Int. J. Pure Appl. Sci. Technol., 22(2) (2014), pp. 1-9

International Journal of Pure and Applied Sciences and Technology

ISSN 2229 - 6107

Available online at ***www.ijopaasat.in***

Research Paper

Pittosporum Undulatum and *Hedychium Gardnerianum* Nutritive Value and Secondary Metabolites on Cattle Reproductive Performances

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(Received: 6-3-14; Accepted: 6-6-14)

Abstract: *Hedychium gardnerianum (HG) and Pittospporum undulatum (PU) are invasive plants all over the world, being in the Azores supplied to cattle on periods of shortage food. As these plants produce secondary metabolites, including a diverse range of phytochemicals compounds, the aim of the present study is to identify how these metabolites can be related to animal's reproductive performances. For such purpose, plants were harvested on winter, compounds extracted by method of decoction and analysed by combination of liquid chromatography and mass spectrometry as well as high-performance liquid chromatography. For nutritive evaluations, Van Soest and Weende methodologies were used. In HG quercetin-3, 4'-di-O-beta-glucopyranoside, myricetin rhamnoside, quercetin rhamnoside, and gibberellin A1 and A8 were identified, while for PU were found cafeic acid derivatives, including dicaffeoylquinic acid and caffeoylquinic acid. In nutritional terms, these plants can be considered as poor, presenting percentages of dry matter (DM%) of 16.34% and 40.39%, respectively for HG and PU. Values for ash 10.4%, crude protein (CP) 7.75%, neutral detergent fiber (NDF) 64.5, acid detergent fiber (ADF) 34.69%, acid detergent lignin (ADL) 3.47% and ether extract (EE) 2.03% were found for HG. For PU values were ash 6.64%, CP 6.11%, NDF 43.84%, acid ADF 35.57%, ADL 3.56% and EE 2.71%. This study clearly indicated that, besides their low nutritive values, these plants can be used to feed ruminants, especially when pasture lacks. Nevertheless, as some compounds, namely the caffeoylquinic and dicaffeoylquinic acids, are known to be associated to physiological reproductive mechanisms, one could*

speculate that these compounds can be directly or indirectly associated to reproductive performances in bovine fed with these plants.

Keywords: *Hedychium gardnerianum*, Nutritive value, *Pittospporum undulatum*, Reproductive events, Secondary metabolites.

Introduction

In the Azores, animal production is limited by the production cycles of grass, existing two seasons, summer and winter in which a great lack occurs. In these periods it is frequent alternative feed ruminants, mainly goats and bovines with *Pittosporum undulatum* Vent. (Incenso) and *Hedychium gardnerianum* Ker-Gawler (Conteira). These two plant's species are invasive of the Azores, commonly known as *incenso* and *conteira,* respectively. *Pittosporum undulatum* is native to south-east Australia (Cayzer *et al*., 2000), being a tree of temperate 5-15m tall belonging to the family of *Pittosporaceae*, with white flowers and lanceolate acute, glabrous leaves (Dias *et al.* 2007). *Hedychium gardnerianum* Ker-Gawl is a rhizomatous (tuberous roots) herbaceous perennial of the family Zingiberaceae, native to the Himalayas (Carvalho *et al*., 2003; Arruda *et al*., 2012). This plant can reach 2 m height, the leaves are oblong with 30 cm long and aromatic numerous red-orange flowers, with inflorescences 20-30 cm height (Sjögren, 1984; Press and Short, 1994). Both plants were introduced in various countries, or for ornamental purposes or in case of *Pittosporum undulatum*, crop protection against wind, which are easily adaptable to many different media invaded native habitats (Cayzer *et al*., 2000; Harris *et al*., 1996).

With regard to the nutritional value, studies developed *in vivo* in sheep by Borba (1990, 1991), showed both plants are considered poor with low values of digestible and metabolizable energy. For *Conteira*, the same author states that the gross energy value and dry matter degradation are also very low.

