

Investigation of Phytochemical and Antimicrobial Activity of *Huberzia* Species

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Summary

An investigation of phytochemical screening of alkaloids, flavonoids, triterpenes, reducing sugars and tannins was carried out on 8 leaf samples of *Huberzia* species. The *in-vitro* antimicrobial activity of these extract of *Huberzia* species were evaluated against six bacteria indicating that the strong activities against Gram positive and Gram negative strains.

Keywords: Fern allies, *Huberzia*; phytochemicals; antimicrobial activity

Introduction

The medicinal values of fern and fern-allies for man as indicators of healthy environment, horticultural and source of pharmacodynamic compounds are getting realized and with continued research efforts, many more fern and fern-allies based products are sure to appear in the market in the coming years. According to the fossil record, the Lycopods are some of the oldest vascular plants to populate the earth's surface. Dating as far back as the early Devonian period, some members of the Lycopod family dominated the landscape as large trees, but today they're only found lining the forest floor as common club mosses. One of these club mosses, *Huperzia serrata*, (reclassified recently by taxonomists, formerly *Lycopodium serrata*) is gaining attention due to recent research on one of its alkaloid constituents, Huperzine A. Huperzine A has been found in tests to inhibit acetylcholinesterase (AChE) activity in the brain. Acetylcholine is one of the primary neurotransmitters in the brain responsible for facilitating synaptic connections among neurons and is thought to be instrumental in the normal functioning of memory. AChE is an enzyme that functions as a regulator by breaking down excess acetylcholine. Some types of mental impairment, such as that found in Alzheimer's patients, are characterized by degeneration of acetylcholine producing neurons, and it has been found that inhibiting AChE's breakdown of acetylcholine can increase the availability of remaining acetylcholine. Although not a cure for memory deficiencies, Huperzine A has been found to positively affect recall through its AchE inhibition activity (1). There has been no report of the phytochemical and antimicrobial activity of south Indian *Huberzia* species.

Materials and Methods

Plant material

The leaf materials of *Huperzia phyllantha*, *H. hamiltonii*, *H. squarrosa*, *H. nilgirica*, *H. macrostachys*, *H. vernicosa* *H. ceylanica*, and *H. seratta* were collected and identified by senior author.

Extraction method

25gms shade-dried powdered leaves *Huperzia phyllantha*, *H. hamiltonii*, *H. squarrosa*, *H. nilgirica*, *H. macrostachys*, *H. vernicosa* *H. ceylanica*, and *H. seratta* were separately extracted with methanol (100ml) using a cold extraction method for 48h. This extract was filtered, and further studies of identification of phytochemicals and antibacterial activity.

Qualitative phytochemical identification tests

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin (2).

Agar-well diffusion

The agar-well diffusion method briefly described as bacterial strains were cultured on nutrient agar medium incubated at 37°C for 18h. Subculture of tested bacteria were suspended in saline solution 0.85% NaCl and adjusted to a turbidity of 0.5 Mac Farland standards (10^8 cfu/ml). The suspension was used to inoculate 90mm diameter Petri plates with a sterile non toxic cotton swab on a wooden applicator. Six millimeters diameter wells were punched in the agar and filled with 50µl of extract of *Huberzia* species. For negative control methanol was used, while tetracycline used as positive control. For tetracycline 10mL solution was prepared mixing 0.8mL tetracycline solution (prepared by dissolving 500mg tablets of tetracycline in 20mL methanol) with 9.2mL of methanol. Plates were incubated at 37°C for 24h. Antibacterial activities were evaluated by measuring inhibition of zone in diameters. This experiment was conducted with twice time.

Results and Discussion

Identification of the phytochemical diversity of secondary metabolism such as alkaloids, glycosides, steroids and terpenoids, flavonoids, reducing sugars and tannin are found to be *Huberzia* species presented in Table -1. Maximum amount of alkaloids content was found to be three species out of 8 *Huperzia* species and minimum content was five species out of eight *Huperzia* species presented in table-1. Alkaloids are a diverse group of compounds and they are known to have a variety of marked effects on animals. Alkaloids often act on the nervous system as stimulators, and sometimes as poisons. Several alkaloids, which occur naturally in *Lycopodium* and other pteridophytes, have been investigated for their medicinal properties. Alpha-onocerin and lycoperine A, were exhibit acetylcholinesterase inhibition activity (3-5)). *Lycopodiella cernua* is antivirally active and has been patented as a treatment for Hay fever (5-6).

