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Secondary compounds characterization in some autochtonous species from a North-Eastern region of Tunisia

A. Bettaieb[1], N. Moujahed[1]* and R. Ksouri[2]

[1]Institut National Agronomique de Tunisie, LRAA, 43 Av Ch. Nicolle, 1082 Tunis (Tunisia)
[2]Centre de Biotechnologie de Borj Cédria, LPE, BP 901, 3050 Hammam-lif (Tunisia)
*E-mail: nizar.moujahed@yahoo.fr

Abstract. Several native species were collected in the region of Zaghouan (eastern-north of Tunisia, semi-arid) in order to evaluate their chemical diversity for an eventual animal use context, during the period of spring 2011. Some secondary compounds were determined by colorimetric quantification in leaves and twigs. Essential oils from these species were extracted by hydro-distillation and then analyzed using gas chromatography technique, coupled with mass spectrometry (GC/MS). Results showed that among the studied species, *Myrtus communis* presented the highest total polyphenol (101.3 mg GAE/g DM), while *Artemisia herba-alba* and *Ruta Chalepensis* were the lowest ones (13.2 and 13.7 mg GAE/g DM respectively). Condensed tannin content in *Juniperus phoenicea* (43.8 mg CE/g DM) was largely higher than in all the other species, in which it ranged approximately between 1 and 5 mg/g DM. In addition, saponin content was the utmost in *J. phoenicea* (25.2 mg/g DM) as compared to the others species, where it varied between almost 6 and 13 mg/g DM. The amount of flavonoids varied widely between 28.6 and 5.1 mg/g DM in *Mentha pelagium* and *M. communis* respectively. Essential oils of all species were composed of wide pools of identified components. For instance, the main compounds were 1.8-cineol in *Rosmarinus officinalis* (39%), thymol (61.3%) in *Thymus vulgaris*, and carvacrol (64.8%) in *A. herba-alba*. It was concluded that the vegetal diversity in the studied area presented a consequent chemical diversity, available for animals as fed in grazing or in housing conditions. These bioactive molecules, mainly essential oils, may be used for their antimicrobial actions to improve animal health and performance. In this connection, *in vitro* studies are currently carried out in our laboratory.

Keywords. Secondary compounds – Polyphenols – Condensed tannins – Saponins – Flavonoids – Essential oils.

Caractérisation des composés secondaires de certaines espèces autochtones d'une région du Nord-Est de la Tunisie

Résumé. *Plusieurs espèces natives ont été recueillies dans la région de Zaghouan (nord-est de la Tunisie, semi-aride) au cours de la période de printemps 2011, afin d'évaluer leur diversité chimique pour une éventuelle utilisation dans un contexte d'alimentation animale. Certains composés secondaires dans les feuilles et les brindilles ont été déterminés par quantification colorimétrique. Les huiles essentielles de toutes les espèces ont été extraites par hydro-distillation puis analysées à l'aide de la chromatographie en phase gazeuse, couplée à la spectrométrie de masse (GC/MS). Les résultats ont montré que, parmi les espèces étudiées, la teneur en polyphénols totaux était la plus élevée chez* Myrtus communis *(101,3 mg/g MS), tandis qu'elle était la plus basse chez* Artemisia herba-alba *et* Ruta chalepensis *(13,2 et 13,7 mg/g MS, respectivement). La teneur en tanins condensés chez* Juniperus phoenicea *(43,8 mg/g MS) a été largement supérieure à celle de toutes les autres espèces et variait approximativement entre 1 et 5 mg/g MS. La teneur en saponines étaient la plus basse chez* J. phoenicea *(25,2 mg/g MS) et variait entre près de 6 et 13 mg/g MS chez les autres espèces. Les flavonoïdes variaient considérablement entre 28,6 et 5,1 mg/g MS dans* Mentha pelagium *et* M. communis*, respectivement. Toutes les huiles essentielles sont composées d'une large gamme de composants identifiés. Les principaux d'entre eux étaient, par exemple, 1.8-cinéole dans* Rosmarinus officinalis *(39%), le thymol (61,3%) dans* Thymus vulgaris *et le carvacrol (64,8%) dans l'*A. herba-alba*. Il a été conclu que la diversité végétale dans la zone étudiée a présenté une diversité chimique considérable, disponible pour les animaux nourris que ce soit au pâturage ou dans des conditions de stabulation. Ces substances, principalement des huiles essentielles, peuvent être utilisées pour leurs actions antimicrobiennes pour améliorer la santé des animaux et leurs performances. À cet égard, des études* in vitro *sont actuellement menées dans notre laboratoire.*

Mots-clés. *Composés secondaires – Polyphénols – Tannins condensés – Saponines – Flavonoides – Huiles essentielles.*

I – Introduction

The interest for secondary metabolites (tannins, essential oils...) in native plants is more and more growing in animal production, particularly after the removal of antibiotic growth promoters. This led to an increased interest in alternative means of manipulating rumen fermentation. Indeed, recent researches showed several secondary metabolites, such as tannins and saponins, to improve feed nutritional value, digestion, production and reproduction of ruminants (Min *et al.*, 2003; Makkar *et al.*, 2007; Mao *et al.*, 2010) when used in particular doses. For essential oils, their main effects were to reduce protein and starch degradation and to inhibit the amino acid degradation due to selective action on certain rumen microorganisms (Benchaar *et al.*, 2008). The aim of this work was to determine, as a first step, the content of secondary compounds (some secondary metabolites and essential oils) in some of these native species from a North-Eastern region of Tunisia, in order to study at a second step their effects in small ruminant nutrition, mainly in extensive conditions.