As plant secondary metabolites are a large group of compounds not directly involved in the metabolism of essential plant, deriving mainly from the metabolism of amino acids or acetate (McNaughton, 1983), a comprehensive overview of their biochemistry, bioactivity and chemistry, is not described till now. These compounds can be divided into three major groups: terpenes, phenolic compounds and alkaloids. In some cases secondary metabolites can be toxic to animal's metabolism, affecting nervous system, immune, endocrine and reproductive (D'Mello and Devendra, 1995; Furlan *et al*., 2007; Stegelmeier, 2007). These compounds are also a significant source of anti-inflammatory and antioxidants, playing an important role as inhibitors of enzymes (Falé *et al*., 2011). Although no study on the influence of secondary metabolites produced by these plants at the reproductive performances in animals could be found, some breeders have the perception that they may cause reproductive decline level, reflecting an increase of early and late embryonic mortality or even abortions.

Thus, the present study aims, beyond the determination of nutritive values of *Pittosporum undulatum* and *Hedychium gardnerianum,* also identify secondary metabolites in these plants that might influence physiological mechanisms in ruminant's reproductive performance

Materials and Methods

Plant Material

Plant materials (*Pittosporum undulatum* and *Hedychium gardnerianum*) were collected in December, since these plants are mainly provided to the animals during winter.

Nutritive Value

Their nutritive characteristics were performed according Van Soest (1970) and Weende (1975) methodology, evaluating the following parameters: dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and ether extract (EE).

Extraction of Phenolic Compounds

For secondary compounds extraction was used conventional method decoction. Briefly, 10g of fresh samples were boiled for 1h in 300 ml of distilled water at 100° C. The extracts were filtered using Whatman filter paper (No. 1). Then, the filtrate was frozen and subsequently lyophilized. The collected filtrates were concentrated in vacuum at room temperature using a rotary evaporator. The residue obtained with both methods was stored in a freezer at −20 °C to further evaluation.

Phenolic Compounds Analysis

The LC–MS and LC–MS/MS analysis were carried out on a liquid chromatography Surveyor Plus Modular LC system connected to a LCQ Duo ion trap mass spectrometer equipped with an electrospray ionisation (ESI) source, from Thermo Scientific (Bremen, Germany). A column LiChroCART® 250-4 LiChrospher® 100 RP-8 (5 µm) was used. The extract was analysed by HPLC injecting 25 µl (10mg/mL) with an auto injector, and using a gradient composed of solution A (1.0% formic acid), and solution B (methanol) as following: 0 min, 80% A, 20% B; 20 min 20% A, 80% B; 25 min, 20% A, 80% B, with a flow of 1,000 mL/min. The mass spectrometer was operated in both positive and negative ion modes in the range m/z 120–1000 and the parameters were adjusted in order to optimize the signal-to-noise ratios (S/N) for the ions of interest. The nebulizing and auxiliary gas (nitrogen) flow rates were, respectively 40 and 20 (arbitrary units) and the capillary temperature was set to 250 ^{o}C. Collision induced dissociation (CID) experiments were performed by isolating the ions within the ion trap and accelerating them in order to suffer multiple collisions with the background gas present in the ion trap (helium) using a data dependent acquisition mode. The ions of interest were activated by applying a percentage of a supplementary a.c. potential in the range of 0.75–1.75 Vp–p (peak-to-peak) to the end cap electrodes of the ion trap at the resonance frequency of the selected ion (referred to as the Normalized Collision Energy, NCE). The injection times were 50 ms in a full scan and 200 ms in an MS/MS scan. Xcalibur™ software from Thermo Scientific was used to acquire and process the data.

Results

Nutritional Value

As it can be observed in Table 1, Pittosporum undulatum showed a higher percentage of DM than Heddychium gardneriaum (40.39% vs. 16.34%). For crude protein it was observed low values in both plants 7.45% and 6.11% per 100g DM respectively in Heddychium gardneriaum and Pittosporum undulatum. Regarding ADF and ADL, both plants presented identical values, differences of less than 1%. Relatively to NDF the values obtained in Hedychium gardnerianum were higher than the obtained to Pittosporum undulatum (64.5 vs 43.84% in DM%).

