Aquatic Plants from Minnesota
Part 1 - Chemical Survey

by

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FOREWORD

This Bulletin is published in furtherance of the purposes of the Water Resources Research Act of 1964. The purpose of the Act is to stimulate, sponsor, provide for, and supplement present programs for the conduct of research, investigations, experiments, and the training of scientists in the field of water and resources which affect water. The Act is promoting a more adequate national program of water resources research by furnishing financial assistance to non-Federal research.

The Act provides for establishment of Water Resources Research Centers at Universities throughout the Nation. On September 1, 1964, a Water Resources Research Center was established in the Graduate School as an interdisciplinary component of the University of Minnesota. The Center has the responsibility for unifying and stimulating University water resources research through the administration of funds covered in the Act and made available by other sources; coordinating University research with water resources programs of local, State and Federal agencies and private organizations throughout the State; and assisting in training additional scientists for work in the field of water resources through research.

This Bulletin is number 46 in a series of publications designed to present information bearing on water resources research in Minnesota and the results of some of the research sponsored by the Center. The Bulletin is concerned with the results of a survey of selected aquatic plants in Minnesota conducted in anticipation of finding compounds which might be useful in medicine. The survey is part of a research project aimed at finding a nutritional, medicinal or industrial use for the unwanted aquatic plants in lake shoreline areas. It is possible that some aquatic plants may contain industrially useful gums-mucilages, or new useful antimicrobial, anticoagulant, or antineoplastic therapeutic principles. If a good industrial, medicinal, or nutritional use for aquatic plants can be discovered, the results of the research could provide an economic incentive for aquatic plant collection and control. The successful completion of the project may significantly assist the State and Nation in partially solving their lake pollution problems.

This Bulletin is related to the following research project:

OWRR Project No.: A-025-Minn.

Project Title: Alleviation of Lake Pollution by Utilization of Aquatic Plants for Nutritional, Medicinal or Industrial Purposes

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Publication Abstract:

The aquatic plants in Minnesota have not been surveyed medicinally for useful chemical compounds. A study was conducted with a reasonable anticipation of finding compounds such as alkaloids, flavonoids, tannins, saponins, steroids and lipids which might be useful in medicine. Examination of chemical constituents was accomplished on the following plants collected from

various lakes in Minnesota: Anacharis canadensis, Calla Polustris, Carex lacustris, Ceratophyllum demersum, Chara vulgaris, Eleocharis smallii, Lemna minor, Myriophyllum exalbescens, Nuphar variegatum, Nymphaea tuberosa, Potamogeton amplifolius, P. natans, P. pectinatus, P. richardsonu, P. zosteriformis, Sagittaria cuneata, S. latifolia, Sparganium eurycarpum, S. fluctuans, Typha angustifolia, Vallisneria americana, and Zizania aquetica.

Taxonomic identification of aquatic plants was made and exhaustive extraction using solvents ranging from the non-polar to polar type was followed to determine the nature of the various constituents present in the aquatic plants. Detection for compounds involved purification of extracts, thin-layer chromatography, hemolysis test, froth test, gravimetric determination, methanolysis, and hydrogenation.

Thin-layer chromatographic detection studies indicated original extracts did not appear to contain alkaloids. Several plant species demonstrated Dragendorff positive spots. Flavonols were most widely distributed in the plant extracts studies. Tannins, especially the condensed type, were widely distributed in the plants screened. Five species of plants are saponin positive. Beta-sitosterol was tentatively identified as being present 8 species. The lipid contents of 3 species may be considered for their nutritional value.

Publication Descriptors: *Aquatic Plants/ *Chemical Compounds/ *Medicine/ Minnesota/ Alkaloids/ Flavonoids/ Tannins/ Saponins/ Steroids/ Lipids/ Phytochemical Screening/ Chromatography

Publication Identifiers: *Hemolysis Test/ *Froth Test/ *Methanolysis/ Hydrogenation/ Dragendorff Positive Spots/ Beta-sitosterol

I. INTRODUCTION

A. Objective of the Study

Higher and lower forms of plants have and are contributing important drugs for the physician's use (1). Higher plants are being studied for their anti-neoplastic (2-5), antimicrobial (6-22) and other pharmacological activities (23,24). The lower forms such as the plankton algae are known to produce antibiotic substances (25,26), the blue-green algae are believed to produce a cyclic polypeptide endotoxin (27). Aquatic plants can be considered in some ways to be botanically and ecologically related to both the higher plants and the algae. They may also be considered nuisances which often bring about biological excesses that are inimical to recreational and other water uses (28).

The aquatic plants in general and the Minnesotan aquatic plants in particular, have not been surveyed medicinally for useful chemical compounds. Therefore, this series study was done with a reasonable anticipation of finding compounds (such as alkaloids, flavonoids, tannins, saponins, steroids and lipids) which might be useful in medicine.

Examination of chemical constituents were done on the following plants collected from various lakes in Minnesota: Anacharis canadensis, Calla palustris, Carex lacustris, Ceratophyllum demersum, Chara vulgaris, Eleocharis smallii, Lemna minor, Myriophyllum exalbescens, Nuphar variegatum, Nymphaea tuberosa, Potamogeton amplifolius, P. natans, P. pectinatus, P. richardsonii, P. zosteriformis, Sagittaria cuneata, S. latifolia, Sparganium eurycarpum, S. fluctuans, Typha angustifolia, Vallisneria americana, and Zizania aquatica.

B. Phytochemical Screenings

Phytochemical screening programs have most often been directed toward finding alkaloids, flavonoids, tannins, saponins, steroids and lipids. The extraction schemes for screening are normally one of the following two:

i) Selective sequential extraction dependant upon the polarity of the solvent. For example, the dried powdered plant materials are extracted first with petroleum ether (non-polar solvent), then with chloroform, ethanol and finally with water (polar solvent).

ii) Non-selective extraction with hydroalcoholic solvents such as 50% or 80% ethanol, and subsequent fractionation of the ethanolic extract with organic solvents under different conditions.

Artifacts caused by thermo-oxidation, chemical oxidations and enzymatic hydrolysis should be avoided during the extraction.

In order to explain the complexity of screening programs, an introduction to the chemistry of constituents most often found in plants together with the problems which might be encountered in the screening are presented.

1. Alkaloid

Alkaloids are widely distributed in the plant kingdom (29-35) and even in some bacteria (36). Wall (37-40), Webb (35) and Hultin (41,42) are among those who have extensively screened vascular plants for alkaloids.

Alkaloids are natural products that are physiologically active, possess basic or sometimes neutral properties, and often contain heterocyclic nitrogen. The definition is broad and great variations in chemical structures are possible, ranging from simple primary amines to very complex indole compounds. The chemical groups of alkaloids include: phenylalkylamine (i.e., ephedrine), purine (caffeine), pyridine (evonine), pyrrolidine (tropinone), pyridine-pyrrolidine (nicotine), condensed piperidine-pyrrolidine (atropine), quinoline (cinchonine), isoquinoline (morphine) and indole (ergoclavine).

Therefore, in general, the plant materials are extracted either with acidic water to extract the alkaloids as salts, or by addition of base to the plant material in order to extract the free base by an organic solvent. Plants containing weakly basic or neutral alkaloids, such as rutaecarpine, colchicine and ricinine, would not be extracted into the organic solvent and special approaches must be developed for those alkaloids. Further purification of the plant extract may be necessary to avoid the extraction of false-positive reacting "alkaloids", such as those reported for some proteins and non-nitrogenous compounds (43,44).

Alkaloid color reagents are used to spray on chromatograms in order to visualize alkaloid spots. The most commonly used ones are Dragendorff's, iodoplatinate, antimony trichloride, and cerium sulfate (in sulfuric acid or in phosphoric acid) (45).

2. Flavonoid

The flavonoid compounds in the plant kingdom are confined almost entirely to the flowering plants and ferns (46). Chemically, flavonoids may be described as plant pigments containing two C_6 groups (substituted benzene rings) connected by a three carbon aliphatic chain (C_6-C_3-C_6). Examples of the chemical classes of flavonoids are the catechins, leucoanthocyanidines, flavanones, flavanonols, flavones, flavonols, and anthocyanidins. A slight variation of C_6-C_3-C_6 patterns is seen in chalcones, dihydrochalcones, aurones and isoflavones where the central pyran ring is open, modified into a benzalcoumaranone ring, or the ring substitution is shifted from C_2 to C_3. Biologically, flavonoids possess extremely diverse pharmacological activities, for instance, quercitrin has an antiviral effect (47), rutin is a capillary antihemorrhagic, eupatomin has anticancer activity (48), and flavone has bactericidal properties (49).

Flavonoids are often extracted by methanol, aqueous ethanol of acetone (50). Many color reactions can be used to characterize the different classes of flavonoids, and many of those color reactions have been reviewed by Seikel (51).

3. Tannin

According to Swain (52), the term "tannin" can be applied to any naturally occurring compound of high molecular weight (between about 500 to 3,000) containing a sufficiently large number of phenolic hydroxyl or other suitable groups (1-2 per 100 M.W.) to enable it to form effective cross-links between proteins and other macromolecules.

Tannins may be divided structurally into the following two distinct classes:

i) Hydrolyzable tannins consist of a polyhydric alcohol esterified with gallic acid or derivatives of gallic acid, such as ellagic acid. Tannins having this structure can readily be hydrolyzed by acids, bases or enzymes (tannin acylhydrolases). Gallotannins and ellagitannins are included in this group.

ii) Condensed tannins contain phenolic nuclei which when treated with the above hydrolytic reagents do not hydrolyze but instead polymerize to yield insoluable amorphous and often red colored phlobaphenes. An example for tannins of this type is catechin, a polymer of flavin-3-ols.

Both the hydrolyzable and condensed tannins are widely distributed in nature. An extensive survey of the occurrence of tannins in the plant kingdom was done by Bath-Smith (53). One interesting observation found was that ellagic acid is absent from non-vascular plants, ferns, gymnosperms and monocotyledons.

Tannins may be precipitated from their solutions by different salts. Salts of heavy metals (Ag, Zn, Cu, Sn) precipitate tannins indiscriminatively, neutral lead salts precipitate tannins possessing adjacent phenolic hydroxyl groups, while lead subacetate precipitates tannins with non-adjacent hydroxyl groups (54). Wall (37) found that precipitation with lead acetate occurred in every plant extract examined, whether or not tannin was found by other tests. Therefore, tannins are separated from other plant constituents by the solvent extraction method. Tannins are often extracted from plant materials with hot water, and then salted out with sodium choloride. Wall (37) detected tannins in the hot aqueous extracts prepared from the dried 95% alcoholic extract. Persinos et al. (55) directly used the 80% alcohol extract for tannin detection in their pharmacognostical study of Nigerian plants.

Tannins can be detected by using a 1% solution of gelatin containing 10% sodium chloride (56). This test is based on the protein-binding capacity of the tannin. Since the condition of pH and ionic strength are critical, Farnsworth (57) modified the test by using buffered gelatin-salt reagent. Another detecting agent is 1% ferric chloride solution which either precipitates tannins or forms different colors, ranging from blue, blue-black, green to blue-green. It should be recognized, that many other phenolic compounds form colors with ferric choloride solution. Because of tannin's polymer and high molecular weight nature, it usually runs as a streak on paper or thin-layer chromatograms (58). As an alternative, the nature of tannin is determined by acid or alkaline hydrolysis of the tannin extract and subsequent chromatographic detection of the building units (gallic, ellagic acid, etc.) in the hydrolysate.

