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Antioxidant Activity and Chemical Constituents of *Pupalia lappacea* (L.) Juss

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Abstract: Phytochemical analysis of foliage of *Pupalia lappacea* (L.) Juss (*Amaranthaceae*) afforded 8 compounds, namely; 1-docosanol, stearic acid, stigmasterol, β-sitosterol, N-benzoyl-L-phenylalaninol acetate, β-sitosterol-3-O-β-D-glucopyranoside, stigmasterol-3-O-β-D-glucopyranoside and 20-hydroxyecdysone. The ecdysteroid, 20-hydroxyecdysone, constitutes nearly 0.3% of the dry weight and 13% of the dichloromethane extracts of the plant, making *Pupalia lappacea* a probable commercial source for this important ecdysteroid. Both the hexane and dichloromethane extracts showed significant antioxidant activity with the dichloromethane exhibiting an IC_{50} comparable to that of BHT, a synthetic antioxidant. All the isolated compounds are reported for the first time in the genus.

Key words: *Pupalia lappacea* (L.) Juss. (*Amaranthaceae*), ecdysteroid, 20-hydroxyecdysone, antioxidant activity, saropeptate

INTRODUCTION

Pupalia lappacea (L.) Juss. (Synonyms: *P. atropurpurea* (Lam.) Moq.; *P. Mollis* (Thonn.) Moq.; *P. distantiflora* A. Rich; *P. tomentosa* Peter), is a large straggling undershrub with flowers in spicate clusters usually growing along hedges and thorny plants (Rajput and Rao, 1999). It is the most widespread of the genus *Pupalia*.

The medicinal uses of *P. lappacea* have been variously documented. For instance, it is used as anti-vomitory, anti-emetic and antalgic in the Southwestern part of Nigeria and the Republic of Benin (Adjanohoun and Adjakidje, 1989) and as antisterility-plant for promoting reproduction in Bukina Faso (Fernandez, 1989). *P. lappacea* decoction in water is used by the people of Niger republic in the treatment of Urethra pain, endometritis, cystitis and leucorrhoea (Adjanohoun and Adjakidje, 1989) and as laxative and purgative in Tanzania (Kokwaro, 1976). In Somalia, 1 handful of crushed roots and leaves of *P. lappacea* are boiled, the decoction is mixed with a decoction of *Panicum deustum* Thunb. (*Poaceae*) and a prescription of one cup, 3 times per day is used for treating diarrhea, dysentery and cholera (Samuelson *et al*., 1991). The people of the northern region of Kenya used the whole plant in the treatment of skin diseases including scabies, ringworm and itching in humans and goats and as tonic, restorative, stimulant and in performance improvement (Timberlake, 1987).

Chemical investigation into the constituents of *P. lappacea* has never been reported. In the present study, we report on the antioxidant activity and phytochemical analysis of the hexane and dichloromethane extracts of the herb.

MATERIALS AND METHODS

Plant material: The foliage of *P. lappacea* used in this investigation were collected in Ibadan, Oyo-state, Nigeria, in July 2005 and identified by Mr. Felix Usang of Forestry Research Institute of Nigeria (FRIN). A voucher specimen has been deposited in the herbarium at FRIN (Voucher No. FHI 107236).

Chemicals and apparatus: 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Butylated Hydroxytoluene (BHT), 1-docosanol and methyl stearate were purchased from Sigma-Aldrich (Ontario, Canada). Analytical TLC was carried out on Silica gel plates (Kieselgel G, F_{254}, type 60, Merck, Germany), eluted with ethyl acetate / hexane (1:3) for all isolates from the hexane extract and ethyl acetate / hexane (2:1) for glycosides and ecdysteroid. Visualization was done by spraying with 10% ethanolic solution of phosphomolybdic acid followed by heating at 110°C for

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1-3 min. Column chromatography was performed on silica gel (type 60, 70-230 mesh, Merck, Germany).

NMR spectra were acquired in $CDCl_3$ and pyridine-d_5 on a Brüker AM-400 spectrometer (Bremen, Germany) using the residual solvent signal as internal standard; standard Brüker pulse programs were used for DEPT, 2D NMR COSY, HMBC, HMQC spectra; EIMS spectra were run on a VG-7070 mass spectrometer (Manchester, UK) operated at an ionization potential of 70eV. GC-MS analysis was on Hewlett Packard 5890 series II; 24 m × 0.2 mm i.d. column coated with DB5 bonded phase (0.33 μm film); temp. prog., 10°/min, then hold at 280°; injector temperature, 250°; detector temperature, 280°; injector volume, typically 1 μL at 70:1 split ratio; flowrate, 0.43 mL min^{-1}.

Extraction and isolation: The air-dried pulverized foliage (350 g) were packed into a Soxhlet extractor and successively extracted with n-hexane and dichloromethane. The solutions of extracts were concentrated *in vacuo* to yield the hexane extract (17.5 g) and dichloromethane extract (8 g).

The hexane extract of *P. lappacea* (3 g) was submitted to open column chromatographic separation on silica gel and eluted with a gradient mixture of diethyl ether and hexane. Sixty eight fractions, 100 mL each, were collected and analyze by TLC on silica gel. Identical fractions were pooled together: 3-20, 21-24, 25-31, 32-35, 36-45, 46-63 and 63-68. Fractions 3-20, coded PHA, 25-31, coded PHB, 36-45, coded PHC and 63-68, coded PHD, adjudged pure by TLC on silica gel, were identified by chromatographic, chemical and spectra methods.

