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# Lipid composition of the water hyacinth *Eichhornia crassipes* (Mart.) Solms

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**Abstract** The water hyacinth *Eichhornia crassipes* was collected in 2004 in the Siak River system, eastern Sumatra. Standard lipid extracts were analysed for *n*-alkanes, *n*-fatty acids, hydroxy fatty acids, *n*-alcohols, steroids and terpenoids. *n*-Alkanes in all plant parts were dominated by $C_{27}$ to $C_{33}$ compounds while the *n*16:0 fatty acid was dominant in the acid fraction. Here, longer chain compounds had only minor contributions. In addition to steroids typical for higher plants ($C_{28}$ and $C_{29}$ compounds) significant contributions from cholest-5en-3*β*-ol were observed. Four unknown pentacyclic triterpenoid alcohols were also present. These could not be detected in river and estuarine sediments thus suggesting either export of *Eichhornia* organic matter to the coastal ocean or rapid degradation.

**Keywords** Lipid composition, water, *Eichhornia crassipes* (Mart.) Solms

## Introduction

The water hyacinth *Eichhornia crassipes* (Pontederiacae) is notorious for its rapid spread in freshwater systems under conditions of eutrophication [e.g. 1-2]. It has become a nuisance for fisheries, navigation, water intake to hydropower plants, irrigation, and recreation in many tropical and subtropical lakes and rivers. Through increased sedimentation and by shading the water column it affects photosynthesis of phytoplankton and benthic macrophytes, thereby leading to deoxygenation of underlying waters with a detrimental impact on aquatic organisms, especially fish. In addition, it facilitates the spread of diseases such as schistosomiasis and malaria [e.g. 1, 3]. Furthermore a possible relation with cholera outbreaks has been established by Feikin et al. for East Africa [4].

Due to the enormous production of the plant attempts have been made to utilise this biomass for various purposes, a.o. for water purification [e.g. 5-10], as fuel source [e.g. 11, 12] or for biogas production [e.g. 13, 14].

Surprisingly, despite its environmental and potential economic importance little is known about the chemical composition of the species and the activities of its constituents.

Allelopathic effects on *Chlorella* sp. and *Scenedesmus obliquus* of root extracts have been described by Jin et al. [15] with the highest activities found for a β-D dehydrated pyranose and pelargonic acid. Sun et al. [16] indicated that the root exudates of *E. crassipes* could significantly inhibit the growth of *Scenedesmus sp.* and *Chlamydomonas reinhardtii*. N-phenyl-1-naphthylamine and N-phenyl-2-naphthylamine acid were found to have good inhibition effects and considered as the main allelochemicals of *Eichhornia crassipes*. Della Greca [17] also conducted a similar study and reported that *Eichhornia crassipes* obviously inhibited *Porphyridium aerugineum* and *Anabaena azollae*, for which phenalene-like allelochemicals such as benzoindenone, dimeric phenalene and phenalene played the major roles.

Antibacterial and antifungal activities of aqueous, ethanolic and methanolic root and leaf extracts against a large number of test organisms have been reported by Fareed et al. [18]. Shanab et al. [19] found similar effects for several bacteria and fungi. A methanolic whole plant extract showed moderate activity against *Pseudomonas syringae* [20], *Agrobacterium tumefaciens* [21] and five pathogenic fungi and bacteria [22] while an ethanolic extract did not show any activity against *Candida albicans* and nine bacterial species [23].

Aqueous leaf extracts provided 13%-65 % protection against lipid peroxidation in rat liver, kidney, and brain tissue homogenates and exhibited a % cytotoxicity reduction against a lung cancer (NCI-H322) cell line [24]. The extract also demonstrated considerable antibacterial activity against *Proteus vulgaris*, *Salmonella typhi*, and *Bordetella bronchiseptica* [24].

Saxena et al. [25] noted that whole plant extracts showed growth inhibitory and juvenile hormone mimicking activities on *Culex quinquefasciatus* larvae without, however, further attempts at structural identification. An aqueous extract had marked and significant histopathological effects on on midgut, integument, fats and muscles of the 2$^{nd}$ instar larvae of *C. pipiens* and the resulting pupae and adults [26].

Devanand and Usha Rani [27] found moderate antifeedant and toxic effects of an acetone leaf extract on third instar larvae of the tobacco cut worm and the castor semilooper, two lepitopderan pest species of *Ricinus communis*.