4. Saponin

Saponins are widely distributed among higher plants (59). Biogenetically, saponins can be divided into two groups:

i) Glycosides of triterpenoid alcohols (normally at the 3-position), such as oleanane.

ii) Glycosides of a particular steroid structure having a spiroketal side chain at the E and F ring juncture. A few of the steroidal sapongenins are distinguished by having a cis-A/B ring juncture.

Due to the sugar residue at 3-beta-hydroxyl group, both types of saponins are soluble in water and ethanol but insoluble in ether. Their aglycones (sapogenins) without the sugar moiety, possess the solubility characteristics of other sterols. Thus saponins are most conveniently extracted from plants with 70-95% hot ethanol or isopropanol.

Saponins are most often detected by:

i) Foam test. Saponins are presumed to be present when the characteristic honeycomb froth persists for at least 30 minutes after shaking an aqueous boiled (3-5 minutes) plant material.

ii) Hemolytic test. Saponin containing plant extracts mixed with defibrinated blood in a buffered physiological saline solution cause the hemolysis of red blood cells. Digitonin, a saponin available in crystalline form, is generally used as the standard. Tannins will interfere with the hemolytic action of saponins, as they form a protective coating around the red blood cells. The removal of tannins with magnesium salt previous to performing the hemolytic test was suggested by Farnsworth (57).

Both triterpenoid and steroid saponins behave the same in those two tests. If necessary, the Liebermann-Burchard test may be used to differentiate them; red, pink or purple colors are developed with triterpenoid saponins whereas blue or blue-green colors are formed with steroidal saponins (60).

5. Steroid

Steroids are derivatives of the tetracyclic hydrocarbon, cyclopentanoperhydrophenanthrene. The steroids thus far discovered in plants include sterols, certain sapogenins, alkaloids, cardenolides and hormones (61).

i) Sterols are hydroxysteroids of C_{27}, C_{28} and C_{29} series (62). An example of C_{27} sterol is cholesterol which is not only present in animals but has also been isolated from algae by Tsuda et al. (63), from potato and Dioscorea plant by Johnson et al. (64). A common C_{28} sterol is ergosterol and widely distributed C_{29} sterols are beta-sitosterol and stigmasterol.

ii) Alkaloids. The occurrence of steroidal alkaloids is limited mostly to Solanum, Veratrum and Holarhena (65) species. They contain a 5-membered E ring and a 6-membered nitrogen containing F ring.

iii) Cardiac glycosides. They are glycosides of either C_{23}(cardenolides) or C_{24} (bufadienolides) steroids with potent cardiac activity. They are structurally characterized by an unsaturated lactone ring at C_{17}, a cis-juncture of ring C and D, a 14-beta-hydroxy group, and by the peculiar sugars (such as D-digitoxose, D-digitalose, D-cymarose and D-sarmentose) attached at C_3.

iv) Hormones. Pregnane (C_{21}), androstane (C_{19}) (66) and estrane (C_{18}) (67) derivatives have been isolated from plants.

Steroids can be extracted with benzene or ether, and the chromatographic technique is of great value to detect plant materials for steroids. A very good article reviewing the application of TLC for steroid detection has been written by Neher (68). Among the reagents most used and most generally applicable are sulfuric acid-alcohol, chlorosulphonic acid-acetic acid and molybdophosphoric acid. Anisaldehyde-sulfuric acid reagent is used for detection of many steroids, it is non-specific but with high sensitivity. Dinitrobenzoic acid (Kedde reagent) and m-dinitrobenzene are specific for the detection of cardenolides and 17-ketosteroids, respectively, cerium sulfate is specific for the detection of nitrogen-containing steroids, Keller (ferric chloride-acetic acid) and Kiliani (ferric sulfate-sulfuric acid) are specific for the deoxy sugar moiety of cardiac glycosides. Frerejacque and DeGraeve (69) had tabulated all the cardiac glycoside detecting agents and their methods of preparation. The use of iodine as a non-destructive location reagent for steroids in TLC has been introduced by Stevens (70). This technique is very valuable for preparative thin- and thick-layer chromatography.

6. Lipid

Since triglycerides have been studied in this thesis, only the saponifiable lipids will be discussed. The saponifiable lipids are classified according to their structures into the following categories: fatty acids, fatty acid esters (triglycerides, waxes, etc.), phospholipids and glycolipids.

Non-polar lipids are extracted with petroleum ether or benzene, and polar lipids are extracted with isopropanol, isopropanol-chloroform, methanol-chloroform, ethanol-chloroform or ethanol-ether. In addition to the avoidance of thermo-, chemical and enzymatic oxidation and hydrolysis, lipid extraction should be carried out under nitrogen to prevent the oxidation of unsaturated compounds. The extracts can be fractionated by TLC into different lipid classes. According to Mangold (71), lipids can be visualized on the chromatograms with iodine vapour, 2', 7'-dichlorofluorescein, Rhodamine B of 6 G, chromic acid-concentrated sulfuric acid and 50% sulfuric acid followed by charring. The last reagent reveals different color changes for different lipids during the heating process, for example, cholesterol and its esters turn red first, then violet, brown and finally black.

Gas-liquid chromatography has been successfully employed in the separation of fatty acid, mono-, di-, and triglycerides (72-74). The technique is also commonly used to analyze the fatty acid content of the triglycerides after methanolysis of the triglycerides. The application of GLC in lipid

screening has been reported by Schlenk et al. (75) in their search for arachidonic, 5,11,14,17-eicosatetraenoic and related acids in plants.

C. Review of the Literature

1. General Considerations

Most studies on aquatic plants are orientated toward either ecology or limnology (76-80). Minerals (81-86), sugars (87-91), organic acids (92,93), chlorophyll and xanthophyll pigments (94) have often been studied for their relationship to plant physiology.

Chara sp. are reported to contain amino acids (95), and uronic acid containing polysaccharides (96). Chara contained many of the important amino acids but none with sulfur. The presence of vitamin B_{12} in Chara may be due to bacteria living epiphytically or in proximity to the algae. Vitamins B_1, B_2 and C were found in some aquatic plants of Moldavia (97).

The branch chain sugar D-apiose was first found in Leman by Duff in 1963 (98), and is believed to be a constituent of the cell wall (99). The biosynthesis of D-apiose was studied by feeding $^{14}CO_2$ or myo-inositol-2-^{14}C to the plant, and observing its localization in the cell wall (100-102).

2. Alkaloids

From the rhizome of Nuphar luteum, five thioalkaloids, i.e., thiobinupharidine, allothiobinupharidine, pseudothiobinupharidine, thiobisdeoxynupharidine and neothiobinupharidine, were isolated (103). It also contains nupharine (104). Mass spectroscopy of their structures were recorded, and structures assigned according to an NMR study (105,106).

From the rhizome of Nuphar japonicum, the following lupine alkaloids were isolated: deoxynupharidine (107), nupharamine (108-110), nupharane (111), nupharidine (112), nuphamine (113) and an unstable base, dehydrodeoxynupharidine (114). The abolute configuration of the identified structures have been reported (115-117). Arata (118,119) succeeded in synthesizing dl-deoxynupharidine.

A piperidine alkaloid, nuphenine, has been isolated from Nuphar variegatum (120).

Paper chromatographic analysis of Nymphaea alba extracts showed two alkaloids to be present in the leaves and flowers and one in the root (121). Their structures remain undetermined, but all are believed different from the Nuphar alkaloids.

Carex brevicollis contains the indole alkaloids brevicolline, harman, brevicarine and in addition, four other alkaloids (122-124). The structure of brevicolline was studied and determined by Terent'eva (125,126).

Table 1. Composition of Fatty Acids from Some Aquatic Plants (GLC Area %) (75).

Plant name	14:0	15:0	16:0	16:1	16:2	16:3	17:0	18:0	18:1	18:2	18:3	22:0
Anacharis occidentalis	0.7	0.1	20.0	5.6				3.0	4.4	17.4	48.9	
Lemna	1.4	0.9	21.7	3.3	0.2			1.4	2.8	17.9	45.8	1.5
Myriophyllum	2.2	0.4	17.3	17.6			0.4	0.9	3.0	23.4	36.6	
Nuphar	0.3	0.1	18.7	3.7	1.0	2.6			3.9	16.2	53.6	
Potamogeton	1.2	0.1	21.6	6.3	4.4	2.4	0.3		3.8	11.8	44.8	
Sagittaria	0.5		17.5	2.1		5.9			1.6	8.1	48.0	
Typha latifolia	0.2		16.3	4.2		1.8	0.1		0.6	17.8	56.3	2.5

Table 2. List of Plant Collected[1/].

Class	Family	Genus	Species	Authority	Lake[2/]
Algae	Characeae	Chara	vulgaris		Mellissa*
Monocoty-ledons	Alismataceae	Sagittaria	cuneata	Sheldon	Itasca
	Alismataceae	Sagittaria	latifolia	Willd	Mellissa*
	Araceae	Calla	palustris	L.	Itasca
	Cyperaceae	Carex	lacustris	Willd	Pine*
	Cyperaceae	Eleocharis	smallii	L.	Pine*
	Gramineae	Zizania	aquatica	L.	Itasca
	Hydrocharitaceae	Anacharis	canadensis	(Michx.) Rich.	Minnetonka
	Hydrocharitaceae	Vallisneria	americana	Michx.	Pine*
	Lemnaceae	Lemna	minor	L.	Itasca
	Najadaceae	Potamogeton	amplifolius	Tuckerm.	Pine*
	Najadaceae	Potamogeton	natans	L.	Itasca
	Najadaceae	Potamogeton	pectinatus	L.	Minnetonka
	Najadaceae	Potamogeton	richardsonii	(Benn.) Rydb.	Mellissa*
	Najadaceae	Potamogeton	zosteriformis	Fern.	Minnetonka
	Sparganiaceae	Sparganium	eurycarpum	Engelm.	Pine*
	Sparganiaceae	Sparganium	fluctuans	Robins	Pine*
	Typhaceae	Typha	angustifolia	L.	Pine*
Dicotyle-dons	Ceratophyllaceae	Ceratophyllum	demersum	L.	Minnetonka
	Haloragaceae	Myriophyllum	exalbescens	(Fern.) Jeps.	Minnetonka
	Nymphaeaceae	Nuphar	variegatum	Engelm.	Minnetonka
	Nymphaeaceae	Nymphaea	tuberosa	Paine	Minnetonka

1/Total plants were collected and studied except in case of Nymphaea tuberosa and Nuphar variegatum where only stems and leaves were collected.
2/Asterisk - Collection made in September 1968; Non-asterisk - Collection made in August, 1968.

3. Flavonoids

Carexidin (a 3-deoxyanthocyanidine) was discovered in Carex riparia and Carex acutiformis (127). Flavonoid A (5,7,3',4'-tetra-hydroxyl-flavone-beta-D-glucopyranoside) and flavonoid B (luteolin) were isolated by means of a polyamide column from Lemna minor (128,129).

