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ULTRAVIOLET-VISIBLE SPECTROSCOPIC EVALUATION OF COMPLEXATION EQUILIBRIA OF SOME COMPLEXES OF *LORANTHUS MICRANTHUS* LINN PLANT PARASITIC ON *KOLA ACUMINATA*

***I. C . Uzochukwu and P. O. Osadebe[2]**

[1]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*

[2]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.*

Abstract

Some complexes of crude methanol extract of *Loranthus micranthus* Linn parasitic on *Kola acuminata* were evaluated using ultraviolet-visible absorption spectrophotometry. The crude methanol extract was complexed with ferric chloride solution, borate mixture, methanolic aluminum nitrate and aluminum chloride solutions respectively and the absorption spectra of the resulting complexes obtained using UV-Vis spectrophotometer. The numbers of complexation equilibria of the formed complexes were determined between the pH of 1 and 12. The formed complexes, except the borate complex, showed bathochromic shift in their absorption spectra. While the borate and aluminum chloride complexes had two inflections each, the ferric chloride and methanolic aluminum nitrate complexes had three inflections and one inflection respectively in the pH-absorbance graph. The use of methanolic aluminum nitrate as a complexing agent is recommended for the development of UV-Vis spectrophotometric assay method for the constituents, formulations or extracts of *L. micranthus* leaves.

Keywords: *Loranthus micranthus*; complexation; phytomedicine; ultraviolet-visible spectrophotometry; methanolic, aluminum nitrate.

INTRODUCTION

Loranthus micranthus Linn (African mistletoe) is a plant traditionally employed in the management of diabetes mellitus and respiratory infections. The safety, antidiabetic and antimicrobial activities of the leaves have been scientifically demonstrated (Osadebe *et al.*, 2004; Osadebe and Ukwueze, 2004; Osadebe and Akabogu, 2005). The formulation and commercialisation of the leaves of *L. micranthus* or its extracts will contribute to better management of diabetes mellitus, create employment as well as generate income for the indigenous communities. The formulation and commercialization of *L. micranthus* is hampered by the absence of validated *in vitro* assay protocol.

Complexation of certain plant biomarker constituents with appropriate complexing

Complexation of certain plant biomarker constituents with appropriate complexing agents may be employed in the development of detection and assay methods for phytomedicines (Harbourne, 1984; Crozier, 2000). The identification of the ideal ligand for the biomarker of *L. micranthus* will afford a convenient method for ultraviolet-visible (UV-Vis) spectrophotometric analysis of the plant material, extracts or formulations. UV-Vis absorption spectrophotometry remains one of the most useful tools for elucidating the composition of complex ions in solution (Skoog and West, 1986).

Since no studies have yet been reported on the complexation of *L. micranthus* constituents with the commonly used complexing agents, our study evaluates some complexes of the crude methanol extract (CME) of *L.*

*Corresponding author: icuzochukwu@yahoo.com

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micranthus leaves parasitic on *Kola acuminata*.

MATERIALS AND METHODS

Preparation of reagents

Preparation of 0.4 M boric acid solution

Boric acid (2.47 g) was weighed and dissolved in 70 ml of distilled water in a beaker. The solution was transferred to a volumetric flask and the volume made up to 100 ml with distilled water.

Preparation of 0.1 M phosphoric acid solution

Phosphoric acid (0.58 ml) was added to 50 ml of distilled water in a volumetric flask. The volume was made up to the 100 ml mark with distilled water.

Preparation of aluminum chloride solution (1 %)

One gram of aluminum chloride was dissolved in sufficient distilled water to make 100 ml.

Preparation of ferric chloride solution (2.7 %)

Anhydrous ferric chloride (2.7 g) was dissolved in sufficient 2 M HCl to make 100 ml.

Preparation of 0.05 M methanolic aluminum nitrate solution

Aluminium nitrate (3.7513 g) was dissolved with 80 ml of distilled water in a volumetric flask. The resulting solution was made up to 100 ml mark with distilled water (0.1 M aluminum nitrate solution). 10 ml of the prepared 0.1 M aluminum nitrate solution was added to 10 ml of absolute methanol.

Plant material and preparation of crude methanol extract of *L. micranthus*

Loranthus micranthus Linn leaves parasitic on *Kola acuminata* were collected from Akwaeze, Eastern Nigeria in January 2005. Mr Ekekwe, J. M. C., a plant kingdom scientific analyst, formerly at the Botany Department of the University of Nigeria, Nsukka, identified the plants. The leaves were dried under the shade to a constant weight and pulverized with a Corona ® grinder. The powder was sieved with a 1 mm sieve.

