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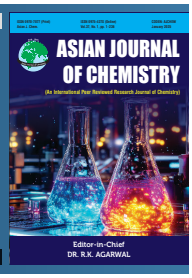

A Comprehensive Assessment and Therapeutic Potential Evaluation of Ethanolic Extract Derived from *Origanum vulgare* Leaves

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The purpose of this study was to investigate the multifaceted bioactive potential of the ethanolic extract obtained from *Origanum vulgare* leaves. GC-MS analysis has been undertaken to identify the phytoconstituents, which unveiled the intricate profile of components of the extract. In addition, the study further explores the biological activities, including antioxidant, antimicrobial, antidiabetic, anti-inflammatory and cytotoxic assays. The results revealed that the ethanolic leaf extract showed strong antimicrobial action against different human pathogens. Furthermore, a remarkable antioxidant potential was shown by the extract as evidenced by its ability to scavenge free radicals. With respect to antidiabetic properties, the extract demonstrated notable inhibitory effects on key enzymes implicated in glucose metabolism. Anti-inflammatory assay exhibited a significant suppression of inflammatory mediators, suggesting its potential therapeutic applications. Moreover, the cytotoxicity assessments indicated promising results, emphasizing the potential of ethanolic extract of *O. vulgare* leaves in cancer research. Such evaluation provides valuable insights into the diverse bioactivities of *O. vulgare*, laying the groundwork for further exploration and utilization of this natural resource in pharmaceutical and therapeutic applications.

Keywords: *Origanum vulgare*, Soxhlet extraction, Rotary evaporation, Bioactive properties, Biological activities, Cytotoxicity.

INTRODUCTION

Exploring herbal sources towards investigation on their bioactive compounds gains considerable momentum in the fields of pharmacology, medicine and nutraceutical research [1,2]. Bioactive compounds such as flavonoids, alkaloids, terpenoids and glycosids found in medicinal plants have been proven as effectual alternative over synthetic materials due to their many advantageous properties [3]. Moreover, pharmaceuticals derived from natural resources also have an impact on the effectiveness of contemporary medical sciences [4,5]. The WHO reports that 80% of people in the developing nations, especially in Asia and Africa, use traditional medicine, which are typically affordable, safe, effective and dependable. In this vein, plants and other natural resources are essential for exploring novel and practical solutions [6].

In this light, *Oregano*, a member of the *Lamiaceae* family and prominently represented by *Origanum vulgare*, has long been recognized for its diverse pharmacological potential [7]. *O. vulgare*, colloquially known as oregano, possesses a rich reservoir of secondary metabolites, including polyphenols, flavonoids and essential oils. These compounds have numerous biological factors, making *O. vulgare* an attractive candidate for in-depth investigation [8]. Essential oil from *O. vulgare* has been used for several decades to flavour food products, such as wine, fish and treat different ailments. The plant aerial portions of *O. vulgare* are used in traditional medicine to treat rheumatoid arthritis, stomach aches, painful menstruation, respiratory illnesses, nutritional disturbances and urinary issues [9]. Based on the symptoms, there is a different range of intake forms, such as tinctures or teas that are used to treat respiratory, digestive and cold ailments and enhance overall body health. *O. vulgare* decoctions have been utilized for their expectorant, antiseptic, digestive assistance and antispasmodic qualities [10].

In a study elsewhere, smoke inhalation for toothache relief was described by Pieroni *et al.* [11]. Bioactive compounds

like alkaloid (1.5%) and flavonoid (2.5%) of methanolic extract of *O. vulgare* shown significant activities against multidrug-resistance strains, such as *S. aureus*, *P. aeruginosa* and *E. coli* isolated from patients with sore throats [12]. In another investigation, it was found that a high proportion of phenolic components, specifically, rosmarinic acid found in the ethanol extract of *O. vulgare* were necessary for considerable antibacterial action against ulcer-associated pathogen [13]. The water extract showed a possible inhibitory action against different bacterial strains. They observed that all the extracts of *O. vulgare* including water and acetone have exhibited the minimum inhibitory concentrations of 0.16-0.6 mg/mL against *S. aureus* and *Bacillus* spp. strains. Contrarily, Licina *et al*. [14] reported that the aqueous extract has minimal antibacterial activity (MIC < 12% v/v) against *P. mirabilis*, *K. pneumonia*, *E. coli* and *P. aeruginosa*. Considering these findings, *O. vulgare* can be a viable option for more research due to its well-established traditional uses and fragrant leaves. Thus, this study aims to close the gap between conventional knowledge and contemporary scientific analysis by thoroughly assessing the bioactive potential present in the *O. vulgare* extracts.

