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Chemical comparison of *Prunus africana* bark and pygeum products marketed for prostate health

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ABSTRACT

The bark of *Prunus africana* may contain atranorin, atraric acid, beta-sitosterol and its esters, ferulic acid and its esters, and N-butylbenzene sulfonamide, compounds that have been shown to improve the conditions of benign prostatic hyperplasia, enlarged prostate. An analytical scheme, involving liquid-solid extractions, saponifications, and LC-APCI-MS (triple quadrupole) analysis, was developed, optimized, and validated to determine the compounds at μg/g levels. Limits of quantification were in the low ng/mL range except for beta-sitosterol. All of the compounds plus two internal standards eluted in under 10 min on a phenyl-hexyl column with gradient elution involving water-methanol and acetonitrile.

The mass fraction of the compounds in *Prunus africana* bark (four samples) and commercial pygeum products (seven samples), derived from bark, were compared. Bark and pygeum were similar in their content of atranorin and atraric acid, found at low μg/g levels, and in the fact that ferulic acid was almost totally (> 90%) in the form of esters. In contrast, the total amount of ferulic acid was on average four times higher in bark (450 μg/g) than in pygeum while the opposite was true for total beta-sitosterol. Some pygeum samples had levels of total beta-sitosterol above 10,000 μg/g while the compound in bark was relatively invariant at about 680 μg/g. The fraction of free beta-sitosterol varied significantly between bark (33%) and pygeum (nearly all). In pygeum, the measured total beta-sitosterol concentration generally followed the labeled values for phytosterol content. No N-butylbenzene sulfonamide was found in any of the bark and pygeum samples.

1. Introduction

Enlarged prostate or benign prostatic hyperplasia (BPH) is common disorder in older men and may be a harbinger of prostate cancer. Activation of the α_1 androgen receptors in the prostate is key for abnormal growth of the prostate [1]. These receptors are strongly activated by dihydrotestosterone, a compound derived from testosterone through a reduction reaction catalyzed by the enzyme 5-alpha-reductase. Thus, treatment of the androgen receptor with antagonists and inhibition of 5-alpha-reductase are approaches to medical treatment. In addition, α-blockers that target and relax the bladder have proven effective. Patients who are between a "wait and see" program and a surgical program of treatment often take over-the-counter supplements or prescribed pharmaceuticals in order to slow the growth of the prostate, or at least to ameliorate the symptoms of the disease. Pharmaceuticals include flutamide, an androgen receptor antagonist, finasteride, a 5-alpha-reductase inhibitor, and prazosin, an alpha-blocker. Botanical dietary supplements sold commercially for treatment of BPH include saw palmetto, pumpkin seeds, *Usnea*, products containing beta-sitosterol, and pygeum. The range of phytotherapeutics for BPH was reviewed by Allkanjari and Vitalone [1].

Pygeum is the powdered bark of *Prunus africana* (also called *Pygeum africanum*), an evergreen tree that grows across the mountainous regions of Africa. Both the powder and a lipophilic extract are sold commercially under the same name. The composition and pharmacology of pygeum has been described [2]. Ingestion of pygeum has been found to inactivate the androgen receptor and inhibit prostate cancer cell growth [3]. Pygeum's major BPH-active components are thought to be atraric acid, N-butylbenzenesulfonamide, ferulic acid and its esters, and beta-sitosterol and its esters [4,5]. The structures of these compounds are given in Fig. 1.

Atraric acid was the first natural compound identified as an androgen receptor antagonist [6]. Atraric acid has been found in pygeum [7], lichens, such as *Usnea* [8,9], and mosses [10] at μg/g

Abbreviations: BPH, benign prostatic hyperplasia; NBBSA, *N*-butylbenzene sulfonamid; DOE, Design of Experiments.

