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Research Article

Antibacterial Capacity and Inhibitory Effect of Rosemary (*Rosmarinus officinalis* L.) Essential Oils on Gastrointestinal Transit in Rats

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Abstract

Objectives: The present study aims to determine the inhibitory effects of Rosmarinus officinalis essential oils (ROEO) on gastrointestinal transit in rats as well as the involvement of its antimicrobial properties in such protection.

Methodology: The antibacterial power of the oils was evaluated against bacteria (2 Gram - bacteria, 2 Gram + bacteria) by the diffusion method on agar medium. The gastrointestinal transit (GIT) was assessed using charcoal meal method. In this respect, female Wistar rats were used and divided into six groups of eight each: control, ROEO treated groups. The last two groups were pretreated with clonidine and yohimbine, two reference molecules, which accelerate and delay TGI, respectively.

Results: Our results demonstrated that the SOEO exhibited a broad spectrum of antibacterial activity, with a great effect against Gram+ strains compared to the Gram-. On the other hand, in vivo study showed that the ROEO significantly, and dose dependently, decreased the GIT activity. However, clonidine and yohimbine respectively decreased (39.76%) and increased (13.93%) the GIT.

Conclusion: In conclusion, we suggest that ROEO had a potent inhibitory effect on gastrointestinal transit due in part to its antimicrobial properties.

Keywords: Antimicrobial Activity; Transit Gastrointestinal; Rosmarinus Officinalis; Essential Oils; Rat

Introduction

According to scientific progress, it has become interesting to determine the chemical compositions of plants and the nutritional value before being incorporated into human and animal food [1]. In this context, the determination of nutrient concentrations and the quantitative and qualitative characterization of bioactive molecules in plants help to create useful databases for nutritionists, physiologists and in the fields of pharmacy [2,3]. These data allow the precise inclusion of plant material that improves not only the nutrition of humans and animals, depending on the specific characteristics of the plant, but also the choice of the dose to control/prevent against several pathologies [2,4].

Several medicinal plants rich in bioactive molecules have been largely used to fight/prevent against digestive diseases. The rosemary (*Rosmarinus officinalis* L.) is a wild plant and has been widely and cultivated for years in Mediterranean countries including Tunisia. Due to its antioxidant and anti-inflammatory properties, rosemary extracts and essential oils exhibit many beneficial health effects such as anti-microbial [5], antidiabetic [6], antiviral [7], anticancer [8], and antinociceptive [9] activities. More importantly, this plant has been widely used in the treatment of most gastrointestinal diseases like ulcerative colitis and peptic ulcers [10,11].

Gastrointestinal pathologies result from the alteration of different parts of the gastrointestinal tract such as diarrhea, constipation, delayed gastric emptying, peptic ulcer, ulcerative colitis and Crohn's disease [12]. Among these, diarrhea is defined as the sudden onset of stools that exceed three unformed excretions per day. Diarrheal disease is one of the most common causes of morbidity

and mortality in many developing countries [13]. According to the World Health Organization (WHO), it is considered acute when it lasts less than 14 days, persistent between 14 and 28 days and chronic beyond that [14]. In addition, *Shigella flexneri*, *Staphylococcus aureus*, Escherichia coli and *Salmonella typhi* are the main bacterial agents responsible for infectious diarrhea in humans [15].

Intestinal motility disorders play an important role in the pathogenesis of diarrhea. For this reason, the use of intestinal motility modifiers during diarrhea is considered an effective solution to combat this pathology. These substances have been shown to significantly reduce faecal water content by decreasing crypt fluid secretion and promoting fluid uptake by intestinal enterocytes [16].

Anti-secretory, anti-ininflammatory and antibiotic agents and some rehydration may be recommended [17-19] However the majority of these drugs induce a complication in the gastrointestinal tract of severe diarrhea or constipation leading in some cases to constipation, colitis and colorectal cancer [20,19].

Generally, secretory diarrhea is accompanied by an acceleration of gastrointestinal transit [21-23]. Hence, in the present investigation we evaluated the antibacterial capacity, as well as the Inhibitory Effect of *Rosmarinus officinalis* L. essential oils administration on gastrointestinal transit.

Materials and Methods

Chemicals

Yohimbine hydrochloride, clonidine, charcoal meal, sodium pentobarbital, Tween 40, Dimethylsulfoxyde (DMSO); ethanol; methanol were obtained from Sigma-Aldrich Co.

