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The lipoid matter of *Syzygium australe* leaves exhibit a promising anti-diarrheal activity: GC/MS analysis and *in-vivo* study.

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Abstract: This study examined the antidiarrheal activity of the lipoid matter of *Syzygium australe* leaves. The antidiarrheal effect was assessed through the castor oil-induced diarrhea model. Additionally, we investigated the acute toxicity of the *S.australe* hexane leaves extract. Hexane extract demonstrated a dose-dependent and statistically significant protective effect on rats against castor oil-induced diarrhea. It also suppressed intestinal transit and postponed gastric emptying in comparison to the control group. Regarding acute toxicity, the study findings suggested that the crude hexane extract was relatively safe and non-toxic to rats. The chemical composition of the lipoidal matter (n-hexane fraction) was identified using gas chromatography-mass spectrometry (GC-MS). Examination of the saponifiable matter using GC-MS unveiled the existence of 13 compounds; with saturated and unsaturated fatty acids making up (14.43% and 50.01%); respectively. Among these, the major compound identified was oleic acid, accounting for (30.43%) of the total composition. Meanwhile, the examination of unsaponifiable matter identified 35 compounds, these identified compounds were categorized as hydrocarbons (43.17%), triterpenoids (27.23%) and steroids (13.19%). squalene (16.84%) was regarded as a major compound, followed by ç-sitosterol (13.9%) and à-amyrin (12.94%) respectively. This represents the initial endeavour to explore the GC-MS analysis of the n-hexane extract obtained from this plant.

Keywords: *Syzygium australe*; Antidiarrheal; Castor oil; Intestinal motility; n-hexane extract; GC-MS analysis

1. INTRODUCTION

Diarrhea has historically been known as a serious worldwide health issue, that humanity has ever faced, especially for people living in underdeveloped and resource-constrained nations. Approximately 2.2 million people die from diarrhea each year worldwide, most of them are babies and young children under 5 years old [1.] Diarrhea is an intestinal illness marked by the repeated passage of semisolid or watery stool typically exceeding three times a day. It includes an escalation in the regularity of bowel motions, abdominal pain, heightened bowel noises, and alterations in fluid secretion and absorption[2]. Diarrhea is mostly caused by pathogenic microbes, including bacteria, viruses, and parasites, as well as digestive tract problems, food poisoning, some medications and stress factors [3]. Even though there are numerous anti-diarrheal treatments available in modern medicine, these drugs have drawbacks, side effects, and resistance. Therefore, the introduction of

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a medication which is safe, efficient, and reasonably priced is necessary to treat diarrhea. *World Health Organization (WHO) has established a protocol for diarrhea management that includes the usage of* conventional herbal remedies. *Numerous medicinal plants have been identified for their potential in treating and controlling diarrhea*[4].

Genus *Syzygium*, Myrtaceae family, comprises around 1200 species[5]. *Syzygium australe (S. australe)* is known as Bruch cherry and was originally native to Eastern Australia [6]. It has now become widely distributed in tropical regions. It is an evergreen shrub with flaky bark [7]. *S. australe* comprises several phytoconstituents, including, phenolics, flavonoids [8], saponins, tannins and triterpenoids [9]. In previous studies, *S. australe* has been reported for its antioxidant, anti-microbial 6 and anti-inflammatory properties, as well as for treating fungal skin infections [9]. Research has documented the individual antidiarrheal activities of several *Syzygium* species, including *S. cordatum* [10], *S. myrtifolium* [11] , *S. polianthum*[12], and *S. cumini*[13].

This research is carried out to identify the phytochemical constituents found in the lipoid matter of *Syzygium australe* leaves (LSA) using gas chromatography-mass spectrometry (GC-MS) analysis and to evaluate its anti-diarrheal activity. This work seeks to provide a scientific foundation for the utilization of *Syzygium australe* in addressing diarrhea-related issues.

2. METHODS

2.1. Plant material.

Syzygium australe leaves were collected in Jan. 2022 from Shihab Mazhar Botanical Garden, Barageel, Giza, Egypt. The identity of the plant was established by Dr. Abd-Elhalim Abd-Magli, Doctor of Plant Taxonomy, Agriculture Research Center, Doki, Egypt. The Voucher specimen (No.SE 2022-265) was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, October 6 University.

2.2. Preparation of Extract

Pulverized leaves (1 kg) were extracted with methanol (3 × 3L, 70%) under reflux (65 ºC). The combined methanol extract was concentrated under vacuum (60 °C) to obtain a brown crude extract of 300g. Subsequently, the methanol extract was suspended in 600 ml distilled water and underwent liquid-liquid partitioning using n-hexane (3 × 4L) then the solvent was evaporated using a rotary evaporator (Rotavapor®, BÜCHI, Switzerland) to yield 14g of semi-solid extract.