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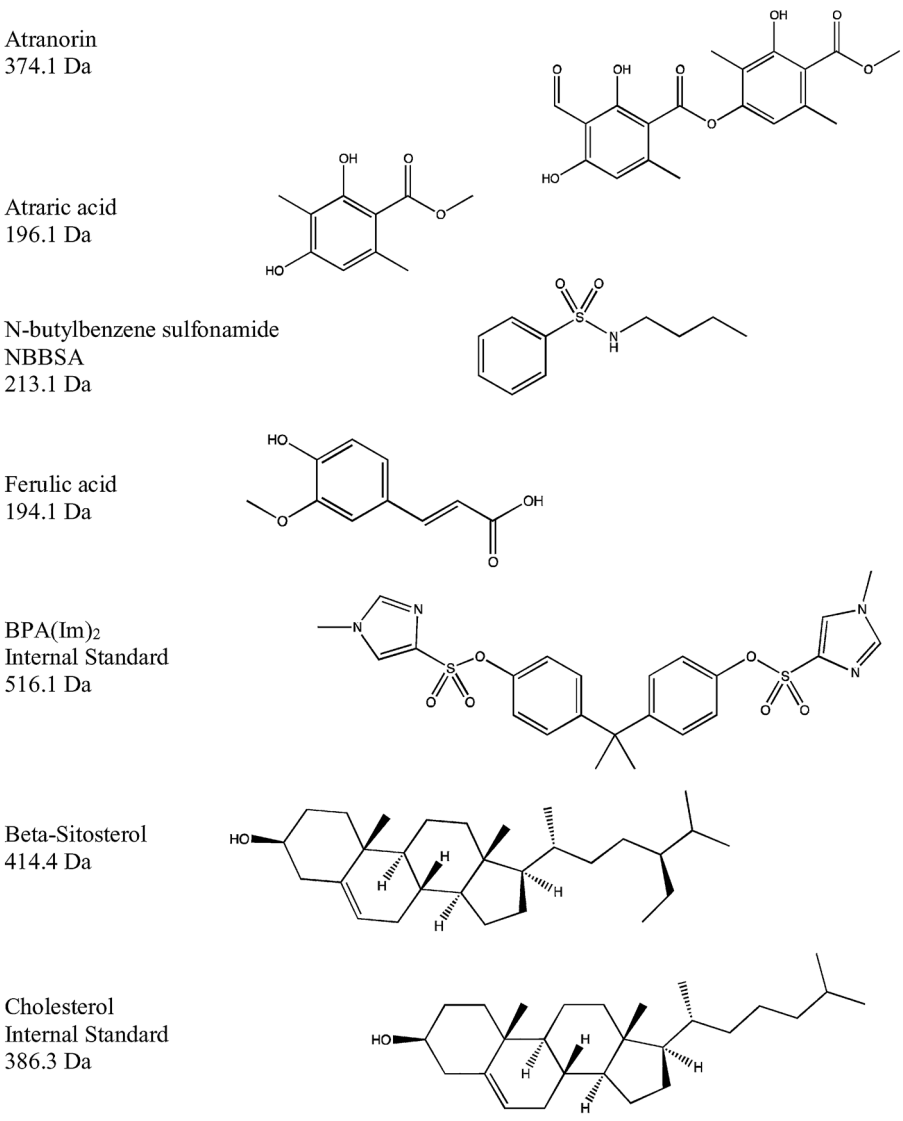

Fig. 1. Structures and exact masses of the analytes and internal standards.

levels. The chemical precursor of atraric acid is thought to be atranorin, a bitter depside and secondary metabolite, also found in lichens and mosses [8–10]. The premise is reasonable since atranorin is chemically and thermally labile [8], decomposing to two main products, one of which is atraric acid [11]. Logically, since lichens are often found on trees, *Prunus africana* bark may be expected to contain both atranorin and atraric acid, and ingesting atranorin would presumably provide atraric acid to the body. Atranorin itself has been identified as a natural anti-prostate cancer compound [12,13]. It is interesting to note that Shukla, et al. found more atranorin in lichens at higher altitude, where *Prunus africana* grows, and reasoned that this was due to atranorin's role as a photoprotectant [14]. In conclusion, though there are no literature references in the *Chemical Abstracts* database on the topic of atranorin in *Prunus africana* bark or pygeum, atranorin should be added to the list of BPH-active components of bark/pygeum.

N-butylbenzenesulfonamide (NBBSA) is a common plasticizer in manufactured polyamide and other plastics and is an herbicide precursor. It has found its way into wastewater, where it has been quantified [15]. NBBSA is also a natural product, a rare natural aromatic sulfonamide [16]. The compound has been found in soil bacteria with antibiotic activity [17] and identified in *Prunus africana* extracts by NMR and IR of isolated material [18]. The compound has been shown to be an androgen receptor antagonist [19], and so is active against BPH.

Esters of ferulic acid with long chain alcohols, such as docosanol and tetracosanol, are present in *Prunus africana* and other botanicals. Ferulic acid and its esters can inhibit the production of cholesterol, leading to decreased levels of testosterone and dihydrotestosterone [20]. In addition, the compounds have been found to be prostate cancer chemopreventatives [21], limiting the development of vessels supplying the cancer [3].

Beta-sitosterol is a phytosterol found in many plants and plant products, including pumpkin, saw palmetto, and *Prunus africana* [1,22,23]. Typical total concentrations, including its esters with common carboxylic acids, are in the hundreds of micrograms per gram. The compound inhibits 5-alpha-reductase and, therefore, can reduce the amount of dihydrotestosterone in the prostate and thereby prostate growth [1]. The compound also exhibits general anti-inflammatory and muscle-relaxing activity and may act as an alpha blocker [5].

In the United States, dietary supplements, including botanicals, are not strictly regulated for efficacy or safety prior to sale. Supplements may contain toxins, colorants, or chemical residues and may be adulterated with active pharmaceuticals. As a consequence, it is essential that the consumer have some information regarding the