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Medicinal Properties of Cyperus Species (Sedge Family, Cyperaceae)

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Medicinal Properties of *Cyperus* Species (Sedge Family, Cyperaceae)

by

Lata Maishaya Udari

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Abstract

The grasses that yield therapeutically important products are among the least studied in the Cyperaceae family. Herb-based medicine from ancient times have played a vital role in ailments of various disease and nowadays it has been a particular area of interest in medicine. Phytochemicals are inherent compounds derived from plants that are biologically active, non-nutritive chemicals that act as a defensive or prophylactic medicine in humans. Their extracts have proven to show inhibition properties against different microbes. Despite the prevalence of *Cyperus* species and their traditional use in medicine, the chemical components responsible for their attributes remain largely unknown. This thesis research compared ten species of sedges (*Cyperus rotundus*, *Cyperus esculentus*, *Cyperus diffusus*, *Cyperus involucratus*, *Cyperus helferi*, *Cyperus prolixus*, *Cyperus papyrus*, *Cyperus surinamensis*, *Cyperus hortensis*, *Cyperus squarrosus*) with respect to their phytochemical constituents by examining their chemical profiles using HPLC. Methanol-based leaf and root extracts between species were shown to have significantly unique phytochemical profiles, with many extracts containing phytochemicals that support mammalian health (*e.g.* asiatic acid). In addition, comparing fresh plant extracts with dried samples (historical specimens) revealed that some of the phytochemical components appeared to be reasonably stable over decades. This study also examined antimicrobial properties of extracts from these plants, although results were inconclusive due to solvent incompatibility with either the phytochemicals or microbial species. Overall, this research revealed the unique phytochemical profiles of *Cyperus* species, and underscored the importance of considering each species and tissue type individually when investigating the medicinal properties of this genus.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

I. Introduction

Since ancient times plant-based medicines have played a pivotal role in treatment of disease (Fransworth et al., 1985). Recently, according to reports from the World Health Organization it was found that nearly 80% of the population in the world chiefly depend on herbal medicines for their basic wellness treatment (Ganesan et al., 2004). In fact, it was found that in the USA alone around 25% of medical prescriptions from 1959 to 1980 were derived from plant extracts or their derivatives (Fransworth et al., 1985). Among the families of plants, the grasses (family Poaceae) and sedges (family Cyperaceae) are well-known for containing medically relevant chemicals (Ahmed and Urooj, 2010). Few species from these families have been studied in detail, especially those within the Cyperaceae family.

For any plant extract, its curative or healing properties primarily depends on the presence of biologically active chemicals called phytochemicals that have the potential to alter physiological processes within the human body. Phytochemicals are inherent compounds derived from plants that are biologically active, non-nutritive chemicals that act as defense agents or prophylactics in humans (Ahmed and Urooj, 2010; Zheng and Wang, 2001). These natural compounds form the basis of numerous modern drugs that we use today (Rout et al., 2009). For example, for ailments of different disorders, such as respiratory disease, brain disorders (Kartnig et al., 1988), lesions, diarrhea, mucus secretion, etc. Asiatic acid found in *Centella asiatica* (Family Araliaceae) has been commonly used for wound healing in many places (Hong et al., 2005; Shetty et al., 2006). Specifically, in India and Madagascar for the ailment of leprosy, *Centella* extract has been commonly used (Swapna et al., 2011). This extract was found to be beneficial

not only for leprosy but also to have the capability to induce broader physiological effects within the human body (Chopra et al., 2000).

Deadly and chronic diseases, such as cancer and heart attacks, are often associated with oxidative stress (Weiss and Fintelmann, 2000). Oxidative stress is also responsible for many neurological disorders in part because the human brain utilizes 20% of oxygen consumed by the body (Weiss and Fintelmann, 2000). In fact, there has been many studies and literature which had proven beneficial effects of phytochemicals against various neurodegenerative disorders (Phani Kumar and Khanum, 2012). Herbal medicines have also been shown to be beneficial against inflammation. For instance, it was found that inflammatory bowel disease was relieved by usage of *Cyperus rotundus* extract. Specifically, the methanol extract of the rhizome of this plant was shown to reduce inflammatory bowel disease (Seo et al., 2001). Similarly, in another study it was found that this same plant extract had anti-inflammatory effects against carrageenan-induced swelling in albino rats (Sivapalan, 2013).

Of particular significance in herbal medicine are common weeds around homes and wastelands, as well as farmlands where sedges form important components. Among these common weeds are members of the family Cyperaceae, which are present nearly everywhere, but they are hard to differentiate due to their close similarity to grass family, except for their characteristically triangular-shaped stems (Adeniyi et al., 2014). Lowe and Stanfield (1974) remarked that about 80% of species from this family grow in damp or wet environments (including a few submerged aquatics), whereas around 20% of its species were discovered in much drier locales (Adeniyi et al., 2014).

The Cyperaceae family consists of about 4000 species (within 70 genera). Twenty of these species are commonly used as medicinal herbs in the Asia-pacific region due to their phytochemicals, such as indole alkaloids and quinones (Wiart, 2006). Despite having medicinal value, these sedges were also used for many domestic purposes. For example, *Cyperus papyrus* has been used for making boats and paper, and other species have been a source of fuel and been cultivated as ornamentals (Archer et al., 2000). The genus *Cyperus* includes about 950 species that are widely distributed across many biomes (Larridon et al. 2011a-c) and are found on all continents except Antarctica and Europe (Tucker, 2001). In the New World, areas of high diversity and endemism include the southeastern United States, Mexico, the Greater Antilles, and eastern Brazil (Tucker 2007, 2013; Tucker et al. 2002). Molecular studies by Larridon et al. (2011a-c) demonstrated that the Cyperus Clade actually consists of 13 or more genera.

Despite the prevalence of *Cyperus* species and their traditional use in medicine, the chemical components responsible for their efficacy are poorly understood. Furthermore, comparisons between the chemical profiles of *Cyperus* species are particularly lacking. Accordingly, the present study compared the phytochemical constituents of ten species of sedges (*Cyperus rotundus*, *Cyperus esculentus*, *Cyperus diffusus*, *Cyperus involucratus*, *Cyperus helferi*, *Cyperus prolixus*, *Cyperus papyrus*, *Cyperus surinamensis*, *Cyperus hortensis*, *Cyperus squarrosus*) by examining their chemical profiles using HPLC (high performance liquid chromatography). This study also attempted to examine antimicrobial properties of solvent-based extracts from these plants. In addition, we compared fresh plant extracts with dried samples (historical specimens) to determine if their phytochemical profiles were stable over time.

II. Objectives

1. Extract and identify phytochemicals from the leaves and roots of ten species of *Cyperus* and compare the chemical profiles between tissue types and species.
2. Examine the stability of phytochemical profiles by comparing extracts of freshly harvested leaves and roots with those from historical (dried) specimens.
3. Explore the antimicrobial properties of *Cyperus* extracts on common Gram-positive and Gram-negative bacterial species.

III. Materials and Methods

A. Plant Specimens

The *Cyperus* species used in this study (*C. diffusus, C. esculentus, C. surinamensis, C. hortensis, C. involucratus, C. papyrus, C. prolixus, C. rotundus, C. squarrosus, and C. helferi*) were sourced locally (Charleston and Mattoon; **Figure 1**), within the state (Shelby County), or outside of the state (*e.g.* Mississippi). Herbarium specimens of *C. involucratus* used for examination of phytochemicals in preserved material were from Brazil (1980 and 1985), Paraguay (1999), France (2000), Palaestina (2009), and Puerto Rico (1982).

B. Phytochemical Extraction and HPLC Analysis

HPLC analysis was used to characterize leaf and root metabolites and examine the variation in the amount or type of chemicals present. Metabolites were extracted using 1 mL of HPLC-grade methanol per 100 mg of fresh leaf, fresh root, and dried leaf samples that were ground to a fine powder with a mortar and pestle using liquid nitrogen. This mixture was centrifuged at 10,000 rpm for 5 min to pellet the insoluble material. After centrifugation, the supernatant was passed through a 0.22 μm filter and 10 μL per sample

was analyzed using a Hitachi Chromaster HPLC with a 5430 Diode Array and Hypersil Gold column as per Meiners et al. (2017). The mobile phase (0.7 mL/min) was a mixture of acetonitrile:water (v/v) at 20:80 from 0–5 min, a linear gradient of 20:80 to 95:5 from 5-45 min, 95:5 from 45–55 min, a linear gradient of 95:5 to 20:80 for 55-60 min, and 20:80 for 60–70 min. Peak areas were quantified using R program and recorded in Excel (Meiners et al., 2017).

C. Antimicrobial Assays

Antimicrobial activity tests were carried out on two representative Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and two representative Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) sourced from the microbiological inventory at Eastern Illinois University. All bacteria were maintained on commercially-produced Tryptic Soy Agar (TSA) at 37°C and transferred to fresh media every 1-2 days. The plant material was prepared by grinding plant tissues in liquid nitrogen using a mortar and pestle. Water-based extracts were prepared by adding 50 g of powdered tissue to 500 mL of ice-cold distilled water in a conical flask. The solution was left undisturbed for 48 h, and then filtered using Whatman No.1 filter paper under vacuum. Methanol-based extracts were prepared by adding 50 g of powdered tissue to 500 mL of methanol in a conical flask. The solution was left undisturbed for 48 h, and then filtered using Whatman No.1 filter paper under vacuum. Both water-based and methanol-based extracts were stored in sterile bottles at 4°C until further use (Adeniyi et al., 2014).

i. Disc Diffusion Method

The disc diffusion assay was performed according to Bauer et al. (1969). A total of 10 mL of TSA was poured into Petri dishes and allowed to solidify. Petri dishes were inoculated by streaking bacteria over their surfaces with the aid of an inoculation loop. To prepare discs, Whatman No.1 filter paper was cut using a paper punch to form paper discs of 6 mm in diameter. The discs were wrapped in aluminum foil and sterilized in an autoclave at 121°C for 15 min. After sterilization, they were soaked in 100 mL of appropriate plant extract. Four discs from each extract, methanol control, ethanol control and water control were then aseptically placed with the aid of sterile forceps on a TSA plate containing one of the four bacterial species. For each bacterial species, the experiment was performed in duplicate. The plates containing the discs and bacteria were then incubated at 37°C. The width of the zones of inhibition around the disc were measured after 24 h.

ii. Liquid Culture Method

Starter liquid cultures of each bacterial species (*P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. aureus*) were prepared in 2 mL of Tryptic Soy Broth (TSB) in a 10 mL test tube by adding a small amount of plate-grown on TSA using a sterile transfer loop. The starter cultures were grown overnight at 225 rpm at 37°C the day prior to the antimicrobial assay. The assay consisted of a series of culture tubes with 2 mL of TSB that had 200 µL of methanol-based extract from the plant species and tissue of interest along with 10 µL of starter culture of the bacterial species of interest. Controls with only methanol were also included to assess the antimicrobial effects of ethanol alone. After 16 h of growth at

225 rpm and 37°C, the optical density of the cultures was measured using a spectrophotometer at 600 nm (zeroed with uninoculated TSB).

