The African Journal of Plant Science and Biotechnology ©2007 Global Science Books

Physicochemical Properties of Choloform Extract of Water Hyacinth (*Eichhornia crassipes*)

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ABSTRACT

Physiochemical examinations were carried out on the chloroform extract of *Eichhornia crassipes* leaves. Preliminary phytochemical analysis of the extract revealed the presence of saponins, alkaloids, glycosides and anthraquinones in the extract. Fractionation of the extract constituents by column chromatography and thin layer chromatography yielded three distinct compounds designated I, II and III with R_f values 0.89, 0.69 and 0.49 respectively. The UV/visible spectra for compounds I, II, and III showed maximum absorbance at three-point wavelength maxima respectively. Chemical tests for the presence of alkaloid and phenolic hydroxide in the three compounds were negative. The IR absorption bands for compound I, II, and III respectively were recorded. Relating the UV/visible and IR results, the presence of unique nitro aromatic esters were revealed in compounds I, II, and III, hence their distinct R_f values.

Keywords: column chromatography, infra red spectroscopy, phytochemical analysis, thin layer chromatography, UV/visible spectroscopy

INTRODUCTION

The genus *Eichhornia*, tropical and subtropical in distribution, belongs to the family *Eichhorniacae*. Water hyacinth, *E. crassipes* (Hiram 1961), is indigenous to South America and has become a household name in several countries around the world. This is because it has spread far and wide, creating problems whenever and wherever it becomes established (Ukiwe and Ogukwe 2007). Nigeria has since 1984 become a victim of water hyacinth invasion. Its rate of growth and spread is terrific. For example, water hyacinth plant can divide into 1200 offspring in 120 days (Oke *et al*. 1988). A possible biological explanation for this behaviour lies in the fact that the plant usually grows in places with high solar energy constants, ranging from 450 to 550 W/m^2 of the land mass (Edewor 1988). Water hyacinth also has a high photosynthetic fixation efficiency (1.52%), and when compared to typical crops in Nigeria like maize (1.0%), cocoa (0.5%) and groundnut (0.29%), it can be readily appreciated why water hyacinth has such a rapid growth rate (Edewor 1988).

Water hyacinth is not new in the ecological history of man. In fact nations have been faced with the problem of having to destroy the weed, while at the same time ignoring the restraining factor that water hyacinth is almost 95% water content. Investment in water hyacinth processing is discouraged by some nations due to the huge amount of money needed and the low turn over. The possible beneficial uses of water hyacinth include: soil additives (Edewor 1988), processed fish and animal feeds (Kuzemiju 1988), pulp, paper and fibre (Udohitinah *et al*. 1988); amino acid production (Edewor 1988) and waste-water treatment (Ukiwe and Ogukwe 2007).

Extraction of active components from plants is one of the most intensive areas of natural product research today, yet the field is far from exhausted and further investigation is worthwhile. The present research investigates chloroform extracts from the leaves of water hyacinth in order to determine their phytochemical content with a view to identifying the active components and to suggest the possible structure of the pure isolated components of the leaf extracts through physiochemical means. The results will form part of an extensive database in the physiochemical characteristics of the plant, which is to be analysed side-by-side with the physiochemical characteristics of aquatic environments that promote growth of the plant.

MATERIALS AND METHODS

Plant material preparation

Fresh leaves of *Eichhornia crassipes* were collected from the Ogun River, near Ikorodu, Lagos, Nigeria. The leaves were dried to a constant weight at 50°C and ground into coarse powder and stored in an airtight moisture-free container.

Reagents

Dragendoff's reagent was prepared using the method described by Egon and Antherder (Egon 1962; Antherden 1969). Meyer's reagent and Hagger Wagner's reagents were also prepared according to a standard procedure (The Pharmaceutical Codex 1996). Fehling solution A and B were prepared according to a standard procedure (British Pharmacopoeia 1993).

Chromatography analysis

The leaf extract was subjected to column chromatography and thin layer chromatography with a solvent system using cyclohexane/acetone (9:1). The chromatographic column of dimensions 50 cm x 4 cm, with a flat, sintered base filter was cleaned with acetone and air-dried. The column was prepared using the method described by Olaniyi *et al*. (1991). The plates for thin layer chromatography (TLC) were 20 cm x 10 cm in dimension. They were prepared from a paste of silion gel (British Drug House, BDH; TLC standard grade) with gypsum binder and fluorescent indicator by using a Dasaga Beldelberg spreader. The thickness of the plates was 0.5 mm and 1 mm (for preparative TLC). The plates were activated by drying in an oven for 2 hrs at 110°C.

