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Research Article

BIOACTIVE MAMMEA-TYPE COUMARINS AND BENZOPHENONES FROM TWO CLUSIACEAE PLANTS

Magadula, Joseph Jangu*
Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania
*Email: jmagadula@gmail.com

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ABSTRACT
The phytochemical isolation of the ethanol extract of the stem bark of *Allanblackia ulugurensis* led to the isolation and identification of two polycyclic polyprenylated acylphloroglucinols (PPAPs), named guttiferone F (**1**) and 30-*epi*-cambogin (**2**) and a biphenyl compound, 3-hydroxy-5-methoxybiphenyl (**3**). The ethanolic crude extract of *A. ulugurensis* exhibited high activity in anti-HIV protease assay with IC_{50} of 4.1 µg/ml, while the standard drug has IC_{50} of 2.2 µg/ml. However, compounds **1** and **2** indicated lower activity in anti-HIV protease assay with IC_{50} values of 11.3 and 22.7 µg/ml respectively. The ethanolic crude extract of the stem bark of *Mammea usambarensis* indicated potent antioxidant activity in the DPPH assay with a value of 6,165 ± 152 μmol TE/g, which is more than twice as higher as that of the standard (Chlorogenic acid; 3,056 ± 157 μmol TE/g). Two compounds namely mammea B/BB (**4**) and mammea B/BD (**5**) were isolated from the stem bark extract of *M. usambarensis* showing antioxidant activities in DPPH assay at 4,012 ± 117 and 2,176 ± 102 μmol TE/g) respectively. The lower activities of the isolated compounds compared to the crude extracts may be attributed by the synergistic effect. Interestingly, the activities of the isolated compounds were higher than the corresponding standard compounds
Keywords: Clusiaceae Plants, Mammea-type, Benzophenones, Anti-HIV-1 PR, Antioxidant activities

INTRODUCTION

The family Clusiaceae (formerly called Guttiferae) has been extensively investigated from phytochemical and biological points of view. Many genera of this family have been reported to be the source of many important compounds with others been developed into pharmaceutical drugs that are currently in use. For instance, the genus *Garcinia* has been a major source of varieties of oxygenated and prenylated phenol derivatives such as prenylated xanthones, polyisoprenylated benzophenones, biflavonoids and triterpenoids.[1-4] Some of them exhibited novel and intriguing chemical structures as well as a wide range of biological and pharmacological properties.[5] *Garcinia* plants showed various biological properties including anti-HIV activity[6], antioxidant activity[7], antimicrobial activity[8], anticancer[9] and antimalarial activity[10]. Other genera like *Symphonia*, *Clusia* and *Allanblackia* have also been investigated phytochemically to produce a series of polyisoprenylated benzophenones and prenylated xanthones[6,8] while the genera *Calophyllum* and *Mammea* are reported to produce bioactive mammea-type coumarins[11]

This current phytochemical and pharmacological investigation of two Clusiaceae species, *A. ulugurensis* and *M. usambarensis* led to the isolations of prenylated benzophenones and mammea-type compounds respectively. The isolation, identification and biological activity of the isolates obtained are reported herein.

MATERIAL AND METHODS:

General:

Optical rotation was measured on a Schmidt Haensch Polartronic-D polarimeter with the sample dissolved in MeOH (*c* 0.16). ^{1}H- and ^{13}C-NMR, COSY, HMQC, HMBC and NOESY spectra were recorded on a Bruker Avance DRX-500 (500MHz) NMR spectrometer. Chemical shifts are expressed relative to the deuterated solvent (MeOH-*d4*: δH/δC, 3.31/49.01 ppm). Mass analyses were performed using a Bruker ESI-TRAP Esquire 3000 Plus spectrometer

Materials:

Dichloromethane was obtained from UNILAB (UNILABR, Nairobi, Kenya), ethanol (absolute) from FlukaChemie GmbH (Sigma-AldrichR, Zwijndrecht,Netherlands) Methanol and Acetone from Sigma-Aldrich GmbH, Germany) whereas Dimethylsulfoxide (DMSO) was from SigmaR (Poole, Dorset,UK). Tryptone Soya broth was obtained from HIMEDIAR (Himedia Laboratories Pvt Ltd, Mumbai, INDIA). 1,1- Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich (L'Isle d'Abeau Chesnes, France). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylique acid (TroloxR), 5′-caffeoylquinic acid (Chlorogenic acid), 2,2′-Azobis (2- methylpropionamidine) dihydrochloride (AAPH), Aminoguanidine and fluorescein (FL) were obtained from Acros Organics (Illkirch, France). Iodonitrotetrazolium chloride was bought from SIGMAR (Sigma- AldrichR, St Louis, USA).

