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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Chemical Composition and Phytochemical Screening of the Leaves of *Hymenocardia ulmoides* and *Vitex ferruginea*

Marcel Andzouana¹ and Jean Bienvenu Mombouli²

¹Department of Chemistry, Faculty of Sciences,

²Department of Agronomy, Institute of Rural Development,
Marien Ngouabi University, P.O. Box 69, Republic of Congo-Brazzaville

Abstract: The chemical and phytochemical analysis of *Hymenocardia ulmoides* and *Vitex ferruginea* leaves, used as medicinal plants in Congo-Brazzaville, was carried out. The proximate analysis revealed that the moisture, proteins, fat, fatty acid, carbohydrate and ash content of the leaves were respectively 67.20; 15.12; 2.75; 2.20; 8.57 and 3.29% for *H. ulmoides*. For *V. ferruginea* leaves, these content values were 55.41; 22.87; 1.38; 1.10; 4.53 and 6.84%. The energy values of the samples were 504.48 and 516.80 kJ/100 g for *H. ulmoides* and *V. ferruginea* respectively. In *H. ulmoides* leaves, potassium (1.33%) was the major element, followed by calcium (0.75), phosphorus (0.55) and magnesium (0.33). The most abundant minerals detected in *V. ferruginea* leaves were potassium (4.39%), calcium (2.05%), magnesium (1.45%), aluminium (0.13) and phosphorus (0.91). Sodium, manganese, iron and aluminium in the case of *H. ulmoides* were detected as trace elements. The phytochemical screening of the leaf extracts revealed the presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids in ethanol-water extracts of both plant samples. In methanol-chloroform extracts of *H. ulmoides* leaves, glycosides and saponins were not detected, while flavonoids, saponins and tannins were not found in *V. ferruginea*. Anthraquinones were absent in all the extracts of both plants. The plant leaves may be important in nutrition as leafy vegetables and in ethnomedicine as a remedy for their nutrient and non-nutrient bioactive compounds.

Key words: *Hymenocardia ulmoides*, *Vitex ferruginea*, phytochemical, chemical analysis

INTRODUCTION

Herbal medicine has been practised worldwide (Ameyaw *et al.*, 2005) and a vast store of knowledge concerning the therapeutic properties of different plants has been accumulated (Felix *et al.*, 2009). Approximately 10% of these plants are used either as food or for medical purposes (Borris, 1996). Medicinal plants have been found to contain bioactive compounds called phytochemicals (Fasuyi, 2006) that can protect humans against diseases (Kumar *et al.*, 2009). Among these medicinal plants, *Hymenocardia ulmoides* Oliv. (Euphorbiaceae) and *Vitex ferruginea* Schum and Thonn (Lamiaceae) are used in folk medicine and in nutrition by the people of Congo-Brazzaville.

H. ulmoides is a tree up to 20 m in height, with a spreading crown or a shrub branched from the base, sometimes scandent (Radcliffe-Smith, 1996). The plant is found in sandy soils, riverine fringes, coastal forests, high rainfall woodland, on rocky out-crops and in forested gullies. It is known from Cameroon to the Sudan, south to Angola and Kwazulu Natal, South Africa, (Hyde and Wursten, 2011a,b) and also in Zaire, Gabon and Congo (Bouquet, 1969). Usually, *H. ulmoides* is a vital component of dietary food. Its leaves are used as an ingredient of soup or sauce by the rural people, e.g. the

Bangangulu from the District of Gamboma, in the northern area of Congo. This agreeable and tasty dietary component is taken in the form of acidic leafy vegetables, such as hibiscus varieties. In herbal medicine, the leaf decoction is known in the treatment of diabetes, hypertension and is used per os to treat neuralgia, dysuria and asthma. It is also taken orally as a mixture with *Nauclea latifolia* to treat angina (Haxaire, 1979; Bouquet, 1969). The leaf, the root and root bark decoction are used per os against blenorragia (Bouquet, 1969), in the treatment of respiratory afflictions, diarrhoea, aches, anaemia and for sore throats. The leaf infusion, as with the decoction, is taken orally as a stimulating treatment for intestinal pains (Haxaire, 1979; Bemba and Remacle, 1992).

