/l, 0.81mg/l, 0.87mg/l, and 0.22mg/l) for *Boerhavia diffusa, Cissus araliodes, Commelina benghalensis, Leucas martinicensis*, and *Senna occidentalis*, respectively. Since these phytochemicals possess biological activities, they are therefore responsible for the medicinal uses of these plants .We recommend for further investigation of the detailed therapeutic properties of these plants and also the isolation, structural elucidation and full characterization of the specific phytoconstituents of the study plants.

KEY WORDS: Evaluation, phytochemistry, medicinal plants, Nigeria.

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Introduction

Plants are of paramount importance in the life of humans. They are sources of food, clothing, shelter, medicine etc. The plants that yield medicinal materials are the medicinal plants. These plants are used in herbal or traditional medicine because they are rich in biologically active compounds. About 70% of human population is dependent wholly or partially on plant-based medicines (Aja, Okaka, Onu, Ibiam and Uraku, 2010).However, World Health Organization (2008) reported that 80% of the world population use herbal medicine for some aspects of primary healthcare.

There are over 8000 species of known medicinal plants in Africa considered as an important part of traditional healthcare systems (Aja, Ugwu Okechuku, Keke, Ibere and Ekpono, 2017).It has been reported that more than 80% of African population is dependent on the cheap and effective traditional medicines used against many diseases and infections (Neuwinger, 2000).Many of these medicinal plants occur in Southeast zone of Nigeria. Typical examples of these plants include: *Boerhavia diffusa* L., *Cissus araliodes* (Welw.) Planch, *Commelina benghalensis* L., *Leucas martinicensis* (Jacq.)R.Br. and *Senna occidentalis* (L.)Link (syn.*Cassia occidentalis* L.).

Boerhavia (*Boerhaavia*) *diffusa* ,commonly known variously as red spiderling, spreading hogweed, or pigweed and locally called "Akandia"or "Azeigwe" or "Anyado-akwa"(Igbo),"Etiponnla"or "Enemo"(Yoruba),and "Sarkinjidjii" or "Babbajuji"(Hausa),is a species of flowering plant in the four o'clock family ,Nyctaginaceae. It is a pantropical weed that is sub-erect perennial herb, growing up to 50cm high (Akobundu and Agyakwa,1996).Medicinally, pigweed is usually used to treat afflictions such as skin rashes, scabies, small pox, yaws, jaundice, asthma, chest pains, cough, cataract, chronic ophthalmia, diseases of the heart, liver, and kidneys, conjunctivitis, blepharitis, anemia, oedema, gonorrhea, and dropsy(Gill,1992;Okujagu,2008).It is also used as a remedy for internal inflammations, dyspepsia, menstrual disorders, enlargement of spleen, abdominal pains, abdominal tumors, and cancerts (Shisode and Kareppa,2011).The plant is equally a remedy for epilepsy, dysentery, pneumonia, urinary troubles, uterine bleeding, thoracic haemorrhage, constipation, rheumatism, hypertension, eczema, and wounds(Desai, Gawali, Naik, and D'Souza,2008; Agrawal ,Sunanda and Pandey,2011).

Cissus araliodes is a gracefully, strong, pendulous, climbing shrub belonging to the angiosperm family Vitaceae, with stem growing up to 25m long (Verdcourt,1993).It is common in the deciduous forests and fringing jungles of tropical West Africa(Jacqueline,2014).The plant is used in the treatment of a number of diseases including swellings, rheumatism, arthritis, veneral diseases, gastrointestinal problems, wounds, cuts, dropsy, oedema, gout, pulmonary troubles, eye problems, fever and cough(Gill,1992;Fernandes and Banu,2012;Omotayo and Borokini,2012;Osakwe and Maduanusi,2019).The plant is commonly known as edible-stemmed vine and locally called "Ogbakiikii"(Igbo),"Avun" or "Evun" or "Eyunororo"(Yoruba), and "Da'ddori"or "Gewaya"or "Tsamoya"(Hausa).

