Chemistry of the stem bark of *Amburana cearensis* (Allemão) (A.C.SM.)

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RESUMO: Constituintes químicos da casca do caule de *Amburana cearensis* (Allemão) (A.C.SM). Extratos da casca do caule de *Amburana cearensis* (Fabaceae) foram purificados por cromatografia em coluna de gel de sílica e analisados através de CG/EIMS, com o objetivo de analisar e identificar os seus constituintes principais. Duas substâncias (cumarina e *trans*-3,4-dimetoxi-cinamato de metila) foram isoladas e identificadas por métodos espectrofotométricos tais como, espectros de massas, IV e UV, além de terem sido co-injetados com os respectivos padrões. Substâncias fenólicas predominaram nos extratos, principalmente cumarinas (cumarina, diidrocumarina e escopoletina). Outras substâncias fenólicas encontradas foram fenilpropanóides (ex. *trans*-3,4-dimetoxi-cinamato de metila), ácidos benzóicos (ex. 3-metoxi-4-hidroxi benzoato de metila), fenóis simples (ex. catecol e guaiacol) e a antraquinona crisofanol. Triterpenóides (ex. lupeol e amirinas), esteróides (ex. γ-sitosterol), ésteres alifáticos (ex. palmitato de metila) e outras substâncias minoritárias foram também detectadas.

Palavras-chave: *Amburana cearensis*, cumarinas, fenilpropanóides.

ABSTRACT: Chemistry of the stem bark of *Amburana cearensis* (Alemão) A.C.SM). Extracts from the stem bark of *Amburana cearensis* (Fabaceae) submitted to silica gel column chromatography were analyzed by GC/EIMS with the objective of identifying their principal constituents. Two compounds (coumarin and *trans*-methyl-3,4-dimethoxy cinnamate) were isolated and identified by MS, UV and IR spectrophotometry, besides being also injected in GC/EIMS together with their respective standards. Phenolic compounds predominated in the extracts, in particular coumarins (coumarin, dihydrocoumarin and scopoletin). Other phenolics found are phenylpropanoids (*e. g. trans*-methyl-3,4-dimethoxy cinnamate), benzoic acids (*e. g.* methyl-3-methoxy-4-hydroxy benzoate), simple phenols (*e. g.* cathecol and guaiacol) and the anthraquinone chrysophanol. Triterpenoids (*e. g.* lupeol and amyrins), steroids (*e. g.* g-sitosterol), aliphatic esters (*e. g.* methyl palmitate) and other minor compounds were also detected.

Key words: *Amburana cearensis*, coumarins, phenylpropanoids.

INTRODUCTION

Amburana cearensis (Allemão) A.C.SM. (syn. *Torresea cearensis* Fr. All., Sophoreae, Fabaceae) is a tree native to South America, ranging from northeast Brazil to Peru. It is commonly used in folk medicine and, together with barks from other sources, its stem bark is used to concoct home made syrups for the treatment of cough and cold (Bravo *et al.*, 1999). The use of its crushed seeds has been recommended for the treatment of toothache (Bravo *et al.*, 1999). There are also reports on the use of its stem bark against fever and headache (Muñoz *et al.*, 2000). Antinociceptive, antiinflammatory and bronchodilator activities were demonstrated in extracts from stem bark and seeds of *A. cearensis*, which are largely used as spasmolytic, and also to treat respiratory tract diseases (Leal *et al.*, 1997). Recently the inhibition of acetylcholinesterase activity of ethanolic extract from the stem bark of *A. cearensis* was verified (Trevisan *et al.*, 2003). From the stem bark several compounds have been isolated, including coumarin and isokaempferide (Leal *et al.*, 2000). The seeds of *A. cearensis* contain 22 – 28% of oil, known as amburana or coumaru oil, with the predominance of saturated C_{10}-C_{24} acids (Badami & Gunstone, 1963). In addition to coumarin, the stem bark of *A. cearensis* growing in Bolivia yielded two phenolic glycosides (Bravo *et al.*, 1999), which have been shown to be active against malarial parasites (Gibbons *et al.*, 1995).

Besides coumarin previously detected in the species, this paper describes the detection of several unreported compounds for the *A. cearensis*.

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MATERIAL AND METHOD

Plant material

Stem bark from *A. cearensis* was collected in January of 1998 from trees growing spontaneously in Aracatu (state of Bahia, northeast Brazil). Voucher specimens are deposited in the Alexandre Leal Costa Herbarium (ALCB-IB/UFBA).