Lalitha et al. [28] presented a review of several compound classes present in *Eichhornia crassipes*. Matai and Bagchi [29] report on the composition of amino acids in hydrolysates of the crude water hyacinth protein. This compound group was also found by class reactions in the stigmatic exudate of *E. crassipes besides s*oluble sugars, phenols, hydroxy phenols, free amino acids, and free fatty acids [30]. Quantitative data on carotenoids, carbohydrates, lipids, phenols and proteins have been given by Vasu et al. [31].

Various phenols have been found to be present by Lata et al. [32] who relate the antimelanoma activity of methanolic extracts of *E. crassipes* [33] to the presence of these compounds as reactive oxygen scavenging compounds.

Sanseverino et al. [34] concentrated on C16 and C18 fatty acids with different degrees of unsaturation. While Arayana et al. [35] provide information on the relative contributions of a number of lipid classes (non-polar lipids, glycolipids, phospholipids) including a.o. pigments, ester waxes or sterols the only group for which detailed information is given are the fatty acids, i.e. free ones, methyl esters as well as di- and triacylglycerols in roots, stalks, leave4s and flowers. All four groups are dominated by the C16 acid followed by C18:2 and C18:1 compounds.

The presence of alkaloids was established by Lata and co-workers [36, 37] and Vasu et al. [31]. Yohimban derivatives have been isolated by Aboul-Enein et al. [38, 39] together with various phthalic acid esters and fluorine and chlorine bearing compounds.

Alkaloids, phenols, steroids, tannins, triterpenoids, saponins and ellagic acid have been determined by class reactions with flavonoids being shown to be absent [31]. On the other hand, flavonoids have been identified by Thamaraiselvi et al. [40] besides alkaloids, phenols, sterols, terpenoids, anthraquinones and protein, again by class reactions.

The presence of phytoalexins of the phenylphenalone type and related compounds has been reported by Della Greca et al. [17, 41-44] and Wang et al. [45]. Five of the initially suggested structures were corrected later based on 2D-NMR analyses [44]. Hölscher and Schneider [46] reported on the presence of phenylphenalenones in *E. crassipes* which are considered to be precursors of phenylphenalones. The compounds found are unusual as they carry the phenyl moitie in the 8 position rather than at C4, C6, C7 or C9 as is common in previously described phenylphenalenones from other plant species.

Anthocyanins with a common delphinidine chromophor have been isolated from the flowers [47, 48].

Little is also known about *E. crassipes* from an ethnobotanical point of view. Perry [49] summarising older literature mentions that in Kedah, NW Malaysia, the flowers are used in skin treatment of horses. According to Grenand et al. [50] inflated petioles are used in Guayana in a decoction for febrifugous baths while the petioles themselves are eaten as diarrhoea treatment. Kunkel [51] reports that young leaves and petioles are occasionally consumed in

cooked form although they are virtually tasteless. Oudhia [52] notes that in India *E. crassipes* is used to treat goiter and that its fresh juice also used as styptic agent. The use of *E. crassipes* against gonorrhoea has been reported for Bangladesh [53]. Use of the dried biomass in handicraft has also been reported [54].

Within the framework of a programme investigating the potential of plant lipids as indicators for the origin of sedimentary organic matter in the Siak River, E Sumatra, we determined the lipid composition of *E. crassipes* in terms of hydrocarbons, fatty acids, steroid and triterpenoid compounds.

## Material and Methods

Plants were collected in March and September 2004 in the Siak River, Province Riau, Sumatra, Indonesia, upstream of the province capital Pekanbaru and the Mandau River, a major tributary to the Siak. Field observations in the Mandau showed extensive coverage of the water surface estimated at about 60 to 70 % in October 2003, being considerably less during the actual collections in 2004. In the Siak only floating mats of the plant were encountered. They originated largely from the Tapung kanan, one of the two major Siak sources.

Plants were separated into leaf, stem and roots immediately after sampling and air-dried. After additional freeze drying the plant material was ground in an agate ball mill at 200 rpm for 30 minutes. Extracts were prepared by ultrasonic extraction using solvent systems of sequentially increasing polarity: 1$^{st}$ fraction *n*-hexane, 2$^{nd}$ fraction - *n*-hexane/dichloromethane (50:50 v/v), 3$^{rd}$ - 5$^{th}$ fractions - dichloromethane/methanol (90:10 v/v) corresponding in polarity to hydrocarbons, alcohols and polar N,S,O compounds, respectively. The combined lipid extracts were rotary-evaporated to dryness and a mixture of squalane, 5α-androstanol, 5α-androstanone and erucic acid was added as internal standards. The *n*-alkanes were separated from the total extracts using a 1.0 * 20 cm glass chromatography column packed with activated silica gel (100-200 mesh). On top of the silica gel, about 10 mm anhydrous $Na_2SO_4$ was added to retain remaining water. After adding an aliquot of the total lipid extract redissolved in dichloromethane to the column, *n*-alkanes were eluted with 15 ml of hexane while polar compounds were eluted with a mixture of 40 ml dichloromethane/methanol (9:1 v/v).