Commelina benghalensis is an annual or perennial weed belonging to the flowering plant family Commmelinaceae. It has the common names Tropical Spiderwort,Benghal dayflower or Wandering Jew ,and with local names "Obogwu" or "Mbogwu" (Igbo),"Godogbo" or "Gbogodo" or "Godogbo-odo" or "Itopere"(Yoruba),"Balaasaana" or "Balasa" or "Balasa" or "Bulabula"(Hausa) .The plant has wide range of medicinal uses including for treatment of leprosy, burns, indigestion, inflammations, epilepsy, nose blockage in children, snakebites, night blindness, skin diseases, respiratory tract diseases(Faden,2006;Mollik,Hossan and Paul,2010),fever, jaundice, mouth thrush, insanity, psychosis, infertility in women, digestive disorders, insomnia, cataract, otitis media, suppurative sores, conjunctivitis, toothaches, and mental disorders(Niluna, Saujan, Kaveri and Shushal,2016;Pooja and Devang,2019).

Leucas martinicensis is an erect, strong, unbranched annual herb belonging to the mint family Lamiaceae (Labiatae).It grows up to 1.5m high, and widely distributed in tropical and subtropical Africa(Anne-Cathrine,2009).The plant is commonly known as wild tea bush and locally referred to as "Keke-owu"(Yoruba).Wild tea bush is medicinally used in the

treatment of such ailments like gastrointestinal troubles, wounds, sores(especially those of the eyes and nose),chronic diseases, mild fevers, colds, rheumatism, snake bites, and roundworms(Agra, Freitas and Barbosa-Filho,2007;Kausole,Hibu,Millogo and Ncoulina,2015),diarrhoea, epilepsy, cholera, pains during pregnancy, and malaria(Musa,2017).

Senna occidentalis, with various common names including septic weed, coffee senna, coffee weed, Magdad coffee, negro-coffee, senna coffee, Stephanie coffee, stinging weed or stytic weed, is a flowering plant in the legume family Fabaceae(Subfamily Caesalpiniodeae).It is an annual undershrub, subglabrous plant, growing to the height of few meters(Burkill,2015).The local names include "Akidi agbara"or "Odudu-emerema" or "Akamuo"(Igbo), "Rere" or "Abo-rere"(Yoruba),and "Rai-dore" or "Tafasar" or "Kwarkwati"(Hausa).Coffee senna is medicinally important as remedies for hemorrhoids, gout, rheumatism, diabetes, bone fractures and bone dislocation, and reported to be poisonous (Vashishtha, Kumar, Jolin and Nayak,2007).Other medicinal uses include the treatment of typhoid fever, yaws, scabies, itches, ringworm, jaundice,headache,toothache,wounds,sores,cutaneous diseases, throat infections, hematuria, cancer, diabetes, and tuberculosis(Sharma, Trikha, Athar and Raisuddin,2010;Tahani,Ahmed,Yahya,Sakina,Abdelradie and Tang,2020).From the foregoing, it is obvious that these plants are of immense ethnomedicinal applications. No wonder then that they are used as important medicinal plants in Southeast zone of Nigeria. A large number of works have been conducted on the phytochemistry as well as pharmacology of the study plants(Usha, Kasturi and Hamalatha, 2007; Rahman, Sadhu and Hassan, 2007; Baskaran, Sivamani and Bai, 2011;Assob,Kamga and Penlap, 2020; Tahani, Ahmed, Yahya, Sakina, Abdelradie and Tang,2020).A survey of the literature indicated that no serious detailed quantification of the bioactive constituents of the study plants have been carried out .Therefore, the present investigation was conducted to study the detailed quantifications of the phytoconstituents of the plants with modern techniques.

Materials

The fresh leaves of the study plants were collected from different parts of the South East zone of Nigeria and identified using Okujagu (2008).

Methods

The leaves were washed and air-dried on a laboratory bench and ground into fine powders using an electric grinding machine. Methanol extracts of the powders were prepared by soaking 50g of each powdered sample in 100ml of 70% methanol for 72hrs.The mixture was filtered through Whatman No.42 filter paper. The filtrates (extracts) and powdered samples were subjected to phytochemical evaluation.