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The chemical components of LSA were determined using GC-MS (Thermos Scientific, Austin, TX, USA), coupled with a thermo-mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The LSA was saponified by refluxing with N/2 alcoholic KOH. The unsaponifiable components were extracted by a partition with successive portions of diethyl ether (4×100 ml)[14]. The same methodology as Elkhouly *et al*, 2023[15] was applied for the chromatographic separation of constituents on a capillary column, with the exception that electron ionization (EI) was used to acquire mass spectra in full scan mode, covering the m/z range of 50–650 m/z. The analysis was conducted at Nawah Scientific Research Centre (https://nawah-scientific.com), Cairo, Egypt. The compounds were recognized by comparing their mass spectra with those stored in the WILEY 09 and NIST 14 mass spectral databases.

2.4. Antidiarrheal activity

Animals

Adult male Sprague-Dawley rats with weights ranging from 150 to 200 grams were housed under the same hygienic conditions, a well-balanced meal, and water in the animal house of Faculty of Pharmacy, Al-Azhar University, Egypt. These rats were kept under a 12-hour light/dark cycle and provided with free access to a standard pellet diet and water. A two-week acclimatization period was allotted to the rats before the experiment. All handling procedures for the animals adhered to the committee guidelines and aligned with internationally recognized standards for laboratory animal care and use. Experimental cages were constructed using plastic material, with the cage floor padded with white blotting paper and wire mesh positioned approximately at a height of 2 cm above the surface of the floor. To familiarize the rats with the experimental setting, they were placed in these cages for 2 hours daily over a week.

Table 1. Chemical composition of the saponifiable matter of *S.australe*

Peak no.	Identified compounds	Chemical formula
1	Octadecadiynoic acid	$C_{18}H_{28}O_2$
2	Dihydroxytetradecynoic acid	$C_{14}H_{24}O_4$
3	Heptadecenynoic acid	$C_{17}H_{28}O_2$

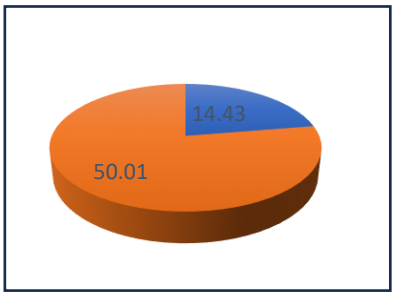
14.43
50.01

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Table 2. Chemical composition of the unsaponifiable matter (USM) of the LSA

Peak No.	Identified compounds	Chemical formula	M.W	Rt(min)	RTT	*Area%
1	Benzene, (1-butylhexyl)	$C_{16}H_{26}$	218	9.14	0.31	0.25
2	Benzene, (1-propylheptyl)	$C_{16}H_{26}$	218	9.32	0.36	0.23
3	Benzene, (1-ethyloctyl)	$C_{16}H_{26}$	218	9.67	0.33	0.30
4	Benzene, (1-methylnonyl)	$C_{16}H_{26}$	218	10.37	0.36	0.46
5	Benzene, (1-butylheptyl)	$C_{17}H_{28}$	232	10.92	0.38	1.14
6	Benzene, (1-propyloctyl)	$C_{17}H_{28}$	232	11.11	0.38	0.75
7	Benzene, (1-ethylnonyl)	$C_{17}H_{28}$	232	11.50	0.40	0.92
8	Benzene, (1-methyldecyl)	$C_{17}H_{28}$	232	12.19	0.42	1.39
9	Benzene, (1-pentylheptyl)	$C_{18}H_{30}$	246	12.59	0.44	0.81
10	Benzene, (1-butyloctyl)	$C_{18}H_{30}$	246	12.67	0.44	0.85
11	Benzene, (1-propylnonyl)	$C_{18}H_{30}$	246	12.89	0.45	0.82
12	Benzene, (1-ethyldecyl)	$C_{18}H_{30}$	246	13.29	0.46	1.03
13	Benzene, (1-methylundecyl)	$C_{18}H_{30}$	246	13.97	0.48	1.44
14	Benzene, (1-pentyloctyl)	$C_{19}H_{32}$	260	14.29	0.50	1.03
15	Benzene, (1-butylnonyl)	$C_{19}H_{32}$	260	14.41	0.5	0.69
16	Benzene, (1-propyldecyl)	$C_{19}H_{32}$	260	14.62	0.51	0.83
17	Benzene, (1-ethylundecyl)	$C_{19}H_{32}$	260	15.03	0.52	0.81
18	Benzene, (1-methyldodecyl)	$C_{19}H_{32}$	260	15.69	0.54	1.13
19	Phytol	$C_{20}H_{40}O$	296	18.84	0.65	4.78
20	Octadecane, 3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	366	27.02	0.94	0.39
21	**Squalene**	$C_{30}H_{50}$	410	28.58	1	**16.84**
22	Hentriacontane	$C_{31}H_{64}$	436	29.82	1.04	2.94
23	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentam ethyl-3,7,11,15,19-heneicosapentaenyl)	$C_{30}H_{50}O$	426	30.15	1.05	0.52
24	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl	$C_{30}H_{50}O$	426	30.33	1.06	0.65
25	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428	31.12	1.08	0.70
26	ç-Tocopherol	$C_{28}H_{48}O_2$	416	31.46	1.1	1.05
27	Octacosane	$C_{28}H_{58}$	394	32.47	1.13	8.12
28	2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol	$C_{29}H_{50}O_2$	430	32.68	1.14	0.35
29	**ç-Sitosterol**	$C_{29}H_{50}O$	414	34.88	1.22	**13.19**
30	Spirost-8-en-11-one, 3-hydroxy-,	$C_{27}H_{40}O_4$	428	35.04	1.22	0.38
31	Lupeol	$C_{30}H_{50}O$	426	35.38	1.23	4.64
32	**α-Amyrin**	$C_{30}H_{50}O$	426	35.85	1.25	**12.94**
33	9,19-Cyclolanostan-3-ol, 24-methylene	$C_{31}H_{52}O$	440	36.48	1.27	2.43
34	Betulin	$C_{30}H_{50}O_2$	442	36.90	1.29	0.54
35	Friedelan-3-one	$C_{30}H_{50}O$	426	37.50	1.31	4.81

