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Chemical profile of *Retama raetam* Forssk. (Webb) aerial parts, appraisal of the possible antioxidant and antidiarrheal effects

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Retama genus has been used traditionally as an antiseptic, antirheumatic, and antidiabetic agent. The study aims to examine the phytoconstituents of *R. raetam* aqueous ethanol extract (AEE) and evaluate its anti-diarrheal and antioxidant activities. Polyphenolics and lipid content were detected using HPLC and GC-MS, respectively. The anti-diarrheal activity was determined using a castor oil-induced enter-pooling animal model for the first time. Hesperidin and kaempferol were the major identified flavonoids. Linoleic and eicosanoic acids were the major unsaturated and saturated fatty acids (FA). The identified sterols were found to have a higher percentage than hydrocarbons. The AEE showed anti-diarrheal activity, increasing the onset of induced diarrhea and decreasing the frequency of defecation and the weight of feces. AEE reduces the intestinal fluid volume and exerts an inhibitory activity on gastrointestinal motility and peristaltic index. Moreover, AEE showed a radical scavenging power compared to Trolox as a positive control. These findings support the traditional use of *R. raetam* aerial parts for managing diarrhea owing to the synergistic effect of polyphenolics.

Keywords: Retama raetam, Anti-diarrheal, Antioxidant, Polyphenolics, HPLC, Fatty acids

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INTRODUCTION

Herbs and their secondary metabolites have been used extensively to manage several diseases (Sendi et al., 2020; Elzawawy et al., 2022; Metwally et al., 2022). In 2019, the WHO stated that around 80% of the population worldwide uses folk medicine owing to its safety and acceptable effectiveness in treating several symptoms and diseases (WHO, 2019). Since oxidative stress is one of the leading causes of several diseases, most research has focused on the antioxidant effect of natural products owing to their chemical diversity (Huang, 2018). Genus *Retama,* family Fabaceae, a well-known plant in the Mediterranean region and also distributed in the Canary Islands and North Africa, includes four species, namely, *R. raetam*, *R. sphaerocarpa*, *R. dasycarpa,* and *R. monosperma*, (Fdil and El Hamdani, 2015). The Egyptian Retama species' natural habitat is the Wadis Desert and maritime sandy regions; they are consumed as cattle food in deserted Mediterranean regions (Youssef et al., 2023). Plants belonging to the genus *Retama*, known as white broom, are used traditionally for the treatment of diabetes (Maghrani et al., 2003), rheumatism, and skin diseases (Awen et al., 2011); besides, they act as an antiseptic agent (Benkhouili et al., 2022). *R. raetam* is native to North Africa and the Middle East countries such as Saudi Arabia and Egypt (Mittler et al., 2001).*R. raetam* has been commonly used to tackle hypertension and diabetes (Maghrani et al., 2003). Biological reports of *R. raetam* have illustrated the antioxidant (Papadopoulos et al., 2013), anti-inflammatory (González-Mauraza et al., 2016), antimicrobial (Mariem et al., 2014), antifungal and antiviral activities through several *in vivo* and *in vitro* assays (Hammouche-Mokrane et al., 2017). The biological effects of *R. raetam* were observed to be attributed to their secondary metabolites, mainly the polyphenolic contents (Ghani et al., 2019).

Diarrhea is a disease or symptom characterized by a frequent discharge of intestinal content in a loose, watery form. Several mechanisms are involved in diarrhea, including the increase in the contraction of the intestinal wall, which leads to inadequate fluid absorption, causing the formation of watery stool (Guarner et al., 2011). Diarrhea, non-infectious or infectious, is one of the main causes of mortality in developing countries (Okolo and Garba, 2013). Diarrhea is categorized into five types. First, secretory diarrhea occurs when the intestine is over-extracting intestinal fluids more than in normal situations, or it cannot absorb fluids properly. Second, motility-related diarrhea happens when ingested food does not have an adequate period for good absorption as it moves so fast. Third, inflammatory diarrhea is experienced when the intestinal epithelial lining becomes injured or inflamed. Fourth, osmotic diarrhea can be contracted when too much water is present inside the bowels. Fifth, dysentery is developed when blood is present in the produced stool (Das et al.,

