

Ultra-violet spectroscopic studies of extracts of *Loranthus micranthus* Linn parasitic on *Kola acuminata*

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Abstract

The extracts of L. micranthus Linn parasitic on Kola acuminata were studied using ultraviolet-visible spectrophotometer. The dried powdered leaves of L. micranthus were extracted with aqueous methanol (90 %), absolute methanol, absolute ethanol, distilled water and ethyl acetate. The respective extracts were scanned between 200 and 700 nm wavelength and their absorption spectra plotted in Microsoft Excel[®] toolpack. The λ_{max} were also determined. Absorbance values of the extracts were determined at 275 nm wavelength. The absorbance spectra of flavonoids fraction of L. micranthus were also similarly obtained in acidic and basic media. Extractive yields of 14.63 % and 13.92 % were obtained for the crude methanol extract and flavonoids fraction respectively. Aqueous methanol (90 %) afforded the best solvent, among the tested solvents, for the extraction of the flavonoid constituents of powdered leaves of L. micranthus. The flavonoids fraction experienced bathochromic shift in the alkaline medium. The obtained UV-Vis absorption spectra may serve as a useful basis for the identification and characterization of products, extracts or formulations of L. micranthus leaves.

Keywords: *Loranthus micranthus*, Phytomedicines, Flavonoids fraction, Methanol extract

Introduction

Loranthus micranthus Linn (African mistletoe) is a medicinal plant traditionally employed in Nigeria for the management of diabetes mellitus and respiratory infections. Its safety, antidiabetic and antimicrobial activities have been substantiated (Osadebe *et al.*, 2004; Osadebe and Ukwueze, 2004; Osadebe and Akabogu, 2005).

Although the World Health Organization (WHO) has urged member states to ensure quality control of drugs derived from traditional plant remedies by using modern techniques and applying suitable standards and good manufacturing practices (WHO, 1996), this is yet to be actualized in most developing nations including Nigeria. In such cases where the isolation of the active constituents has not been done or isolated constituents characterized, the whole plant extract may be considered as one active constituent and the assay of marker substances may be adopted for its assay (EMA, 2001). If a reproducible fingerprint (chromatographic or UV-Vis spectroscopic) of the authentic herbal plant can be determined, such fingerprint can serve as a good identification and quantitation basis. The assay developed from such fingerprint may be considered suitable as an overall method of assay (EMA, 2001).

Uv-vis absorption spectrophotometry is about the most versatile modern analytical instrument and can be applied in the study of several properties of the absorbing species (Harbourne, 1984). The absence of validated chemical assay methods for many phytomedicines constitutes a limiting factor to their standardization, formulation, commercialisation and acceptance by healthcare providers and consumers. This study therefore attempts to characterize some extracts of *L. micranthus*

parasitic on *Kola acuminata* by employing UV-Vis spectrophotometry.

Materials and Methods

Plant material: *Loranthus micranthus* (Linn) leaves parasitic on *Kola acuminata* were collected from Akwaeze in Eastern Nigeria in January 2005. Mr J. M. C. Ekeke, 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 passed through a 1 mm sieve.

Determination of best solvent for extraction of *L. micranthus* constituents:

Five grams each of the powdered leaves of *L. micranthus* were weighed and wetted with 10 ml each of 90 % aqueous methanol, absolute methanol, absolute ethanol, distilled water and ethyl acetate. The wetted powders were warmed and macerated in a water bath (60°C) for 10 minutes. The mixtures were filtered with Whatman No 1 filter paper. The resulting filtrates (0.5 ml each) were diluted with the respective solvents to make 10 ml and the absorbance values determined at 275 nm (the benzenoid absorption band in UV region) using UNICO UV-Vis 2102 PC spectrophotometer.

Preparation of n-hexane extract and flavonoids fraction of *L. micranthus*:

The dried powdered plant leaves (80 g) of *L. micranthus* was first extracted with n-hexane for 5 h and the resulting marc further extracted with absolute methanol for another 5 h using a Soxhlet apparatus (Antri *et al.*, 2004). The resulting methanol extract and the n-hexane extracts were each evaporated in a water bath maintained at 70 ± 5 °C and dried for 24 h in a

hot air oven set at 50 ± 1 °C. The resulting methanol extract was confirmed to contain flavonoids using aluminum chloride solution and thus designated as flavonoids fraction (FF)

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

Determination of λ_{\max} of extracts of *L. micranthus*: Stock solutions of the crude methanol extract (CME), flavonoids fraction (FF) and n-hexane extract of *L. micranthus* were prepared in 90 % aqueous methanol. About 4 ml of the resulting solutions were scanned using a UNICO UV-Vis 2102 PC spectrophotometer between the wavelengths of 200 and 700 nm at 1 nm interval against a blank (90 % aqueous methanol). Similarly, the filtrates from the solvents extraction (90 % aqueous methanol, absolute methanol, absolute ethanol, distilled water and ethyl acetate) were diluted appropriately and scanned against the respective blank solvents. The obtained scan data were transferred to Microsoft Excel® tool-pack and the data used to plot the graphs of absorbance against the wavelength. The respective λ_{\max} of the obtained absorption spectra were determined from the respective graphs.