D. Statistical Analysis

HPLC peak areas for all peaks from each sample were analyzed using metaMDS in R (R Core Team, 2013). The supplemental package "vegan" was required. The command line "metaMDS(peaks.dat, distance = "bray", k=3)" was used to conduct the metabolic profile comparisons, and the command lines "adonis(peaks.dat~sample*treatment, data= sam.dat)" and "adonis(peaks.dat~sample*treatment*replicate, data=sam.dat)" were used for the statistical analyses. Data were exported as CSV files, and the resulting data tables were moved to Excel for graphing purposes.

IV. Results and Discussion

A. Objective 1: Extract and identify phytochemicals from the leaves and roots of ten species of *Cyperus* and compare the chemical profiles between tissue types and species

i. Phytochemical Profile Analysis

To explore the phytochemical profiles of the *Cyperus* species, methanol-based extracts were analyzed using HPLC. This analytical approach detected polar-type molecules, though the resolution for these compounds was limited. Non-polar chemicals were resolved best over the entire duration of each run using this method because of the nature of the column used for this analysis (C18-based chemistry).

To explore the broad phytochemical patterns across all species and both tissue types (leaves and roots), an nMDS plot was generated to visualize differences in phytochemical profiles holistically (**Figure 2**). Visually, the leaves and root samples from

each species cluster relatively closely to each other. For example, the RR and RL (*C. rotundus* root and leaf, respectively) samples (upper left of **Figure 2**). Nevertheless, statistical analysis suggests significant differences (p= 0.001) between species irrespective of tissue type (**Table 1**). Moreover, when we look at root and leaf tissues collectively, we also see a significant difference (p= 0.001; **Table 1**). As expected, analysis of replicates shows no significant difference (p=0.042; **Table 1**), which indicates that the three replicates within each species and tissue type were reliably similar. This is further evidenced by the tight clustering of replicates observed in **Figure 2**.

To better understand the phytochemical profiles between species, the data were separated into leaf and root samples for analysis. The nMDS plot of only the leaf samples is shown as **Figure 3**, which clearly shows separation between the different species. The statistical analysis confirms that the leaf samples across all species are significantly different (p=0.001; **Table 2**). Similar to the previous analysis, the replicates themselves were not significantly different as expected (p=0.125; **Table 2**). When examining roots rather than leaves in a similar fashion there was also clear separation between species (**Figure 4**) with statistical significance between samples (p=0.001) but not replicates (p=0.378) as expected (**Table 3**).

These nMDS analyses indicated that the phytochemical profiles between distinct but very much related species of *Cyperus* are unique at the whole plant level (leaf and root) and between the two tissue types. It is therefore important in medicinal studies with extracts from this genus to carefully consider the unique nature of the chemicals available from the different species, and to appreciate the unique phytochemical mixtures between leaf and root samples within a particular plant of interest.

ii. Bioactive Phytochemicals

a. Asiatic Acid

Asiatic acid's major source is from *C. asiatica*, but is also found in another plant extracts that have been widely used in medicines. It was found to have anti-inflammatory properties and has been used for wound healing by inducing collagen production (Maquart et al., 1999). Asiatic acid has also been shown to induce cell cycle arrest and apoptosis in breast cancer cells, block angiogenesis in cells and tumors from glioblastomas (Hsu et al., 2005; Kavitha et al., 2011), and reduce neuronal damage and cognitive defects from glutamate-induced apoptosis in isolated human neuroblast cells (Xu et al., 2012).

In our analysis, ten cyperaceous species (*Cyperus rotundus, Cyperus esculentus, Cyperus diffusus, Cyperus involucratus, Cyperus helferi, Cyperus prolixus, Cyperus papyrus, Cyperus surinamensis, Cyperus hortensis, Cyperus squarrosus*) and a commercial standard of asiatic acid were analyzed using HPLC. The retention time of the asiatic acid standard was determined to be 25.5 min (**Figure 5**) using the parameters outlined in the Materials and Methods. Based on the common retention time a peak representing asiatic acid was found in the leaf and root samples of only *C. rotundus*, *C. squarrosus*, and *C. esculentus* (**Figure 6**).

b. Coumarin

Coumarin (1,2-benzopyrone) is normally a white crystal at ambient temperature that is found in various plant extracts, such as woodruff, yellow sweet clover, lavender, and cassia (Hoult and Payd, 1996). Coumarin was found to have anticarcinogenic properties

due to inhibition of growth in both benzopyrene-induced tumors (Sparnis and Wattenberg, 1981) and spontaneous tumors (Tseng, 1991) in mice.

HPLC analysis of coumarin (17.5 min retention time; **Figure 7**) revealed that it may be present in a greater diversity of *Cyperus* species than asiatic acid. Specifically, in addition to *C. rotundus* (root), *C. squarrosus* (leaf and root), and *C. esculentus* (leaf and root), appreciable levels of coumarin were also found in *C. papyrus* (leaf), *C. hortensis* (root), *C. diffusus* (leaf), and *C. involucratus* (leaf), with the highest level found in the leaf tissue of *C. squarrosus* (**Figure 8**).

c. Curcumin

Curcumin is a commonly found in turmeric and has similar properties, such as being stable in the acidic pH of human stomachs (Wang et al., 1997) and being soluble in organic solvents (Aggarwal et al., 2003). It has anti-inflammatory properties (Arora et al., 1971; Chandra and Gupta, 1972; Chainani-Wu, 2003; Mukhopadhyay et al., 1982) as well as antioxidant properties (Toda et al., 1985). It has also been shown to be beneficial for brain conditions, such as Alzheimer's (Lim et al., 2001; Travis, 2001). In addition, the rhizome extract of *Curcuma longa* showed anticancer activity (Singh et al., 2012). In the present study, curcumin had a retention time of 26 min with our analysis system (**Figure 9**). The compound was shown to be present in *C. rotundus* (leaf and root), *C. squarrosus* (leaf), *C. esculentus* (leaf), *C. diffusus* (leaf), and *C. involucratus* (root), but not in detectable quantities in all other species tested (**Figure 10**).

d. Quercetin

Quercetin is a compound that cannot be synthesized in the human body, and belongs to the flavanol group (Lakhanpal and Rai, 2007). It was found to have many medicinal properties, such as protecting erythrocytes from damage caused by smoking (Begum and Terao, 2002), decreasing inflammatory mediators (Warren et al., 2009; Xiao et al., 2011), as well as lowering blood pressure and preventing cardiac hypertrophy (Edwards, 2007). Other medical properties discovered include reduced LDL oxidation (Chopra et al., 2000), inhibition of fat accumulation (Chopra et al., 2000; Park et al., 2008), anticarcinogenic properties (Akan and Garip, 2013; Vásquez-Garzón et al., 2009), and beneficial effects against neurodegenerative diseases (Denny and Muralidhara, 2013; Graefe et al., 2001).

In the present study, the presence of quercetin (18 min retention time; **Figure 11**) was explored across the ten *Cyperus* species, with detectable levels found in *C. squarrosus* (root), *C. esculentus* (leaf and root), *C surinamensis* (root), *C. diffusus* (leaf and root), and *C. prolixus* (root). There was a noticeable abundance of quercetin in the *C. surinamensis* root sample compared with all other tissues across all other samples (**Figure 12**). In addition, it is noteworthy that quercetin was not detected in the leaf tissue of *C. surinamensis*, which suggests that efforts to exploit quercetin in this species should focus on the root tissue.

e. Flavanones

Flavonoids are a common compound found in all vascular plants, especially in fruits and fruit juices of the *Citrus* genus (Peterson et al., 2005). Some putative medicinal benefits include the prevention of cardiovascular disease (Dauchet et.al., 2005, 2006; He et al.,

2006, 2007) and cerebrovascular diseases (Pnekt et al., 2002), reduced incidence of hypertension (Cassidy et al., 2012), and beneficial roles in atherosclerosis progression (Choe et al., 2001; Lee et al., 2001). An examination of flavanone levels (28 min retention time; **Figure 13**) in the present study revealed relatively high levels in several species: *C. rotundus* (leaf and root), *C. squarrosus* (leaf and root), *C. hortensis* (root), *C. esculentus* (leaf), *C. surinamensis* (leaf), and *C. diffusus* (leaf and root). The highest levels in the root tissue were found in *C. rotundus*, which also had the second highest levels in the leaf tissue after *C. diffusus* (**Figure 14**).

f. Madecassic Acid

Madecassic acid is commonly found in *Centella asiatica* (Hashim et al., 2011) and is a pentacyclic triterpenic acid (Yin et al., 2012). Some possible medicinal benefits include anti-oxidative stress in cerebral artery occlusions in rats (Tabassum et al., 2013) and inhibited cancer growth in mouse colons (Zhang et al., 2014). Madecassic acid had a retention time of23.5 min in our HPLC-based analytical system (**Figure 15**). Just two species (*C. rotundus* and *C. squarrosus*) had detectable levels of madecassic acid in root tissue, while just three species (*C. helferi*, *C. surinamensis* and *C. diffusus*) had detectable levels in leaves (**Figure 16**). The highest levels found were in *C. diffusus* leaf tissue, and overall the leaves in this study had much greater levels of this compound than the roots (**Figure 16**).

B. Objective 2: Examine the stability of phytochemical profiles by comparing extracts of freshly harvested leaves and roots with those from historical (dried) specimens

In this approach the goal was to examine the stability of secondary metabolites in freshly harvested plant samples compared to preserved specimens. For the purposes of this analysis, freshly harvested leaf samples were from locally sourced *C. involucratus* and the preserved specimens were dried *C. involucratus* leaves from a group of samples from a variety of locations. The fresh and dried samples were extracted and analyzed by HPLC as described previously.

When examining all peaks in a holistic fashion, the fresh *C. involucratus* samples showed relatively small peaks in the non-polar range (8-55 min; **Figure 17**) compared to preserved specimen samples (**Figures 18 and 19**). For example, there is a relatively large peak at retention time 20 minutes and near 28 minutes that is comparatively small in freshly harvested leaf samples than in dried leaf samples (**Figures 17-19**). Among the compounds that could be identified that appeared to be more highly concentrated in the preserved specimens were asiatic acid, coumarin, vanillin, curcumin, quercetin, flavanone, and madecassic acid (**Figure 20**). The apparent higher concentration of these chemicals in the preserved specimens may be attributable in part to the fact that the fresh and dried (preserved) samples were prepared according to equivalent starting masses, where the fresh leaf samples were hydrated, and the dried samples were dehydrated. This difference in water mass would cause the dried samples to be effectively more concentrated, which would lead account for the apparent increased concentration of these

phytochemicals observed through HPLC analysis. Nevertheless, it is clear that preserved samples are able to retain these important chemicals after many years in a dried state.