The dried powdered plant leaves (80 g) of *L. micranthus* was extracted with 90 % aqueous methanol for 5h using a Soxhlet apparatus (Osadebe *et al.,* 2004). The resulting methanol extract was evaporated in a water bath maintained at 70 ± 5 ºC and dried for 24 h in a hot air oven set at 50 ±1 ºC.

Determination of λ_{max} of crude methanol extract and complexes of *L. micranthus*

A stock solution of the CME of *L. micranthus* was prepared by dissolving 124 mg of the CME in sufficient volume of 90 % aqueous methanol to obtain 100 ml of the solution. Two dilutions of the CME solution (1.24 and 0.124 mg %) were subsequently prepared by appropriate dilution of the stock solution with 90 % aqueous methanol. One millilitre each of the 0.124 mg % solution of CME was mixed with 9 ml of ferric chloride solution and 9 ml of equal volumes (1:1) of boric acid and phosphoric acid respectively. Similarly, 1 ml each of the 1.24 mg % solution of CME was mixed with 9 ml each of methanolic aluminum nitrate and aluminum chloride solutions respectively. The mixtures were vigorously shaken and about 4 ml each of the resulting mixtures and the 1.24 mg % of the CME solution scanned using a UNICO® UV-Vis 2102 PC spectrophotometer between the wavelength of 200 and 700 nm at 1 nm interval against a blank (90 % aqueous methanol). The obtained scan data were transferred to Microsoft Excel® toolpack and the data used to plot the graphs of absorbance against the wavelength. The λ_{max} of the CME and the respective complexes were determined from the resulting graphs.

Determination of number of complexation equilibria of complexes of *L micranthus*

Buffer solutions (pH 1, 3, 5, 6, 7, 8, 9, 10, 11 and 12) were prepared. 100 mg of the CME of *L. micranthus* was dissolved in enough 90 % aqueous methanol to make 100 ml. A 10 mg % solution of the CME was prepared appropriately by dilution of the stock solution with 90 % aqueous methanol. The respective buffer solutions (2 ml each) were mixed with 4 ml of the complexing agents (methanolic aluminum nitrate, boric acid: phosphoric acid mixture and aluminum chloride solutions). Four millimeters of the CME solution (10 mg %) were thoroughly mixed with the buffered agents respectively. The absorbance values of the resulting complexes were determined at the λmax of the respective complexes using UNICO® UV-Vis 2102 PC spectrophotometer. A graphical plot of the absorbance versus pH was drawn using Microsoft Excel® tool pack.

Similarly, 2 ml of the respective buffer solution were mixed with 0.5 ml of ferric chloride solution. The respective buffered ferric chloride were mixed with 1 ml each of the crude methanol extract (10 mg %). The resulting complex (0.2 ml) was mixed with 2 ml each of 90 % aqueous methanol and the absorbance values determined at 439 nm using UNICO UV-Vis 2102 PC spectrophotometer. A graphical plot of the absorbance versus pH was also drawn using Microsoft Excel® tool pack.

RESULTS AND DISCUSSION

The results of determination of λ_{max} of *L micranthus* and its complexes are shown in Table 1. The resulting absorption spectrum of CME displays three absorption bands at 223, 275 and 663 nm respectively pointing to the existence of at least three different absorbing species. The absorption band at 275 nm is characteristically a benzenoid band, which generally occurs between 250 and 280 nm (Finar, 1986). The benzenoid band represents the Π to Π* transition in the absorbing group of molecules upon irradiation with ultraviolet radiation. *L. micranthus* has been demonstrated to contain flavonoids (Osadebe *et al.*, 2006). Flavonoids are polyphenols containing conjugated systems and thus show intense absorption in the ultra-violet region of the electromagnetic spectrum (Harbourne, 1984). The absorption of the CME at 663 nm in the visible region suggests the presence of coloured compound(s) in the crude plant extract, the colour of the absorbing compound(s) being red. Materials that reflect red light absorb maximally in the visible region between 605 and 750 nm (*Anon*, 2006).

The result shows that the absorption maxima of the borate complexation are similar to the absorption maxima of the CME. This implies that the study could not detect any complexation between the CME and the phosphoric acid: boric acid mixture at the tested concentrations.