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Hydrocarbons	**43.17%**
Triterpenoids	**27.23%**
Steroids	**13.19%**
Miscellaneous	**6.56%**
Total identified compounds	**90.15%**

The comparative proportions of the components in the LSA were established based on the overall peak area of the detected components.

@RTT, retention time relative to squalene; MW, molecular weight; and RT, retention time

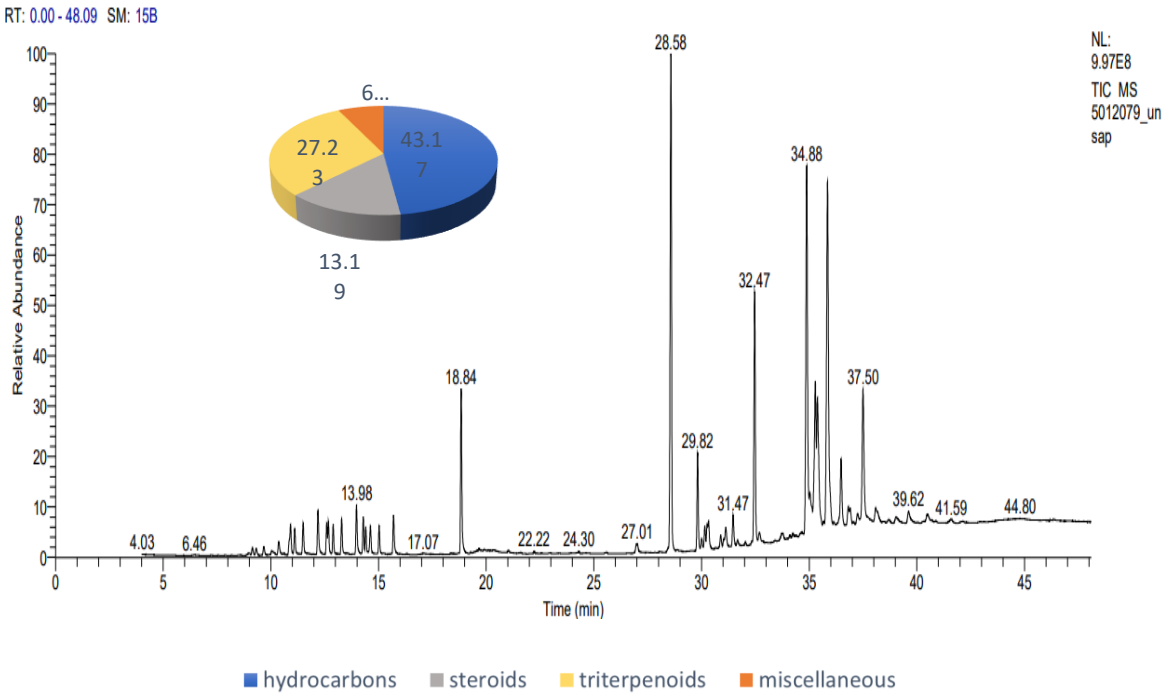

Figure. 2. GC/MS chromatogram of the unsaponifiable matter of *S.australe*

2.4.1 Acute Oral Toxicity Test

The acute oral toxicity assessment was conducted following the OECD 425 guideline [16]. Rats were randomly distributed into control and experimental groups each containing six rats, where each rat received either 1% Tween 80 (as control) or a single oral dose of LSA at varying concentrations (600, 800, 1000, 2000, or 4000 mg/kg body weight). Before the experiment, rats underwent a nightlong fasting period, with food withheld for four hours post-extract administration. Observations were made on each treated rat individually for any unforeseen reactions or mortality over the subsequent 72-hour period.