2012). Even though diarrhea can be controlled and treated permanently, Jaradat et al. (2016) stated that diarrhea is considered the second leading cause of death among children worldwide (Jaradat et al., 2016). The estimated annual diarrhea mortality among children worldwide was around nine million (de Wet et al., 2010). The statistics revealed that diarrhea death occurs in African regions due to inadequate health care and people's limited access to clean water, which is considered a major cause of diarrhea (Forsberg et al., 2009). People in developing countries rely on medicinal plants to recover from diarrhea (Park, 2021) because they are favorable, available, and cost-effective compared to modern medicine. The previous reports have demonstrated the efficacy of medicinal plants as antidiarrheal through several mechanisms, such as induction of water absorption and arresting intestinal motility (Czigle et al., 2022). Plants rich in flavonoids were reported to have the ability to inhibit intestinal motility (Venkatesan et al., 2005). Moreover, plants rich in gallic acid were noted to have significant antidiarrheal activity in a trinitrobenzene sulfonic acid (TNBS) induced rat colitis model owing to its astringent, anti-inflammatory, and antioxidant activities (Marinov et al., 2019). Therefore, the present study aimed to identify the phytoconstituents, polyphenolics, and lipid contents in *R. raetam* aerial parts and estimate the antioxidant and anti-diarrheal activities through *in-vitro* and *in-vivo* assays.

MATERIALS AND METHODS

Chemicals

Authentic references of phenolics and flavonoids were obtained from Merck, Darmstadt, Germany. Loperamide HCl (Sigma-Aldrich, USA), activated charcoal (Labchem Industries, India), and castor oil were collected from a local pharmacy in Egypt. Solvents and other chemicals of the highest quality were attained from El Nasr Pharmaceutical Chemicals Co., Egypt.

Plant materials

R. raetam aerial parts were collected from Wadi Hagol, Egypt in November 2022. Dr. Therese, a taxonomy specialist at Mazhar Garden, El-Giza, Egypt, identified the plant sample. A voucher sample of 06*Rra*/2024 was deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Egypt. Using the Soxhlet method, the powdered plant material (2 kg) was extracted with aqueous ethanol (6 x 500 mL, 80%, for 2 h). The extract was filtered and subsequently evaporated (Rotavapor®, BÜCHI, Flawil, Switzerland) (Boshra and El-Haddad, 2018).

Estimation of total phenolics and flavonoids

Total phenolic content (TPC) as gallic acid equivalent (GAE/g DR) was determined using the Folin–Ciocalteu method (Attard, 2013), while total flavonoid content (TFC) as rutin equivalent (RE/mg DR) was identified using the aluminum chloride method (Kiranmai et al., 2011) with minor modifications. In TPC, the procedure consisted of mixing sample/standard (10 µL) with Folin-Ciocalteu (100 µL, Diluted 1:10) in a 96-well microplate. Next, sodium carbonate (80 µL, 1M) was added and incubated (25±2 °C, 20 min) in the dark. Briefly, in TFC, in a 96-well microplate, sample/standard (15 µL) was placed with methanol (175 µL), followed by $AlCl_3$ (30 µL, 1.25 %). Finally, sodium acetate (30 µL, 0.125 M) was added and incubated (5 min). The color formed between phenolics and flavonoids with folin-ciocalteu and $ALCl_3$, respectively, was measured using a spectrophotometer (Shimadzu, Japan) at 630 and 420 nm, compared to the standard calibration curves (El-Haddad et al., 2019). Data was represented as means ± SD, and the results were recorded using a FluoStar Omega microplate reader.

HPLC analysis of phenolics

The estimation of phenolics was performed using HPLC analysis, employing a Waters 2695 Alliance system set with a Waters 996 photodiode array detector. Kromasil column C8 (4.6x250mm, 5µm) was used with a mobile phase of phosphoric acid (0.1%) in water: acetonitrile at 1.0 ml/min flow rate. The detector was adjusted at 280 nm. The column temperature was set at room temperature. Both AEE (0.5g, 50 ml in methanol) and standards were filtered using a syringe filter, then separately injected (10 µl) for analysis into the HPLC. Compounds were identified through a comparison of their obtained results with the authentic (El-Haddad et al., 2019).

Investigation of the lipid content

Gas Chromatography Mass Spectrometry (GC-MS) was performed using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a capillary column TG–5MS (30x0.25 mm x 0.25 µm). The column temperature was first set at 50°C, then increased by 5°C /min to 250 °C and kept for 2 min., and increased to 300°C by 30°C /min and remained for 2 min. The injector and MS transfer line temperatures were kept at 270° and 260°C,

