Chemical Profiling and Antioxidant, Anti-Inflammatory, Cytotoxic, Analgesic, and Antidiarrheal Activities from the Seeds of Commonly Available Red Grape (*Vitis vinifera* L.)

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ABSTRACT

OBJECTIVES: The current study aimed to conduct a phytochemical screening of commonly known fruit red grape (*Vitis vinifera* L.) seed methanolic extract through gas chromatography and mass spectrometry (GC-MS) to identify the bioactive compounds responsible for its health benefits and evaluate the pharmacological potentialities of the extract and its fractions against oxidation, inflammation, pain, and diarrhea.

METHODS: The *in vitro* antioxidant, anti-inflammatory, and cytotoxic characteristics of methanolic extracts and various solvent fractions of *V. vinifera* were evaluated using the DPPH free radical scavenging assay, membrane stabilizing, and brine shrimp lethality bioassay. Furthermore, the study assessed the effects of crude extracts (200, 400, and 600 mg/kg of body weight) on pain relief and reduction of diarrhea in animals using methods such as tail immersion, the acetic acid-induced writhing technique, and a diarrheal mouse model induced with castor oil.

RESULTS: A total of 73 phytoconstituents were predominantly found in the seed extract based on the GC-MS analysis. Among the identified compounds, 9-octadecenamide (13.7%), and (*9E,11E*)-octadeca-9,11-dienoate (11.07%) are most abundant. Several notable constituents, such as gamma-sitosterol, stigmasterol, paromomycin, 4,6-cholestadienol, gamma-tocotrienol, 24-Propylidenecholest-5-en-3beta-ol, and alpha-tocopherol acetate, are also present. The methanolic extract of *V. vinifera* seed and its different solvent fractions showed promising antioxidant properties (IC_{50} = 1.19-17.42 µg/mL) compared to the standard antioxidant butylated hydroxytoluene (IC_{50} = 20.46 µg/mL). Aqueous soluble fraction exerted inhibition of nearly 50% heat-induced hemolysis compared to the standard acetylsalicylic acid (42%). Besides, all the tested doses (200, 400, and 600 mg/kg bw) of the crude extract showed significant ($P < .05$) analgesic and antidiarrheal effects.

CONCLUSION: The current findings endorsed the health benefits of *V. vinifera* by revealing potent antioxidant, anti-inflammatory, analgesic, and antidiarrheal effects. Nevertheless, further in-depth analysis of the plant's chemical constituents and pharmacological effects on health is warranted for novel drug discovery from *V. vinifera*.

KEYWORDS: Red grape seed, GC-MS analysis, antioxidant, anti-inflammatory, cytotoxicity, analgesic, antidiarrheal

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Introduction

Fruit is classified as a botanical product originating from the reproductive structures of plants, constituting a vital component of the human and animal diet. Fruits serve as a primary energy source while offering essential nutrients and an array of bioactive compounds crucial for maintaining optimal health. Furthermore, fruits exhibit health-promoting properties, mitigating susceptibility to particular ailments and age-related functional degenerations.[1] A significant correlation between a diet rich in antioxidants and diminished susceptibility to chronic illnesses was established in various research.[2] Cereals, legumes, oilseeds, as well as fruits and vegetables, serve as primary sources of dietary polyphenols and numerous bioactive constituents.[3,4] These components play pivotal roles in enhancing the functional and nutraceutical qualities of the diet.[3] Moreover, it is noteworthy that not only the consumable portions of fruits but also the residual byproducts amount contain substantial well-known for of phenolic compounds and bioactive phytochemicals. These compounds are their notable antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties.[3,5,6]

Food loss and waste have emerged worldwide, manifesting at different points along the food supply chain. In 2019, hunger or insufficient access to nutritious food affected around 2 billion people, equivalent to about a quarter (25.9%) of the global population. Conversely, each year, about one-third (1.3

billion metric tons) of the food produced for human consumption goes to waste throughout the food supply chain.[7] This wastage also entails substantial nutritional losses, with foods rich in nutrients like fruits and vegetables being the most commonly discarded. Consequently, reducing or reallocating food waste has the potential to enhance food accessibility and simultaneously elevate nutritional and dietary standards.

The most common fruits consumed globally encompass apples, grapes, and exotic fruits indigenous to their respective cultivation regions. Grapes notably constitute the largest fruit crop worldwide, boasting an annual production exceeding 75 million tons globally.[8] Within this framework, grapes and their derivatives, including wine, grape juice, and preserves, have significant economic importance and exert a substantial influence on waste generation. Residues such as marc, peels, and grape seeds, persist due to inadequate management practices, thereby posing a contamination risk.[8] Conversely, grape byproducts serve as a valuable reservoir of essential nutrients, encompassing vitamins, minerals, lipids, proteins, carbohydrates, and polyphenolic compounds.[6]

The red grape (*Vitis vinifera* L.; Family: Vitaceae), which grows in the Mediterranean and Central Asia, is a commonly consumed fruit rich in nutrients, which offers many health advantages.[9] However, the winemaking sector is renowned for producing significant quantities of byproducts, and managing their disposal presents economic and environmental challenges. This issue of food waste has drawn considerable public health concern. Hence, there is value in conducting research to transform food waste into a usable resource and tackle worldwide nutritional deficiencies.[10,11]

In several experimental investigations, red grape (*Vitis vinifera* L.) seed extract, which is a byproduct of manufacturing wine and is high in pro-anthocyanidins, has demonstrated promise. Research indicating its effectiveness against hypertension, inflammation, peptic ulcers, microbiological infections, cardiovascular disease, cancer, and diabetes has confirmed its pharmacological activity and beneficial health effects. Extracts of grape seeds are poised to be turned into a potential therapeutic due to their wide variety of applications.[12] In animal experiments, grape seed extract has demonstrated preventive properties, including suppression of DNA breakage, lipid peroxidation, and reduction of cardiac infarct size. It has been shown to inhibit lineages of human cancer cells *in vitro*.[13]

The current investigation utilizes GC-MS analysis to identify bioactive components from the methanolic extract of red grape (*V. vinifera*) seeds. This research additionally explored the pharmacological potentialities of the fruit seeds against oxidation, inflammation, pain, and diarrhea. These outcomes of the research may be helpful in spreading the nutraceuticals and medicinal value of the *V. vinifera* seeds, which is commonly available in Bangladeshi market.

Methods and Materials

Collection of V. vinifera *seeds and drying*

The *Vitis vinifera* (Family: Vitaceae), locally known as red grapes, were purchased from the local market of West Dhanmondi, Dhaka, Bangladesh, in January 2022. The batch was collected from Yantai, Shandong province, China. Following the acquisition of the fruit from the market, the process entailed the manual collection of fresh seeds, which were subsequently subjected to washing with fresh water. The seeds underwent a drying phase through natural shedding, facilitated by exposure to open air for an extended period. During this drying regimen, stringent measures were implemented to consistently maintain an environmental temperature below 30°C. This precautionary measure was adopted to ensure the preservation of heat-sensitive compounds and to preempt any possibility of degradation. Supplemental Figure S1 illustrates the dried seeds of *V. vinifera*.

Chemicals and reagents

Reagents of Analytical-level and substances were utilized in this investigation. All the chemicals such as Tween 80, tert-butyl-1-hydroxytoluene (BHT), Folin-Ciocalteau reagent, and Gallic acid were collected through Merck (Germany). Sigma-Aldrich (USA) supplied 2,2-diphenyl-1-picrylhydrazyl (DPPH). BEXIMCO Pharmaceuticals Ltd., Dhaka, Bangladesh, delivered the glibenclamide, normal saline solution, and loperamide as gift samples.

Extraction

A high-capacity grinding apparatus was employed to transform the desiccated seeds into a coarse powder. The extraction process followed the solid-liquid extraction method. It is noted that 500 g of powdered seeds were placed inside a sanitized amber bottle with a 3.0 L capacity. Subsequently, the powder was immersed in 2.0 L of methanol and allowed to soak for a period of 15 days at room temperature, with intermittent shaking and stirring. After that, a fresh cotton pad and filter paper were used to filter the solvent mixture. In order to produce a concentrated crude extract, the methanol was subsequently separated using an "EYELA Rotavapor" rotary evaporator at smaller pressures and about 40 to 50°C. Ultimately, a quantity of 67 g of gummy exudate was acquired from *V. vinifera*, signifying a yielding rate of 13.4%.

