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European Journal of Medicinal Plants
4(11): 1336-1344, 2014

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Polyphenolic Compounds with Anti-Ages Activity from Three Clusiaceae Plants

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Authors' contributions

This work was carried out in collaboration all authors. Author JJM performed the experiments, analyzed the data, managed the literature searches and wrote the first draft of the manuscript. Author ZHM performed the experiments, analyzed the data, and managed the literature. Author JG performed the experiment of anti-AGEs activity and analyzed the data. Author SD performed the experiment of anti-AGEs activity and analyzed the data. Author PR managed the study and corrected the manuscript and managed the literature. All the authors read and approved the final manuscript.

Original Research Article

Received 10th April 2014
Accepted 1st July 2014
Published 26th July 2014

ABSTRACT

Aim: This study focused on finding molecules with inhibitory effects on Advanced Glycation End-products (AGEs) formation from Tanzanian some Clusiaceae plant species
Study Design: Field study and Laboratory experimental tests.
Place and Duration of Study: Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania and PRES LUNAM, Université d'Angers, EA 921 SONAS, 16 Bd Daviers, 49045 Angers, France, between June 2011 and July 2013.
Methodology: Three Clusiaceae plant species (*Garcinia semseii, G. volkensii* and *Allanblackia ulugurensis*) were collected and dried in the field with the assistance of a botanist. Extraction and concentration of plant samples to obtain crude extracts were done in the laboratory following standard procedures. The isolation of the phenolic compounds was carried out by using normal phase column chromatography as well as High-

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performance liquid chromatography (HPLC). The isolated compounds were tested for anti-AGE activity using the *in vitro* automated assay.
Results: Two polyphenolic compounds exhibiting phloroglucinol moieties [e.g. polyprenylated benzophenones, such as guttiferone F, 2 (18 mg)] or biflavonoids [such as morelloflavone, 1 (22mg)] were isolated and identified from *A. ulugurensis* and *G. volkensis* respectively. The results further indicated that compound 1 is an excellent inhibitor of AGE formation exhibiting an IC_{50} values of 78 and 64 µM at wavelength of 370/440 (vesperlysines-like AGEs) and 335/385 (pentosidine-like AGEs) respectively.
Conclusion: Plants belonging to the Clusiaceae family commonly used in Tanzanian traditional medicine need to be considered as a potential source of molecules exhibiting pharmacological activities such as anti-AGE activity. Morelloflavone (1) and other biflavonoids prove to be very good anti-AGE compounds using our automated screening assay. Hence, our automated *in vitro* assay allows a fast, effective and quite inexpensive screening of natural compounds and can therefore be applied to high throughput screening projects.

Keywords: Clusiaceae plants; Isolation; Polyphenolic compounds; anti-ages activities.

1. INTRODUCTION

Advanced glycation end-products (AGEs) are involved in the pathogenesis of diabetes and neurological diseases such as the Alzheimer [1-2]. They also contribute to the development of atherosclerosis [3], joint diseases [4] as well as responsible for aging and tissue damage [5].

AGEs are formed endogenously, during so-called Maillard's reaction, when the carbonyl groups of reducing sugars non-enzymatically react with the free amino groups on proteins [6]. They are generated *In vivo* as a normal consequence of metabolism, but their formation is accelerated under conditions of hyperglycemia, hyperlipidemia and increased oxidative stress. AGEs are involved in inflammation processes which result in pathological disorders.

The interaction of AGEs with specific receptors (RAGEs) leads to an increase in production of reactive oxygen species (ROS) and activates the transcription factor NF-kB. In various types of cells, the transcription of NF-kB regulated genes induces an inflammation process [7] and pathological changes [8]. Therefore, compounds that are able to break AGEs, or inhibit their formation, may be considered as potential drugs, dietary supplements or other bioactive ingredients [9]. AGEs inhibitors may follow different inhibition pathways in order to prevent AGE formation. For example, they can prevent glycation *via* reaction with free amino groups in proteins or carbonyl groups on reducing sugars. In addition, compounds with antioxidant (AO) properties (free radical scavengers or transition metal chelators) may reduce reactive oxygen species (ROS). Finally, they can also scavenge α,β-dicarbonyl intermediates or even break them (AGE breakers) [10].