V. ferruginea is a scrambling multi-stemmed shrub 15m in height with a grey to brown bark, finely striated and with leaves 3-5 foliolate, of which the lower-most leaflets are often undeveloped. The plant is found on sandy soils, in deciduous woodland and along the banks of streams, often among rocks. It has been found in tropical Africa, southwards to Kwazulu-Natal, South Africa (Hyde and Wursten, 2011a,b) and from Guinea to Congo (Bouquet, 1969). Commonly, the leaf decoction of *V. ferruginea* is taken as tea, drunk alone or as a

mixture with milk, or the leaf decoction of anisophyllea quangensis, afromomum stipulatum, during convalescence. Thus, in Congo-Brazzaville it is a so-called "vitamin" for the above uses. In herbal medicine, the water decoction of the bark is prescribed in the treatment of skin dermatoses (Bouquet, 1969). The aim of this study is to investigate the chemical and phytochemical composition of the leaves of *H. ulmoides* and *V. ferruginea* in order to determine their nutritive and medicinal value.

MATERIALS AND METHODS

Plant materials: The experimental leaves of *H. ulmoides* and *V. ferruginea* were collected from Makabandilou (North-Brazzaville) on 14th March, 2010. The plant materials were identified and authenticated by Nkouka Saminou from the National Herbarium of the Vegetal Research Centre of Brazzaville (ex-OROSTOM-Congo), where voucher specimens are conserved. The leaves were air-dried for 15 days and milled into a powder. The powder was stored under dry conditions before analysis.

Chemical analysis

Proximate analysis: The moisture contents were determined by drying at 105°C in an oven, until a constant weight was reached. For ash determination, the plant samples were weighed and converted to dry ash in a muffle furnace at 450°C and at 550°C for incineration. The Kjeldahl method was used for crude protein determination. Total fat contents were determined by extraction with hexane, using a soxhlet apparatus. Carbohydrates were determined by the difference of the sum of all the proximate compositions from 100%. Energy values were obtained by multiplying the carbohydrate, protein and fat by the Atwater conversion factors of 17, 17 and 37 respectively (Kilgour, 1987). The crude fat was converted into fatty acid by multiplying with a conversion factor of 0.80 (Greenfield and Southgate, 2000).

Mineral analysis: Mineral analyses were carried out according to Martin-Prevel *et al.* (1984).

Elemental analyses were carried out using an atomic absorption spectrophotometer and a flame photometer to determine calcium, sodium, potassium, magnesium and manganese content. Aluminium, iron and phosphorus were determined colorimetrically. The concentration of each element in the sample was calculated on a dry matter basis.

Preparation of extracts: The extraction of active principles was carried out in the solvent mixture (methanol-chloroform and ethanol-water) using a percolation method, according to the procedures described by Harborne (1973) and Biyiti *et al.* (1988). 20

g of an air-dried, powdered sample were weighed and transferred into a beaker. 200 ml and 300 ml of a methanol-chloroform (1:1) and ethanol-water (1:2) mixture respectively were added and after agitation were allowed to extract at laboratory temperature for 72h. The mixture was then filtered and the filtrate evaporated to dryness on a boiling water bath. The extracts' yields were 4.90% and 6.50% in the methanol-chloroform (CH₃OH/CHCl₃) mixture and 10.70% and 13.80% in the ethanol-water (C₂H₅OH/H₂O) mixture for *H. ulmoides* and *V. ferruginea* leaves respectively.

Preliminary phytochemical screening: The solvent extracts of *H. ulmoides* and *V. ferruginea* were dissolved in each of their own mother solvents until total dissolution. The extracts thus obtained were subjected to qualitative analysis following the methods described by Trease and Evans (1989); Harborne (1998) and Kokate (2001). Phytochemical analysis was conducted to determine the presence of Alkaloids, Anthraquinones, Flavonoids, Glycosides, Saponins, Steroids, Tannins and Triterpenoids.