Fractionation

For the solvent-solvent differentiation of the extract, S. Morris Kupchan's partitions (1970) technique, modified by VanWagenenet et al[14] as used. It was accomplished by extracting 5 g of crude extract with water in methanol (1:9), petroleum ether (PESF), dichloromethane (DCMSF), ethyl acetate (EASF), and water

(AQSF) to produce 4 distinct fractions. Each fraction also continued to evaporate until it was completely dried. The yields for the fractions produced by petroleum ether, dichloromethane, ethyl acetate, and water solubility were 25%, 24%, 18%, and 27%, respectively.

Gas Chromatography Mass Spectrometry (GC-MS) analysis

With an auto-sampler, Shimadzu, Japan, GC/MS-QP2010 ultra was used to analyze the phytoconstituents of the *V. vinifera* seeds' methanolic extract. In a 5 MS/HP column (30 m, 0.25 mm, and 0.25 m), extremely pure helium was used as the mobile phase. The linear speed of the helium was 39 cm/s, and the helium circulation rate was 1.12 mL/min. The oven temperature was kept constant at an average rate of 10°C per minute, ranging from 110°C to 280°C. The needle temperature was adjusted to 250°C. The injection volume (50 µL) was entered in splitless mode (ratio of 10:1). The detector voltage was maintained at 0.94 kV. The ambient temperatures of the ion source, MS transfer line, and the ion source were all kept at 200 and 250°C, respectively. Full-scan mass spectra with a (m/z) range of 85 to 500 were captured at 10 000 u/s. Searching in the National Institute of Standards and Technology (NIST) collection yielded to identify peaks and chemical constituents.

Antioxidant properties

Total phenolic content (TPC). The TPC of the seeds' methanolic extract and fractions was determined using the Folin-Ciocalteu method.[15] One milliliter of each fraction and extract (2 mg/mL) was combined with Na_2CO_3 (2.5 mL, 7.5% w/v) and a 10-fold diluted Folin-Ciocalteu reagent (2.5 mL). The solution was incubated in the dark at room temperature for 30 minutes. A UV-Vis spectrophotometer (760 nm) was used to test the sample's absorbance. The calibration standard curve (Gallic acid) was created by placing the absorbance against various Gallic acid concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 µg/mL) to calculate the TPC of the samples stated on the label as GAE mg (Gallic acid equivalent)/g of the study's samples. The Gallic acid calibration curve formulations are as follows:

$$y = 0.0162x + 0.0215, R^2 = 0.9985$$

DPPH scavenging assay. Antioxidant activity was qualitatively and quantitatively assessed using thin-layer chromatography (TLC) and the stable free radical 0.04% DPPH (1,1-diphenyl-2-picrylhydrazyl).[16] To separate the polar and non-polar components of the extract, diluted stock solutions were applied to a dyed silica gel TLC plate, which was then placed in chambers containing solvents of varying polarities (polar, medium polar, and non-polar). The plate was subsequently sprayed with 0.02% DPPH in ethanol and incubated at room temperature. Following the DPPH treatment, color shifts (yellow on a purple background) were observed on the resolved bands for 10 minutes.[16]

The DPPH approach was employed to assess the antioxidant potential of MECE and various fractions.[17] Initially, 3.0 mL of freshly made methanol and DPPH solution (20 µg/mL) was thoroughly combined with 2 mL of the samples' various strengths (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.93, and 0.97 µg/mL) before being placed in a dark environment at room temperature for 30 minutes. The samples' absorbance was then measured with a UV-Vis spectrophotometer calibrated to 517 nm. The inhibition percentage (I%) of the DPPH free radical was calculated using the following formula[17]:

$$I\% = \left(A_{control} - A_{sample}\right)/A_{control} * 100$$

$A_{control}$ indicated the absorbance of the solution that had all reagents but lacked the test components. A_{sample} indicated the absorbance of the samples being examined or the standard (BHT) solution. Every test sample generated a graph showing the percentage of inhibition versus the concentrations of the tested substances, and the IC_{50} (half concentration of the greatest inhibitory concentration) values were then calculated using a linear regression approach.

Anti-inflammatory effects

The effectiveness of *V. vinifera* seed extract and fractions to stabilize human erythrocyte membranes was investigated using hypotonic and heat-induced techniques.[18,19]

Hypotonic solution-induced hemolysis. Red blood cells (RBCs) were given to a 70 kg adult with fair skin and no hidden disorders. The RBCs were put in a sterile container containing an anticoagulant, EDTA. A buffer solution with a pH of 7.4 was created using disodium phosphate and its conjugate acid, monosodium phosphate. 4.5045 g of NaCl was dissolved in sterile distilled water to make an isotonic solution (500 mL, 154 mM). 1.4625 g of NaCl was dissolved in sterile distilled water to make a hypotonic solution (500 mL, 50 mM). The blood was cleaned 3 times with sodium phosphate buffer and isotonic solution before centrifugation (3000 rpm, 10 minutes) to get the erythrocyte suspension. A stock RBC suspension (0.50 mL), a buffer of 10 mM sodium phosphate (4.5 mL), and a hypotonic solution (50 mM NaCl) were used as test samples, with different proportions of MECE (2.0 mg/mL) or standard ASA (0.10 mg/mL) as the reference standard. Before centrifugation (3000 rpm, 10 minutes), the resulting solutions were incubated at ambient temperature for 10 minutes. The supernatant's absorbance (optical density (OD)) was then measured at 540 nm. The following calculation was used to compute the hemolysis inhibition percentage or membrane stabilization of the tested samples and standard ASA:

$$\%\ \text{Hemolysis inhibition}\ (\text{hypotonic solution induced}) = 100 \times (OD_1 - OD_2)/OD_3$$

OD_1: hypotonic buffered saline solution optical density (Control)

OD_2: optical density of the examined sample in hypotonic solution

Heat-induced hemolysis. Two batches of centrifuge tubes containing 5 mL of isotonic buffer and 1.0 mg/mL of seed extract and various fractions were made. One tube with the same amount of material was used as a control. Each tube was filled with 30 µL of erythrocyte suspension and stirred slowly by inversion. A single set of tubes was submerged in a 54°C water bath for 20 minutes, whereas the additional set was maintained in a 0 to 5°C cold bath. Following incubation, the supernatant was turned to a centrifuge (1300 rpm, 3 minutes) and the optical density (OD) was measured at 540 nm. The percentage inhibition of hemolysis in the study was estimated using the following equation:

$$\%\ \text{hemolysis inhibition}\ (\text{heat-induced}) = 100 \times \left(\frac{1 - OD_2 - OD_1}{OD_3 - OD_1}\right)$$

Here, OD_1 = optical density (OD) of the test sample unheated, OD_2 = OD of test sample heated, and OD_3 = OD of control sample heated.

Cytotoxicity: brine shrimp lethality bioassay

The methanolic extract and its fractions of *V. vinifera* seeds were assessed for their cytotoxic effects through a brine shrimp lethality test described by Rashid et al,[19] utilizing vincristine sulfate (VS) as a positive control. To create varying concentrations (ranging from 400.0 to 0.781 µg/mL) of the samples, a serial dilution of each 4 mg test sample was prepared in 99% DMSO (dimethyl sulfoxide). These solutions were then exposed to simulated seawater containing around 10 live brine shrimp nauplii. After a 24-hour period, the surviving nauplii were scrutinized using a magnifying glass. The toxicity level of the shrimp in response to each concentration of the sample was determined to estimate the LC_{50} value. The LC_{50} value, representing the concentration at which 50% of the shrimp were not viable, was calculated from a graph plotting the percentage of non-viable shrimp against the logarithmic concentration of the plant extract, utilizing the vincristine standard curve as reference.