Long chain *n*-alkanes (*n*-$C_{16-39}$) were analysed with a Hewlett Packard 5890 series II gas chromatograph equipped with a cold injection system (KAS 3, Gerstel), a FID detector and a J&W DB 5 capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) programmed from 60 °C to 300 °C at a rate of 6 °C/min and held at 300 °C for 30 min. Helium was used as carrier gas with a flow rate of 1.2 ml/min. Individual *n*-alkanes were identified based on the retention times of authentic standards. Concentrations were calculated by comparison to the response of the corresponding internal standard (squalane).

Polar compounds were analysed using a Agilent 5973 GC-MS System operating at 70 eV with a mass range of *m/z* 50-650 in the scan modus. The GC was equipped with a fused silica capillary column of the same specifications as described above. The carrier gas was helium. The same temperature program as above was used. Before measurement the polar compounds were derivatised to trimethylsilyl ethers by adding 50 µl of *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) to each sample. Components were identified by comparison of their mass spectra and retention times with synthetic standards or published data. The different internal standards added prior to the sample extraction were used for quantification.

A voucher specimen has been deposited at the Herbarium Universitas Andalas, Padang, Sumatra under number 97.

## Results and Discussion

*Hydrocarbons*

The *n*-alkane distributions of the different plant parts of *E. crassipes* are typical of higher terrestrial plant patterns showing high CPI values (**C**arbon **P**reference **I**ndex, [55]), reflecting an odd-numbered carbon dominance [56]. In contrast to other submerged and floating freshwater aquatic macrophytes the *n*-alkane distribution patterns maximise at *n*-$C_{31}$ in leaf and root samples, while the distribution pattern of stems maximises at *n*-$C_{27}$ (Fig. 1). Odd carbon-numbered dominant distributions of long chain *n*-alkanes, typically in the range of $C_{25}$ to $C_{35}$, are characteristic components of the epicuticular leaf waxes of higher plants [56].

