Comparative study of biological activities of *Crocus sativus* L. extracts and *Lamiaceae* plants' extracts

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The use of aromatic plants and herbs in medicines is since humans inhabited earth. The Mediterranean basin has been distinguished throughout generations with a rich record of medicinal herbs. Aromatic plants are excellent sources of bioactive compounds that can be extracted using several processes. In the current study, different extracts of *Origanum dictamnus* L. leaves (dittany), *Melissa officinalis* L. leaves (lemon balm) and *Crocus sativus* L. stigmas (saffron) were tested as potential natural antoxidant and antimicrobial agents.

Sample preparation.

Plants were subjected to sequential extraction with petroleum ether, diethyl ether and methanol, as shown in figure 1.

All extracts were evaporated under reduced pressure and dried using rotary evaporator. Dried extracts were stored in labeled screw capped bottles at -20°C.

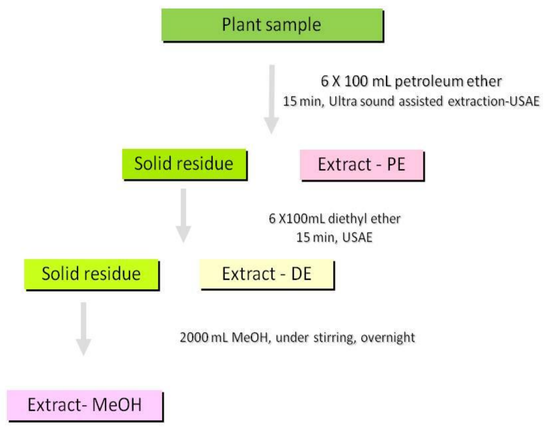

Figure 1. *Schematic plan of extraction procedure*

Antioxidant activity.

Antioxidant activity of extracts were tested applying both the ABTS assay -2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) and the DPPH assay (2, 2-diphenyl-1-picrylhydrazyl radical). Results were expressed as TEAC in μmol of Trolox/100g extract..

Methanolic extracts of all plants were the most potent antioxidant followed by the diethyl and petroleum ether extracts. However, *Lamiaceae* methanolic extracts had higher antioxidant activity than the corresponding *Crocus sativus* L. extracts.

Table 1*. Antioxidant activity of plant extracts*

		TEAC (μmol TROLOX/100 g extract)	
Plant	**Extract**	**ABTS**	**DPPH**
Dittany	Petroleum ether	19,4±1,0	8,7±2,8
	Diethyl ether	9,8±2,4	6,7±0,3
	MeOH	88,9±12,0	65,9±9,0
Lemon balm	Petroleum ether	1,1±0,5	1,2±0,4
	Diethyl ether	22,2±1,2	47,1±0,6
	MeOH	135,8±7,7	183,0±2,0
Saffron	Petroleum ether	2,1±0,5	2,6±0,5
	Diethyl ether	0.22±0.05	*
	MeOH	7,0±0,3	**

* Pro-oxidant behavior ** Interference

The chemical composition of methanolic extracts of *Lamiaceae* revealed that the major compound was rosmarinic acid. The antioxidant properties reported for lemon balm and in general for *Lamiaceae* plants relate primarily to the rosmarinic acid content [1,2]. Its methanol extract contained 90% rosmarinic acid, while the dittany's extract only 58,4%, reflecting the lower TEAC values of the latter.

Their diethyl ether extracts were rich in flavones (luteolin, apigenin, genkwanin, cirsimaritrin) and flavonols (kaempheride, kaempherol). As for diethyl ether saffron extract, the predominant compound was picrocrocin, however this compound has not been reported as an antioxidant agent. Moreover, other compounds not been indentified in the extract acted as pro-oxidant agents [3,4]. Crocins are the major constituents isolated from the methanolic extracts of saffron stigmas. Ambiguous studies exist for antioxidant activity of saffron stigmas[4-7]. The structure of the crocins provides a chromophoric system which leads to interference in the DPPH assay, that was also evident in the current study and it has also been cited as a drawback of the assay [8].

In petroleum ether extracts the major compounds were carvacrol, citral (neral and geranial) and safranal in ditanny, lemon balm and saffron, respectively.

Antibacterial activity.

A concentration of 25mg/mL of all extracts was tested against oral pathogens bacteria (*Streptococcus gordonii* LMG 14518^T, *Str. mutans* LMG 14558^T, *Str. oralis* LMG 14532^T, *Str. salivarius* LMG 11489^T, *Str. sanguinis* DSM 20068, *Str. sobrinus* LMG 14641^T), food pathogens bacteria (*Escherichia coli* CFA-I, *Escherichia coli* C1845, *Salmonella typhimurium* SL1344) and food spoilage bacteria (*Bacillus cereus* LMG 6923^T, *Bacillus licheniformis* FMCC B-91, *Bacillus subtilis* FMCC B-109) by the well diffusion assay -WDA.

Screening of plants extracts against oral pathogens.

Diethyl ether and methanol extracts of saffron stigmas induced the highest bactericidal effect against the tested bacteria, followed by petroleum ether extracts. The same tendency was also observed in lemon balm extracts. The petroleum ether extract of dittany was more potent followed by diethyl and methanolic extracts. *Str. mutans* LMG 14558^T and *Str. salivarius* LMG 11489^T were more resistant to methanolic extracts.