Experimental design and sample size determination for in vivo studies

Swiss albino adult mice that were healthy, weighed between 25 and 30 g, and were between 4 and 5 weeks' old were collected from the Animal Division of the International Center for Diarrheal Diseases and Research in Bangladesh (ICDDRB). The mice were kept in polypropylene cages under standard climatic settings, including a 25°C ambient temperature, a 55°% relative humidity level, and a 12-hour light/dark cycle before the animal study began. During this time, ICDDRB prepared food and water for them to consume. The study followed the rules established by the Federation of European Laboratory Animal Science Associations (FELASA) for the moral treatment of animals in investigations. The Animal Ethics Committee of State University of Bangladesh also carefully examined and confirmed the research's ethical standards and procedures. In every test, mice were divided into 5 distinct groups; negative control, positive control, and 3 different (200, 400, and 600 mg/kg bw) sample groups I-III. Every group had 2 male and 2 female mice. Since we could not determine the standard deviation and effect size, we employed the "resource equation" method outlined by Mukta et al[5] and Bulbul et al[20] to estimate the sample size. While working with a 1-way analysis of variance (ANOVA), we calculated the sample size for the degrees of freedom associated with the variability between subjects, also known as within-subject degrees of freedom, using the formula:

$$n = DF/k + 1$$

In this formula, "n" represents the number of animals in each group, "DF" signifies the degrees of freedom, and "k" denotes the total number of groups. We considered the allowable range for degrees of freedom (DF) to establish the minimum and maximum number of animals per group. We obtained the respective minimum and maximum numbers of animals per group by using the minimum (10) and maximum (20) values for DF. In this case, we excluded the normal control group by setting 'k' to 4, indicating 4 groups (comprising 3 test groups I-III with doses of 200, 400, and 600 mg/kg bw, and 1 positive control group with the standard drug). Therefore, the minimum and maximum numbers of animals per group should be 4 ($10/k + 1 = 10/4 + 1$) and 6 ($20/k + 1 = 20/4 + 1$), respectively. Following these ethical principles, we carried out this preliminary investigation with the minimal number of animals per group (n = 4). At the end of the study, according to the AVMA (American Veterinary Medical Association) guidelines for the Euthanasia of Animals, all mice were compassionately euthanized while under general anesthesia.[20]

Central analgesic activity

The central analgesic effects of the MECE were evaluated using a heat approach and the tail immersion experiment.[21,22] The given morphine (15 mg/mL) was diluted with saline water to form the standard sample (subcutaneous, 2 mg/kg). The test medications were orally administered to the mice using a feeding syringe. The test included submerging a mouse's tail in water heated to 55°C. At 0, 30, 60, and 90 minutes after giving the test samples to each mouse, the PRT or latency duration to move its tail in hot water was assessed.

Peripheral analgesic activity

The acetic acid-induced writhing method was employed to assess the peripheral analgesic effects of MECE.[22] Each animal group received glacial acetic acid, a substance that induces pain. Tween 80 was used as a negative control, Diclofenac sodium (50 mg/kg) as a positive control, and MECE (200, 400, and 600 mg/kg) were given to the distinctive mouse groups. Acetic acid is in charge of writhing. The number of writhes was counted after delivering acetic acid intraperitoneally for 10 minutes. The following percentage of writhing inhibition was calculated:

$$\text{Writhing inhibition } \% = \left(N_{Control} - N_{test}\right)/N_{Control} \times 100\%$$

where N = average amount of stomach writhing for every group.

Anti-diarrheal activity

A castor oil-induced diarrhea model in mice was implemented to evaluate the anti-diarrhea activity of MECE, as stated by earlier investigation.[23] Each of the 3 groups—the control, positive control, and test groups—contained 4 mice each. The control group received 1% Tween 80 in normal saline as a carrier solution and only orally at a specific dose (10 mL/kg). While different doses (200, 400, and 600 mg/kg) of MECE were provided to the 3 test groups with Tween 80 (1% in normal saline) as a carrier, loperamide (50 mg/kg) was administered orally to the positive control group. Each mouse was administered 1 mL of pure castor oil to induce diarrhea after 1 hour. Each mouse was housed in an individual cage placed on the floor, lined with blotting paper. The quantity of diarrheal stools for each mouse was recorded at the conclusion of every hour for up to 4 hours following the administration of castor oil. The blotting paper was replaced at the beginning of each hour. Observations from the test groups were compared with those from the negative and positive control groups to evaluate the potential of MECE as an antidiarrheal agent. The percentage reduction in diarrhea was calculated using the following formula to assess the antidiarrheal activity.[23]

$$\%\text{ inhibition of defecation} = \left(D_{control} - D_{test}\right)/D_{control} \times 100\%$$

where D = Average diarrheal episode/number in every group.

Statistical analysis

MS Excel (version 10.0) was used for processing the graphs and data related to in vitro investigation. Because the *in vitro* data was derived from a single experiment, there was no statistical analysis conducted for comparing between groups; instead, a numerical comparison was carried out. Instead, statistical analysis was done on the data collected from the *in vivo* tests. We compared the treatment groups to the control (vehicle) group. The average values with their associated standard errors of the mean was expressed as mean ± SEM to summarize the *in vivo* results. To analyze the *in vivo* data, we used Statistical Package for Social Sciences (SPSS) Software (version 26.0), conducting a 1-way analysis of variance (ANOVA) on all variables, followed by a student's t-test. Any *P*-values below .05 were considered statistically significant.

Results

Identification of phytoconstituents by GC-MS analysis

A total of 73 phytoconstituents were predominantly found in the MECE based on their GC-MS analysis. The spectrum obtained from GC-MS analysis is available as Figure 1. Among the isolated compounds, 9-Octadecenamide (13.7%), (*9E,11E*)-octadeca-9,11-dienoate (11.07%), 1,3-dihydroxypropan-2-yl(*9Z,12Z*)-octadeca-9,12-dienoate (9.06%), and Tris(hydroxymethyl)nitromethane (7.93%) were most abundant (Table 1), while squalene (0.08%), 13-Docosenoicacid, methyl ester (*Z*)- (0.1%), and Vitamin E (0.1%) were the least amount compounds (Table 1). Some bioactive plant steroids such as gamma-sitosterol, stigmasterol, Ergost-5-en-3-ol, (3beta, 24R)-, and 24-Propylidenecholest 5-en-3beta-ol were also detected from the MECE. In addition, some notable compounds are paromomycin, 4,6-cholestadienol, gamma-tocotrienol and alpha-tocopherol acetate. According to GC-MS analysis, all identified compounds' names, retention time (RT), % area, molecular formula, molecular weight, PubChem CIDs, and chemical structures were tabulated in Table 1. In addition, the reported bioactivities of these identified compounds were also tabulated with their corresponding references (Table 1).

Assessment of antioxidant properties

Total phenolic content (TPC). The findings revealed that the MECE expressed the most TPC (223.31 mg of GAE/g of dried extract), then the EASF and DCMSF fractions at 190.25 mg of GAE/g and 135.25 mg of GAE/g, respectively. In contrast, the PESF fraction indicated the least TPC, with 11.00 mg of GAE/g (Figure 2). These results suggest that the phenolic profiles of the various fractions of the seed extract are diverse, which may have consequences for their prospective bioactivity and use.

DPPH scavenging activity. In this study, the antioxidant potency of methanolic extract of *V. vinifera* seed and its different solvent fractions showed promising antioxidant properties (IC_{50} = 1.19-17.42 µg/mL) compared to that of the standard antioxidant butylated hydroxytoluene (BHT; IC_{50} = 20.46 µg/mL; Figure 3). The EASF has the highest free radical scavenging potency (IC_{50} = 1.19 µg/mL), followed by MECE (IC_{50} = 2.94 µg/mL), AQSF (IC_{50} = 6.55 µg/mL), PESF (IC_{50} = 16.81 µg/mL), and DCMSF (IC_{50} = 17.42 µg/mL).