Among the traditional existing extraction methods employed to extract the bioactive compounds from botanical sources, Soxhlet extraction coupled with rotary evaporation is one of the emerging techniques that has been proven as efficient and widely utilized method [15]. Accordingly, the primary goal of this experiment is to recognize and characterize the chemical components present in the ethanolic extract by GC-MS analysis [16]. Subsequently, the examinations were carried for assessing its antimicrobial efficacy, antioxidant capacity, antidiabetic properties, anti-inflammatory activity and cytotoxic and apoptosis potential against Hep G2 cancer cell line [17]. This extensive evaluation attempts to reveal the complex pharmacological properties of *O. vulgare* and enhance natural product-based drug discovery and development [18].

EXPERIMENTAL

Fresh leaves of *Origanum vulgare* plant were collected from Ukkadam locality, Coimbatore, India. It was rinsed in clean water, then, left to air dry for 8 h to use in later studies.

Preparation of plant extract: The plant extract was prepared using ethanol (99.9%) as a solvent. In brief, the dried leaves were manually crushed and 30 g of the crushed material was extracted with 150 mL of ethanol at 70 ºC using a Soxhlet extraction apparatus per cycle. The extract was rotary evaporated at 60 ºC to remove remaining residues and the resultant concentrated extract was stored for further analysis.

Phytochemical examinations: The therapeutic properties of plants are often related to the bioactive compounds, making phytochemical testing crucial in the initial evaluation of plants for their medicinal and therapeutic capabilities. Therefore, the investigation focused on the presence of flavanoids, phenols, saponins, terpenoids, and carbohydrates.

GC-MS analysis: In this study, the GC-MS study was executed to identify different bioactive volatile constituents found in *O. vulgare* extract. An instrument, Agilent GCMS-QP2010 Plus and 7000 GC with columns measuring 30 m × 250 µ × 5.0 µm were employed during the analysis. Helium was used as gas carrier and diluent-ethanol made up was utilized as the solvent system. However, determining the concentration of individual constituents was unfeasible due to the qualitative nature of the analysis. The entire running time of the analytical process for all of the samples was set to 38 min [15,19].

Antibacterial, antifungal and antioxidant assays: The antibacterial activity against five different pathogenic bacterial samples through the well diffusion method was carried out [14]. The bacterial strains like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*, as they are clinically significant pathogenic isolates and known for their multi-drug resistance. An antifungal potency of ethanolic extract of *O. vulgare* was ascertained against two fungi (*Aspergillus niger* and *Fusarium oxysporum*) through a well diffusion method [8]. The DPPH method was carried out to investigate the scavenging action of the antioxidants [17]. In brief, 0.5 mL of plant extract was mixed with 0.2 mL of 0.1 mM DPPH and then left to stand at room temperature for 5 min. Then, 0.4 mL of 50 mM Tris HCl was added followed by the mixture was incubated for 30 min at room temperature. The optical density was recorded for control, blank and test samples by UV-Vis spectrophotometer (Labtronics LT29, Microprocessor) at 517 nm [18,20]. The percentage of antioxidant activity was calculated using eqn. 1:

$$\text{Antioxidant activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \times 100 \quad (1)$$

Free radical scavenging activity was also assessed using DPPH method. The sample (1 mL) was dissolved in an equivalent volume of phosphate buffer solution and 0.1% potassium ferric cyanide solution. The test solutions were incubated at 50 ºC for 20 min. The sample was then mixed with 1 mL of 10% TCA solution, 1 mL of distilled water and 0.1% $FeCl_3$ solution. A spectrophotometer was then used to record the OD readings at 700 nm (Labtronics LT29, Microprocessor). As standard, ascorbic acid has been utilized to determine mg/g of FRAP content [21].

Total phenolic content (TPC) assay: In this study, TPC was determined as per the procedure documented by Dejene *et al.* [22]. In brief, with 1 mL of sample extract, 0.1 mL of Folin's phenol reagent and 0.5 mL of Na_2CO_3 (20%) were added and incubated at 45 ºC for 45 min. The optical density (OD) value measured at a wavelength of 765 nm was analyzed using standard curve values derived from gallic acid as the standard [23].

Anti-inflammatory activity assay: In present work, the anti-inflammatory assay was performed by adopting the procedure reported by Zhao *et al.* [24]. Herein, the two different assays, protein denaturation and trypsin method, were considered for asserting the anti-inflammatory potential.