C. Objective 3: Explore the antimicrobial properties of *Cyperus* extracts on common Gram-positive and Gram-negative bacterial species

Phytochemicals have been linked to antimicrobial properties in the past. For example, coumarins have shown antimicrobial properties against *Staphylococcus aureus* and *Candida albicans* (Sardari et al., 1999). Curcumin has shown antimicrobial properties for different strains of bacteria and fungi (Lutomski et al., 1974; Negi et al., 1999), and has also shown anti-mutagenic properties, inhibition of human platelet aggregation, and anti-HIV properties (Hoult and Payd, 1996). The most common methods to assess antimicrobial properties of plant extracts are the disc diffusion method and liquid culture method (Balouiri et al., 2016). In the present study, the disc diffusion method and liquid culture methods were both used to examine the antimicrobial capabilities of *Cyperus* leaf and root extracts against two species each of Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*).

i. Disc Diffusion Method

Table 4 shows the inhibition zones measurements of each of the four bacterial species around the discs from each plant species and tissue type (methanol-, ethanol-, and water-based extractions) from the present study. A representative group of plates is shown in **Figure 21**. Some positive antibacterial activities were apparent in the present study. For example, *Pseudomonas aeruginosa* appeared to be inhibited by the ethanol extracts from *C. surinamensis* leaf, *C. involucratus* leaf, *C. papyrus* root, and *C. diffusus* root, when

compared with discs containing only ethanol (controls; **Table 4**). Similarly, *Staphylococcus aureus* appeared to be inhibited by ethanol extracts from *C. diffusus* and *C. papyrus* roots, compared to the ethanol controls.

Extractions that were based in water did not showed promising antibacterial activities (**Table 4**). One of the primary reasons to explain this may be that the active compounds (many of which are reasonably non-polar) were not extracted and solubilized from the plant tissue into this extremely polar solvent. In methanol, few of the plant extracts inhibited growth, with the exception of *C. surinamensis* roots, which showed antibacterial activities for all four strains of bacteria (**Table 4**). Also, *C. diffusus* leaves in methanol solvent showed antibacterial activity for *Escherichia coli* and *Bacillus subtilis*, while *C. papyrus* root in methanol solvent led to inhibition of *Pseudomonas aeruginosa* and *Bacillus subtilis*. Other extracts appeared to only effect one of the four species of bacteria. For example, *C. helferi* roots in methanol solvent showed positive results only for *Escherichia coli* (**Table 4**). Despite promising results, the disc diffusion method was challenging to obtain conclusion results. One of the major issues with this method is that phytochemicals are often extracted in solvents that have antimicrobial properties by themselves (*e.g.* methanol and ethanol). Water would be an ideal solvent for antimicrobial assays, but water is not well suited for extracting phytochemicals with limited polarity.

ii. Liquid Culture Method

A major benefit to this method compared to the disc diffusion strategy is that the dosage of molecules or whole extracts can be carefully controlled to determine the MIC. A preliminary examination of the liquid culture method used in the present study was

inconclusive. Once again, the need for a solvent such as methanol for sufficient phytochemical extraction makes interpretation of inhibition extremely difficult because methanol (and similar solvents) alone are bactericidal. Our preliminary data (**Table 5**) demonstrate this scenario, where the methanol control samples showed very strong inhibition against *P. aeruginosa* and *E. coli* (both Gram-negative) and *S. aureus* (Gram-positive). Accordingly, these data should be viewed as extremely preliminary. Future studies of this nature will require the use of a solvent for extractions that have limited or no apparent bactericidal properties against the test bacteria.

Table 1. nMDS analysis of leaf and root samples from *Cyperus* species. Bold values indicate statistical significance at the 99.9% level.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	P value
species	9	12.4405	1.38227	20.2759	0.50326	**0.001**
tissue	1	2.1166	2.11661	31.0475	0.08562	**0.001**
replicate	1	0.0715	0.07151	1.049	0.00289	0.402
species:tissue	9	7.3842	0.82047	12.0351	0.29872	**0.001**
species:replicate	9	0.5458	0.06064	0.8895	0.02208	0.807
tissue:replicate	1	0.1042	0.10417	1.528	0.00421	0.097
sample:treatment:replic	9	0.6934	0.07705	1.1302	0.02805	0.18
Residuals	20	1.3635	0.06817		0.05516	
Total	59	24.7196			1	

Table 2. nMDS analysis of leaf samples from *Cyperus* species. Bold values indicate statistical significance at the 99.9% level.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	P value
species	9	9.3974	1.04415	17.4368	0.87502	**0.001**
replicate	1	0.0949	0.09494	1.5854	0.00884	0.125
species:replicate	9	0.6484	0.07205	1.2032	0.06038	0.185
Residuals	10	0.5988	0.05988		0.05576	
Total	29	10.7396			1	

Table 3. nMDS analysis of root samples from *Cyperus* species. Bold values indicate statistical significance at the 99.9% level.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	P value
species	9	10.4273	1.15859	15.1521	0.87895	**0.001**
replicate	1	0.0807	0.08074	1.056	0.00681	0.378
species:replicate	e 9	0.5907	0.06564	0.8584	0.0498	0.833
Residuals	10	0.7646	0.07646		0.06445	
Total	29	11.8635			1	

Table 4. Inhibition data from disc diffusion study reported as centimeters from the disc center. Minus sign indicates no effect and plus sign indicates slight inhibition.

	species and tissue	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*
ethanol	*C. diffusus* roots	1	1.3	0.9	0.7
	C. involucratus leaves	1.1	1	1.3	0.7
	C. papyrus roots	0.9	1.4	1.2	1.3
	C. prolixus leaves	0.4	0.7	0.8	1.2
	C. prolixus roots	1	0.9	-	0.1
	C. surinamensis leaves	-	-	1	1.1
	control	1.4	1.2	0.8	1.5
water	*C. prolixus* leaves	+	-	-	-
	C. prolixus roots	-	-	-	-
	C. surinamensis roots	-	-	-	-
	control	-	-	-	-
methanol	*C. diffusus* leaves	1	0.8	+	1
	C. diffusus roots	0.9	-	0.8	0.8
	C. helferi leaves	0.7	+	-	0.85
	C. helferi roots	0.7	0.7	-	0.95
	C. involucratus leaves	0.1	-	-	0.9
	C. involucratus roots	0.8	0.8	-	1
	C. papyrus leaves	0.9	0.8	+	0.7
	C. papyrus roots	1.2	0.8	0.9	0.8
	C. prolixus leaves	0.7	0.9	-	0.8
	C. prolixus roots	0.9	0.8	+	0.8
	C. surinamensis leaves	0.9	0.8	0.4	0.9
	C. surinamensis roots	1.1	1.25	0.9	1.1
	control	0.9	0.9	0.8	0.8

Table 5. Optical density (600 nm) readings for the liquid culture inhibition study that used methanol extracts of various *Cyperus* species.

	P. aeruginosa	*B. subtilis*	*S. aureus*	*E. coli*
C. hortensis	0.136	1.247	0.360	0.602
C. esculentus	0.158	1.345	0.507	0.745
C. papyrus	0.170	1.293	0.652	0.187
C. surinamensis	0.220	1.172	1.394	0.828
C. involucratus	0.051	1.271	0.215	0.196
C. diffusus	0.158	1.251	1.044	0.614
C. helferi	0.200	0.986	0.619	0.879
C. prolixus	0.206	1.001	0.341	0.461
C. squamosa	0.190	0.485	1.021	0.785
C. rotundus	0.175	1.157	1.041	0.595
methanol control	0.021	1.244	0.438	0.024

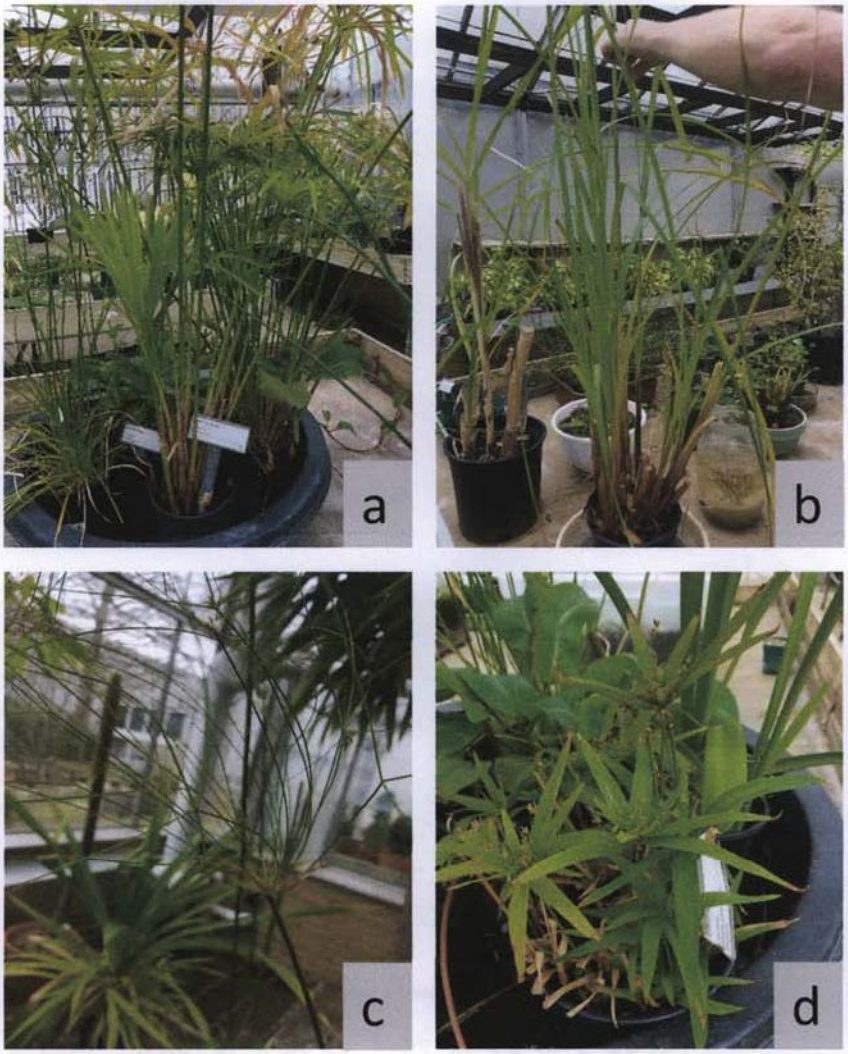

Figure 1. Representative samples of *C. involucratus* (a), *C. prolixus*(b), *C. papyrus* (c), *C. diffusus* (d), that were grown in the Thut Greenhouse at Eastern Illinois University.

