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Exploration Of Antidiabetic Potentials Of Origanum Vulgare Plant Extract

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ABSTRACT

Origanum vulgare Linn., commonly known as oregano, is a perennial herb from the Lamiaceae family, widely recognized for its culinary and medicinal applications. This study focuses on the morphological characteristics, phytochemical composition, and pharmacological potential of *O. vulgare*. Morphological analysis revealed that the plant grows between 20 cm to 80 cm (7.9–31.5 inches), with dark green oblong-ovate leaves that turn yellowish-green upon drying. The corolla and calyx are white and green, respectively, with a smooth texture and sparse hairs. The taste was found to be astringent. Oregano is rich in bioactive compounds such as carvacrol and thymol, contributing to its antimicrobial, antioxidant, and therapeutic properties. Traditionally, it has been used for treating colds, indigestion, and stomach disorders. The plant thrives in full sun and well-drained soil, making it an easily cultivable herb with significant economic and medicinal value. This research highlights the morphological attributes, phytochemical potential, and pharmacological significance of *O. vulgare*, supporting its widespread use in traditional medicine and the food industry.

Keywords: *Origanum vulgare, Oregano essential oil, Medicinal plants, Antioxidant properties, Phytochemical composition, Traditional medicine*

1. INTRODUCTION

Origanum vulgare Linn., commonly known as oregano, is a widely recognized medicinal and culinary herb belonging to the Lamiaceae (mint) family. Native to the Mediterranean region, Europe, North Africa, and parts of Asia, oregano has been cultivated and utilized for centuries for its flavor, aroma, and therapeutic properties (Kintzios, 2002). The name "oregano" is derived from the Greek words *oros* (mountain) and *ganos* (joy), reflecting its historical association with health and well-being in ancient civilizations (Nurzyńska-Wierdak, 2015).

Oregano is a perennial herb that grows to a height of 20–80 cm (7.9–31.5 inches), featuring oblong-ovate leaves that are dark green when fresh and yellowish-green upon drying. The plant produces small white or green flowers, and its texture is smooth with sparse hairs. Due to its adaptability, it thrives in full sun and well-drained soil, making it a popular choice for both commercial cultivation and home gardens (Kulisic et al., 2004).

Oregano has a long history of medicinal use, particularly in traditional European, Greek, and Chinese medicine, where it has been employed to treat respiratory infections, digestive disorders, and inflammatory conditions (Lagouri & Boskou, 1996). The plant is rich in bioactive compounds, particularly essential oils such as carvacrol, thymol, and rosmarinic acid, which contribute to its antimicrobial, antioxidant, and anti-inflammatory properties (Burt, 2004).

In modern medicine and pharmacology, oregano is being studied for its potential to combat bacterial and fungal infections, oxidative stress, and chronic diseases. Research suggests that oregano extracts may help in preventing foodborne illnesses and extending the shelf life of food products due to their antioxidant and antimicrobial properties (Ruberto et al., 2000).

Economic and Agricultural Significance

Oregano is widely cultivated for its culinary and medicinal applications, and its essential oil is commercially valuable in the pharmaceutical, cosmetic, and food industries. The growing interest in natural remedies and plant-based medicine has further increased the demand for high-quality oregano extracts. Additionally, due to its ability to repel certain pests and improve soil quality, oregano is also used in companion planting in organic farming systems (Kintzios, 2002).

This study aims to explore the morphological characteristics, phytochemical composition, and medicinal applications of *Origanum vulgare*, providing scientific insight into its pharmacological potential and economic significance.

2. MATERIAL AND METHOD

Plant Collection and Authentication

Leaves of *Origanum vulgare* were collected from Yamunanagar, Haryana, and authenticated by the Department of Botany on November 28, 2021. A voucher specimen was deposited at Baba Mastnath University, Rohtak, for future reference (Kumar et al., 2021).

Pharmacognostical Studies

Morphological and Microscopical Analysis

Macroscopic evaluation was carried out by observing the color, shape, size, odor, and texture of fresh leaves under diffused daylight (Evans, 2009). Microscopical studies involved the preparation of transverse sections of fresh leaves, cleared with chloral hydrate, and stained with phloroglucinol and dilute HCl for lignified tissue identification. Powdered samples were treated with iodine for starch grains, ruthenium red for mucilage, and safranin for general staining (Khandelwal, 2016).

Physicochemical Analysis

Standard physicochemical parameters were determined according to the Indian Pharmacopoeia (IP, 2018) and WHO guidelines (WHO, 2011):

- Ash Values: Total ash, acid-insoluble ash, and water-soluble ash were determined by incinerating the powdered sample in a muffle furnace at 550°C.
- Foreign Matter & Moisture Content: Assessed using a 10X magnifying lens and drying in an oven at 105°C until a constant weight was achieved.
- Swelling and Foaming Index: The swelling index was determined by measuring the volume increase over 24 hours, while the foaming index was analyzed by observing the height of foam produced (Anonymous, 1996).
- Extractive Values: Ethanol and water-soluble extractive values were determined using maceration and subsequent drying of the extracts.
- Heavy Metal & Aflatoxin Analysis: Pb, Cd, and Hg levels were analyzed using Atomic Absorption Spectroscopy (AAS) following the AOAC method (AOAC, 2005). Aflatoxins were quantified using Liquid Chromatography-Mass Spectrometry (LC-MS).
- Microbial Infestation Assay: The total aerobic count and presence of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Salmonella* species were assessed using standard culture methods (Indian Pharmacopoeia, 2018).

Preparation of Extracts

Soxhlet extraction was performed using aqueous, methanol, ethyl acetate, and petroleum ether solvents at 50-75°C over 32 cycles. Extracts were concentrated under reduced pressure, dried, and stored in airtight containers at 4°C (Harborne, 1998).

Preliminary Phytochemical Screening

- Qualitative Analysis: Standard tests were conducted for alkaloids (Mayer's test), carbohydrates (Molisch's test), saponins (frothing test), phenolic compounds (Ferric chloride test), flavonoids (Shinoda test), proteins (Biuret test), and sterols (Salkowski test) (Trease & Evans, 2002).
- Quantitative Analysis: Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method, while Total Flavonoid Content (TFC) was assessed using the aluminum trichloride method (Singleton et al., 1999).

Thin Layer Chromatography (TLC) Analysis

TLC was performed using preparatory plates (Merck) with the following solvent systems:

- Toluene-ethyl acetate-glacial acetic acid (30:40:5)
- Toluene-acetone-formic acid (4.5:4.5:1) Retention factor (Rf) values were calculated and compared with standards (Wagner & Bladt, 2001).

