



## Research article

Hypoglycemic and antioxidant activities of *Jasminum officinale* L. with identification and characterization of phytochemicals

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## ARTICLE INFO

## Keywords:

*Jasminum officinale*

Glucose uptake

Phytochemicals

Diabetes

Antioxidant

Anti-inflammatory

## ABSTRACT

The utilization of plant-derived chemicals with anti-diabetic properties is widely promoted for its advantageous tactics in managing diabetes, as they are cost-effective and have minimal or no adverse effects. Therefore, this work investigates the medicinal plant *Jasminum officinale* L. leaves by extraction and bio-guided fractionation. The ethyl acetate fraction showed a higher yield of 36.4%. A phytochemical test on *Jasminum officinale* confirmed flavonoids, saponins, phenols, and tannins. The highest total phenol and flavonoid contents in the ethyl acetate fraction of *J. officinale* are  $103.01 \pm 1.1$  mg GAE/g and  $80.29 \pm 1.03$  mg QUE/ value found in methanol crude extract. Furthermore, HPTLC analysis of the ethyl acetate fraction detected the existence of flavonoids (kaempferol) and phenols (gallic acid, quercetin, and rutin). The compounds detected at the greatest concentrations in the LC-M/MS analysis of the ethyl acetate fraction were cirsiolol, kaempferol, and 2-tridecanone. Additionally, *J. officinale* ( $IC_{50}$   $33.845 \pm 1.09$   $\mu$ g/mL) demonstrated the highest DPPH scavenging activity in EAF like that of ascorbic acid ( $IC_{50}$   $22.27 \pm 0.96$   $\mu$ g/mL). Also, in the FRAP assay, the  $IC_{50}$  of this fraction is  $15.14 \pm 0.25$   $\mu$ M Fe equivalents. In the range of alpha-amylase deactivating action, from 13.25% to 74.51%, and  $IC_{50}$  value ( $47.40 \pm 0.29$   $\mu$ g/mL) was significantly higher in the ethyl acetate fraction of *J. officinale* leaf extract. Moreover, *J. officinale* leaf extract had a substantially higher retention of glucose level ( $23.92 \pm 0.85$  % to  $87.21 \pm 0.6$  %), significantly higher anti-inflammatory activity with the lowest  $IC_{50}$  value ( $66.00 \pm 1.84$ ), and lipid peroxidation ( $IC_{50}$  value  $34.67 \pm 1.69$ ) by utilizing egg yolk as a substrate for lipids. Overall, the study revealed that *J. officinale* has considerable anti-diabetic characteristics. However, further comprehensive research is necessary to ascertain the

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<https://doi.org/10.1016/j.heliyon.2024.e39165>

Received 31 May 2024; Received in revised form 6 October 2024; Accepted 8 October 2024

Available online 10 October 2024

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medicinal purposes of *J. officinale* and its chemical components, pharmacological effects, and clinical uses.

## 1. Introduction

Phytochemicals have biological qualities that can improve health and lower the chances of chronic diseases. The most prominent phytoconstituents in plants are alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids [1]. The quest for plant materials rich in bioactive chemicals is being stepped up in response to the rising popularity of functional foods and herbal supplements [2]. Multiple studies have emphasized the challenge of replacing artificial antioxidants with natural alternatives and integrating them into meals as a better choice. Increasing drug development costs, decreasing hits in contemporary pharmacological development, and negative effects associated with synthetic drugs [3].

Numerous of these phytochemicals act as antioxidant defence mechanisms against free radicals and are widely employed for treating and preventing a variety of diseases. These substances are known for having medical benefits, such as antidiabetic and antioxidant characteristics [4,5]; [6]. Various ethnomedicinal plants have traditionally been used to cure diabetes, and certain plants have been revealed to have bioactive chemicals that aid in the manufacture of synthetic diabetics [7]. The phytochemical content of the plant has been studied and examined for various biological attributes, including its antioxidant properties and anti-diabetic characteristics. Diabetes is defined by either insufficient insulin secretion in pancreatic cells (type 1) or inaccurate insulin secretion and exertion (type 2) [8]. However, lowering blood glucose levels with most drugs has its limitations, including the possibility of hypoglycemia, weight gain, and medication resistance, and adverse consequences for the liver and kidneys [9]. The indicators of diabetes are inflammation, glucose uptake, oxidative stress [10], lipid profile, and genetics [11], which demonstrate the various diabetes treatment methods. Oxytocin influences the balance of glucose and insulin in the body, as well as controlling body weight. According to reports, oxytocin enhances the absorption of glucose and triggers the release of insulin. It induced insulin release in pancreatic islets regardless of changes in plasma glucose levels, and it also triggered glucagon release in the pancreas. This implies that oxytocin may play a role in diabetes development. A recent study showed that the treatment of oxytocin decreased obesity-related diabetes symptoms, including glucose intolerance, insulin resistance, and enlargement of the pancreatic islets. Diabetic instances may result in lipolysis, the breakdown of fats, and the release of free fatty acids from adipose tissues [12].

Diabetes is believed to cause problems with triglyceride storage (TG) and increased fat breakdown in adipose tissue. This results in an excess of circulating TG, potentially disrupting the energy production process in mitochondria and the insulin-activated glucose transportation in muscle cells. The process begins with a high-calorie diet and ends with the accumulation of fat as triglycerides in the body by adipocytes [13].

As to the latest report by the International Diabetes Federation (IDF), the global population with diabetes is estimated to be 537 million, signifying that over 10 % of people suffer from the disease, and this figure is expected to rise [14]. Thus, there is a pressing need to discover safe, efficient drugs that have fewer side effects to treat type 2 diabetes (T2DM). The genus *Jasminum* consists of more than 200 species that belong to the Oleaceae family [15]. *J. officinale* is an evergreen shrub that climbs up as a vine and reaches a height of around 8–10 feet. It is a vibrant deciduous climber with leaves that are 6–10 cm long, finely pointed, and pinnate. It arranges its leaves in opposite directions. This plant produces big clusters of starry, pure white flowers that emit a strong and pleasant fragrance. Fruits have the characteristics of being small, dark-colored, and containing a substantial amount of red-colored liquid [16].

1.1 One of the ethnomedicinal plants utilized in traditional medicine in South China for hepatitis, diabetes, and duodenitis is jasmine. Historically, in Western Himalaya people have used *J. officinale* for the treatment of urinary tract infections and its properties as a sedative, mild anesthetic, and astringent agents [17]. Furthermore, people used it to treat depression, nervousness, and stress-related disorders. Folklore uses the whole plant to induce feelings of optimism, confidence, and exhilaration. It is particularly effective in treating conditions of apathy, indifference, or listlessness. Despite its use as a traditional medicine, *J. officinale* received the least attention for the treatment of diabetes [18]. Leaf samples from *J. officinale* were tested for phytochemicals, and their connections to the plant's bioactivities were investigated [19]. Phytochemical analysis revealed that secoiridoid glycosides are compounds found in this plant [20]. The previous chemical studies of the genus *Jasminum* has resulted in the isolation of iridoid with some biological activities [14,21]. Similarly, a chemical named jasminol was extracted from the stems of *J. officinale* [15].

There have been few studies on its chemical ingredients and pharmacological activities [22]. So, *J. officinale* works in many ways, such as by lowering calorie intake, stopping lipogenesis, blocking absorption, stopping lipid absorption, and stopping obesity gene product inhibitors [23]. The plant species *J. officinale* shows promise as a herbal medicine due to its high content of phenols and flavonoids [24]. Despite its use as a traditional medicine, *J. officinale* received the least attention for the treatment of diabetes. Further investigation is required to conduct preclinical and clinical trials on the potential benefit. Considering the plant's widespread use in folklore, the study aims to assess the effectiveness of medicinal herbs for their potential in treating diabetes using glucose uptake inhibition and amylase inhibition tests. The study targeted evaluating the impact of *J. officinale* on secondary metabolites and antioxidant activity. The study intends to evaluate the efficacy of medicinal herbs in treating diabetes through glucose uptake inhibition and amylase inhibition tests. Multiple studies have emphasized the challenge of replacing artificial antioxidants with natural alternatives and integrating them into meals as a better choice. We also analyzed liquid chromatography-mass spectroscopy (LC-MS) and high-performance thin layer chromatography (HPTLC) in the ethyl acetate fraction (EAF) because it has the most phytoconstituents and the highest percentage of inhibition. In addition, the antioxidant, and anti-inflammatory activities of *J. officinale* were assessed *in-vitro* by their respective reported therapeutic activities, and the findings were subsequently compared. The percentage yield,

phytochemicals, and presence of quercetin, rutin, gallic acid, and kaempferol in the various fractions (methanolic crude extract (MCE), chloroform fraction (CF), ethyl acetate fraction (EAF), and an aqueous residual fraction residual (AF) were compared and documented in the study. Antioxidant molecules with varying chemical characteristics and polarity may exhibit solubility in diverse solvents. The solvent's composition significantly influences the quantity of extract generated and the antioxidant activity of the sample.

## 2. Materials and methods

### 2.1. Identification of a plant species

We collected the leaves of the *J. officinale* plant from the Sirmaur district of Himachal Pradesh, India. *J. officinale* leaves were authenticated at the Botanical Survey of India, Nauni, using the accession number (Acc-00040) and voucher number (SUBMS\BOT-4840). The leaves (4 kg) of the selected medicinal plant were collected in June–August 2022. After that, *J. officinale* leaves were collected. Subsequently, the dried leaves were pulverized using a mixer grinder (Philips HL7707/00) and stored in tightly sealed glass containers.