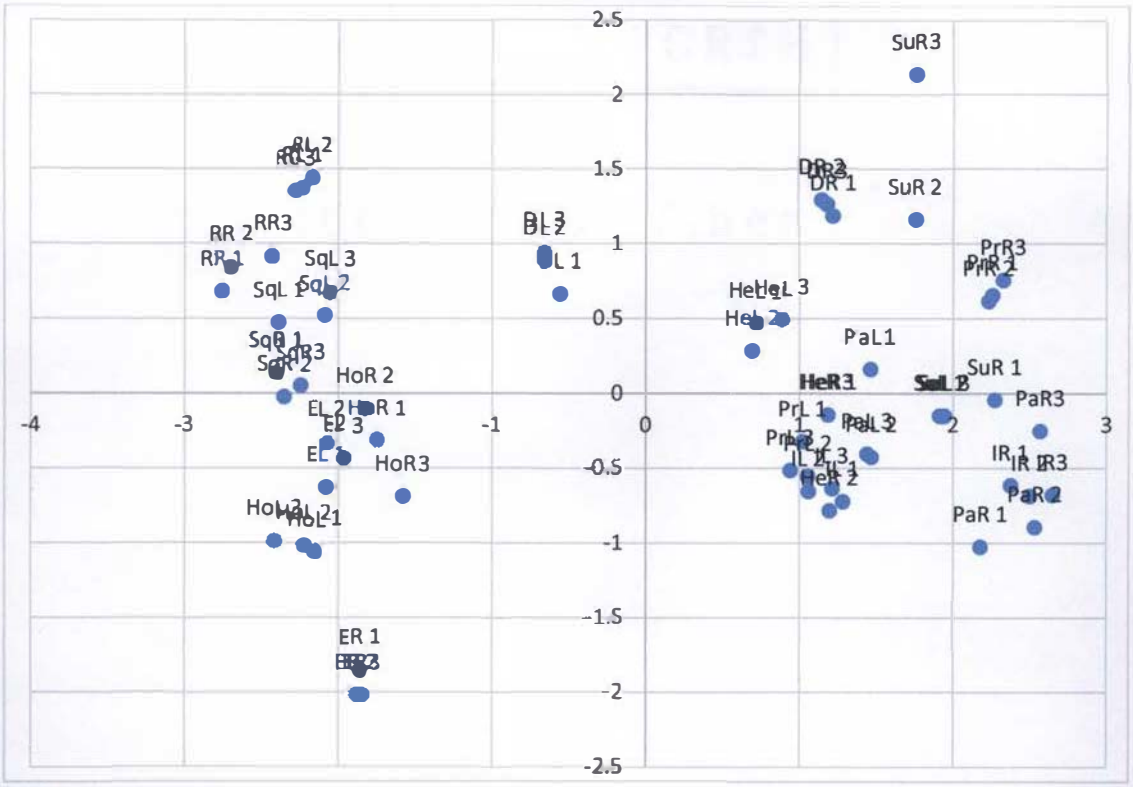

Figure 2. nMDS plot of extracts from *Cyperus rotundus* (R), *Cyperus esculentus* (E), *Cyperus diffusus* (D), *Cyperus involucratus* (I), *Cyperus helferi* (He), *Cyperus prolixus* (Pr), *Cyperus papyrus* (Pa), *Cyperus surinamensis* (Su), *Cyperus hortensis* (Ho), *Cyperus squarrosus* (Sq), from the leaf (L) and root (ending letter R) tissues. The number at the end of the code indicates the replicate number for that species and tissue type.

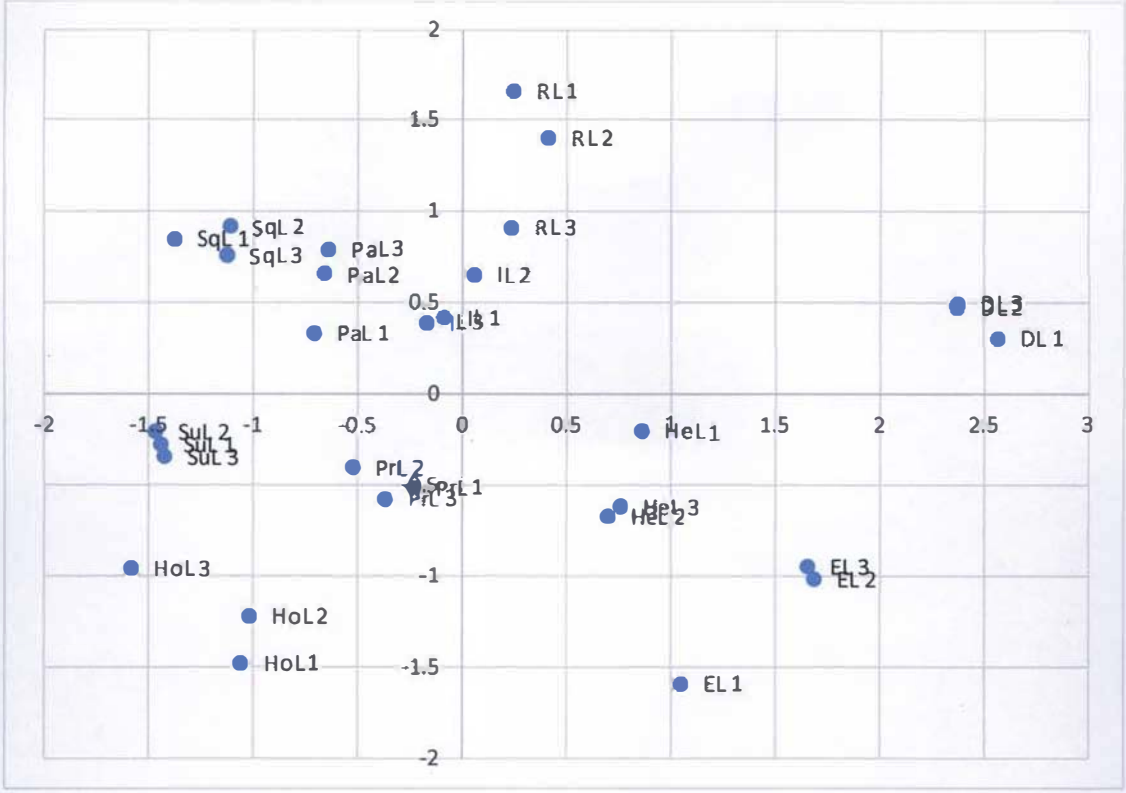

Figure 3. nMDS plot of extracts from *Cyperus rotundus* (R), *Cyperus esculentus* (E), *Cyperus diffusus* (D), *Cyperus involucratus* (I), *Cyperus helferi* (He), *Cyperus prolixus* (Pr), *Cyperus papyrus* (Pa), *Cyperus surinamensis* (Su), *Cyperus hortensis* (Ho), *Cyperus squarrosus* (Sq), from the leaf (L) tissues. The number at the end of the code indicates the replicate number.

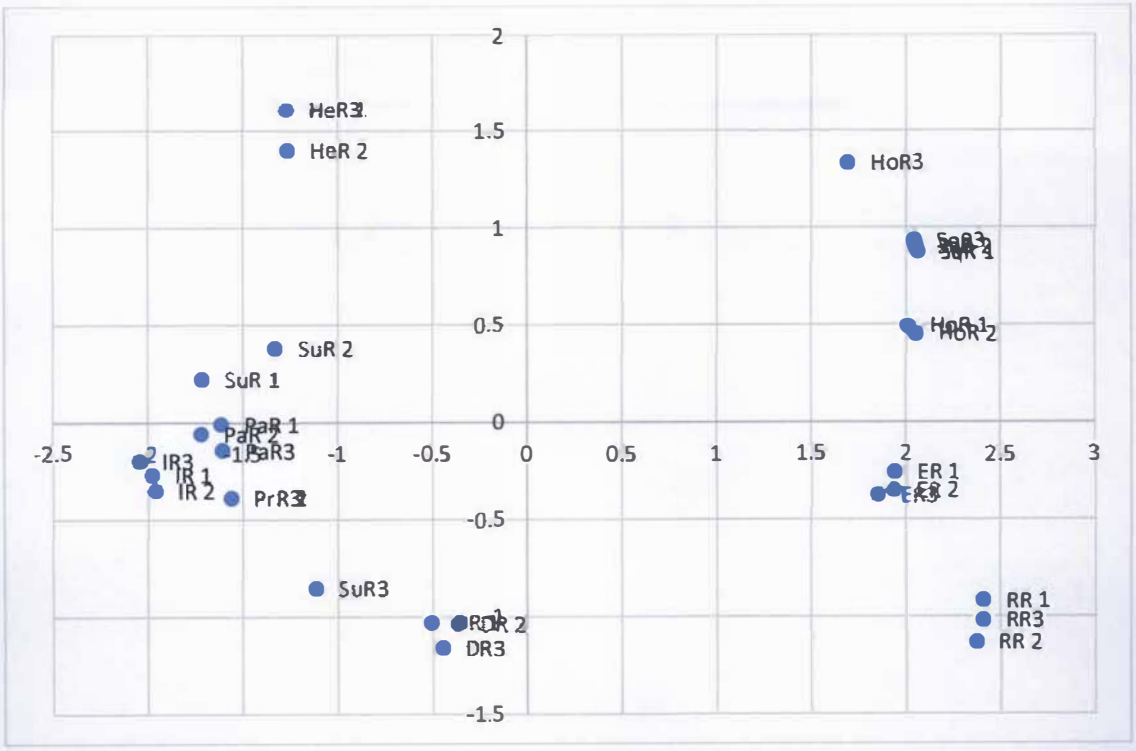

Figure 4. nMDS plot of extracts from *Cyperus rotundus* (R), *Cyperus esculentus* (E), *Cyperus diffusus* (D), *Cyperus involucratus* (I), *Cyperus helferi* (He), *Cyperus prolixus* (Pr), *Cyperus papyrus* (Pa), *Cyperus surinamensis* (Su), *Cyperus hortensis* (Ho), *Cyperus squarrosus* (Sq), from the root (R) tissues. The number at the end of the code indicates the replicate number.

