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Phytochemical Profile of Hydrostachys Insignis (Hydrostachyaceae)

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PHYTOCHEMICAL PROFILE OF *HYDROSTACHYS INSIGNIS* (HYDROSTACHYACEAE)

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ABSTRACT

Foliar material of *Hydrostachys insignis* contains kaempferol 3-glucoside and kaempferol 3-sophoroside. Iridoids, proanthocyanidins, acteoside, alkaloids, hydrolyzable tannins (ellagic acid), cyanogenic glycosides, and saponins could not be detected. This chemical profile does not support proposed relationships between Hydrostachyaceae and either Podostemaceae or members of the Scrophulariales. It is proposed that the chemical profile of Hydrostachyaceae reflects phylogeny, not convergence to an aquatic habitat; but the profile does not suggest any accurate systematic alignment of Hydrostachyaceae.

Key words: *Hydrostachys insignis,* Hydrostachyaceae, flavonoids, kaempferol 3-glucoside, kaempferol 3-sophoroside, comparative phytochemistry, aquatic plants.

INTRODUCTION

The family Hydrostachyaceae consists of the single genus *Hydrostachys* Thou., comprising about 22 species (Cusset 1973). Members of the family are all submerged, freshwater-aquatic, perennial herbs. A few species occur throughout southern Africa, but most of the species are endemic to Madagascar.

The floral morphology of Hydrostachyaceae is highly reduced, as is typical of aquatic angiosperms, and has made accurate systematic assignment of the family problematic. Hydrostachyaceae have been suggested to be closely related to Podostemaceae (Mauritzon 1933). More recently, morphological and embryological studies have suggested a relationship to the "Tubiflorae" families, especially members of the Scrophulariales (Rauh and Jäger-Zürn 1967).

Nothing has been reported regarding phytochemical features of Hydrostachyaceae although Gibbs (1974) noted that such studies should yield fruitful results. In the present study, a phytochemical profile of *Hydrostachys insignis* Mildbr. et Reim. was determined and the flavonoid phenolic constituents were identified in an effort to clarify the systematic relationships of Hydrostachyaceae.

MATERIALS AND METHODS

Dried plant material was removed from RSABG herbarium accession #266240 of *Hydrostachys insignis.* This material was ground to a fine powder and exhaustively extracted with 85% MeOH for 48 hr. The filtered and concentrated extract was subjected to 2D PC using TBA and 15% HOAc as solvents and the resulting chromatogram was examined under UV-light (Mabry, Markham, and Thomas 1970). Phenolic compounds so detected were separated by 1D PC using 15% HOAc, eluted into MeOH, and characterized chromatographically and spectroscopically using standard methods (Mabry et al. 1970).

Screening for additional classes of secondary compounds was performed using the methods of the following workers: alkaloids (Hultin and Torssell 1965); iridoids (Weiffering 1966); cyanogenic glycosides, proanthocyaninidins, and saponins (Gibbs 1974).

RESULTS

Several classes of secondary compounds were not detectable in *Hydrostachys insignis* using the methods described. These include iridoids, proanthocyaninidins, cyanogenic glycosides, alkaloids, phenylpropanoid glycosides, hydrolyzable tannins (as ellagic acid) and saponins.

Two flavonoid compounds were present and identified as kaempferol 3-glucoside and kaempferol 3-sophoroside. The chromatographic and UV-spectroscopic properties of the *Hydrostachys* flavonoids agree with published values in all respects (Harborne 1963, 1967). Acid and β-glucosidase hydrolysis of both compounds yielded kaempferol.

DISCUSSION

Depauperate Secondary Compound Chemistry

Hydrostachys insignis is depauperate of secondary compounds. This feature is not attributable to the aquatic habitat in which Hydrostachyaceae occur since other aquatic angiosperms in both the freshwater and marine environments maintain a rich suite of phylogenetically determined secondary compounds. Classes of secondary compounds which have been retained in aquatic taxa include alkaloids (Ostrofsky and Zettler 1986), iridoids (Hegnauer 1969), phenylpropanoid glycosides (Scogin 1992), and tannins (McMillan 1984; Williams and Harborne 1988). Such results from other plant groups suggest that the depauperate secondary compound chemistry of Hydrostachyaceae is not due to occurrence in the aquatic habitat, but rather a reflection of phylogenetically based biosynthetic constraints within the genome.