4. Tannins, saponins, steroids and lipids

Very few studies have been done on aquatic plants for tannins, saponins, steroids or lipids. Tannin was reported to be present in Lemna (128), Nuphar and Nymphaea (ellagic acid) (130), but absent in Elodea (131). Trace amount of steroidal sapogenins were found in Sagittaria sp. (132). Stigmasterol and beta-sitosterol were found in the rhizome and the flower (133) of Nuphar luteum. A lipoprotein complex was detected in Elodea (131).

The fatty acid composition of some aquatic plants have been analyzed by Schlenk (75) by means of the gas-liquid chromatographic technique. The results are summarized in Table 1.

II. Materials and Methods

A. Plant Collection and Identification

Plant materials used in this study were collected from various lakes in Minnesota during August and September 1968. Specimens representing the collections were pressed between blotters and carefully dried at 50°C. Taxonomic identification was made by Dr. Robert C. Bright, Assistant Professor in Limnology, University of Minnesota, in the field and later confirmed by Dr. Gerald B. Ownbey, Professor and Curator of Herbarium, University of Minnesota. One voucher herbarium for each plant has been deposited at the Botanical Museum, Harvard University, Cambridge, Massachusetts. Representative plants were also preserved in jars containing water, 95% ethanol, formaldehyde (6:3:1) and 5% glycerine. Copper ion at 0.02 ppm was added to help retain the original color of plants.

A list of those plants collected, representing one algae, seventeen monocots and four dicots, is shown in Table 2. The gross appearance and general ecology of the plants are discussed alphabetically. Abbreviations in the parentheses will be utilized in tables throughout this dissertation instead of the full names of plants.

Anacharis canadenis (Michx.) Rich. (Ac): Submerged. Stems are so branched that they often form large masses. Leaves (2 mm) are whorled, usually three in each whorl.

Calla palustris L. (Water arum) (Cp): Emergent. Perennial herbs with petioled basal leaves and solitary spathes, leaves are either ovate or subrotund (5-10 cm).

Carex lacustris Willd (Cl): Emergent plants grown in swamps and marshes, leaves are stout usually with conspicuous cross-septate and with numerous elevated nerves.

Ceratophyllum demersum L. (Coontail) (Cd): Submerged. Stems with whorls of stiff, forked leaves and leaflets with toothed or serrated margins on one side only. They are freely branched, forming large masses and are found in quiet water.

Chara vulgaris (Cv): Submerged green algae (Muskgrass). The plant possesses a musky odor and is made up of stems bearing whorled, smooth brittle branches, easily snapped with a slight pressure.

Eleocharis smallii L. (Spike rush) (Es): Erect and emergent. Rhizomes are often conspicuous and the leaf sheaths are obliquely truncate and firm.

Lemna minor L. (Duckweed) (Lm): They represent the smallest of the aquatic plants (2-4 x 1.5-3 mm). They have no true leaves nor stems, but the floating green plant body usually possessing a tiny root that penetrates the water. They may grow sufficiently dense to prohibit sunlight from penetrating the water, thus killing algae and other aquatic plants.

Myriophyllum exalbescens (Fern.) Jeps (Water-milfoil) (Me): Submerged herbs in quiet water. Leaves are feather-like with one central axis and branches in whorls around the stem.

Nuphar variegatum Engelm (Yellow water lily) (Nv): Floating leaves are heart-shaped with veins radiating from the mid-rib nearly to the margin without forking. The floating flowers are attractive and yellow.

Nymphaea tuberosa Paine (Water lily) (Nt): Floating circular leaves possess much-forked veins radiating to the margin. Flowers with green sepals and white petals and are long-petioled (usually striped).

Potamogeton amplifolius Tuckerm. (Pa): Those belonging to Potamogeton sp. (Pondweed) are plants with usually both floating and submerged leaves scattered along the stem, and with midribs evident when held against bright light. For Potamogeton amplifolius, the submerged leaves (8-20 cm) are falcately folded, and floating leaves (5-10 cm) are elliptic.

Potamogeton natans L. (Pn): Stems are simple or sparingly branched. Submerged leaves are phyllodial, narrowly linear (1-4 cm x 102 mm), floating leaves are elliptic (5-10 x 2-4.5 cm). Petiole usually exceeding the blade and flexibly attached to it.

Potamogeton pectinatus L. (Pp): Leaves are all submerged, narrowly linear (3-10 cm x 0.5-1.5 mm). Lower part of stem is simple or sparingly branched with elongated internotes, while the upper part is freely dichotamously branched. Therefore, the appearence of a bounch of rounded tread-like leaves as they float in the water is very characteristic.

Potamogeton richardsonii (Benn.) Rybd. (Pr): Stems are freely branched and densely leafy. Leaves are lanceolate to nearly linear (3-12 cm x 5-20 mm).

Potamogeton zosteriformis Fern. (Pz): Stems are freely branched, flattened and winged (1-3 mm wide). Leaves are linear (1-2 cm x 2-5 mm). They are most usually found in slow streams.

Sagittaria cuneata Sheldon (Water plantain) (Sc): Emergent plants rooted to the substratum and extending upward out of the water. Leaves are long-petioled and sagittate with variable sizes (6-18 x 1-10 cm).

Sagittaria latifolia Willd (Arrow-head) (Sl): Emergent plants usually found in swamps or ponds, leaves are sagittate (5-40 x 2-25 cm).

Sparganium eurycarpum Engelm (Se): Stout and emergent (5-12 dm). Leaves are shallowly and broadly triangular in cross section (8 dm x 6-12 mm). They are grown in mud or shallow water.

Sparganium fluctuans (Morong) Robins (Sf): Floating plants with slender, elongate stems (15 dm), leaves are flat, thin, alternate, translucent with cross reticulate and with sheathing bases.

Typha angustifolia L. (Cat-tail) (Ta): Erect, colonial herbs with long, linear leaves sheathing at the base. Flowers are densely crowded in long, cylindric, terminal spikes. They are grown in marshes.

Vallisneria americana Michx. (Va): Perennial herbs with very thin, long, ribbon-like, basal submersed leaves (2 m x 3-10 mm). They are found in quiet water area.

Zizania equatica L. (Wild rice) (Za): Robust annual grasses usually 2-3 m high. They are found in marshes and shallow water with tall culms and wide flat blades.

B. Extraction

Plants were rinsed thoroughly, and adherring foreign materials, such as sand, leeches and other plant species were removed. The plants were then spread on metal screens and dried in a well-ventilated oven at 50°C. When dry, each plant species was milled (Fitz Mill Model D, Chicago, Ill.) to pass a No. 14 seive, weighed and stored in a tightly closed polyethylene bag until being extracted.

In order to study the nature of the various constituents present in those aquatic plants, exhaustive extraction using solvents ranging from the non-polar to polar type was followed. The solvent sequence used was skellysolve F (b.p. 30-60°C), chloroform, 80% ethanol, acidic water and lastly, basic water. For each extraction, 150 gm of dried powdered plant material was used. All the concentrated extracts prepared were stored under nitrogen in amber colored bottles, sealed with paraffin and freezed.

Dried powdered plant material was extracted continuously with skellysolve F and then with chloroform in a Soxhlet extractor until the fresh extract no longer had a green color. Each extract was concentrated to a volume of 20 ml in a flash evaporator.

The skellysolve F and chloroform extracted plant material was air dried and placed in a Waring blender containing 1500 ml of 80% ethanol. After homogenizing twice for 30 seconds, the material was allowed to macerate overnight. The mixture was then vacuum filtered through a layer of cheesecloth and then through a Whatman No. 1 filter paper. The residual plant material was transferred to the Waring blender and the extraction procedure repeated. The filtrates were then combined and concentrated to a volume of 40 ml in a flash evaporator.

The solvent exhausted plant material was then macerated for 24 hours with warm (60°C) water that had been adjusted with 10% HCl to pH 3. The mixture was vacuum filtered and flash evaporated to a volume of 20 ml. The same procedure was followed for basic water extraction except that 10% KOH was used to adjust the mixture to pH 10.

C. Detection for Alkaloids

1. Purification of extracts

Skellysolve F, chloroform and 90% ethanol extracts were purified according to the flow chart in Fig. 1 before spotting on the silica gel H plates.

2. Thin-layer chromatography

Preparation of plates

Cleaned and dried glass plates (10 x 20 cm) were coated (0.25 mm) with silica gel H (prepared according to Stahl for thin-layer chromatography, E. Merck Ag. Darmstadt, Germany) on a DeSaga apparatus (Brinkmann Instruments Inc., Great Neck, Long Island). The slurry was made by mixing 25 gm of silica gel H with 75 ml of the mixture of methanol and water (3:1) for 20 seconds. The plates were air dried for 20 minutes, activated at 110°C for 30 minutes, and stored over calcium sulfate in a desiccator.

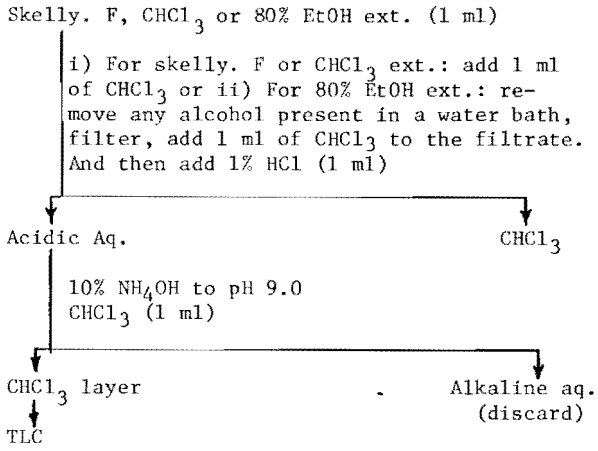
Application of purified samples and standards

Each purified extract (10 µl) was applied to the silica gel H plate and developed in chloroform:acetone:diethylamine (5:4:1) for the skellysolve F extracts, or in n-butanol:acetic acid:water (4:1:1) for the chloroform and 80% ethanol extracts. The following alkaloids, which represent different chemical classes, were used as standards at a concentration of 10 mg/ml in 50% ethanol:caffeine (purine), L-hyoscyamine (tropane), and ergonovine maleate (indole).

Detection

The following methods were used for the visualization of colorless substances on chromatograms: i) Observation under ultraviolet light (254 mµ); ii) Spraying with Dragendorff's reagent (134); iii) Spraying with 1% p-dimethylaminobenzaldehyde (PDAB, Ehrlich's reagent) followed by freshly prepared 0.1% $NaNO_2$ in 50% ethanol (135).

Figure 1. Purification of skellysolve F, chloroform and 80% ethanol extracts for the alkaloid detection.



