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Phytochemical screening and antimicrobial potential of *Philonotis hastata* (Duby) Wijk & Margad.

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Abstract: *The present study was carried with a view to investigating the phytochemical constituents and the antimicrobial potentials of acetone and ethanol extracts of Philonotis hastata. The moss plant was collected from the Obafemi Awolowo University Campus, Ile-Ife, Nigeria. The extracts were procured using acetone and ethanol separately. The extracts were screened for the presence of some secondary metabolites and the antimicrobial potentials tested against Escherichia coli, Staphylococcus aureus, Aspergillus flavus and Candida albicans using standard methods. The phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids and saponins. The test plant extracts showed potential antimicrobial activity against Staphylococcus aureus, Aspergillus flavus and Candida albicans.*
Key words: *antimicrobial, metabolites, phytochemicals, Philonotis hastata, potentials*

I. Introduction

Bryophytes (comprising hornworts, liverworts and mosses) are the second largest group of land plants after flowering plants, with estimated 15,000 to 25,000 species worldwide [1]. Nigeria in West Africa, is a home to varieties of these bryophytes and some of the common species in Nigeria include; *Jaegerina scariosa* (Lor.) Arz., *Octoblepharum albidum* Hedw. *Racopilum africanum* Mitt., *Barbula lambarenensis* C. Mull and *Philonotis hastata* (Duby) Wijk & Margad., [2]. Other common species include; *Archidium ohioense* Schimp ex. C. Muell, *Bryum coronatum* Schwaegr., *Bryum crugeri* Hampe, *Hyophila involuta* (Hook) Jaeg, *Calymperes erosum* C. Muell, and *Thuidium gratum* (P. Beauv) Jaeg.

Bryophytes contain cocktails of different active chemical compounds that they use as weapons that are part of their alternative poikilohydric life strategy. If a fungal spore falls on a bryophyte thallus or leaves, phenolic compounds are released by the plant when the surface becomes wet inhibiting spore germination. This may be the least one important factor for the evolutionary success of bryophytes and the fact that, they survived for more than 350 million years [3]. Bryophytes are able to produce diverse secondary metabolites to cope up with a number of biotic and abiotic stresses such as predation, ultraviolet radiation, extreme temperature and microbial decomposition [4].

The use of bryophytes in traditional system of medicine has also been recorded [5]. The doctrine of signatures (based on the concept that God provided visual clues through the characteristics of the plants), highly developed during the European Renaissance has dictated the use of variety of bryophytes, especially liverworts, in herbal medicine. Numerous compounds including oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, phenylquinones, aromatic and phenolic substances were found in bryophytes but few links have been made between any medical effect and specific bryophyte species or compounds [6].

As an oldest land plant, bryophytes posses medicinally important bioactive compound and are traditionally used in Chinese, Europeans, North American and Indian medicine, to treat illness of cardiovascular system, tonsillitis, bronchitis, tympanitis (inflammation of inner ear), skin disease and burns [7]. Microbial infections pose a health problem throughout the World, and plants are a possible source of antimicrobial agents [8][9].

In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal [10]. The development of drug resistance in pathogenic bacteria and fungi due to the use of conventional antibiotics could be dealt-with with diverse mechanisms of action from some unique sources like bryophytes. This study therefore focused on *Philonotis hastata* with a view to investigating its phytochemical constituents and antimicrobial activities of its acetone and ethanolic extracts.

II. Materials and Methods

2.1 Plant Sample Collection and Preparation

The plant was collected from the pavement which serves as retaining wall in front of Intecu Building, Obafemi Awolowo University, Ile-Ife, Nigeria (7^0 3' and 7^0 34' N & 4^0 30' and 4^0 32' E). The plant was identified by Dr. A. M. Makinde, Department of Botany of the same University. Thereafter, the plant was

collected in large quantity and carefully sorted out from other extraneous materials, air dried and stored in a cool dry place for further laboratory work.

2.2 Extraction and Screening for Phytochemical Substances

The extracts of the mosses were procured by soaking the moss plants in acetone and ethanol separately for seventy-two (72) hours. The resulting solutions from these were filtered and the filtrates evaporated to dryness to obtain acetone and ethanol extracts. The extract was then screened for the presence of secondary metabolites like alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatanins, saponins, steroids, tannins, triterpenes and xanthoproteins using the methods of [11][12][13] as reported by Isa *et al*., [14].

2.2.1 Alkaloids: Acidic extracts were prepared by mixing 50 mg of each extract (ethanol and acetone) with 10 ml of 10 % (v/v) HCl separately. These were heated and thereafter filtered. To 1 ml of the filtrate, few drops of Mayer's reagent, Dragendorff's reagent and Wagner's reagent were added separately. The mixtures were then examined for colour change, turbidity or formation of precipitate. The formation of precipitate indicated the presence of alkaloids.