Phytochemical Evaluation

The qualitative and quantitative evaluations of leaf extracts of the study plants were carried out for alkaloids, tannins, flavonoids, saponins, phenols, terpenoids, and cardiac glycosides using the standard procedures of Harborne (2016).

Qualitative Evaluation

The leaf extracts and powders were subjected to qualitative evaluation to determine the presence or absence of phytochemicals as listed below.

Test for Alkaloids

About 0.5g of each powdered sample was dissolved in 5ml of 1% Hcl on a water bath. The mixture was filtered through Whatman No.42 filter paper.1ml of the filtrate was treated with 3 drops of Dragendorff's reagent. The appearance of turbidity or precipitation indicated the presence of alkaloids.

Test for Tannins

One gram (1g) of each leaf powder was dissolved in 20ml of distilled water and filtered. Then 3 drops of 10% of $FeCl_3$ was added to 2ml of the filtrate. The occurrence of blackish-blue coloration was indicative of presence of tannins.

Test for Flavonoids

About 0.2g of each leaf powder was dissolved in 2ml of methanol and heated over water bath. A chip of magnesium metal was added to the mixture (as a catalyst) followed by the addition of few drops of conc. Hcl. The appearance of a red coloration indicated the presence of flavonoids.

Test for Saponins

One milliliter (1ml) of each leaf extract was added into a test tube containing small amount of NaH_2CO_3 and distilled water and shaken vigorously. The formation of honey comb-like froth indicated the presence of saponins.

Test for Phenols

Fifty grams (50mg) of each extract was added to 5ml of distilled water and 3ml of 10% lead acetate solution and mixed properly. The occurrence of a bulky white precipitate showed the presence of phenols.

Test for Terpenoids

Ten milliliters (10ml) of each leaf extract was mixed with 4ml of chloroform followed by careful addition of 5ml of conc.H_2SO_4.A layer of reddish-brown coloration at the interface was indicative of the presence of terpenoids.

Test for Cardiac Glycosides

2ml of glacial acetic acid and 2 drops of $Fecl_3$ were added to a test tube containing 1ml of each extract and 10ml of conc.H_2SO_4 and shaken vigorously. The formation of red-brownish rings revealed the presence of cardiac glycosides.

Quantitative Evaluation

The leaf extracts and powders of the five study plants were evaluated quantitatively for the determination of the crude contents of their phytoconstituents.

Alkaloid Determination

Five grams (5g) of each leaf powder(W) was weighed into 250ml beaker and 20ml of 10% acetic acid in ethanol was added, covered, and allowed to stand for 4hrs.The mixture was filtered and the filtrate concentrated on a water bath to one quarter of the original volume.Conc.NH_4OH solution was added drop wise to the extract until precipitation was completed. The whole solution was allowed to settle and the precipitate collected, washed with dilute NH_4OH solution and filtered. The residue was the alkaloid which was dried and weighed (W_2) and subjected to percentage calculation thus:

$$\% \text{ Alkaloid} == \frac{W_1 - W_2}{W} \times \frac{100}{1}$$

Where: W1=Weight of filter paper: W2=Weight of filter paper +alkaloid precipitated;

W=Weight of sample used.

Tannin Determination

One gram (1g) of each powdered sample (W) was weighed into a conical flask, 50ml of distilled water was added and shaken for 4hrs in a mechanical shaker. The mixture was filtered, then 1ml of Folin Denis reagent and 2.5ml of saturated $NaCO_3$ were added to the filtrate. The volume was diluted with distilled water after mixing and filtered through Whatman No.42 filter paper (of known weight,W_1).The filtrate was evaporated to dryness in an oven at 80^0C-100^0 C. It was cooled in a desiccator and weighed to a constant weight (W_2) and percentage concentration of tannins determined as follows:

$$\% \text{ Tannins} == \frac{W_1 - W_2}{W} \times \frac{100}{1}$$

Where: W1 =Weight of filter paper; W2 =Weight of filter paper +Tannin precipitated=Weight of sample used.