2.4.2 Induction of diarrhea by castor oil

Following the procedures outlined by Roy and Anjum [17 -18] for conducting antidiarrheal experiments, 24 rats underwent a 24-hour fasting period and were subsequently divided into 4 groups (each with n = 6). All groups were orally administered castor oil at a dosage of 3 ml per animal. After thirty minutes of the administration of castor oil, Group 1 (control) received a vehicle solution consisting of 1% Tween 80 in distilled water, while Group 2 took the standard drug

loperamide orally administered in a dose of 3 mg/kg. Group 3 and Group 4 were given oral doses of LSA at 150 mg/kg and 200 mg/kg, respectively. After that, each rat was retained in a separate cage padded with translucent paper, with the padding being altered every 2 hours. The entire number of fecal outputs was recorded over 24 hours after the beginning of diarrhea, and the entire fluid content of the feces was assessed by calculating the variance in weight between moist and dry feces (desiccated for 24 hours at room temperature in a shaded area). The evacuation category was determined by assessing stool consistency and the overall count of solid, semi-solid, and liquid feces. A numerical grading system was employed: normal stool was assigned a score of 1, semi-solid stool a score of 2, and watery stool a score of 3. Subsequently, the mean evacuation index (EI) was computed for every group to assess the extent of diarrhea severity.

Evacuation index (EI) = **n** solid feces x 1 + **n** semi−solid feces x2 + **n** liquid feces x 3

The performance of each group was indicated by the (%) inhibition of diarrhea, which was determined by assessing the results of all groups in comparison with those of the control group. The percentage of inhibition in evacuation was calculated as follows:

$$\text{Inhibition of evacuation (\%)} = \frac{\text{A (control)} - \text{B(treated)}}{\text{A (control)}} \times 100$$

where A represents the average number of evacuations caused by castor oil (control); B represents the average number of evacuations caused by the tested agents.

2.4.3 Intestinal motility

The method of Adeniyi[19] was employed to assess intestinal motility. A novel number of rats was grouped into 5 sets, with each group comprising 6 rats. Rats in group 1 were administered 1 mL of 1% Tween 80, while those in group 2 received 3 mL of castor oil. Group 3 was treated with a standard drug; atropine, at a dose of 5 mg/kg. Groups 4 and 5 were administered 1 mL equivalent to 150 mg/kg and 200 mg/kg of LSA, respectively. Following a 30-minute interval, rats received 1 mL of freshly prepared charcoal meal containing deactivated charcoal (10%, in 1% Tween 80). Thirty minutes later, the rats were euthanized through cervical dislocation, and their anterior abdominal walls were dissected to retrieve the complete small intestine from the pylorus to the caecum. The length of the small intestine and the distance from the pylorus region to the leading edge of the charcoal meal were assessed and represented as a percentage of the total length of the small intestine.

$$\text{Charcoal transient time (\%)} = \frac{\text{Distance traveled by charcoal meal}}{\text{Total length of the small intestine}} \times 100$$

$$\text{Inhibition (\%)} = \frac{\text{D control – D treated}}{Dcontrol} \times 100$$

Where $D_{control}$ indicates the distance traveled in the control group; $D_{treated}$ indicates the distance traveled in the treated group.

2.4.4 Statistical Analysis

The mean ± standard error (SE) is used to express the results. Several comparisons were carried out using analysis of variance test (one-way ANOVA) followed by Tukey's post-hoc test. Statistical Significance was determined at a p-value lower than 0.05. Using GraphPad Prism software version 5 (San Diego, CA, USA). All graphs were created with the Graph Pad Prism (version 5).

3. RESULTS

3.1. GC/MS analysis of n-hexane leaves extract of *Syzygium australe*

The LSA yielded 57.91% and 42.08% for unsaponifiable matter (USM) and saponifiable matter (SAP) respectively**.** The various components were identified by comparing the spectral fragmentation patterns with data from the Wiley and NIST Mass Spectral Libraries and their comparison with published data. The GC chromatograms are displayed in (**Fig.1 and Fig.2**). The structures and spectra of the main identified compounds were illustrated in (**Fig.3**)

3.1.1. Chemical Profile of SAP Matter of *S.australe*

The result of GC/MS analysis of the SAP constituents of *S. australe* leaves was displayed in Table (1) and Figure (1), which unveiled the presence of 13 compounds. Among these, the relative percentages of saturated and unsaturated fatty acids derivatives were 14.43% and 50.01%, respectively. The major identified saturated fatty acid was oxiraneundecanoic acid, 3-pentyl (8.78%). Moreover, the main unsaturated fatty acids were oleic acid (30.46%) and arachidonic acid (4.29%).

3.1.2. Chemical Profile of USAP Matter of *S.australe*

The findings of the GC/MS analysis of the USAP matter of *S.australe* were depicted in Table (2) and Figure (2), indicating the existence of 35 compounds. These identified compounds were categorized as hydrocarbons (43.17%), triterpenoids (27.23%), steroids (13.19%) and miscellaneous (6.56%). Notably, the major identified compound within the USAP matter was squalene, followed by ç-Sitosterol and α -Amyrin representing 16.84%, 13.9% and 12.94; respectively of the total identified compounds.