Antioxidant Activity

- DPPH Radical Scavenging Assay: Free radical scavenging activity was analyzed using the DPPH method. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Blois, 1958).
- Hydrogen Peroxide Scavenging Assay: The scavenging effect of hydrogen peroxide was assessed by measuring absorbance at 230 nm after incubation with phosphate-buffered saline (Gulcin et al., 2003).

Formulation Development and Optimization

Tablets were formulated using direct compression and optimized using the Box-Behnken design. Evaluations included:

- Weight variation, diameter, thickness, hardness, friability, disintegration time, and dissolution studies following USP guidelines (USP, 2020).

Antimicrobial Activity

- Microorganisms Tested: *Pseudomonas aeruginosa, Enterococcus faecalis, Candida albicans*, and *Aspergillus niger* were tested.
- Zone of Inhibition: Determined using the cup-plate method.
- Minimum Inhibitory Concentration (MIC): Assessed using broth dilution techniques (CLSI, 2012).

Anti-Diabetic Activity

- α-Amylase Inhibition Assay: Carried out using DNS reagent with absorbance at 540 nm (Miller, 1959).
- α-Glucosidase Inhibition Assay: Conducted using *p*-Nitrophenyl-α-D-glucopyranoside (pNPG), with absorbance measured at 405 nm (Kim et al., 2000).

3. RESULT AND DISCUSSION

Morphological Studies of *Origanum vulgare* Linn.

Origanum vulgare Linn., commonly known as oregano, belongs to the mint family (Lamiaceae). It is a perennial herb that grows between 20 cm to 80 cm (7.9–31.5 inches) in height. The name "oregano" is derived from the Greek words "oros" (mountain) and "ganos" (joy). The plant has a fragrant and slightly bitter aroma, with fresh leaves appearing dark green, while dried leaves turn yellowish-green. The leaves are oblong-ovate, smooth in texture, and lack hairs. The corolla and calyx are white and green, respectively, with sparse hairs. The taste is astringent. Oregano is widely used as a culinary herb, especially in Italian cuisine, and has medicinal applications for colds, indigestion, and stomach ailments due to its high carvacrol and thymol content. It thrives in full sun and well-drained soil, making it an easy-to-grow herb. The morphological characteristics are illustrated in **Figure 1**.

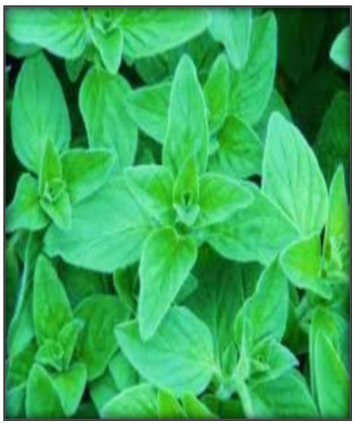

Fig. 1: Representative photomicrograph of *Origanum vulgare* Linn.

Microscopical studies

Microscopy of the fresh leaves and dried fine powder of plant was carried out.

***Origanum vulgare* Linn.:** Transverse section of leaf showed the presence of single layered epidermis with multicellular epidermal hairs and a layer of cuticle were observed. The palisade cells were single layered and columnar. Spongy cells were found to be scattered and loosely arranged. Vascular bundles were found and closed shape.

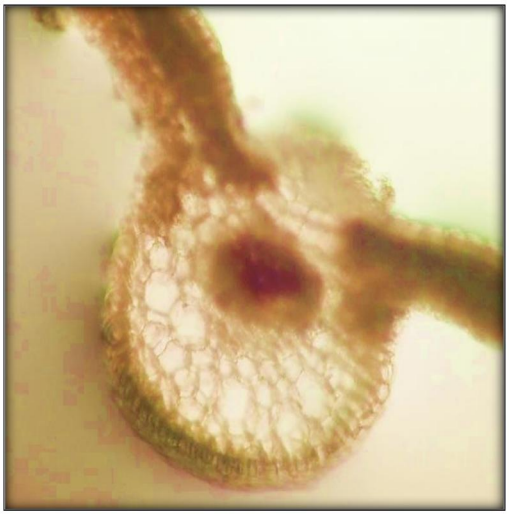

Fig. 2: Representative photomicrographs of the transverse section of *O. vulgare* Linn. Leaves.

Physicochemical Constants

Plant leaves were subjected to the physicochemical constants *i.e.*, total ash value, acid-insoluble ash value, water-soluble ash value, loss on drying, swelling index, water-soluble and alcohol-soluble extractive values were calculated by per procedures mentioned in section The results for various physicochemical parameters are presented below (**Table 1** to **Table 4**):

Table 1: Ash values of the *O. vulgare* Linn. Leaves.

Particulars	**Total Ash (%)**	**Acid Insoluble Ash (%)**	**Water Soluble Ash (%)**
O. vulgare	11.5 ± 0.32	10.1 ± 0.24	5.4 ± 0.45

Values represented as mean ± SEM (n=3)

Table 2: Extractive values of powdered *O. vulgare* Linn. Leaves.

S. No.	**Plants**	**Alcohol Soluble Extractive Value (%)**	**Water Soluble Extractive Value (%)**
1	***O. vulgare***	1.2 ± 0.55	0.88 ±0.72

Values represented as mean ± SEM (#n=3)

Table 3: Presence of heavy metal in leaves of *O. vulgare.*

Trace metal	***O. vulgare***	**WHO limits**
Lead (mg/kg)	0.04	10 (PPM)
Cadmium (mg/kg)	-	0.3 (PPM)
Arsenic (mg/kg)	-	3 (PPM)
Mercury (mg/kg)	-	1(PPM)
Iron	1.44	Below 200 mg/kg
Copper	0.01	20-150 mg/kg

Magnesium	0.35	200 mg/kg
Zinc	0.034	Below 50 mg/kg

Where; - denotes not detected

Table 4: Aflatoxin analysis of *O. vulgare* leaves.

Aflatoxin (µg/kg)	**WHO Limits (ppm)**
B1	**0.45**
B2	**0.1**
G1	**0.5**
G2	**0.1**

Where – denotes, absent or not found

Microbial Infestation Assay

Results for the microbial content of stems of the both plants are represented in **Table 5**.

Table 5: Result for microbiological analysis of *O. vulgare* leaves.

Sample name	***O. vulgare* leaves**	**WHO limits**
TPC (CFU/g)	157	10^5 per gram
Yeast & Mold (CFU/g)	112	10^3 per gram
***Salmonella* (per g)**	Negative	absence per gram
***S. aureus* (per g)**	Negative	absence per gram
***E. coli* (per g)**	Negative	absence per gram
***P. aeruginosa* (per g)**	Negative	absence per gram

The various standardization parameters of *O. vulgare* leaves were observed *i.e.*, macroscopic, microscopic examination, ash value, foreign organic matter, moisture content, swelling index, foaming index, extractive values, elemental analysis, aflatoxin content, and microbial infestation assay were carried out according to the methods mentioned in WHO 2007 and API 2001 were also found within limit.