In protein denaturation assay, 1 mL of sample and 0.5 mL of diclofenac was mixed with 3 mL phosphate buffer saline followed by the addition of 1mL of 1% egg albumin solution. The resultant mixture was incubated at 37 ºC for 20 min, then again incubated at 90 ºC for 2 min for denaturation process. After cooling, the absorbance was taken at 660 nm [25]. Eqn.

2 was used to ascertain the percent inhibition in terms of protein denaturation assay.

$$\text{Inhibition (\%)} = 100 - \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \times 100 \right) \quad (2)$$

In the trypsin method, 1 mL of the sample and 1 mL of 20 mM Tris-HCl solution were mixed with 0.06 mg of trypsin. The mixture was then incubated at 37 ºC for 10 min. After incubation, 1 mL of 0.8% casein solution was added and the mixture was left for 20 min. To stop the reaction, 2 mL of 70% perchloric acid was added. The cloudy suspension was centrifuged at 5000 rpm for 5 min followed by the collection of supernatant. Absorbance was measured using a spectrophotometer at 210 nm [23,26] and eqn. 2 was used to ascertain the percent inhibition.

Antidiabetic assay: In this study, an antidiabetic assay was examined with two different approaches, namely, α-amylase inhibition and α-glucosidase inhibition [27,28]. The procedure was adopted as reported by Sarian *et al.* [25]. In brief, 0.2 mL of α-amylase enzyme and 1 mL of extract were mixed with 0.1% starch solution prepared in 16 mM of sodium acetate buffer [29]. Utilizing a spectrophotometer, the absorbance measurement at 540 nm was made [30,31].

For α-glucosidase assay, 1 mL of 2% starch solution, 1 mL of sample and 1 mL of 0.2 M tris buffer (pH-8) were mixed and then incubated at 37 ºC for 5 min to ascertain the inhibitory action. Further, the α-glucosidase enzyme (1U/mL) was added to 1 mL and the reaction was allowed to react for 40 min at 35 ºC. A 2 mL of HCl (6 N) was added to stop the reaction and then measured the absorbance at 540 nm [30,31].

Cytotoxic assay: MTT assay was carried out to examine the anticancer properties of the specimen utilizing the HepG2 cell line. The cell line was acquired from the NCCS, Pune, India and cultured in Roswell Park Memorial Institute medium added with 10% BSA, glucose and sodium carbonate after procurement. The cells were cultured for 24 to 72 h at 37 ºC, pH 7 to 7.5 and humidity 70 to 80% in a CO_2 incubator after all the chemicals had been added to the T-flask [32]. For a cell line with varied sample concentrations (6.25, 12.5, 25, 50 and 100) in µg/mL, 24 h of incubation was needed. Doxorubicin (12.5 µg) was used as a positive control. The blank chosen was DMSO. Following incubation, DMSO was used to wash the cells. The plates were carefully combined following the introduction of trypsin to each well and subsequently placed in a CO_2 incubator for 24 h incubation period at 37 ºC. Once the reaction mixture was withdrawn from each well, 100 µL of absolute ethanol was added and the mixture was well mixed to dissolve the formazan crystals [33]. A microtiter plate ELISA reader (Robonik, India) was used to assess the absorbance of the purple colour at 570 nm after 24 h. The percentage of cell viability was computed by using eqn. 3:

$$\text{Cell viability (\%)} = \frac{\text{Mean abs of cells treated}}{\text{Mean abs of cells untreated}} \times 100 \quad (3)$$

Apoptosis (ROS): In this study, the procedure reported by Czabotar & Garcia-Saez [34] was adopted. In order to induce ROS-mediated apoptosis, cells were cultured under standard conditions and treated with hydrogen peroxide (H_2O) at different concentrations (100-500 µM). Intracellular ROS levels were assessed using DCFH-DA dye and quantified using fluorescence microscopy after treatment. Analyzed by flow cytometry, apoptotic cells were identified using Annexin V-FITC/PI staining. JC-1 dye was used to measure the potential of the mitochondrial membrane. A fluorometric assay was used to measure the activity of caspase-3/7. DNA fragmentation was detected using a TUNEL assay. The cells used as negative and ROS-inhibition controls were untreated and antioxidant-pretreated, respectively.