Plant collection and Isolation of essential oils

The rosemary *(Rosmarinus officinalis* L.) aerial parts were collected from the region of Tabarka (North-West of Tunisia) during April 2020 and identified by Dr. Chokri HAFSI, associate professor (University of Jendouba, Tunisia). The voucher specimens (No. R101) have been deposited with the herbarium of the Higher Institute of Biotechnology of Béja.

The extraction of essential oils from aerial parts of rosemary was realized by steam distillation for 3 h. The technique consists on putting 15 L of water with 20 kg of plant material in a still and then heating under at 100 °C. Te heated water produces steam enriched in volatile constituents. Finally, the obtained essential oil was collected in an opaque glass bottle. The average oil yield was estimated on the basis of the fresh weight of the plant material.

Antibacterial activity

Bacterial species

Antimicrobial activities of ROEO were inspected against four bacteria strains: two Gram negative: *Escherichia coli* and *Salmonella enterica* and two Gram-positive: *Listeria monocytogenes and Bacillus subtilis*. Bacterial strains were grown in tryptocaseinesoja agar (TSA) and incubated at 37 °C for 24h.

Disc diffusion assay

The antimicrobial potentials of ROEO were investigated using disk diffusion assay according to Sacchetti., *et al.* [24]. Briefly, various doses of ROEO (12.5 to 50 µg/mL) were placed into the wells, then the Petri dishes were incubated at + 4°C for 3 to 4 hours in order to allow the diffusion of the essential oils. Finally, they were incubated at 37°C in a BIOBAE microbiological oven model BJPX-H64II for 48h. The antibacterial activity was evaluated by measuring zone of inhibition (mm).

Animals and treatment

Wistar albinos female rats (weighing 200–220 g and housed four per cage) were purchased from SIPHAT (Tunisia) and used in accordance with the local ethics committee of the Tunis University for use and care of animals in conformity with the NIH recommendations. The animals were housed in standard cages with food and water ad libitum under controlled conditions: 12-h light-12-h dark, 22 ± 2°C.

Gastrointestinal transit (GIT)

The study of gastrointestinal transit (GIT) was carried using charcoal meal as a marker diet [25]. Briefly, rats were divided in 6 groups of eight animals each, fasted for 18 h and pre-treated as following:

Group 1 served as a control and received physiological solution (NaCl, 0.9%, p.o.).

Groups 2, 3, and 4 were pretreated with various doses of ROEO (20, 40, and 80 mg kg^{-1}, b.w., p.o.).

Groups 5 and 6 were, respectively, pretreated with clonidine (1 mg kg^{-1}, b.w., p.o.) and yohimbine (2 mg kg^{-1}, b.w., p.o.).

Two hours after treatment, different groups of rats received the standard charcoal meal (10% charcoal in 5% gum arabic). After 30 min, rats were anesthetized, and a laparatomy was performed followed by examination of stomach and small intestine. The gastrointestinal transit (GIT) was determined using the following formula

GIT (%) = Distance traveled by charcoal (cm)/Total intestinal length (cm) × 100.

Statistical analysis

The data are expressed as means ± standard error of the mean (SEM). The statistical analysis for the animal experiments was carried out using one-way ANOVA. The results were compared with the control group. A value of P less than 0.05 were considered statistically significant.

Results and Discussion

Antibacterial activity

The antibacterial activity of ROEO obtained by the disc diffusion method is presented in table 1. We firstly showed that the percentages of inhibition increased in a dose-dependent manner in the four tested strains. The ROEO showed a significant effect against the entire tested bacterial flora and the highest zone of inhibition was found against *Bacillus subtilis* (17.07 ± 0.65mm) at the high dose of ROEO (50 µg/mL). A variation in the antimicrobial properties of SOEO according to the microorganism species was observed.

ROEO (µg/mL)	*Listeria monocytogenes*	*Bacillus subtilis*	*Salmonella enterica*	*Escherichia coli*
12.5	11.54 ± 0.28^{b}	10.45 ± 0.18^{c}	10.11 ± 0.06^{c}	9.87 ± 0.24^{c}
25	12.53 ± 0. 41^{b}	12.97 ± 0. 42^{c}	10.87 ± 0.04^{c}	11.87 ± 0.91^{b}
50	16.45 ± 1.02^{b}	17.07 ± 0.65^{b}	13.37 ± 0.17^{b}	12.88 ± 1.52^{b}
Gentamicin	26.93 ± 1.16^{a}	24.72 ± 1.17^{a}	18.94 ± 0.15^{a}	20.91 ± 0.92^{a}

Table 1: Inhibition zone diameter (IZD, mm) of *Rosmarinus officinalis* essential oils (ROEO) against four reference strains. Data are represented as means ± SD (n = 3). Means in the same column with no common superscript differ significantly (P < 0.05). IZD are expressed in mm.