The chromatographic analysis of the extracts revealed that dittany's petroleum extract was rich in carvacrol, a phenolic terpene that posses strong antimicrobiological activity [9]. The respective extracts of the other two plants showed the existence of neral for lemon balm and safranal for saffron as the major compounds, however, these two compounds aren't so potent as carvacrol [9,10]. Rosmarinic acid found at high levels in methanol extract of *Lamiaceae* plants did not show antibacterial activity against *Str. mutans*, *Str salivarius* and *Str. sanguinis*. Diethyl ether extracts of lemon balm and dittany exhibit also antibacterial activity mostly due to flavones and flavonols predominant on these extracts. Scarce literature exist for saffron stigmas. The methanol extract rich in crocins showed better results compared to petroleum ether extract, but both lag besides to diethyl ether extract.

Table 1*. Antimicrobial activity of plant extracts towards six Streptococcus strains as determined by the well diffusion assay*

		Inhibition (diameter, mm)					
Plant	**Strain/ Extract**	***Streptococcus gordonii* LMG 14518^T**	***Streptococcus mutans* LMG 14558^T**	***Streptococcus oralis* LMG 14532^T**	***Streptococcus salivarius* LMG 11489^T**	***Streptococcus sanguinis* DSM 20068**	***Streptococcus sobrinus* LMG 14641^T**
Dittany	Petroleum ether	25	25	18	24	16	25
	Diethyl ether	25	25	22	17	12	14
	MeOH	13	0	13	7	10	12
Lemon balm	Petroleum ether	8	0	7	0	0	9
	Diethyl ether	14	12	21	11+6	14	10
	MeOH	7	0	9	9	9	0
Saffron	Petroleum ether	8	12	8	0	0	8
	Diethyl ether	20	25	22	20	23	25
	MeOH	12	7	13	8	13	14

Screening of plants extracts against food pathogens and food spoilage bacteria.

Table 2*. Antimicrobial activity of plant extracts towards food pathogens bacteria*

		Inhibition (diameter, mm)		
Plant	**Strain/ Extract**	***Escherichia coli* CFA-I**	***Escherichia coli* C1845**	***Salmonella typhimurium* SL1344**
Dittany	Petroleum ether	11	13	10
	Diethyl ether	11	12	12
	MeOH	0	0	0
Lemon balm	Petroleum ether	0	0	0
	Diethyl ether	7	7	7
	MeOH	0	0	0
Saffron	Petroleum ether	0	0	0
	Diethyl ether	18	18+7	15
	MeOH	0	0	0

Table 3*. Antimicrobial activity of plant extracts towards food spoilage bacteria assay*

		Inhibition (diameter, mm)		
Plant	**Strain/ Extract**	***Bacillus cereus* LMG 6923^T**	***Bacillus subtilis* FMCC B-109**	***Bacillus licheniformis* FMCC B-91**
Dittany	Petroleum ether	16	24	24
	Diethyl ether	18	14	12
	MeOH	7	0	7
Lemon balm	Petroleum ether	8	10	10
	Diethyl ether	8	10	11
	MeOH	7+8	0	8+4
Saffron	Petroleum ether	0	0	0
	Diethyl ether	20	20	20
	MeOH	9	7	7

In case of pathogens, only the diethyl ether extracts of all plants, as well as the petroleum ether extract of dittany posed antibacterial activity. Studying the effect of saffron on different *Salmonella* strains during storage at room temperature, Pintado et al., 2011 [11] concluded that safranal and crocin concentrations in saffron are not high enough to be the only determinants of antibacterial activity. The above is in accordance with the results on Gram negative bacteria, indicating that other compounds, extracted with the diethyl ether, are responsible for its bactericidal effect.

Diethyl ether extracts of *Lamiaceae* plants possessed activity in contrast to methanol ones, revealing that among the phenolic compounds found in the extracts, flavonoids were active against the pathogens tested, while the hydroxycinnamic acids had no or low activity. The same resulted when *Rosmarinus officinalis* extracts were investigated for antimicrobiologiacl activity [12].

The activity of dittany petroleum ether extract is strongly correlated to its high concentration of carvacrol [9,10].

As for the food spoilage bacteria, all *Lamiaceae* plants extracts showed activity, apart from the methanolic extracts that did not have any activity against *Bacillus subtilis*. Petroleum ether extract of saffron had no activity against all three *Bacillus* strains.

It is interesting that among *Bacillus sp.*, the extracts have different behavior.

Overall, major bacteriostatic effects were exerted against Gram positive bacteria. The double layer of phospholipids on the cell wall of Gram negative bacteria, as well the level of hydrophobicity of each compounds of extracts seem to be important to the inhibitory effect on bacteria growth.

Conclusions.

No correlation was detected between antioxidant and anti microbiological activity, indicating the different secondary metabolites of each plant and extract are responsible for the above activities. Further phytochemical studies are also warranted to isolate and characterize active ingredients that are responsible for the antimicrobial and antioxidant activities, and to explore the existence of synergism or/and antagonism among the compounds.

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