respectively. Helium was used at a flow rate of 1 ml/min. The solvent delay was 4 min, and diluted samples were injected (1 µl) automatically using Autosampler AS1300 in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 50–650 in full scan mode. The components were identified by comparing their mass spectra to those of WILEY 09 and NIST 14 database (Ghani et al., 2019). The lipid content was obtained by macerating the dried *R. raetam* (1.0 kg) aerial parts with light petroleum ether. Then, the solvent was filtered, and the residue was macerated in ethanol (80%). Ethanol extract was filtered, evaporated, and then extracted with petroleum ether. Lipids were extracted from petroleum ether fraction by chloroform: methanol (2:1 v/v), according to (Mohammed et al., 2021). The associated non-lipids were removed by washing the lipoidal extract three times with an equal ratio of methanol to water. The lipid content in chloroform was then dehydrated over anhydrous sodium sulfate and evaporated under a vacuum. The lipoidal fraction was saponified overnight with alcoholic potassium hydroxide (20%) at room temperature (Ahmed et al., 2019). After distilling ethanol and diluting it with water, the unsaponifiable matter (USM) was extracted with ether. Then, the aqueous mother liquor was acidified with hydrochloric acid (5N), and the liberated fatty acids (FAs) were extracted with ether. The resulting extract was washed three times with distilled water and dehydrated over anhydrous sodium sulphate. The residue obtained after evaporation of ether was maintained for analysis of USM and FAs.

Antioxidant activity

Serial dilutions of AEE of *R. raetam* were prepared (62.5, 125, 250, 500, and 1000 µg/mL). Moreover, Trolox dilutions (1.25, 5, 7.5, 10, and 12.5 µg/mL) were prepared in methanol. Radical scavenging assay: DPPH (1,1-diphenyl-2-picryl-hydrazyl-hydrate) was carried out (Ahmed et al., 2019; Raïnatou et al., 2016). DPPH (100 µL, 0.1% in methanol) was added to AEE of *R. raetam* (100 µL) in 96 wells plates, and the reaction was incubated (25±2 °C, 30 min) in the dark. After that, the color intensity was measured at 540 nm., and data was represented as mean ±SD according to the following equation: % inhibition= (Average absorbance of blank-average absorbance of the test)/ (Average absorbance of blank) *100. The results were recorded using a microplate reader FluoStar Omega. Data was analyzed using Microsoft Excel®, and the IC_{50} value was calculated using Graph pad Prism 6®.

Animals

Swiss albino mice of both sexes aged 6-8 weeks (25-30g each) were purchased from the breeding unit of the Egyptian Organization of Biological Products and Vaccines (Helwan, Egypt). The mice were housed and acclimatized for a week in standard conditions (room temperature and light/dark cycle, standard pellet, and water ad libitum). The research followed the protocols of the Ethics Committee of Scientific Research (Protocol No. 04A2023).

Acute oral toxicity test

Mice were loaded with gradual doses of the AEE of *R. raetam* up to 3000 mg/kg. The mice were observed for behavioral and physical changes, mortality rate, and any signs of toxicity for 24 h.

In vivo anti-diarrheal assessment

Castor oil-induced diarrhea in mice: After 18 hours of fasting, thirty mice were randomly divided into six groups (n=5). The control group (gp 1) was administered with distilled water. The standard group (gp 2) was orally administered loperamide (5mg/kg). Group 3-5: Mice were orally administered with the AEE of *R. raetam* 250, 500, and 1000 mg/kg, respectively. Castor oil (0.2 mL) was administered orally for one hour following the AEE or loperamide, and each mouse was kept individually in a plastic cage covered with white paper. The onset of diarrhea was measured, and the total number of normal, watery, and wet feces was counted for four hours (Mady et al., 2023). The percentage of diarrhea inhibition was estimated according to the following equation:

$$\text{Percentage of diarrhea inhibition} = \frac{\text{Mean of WFC} - \text{Mean of WFT}}{\text{Mean of WFC}} \text{X100},$$

Where, WFC = wet feces of the control gp, WFT = wet feces of the test gp.

Castor oil-induced enter-pooling in mice: Five fasted mice groups (n=6) were divided as follows: The control group (gp 1) received distilled water; the standard group (gp 2) received oral loperamide (5 mg/kg); and the mice in Group 3-5 were administered the AEE of *R. raetam* 250, 500, and 1000 mg/kg, respectively. Castor oil (0.5 mL) was administered orally one hour after loperamide or AEE. After an hour, the mice were sacrificed by cervical dislocation, and the intestine was isolated after ligation from the pylorus to the caecum end.

The intestinal content was collected and measured according to (Andargie et al., 2022).