Systematic Affiliation

The systematic affiliation of Hydrostachyaceae is problematic. Three possible associations have been suggested. An early suggestion relating Hydrostachyaceae to Podostemaceae was based largely on the similarities of growth form and aquatic habitat (Kerner von Marilaun 1891). Support for this alliance has been weakened by recent morphological and embryological studies (Rauh and Jäger-Zürn 1967). Further, association with Podostemaceae doesn't solve the problem of relationships, because Podostemaceae too are systematically isolated and equally problematic. A second suggestion as to systematic relationships of Hydrostachyaceae emerged from the morphological and embryological studies of Rauh and Jäger-Zürn (1967) from which an alliance with the Tubiflorae was indicated, especially the Plantaginaceae or Solanaceae. This suggestion has been taken up by several contemporary phylogenists who place Hydrostachyaceae close to the Scrophulariales either in their own order (Hydrostachyales) (Dahlgren, Rosendal-Jensen, and Nielsen 1981; Takhtajan 1986) or in an order containing several aquatic families (Callitricales) (Cronquist 1981). The third suggestion as to systematic affiliates of Hydrostachyaceae is the proposal by Thorne (1992) that Hydrostachyaceae are allied with the superorder Rosanae, probably related to other south African families of the order Bruniales.

Chemical data do not strongly support a relationship between Hydrostachyaceae and any of these three putative associates; however, it does weigh against two of the possibilities, Podostemaceae and Scrophulariales. The Podostemaceae is phytochemically unstudied, but is currently under investigation by Romo and Scogin. Preliminary results indicate a very characteristic phenolic chemistry for the family which includes the presence of proanthocyanidins and xanthones, but the absence of flavonoids (Romo and Scogin, unpubl. data). The Hydrostachyaceae share none of these chemical features and thus exhibit a very low chemical similarity to Podostemaceae.

The Scrophulariales are chemically well characterized. Members produce flavonoids, usually flavones which are often 6- or 8-hydroxylated (Tomas-Barberan, Grayer-Barkmeijer, Gil, and Harborne 1988). Members of the Scrophulariales also characteristically produce iridoids (Dahlgren et al. 1981) and the phenylpropanoid glucoside, acteoside (Scogin 1992). *Hydrostachys insignis* produces two kaempferol glucosides only and, significantly, lacks iridoids and acteoside. The loss of occurrence of a class of secondary compounds is a more frequent occurrence than the gain of a compound class presence. Thus, the absence in *Hydrostachys insignis* of iridoids and acteoside is less significant than would be the presence of a diagnostic secondary compound. However, the coordinate loss of two compound classes characterizing the Scrophulariales (iridoids and acteoside) appears unlikely. Further, such a loss cannot be attributed to the aquatic habitat since other aquatic representatives of the Scrophulariales (*Hippuris* and *Callitriche*) still exhibit both of these compound classes (Weiffering 1966; Scogin 1992). Chemical features argue against a close association of Hydrostachyaceae with members of the Scrophulariales.

Among members of the Bruniales, the third suggested affiliate of Hydrostachyaceae, only Bruniaceae have been examined phytochemically. Jay (1968) surveyed six genera of Bruniaceae and reported the presence in leaves of flavonols and proanthocyanidins and the absence of ellagic acid. Quercetin was present in all genera examined, but kaempferol was absent. Proanthocyanidins were present in all genera examined, but are absent in Hydrostachyaceae. Loss of such condensed tannin compounds cannot be attributed to the aquatic habitat, since other aquatic angiosperms retain proanthocyanidin production (McMillan 1984). Thus, *Hydrostachys* could be chemically accommodated in the Bruniales, but the phytochemical evidence is not compelling.

In summary, phytochemical constituents of *Hydrostachys insignis* argue strongly against two suggested associations (Podostemaceae and Scrophulariales) and weakly against a third (Bruniales). The two kaempferol glycosides present in *Hydrostachys insignis* occur commonly and are widely distributed among angiosperms. In the absence of other diagnostically useful classes of secondary compounds, the chemical evidence does not make any productive suggestion as to a more likely systematic alliance.

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