

Aromatic plants rich in phenolic compounds as a valuable basis for dietary supplements in intestinal motility disorders

M.D. García-Giménez

University of Seville

A. Quilez-Guerrero

University of Seville

M.A. Fernandez-Arche

University of Seville

R. De la Puerta (✉ puerta@us.es)

University of Seville

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Abstract

Plant extracts can be an important adjuvant treatment in gastrointestinal diseases where intestinal transit is involved. Because transit disorders are often associated with infections and inflammation, in our investigation we have selected five aromatic Mediterranean plants with antimicrobial, antioxidant and anti-inflammatory activities which in certain areas are also used for culinary reasons. We evaluated the intestinal transit in mice after oral administration of hydro-alcoholic extracts of *Achillea millefolium* L., *Sideritis angustifolia* Lag., *Rosmarinus officinalis* L., *Matricaria chamomile* L., and *Aloysia citriodora* Palau.

Total content of phenols and flavonoids and their antioxidant activity were previously determined. *Rosmarinus officinalis* showed the highest antioxidant capacity ($p < 0.001$) in the DPPH and ABTS methods with IC_{50} of 48.89 ± 2.98 and 27.28 ± 1.83 $\mu\text{g/mL}$ respectively, in agreement with the highest phenol content.

Oral administration of the extracts to mice and rats showed no signs or symptoms of toxicity in any case. The extracts of *A. millefolium*, *R. officinalis*, and *M. chamomilla* significantly inhibited intestinal transit ($p < 0.01$ and $p < 0.05$) when compared to the castor oil control group, with a percentage of intestinal transit similar to that of the reference antidiarrheal loperamide drug ($47.8 \pm 3.6\%$).

These non-toxic plant extracts constitute a valuable basis to produce dietary supplements for intestinal motility disorders.

Introduction

Diarrhoea is a consequence of alteration in the transport of water and electrolytes in the intestine and has the risk of suffering dehydration and disorders in the balance of hydro-electrolytes. An alteration of the intestinal mucosa, due to an underlying inflammatory process, alters permeability and increases motility.

Acute diarrhoea is normally caused by an infection caused by viruses, bacteria, or parasites, but in the case of chronic diarrhoea, various pathologies can be its cause, including celiac disease, Crohn's disease, irritable bowel syndrome, other inflammatory disorders of the gastrointestinal tract, overgrowth of bacteria in the small intestine, and ulcerative colitis. Prolonged consumption of medications, especially antibiotics, taken therapeutically or ingested in the diet, is also a common cause of diarrhoea.

Medicinal plants constitute the major component of traditional medicine as practised throughout the world. This is due to their economic viability, accessibility and ancestral experiences. Molecules and extracts of natural origin, such as opium and its derivatives or anticholinergics from tropane alkaloids, are widely used in diarrhoeal processes. Thus, for gastrointestinal disorders such as diarrhoea, research on natural products and plant extracts is becoming very important in the search for new therapies. Some aromatic plants with culinary uses as fennel and peppermint and its essential oils have demonstrated to be beneficial in the treatment of irritable bowel syndrome in clinical trials [1, 2]. Furthermore, many herbs

with medicinal and culinary properties containing phenolic such as phenolic acids, phenolic terpenes, flavonoids, tannins, and catechins, have potent antioxidant power and may be effective therapeutic agents [3].

In this study, five vegetal species rich in essential oils and phenolic compounds have been selected for their potential antispasmodic and antiseptic properties that could favour the digestive process and improve intestinal pathologies. These species grow in the Mediterranean region and play an important role in traditional medicine and culinary purpose in some areas. Regarding phenol composition, *Achillea millefolium* L. (Asteraceae) is especially rich in phenolic substances, such as caffeic and salicylic acid, in addition to numerous flavonoids [4]. *Sideritis angustifolia* Lag. (Lamiaceae), in addition to diterpenes, contain important flavonoids, such as sideritoflavone and xanthomicrol [5]. *Rosmarinus officinalis* L. (Lamiaceae) particularly presents phenolic acids, such as carnoside, rosmarinic acid, rosmanol, and flavonoids [6]. *Matricaria chamomilla* L. (Asteraceae) is rich in flavonoids and contains flavones, such as apigenin and quercetin [7] and *Aloysia citriodora* Palau (Verbenaceae) contains, in addition to many flavonoids, interesting phenylpropanoids, such as verbascoside and isoverbascoside [8].