Gastrointestinal motility in mice: Thirty fasted mice were categorized into 5 groups, like the previous experiments. One hour after administering the AEE of *R. raetam* or loperamide, castor oil (0.5 mL) was administered to all mice. One hour later, activated charcoal (0.5 ml, 5%) was administered in distilled water. After 30 mins, mice were sacrificed, and the small intestine was isolated. The peristaltic index was calculated by determining the intestinal length traveled by the charcoal, expressed as a % of the total length of the small intestine (Mady et al., 2023).

$$\text{Peristalsis index} = \frac{\text{Distance traveled by charcoal}}{\text{Length of small intestine}} X100$$

$$\text{Percentage of inhibition} = \frac{\text{Mean Peristalsis index of control} - \text{Mean Peristalsis index of test}}{\text{Mean Peristalsis index of control}} \text{X100}$$

Statistical analysis

Nonlinear regression analysis of residuals and statistical analysis for mean±SE was conducted by one-way ANOVA test followed by Tukey's post hoc tests using version 8 of Graph Pad Prism (GraphPad Software Inc., USA). A significant difference is reported for $P < 0.05$.

RESULTS AND DISCUSSION

Phenolics and flavonoid contents of R. raetam aerial parts

R. raetam contained 67.399 µg GAE/g and 12.586 µg RE/g for total phenolic and flavonoid contents, respectively. HPLC analysis showed a chemical profile for flavonoid content, where hesperidin is the major identified flavonoid, followed by kaempferol (37.33 and 31.96 %, respectively) compared to authentic references (Table 1 and Figure 1).

GC analysis of FA and USM of R. raetam aerial parts

The GC analysis of FAs revealed the presence of 8 major compounds (Table 2). The percentage of identified saturated FAs was higher than that of the unsaturated ones (28.37% and 5.31 %, respectively). The GC analysis of USM revealed the presence of 21 major compounds (Table 3). The percentage of the identified sterols was higher than that of hydrocarbons (28.11% and 20.3 %, respectively).

DPPH radical-scavenging activity

The radical scavenging power was estimated using the linear regression equation from the prepared calibration curves. According to the DPPH assay, AEE showed radical scavenging power compared to Trolox as a positive control (283.6±8.6 and 7.21±0.309 µg/mg, respectively).

Investigation of antidiarrheal activity of AEE of R. raetam

Acute oral toxicity test: The mice showed no behavioral or physical changes such as motor activity, convulsions, or diarrhea at the dose of 3 g/kg. Moreover, the mortality rate was equal to zero.

Effects of the AEE of R. raetam on fecal features in castor oil-induced diarrhea: Oral administration of the AEE of *R. raetam* showed a significant increase in the onset of diarrhea in a dose-dependent manner (Figure 2A). Moreover, the normal feces numbers were increased with the standard drug and AEE compared to the control group (Figure 2B). However, the numbers of both wet and watery feces were significantly decreased after treated with loperamide and AEE; 250, 500, and 1000 mg/kg with inhibition % equal 68%, 16%, 52%, and 60%, respectively, compared to the control group (Figure 2C, D). Remarkably, the AEE of *R. raetam* at a dose of 1000 mg/kg showed a non-significant change from the standard group in the numbers of wet and normal feces.

Effects of the AEE of R. raetam on castor oil-induced enter-pooling: AEE of *R. raetam* and loperamide significantly declined the volume of intestinal content compared to the control group, with % inhibition of 66.6%, 22.6%, 39.4%, and 53.8%, respectively (Figure 3A).

Effects of the AEE of R. raetam on gastrointestinal motility and peristaltic index: The AEE of *R. raetam* 250, 500, and 1000 mg/kg significantly decreased gastrointestinal motility and peristaltic index compared to the control group with inhibition percentages 20.9%, 31.9%, and 41.1%, respectively (Figure 3B, C, and D). The AEE of *R. raetam* at a dose of 1000 mg/kg demonstrated the most substantial effect, but it was still significant from the standard group (54.6%). Phenolic compounds are considered the most explored natural chemical entities owing to their significant health benefits, as confirmed in many studies (da Silva et al., 2013). The utilized Folin–Ciocalteu method and aluminum chloride in the studied *R. raetam* extract showed that the flavonoid content is lower than the phenolic content, indicating that the potential pharmacological activity of *R. raetam* aqueous alcoholic extract is attributed to the level of phenolic acid along with the synergism

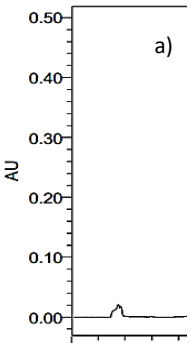
Table 1. Compounds detected by HPLC in aqueous ethanol extract of *R. raetam* aerial parts