3.2. Oral Acute Toxicity:

In acute oral toxicity assessments conducted on rats, LSA demonstrated safety. No adverse effects or alterations in behavior were observed following the administration of a single oral dose. Physical examinations of the rats revealed no signs of physiological changes such as weakness, diarrhea, abnormal breathing sounds, convulsions, or lethargy. Furthermore, there were no instances of mortality or weight loss detected.

3.3. Anti-diarrhea Activity Evaluations:

3.3.1 Effect of LSA on castor oil-induced diarrhea

Results indicated that castor oil administration led to diarrhea in all rats. Notably, all doses of LSA resulted in a notable reduction in the total count of wet fecal pellets expelled compared to the control group (castor oil), with this effect being dose-dependent. Specifically, LSA at 150 mg/kg and 200 mg/kg demonstrated % of inhibitions of 33.04% and 47.25%, respectively. The standard drug loperamide exhibited the highest efficacy, with an inhibition % of 54.2% (Table 3).

As shown in Fig. 4, The intake of castor oil led to an evacuation index reaching 62.33, suggesting the occurrence of liquid and semi-solid stools. Conversely, the group administered with loperamide (3 mg/kg, orally) decreased the evacuation index to 14.6, LSA at 150 and 200 mg/kg abolished diarrhea and reduced the evacuation index to 27 and 21.8, respectively compared to the control group.

Each value indicates the mean of 6 animals ± S.E. One-way ANOVA was used for statistical analysis, followed by the Tukey- multiple comparison test as a post-hoc test. $^{a}p< 0.05$ *vs* the control group, $^{b}p< 0.05$ *vs* the Loperamide group.

3.3.2. Gastrointestinal motility test

Table 4 indicates that the charcoal meal traveled an average of 41.6%of the total length of the small intestine in the group of rats that received the vehicle. LSA demonstrated a noteworthy reduction in the relative distance traveled by the charcoal meal, with the reduction being dose-dependent. Specifically, the highest dose of LSA (200 mg/kg) resulted in a movement of only 26.6% of the small intestine, representing a decrease in transit distance by 36% compared to the control gp. Atropine exhibited a more substantial decline in intestinal movement compared to LSA, while the administration of castor oil notably accelerated intestinal motility relative to the control, as depicted in Table 4.

Each value indicates the mean of 6 animals ± S.E. One-way ANOVA was used for statistical analysis, followed by the Tukey- multiple comparison test as a post-hoc test. $^{a}p< 0.05$ *vs* the control group, $^{b}p< 0.05$ *vs* the castor oil group and $^{c}p< 0.05$ *vs* the atropine group.

4. DISCUSSION

Diarrhea continues to be a serious gastrointestinal illness that kills 2.2 million people a year, especially children under five, with the lack of benefits and increased risk of adverse effects of antidiarrheal and antimotility agent therapies aggravating the problem[20]. Historically, a range of herbal remedies have been used in traditional medicine to treat diarrheal disease. Our current work aims to evaluate the chemical profile of LSA and investigate its antidiarrheal capabilities and mechanism of action.

The acute oral toxicity analysis is an essential component in determining the therapeutic index of drugs and dietary supplements [21]. Oral administration of doses (600–4000 mg/kg bw) of LSA to mice was relatively safe and had a wide safety margin. For this, the LD_{50} of LSA was determined to be higher than 4000 mg/kg bw.

Castor oil-induced diarrhea in animals is a well-known model. Ricinoleic acid is a bioactive metabolite of castor oil, induces the release of prostaglandin and consequently inflames the

mucosal tissues. GIT motility and secretion are subsequently enhanced [22]. In this research, LSA showed dose-dependent protection for the animal model of diarrhea produced by castor oil and