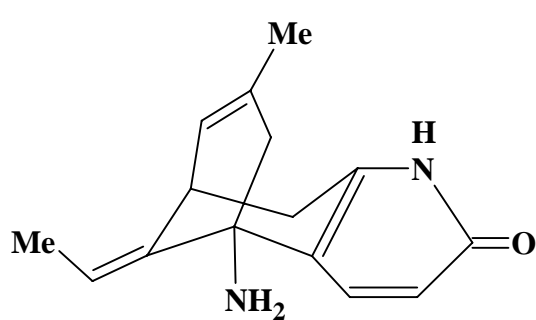
Table 1: Bioactive constituents of identification from *Huperzia* species

Fern-allies	Alkaloid	Flavonoid	Glycosides	Terpenoids and steroid	Reducing Sugar	Tannins
<i>H. ceylanica</i>	++	++	+++	++	+	+
<i>H. hamiltonii</i>	+++	++	+	++	+++	+
<i>H. nilgirica</i>	++	++	+	++	+	+
<i>H. macrostachys</i>	++	+	++	++	+	+
<i>H. phyllantha</i>	+++	+++	+	+	+++	+
<i>H. seratta</i>	+++	+	+	++	+	+
<i>H. squarrosa</i>	++	++	+++	++	+	+
<i>H. vernicosa</i>	++	+++	+++	+	++	+