### 2.2. Preparation of crude methanolic extracts

The coarse leaf powder (20 g) was subjected to extraction with 95 % methanol (200 mL) using an orbital shaker set at 37 °C for 72 h each, as described by Ghosh and Chandra [25]. Separately, the leaves' concentrated crude extracts (MCE) were gathered and kept at 4 °C. The filtrate was separated using Whatman filter paper no. 1 and evaporated at 37 °C in a hot air oven. The MCE of leaves was collected individually and then kept at 4 °C.

#### 2.2.1. Fractionation of crude methanolic extracts

A total of 5 g leaves of *J. officinale* were dissolved individually in 100 mL of distilled water by stirring for 10 min. After that, the mixture was placed in the separating funnel (until the solvent-soluble layer was colorless.) and extracted with 100 mL of petroleum ether for defatiation, thereafter portioned with chloroform and ethyl acetate solvents [26]. The ethyl acetate fraction (EAF) and chloroform fraction (CF) as well as an aqueous residual (AF) of leaves. They were collected individually, dried, and kept (4 °C) in the refrigerator for later use (Fig. 1)

### 2.3. Extraction yield (%)

Gonfa et al. [27] used the subsequent equation to calculate the extraction yield (%) for each extract:

$$\text{Extraction yield (\%)} = \text{Weight of the crude} \frac{\text{extract}}{\text{Weight}} \text{ of the powdered sample} \times 100$$

#### 2.3.1. Phytochemical assessment

2.3.1.1. *Total phenol estimation.* Alam et al. [28], described a method for total phenolics by the Folin-Ciocalteu reagent (5 mL) was well mixed with 1 mg/mL samples. After 10 min, 4 mL of 7.5 % Na<sub>2</sub>CO<sub>3</sub> was included, and after an hour of reaction at room temperature, the absorbance at 765 nm was determined. Samples were measured three times. A similar procedure was used to create a standard curve by gallic acid (20–100 µg/mL). The results were represented as mg GAE/g extract. The total phenol content in all

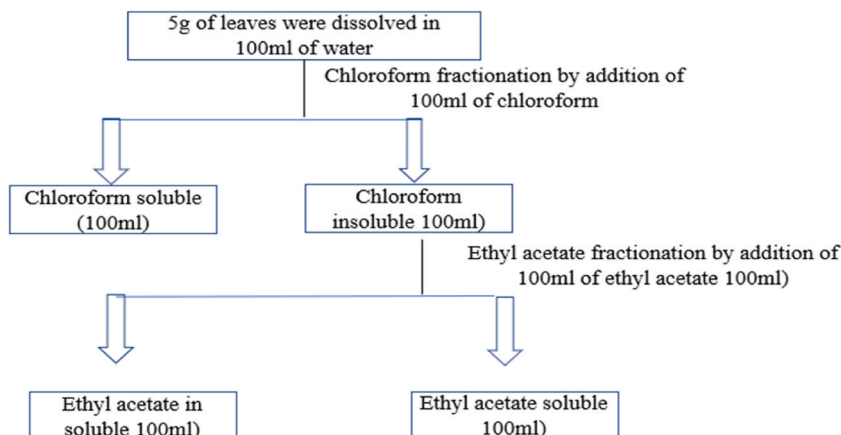


Fig. 1. General methodology used for fractionation of crude methanolic extracts of *J. officinale*.

fractions and extract was determined by applying the following formula:

$$A = \frac{\text{Conc.} \times \text{Vol.}}{W}$$

within this formula, A- TPC in  $\mu\text{g/mL}$  GAE; Conc.- the amount of GAE determined from the linear regression graph in  $\text{mg/mL}$ ; Vol.- volume of extract in  $\text{mL}$ , and W- extract weight in  $\text{g}$ .

**2.3.1.2. Total flavonoid estimation.** Bondonno et al. [29], method was used for total flavonoid concentration using colorimetric aluminium chloride. Add mixed 1.5 mL of 95 % ethanol with 0.5 mL of plant-based extract (1  $\text{mg/mL}$ ) and swirled for 5 min. Then, 0.1 mL of 10 % aluminium chloride mixed with 1 M potassium acetate were introduced. The reaction was diluted by adding distilled water until it reached a total volume of 5 mL. The mixture was stirred and then left to stand at room temperature for 30 min. Also, quercetin used as a standard (20–100  $\mu\text{g/mL}$ ) and analyzed at 415 nm. Flavonoid concentrations in all tested extracts were reported as  $\text{mgQE/g DW}$  using the calibration curve. Distilled water was replaced with 10 % aluminium chloride in the blank. A similar method was repeated three times.

**2.3.1.3. Total tannin estimation.** Morsy et al. [30] provided a method for calculating tannin concentration. Each 0.5 mL plant extract was handled with 1 % potassium ferricyanide and 1 % ferric chloride ( $\text{FeCl}_3$ ). The solution was diluted by adding distilled water until it reached a volume of 10 mL. The same procedure was used to make (5–25  $\mu\text{g/mL}$ ) tannic acid which was used as standard, which were absorbed at 720 nm. All extracts were measured in  $\text{mg TAE/g DW}$  for tannins.

**2.3.1.4. Total saponins estimation.** The vanillin-sulphuric acid colorimetric method by Goel et al. [31], was used to test all the extracts for saponin. Diosgenin was used as a control. This approach was treated with a solution containing plant extract (0.5 mL of a 1  $\text{mg/mL}$ ), 0.5 mL of vanillin in 8 % ethanol, and 5 mL of concentrated sulphuric acid at 72 % concentration. After rapidly stirring for 15 min in a 60°C-water bath, the solution was cooled for 5 min. Standard diosgenin (5–60  $\mu\text{g/mL}$ ) were also made similarly. After cooling for 5 min, the absorbance at 560 nm was measured to determine the saponin concentration of the extract and standard.

**2.3.1.5. Total alkaloid estimation.** Kennedy et al. [32], provided a method for the quantification of alkaloid using bromocresol. The concentration of the plant extract was (1  $\text{mg/mL}$ ), and after dissolving it in 2 N hydrochloric acids, and filtered with 0.1 N NaOH, and adjusting the phosphate buffer solution's pH to a neutral level. 1 mL of solution was put into a separating funnel, followed by the addition of 5 mL of BCG and 5 mL of phosphate buffer. The complex was isolated using chloroform following intense agitation. A 10–20-mL volumetric flask was used to collect and dilute the extract using chloroform. The absorbance of the sample was determined at a wavelength of 470 nm. The experiment was conducted three times using precisely measured quantities of a caffeine standard. This was done to find out how much caffeine was in the extracts ( $\text{mg/g}$ ).

**2.3.1.6. Total terpenoid estimation.** Truong et al. [33], process was utilized with few changes for total terpenoid content using linalool as the standard. Plant extract (1  $\text{mg/mL}$ ) was treated with 2 mL of chloroform and 200  $\mu\text{L}$  of pure sulphuric acid. The mixture was left to settle at room temperature in the absence of light for 1.5–2 h. Incubation produced a reddish-brown precipitate. After 2 h, the reaction mixture's supernatant was filtered, 3 mL of a methanol solution containing 95 % methanol was stirred. The mixture was vigorously stirred until solid particles formed dissolved in methanol. The same process was used to make standard linalool (5–25  $\mu\text{g/mL}$ ) and test absorbance at 538 nm. The equation line approach  $y = mx + b$  was used to calculate unknown sample values in each test sample.

## 2.4. Evaluation of antioxidant activity of *J. officinale*

### 2.4.1. Free radical scavenging assay using DPPH

The DPPH assay was performed as described by Hu-Qi et al. [34] with minor modifications. Initially, 3.94 mg of DPPH were mixed with 100 mL of methanol. After that 1 mL of DPPH solution was mixed with 3 mL of samples at concentrations ranging from 20 to 100  $\mu\text{g/mL}$ . The control test involved the addition of the same methanol. Stirring rapidly mixed the ingredients, which were then allowed to rest for 30 min at room temperature. The absorbance of every mixture was observed at 517 nm. Ascorbic acid was used as a positive control. The percentage of inhibition (I%) of the DPPH radical is calculated using the following Eq. (1).

$$I\% = \frac{A_o - A_s}{A_o} \times 100 \quad (1)$$

where,  $A_s$  is sample absorbance and  $A_o$  is the control reaction absorbance (all reagents except the sample). We graphed inhibition percentages (I%) against sample concentrations.

### 2.4.2. Free radical scavenging assay using FRAP (ferric reducing antioxidant power)

This approach determines the antioxidant's ferric iron-lowering ability. At low pH, ferric ion and the 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride complex (TPTZ) become ferrous following the methodology described by Benzie and Strain [35]. The 0.02 M  $\text{FeCl}_3$  solution was prepared by dissolving 1 mL of 1 M hydrochloric acid in 50 mL of distilled water. A fresh FRAP reagent

was made (300 mM CH<sub>3</sub>COONa buffer, 10.0 mM TPTZ solution, and 20.0 mM ferric chloride solution in a 10:1:1 ratio with pH 3.6). Ferrous sulfate was utilized as a reference for 593 nm absorbance after 30 min at 37 °C. Ferrous sulfate was used as a standard. The linear ferrous sulfate, or standard calibration curve, assessed the sample's antioxidant potential.

