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Original Article

Phytochemical, Proximate and Antimicrobial Screening in the aerial part plant of *Drynaria sparsisora*

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ABSTRACT

After being gathered from the Uviwe local government area in Delta state, the leaves of *Drynaria sparsisora* were mixed, air-dried, and then extracted using a soxhlet apparatus with n-hexane and methanol as the solvents. Using the standard procedures of the Association of Official Analytical Chemists (A.O.A.C.), the phytochemical and proximate analysis was performed on the extract. Results of the phytochemical screening carried out on the combined extracts (n-hexane and methanol) showed the presence of alkaloids, proteins, sugars, glycosides, tannins, phenolic compounds, flavonoids, and steroids. To ascertain the moisture content, carbon, lipid content, ash, fat, and nitrogen, proximate analysis was used. According to the results, there was 1.2% fat and 50.4% carbon in the sample. The following were obtained for the others: lipid content (34%), ash content (6.5%), and nitrogen (6.69%). Mueller Hinton agar medium was used to test the antimicrobial activities of both extracts against a variety of clinical pathogenic microorganisms, including Helico bacterpylori, Campylo bacterjejuni, Escherichia coli, Salmonel platypi, Proteus mirabilis, Candida albicans, Candida krusei, and Candida tropicalis. The zones of inhibition were determined, and at various concentrations, the n-hexane and methanol extract demonstrated resistance to Escherichia coli, vancomycin-resistant enterococci, methicillin-resistant Staph aureus, candida krusei, and candida tropicalis. Methanol and n-hexane extracts have MICs of 25 µg/m and 50 µg/m, respectively. For the extracts of methanol and n-hexane, the MBC/MFC are 50 µg/m and 25 µg/m, respectively.

1. Introduction

Plant-based medicines to advance Man has always sought to protect himself from the elements, including cold, heat, and other ailments. Any substance having properties in one or more of its organs that can be used for medicinal purposes, or that serve as a precursor to the integration of different drugs, is considered a medicinal plant. Medicinal plants have been found to be useful in the treatment and management of a number of illnesses over time. The phytochemical components of the medicinal plant, which give it its pharmacological properties, include flavonoids, alkaloids, tannins, saponins, quinines, terpenoids, glycosides, polyphenols, fats, and oils [1].

Given that this is the first report of its kind, the goal of the study is to determine, if not establish, the medicinal uses of the plant's extract and to investigate the chemical composition of Drynaira sparsisora

2. Materials and Methods

2.1. Collection and identification of plant materials

Effurun, Delta State, Nigeria's Ugbomro community is home to a small forest from which fresh mature *Drynaria sparsisora* leaves were collected in January 2022. The plant was recently transplanted and given a voucher number, ABU05231, for identification at Ahmadu Bello

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University's Department of Botany. Abubakar B.Y. made the identification.

2.2. Preparation of the plant extracts

Knife and gloves were used to carefully remove the leaves of *Drynaria sparsisora* from their source. The plant was carefully cleaned after being gathered in large quantities to get rid of dust and other unidentified particles from the leaf's surface. The leaves were then laid out flat in the lab and allowed to air dry for eleven days at room temperature. After chopping the dried leaves into smaller pieces and pulverizing them into a fine powder, the extraction process started.

2.3. Extraction of the plant

The process of soxhlet extraction was applied. Three (2) beakers containing 25 g of the blended leaf were weighed and covered with white cloths before being placed inside the soxhlet extractor, and the extract was collected using three (2) different solvents (n-hexane and Methanol) [2].

2.4. Screening for phytochemicals

The contents of the extract were examined for the presence of proteins, anthraquinones, tannins, phenolic, steroids, glycosides, carbohydrates, terpenoids, alkaloids, and flavonoids.

2.5. Alkaloids screening

In a water bath, evaporation was used to dry the aqueous extract of the crude dry powder of the leaves. The residue was then dissolved using about 1.0M HCI. The mixture was divided into three equal portions after being filtered. A few drops of Mayer's Reagent were added to one portion. Dragon Droff's Reagent was used on one of the parts, and Wagner's Reagent was used on the other. Reparative alkaloid presence is indicated by the presence of a creamy, orange, or brownish precipitate, respectively.