Polyphenolic compounds are well reported to be biosynthesized in different genera of the family Clusiaceae [11-12]. These compounds displayed different biological activities against malaria, HIV, cancer, oxidation stresses [13-14]. The chemodiversity of natural products such as plant secondary metabolites could be exploited in order to identify molecules with inhibitory effects on AGE formation. In Tanzanian traditional pharmacopeia, there are six genera of Clusiaceae plants (*Allanblackia*, *Calophyllum*, *Garcinia*, *Mammea*, *Pentadesma*

and *Symphonia*) which are known to be the source of diverse polyphenolic derivatives such as benzophenones, biflavonoids, coumarins, phloroglucinols and xanthones [15-16]. All these classes of compounds may be potential anti-AGE agents. The plants (*G. semseii* Verdc., *G. volkensii* Engl. and *A. ulugurensis* Engl.*)* in this investigation were selected based on chemotaxonomical information only [17], as there is limited or no report on the ethnomedical use in traditional medicine. Previous studies on these plants reported the crude extracts to indicate significant anti-HIV-protease inhibitory effects as well as antioxidant activities [18-20]. Our study aimed to conduct the phytochemical study on these three Tanzanian Clusiaceous species and determine their anti-AGE activities.

2. MATERIALS AND METHODS

2.1 Plant Materials

Plant materials were collected in Tanzania. Stem barks of *G. volkensis* were collected from Shagayu forest reserve in Lushoto district, Tanga region (voucher specimen number HOS 3409). The root barks of *A. ulugurensis* were collected from Morning Site in Morogoro region (voucher specimen number BM 6401) while the Fruit hulls of *G. semseii* were obtained from Kihansi forest reserve in Iringa region (voucher specimen number FM 1629).

2.2 Preparation of Extracts

Stem and root barks and fruit hulls of plants under investigations were dried under shade for 14 days and then milled to obtain pulverized powders. 200g of the pulverised powder of fruit hulls of *G. semseii* and stem bark of *A. ulugurensis* were soaked in 1L of ethanol and 1L dichloromethane/Methanol for stem bark of *G. volkensis* for 48h. The crude extracts were obtained after evaporation of the solvent under reduced pressure at 40ºC on a rotary evaporator.

2.3 Anti-AGE Activity Screening

The AGE inhibition assay was carried out according to the method by Séro et al. [21]. This assay measures the ability of extracts or compounds to break advanced glycation end-products or inhibit their formation and hence deterring pathogenesis of conditions like diabetes and neurological disorders. The assay involved incubating bovine serum albumin (BSA) (10 mg/ml) with D-ribose (0.5M) and the tested compound (10^{-5}–10^{-2} M) in a Na-phosphate buffer (50mM pH 7.4). Solutions were incubated in black microtitre plates (96 wells) at 37ºC for 24h in a closed system. AGEs fluorescence (excitation: 370 nm; emission: 440 nm and excitation: 335nm; emission: 385 nm) was measured using a spectrofluorimeter Infinite M200 (Tecan, Lyon, France). To avoid quenching phenomena, the fluorescence resulting from incubation in the same conditions with BSA (10mg/ml) and the tested compound or extract was subtracted from each measure. Tests were performed in triplicate. The negative control, i.e. 100% inhibition of AGEs formation consisted of wells with only BSA. The positive control, i.e. no inhibition of AGEs formation consisted of wells with BSA (10mg/ml) and D-ribose (0.5 M). The final volume assay was 100µl. The compound concentration for 50 percent inhibition (IC_{50}) was calculated from the data and compared with that of the reference compound. The intrinsic fluorescence of tested product is also evaluated and subtracted for each measurement. Results are compared with the reference compounds, aminoguanidine and quercetin.

2.4 Isolation and Determination of Pure Compounds

2.4.1 Open Column Chromatographic (CC) technique

The crude extract was analyzed by TLC to establish the solvent system that will be used in open column chromatography method. Then, the column (5 cm diameter) was packed with silica gel and 15g of each crude plant extract was introduced separately and eluted with solvents of increasing polarities such as Petroleum ether (PE), Dichloromethane (DCM) and Methanol (MeOH).

Through the conventional Column chromatography (CC) the crude extract of *G. volkensis* (2.9 g) was eluted using DCM: PE (4:1 vv) to afford compound **1** (22 mg). The crude fraction from *A. ulugurensis* (4.2g) was packed over silica gel on CC and eluted with solvent systems of DCM:PE (3:7 v/v) to yield compounds **2** (18 mg), **3** (15 mg) and **6** (2 mg). The fruit extract (2.4 g) of *G. semseii* was subjected to the CC and eluted with DCM: PE (4:1 v/v) to get compounds **4** (21mg) and **5** (12mg).

The fractions obtained were further analyzed by TLC and thereafter taken to HPLC for further purifications.

2.4.2 Chromatographic analyses by HPLC technique

An amount of 1-2mg of each tested extract was dissolved in 1.5mL of methanol HPLC grade. They were centrifugated prior to analysis. High-performance liquid chromatography (HPLC) equipped with a diode array detector (HPLC-DAD) was performed on an AGILENT model (AGILENT series 1100). The chromatographic separation (25ºC) was done on a reversed-phase C18 analytical column (Lichrospher® 100, 125mm x 4mm x 5µm Merck KGaA). A gradient elution of 0.1% formic acid in water (A) and methanol (B) was used (gradient: t=0 min 80% B; t=15 min 100% B; t=20 min 100% B) at a flow rate of 1mL/min. The injection volume was 20µL. Data acquisition was performed using Chemstation for LC 3D (AGILENT).