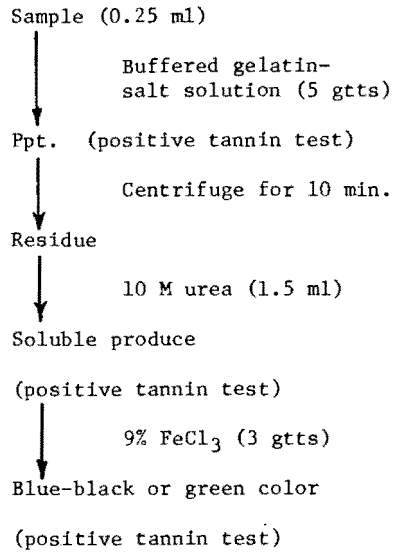
D. Detection for Flavonoids

Skellysolve F and chloroform extracts were applied (10 µl) to silica gel H thin-layer plates prepared as previously described (p. 50), developed in chloroform:methanol (95:5), whereas, 80% ethanol extracts were applied (10 µl) on 20 x 20 cm cellulose sheets (No. 6064, Eastman Kodak Co., N.Y.) and developed in n-butanol:acetic acid:water (4:1:5). The chromatograms were examined by i) Direct observation of yellow to pink compounds; ii) Fluorescent pattern (254 mµ); iii) Exposure to ammonia vapour and iv) Spraying with p-nitrobenzene diazonium fluoroborate (0.5% aqueous). The standards (4 mg/ml in methanol) used were rutin, umbelliferone, visnagin and provismine.

E. Detection for Tannins

An aliquot (0.2 ml) of 80% ethanol, acidic water or basic water extract was mixed with distilled water (1.8 ml), filtered, and the filtrate tested for the presence of tannins. Aqueous tannic acid U.S.P. (1%) was used as the standard. The test procedure used is shown in Fig. 2. The reagents used were i) Buffered gelatin salt solution- dissolve gelatin (1 gm) and NaCl (5 gm) in acid phthalate buffer (pH 3.0, 100 ml) (136); ii) 10 M Urea- dissolve urea (30 gm) in water (50 ml); and iii) 9% Aqueous ferric chloride solution.

Figure 2. Procedure for the detection of tannins.



F. Detection for Saponins

1. Hemolysis test

As aliquot (0.5 ml) of 80% ethanol, acidic water or basic water extracts was freed of tannin by adding 80% ethanol (4.5 ml) or 0.9% normal saline for water extracts, filtered and heated at 70°C for 15 minutes, Magnesium oxide (1 gm) was added to the filtrate, and heated at 70°C for 15 minutes to form a tannin-MgO complex. Ethanol (95%, 5 ml) was then added, and the tannin-free filtrate used for the hemolysis test. A digitonin solution (0.1 gm/ml in 80% ethanol) was used as the standard.

The hemolysis test consists of mixing 1 ml of detanned filtrate with 1 ml of standarized red blood cells. The hemolytic activity was recorded as the time, in minutes, required to completely hemolyze the red blood cells. On each experimental day, an aliquot of stock red blood cells (5 ml) was withdrawn and standarized with 0.5 ml of digitonin solution (0.1 mg/ml). Indication of hemolysis of the red blood cells was observed microscopically and by centrifugation after a ten-minute reaction period. A colorless upper layer after centrifugation indicated a negative saponin test, whereas a red upper layer indicated a positive saponin test. The stock red blood cells were diluted with isotonic phosphate buffer (pH 7.4) in a dilution of 1:5 to obtain a positive digitonin hemolysis test.

The standarized red blood cells were prepared by defibrinating fresh human whole blood (6 ml) with sealed fine capillary tubes. The fibrin-free blood was suspended in normal saline (35 ml) and centrifuges (speed

six, serial No. 37618 P-2, International Equipment Co., Needham Hts., Mass.). The supernatant was discarded and the packed red blood cells washed three times with normal saline. The washed packed red blood cells were resuspended in normal saline (100 ml), stored at 10°C overnight, and used within 2-3 days. The isotonic phosphate buffer used was prepared by dissolving 0.44 gm of NaCl in the mixture of 20 ml of sodium biphosphate solution (0.8% in water) and 80 ml of sodium phosphate solution (0.94% in water).

2. Froth test

A freshly prepared hot water extract was made by heating a mixture of 1 gm of dried plant material and 15 ml of distilled water on a water bath. After cooling, the mixture was filtered through four layers of cheese-cloth and the filtrate was shaken vigorously for exactly one minute. The honeycomb froth geight formed after exactly 5 and 30 minutes were recorded. Digitonin (1 ml, 0.1 mg/ml) was used as the standard.

G. Detection for Steroids

Skellysolve F, chloroform and 80% ethanol extracts were applied (10 µl) to silica gel H thin-layer plates as previously described (p. 50), developed in cyclohexane:ethylacetate (1:1). The plates were examined by i) Fluorescent pattern (254 mµ); ii) Heating at 100°C for 15 minutes after spraying with freshly prepared anisaldehyde reagent (1% v/v of anisaldehyde in 2% v/v concentrated sulfuric acid in glacial acetic acid) (137); and iii) Spraying with Kedde reagent (1% 3,5-dinitrobenzoic acid in 0.5 N of 50% v/v aqueous methanolic KOH) (138) followed by Zimmermann reagent (mix 1 vol. of 2% m-dinitrobenzene in ethanol with 1 vol. of 1.25 N of ethanolic KOH) (139). Androstandiol, digitoxigenin, digitoxin, progesterone and beta-sitosterol (10 mg/ml in 1:1 misture of methanol and chloroform) were used as standards.

H. Lipid Analysis

1. Gravimetric determination of total lipids

Skellysolve F and chloroform extracts, representing 110.25 gm and 102.75 gm of dry plant material, respectively, were brought up to a volume of 25 ml with their respective solvents. An aliquot of 1 ml was transferred to a preweighed aluminum dish which was then freed of solvent in a high vacuum desiccator until a constant weight was achieved. Weight percent (w/w) content of lipids extractable by skellysolve F or chloroform with respect to the original dried powdered plant material was obtained by the following formula:

$$\frac{\text{concentration (g/ml)} \times \text{(ml)}}{\text{110.25 (gm, for skellysolve F ext.) or 102.75 (gm, for } CHCl_3 \text{ ext.)} \times 100}$$

2. Systemic analysis of lipid distribution

Thin-layer chromatography

Skellysolve F and chloroform extracts (5 µl each) were applied to thin-layer Chromagram Sheets (20 x 20 cm, 6061 silica gel without fluorescent indicator, Eastman Kodak Co., N.Y.). The solvent system skellysolve F:ether (95:5) was used for the skellysolve F extracts, and that of skellysolve B:ether:glacial acetic acid (70:30:1) for the chloroform extracts. The lipids were detected by exposing the chromatograms to iodine vapour.

The reference standard used was lipid mixture S8 (Lipids Preparation Laboratory, The Hormel Institute, Austin, Minn.) which consists of oleic acid, methyl oleate, alkyl diglyceride (primarily dioleate), triolein, oleyl palmitate, octadecene-9, neutral plasmalogen, cholesterol and cholesterol oleate.

3. Fatty acid constituents of triglycerides isolated from selected plant extracts

Triglycerides were first separated from the skellysolve F and chloroform extracts of Nymphaea tuberosa, Ceratophyllum demersum, Anacharis canadensis and Carex lacustris. Methyl esters of constituent fatty acids obtained by methanolysis of pure triglycerides were analyzed by GLC. Confirmation of chain lengths of fatty acids and their degree of unsaturation was achieved by hydrogenation with subsequent GLC analysis of hydrogenated methyl esters of fatty acids.

a. Isolation of triglycerides

Preparative thin-layer chromatography

Aliquots of skellysolve F and chloroform extracts of each plant were applied as a narrow band (5 mm) on silica gel G plates (1 mm) under a stream of nitrogen. A C-16 triglyceride standard was spotted on both sides of the band. The plates were developed in the solvent system of skellysolve F:diethyl ether (80:20). Triglyceride appeared as a darker band on the chromatograms which was easily seen by observing the plates against a strong light source in a dark room. The standard triglyceride co-chromatographed on both sides served as an additional guide-line.

The triglyceride fractions from the skellysolve F and chloroform extracts were combined and transferred to a screw-capped vial and extracted three times with peroxide-free diethyl ether previously saturated with oxygen-free water. The ethereal extracts were brought down to almost dryness (trace of water left) under a stream of nitrogen. A small amount of skellysolve F was then added, and the extract dried over anhydrous sodium sulfate and under nitrogen to remove the water. If necessary, a second solvent system of skellysolve F:diethyl ether (90:10) was used for the thin-layer chromatographic purification of triglyceride.

b. Methanolysis

The pure triglyceride (5 mg) and 25 ml of reaction mixture (absolute methanol, benzene and concentrated sulfuric acid, 86:10:4) were reacted under nitrogen at 80-90°C for 2 hours to form the fatty acid methyl derivatives. Water (40 ml) was added at the end of the reaction period, and the mixture extracted three times with hexane (5 ml). The combined hexane extract was dried over anhydrous sodium sulfate, and stored under nitrogen until ready for gas-liquid chromatographic analysis.

TLC of methyl esters of fatty acids which after spraying with 0.2% of 2,7-dichlorofluorescene in 95% ethanol form a characteristic yellow fluorescent spots under ultraviolet light.

c. Hydrogenation

Hydrogenated methyl esters of fatty acids were prepared by mixing 4 ml of methyl esters in skellysolve F (1 mg/ml) with a few crystals of platinum dioxide and then subjected to hydrogenation for 3 hours at 40 pounds in a hydrogenator (Parr Instrument Company, Inc., Moline, Ill.).

d. Gas-liquid chromatography (GLC)

Instrument and the experimental conditions

The instrument employed was a Beckman/GC-2 Gas Chromatograph (Beckman Scientific Instrument Division, Fullerton, Calif.) equipped with a flame ionization detector. The aluminum column used (6 feet in length and 1/8 inch I.D.) was packed with 20% w/w diethylene glycol succinate (DEGS) on Anakrom A (100/110 mesh) and purchased from Analab. Inc., Hamden, Conn. The column temperature used was 175°C, the injection-port temperature was 200°C and helium at 25 psi was used as the carrier gas.

The standard used was GLC Reference Mixture No. 1 (Lipids Preparation Laboratory, The Hormel Institute, Austin, Minn.) which is a mixture of methyl esters of palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linoleic acid (18:3).

Sample peaks were identified with the aid of the standard graph of log retention time vs. carbon number (Fig. 3). Assignment of the carbon chair length of each unknown peak was made possible through its retention time. The area of each peak was calculated by multiplying its peak height with one-half of its base width and the relative percentage of each fatty acid ester or that of its hydrogenated compound was obtained by dividing its peak area with total peak areas on the chromatograph.

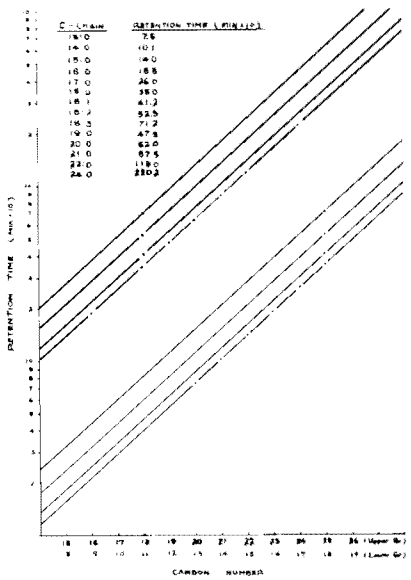


Figure 3. Standard graph of log retention time vs. carbon number of reference fatty acids.