2.6. Examine the saponins

The frothing test verified that saponin was present. One gram of dry leaf powder and five milliliters of distilled water were mixed well and left for twenty minutes. A stable froth more than 2.0 cm thick after being classified as saponin is indicative of saponin presence.

2.7. Examine your flavonoids

One milliliter of the leaf extract in water was mixed with two drops of diluted sodium hydroxide. Acid that has been diluted first turns a bright yellow color before becoming colorless, which indicates the presence of flavonoids.

2.8. Use Millon's test to check for protein

One milliliter of the leaf extract in water was mixed with two drops of diluted sodium hydroxide. Diluted acid causes an intense yellow color to initially appear before turning colorless, indicating the presence of flavonoids.

2.9. *Anthraquinones* (using Borntrager's test)

After boiling 2 g of the plant extract in 5% HCl for 10 minutes, it was filtered and given time to cool. After adding the same volume of HCl to the filtrate, a few drops of 5% ammonia were added. Following heating, the mixture took on a pinkish hue, signifying the presence of anthraquinones.

2.10. Tannin detection (ferric chloride test)

The extract was filtered following a brief boil in some distilled water. To the filtrate, two drops of a 5% FeCl solution were added. It is Upon adding diluted acid, an intense yellow color first appears before going colorless, suggesting that flavonoids are present.

2.11. Phenolic compound test: Filtration was carried out on the extract after a small quantity of distilled water was brought to a boil. Two drops of a 5% FeCl solution were added to the filtrate the filtrate, two drops of a 5% FeCl solution were added. A blue-black or greenish-black hue that appears indicates that tannins is in the extract.

2.12. Examine for steroid use

After mixing 1 ml of acetic acid anhydride with 2g of the plant extract, a concentrated sulfuric acid was added in drops. Steroids are present when there is bluish-green coloring present.

2.13 Glycoside Assay (Salkowski's Test)

Five milliliters of plant extract were mixed with chloroform. 5ml of concentrated H2SO4 was then added and given a gentle shake. Examine for phenolic compounds: The extract was filtered after a tiny volume of distilled water was brought to a boil. The filtrate received two drops of a 5% FeCl solution added to it. It is a blue-black or greenish-black color that indicates the presence of tannins.

2.12 Examine for steroid use

2g of the plant extract and 1ml of acetic acid anhydride were combined, and then a few drops of concentrated sulfuric acid were added. Steroids are indicated by the presence of bluish-green coloration.

2.13 Use Salkowski's test to check for glycosides

A volume of 5 milliliters was mixed with chloroform and plant extract. Thereafter, 5ml of concentrated H2SO4 was added with caution and shaken gently.
The presence of a steroidal ring, or the glycone portion of the glycoside, was indicated by a reddish-brown color.

2.14. Use Fehling's test to check for carbs

Fehling A and Fehling B reagents were mixed in equal amounts, and 2 milliliters of each was added to 5 milliliters of plant extract before being slowly cooked. 7g of CuSO4.5H2O was dissolved in 100 ml of distilled water along with 2 drops of diluted sulfuric acid to create Fehling A reagent. A red precipitate at the test tube's bottom indicated the presence of reducing sugars.

2.15 Terpenoids Test: Five milliliters of water extract were dissolved in two milliliters of chloroform and then dried by evaporation. Then, this was used to heat 2 ml of concentrated H2SO4 for approximately 2 minutes. Terpenoids were distinguished by their grayish hue.

2.16. Proximate Analysis

The protocol was developed by the Association of Official Analytical Chemists (A.O.A.C., 1990) for the precise measurement of moisture content, ash content, crude protein content, and fiber content. By minusing the total (g/100g of the dry matter) of crude fat, crude protein, ash, and fiber from 100g of dry matter, the difference was utilized to compute the carbohydrate technique (A.O.A.C., 1990). The water factor was met by the energy content (FAO, 2003). Macroscopic nutrients are fats, proteins, and carbohydrates; micronutrients are vitamins and minerals, depending on the daily requirements [3]. The next steps in the proximate analysis are to determine the ash content, moisture content, crude fat, crude and protein, fiber, and carbohydrate composition using plant extracts. In 2020, Ogwuche et al., among other researchers, reported some preliminary research on the components of medicinal plants [4].