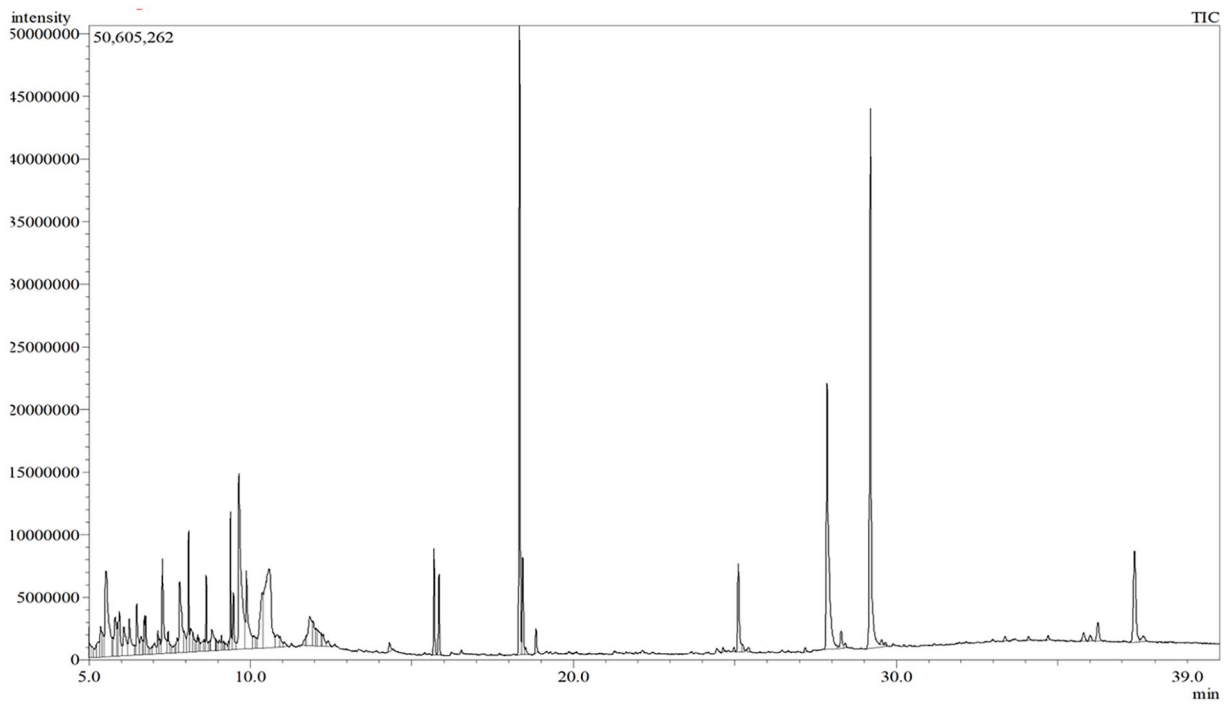


Figure 1. GC-MS spectrum of the crude extract obtained from *V. vinifera* L.

Anti-inflammatory effects

Hypnotic medium-induced hemolysis has been diminished as below: AQSF (22.62%), DCMSF (13.62%), PESF (10.39%), MESF (6.53%), and EASF (7.10%). The standard acetylsalicylic acid (ASA) inhibition rate was significant (61.90%; Figure 4). The most efficacious inhibitor of heat-induced hemolysis was AQSF (44.9%), followed by DCMSF (31.15%), PESF (21.65%), MESF (13.43%), and EASF (13.50%). The inhibition of the standard ASA was 42.00% (Figure 4).

Cytotoxicity

Each sample that underwent testing displayed noteworthy lethality against brine shrimp larvae, as evidenced by their LC_{50} values (15.95-83.24 µg/mL), which were comparable to the established benchmark set by vincristine sulfate with an LC_{50} of 0.45 µg/mL (Figure 5).

Central analgesic activity

As stated in Figure 6, all the doses of the tested sample expressed significant ($P < .05$) dose- and time-dependent analgesic properties compared to standard morphine. The groups of ingesting MECE at various doses demonstrated a rise in pain reaction latency compared to the control, whereas the negative control possessed no analgesic impact.

Peripheral analgesic activity

The study found that MECE showed noticeable and dose-dependent activity in peripheral analgesic assessment compared to the negative control group. The outcomes demonstrated that the seeds extracted from groups I to II exhibited a time- and dose-dependent reduction in writhing activity in mice (Figure 7). The seeds extract at group II and group III exerted 36.78% and 43.68% writhing inhibition in mice, around half of the standard diclofenac sodium (77.01% inhibition; Supplemental Figure S2). All the sequels were statistically significant with $P < .05$, indicating that the MECE might have a peripheral analgesic effect in mice in response to acetic acid-induced stomach writhing.

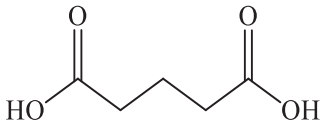
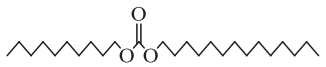
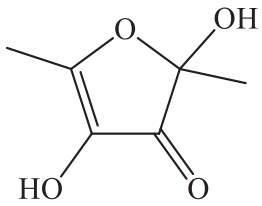
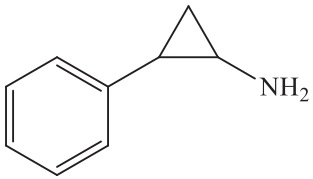
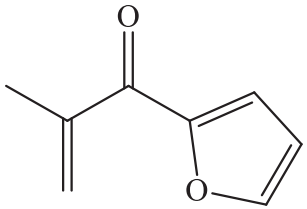
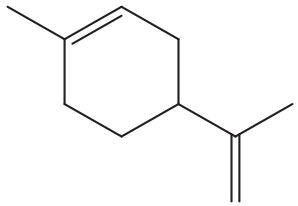
Anti-diarrheal activity

The results of the MECE indicated a statistically significant reduction in the total amount of diarrheal feces at all doses of MECE (Figure 8). Notably, the 400 mg/kg dose of MECE demonstrated the topmost degree of activity (80.43%) compared to the positive control Loperamide (78.26%) for the reduction of diarrhea (Supplemental Figure S3).

Discussion

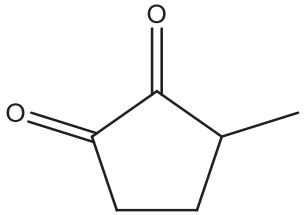
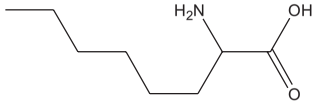
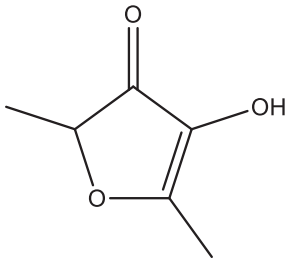
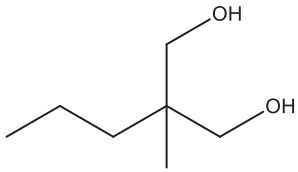
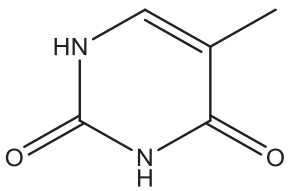
Herbal remedies, including parts like fruit, seeds, bark, fruit peel, and leaves, are frequently viewed as valuable sources of bioactive phytochemicals for addressing various health issues such as oxidative stress, diabetes, pain, fever, cancer, hypertension, and many more human illness.[77] The present research examined the chemical components within the seed extract of *V. vinifera*. This analysis revealed a range of potentially valuable bioactive elements. These elements could play a crucial role in driving the diverse bioactive effects observed in the red grape seed extract and its different solvent-based fractions. The GC-MS analysis of the MECE expressed the presence of several phytoconstituents, such as organic acids (pentanedioic acid, 2-amino octanoic

Table 1. List of Detected Constituents by Gas Chromatography Mass Spectrometry (GC-MS) from Methanolic Extract of *V. vinifera*.

SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
1	Pentanedioic acid	5.027	0.39	132.11	$C_5H_8O_4$	743		Anti-neoplastic activity	Goswami et al[24]
2	Decyl tetradecyl ester carbonic acid	5.105	0.32	272.423	$C_{25}H_{50}O_3$	91693142		Antioxidant, Antibacterial activity	Gopal Pandit et al[25]
3	2,4-dihydroxy-2,5-dimethylfuran-3(2H)-one	5.365	0.82	144.12	$C_6H_8O_4$	538757		Anti-oxidative	Cerny[26]
4	Tranylcypromine	5.41	0.78	133.19	$C_9H_{11}N$	5530		MAO inhibitor	Tranylcypromine[27]
5	1-(furan-2-yl)-2-methylprop-2-en-1-one	5.532	4.56	136.15	$C_8H_8O_2$	3007408		Anti-fungal activity	Ogata et al[28]
7	Limonene	5.817	1.46	136.23	$C_{10}H_{16}$	22311		Anti-bacterial activity	Han et al[29]