Plant material:

The stem barks of *A. ulugurensis* were collected from Morningsite in Morogoro district, Tanzania while the stem barks *M. usambarensis* were collected from Shagayu forest reserve, Lushoto district, Tanzania. The plant materials were identified by Mr B. Mhoro and Mr H.O. Suleiman and the voucher specimen numbers BM 6401 and HOS 3429 have been deposited at the Institute of Traditional Medicine, University of Dar es Salaam, Tanzania.

Biological assays:

Two biological assays involved in this study were HIV-1 protease and antioxidant (DPPH) activities. The crude extracts of both *A. ulugurensis* and *M. usambarensis* were evaluated as previously reported[12]. Testing of the isolated compounds has also followed the same procedures as reported[12].

Extraction and isolation:

Air-dried and milled stem bark of *A. ulugurensis* (1kg) was extracted by maceration with MeOH: CHCl3 (1:1) twice at room temperature and then evaporated in *vacuo* to afford 210 g brownish gum of crude extract. A 110 g was suspended in

20% aqueous ethanol (400 ml) and partitioned sequentially with Pet ether, CH_2Cl_2 and EtOAc, to give 36.1 g, 16.0 g and 43.2 g of dry extracts respectively. The TLC analysis indicated presence of fatty acids in the Pet ether extract that was not followed further, two spots with different Rf values in CH_2Cl_2 extract and three spots with close Rf values in EtOAc.

The dichloromethane extract (14 g) was packed in the column chromatography (CC) in silica gel eluting with Pet ether:CH_2Cl_2 (2:3) to afford compound **3** (16.1 mg). The ethyl acetate extract (20 g) was stacked in Si gel and subjected to column chromatography eluting with gradient solvent mixtures, CH_2Cl_2/MeOH(4:1). A total of 60 fractions of 15 ml each were collected and through TLC profiles, subfractions 35-60 were pooled together to give 3.5 g. This was rechrommatographed in Sephadex LH-20 column and eluted with $CHCl_3$:MeOH (1:9) to afford compounds **1** (12.6 mg) and **2** (6.2 mg). Fractions 1-34 were combined and packed on CC under Si gel to give a steroidal compound, namely stigmasterol (7.8 mg).

Phytochemical work on the stem bark of *M. usambarensis* was achieved by packing 10 g of the crude plant material in the CC under Si gel eluting Pet ether, increasing amount of CH_2Cl_2 and MeOH. A total of 123 fractions were collected with 20 ml each. TLC analysis indicated similarity for some fractions and combined as follows; fractions 1-34 showed presence of long chain fatty acids and were not followed further, fractions 35-41 indicated two UV positive spots while fractions 42-60 indicated sugars and were not followed up. CC under Si gel of fractions 35-41 (6.2 gm) eluted with Pet ether: CH_2Cl_2 (2:3) yielded compounds **4** (38.1 mg) and **5** (14.1 mg).

RESULTS AND DISCUSSION:

The identification of the isolated pure compounds was achieved by the use of combined 1D and 2D NMR techniques, MS as well as chemical and physical properties. From *A. ulugurensis*, three pure compounds were isolated and identified as polycyclic polyprenylated acylphloroglucinols (PPAPs) namely guttiferone F (**1**) and 30-*epi*-cambogin (**2**) together with a 3-hydroxy-5-methoxybiphenyl (**3**). The literature indicated that these compounds were formerly reported from other Clusiaceae plant species. For instance, compound **1** and **2** were isolated from *Allanblackia stuhlmannii*[13]. The NMR data of compound **3** were consistent with those obtained from the literature, being isolated from the root bark of *Lindera fruticosa,* family Lauraceae[14]. Although its closely related geranylated biphenyl derivative, 3-hydroxy-4-geranyl-5-methoxybiphenyl has been reported from the genus *Garcinia* of the family Clusiaceae[15], this is the first time that compound **3** has been isolated from the genus *Allanblackia.*

From *M. usambarensis*, the isolated compounds were identified as mammea B/BB (**4**) and mammea B/BD (**5**). In the literature, compounds **4** and **5** were reported from two Clusiaceae plant species such as *Mammea americana*[16] and *Calophyllum brasiliense*[11].