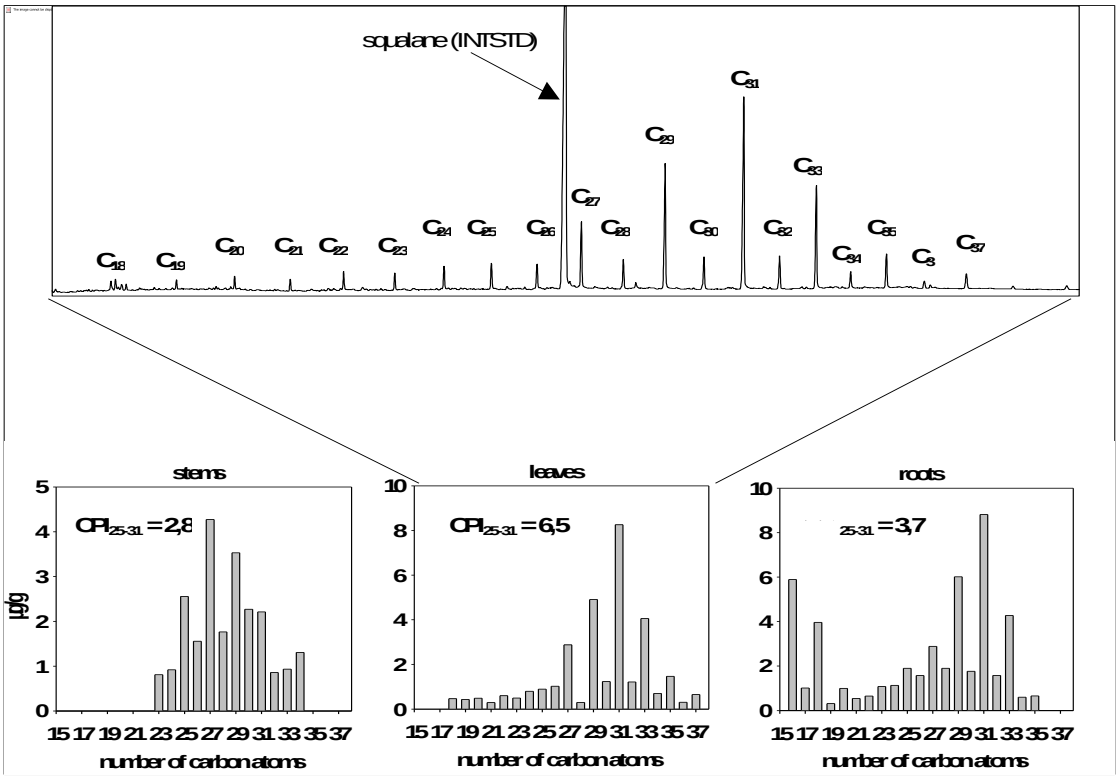


*Figure 1: GC spectrum of a hydrocarbon fraction and n-alkane distribution patterns of Eichhornia crassipes. INTSTD–internal standard).*

Studies of other submerged or floating plants such as *Najas marina*, *Potamogeton spp*., *Vallisneria gigantea*, *Elodea nuttali* and *Posidonia oceanica* indicate that the *n*-alkane distributions of these plants maximise at $C_{21}$, $C_{23}$ or $C_{25}$ [57, 58]. The shift in the *n*-alkane distribution pattern from $C_{21}$ to $C_{25}$ in the floating and submerged plants listed above to a $C_{31}$ dominated distribution pattern in *E. crassipes* could also reflect different climatic conditions. Do Amaral et al. [59] also report an alkane maximum at $C_{29}$ or $C_{31}$ in aquatic angiosperm specimens with air-exposed leaves from southern Brazil. While *N. marina*, *Potamogeton spp*., *V. gigantean*, *E. nuttali* and *P. oceanica* were sampled in Afroalpine communities from lakes at Mt. Kenya (1,820-3,500 m above sea level) and grow under moderate temperatures [58], the analysed specimens of *E. crassipes* were growing in hotter subtropical to tropical climates suggesting an adaptive response to higher ambient temperatures.

Straight chain alkanes of *Eichhornia crassipes* occur in relatively low concentrations (31, 23 and 48 µg/g dry weight (dw) - leaf, stem, root) compared to other higher plants. For instance, Maffei [60] found total alkane contents to range from 6.9 to 1860 with a mean value of 164.1 µg $g^{-1}$ fresh weight in 93 species of graminae. Similarly Conte et al. [61] report alkane contents in the $C_{25}$ to $C_{35}$ range from 263 to 11,831 µg/g dw for prairie vegetation in Alberta, Canada. The low values found for *E. crassipes* may thus be due to the aquatic habitat of *E. crassipes* in which a protection mechanism against evapotranspiration might not be necessary and a thick leaf wax coating is hence missing.

The unusual occurrence of $C_{16}$ and $C_{18}$ *n*-alkanes in the roots of the analysed plants could reflect an input from a different source or originate from oil-derived contamination. In fact, root samples collected downstream of Pekanbaru had more often a *n*-alkane distribution with CPI values of 1,5 or less suggesting a contribution from fossil fuels [62]. Tang and Lu [63] found a reduction in oil content from 150 to 4 mg/L of oil refinery wastewater within two to four days in aeration ponds although in this study no detailed analyses were carried out. Ndimele [64] also reports that *E. crassipes* significantly accumulated petroleum hydrocarbons.

*n-Fatty acids*

*n*-Fatty acids are one of the major compounds of plant epicuticular waxes usually having straight chains with an even number of carbon atoms and a wide chain length range from $C_{14}$-$C_{36}$ [65]. Straight-chain fatty acids are predominantly present in leaf material whereas roots and stems contain comparatively little of these compounds (Fig. 2).