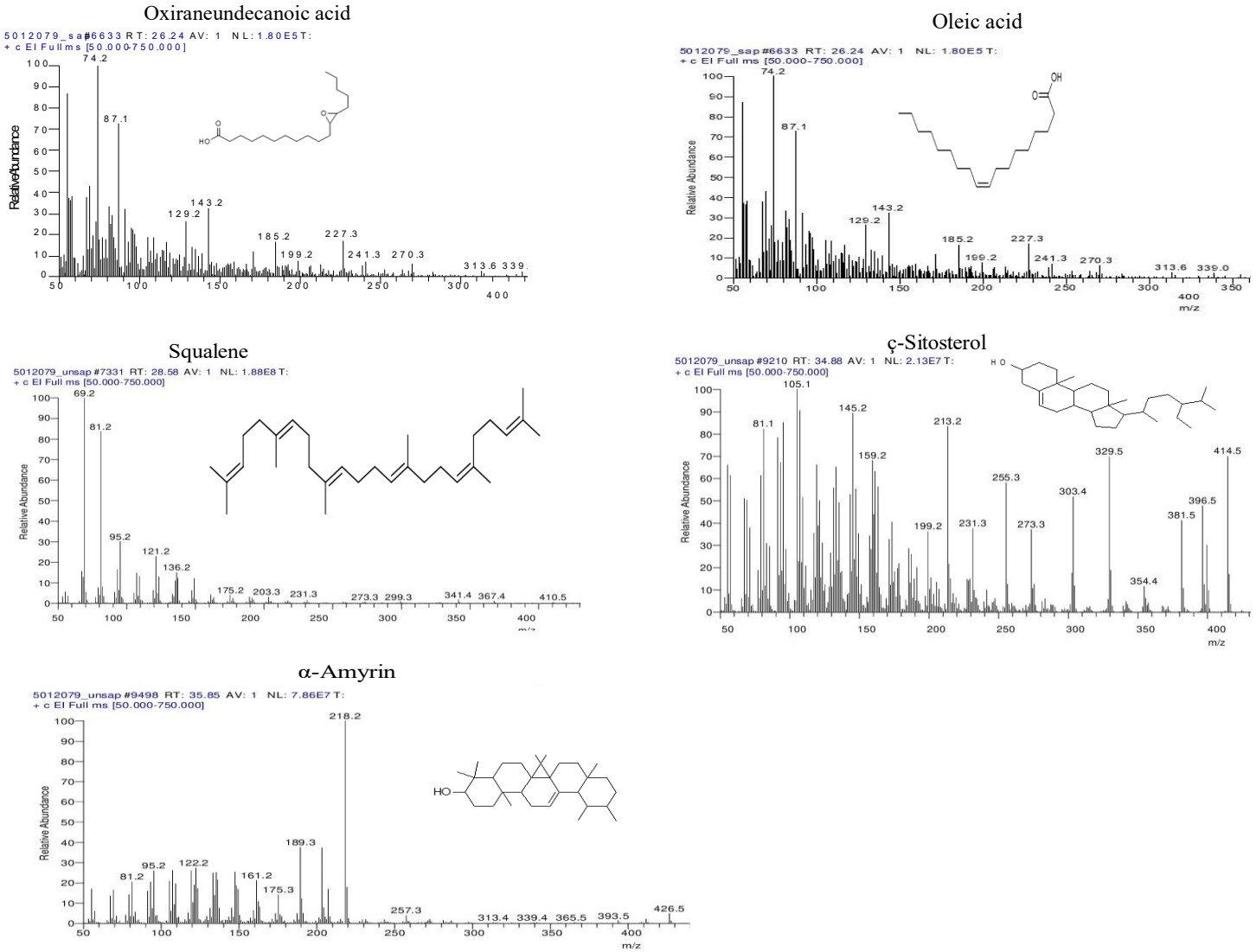

Figure. 3. Mass spectra of major compounds identified in lipoidal matter of *S.australe*.

Table 3: Effect of LSA on castor oil-induced diarrhea in rats.

Group	Total number of feces in 24 h (frequency of defecation in 24 h	Weight (g) of Wet Faces	Weight (mg) of Dry Faces	The fluid content of the feces	Mean evacuation index	% Inhibition of Defecation
Control	22.17±0.6009	5.750±0.2045	3.450±0.1335	2.300±0.1826	62.33±2.319	--------
Loperamide 3mg/kg	12.17±0.4773 [a]	2.633±0.1874 [a]	2.100±0.1844[a]	0.5333±0.06667 [a]	14.67±0.9888 [a]	54.21%
LSA 150 mg/kg	18.17±0.4773 [a b]	3.850 ±0.1765 [a b]	2.600±0.2463 [a b]	1.250±0.2156 [a b]	27.17±1.400 [a b]	33.04%
LSA 200 mg/kg	14.50±0.4282 [a b]	3.033 ±0.2155 [a]	2.100±0.1826 [a]	0.9333 ±0.1820 [a]	21.83±1.167 [a b]	47.25%

Each value indicates the mean of 6 animals ± S.E. One-way ANOVA was used for statistical analysis, followed by the Tukey- multiple comparison test as a post-hoc test. [a]p< 0.05 *vs* the control group, [b]p< 0.05 *vs* the Loperamide group.

significantly inhibited various diarrheal parameters, including the onset, frequency, total stool count, and fluid content, among others. It may be assumed that this antidiarrheal effect of the examined extract is owing to its inhibition of gastrointestinal motility, reduction of intestinal fluid secretion, and delay of gastric emptying, a similar mechanism produced by loperamide, the standard antidiarrheal drug used in the study [1].

The gastrointestinal motility test model demonstrated that the LSA decreased the gastrointestinal motility of rats fed with castor oil, as evidenced by the decrease in the gastrointestinal movement of the charcoal meal. At 150 and 200 mg/kg doses, LSA considerably lowered intestinal charcoal meal transit concerning the control group. These results show that when the LSA dose was increased, the gastrointestinal motility was significantly reduced. The lengthening of the GI contents' presence in the intestine due to a decrease in motility may facilitate the absorption of water and electrolytes in the intestine. Therefore, the plant extract's antimotility action may be responsible for some of its antidiarrheal effects.