Most of them have been investigated *in vitro* to demonstrate their spasmolytic activity and the possible compounds responsible for this activity, but there are no *in vivo* studies comparing their antimotility effects. In this work, we have focused on determining and comparing the antioxidant power of the hydro-alcoholic extracts from these species, quantifying their content in total phenols and flavonoids, and testing the effect on intestinal motility in mice. Furthermore, the safety of oral administration of ethanolic extracts in experimental animals has been investigated. The aim is to quantify and compare the capacity of these aromatic species rich in phenols to improve intestinal transit and highlight their potential as food supplements for intestinal motility disorders.

Materials And Methods

The Materials and Methods section is presented as *supplementary material*.

Results And Discussion

Antiradical activity; total phenols and flavonoids

The results of the DPPH and ABTS radical scavenging assays are presented in Table 1.

In this comparative measurement of antiradical activity, the extracts showed different orders of potency, which coincided in the two methods performed *Rosmarinus* > *Sideritis* > *Achillea* > *Matricaria* > *Aloysia*.

Table 1
Antiradical potential of extracts and references expressed as IC₅₀

Extracts and Standards	DPPH IC ₅₀ (µg/ml)	ABTS IC ₅₀ (µg/ml)
<i>Achillea millefolium</i>	158.65 ± 4.22	87.58 ± 2.78
<i>Aloysia citriodora</i>	269.06 ± 10.37	111.83 ± 6.56
<i>Rosmarinus officinalis</i>	48.80 ± 2.98 ***	27.28 ± 1.83***
<i>Matricaria chamomilla</i>	205.06 ± 11.87	104.43 ± 8.76
<i>Sideritis angustifolia</i>	142.18 ± 9.34	62.22 ± 1.02
Caffeic acid	7.64 ± 0.20	
Quercetin	8.72 ± 0.39	
Trolox		19.07 ± 0.87
The results are expressed as mean ± error of n = 3. For clarity purposes, the statistical significance of the most active extract is shown compared to the remaining extracts. ANOVA + Tukey's comparison test: ***p < 0.001: <i>Rosmarinus</i> vs. the others.		

The greater and significant antiradical potency of the *Rosmarinus officinalis* L. extract versus the other tested extracts (***p < 0.001) is highlighted. A significant antiradical potency of rosemary extract has been demonstrated in both the experimental antiradical assays DPPH and ABTS (IC₅₀ values of 48.80 ± 2.98 and 27.28 ± 1.93 µg/ml respectively). The total phenol and flavonoid contents of each extract are shown in Fig. 1 and it is referred to gram of dry extract. *Rosmarinus* extract also showed the highest content of phenolic compounds supporting its higher antioxidant activity.

In the quantification of flavonoids, the extracts of *Rosmarinus officinalis* and *Sideritis angustifolia* presented very similar values. It can be deduced that flavonoids are not the only phenols present in the extracts; other phenolic compounds, such as tannins and phenolic acids, would also be present.

The antioxidant and antimicrobial activity of *R. officinalis* demonstrated by other authors it could be related to its high content of phenols such as carnosol, rosmarinic acid, or rosmanol [6]. These phenolic compounds have shown considerably higher antioxidant activity than synthetic butylhydroxytoluene (BHT) and α-tocopherol [9]. In addition, volatile compounds can also contribute to the antioxidant activity of this plant [10]. Many studies have shown the effectiveness of this plant against numerous pathological disorders. An extensive and comprehensive review of the therapeutic properties of this plant is described elsewhere [11].

Acute And Subacute Toxicity

In the experimental toxicity studies, all animals survived the study period, and no signs of mortality were observed with acute administration of 1000 mg/ bweight (bw) and repeated administration dose of 500 mg/bw/day for 28 days with different plant extracts. In the macroscopic analysis of the organs, no damage was observed in the livers, although only in two animals belonged to the *Sideritis* group, 2 or 3 very small ulcers were detected in their stomachs. Moreover, there were no significant differences in food daily intake and weight gain among the groups (supplementary material SM Table 2).