Preparation of extracts

The extraction was performed through Soxhelation process. The percentage yield of extracts of *O. vulgare* Linn. leaves were found with various solvents *i.e.*, petroleum ether 2.1%, ethyl acetate 3.7%, methanol 8.6%, aqueous 9.2%, and the extracts color observed were found dark green to light green.

Phytochemical Screening

Qualitative Phytochemical Screening

Phytochemical screening of *O. vulgare* leaves were carried out for different extracts using chemical methods employed for identification of various components. Results for screening of various phytoconstituents are represented in **Table 6**.

Table 6: Phytochemical screening of *O. vulgare* Linn. leaves extracts.

S. No.	Tests	Compounds	*O. vulgare* leaves extracts			
			Petroleum ether	Ethyl acetate	Methanol	Aqueous
1.	Alkaloids	Mayer's reagent	+	+	+	+
		Hager's reagent	+	+	+	+
		Wagner's reagent	+	+	+	+
2.	Carbohydrates	Molish's test	+	-	+	+
		Fehling's test	+	-	+	+
		Benedict's test	+	-	+	+
3.	Saponins	Frothing test	-	-	+	+
4.	Phenolic compound and tannins	Ferric chloride test	-	+	+	+
		Silver nitrate test	-	+	+	+
5.	flavonoids	Ammonia test	+	+	+	+
		Shinoda test	+	+	+	+
6.	Proteins and amino acids	Millon's test	-	+	+	+
		Biuret test	-	+	+	+
		Ninhydrin test	-	+	+	+
7.	Sterols	Liebermann-Burchard's test	+	-	+	+
		Salkowski reaction	+	-	+	+

#+ showed present and – showed absent

Qualitative Phytochemical Screening revealed that the presence of alkaloids, glycosides, carbohydrates, saponins, phenolic, sterols, flavonoids, protein and free amino acids in the selected plants methanol and aqueous extract. These secondary metabolites were responsible for various pharmacological activities.

Quantitative Analysis

Total Phenolic Content (TPC)

The mg of Gallic acid equivalents (GAE) per g of *O. vulgare* Linn. leaves methanol and aqueous extracts were found to be 7.42± 0.83 and 5.03± 0.04, respectively (**Figure 3**). The mg of Gallic acid equivalents (GAE) per g of *E. longifolia* Jack roots ethanol and aqueous extracts were found to be 6.03± 0.44 and 4.82± 0.67, respectively. The ethanol extract of *E. longifolia* Jack roots product contained maximum total phenolic content (7.42 mg GAE/g) than other extract. As shown in figure, Gallic acid was used to prepare a standard curve as linearity curve. $y = 0.1613x - 0.1089$ ($R^2 = 0.9971$) y is the absorbance; x is the solution concentration). The results were expressed as mg of gallic acid equivalents (GAE) per g of powdered crude drug as in **Table 7**.

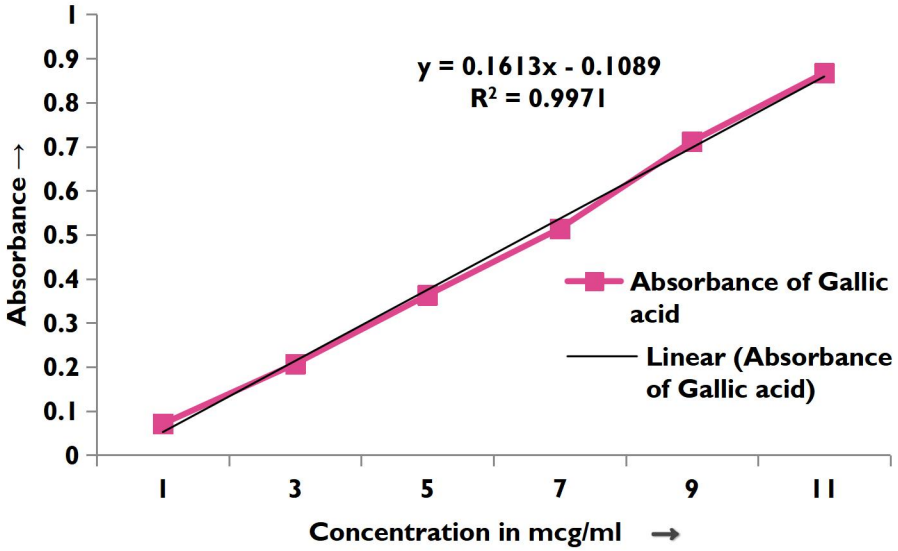

Figure 3: Linearity for Gallic acid.

Table 7: Total Phenolic content and Total flavonoid content of *O. vulgare* leaves methanol and aqueous extracts.

S. No.	Plants	TPC		TFC	
		Methanol	Aqueous	Methanol	Aqueous
1	*O. vulgare*	7.42± 0.83	5.03± 0.04	2.63± 0.22	1.64± 0.26

Total flavonoid content

The mg of quercetin equivalents (QE) per g of *O. vulgare* Linn. leaves methanol and aqueous extract were found to be 2.63± 0.22 and 1.64± 0.26, respectively (**Figure 4**).

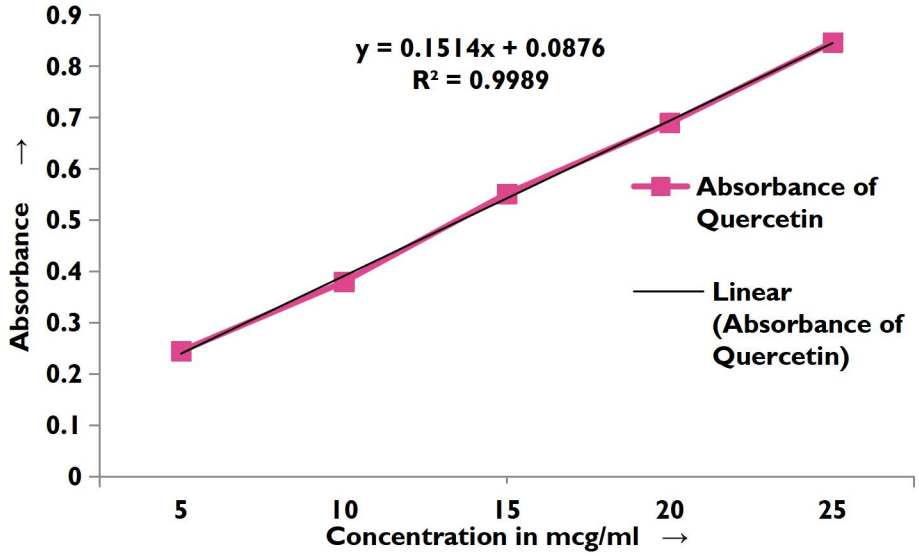

Figure 4: Linearity for Quercetin.

