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Research Article

Phytochemical profiling and *in vitro* pharmacological evaluation of aerial parts of *Ocimum basilicum*

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Abstract
Ocimum basilicum is a potential medicinal plant which possess distinct herbal properties with diverse phytochemical composition and *in vitro* biological activities. In the present study leaves, stems, and seeds of *O. basilicum* were collected, dried, powdered, and extracted using the Soxhlet method in methanol (15 cycles at 40°C) and stored after the lypholization. the extract were subjected to phytochemical screening along with DPPH (2,2-diphenyl-1-picrylhydrazyl), radical scavenging, Protein denaturation assay and α-Amylase inhibition for comparative assessment of biological potential of prepared extracts. Methanolic extracts from leaves, stems, and seeds observed with the phytochemical compounds including alpha-amyrin, tau-cadinol, and gamma-sitosterol. DPPH radical scavenging activity found to be highest in seed extract (63.65 ± 0.66%) followed by Butylated Hydroxy Toluene (BHT). Protein denaturation assay of leaf extract depicted superior inhibition of the protein (68.32 ± 1.41%) compared to seed and stem. α-Amylase inhibition assay also resulted in higher activity in seed extract compared to leaves and stem, however the activity of all three extract was comparable to acarbose. One-way ANOVA confirmed significant concentration-dependent effects across all assays ($p < 0.05$). The findings of the study, i.e. biological activities of the leaves, seeds, and stems of *O. basilicum*, which supports further exploration and utilization of the plant in healthcare and allied industries.

Keywords: *Ocimum basilicum,* Plant extract, Phytocompounds, α-amylase inhibition, Protein denaturation activity

INTRODUCTION

Medicinal plants are valued globally for their therapeutic potential, containing potential photochemical compounds including terpenoids, phenolics and flavonoids that exhibit diverse pharmacological properties (Rawat *et al*. 2016). This rich diversity of secondary metabolites imparts the characteristic medicinal potential to the plant (Bensid *et al*. 2022). *Ocimum basilicum,* is a key species within the diverse *Ocimum* genus (family *Lamiaceae)*, native to Asia, Africa, and the Americas, valued for its varied morphology, chemical composition, and culinary significance in Iranian, Italian, Chinese, and Indian cuisines. It is popular under local names such as *Marua-Phool* and *Ram-Tulsi* in India and Nepal (Bilal *et al*. 2012). Its dominant volatile compounds, such as linalool, eugenol, methyl chavicol, bergamotene, methyl cinnamate, contribute to characteristic aroma and therapeutic potential (Shahrajabian *et al*. 2020). The plant possesses several medicinal properties, such as anti-anxiety, anticonvulsant, antidiabetic, anti-gout, antimutagenic, antiulcer, antibacterial, antifungal, antiprotozo-

al, and insecticidal (Ahmed *and* Aujla 2012; Nadeem *et al*. 2022). Essential oil obtained from *O. basilicum* is also of medicinal significance with antiseptic, antibacterial, antiviral, and antifungal properties, along with utilization for stress relief. In addition, the plant is used to treat food poisoning, staph and tetanus infections, typhoid, malaria, flu, common cold, mumps, and measles (Marwat *et al*. 2011; Sekar *et al*. 2009; Campinho *et al*. 2023). *O. basilicum* possess medicinal propertiesdue to the presence of its rich phytochemical composition, comprising of diverse type of secondary metabolites. Eugenol, citral, geranyl acetate, cadinene, ocimene, linalyl acetate, carvacrol, terpineol, linalool, other terpenes represent major active constituents of oil of *O. basilicum*. These compounds act through different mechanisms, targeting various pathways to exert their medicinal effect (Salehi *et al*. 2020). In addition, *O. basilicum* is a popular ingredient in several traditional homemade recipes, again owing to its medicinal properties and flavour. The present study investigate the comparative the phytochemical profiling of the extracts and biological activities of methanolic extracts of aerial parts of *O. basilicum.*

MATERIALS AND METHODS

Plant material

The plant of *O. basilicum* was identified at the Patanjali Research Foundation Herbarium (PRFH), accession number *PRFH-25199.* The leaves, stems, and seeds of *O. basilicum* were collected from the plants maintained in the herbal garden of Uttaranchal University, Dehradun, India.

Preparation of extracts

Plant parts were washed, dried, ground to obtain a fine powder. In separate Round bottom flask (RBF), 100 gm of powdered leaves, stem and seeds were dipped in 250 ml methanol and left overnight. The next day the Soxhlet method was utilized for extraction with 15 cycles at 40°C. Lyophilized extracts for further studies stored at 4°C

Phytocompound profile by Gas chromatographic - mass spectroscopy (GC-MS) analysis

Identification of different types of phytocompounds and secondary metabolites were conducted by gas chromatography-mass spectrometry analysis. The methodology reported by Raghav *et al*. (2022) was adopted for GC-MS analysis. The GC-MS analysis was conducted using an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass selective detector, with separation on an HP-5MS Ultra Inert capillary column, helium carrier gas at 1 mL/min with electron ionization (70 eV) at source (230 °C) and quadrupole (150 °C) temperatures. These additions provide full details for reproducibility while maintaining consistency with the referenced method.