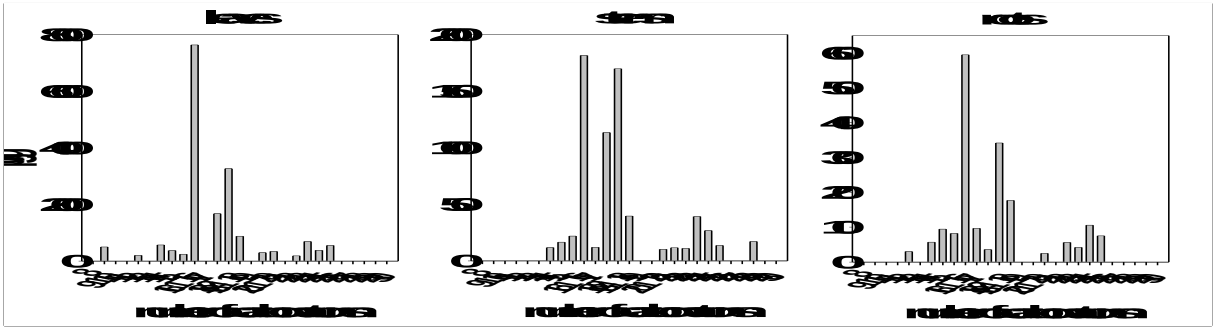

*Figure 2: n-Fatty acid distribution patterns of Eichhornia crassipes.*

All distributions show a predominance of the 16:0 saturated acid together with moderate amounts of C18:1 and C18:2 mono- and di-unsaturated fatty acids and C18:0 in the leaves and roots of the plant. These compounds are also dominant in stems of *E. crassipes*. Small amounts of long-chain saturated fatty acids were also present accounting for less than 20 % of the total fatty acids. Some samples displayed surprisingly little even over odd carbon number predominance in the higher molecular weight region (>C20). In leaves and stems of *E. crassipes* the 23:0 fatty acid was the most abundant fatty acid in this carbon number range, only the roots showed a predominance of the 24:0 homologue. The 28:0 fatty acid was only detected in stems of *E. crassipes*.

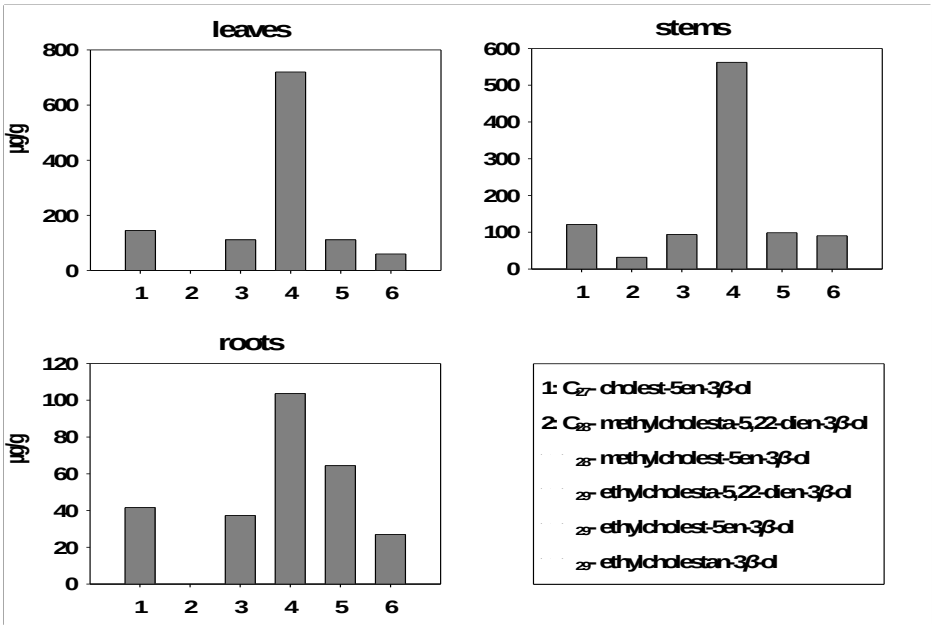


*Figure 3: Sterol distribution patterns of Eichhornia crassipes.*

*Steroids*

The distribution pattern of sterols in the different parts of *E. crassipes* are similar (Fig. 3) and clearly dominated by stigmasterol (ethylcholesta-5,22-dien-3*β*-ol, compound 4). In contrast to most other higher plants, the distribution pattern is not dominated by *β*-sitosterol (ethylcholest-5en-3*β*-ol, compound 5). In addition, *E. crassipes* contains a significant amount of cholest-5en-3*β*-ol (compound 1), a compound of widespread occurrence also found in plants from aquatic/lacustrine environments (e.g. [66, 67]. In general the total amount of steroids are highest in leaves and stems of *E. crassipes*, while the content in roots is significantly lower by a factor of ~6.

Goswami et al. [68] also reported on the dominance of stigmasterol accounting for 55.6 % of total sterols followed by *β*-sitosterol (33.4 %9 and campesterol (11.0 %). Cholesterol was not reported to be present.