Total phenolic content and flavonoid content estimation

TLC of the plant extracts were carried out for qualitative analysis of various components using various solvents. The results of TLC analysis are represented in **Table 8** and **Figure 5**.In TLC profile of *O. vulgare* leaves extract in mobile phase Toluene-Acetone-Formic acid (4.5: 4.5: 1) v/v, petroleum extract showed no spot Under UV light 254 nm, and

Under UV light 365 nm. Ethyl acetate extract showed one spot Under UV light 366 nm, and showed one spot Under UV light 254 nm. Methanol extract showed one spot Under UV light 366 nm, and showed 2 spots Under UV light 254 nm. Aqueous extract showed one spot Under UV light 366 nm, and showed 2 three spots Under UV light 254 nm (**Figure 6**).

Table 8: TLC profile of *O. vulgare* leaves in mobile phase Toluene-Acetone-Formic acid (4.5: 4.5: 1) v/v/v.

Samples	**Observation**	**No. of spots observed (R_f value)**
Petroleum extract	**Under UV light 366 nm**	No spot
	Under UV light 254 nm	No spot
Ethyl acetate extract	**Under UV light 366 nm**	One spot (0.33)
	Under UV light 254 nm	One spot (0.33)
Methanol extract	**Under UV light 366 nm**	One spot (0.33)
	Under UV light 254 nm	Two spots (0.33, 0.42)
Aqueous extract	**Under UV light 366 nm**	One spot (0.33)
	Under UV light 254 nm	Two spots (0.33, 0.42)

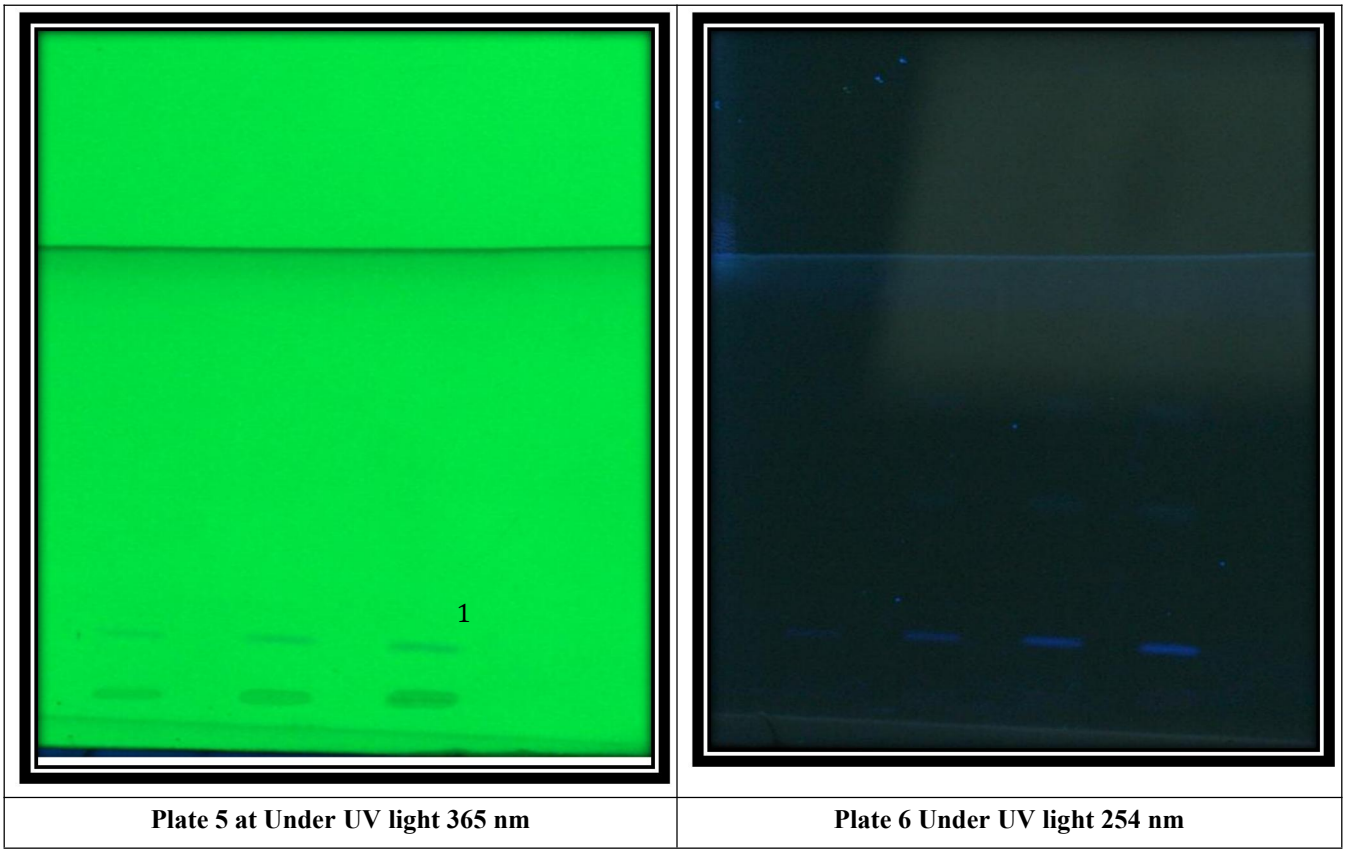

Plate 5 at Under UV light 365 nm | **Plate 6 Under UV light 254 nm**

Figure 5: Representative images of TLC analysis of *O. vulgare* leaves: in Solvent System: Toluene-Acetone-Formic acid (4.5: 4.5: 1) v/v.