( $y = mx + b$ ) represents antioxidant concentration with ferric reducing capacity equal to 1 μM FeSO<sub>4</sub>, usually expressed as mol Fe (II) equivalent/g dry weight sample [28].

## 2.5. In-vitro antidiabetic activities of *J. officinale*

### 2.5.1. Inhibition of lipid peroxidation

Based on the method described by Das et al. [36,37] with minor modifications, the assay was conducted with samples prepared at various concentrations (20–100 μg/mL). 1 mL of egg homogenate (0.5 ml of 10 % v/v) and extract (0.1 mL) were mixed with distilled water. Then samples were treated with 0.05 mL of FeSO<sub>4</sub> (0.07 M) for 30 min to induce lipid peroxidation. After adding 1.5 mL of 20 % acetic acid (pH adjusted to 3.5 with NaOH), 1.5 mL of 0.8 % (w/v) thiobarbitric acid (TBA) in 1.1 % sodium dodecyl sulfate, and 0.05 M of trichloroacetic acid (20 %), the mixture was agitated vigorously and subjected to a temperature of 95 °C for a duration of 60 min. After cooling, each test tube received 5.0 mL of butan-1-ol and was centrifuged for 10 min at 3000 rpm. The absorbance of the organic top layer was measured at 532 nm. The positive control was ascorbic acid. The extract's ability to prevent lipid peroxidation was quantified as a percentage using the following Eq. (2).

$$I\% = \frac{A_c - A_s}{A_c} \times 100 \quad (2)$$

where,  $A_s$  is sample absorbance and  $A_c$  is the control reaction absorbance.

### 2.5.2. Glucose uptake assay

The glucose absorption was determined following the methods described by Nair et al. [38]; Sidhu et al. [39] with minor modification. Subsequently, 1 mL of the extract was introduced into a dialysis membrane containing a glucose solution (2000 MW, HiMedia International Scientific and Surgical, Solan, India). After knotting both ends with thread, it was submerged in 40 mL of 0.15M sodium chloride and 10 mL of distilled water in a beaker. Control was 1 mL of 0.15M sodium chloride, 22 mM glucose, and pure water. Subsequently, the beakers were stored at ambient temperature on an orbital shaker. Every half hour, glucose flow into the external solution was measured. To stop the reaction, 1 mL of dinitro salicylic acid (DNSA) color reagent was introduced. Metformin was used as a standard. The % Inhibition calculated by this Eq. (3).

$$I\% = \frac{A_o - A_s}{A_o} \times 100 \quad (3)$$

where  $A_o$  represents the absorbance of the control and  $A_s$  represents the absorbance of the sample.

### 2.5.3. Inhibition of protein denaturation method

A modified version of the bovine serum albumin (BSA) assay was used to find out how well the raw and fractionated plant extracts reduced inflammation [40]. We used a concentration range of 20–100 μg/mL of *J. officinale* extract as the test sample and diclofenac sodium, which served as the standard drug. A solution of BSA was created by dissolving one tablet in 15 mL of deionized water to generate a concentration of 0.4 % (w/v). The solution was made in tris buffered saline, resulting in a final concentration of 0.05M tris and 0.15M sodium chloride. The pH of the solution was adjusted to 7.6 at 25 °C. The procedure for testing the positive (diclofenac sodium) and negative (methanol) controls was the same. The solutions were subsequently subjected to a 10-min water bath heating at 72 °C and a 20-min cooling period under controlled laboratory conditions. The absorbance was taken at 660 nm. The following Eq. (4) was used to determine the percentage inhibition of precipitation (protein denaturation) relative to the negative control:

$$\text{The \% Inhibition : } I\% = \frac{V_t}{V_c} - 1 \times 100 \quad (4)$$

Where,  $V_t$  depicts the absorbance of the test sample, whereas  $V_c$  depicts the absorbance of the control. The concentration of the plant extract required to achieve 50 % inhibition ( $IC_{50}$ ) was calculated using a dose-response curve.

### 2.5.4. Inhibition of $\alpha$ -amylase

The plant extract was diluted in double-distilled water to produce five distinct concentrations. The concentrations (20–500 μg/mL) of plant extract and 500 μL of 0.02M sodium phosphate buffer (pH 6.9) containing  $\alpha$ -amylase solution (0.5 mg/mL) were subjected to incubation for 10 min at 25 °C. After a period of pre-incubation, 500 μL of a 1 % starch solution in a sodium phosphate buffer with a pH of 6.9 and a sodium chloride concentration of 0.006M were added to each tube at 5-s intervals. The reaction mixture was thereafter placed in an incubator at a temperature of 25 °C for 10 min. To stop the reaction, 1 mL of DNSA color reagent was introduced. After being submerged in a boiling water bath for 5 min, the test tubes were then cooled to the ambient temperature. Afterwards, the reaction mixture was additionally diluted with 10 mL of distilled water, and the absorbance was quantified at a wavelength of 540 nm [41]. Metformin was used as a standard. The percentage (%) of inhibition was calculated using the following Eq. (5).

$$I\% = \frac{A_o - A_s}{A_o} \times 100 \quad (5)$$

where,  $A_o$  is the control absorbance and,  $A_s$  is test sample absorbance.

## 2.6. HPTLC analysis

HPTLC was carried out using CAMAG HPTLC equipment. It includes an automatic Linomat V sample applicator, a chamber for creating TLC, and a CAMAG TLC scanner for densitometric chromatogram evaluation. Through HPTLC fingerprinting, quantification of the phytochemicals like kaempferol, rutin, gallic acid, and quercetin in ethyl acetate fractions was done, and the results were interpreted by CATS 4 Software. 15 mg EAF were prepared in 1 mL of methanol chromatographic grade solvents and then filtered by Whatman filter paper No. 1. Rutin, kaempferol, gallic acid, and quercetin were dissolved in methanol (1 mg/mL). Several solvent solutions were attempted for TLC, each fraction for higher resolution and maximal spots found in EAF. In HPTLC, different markers were selected, like kaempferol, rutin, gallic acid, and quercetin. Gallic acid was achieved with the mobile phase comprising Toluene: Ethyl Acetate: Formic Acid: Methanol (5: 3: 1: 0.5); rutin was achieved using mobile phase comprising Butanol: Acetic Acid: Water (4: 1: 5); quercetin was achieved with the mobile phase comprising Toluene: Ethyl acetate: Formic acid (5:4:0.2); for kaempferol, Toluene: Ethyl acetate: Formic acid 5: Methanol (5: 3: 1: 5: 0.5) [42]. Each fraction sample (3  $\mu$ l) was applied over a TLC plate. A calibration curve was established using standard samples: quercetin, gallic acid, kaempferol, and rutin, at values ranging from 1 to 8  $\mu$ g/mL. Before derivatization, spots could be seen at 254 and 366 nm wavelengths. WIN-CATS recorded the  $R_f$  values and fingerprint profile photo for the resolved band at 254 and 366 nm [43].

## 2.7. LC-MS analysis for secondary phytochemical profiling

To investigate the LCMS/MS of EAF (5 mL), the Tambunan et al. [42] methodology was used. On an ACQUITY UPLC BEH C18 Column (1.7 m 2.1 mm 50 mm) at 40 °C, sample extract (5  $\mu$ L) was separated. A gradient elution technique was utilized with acetonitrile-formic acid flowing at 0.3 mL/min as solvent B and water-formic acid at 0.1 % (%v/v) as solvent A. It began with A: B at a ratio of 95:5 for the first couple of minutes, raised the linearity solvent B for 6 min, and then held at that solvent for 7.5 min. The overall chromatographic run time was 9 min. To identify secondary metabolite chemicals, high-definition mass spectrometry was carried out using an XEVO-G2-Quadrupole (Q)-Time of Flight Mass (ToF) system (Waters, Milford, MA) in V-optics and operating in Electrospray Ionisation (ESI) positive (resolution mode). The following were the ideal analytical conditions: 3 kV in the capillary, 38 V in the sample cone, 300 °C for the desolvation process, 110 °C for the source, 500 L per hour for the desolvation gas flow, and 16 L per hour for the cone gas flow.

## 2.8. Statistical analysis

The mean  $\pm$  SEM was used to show the results. One-way ANOVA by tukey's test ( $p < 0.05$ ) was carried out to determine significance between extract and fractions. Significant values utilize different letters, while non-significant values use the same. The superscripts (a–e) on the bars show these differences. Distinct subscripts imply substantial diversity, whereas identical subscripts indicate a lack of significant change. The error bars are shown in black.

## 3. Result

### 3.1. Yield percentage

Results showed that the *J. officinale* extract and its fraction had distinct extraction yields. Methanolic crude extracts were separated by increasing relative polarity with different solvents, as their coding and percentage extract are mentioned in Table 1. According to observations, the EAF fraction of *J. officinale* (36 %) had the highest yield among the various fractions, followed by methanol crude extract (29.52 %), chloroform (16.06 %), and an aqueous residual fraction (12.8 %).

**Table 1**  
Description of collected plant, their coding, and the yield percentage.

Plant Extract	Coding	Yield%
<i>Jasminum officinale</i>	MCE	29.52 %
Chloroform fraction	CF	16.06 %
Ethyl acetate fraction	EAF	36.42 %
Aqueous fraction residue	AF	12.98 %

\* MCE – Methanolic crude extract, CF – Chloroform fraction, EAF – Ethyl acetate fraction and AF – Aqueous residual fraction.