Received: 27 June, 2007. Accepted: 20 September, 2007.

Short Communication

Spectrophotometric analysis

The distinct compounds isolated via chromatographic separations were each dissolved in methanol and the resulting solution analysed using UV/visible (Pye-unicam 8700) and infrared (Pye-unicam 9706) spectrophotometry.

Phytochemical analysis

Phytochemical analysis for alkaloids, saponins, tannins, phlobatannins and glycosides were performed on the isolated compounds as well as the crude extract as reported elsewhere (Sofowora 1984).

RESULTS AND DISCUSSION

Chromatographic analysis

Three compounds designated I, II, and III respectively could be isolated out of the eight different separations exhibited on the TLC plates. The three compounds showed no presence of alkaloids and phenolic-OH unlike the crude extract, which contained alkaloids, glycosides, saponins and anthraquinones. This suggests that the main chemical components of *E. crassipes* have little or no medicinal and essence values. Hence the solution of a biological problem in a plant like water hyacinth depends on identifying a range of complex chemical structures, which may be available for study in microgram amounts (Harbone 1984).

Phytochemical analysis

Several authors reported on the analgesic, antipyretic and antitussive properties of alkaloids (Olaniyi 1989) as well as cathartic properties of anthraquinones (Skim 1967). The search in plants for saponins has been stimulated by the need for a readily accessible source of therapeutic use such as cortisones, contraceptive estrogens (Skim 1967). The results obtained in the study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of *E. crassipes* as an analgesic and cathartic and thus lead to potent steroid hormones.

Spectrophotometric analysis

The isolated compounds were subjected to ultra violet (UV)/ visible spectrophotometric analysis using a Pye-unicam 8700 UV/visible spectrophotometer. All the compounds gave three distinct absorption maxima as illustrated in **Table 1**. For compound I, the absorption peaks around 233.1 nm may suggest the presence of an aromatic nucleus (Stanley *et al*. 1991). The absorption at 325.0 nm may be due to chromophone or anxochrome present in the compound.

The absorption peaks in compound II suggest a structural relationship or the same chromophoric system (Stanley *et al*. 1991) between compounds I and II.

The absorption peak at 400.2 nm in compound III suggests the presence of a strong anxochrome or extensive conjugation (Stanley *et al*. 1991).

Conjugation increases the absorption wavelength by decreasing the energy difference between ground and excited states. UV/visible spectra arise only from the ground or conjugated compound that absorbs radiation (the chromophore). They are little influenced by the rest of the molecular skeleton. Therefore different compounds having the same chromophoric system show a very similar UV/visible spectra (Stanley *et al*. 1991). The absorption bands were observed to be broad. It may be due to the transition to a higher electronic level by the molecules, coupled with the vibrations and rotations.

The IR spectra of the three compounds were determined on a Pye-unicam 9706, IR spectrophotometer using chloroform as solvent. The spectrophotometer used in the operations worked between 4000 cm^{-1} and 200 cm^{-1}. The observed bands for the various compounds are also shown in **Table 1**.

Table1 Retention factor, absorption peaks and absorption bands of three compounds detected.

Compound	R_f	λ_{max} (nm)	V (cm^{-1})
I	0.89	215.2, 233.1, 325.0	1400-1460,1740, 2920
II	0.69	212.8, 232.7, 324.9	500-750,1160, 1450, 1750, 2920
III	0.47	212.3, 233.6, 400.2	1460, 1730, 2920

Compound I showed bands around 1400–1460 cm^{-1} which may be due to the presence of a nitro-compound that was relatively unaffected by conjugation (Stanley *et al*. 1991). Absorption band around 1740 cm^{-1} suggested the presence of carbonyl compound. Hence the absorption range of a carbonyl group is between 1650 and 1750 cm^{-1}. An absorption band around 2920 cm^{-1} might suggest the presence a C-H (saturated bonds) or over lap of the solvent chloroform. The Infra Red Absorption peaks in compound III suggest a structural relationship between compound I and compound III. Compound II showed bands around 500–750 cm^{-1} due to single bond stretching. This may be due to the presence of C-X (halo alkanes). Also band at 1160 cm^{-1} may be due to the presence of C-O-C (Single bond stretching). Absorption band around 1450 cm^{-1} may be due to the presence of nitro-compound. Absorption band at 1750 cm^{-1} may reveal the presence of carbonyl group. Absorption band at 2920 cm^{-1} may be due to C-H (saturated bonds) compound or the interference of the solvent chloroform.