Sterols with 27 to 29 carbon atoms per molecule are present in all plants and animals. Although a multitude of combinations of structural and optical isomers of C-27 to C-29 alcohols is theoretically possible, less than ten distinctively structured C-27 to C-29 sterols comprise more than 90 % (by weight) of biological sterols. C-27 and C-28 sterols are the most abundant sterols in plankton and marine invertebrates whereas C-29 sterols are the predominant sterols in higher plants and animals.

The finding of a significant relative contribution from a $C_{27}$ compound in a higher plant might be taken as an indication that this is an adaptation to the particular mode of life. However, other floating macrophytes such as e.g. *Potomageton* or *Lemna* have to be investigated to confirm this.

*α-Hydroxy-fatty acids*

A significant amount of *α*-hydroxy-fatty acids was only detectable in leaves of *E. crassipes* (Fig. 4).

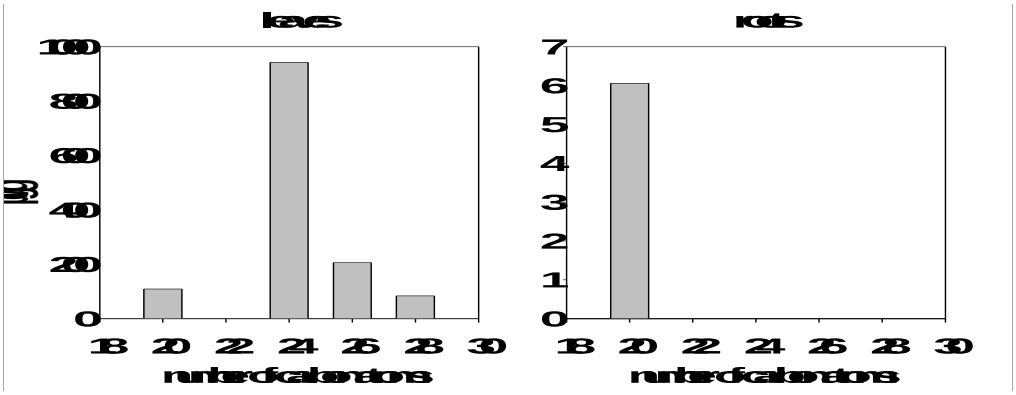


*Figure 4: α-Hydroxy-fatty acid distribution pattern in Eichhornia crassipes.*

The distribution pattern is dominated by even carbon numbered homologues with 20 to 28 carbon atoms and a high amount of the $C_{24}$ *α*-hydroxy-fatty acid in leaves of *E. crassipes* (943 µg/g). The roots contain only a minor amount of the $C_{20}$ *α*-hydroxy-fatty acid (6 µg/g), while *α*-hydroxy-fatty acids are completely absent in the stems of the plant.

*n-Alcohols*

*n*-Alcohols occur only in leaves and roots of *E. crassipes* in the range $C_{22}$-$C_{28}$ and are predominantly composed of even-carbon numbered components. Only four compounds were found, i.e. the $C_{22}$ and $C_{24}$ in leaf and $C_{22}$, $C_{24}$, $C_{26}$ and $C_{28}$ in the root (Fig. 5). The distributions are dominated by the $C_{24}$ homologue in leaves and the $C_{26}$ one in roots of *E. crassipes*.

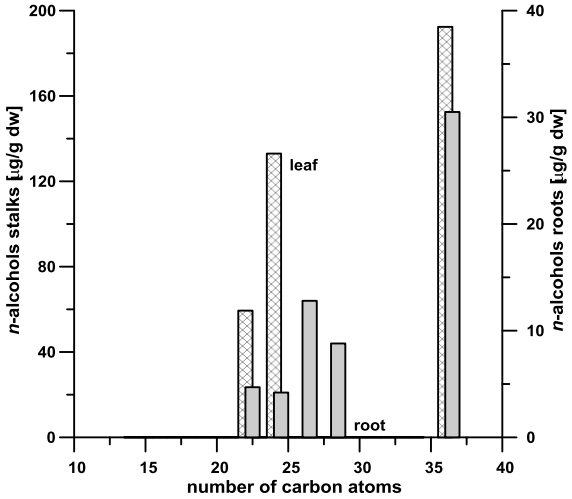


*Figure 5: n-Alcohol distribution in Eichhornia crassipes*

*Triterpenoids*

Triterpenoids are a widespread class of compounds in the plant kingdom where they occur free or as glycoconjugates [69]. Unconjugated triterpenes are often found in the epicuticular waxes of plants [70, 71] where their main function is to prevent water loss and to constitute the first defensive barrier against bacteria, fungi or insects [71]. The presence of triterpenoids has been reported previously with, however, no structural characterisation [31]. All plant parts of the analysed *E. crassipes* specimens contain significant amounts of four triterpenes with similarly high contents in the leaves and stems of the plant (Fig. 6).