RESULTS AND DISCUSSION

The results from the phytochemical screening of the ethanolic extract of *Origanum vulgare*, as shown in Table-1, indicate the presence of various bioactive compounds. The flavonoid test yielded a pinkish-red colouration, confirming its positive presence. Similarly, the phenol test resulted in the formation of a greenish-blue colour, indicating the presence of phenolic compounds. The saponin test demonstrated foam formation, confirming a positive result. Additionally, the presence of terpenoids was verified by the appearance of a reddish-brown colour. Furthermore, the carbohydrate analysis using Fehling's test produced a red precipitate, confirming the presence of reducing sugars. These findings highlight the diverse range of phytochemicals present in the sample.

TABLE-1
RESULTS FROM PHYTOCHEMICAL SCREENING

Test name	Observation	Result
Flavanoid	Pinkish red	+ve
Phenol	Formation of greenish blue	+ve
Saponins	Formation of foams	+ve
Terpenoids	Reddish brown colour	+ve
Test for carbohydrates		
Fehling's test	Red precipitate	+ve

GC-MS chromatographic analysis: The GC-MS chromatographic analysis of ethanolic extract of *O. vulgare*, as illustrated in Fig. 1, revealed the presence of a diverse array of bioactive compounds (Table-2). The analysis identified a total of 38 compounds with varying retention times and peak intensities, indicating their relative abundance. Among the major constituents, 9-octadecenoic acid, 1,2,3-propanetriyl ester (glyceryl triacetyl ricinoleate) exhibited the highest peak percentage (13.07%), followed by 2-cyclohexen-1-one, 2-methyl-5- (cis-carvyl acetate, 9.76%) and palmitic acid (8.00%). Other notable compounds include 2-oxabicyclo[2.2.2]octane, 1,3,3-(2-hydroxycineol, 7.76%), 3-pentanol, 2,3-dimethyl- (7.63%), and 2,3-dihydro-benzofuran (coumaran, 6.67%). The presence of various fatty acids, esters, phenols, alcohols and terpenoids highlights the complex chemical profile of oregano extract, contributing to its potential pharmacological and therapeutic properties. These findings provide valuable insights into the composition of *O. vulgare*, supporting its traditional medicinal applications and potential for further biotechnological and pharmaceutical exploration.