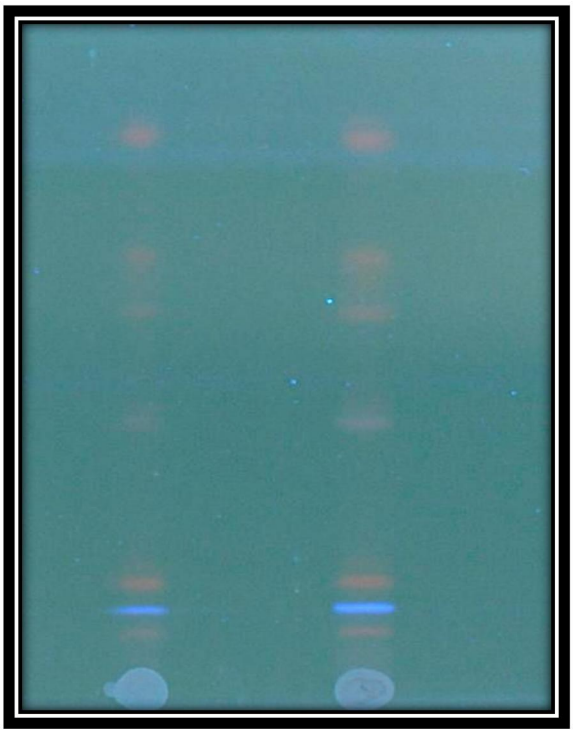

Plate 7 *O. vulgare* leaves methanol extract

Figure 6: Representative images of TLC analysis of *O. vulgare* leaves methanol and aqueous extracts: under UV light (365 nm); Solvent System: Toluene-ethyl acetate-Glacial acetic acid (3:4:0.5) v/v/v.

In TLC profile of *O. vulgare* leaves extract in mobile phase Toluene-ethyl acetate-Glacial acetic acid (3:4:0.5) v/v/v, Methanol extract showed five (0.19, 0.22, 0.26, 0.53, and 0.90) spot and aqueous extract showed seven (0.19, 0.22, 0.26, 0.38, 0.49, 0.53, and 0.90) spots under UV light 254 nm.

Antioxidant activity

DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity

DPPH radical scavenging activity of *O. vulgare* Linn. leaves extract and ascorbic acid (positive control) was observed with a range concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml (**Figure 7**), the results are shown in **Table 9**.

Table 9: Effect of *O. vulgare* Linn. leaves extracts on DPPH radical scavenging activity.

Concentration (µg/ml)	Ascorbic acid	Methanol	Aqueous
10	43.31±0.45	22.87±0.44	19.9±0.45
20	55.73±0.05	30.73±0.87	23.65±0.34
40	80.45±0.67	36.02±0.55	30.86±0.86
80	100.46±0.21	41.76±0.23	37.45±0.65
160	137.98±0.86	52.86±0.66	45.76±0.34
320	200±0.05	88.27±0.27	77.97±0.75
IC_{50}	17±0.87	159.45±0.74	188.31±0.46

All values are expressed as mean±SEM (n=3), extracts groups were compared by One-way ANOVA followed by Dunnett's test.

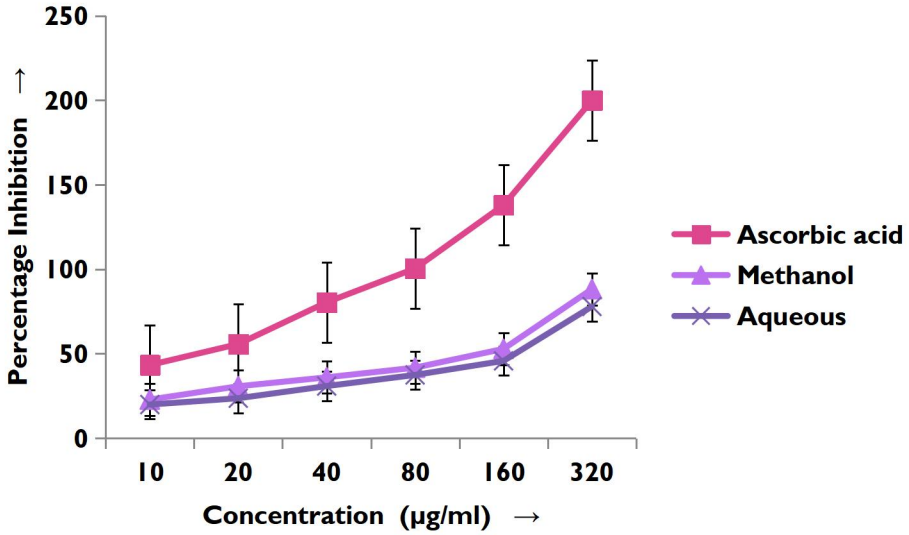

Figure 7: Effect of *O. vulgare* leaves extracts on DPPH radical scavenging activity.

All values are expressed as mean±SEM (n=3), extracts groups were compared by One-way ANOVA followed by Dunnett's test.

Hydrogen Peroxide scavenging activity

Hydroxyl radicals scavenging activity was quantified by measuring inhibition of the degradation of the deoxyribose by free radicals. Studies have attributed that antioxidant properties are due to the presence of phenols and flavonoids. The hydrogen peroxide scavenging activity of petroleum ether, ethyl acetate, methanol and aqueous extracts of *O. vulgare* Linn. leaves extracts are depicted in **Table 10** and **Figure 8**.

Table 10: Effect of *O. vulgare* leaves extracts on hydrogen peroxide scavenging activity.

Concentration (µg/ml)	Ascorbic acid	Methanol	Aqueous
10	40.35±0.87	25.67±0.65	21.88±0.76
20	53.89±0.66	31.9±0.32	26.86±0.51
40	70.86±0.07	36.87±1.86	32.97±0.65
60	80.24±0.56	42.46±1.72	38.33±0.34
80	93.46±0.35	50.87±0.65	44.77±0.87
100	105.45±0.82	61.56±0.43	50.92±0.77
IC_{50}	17.06±0.71	80.1±0.21	100±0.24

*All values are expressed as mean± SEM (n=3), extracts groups were compared by One-way ANOVA followed by Dunnett's test *** P < 0.001, ** P < 0.01, *P < 0.05.*

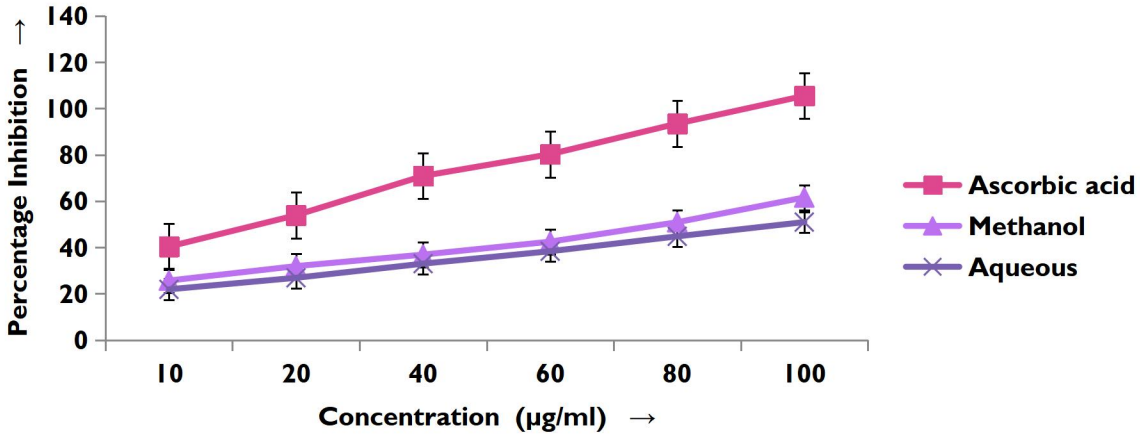

Figure 8: Effect of *O. vulgare* leaves extracts on hydrogen peroxide scavenging activity.