Extraction, purification and isolation of compounds

Dried and powdered material (2 Kg) was extracted in soxhlet with 3 L of each of the solvents hexane, chloroform, ethyl acetate, acetone and methanol. The amounts of residue obtained in each extract were, respectively, 2.4, 3.1, 3.8, 3.0 and 6.4 g.

The extracts were concentrated and purified over silica gel columns using mixtures of hexane, chloroform and methanol with increasing polarity. Coumarin and *trans*-methyl-3,4-dimethoxycinnamate were isolated and were purified using thin layer chromatography (CD plates) and chloroform-methanol (1%) as mobile phase.

GC/EIMS and identification of compounds

Pure compounds or purified extracts eluted from the columns were concentrated and then diluted with a suitable solvent. 1 mL of a chloroform, ethereal, acetone or methanol solution was injected into a 5890 series II GC chromatograph, interfaced with a 5989B ChemStation System Mass Spectrometer (Hewllett-Packard), operating with the EI mode at 70 eV. A HP-5MS fused silica capillary column (30 m x 0.25 mm) was used, with mass selective detector and He as carrier gas, at 32 cm min^{-1} and split ratio 1:10. Oven temperatures ranged from 100 to 300°C at 10°C min^{-1}, followed by an isothermal period of 15 minutes. Injector and detector temperature was 300°C. The relative percentages of each component in the corresponding chromatogram were estimated by integration of the areas under the corresponding GC peaks. Such data are given in Table 1 as relative amounts in relation to the total amount of the corresponding extract.

Identification of the substances followed computer searches over library Wiley 275L, and the fractions were also injected with reference compounds (standards) in order to assist in the identification. The principal substances were identified in eluates from chromatographic columns using standards (such as, coumarin, amyrins and their acetates, *trans*-methyl-3,4-dimethoxycinnamate, etc) as reference compounds in the analysis made by GC/EIMS. Together with known compounds were found some unknown compounds that have not yet been identified.

Coumarin: UV (methanol) λ max. 274 nm. IR (nujol) 1700 cm^{-1} (C=O – lactone ring carbonyl).

trans-Methyl-3,4-dimethoxycinnamate: UV (methanol) λ max. 234 nm and 321 nm.

RESULT AND DISCUSSION

The analysis of the extract of steam bark of *A. cearensis* revealed that the most abundant compound identified in the present work is coumarin (2H-1-benzopyran-2-one or 1,2-benzopyrone, Table 1), which was previously described (Bravo *et al.*, 1999; Leal *et al.*, 2000). Coumarin, dihydrocoumarin and *trans*-methyl 3,4-dimethoxy cinnamate were the principal compounds found in this plant and were isolated in order to carry out their identification with accuracy. Taking into account the relative estimaties of coumarin in Table 1 and the yields of the extracts, the content of this compound in the stem bark analyzed is 0.36%. Coumarins are not often found in Fabaceae. With the exception of psoralen (a furanocoumarin), coumarins so far identified in Fabaceae are simple compounds, such as the ones here reported (coumarin, dihydrocoumarin and scopoletin, Table 1). Coumarins are present in species widespread in northeast Brazil and included in different botanical families (Leal *et al.*, 2000). Activities ascribed to coumarins include antibacterial (Tada *et al.*, 2002) and cholinesterase inhibition (Choudhary *et al.*, 2002). In folk medicine, coumarin bearing plants are used for the treatment of respiratory tract diseases (Moura *et al.*, 2002) and malaria (Muñoz *et al.* 2000). Scopoletin (7-hydroxy-6-methoxy coumarin), has been shown to have antimicrobial effects (Kwon *et al.*, 2002), anti-inflammatory activity (Muschietti *et al.*, 2001) and monoamine oxidase inhibition (Yun *et al.*, 2001).