The dichloromethane extract (2 g) was adsorbed on silica gel and introduced into a column packed with the same adsorbent in 100% hexane. The system was eluted under atmospheric pressure with gradient mixture of hexane / diethyl ether and 10-20% methanol in chloroform. Fractions collected (100 mL each) were bulked based on TLC analysis. Fractions 52-79, coded PDA and fractions 81-86, coded PDB (eluted with 15% methanol in chloroform) were submitted for spectroscopic analyses.

Antioxidant assay: The hydrogen atom or electron donation abilities of the corresponding extracts and BHT were measured from the bleaching of the purple-coloured methanol solution of 1, 1-Diphenly-2-Picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000; Cuendet *et al*., 1997). One mililitter of various concentrations of the extracts in ethanol was added to 4 mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 520 nm. Percentage inhibition of free radical was calculated using the formula:

$$\% \text{ Inhibition} = (A_{blank}-A_{sample}/ A_{blank}) \times 100$$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract) and A_{sample} is the absorbance of the test extract. Extract concentration proving 50% inhibition (IC_{50}) was calculated from the plot of % inhibition against extract concentration. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

Spectroscopic data of FHA were in perfect agreement with those of 1-docosanol in the Aldrich Library of ^{13}C- and ^{1}H-NMR (FT spectra). The identity was further established by co-TLC with authentic compound from Sigma-Aldrich. FHB was revealed by its NMR spectra as a long-chain fatty acid and was subsequently identified by GC-MS analysis of its methyl ester derivative as stearic acid. This was further confirmed by comparison of retention time with authentic sample. FHC was identified as a mixture of stigmasterol and β-sitosterol in the ratio 1.2:1.0 from its NMR spectra and its spectroscopic data were in perfect agreement with a similar mixture isolated from *Croton sublyratus* Kurz (De-Eknamkul and Potduang, 2003). Careful interpretation of the spectroscopic data of FHC revealed FHD as the biologically active peptide analogue, saropeptate (N-benzoyl-L-phenylalanyl-L-phenylalaninol acetate). The structure was confirmed by comparison of spectroscopic data with those recorded in literature for the same compound (Ishiguro *et al*., 1991). To the best of our knowledge, this is the second mention of this compound in the *Amaranthaceae* and the first in this genus.

PDA was identified as β-sitosterol-3-O-β-D-glucopyranoside and its spectroscopic data agreed perfectly with those recorded for the same compound isolated from *Prunella vulgaris* (Kojima *et al.,* 1990). Furthermore, weak (about 20%) signals at d_H 5.01 (dd, J = 15, 8.2 Hz) and d_H 5.23 (dd, J = 15, 8.2 Hz) assignable to H-22 and H-23 of stigmasterol, coupled with the presence of signals in the 13C NMR spectrum corresponding to documented signals for stigmasterol-3-O-β-D-glucopyranoside indicated that PDA has trace of stigmasterol-3-O-β-D-glucopyranoside as impurity. The identity of PDB followed from a careful interpretation of its ^{1}H NMR, ^{13}C NMR, DEPT 90, DEPT 135, COSY, HMBC and HMQC spectra, with good agreement from EIMS and

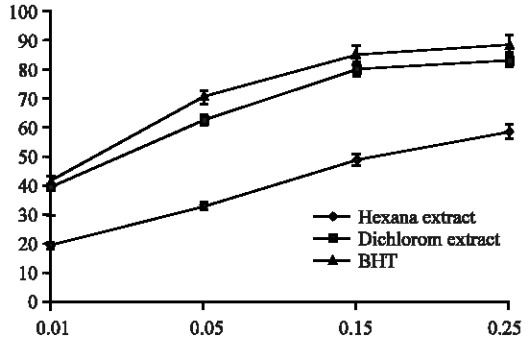

Fig. 1: Antioxidant responses of extracts of *P. lappacea* and standard

ESMS. A search in the Ecdybase, a free on-line listing of ecdysonoids (www.ecdybase.org) confirmed the identity of PDB as 20-hydroxyecdysone.

DPPH, a stable free radical with a characteristic absorption at 520 nm, was used to study the radical scavenging effects of extracts. Free radical scavenging capacities of the hexane and dichloromethane extracts and BHT, a synthetic antioxidant, are shown in Fig. 1. All concentrations studied showed free radical scavenging activity, with the dichloromethane extract exhibiting significant antioxidant activity (IC_{50} 0.029 mg mL^{-1}) comparable to BHT (IC_{50} 0.018 mg mL^{-1}).

CONCLUSION

The presence of 20-hydroxyecdysone in *P. lappacea* further supports the claims that the *Amaranthaceae* family is one of the largest 20-hydroxyecdysone-producing plants families (Robyn, 2004) and enlisted the genus *Pupalia* as a new member of the 20-hydroxyecdysone-producing genera of the family. This compound makes up nearly 13% of the chemical constituents of the dichloromethane extract of the plant and about 0.3% of the dry weight. The presence of these 2 biologically a ctive compounds, namely, 20-hydroxyecdysone and N-benzoyl-L-phenylalanyl-L-phenylalaninol acetate in *P. lappacea* makes the plant promising for further pharmacological and biochemical experiment.

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