Formulation development and optimization

Development of tablet of methanol extracts of *O. vulgare* Linn. leaves by using formula:

Microcrystalline cellulose (MCC) - 100-150 mg

Sodium starch glycolate -10 mg-20 mg

Magnesium stearate - 1 mg- 2 mg

Lactose -30 mg

Polyvinyl pyrolividine (binder) – 3 mg

Talcum (1%) and Fumed silica (1%), 1.5% HPMC

Optimization of the tablet formulation Box-Behnken design

Table 11: Variable selected to perform optimization.

S. No.	Variables	Low level	Middle level	High level
1	Quantity of MCC (mg) X1	100 (-1)	125 (0)	150 (+1)
2	Sodium starch glycolate X2	10(-1)	15 (0)	20 (+1)
3	Magnesium stearate (mg) X3	0.5(-1)	0.8 (0)	1 (+1)

Table 12: Different formulations suggested by Design Expert® software using Box-Behnken design.

	Factors			Responses	
Formulation code	X1	X2	X3	Y1	Y2
	Microcrystalline cellulose (mg)	Sodium starch glycolate	Magnesium stearate (mg)	Hardness	Disintegration time (min)
F-1	100	10	1	6.83	2.44
F-2	150	20	1.5	7.45	3.21
F-3	125	15	1.5	7.01	2.11
F-4	100	20	1	6.78	2.24
F-5	125	15	2	6.88	2.22
F-6	100	20	2	7.55	3.01

F-7	100	10	1	6.74	3.42
F-8	125	15	1.5	6.88	2.34
F-9	150	10	1.5	7.74	1.50
F-10	150	20	2	7.01	2.10
F-11	125	10	1	6.89	2.23
F-12	100	15	1	6.84	2.13
F-13	150	15	2	7.23	2.21

The disintegration time of developed tablet formulations (F-1 to F-13) was reported using incorporating developed tablets in disintegration test apparatus and time taken for disintegration was recorded (**Table 12**). F9 were obtained as optimized condition having good responses.

Response (R1) - Hardness

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob > F**	**Inference**
Model	234.05	5	55.32	381.29	< 0.0001	Significant
A-PVP	24.21	1	31.03	213.66	< 0.0001	
B-SSG	60.02	1	71.17	417.73	< 0.0001	
AB	20.33	1	20.21	180.15	< 0.0001	
A^2	175.36	1	105.36	970.64	< 0.0001	
B^2	31.10	1	31.10	182.53	< 0.0001	
Residual	1.36	5	0.17			
Lack of Fit	1.36	3	0.45	844.86	< 0.0001	Significant

% CDR (feno) = +88.2-0.54A+1.03B-2.86AB -3.64A^2 – 2.05B^2

Response (R2) - Disintegration time

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob > F**	**Inference**
Model	75.4	5	1.67	23.87	0.0001	Significant
A-PVP	22.8	1	3.56	50.87	< 0.0001	
B-SSG	4.46	1	4.54	64.74	< 0.0001	
AB	0.042	1	0.099	1.42	0.2682	
A^2	2.64	1	0.11	1.53	0.2507	
B^2	0.068	1	0.068	0.96	0.3550	
Residual	0.56	8	0.070			
Lack of Fit	0.15	3	0.049	0.58	0.6506	Not significant

Disintegration time = +5.25 +0.67A- 0.75B -0.16 AB -0.12A^2 – 0.096B^2

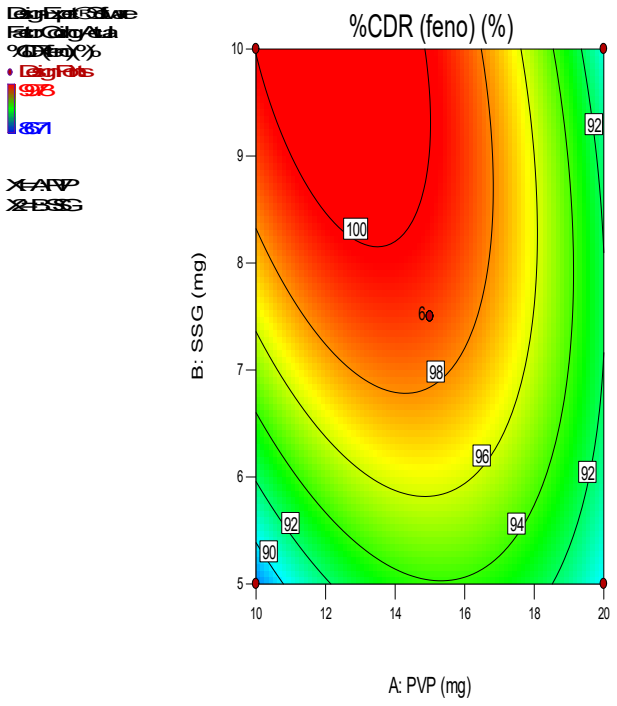

Figure 9: Illustration of actual vs. predicted plot for selected response disintegration time.

Statistical analysis was done for the obtained results. Based on coded equations and Desirability index optimized formulation was selected. Furthermore, optimized formulation was characterized for various parameters such as organoleptic properties, dimensions (Vernier calliper), weight (weighing balance), diameter or thickness (Vernier calliper), hardness (Digital Hardness Tester), Friability testing (Friability tester), Disintegration time, and Dissolution time.

Evaluation of optimized Tablet

The optimized tablets were developed and evaluated for different parameters (**Table 13**).

Table 13: Evaluation of optimized tablets from different parameters.

