ALKALOIDS AS TAXONOMIC MARKER OF FOUR SELECTED SPECIES OF Eupatorium WEEDS

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ABSTRACT Chemotaxonomy provides information as to additional taxonomic character to some selected plants. In the case of the four selected weed species of the genus Eupatorium, Alkaloid, a secondary metabolite is present in all the weed plants. Moreover, the functional groups of the alkaloid present was analyzed through Infrared spectroscopy. It was found out that based on the clustering of the functional groups there are two types of alkaloids that the weed plants possess. Eupatorium adenophorum and Eupatorium coelestinum contain one type of alkaloid while Eupatorium odoratum and Eupatorium riparium have another type. Thus adenophorum and coelestinum can be clustered to one group while odoratum and riparium belong to another cluster. The alkaloid extracted from the weed specimens were tested against Staphylococcus aureus and Escherichia coli to determine their antibacterial activity. When tested against S. aureus, a gram positive bacterium, the extracts from the weed plants were not as effective as the commercial antibiotic, Imipinem. However, the alkaloid extracts from E. adenophorum and E. riparium were as effective as synthetic Imipinem when tested against E. coli, a gram negative bacterium. Seemingly, the alkaloid extracts were bactericidal in activity.

INTRODUCTION

There are so many variabilities of living components of the environment. To know and understand better these wide arrays of living organisms, they have to belong to discrete groups and carry specific names. Through a classification system, these organisms take a definite stand as to their identity. Thus came about the science of taxonomy which occupies a unique position in biology. Taxonomy takes data from other areas of biological research to construct such a classification system and eventually provide the correct identification of organisms under study along with their probable relatives. In this sense, classification systems are effective mechanisms for storage of information of all types on the various taxonomic groups (Sivarajan, 1991).

In the field of taxonomy, some traditional sources of characters have been widely used in the intent to classify plants. Such a traditional scheme made use of comparative external morphology and anatomy, together with palynology which studies plant spores and pollen. To augment to the stability of the structure, embryological characters have also been considered. For more recent scheme in the characterization of plants for purposes of classification, as cited by Raven, et al., (1986), chromosome number and morphology have been used for almost 80 years in evaluating relationships and deducing phylogenetic sequences in Angiosperms. In such case, closely related species are usually similar in these aspects while distinctly unrelated species are conspicuously different.

Another recent pattern used to establish relationships of plants is using phytochemical properties. This scheme of classification is known as chemotaxonomy which incorporates the principles and procedures involved in the use of chemical evidence or biochemical composition of plants. Chemical constituents in plants may serve as reliable guide that may determine the relationships in plants which may be evolutionary or phylogenetic in nature. The principles and results of investigations into the chemical variation of plant taxa can be applied in two different purposes: first to provide taxonomic characters which may improve existing plant classification and second, to add knowledge of phylogeny or evolutionary relationship (Smith, 1976).

In plants, the more popular families that have been studied through chemotaxonomy are Malvaceae, Ranunculaceae, Magnoliaceae, Polygonaceae, and Solanaceae (Sivarajan, 1991).
There have been studies on the chemotaxonomic features of some selected Compositae plants like *Flaveria Dubautia*, and *Achillea*; all of which are non-weed plants. The presence of thiopene derivatives in two species of Argentinian *Flaveria* of Family Compositae was investigated to expand on the existing chemical information on both taxa (Agnese, et al., 1999). A medicinal plant of Cordoba in Argentina, *Tagetes lucida* was studied for its flavonoid content namely: patuletin, isorhamnetin, quercetagetin 3-O-rabionosyl galactoside and isorhamnetin 7-O glucoside (Abdala, 1999). Furthermore plants of Family Polygonaceae were studied as to the presence of secondary metabolites anthraquinones and flavonoids as chemotaxonomic markers (Vysochina, 1998). In line with this, such secondary metabolites serve for taxonomic purpose in describing the biochemical profile of the plants studied. Chemical constituents in plants, they are primary or secondary metabolites play a vital role in providing chemotaxonomic markers to augment existing characters for taxonomic descriptions (Roslinska, et al., 2001).

With the development of natural product chemistry, scientists have shown that phytochemical constituents can be used to characterize and classify species into taxa (Fang, et al., 2001). Three broad categories of plant chemical constituents are used for systematic purposes and they are: primary metabolites, secondary metabolites, and semantides. Metabolites such as carbohydrates, proteins, and lipids are the products of primary metabolism. Secondary metabolites are by products of basic metabolism of the cell generating secondary products that give plants the colors, flavors, and smells. These products are the sources of drugs, insecticides, dyes, flavours, fragrances, and the phytomedicines found in medicinal plants. Some of the secondary plant products perform signaling function as plant hormones. Semantides, on the other hand, are information-carrying molecules such as DNA, RNA, and proteins (Smith, 1976).

For weeds belonging to Family Asteraceae studies on the secondary metabolites as a chemotaxonomic character have rarely been undertaken. This study then endeavors to find out if there are predominant physiologically active substances present in the weeds of the Genus *Eupatorium* which can serve as chemotaxonomic markers of the taxa. The test plants were four species of *Eupatorium* weeds namely: *adenophorum*, *coelestinum*, *odoratum*, and *riparium*. These are herbaceous plants but a few are shrubs. Most commonly they called boneset, thoroughwort, or snakeroots.