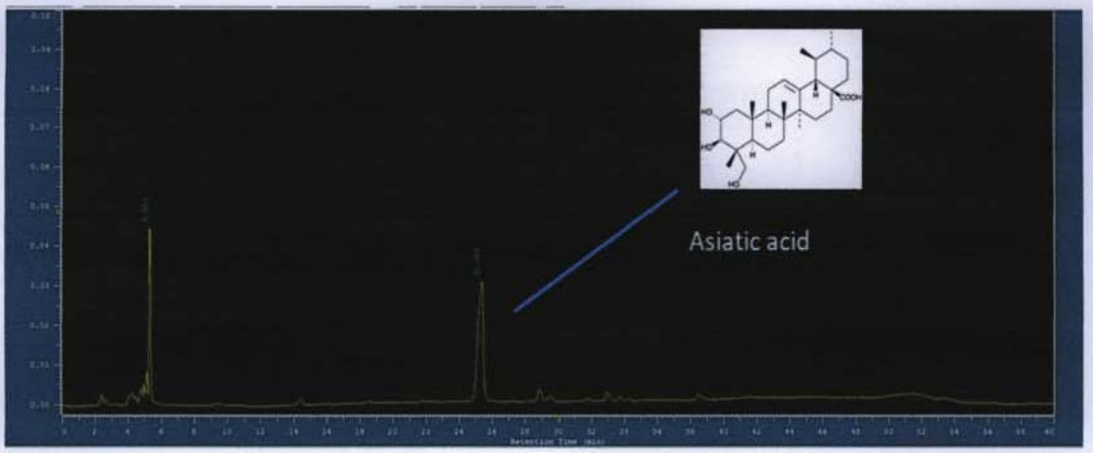

Figure 5. HPLC chromatogram from a pure asiatic acid sample diluted in methanol.

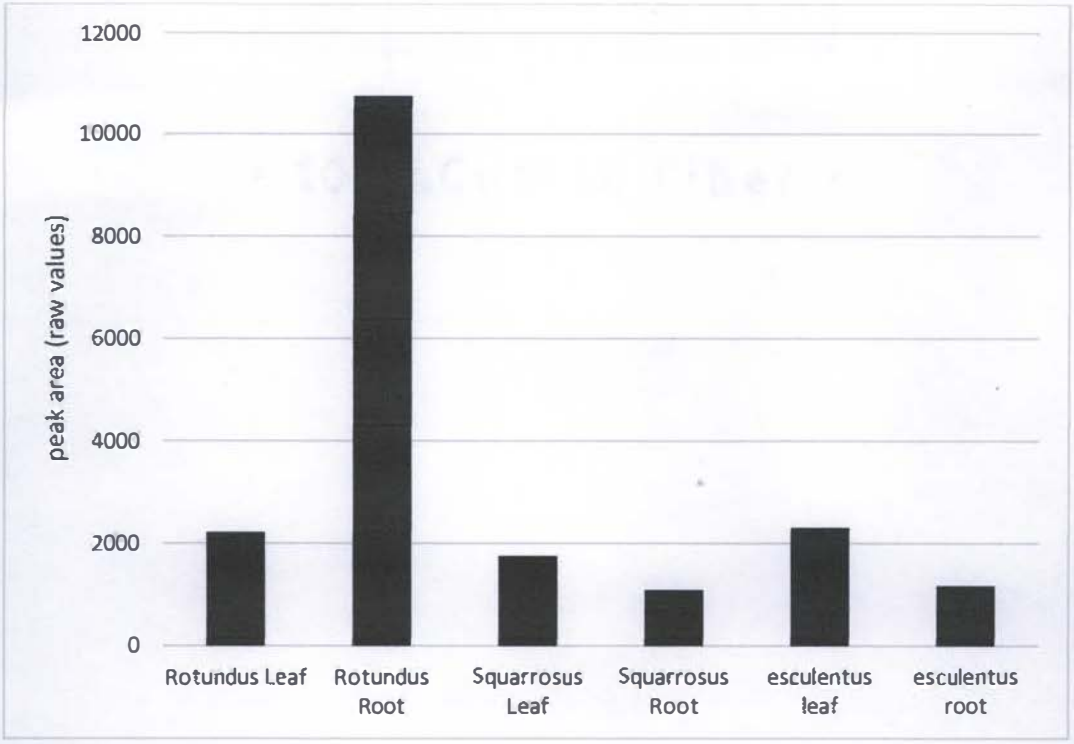

Figure 6. Asiatic acid concentrations in the leaves and roots of *Cyperus* species as determined by HPLC analysis.

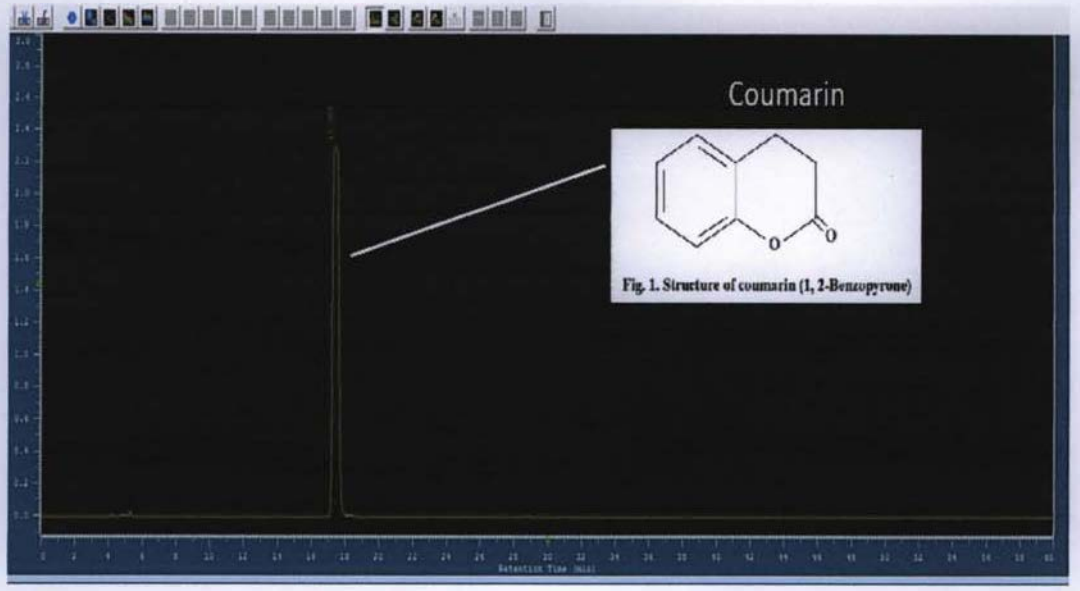

Figure 7. HPLC chromatogram from a pure coumarin sample diluted in methanol.

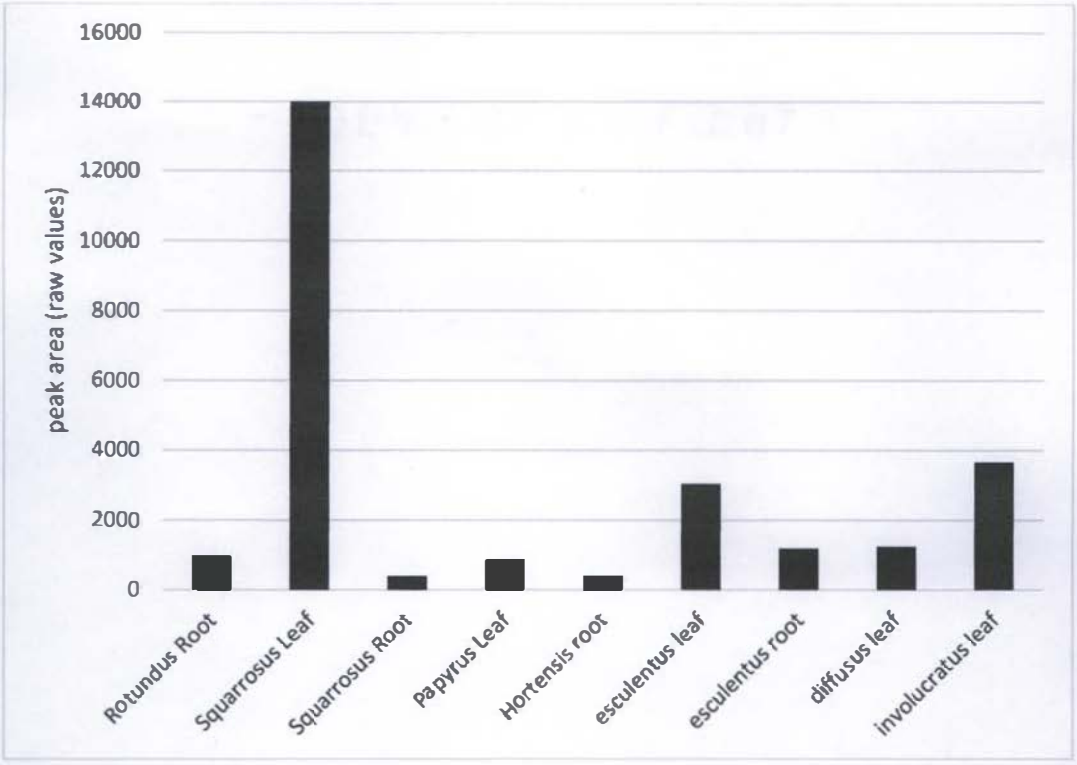

Figure 8. Coumarin concentrations in the leaves and roots of *Cyperus* species as determined by HPLC analysis.

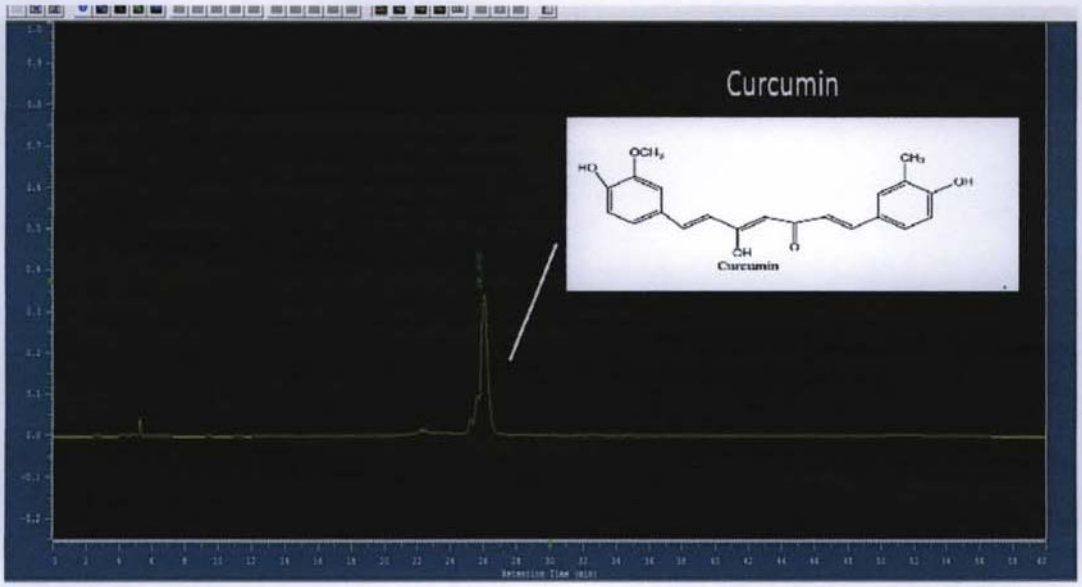

Figure 9. HPLC chromatogram from a pure curcumin sample diluted in methanol.

