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Full Length Research Paper

Steroids isolated from *Millettia versicolor* Baker (Fabaceae)

Ongoka, P. R.[1,2]*, Banzouzi, J. T.[3,4], Poupat, C.[3], Ekouya, A.[2], Ouamba, J. M.[2] and Moudachirou, M.[5]

[1]Département des Sciences Exactes, Ecole Normale Supérieure, Université Marien Ngouabi, BP 69, Brazzaville – Congo.
[2]Unité de Chimie du Végétal et de la Vie, Faculté des Sciences, Université Marien Ngouabi, BP 69, Brazzaville – Congo.
[3]Institut de Chimie des Substances Naturelles (CNRS), 1, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex – France.
[4]Centre d'Etudes et de Recherches Médecins d'Afrique (CERMA), BP 45, Brazzaville – Congo.
[5]Université d'Abomey Calavi, 01 BP 526, Cotonou – Bénin.

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The objective of this investigation was to isolate and determine the chemical constituents of the leaves of *Millettia versicolor* Baker, a medicinal plant used in the traditional pharmacopoeias of Central Africa, essentially for its pain-relieving and anti-parasitic properties. A methanol extract of the leaves was made. The chemical compounds isolated were analyzed by HPLC/MS and GC/MS. The structures were elucidated on the basis of spectral studies (IR, RMN ^{1}H, ^{13}C) and confirmed by comparison with published data. Seven known compounds (two sterols, one stanol and four triterpene alcohols) were determined, the major compound being stigmasterol. Except lupeol, previously isolated from *M. versicolor* aerial parts, these compounds are isolated from this plant for the first time. Their presence supports the pain-relieving use of the plants, since 5 of the 7 compounds have reported anti-inflammatory activity, and 2 of these 5 had also an anti-nociceptive action.

Key words: Medicinal plant, *Millettia versicolor*, anti-inflammatory, anti-nociceptive, phytosterols.

INTRODUCTION

Millettia versicolor Baker (Fabaceae) is a medicinal plant used in African traditional medicine (Angola Congo, D.R. Congo and Gabon) to relieve pain and cure parasitosis. An aqueous decoction of stem bark is employed in Congo for intestinal parasitoses, kidney pains, cough, female sterility, senile impotence of men. An infusion is used in DR Congo to rub the syphilitic wounds. The aqueous decoction of leaves is taken against feverish rheumatisms, headache, kidney pains, intestinal parasitoses, and cough (Congo). It is also used in bath against syphilis (Gabon). The trunk bark has anthelminthic applications (Angola, Congo, and Gabon) (Bouquet, 1969; Adjanohoun et al., 1988).

Pharmacological studies confirmed the anthelminthic potential of the plant roots and leaves (Kasonia et al., 1989; Ongoka et al., 2004) but the active compounds responsible for this activity have not yet been determined. The stem bark has reported anti-inflammatory properties, attributed to a furoquinone (Fotsing et al., 2003). From the leaves, which are the major plant part used for relieving pain, only lupeol had yet been isolated (Ekouya et al., 1990). However, our preliminary chemical screening (Ongoka et al., 2004) indicated the presence of numerous secondary metabolites in the aqueous and alcoholic fractions: flavonoids, tanins, polyphenols, saponins, terpenes and steroids. The present study aims to separate and identify the chemical constituents of the leaves of *M. versicolor*, to try and support its traditional use.

MATERIAL AND METHODS

Plant material

M. versicolor leaves were collected from Mossaka area in the Cuvette region (North of Republic of Congo) in 2004. Botanical identification was confirmed by the Department of Plant Biology and

*Corresponding author. E-mail: ongokapascal@yahoo.fr.

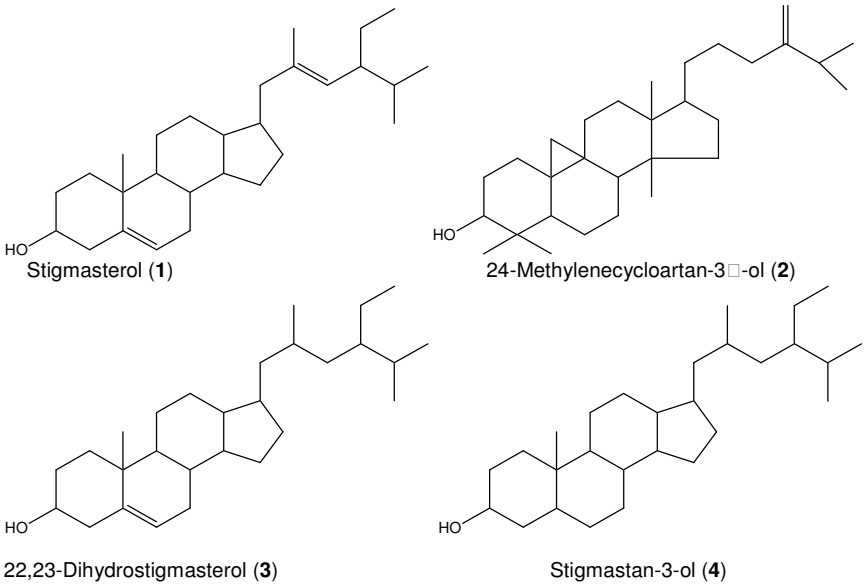

Figure 1. Structures of the compounds isolated from the leaves of *Millettia versicolor.*

Physiology of Université Marien Ngouabi (Brazzaville, Congo) where a voucher specimen has been deposited under number OP2004-1. The leaves were dried under shed then ground to powder prior to extraction.