II – Material and methods

Leaves and stems of 9 native species (*Rosmarinus officinalis, Thymus vulgaris, Mentha pelagium, Myrthus communis, Juniperus phenicea, Ruta chalepensis, Pistacia lentiscus, Lavandula stoechas*, and *Artemisia herba-alba*) were collected from the region of Zaghouan (North-East of Tunisia, semi-arid) during the period of spring 2011.

For secondary compounds determinations, each specie sample was dried at 40°C during 48 h and then ground and stored in obscurity. Extracts were obtained by using methanol/water (80v/20v). Methanolic secondary metabolites were analyzed for their total phenolic, flavonoid, condensed tannins and saponin contents.

Total phenolic, flavonoid, condensed tannin and saponin contents were determined as described by Dewanto *et al.* (2002), Zhishen *et al.* (1999), Sun *et al.* (1998) and Hiai *et al.* (1976), respectively.

Essential oils from the studied species were extracted using hydro-distillation on 10-days air-dried biomass. Their chemical composition was assessed using gas chromatography coupled with mass spectrometry (GC/MS). This analysis was performed on an HP 5972 mass spectrometer (Agilent technologies, Palo Alto, California, USA) with electron impact ionization (70 eV). An HP-5MS capillary column (30 m-0.25 mm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 lm film thickness) was used.

III – Results and discussion

1. Secondary metabolites

Secondary compounds contents are given in Table 1. *Myrtus communis* was the highest in polyphenols (101.30 mg GAE/g DM) while *Ruta chalepensis* and *Artemisisa herba-alba* were the lowest (about 13 mg GAE/g DM). Condensed tannins content in *Pistacia lentiscus* (56.82 mg CE/g DM) and *Juniperus phoenicea* (43.75 mg CE/g DM) were largely higher than in all the other species, where the proportion ranged approximately between 1 and 6 mg CE/g DM. *Juniperus phoenicea* exhibited the highest saponin content (25.17 mg DE/g DM), which varied in the other species between 6 and 19.5 mg DE/g DM in *Artemisia herba-alba* and *Pistacia lentiscus,* respectively. Flavonoïds concentrations were comprised between 28.6 and 5.1 mg CE/g DM in *Mentha pelagium* and *Myrtus communis* respectively.

When compared with literature, Gardely *et al.* (2008) mentioned 352 mg and 588 mg GAE/g DM of total phenolic for *Myrtus communis* and *Pistacia lentiscus* respectively, while Abidi-Wannes *et al.* (2010) found a variation between different myrtle parts ranging from 33.67 and 11.11 mg

GAE/g for flower and steam respectively. The authors found that for flavonoids and condensed tannin, the highest values were observed in steam (5.17 and 1.99 mg GAE/g respectively).

Table 1. Secondary compounds contents of the studied species

Species	Vegetative stage	Total polyphenols mg GAE†/g DM	Flavonoïds mg CE††/g DM	Condensed tannins mg CE/g DM	Saponins mg DE†††/g DM
Rosmarinus officinalis	Flowering	22.74	22.40	1.37	7.85
Thymus vulgaris	Flowering	20.50	10.71	1.34	7.18
Mentha pelagium	Flowering	56.20	28.56	2.18	7.01
Myrtus communis	Flowering	101.30	5.14	6.39	7.84
Juniperus phenicea	Fruiting	47.42	14.37	43.75	25.17
Ruta chalepensis	Fruiting	13.68	6.50	1.41	12.36
Pistacia lentiscus	Fruiting	95.19	13.85	56.82	19.46
Lavandula stoechas	Flowering	23.10	25.78	3.30	12.01
Artemisia herba alba	Flowring	13.20	15.41	1.91	6.37

†GAE: gallic acid equivalent.
††CE: catechin equivalent.
†††DE Diosgenin equivalent.

2. Chemical composition of essential oils

A wide variety of components was identified for the different substrates. As an example, for *Ruta chalepensis,* more than 70% of EO was composed of 2-undecanone. Carvacrol and thymol represented a mean of 63% of *Artemisia herba-alba* and *Thymus vulgaris* EO. Finally, about 30% of essential oils consisted of α-pinene for *Myrtus communis* and *Pistacia lentiscus* species.

When compared to earlier studies, our results concerning rosemary, confirmed those of Moujahed *et al.* (2010) and Zaouali *et al.* (2008) about the presence of 1-8 cineol, camphor, and α-pinene. For *Thymus vulgaris,* our results are on line with several other findings in which thymol was the chimiotype (about 45%), with the abundance of Q-terpinene, b-cymene, and b-caryophyllene (Hudaib *et al.,* 2002; Ozcan and Chalchat, 2004). Our finding relative to *Myrtus communis* confirmed those of Yadegarinia *et al.* (2006) and Gardely *et al.* (2008) who found that α-pinene, limonene and linalool are the major molecules in the EO of this specie. In connection with the studies of Mansouri *et al.* (2011) on *Juniperus phenicea*, we found α-pinene to largely dominate the composition of *Juniperus* EO. In other studies (Mejri *et al.*, 2010), the analysis of *Ruta chalepensis* EO showed that 2-undecanone and 2-decanone exhibited the highest rate, confirming thereby our findings. For *Pistacia lentiscus*, our results are similar to those of Gardely *et al.* (2008) concerning the abundance of α-pinene. Simultaneously, the last authors mentioned a higher rate of limonene (17.8%).

IV – Conclusions

The vegetal diversity in the studied area presented a consequent chemical diversity, available for animals as fed in grazing or in housing conditions. These substances, mainly essential oils, may be used for their antimicrobial actions to improve animal health and performances. In this connection, *in vitro* studies are currently carried out in our laboratory.

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