2.17. Ash content calculation

2.0 g of the sample was weighed and then put into a sterile petri dish. After that, the petri dish and its contents were heated in a muffle furnace for eight hours at 550 C. Once the material had cooled in the desiccator, the procedure was carried out again and again until the weight remained constant. The ash content was computed with the aid of the weights of the residue and that of the petri dish recorded.

2.18. Carbohydrate composition determination

The percentage values of the other components (moisture, protein, fat, ash, and crude fiber) were added up, and then the total amount of carbohydrates was subtracted by 100.

2.19. Protein composition determination

The following ingredients were added to a 50 ml Kjeldahl digestion flask: 0.5 g of the sample, 3 g of Kjeldahl digestion catalyst, 10 ml of 10% concentrated H2SO4, and a tiny quantity of anti-bumping agents. The material within liquefied when the flask was heated until the foaming stopped. Up until the substance's color transitioned from ash to blue-green or pale green, the heating was increased further while the flask was rotating sporadically. After the material had cooled, it was moved to a 100 ml volumetric flask and filled up with one inch of distilled water.20 milliliters of the diluted digest were added to a distillation flask that contained 100 milliliters of the diluted digest and anti-bumping chips. A Buchner funnel was put inside a 250 ml beaker, and the flask was then connected to the funnel via the condenser's receiver. which has 15ml of a 2% boric acid solution added to two drops of methyl red-methylene blue (double indicator). The flask was filled with a 20 ml 50% NaOH solution. When nearly the same volume remained in the beaker as before and the boric acid in the receiver flask turned pale green instead of purple, the distillation was stopped. The ammonia was then added to the boric acid solution. The distillate of boric acid and ammonia was titrated with 1.0M HCI acid. When a pink coloration developed, the titration was stopped.

2.20. Composition analysis of fat

This estimation was carried out sequentially using Soxhlet extraction techniques. The powdered plant sample weighed 5g and was placed in a thimble after being wrapped in filter paper. The extraction column, which was connected to a condenser, held the thimble, which was then wrapped in cotton wool. Hexane (100 ml) was used for the lipid extraction.

2.21. Moisture content assessment Contents

2g of the sample were added to an empty, pre-weighed silica dish, which was then baked for 12 hours at roughly 55°C. After letting it cool in a desiccator and weighing it once more, this was put back in the oven for a further twelve hours. The cooling procedure was carried out once a steady weight was reached. Next, a composition prediction is made for the moisture.

2.22. Composition analysis of crude fiber

A 1L conical flask containing 100 ml of 1% H2SO4 and 1g of the sample was filled and allowed to boil for 20 minutes.

Deionized water was used to rinse the contents after a Buckner funnel filter. After that, the filtrate was gently cooked for a further twenty minutes in 100 milliliters of 1% boiling NaOH, and then it was filtered again. The residue was dried in an oven at 55°C for six hours after being cleaned with hot deionized water, 5% HCL, and dimethyl ether. After cooling, weighing, and burning the remaining material, 200°C for 50 minutes with ash. The ash was weighed and allowed to cool before the fiber content was determined.

2.23. The screening of microbes

The antimicrobial properties of plant extracts containing methanol and n-hexane were tested against pathogenic microbes. Microbes from the ABU Teaching Hospital in Zaria's Department of Medical Microbiology were used. This work was conducted using standard operating procedures as outlined by certain researchers [5] and [6]. Into 10 ml of DMSO was dissolved 0.1 mg of the weighed extract to achieve a concentration of 10 mg/ml, the starting concentration of the extract used to evaluate its antimicrobial activities. To filter the extracts, the diffusion method is used. Mueller Hinton agar medium, which was made in compliance with the manufacturer's instructions and sterilized for 15 minutes at 121 degrees Celsius and then placed in Petri dishes which were sterile and given time to cool and solidify, was used to cultivate the microbes. A standard 0.1 ml bacterial inoculum was added to the sterilized medium. Test microbe: A sterile swab was used to evenly distribute the inoculum throughout the medium's surface. A conventional cork borer with a 6mm diameter was used to drill a well in the center of each inoculation medium. Following that, 1 milliliter of the extract solution at a concentration of 10 mg/ml was added to the well on the inoculation medium. The medium-coated plates were inspected for the zone that appeared after 24 hours of incubation at 37°C.