III. RESULTS AND DISCUSSIONS

A. Alkaloids

The results for the thin-layer chromatographic detection of alkaloids are shown in Table 3.

Most of the original extracts did not appear to contain alkaloids after examining the TLC of 10 µl aliquots of the extracts (equivalent to 750 mg of dry plant powder for skellysolve F and chloroform extracts, and 375 mg for 80% ethanol extracts). Therefore, the extracts were further purified to remove water soluble organic materials which might have interfered with the alkaloidal detection.

The following plant species demonstrated Dragendorff positive spots: Anacharis canadensis, Ceratophyllum demersum, Carex lacustris, Lemna minor, Nymphaea tuberosa, Nuphar variegatum, Potamogeton amplifolius, P. pectinatus, P. richardsonii, P. zosteriformis, Sagittaria latifolia and Vallisneria americana. Among those, Nymphaea tuberosa and Nuphar variegatum gave the most prominent Dragendorff reactions. P. richardsonii seems to contain various Dragendorff positive spots in relatively lower concentrations. Nuphar variegatum, N. japonicum and N. luteum have been reported to contain piperidine (120), lupine (107-119) and thio-alkaloids (103,105,106), respectively. The nature of two alkaloids (121) found in Nympheae alba is unknown. However, the alkaloid in Nymphaea tuberosa in this study exhibited a positive Ehrlich reaction and is suspected to be an indole-type alkaloid. Although indole alkaloids are reported present in Carex brevicollis (122-126), they are not present in Carex lacustris.

Dragendorff's reagent can detect very small concentrations of alkaloids (140) by forming an orange to pink metal complex. Farnsworth (44) found that conjugated carbonyl (ketone or aldehyde) or lactone functions was the minimum structural requirement for a Dragendorff reaction to occur. By this criterium, natural products such as coumarins, anthraquinones, etc., will also give an alkaloid-like reaction. Ehrlich's reagent has been widely used for the detection of indole compounds. Any electron-withdrawing group (e.g., -CN, -C=O) decreases the electron density around the 2-carbon atom of the indole nucleus and will repell the attacking electrophilic aldehyde. Ehrlich's reagent will react with simple indoles (tryptophan, 5-OH tryptophan etc.), aromatic amines (anthranilic acid), ureides (thiourea) and phloroglucinol (141). The color of the condensation products formed between indole and aldehydes may be purple, pink, blue, green to brown, orange, or yellow. Most indole alkaloids will form a purple blue to gray color with p-dimethylaminobenzaldehyde and these colors may be stabilized by freshly prepared 0.1% $NaNO_2$ in ethanol solution.

The interpretation symbols (+++, ++, + or t) for the Dragendorff reactions are only approximate, as the surface area represented by a single alkaloid spot increases with an increase in Rf value.

Table 3. Thin-layer Fluorescent and Alkaloid Patterns[1/].

I[2/]	II[3/]	III[4/]			IV			V		
		a	b	c	a	b	c	a	b	c
Ac-1	S	0.30	bl ye	+				0.30	gr ye	+
	C									
Ac-2	A	0.40	bl	++	0.08	pi	+	0.49	br	+
	S	0.09	or	++						
	C									
	A	0.08	ye	++	0.01	or	t			
Cd	S	0.73	bl	+						
		0.82	re	+						
	C	0.02	ye	++				0.02	pu bl	t
		0.30	ye	++						
	A	0.37	pu	+	0.16	pi	+			
Cl	S							0.80	gy	t
	C	0.02	pi	+						
	A				0.16	or br	+			
Cp	S									
	C	0.43	bl	+						
		0.62	bl	+						
	A	0.02	bl	++	0.52	pi	t			
		0.42	bl	++						
Cv	S	0.76	re	+						
	C				0.79	pi	t	0.79	ye	+
	A	0.70	or	+				0.70	gr ye	+
Es	S	0.03	gr	+						
	C									
	A									
Lm	S									
	C	0.48	bl	+	0.60	or	t			
	A	0.41	bl	+	0.04	pi	+			
Me	S	0.04	wh	++						
		0.15	br	+						
	C									
	A									
Nt	S	0.36	re	+						
lv	C									
	A	0.43	bl	+	0.60	pu	++	0.60	gy	+
Nt	S									
st	C									
	A	0.39	pu	++	0.05	or	+	0.66	gy	+
		0.49	bl	++	0.66	pu gy	+			
Nv-1	S									
lv	C									
	A				0.44	or	+	0.30	yg	+
Nv-1	S	0.07	or	+						
st	C				0.43	or	++			
	A	0.20	gr ye	+	0.21	or	+++			
Nv-2	S									
lv	C	0.24	gr ye	+	0.23	or	++			
	A				0.40	or pi	++			

(Table 3 continued)

I	II	III			IV			V		
		a	b	c	a	b	c	a	b	c
Nv-2	S	0.05	ye	+						
st		0.85	ye	+						
	C	0.70	gr ye	+	0.28	or	+	0.70	ye	+
	A	0.20	bl ye	++	0.20	or	+++			
Pa	S	0.73	re	t	0.73	or	+			
	C									
	A	0.43	bl	+	0.53	br	+			
Pn	S									
	C	0.48	bl	+	0.67	br	t			
	A	0.44	bl	+				0.59	br	t
		0.53	bl	+						
Pp	S	0.15	br	+						
	C									
	A	0.43	bl	t	0.11	pi	+			
Pr	S	0.80	re	+						
	C				0.48	or	+	0.72	ye	+
					0.67	or	+			
					0.72	pi	t			
	A	0.43	bl	t	0.48	pi	+			
Pz	S	0.52	re	t						
	C									
	A	0.41	bl	+	0.05	or pi	+			
Sc	S									
	C							0.72	br	+
	A	0.43	bl	++						
Se	S	0.06	gr ye	+						
	C	0.25	bl	+						
		0.44	bl	+						
		0.55	gr ye	+						
	A	0.61	gr bl	+						
Sf	S	0.03	pu	+						
		0.07	ye	+						
		0.15	ye	+						
		0.75	bl	+						
	C				0.77	pi	t	0.77	ye	+
	A	0.46	bl	+						
		0.58	ye	+						
		0.68	gr bl	+						
Sl	S	0.05	pi	+						
	C				0.09	pi	+	0.46	br	+
	A	0.36	bl	t	0.36	br	t			
Ta-1	S	0.66	ye wh	+++						
	C							0.60	bl pu	t
	A	0.44	bl	+				0.62	pu	t
Ta-2	S	0.08	gr	++						
		0.64	bl	+						
	C									
	A	0.43	bl	++						

(Table 3 continued)

I	II	III			IV			V		
		a	b	c	a	b	c	a	b	c
Va	S	0.06	ye	++	0.23	or	+			
		0.46	ye	t	0.06	gr or	+			
	C							0.02	by	+
	A	0.30	bl	+						
		0.46	bl	+						
Za	S									
	C									
	A	0.43	bl	+				0.59	br	t
Std.[3,5]										
Er	a)	0.05	pu	++	0.02	or	++	0.02	gy	+
	b)	0.29	pu	++	0.29	gy or	++	0.29	pu	++
Hy	a)				0.19	ye or	++			
	b)				0.24	or	++			
Ca	a)				0.52	or	++			
	b)				0.28	or	+			

1/Roman numerials I- Plant names; II- Extracts; III- Fluorescent pattern (254 mμ); IV- Dragendorff's positive spots; V- p-Dimethylaminobenzaldehyde positive spots.

2/Plant names Ac- Anacharis canadensis (1- collected at Lake Minnetonka; 2- collected at Lake Itasca); Cd- Ceratophyllum demersum; Cl- Carex lacustris; Cp- Calla palustris; Cv- Chara vulgaris; Es- Eleocharis smallii; Lm- Lemna minor; Me- Myriophyllum exalbescens; Nt- Nymphaea tuberosa (lv- leaf; st- stem); Nv- Nuphar variegatum (1- collected at Lake Minnetonka; 2- collected at the Pine Lake); Pa- Potamogeton amplifolius; Pn- P. natans; Pp- P. pectinatus; Pr- P. richardsonii; Pz- P. zosteriformis; Sc- Sagittaria cuneata; Se- Sparganium eurycarpum; Sf- Sparganium fluctuans; Sl- Sagittaria latifolia; Ta- Typha angustifolia (1- collected at the Silver Lake; 2- collected at the Pine Lake); Va- Vallisneria americana; Za- Zizania aquatica.

3/Extracts and solvent systems A- 80% Ethanol; C- Chloroform; S- Skellysolve F. a)- Solvent system - chloroform:acetone:diethylamine (5:4:1) for Skellysolve F extracts; b) Solvent system- n-butanol:acetic acid: water (4:1:1) for Chloroform and 80% ethanol extracts.

4/a- Rf values; b- Color bl-blue; br-brown; gr-green; gy-gray; or-orange; pi-pink; pu-purple; re-red; wh-white; ye-yellow; c- Intensity +++=high; ++= medium; += low; t= trace.

5/Standards Er- Ergonovine maleate; Hy-Hyoscyamine; Ca-Caffeine.

B. Flanonoids

The results for the thin-layer chromatographic detection for flavonoids are shown in Table 4.

Flavonols were most widely distributed in the plant extracts studied. These compounds were present in Calla palustris, Lemna minor, Myriophyllum exalbescens, Nuphar variegatum, Potamogeton natans, P. pectinatus, P. richardsonni, P. zosteriformis, Sagittaria cuneata, S. latifolia, Typha angustifolia and Zizania aquatica. Flavonones appear to be present in Potamogeton amplifolius, P. natans, P. pectinatus, P. zosteriformis, Sagittaria cuneata, S. latifolia, Sparganium fluctuans and Vallisneria americana. Flavones were present in Lemna minor, Myriophyllum exalbescens and Nymphaea tuberosa. Anthocyanidine and aurones are not present in 80% ethanol extracts as indicated by the absence of visible orange to pink spots on the chromatograms. Catechin was present in Carex lacustris and Potamogeton richardsonii. The flavonol spots fluorescent blue-purpoe before ammonia vapour treatment, turned yellow instantly in the ammonia vapour chamber and their fluorescence intensified. According to the patterns of ring substitutions, the Rf values for those flavonols varies from 0.44 to 0.76 in the solvent system of n-butanol:acetic acid:water (4:1:5). The pale blue fluorescent spots (Rf values 0.88-0.90 in BAW 4:1:5) of flavanones showed no conspicuous color change by ammonia vapour or only being slightly enhanced. The orange-yellow fluorescent spots of flavones (Rf value 0.35 in BAW 4:1:5) were intensified to dull brown or bright yellow after fuming with ammonia. The blue fluorescent spots of catechins (Rf values 0.90-0.94 in BAW 4:1:5) appear only after ammonia vapour treatment. The flavonoid, 3-deoxyanthocyanidine previously found in Carex riparia and C. acutiformis (127) was not in C. lacustris, whereas the presence of the flavone in Lemna minor reported (128,129) was evident.