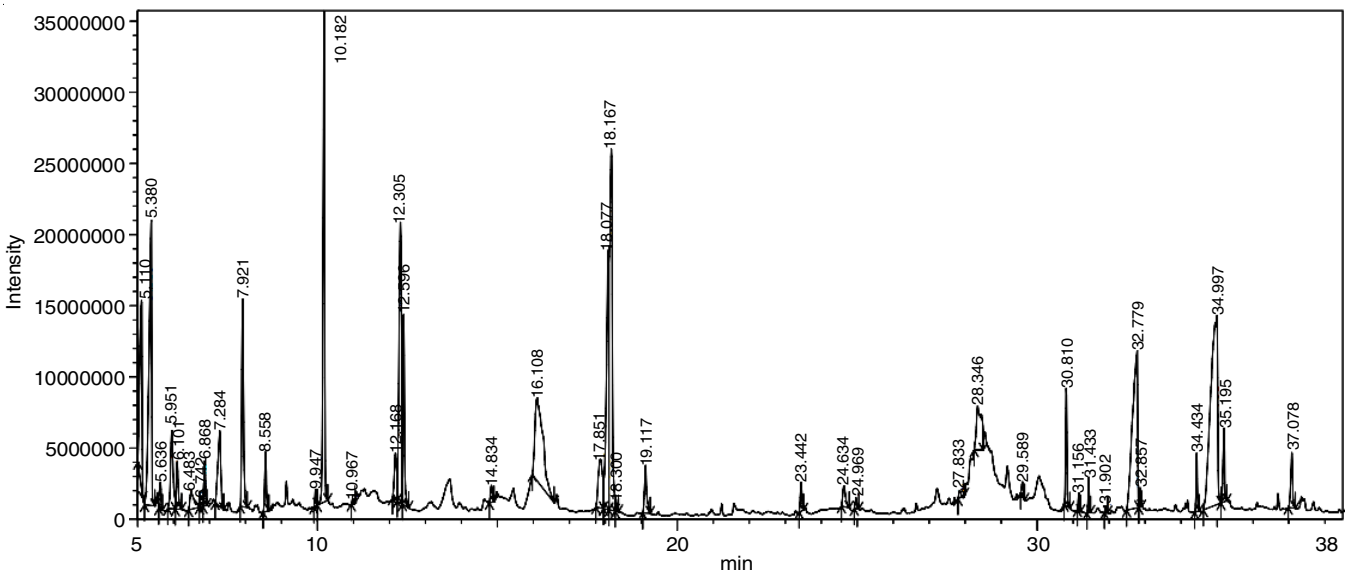

Fig. 1. Chromatograph of GCMS

TABLE-2
COMPOUNDS DETECTED FROM THE ETHANOLIC EXTRACT OF *O. vulgare*

P. No.	Retention time	Name of the compound	Common name	Peak (%)	m.f.	m.w. (g/mol)
1	5.110	2-(Trimethylsilyl)ethanol	Ethylene glycol	4.37	$C_5H_{14}O_2Si$	134.25
2	5.380	3-Pentanol, 2,3-dimethyl	2,3-Dimethylpentan-3-ol	7.63	$C_7H_{16}O$	116.20
3	5.636	Ethyl but-2-enoate	Crotonic acid ethyl ester	0.44	$C_6H_{10}O_2$	114.14
4	5.951	2-Furanmethanol	α-Ethyl-2-furanmethanol	2.02	$C_7H_{10}O_2$	126.15
5	6.101	Protoanemonine	5-Methylene-2(5*H*)-furanone	0.92	$C_5H_4O_2$	96.08
6	6.483	α,β-Crotonolactone	Butenolide	0.75	$C_4H_4O_2$	84.07
7	6.742	2-Propenoic acid, butyl ester	1-Dodecanethiol	0.25	$C_{37}H_{56}O_2S$	564.9
8	6.868	1,2,4-Butanetriol	2-Deoxyerythritol	0.70	$C_4H_{10}O_3$	106.12
9	7.284	2-Hydroxycyclopent-2-en-1-one	2-Hydroxy-2-cyclopenten-1-one	2.17	$C_5H_6O_2$	98.10
10	7.921	Benzaldehyde	Benzoic aldehyde	3.75	C_7H_6O	106.12
11	8.558	2,4-Dihydroxy-2,5-dimethyl-3(2*H*)-furan-3-one	2,4-Dihydroxy-2,5-dimethylfuran-3-one	0.99	$C_6H_8O_4$	144.12
12	9.947	Benzene, 1-methyl-3-(1-methyle	Isopropyl *m*-tolyl sulphide	0.25	$C_{10}H_{14}S$	166.29
13	10.182	2-Oxabicyclo[2.2.2]octane, 1,3,3	2-Hydroxycineol	7.76	$C_{10}H_{18}O_2$	170.25
14	10.967	1,4-Cyclohexadiene, 1-methyl-4	2,5-Dihydrotoluene	0.04	C_7H_{10}	94.15
15	12.168	6-Dien-3-yl formate	Coriandrol	1.10	$C_{10}H_{18}O$	154.25
16	12.305	1,1,3-Triethoxybutane	Butyraldehyde	7.14	$C_{10}H_{22}O_3$	190.28
17	14.834	3-Cyclohexene-1-methanol	1,2,3,6-Tetrahydrobenzyl alcohol	0.32	$C_7H_{12}O$	112.17
18	16.108	2,3-Dihydro-benzofuran	Coumaran	6.67	C_8H_8O	120.15
19	17.851	Phenol, 2-methyl-5-(1-methylet	Ethyl-5-isopropyl-*o*-cresol	2.11	$C_{12}H_{18}O$	178.27
20	18.077	2-Methoxy-4-vinylphenol	4-Hydroxy-3-methoxybiphenyl	6.54	$C_{13}H_{12}O_2$	200.23
21	18.167	2-Cyclohexen-1-one, 2-methyl-5	*cis*-Carvyl acetate	9.76	$C_{12}H_{18}O_2$	194.27
22	18.300	Octadecanoic acid, 9,10-dihydr	Dioxystearinsaeure	0.15	$C_{18}H_{36}O_4$	316.5
23	19.117	Benzoic acid, 4-formyl-, methyl ester	4-Carbomethoxybenzaldehyde	1.05	$C_9H_8O_3$	164.16
24	23.422	2-Cyclopenten-1-one, 2-(2-butenyl)-4-hydr	Cinerolone	0.46	$C_{10}H_{14}O_2$	166.22
25	24.634	Furan, 2,3-dihydro-2,2-dimethyl	Carbofuran phenol	0.59	$C_{10}H_{12}O_2$	164.20
26	24.969	1,3,3-Trimethyl-2-(2-methylcycl	1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene	0.19	$C_{13}H_{22}$	178.31
27	24.833	3-Oxabicyclo[3.3.0]octan-2-one, 7-neopen	5-Methylenehexahydro-1*H*-cyclopenta[*c*]furan-1	0.10	$C_8H_{10}O_2$	138.16
28	28.346	Benzoic acid, undecyl ester	Undecyl benzoate	2.23	$C_{18}H_{28}O_2$	276.4
29	29.589	Myristic acid P1035	Tetradecanoic acid	0.19	$C_{14}H_{28}O_2$	228.37
30	30.810	2,6,10-Trimethyl,14-ethylene-14-	Norpristane	1.53	$C_{18}H_{38}$	254.5
31	31.433	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3,7,11,15-Tetramethylhexadec-2-en-1-ol	0.38	$C_{20}H_{40}O$	296.5
32	31.902	Eicosanoic acid, methyl ester	Methyl arachidate	0.09	$C_{21}H_{42}O_2$	326.6
33	32.779	Palmitic acid	Hexadecanoic acid	8.00	$C_{16}H_{32}O_2$	256.42
34	32.857	Hexadecanoic acid, ethyl este	Ethyl palmitate	0.23	$C_{18}H_{36}O_2$	284.5
35	34.437	Phytol	*trans*-Phytol	0.71	$C_{20}H_{40}O$	296.5
36	34.997	9-Octadecenoic acid, 1,2,3-propanetriyl es	Glyceryl triacetyl ricinoleate	13.07	$C_{63}H_{110}O_{12}$	1059.5
37	35.195	Octadecanoic acid	*trans*-2-Oleic acid	1.17	$C_{18}H_{34}O_2$	282.5
38	37.078	1,6,10-Dodecatrien-3-ol, 3,7,11-tr	-Nerolidyl acetate	1.02	$C_{17}H_{28}O_2$	264.4