It may be inferred from the table above that the UV absorption wavelength at 233.1 nm, 232.7 nm, and 233.6 nm for compounds I, II and III, respectively may suggest the presence of aromatic carbonyls with ester linkage using the Woodward-Feiser Rules (Stanley 1987). The ester linkage may have become established by the IR values of compound I, 1750 cm^{-1} for compound II, 1750 cm^{-1} and for compound III, 1730 cm^{-1}, respectively. Since in each case the attachment is due to an aromatic ring (Stanley 1987).

The presence of conjugation in UV readings for the three compounds were evident. These were further noted in the IR readings of the three compounds, which showed the presence of nitro compounds that were relatively unaffected by conjugation. The nitro groups may be attached to the aromatic ring. It may be that the differences in R_f values of the three compounds are because of the differences in branch chains attached to the ester linkage.

The student's *t*-test was applied to analyse the absorption peaks in compounds I, II and III, respectively. The t-value calculated for compound I was 0.08, while that for compounds II and III were 0.78 and 1.1, respectively. At 2 degrees of freedom and at a 5% and 1% confidence level, these values were not significant and hence there is no particular bias in the analytical procedure.

The plant has been reported to be an efficient remover of very low concentration of metal ions and toxic organic molecules from aqueous medium (Rahman *et al*. 1988). Silver, the treasured metal in jewelry and fine flatware can be recovered by water hyacinth biomass. This may be possible due to the presence o nitro groups, which easily solubilize silver ions when the nitro groups have been reduced to amine group. Hence, there may be hope for environment polluted with solutions used in developing, fixing and washing photographic films, which contain a lot of silver ions.

REFERENCES

Ajibola AO (1989) *Essential Medicinal Chemistry* (Student Edn), Shaneson C. I. Limited, Ibadan, pp 150-151

Ajibola AO, Francis OO (1991) *Experimental Pharmaceutical Chemistry*, Odu's Printing Company, Eleyele, Ibadan, pp 172-173

Antherden LM (1969) *Textbook of Pharmaceutical Chemistry* (8th Edn), Oxford University Press, London, pp 813-814

British Pharmacopoeia (Volume II) (1993) Cambridge University Press, Cambridge, pp 557-570

Edewor JC (1988) Developing water hyacinth from menace status to national profitability level. *Proceedings of an International Workshop on Water Hyacinth*, Lagos, Nigeria, pp 175-176

Egon S (1962) *TLC, A Laboratory Hand-book*, Academic Press, New York, pp 8-10

Haborne JB (1984) *Phytochemical Methods: A Guide to Modern Techniques to Plant Analysis*, Academic Press, London, pp 124-130

Hiram W (1961) Harmful aquatic plants in Africa and Madagascar. Joint CCTA/CSA, Project No, 14, pp 7-8

Kusemiju K (1988) Aspect of the ecology of water hyacinth, *Eichhornia crassipes* (Mart Solms) in the Lagos Lagoon system. *Proceedings of an International workshop held in Lagos*, pp 80

Morrison RT, Boyd RN (1983) *Organic Chemistry* (4th Edn), Allyn and Bacon Inc., Massachusetts, pp 168-169

Oke OL, Imevbore AMA, Farri AT (1988) Water hyacinth, menace and resource. *Proceedings of an International Workshop on Water Hyacinth*, Lagos, Nigeria, pp 12-13

Rahman AT, Akueshi CO, Umoru AI (1988) Possible application of symbiotic growth of water hyacinth, (*Eichhornia crassipes*) and *Azzolla africana*. *Proceedings of an International Workshop on Water Hyacinth*, Lagos, Nigeria, pp 204-205

Skim SK (1971) *Medicinal Plant Glycosides*, University of Toronto Press, Toronto, pp 20-25

Sofowora A (1984) *Medicinal Plants and Traditional Medicine in Africa*, John Wiley and Sons Ltd., Pitman Press Limited, Bath, Avon, pp 142-145

Stanley HP (1987) *Organic Chemistry* (International Edn), McGraw-Hill, New York, pp 1097-1117

Stanley HP, Hendrichson JB, Cram DI, Hammond GS (1991) Organic Chemistry (4th Edn), McGraw-Hill, New York, 585 pp

The Pharmaceutical Codex 11th Edn (1979) The Pharmaceutical Press, London, pp 1005-1006

Udohitinah JS, Koleoso OA (1988) Utilization of water hyacinth (*Eichhornia Crassipes*) for pulp and paper production. *Proceedings of an International Workshop on Water Hyacinth*, Lagos, Nigeria, pp 189-191

Ukiwe LN, Ogukwe CE (2007) Ion uptake by water hycinth (*Eichhornia crassipes*) on the lower reaches of the Niger River, Nigeria. *The African Journal of Plant Science and Biotechnology* **1**, 36-39