In the histological study of liver sections stained with hematoxylin-eosin (Fig. 2), no abnormalities were observed with respect to the control group. Normal undamaged hepatocytes were observed in the images (10x) (Fig.2). These data further confirmed the low toxicity of the extract, as it did not show suggestive findings of toxic effects.

The values of the biochemical analysis of plasma samples from the different groups are shown as supplementary material in SM Table 3. Biochemical parameters are essential, as they provide information on the main toxic effects on tissues, specifically on the kidneys and livers. Few significant differences appeared in the groups treated with extracts compared to the control. There was a significant increase in HDL cholesterol in the group treated with *Matricaria* and a slight increase of this factor with *Rosmarinus*. In the data corresponding to glucose values, a significant decrease was also observed in the *Achillea*, *Matricaria* and *Rosmarinus* groups compared to the control group ($p < 0.05$). This hypoglycemic effect has been detected by other authors [12, 13, 14].

The low acute toxicity of rosemary agrees with other studies in which the median oral lethal doses (LD50) were greater than 2000 mg/kg of body weight (bw) [15]. No data were found in the literature on the acute and subacute toxicity of *Achillea millefolium* (yarrow), but for *Achillea wilhelmsii*, the LD50 was greater than 5000 mg/kg [16], although no data were found for repeated doses. For lemon verbena (*Aloysia citrodora*) a study reported that the LD50 of the aqueous extract was greater than 5 g/kg in acute toxicity evaluation and in subacute analysis, no toxic effect was observed after 21 days of administration [17]. However, the data found for an ethanolic flower extract of *Chamomile recutita* (synonym of *Matricaria chamomile*) demonstrated a high safety margin, as the animals tolerated up to 10,000 mg kg body weight of the extract orally in the acute toxicity study and tolerated repeated doses of up to 500 mg kg for 28 days [18]. Regarding *Sideritis angustifolia* (cat's tail), no animal toxicity studies were found; however, the *Sideritis scardica* ethanol extract was not toxic after administration of a single dose of 2000 mg/kg bw and repeated doses of 1000 mg/kg bw for 28 days [19]. From the data obtained in our experiments and those reviewed in the literature, we can conclude that these species have a high safety profile and the dose we used for the intestinal motility assay (300 mg/kg bw) is well below LD50.

Intestinal transit results

The intestinal transit in mice is a preclinical pharmacological model, which supports the hypothesis that extracts that decrease it may contain an antidiarrhoeal action. The reference loperamide used in the experiment is an opioid derivative that decreases faeces production by reducing intestinal activity, thus

increasing faecal transit time in the intestinal tract, favouring fluid and electrolyte retention [20]. The effects obtained with the various extracts on intestinal transit are shown in Table 4.

Table 4
Percentage of intestinal transit and percentage of inhibition of intestinal transit of extracts and loperamide.

Groups	Intestinal transit %	Inhibition %
Control	66.7 ± 8.6	
Loperamide	47.8 ± 3.6 ^{**} . #	27.6 ± 3.5
Castor oil	79.5 ± 3.0	-28.2 ± 1.7
<i>Achillea</i>	45.2 ± 6.7 ^{**} . #	22.4 ± 5.1
<i>Sideritis</i>	58.1 ± 3.5	5.54 ± 1.9
<i>Rosmarinus</i>	46.2 ± 4.7 ^{**} . #	30.0 ± 9.1
<i>Matricaria</i>	48.8 ± 2.1 [*]	20.3 ± 6.4
<i>Aloysia</i>	55.1 ± 4.4	15.4 ± 3.4
ANOVA + Dunnett's comparison test. * p < 0.05, ** p < 0.01 vs. castor oil; #p < 0.05 vs. control.		