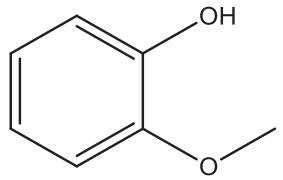
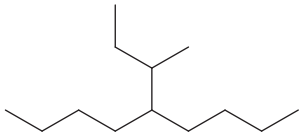
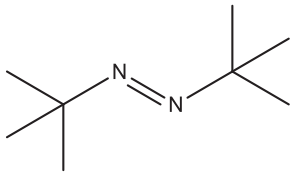
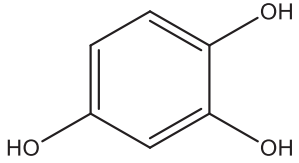
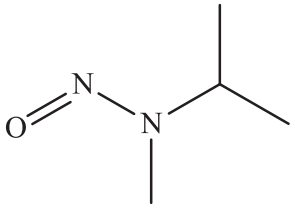
(Continued)

Table 1. (Continued)

SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
8	Maple lactone	5.91	0.55	112.12	$C_6H_8O_2$	6660		Anti-oxidant activity	Kim et al[30]
10	2-amino octanoic acid	6.09	1.36	159.22	$C_8H_{17}NO_2$	69522		Antibiotic activity	Almahboub et al[31]
11	Furaneol	6.251	1.46	128.13	$C_6H_8O_3$	19309		Anti-microbial & Anti-fungal activity	Sung et al[32]
12	1,3-Propanediol, 2-methyl-2-propyl	6.435	0.26	370.38	$C_{11}H_{22}N_4O_8S$	117593		Sedative, anticonvulsant and muscle relaxant effect	Yale et al[33]
13	Thymine	6.489	1.19	126.11	$C_5H_6N_2O_2$	1135		Anti-cancer & Anti-microbial activity	Kumar et al[34]

(Continued)

Table 1. (Continued)

SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
14	2-methoxy phenol	6.617	0.6	124.13	$C_7H_8O_2$	460		Antibacterial and antioxidant activity	Rubab et al[35]
15	Nonane,5-(1-methylpropyl)	6.683	0.21	184.36	$C_{13}H_{28}$	43943		Antioxidant and Antimicrobial activity	Ashraf et al[36]
16	Diazene, bis(1,1-dimethylethyl)-	6.725	0.61	142.24	$C_8H_{18}N_2$	70227		Anti-phytopathogenic	Singh et al[37]
19	1,2,4-Benzenetriol	6.94	0.22	126.11	$C_6H_6O_3$	10787		Anti-oxidant	Zhang et al[38]
21	2-Propanamine, N-methyl-N-nitroso	7.141	0.48	102.14	$C_4H_{10}N_2O$	92271		Anti-bacterial	Kadiri et al[39]

(Continued)

Table 1. (Continued)

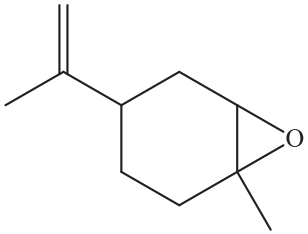
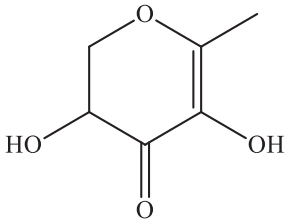
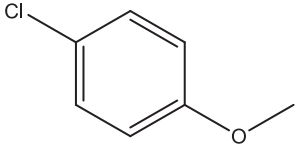
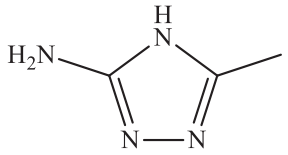
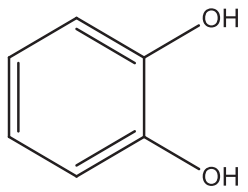
SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
22	Limonene oxide	7.197	0.3	152.23	$C_{10}H_{16}O$	8029780		Bactericidal, anticancer,	Junior and Pastore[40]
23	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	7.282	2.56	144.12	$C_6H_8O_4$	119838		Anti-oxidant and mutagenic	[41]
24	4 chloroanisole	7.459	0.49	142.583	C_7H_7ClO	11137567		Antibacterial activity	Boubakri et al[42]
25	5-methyl-4H-1,2,4-triazol-3-amine	7.545	0.2	98.11	$C_3H_6N_4$	234610		Antiviral & Anti-infective activity	[43]
28	Catechol	7.81	2.88	110.11	$C_6H_6O_2$	289		Anti-microbial, Anti-fungal activity	Kocaçalışkan et al[44]

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Table 1. (Continued)

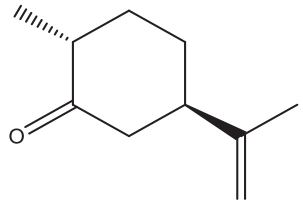
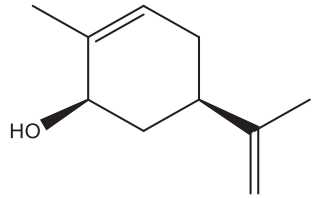
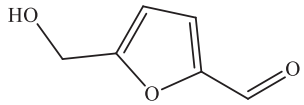
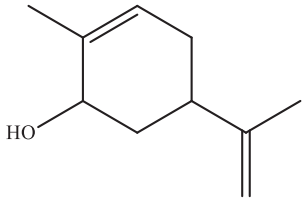
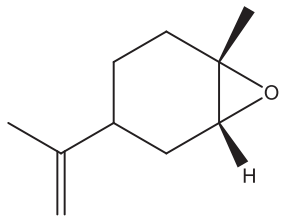
SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
29	2-methyle-5(1-methylethyl)-cyclohexanone	7.957	0.63	154.25	$C_{10}H_{18}O$	10362		Weak fungicidal activity	Xia et al[45]
30	(1R,5R)–2-methyl-5-(prop-1-en-2-yl)cyclohex-2-enol	8.09	1.86	152.23	$C_{10}H_{16}O$	11084068		Anti-bacterial & Anti-fungal activity	Aberchane et al[46]
31	5-(hydroxymethyl) furan-2-carbaldehyde	8.152	0.86	126.11	$C_6H_6O_3$	237332		Anti-oxidant & Anti-proliferative	Qiu et al[47]
32	Carveol	8.23	0.36	152.23	$C_{10}H_{16}O$	7438		Anti-oxidant, Anti-cancer & vasorelaxation	Lacerda-Neto et al[48]
35	*cis*-Limonene oxide	8.638	1.05	152.23	$C_{10}H_{16}O$	6452061		Anti-microbial & insecticidal activity	He et al[49], Aggarwal et al[50]

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Table 1. (Continued)

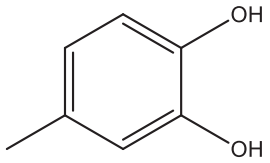
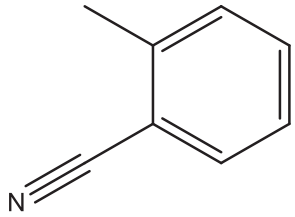
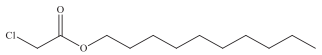
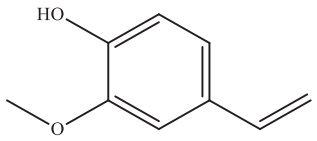
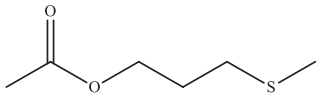
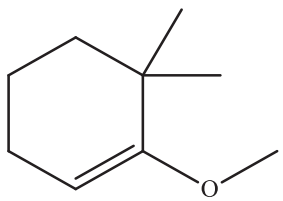
SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
36	4-Methyl catechol	8.807	0.88		$C_7H_8O_2$	9958		Antioxidant	Ito et al[51]
37	o-Tolunitrile	8.91	0.13	117.15	C_8H_7N	10721		NA	
38	Cyclopropane	8.94	0.16	42.08	C_3H_6	6351		NA	
39	Decyl 2-chloroacetate	8.975	0.18	234.76	$C_{12}H_{23}ClO_2$	229381			
40	2-Methoxy 4-vinylphenol	9.046	0.29	150.17	$C_9H_{10}O_2$	332		Antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination	Ibibia et al[52]
41	3-(Methylthio)propyl acetate	9.165	0.13	148.23	$C_6H_{12}O_2S$	85519		Flavor activity	Aoki and Uchida[53]
43	1-Methoxy-6,6-dimethylcyclohex-1-ene	9.24	0.18	140.22	$C_9H_{16}O$	580527		NA	

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Table 1. (Continued)