The second abundant class of compounds listed in Table 1 correspond to phenylpropanoids, in particular *trans*-methyl 3,4-dimethoxy cinnamate. The UV spectra of a series of *trans*-cinnamic acids have two regions of high absorption intensity, at 215-230 nm and 270-320 nm (Wheeler & Covarrubias, 1963). The UV spectrum of the phenylpropanoid isolated in this investigation has regions of maximum intensities at 234 nm and 321 nm and corresponds to *trans*-methyl-3,4-dimethoxy-cinnamate, according to the comparison made using the standard as reference compound. The *cis* isomer was also found in low concentration. Phenylpropanoids are important constituents of propolis, and have been recognized as having several of the biological activities attributed to the bee product, chiefly antimicrobial (Bankova *et al.*, 2000). Benzoic acids (*e. g.* methyl-3,4-dihydroxy benzoate), benzylic derivatives (*e. g.* 4-methoxy-methylphenol) and simple phenols (*e. g.* catechol, guaiacol) were also detected, some of them (*e. g.* 4-hydroxy-benzenemethanol) in considerable amounts (Table 1).

The anthraquinone chrysophanol (Table 1)

TABLE 1 - Compounds identified by GC/EIMS and respective standards in hexane (C_6H_{14}), chloroform ($CHCl_3$), ethyl acetate (EtOAc), acetone (Me_2CO) and methanol (MeOH) extracts of stem bark of *Amburana cearensis*, after purification by silicagel column chromatography. Digits correspond to apparent percentages in the corresponding extract, as estimated by GC.

COMPOUNDS	EXTRACTS				
	C_6H_{14}	$CHCl_3$	EtOAc	Me_2CO	MeOH
Coumarins					
Coumarin	15.0	26.0	16.0	64.0	56.0
Dihydrocoumarin		17.0		32.0	9.0
Scopoletin			4.0		
Phenylpropanoids					
Methyl 3-methoxy-4-hydroxy cinnamate			6.5		
trans-Methyl 3,4-dimethoxy cinnamate	9.5	26.0	6.0		5.0
cis-Methyl 3,4-dimethoxy cinnamate		5.0			
Methyl 4-hydroxy benzoate			1.0		
Methyl 3,4-dihydroxy benzoate			5.0		
Methyl 3-hydroxy-4-methoxy benzoate			4.0		
1,2-Benzenediol (catechol)	3.0				
2-Methoxy-phenol (guaiacol)			10.0		
α-Ethoxy-*p*-cresol					3.0
4-Hydroxy-benzenemethanol	4.5	14.0			4.0
4-Methoxy-methylphenol		11.0			5.0
2,3-Dihydrobenzofuran	2.5				
Anthaquinone					
Chrysophanol			4.0		
Triterpenoids or similar					
Squalene	4.0				
α-Amyrin	19.0				
β-Amyrin	7.5				
D:C-Friedooleanan-3-one	7.5				
Steroids					
Ergost-5-en-3β-ol	3.0				
24,26-Dimethylcholesta-5,22-dien-3β-ol	10.0				
γ-Sitosterol	12.0				
Aliphatics					
n-Undecene			2.0		
Ethyl hexadecanoate (ethyl palmitate)			8.5		
Methyl eicosanoate			2.5		
Methyl 9(Z)-Octadecenoate (oleic acid methyl ester)	2.0				
Hexanedioic acid dioctyl ester			7.0		
9,17(Z)-Octadecadienal			1.0		

has antioxidant and antiplasmodial activities (Bezabih *et al.*, 2001) and may have laxative and anti-gonorrheal effects (Qhotsokoane-Lusunki & Karuso, 2001). Other important classes of stem bark constituents of *A. cearensis* are the triterpenoids and steroids (Table 1). Lupeol, a-amyrin and their esters have been recognized as protease inhibitors (Rajic *et al.*, 2000) and anti-inflammatory (Miranda *et al.*, 2000). Amyrins exerted gastroprotection in experimental rat ulcer models (Navarrete *et al.*, 2002) and b-amyrin has antibacterial activity (Ramesh *et al.*, 2001; Singh & Dubey, 2001). The compounds a- and b-amyrin acetates have been shown to be antinociceptive (Krogh *et al.*, 1999).

Several aliphatic compounds were detected predominantly in the ethyl acetate extract, namely hydrocarbons (*n*-undecene), esters (*e. g.* methyl palmitate) and a long chain linear aldeyde (9,17(Z)-octadecadienal) (Table 1).

CONCLUSION

The technique of GC/EIMS provided a fast evaluation of the chemical composition of *A. cearensis*, allowing the identification of various known compounds, which were identified using the library data and standards. In the extracts unknown compounds that were also found have not yet been identified. Thus, this method of analyse is efficient for identify known compounds and making a quick analyse of chemical composition of medicinal plants.

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