2.4.3 NMR analyses

^{1}H- and ^{13}C-NMR, COSY, DEPT, HSQC and HMBC spectra of pure compounds were recorded on a Bruker Avance DRX-500 (500MHz) NMR spectrometer. NMR data were collected in $CHCl_3$-d. Chemical shifts are expressed relative to the deuterated solvent ($CHCl_3$-d: δ_H/δ_C, 7.26/77.0ppm). Results were analyzed using MestReNova (Mestrelab Research S.L.).

2.4.4 Mass analysis

Mass analyses were carried out by constant infusion to a Bruker ESI-TRAP Esquire 3000 Plus spectrometer. Results were analyzed using Data Analysis software (Bruker Daltonik).

2.4.5 Optical rotations

Optical rotations were measured on a Schmidt Haensch Polartronic-D polarimeter with samples dissolved in MeOH (*c* 0.16).

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Pure Compounds

Both CC and HPLC purifications of sub-fractions of *G. volkensis* and structural characterization using combined spectroscopic techniques (UV, IR, MS and NMR) revealed the presence of the flavanone-(3-8'')-flavone type biflavonoid named morelloflavone (1) [22]. From the active fraction of *A. ulugurensis*, three compounds were isolated including guttiferone F (2) [23], 30-*epi*-cambogin (3) [23] and 3-hydroxyl-5-methoxybiphenyl (6) [24], while from the fruits of *G. semseii*, two polyprenylated benzophenones, named guttiferones A (4) [12] and K (5) [25] were isolated (Fig. 1).

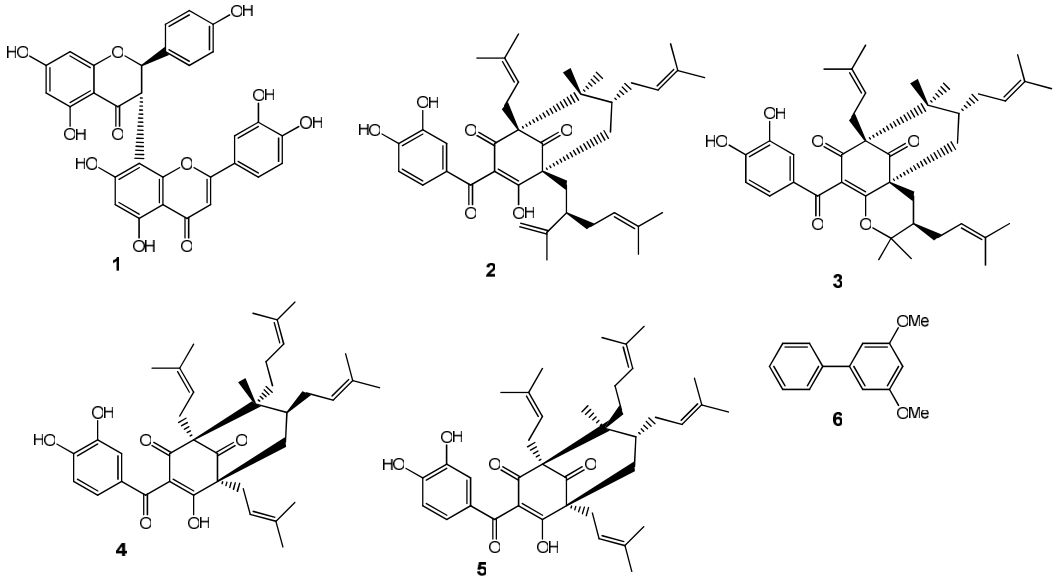

Fig. 1. Structures of compounds (1-6) isolated from three *Clusiaceae* plant species

3.2 Anti-AGE Activity

Using an automated liquid handling workstation, samples are diluted at different concentrations in a 96 well-microplate to determine the 50% inhibition concentration. The intrinsic fluorescence of tested product is also evaluated and subtracted for each measurement. Results are compared with the reference compounds aminoguanidine and quercetin and presented in Table 1. The results indicate morelloflavone (1) to be an excellent inhibitor of AGE formation as compared to other tested compounds.