Extracts that showed an intestinal transit percentage close to that of loperamide (47.8 ± 3.6%) were: *Achillea* (45.2 ± 6.7%), *Rosmarinus* (46.2 ± 4.7%), and *Matricaria* (48.8 ± 2.1%). The other groups showed higher rates, but in no case did they exceed that shown by the control group (66.77 ± 8.6%). Furthermore, the extracts of *Achillea*, *Rosmarinus*, and *Matricaria* can be considered to show a significant percentage of inhibition of intestinal transit compared to the castor oil group.

Most of these species have previously been investigated *in vitro* in organ bath for their spasmolytic effect. The hydroalcoholic extract of *A. millefolium* showed a reduction in contraction by acetylcholine and KCl in isolated rat ileum and its use has been indicated to eliminate intestinal spasms [21]. In the spasmolytic activity of a flavonoid fraction of a commercial sample of yarrow (*Achillea millefolium*), the aglycones quercetin, luteolin, and apigenin exhibited the highest antispasmodic activities with IC₅₀ values of 7.8 micromol/L, 9.8 micromol / L, and 12.5 micromol / L, respectively, in isolated guinea-pig ilea [22]. In our experiment, *A. millefolium* extract produced a significant inhibition of motility compared to loperamide (p < 0.05) and castor oil (p < 0.001) likely due to its flavonoids content. Although the essential oil of the plant may also influence its spasmolytic effect in the rat duodenum, as is the case with the closely related species *Achillea ageratum* [23].

No data on the spasmolytic activity of *Sideritis angustifolia* species were found; however, the ethanol extract of *S. raeseri* known as 'mountain tea' produced inhibition of spontaneous contractions of the rat

ileum and contractions induced by different spasmogens, which justifies its use in gastrointestinal disorders [24].

The aqueous extract of *Aloysia citrodora* has been reported to decrease the contraction of acetylcholine in the isolated duodenum in a noncompetitive manner (IC_{50} of 1.34 ± 0.49 mg lyophilized/mL). The spasmolytic effect was said to be due in part to its content in the flavonoid vitexin and probably to other flavones such as luteolin and apigenin detected in its composition [25]. Other authors evaluated a crude methanolic extract of *A. citrodora* in charcoal-gum acacia-induced hyperperistalsis in rats and found a 32% inhibition of hyperpropulsive movement in the small intestine, which supports the use of the plant as an antidiarrhoeal agent [26]. The weaker effect on intestinal transit inhibition observed in our experiment ($15.4 \pm 3.4\%$) could be due to the fact that mice instead of rats were used in our experiment and a different type of extract was assayed.

On the other hand, the extracts, and some components of *Matricaria chamomilla* showed a dose-dependent antispasmodic effect in vitro. The chamomile flavones of apigenin, luteolin, patuletin, and quercetin showed marked spasmolytic activity on the isolated pig ileum. This effect was also shown to be produced by its essential oil, particularly by (-)- α -bisabolol [27]. More recently, research on antidiarrhoeal, antisecretory, and antispasmodic activities of *M. chamomilla* has shown that the effect was predominantly mediated through K(+) -channel activation [28]. In a similar experiment like that carried out here, oral administration of an herbal formulation containing *Matricaria recutita* flowers caused a delay in intestinal transit in mice, significantly reducing intestinal motility [29].

Rosmarinus officinalis is widely acknowledged to be carminative and is used internally as an antispasmodic. Certain publications have shown the inhibitory effect of acetylcholinesterase by *Rosmarinus officinalis*, which could influence its inhibitory action on intestinal transit [30].

Despite these promising results, clinical trials are needed to verify antimotility efficacy in humans to determine the most effective dose of each of them.

Conclusions

The effectiveness of the extracts in inhibiting intestinal transit, the lack of toxicity of the plant extracts, together with the high concentration in phenolic compounds with anti-inflammatory, spasmolytic, and antioxidant action, allow us to support the hypothesis that the species tested herein may be useful in gastrointestinal pathologies. This is especially relevant for the most active species among them: Extracts of *A. millefolium*, *R. officinalis* and *M. chamomilla* that could be potentially valuable as food supplements in the treatment of intestinal motility disorders and could be included individually or in combination for the manufacture of nutraceutical products or dietary supplements.