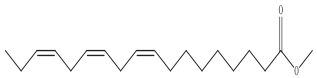
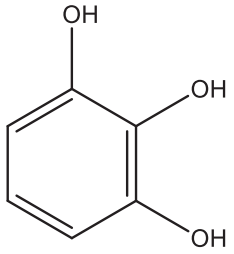
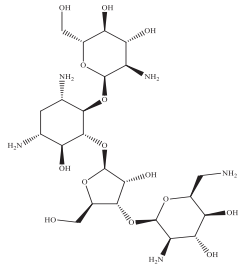
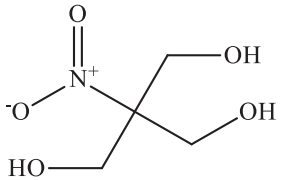
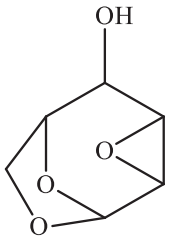
SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
44	9,12,15-Octadecatrienoic acid, methyl ester, (9Z,12Z,15Z)-	9.329	0.15	292.5	$C_{19}H_{32}O_2$	9316		Antioxidant, anti-carcinogenic, anti-inflammatory, and anti-obese	Yuan et al[54]
45	1,2,3-Benzenetriol	9.646	7.39	126.11	$C_6H_6O_3$ or $C_6H_3(OH)_3$	1057		antibacterial and antioxidant	Cynthia et al[55]
46	Paromomycin	10.2	0.22	615.6	$C_{23}H_{45}N_5O_{14}$	441375		Antibiotic activity	Davidson et al[56]
48	Tris(hydroxymethyl) nitromethane	10.575	7.93	151.118	$C_4H_9NO_5$	31337		Preservative, Disinfectant & Bactericidal activity	Izzat and Bennett[57]
49	Anhydro-d-mannosan	10.839	0.65	162.14	$C_6H_{10}O_5$	11947765		Muscle-relaxing and pain relieving	Yellu and Bhukya[58]

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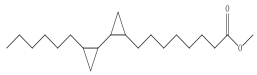
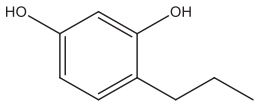
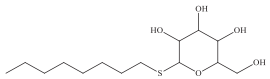
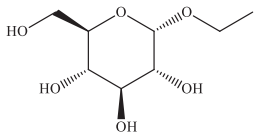
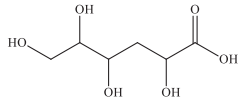
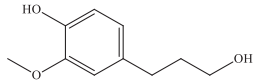
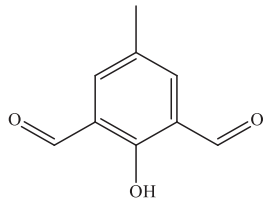
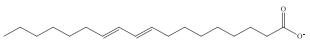
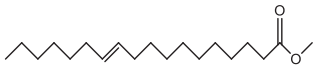
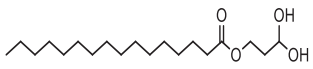
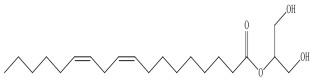
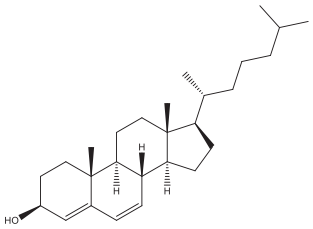
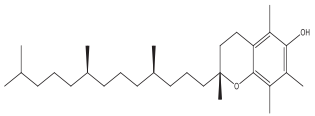
Table 1. (Continued)

SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
50	2′ -Hexyl-1,1”-bicyclopropane-2-octanoic acid methyl ester	10.93	0.34	322.5	$C_{21}H_{38}O_2$	552098		Anti-microbial activity	Srivastava et al[59]
52	4-Propylresorcinol	11.842	1.73	152.19	$C_9H_{12}O_2$	87874		Anti-tyrosine activity	Matsubara et al[60]
53	2-(hydroxymethyl)-6-octylsulfanyloxane-3,4,5-triol	12.04	0.4	308.44	$C_{14}H_{28}O_5S$	363418		NA	
54	Ethylalpha-d-glucopyranoside	12.105	0.69	208.21	$C_8H_{16}O_6$	91694274		Preservative	Rajalakshmi and Mohan[61]
55	3-Deoxyhexonic acid	12.19	0.25	180.16	$C_6H_{12}O_6$	10350		NA	
56	Dihydroconiferyl alcohol	12.256	0.39	182.22	$C_{10}H_{14}O_3$	16822		Stimulating cell growth	Lee et al[62]
57	2-Hydroxy-5-methylisophthalaldehyde	14.311	0.28	164.16	$C_9H_8O_3$	81744		NA	
58	(9E,11E)-octadeca-9,11-dienoate	18.337	11.07	279.4	$C_{18}H_{31}O_2$			Metabolic regulator, hypocholesterolemia, anti-atherogenic, anti-carcinogenic, antioxidant,	Fagali and Catalá[63]

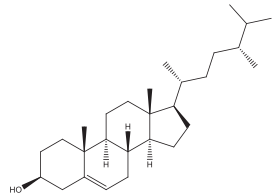
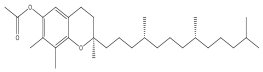
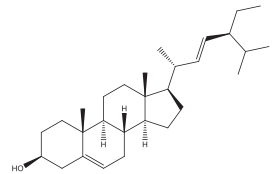
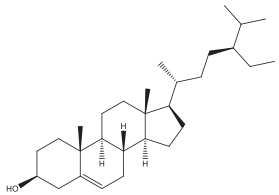
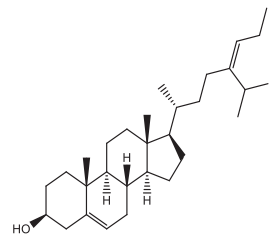
(Continued)

Table 1. (Continued)

SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
59	(*E*)-methyl octadec-11-enoate	18.436	1.95	296.5	$C_{19}H_{36}O_2$	74738		Anti-diarrheal activity	
60	13-Docosenoicacid, methyl ester (Z)-	24.971	0.1	352.6	$C_{23}H_{44}O_2$	5363109		Anti-cancer activity	Paudel and Pant[64]
61	3,3-dihydroxypropyl palmitate	25.108	2.24	689.1	$C_{40}H_{80}O_8$	24698		Anti-cancer	Zhu et al[65]
62	1,3-dihydroxypropan-2-yl (9Z,12Z)-octadeca-9,12-dienoate	27.855	9.06	354.5	$C_{21}H_{38}O_4$	5365676		Analgesic, anti-inflammatory and Anti-ulcerogenic	Mohammed et al[66]
63	2,3-Dihydroxypropyl 12-hydroxyoctadecanoate	28.288	0.55	374.6	$C_{21}H_{42}O_5$	95408		NA	
64	9-Octadecenamide	29.195	13.7	281.5	$C_{18}H_{35}NO$	1930		Antioxidative and hypolipidemic properties	Cheng et al[67]
65	Squalene	29.654	0.08	410.7	$C_{30}H_{50}$	638072		anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient	Kim and Karadeniz[68]
66	4,6-cholestadienol	33.363	0.13	384.6	$C_{27}H_{44}O$	14795191		Broad spectrum antimicrobial activity	Zhu et al[69]
67	Vitamin E	34.093	0.1	430.7	$C_{29}H_{50}O_2$	14985		Antioxidant activity	Stanner and Weichselbaum[70]

(Continued)

Table 1. (Continued)

SL. NO.	COMPOUND	RT	AREA %	MOL. WEIGHT (G/MOL)	MOLECULAR FORMULA	PUBCHEM CID	CHEMICAL STRUCTURES	BIOLOGICAL ACTIVITY	REF
68	Gamma-Tocotrienol	34.698	0.13	410.63	$C_{28}H_{42}O_2$	5282349		Antitumor activity	Suman et al[71]
69	Ergost-5-en-3-ol, (3beta,24R)-	35.798	0.31	400.7	$C_{28}H_{48}O$	6428659		Antioxidant, Anti-cancerous properties	Kang et al[72]
70	Alpha-tocopherol acetate	36	0.18	472.7	$C_{31}H_{52}O_3$	86472		antioxidant	Tucker and Townsend[73]
71	Stigmasterol	36.234	0.68	412.7	$C_{29}H_{48}O$	5280794		Antimicrobial, anticancer, diuretic, anti-inflammatory, antioxidant	Khalid et al[74]
72	Gamma sitosterol	37.372	3.54	414.7	$C_{29}H_{50}O$	457801		Anti-hyperglycemic	Sirikhansaeng et al[75]
73	24-Propylidenecholest-5-en-3beta-ol	37.647	0.29	426.7	$C_{30}H_{50}O$	193212		Anti-bacterial	Kavita et al[76]