**METHODOLOGY**

Leaves from the four species of *Eupatorium* weeds were collected from the crop fields, waste lands, and roadsides of nearby municipalities of Benguet as well as in Baguio City. The four selected species were *adenophorum*, *coelestinum*, *odoratum*, and *riparium*.

Extracts from the four weed samples were prepared using 80% ethyl alcohol. These crude alkaloid extracts were subjected to a confirmatory test through thin layer chromatography then fractionated and isolated using a series of organic solvents: tartaric acid and chloroform. The isolated alkaloids were purified using ethanol.

The purified alkaloid crystals were subjected to Infrared spectroscopy analysis to reveal the functional groups present.

**RESULTS AND DISCUSSION**

Preliminary test through phytochemical analysis revealed the presence of the following secondary metabolites. Table 1 summarizes these secondary plant products.
Table 1. Results of the phytochemical analysis of the four species of *Eupatorium* weeds

<table>
<thead>
<tr>
<th>Weed sample</th>
<th>Alkaloids</th>
<th>Saponins</th>
<th>Flavonoids</th>
<th>Tannins &amp; Polyphenols</th>
<th>Anthraquinones</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. adenophorum</em></td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>E. coelestinum</em></td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>E. odoratum</em></td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><em>E. riparium</em></td>
<td>Present</td>
<td>Present</td>
<td>present</td>
<td>Absent</td>
<td>absent</td>
</tr>
</tbody>
</table>

The three species namely: *adenophorum*, *coelestinum*, and *riparium* contain alkaloids except *odoratum*. Saponins are found in *odoratum* and *riparium* except in *adenophorum* and *coelestinum*. *Adenophorum*, *coelestinum*, and *riparium* all contain flavonoids except *odoratum*. Tannins and polyphenols are found in *adenophorum*, *coelestinum*, and *odoratum* but not in *riparium*. All the four species do not contain anthraquinones.

To confirm the presence of alkaloids in the weed samples, thin layer chromatography was performed and Table 2 summarizes the results.

Table 2. Results of the confirmatory test for the presence of alkaloids in the weed samples through thin layer chromatography

<table>
<thead>
<tr>
<th>Weed Samples</th>
<th>Solution A (Dragendorff’s spray)</th>
<th>Solution B (Dragendorff’s spray)</th>
<th>Solution C (Dragendorff’s spray)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. adenophorum</em></td>
<td>Brown spots</td>
<td>Brown-orange spots</td>
<td>Absence of color</td>
</tr>
<tr>
<td><em>E. coelestinum</em></td>
<td>Brown spots</td>
<td>Absence of color</td>
<td>Absence of color</td>
</tr>
<tr>
<td><em>E. odoratum</em></td>
<td>Brown spots</td>
<td>Absence of color</td>
<td>Absence of color</td>
</tr>
<tr>
<td><em>E. riparium</em></td>
<td>Brown spots</td>
<td>Absence of color</td>
<td>Absence of color</td>
</tr>
</tbody>
</table>

While the preliminary test revealed the absence of alkaloids in *E. odoratum*, a confirmatory procedure, the thin layer chromatography showed all weed specimens to contain alkaloids. The presence of brown spots on the TLC plate gave a positive reaction for the presence of alkaloids in the weed extracts because the Dragendorff’s spray served as a developer to make visible the secondary metabolite on the plate (Aguinaldo, *et al*., 2004).

Through Infrared analysis, the alkaloid crystals yielded functional groups. All the four weed samples contain the following functional groups: amide, amine, alcohol, aldehyde, aromatic rings, benzene rings, carboxylic acid, ethers, phenol, sulphone sulphonamide, sulphonic acid, urethane, (C-CH₂), CH₂, P-O-C and P-O-P. The presence of these functional groups in all plant weeds mean that they belong to the same genus. The following functional groups are contained in three weed samples but absent in one species: anhydride, beta lactone, butyl, carboxyl acid OH, epoxide, halogens like bromine and iodine, nitro aromatic, sulphone, (C=O), CH=CH, Si-O-C, CH-CH₂, (C-Cl). The other set of functional groups are found in two species but absent in the other two species. They are: amino acids, alkynes, amine hydrohalide, benzoate, carboxylate salt, daizonium salt, isocyanate, ketones, mercaptan, hydroxide, oxime, hydrocarbon, sulphate, CF₃, C-C-H, C=N, O-CH₃, P-O-H, P-H, P-O, Si-O-Si. The last sets of functional groups are present in one weed species but absent in the other three species. They are: ammonium ion, benzophenone, esters, isothocyanate, nitrile, sulphonyl chloride, C₅, C-S, N-CH₃, Si-CH₃.

There is a pattern that can be observed in the presence or absence of the functional groups of the alkaloids extracted from the weed samples. Two types of alkaloids are present in the four weed samples and the weed species are clustered as *adenophorum and coelestinum* belonging to one group and *odoratum and riparium* belonging to another group.
REFERENCES

SIVARAJAN, V. V. 1987. Introduction to the Principles of Plant Taxonomy.
SMITH, P. M. 1976. The Chemotaxonomy of Plants.