2.2.2 Anthraquinones: Each extract (0.5 g) was boiled in about 2 ml of diluted sulphuric acid and then filtered while it was hot. To the filtrate, about 2.5 ml of benzene was added, shaken and the benzene layer was separated. Few drops of 10 % (v/v) ammonia solution were then added and the mixture was observed for colour change. Formation of a pink, red or violet colouration in the ammonia layer indicated the presence of anthraquinones in the extract.

2.2.3 Cardiac glycosides: Each extract (0.5 g) was dissolved with 2 ml of chloroform, filtered and concentrated sulphuric acid was carefully layered at the bottom of the tube to form a lower layer. The chloroform/sulphuric acid interphase was then observed for the formation of a reddish brown colour ring indicating the presence of cardiac glycosides in the extract.

2.2.4 Flavonoids: About 5 ml of ethanol was added to 5 mg of each of the extracts, shaken and then filtered. To 1 ml of the filtrate, few drops of 0.5 N ethanolic potassium hydroxide solution were added. Formation of suspension, cloudiness or precipitate indicated flavonoids presence in the extract.

2.2.5 Phlobatanins: About 0.5 g of the extracts was heated with 10 % (v/v) HCl in boiling water. The solution was then observed for formation of red precipitate which signified presence of phlobatanins.

2.2.6 Saponins: Each extract (0.1 g) was suspended in water in a test tube, shaken vigorously and noting the frothing. The solution was warmed at 70 ^{0}C for about 15 min. in a water bath. The mixture was shaken vigorously after warming. Persistence of frothing after warming indicated the presence of saponins.

2.2.7 Steroids: To 1 ml of concentrated sulphuric acid was added 1 ml of aqueous extract. It was allowed to stand for 5 min. and then examined for the formation of reddish brown precipitate which was an indication of the presence of steroids.

2.2.8 Tannins: The extract (10 mg) was dissolved in 10 ml distilled water and then filtered. To 1.0 ml of the filtrate was added few drops of 0.5 M ferric chloride in glacial acetic acid. The mixture was examined for the formation of blue, blue-black or greenish precipitate which indicated the presence of tannins.

2.2.9 Triterpenes: Extracts (20 mg) was suspended in 10 ml of chloroform, warmed slightly in water bath and then filtered. About 5 ml of concentrated sulphuric acid was then added to the chloroform filtrate and was properly mixed. The mixture was examined for the formation of red colour which indicated the presence triterpenes in the extract.

2.2.10 Xanthoproteins: Few drops of nitric acid were added to 1 ml of aqueous extract followed by the addition of few drops of ammonia solution. Formation of reddish or slightly brown precipitate indicates the presence of xanthoproteins.

2.3 Investigation of Antimicrobial Potential

2.3.1 Test Organisms:

The organisms used which include *Escherichia coli* (NCIB 86) and *Staphylococcus aureus* (NCIB 8588); *Aspergillus flavus* and *Candida albicans*; were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.3.2 Preparation of Media

The appropriate quantity of each medium used was accurately weighed and dissolved in the appropriate amount of distilled water according to the recipe for use. Heated to boil when necessary, dispensed into McCartney bottles or Bijou bottles and then autoclaved. After autoclaving, the nutrient agar (for the bacteria), were kept in molten form for subsequent use, while the broth were allowed to cool before sub-culturing. The eighteen hour peptone broth cultures were used. With the aid of a sterile 1 ml pipette, 0.1 ml of the broth culture of each test organism (*E. coli* and *S. aureus*) was added to the 20 ml sterile molten nutrient agar (sabourand dextrose agar in the case of *A. flavus* and *C. albicans*) which had already cooled to 44 ^{0}C. Each bottle was gently rotated to mix the inoculums with the medium and then poured into a properly labeled sterile Petri-dish and allowed to set.

2.3.3 Determination of antibiotic activity of the extracts

The agar diffusion method by Kudi *et al*. [15] and Ogundipe *et al*. [16] as reported by Isa *et al*. [14] was used to determine the growth inhibition of the test organisms by the plant extracts. The tests were carried out using different concentrations; 3.330, 1.110, 0.370, 0.123, 0.041 and 0.014 ppm which were prepared by re-dissolving the extracted powder in the same solvent. Thus, acetone extract was re-dissolved in acetone and the ethanolic extract in ethanol.

Using a sterile cock-borer of 0.5 mm diameter, six equi-distant holes per plate were made in the set agar with the control at the centre. Thereafter, the wells and the ditches were filled with the prepared different concentrations of the extract solutions, using sterile Pasteur pipettes. These were done in duplicates. The culture plates were incubated at 35 ºC for 24 hours and the relative susceptibility of each organism to the extracts as indicated by clear zones of growth inhibition around the wells were examined and recorded.