Abbreviations

ABTS: Azino-bis(3-ethylbenzothiazoline-6 sulfonic acid) diammonium salt

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

GAE: Gallic acid equivalent

IC₅₀: Median inhibitory concentration

LD₅₀: Median lethal doses

OECD: Economic Cooperation and Development

QE: Quercetin equivalent

Declarations

Ethical Approval

Animal-related experimental procedures were carried out in accordance with the Ethical Committee of the University of Seville (reference CEEA-US2016-13/2) that followed the European Directives 2016/63/EU.

Consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

All authors agree with the submission, and there is no ethical problem or conflict of interest with respect to this manuscript.

Author's contributions

R.D.L.P and M.D.G.G. conceptualized the objective; R.D.L.P, M.D.G.G., M.A.F.A. and A.Q.G. reviewed the methodology; R.D.L.P, M.D.G.G., M.A.F.A. and A.Q.G. performed the laboratory work; R.D.L.P, M.D.G.G. and M.A.F.A. worked up and analysed the results; R.D.L.P, M.D.G.G., wrote the draft manuscript; R.D.L.P and M.D.G.G. wrote, reviewed and edited the final manuscript. All authors provided critical comments on the manuscript.

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Data availability

Data supporting the findings are available from the corresponding author.

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Figures

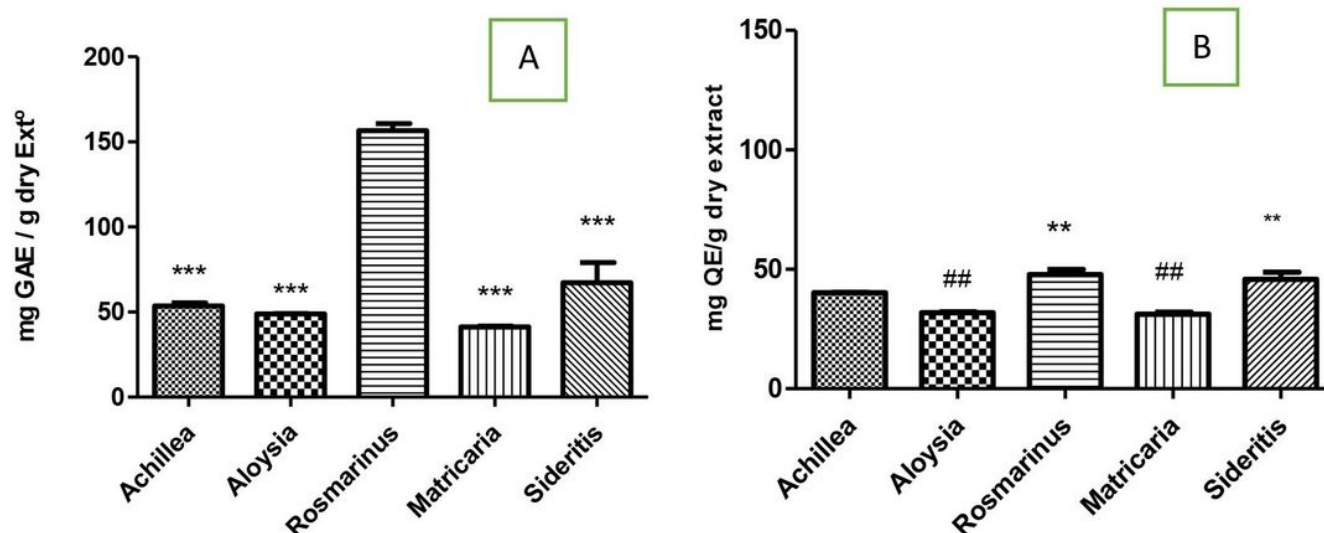


Figure 1

A) Total phenol content expressed as mg GAE / g dry Ext°. Results are expressed as mean $\pm$ error of n = 3. *** p < 0.001 vs. *Rosmarinus*. B) Flavonoid content expressed in mg QE/ g dry Ext°. Results are expressed as mean $\pm$ error of n = 3. ** p < 0.01; ** p < 0.01 versus *Aloysia* and ## p < 0.01 vs *Sideritis* (Anova followed by Tukey's multiple comparison test).