Screening procedure

Alkaloid determination: 5 ml of each extract was added to 2 ml of dilute HCl. To this acidic medium, a few drops of Wagner reagent were added. An orange or red precipitate produced immediately in the extract indicated the presence of alkaloids.

Test for anthraquinones: 5ml of each extract solution was hydrolysed with diluted concentrated H₂SO₄, extracted with benzene. 1 ml of dilute ammonia was added to the acidic solution. A rose pink coloration suggested the positive response for anthraquinones.

Test for flavonoids: To 1 ml of each extract a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the extract solution which became colourless on addition of a few drops of dilute acid, indicating the presence of flavonoids.

Test for glycosides: 5 ml of each extract was dissolved in 2 ml of chloroform. Dilute H₂SO₄ was added to form a layer and the colour at the interface was recorded. A brown ring at the interface is characteristic of de-oxy sugars in cardiac glycosides (Trease and Evans, 1989).

Test for saponins: The extracts were diluted with 20 ml of distilled water in a test tube and shaken vigorously for 15 mn. The formation of 1cm of a persistent foam layer showed the presence of saponins.

Test for steroids: 1 ml of each extract was dissolved in 10 ml of chloroform and an equal volume of

concentrated sulphuric acid was added down the side of the test tube. The upper layer turned red and the sulphuric acid layer turned yellow, with a green fluorescence.

Test for tannins: To 5 ml of each extract a few drops of 1% lead acetate were added. A yellow precipitate was formed, indicating the presence of tannins.

Test for triterpenoids: 10 ml of each extract was dissolved in 1 ml of chloroform. 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated H₂SO₄. The formation of a reddish violet colour indicates the presence of triterpenoids.

RESULTS

Table 1 summarizes the proximate composition of the powdered *H. ulmoides* and *V. ferruginea* leaves. The results revealed high moisture contents of 67.20% and 55.41% for the leaves of *H. ulmoides* and *V. ferruginea* respectively. The protein values of 15.12% and 22.87% in both samples were relatively high. The results showed that the fat (2.75% and 1.38%), fatty acid (2.20% and 1.10%) and ash contents (3.29% and 6.84%) in these leaves were low.

The carbohydrate content of the samples was quite low (8.57% and 4.53% respectively for *H. ulmoides* and *V. ferruginea*).

The caloric values of 504.48 and 516.80 KJ/100 g calculated for *H. ulmoides* and *V. ferruginea* leaves respectively were significantly high.

The mineral composition is presented in Table 2. The minerals detected in both plant leaves were calcium, magnesium, potassium, sodium, manganese, iron, aluminium and phosphorus. Analysis of the leaves showed that potassium was the most abundant mineral (1.33%) in *H. ulmoides* leaves, followed by calcium (0.75%), phosphorus (0.55%) and magnesium (0.33%), which were detected in very low concentrations. Potassium (4.39%), calcium (2.05%) and magnesium (1.45%) were the most abundant minerals detected in *V. ferruginea* leaves. Phosphorus (0.90%) and aluminium (0.13%) were detected in very low amounts. However sodium, manganese, iron and also aluminium (in the case of *H. ulmoides*) were detected as trace elements (0.01%-0.04%) in both analyzed samples. The results showed that *H. ulmoides* can be ranked as a poor mineral plant due to the low level of elemental content of its leaves, while *V. ferruginea* leaves showed relatively high amounts of magnesium and calcium.