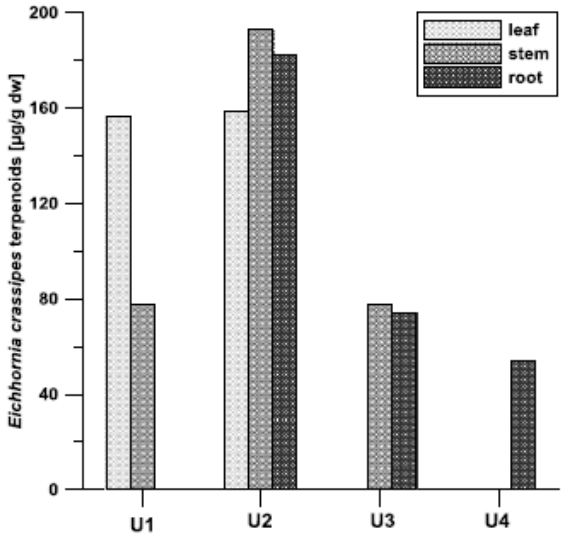


*Figure 6: Distribution patterns of unknown pentacyclic triterpenoids of Eichhornia crassipes*

The unidentified compound U2 is the only one which occurs in all parts of the plant, while U1 occurs only in leaves and stems, U3 in stems and roots and U4 in roots of *E. crassipes* (for mass spectral data see Fig. 7).

Mass spectra of a large number of triterpenoids are available (e.g. [72, 73]. The base peak and the molecular ion in mass spectra of triterpenoid classes have been found to be characteristic of the basic stereoskeleton [74]. The mass spectral fragmentation patterns of the unknown compounds U1 and U3 suggest a pentacyclic triterpenoid alcohol with 30 carbon atoms ($M^+$ 498). The fragmentation patterns of compounds U2 and U4 show significant similarities only distinguishable by a mass shift of two mass units, indicating a double bond in compound U2. Both compounds contain also 30 carbon atoms, again typical for a pentacyclic triterpenoid alcohol. The mass spectral data of all unknown compounds show an unusual fragmentation pattern which does not fit analytical reference standards and literature data of the most common triterpenoids of the oleanan, ursan, lupan or friedelan groups. Thus, the mass spectral data alone do not provide sufficient information for an unambiguous identification of the triterpenoid alcohols present in *E. crassipes*.

Nevertheless, compounds U1 to U4 appear to be unique to *E. crassipes* and have not been found in any other of the 43 plant species analysed from the river bank vegetation of the Siak (own unpubl. results). They may thus be used to elucidate whether organic matter from this floating macrophyte is incorporated into sedimentary organic matter either of the river and estuary or the adjacent coastal waters. Field observations show that water hyacinths may reach an extensive coverage (up to 70 % of the water surface in the Mandau, a tributary to the Siak) in the freshwater realms. As soon as the plant comes into contact with saltwater, however, it rapidly disappears and is no longer observed floating. According to Duke [75] the leaves show epinasty and chlorosis and eventual death in brackish waters. According to Casabianca and Laugier [76] plants are cankered at salt contents >6 g/L. Similar salinity effects were also observed in other subtropical and tropical areas such as the Bay of Paranaguá, southern Brazil, or the Ciénaga Grande de Santa Marta in the Columbian Caribbean (own field observations).