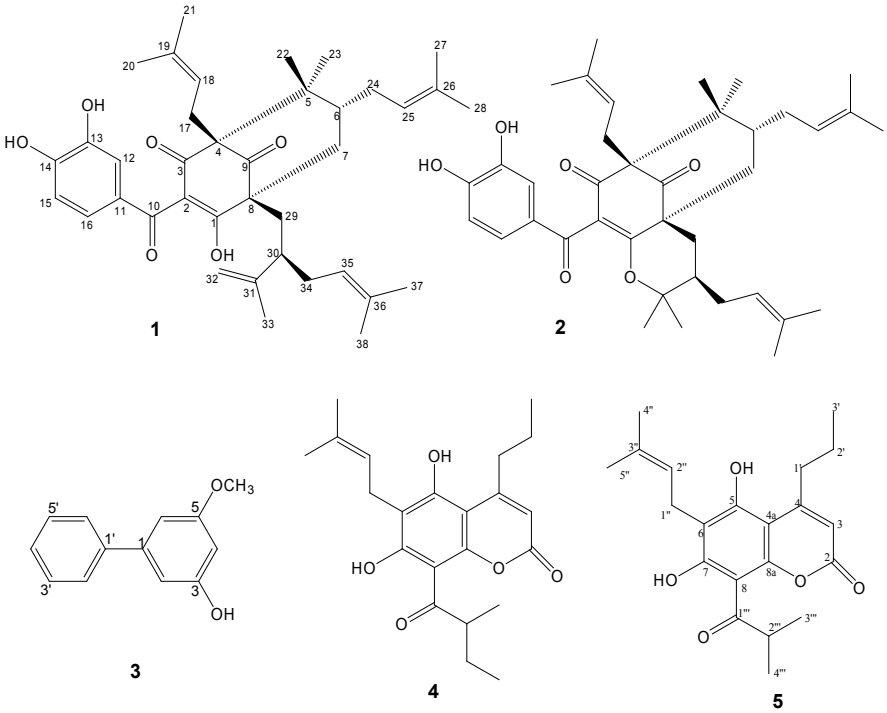

Fig 1: Structures of compounds isolated from the stem barks of *A. ulugurensis* and *M. usambarensis*

The crude extract of the root bark of *A. ulugurensis* indicate high activity in an anti-HIV protease assay with IC_{50} of 4.1 µg/ml. In this assay compounds **1** and **2** were tested indicating IC_{50} values of 11.3 and 22.7 µg/ml respectively. The activities of individual compounds are lower than the crude extract indicating the possibility of synergism. These results are in agreements with the reported anti-HIV activity for compound **1** previously isolated from A. *stuhlmannii*[13]. Compound **1** exhibited partial cytoprotection against HIV-1 *in vitro* at EC_{50} value of 23 µg/ml, however it was not followed further as it indicated direct cytotoxicity to the host cells at a significant IC_{50} value of 82 µg/ml[13]. On the other hand, the crude extract of the stem bark of *M. usambarensis* indicated potent antioxidant activity at 6,165 ± 152 μmol TE/g, which is more than twice as higher as that of the standard (Chlorogenic acid, 3,056 ± 157 μmol TE/g)

(Magadula *et al.*, 2011). The antioxidant activity of compounds 4 and 5 indicated the same trend of having lower activity than the crude extract. When tested in DPPH free-radical assay compounds **4** and **5** exhibited an antioxidant capacity at 4,012 ± 117 and 2,176 ± 102 µmol TE/g respectively (Table 1). These results are in agreement with previous reports of antioxidants prenylated coumarins[16]. The potential antioxidant activities of these compounds may have been attributed by the presence of hydroxyl groups which acts as good hydrogen donors[17]. The lower activities of the isolated compounds compared to the crude extracts may be attributed by the synergistic effect. Interestingly, the activities of the isolated compounds were higher than the corresponding standard compounds. This implies that the plant extracts can be developed and formulated further into standardized herbal products

Table 1. Biological activities of crude extracts and pure compounds from *A. ulugurensis and M. usambarensis*

Plant	Extract/Compound	Anti-HIV-1 protease (µg/ml)	Antioxidant (*µ*mol TE/g), DPPH assay
A. ulugurensis	Ethanol extract (crude)	4.1	NT
	1	11.3	NT
	2	22.7	NT
	3	NT	NT
Standard	**Acetyl pepstatin**	**2.2**	
M. usambarensis	Ethanol extract (crude)	NT	6,165 ± 152
	4	NT	4,012 ± 117
	5	NT	2,176 ± 102
Standard	**Chlorogenic acid**		**3,056 ± 157**

CONCLUSION:

It is of significant importance to note that both crude extracts and pure compounds from the plants under this study exhibited activity level greater than the standard compounds. Hence, further studies on toxicity/acute toxicity is proposed as well as mixing pure compounds and active extracts in standardized ratios can led to active herbal products.