S. No.	Different tests	*O. vulgare* leaves
1	Organoleptic properties	Round shape, light green colour
2	Weight and weight variation	505±5.5
3	Measurement of diameter and thickness	5.142 ± 0.051
4	Hardness test	7.745 ± 0.63
5	Friability test	0.02±0.01%
6	Disintegration time	1 min. 50 Sec
7	Dissolution test	98.997

Results of optimized developed formulation were found within the acceptable ranges of values as given in official compendia but it was observed that the optimized tablet weight variation was found 505±5.5, 510±6.13 which is lowest with tablets. It is a well-known fact that the weight variation has a direct impact on the assay of the tablets. Moreover, it also indicates that the distribution of excipients is not right or homogenous. Both formulations disintegrated very rapidly and were well within official limits. The time of disintegration ranged from 1min to 2minutes. While the USP and BP have official limits of not more than a time period of 2 minutes disintegration time for uncoated tablets. Dissolution test was found not less than 90%.

Antimicrobial Activity

Antimicrobial activity of a substance is the ability to stop or kill the growth of microorganisms *i.e.*, bacteria, viruses, fungi etc. We studied antibacterial and antifungal activity of *O. vulgare* Linn. leaves and Selected two strains *i.e.*, was MTCC 424 i.e., *Pseudomonas aeruginosa* and MTCC 2729 *Enterococcus faecalis* for antibacterial activity of selected plants and Selected two strains i.e., MTCC 227 *Candida albicans* and NCIM 501 *Aspergilus niger* for antifungal activity.

Zone of Inhibition and Minimum Inhibitory Concentration

The zone of inhibition is microorganisms inhibited or killed a circular area around a test substance. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that can stop the growth of a microorganism.

i) Bacterial Strains:

Table 14: Percentage of the zone of inhibition (mm) for the tablets of methanol extract of *O. vulgare* leaves.

S. No.	Concentration	Ofloxacin	OVLMT
1	*Pseudomonas aeruginosa* (MTCC 424)	23.34±0.93	18.22±0.76
2	*Enterococcus faecalis* (MTCC 2729)	25.05±0.56	19.87±0.57

Where, OVLMT- *O. vulgare* leaves Methanol extract tablet;

Table 15: Minimum Inhibition Concentration for the tablets of methanol extract of *O. vulgare* leaves.

S. No	Bacterial Strains	Ofloxacin	OVLMT
1	*Pseudomonas aeruginosa* (MTCC 424)	50	300
2	*Enterococcus faecalis* (MTCC 2729)	50	300

Where, OVLMT- *O. vulgare* leaves Methanol extract tablet;

Table 16: Percentage of the zone of inhibition (mm) for the tablets of methanol extract of *O. vulgare* leaves.

S. No.	Fungal strain	Clotrimazole	OVLMT
1	*Candida albicans (MTCC 227)*	23.34±0.07	18.22±0.59
2	*Aspergilus niger (NCIM 501)*	25.05±0.46	19.87±0.34

Where, OVLMT- *O. vulgare* leaves Methanol extract tablet;

Table 17: *O. vulgare* leaves.

S. No.	Fungal strain	Ofloxacin	OVLMT
1	*Candida albicans (MTCC 227)*	50	300
2	*Aspergilus niger (NCIM 501)*	50	300

Where, OVLMT- *O. vulgare* leaves Methanol extract tablet;

Anti-Diabetic activity

i) α-amylase inhibition assay

Table 18: Percentage Inhibition of α-amylase at different concentrations of tablets from *O. vulgare* leaves methanol extracts.

Concentration	Standard	OVLMT
100	50.2±0.34	40.7±0.76
200	68.5±0.87	47.3±0.45
300	90±0.34	53±0.87
400	95±0.87	57.7±0.56
500	100±0.34	72.3±1.06
IC 50	100±2.8	251.3±1.67

Where, OVLMT- *O. vulgare* leaves Methanol extract tablet

Table 19: Percentage Inhibition of α- glucosidase at different concentrations tablets of *O. vulgare* leaves methanol extract.

Concentration	Standard	OVLMT
100	56.3±0.23	42.8±0.27
200	70.4±0.77	49.2±0.41
300	86.2±0.59	53.7±0.8
400	97.8±0.84	62±1.3
500	100±0.04	70.2±0.67
	80±1.2	226.7±2.65

Where, OVLMT- *O. vulgare* leaves Methanol extract tablet

iii) Glucose uptake assay

3T3 L1 cells are extensively used as a model to study adipogenesis 3T3 L1 cells were differentiated with different combinations of chemical stimulants. These cells were triggered with Insulin (diluted to different concentrations using low glucose medium) and the resultant uptake of glucose was measured (**Table 20**).

Table 20: Percentage of glucose uptake on 3T3-L1 adipocyte cell.

S. No.	Tests	Concentration (ng/ml)
1	Insulin	92.54±1.44
2	Dexamethasone	55.1±2.6

Table 21: Percentage of glucose uptake after treatment with various concentrations of tablets from *O. vulgare* leaves methanol extract on 3T3-L1 adipocyte cell.

Concentration (ng/ml)	OVLMT
100	40.3
500	65
750	78.2
1000	85.5
IC_{50}	153.72

For an Insulin-related glucose uptake study, we worked with radio-active methods and to investigate the most sensitive. Differentiated 3T3L1 cells were charged with different concentrations of tablets from *O. vulgare* leaves methanol extract and assessed for glucose uptake. **Table 21** showed that 3T3 L1 cells differentiated using the Insulin and Dexamethasone showed a higher fold in uptake of glucose than the cells treated with the other combinations. From the results obtained, it

was clear that there was a drug dose-dependent increase in the signal obtained. Earlier studies have hypothesized that Dexamethasone, a synthetic glucocorticoid enhances pre adipocyte differentiation

4. CONCLUSION

The present study comprehensively evaluated the pharmacognostical, phytochemical, antioxidant, antimicrobial, and anti-diabetic properties of *Origanum vulgare*. Morphological and microscopical analyses confirmed its characteristic features, while physicochemical parameters established quality control standards. Phytochemical screening revealed the presence of bioactive compounds, including flavonoids, alkaloids, and phenolic compounds, which contribute to its pharmacological activities. TLC profiling provided insights into its chemical fingerprint, supporting its standardization. The antioxidant assays demonstrated significant free radical scavenging activity, reinforcing its role as a natural antioxidant. The antimicrobial studies confirmed its efficacy against various bacterial and fungal strains, while the anti-diabetic assays indicated potential enzyme inhibition, supporting its traditional use in metabolic disorders. Additionally, the formulation and optimization of *O. vulgare*-based tablets provided a stable dosage form for potential therapeutic applications. These findings validate the medicinal importance of *O. vulgare* and encourage further studies to explore its clinical applications and mechanistic pathways.

CONFLICT OF INTEREST

No conflict of interest is declared.

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REFERENCES

[1] Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*(3), 223-253.

[2] Kintzios, S. E. (2002). *Oregano: The Genera Origanum and Lippia*. CRC Press.

[3] Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry, 85*(4), 633-640.