Phenolic Compounds

The extracts obtained from the *Pittosporum undulatum* and *Hedychium gardnerianum* were analysed by LS-MS, Figure 1 and 2 respectively. LC-MS identifications for both plants are presented in Table 2, for *P. undulatum* and Table 3, for *H. gardnerianum*. The extract of *P. undulatum* is composed mainly of caffeoylquinic and dicaffeoylquinic acids. In figure 1, peak 4 was identified as chlorogenic acid, peak 10 was identified as 1,3-dicaffeoylquinic acid (cynarin). Peaks 6,7,8,9 and 11 were

identified generically as isomers of the dicaffeoylquinic acid. Peaks 1, 4, and 5 were recognized as isomers of caffeoylquinic acid. Peaks 2 and 3 are caffeic acid derivatives.

Regarding *Hedychium gardnerianum's* extract, in the chromatogram represented in Figure 2 five peaks were subsequently identified as quercetin-3,4'-di-O-beta-glucopyranoside, myricetin rhamnoside, quercetin rhamnoside, gibberellin A8 , gibberellin A1, respectively as peak 1 to 5 (Table 3).

Discussion

In the present study, secondary metabolites produced by *Pittospporum undulatum* and *Hedychium gardnerianum* that might influence animal reproduction at physiological level, as well as their nutritional value were evaluated. Relatively to *Pittosporum undulatum* values found for DM were higher than those described by Borba (2007), but similar to those related by Oliveira (1996). Both of these authors found higher values of crude protein compared to that observed in this study. For NDF, values obtained for *Pittosporum undulatum* were higher than those found by other authors (e.g. Oliveira 1996), being, however values for ADF similar to that found by Borba (2007), but higher than that found by Oliveira (1996). For ADL, it was found a higher value than the found by Oliveira (1996), but much lower than found for Borba (2007); both authors reported higher values of crude ash. These different results can be explained by different season of the year in which plants were harvested or having been harvested in different geographical areas. As known, in summer, DM of plants is much higher than in winter, altering substantially their chemical compounds. By the other hand, the place in which plants were removed from soil can play an important role in their chemical composition. Plant-soil feedback is the phenomenon by which a plant influences biotic or abiotic properties of the rhizosphere which, in turn, influences the performance of that individual or another plant (Ehrenfeld *et al*., 2005).

Changes in the composition of plant species (or of functional groups) modify resource availability for heterotrophic microbial communities in the soil, which, in turn, also modifies their composition (Zak *et al*., 2003). According to Van Soest (1987) the nutritive value and forage quality are consequences of conditions of plant growth, namely, the state of maturity of the plant, the type of soil, diseases and environmental conditions, temperature, light, fertilizer and water available, then each author can have different results from the same plant. In the present study, for *Hedychium gardnerianum*, the results observed for ADF (34.69%) and crude ash (10.04%) were similar to those found by Borba (2007). However, for dry matter, crude protein, and NDF, ADL, it was observed that the values obtained by Borba (2007) were higher than those determined in this study. When compare the results obtained by Oliveira (1996) observed that ADL, NDF and crude ash results were identical. However, the values of CP and ADF were lower than those found by Oliveira (1996). Regarding the dry matter values obtained in this study were higher than those referenced by Oliveira (1996).

Analyzing the results of the nutritional value of these forages, when comparing the results obtained with the values of a straw and ryegrass, referenced to INRA (2007), we observed that both plants have higher crude protein content than straw. As expected the fibber values in these plants over are lower the straw.

If we compare the ryegrass with *Pittosporum undulatum* and *Hedychium gardnerianum* observed that in terms of crude protein values obtained for plants (Table 1) are much lower than those referenced by INRA (2007) for ryegrass (22.3%). Regarding found that NDF values found in *Pittosporum undulatum* (43.84%) is lower than ryegrass (48.2%) the *Hedychium gardnerianum* has a higher content of cell wall (NDF) of the ryegrass.

