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Influence of harvest date and material quality on polyphenolic content and antioxidant activity of *Cymbopogon citratus* infusion

Gustavo Costa[a,b], Helena Grangeia[b], Artur Figueirinha[b], Isabel Vitória Figueiredo[c], Maria Teresa Batista[a,b,*]

[a] Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal
[b] Group of Bromatology, Pharmacognosy and Analytical Sciences, Faculty of Pharmacy, University of Coimbra, Portugal
[c] Institute for Biomedical Imaging and Life Sciences, University of Coimbra, Portugal

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ABSTRACT

Cymbopogon citratus (Cc) leaves are a product of high economic value because of its use in perfumery, cosmetic, pharmaceutical and agriculture industries. Polyphenols are most frequently present in combined form, and their structure and contents are often dependent of several factors like: plant varieties, culture conditions, maturation and processing. In this study, we aimed to evaluate the effect of the harvesting date and the quality of the plant material on the phenolic composition and the antioxidant activity of Cc leaves.

The plant material was classified accordingly to its harvest month for the same year: April–September and also accordingly to its quality (leaves aging state): high, medium and low. A lipid-free infusion was prepared. Total hydroxycinnamic acids, flavonoids and tannins were evaluated by spectrophotometric methods and polyphenolic profile was attained by HPLC-DAD. Antioxidant capacity was assessed using DPPH, ABTS and FRAP assays.

Regarding the harvest date influence, total phenols were fairly constant from April to August, with a little decrease in September. Higher concentration of hydroxycinnamic acids was found in April and June, but much lower on the following months. With exception of August, almost the same percentage of flavonoids was registered for all months. On the other hand, we found that the best months for tannins biosynthesis were June, July and August. The samples collected in April and June were the most effective against all the oxidant species tested, suggesting a correlation between this parameter and amount of hydroxycinnamic acids.

In what concerns the contents of each polyphenol, evaluated by HPLC-DAD, some significant differences were observed for the hydroxycinnamic acids, which seem to be more influenced by the conditions studied (harvest month and material quality).

In conclusion, for *C. citratus* leaves, both harvest date and plant quality are key criteria that have to be taken into account when selecting the material for human consuming, in order to receive benefits that have been associated with phenolic compounds, namely to those who are present in this plant.

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1. Introduction

Medicinal plants are commonly used to obtain phytotherapeutic medicines, food and cosmetics (Martinazzo et al., 2009). However, their use is limited by several factors including: cultivation, harvest period, climatic factors, humidity, brightness, part of the plant, transportation method, storage, drying process and extraction process, which may all modify the composition of the products, directly affecting its safety and efficiency (Calixto, 2000).

Phenolic compounds are ubiquitous in the plant kingdom exerting several functions on plant. Many factors influence phenolic accumulation in plants although some are more significant than others. The hydric stress and other exogenous growth factors, exposure to various light sources and the presence of fungal or predatory pressures are shown to alter plant biosynthesis (Cohen and Kennedy, 2010). During plant growth, phenolic compounds protect fruits and vegetables from microbial damage, ultraviolet (UV) radiation, and predation among other environmental stresses. The sun exposure is credited with being a major element relating to

* Corresponding author at: Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal. Fax: +351 239 488 503.
E-mail address: mtpmb@ff.uc.pt (M.T. Batista).

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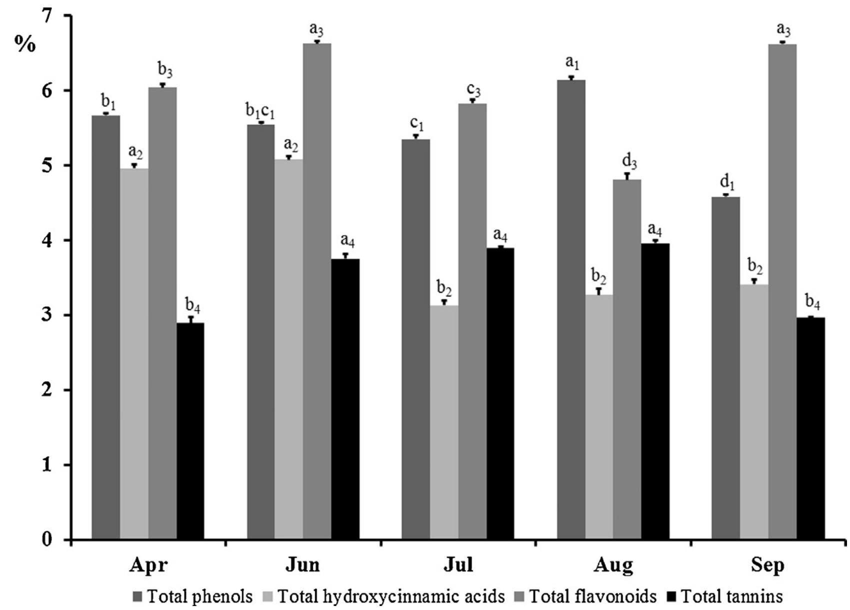