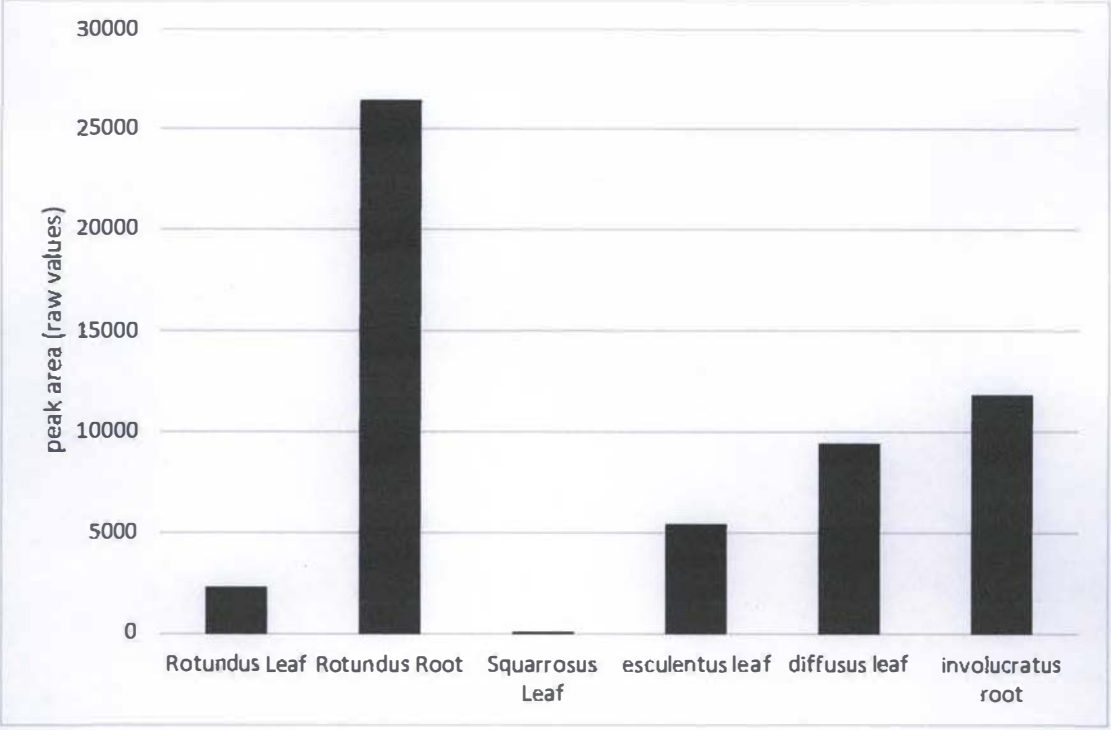

Figure 10. Curcumin concentrations in the leaves and roots of *Cyperus* species as determined by HPLC analysis.

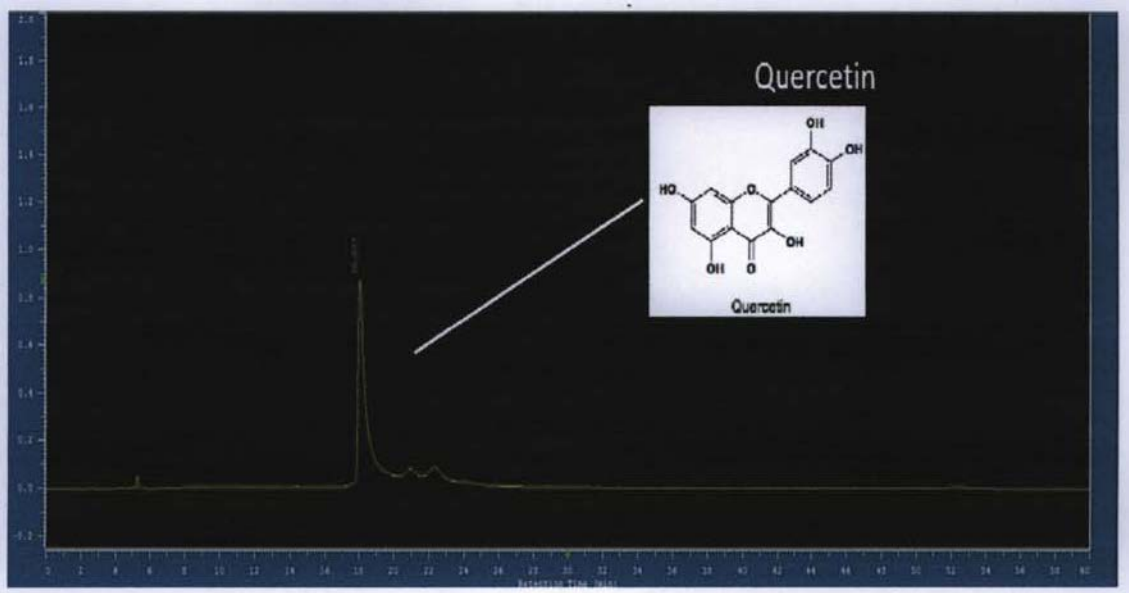

Figure 11. HPLC chromatogram from a pure quercetin sample diluted in methanol.

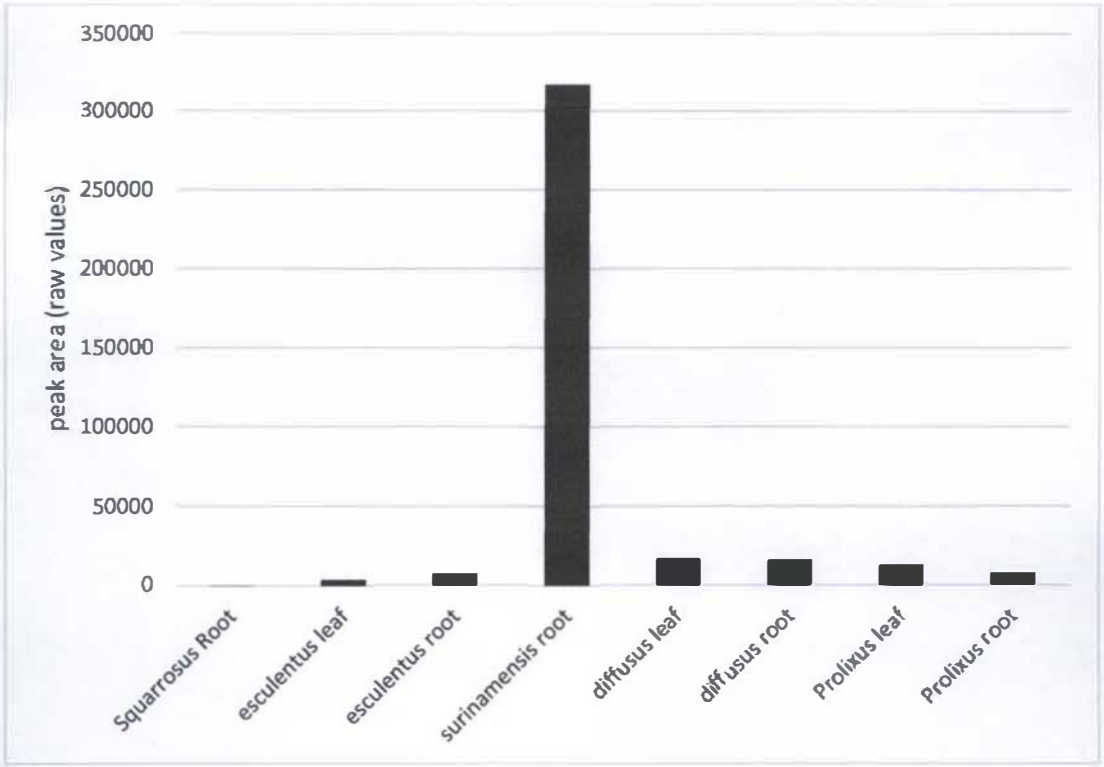

Figure 12. Quercetin concentrations in the leaves and roots of *Cyperus* species as determined by HPLC analysis.

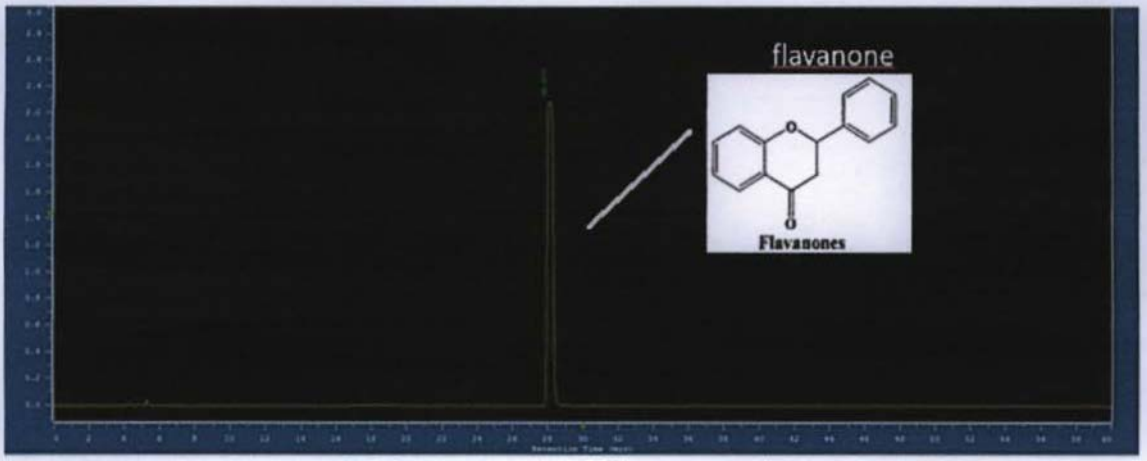

Figure 13. HPLC chromatogram from a pure flavone sample diluted in methanol.

