Effect of Watery and Alcoholic Medicinal Plants Extractions on *In-Vitro* Ruminant Digestibility

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Received (Geliş): **11.06.2014** **Accepted** (Kabul): **27.10.2014**

Abstract: Two methods of extraction of some medicinal plants, water and alcoholic extractions for *Nigella sativa*, rosemary, borage, lemon balm and licorice root (*Glycyrrhiza glabra*) were employed to test their effects on *in-vitro* rumen digestion trial. Total phenolics contents significantly increased in their water extractions in contrast to their alcoholic extractions. Dry matter digestibility (DMD) significantly increased the water extractions of lemon balm, rosemary and licorice root: 68.47, 70.16 and 73.49 % compared to the control with 76.92%, respectively. Similar trend was observed with organic matter digestibility values (OMD): 69.71, 72.93 and 74.11% compared to the control with 77.51%, respectively. However, there was an overall significant increase in the studied digestion coefficient values with alcoholic extractions of the same plants. In conclusion, the alcoholic extractions of some medicinal plants can be recommended to be used in ruminant nutrition when these medicinal plant extracts are to be included in their feed for better digestibility values of dry matter and organic matter.
Key Words: Medicinal plants, in vitro, digestibility

INTRODUCTION

Most of medicinal plants have been used as remedies for human diseases because of therapeutic value. In addition some medicinal plants such as *Nigella sativa* (Black Cumin or Kalonji) oils 36-37%, crude protein 20-23% and rosemary were used as additive feed in ruminant animals to improve growth rate and carcass characteristics of Awassi lambs (Hassan and Hassan, 2010; Hassan et al., 2011). Nowadays, medicinal plants extract used as natural preservative in food, cosmetic and pharmaceutical industries. Rababah et al. (2004) work on plant extracts such as green tea and grape seed, the extracts can be used to retard lipid oxidation in a variety of food products. The importance of extracts was to high antioxidants content. Phenolics are the highest contents and most important as antioxidant effect in the medicinal plant extractions, the variety of its concentration due to different factors: Way and time of extraction, harvesting time and the part of plant using for extraction. Phenolics compound reacts as antioxidants because of its ability to be resonance-stabilized. There are a lot of phenolics, there effect to the kind of phenolic composition (quality) does not depend on the high content of total phenolics (quantity) in plant or solution (Mhamdi et al., 2010). While ruminant grazing on different kinds of medicinal plants in pasture, phenolics affect negatively on rumen microorganisms activity (Hassan and Tawffek, 2009 and Hassan et al., 2011). So, we select the most common wiled medicinal plants with two extraction methods, watery and alcoholic extraction, to investigate the nutritive value by *in-vitro* ruminant digestion coefficient. The medicinal plants were: *Nigella sativa*, rosemary, borage, lemon balm and licorice root (*Glycyrrhiza glabra*).

MATERIAL and METHODS

Preparation and Extraction of Samples

All medicinal plants used in this research were harvested from the department of medicinal plant in Agriculture College/Baghdad University, sampled and dried at 65°C to loss most of the wet then grounded at sieving 1mm. Two methods of extractions were used at room temperature: watery and ethanol extraction (Al-Sarraj et al., 1985). In watery method, take 50gm of samples and put each one in beaker 500ml, fill it with boiled distil water for 30 minutes in stirrer then filtered with cheese cloth extraction, take 50gm of samples with 250 ml of absolute ethanol for 24 hours, filtered and concentrated to dryness using a rotary evaporator at 50 – 60 ^{0}C.

Preparation of Ration

General ruminants ration for in vitro trial was used as in table 1 and applied at two stages (96h.) of in vitro trial as in Tilley and Terry (1963) as following: Rumen fluid was collected from slaughtered sheep and incubated in water bath at 38°C. Preparing artificial saliva or buffer solution which consisted of two solutions, first: McDougall's Buffer solution, 49gm $NaHCO_3$ + 18.6gm Na_2HPO_4 dissolve in 800ml distilled water. Second: Cloride solution, 28.5gm KCl + 23.5gm NaCl + 6gm $MgCl_2.7H_2O$ + 2gm $CaCl_2$ dissolved in 1000ml distilled water. Add 100ml from the second solution to the first one (800ml) and complete the volume up to 1000ml then incubated in 38°c water bath. Preparing *in-vitro* digestion tubes with 0.5gm milled rations through a 1mm sieve, add 50 ml of solution consisted of 40ml artificial saliva and 10ml rumen fluid to each tube then incubated for 48h in shaker water bath at 38°c with gentle shaking two times a day. After finished 48h centrifuged the tubes and re-incubated the

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supernatant 48h with 50 ml. of 0.2% pepsin solution (dissolved 0.2gm pepsin in 100 ml. 0.1N HCl solution). After finished the second 48h., centrifuged the tubes and dry it 105°c overnight. Weight then ash the supernatant at 600°c for 5 h. And determine *in-vitro* dry matter digestibility and organic matter digestibility.

The chemical analysis of grounded ration through a 1mm screen was determined according to AOAC (2005) for dry matter (DM), wet, organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), ash and nitrogen free extract (NFE).