### 3.2. Qualitative screening of phytoconstituents in *J. officinale*

The phytochemical analysis of leaf extracts from *J. officinale* in MCE, EAF, CF, and AF showed that all phytoconstituents were present in all of them. Our research on *J. officinale* leaves revealed the existence of many phytoconstituents, including, saponins, flavonoids, phenols, alkaloids, tannins, and, terpenoids. The results of phytoconstituents were validated, and it was established that phytoconstituents exist in many solvents as represented in Table 2. The current work has demonstrated that Phytochemical analysis of *J. officinale* detected saponin, flavonoid, alkaloid, tannin, and phenol compounds.

### 3.3. Quantitative screening of phytoconstituents in *J. officinale*

Phytochemicals are biologically active substances that plants make and are responsible for activities such as antioxidants, anti-inflammatory agents, and antidiabetic agents. Additionally, the leaves of *J. officinale* were examined using four different solvents to determine the quantitative estimation of phytochemicals. The standard graph of gallic acid was used to determine it by plotting the linear regression line ( $y = 0.0089x + 0.0067$   $r^2 = 0.9951$  mg/mL). Also, total phenol content (TPC) was significantly ( $p < 0.05$ ) higher in the EAF of *J. officinale* ( $151.25 \pm 2.31$  mg/g), as shown in Fig. 2 (A).

The total flavonoid content (TFC) was quantified by creating a linear regression line based on the standard curve of quercetin ( $y = 0.0036x + 0.1113$   $r^2 = 0.9954$  mg/mL). The findings indicated that out of all the fractions, EAF of *J. officinale* ( $103.01 \pm 1.1$  mg/g) showed the maximum quantity of flavonoids, followed by methanolic crude extract ( $77.01 \pm 4.1$  mg/g), chloroform ( $41.01 \pm 2.1$  mg/g), and an aqueous residual fraction as shown in Fig. 2 (B) ( $57.01 \pm 2.1$  mg/g). However, the least amount of TFC was found in the chloroform fraction of *J. officinale* ( $47.5 \pm 0.2$  mg/g). The tannin components in the extracted substance were determined applying the regression equation obtained from the calibration curve,  $y = 0.0138x + 0.1828$ , where  $r^2 = 0.9922$ . In tannins, the EAF fraction of *J. officinale* showed substantially higher tannin content, as shown in Fig. 2 (C) ( $147.38 \pm 1.28$  mg/g DW), followed by other fractions.

The quantification of saponin levels in *J. officinale* leaves revealed significant variations in the EAF, with the highest level of concentration as shown in Fig. 2(C) ( $71.27 \pm 3.81$  mg/g DW) discovered in this fraction. Moreover, in the case of terpenoids, the equation of the standard curve ( $y = 0.0059x + 0.4163$ ), where  $r^2 = 0.9664$ , was used to determine the terpenoid content in *J. officinale*. Furthermore, terpenoids were found to be substantially higher in the CF of *J. officinale* ( $35.21 \pm 1.05$  mg/g DW), which demonstrates a relatively insignificant difference with other fractions except the aqueous residual fraction, as shown in Fig. 3 (B). In the case of alkaloids, the equation of the standard curve was ( $y = 0.1437x + 0.1362$ ), where  $r^2 = 0.9806$  was utilized to ascertain the alkaloid concentration. The EAF fraction showed considerably higher alkaloid content ( $6.184 \pm 1.29$  mg/g) when compared to the other fractions, as illustrated in Fig. 3 (C). The study also revealed significant variations among all fractions. As illustrated and summarised in Figs. 2 and 3. The variation in polyphenolic content (TPC and TFC) among various fractions may be attributed to increased solubility of the phytoconstituents in mid-polar solvent EAF than in non-polar solvent CF, polar solvent MCE, and AF residual.

### 3.4. Evaluation of the anti-oxidant activity of *J. officinale*

#### 3.4.1. Antioxidant activity using DPPH assay

The extract and fractions were evaluated for their antioxidant activity by comparing them to ascorbic acid, a standard reference known for its ability to inhibit free radicals. The assessment was based on the percentage inhibition, as depicted in Fig. 4 (A), and the IC<sub>50</sub> value mentioned in Table 3. The data suggest that range of inhibition varied from  $13.2 \pm 0.1$  to  $82.56 \pm 0.1$  in the EAF of *J. officinale* when compared to the other fractions. And ascorbic acid, which was used as a positive control, ranged from  $13.2 \pm 0.2$  to  $91.1 \pm 0.1$ . For example, the EAF of *J. officinale* had a significantly ( $p < 0.05$ ) higher inhibition than the other fractions.

#### 3.4.2. Antioxidant activity of *J. officinale* using FRAP assay

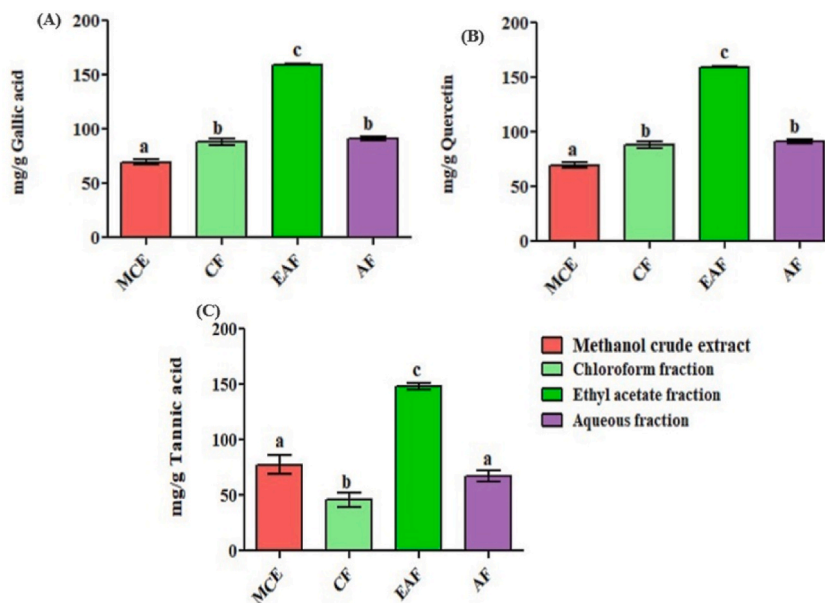
The FRAP test relies on the capacity of an antioxidant to convert Fe<sup>3+</sup> to Fe<sup>2+</sup> in the presence of TPTZ, leading to the formation of a vivid blue Fe<sup>2+</sup>-TPTZ complex that exhibits its highest absorption at 593 nm. It is important to note that this reaction is influenced by pH, with an ideal pH of 3.6. Similarly, the range of inhibition of the FRAP assay of EAF of *J. officinale* was  $16.53 \pm 0.81$  to  $81.25 \pm 0.36$  when compared to the ferrous sulfate  $10.25 \pm 0.12$  to  $97.25 \pm 1.3$ . IC<sub>50</sub> value mentioned in Table 3. The results of FRAP assay of extracts and fractions are illustrated in Fig. 4 (B). The EAF of *J. officinale* exhibited a considerably higher percentage ( $p < 0.05$ )

**Table 2**

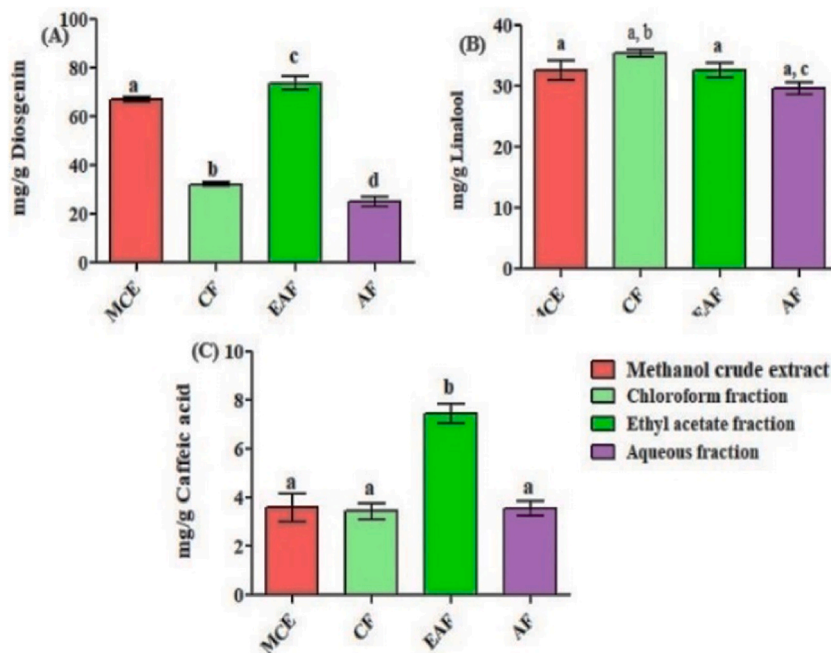
Qualitative analysis of phytochemicals from leaf extract of *J. officinale* in different solvents.