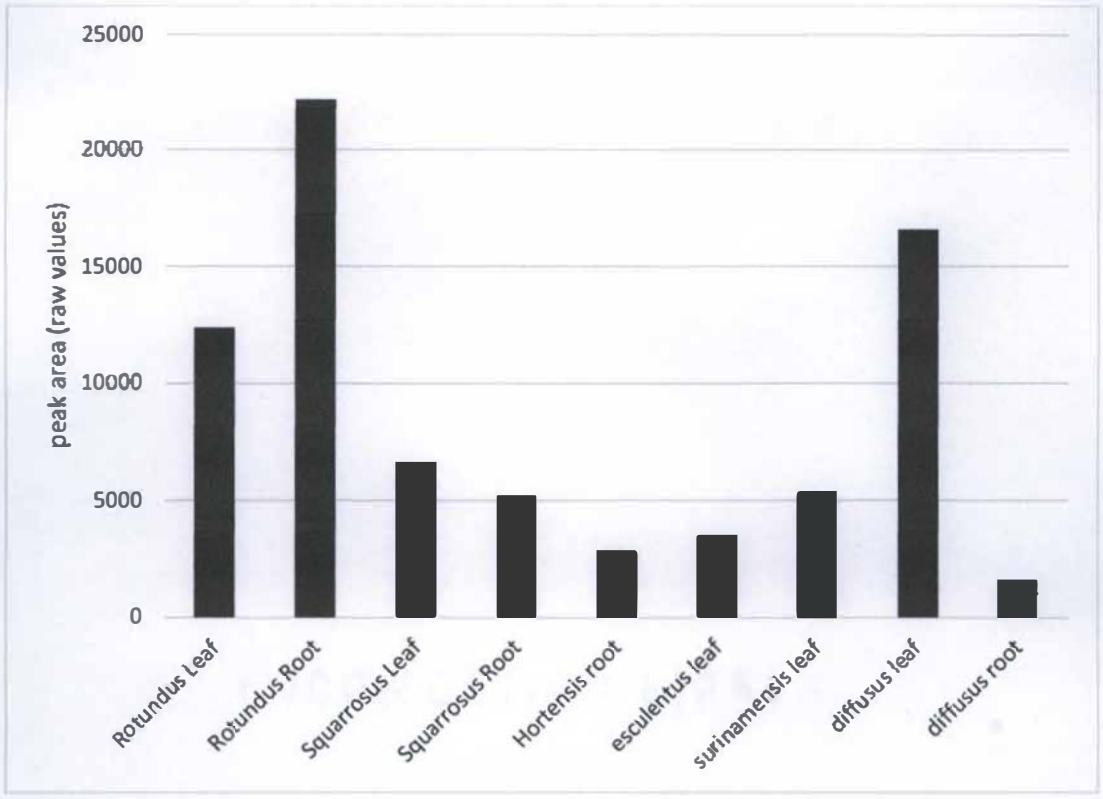

Figure 14. Flavanone concentrations in the leaves and roots of *Cyperus* species as determined by HPLC analysis.

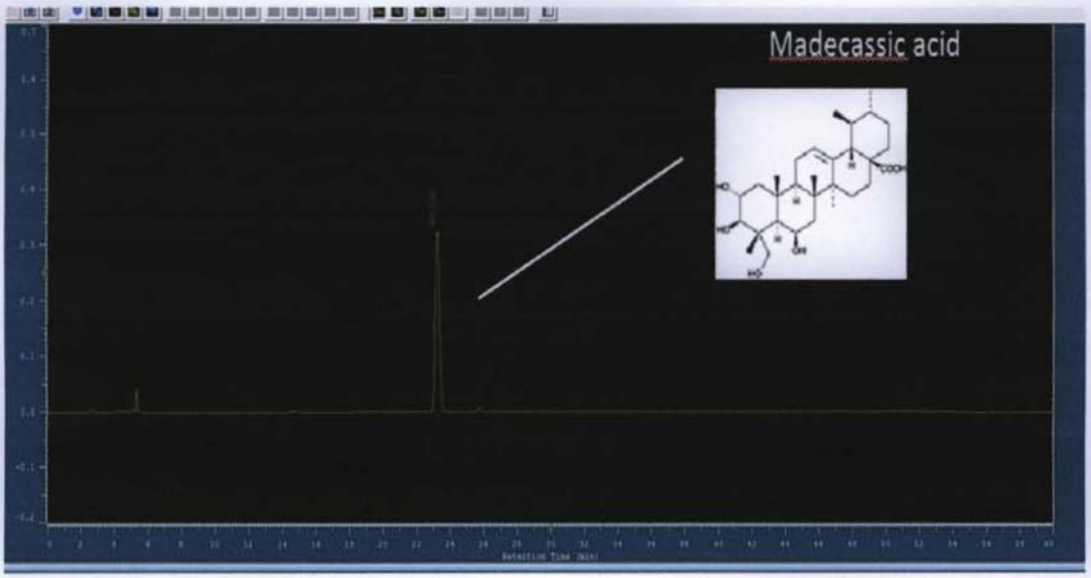

Figure 15. HPLC chromatogram from a pure madecassic acid sample diluted in methanol.

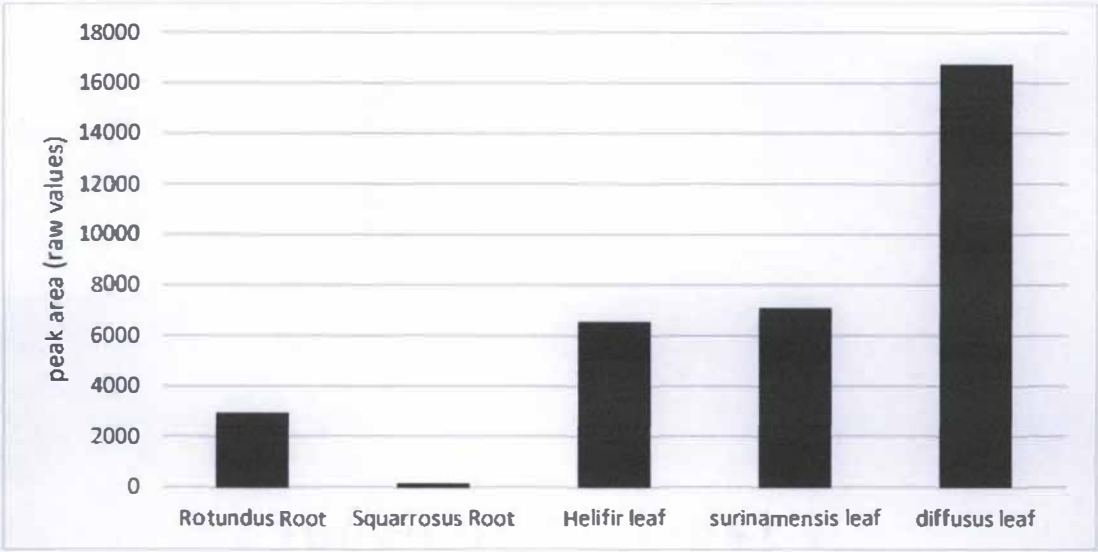

Figure 16. Madecassic acid concentrations in the leaves and roots of *Cyperus* species as determined by HPLC analysis.

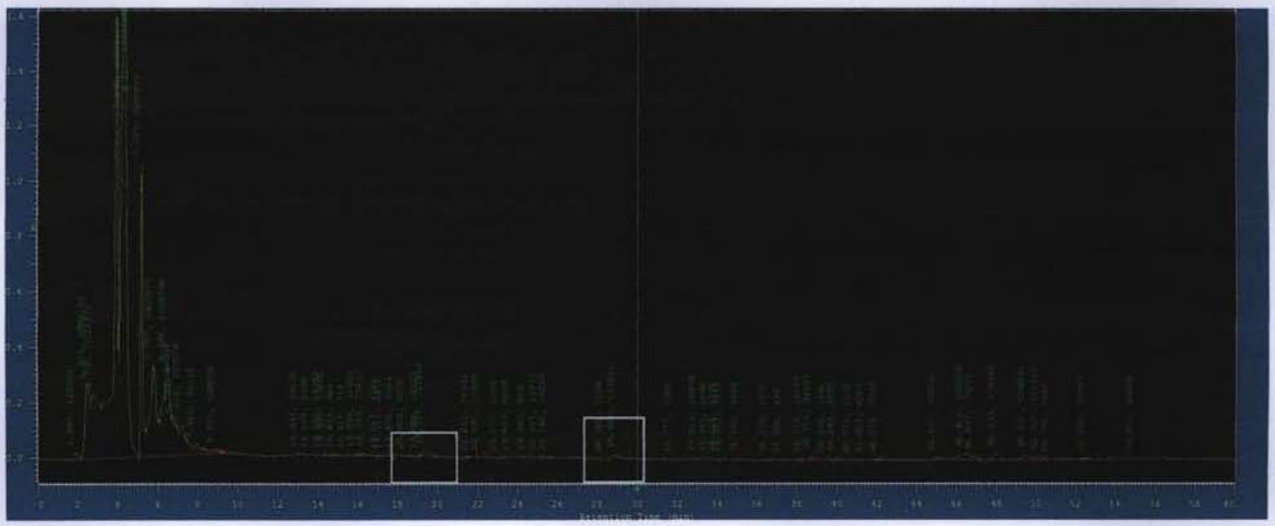

Figure 17. HPLC chromatogram of fresh *C. involucratus* leaf sample.

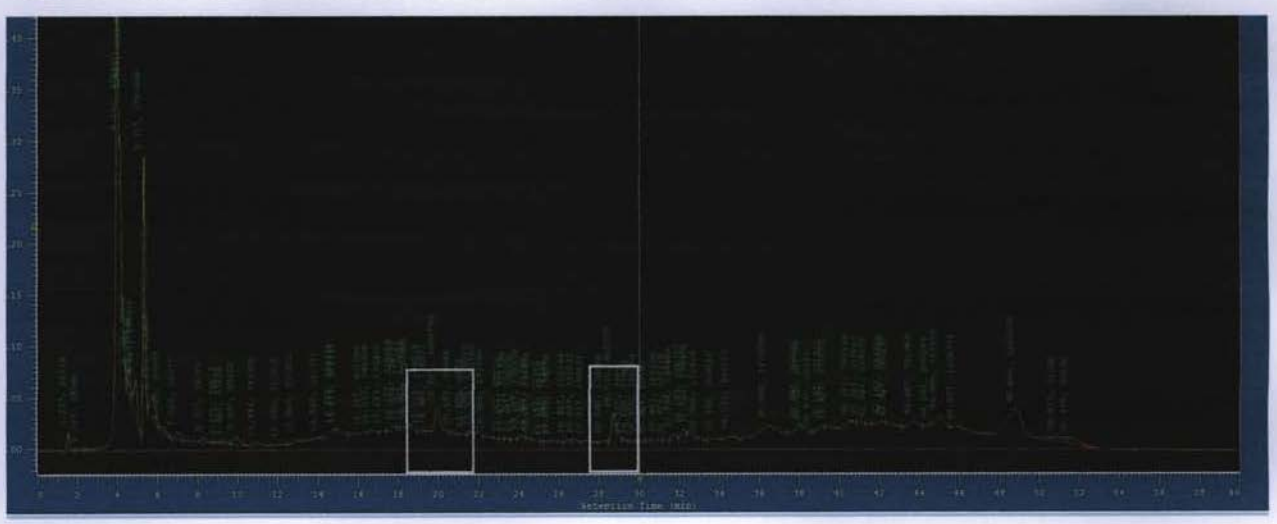

Figure 18. HPLC chromatogram of preserved *C. involucratus* leaf sample (1980).