Table 1. Formulation & chemical composition of ingredients and concentrate diet used in the *in-vitro* trail (% as dry matter basis)

Chem. Comp.	%	DM	OM	CP	EE	CF	NFE
Ingredients							
Barley	45	95.15	94.31	12.11	2.31	4.18	75.71
Soybean meal	12	96.73	93.91	46.10	2.19	3.35	42.27
Wheat bran	40	95.33	95.88	14.89	2.52	15.89	62.58
Bentonite	1	------	------	------	------	------	------
Min. & Vit.	2	------	------	------	------	------	------
Concentrate	100	95.41	94.89	16.22	2.38	10.19	66.10

Determination of Total Phenolics

The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent. Tannic acid was used as a standard (figure 1.) and the total phenolics were expressed photometrically as tannic acid equivalents to 100ml of extract using Lambert-bear law "$A = A_s*B*C$" Where: A(absorption), A_s(0.966) from the standard curve, B(cell diameter =1) and the C (total concentration of phenolics) then recalculated as %.

The Folin-Ciocalteu reagent reducing compounds including polyphenols and producing blue color which measured spectrophotometrically at 760nm (Folin, and Denis, 1912).

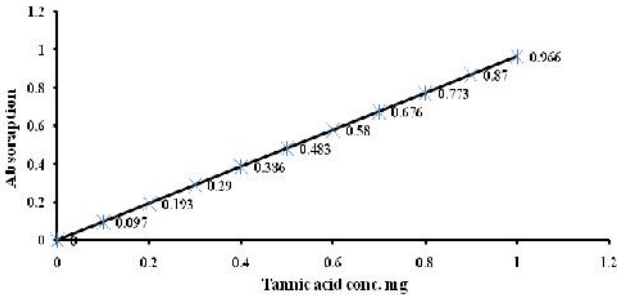

Figure 1. Phenolics standard curve as tonic acid conc.

Statistical Analysis

For statistical analysis, 2×5 factorial experiments were used in a completely randomized factorial design (SAS, 2001) and Duncan's multiple ranges tested was used to determine the significant differences between extractions means (Duncan, 1955).

RESULTS and DISCUSSION

Method of Medicinal Plant Extraction

In this study, the watery extractions having high significance content of total phenolics in contrasting with alcoholic extractions (Table 2). Phenolics don't soluble in ether or chloroform and other hydrophobic solvents but high soluble in water, alcohol and other hydrophilics (Joslyn, 1950). Water one of the very strong solvents because of its negative and positive charges. So, it can dissolve high amount of phenolics in contrast of alcohol. Sunita and Dhananjay (2010) found the maximum phenolic content in the aqueous extract (92.4±0.14 mg/g).

***In-vitro* dry Matter and Organic Matter Digestibility**

In-vitro ruminants digestion trial was studied with or without addition watery extractions for some medicinal plants: *Nigella sativa*, rosemary, borage, lemon balm and licorice root in contrast with general ruminants ration as control diet (Table 3) then reinvestigated with or without alcoholic extractions for the same medical plants (Table 4). Results indicated that the addition of watery extractions affected negatively ($p<0.01$) on dry matter digestibility (DMD) with lemon balm, rosemary and licorice root 68.47%, 70. 16%, 73.49% vas control (76.92%) and same effect for organic matter digestibility (OMD) 69.71%, 72.93%, 74.11% in contrast with control (77.51%). Negatively increasing the concentration of phenolics affected on rumen microorganisms activity and depress the digestion coefficient in contrast with control. Tanja and Barbara (2009) using rosemary extracts as antimicrobial natural additives to prevent the proliferation of microorganisms or protect food from oxidation by using essential oils or plant extracts as natural additives in foods.

In contrast, the addition of alcoholic extraction (table 4.) increasing digestion coefficient (DMD) from 77.49% for the control diet up to 80.72%, 83.04%, 86.98% and 88.11% for rosemary, licorice root, borage and nigella sativa respectively and the same for OMD. This because of two rezones: First, low total phenolics content by alcoholic extraction (Table 2) and second, alcohol can dissolve low chain fatty acids which having great positive effect on increasing ruminant microflora as source of energy (Tawffek and Hassan, 2014) and 60.8% of oil in *Nigella sativa* seeds as linoleic acid (essential fatty acids) Nergiz and Otles (1993). No

differences in digestion coefficient between two methods of extraction, but there are a significance increasing in digestion coefficient with the addition of alcoholic extractions for dry matter content (figure 2.) and for organic matter (Figure 3.). The results agree with Zanouny et al. (2013) to increase digestibility and body weight when *Nigella sativa* added to the ration of Ossimi male lambs.