Determination of differential absorption spectra of FF of *L. micranthus*: A stock solution of FF of *L. micranthus* was prepared in 90 % aqueous methanol. A 0.1N HCl (pH, 2.0) and 0.1N NaOH (pH, 10.0) solutions were added respectively to an equal volume of the FF solution. About 4 ml of the acidic and basic FF solutions were each 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® tool-pack and the data used to plot the graphs of absorbance against the wavelength. The wavelengths of maximum absorption (λ_{\max}) of FF in the acidic and basic media were determined from the respective graphs.

Results and Discussion

The yield of the extraction of CME and FF were 14.63 % and 13.92 % respectively. The results of determination of best solvent (absorbance values) for the extraction of *L. micranthus* are shown in Table 1. The absorbance value of the different extracts at 275 nm reflects the quantity of benzenoid constituents (flavonoids) of *L. micranthus* present in the extracts (Finar, 1986). Higher absorbance values at 275 nm wavelength correspond to higher amount of flavonoids constituents present in the extract. The results show that while 90 % methanol is the best solvent, ethylacetate was the worst solvent for the extraction of the flavonoid constituents of *L. micranthus* leaves. This finding is understandably due to the polarity of the respective solvents. Ethyl acetate is

the least polar of the five tested solvents and consequently extracted the polar flavonoid constituents in the least capacity. Proportions of polar solvents such as methanol and water have been reported to increase extractive yields for polyphenols such as flavonoids (Harbourne, 1984). This also explains the higher absorbance value obtained for the aqueous methanol (90 %) extract compared to the absorbance value obtained for the absolute methanol extract.

Table 1: Absorbance values of extracts of *L. micranthus*

Extract	Mean absorbance* ± sd at 275 nm
90% methanol	1.7287 ± 0.0060
Absolute methanol	1.4093 ± 0.0050
Absolute ethanol	0.7338 ± 0.0014
Water	0.6602 ± 0.0040
Ethyl acetate	0.1552 ± 0.0033

*n=6

The results of determination of λ_{\max} of some extracts of *L. micranthus* are shown in Table 2. The results show that while the principal peaks occurred between 220 and 230 nm wavelengths, the secondary peaks occurred between 270 and 280 nm wavelengths. Only the n-hexane extract had a peak at 534 nm wavelength presumably attributable to chlorophyll. A characteristic absorption band occurred between 663 and 669 nm in the CME, FF and n-hexane extract. This absorption in the visible region suggests the presence of red coloured compound(s) in the extracts. Materials that reflect red light absorb maximally in the visible region between 605 and 750 nm (Anon, 2006).

Table 2: Absorption maxima (λ_{\max}) of extracts of *L. micranthus*

Sample	Wavelengths of maximum absorption (nm)
Aqueous methanol extract (90 %)	223, 275, 663
Absolute methanol extract	221, 258, 665
Ethanol extract	231, 259, 667
Water extract	224, 252, 462
Ethyl acetate extract	327, 412, 667
n-hexane extract	456, 534, 669
Flavonoids fraction	230, 275, 664

These results show that it is possible to obtain a reproducible absorption UV-Vis spectrophotometric fingerprint for the extracts of *L. micranthus* leaves. The obtained UV-Vis spectrophotometric fingerprint could serve as a reference spectrum for the authentic phytomedicine. Quality control of other samples can thus be assessed based on reasonable conformity or non-conformity to the reference UV-Vis spectrophotometric fingerprint. Moreso, quantitative UV-Vis spectrophotometric analysis method can also be developed, as long as the developed method is appropriately validated.

The result of determination of differential absorption spectra FF of *L. micranthus* is shown in Fig. 1. Ultraviolet spectral comparison in different pH media (differential spectrophotometry) can be employed in characterization of absorbing species such as flavonoids (Harbourne, 1984).

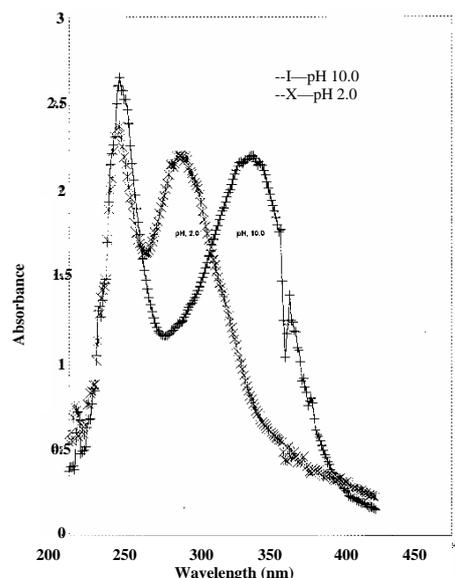


Fig. 1: Graph of differential absorption spectra of flavonoids fraction of *L. micranthus*

The result shows that the benzenoid band of the FF experienced a marked bathochromic shift (275 to 319 nm) in the alkaline medium. Phenolic compounds characteristically exhibit bathochromic shifts in their spectra in the presence of alkali and confirmation of phenolic moiety is readily achieved by UV spectral comparison in alcoholic and alkaline solutions (Harbourne, 1984). Conversely, the benzenoid band experienced a slight shift (275 to 272 nm) in the acidic medium. Alkaline solutions generate an additional lone pair of electron which can interact with the π electrons of the aromatic ring of the phenolic moiety thereby extending the chromophore. These findings confirm that the FF obtained from the *L. micranthus* leaves are composed of phenolic flavonoids.

Conclusion: The obtained ultraviolet-visible absorption spectra of crude methanol extract and flavonoids fraction of authentic sample of *L.*

micranthus can be employed in the identification and characterization of products, extracts or formulations of *L. micranthus* leaves.

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