Antibacterial activity: The antibacterial activity of the ethanolic extract of *O. vulgare* was evaluated against five pathogenic bacterial strains using an inhibition zone assay. The results indicate varying degrees of susceptibility among the tested pathogens (Table-3). The ethanolic extract exhibited the highest inhi-bition zone against *S. aureus* (21 mm), followed by *K. pneumoniae* (15 mm), *E. coli* and *P. aeruginosa* (11 mm each), and *B. cereus* (6 mm). Comparatively, methicillin (positive control) demonstrated greater inhibitory effects against all strains, with the largest inhibition zone observed for *S. aureus* (30 mm), whereas ethanol (negative control) showed no antibacterial activity. These findings suggest that *O. vulgare* possesses significant antibacterial potential, particularly against Gram-positive bacteria like *S. aureus*, highlighting its possible application as a natural antimicrobial agent.

Antifungal activity: The action of the volatile phase of ethanolic extract of *O. vulgare* against the fungal growth of *A. niger* and *F. oxysporum* does not exhibit inhibition in antifungal activity against these two distinct fungi.

Antioxidant activity

DPPH assay: The DPPH free radical scavenging abilities of an ethanol extract of *O. vulgare* leaves were assessed and compared with those of the standard ascorbic acid. At 517 nm, the sample has an absorbance of 0.176 while the positive control has an absorbance of 0.675. The results of the ethanolic extract expressed a 74% decrease in the initial DPPH concentration, known as IC_{50}, and it is used to express the antioxidant activity of the extract.

FRAP assay: The reduction of ferric ions into ferrous ions in an ethanolic extract of *O. vulgare* leaves was assessed and compared with those of the standard ascorbic acid. The absorbance of a sample at a wavelength of 700 nm was 0.176 and the absorbance of a blank at a wavelength of 700 nm was 0.153. Using ascorbic acid as standard, the reducing power of an antioxidant in reaction with ferric cyanide was estimated. The antioxidant concentration in ethanolic leaf extract was found to be 135.2 mg/g of the ethanolic extract.

Total phenolic content (TPC): Plants require phenolic components because of their hydroxyl group-mediated scavenging action. The standard curve was used to calculate the total phenol concentrations, with gallic acid serving as the standard. The absorbance value of the sample was recorded as 0.231, it was compared with the standard graph from which the total phenol content was calculated to be 2.3 mg/g of the ethanolic extract.

Anti-inflammatory activity: The activity against the inflammation was assessed using the trypsin and protein denaturation method against egg albumin protein. The ethanolic extract of *O. vulgare* shows 83% inhibition of protein denaturation and 56% inhibition by the trypsin method. This finding correlates with the GC-MS screening result showing the various anti-inflammatory phytoconstituents in the plant extract might have contributed to this activity.