Table 2. Total phenolics content in watery and alcoholic extractions for some medicinal plants (mg/ 100ml of extraction)±SD

Treatments	Watery extractions phenolics content	Alcoholic extractions phenolics content
Nigella sativa	0.279 [a] ±0.06	0.155 [b] ±0.07
Rosemary	0.408 [a] ±0.04	0.283 [b] ±0.05
Borage	0.271 [a] ±0.02	0.107 [b] ±0.04
Lemon balm	0.327 [a] ±0.02	0.113 [b] ±0.01
Licorice root	0.795 [a] ±0.02	0.355 [b] ±0.01

Different letters in the same raw indicate differences at 0.01 significance level

Table 3. Effect of watery extractions for some medicinal plants on *in-vitro* dry and organic matter digestibility (%)±SD

Treatments	*In-vitro* dry matter digestion coefficient	*In-vitro* organic matter digestion coefficient
Control	76.92 [a] ±0.42	77.51 [a] ±0.75
Nigella sativa	77.64 [a] ±0.71	78.55 [a] ±0.34
Rosemary	70.16 [b] ±0.76	72.93 [b] ±0.78
Borage	76.51 [a] ±0.49	77.37 [a] ±0.23
Lemon balm	68.47 [b] ±0.61	69.71 [b] ±0.24
Licorice root	73.49 [b] ±0.42	74.11 [b] ±0.45

Different letters in the same column indicate differences at 0.01 significance level

Table 4. Effect of alcoholic extractions for some medicinal plants on *in-vitro* dry and organic matter digestibility (%)±SD

Treatments	*In-vitro* dry matter digestion coefficient	*In-vitro* organic matter digestion coefficient
Control	77.49±0.34[c]	79.08±0.58[c]
Nigella sativa	88.11±0.67[a]	90.02±0.72[a]
Rosemary	80.72±0.75[b]	82.50±0.37[b]
Borage	86.98±0.56[a]	88.84±0.29[a]
Lemon balm	78.98±0.70[c]	81.23±0.34[c]
Licorice root	83.04±0.38[b]	87.78±0.35[a]

Different letters in the same column indicate differences at 0.01 significance level

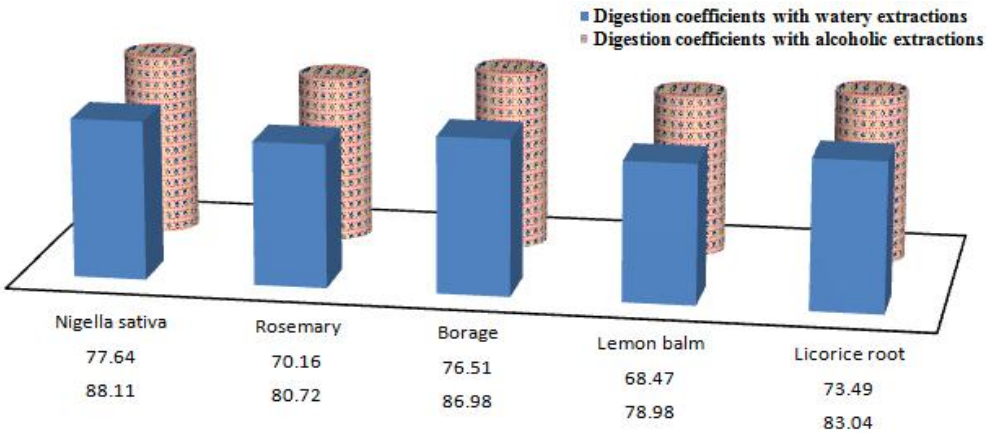

Figure 2. In-vitro dry matter digestion coefficient with watery or alcoholic extractions for some medicinal plants

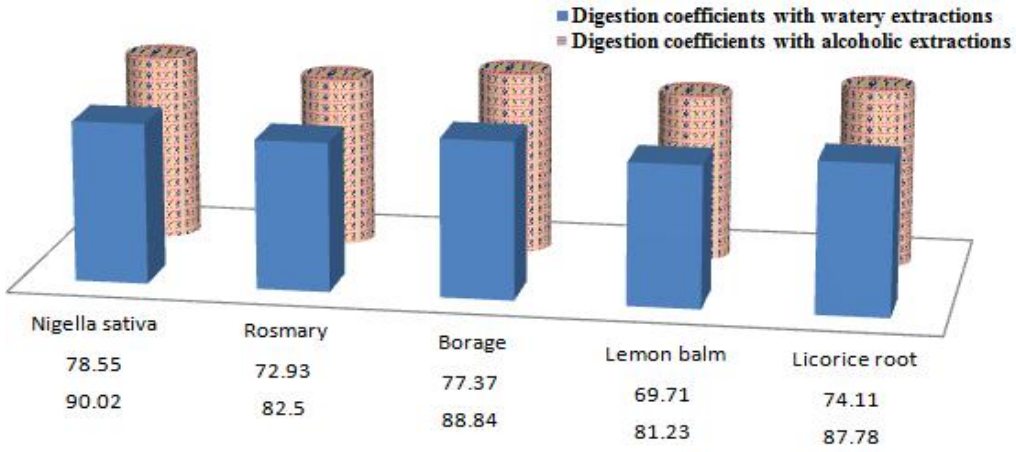

Figure 2. In-vitro organic matter digestion coefficient with watery or alcoholic extractions for some medicinal plants

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