Importantly, we showed in the present study that Gram positive bacteria were more sensitive than Gram negative bacteria. These data are in line with previous published reports which demonstrated that gram (+) bacteria are more sensitive to plant extracts than gram (-) [26-28].

The resistance of Gram- bacteria was mainly attributed to the presence of hydrophobic lipopolysaccharide in the outer membrane which provides protection against different agents [29-31].

Phytochemical study of ROEO by GC-MS analysis allowed to the identification of 31 compound, and the main constituents were 1,8-cineole (20.07%), eucalyptol (18.87%), caryophyllene (9.49%), β-pinene (8.52%), β-thujone (7.42%) and ledol (6.39) [32].

The observed antibacterial activity of ROEO can be in part attributed to its richness in phenolic compounds particularly β-myrcene, β-pinene, camphene, α-pinene, α1,8-cineol, camphre, borneol, caryophyllene [33,34].

Gastrointestinal transit (GIT) inhibition

We further looked at the effects of ROEO as well as pharmacological agents on gastrointestinal transit (GIT), assessed by the distance covered by the charcoal meal, indicated the speed of food traversing throughout the gastrointestinal tract and particularly peristaltic frequency. Clonidine (an alpha-2 adrenergic agonist, 1 mg kg^{-1} b.w., p.o.) significantly inhibited ($p < 0.05$) the gastrointestinal transit (GIT), while yohimbine (an alpha-2 adrenergic antagonist, 2 mg/kg b.w., p.o.) significantly reduced ($p < 0.05$) it. More importantly, compared to the control group, ROEO significantly increased ($p < 0.05$) GIT in a dose-dependent manner (Table 2).

Groups	GIT (%)	% of increase or decrease
Control	71.26 ± 5.43	--
ROSO-20 mg kg^{-1}	66.63 ± 4.32*	6.75 (-)
ROSO-40 mg kg^{-1}	59.31 ± 3.26*	16.85 (-)
ROSO-80 mg kg^{-1}	52.81 ± 2.87*	25.96 (-)
Clonidine (1 mg kg^{-1})	42,97 ± 3.24*	39.76 (-)
Yohimbine (2 mg kg^{-1})	82,89 ± 4.56*	13.93 (+)

Table 2. Effects of various doses of *Rosmarinus officinalis* essential oils (ROEO), Clonidine and Yohimbine on gastrointestinal transit (GIT) in rats.

Animals (n = 8) were pretreated with various doses of ROEO (20, 40 and 80 mg kg^{-1}, b.w., p.o.), and reference molecules (clonidine, 1 mg kg^{-1}, b.w., p.o. and yohimbime, 2 mg kg^{-1}, b.w., p.o.) or vehicle (NaCl 0.9%). Two hours after, rats received the standard charcoal meal (10% charcoal in 5% gum arabic) by gavage for 30 min. *: $P < 0.05$ compared to control group. Abbreviations: GIT, gastrointestinal transit; (-), transit decrease; (+), tansit increase; NaCl, sodium chloride.

The anti-motility effect of ROEO appears to be due to mechanisms independent of activation of cholinergic receptors, since atropine, a cholinergic antagonist, failed to reverse its action in the gastrointestinal transit test. Other mechanisms such as adrenergic and/or nonadrenergic-noncholinergic (NANC) systems are also suspected.

GIT inhibition were previously reported for many medicinal plant extracts, such *Myrtus communis* [35], *Ceratonia siliqua* [22] and *Salvia officinalis* [23]. Clonidine and yohimbine, used as reference molecules, respectively, inhibited and stimulated the GIT were previously demonstrated by Rtibi., *et al.* [22].

The 1,8-cineole was the principal chemical components of ROEO (which has been identified in our previous study). The presence of such terpenes in the essence could be attributed to the GIT inhibitory and consequently antidiarrheal activity of ROEO. In this respect, it was demonstrated a positive correlation between antidiarrheal activitties and GIT inhibitory effects exerted by plant extracts and/or essential oils [21,23,36]. In addition, on the isolated ileum, the cineole provided an antispasmodic activity [37]. In addition, there is probably an involvement of cineole, which has been shown to block nerve excitability [38].

Importantly, besides their antioxidant activity, these molecules are implicated in the regulation of GIT [39]. Indeed, anti-inflammatory activity of cineole through the inhibition of inflammatory mediators releasing was previously demonstrated [40,41]. In fact, this terpens blockade prostaglandin and other inflammatory mediator's formation in diarrhea which may in part explain its antisecretory effect.