Antidiabetic assay: The antidiabetic assay was assessed using the α-amylase inhibition and α-glucosidase inhibition assay. The sample showed 45.59% inhibitory activity through the α-amylase inhibition assay and 63.60% inhibitory activity through the α-glucosidase inhibition assay.

Cytotoxicity: The cytotoxic effects of ethanolic extract of *O. vulgare* were evaluated against the HEP-G2 cancer cell line using the MTT assay. The results indicate a dose-dependent reduction in cell viability (Fig. 2). At the lowest concentration (6.25 µg/mL), 89% of the cells remained viable, while increasing the concentration to 12.25 µg/mL and 25 µg/mL reduced cell viability to 80% and 69%, respectively. A further decline was observed at 50 µg/mL (55% viability), with the highest tested concentration (100 µg/mL) resulting in only 53% viability. These findings confirmed the cytotoxic potential of *O. vulgare* extract, demonstrating a significant inhibitory effect on HEP-G2 cells at higher concentrations. This suggests that oregano extract possesses promising anticancer properties, affirming further investigation into its bioactive components and mechanisms of action.

Apoptosis: Apoptosis plays a critical role in cellular homeostasis and cancer therapy, with key hallmarks including phosphatidylserine (PS) externalization and membrane integrity loss. The Annexin V/PI staining assay was performed to evaluate the apoptotic effects of the ethanolic extract of *O. vulgare* on HepG2 cells. The results revealed a significant increase in apoptosis upon treatment with the sample extract (Fig. 3). The untreated control exhibited only 4.12% apoptosis, indicating normal cell survival. In contrast, standard control (doxorubicin, 5 µM/mL) induced 59.47% apoptosis, confirming its cytotoxic efficacy. Treatment with the sample extract at 72 µg/mL resulted in 49.55% apoptosis, demonstrating the substantial apoptotic activity. These findings suggest that ethanolic extract of *O. vulgare* effectively triggers apoptosis in HepG2 cells, though slightly less than the standard drug. The observed apoptotic induction highlights the potential of *O. vulgare* as a natural anticancer agent, necessitating further mechanistic studies to elucidate its molecular pathways.

Conclusion

The ethanolic extracts of *O. vulgare* for their antioxidant, antimicrobial, antidiabetic, anti-inflammatory and cytotoxic assays using total phenolic contents (TPCs) and DPPH, disc diffusion method and MTT cytotoxicity assays were evaluated

TABLE-3
RESULTS OBTAINED FROM ANTIBACTERIAL INHIBITION TESTS

Name of the pathogen	Ethanol (-ve control)	Zone of activity (mm)	Zone of activity (mm)	Methicillin (+ve control)
E. coli	Nil	11	12	Nil
Pseudomonas aeruginosa	Nil	11	26	Nil
Staphylococcus aureus	Nil	21	30	Nil
Klebsiella pneumoniae	Nil	15	25	Nil
Bacillus cereus	Nil	6	12	Nil