With the exception of catechins and leukoanthocyanines, the flavonoid compounds fluorescent under ultraviolet light, and their fluorescence may be intensified or changed by exposing the chromatogram to ammonia fumes. The action of ammonia on flavonoids is reversible. p-Nitrobenbenzene diazonium fluoroborate is a very effective coupling reagent forming with phenols yellow, orange or brown colors. The spray is non-specific, and will not only detect flavonoids but also aryl amines, tannins, etc. (142).

C. Tannins

The results for the color detection of tannins are shown in Table 5.

Tannins, especially the condensed type, were widely distributed in the aquatic plants screened. The following plant species were found to contain condensed tannins: Carex lacustris, Eleocharis smallii, Lemna minor, Myriophyllum exalbescens, Potamogeton amplifolius, P. natans, P. richardsonii and Sparganium fluctuans. The extracts formed precipitates with gelatin-salt solution, and the resulting urea solubilized precipitates showed a blue-green or green-yellow color with the ferric chloride reagent.

Nuphar variegatum and Numphea tuberosa contain hydrolyzable tannins which after solubilization with urea formed a blue-black or purple-blue color with the ferric chloride reagent. Acidic and basic water extracts

Table 4. Thin-layer Fluorescent and Flavonoid Patterns[1/].

I	II[2/]	III	IV[3/] Daylight	IV[3/] UV	V[3/] Daylight	V[3/] UV	VI
Ac-1	S						
	C	0.43	ye +				
		0.67		re t			or +
	A	0.60		bl t		gr +	
		0.92		ye t		ye +	
Ac-2	S						
	C	0.08					or +
		0.40	or +				
	A	0.60		bl t		bl +	
		0.92		or +		or +	
Cd	S	0.78					ye t
	C	0.17					or +
		0.43	ye +				
		0.66		re t			or +
	A	0.41			ye +	br +	br t
		0.60		bl +		+	br +
Cl	S	0.76	ye +			gr ye	
	C	0.15		re t			
		0.23			ye +		
		0.40	or ++				
		0.72		re t	ye +		
	A	0.54		bl t		bl t	
		0.72		bl +		bl +	
		0.90				bl +	or +++
Cp	S	0.51	ye +				
	C	0.17	gy gr +				
		0.45	ye ++				
	A	0.36				br +	
		0.59			ye +		br +
		0.66		bl t		bl +	br +
Cv	S	0.85	ye +				
	C	0.06		re or +			
		0.44	ye +				
	A						
Es	S	0.52		bl +			
		0.77	ye +				br +
	C	0.26		bl +			br +
		0.44	ye +				
		0.50		bl +			
		0.73		re t			
	A	0.41		ye t		ye t	ye +
		0.52		bl +		pu +	ye +
Lm	S	0.68	ye ++				
	C	0.07					or +
		0.16	gr +				
	A	0.35		pu +	ye ++	br +	br +
		0.62		pu ++	ye ++	br pu +	br +
		0.86		bl +			

(Table 4 continued)

I	II	III	IV Daylight	IV UV	V Daylight	V UV	VI
Me	S	0.40					ye t
	C	0.13	ye +				
	A	0.35		ye t		ye +	
		0.57		pu +	ye +		
		0.62		bl +		pu t	br +
Nt	S	0.37	ye ++				
lv	C	0.26					br +
		0.41		re ++			
		0.50	ye +				
		0.80		re +			
	A	0.35		or +		or +	
		0.71		pu t		pu t	ye +
Nt	S	0.37	ye ++				
st	C	0.21					or +
	A	0.35		or t		ye +	
		0.71		pu +		pu +	ye ++
Nv-1	S	0.43	ye ++				
lv	C	0.40	ye ++				
	A	0.35		ye t		or +	
		0.57		pu ++	ye ++	pu ++	br +
		0.66		bl +	ye t	bl +	
Nv-1	S	0.20	ye +				
st	C	0.21	ye +				
	A	0.35		ye t		br +	
		0.57		pu ++	ye ++	pu ++	br +
		0.66		bl t			
Nv-2	S	0.43	ye +				
lv		0.68	ye ++				
	C	0.03		ye t			
		0.45	ye ++				
	A	0.35		ye t			br +
		0.57		pu +	ye +	pu ++	br ++
		0.66		bl t			
Nv-2	S	0.85		re +			
st	C	0.24					or +
		0.51	ye +				
		0.80		re t			ye +
	A	0.35		ye t			
		0.57					br t
Pa	S	0.78	re ++				
	C	0.52	re gr +				
	A	0.90		bl t		bl t	br t

(Table 4 continued)

I	II	III	IV Daylight	IV UV	V Daylight	V UV	VI
Pn	S	0.66	re or ++				
	C	0.07					or +
		0.45	or ++				
	A	0.57		pu t	ye +	pu +	br t
		0.66		pu +	ye ++	bl ++	br +
		0.90		pu ++	+	bl ++	br ++
Pp	S						
	C	0.08					or ++
	A	0.66		pu t	ye t		
		0.90		bl +	ye t	bl +	br +
Pr	S	0.76	re +				
		0.84	ye +				
	C	0.45	ye +				br ++
		0.67	re +				
	A	0.57		pu t	ye t	pu +	
		0.94				bl pu +	br +
Pz	S	0.38	br +				
	C	0.21		re t			or +
	A	0.57		pu +	ye ++	pi +	br +
		0.90		bl t			br +
Sc	S	0.82	or ++				
	C	0.42	or ++				
	A	0.66		pu +	ye ++	pi +	br +
		0.90		bl t		bl t	br +
Se	S	0.46	ye +				
		0.76	gy +				ye +
	C						
	A	0.67			ye +		br +
Sf	S						
	C	0.44	ye +				
		0.48		ye +			
		0.58		wh +			
	A	0.90		gr bl ++		gr bl ++	
	S	0.49	ye ++				br +
		0.84	ye ++				
	C	0.03		ye +			
	A	0.66		pu +	ye +	br +	br +
		0.89		bl t		bl +	br +
Ta-1	S	0.82		ye t			br +
	C	0.35		ye gr +			or +
	A	0.44		pu +	ye t	br t	
		0.57		bl t	ye t	br t	br +
		0.74		pu ++	ye ++	br ++	ye br ++

(Table 4 continued)

I	II	III	IV Daylight	IV UV	V Daylight	V UV	VI
Ta-2	S						or +
	C	0.21	ye +				
	A	(Identical with those for Ta-1 A)					or +++
Va	S	0.11		br t			
	C	0.56		re t			
	A	0.88		ye +		ye br +	
Za	S	0.81	or +				
	C	0.50	or +				
	A	0.45		ye wh ++			
		0.57		bl t	ye t	bl t	ye +
		0.66		bl +	ye +	bl t	
Std.							
Ru	a)	0.02		gr ye ++	ye ++	pu +++	br ++
Ru	b)	0.66	ye t	pu ++	ye ++	pu +++	br +
Um	a)	0.31		bl ++			or ++
Vi	a)	0.53		gr ++			br +++
Pr	a)	0.74		pu ++			ye +

1/I- Plant names: (refer to Table 3, footnote 2); II- Extracts, S- skellysolve F; C- chloroform; A- 80% ethanol; III- Rf values; IV- visible and fluorescent patterns without any treatment; V- visible and fluorescent patterns after exposure to ammonia vapour; VI- Nitrobenzene diazonium fluoroborate positive spots.

2/Solvent systems for samples and standards: a) Chloroform:methanol (95:5) for skellysolve F and chloroform extracts; b) n-Butanol:acetic acid:water (4:1:5) for 80% ethanol extracts; Standards: Ru- Rutin; Um- Umbelliferone; Vi- Visnagin; Pr- Provismine.

3/Color and intensity of spots under daylight and ultraviolet light bl- blue; br- brown; gr- green; gy- gray; or- orange; pi- pink; pu- purple; re- red; wh- white; ye- yellow; +++= high; ++= medium; += low; t= trace.

Table 5. Presence of Tannins in Minnesotan Aquatic Plants[1/].

I	II	III			IV[2/]			I	II	III			IV		
		a	b	c	a	b	c			a	b	c	a	b	c
Ac-1	A	t	wh	t				Nt	A	+	br	+		pu bl	+++
	AW							st	AW					gr	+
	BW								BW					pu	+++
Ac-2	A							Nv-1	A		gr br	+		bu gr	++
	AW							lv	AW				+	gr	+
	BW				+	gr	+		BW				+	gr	+
Cd	A	t	wh	t				Nv-1	A	t	gr	t		ye	+
	AW				+	ye	+	st	AW						
	BW				+	ye	+		BW	t	bl	+	+	pu gr	+
Cl	A	+	br	+++		br gr	+	Nv-2	A	+	ye br	++		gy bl	+++
	AW					gr br	+	lv	AW				+	gr	++
	BW				+	br	++		BW				+	gr	++
Cp	A							Nv-2	A						
	AW							st	AW						
	BW					gr br	t		BW				+	ye gr	+
Cv	A							Pa	A	+	br	+		gr	+
	AW								AW	t	br	+	+	br gr	+++
	BW								BW				+	gr br	++
Es	A	+	ye gr	+		gr ye	+	Pn	A	+	gr	+		ye gr	+++
	AW					gr	t		AW	+	gr	+		gr	+++
	BW					gr	+		BW				+	gr br	++
Lm	A	+	br gr	++		ye gr	++	Pp	A	t	wh	+		ye	+
	AW				+	gr	++		AW	+	gr	+		bl gr	++
	BW				+	gr br	++		BW				+	gr	+++
Me	A	+	gr	+	+	ye gr	+++	Pr	A	+	gr ye	+		gr ye	+
	AW					gr br	+		AW					gr	+
	BW				+	gr br	++		BW				+	gr br	+
Nt	A	+	br	+++		gy bl	+++	Pz	A						
lv	AW					bl gr	++		AW				+	ye	+
	BW				+	br pu	+++		BW						

(Table 5 continued)

I	II	III			IV			I	II	III			IV		
		a	b	c	a	b	c			a	b	c	a	b	c
Sc	A							Ta-1	A	+	br gr	+		ye	+
	AW				+	gr ye	+		AW						
	BW				+	gr	++		BW					br	t
Se	A	+	br	+		ye	+	Ta-2	A	+	wh br	+		br gr	++
	AW					gr	t		AW					ye	t
	BW				+	gr br	+		BW				+	br	++
Sf	A	+	br	+		ye gr	+	Va	A	+	ye gr	+		ye	+
	AW					gr	t		AW				+	gr	+
	BW				+	gr br	++		BW				+	gr	+
Sl	A							Za	A	+	gr br	+		ye	t
	AW				+	gr	++		AW						
	BW					gr	++		BW				+	br	+

1/I- Plant names (refer to Table 3, footnote 2/; II- Extracts A- 80% Ethanol; AW- Acidic water; BW- Basic water; III- Gelatin-salt solution, a- Ppt (+); no ppt (blank); trace ppt (t); b- color bl- blue; br- brown; gr- green; gy- gray; pu- purple; wh- white; ye- yellow; c- Intensity +++= high; ++= medium; += low; t= trace; IV- Ferric chloride test.

2/ Underlined colors represent those precipitants dissolved by urea (positive tannin test).

of Nuphar variegatum and Nymphaea tuberosa gave a positive ferric chloride test. The hydrolysis products of the action of the acid or the base on tannins, gallic or ellagic acid, account for the blue or green ferric chloride test.