2.23. Determination of minimum bactericidal and minimum fungicidal concentration

To ascertain whether the test microbes were actually killed or just had their growth slowed down, MBC/MIC was performed. In sterile Petri dishes, Mueller Hinton agar was prepared, sterilized at 121 c for 15 minutes, and then allowed to cool and solidify. The contents of the MIC were then sub cultured onto the prepared medium, which was incubated at 37°C for 24 hours before the plates of the medium were checked for colony growth. The MBC/MC plates, which had the lowest concentration of extract and no colony growth, were the results.

2.24. Determination of minimum inhibition concentration

Using the broth dilution method, the extract's minimum An inhibitory concentration was found. The Mueller Hinton broth was prepared, 10 ml was pipetted into test tubes, and the broths were cooled after 15 minutes of sterilization at 121 °C. MC-Farland's turbidity standard scale number 0.5 was created as a remedy. After 10 ml of prepared normal saline was dispensed into a sterile test tube, the test microbe was added. The sample was then incubated for six hours at 3 °C. After being diluted in regular saline until the turbidity matched that of the MC-Farland scale, the test microbe now has a concentration of roughly 1.5Xc/ml. In the sterile broth, the extract was serially diluted twice in order to obtain the concentrations..

3. Results and Discussion

Upon phytochemical screening of the n-hexane extract of *Drynaria sparsisora*, the following compounds were found to be present: proteins, flavonoids, glycosides, tannins, and saponin. Methanol, on the other hand, contains proteins, glycosides, alkaloids, tannis, and saponin (Table 1). These bioactive ingredients account for their significance in diet and nutrition. It is probable that saponins have the following characteristics: antibacterial, anti-inflammatory, anti-cancer, and antidiabetic. Terpenoids possess antibacterial properties, as stated by Urzua et al. (2008) [9].

The proximate analysis of the Drynaira sparsisoria results shown in Table 2 was examined using the standard reference method. Carbon had the highest value at 50.4 in the quantitative analysis of *Drynaria sparsisora's* proximate analysis, as shown in the table. %), nitrogen fats, at 1.20%, placed third, and came in second at 6.69%. The figure (3.2). The original food's mineral content is usually ascertained by its ash content [10]. The fat content that was found to be the lowest was 1.2%. The activity of coenzymes and water-soluble enzymes, which are aided by the moderate moisture content, is essential to the plant's metabolism [11].

The antibacterial properties of Drynaria spasrsisora are similar to those found in conventional medicine. Tables 3-5 contain the results of the antimicrobial screening. The extracts' efficiency against the selected clinical pathogens. According to a methanol extract from Drynaira sparsissora, Staph aureus is resistant and does not exhibit zone inhibition. When compared to n-hexane's zero zone inhibition, vancomycin-resistant enterococci exhibit sensitivity to methanol extract, with a value of 26. With a zone inhibition of 22, Staphylococcus aureus was sensitive to methanol, but it had a zone inhibition of 26 for n-hexane. For Escherichia coli, n-hexane was resistant with no zone inhibition, while methanol was sensitive with a

zone inhibition of 26. Helicobater pylori exhibited zone inhibition of 24 for methanol and 26 for n-hexane, indicating its sensitivity to the former. Methanol was resistant to and lacked zone inhibition from *Campylobacter jejuni*, whereas n-hexane did. It had a 23-zone inhibition, making it sensitive. Methanol was sensitive to Proteus mirabilis, with a no zone inhibition of 23 and a zone inhibition of 27 for n-hexane. Methanol is sensitive with no zone inhibition for *Candida albicans*, whereas N-hexane is sensitive with no zone inhibition. The Antimicrobial Activity of Methanol Extract and n-hexane states that where there is (S) it means that it is Sensitive to the bacteria while (R) act as a resistance to the bacteria. For *candida krusei*, methanol was resistance with zone inhibition of 23 while for n-hexane it was resistance with no zone inhibition.