Sr. No	Phytochemicals	MCF	CF	EAF	AF
1	Phenols	++	+	++	++
2	Flavonoids	+	+	+++	+
3	Tannins	+	+	+++	+
4	Saponins	++	++	++	+
5	Terpenoids	+	+	+++	+
6	Alkaloids	+	+	+	+

\*MCE-methanol crude extract, CF- chloroform fraction, EAF- ethyl acetate fraction, AF- aqueous fraction. Where ‘+++’ (highly present); ‘++’ (moderately present); ‘+’ (present). Where, MCE: Methanolic crude extract, CF: Chloroform fraction, EAF: Ethyl acetate fraction, and AF: Aqueous fraction.



**Fig. 2.** (A) Total phenol content was shown as mg of gallic acid per gram of extract (dry weight) (B) Total flavonoid content (C) Total tannin content. The format of the data displays the mean and standard deviation (SD). Tukey's one-way ANOVA was used to find the significant and non-significant differences between the crude extract and fraction from all three locations. The superscripts (a–d) on the bars show these differences. Distinct subscripts imply substantial diversity, whereas identical subscripts indicate a lack of significant change. The error bars are shown in black.



**Fig. 3.** (A) Total saponin content, (B) Total terpenoid content, and (C) Total alkaloid content. Bars with a unique letter at the top indicate notable variations between fractions and extract. ( $p < 0.05$ ), As demonstrated by Tukey's One way ANOVA test. The format of the data displays the mean and standard deviation (SD). One-way ANOVA was used to find the significant and non-significant differences between the crude extract and fraction from all three locations. The superscripts (a–d) on the bars show these differences. Distinct subscripts imply substantial diversity, whereas identical subscripts indicate a lack of significant change. The error bars are shown in black.



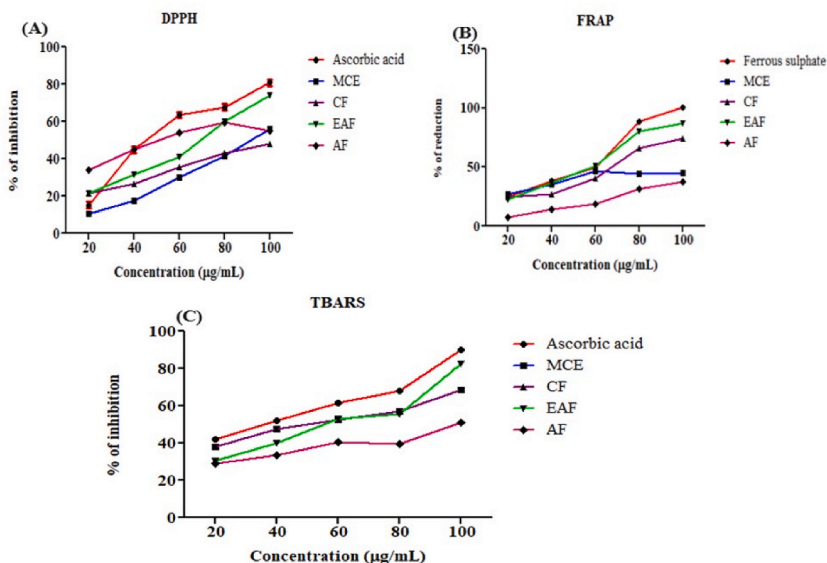


Fig. 4. (A) Antioxidant activity or percentage of inhibition of DPPH, (B) FRAP and (C) lipids peroxidation assay.

Table 3

IC<sub>50</sub> value of *J. officinale* and their fractions.

Biological Activities	Extracts/Standard	IC <sub>50</sub> value of <i>J. officinale</i> (µg/mL)
DPPH (Antioxidant activity) (µg/mL)	MCE	90.85 ± 1.17 <sup>a</sup>
	CF	133.64 ± 1.29 <sup>b</sup>
	EAF	33.845 ± 1.09 <sup>c</sup>
	AF	56.04 ± 0.98 <sup>d</sup>
	Ascorbic acid	22.27 ± 0.96 <sup>e</sup>
FRAP (µM Fe equivalents)	MCE	56.08 ± 1.28 <sup>a</sup>
	CF	24.03 ± 0.09 <sup>b</sup>
	EAF	15.14 ± 0.25 <sup>c</sup>
	AF	50.22 ± 0.17 <sup>d</sup>
	Ferrous sulfate	9.73 ± 0.24 <sup>e</sup>
Antiinflammatory activity (µg/mL)	MCE	133.13 ± 1.27 <sup>a</sup>
	CF	90.82 ± 1.63 <sup>b</sup>
	EAF	66.00 ± 1.84 <sup>c</sup>
	AF	135.79 ± 1.61 <sup>d</sup>
	Diclofenac sodium	50.39 ± 0.65 <sup>e</sup>
Alpha amyalse activity (µg/mL)	MCE	75.81 ± 0.01 <sup>a</sup>
	CF	114.22 ± 0.01 <sup>b</sup>
	EAF	47.40 ± 0.29 <sup>c</sup>
	AF	240.40 ± 0.11 <sup>d</sup>
	Metformin	19.92 ± 0.14 <sup>e</sup>
Glucose assay (µg/mL)	MCE	7.29 ± 0.49 <sup>a</sup>
	CF	8.10 ± 0.13 <sup>a</sup>
	EAF	4.28 ± 1.28 <sup>b</sup>
	AF	9.16 ± 1.31 <sup>a</sup>
	Metformin	1.94 ± 0.36 <sup>c</sup>
Lipids peroxidation (µg/mL)	MCE	51.02 ± 1.59 <sup>a</sup>
	CF	45.56 ± 0.36 <sup>b</sup>
	EAF	34.67 ± 1.69 <sup>c</sup>
	AF	40.23 ± 1.31 <sup>d</sup>
	Ascorbic acid	26.58 ± 0.41 <sup>e</sup>

\*Data are shown as mean ± SD (n = 3). \*a-e Means with the same subscript in column do not differ substantially (p < 0.05) from each other. The Tukey one-way analysis of variance (ANOVA) was used to assess the statistical significance of the activity levels in the plant samples. When two values have the same superscript in a column, it indicates that they do not differ substantially (p < 0.05) from each other.

compared to the other fractions.

### 3.5. In- vitro antidiabetic activities of *J. officinale*

#### 3.5.1. Lipid peroxidation assay

The TBARS assay is a widely used approach to assess lipid peroxidation via spectrophotometric analysis of TBARS-MDA adducts [44]. So, we investigated how *J. officinale* affected lipid peroxidation that was not caused by enzymes. As represented in Fig. 4 (C). The extract and fractions of *J. officinale*'s lipid peroxidation assay results are shown in terms of percentage inhibition ( $\mu\text{g/ml}$ ). The results indicate that the EAF of *J. officinale* ( $20.30 \pm 0.24$  to  $89.36 \pm 0.21$ ) showed the higher % of inhibition. Ascorbic acid ( $19.26 \pm 0.1$  to  $91.18 \pm 0.11$ ) was used as a positive control. The  $\text{IC}_{50}$  value is represented in Table 3.

#### 3.5.2. Glucose uptake assay

Diabetes is characterised by an imbalance in energy and a reduced ability to tolerate glucose, both of which are contributing factors to the disease. Thus, *J. officinale* leaves were examined to determine their ability to improve glucose levels. The  $\text{IC}_{50}$  value of the glucose uptake assay for plant extracts are presented in Table 3, and inhibitory range is depicted in Fig. 5 (A). The glucose uptake test results for the extract and fractions of *J. officinale* are shown in terms of % inhibition ( $\mu\text{g/ml}$ ). The EAF of *J. officinale* had the highest % inhibition ( $17.40 \pm 0.04$  to  $74.36 \pm 0.01$ ) compared to the other fractions. The results indicate that the EAF of *J. officinale*, followed by other fractions, has significant ( $p < 0.05$ ) variations and properties for quenching glucose molecules and improve high glucose concentrations.

#### 3.5.3. BSA protein denaturation assay

Protein denaturation refers to the loss of tertiary and secondary structures. The effect arises from the modification and disturbance of hydrogen, electrostatic, disulfide bonds, and covalent caused by chemical and physical factors [45]. *J. officinale* in terms of % inhibition ( $\mu\text{g/ml}$ ) are presented in Fig. 5 (B). The findings shows that the EAF from *J. officinale* had the strongest anti-inflammatory effect against great-induced protein denaturation ( $22.23 \pm 0.24$  to  $76.26 \pm 0.22$ ), with the other fractions coming in second and third. Diclofenac sodium ( $15.26 \pm 0.1$  to  $92.28 \pm 0.1$ ) was used as a positive control. The  $\text{IC}_{50}$  value of the anti-inflammatory efficacy of plant extracts is mentioned in Table 3, and the range of inhibition is represented in Fig. 5 (B). The anti-inflammatory activity of crude extract and fractions increased from 20 to 100  $\mu\text{g/ml}$ , indicating a dose-dependent response.

#### 3.5.4. Alpha- amylase inhibition assay

The most effective method for lowering blood glucose levels after a meal is to inhibit the activity of  $\alpha$ -amylase. The results indicate that EAF of *J. officinale* showed the maximum percentage of inhibition, whereas AF of *J. officinale* showed the least significant percentage of amylase inhibition assay. Fig. 5 (C) illustrates the alpha-amylase inhibition finding, while Table 3 mentions the  $\text{IC}_{50}$  value. EAF of *J. officinale* extract varied in the range of  $17.4\% \pm 0.12$ – $74.7\% \pm 0.11$  as compared to metformin ( $19.6\% \pm 0.05$ – $87.9\% \pm 0.02$ ). Whereas, CF ( $\text{IC}_{50}$   $114.22 \pm 0.01$ ) had the least % of inhibition. The extract maximally inhibited the enzyme at a dose of 100  $\mu\text{g/ml}$ , with an inhibition rate of 74 %.