Preparation of the extracts

70 g of dried leaves powder were extracted with methanol (soxhlet). The solvent was evaporated *in vacuo* and yielded 10 g of crude extract.

Isolation and determination of the steroids and triterpenes

5 g of the crude extract were separated on Sephadex LH20 column (200 g, methanol) and 5 fractions were obtained. Fraction 3 was chromatographed on silica gel column (200-300 mesh) with the following solvent systems: heptane-EtOAc (50:50), EtOAc (100) and EtOAc-MeOH (80:20), then by preparative silica TLC (Merck). Isolated compounds were analyzed by HPLC/MS (Symmetry column (C18), isocratic: H_2O-ACN (60:40), flow rate 1 ml/min) and GC/MS (GC TRACE Thermo 2000, solvent: CH_2Cl_2; Supelco Equity 5 silica capillary column 28089-U (30 m x 0.25 mm x 0.25 □m), carrier gas: helium, at a flow rate of 2 ml/min, column held initially at 160°C for 2 min and then increased to 280°C with a 5°C/min heating ramp, injection performed in split mode (50:1) at 280°C) to assess their purity. The chemical structures were elucidated on the basis of IR, RMN ^1H, ^{13}C spectral studies and confirmed by comparison with published data.

RESULTS AND DISCUSSION

The spectral analysis enabled us to identify 4 known compounds: 2 phytosterols: stigmasterol (1), 24-methylenecycloartan-3β-ol (2), 22,23-dihydrostigmasterol (3) and a phytostanol, stigmastan-3-ol (4) (Figure 1). Three triterpenes were also identified: lupeol, taraxasterol and β-amyrin. We have isolated these triterpenes from extracts of *M. versicolor* in previous studies (Ekouya et al., 1990; Alphonse et al., 2006).

Compound 1 is the major composite of the leaves methanol extract. The structure of these compounds was confirmed by comparison with published data (Toshihiro et al., 1988; Kojima et al., 1990) and by the use of authentic samples. With the exception of lupeol, all these compounds are isolated from *M. versicolor* for the first time and compounds 1, 2, 3, 4, had not yet been isolated in the *Milletia* genus, though all are already known from the *Fabaceae* family.

Pain-relieving activity is well supported by our study, since 5 of the 7 isolated compounds have a reported anti-inflammatory effect (stigmasterol) (Garcia et al., 1999; Gomez et al., 1999), (lupeol) (Akihisa et al., 1996; Della et al., 1994; Ramirez et al., 2004; Fernandez et al., 2001; Fernandez et al., 2001; Geetha and Varalakshmi, 2001), (24-methylene-cycloartan-3β-ol) (Akihisa et al., 1996; Yasukawa et al., 1998), (taraxasterol and β-amyrin) (Akihisa et al., 1996; Della et al., 1994) and 2 of these have also antinociceptive activity (3□-amyrin) (Lima et al., 2006; Oliveira et al., 2005; Otuki et al., 2005), (stigmasterol) (Santos et al., 1995). Lupeol and β-amyrin both have a hepatoprotective effect (Preetha et al., 2006; Sunitha et al., 2001; Oliveira et al., 2005) and lupeol also has a nephroprotective effect (Nagaraj, 2000). None of the 7 determined compounds had a reported anthelmintic activity.

Some biological activities that do not appear in traditional medicine are also reported, such as chemoprevention (taraxasterol) (Yasukawa et al., 1996; Takasaki et al., 1999; Ovesna et al., 2004), lupeol (Sultana et al., 2003; Saleem et al., 2004; Saleem et al., 2003; Hata et

al.,2000; Miles and Kokpol, 1976), stigmasterol (Awad and Fink, 2000; De Stefani et al., 2000), anti-hypercholesterolemia (stigmastan-3-ol) (Plat and Mensink, 2000; Plat and Mensink, 2001; Ramjiganesh et al., 2001; Jones et al., 1999) and 24-methylenecycloartan-3□-ol (Kiribuchi et al., 1983), and anti-malaria (lupeol) (Ziegler et al., 2004; Ziegler et al., 2002; Alves et al., 1997). It would be therefore interesting to test *M. versicolor* for hypocholesterolemic and chemopreventive activity. Antimalarial assay has already been done but revealed only a moderate activity against *Plasmodium falciparum in vitro* (Mbatchi et al., 2006).

Conclusion

This study permitted the isolation of seven known compounds from the methanolic extract of the leaves of *M. versicolor* Bak, six of them being new for the species. A majority of them has reported analgesic or anti-inflammatory activities, which support the traditional use of the plant for pain relief. Some of the compounds have other interesting biological effects, for which the leaves of *M. versicolor* could be investigated.

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