At the second part of the present study, different secondary metabolites of these plants have been investigated. For *Hedychium gardnerianum* five compounds were identified in which three are flavonoids, quercetin-3,4'-di-*O*-beta-glucopyranoside, myricetin rhamnoside, quercetin rhamnoside,

the latter two compounds are gibberellin A1 and A8. Gibberellins comprise a large group of diterpenoid carboxylic acids, ubiquitous in higher plants. These compounds are defined as a class of related plant hormones stimulating growth in stem and leaves, triggering the germination of seed and breaking bud dormancy. They can be also involved, with auxins, stimulating fruit development (Hedden and Thomas, 2012). As functions of reproductive system of animals are regulated by hormonal control, one can speculate that this natural hormonal status can be disturbed by the ingestion of these plants, resulting in morphological changes of animal's reproductive hormones, stimulating/inhibiting physiological processes of their reproductive functions (Kistanova, 2003). Flavonoids are found in a huge variety of edible plants, fruits and vegetables. As phenolic compounds, which nature and action are similar to animal steroids hormones (Kistanova, 2003), may have negative influences on sexual system of female animals, reducing thus conception rates and increasing embryonic loss (Adams, 1995).

In *Pittosporum undulatum* two of the identified compounds are caffeic derived: chlorogenic acid (caffeoylquinic acid) and cynarin (dicaffeoylquinic acid), which have different properties of which we highlight the anti-inflammatory, antioxidant (Perez-Garcia *et al*., 2000) and enzyme inhibition activity (Koshihara *et al*. 1984; Falé *et al*., 2013). However, according Shan (2009) only have a chlorogenic acid in physiological mechanisms of action acting on the reproductive metabolism of arachidonic acid by inhibition of cyclooxygenase (COX) (Ringbom, 2001). Thus, taking into account that COX is the key enzyme in the biosynthesis of prostaglandins (PG) in the production play a key role for the regulation of oestrus, the recognition of pregnancy and childbirth (Arosh *et al*, 2002). The COX presented two isoforms COX-1 and COX-2. According Langenbach *et al* (1995) COX-1 does not interferes apparently reproductive metabolism in rats. However, Lim *et a*l (1997) disturbed metabolism of COX-2 in rats and found that there were many failures of breeding females, especially in ovulation, fertilization and implantation of embryos. Having regard to the bibliography, we think it is possible that excessive consumption *Pittosporum undulatum* may lead to some changes occurring in the reproductive physiologic mechanism of ruminants, including by way of fertilization of the egg or the difficulty of the implementation of the embryos. Thus producers supplying these shrub animals may have empirically observed a decline in reproductive performance of dairy cattle.

Figures and Tables:

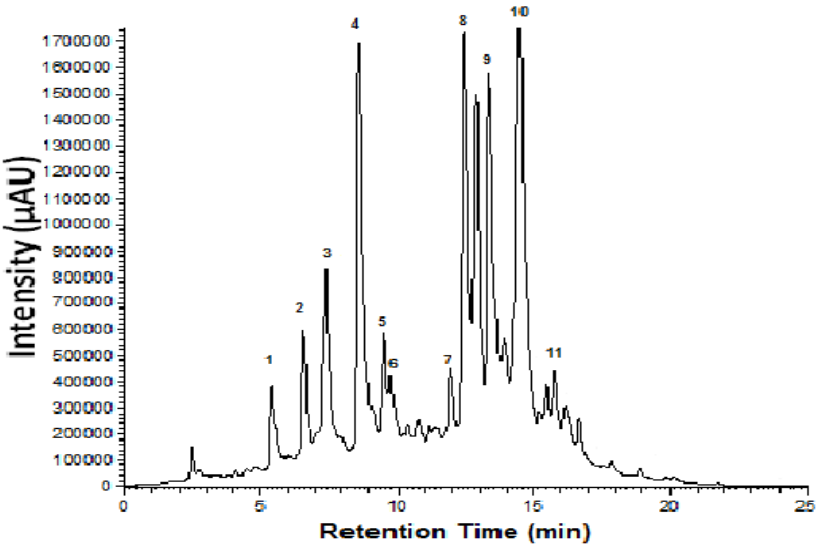

Figure 1: Chromatograms of the extracts of *Pittosporum undulatum* in aqueous medium