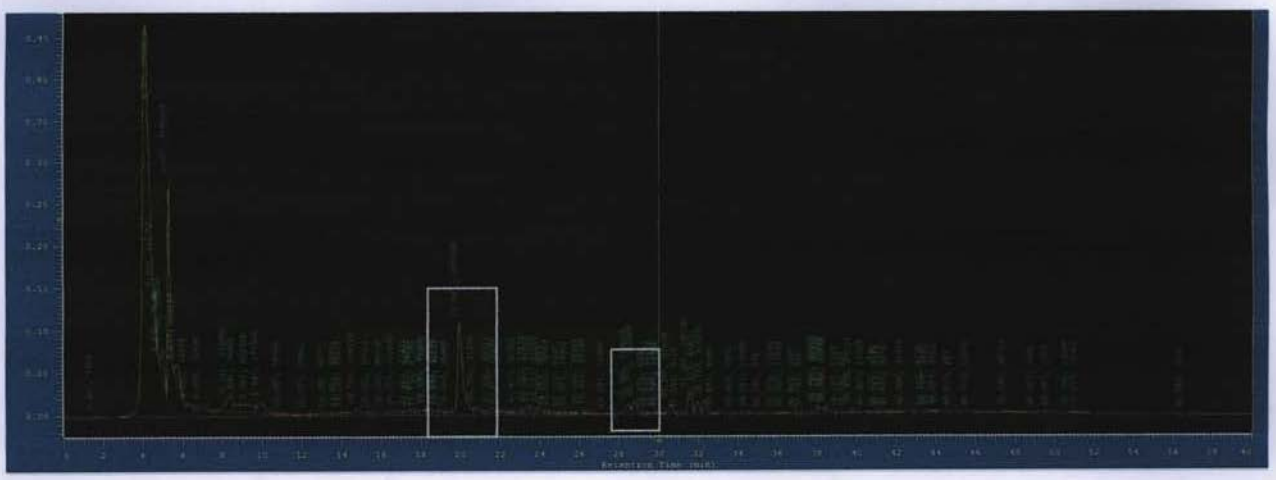

Figure 19. HPLC chromatogram of preserved *C. involucratus* leaf sample (1995).

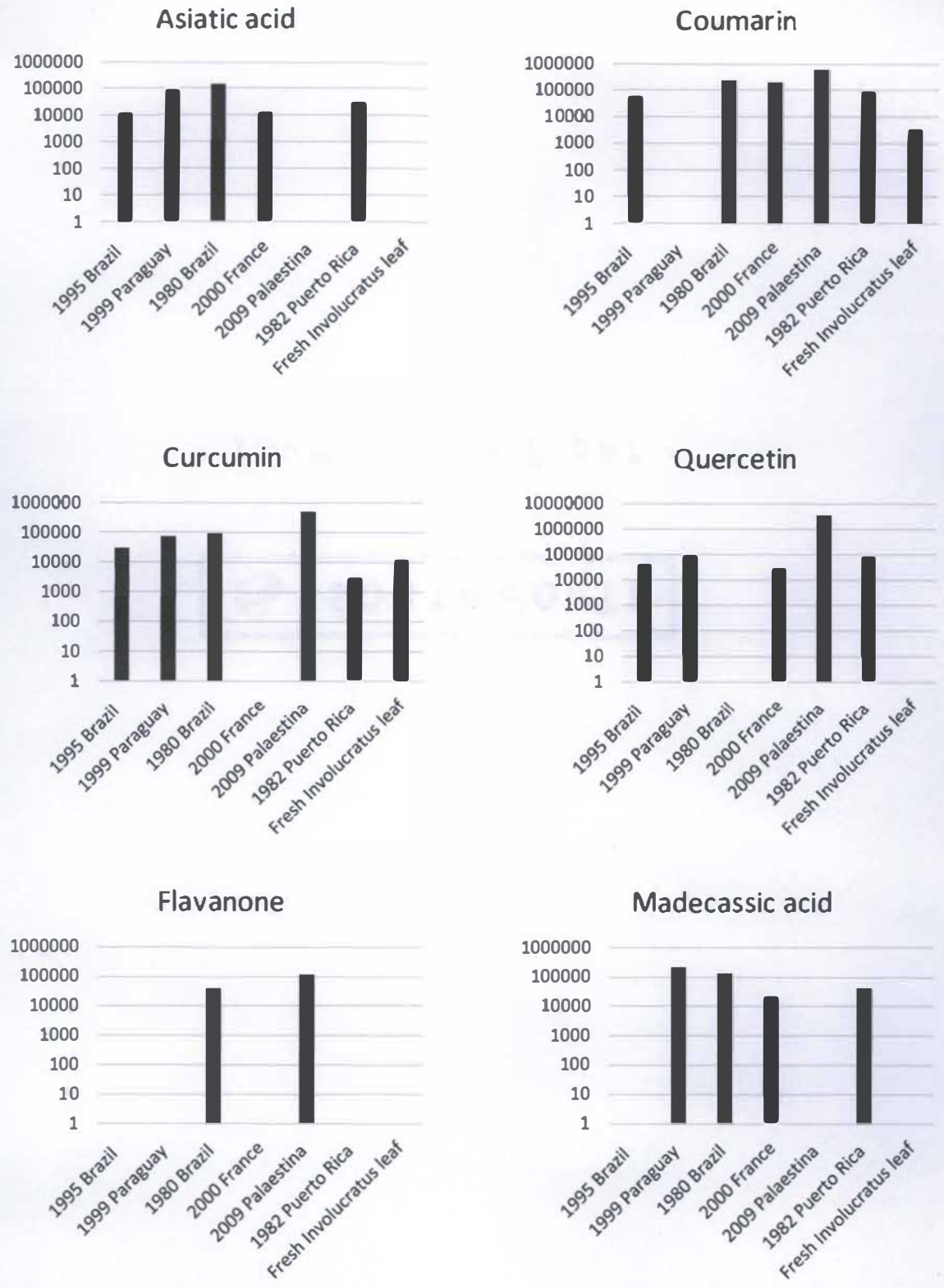

Figure 20. Phytochemical comparisons within preserved (dried) leaf tissues from various *C. involucratus* specimens. Y-axes are HPLC raw value peak areas (Log_{10} transformed).

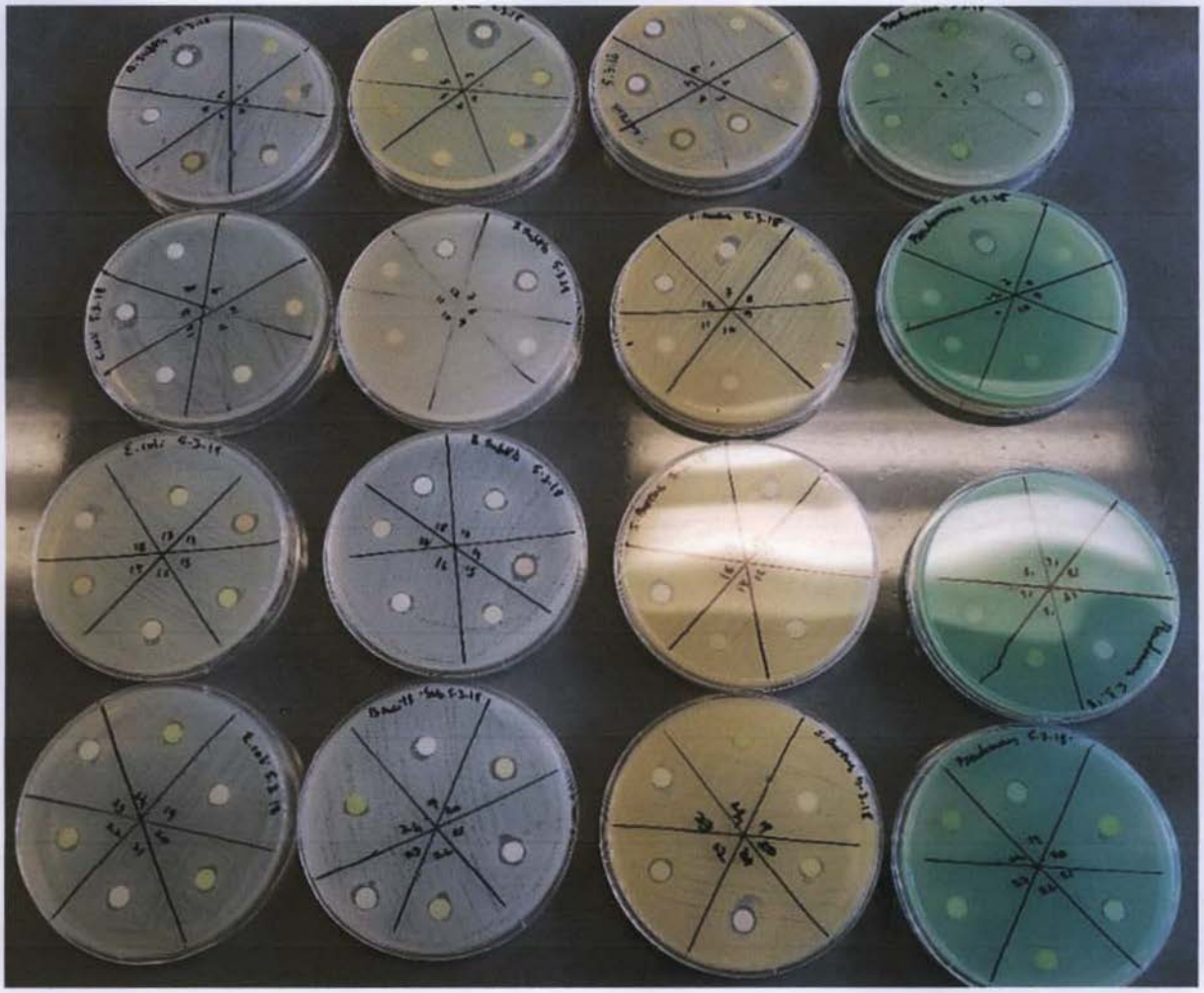

Figure 21. Example plates from a disc diffusion experiment showing inhibition of *E. coli, B. subtilis, S. aureus, and P. pseudomonas* (left to right).

V. References

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