Compounds	RT (min)	% area in authentic	% area in *R. raetam*
Gallic acid	10.006	55.72	-
Catechin	23.463	1.02	7.16
Chlorogenic acid	27.028	1.92	-
Caffeic acid	30.098	16.72	-
Ellagic acid	42.493	2.05	-
Rutin	43.567	2.76	-
Hesperidin	45.902	4.23	37.33
Quercetin	54.437	5.70	-
Kaempferol	57.611	0.55	31.96
Apigenin	58.161	9.32	23.55

Table 2. GC analysis of fatty acids (FA), compounds

R_T	Compound	Area %	Molecular Formula	Molecular Weight
17.55	Lauric acid methyl ester (Dodecanoic acid, methyl ester)	2.64	$C_{13}H_{26}O_2$	214
26.21	Palmitic acid methyl ester (Hexadecanoic acid, methyl ester)	3.38	$C_{17}H_{34}O_2$	270
29.35	Linoleic acid methyl ester (Octadecadienoic acid, methyl ester)	13.97	$C_{19}H_{34}O_2$	294
29.45	Linolenic acid methyl ester (Octadecatrienoic acid, methyl ester)	4.23	$C_{19}H_{32}O_2$	292
30.00	Stearic acid methyl ester (Octadecanoic acid, methyl ester)	1.08	$C_{19}H_{38}O_2$	298
33.48	Eicosanoic acid, methyl ester.	6.17	$C_{21}H_{42}O_2$	326
36.69	Docosanoic acid, methyl ester	1.40	$C_{23}H_{46}O_2$	354
39.71	Tetracosanoic acid, methyl ester.	0.81	$C_{25}H_{50}O_2$	382
% Identified saturated fatty acids		28.37		
% Identified unsaturated fatty acids		5.31		

Table 3. GC analysis of hydrocarbons and sterols (USM), compounds



R_T	Compound Name	Area %	Molecular Formula	Molecular Weight
7.47	Benzene (1-methylnonyl)	0.33	$C_{16}H_{26}$	218
7.91	Benzene (1-butylheptyl)	0.77	$C_{17}H_{28}$	232
8.12	Benzene (1-propyloctyl)	0.45	$C_{17}H_{28}$	232
8.47	Benzene (1-ethylnonyl)	0.61	$C_{17}H_{28}$	232
9.10	Benzene (1-methyldecyl)	1.37	$C_{17}H_{28}$	232
9.47	Benzene (1-pentylheptyl)	0.70	$C_{18}H_{30}$	246
9.54	Benzene (1-butyloctyl)	0.67	$C_{18}H_{30}$	246
9.76	Benzene (1-propylnonyl)	0.61	$C_{18}H_{30}$	246
10.13	Benzene (1-ethyldecyl)	0.79	$C_{18}H_{30}$	246
10.78	Benzene (1-methylundecyl)	1.58	$C_{18}H_{30}$	246
11.19	Benzene (1-butylnonyl)	0.60	$C_{19}H_{32}$	260
11.40	Benzene (1-propyldecyl)	0.66	$C_{19}H_{32}$	260
11.79	Benzene (1-ethylundecyl)	0.66	$C_{19}H_{32}$	260
12.45	Benzene (1-methyldodecyl)	1.36	$C_{19}H_{32}$	260
27.75	Vitamin E	6.57	$C_{29}H_{50}O_2$	430
28.54	Campesterol	8.27	$C_{28}H_{48}O$	400
28.78	Stigmasterol	6.53	$C_{29}H_{48}O$	412
29.35	β-Sitosterol	6.47	$C_{29}H_{50}O$	414
29.48	Stigmastadien-3-ol	1.47	$C_{29}H_{48}O$	412
29.73	α-Amyrin	5.37	$C_{30}H_{50}O$	426
30.84	Docosanediol	2.57	$C_{22}H_{46}O_2$	342
% Identified sterols		28.11		
% Identified hydrocarbons		20.3		