The results of the phytochemical screening (Table 3) showed the presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids in an ethanol-water extract of *H. ulmoides* and *V. ferruginea* leaves. In a methanol-chloroform extract of *H. ulmoides* leaves, glycosides and saponins

Table 1: Proximate composition of *H. ulmoides* and *V. ferruginea* leaves

Parameters	Percentage composition (%)	
	<i>H. ulmoides</i>	<i>V. ferruginea</i>
Moisture	67.20	55.41
Total fat	2.75	1.38
Crude proteins	15.12	22.87
Carbohydrate	8.57	4.53
Total ash	3.29	6.84
Fatty acid	2.20	1.10
Energy (Kj/100 g)	504.48	516.80

Table 2: Mineral composition of the plant samples

Element	Content (%)	
	<i>H. ulmoides</i>	<i>V. ferruginea</i>
Calcium	0.75	2.05
Magnesium	0.33	1.45
Potassium	1.33	4.39
Sodium	0.04	0.01
Manganese	0.01	0.01
Iron	0.03	0.02
Aluminium	0.03	0.13
Phosphorus	0.55	0.91

Table 3: Phytochemical analysis of *H. ulmoides* and *V. ferruginea* leaves

Phytochemical	<i>H. ulmoides</i>		<i>V. ferruginea</i>	
	CH ₃ OH/ CHCl ₃	C ₂ H ₅ OH/ H ₂ O	CH ₃ OH/ CHCl ₃	C ₂ H ₅ OH/ H ₂ O
Alkaloids	+	+	+	+
Anthraquinones	-	-	-	-
Flavonoids	+	+	-	+
Glycosides	-	+	+	+
Saponins	-	+	-	+
Steroids	+	+	+	+
Tannins	+	+	-	+
Triterpenoids	+	+	+	+

+ = Presence, - = Absence

were not detected, while flavonoids, saponins and tannins were not found in the *V. ferruginea* extract. Furthermore, the absence of anthraquinones was noticed in both extracts of the two leaves.

DISCUSSION

The moisture content of 67.20% recorded in the present study for *H. ulmoides* was within the range for fruits and vegetables (68-83 g/100 g) (FAO, 1968). That for *V. ferruginea* (55.41%) was found to be slightly lower than this range. High moisture content values were also recorded for the leaves of the *H. indigo* (51.00%) (Gafar *et al.*, 2011) and some leafy vegetables ranged from 76-93.4% (Mensah *et al.*, 2008). The moisture values of the studied leaves were higher than those reported for the leaves of *C. petiolata* (6.82%) (Omoyeni and Aluko, 2010), *M. oleifera* (5.9%) (Yameogo *et al.*, 2011) and some edible fruits and seeds ranging between 8.82% and 12.66% (Dike, 2010).

The high moisture percentage of the leaves indicated that they would probably not be susceptible to microbial attack during a storage period (Ogungbenle, 2006).

The protein content of *H. ulmoides* leaves (15.12%) fell within the range of 14.69% to 15.05% for *L. cupanioides* and *G. africanum*, while that of *V. ferruginea* (22.87%) was comparable to the value of 21.70% obtained for *V. amygdalina* (Dike, 2010). The protein values recorded for *C. zenkeri* (20.53%) (Ujowundu *et al.*, 2010), *S. rebaudiana* (20.42%) (Manish and Subhash, 2006) and *C. petiolata* (20.03%) leaves, (Omoyeni and Aluko, 2010) were higher when compared with those of *H. ulmoides*, recorded in the present study, but lower than the value of *V. ferruginea* leaves. All these values recorded for both the plant leaves studied were lower than those of *P. mildbraedii* (26.45%) (Akinyeye *et al.*, 2010) and *M. oleifera* (27.2%) (Yameogo *et al.*, 2011). However, they were found to be higher than the protein contents recorded for *T. triangulare* (2.406%), *T. occidentalis* (4.20%) (Dike, 2010) and some leafy vegetables, such as *B. rubra*, *C. argentea* and *O. gratissimum*, which ranged from 1.60-4.70% (Mensah *et al.*, 2008).

As reported by Ramirez-Mares *et al.* (2010), for the *Cyrtocarpa procera* pulp (1.61%) the protein value of 15.12% and 22.87% recorded for both plant samples will satisfy the daily requirement of an adult man of 70 kg (0.8 g/kg body weight per day). The high protein content suggests that the plants are a potential source of protein and could also be used as protein supplements in low protein diets.