The chemical compounds of the LSA were examined via the GC/MS analysis, with the identification of forty-eight phytochemical compounds. Peak area, retention time, molecular formula, and molecular weight are used to identify these compounds. For lipid constituents (fatty acids and unsaponifiable matter) of *Syzygium aystrale* leaves, the main classes are unsaturated fatty acids with oleic acid as the main acid, triterpenes in which squalene and α-amyrin constitute the highest percentage of triterpenes, on the other hand ç-sitosterol represents the main steroid. Previous studies have shown that oleic acid can reduce diarrhea by slowing down the small intestine's transit[23, 24].

Additionally, oleic acid exhibits antibacterial efficacy against a variety of diarrhea-causing pathogens, including Candida stellatoidea, Micrococcus pyogenes, and S. aureus[25]. Remarkably, the GC/MS analysis's findings aligned with earlier studies on *S. calophyllifolium*, which likewise revealed the plant's high triterpene content with squalene as a major constituent[26]. rene *et al*., showed a growing proportion of antidiarrheal activity when the triterpene mixture was given at doses of 100 and 250 mg/kg BW of mice, respectively[27]. Moreover, Triterpenes were most likely the components of the chaenomeles fruit that suppressed the diarrhea caused by Heat-labile enterotoxin (LT)[28]. When there is inflammation, food normally passes through more rapidly and there is less time for water absorption, which leads to watery stools. As a result, taking an anti-inflammatory medication helps reduce diarrhea. α-amyrin possesses strong and sustained anti-inflammatory properties, as demonstrated by multiple investigations.

Additionally, findings demonstrated the various ways in which β-sitosterol combats harmful bacteria, offering a fresh method for treating colonic inflammation[29]. Numerous *Syzygium* species, including *Syzygium alternifolium, S. cordatum, S. cumini, S. gratum, S. guineense, S. nervosum, and S. Syzygium polyanthum*, have been documented as effective in managing diarrhoea[26]. Considering previously mentioned data, this could account for *Syzygium australe* antidiarrheal properties.

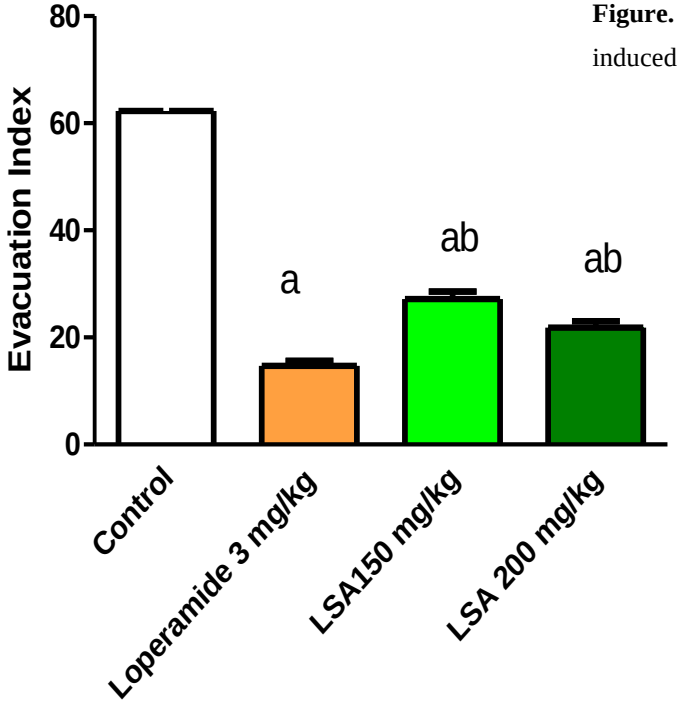

Figure. 4. Effect of LSA on evacuation index in castor oil-induced diarrhea in rats**.**

Table 4: Effects of LSA on small intestine transit

Group	Total intestinal length (cm)	Distance traveled by charcoal (cm)	%Intestinal transit of charcoal	%Inhibition rate
Control	82.67±1.453	34.33±2.333	41.67±2.333	---------
Castor oil	96.00±0.5774	51.0±1.528	53.33±1.453 [a]	-28%
Atropine 5 mg/kg	100.7±2.333	19.67±1.453	19.67±1.202 [a b]	53%
LSA 150 mg/kg	109.7±3.528	32.33±1.453	29.67±1.202 [a b c]	29%
LSA 200 mg/kg	108.0±2.309	29.00±2.082	26.67±1.202 [a b]	36%

Each value indicates the mean of 6 animals ± S.E. One-way ANOVA was used for statistical analysis, followed by the Tukey- multiple comparison test as a post-hoc test. [a]$p< 0.05$ *vs* the control group, [b]$p< 0.05$ *vs* the castor oil group and [c]$p< 0.05$ *vs* the atropine group.