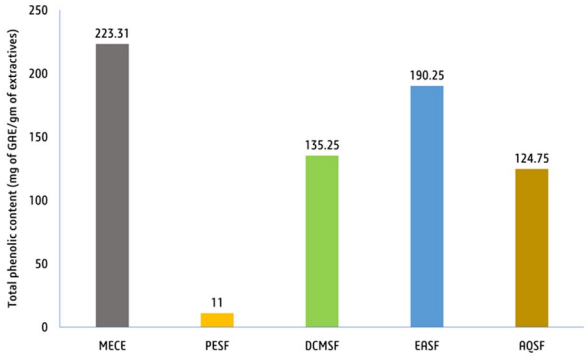


Figure 2. Total phenolic content of the various fractions and methanolic extract of *V. vinifera* seeds. (Here, MECE = methanolic crude extract of *V. vinifera*, PESF = petroleum ether soluble fraction, DCMSF = dichloromethane soluble fraction, EASF = ethyl acetate soluble fraction, and AQ = aqueous or water-soluble fraction).

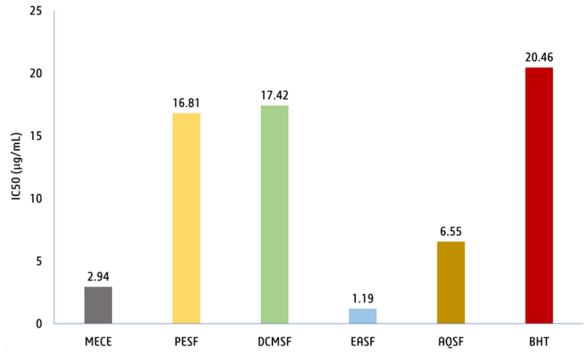


Figure 3. DPPH free radical scavenging activity (IC_{50}, µg/mL) of various solvent fractions and methanolic extract of *V. vinifera* seeds. (Here, MECE = methanolic crude extract of *V. vinifera*, PESF = petroleum ether soluble fraction, DCMSF = dichloromethane soluble fraction, EASF = ethyl acetate soluble fraction, and AQ = aqueous or water-soluble fraction).

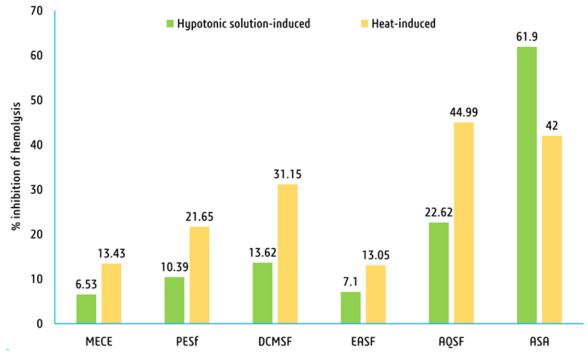


Figure 4. Membrane stabilizing impact of MECE and various fractions on hypotonic medium hemolysis and heat-induced hemolysis. (Here, MECE = methanolic crude extract of *V. vinifera*, PESF = petroleum ether soluble fraction, DCMSF = dichloromethane soluble fraction, EASF = ethyl acetate soluble fraction, and AQ = aqueous or water-soluble fraction).

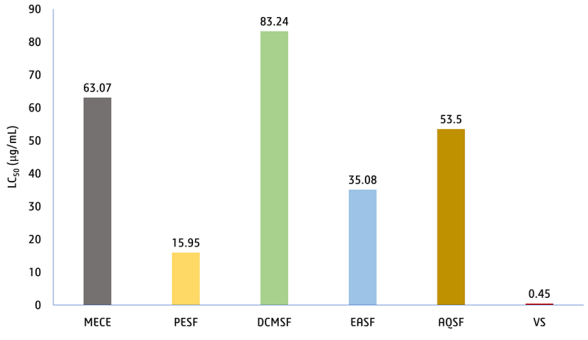


Figure 5. Cytotoxicity of methanolic extract and its fractions of *V. vinifera* seeds in terms of LC_{50} (µg/mL). (Here, MECE = methanolic crude extract of *V. vinifera*, PESF = petroleum ether soluble fraction, DCMSF = dichloromethane soluble fraction, EASF = ethyl acetate soluble fraction, and AQ = aqueous or water-soluble fraction).

acid), esters (Decyl tetradecyl ester carbonic acid), aliphatic hydrocarbons (Limonene, Nonane,5-(1-methylpropyl), Cyclopropane, Squalene), ketones (2,4-dihydroxy-2,5-dimethylfuran-3(2H)-one, 2,4-dihydroxy-2,5-dimethylfuran-3(2H)-one, 1-(furan-2-yl)-2-methylprop-2-en-1-one, Maple lactone), alcohols (1,3-Propanediol,2-methyl-2-propyl, carveol), amine (Tranylcypromine), phenols (2-methoxy phenol, 1,2,4-Benzenetriol, Catechol, 4-Methyl catechol), steroids (gamma sitesterol, 24-Propylidenecholest-5-en-3beta-ol, stigmasterol, ergost-5-en-3-ol, (3beta, 24R)-), and tocopherols (alpha-tocopherol, gamma-tocotrienol; Table 1). These 73 compounds from the MECE may contribute to the medicinal activities of the fruit. From the previous literature study, most of the detected compounds in this study have anti-inflammatory, antioxidant, antibacterial, and anticancer effects (Table 1). The availability of cyclic unsaturated compound especially which has an aromatic ring such as catechol and 1,2,4-Benzenetriol may play a role for antioxidant properties of the MECE.[38,44] Another molecule squalene was also identified from this plant which have potential anticancer and antioxidant activities that are matched with the grape fruits bioproperties.[68]

Free radicals and oxidants are harmful to the body because they are produced by both natural cellular processes and external causes such as emissions, smoking, medicines and radiation. The buildup of free radicals exceeds the body's ability to remove them, resulting in oxidative stress. This mechanism has a crucial involvement in the onset of degenerative and chronic illnesses such as cardiovascular disease, autoimmune disorders, carcinoma, rheumatoid arthritis, cataracts, aging, and neurological diseases. The human body uses a variety of strategies to resist oxidative stress, including the creation of antioxidants. These antioxidants can be produced naturally by the body or obtained from food sources or supplementation.[77] In this study, the MECE showed the highest total phenolic content and EASF showed the highest scavenging capacity against DPPH freer radical. Zeghad et al[78] showed that whole fruits with seeds had tremendous antioxidant activity. Another study revealed that *V. vinifera* is the most abundant source of various flavonoids, polyphenols, and caffeic acid derivatives, while *V. vinifera* showed positive outcomes in the DPPH and ferric reducing antioxidant power (FRAP) assays.[79]

Due to the harmful nature of a crude medicinal substance which is a significant worry, a low-cost and dependable method,

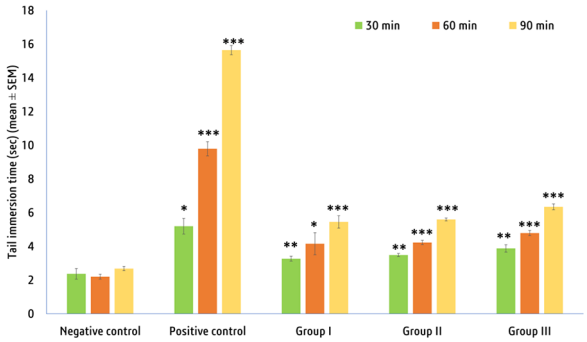


Figure 6. Central analgesic properties of crude methanolic extract of *V. vinifera* seeds. Values are expressed as Mean ± SEM (n = 4). ***$P < .001$, *$P < .05$ compared to negative control group. Positive control (morphine at 2 mg/kg b.w.), Groups I, II, and III = 200, 400, and 600 mg/kg bw = Methanol extract *V. vinifera* seed, respectively.