The low fat contents of 2.75% and 1.38% in *H. ulmoides* and *V. ferruginea* leaves respectively were similar to the content recorded for the leaves of *V. amygdalina* (2.74%), *A. digitata* (1.15%) (Dike, 2010) and *C. zenkeri* (2.27%) (Ujowundu *et al.*, 2010). These fat values were higher than those recorded for *T. triangulare* (0.40%) and *T. occidentalis* (0.68%) (Dike, 2010). In comparison, a high fat content was recorded for the leaves of *M. oleifera* (17.1%) (Yameogo *et al.*, 2011) and for seed kernels (48.61%) (Lohlum *et al.*, 2010).

The fat content showed that these plants can be considered as poor oil species and therefore could not be used as a source oil for industrial or domestic purposes (Manish and Subhash, 2006; Akinyeye *et al.*, 2010).

The low values of carbohydrate content (8.57% and 4.53%) recorded in the present study were similar to those recorded for leafy vegetables such as *A. cruentus* (7.0 g/100 g) and *T. triangulare* (4.4 g/100 g), but slightly higher than that of *O. gratissimum* (3.2 g/100 g), (Mensah *et al.*, 2008). These values were lower than those reported by Mensah *et al.* (2008) for some vegetables like *V. amygdalina* (47.9 g/100 g), Ujowundu *et al.* (2010) for *O. viridis* (48.02%) and *C. zenkeri* (65.72%), Omoyeni and Aluko (2010) for *C. petiolata* (47.31%) and Gafar *et al.* (2011) for *I. astragalina* (75.94%).

It is known that carbohydrates contribute the greatest quota of energy required by man and animal (Oko and Onyekwere, 2010). The low fat and carbohydrate contents of the samples recorded in the present study suggested a low contribution of these nutrients to the energetic values. However the high energetic values of 504.48 and 516.80 KJ/100 g calculated for *H. ulmoides* and *V. ferruginea* leaves respectively are mainly linked to the contribution of their high protein contents.

The low ash values of these leaves (3.29% and 6.84%) reflect their low mineral and high organic contents. These values are comparable to those recorded by Dike (2010) for the seeds and fruits of *Carpobolbia lutea* (3.74%), *Canarium schweinfurthii* (6.12%) and *Garcinia kola* (6.52%). However, they were lower than those reported for *P. mildbraedii* (20.63%) (Akinyeye *et al.*, 2010) and *T. triangulare* (20.05%), (Omoyeni and Aluko, 2010).

The presence of potassium, calcium and magnesium as the major elements in the plant leaves correlates with earlier reports on the leaves of *M. oleifera* (Yameogo *et al.*, 2011) and *H. indigo* (Gafar *et al.*, 2011). The percentages of potassium and calcium recorded in the present study were lower than those reported for *S. rebaudiana* (potassium 2.51%; calcium 1.55%) (Manish and Subhash, 2006), while that of phosphorus was higher than the 0.35% of *S. rebaudiana*. However, the magnesium content (0.50%) was lower than the value found in *V. ferruginea* leaves (1.45%), but slightly higher than the 0.33% recorded in *H. ulmoides* leaves. Besides potassium, calcium, magnesium, recorded as the major elements in the present study, sodium and zinc were detected in high amounts in *H. opposita*, *L. camara* and *C. citratus* (except phosphorus) (Ojo and Ajayi, 2009). Contrary to the present results, Omoyeni and Aluko (2010) reported that the most abundant minerals of *C. petiolata* leaves were phosphorus and zinc, followed by calcium and magnesium. This trend was also observed when comparing the present results with those reported for *P. mildbraedii* leaves (Akinyeye *et al.*, 2010), which revealed the presence of phosphorus, sodium, calcium and zinc as the major elements. Dike (2010) recorded a high amount of calcium and phosphorus, followed by magnesium and potassium in the leaves of *Spondias mombin* and *Dennettia tripetata*. The variation of the mineral composition, especially the quantitative difference observed for each species when comparing the present results with earlier reports, could be linked to the specificity and the nature of the leaves.