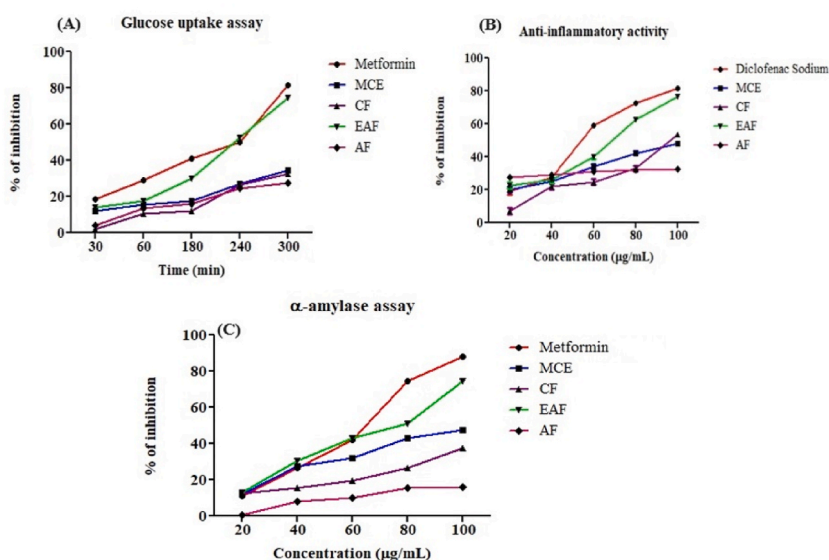


Fig. 5. (A) In-vitro glucose uptake assay, (B) anti-inflammatory and (C) alpha amylase inhibition of crude extract and fractions of EAF of *J. officinale*.

### 3.6. Identification and characterization of phytoconstituents present in the most bioactive fraction of EAF of *J. officinale*

According to the results of all biological activities, EAF of *J. officinale* has the most biological potential and stands out as a promising bioactive fraction. Therefore, we have analyzed EAF using quantitative methods like LCMS and HPTLC.

#### 3.6.1. HPTLC chromatogram of standard compounds gallic acid in EAF of *J. officinale*

HPTLC is an important analytical method for estimating phytochemicals in plants, both qualitatively and quantitatively. Many phytochemicals were detected by analysing the HPTLC fingerprinting profile of the EAF of *J. officinale*. The HPTLC chromatogram at various nm wavelengths showed peaks at various retention factor (Rf) levels and peak areas (Figs. 6–13; Table 4). In the current study, thin-layer chromatography was performed to evaluate the phytoconstituents that are currently present in the EAF of *J. officinale*.

HPTLC was used to detect biomarkers such as gallic acid (270 nm), rutin (415), quercetin (256), and kaempferol (365 nm) in EAF of *J. officinale* at different wavelengths (270, 415, 256, and 365 nm), which showed the chromatographic pattern of gallic acid in the EAF of *J. officinale*. For HPTLC analysis, different mobile phases were used to separate gallic acid, rutin, quercetin, and kaempferol from *J. officinale*. Typical HPTLC chromatograms of standards like gallic acid, rutin, kaempferol, and quercetin with different percentage yields are shown below in Table 4 and area, as well as area percentage. Also, different compositions of the mobile phase were tried, as mentioned in Table 4. The higher percentage yields of gallic acid, quercetin, and kaempferol were found in the EAF of the *J. officinale* fraction.

Effective separation of gallic acid was accomplished using a mobile phase comprised of Toluene: Ethyl acetate: Formic acid: Methanol (5: 3: 1: 0.5) as shown in Fig. 6. The Rf values of *J. officinale* extracts for gallic acid, was 0.46 respectively as in Fig. 7. Separation of rutin was achieved with the mobile phase in *J. officinale* comprising Butanol: Acetic acid: Water (4: 1: 5) as shown in Fig. 9.

The Rf values of *J. officinale* leaves extracts for rutin, were 0.70, 0.61 and 0.61 respectively. From the different tried mobile phases, the mobile phase for rutin comprising of Butanol: Acetic acid: Water (4: 1: 5) was found to be satisfactory as shown in Fig. 8. Quercetin was achieved using the mobile phase comprising Toluene: Ethyl acetate: Formic acid (5: 4: 0.2) as shown in Fig. 10. The Rf values of *J. officinale* extracts for quercetin, was 0.44 respectively as shown in Fig. 11. Good separation of Kaempferol (Figs. 11–12) was achieved by the mobile phase is Toulene: Ethyl acetate: Formic acid: Methanol (5: 3: 1 5: 0.5). The Rf values of *J. officinale* extracts for kaempferol, was 0.76 respectively. From the different tried mobile phases, mobile phase comprised for kaempferol is Toulene: Ethyl acetate: Formic acid 5: Methanol (5:3:1:5:0.5).

#### 3.6.2. LC-MS/MS analysis of the EAF of *J. officinale*

The chromatograms of various phytochemicals present in EAF of *J. officinale* obtained using LCMS/MS analysis. A LC-MS/MS of plant extract was done to identify the major phytoconstituents present in *J. officinale*. A total of 5 major compounds (Table 5) with the prominent peaks were identified in the EAF of *J. officinale* (Kaempferol, 2-Tridecanone, Cirsiliol, etc). LCMS analysis of the EAF of *J. officinale* showed the presence of different molecules with different molecular weights and antidiabetic property. The LCMS scan revealed the existence of several chemicals exhibiting a retention duration (RT) ranging from 2 to 10 min. The scan displays the distinct RT of the chemicals found in the extract sample. The data were graphed based on the RT, peak area, and mass ratio ( $m/z$ ) of the peaks. The extraction process revealed variations in the separated compounds, as shown by their distinct characteristics such as RT, peak area, and  $m/z$  values and values (Table 5). The diverse pattern of chemical distribution may account for the varying degree of biological activities exhibited by the test extracts.

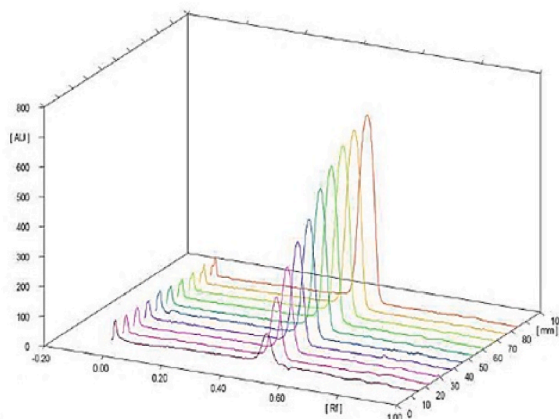


Fig. 6. The HPTLC chromatogram displays the standard compound of gallic acid at different concentration.

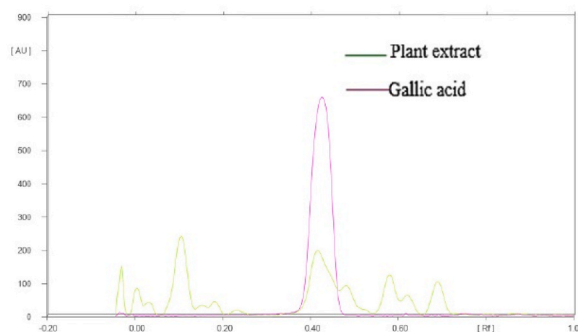


Fig. 7. HPTLC chromatogram of gallic acid from the EAF of *J. officinale*.

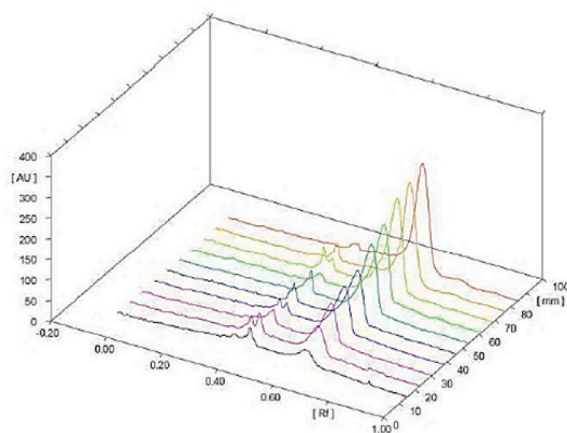


Fig. 8. The HPTLC chromatogram displays the standard compound of rutin.

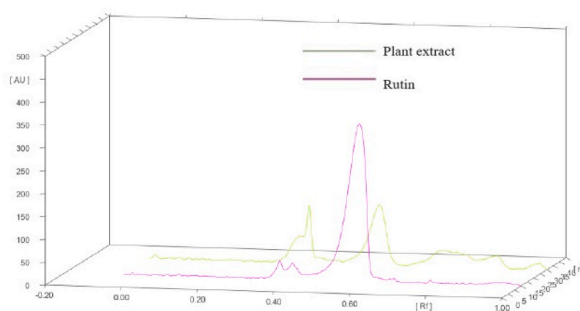


Fig. 9. HPTLC chromatogram of rutin from the EAF of *J. officinale*.