[4] Lagouri, V., & Boskou, D. (1996). Nutrient antioxidants in oregano. *International Journal of Food Science & Nutrition, 47*(6), 493-497.

[5] Nurzyńska-Wierdak, R. (2015). Essential oil composition of *Origanum vulgare* L. ssp. vulgare from Poland. *Natural Product Research, 29*(5), 494-497.

[6] Ruberto, G., Baratta, M. T., Deans, S. G., & Dorman, H. J. (2000). Antioxidant and antimicrobial activity of foeniculum vulgare and *Origanum vulgare* essential oils. *Journal of Agricultural and Food Chemistry, 48*(11), 4973-4976.Anonymous. (1996). *Quality Control Methods for Medicinal Plant Materials.* WHO.

[7] AOAC. (2005). *Official Methods of Analysis of AOAC International.* 18th Edition.

[8] Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181(4617), 1199-1200.

[9] CLSI. (2012). *Performance Standards for Antimicrobial Susceptibility Testing.* 22nd Informational Supplement, M100-S22.

[10] Evans, W. C. (2009). *Trease and Evans' Pharmacognosy.* 16th Edition.

[11] Gulcin, I., et al. (2003). Antioxidant activity of *Centaurea* species. *Phytotherapy Research*, 17(1), 83-87.

[12] Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* Springer Science & Business Media.

[13] Indian Pharmacopoeia (IP). (2018). *The Indian Pharmacopoeia Commission.*

[14] Khandelwal, K. R. (2016). *Practical Pharmacognosy.* 23rd Edition, Nirali Prakashan.

[15] Kim, J. S., et al. (2000). Inhibition of α-glucosidase and α-amylase by luteolin. *Journal of Nutritional Science and Vitaminology*, 46(2), 108-113.

[16] Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426-428.

[17] Singleton, V. L., et al. (1999). Analysis of total phenols and antioxidants. *Methods in Enzymology*, 299, 152-178.

[18] Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy.* 15th Edition.

[19] USP. (2020). *United States Pharmacopeia and National Formulary.*

[20] Wagner, H., & Bladt, S. (2001). *Plant Drug Analysis: A Thin Layer Chromatography Atlas.* Springer.

[21] Husain GM, Singh PN, Singh RK, Kumar V. Antidiabetic activity of standardized extract of *Quassia amara* in nicotinamide–streptozotocin-induced diabetic rats. Phytother Res. 2011;25(10):1451-1454.

[22] Ferreira SF, Azevedo SCSF, Vardanega-Peicher M, Pagadigorria CLS, Garcia RF. Anti-hyperglycemic effect of *Quassia amara* (Simaroubaceae) in normal and diabetic rats. Rev Bras Plantas Med. 2013;15(4):469-475.

[23] Bertani S, Houël E, Stien D, Chevolot L, Jullian V, Bourdy G, et al. Simalikalactone D is responsible for the antimalarial properties of an Amazonian traditional remedy made with *Quassia amara* L. (Simaroubaceae). J Ethnopharmacol. 2006;108(1):155-157.

[24] Ajaiyeoba EO, Abalogu UI, Krebs HC, Oduola AMJ. In vitro antimalarial activities of *Quassia amara* and *Quassia undulata* plant extracts in mice. J Ethnopharmacol. 1999;67(3):321-325.

[25] Mishra K, Chakraborty D, Pal A, Dey N. *Plasmodium falciparum*: in vitro interaction of quassin and neo-quassin with artesunate, a hemisuccinate derivative of artemisinin. Exp Parasitol. 2010;125(4):296-300.

[26] Raji Y, Oloyo AK, Morakinyo AO, Bolarinwa AF. Effects of bioactive principles from stem bark extract of *Quassia amara*, quassin and 2-methoxycanthin-6-one, on haematological parameters in albino rats. Niger J Physiol Sci. 2013;28(1):107-111.

[27] Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS, et al. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia. 2008;79(7-8):557-561.

[28] Abdullah Z, Hussain K, Zhari I, Rasadah MA, Mazura MP, Fadzelly AB, et al. Anti-inflammatory activity of standardized extracts of leaves of three varieties of *Ficus deltoidea*. Int J Pharm Clin Res. 2009;1(3):100-105.

[29] Choo CY, Sulong NY, Man F, Wong TW. Vitexin and isovitexin from the leaves of *Ficus deltoidea* with in-vivo α-glucosidase inhibition. J Ethnopharmacol. 2012;142(3):776-781.

[30] Cahyanto HA, Supriyatna N. Anti-diabetic activity of Tabat Barito leaves (*Ficus deltoidea*, Jack) extract in rats. Biopropal. 2013;4(1):1-6.

[31] Nurdiana S, Goh YM, Ahmad H, Dom SM, Azmi NS, Rajikin MH. Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with *Ficus deltoidea* and vitexin. BMC Complement Altern Med. 2017;17(1):290.

[32] Nurdiana S, Goh YM, Ahmad H, Dom SM, Azmi NS, Rajikin MH. Improvement of spatial learning and memory, cortical gyrification patterns and brain oxidative stress markers in diabetic rats treated with *Ficus deltoidea* leaf extract and vitexin. J Tradit Complement Med. 2018;8(2):190-202.

[33] Nurdiana S, Goh YM, Ahmad H, Dom SM, Azmi NS, Rajikin MH. *Ficus deltoidea* promotes bone formation in streptozotocin-induced diabetic rats. Pharm Biol. 2017;55(1):114-123.

[34] Suhaimi NA, Hashim N, Nurdiana S. *Ficus deltoidea* ethanolic leaves extract improves hormonal balance among letrozole-induced polycystic ovarian syndrome (PCOS) rats. Malays Appl Biol. 2018;47(1):137-144.

[35] Haslan MA, Nurdiana S, Hashim N, Zin NSNM, Shirazi FH. *Ficus deltoidea* ameliorates biochemical, hormonal, and histomorphometric changes in letrozole-induced polycystic ovarian syndrome rats. BMC Complement Med Ther. 2020;20(1):1-12.

[36] Nurdiana S, Azizan MI, Abdullah Z, Mohd Luqman Hakim MN. Effect of *Ficus deltoidea* leaves extracts on blood clotting, sperm quality and testosterone level in alloxan-induced male diabetic rats. Int J Pharm Sci Rev Res. 2018;50(1):1-7.

[37] Elkarim AS, Ahmed AH, Elgamal AM, Haggar RE, Khedr A, Shalaby MF, et al. Evidence-based evaluation of pharmacological activity and herb-herb interaction of organic extracts of certain natural anti-diabetic mixtures. Pharmacogn J. 2021;13(6):1366-1376.

[38] Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS, et al. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia. 2008;79(7-8):557-561.