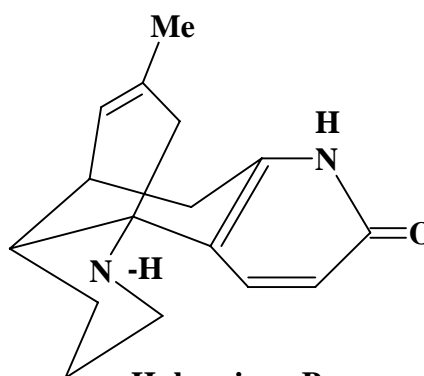
Color indication "+" trace

"++" minor

"+++""Maximum

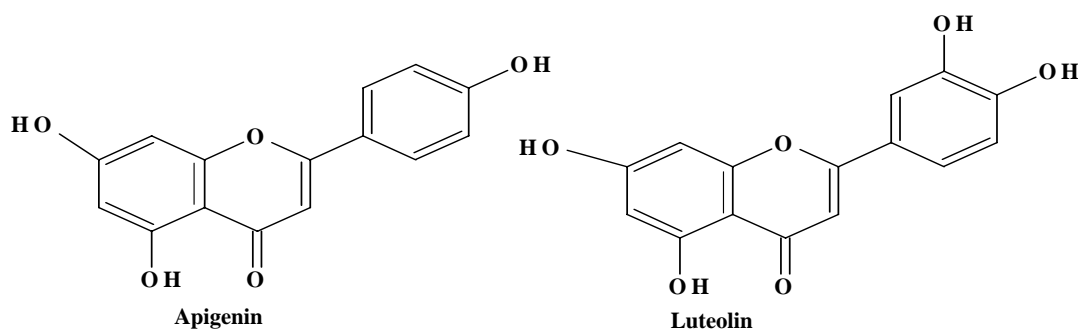


Huberzine - A



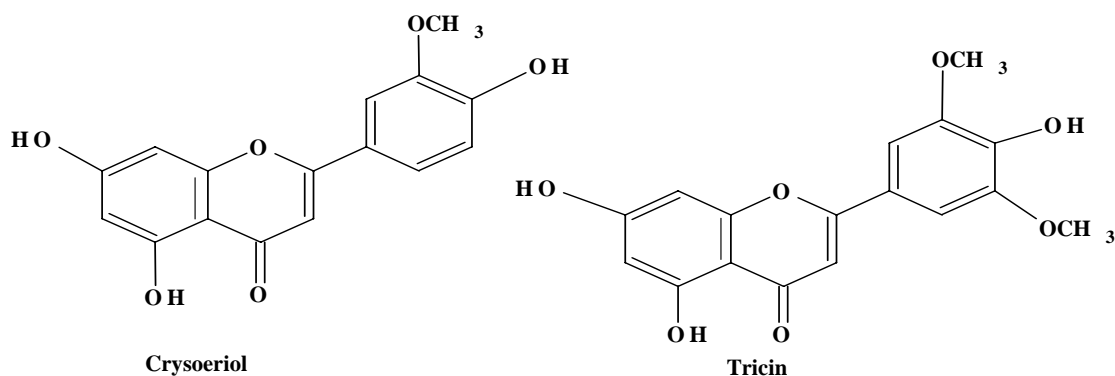
Huberzine - B

Flavonoids content was observed in high colour indication of both species of *H. phyllantha* and *H. vernicosa*. Medium content of flavonoids were found to be *H. ceylanica*, *H. hamiltonii*, *H. nilgirica*, and *H. squarrosa* and trace amount observed in *H. macrostachys* and *H. seratta*. Earlier, Voirin and Jay reported the flavonoids constituents of chrysoeriol, apigenin, luteolin, selgin, and triclin were presence in the *Huperzia* species (7).



Apigenin

Luteolin



Chrysoeriol

Tricin

The results of glycosides were found to be maximum amount of *H. ceylanica*, *H. squarrosa* and *H. vernicosa*. While minor quantity of glycoside was observed in four species of *H. hamiltonii*, *H. nilgirica*, *H. phyllantha* and *H. seratta*. Terpenoids and steroid were preliminary identified from the minor quantity was observed in six species out of eight species of *Huberzia* presented in table-1. Trace amount of terpenoid was observed in both species of *H. phyllantha* and *H. vernicosa*. Reducing sugar was preliminary identified by maximum color indication of two species of *H. hamiltonii* and *H. phyllantha*. While trace a quantity was observed in *H. ceylanica*, *H. nilgirica*, *H. macrostachys*, *H. seratta* and *H. squarrosa*. Tannin was identified in trace quantity of all tested *Huberzia* species seen in Table-1.

Table-2: Antibacterial activity of Leaf extract of *Huberzia* species

Fern-allies	Tested bacteria					
	<i>P. a</i>	<i>S. e</i>	<i>S. t</i>	<i>S. a</i>	<i>B. s</i>	<i>B. c</i>
<i>H. ceylanica</i>	12	16	8	14	15	11
<i>H. hamiltonii</i>	16	8	12	9	11	14
<i>H. nilgirica</i>	17	11	13	9	17	9
<i>H. macrostachys</i>	14	13	19	12	14	15
<i>H. phyllantha</i>	13	10	14	18	16	8
<i>H. seratta</i>	12	15	9	9	14	14
<i>H. squarrosa</i>	9	16	11	12	9	12
<i>H. vernicosa</i>	8	11	16	16	11	17

P. a- *Pseudomonas aeruginosa*; *S. e* - *Salmonella enteritidis*; *S.t*- *Salmonella typhimurium*; *S. a*- *Staphylococcus aureus*; *B.s*- *Bacillus subtilis*; *B.c*- *Bacillus cereus*

Extract of *Huberzia* species used in the well- diffusion method varied in their antibacterial activity. According to the results given in Table -2, the extract of *Huberzia* species had great antimicrobial activity against all investigated microorganism. The diameters of growth inhibition zone ranged from 8 to 19mm (including the diameter of the well - 6 mm) with the highest inhibition zone values observed against *Salmonella typhimurium* (19 mm). When compared to the reference antibiotic, (tetracycline, at recommended doses), *H. macrostachys* extract showed better anti-microbial activity against *Salmonella typhimurium* and *S. aureus* although *H. phyllantha* seems to resist the doses of antibiotic (tetracycline) assayed.

Conclusion

The results of preliminary phytochemical screening of methanol extract of *Huberzia species* leaves showed the presence of alkaloids, flavanoids, glycosides and terpenoids and the seldom presence of tannin.

Our results suggest that antimicrobial effect of *Huberzia* species were confirm their potential application in the treatment and prevention of diseases causing bacteria are *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus*. Further work is in progress to isolate and to characterize the active compounds present in leaves extract of *Huberzia* species.

Acknowledgment

Authors are thankful to the Principal, Secretary and Management of Pioneer Kumaswamy College, Nagercoil, Tamil Nadu for providing lab facilities. Authors also acknowledge the financial support provided by the Dr. K. S. Kothari Postdoctoral Fellowship, University Grant Commission, New Delhi for this work.

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