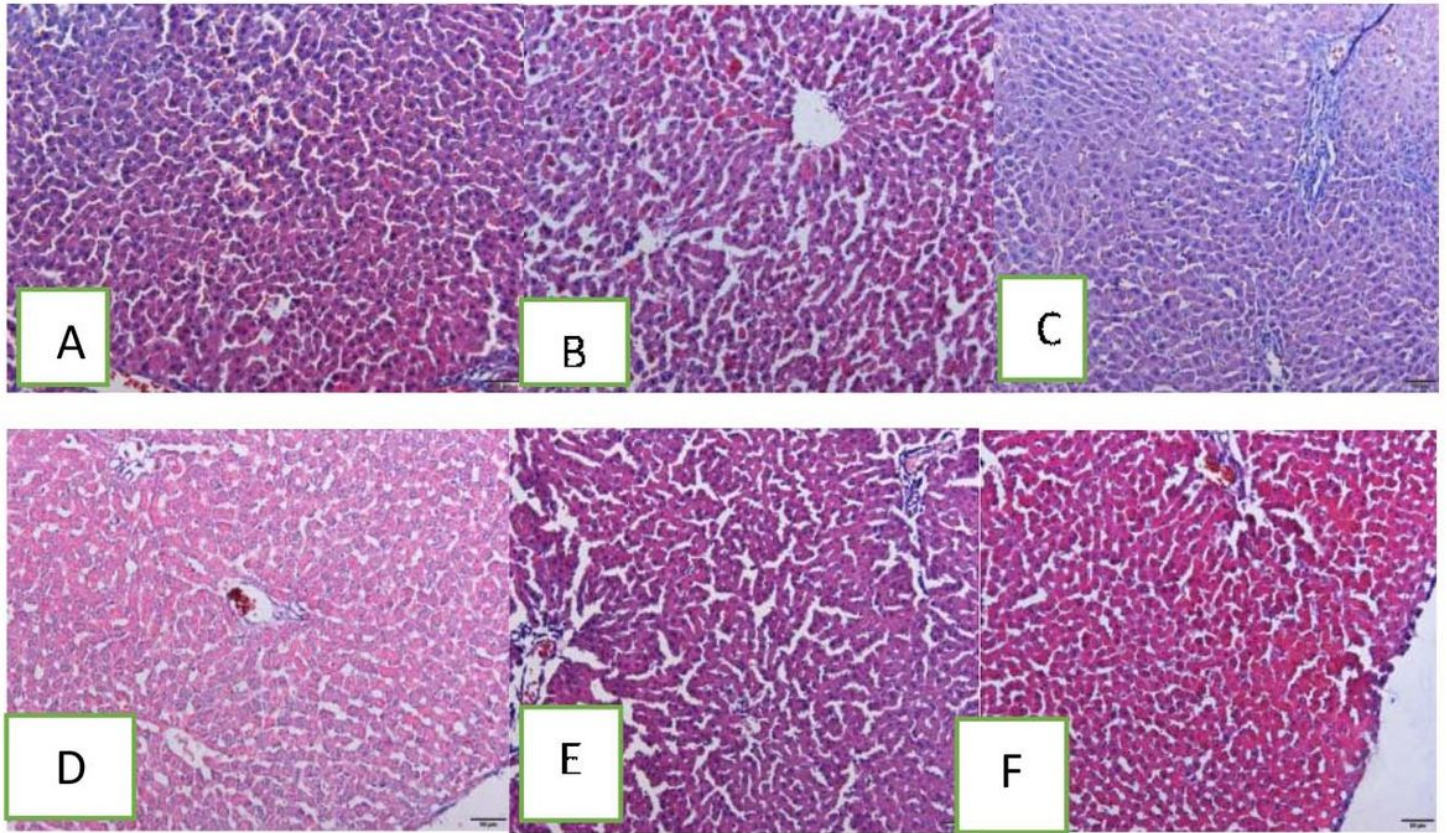


Figure 2

Histological images of liver sections from rats from different groups: A control; B: *Sideritis* C: *Achillea*. D: *Rosmarinus*, E: *Matricaria*, F: *Aloysia*. (H&E, 10X).

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