The results indicate that complexation with ferric chloride, aluminum chloride and methanolic aluminum nitrate led to shift in the wavelength of maximum absorption to a higher wavelength (bathochromic shift). While the shift of wavelength by complexation with methanolic aluminum nitrate occurred in the ultraviolet region (300 nm), the shift in wavelength on complexation with ferric chloride and aluminum chloride occurred in the visible region (439 and 415 nm) respectively. This finding is consistent with the use of ferric chloride solution and aluminum chloride solution in the detection of plant phenolic compound and flavonoids respectively (Harbourne, 1984). Some authors have also employed aluminum chloride and aluminum chloride/HCl as ultra-violet diagnostic shift reagents in the identification of flavonoids (Antri *et al.*, 2004; Mabry *et al.*, 1970). Qualitative analysis of plant materials, extracts or formulations of *L. micranthus* can therefore be based on the development of colour on mixing of the test sample with ferric chloride and aluminium chloride solutions. These qualitative tests are however not exclusive to *L. micranthus*.

Table 1: Result of wavelength of maximum absorbance of CME and its complexes

Extract/Complex	Principal peaks (nm)	Other Peaks (nm)
Crude methanol extract	223, 275	663
Aluminum nitrate complex	240, 300	
Ferric chloride complex	439	
Borate complex	225, 273	
Aluminum chloride complex	415	

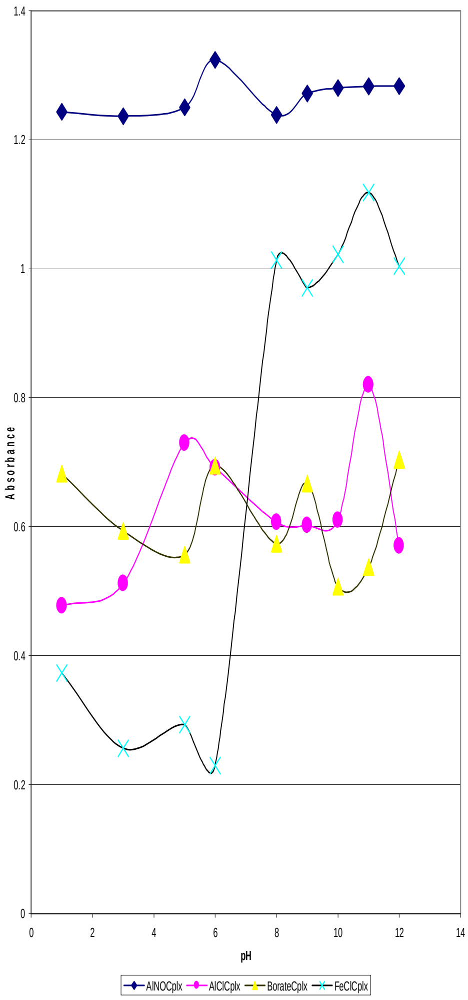

Fig 1: Result of determination of number of complexation equilibria of complexes of L. micranthus

The bathochromic shift to 300 nm wavelength seen on complexation with methanolic aluminum nitrate solution is highly characteristic and lends itself to the UV-Vis spectrophotometric identification and assay of *L. micranthus* leaves or its extracts and formulations.

The result of determination of number of complexation equilibria of *L micranthus* complexes is shown in Figure 1. The result of the absorbance-pH graphs show two sufficiently separated inflections for both the borate and aluminum chloride complexes. Three inflections are seen in the absorbance-pH graph for the ferric chloride complex of *L. micranthus*. The absorbance-pH graph of the complex formed between the *L. micranthus* extract and methanolic aluminum nitrate solution had one inflection only.

The two inflections each for the borate and the aluminum chloride complexes denote the existence of two ranges of complexation equilibria in solution (Arifien, 2004). This is probably a consequence of a stepwise transition of the respective borate and aluminum chloride complexes at two different pH ranges. Similarly, three inflections for the ferric chloride complex of *L. micranthus* denote the existence of three complexation equilibria in solution. There is also probably a stepwise transition from one equilibrium to another at three different pH ranges.

Finally, the one inflection seen in methanolic aluminum nitrate complex of *L. micranthus* denotes the existence of just one complexation equilibrium in solution. This finding suggests that methanolic aluminum nitrate is the best out of the four complexing agents studied in the complexation with constituents of *L. micranthus*. This finding is also consistent with the findings by Crozier *et al.*, 2000, that plant flavonoids such as quercetin, myricetin, morin, kaempferol and isorhamnetin can be chelated with methanolic aluminum nitrate and the resulting complexes subsequently detected fluorimetrically.

CONCLUSION

The use of methanolic aluminum nitrate solution as a complexing agent is recommended for the development of a UV-Vis spectrophotometric assay method for products, formulations or extracts of *L. micranthus* leaves.

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