III. Results

3.1 Extraction and Screening for Phytochemical Substances

The results of the phytochemical screening are presented in Table 1. Only ethanol extract tested positive for the presence of alkaloid and that was with the use of Mayer's reagent, while, acetone extract did not. Cardiac glycosides tested positive in both extracts (acetone and ethanol). Flavonoids tested positive only in acetone extract and saponins tested positive only in ethanol extract.

Anthraquinones, Phlobatanins, Steroids, Tannins, Triterpenes, and Xanthoproteins were not detected in both extracts.

Table 1: The result of phytochemical screening of *P. hastata*

Phytochemicals	**Acetone Extract**	**Ethanol Extract**
Alkaloids: Mayer's reagent Wagner's reagent Dragendorff's reagent	 - - -	 + - -
Anthraquinones	-	-
Cardiac glycosides	+	+
Flavonoids	+	-
Phlobatanins	-	-
Saponins	-	+
Steroids	-	-
Tannins	-	-
Triterpenes	-	-
Xanthoproteins	-	-

Key: + = Present; - = Absent

3.2 Antibiotic activity test

The summary of the results of antibacterial and antifungal potentials of the extracts is presented in Table 2 below.

Table 2: Summary of the results of antibiotic activity test of *P. hastata* extracts

Organisms	Resistance to Crude Extracts	
	Acetone Extract	Ethanol Extract
Aspergillus flavus	S	R
Candida albicans	SR	SR
Escherichia coli	R	R
Staphylococcus aureus	R	S

Key: R = Resistant; S = Sensitive; SR = Slightly Resistant

The study showed different reactions to the extracts by each of the test organisms. The acetone extract totally inhibited the growth of *A. flavus* but, only slightly inhibited the growth of *C. albicans*. *E. coli* and *S. aureus* showed resistance to the concentrations of the acetone extract used. The ethanol extract also only slightly inhibited the growth of *C. albicans* but did not inhibit that of *A. flavus* and *E. coli*, while it totally inhibited the growth of *S. aureus*.

IV. Discussion

This study showed that *Philonotis hastata* has alkaloid, cardiac glycosides, flavonoids and saponins as part of its chemical constituents. Generally, bryophytes have been reported to serve as a source for a wide variety of chemical compounds that are known to have numerous potentials. For example, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, phenylquinones, aromatic and phenolic substances are reported to be present in bryophytes [6]. The presence of alkaloid, cardiac glycosides, flavonoids and saponins as part of *P. hastata* (a bryophyte species) chemical constituent is an indication that, the plant has some pharmacological potential in it.

It has been reported that alkaloids and flavonoids from bryophyte extract possess effective antibacterial, antifungal activity against pathogenic microorganisms [17][18][19]. Cardiac glycoside is reported to be useful in treating heart problems [20]. Saponins were reported to possess expectorant action, which is very useful in the management of upper respiratory tract inflammation; it is also reported to have antidiabetic properties [21].

Systemic screening of plant extracts for antibiotic activity is a continuous effort to find new antibiotic compounds. The test organisms used in this study are related with diverse forms of human infections. From a medical viewpoint, *E. coli* results into septicemia and infection of lungs, gall bladder, skin lesions and meninges, and also a number of food related diseases that manifest themselves in the form of diarrhoea[22][23]. *S. aureus* causes boil, ulcers, food poisoning, toxic shock and pneumonia e.t.c. *C. albicans* is often associated with opportunistic oral or genital infection infections in human. It can also be the causal agent for severe fungaemias (systemic fungal infections), particularly common in immunocompromised patients.

Lack of activity by the extracts recorded for some of the tested organisms may be ascribed to low concentration of the extract or its active substances. Acetone extract of *P. hastata* exhibited activity against *A. flavus* by inhibiting its growth and only slightly inhibited the growth of *C. albicans*. Similarly, the ethanolic extract also, only slightly inhibited the growth of *C. albicans* just like that of acetone extract; but, totally inhibited the growth of *S. aureus*. Generally, different organisms react differently to different factors, although, there may still be some kind of similarities of reaction to some others factors. The way gram positive bacteria would react to some dye will be different from the way a gram negative one will react to it. Thus, the acetone extract did not inhibit the growth of *E. coli* and *S. aureus*. No inhibition of *E. coli* and *A. flavus* was also recorded for the ethanolic extract in the study. According to Basile *et al*. [24], absence of antifungal activity could be due to inability of the active molecule(s) present in the extract to cross the fungal cell wall.

V. Conclusion

It is concluded that, both acetone and ethanolic extracts of *P. hastata* showed the presence of pharmacological useful class of compound in them and inhibition of the growth of *A. flavus*, *C. albicans* and *S. aureus*. So, it is promising to say that *P. hastata* is a potentially valuable source of medicine as well as source of bioactive material.

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