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Seasonal variation of naphthoquinones in *Euclea natalensis* subspecies *natalensis*

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Abstract

Seasonal variation of naphthoquinones was investigated from eleven wild plants of *Euclea natalensis* A.DC. subsp. *natalensis* belonging to the same population. The study was carried out in Tembe National Park, KwaZulu-Natal, South Africa. Quantitative analysis of bioactive naphthoquinones (shinanolone, 7-methyljuglone, diospyrin, isodiospyrin and neodiospyrin) from the root extracts was conducted using High Performance Liquid Chromatography (HPLC). All these naphthoquinones have potent antituberculosis activity. The concentration of the best antituberculosis compound 7-methyljuglone, ranged from 0 g/kg to 3.77 g/kg between individuals of a population and among seasons. Highest amount of 7-methyljuglone was found in winter in most of the trees. Accumulation of isodiospyrin and neodiospyrin varied significantly with seasonal changes ($P<0.05$). Maximum levels of isodiospyrin and neodiospyrin recorded per individual plant were 1.72 g/kg in summer and 0.48 g/kg in winter respectively. A statistically significant variation ($P<0.05$) was established between the mean levels of diospyrin and seasonal changes. Diospyrin was detected at a mean concentration of 3.19 g/kg during spring, which was the highest mean value quantified in all the four seasons. The highest amount of diospyrin quantified per individual plant was 6.79 g/kg during spring. The study showed that there is tremendous variation in the accumulation of naphthoquinones by natural populations of *E. natalensis* subsp. *natalensis*. Depending on the requirement of a particular naphthoquinone for research, one could harvest the roots from a given population in seasons recommended from this study.

Keywords: 7-Methyljuglone; Diospyrin; *Euclea natalensis* subspecies *natalensis*; Isodiospyrin; Naphthoquinones; Neodiospyrin; Shinanolone

1. Introduction

Euclea natalensis A.DC. subsp. *natalensis* is a member of the Ebenaceae family and is native to the eastern coast of southern Africa. It is a perennial, woody plant species that is commonly found as a shrub, or a medium sized tree of about 2–4 m in height (Palgrave, 1991). *E. natalensis* subsp. *natalensis* and the other subspecies of *E. natalensis* (*E. natalensis* A.DC. subsp. *anguistifolia*, *E. natalensis* A.DC. subsp. *magutensis*, *E. natalensis* A.DC. subsp. *capensis*, *E. natalensis* A.DC. subsp. *obovata* and *E. natalensis* A.DC. subsp. *rotundifolia*) display a complicated ecogeographical variation and their delimitations may be arbitrary due to the presence of intermediates and their susceptibility to hybridization (personal communication, Ms. Snyman, National Botanical Institute, Pretoria). Species of *Euclea* display an extensive flowering period ranging from May to January (Palgrave, 1991). *E. natalensis* subsp. *natalensis* is generally delineated from the other subspecies by virtue of their leaves, which are hairy, elliptic, and have pronounced wavy margins with tapering apices. Indigenous people of southern Africa harvest *Euclea* species for a number of horticultural and medicinal applications. Twigs of these plant species are locally used as toothbrushes for oral hygiene and their extracts are used for colouring purposes (Van Wyk and Van Wyk, 1997). The Zulus use its root bark to treat respiratory complications; Shangaans apply the ground powder to the skin in cases of leprosy and the powdered plant material is also used by various ethnic groups of southern Africa to relieve headache and toothache (Bryant, 1966; Palgrave, 1991).

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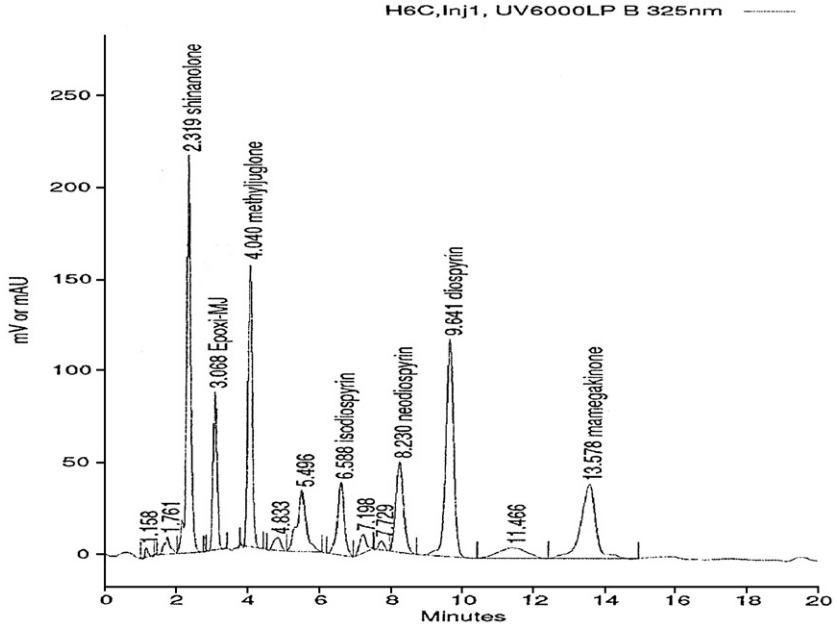