#### 4. Discussion

Medicinal herbs have been used for an extended period and are known for their significant healing properties and few side effects [46,47]. The current work is a detailed investigation of *J. officinale* leaves to determine their phytoconstituents, antioxidant, and anti-diabetic properties. The study was developed based on previous publications and its traditional usage for diabetic therapy. The leaves were selected for this study since they are the most often used part of the plant. Notably, higher extraction rate was found in the EAF of *J. officinale*, which correlates with a greater abundance of bioactive chemicals found in herbs.

A difference in the polarity of the solvent could explain the change in yield (%). This could affect how well the phytoconstituents dissolve in the sample and how they are extracted [48]. Similarly, several elements impact the yield %, including the composition of chemicals, the polarity of solvents, phytoconstituent nature, plant part changes, storage durations, and temperature [46]. As stated by Ngo et al. [49], extraction conditions and solvent are acknowledged as two of the most essential parameters. The current study

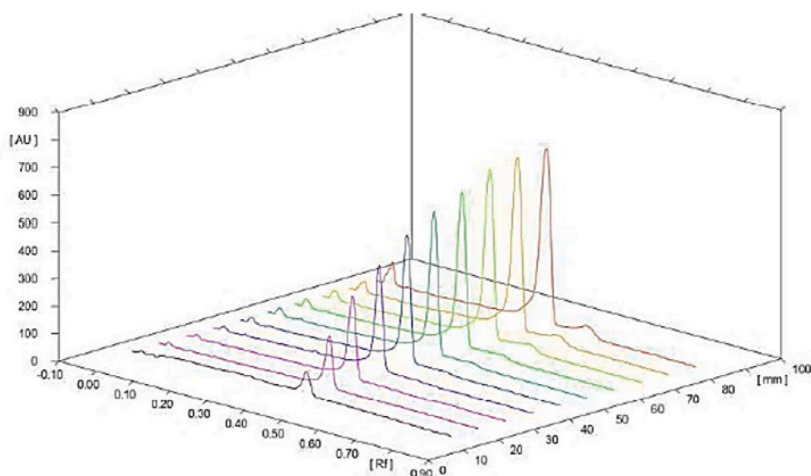


Fig. 10. The HPTLC chromatogram displays the standard compound of quercetin.

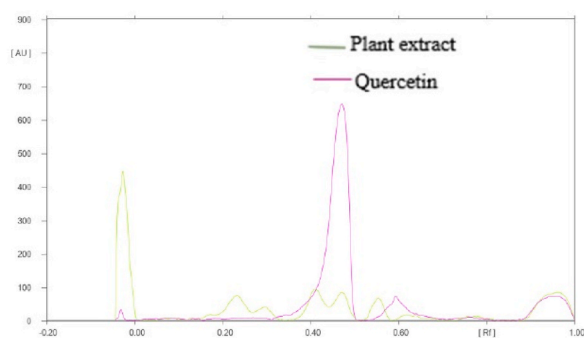


Fig. 11. HPTLC chromatogram of quercetin from the EAF of *J. officinale*.

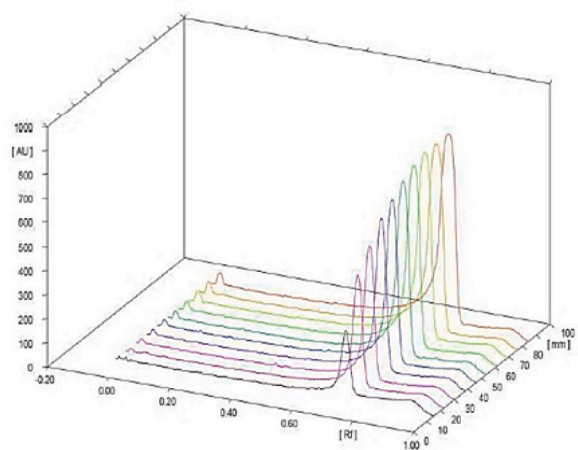


Fig. 12. The HPTLC chromatogram displays the standard compound of kaempferol.

discovered that various solvents produced variable extraction yields. Variations in the polarity of extraction solvents can produce a considerable change in the amount of bioactive chemicals in the extract. The most significant class of phytochemicals having anti-inflammatory, antioxidant, and antihyperglycemic properties is the phenolic group. The findings demonstrated that the EAF of *J. officinale* leaves contains a variety of high phytoconstituents, which indicates that they have variable effects on several antidiabetic

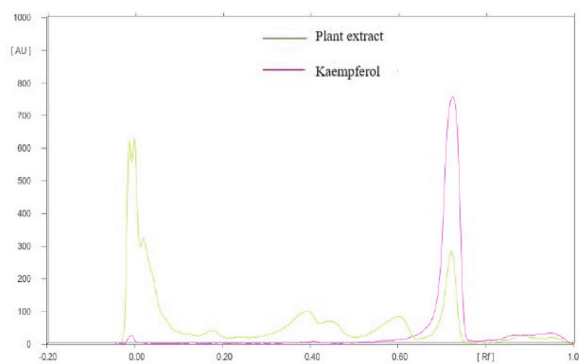


Fig. 13. HPTLC chromatogram of kaempferol from the EAF of *J. officinale*.

Table 4

Area of *J. officinale* extract and composition of mobile phase.

S. No	Compounds	Area of <i>J. officinale</i> (EAF) Rf 0.46	Mobile phase	Quantification ( $\mu\text{g}/\text{mg}$ )
1	Gallic acid	7283.8	Toluene:Ethylacetate: Formic acid Methanol (5: 3: 1: 0.5).	12.1
2	Rutin	4775.9	Butanol: Acetic acid: Water (4: 1:	15.5
3	Quercetin	2663.2	Toulene: Ethyl acetate: Formic acid (4: 0: 2)	10.8
4	Kaempferol	22641.1	Toulene: Ethyl acetate: Glacial ace acid (30: 40: 4)	18.4

Table 5

LCMS/MS based identification and characterization of prominent phytoconstituents from EAF of *J. officinale*.

Compounds	Molecular weight	Chemical formula	Spectra peak ( $m/z$ )	Retention time (min)
Kaempferol	286.24	$\text{C}_{15}\text{H}_{10}\text{O}_6$	287.75	9.51
2-Tridecanone	198.34	$\text{C}_{13}\text{H}_{26}\text{O}$	237.16	2.10
Cirsiliol	330.29	$\text{C}_{17}\text{H}_{14}\text{O}_7$	367.12	9.51

markers. The findings in a qualitative study of phytochemical components present in several extracts, including methanol chloroform and petroleum ether extracts, demonstrated the presence of chemicals such as phenols, flavonoids, and saponins [41]. As per Patil et al. [16], in the ethanolic extract of *J. officinale*, alkaloids, terpenoids, and tannins are absent and saponin is present. In the same way, Prachee et al. [19] found that different parts of *J. officinale* leaves, including hexane, chloroform, ethanol, methanol, and water, all contained tannins, alkaloids, flavonoids, terpenoids, and saponins. The results of this investigation demonstrate that phytoconstituents, including flavonoids, tannins, terpenoids, and saponins, may be determined in both polar and non-polar solvents.

Lausevic et al. [50], [51] found that *J. officinale* has high amounts of TPC and TFC, and an ethanolic extract of the leaves worked best ( $31.58 \pm 1.61$  mg/g and  $13.54 \pm 0.69$  mg/g), followed by an ethyl acetate extract of the leaves ( $25.64 \pm 1.27$  mg/g and  $9.36 \pm 0.46$  mg/g), and then petroleum ether ( $12.48 \pm 0.54$  mg/g and  $6.47 \pm 0.28$  mg/g). However, the aqueous extracts had significantly higher amounts of phenols and flavonoids. Similarly, Tharakan [52] performed a quantitative analysis on the extract of *J. officinale* and found that the ethanolic extract contains the highest level of phenols ( $133.4 \pm 0.28$   $\mu\text{g}$  GAE/mg).

This might be attributable to the existence of several polar chemicals in the plant material, which can dissolve in polar solvents such as water, methanol, and ethyl acetate [33]. Collectively, these findings indicate that utilizing EAF and AF as solvents yields the most favourable results in extracting bioactive components from this medicinal plant. Various variables, including the duration of extraction, pH level, polarity of the solvent, and temperature, might influence the quantity of phenolics extracted [53]. The different amounts of phenols, flavonoids, flavanols, and tannins found in different parts of *J. officinale* may be because different polarity solvents were used to extract the phytocompounds.

Furthermore, the antioxidant properties of all fractions and extracts of *J. officinale* were assessed using DPPH and FRAP. All the extracts exhibited antioxidant activity that varied depending on the dosage. This might be since polyphenolic chemicals, like flavonoids and phenols, are physiologically active plant components with antioxidant activity. The highest antioxidant activity was detected in the EAF of *J. officinale*. This might be since it contains a lot of TPC and TFC. As these chemicals are more soluble in ethyl acetate than the other solvents [54], it seems that this is the first scientific study that describes how *J. officinale* leaves from the western Himalayas might be used as an antioxidant and diabetes-fighting substance. According to this study, the antioxidant activities and variations in antidiabetic activities are linked to the beneficial effects on beta cells.