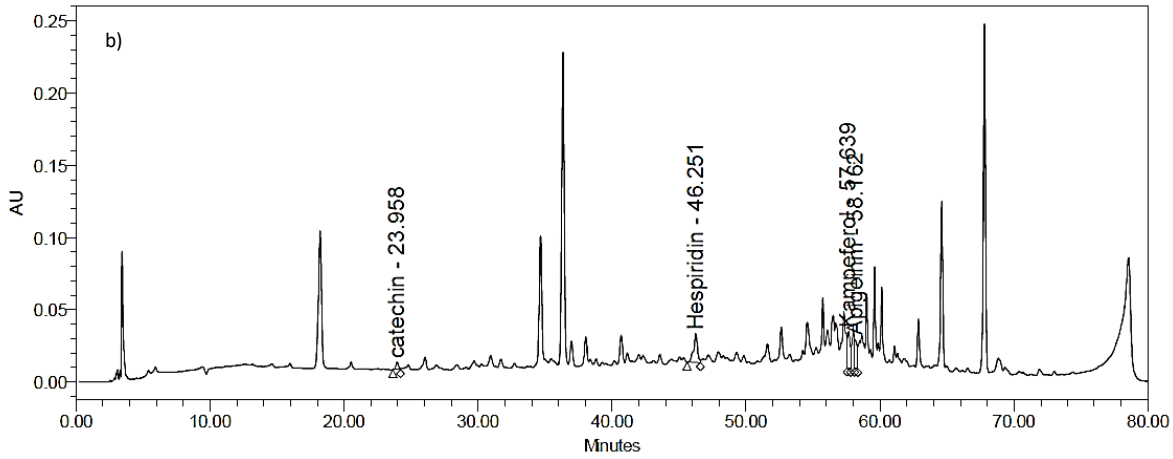


Figure 1. HPLC profile; (a) authentic (b) aqueous ethanol extract of *R. raetam* aerial parts.

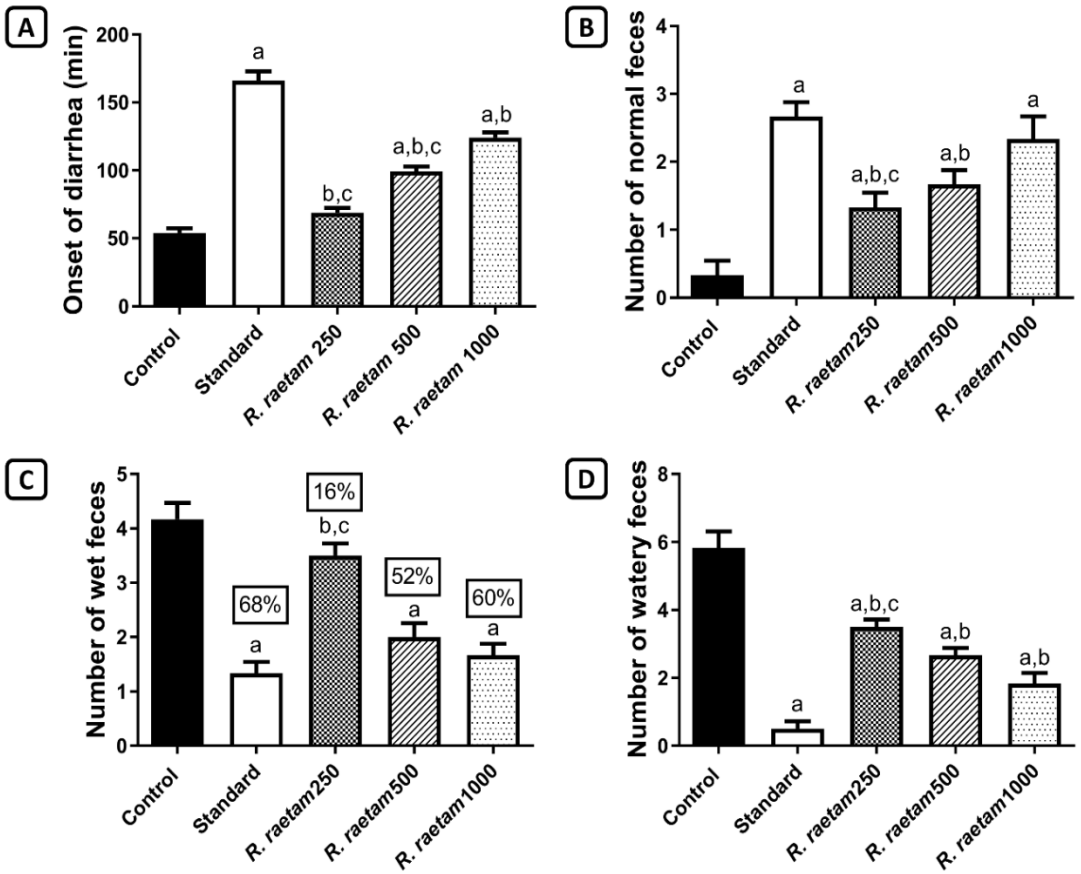


Figure 2. Effects of the AEE of *R. raetam* on fecal features in castor oil-induced diarrhea in mice (A): Onset of diarrhea. (B): Number of normal feces. (C): Number of wet feces. (D): Number of watery feces, Data presented as Mean ± SE (n =5); a: significant from control; b: significant from standard; c: significant from 500 mg; d: significant from 1000 mg.