3.3 Dose Dependent

Dose-response curves illustrating the effects of tested compounds on AGE formation (top: effect on vesperlysines-like AGEs; down: effect on pentosidine-like AGEs)

Table 1. Anti-AGE activities for compounds 1–6

Class of compound	Compound	Source plant	IC_{50} [mM]	
			370/440 nm	335/385nm
Biflavonoid	Morelloflavone (1)	*G. volkensis*	0.078	0.064
Polyprenylated benzophenones	Guttiferone F (2)/30-*epi*-cambogin (3)	*A. ulugurensis*	>3	1.2
	30-*epi*-cambogin (3)	*A. ulugurensis*	2.2	0.7
	guttiferone A (4)/K (5)	*G. semseii*	0.8	0.1
Biphenyl	3-hydroxyl-5 methoxybiphenyl (6)	*A. ulugurensis*	>3	> 3
Standard	Aminoguanidine	-	10	1.1
Standard	Quercetin	-	0.2	0.2

(IC_{50} = 1mg/mL), for aminoguanidine

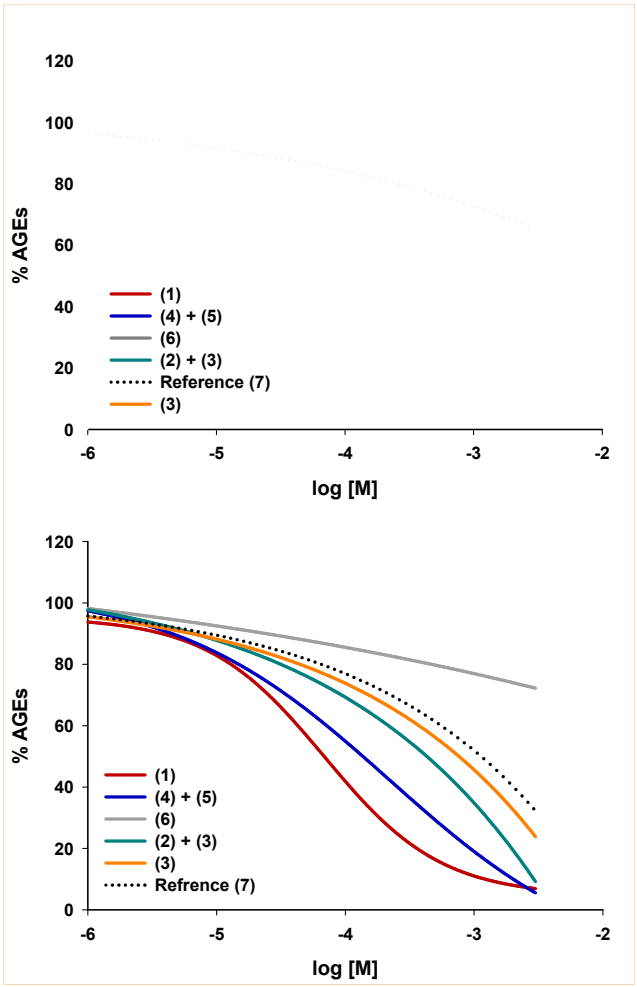

Fig. 2. Dose-response curves (top: effect on vesperlysines-like AGEs; down: effect on pentosidine-like AGEs)

Advanced glycation end-products (AGEs) are associated with many pathogenic disorders such as Alzheimer's disease, pathogenesis of diabetes, atherosclerosis or endothelial dysfunction leading to cardiovascular events. Clusiaceae family is rich in compounds like polyphenols which are able to inhibit their formation and are therefore of great interest.

Our results indicated that, morelloflavone (1) Appears as an excellent inhibitor of AGE formation as compared to other tested compounds. The anti-AGE properties of numerous biflavonoids, structurally related to compound 1 were previously reported [26-27]. The activity shown by these compounds could be associated to an efficient anti-inflammatory activity.

In polyprenylated benzophenones, the cyclisation of prenylated side chains (as for 30-epi-cambogin) leads to a loss of activity while no significant activity is observed for the biphenyl compound 3-hydroxy-5-methoxybiphenyl.

Anti-AGE compounds are classified into three categories: Anti-oxidants, dicarbonyl scavengers and crosslinks breakers [10]. As far as clusiaceous polyphenols are concerned, our results showed that the number of free phenols seems to be a crucial parameter to maintain a good anti-AGE activity. More precisely, biflavonoids such as morelloflavone (1) were previously described as strong antioxidant (AO) able to scavenge free radicals and to reduce metallic cations involved in Fenton reaction [28]. As illustrated in Fig. 2 above, these observations could illustrate that powerful AO such as biflavonoids are usually able to prevent efficiently the formation of advanced glycation end-products (AGEs).

4. CONCLUSION

The present study demonstrated that pure compounds from three Clusiaceae species under this investigation can inhibit AGES formation. In addition to showing significant anti-AGEs activity, these compounds are also regarded as powerful anti-inflammatory as well as antioxidant compounds.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

The authors wish to thank Vegepolys Innovation (7 rue Jean Dixmeras, 49044 Angers Cedex 01, France) to access for the liquid handling workstation and the microplate spectrofluorometer. We also wish to thank Patricia Blanchard for her assistance in performing the anti-AGE assays as well as the Sida-MUHAS for granting the study through the Directorate of Research and Publications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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