According to Shekhar and Prasad [55], the ethanolic extract of *J. officinale* exhibited higher antioxidant activity compared to other samples extracted with ethanolic solvents. Similarly, Saraswathi and Palayyan [56] examined the antioxidant capacity of a hydroalcoholic extract derived from leaves using many *in vitro* techniques. They discovered that *jasminum sambac* had a modest scavenging



effect on the concentrations of DPPH radicals, nitric oxide, and hydrogen peroxide (122  $\mu\text{g}/\text{mL}$ , 173.94  $\mu\text{g}/\text{mL}$ , and 125  $\mu\text{g}/\text{mL}$ ), respectively, in comparison to ascorbic acid. Similarly, Habtemariam [57] stated that the methanol extract showed a strong radical-scavenging effect ( $20.00 \pm 2.67$ ).

Moreover, a lipid peroxidation assay was used to check the inhibition of lipids by spectrophotometric analysis of TBARS-MDA adducts developed when radicals attack the lipid barrier, which has extensive effects on how cells work. So, according to the findings, EAF had the highest percentages of inhibition. This shows that higher levels of phenol and flavonoid are linked to higher lipid peroxidation activity. These chemicals also get rid of free radicals and lower the amount of oxygen in the body. Cell functions are harmed because the cell membrane structure is messed up, which changes the fluidity and permeability, as well as ion transport, and eventually stops metabolic processes [44]. The addition of antioxidants may make oils high in polyunsaturated fatty acids, like flax seed and chia seed oils, more resistant to oxidation. A lot of the time, butylate hydroxyanisole (BHA), butylate hydroxytoluene (BHT), and tertbutyl hydroquinone (TBHQ) are added to processed food oils to make them less likely to oxidize. However, these chemicals may be harmful to our health [58]. Thus, to avoid the lipid oxidation of edible oils, natural antioxidants have garnered a lot of research [59].

Additionally, a glucose uptake assay was used to check the antidiabetic activity of *J. officinale* by using a dialysis membrane. We used four different solvents—methanol crude extract, ethyl acetate, aqueous residual, and ethyl acetate fractions to test the leaves of *J. officinale* for potential effects on glucose diffusion using dialysis membranes, a model system for the gastrointestinal tract. The results indicate that the EAF of *J. officinale* showed the best activity. This assay aids in evaluating the potential of drugs in inducing or inhibiting the entry of glucose into the cells. *J. officinale* leaves are not yet analyzed for their *in-vitro* antidiabetic potential by glucose uptake assay. According to the literature, antidiabetic potential was overestimated by researchers in the *J. officinale* plant genus or related extracts not in particular species. According to Kaviya et al. [60], jasmine oil exhibited an increase in glucose uptake in muscle cells, revealing its insulin sensitivity. Sidhu et al. [39] conducted a study where they evaluated the impact of leaf and callus extract from *Costus pictus* D. Don in four different solvents (ethanol, acetone, aqueous, and ethyl acetate) on the diffusion of glucose across a dialysis membrane. Similarly, Muzammil et al. [61] confirmed the antihyperglycemic efficacy of *Tabernaemontana divaricata* (Jasmine) flowers using *in-vitro* testing (0.2–26.3 %) at 2 mg/ml. Furthermore, Gunathilaka et al. [62] conducted a glucose uptake experiment using the glucose oxidase enzyme.

Additionally, BSA was utilized in the protein denaturation procedure to assess anti-inflammatory efficacy. Protein denaturation is a recognised factor that can lead to inflammation and inflammatory conditions, including rheumatoid arthritis, cancer, and diabetes [63]. The results indicate EAF showed the highest percentage of inhibition and lowest IC<sub>50</sub> value in comparison to crude extracts and fractions. The anti-inflammatory potential and IC<sub>50</sub> values are inversely related, with the lowest IC<sub>50</sub> value indicating the highest anti-inflammatory potential. The IC<sub>50</sub> values are listed in Table 3. This might be due to the various variables, including the duration of extraction, pH level, polarity of the solvent, and temperature, which might influence the quantity of phenolics extracted. According to Prakash et al. [64], *J. officinale* leaf extract has a greater level of anti-inflammatory action compared to the flower extract. Similarly, *J. multiflorum* possessed anti-inflammatory activity with an IC<sub>50</sub> of 67.2  $\mu\text{g}/\text{ml}$  [65]. Similarly in current study EAF of *J. officinale* showed higher % of inhibition as compared to other extract and fraction this may be due the heigher % of phytochemicals in a semi polar solvents. Just like that, in this study, the EAF of *J. officinale* had a higher percentage of inhibition compared to other fractions and extracts. This might be because it had more phytochemicals in a semi-polar solvent. No publications on *J. officinale*'s anti-inflammatory activity were determined *in-vitro* using protein denaturation tests. An alpha-amylase test was also used to see how well each fraction and extract of *J. officinale* stopped alpha-amylase from working. Thus, it was discovered that EAF had the highest percentage of inhibition. Plant-derived phytoconstituents may be responsible for this, as they affect metabolic glucose by decreasing the action of alpha-amylase, improving the effectiveness and production of insulin, preventing cell death, promoting the mobility and presence of glucose transporters, reducing the production of glucose in the body, promoting the growth of pancreatic beta cells, and safeguarding them against oxidative stress and inflammation [66]. These phytochemical pathways improved antidiabetic medication design. Based on the analysis of Schwartsburd [67], the pancreatic beta cells were found to be protected from both antioxidant and hyperglycemic activities.

The current investigation has shown a notably elevated level of phenolic and flavonoid compounds in the EAF followed by AF, CF, and MCE. The results align with previous observations by Wei et al. [68] they reported that *J. officinale* contains phenols like 5-dihydrocaffeoylquinic acid (191  $m/z$ ) and 4-p-coumaroylquinic acid (337  $m/z$ ), as well as flavonoids like quercetin ( $m/z$  301), kaempferol ( $m/z$  285). The same thing happened when Shekhar and Prasad [55] looked at the leaves of *Jasminum grandiflorum* Linn and found that the ethanol and acetone solvent extracts had phytochemicals in them but the benzene solvent extract did not. A study by Watanabe et al. [69] used HPTLC to separate five antioxidant chemicals: rutin, quercetin, hyperin, and 3,4-dihydroxybenzaldehyde. Similarly, Alam et al. [28] used HPTLC to separate active ingredients like protocatechuic acid (13.4 mg/100 g), 3,4-dihydroxybenzaldehyde (6.1 mg/100 g), hyperin (5.0 mg/100 g), rutin (4.3 mg/100 g), and quercetin (2.5 mg/100 g). This includes TLC fingerprint profiles as well as the estimation of chemical markers and biomarkers.

The analysis of these activities may be due to the use of more bioactive phytoconstituents found in HPTLC and LCMS analysis. Some of the probable compounds detected by LC-MS have been documented to have hypoglycemic effects. The chromatograms of the EAF of *J. officinale* reveal several compounds, including kaempferol, cirsiol, and 2-tridecanone. These compounds exhibit various pharmacological properties, such as antimicrobial, anti-inflammatory, antioxidant, antitumor, cardioprotective, neuroprotective, and antidiabetic activities. They also inhibit alpha-amylase and are being investigated for their potential application in cancer chemotherapy [70–73].

## 5. Conclusion

Traditional medicinal bioactive chemicals have recently gained a lot of interest as a possible targeted treatment for a variety of illnesses. Plant-derived antioxidants have been acknowledged as crucial for human well-being in the last three years. The study discovered that the leaf extract of *J. officinale* contains natural phenols and flavonoids that are beneficial antioxidants against DPPH and FRAP. These compounds also have strong antihyperglycemic effects, as shown by tests for alpha-amylase, glucose absorption, and lipid peroxidation. The study's findings indicate that we can use EAF from *J. officinale* extracts to develop new medicines or treatments that effectively block glucose uptake, free radicals, and alpha-amylase. Furthermore, it is possible that *J. officinale*, which has the same phytochemicals, could provide comparable therapeutic outcomes. For instance, kaempferol, which is present in various medicinal plant species, possesses significant antioxidant properties and has the potential to aid in the treatment of various disorders linked to oxidative stress. However, HPTLC analysis found the main phytochemical, kaempferol, that controlled their therapeutic effects, especially against diabetes. Preliminary results indicate that conventional medicine can safely use *J. officinale* to address a variety of health issues without harming the local population. Therefore, an extensive study should be carried out to isolate and differentiate the active components from the EAF of *J. officinale*. Current research has laid the groundwork for identifying new compounds from *J. officinale*'s bioactive fractions. These compounds have the potential to be used to develop plant-based pharmaceutical therapies. Further *in-vivo* research is required to determine this plant's pharmacological significance.

## CRedit authorship contribution statement

**Mehak Thakur:** Writing – original draft, Formal analysis, Data curation. **Rachna Verma:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization. **Dinesh Kumar:** Writing – review & editing, Formal analysis. **Sivakumar Manickam:** Writing – review & editing, Formal analysis. **Riaz Ullah:** Resources, Funding acquisition. **Mohamed A. Ibrahim:** Writing – review & editing, Resources, Funding acquisition. **Ahmed Bari:** Writing – review & editing, Resources, Funding acquisition. **H. Lalhenmawia:** Software, Resources. **Deepak Kumar:** Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

RU, MAI and AB extend their appreciation to the Researchers Supporting Project (RSP2024R171), King Saud University, Riyadh, Saudi Arabia.

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