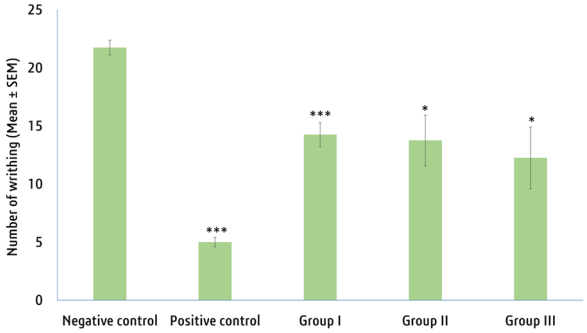


Figure 7. Peripheral analgesic properties of crude methanolic extract of *V. vinifera* seeds. Values are expressed as Mean ± SEM (n = 4). ***$P < .001$, *$P < .05$ compared to negative control group. Positive control (Morphine at 2 mg/kg b.w.), Groups I, II, and III = 200, 400, and 600 mg/kg bw = Methanol extract *V. vinifera* seeds, respectively.

the brine shrimp lethality bioassay using *Artemia salina*, was performed to initially screen for cellular toxicity in plant extracts. A study conducted by Lagartoparra et al[80] discovered a strong connection (with a correlation coefficient of .85 and a significance level of $P < .05$) between the 50% lethal concentration (LC_{50}) derived from the brine shrimp test and the 50% lethal dose (LD_{50}) from animal trials. This indicates that the brine shrimp test could serve as an alternative model. Meyer et al[81] suggested that a bioactive plant compound would generally have an LC_{50} value of <1000 µg/mL. The present research revealed that the LC_{50} values obtained from the brine shrimp bioassay were all below 1000 µg/mL. As demonstrated by previous research (LC_{50} >10 µg/mL), none of the plant's crude extracts or fractions should be deemed extremely toxic or deadly.[82]

The current research has also exerted a promising anti-inflammatory effect in heat-induced membrane stabilizing assays. Previous research has shown that *V. vinifera* seed extract has considerable anti-inflammatory effects by significantly reducing gene expression as well as protein secretion of inflammatory factors like tumor necrosis factor (TNF-α), interleukin-6 (IL-6), the inducible isoform of nitric oxide synthase (iNOS), and nitric oxide (NO).[83] Through histological

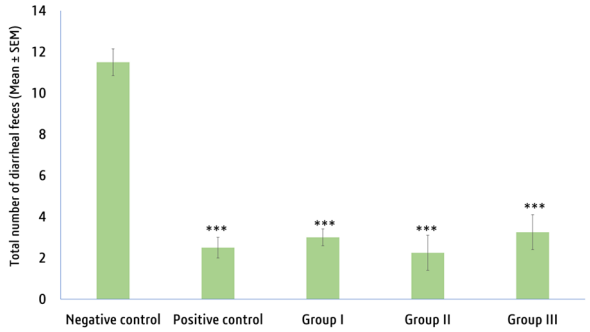


Figure 8. Effects crude methanolic extract on the number of diarrheal feces (mean ± SEM) of mice after 4h of administration of castor oil. "***" means $P < .001$ compared to negative control group. Positive control (loperamide at 50 mg/kg b.w.), Groups I, II, and III = 200, 400, and 600 mg/kg bw = Methanol extract *V. vinifera* seeds, respectively.

examination, another study using mice found that seed extracts successfully diminished the levels of inflammatory molecules such as TNF-α, NF-K, IKK-a, IL-6, and IL-1.[84] However, the findings of this current study imply that the AQSF with heat-induced hemolysis showed a similar significance anti-inflammatory effect compared to the standard drug (44.99% versus 42.00%, respectively). In the GC-MS analysis, some anti-inflammatory compounds such as 2-Methoxy 4-vinylphenol, 9,12,15-Octadecatrienoic acid and 1,3-dihydroxypropan-2-yl (*9Z,12Z*)-octadeca-9,12-dienoate, and stigmasterol were detected, which may responsible for the anti-inflammatory properties of the MECE. Furthermore, large antioxidant molecules detection may also be responsible for the anti-inflammatory action of the fruit. The inflammation is mainly brought on by oxidative stress.[85] Histamine, serotonin, proinflammatory cytokines (including interleukin-1B and tumor necrosis factor-α), and inflammatory cells like leukotrienes and macrophages are the mediators involved in the complicated inflammation process.[85] Thromboxane A2, prostaglandins, and leukotrienes are examples of arachidonic acid metabolic products that also play a role in this process. Finally, these inflammations cause pain and diarrhea.[86] As a result, the presence of antioxidant molecules in *V. vinifera* is advantageous since they aid in controlling inflammation by neutralizing damaging ROS. These may help prevent or treat inflammatory-related disorders and positively impact general health.

Natural remedies for pain relief are being sought as replacements to manmade medications since they are associated with side effects.[87] In this study, we found that the seed extract of *V. vinifera* significantly decreased both central and peripheral pain experience in mice. Several analgesic compounds such as 2-methoxy 4-vinylphenol, Anhydro-d-mannosan, 1,3-dihydroxypropan-2-yl *(9Z,12Z)*-octadeca-9,12-dienoate were also recorded from the presence study. Moreover, the predominant antioxidant molecules also conclude the potential analgesic properties of MECE. Nadia et al showed that *V. vinifera* has dose-dependent central analgesic activity.[88] Aouey et al indicated in their study that some compounds mainly caffeoyltartaric acid and flavonoids

derivatives were detected from the *V. vinifera* extract that may interfere the prostaglandins pathways.[89] Antioxidant molecules effectively inhibit the production of the prostaglandins and COX-2 production, which are responsible for the pain reaction.[90] In addition, endogenous fatty acid amides have been shown in previous research to exhibit significant bioactivity both centrally and peripherally.[87]

The current study also found significant antidiarrheal effects of the MECE. Castor oil induces diarrhea primarily through 3 mechanisms: producing nitric oxide, increasing gastrointestinal membrane calcium permeability, and triggering prostaglandin production, resulting in increased fluid and electrolytes in the intestine, and stimulating peristalsis.[91] The key ingredient in castor oil, ricinoleic acid, is alleged to upset the gut wall by generating prostaglandins and inducing peristaltic movement that might lead to diarrhea.[92-94] Antioxidants molecules have significant prostaglandin inhibition activity.[95] In this current study, although, no significant anti-diarrhea compounds were identified, several antioxidant compounds were detected from this GC-MS analysis, which may be responsible for the inhibition of castor oil-induced gut inflammation diarrhea effects.

Limitations and future research

The study presents certain areas for improvement, notably the need for comprehensive chromatographic isolation, purification, and spectroscopic characterization of the phytoconstituents from the investigated sample. Future research can be done to isolate pure compounds from the fruit's seed extract and evaluate their pharmacological potential via *in vitro*, *in vivo*, and *in silico* analyses against diverse therapeutic targets.

Conclusion

The current study identified 73 phytoconstituents, including 9-octadecenamide, gamma-sitosterol, stigmasterol, paromomycin, 4,6-cholestadienol, gamma-tocotrienol, 24-Propylidenecholest-5-en-3beta-ol, and alpha-tocopherol acetate, from the seed extract of *V. vinifera*. The study also evaluated its pharmacological properties, focusing on antioxidant, anti-inflammatory, cytotoxicity, analgesic, and antidiarrheal activities. The methanolic seed extract and its various solvent fractions exerted promising antioxidant and anti-inflammatory properties, and the 3 tested doses (200, 400, and 600 mg/kg bw) of the crude extract showed significant in vivo effects against pain and diarrhea. However, further investigation is required to completely comprehend the precise processes and to discover the bioactive substances that regulate the actions of *V. vinifera* via in vitro, in vivo, and in silico approaches. Nevertheless, the antioxidant ability of this plant species provides hope for reducing inflammation and enhancing health.

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Author Contributions

JH: Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. KRL: Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation. AS: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. RA: Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation. MAR: Writing – review & editing, Visualization, Validation, Project administration. MAA: Writing – review & editing, Visualization, Validation, Software, Resources, Data curation.

Consent for Publication

Not applicable.

Data Availability Statement

The article contains all necessary information to support the conclusions. Contacting the corresponding author with a fair request will get you further raw data.

Ethical Approval

The work adhered to the standards for the ethical treatment of animals in research set forth by the Federation of European Laboratory Animal Science Associations (FELASA). The Animal Ethics Committee of the State University of Bangladesh thoroughly reviewed and approved the research's ethical standards and practices.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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