Flavonoid Determination

Five grams (5g) of the leaf powder was mixed with 100ml of 30% aqueous methanol ,kept at room temperature for 24hrs and filtered through Whatman No.42 filter paper of known weight (W_1).The filtrate was evaporated in an oven to a constant weight (W_2).The percentage flavonoid content was calculated thus:

$$\% \text{ Flavonoids} = \frac{\text{Weight of Flavonoid}}{\text{Weight of Sample}} \times \frac{100}{1}$$

Where: W1 = Weight of filter paper; W2 =Weight of filter paper+ flavonoid precipitated= Weight of sample used.

Saponin Determination

Five grams (5g) of each powdered sample (W) was weighed into a 250ml conical flask containing 200ml of 20% aqueous methanol. The mixture was heated over a water bath for 4hrs with continuous stirring at about 55^0 C. The mixture was filtered and the residue extracted with another 200ml of 20% methanol. The combined extract was reduced to 40ml over water bath at 90^0 C. Concentrated diethyl ether was added and shaken vigorously, and the aqueous layer was recovered while the ether layer was discarded. The 60ml of n-butanol was added and the extract washed twice with 100ml of 5% Hcl. The remaining solution was heated in a water bath for 5mins and then transferred to a weighed crucible (W1) and the sample dried in an oven to a constant weight (W_2).The percentage content of saponins was expressed thus:

$$\% \text{ Saponin} = \frac{\text{Weight of Saponin}}{\text{Weight of Sample}} \times \frac{100}{1}$$

Where: W1 = Weight of crucible; W2 =Weight of crucible + Saponin precipitated=Weight of sample used.

Phenol Determination

Total phenol content of each leaf extract was determined using Folin-Ciocalteau reagent. One milliliter (1ml) of properly diluted extract of each plant sample was mixed with 0.5ml of Folin-Ciocalteau reagent pre-diluted 10 times with distilled water. After standing for 8mins at room temperature, 2ml of 7.5 %(w/v) $NaCO_3$ solution was added and allowed to stand for 30mins at room temperature. Then, the absorbance was measured with a spectrophotometer at 765nm.A calibration

curve was prepared using standard solution of Gallic acid. The results obtained were expressed in mg of Gallic acid equivalents (GAE)/100Mg dry weight (dw) extract.

Terpenoid Determination

One gram (1g) of each powdered sample was taken into a conical flask (W) and soaked in 9ml of methanol for 24hrs.The mixture was shaken vigorously and filtered and the filtrate re-extracted with 10ml of petroleum ether in a separatory funnel. The ether extract was transferred into a pre-weighed vial (W_1) and allowed for complete drying (W_2).The percentage total terpenoids was determined with the expression:

$$\%\ \text{Total Terpenoids} = \frac{W_1 - W_2}{W} \times \frac{100}{1}$$

Where: W_1 = Weight of empty vial; W2 =Weight of vial +Terpenoids precipitated = Weight of sample used.

Cardiac Glycoside Determination

Eight milliliters (8ml) of each leaf extract was taken into a 100ml volumetric flask in conjunction with 60ml of distilled water and 8ml of 12.5% lead acetate. The mixture was properly shaken and filtered. Then 50ml of the filtrate was transferred into another 100ml flask and 8ml of 47% Na_2HPO_4 was added to precipitate excess lead ions. It was mixed properly and made up to volume with distilled water. The mixture was filtered twice through the same filter paper to remove excess lead phosphate. Then 10ml of purified filtrate was transferred into a clean Erlyn-Meyer flask and treated with 100ml Baljet reagent. This was allowed to stand for 1hr for complete color development. The color intensity was determined calorimetrically at 495nm and the total cardiac glycosides were determined in mg/l thus:

$$\%\ \text{Total Cardiac Glycosides} = \frac{A}{77} \times \frac{100}{1}$$

Where: A = Absorbance

Results

The results of the phytochemical evaluation of leaf extracts of *Boerhavia diffusa, Cissus araliodes, Commelina benghalensis, Leucas martinicensis,* and *Senna occidentalis* (Table 1) revealed that the plants are rich in important bioactive compounds occurring in varying concentrations as: alkaloids (2.42%±0.04, 3.31% ± 0.1, 1.82% ±0.01, 1.21 % 0.02,and 2.23%±0.24); tannins (1.24%±0.08, 10.20%±0.05, 17.78%±0.1, 1.45%±0.01, and 2.04%±0.08); flavonoids (1.80% ± 0.04, 11.13% ± 0.09, 6.50% ± 0.03, 7.46% ±0.04, and 1.67%±0.12); saponins (10.73% ± 0.04, 4.70% ± 0.01, 2.90% ± 0.02, 8.94% ± 0.01,13.48% ± 0.19); phenols (4.12mg/100g ± 0.02, 13.12mg/100g ± 0.05, 5.14mg/100g ± 0.01, 3.21mg/100g ± 0.02, and 4.38mg/100g ± 0.01); terpenoids (4.28%±0.03,15.16%±0.04, 2.68%±0.01,4.85%±0.02, and 8.39%±0.04) and cardiac glycosides (0.13mg/l ± 0.08, 0.28mg/l ± 0.005, 0.81mg/l ± 0.03, 0.80mg/l ± 0.001, and 0.22mg/l ± 0.001), respectively.

Table 1: Phytochemical Evaluation of five medicinal plants of Southeast zone of Nigeria

S/N	Plant Species	Alkaloids(%)	Tannins(%)	Flavonoids (%)	Saponins(%)	Phenols(mg/100g)	Terpenoids (%)	Cardiac Glycosides(mg/l)
1.	Boerhavia diffusa	2.42 ±0.04	1.24± 0.08	1.80 ±0.04	10.73 ±0.04	**4.12 ± 0.02**	**4.28 ±0.03**	**0.13 ± 0.08**
2.	Cissus aralioides	3.31 ± 0.10	10.20 ± o.o5	11.13 ± 0.09	4.70 ± 0.01	**13.12 ± 0.05**	**15.16 ± 0.04**	**0.28 ± 0.005**
3.	Commelina benghalensis	1.82 ± 0.01	17.78 ± 0.10	6.50 ± 0.03	2.90 ± 0.02	**5.14 ± 0.01**	**2.68 ± 0.01**	**0.81 ± 0.03**
4.	Leucas martinicensis	1.21 ± 0.02	1.45 ± 0.01	7.46 ± 0.04	8.94 ± 0.01	**3.21 ± 0.02**	**4.85 ± 0.02**	**0.87 ± 0.001**
5.	Senna occidentalis	2.21 ± 0.24	2.04 ± 0.80	1.67 ± 0.12	13.48 ± 0.19	**4.38 ± 0.01**	**8.39 ± 0.04**	**0.22 ± 0.001**

Discussion

The presence of these secondary metabolites found in the study of these plants may be responsible for their use in traditional medicine for the treatment of various diseases and with a lot pharmacological properties. For instance, alkaloids are reported to be used in the treatment of bronchial asthma, sinusis, common colds, Irregular rhythms of the heartbeat(arrhythmias),acute gout, malaria, and to relieve discomfort of hay fever (Federich, Jacquier, Thepenier, De Mol, Tits and Phipps,2002).Alkaloids have anti-parasitic,anti-plasmodial, anti-corrosive, anti-oxidative, anti-bacterial, anti-HIV, anti-inflammatory, anticancer, analgesic, local anaesthetic, antimicrobial, antifungal, antimutagenic, antitoxic, and hallucinogenic properties(Fernandez, Sykes, Andrews and Avery,2010;Patel, Gadewar, Tripathi, Prasad and Patel, 2012; Zhang, Zhang, Shan Han, Wainber and Yue,2015).