Table 1: Phytochemical screening

Test performed	**Result for n-hexane Extract**	**Result for Methanolic extract**
Total Volume of sample	Mls	Mls
Appearances	Liquid	liquid
colour Description	Dark green	Dark green
flavonoids	+	+
proteins(Amino acids)	-	+
Terpenoids	-	-
Anthraquinones	-	-
Reducing sugar	-	-
Glycosides	+	+
Tannis	+	+
Alkaloids	-	+
saponin	+	+
steroids	-	+
phenolic compounds	+	+
carbohydrates	+	+

Table 2: Percentage contents of Proximates

Test performed	**Results**
Carbon (%)	50.4
Nitrogen (%)	6.69
Lipid Content	34
Fats (%)	1.20
Ash (%)	6.5
Moisture (%)	1.9

Table 3: Zone of Inhibition of the Extract Against the test Microorganism and standard used

Test organism	Methanol	n-hexane	Sparfloxacin	Ciprofloxacin	Fluconazole
Methicillin Resist Staphaureus	0	0	30	0	0
Vanomycin resistant enterococci	26	0	0	34	0

Staphylococcus aureus	22	26	40	0	0
Escherichia coli	26	0	0	37	0
Helicobacter pylori	24	26	0	35	0
Campylobacter jejuni	0	23	35	0	0
Proteus mirabilis	23	27	30	0	0
Candida albicans	26	3	0	0	32
Candida krusei	0	0	0	0	35
Candida tropicalis	23	0	0	0	33

S=>Sensitive, R=>Resistance

Table 1 Minimum inhibition concentration of Methanol and n-hexane Extract

Test organism	100µg/ 50µg/m 25µg/m 12.5µg/ 6.25µg/	100µg/ 50µg/m 25µg/m 12.5µg/ 6.25µg/
Methicillin Resist Staphaureus		
Vanomycin resistant enterococci	- - 0* + ++	
Staphylococcus Aureus	- - 0* + ++	- 0* + ++ ++
Escherichia coli	- - 0* + ++	
Helicobacter	- - 0* + ++	- 0* + ++ ++
Campylobacter jejuni		0* + ++ +++
Proteus mirabilis	- - 0* + ++	- 0* + ++ ++
Candida albicans	- - 0* + ++	0* + ++ +++
Candida krusei		
Candida tropicalis	- - 0* + ++	

Key=- No colony, o*=>/MFC, +=>Scanty colony growth, ++=>Moderate colony growth, +++=>Heavy colony growth

Table 2 Minimum bactericidal/fungicidal concentration of Methanol and n-hexane plant extract

Test organism	100µg/ 50µg/m 25µg/m 12.5µg/ 6.25µg/ml	100µg/ 50µg/m 25µg/m 12.5µg/ 6.25µg/
Methicillin Resist Staphaureus		
Vanomycin resistant enterococci	- - 0* + ++	- - 0* + ++
Staphylococcus aureus		- - 0* + ++
Escherichia coli	- - 0* + ++	
Helicobacter pylori	- - 0* + ++	- - 0* + ++
Campylobacter jejuni		- - 0* + ++
proteus mirabilis	- - 0* + ++	- - 0* + ++

Candida albicans	- - 0* + ++	
Candida krusei		
Candida tropicalis	- - 0* + ++	- - 0* + ++

Key=- No colony, o*=>/MFC, +=>Scanty colony growth, ++=>Moderate colony growth, +++=>Heavy colony growth

4. Conclusion

According to the analysis's findings, Drynaira sparsisoria contains bioactive compounds and proximate contents that can inhibit the growth of microorganisms.

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Conflict of Interest

The authors declare that they have no conflict of interest

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