Finally, it is interesting to highlight that α-pinene of the essential oil of *R. officinalis*, could also enhance the antidiarrheal activity of ROEO, since it has shown anti-inflammatory effects in animal models [42]. Other ROEO constituents also appear to be involved in its GIT inhibition. Further studies, such as the effect of ROEO on diarrhea and fluid accumulation experimentally induced, are needed to clarify the mechanism of action and the components responsible for these pharmacological effects.

Conclusion

In conclusion, ROEO possesses antibacterial properties and inhibitory effects on gastrointestinal propulsion, might be due to the synergistic effect of more than one oil compound. These data give a scientific base justifying the traditional use of ROEO usage as a vegetable and an herbal medicine for the treatment and/or management of infectious diarrhea in Tunisia.

Conflict of Interest

There is no conflict to declare.

Bibliography

1. Ayaz A., *et al.* "Phylogeny and diversity of Lamiaceae based on Rps14 gene in Pakistan". *Genetika* 52 (2020): 435-452.

2. Kholif and Olafadehan. "Essential oils and phytogenic feed additives in ruminant diet: chemistry, ruminal microbiota and fermentation, feed utilization and productive performance". *Phytochemistry Reviews* 20 (2021): 1087-1108.

3. Severin ES and Savvateeva MV. "Molecular and physiological mechanisms of membrane receptor systems functioning". *Acta Naturae* 3 (2011): 20-28.

4. Rahman MM*., et al.* "Role of phenolic compounds in human disease: Current knowledge and future prospects". *Molecules* 27 (2021): 233.

5. Nieto G., *et al.* "Antioxidant and Antimicrobial Properties of Rosemary (*Rosmarinus officinalis*, L.): A Review". *Medicines (Basel)* 5 (2018): 98.

6. Bao T., *et al.* "Antidiabetic effects and mechanisms of rosemary (*Rosmarinus officinalis* L.) and its phenolic components". *The American Journal of Chinese Medicine* 48 (2020): 1353-1368.

7. Al-Megrin WA., *et al.* "Potential antiviral agents of *Rosmarinus officinalis* extract against herpes viruses 1 and 2". *Bioscience Reports* 40 (2020): BSR20200992.

8. Allegra A., *et al.* "Anticancer activity of *Rosmarinus officinalis* L.: mechanisms of action and therapeutic potentials". *Nutrients* 12 (2020): 1739.

9. González-Trujano ME., *et al.* "Evaluation of the antinociceptive effect of *Rosmarinus officinalis* L. using three different experimental models in rodents". *Journal of Ethnopharmacology* 111 (2007): 476-482.

10. Minaiyan M., *et al.* "Effects of extract and essential oil of *Rosmarinus officinalis* L. on TNBS-induced colitis in rats". *Research in Pharmaceutical Sciences* 6 (2011): 13-21.

11. Amaral GP., *et al.* "Protective action of ethanolic extract of *Rosmarinus officinalis* L. in gastric ulcer prevention induced by ethanol in rats". *Food and Chemical Toxicology* 55 (2013): 48-55.

12. Bharucha AE., *et al.* "American gastroenterological association medical position statement on constipation". *Gastroenterology* 144 (2013): 211-217.

13. Shoba FG and Thomas M. "Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhea". *Journal of Ethnopharmacology* 76 (2001): 73-76.

14. DuPont HL. "Guidelines on acute infectious diarrhea in adults". *American Journal of Gastroenterology* 92 (1997): 1962-1975.

15. Toyin YM., *et al.* "Antidiarrheal activity of aqueous leaf extract of ceratotheca sesamoides in rats". *Bangladesh Journal of Pharmacology* 7 (2012): 14-20.

16. Lee JH., *et al.* "Comparison of chemical compositions and antimicrobial activities of essential oils from three conifer trees; *Pinus densiflora*, *Cryptomeria japonica*, and *Chamaecyparis obtuse*". *Journal of Microbiology and Biotechnology* 4 (2009): 391-396.

17. Diniz-Santos DR., *et al.* "Antibiotics for the empirical treatment of acute infectious diarrhea in children". *Brazilian Journal of Infectious Diseases* 10 (2006): 217-227.

18. Barr W and Smith A. "Acute diarrhea in adults". *American Family Physician* 89 (2014): 180-189.

19. Ingle SB., *et al.* "Microscopic colitis: Common cause of unexplained nonbloody diarrhea". *World Journal of Gastrointestinal Pathophysiology* 5 (2014): 48-53.