Both samples could be used in a human diet to supply the required mineral elements, though the amounts of these minerals that they contain are too low for the full amount needed in the management of human health. Minerals are known to play important metabolic and physiologic roles in living systems (Enechi and Odonwodo, 2003). The presence of minerals in

supplementary diets is necessary to avoid metal deficiency syndrome, like rickets and the clarification of bones (Alli, 2009).

A qualitative analysis of solvent extracts showed that most phytochemicals are detected in an ethanol-water extract of both leaves, compared to a methanol-chloroform extract. In a methanol-chloroform extract of *H. ulmoides* leaves, glycosides and saponins were not detected in the present study. Similarly, Sivasankari *et al.* (2010) reported the absence of glycosides in a chloroform extract of *C. pulcherrima*, while saponins were not found in methanol and water extracts. They were absent in all the solvent extracts (ethanol, methanol, water) of *C. bonduc*. Glycosides were also not detected in *C. sinensis*, *A. anthelmintica* (Mariita *et al.*, 2010) and *T. triangulare* (Aja *et al.*, 2010). Mensah *et al.* (2008) reported the absence of saponins in *T. occidentalis*, *C. argentea* and *G. africana*. They were not found in *E. scarlatina*, *S. persica*, *P. dawei* or *A. friesiorum* (Mariita *et al.*, 2010). On the other hand, the results of the present study revealed that flavonoids, saponins and tannins were not detected in a methanol-chloroform extract of *V. ferruginea* leaves. The absence of flavonoids correlated with the observations of Mariita *et al.* (2010) for *A. horrida*. Analogically, the absence of tannins was observed in *V. amygdalina*, *G. latifolium* (Mensah *et al.*, 2008) and *S. persica* (Mariita *et al.*, 2010). Moreover, cardiac glycosides were not found in *H. opposita* and *L. camara*, but saponins were present. Flavonoids and saponins were not found in *C. citratus*, while tannins were present (Ojo and Ajayi, 2009). As observed in the present study for all the extracts of both plant samples, anthraquinones were completely absent in all the screened extracts of *C. pulcherrima* and *C. bonduc* (Sivasankari *et al.*, 2010). The absence of certain phytochemicals observed in this study suggested that they are present in undetectable amounts in the sample, or this is probably due to their low solubility in organic solvents. Phytochemical components are responsible for both pharmacological and toxic activities in plants (Ibrahim *et al.*, 2000). They have been reported to be responsible for protecting them (Smith and Eyzaguine, 2007; Kumar *et al.*, 2009) and for biological properties (Seenivasan *et al.*, 2006). They are used for therapeutic purposes to cure various diseases and to heal injuries (Okwu and Josiah, 2006). The secondary metabolites have been associated with numerous physiological activities in mammalian cells in various studies (Mishra *et al.*, 2009). The variability of the content observed for each nutrient and non-nutrient component analyzed, in comparison with earlier reports, could be attributed to the variability in geographical location of the plants, the availability of soil nutrients, as well as species differences (Oko and Onyekwere, 2010).

Conclusion: The study revealed that each sample contains nutrients and minerals important in human

diet. The phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids in an ethanol-water extract of both samples. In a methanol-chloroform extract, glycosides and saponins were not detected in the case of *H. ulmoides*, while flavonoids, saponins and tannins were absent in *V. ferruginea*. The plant leaves are a potential source of protein, energy, minerals and phytochemicals. They could be used in a human diet to supply the required minerals and proteins in poor diets with low nutritional value for adults and infants. The presence of phytochemicals with various pharmacological and biological properties in the leaves determines the medicinal value of the plants as a useful source of drugs in ethnomedicine. The presence of nutrient in their leaves contributes, therefore, to the nutritional value of *H. ulmoides* and *V. ferruginea*.

This study provides some knowledge of the biochemical and phytotherapeutic potential of the leaves that may be needed for scientists and researchers.

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