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Terpenoids are involved in the treatment of such afflictions like cancer, diabetes, stomachache, cough, urinary problems, diarrhoea, jaundice, inflammations, chest pain, asthma, and food poisoning(Cox-Georgian, Ramadoss, Dona and Basu, 2019).They have many biological activities including antimicrobial,antifungal,antiviral,diuretic,anticancer,antidiabetic,antidepressant,antiseptic,antiplasmodial,antihyperglycemic,anti-inflammatory,antioxidant,antiparasitic,immunomodulatory activities, and as skin permeation enhancer (Brahmkshatriya and Brahmkshatriya,2013).

Furthermore, flavonoids are reported to be applied as cure for cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, plaques, hypertension, oedema, diabetes, and ischemia (Havsteen, 2002). They exhibit some pharmacological properties such antioxidant, anticarcinogenic, radical-scavenging, blood lipid-lowering, and cholesterol-lowering agents, antiobesity, antiallergic, antiviral, and vasodilating actions(Panche, Chandra and Diwan,2015;Srivastava and Bezwada,2015).On the other hand, saponins are known as remedies for cancer, diabetes, dental caries, hypercalciurea, renal stones, rheumatoid arthritis, gout, and haemorrhoids, and inhibit platelet aggregation and as an antidote against acute lead poisoning (Shikonesh, Arunasalam, Yeung, Gauri and Jiang,2004;Hassan,Sule,Musa,Musa,Abubakar and Hassan,2011;El-Aziz, Ashour and Al Sadek,2019). They possess anticatarrhal, hormone modulating, adrenal adaptogenic, hypocholesterolemic, anticarcinogenic, antioxidant, astringent, analgesic, immunostimulant, antitumor, antiviral, antifungal, insecticidal, and molluscicidal effects(Desai *et al*.,2009),antihepatic,hepatoprotective,anti-inflammatory, hypoglycemic, antihyperlipidemic, antiparasitic, antidepressant, diuretic, expectoant, antiplasmodic, and aphrodisiac activities (Srivastava, 2013;Mishra, Rajiput and Mishra, 2017).

Moreover, phenolic compounds have been claimed to be used in the treatment of sore throat, sore mouth, mouth irritation, and pain, cancer, canker sore pains, itching, pharyngitis, and focal spasticity(Chen, Xu, Tang, Xan, Qin, Xu, Hang, Mao, Huo, Xia, Xu and Wang,2013),arthritis, obesity, malaria, cardiovascular diseases, diabetes, viral, bacterial, and protozoal infections, ingrown toe and finger nails(Bhuyan and Basu,2017).They show such pharmacological activities like antifungal, antiviral, antimicrobial, antiseptic, analgesic, arbotifascient (Chen *et al*.,2013), antioxidant, anticancer, anti-inflammatory, anticarcinogenic or antimutagenic (Wu-Yang ,Yi-Zang and Zhang, 2010),as well as antiallergic,antihypertensive,cardioprotective,antiarthritic,and immunomodulatory properties (Bhuyan and Basu,2017).

Also, cardiac glycosides are employed in the management of heart failure and atrial cardiac arrhythmias, cystic fibrosis, itchaemic stroke, cancer, hypertension, and neurodegenerative diseases(Patel,2016),and with anticancer, antihypertensive, and antineoplastic properties(Prassas and Diamandis,2008).It is known that at high concentrations, cardiac glycosides may cause heart failures .However, the study plants contain low concentrations of cardiac glycosides(Table 1).It is reported by Patel(2016) that at low concentrations, cardiac glycosides can positively be used as medicine in the treatment of congestive heart failures and cardiac arrhythmias.

Conclusion

The plants studied are rich in important bioactive compounds including alkaloids, tannins, flavonoids, saponins, phenols, terpenoid, and cardiac glycosides. The use of these plants in traditional medicine for the treatment of various ailments could be as a result of their rich contents of these phytochemicals. This is because the phytochemicals have the capability to attack and destroy a number of pathogens that affect humans.

Recommendations

From the results of this investigation, the following are recommended:

* In view of their rich phytoconstituents, the study plants have great potential in pharmaceutical industry for drug formulation.
* However, we suggest for further investigation of the detailed therapeutic properties of these plants.
* We also recommend for further research into the isolation, structural elucidation, and characterization of the phytoconstituents of these plants.

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