D. Saponins

The results from hemolysis and froth tests for the detection of saponins are shown in Table 6.

Only Nuphar variegatum (stem, collected at Lake Minnetonka), Potamogeton pectinatus, P. richardsonii and Typha angustifolia failed to hemolyze standarized red blood cells in 10 minutes. However, the hemolytic activity of the other plant species may be due to the presence of non-saponin hemolytic plant constituents such as amines, rancid fats or plant acids (57) which affect the permeability and/or membrane integrity of the red blood cells. The following species demonstrated a hemolytic activity in less than 5 minutes and a characteristic honeycomb froth height of more than 5 mm in 5 minutes and, therefore, are saponin positive: Lemna minor, Nuphar variegatum (collected at the Pine Lake), Potamogeton amplifolius, Sparganium fluctuans and Sagittaria latifolia.

E. Steroids

The results for the thin-layer chromatographic detection of steroids are shown in Table 7.

Beta-sitosterol is tentatively identified as being present in Ceratophyllum demersum, Carex lacustris, Nuphar variegatum, Potamogeton amplifolius, P. richardsonii, P. zosteriformis, Sparganium fluctuans and Typha angustifolia. This interpretation is based upon its Rf values of 0.50-0.56 in 1:1 cyclohexane:ethyl acetate and its reaction with anisaldehyde reagent. Beta-sitosterol has also been reported (133) as being present in the rhizome and flower of Nuphar luteum. Sagittaria latifolia may contain a hydroxy steroid (Rf value 0.38 in 1:1 cyclohexane:ethyl acetate) because of its color reaction with anisaldehyde reagent. Cardenolides, 3- or 17-oxosteroids are totally absent in the plant species studied, although brown Kedde-Zimmermann reactions were observed.

Anisaldehyde reagent is a general detecting reagent with high sensitivity for steroids, terpenes, carbonyl compounds, etc. (137). Beta-sitosterol gives purple color with anisaldehyde reagent; 16-hydroxy-steroids and many hydroxy-derivatives of progesterone give yellow or yellow-brown color; and 11-ketosteroids give red or carmin color (143). Kedde reagent forms a blue-violet color with alpha, beta-unsaturated 5-ring lactones (cardenolides) (144). Zimmermann reagent is specific for ketonic steroids with an ortho unsaturated methylene group (144). The m-dinitrobenzene reacts with the methylene group which is activated by the oxo-function, and 3-oxo-steroids appear immediately as blue spots, whereas 17-oxo-steroids with an unsaturated 16 position give violet color after 3 to 6 minutes.

Table 6. Detection of Saponins by Homolysis and Froth Tests[1/].

I	II	III	IV[2/]		V	I	II	III	IV		V
			a	b					a	b	
Ac-1	A	5	4	2		Nv-2	A	4			
	AW	40				st	AW		6	4	66
	BW						BW	60			
Ac-2	A	5	3	1		Pa	A	1			
	AW	29					AW		6	4	66
	BW						BW				
Cd	A					Pn	A				
	AW	6	1				AW	4	1	1	
	BW						BW	60			
Cl	A					Pp	A				
	AW		1				AW		3	1	
	BW	8					BW				
Cp	A					Pr	A				
	AW	6					AW	43	3	2	
	BW						BW				
Cv	A	8				Pz	A				
	AW		8	3	37		AW	5	1		
	BW						BW				
Es	A					Sc	A	4			
	AW	5					AW	17	4	2	
	BW	10					BW				
Lm	A					Se	A				
	AW	4	8	4	50		AW	5			
	BW						BW	60			
Me	A	4	3	1		Sf	A	2			
	AW	16					AW		8	4	50
	BW						BW	60			
Nt	A					Sl	A	5			
lv	AW	6					AW	29	8	4	50
	BW						BW				
Nt	A	5				Ta-1	A	5			
st	AW		4	1			AW				
	BW						BW				
Nv-1	A	12				Ta-2	A				
lv	AW		12	6	50		AW	15	3	2	
	BW	58					BW	50			
Nv-1	A	2				Va	A	3			
st	AW		1				AW	12	4	2	
	BW	12					BW	7			
Nv-2	A	2				Za	A				
lv	AW		10	6	60		AW	9	6	4	66
	BW						BW				
						Digitonin		3	3	12	66

1/I- Plant names (refer to Table 3, footnote 2); II- Extracts A- 80% ethanol, AW- acidic water; BW- basic water; III- Hemolytic activity. The time, in min., required to completely hemolyze the RBCs; IV- Froth height (mm); V- Froth persistency- % ratio of froth height of the fresh hot water extract in 30 minutes as compared to that in 5 minutes.
2/Froth height at a- 5 minutes; b- 30 minutes.

Table 7. Thin-layer Fluorescent and Steroid Patterns[1/].

I	II	III[2/]			IV[2,3/]		
		a	b	c	a	b	c
Ac-1	S						
	C				0.49	pu	+
	A	0.01	wh	++	0.01	ye	+
					0.07	pu gr	+
Ac-2	S						
	C				0.47	bl	+
	A	0.01	ye	+	0.07	gr	+
					0.21	pu	t
Cd	S				0.61	re pu	+
					0.79	br	+
	C				0.22	bl gr	+
					0.51	pu	+
					0.78	re ye	+
	A				0.02	ye	+
Cl	S				0.50	pu	+
	C				0.10	ye	+
					0.26	gy	+
					0.52	pu	+
					0.82	re pu	+
	A	0.01	ye	++	0.04	re br	+
		0.08	bl	+			
Cp	S	0.71	re	t	0.01	ye	+
					0.28	pu ye	+
					0.81	ye	+
	C				0.77	re pu	+
	A				0.02	br	+++
Cv	S				0.62	pu	+
					0.86	pu	t
	C				0.71	gr ye	+
					0.84	br	t
	A	0.01	wh	++	0.01	gr ye	++
Es	S	0.40	bl	+	0.47	pu	+
	C	0.45	bl	+	0.31	gy	+
					0.53	re br	+
					0.82	pu	+
	A				0.01	br	++
					0.05	or	++
					0.10	gr or	++
Lm	S				0.77	re	+
	C						
	A				0.01	ye	++
					0.07	re	t
Me	S						
	C						
	A	0.12	ye	+	0.12	gr ye	++
Nt	S						++
lv	C				0.26	ye pu	
	A	0.20	re	+			

(Table 7 continued)

I	II	III			IV		
		a	b	c	a	b	c
Nt	S						
st	C				0.09	ye pu	+
	A						
Nv-1	S				0.74	re br	+
lv	C						
	A	0.32	re	+			
Nv-1	S				0.75	re	t
st	C						
	A						
Nv-2	S				0.09	ye pu	t
lv					0.50	pu	+
					0.67	pu ye	+
	C				0.27	gy	+
					0.74	re pu	+
	A				0.01	gr ye	++
					0.17	pu	t
Nv-2	S				0.10	pu ye	t
st					0.51	pu	+
					0.77	ye br	+
	C				0.19	pu	+
	A	0.01	ye	++	0.01	ye	++
					0.20	pu	t
Pa	S				0.56	pu	+
					0.80	br	+
	C				0.19	gy	+
					0.27	gy	+
					0.52	pu	+
					0.81	re pu	+
	A	0.01	ye	++	0.01	ye	++
Pn	S	0.46	re	t	0.76	re	+
	C						
	A				0.02	ye	t
Pp	S	0.24	ye	t			t
	C						+
	A				0.07	re bl	t
Pr	S				0.20	pu	+
					0.52	pu	+
					0.82	re	+
	C				0.39	pu	+
					0.59	re pu	++
					0.71	gr ye	++
					0.84	pu	+
	A	0.02	gr ye	+	0.01	ye	t
Pz	S				0.01	ye	
					0.50	pu	++
					0.72	re pu	
	C						
	A	0.01	wh	++	0.02	ye	

(Table 7 continued)

I	II	III			IV		
		a	b	c	a	b	c
Sc	S	0.45	re	t	0.86	br ye	+
	C				0.76	re pu	+
	A	0.01	ye	++	0.01	ye	++
					0.59	pu	t
Se	S	0.12	ye	+			
	C	0.11	gr bl	++			
	A				0.01	ye	++
Sf	S	0.05	bl	+	0.19	pu	t
		0.53	bl	+	0.56	pu	t
	C	0.15	gr bl	++	0.51	pu	++
		0.52	bl	++	0.63	gr ye	+
					0.75	re pu	++
	A	0.15	gr	+	0.01	br	++
	S				0.08	gr re	t
					0.50	gr re	+
	C						
	A	0.21	re	+	0.01	br	++
					0.38	ye	t
Ta-1	S	0.44	bl	+	0.50	pu	+
		0.69	wh	+++	0.78	br	+
	C	0.73	ye	++			
	A	0.07	ye	+	0.01	br	++
					0.07	re br	+
Ta-2	S				0.50	pu	+
	C	0.20	bl	+	0.53	pu	+
	A	0.07	ye gr	+	0.05	re br	+
Va	S				0.20	br	+
	C						
	A	0.01	wh	++	0.01	ye	++
Za	S						
	C				0.74	re pu	++
	A	0.01	ye	++	0.01	br	++
An					0.29	br	+
Dg					0.14	gr	++
Dx					0.02	bl	+++
Pr					0.40	ye	+
Si					0.55	pu	++

1/I- Plant names (refer to Table 3, footnote 2) and standards. An- androstan-diol; Dg- digitoxigenin; Dx- digitoxin; Pr- progesterone; Si- beta-sitosterol; II- Extracts S- Skellysolve; C- chloroform; A- 80% ethanol; III- Fluorescence pattern (254 mμ); IV- Anisaldehyde positive spots.
2/a- Rf values; b- Color and c- Intensity (refer to Table 4, footnote 4).
3/Underlined Rf values represent a positive Kedde-Zimmermann test.

F. Lipids

The results for the gravimetric determination of total lipids obtained for each botanical family studied are summarized in Table 8, those for the systemic analysis of lipid distribution are shown in Table 9, and those for the fatty acid constituents of triglycerides are shown in Table 10.

Total lipids extractable by skellysolve F and chloroform range from 0.68% (Chara vulgaris) to 6.67% (Potamogeton natans) of dry plant material. There is a wide variation of total lipid contents among Najadaceae (1.50-4.89%), Hydrocharitaceae (1.43-4.48%) and Alismataceae (4.78-6.58%). No reliable difference in total lipid contents was found in Cyperaceae (1.18-1.73%), Sparganiaceae (0.74-1.80%) and Typhaceae (1.08-1.73), because of the small number of plants within the genus studied.

Potamogeton natans, Sagittaria latifolia and Nymphaea tuberosa with total lipid contents of 5.35, 5.24 and 3.91%, respectively, may be considered for their nutritional value, but the presence of alkaloid in N. tuberosa might be a drawback to its usefulness.