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REFERENCES:

1. Bennet GJ, Lee HH. Xanthones from Guttiferae. Phytochem, 1989; 28: 967
2. Oliveira CMA, Porto ALM, Biurich V, Marsaioli AJ. Two prenylated benzophenones from the floral resins of three *Clusia* species. Phytochem, 1999; 50: 1073-1079
3. Nyemba AM, Mpondo TN, Connolly JD, Rycroft DS. Cycloartane derivatives from *Garcinia lucida*. Phytochem, 1990; 29: 994-997
4. Babu V, Ali SM, Sultana S, Ilyas M. A biflavonoid from *Garcinia nervosa*. Phytochem, 1988; 27: 3332–3335
5. Sordat-Diserens I, Marston A, Hamburger M, Rogers C, Hostettmann K. Novel Prenylated Xanthones from *Garcinia gerrardii* HARVEY. Helv. Chim. Acta, 1989; *72*: 1001-1007
6. Gustafson KR, Blunt JW, Munro MHG, Fuller RW, McKee TC, Cardellina II JH, McMahon JB, Cragg GM, Boyd MR. The Guttiferones, HIV Inhibitory Benzophenones from *Symphonia globulifera, Garcinia livingstonei, Garcinia ovalifolia* and *Clusia rosea*. Tetrahedron, 1992; 48: 10093-10102
7. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and Anti-Glycation Activity of Garcinol from *Garcinia indica* Fruit Rind, J. Agric. Food Chem., 2000; 48: 180-185
8. Azebaze AGB, Ouahouo BMW, Vardamide JC, Valentin A, Kuete V, Acebe L, Beng V P, Nkengfack AE, Meyer M. Antimicrobial and antileishmanial xanthones from the stem bark of *Allanblackia gabonensis* (Guttiferae), Nat. Prod. Res., 2008; 22: 333-341
9. Protiva P, Hopkins ME, Baggett S, Yang H, Lipkin M, Holt PR, Kennelly EJ, Bernard WI. Growth inhibition of colon cancer cells by polyisoprenylated benzophenones is associated with induction of the endoplasmic reticulum response. Int. J. Cancer, 2008; 123: 687-694
10. Lannang AM, Louh GN, Lontsi D, Specht S, Sarite SR, Flörke U, Hussain H, Hoerauf A, Krohn K. Antimalarial Compounds from the Root Bark of *Garcinia polyantha* Olv., J. Antibiot. 2008; 61: 518-523
11. Reyes-Chilpa R, Estrada-Muñiz E, Vega-Avila E, Abe F, Kinjo J, Hernández-Ortega S. Trypanocidal constituents in plants: 7. Mammea-type coumarins, Mem Inst Oswaldo Cruz. 2008; 103: 431-436
12. Magadula JJ, Tewtrakul S, Gatto J, Richomme P. *In vitro* antioxidant and anti-HIV-1 protease (PR) activities of two Clusiaceae plants endemic to Tanzania. Int. J. Biol. Chem. Sci. 20115: 1096-1104
13. Fuller RW, Blunt JW, Boswel JL, Cardellina II JH, Boyd MR. Guttiferone F, the First Prenylated Benzophenone from *Allanblackia stuhlmannii*, *J. Nat. Prod.* 1999; 62: 130-132
14. Song MC, Nigussie F, Jeong TS, Lee CY, Regassa F, Markos T, Baek NI. Phenolic compounds from the roots of *Lindera fruticosa*, J. Nat. Prod., 2006; 69: 853-855
15. Dharmaratne RW, Piyasena KGNP, Tennakoon SB. A geranylated biphenyl derivative from *Garcinia mangostana*, Nat. Prod. Res., 2005; 19: 239-243
16. Yang H, Protiva P, Gil RR, Jiang B, Baggett S, Basile MJ, Reynertson KA, Weinstein IB, Kennelly EJ. Antioxidant and Cytotoxic Isoprenylated Coumarins from *Mammea americana*, Planta Med., 2005; 71: 852-860
17. Cao C, Sofic E, Prior R. Antioxidant capacity of Tea and common Vegetables. J. Agric Food Chem, 1996; 44: 3426-3431

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