Fig. 1. A chromatogram showing the separation of naphthoquinones using High Performance Liquid Chromatography (HPLC).

The bioactivity of these plant species is directly linked to the presence of naphthoquinones, which are phenolic compounds prevalent in the Ebenaceae family (Mallavadhani et al., 1998; Mebe et al., 1998). Phytochemical analysis of crude extracts from the roots of *Euclea* species revealed the presence of 7-methyljuglone, diospyrin, isodiospyrin, neodiospyrin, mamegakinone, shinanolone as well as pentacyclic triterpenoids, all of which were previously isolated (Orzalesi et al., 1970; Khan, 1985; Mebe et al., 1998). Numerous antimicrobial studies have been conducted in an attempt to evaluate the pharmacological efficacy of naphthoquinones, these including antibacterial (Adeniyi et al., 2000), antiprotozoal (Kayser et al., 2000), antifungal (Sasaki et al., 2002) and anti-inflammatory activities (Kuke et al., 1998). Owing to their cytotoxic properties, many naphthoquinones have displayed inhibitory activities on different cancer cell lines (Hazra et al., 2005; Wube et al., 2005). Various naphthoquinone derivatives are currently being synthesised in an attempt to ameliorate their cytotoxicity. Crude extracts and naphthoquinones isolated from *E. natalensis* exhibited significant activity on a number of bacterial strains (Khan et al., 1978), especially against the drug-resistant strain of *Mycobacterium tuberculosis* (Lall and Meyer, 2001; Lall et al., 2005).

Continual harvest of plant material for both medicinal uses and ethnopharmacological studies necessitate an understanding on how plants synthesize and accumulate bioactive compounds. Many wild species exhibit temporal and spatial variation with regard to the production and accumulation of secondary metabolites. The observed variation is usually ascribed to constantly changing factors such as nutrient availability (Bi et al., 2005), geography (Castells et al., 2005), seasonal changes (Fischbach et al., 2002) and plant soil interactions (Wrobel et al., 2002). Elevated levels of toxin accumulation may possibly correlate with high pathogen attack whereas low concentrations of secondary metabolites could coincide with major phenological processes such as flowering and fruit setting. Qualitative and quantitative variation of these secondary compounds affects the curative potency of medicinal plants (McGaw et al., 2002). Understanding of the underlying variables that influence the synthesis and accumulation of secondary metabolites, will enhance our ability to predict their biosynthetic patterns across heterogeneous landscapes and to further apply strategies that will maximize yields from harvests on a sustainable basis. Our main objective for this study was to establish if any significant interaction exists between seasonal changes and accumulation of bioactive naphthoquinones in a given population of *E. natalensis* subsp. *natalensis*.

2. Materials and methods

2.1. Experimental area

The study was conducted at the Tembe Elephant Park, KwaZulu-Natal Province, along the eastern coast of South Africa which is located at the latitude 26° 51.56′S–27° 03.25′S, longitude 32° 24.17′E–32° 37.30′E and has an altitude of about 129 m above sea level. The climate is moist tropical/subtropical with a mean annual precipitation of approximately 721 mm and most of the rainfall is distributed between October and April. Mean annual temperature is 23.1 °C, the highest maximum temperature being 45 °C and the lowest minimum temperature recorded at 4 °C. Tembe Elephant Park is primarily a Sand Forest occurring with other vegetation types such as woodland, thicket and wetland. The soil of the experimental site is well drained and classified as coarse sandy in texture (sand 96.1%, silt 0.6% and clay 1.9%), with a pH of 5.6 and availability of P, Ca, K, Mg and Na at 8.6, 462, 88, 101 and 64 mg/kg respectively.