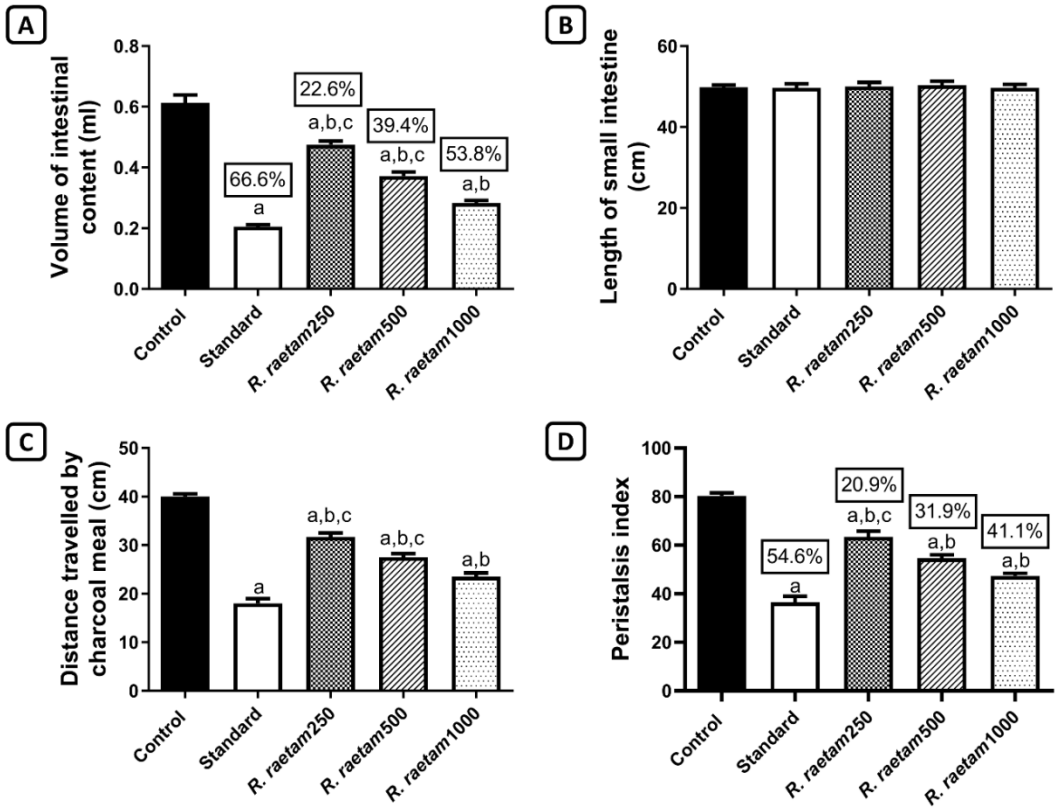


Figure 3. Effects of the AEE of *R. raetam* on castor oil-induced enter-pooling in mice (A), Volume of intestinal content. (B): Length of small intestine. (C): Distance traveled by charcoal meal. (D): Peristalsis index. Data presented as mean ± SE (n =6); a: significant from control; b: significant from standard; c: significant from 500 mg and d: significant from 1000 mg.

with flavonoid content. In addition to the phenolic content, GC was used to investigate the lipid constituents of *R. raetam* lipids fraction. The percentage of identified saturated FAs was found to be higher than that of the unsaturated ones. Linoleic acid is the major detected unsaturated FA, followed by linolenic acid, whereas eicosanoic acid is the major detected saturated FA followed by palmitic acid, which aligns with (Fdil and El Hamdani, 2015) results concerning the lipid content of *R. monosperma* for the percentage of the major fatty acids. On the other hand, identified hydrocarbons were higher than sterols. The percentage of the identified hydrocarbons was higher than that of sterols. Campesterol is the major sterol, followed by stigmasterol and β-sitosterol (Fdil and El Hamdani, 2015).

DPPH and FRAP assays are colorimetric assays that can measure the antioxidant capability of the plant. In both assays, the methanol fraction was the most active among the other fractions compared to standards. Several reports of *R. raetam* plant have illustrated the phytochemical composition of this plant species as having a versatile polyphenolic content, ranging from phenolic acids, flavonoids, and tannins, which justify the reported radical scavenging activity (Benkhouili et al., 2022; El Baakili et al., 2023; Nur-e-Alam et al., 2019). Regarding oral toxicity of the tested extract, the LD_{50} for the AEE of *R. raetam* was expected to be more than 3000 mg/kg; consequently, dose selection of 250, 500, and 1000 mg/kg was considered harmless. Algandaby et al. (2015) reported that LD_{50} for *R. raetam* fruit 1995 mg/kg has a potential hepatotoxic, nephrotoxic effect above 250 mg/kg.