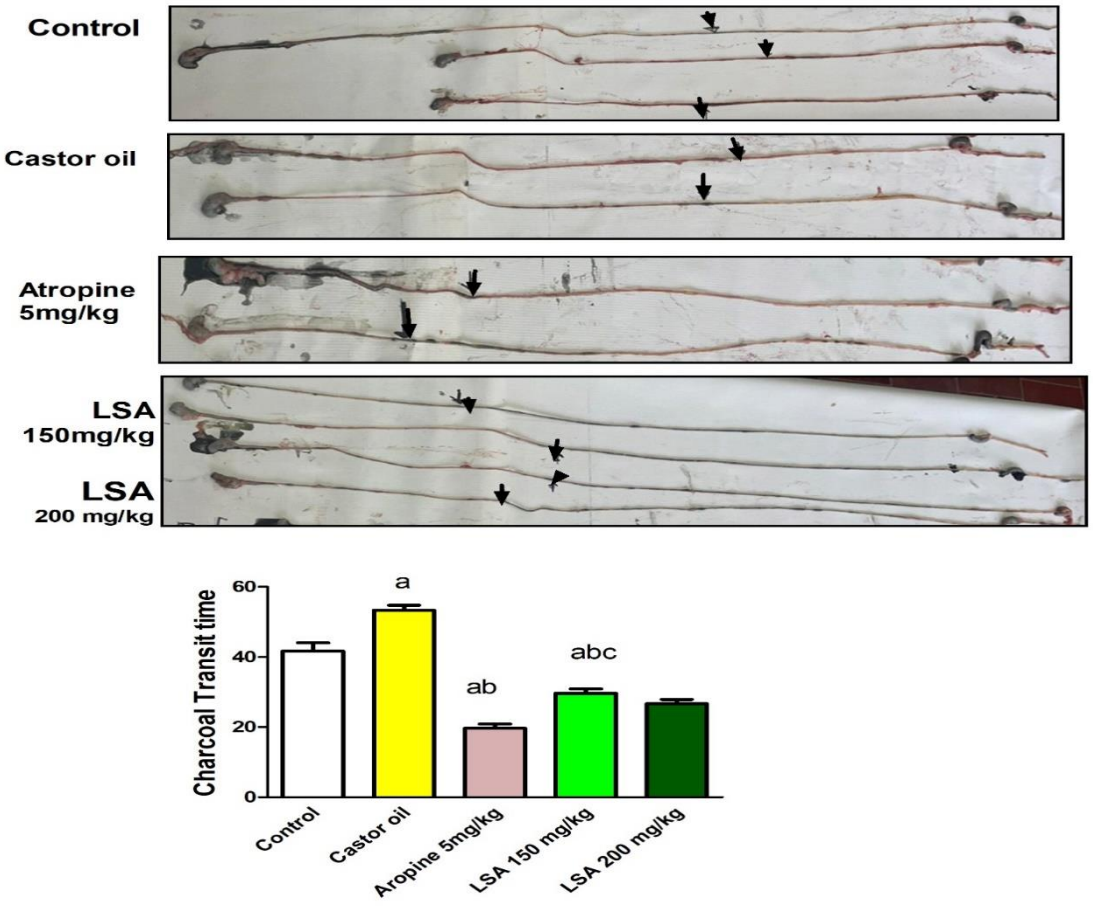

Figure. 5. Effects of LSA on small intestine transit

Each value indicates the mean of 6 animals ± S.E. One-way ANOVA was used for statistical analysis, followed by the Tukey- multiple comparison test as a post-hoc test. $^{a}p< 0.05$ *vs* the control group, $^{b}p< 0.05$ *vs* the castor oil group and $^{c}p< 0.05$ *vs* the atropine group.

5. CONCLUSIONS

While there are medications for treating diarrhea, most of them including loperamide and atropine have adverse effects if taken over an extended length of time. As a result, using herbal remedies to treat diarrhea is becoming more and more popular. In the current study, *Syzygium australe* hexane extract showed antidiarrheal activity in rat models of castor oil-induced diarrhea and gastrointestinal transit delay. GC/MS analysis showed that unsaturated fatty acids, triterpenes, and steroids are the major classes of LSA. The results of this investigation lend credence to the usage of *Syzygium australe* in the treatment of diarrhea. The mechanisms behind this anti-diarrheal effect need to be further investigated in clinical settings to potentially identify naturally occurring chemicals for the treatment of diarrhea.

Declaration of competing interest

The authors state that they have no conflicts of interest.

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Conflict of interest

the authors declare no conflict of interest is associated with this publication.

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Author contribution

Heba H. Elzayat performed extraction to obtain lipoidal matters, participated in GC/MS analysis, and wrote the paper. **Alaadin E. Elhadad** revised the paper and conceived the project. **Shaza A.Mohamed** revised and finalized the paper. **Mona Mansour** shared in the pharmacological study. **Noha A. Seif**

Eldein participated in the interpretation of GC/MS charts, wrote, and revised the paper.

Authors consent of publication Authors declare that this manuscript is original and has not been published before, and not being considered for publication elsewhere.

List of Abbreviation

HE of S. *australe* leaves: n-hexane extract of *Syzygium australe* leaves

LSA: lipoid matter of *Syzygium australe* leaves

GC/MS: Gas Chromatography-Mass Spectrometry

NIST: National Institute of Standards and Technology

OECD: Organization for Economic Cooperation and Development

PO: By mouth (orally)

GI: gastrointestinal

S. aureus: *Staphylococcus aureus*

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