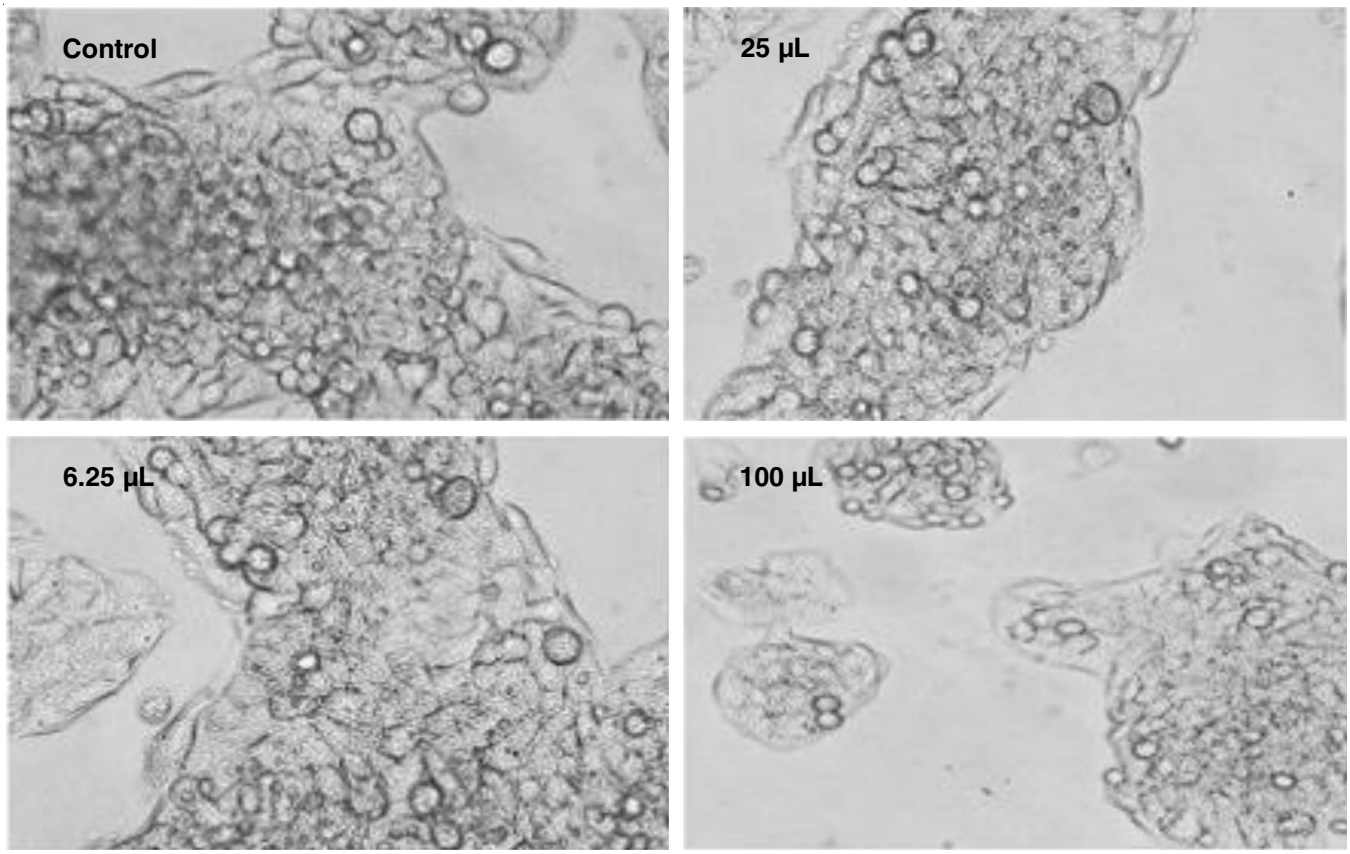

Fig. 2. Result of MTT assay against HEP-G2 cancer cell line

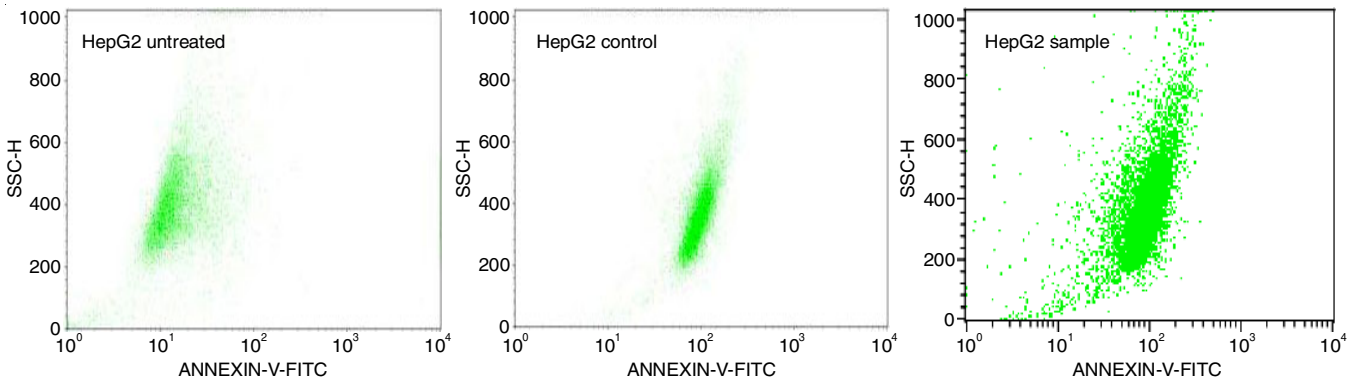

Fig. 3. Result of apoptosis against the HEP-G2 cell line

respectively. These results provide opportunities for more research and use of *O. vulgare* as a significant natural resource, with great potential for the pharmaceutical and therapeutic industries.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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