20. Siegers CP, *et al.* "Anthranoid laxative abuse a risk for colorectal cancer?" *Gut* 34 (1993): 1099-1101.

21. Jalilzadeh-Amin G and Maham M. "Antidiarrheal activity and acute oral toxicity of *Mentha longifolia* L. essential oil". *Avicenna Journal of Phytomedicine* 5 (2015): 128-137.

22. Rtibi K., *et al.* "Phytochemical properties and pharmacological effects of *Quercus ilex* L. aqueous extract on gastrointestinal physiological parameters *in vitro* and *in vivo*". *Biomedicine and Pharmacotherapy* 94 (2017): 787-793.

23. Jedidi S., *et al.* "Phytochemical/antioxidant properties and individual/synergistic actions of *Salvia officinalis* L. aqueous extract and loperamide on gastrointestinal altering motor function". *Journal of Medicinal Food* 22 (2019): 1235-1245.

24. Sacchetti G., *et al.* "Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antibacterials and antimicrobials in foods". *Food Chemistry* 91 (2005): 621-632.

25. Ali BH and Bashir AA. "The effect of alpha 2-receptor agonists and antagonistis on gastrointestinal transit time". *Clinical and Experimental Pharmacology and Physiology* 20 (1993): 1-6.

26. Boukhebti H., *et al.* "Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils". *Der Pharmacia Letter* 3 (2011): 267-275.

27. Aloui F., *et al.* "Chemical composition, anti-radical and antibacterial activities of essential oils from needles of *Pinus halepensis* Mill., P. *pinaster Aiton*., and *P. pinea* L". *Journal of Essential Oil-Bearing Plants* 24 (2021): 453-460.

28. Jedidi S., *et al.* "Antioxidant and antibacterial properties of *Salvia officinalis* essential oils and their effects on *in vitro* ruminal fermentation and methane production in goats". *Animal Nutrition and Feed Technology* 22 (2022): 297-310.

29. Burt S. "Essential oils: their antibacterial properties and potential applications in foods-a review". *International Journal of Food Microbiology* 94 (2004): 223-253.

30. Zarai Z., *et al.* "The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of *Marrubium vulgare* L. essential oil grown in Tunisia". *Lipids in Health and Disease* 10 (2011): 161.

31. Elisha IL., *et al.* "The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts". *BMC Complementary and Alternative Medicine* 17 (2017): 133.

32. Jedidi S., *et al.* "Phytochemical profile and antioxidant properties of essential oils isolated from the aerial parts of *Rosmarinus officinalis* L. cultivated in northwestern of Tunisia". *Moroccan Journal of Agricultural Sciences* 3 (2022): 229-233.

33. Lin PC., *et al.* "Essential oils from Taiwan: Chemical composition and antibacterial activity against Escherichia coli". *Journal of Food and Drug Analysis* 24 (2016): 464-470.

34. Khalil R and Li ZG. "Antimicrobial activity of essential oil of *Salvia officinalis* L. collected in Syria". *African Journal of Biotechnology* 10 (2011): 8397-8402.

35. Jabri MA *et al.* "Role of gastrointestinal motility inhibition and antioxidant properties of myrtle berries (*Myrtus communis* L.) juice in diarrhea treatment". *Biomedicine and Pharmacotherapy* 84 (2016): 1937-1944.

36. Jedidi S., *et al.* "Antioxidant properties, phytoactive compounds and potential protective action of *Salvia officinalis* **flowers against combined gastro-intestinal ulcer and diarrhea experimentally induced in rat".** *Dose Response* 20 (2022): 15593258221102313.

37. Magalhaes PJC., *et al.* "Intestinal myorelaxant and anti-spasmodic effects of the essential oil of *Croton nepetaefolius*, and its constituents cineole, methyl-eugenol and terpineol". *Phytotherapy Research* 12 (1998): 172-177.

38. Leal-Cardoso JH., *et al.* "Effects of estragole on the compound action potential of the rat sciatic nerve". *Brazilian Journal of Medical and Biological Research* 37 (2004): 1193-1198.

39. Biswas S., *et al.* "Antidiarrhoeal activity of *Strychnos potatorum* seed extract in rats". *Fitoterapia* 73 (2002): 43-47.

40. Santos FA and Rao VSN. "Antiinflammatory and antinociceptive effects of 1,8-cineole, a terpenoid oxide present in many plants essential oils". *Phytotherapy Research* 13 (2000): 1-5.

41. Kawata, M., *et al.* "Steroid receptor signalling in the brain-lessons learned from molecular imaging". *Journal of Neuroendocrinology* 20 (2008): 673-676.

42. Guimaraes, AG., *et al.* "Monoterpenes with analgesic activity - a systematic review". *Phytotherapy Research* 27 (2013): 1-15.