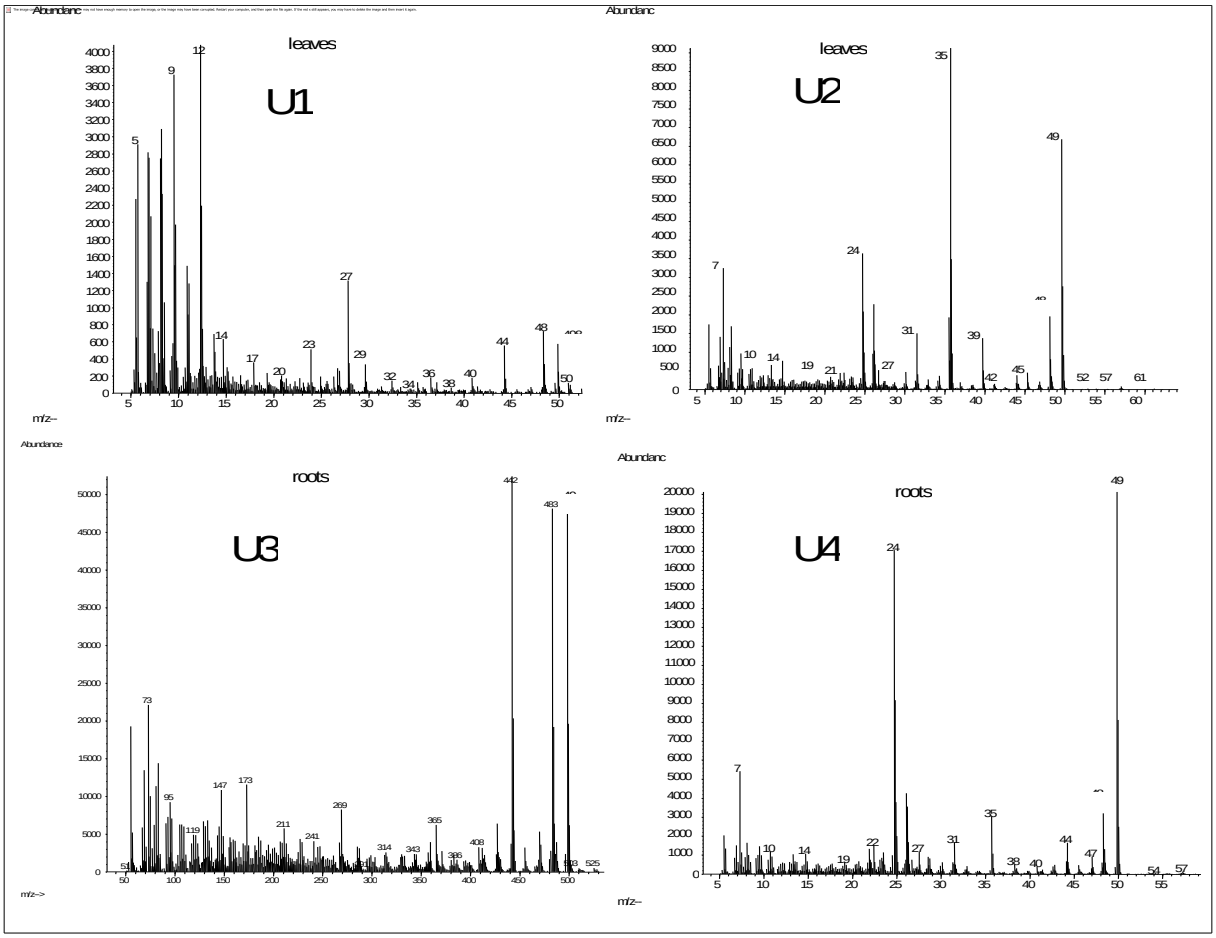


*Figure 7: Mass spectra of the unknown pentacyclic triterpenoids U1–U4.*

Due to the large amounts of biomass produced decaying *Eichhornia* might be a significant contributor to organic matter in sediments and might also fuel macro- and microheterotrophic metabolism both in the water column and in the sediment. Analysis of ten sediment samples – two from the Siak tributary Mandau, five from the Siak estuary, two from the Selat Panjang in front of the Siak estuary and one from the Malacca Strait – did not provide any evidence for the presence of the terpenoid compounds U1 to U4. This suggests that the *Eichhornia* biomass, i.e. particularly leaves and stems, becomes either rapidly disintegrated, is consumed or exported further into the Malacca Strait.

## Conclusions

The distribution patterns of extractable lipid compounds in *E. crassipes* are typical for higher terrestrial plants with some noticeable exceptions. The low absolute content of hydrocarbons is possibly a result of the particular mode of life of the species which does not necessitate the production of organic leaf coatings protecting against desiccation.

In contrast to other submerged and floating plants the *n*-alkane distribution is dominated by C-29 to C-33 compounds. In leaf material the range is even extended to C37. This suggests that the shift to higher carbon numbers might be an adaptation to the tropical environment.

The presence of significant amounts of C-27-cholest-5en-3*β*-ol allows a differentiation from higher land plants, where C27-steroids are generally absent and the distribution patterns are clearly dominated by C29 compounds.

However, to confirm whether these special traits are typical for floating macrophytes requires additional analyses of other species, both from temperate and tropical regions.

**Acknowledgements**

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