Fig. 1. Phenolic compounds amount (%, g of respective standard equivalents per 100 g of extract) quantified in lemongrass infusion of samples harvested from April to September. Statistically significant differences were denoted within each group by $P < 0.05$.

plant metabolism. It is now a well-established knowledge that sunlight itself directly influence the phenolic compounds biosynthesis (Zucker, 1965). The major challenge on studying sun exposure and plants is the dual effect of radiation. As the amount of sun exposure increases there is a concomitant rise in temperature (Spayd et al., 2002). Both flavonoids and proanthocyanidins showed significant increases with sun exposure (Spayd et al., 2002). Also phenolic acids are affected by UV radiation (Booij-James et al., 2000). As seen in sun exposure, temperature can also be correlated to phenolic composition (e.g., anthocyanins) but breaching a critical limit (high and low) has detrimental effects. It is likely that all plants exhibit temperature thresholds (Bradfield and Stamp, 2004). Furthermore, the phenolic composition, the degree of hydroxylation and acylation, is also sensitive to temperature shifts and likely reflects different sensitivity of various genes or enzymes in the flavonoid pathway (Cohen et al., 2008). The phase of postharvest, more precisely the drying process, requires special attention; therefore, the choice of dryer, drying system, air composition, height of layers of leaves in the dryer, and temperature and relative humidity of the room air can provide plants with better quality after harvesting. The rate at which water is removed from the medicinal plant during drying is very important because a rapid process can degrade active ingredients (Coradi et al., 2014).

Cymbopogon citratus (DC.) Stapf, Poaceae, is an aromatic herb known as lemongrass, native from India and tropical Asia and currently it grows worldwide. Lemongrass is a crop of high economic value because of its use in the perfumery, cosmetic, pharmaceutical and food industries (Oyedele et al., 2002; Saddiq and Khayyat, 2010). *C. citratus* is commercially cultivated in the Democratic Republic of Congo, Angola, Madagascar and Comoros Island. However, the leading exporter of this plant is Guatemala, trading about 250,000 kg per year, while the USA imports about 70,000 kg per year (Department of Agriculture, Forestry and Fisheries, 2012). The commercial value of lemongrass species is further enhanced by their ability to grow in moderate and extremely harsh climatic conditions (Padalia et al., 2011). *C. citratus* is commonly used in folk medicine for treatment of nervous and gastrointestinal disturbances, and as antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic and sedative (Santin et al., 2009). Recently, our group has been involved in the identification of functional compounds from lemongrass. We demonstrated that flavonoids, such as luteolin and apigenin glycosides, and proanthocyanidins strongly contributed to antioxidant and anti-inflammatory properties of an essential oil-free infusion from lemongrass (Costa et al., 2015; Figueirinha et al., 2010, 2008; Francisco et al., 2011, 2014, 2013).

Factors such as harvest season and quality grades are usually not taken into account for this plant, since it is harvest along the whole year, except for the winter (Department of Agriculture, Forestry and Fisheries, 2012). Hence, the chemical composition of the plant may vary from one month to another, leading to different industrial final products. Quality grading, performed by expert graders from industry, entails evaluating the appearance of the fresh and/or dry leaves. A literature search was undertaken on the effect of different methods of drying on chemical composition and content of the essential oil. The results showed that drying method had a significant effect on oil content and composition of aromatic plants (Okoh et al., 2008). However, to our knowledge, there are no reports of those effects on the polyphenolic content of lemongrass.

Therefore, the aim of this study was to investigate the effect of the harvest time and plant quality on the qualitative and quantitative phenolic composition of *C. citratus* infusion.

2. Materials and methods

2.1. Plant material

Dry leaves of *C. citratus* were acquired from ERVITAL (Mezio, Castro Daire, Portugal). *C. citratus* was bred in the region of Mezio, Castro Daire (Portugal), located in a greenhouse located 100 m above sea level. A voucher specimen is deposited in the Herbarium of Aromatic and Medicinal Plants of the Faculty of Pharmacy—University of Coimbra (A. Figueirinha 0109). The identity of the plant was confirmed by J. Paiva (Life Sciences Department, University of Coimbra, Portugal). The plant material was harvest within the year of 2011, and classified accordingly to two parameters: its harvest date – April (Apr), June (Jun), July (Jul), August (Aug) and September (Sep) – and its quality grade, by leaves aging state – high (H), medium (M) and low (L) quality, where high quality corresponds to young fully-grown green leaves and

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