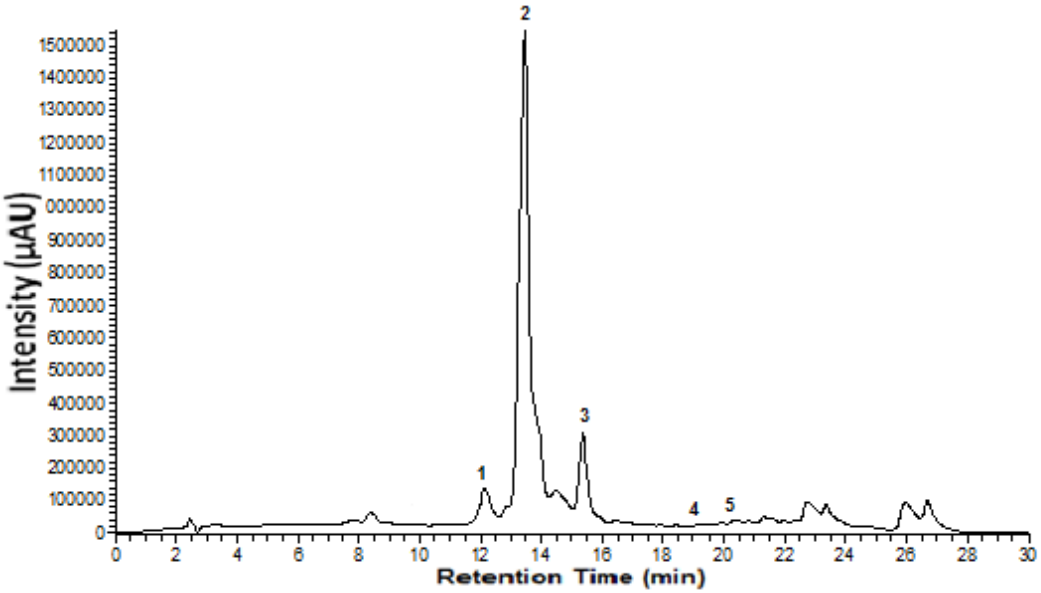

Figure 2: Chromatograms of the extracts of *Hedychium gardnerianum* in aqueous medium

Table 1: Results of chemical analysis of *Pittosporum undulatum* and *Hedychium gardnerianum*

Samples	%DM	For 100g DM					
		Ash	CP	NDF	ADF(%)	ADL(%)	EE
Hedychium gardnerianum	16.34	10.04	7.75	64.5	34.69	3.47	2.03
Pittosporum undulatum	40.39	6.64	6.11	43.84	35.57	3.56	2.71

Table 2: Main components of the of *Pittosporum undulatum* in aqueous medium

Peak	Compound	Ion (m/z)	Product ions m/z
1	Caffeoylquinic acid	353	-
2	Caffeic acid hexoside	341	281;251;222;179;149
3	Caffeic acid hexoside	341	-
4	Chlorogenic acid	353	191; 179
5	Caffeoylquinic acid	353	-

6	Dicaffeoylquinic	515	353;335;179
7	Dicaffeoylquinic	515	353;316;298;203
8	Dicaffeoylquinic	515	353;335;299;255;179
9	Dicaffeoylquinic	515	353;335;179
10	Dicaffeoylquinic (cynarin)	515	353;335;317;299
11	Dicaffeoylquinic	515	353; 317; 299; 173

Table 3: Main components of the of *Hedychium gardnerianum* in aqueous medium

Peak	Compound	Ion (m/z)	Product ions m/z
1	Quercetin-3,4´-O-di-beta-glucopyranoside	625	478;316
2	Myricetin rhamnoside	463	317
3	Quercetin rhamnoside	447	301
4	Gibberellin A8	363	301;275
5	Gibberellin A1	347	303;285;221

Conclusions

Results of the present study indicate that, although these plants can be used as the basis of ruminant nutrition, farmers must pay attention as these plants have low nutritional value, as well as if their secondary compounds can influence reproduction physiological mechanisms. Further studies are, however, necessary to evaluate if plant metabolites can be identified in bloodstream as well as the possible interactions occurring in the rumen.

Acknowledgements

The first author is financed by the Azorean Agency for Science and Technology, Grant BD M3.1.2/F/012/2011. CITA-A is also fully acknowledged.

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