The ingestion of castor oil leads to the liberation of ricinoleic acid, i.e., one of the pharmacologically active constituents of castor oil, by the action of the intestinal lipase enzyme (Kulkarni and Pandit, 2005). The stimulant level of ricinoleic acid changes due to its agonist effect on the prostanoid EP3 receptor, resulting in an increase in prostaglandin level that causes a significant increase in intestinal motility, leading to a mechanical laxative effect (Adeniyi et al., 2017; Mady et al., 2023). Based on the mechanism of castor oil, it has been used in animal protocol to

induce diarrhea to investigate the efficacy of antidiarrheal potential drugs. Conversely, Ezeja et al. (2012) stated that castor oil reduces the absorption of reduced active Na^+ and K^+ and decreases the activity of Na^+, K^+-ATP in the small intestine and colon, causing a lack of absorption and incidence of diarrhea (Ezeja et al., 2012). Several reports have extensively described the high potential of flavonoids, tannins, and phenolic acids as antioxidants and enzyme inhibitors, which constitute the key elements of their anti-inflammatory effect; they can reduce the prognosis of different inflammatory syndromes (Emudainohwo et al., 2015; Kim et al., 2004; Pérez-Cano et al., 2014). Other reported mechanisms of the antidiarrheal effect of natural products are based on controlling gastric motility and suppressing the activity of several gastric parasites (Özbilgin et al., 2013). The current study used the castor oil animal model protocol to induce pathophysiological diarrhea and monitor several parameters, such as the onset of diarrhea and the count of feces produced in both treated and control groups. The findings illustrated that the tested extract exerts a notable antidiarrheal and antisecretory effect. The current phytochemical analysis of *R. raetam* ethanol extract is rich with several phenolics. Pandurangan et al. (2015) reported the effect of gallic acid on colon inflammation induced in mice using dextran sulfate sodium by reporting gallic acid`s ability to suppress the activation of p65-NF-κB and IL-6/p-STAT3Y705 inflammatory mediator (Pandurangan et al., 2015). Hsiang et al. (2013) also added another value of gallic acid as an anti-inflammatory by asserting its downregulating effect on NF-κB pathway after the induction of inflammation in mice using lipopolysaccharide (Hsiang et al., 2013). Regarding the effect of gallic acid and its derivatives, Kim et al. (2020) observed that gallic acid dietary intake for a patient suffering from irritable bowel syndrome significantly increased the presence of several *Lactobacillus* (beneficial bacteria), which reduced the initiation of diarrhea and boosted the excreted butyric acid in feces (Kim et al., 2020). Chen et al. (2006) added another value of gallic acid by reporting its antisecretory effect by inhibiting H^+K^+-ATPase activity (Chen et al., 2006). Also, several flavonoids and phenolic acids investigated in the *R. raetam* ethanol extract function as antidiarrheal, and their antidiarrheal mechanism is reported chiefly due to their inhibitory activity for prostaglandin release (Hämäläinen et al., 2011).

Castor oil in the *in-vivo* induced enter-pooling animal protocol increases intestinal fluid secretion by preventing the water and electrolytes reabsorption after the promotion of prostaglandin production (Mady et al., 2022; Rahman et al., 2015), which indicated that the potential antidiarrheal effect of AEE of *R. raetam* could be attributed to its ability to decrease gastrointestinal secretion volumes by promoting water and electrolyte reabsorption. The charcoal meal animal protocol investigated other potential antidiarrheal mechanisms by investigating its effect on gastrointestinal motility and content transposition (Ezekwesili-Ofili et al., 2016). Several studies have demonstrated the ability of flavonoids and phenolic acids to arrest intestinal motility and control water and electrolyte absorption (Di Carlo et al., 1993). Moreover, Dosso et al. (2011) focused on the ability of flavonoids to control intestinal contractions and secretion induced by prostaglandin E2 through their inhibitory activity to autocoids and prostaglandin release (Dosso et al., 2011). These findings support the idea of the potential antidiarrheal activity of a phenolic-rich extract as they act as prostaglandin biosynthesis inhibitors (Brijesh et al., 2009). One of the potential antidiarrheal mechanisms of polyphenols is also through inhibition of cytochrome P450 systems (Anderson et al., 1991).

CONCLUSION

R. raetam exerted a significant antidiarrheal activity that makes this plant one of the traditional remedies and opened the gate to extensive study for the potential synergism between the polyphenolic and lipoid content that contributes to this activity.

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