Iodine vapour is a sensitive (less than 1 gamma) detector for all unsaturated lipids and some saturated nitrogenous lipids (71). Identification of lipid classes in the plant extracts is tentatively obtained by comparing with the S8 standard (145). Free fatty acid (Rf value 0.00 in 95:5 skellysolve F:diethyl ether) is not present in the extracts studied; free sterol (Rf values 0.03-0.06 in 95:5 skellysolve F:diethyl ether) is quite common and only absent from Sagittaria latifolia; fatty acid esters (Rf values 0.43-0.44 in 95:5 skellysolve F:diethyl ether) are found in Chara vulgaris, Sagittaria latifolia, Calla palustris, Carex lacustris, Eleocharis smallii, Zizania aquatica, Nuphar variegatum and Nyphaea tuberosa; hydrocarbons (Rf value 0.60 in 95:5 skellysolve F:diethyl ether) present only in Nuphar variegatum and Nymphaea tuberosa. Lipid distributions are identical in the family of Najadaceae, Hydrocharitaceae, very similar in Cyperaceae and Numphaeaceae but quite different in Alismataceae and Sparganiaceae. Only a few plant extracts showed the presence of triglycerides (Rf values 0.10-0.12 in skellysolve F:diethyl ether:acetic acid, 70:30:1) by TLC. This is due to the lower concentration of triglyceride as compared to other lipid classes in aquatic plants.

As shown in Table 11 the major fatty acids of triglycerides are 16:0 (carbon number:number of unsaturation), 18:1, 16:1 and 18:2 for Anacharis canadensis; 20:3, 24:1, 18:2 and 16:0 for Ceratophyllum demersum; 18:3, 18:2, 18:1 and 16:0 for Carex lacustris; 16:0, 24:0, 18:2 and 18:1 for Nymphaea tuberosa (leaves) and 18:2, 16:0, 18:3 and 24:0 for Nymphaea tuberosa (stems). High content of not very common 24:0 fatty acid in Nymphaea tuberosa and 20:3, 24:1 fatty acids in Ceratophyllum demersum are interesting. When compared to the fatty acid contents of linseed and soybean oils (cf Table 11), the aquatic plants studied also indicated the lower concentrations of stearic acid. With the exception of Carex lacustris, palmitic acid in aquatic plants studied was present in higher whereas the unsaturated fatty acids (oleic, linoleic and linolenic) in lower percentage than those found in linseed or soybean. Composition of fatty acids from other aquatic plants reported (Table 1) also showed a lower content of stearic acid but with higher percentage of palmitic acid as compared to those found in linseed and soybean oils. Nevertheless, the unsaturated C-18 fatty acid contents are comparable to those for linseed and soybean.

Table 8. Gravimetric Determination of Total Lipids.

Family	Plant[1/]	Ext.[2/]	Wt. % (w/w)[3/]	Total lipids[4/]
Characeae	Cv	S	0.11	0.54
		C	0.43	
Alismataceae	Sc	S	2.48	3.85
		C	1.37	
	Sl	S	2.19	5.24
		C	3.05	
Araceae	Cp	S	1.73	3.82
		C	2.09	
Cyperaceae	Cl	S	0.35	0.93
		C	0.58	
	Es	S	0.46	1.37
		C	0.91	
Gramineae	Za	S	0.37	0.87
		C	0.50	
Hydrocharitaceae	Ac-1	S	0.25	1.14
		C	0.89	
	Ac-2	S	0.60	3.53
		C	2.93	
	Va	S	2.30	2.59
		C	0.29	
Lemnaceae	Lm	S	1.06	2.98
		C	1.92	
Najadaceae	Pa	S	0.21	1.07
		C	0.86	
	Pn	S	3.31	5.35
		C	2.04	
	Pp	S	0.18	0.61
		C	0.43	
	Pr	S	0.37	1.55
		C	1.18	
	Pz	S	0.16	1.14
		C	0.98	
Sparganiaceae	Se	S	1.06	1.40
		C	0.34	
	Sf	S	0.39	1.42
		C	1.03	

(Table 8 continued)

Family	Plant[1/]	Ext.[2/]	Wt. % (w/w)[3/]	Total lipids[4/]
Typhaceae	Ta-1	S	0.46	0.87
		C	0.41	
	Ta-2	S	0.28	1.07
		C	0.79	
Ceratophyllaceae	Cd	S	0.21	1.00
		C	0.79	
Haloragaceae	Me	S	0.32	0.99
		C	0.67	
Nymphaeaceae	Nv-1 lv	S	1.89	3.55
		C	1.66	
	Nv-1 st	S	0.94	1.83
		C	0.89	
	Nv-2 lv	S	1.17	1.96
		C	0.79	
	Nv-2 st	S	0.64	1.19
		C	0.55	
	Nt lv	S	2.25	3.91
		C	1.66	
	Nt st	S	0.83	2.77
		C	1.94	

1/Plant name- (refer to Table 3, footnote 3).
2/Extract S- skellysolve F; C- chloroform.
3/Weight % of lipid extractable by skellysolve F or chloroform.
4/Total weight % of lipid extractable by skellysolve F and shloroform.

Table 9. Systemic Analysis of Lipid Distribution.

Family	Plant[1/]	Extract[2/] Skelly. F Rf	Col.	Int.	Chloroform Rf	Col.	Int.
Characeae	Cv	0.06	br	+	0.03	br	+
		0.43	br	t	0.11	gr	++
Alismataceae	Sc	0.03	br	++	0.06	ye	+
		0.11	gr	+++	0.33	br	t
		0.18	br	+++			
		0.30	br	+++			
	Sl	0.40	br	+	0.06	ye br	+
		0.54	br	+			
		0.63	br	+			
Araceae	Cp	0.03	br	+	0.03	br	+
		0.06	br	++	0.11	gr	++
		0.08	br	+	0.30	br	t
		0.21	br	t			
		0.33	br	t			
		0.43	br	++			
Cyperaceae	Cl	0.06	br	+	0.03	br	+
		0.43	br	t	0.11	gr	+
					0.32	br	+
					0.53	br	t
	Es	0.06	br	+	0.03	br	+
		0.43	br	++	0.11	gr	+
					0.32	br	+
					0.37	br	t
Gramineae	Za	0.05	br	+	0.11	gr	t
		0.38	br	+			
		0.44	br	+			
Hydrocharitaceae	Ac-1	0.05	br	+	0.03	br	+
					0.11	gr	+
					0.37	br	+
	Ac-2	0.05	br	+	0.03	br	+
					0.11	gr	+
					0.37	br	+
	Va	0.10	br	++	0.03	br	+
Najadaceae	Pa	0.05	br	++	0.03	br	+
		0.08	br	+	0.11	gr	+
					0.34	br	t
	Pn						
	Pp						
	Pr	(Identical with those of Pa result)					
	Pz						
Sparganiaceae	Se	0.05	br	+			
	Sf	0.05	br	+	0.03	br	+
					0.11	gr	+
					0.14	br	+
					0.21	br	t
					0.33	br	+
					0.63	br	t

(Table 9 continued)

Family	Plant	Extract Skelly. F Rf	Col.	Int.	Chloroform Rf	Col.	Int.
Typhaceae	Ta-1	0.05	br	+	0.11	gr	+
					0.21	br	+
	Ta-2	0.05	br	+	0.11	gr	+
					0.21	br	+
Ceratophyllaceae	Cd	0.05	br	+	0.11	gr ye	+
					0.20	br	+
					0.26	br	t
					0.34	br	+
Haloragaceae	Me	0.05	br	+	0.03	br	+
					0.11	br gr	+
					0.36	br	+
Nymphaeaceae	Nv-1	0.05	br	++	0.03	br	+
	lv	0.11	br ye	+	0.11	br	+
		0.23	br	t	0.38	br	+
	Nv-2 lv	0.37	br	++	0.56	br	+
		0.44	br	+			
	Nt lv	0.60	br	++			
	Nv-1 st	0.05	br	+	0.03	br	+
		0.11	br ye	+	0.11	gr	+
		0.44	br	t	0.38	br	+
		0.60	br	t			
	Nv-2 st Nt st	(Identical those for Nv-1 st)					
S8 standard		0.00	br	+	0.21	br	+
		0.05	br	+	0.38	br	+
		0.12	br	+	0.56	br	++
		0.19	br	+			
		0.23	br	+			
		0.38	br	t			
		0.44	br	+			
		0.60	br	+			

1/Plant names- (refer to Table 3, footnote 2).
2/Rf- Rf values; Col.- Color br- brown; gr- green; ye- yellow; Int.- Intensity +++= high; ++= medium; += low; t= trace.

Table 10. Constituent Fatty Acids of Triglycerides Derived From Selected Aquatic Plants.

Plant	Chain length & number of double bonds	As methyl esters %	As hydrogenated methyl esters %
Anacharis canadensis	14:0	4.87	3.14
	15:0	7.00	2.90
	16:0	29.70	25.88
	16:1	12.91	
	18:0	3.75	42.19
	18:1	17.50	
	18:2	10.84	
	18:3	7.50	
Ceratophyllum demersum	14:0	1.15	1.97
	15:0	Trace	Trace
	16:0	7.81	18.74
	16:1	3.18	
	18:0	1.46	39.18
	18:1	6.73	
	18:2	12.36	
	18:3	4.63	
	20:3 (?)	29.05	12.18
	24:0	7.24	23.63
	24:1	23.46	
Carex lacustris	14:0	1.13	1.13
	16:0	7.94	16.77
	17:0	5.22	4.79
	18:0	6.90	59.74
	18:1	10.45	
	18:2	23.57	
	18:3	26.80	
	22:0 (?)	1.08	
	24:0	1.01	4.76
Nymphaea tuberosa (lv)	14:0	5.09	3.21
	15:0	Trace	Trace
	16:0	28.24	14.44
	16:1	4.02	
	17:0	Trace	Trace
	18:0	4.50	21.97
	18:1	9.46	
	18:2	9.54	
	18:3	8.57	
	24:0	17.40	44.23
Nymphaea tuberosa (st)	14:0	1.13	2.74
	15:0	Trace	Trace
	16:0	22.32	53.81
	16:1	2.10	
	17:0	1.00	2.11
	18:0	2.89	36.17
	18:1	4.21	
	18:2	43.96	
	18:3	16.48	
	24:0	4.97	1.12

Table 11. Comparison of Major Fatty Acids in Aquatic and Terrestrial Plants (Linseed and Soybean).

Plants[1/]	Chain length & number of unsat.[2/]					Total % of unsat.[3/]	Ref.
	16:0	18:0	18:1	18:2	18:3		
Linseed	7.2	3.4	18.5	17.0	55.2	90.7	(146)
Soybean	13.5	7.0	25.9	48.0	6.6	80.5	(147)
Ac	29.7	3.2	17.5	10.8	7.5	35.8	
Cd	7.8	1.5	6.7	12.4	4.6	23.7	
Cl	7.9	6.9	10.5	23.6	26.8	60.9	
Nt lv	29.2	4.5	9.5	9.5	8.6	27.6	
Nt st	22.3	2.9	4.2	44.0	16.5	64.7	

1/Plant names: Ac- Anacharis canadensis; Cd- Ceratophyllum demersum; Cl- Carex lacustris; Nt lv- Nymphaea tuberosa (leaf); Nt st- N. tuberosa (stem).
2/Percent contents of fatty acids were listed under each fatty acid column.
3/Total % of unsaturated fatty acid (18:1, 18:2, and 18:3).

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