Pharmacological evaluations

DPPH(2,2-diphenyl-1-picrylhydrazyl)- radical scavenging activity

The antioxidant potential of the plant extracts were analyzed through DPPH radical-scavenging assay (William *et al*. 1995). Varying concentrations (200, 400, 600, and 800 µg/mL) of test samples were utilized to assess DPPH activity. The scavenging percentage was calculated using Equation 1 after measuring the absorbance at 517 nm. A methanol control was included in the assay

DPPH radical scavenging percentage = Control (A_0) - Sample (A_1) /Control (A_0) X 100 (1)

Protein denaturation assay

The denaturation potential of protein (albumin) in leaf, stem, and seed extracts was analysed using the protocol reported by Bailey-Shaw *et al*. (2017). Plant extracts and standard salicylic acid were prepared at varying concentrations of 200, 400, 600, and 800 µg/mL. A mixture of each consisting of 200 µl bovine albumin serum (1 mg/ml), 1.4 ml of PBS, and 1 ml extract was prepared, incubated for 15 min at 37°C and then heated for 5 min at 70°C. Sample mixture was cooled, observed the absorbance at 660 nm and calculated by Equation (2). A methanol control was included in the assay.

Protein denaturation percentage = Control (A_0) - Sample (A_1) /Control (A_0) X 100 (2)

α-Amylase inhibition assay

Antidiabetic potential of leaf, stem, and seed extracts of *O. basilicum* were analyzed through α-amylase inhibition assay (Kwon *et al*. 2008). Varying concentration of extracts 200- 800 µg/mL, and acarbose (standard drug), were initially treated with 100 µL α-amylase and 200 µL sodium phosphate buffer. After 20 minutes of incubation, 100 µl of starch solution and 200 µl of sodium phosphate buffer were added. 1 ml of DNS reagent was added to the solution after 20 mins of incubation, followed by boiling the sample mixture for 5 min. Reaction solutions were finally cooled and absorbance measured at 540 nm and calculated by Equation (3). A methanol control was included in the assay.

α-amylase inhibition percentage = Control (A_0) - Sample (A_1) /Control (A_0) X 100 (3)

Statistical evaluation

Triplicate experiments were carried out and the data are presented as mean ± standard deviation (SD). Nonlinear regression analysis were conducted to determine IC50 values using a four-parameter logistic dose-response inhibition model in OriginPro software (2021b

version, OriginLab Corporation, USA). Concentration-dependent effects for each sample were evaluated using One-way ANOVA. One-way ANOVA was performed by Tukey's significant difference (HSD) post-hoc test for pairwise comparisons to compare activities between extracts and standards. This statistical approach was employed in phytochemical and pharmacological studies for evaluating dose-response parameters such as percentage inhibition at fixed concentrations and IC□□ values across plant-derived extracts and positive controls (Ojo *et al*., 2023) Statistical significance was set at $p < 0.05$. All analyses were conducted using OriginPro and Python (with stats models for Tukey's HSD). Detailed post-hoc results for comparisons at the highest concentration (800 µg/mL) are provided in Supplementary Table S1.

RESULTS AND DISSCUSION

Phytochemical screening of the extracts through GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of methanolic extracts from *O. basilicum* leaves, seeds, and stems revealed distinct phytochemical profiles, with different distributions of compound classes across the plant parts (Figs. 1, 2, and 3). Methanolic extracts from leaves, stems, and seeds revealed 62, 83, and 62 phytocompounds, respectively are provided in Supplementary Tables S2, S3 and S4. Monoterpenoids were the most abundant at 36.5%, including compounds like linalool and methyl chavicol, followed by sterols (20.0%), aliphatic amines (10.4%), and phenolic alcohols (4.2%) in the leaf extracts (Fig. 1). Seed extracts showed significant levels of aliphatic alcohols (21.4%), sterols (6.7%), and aliphatic amines (8.0%), with heterocyclic amines present at 3.9% (Fig. 2). Monoterpenoids and terpenoid alcohols were absent in seeds. Stem extracts constitute monoterpenoids (21.4%), aliphatic alcohols (13.7%), terpenoid alcohols (10.7%), and heterocyclic amines (6.7%) (Fig. 3). Specific compounds identified include linalool and methyl chavicol in leaves and stems, contributing to their high monoterpenoid content. The phytochemical profiling of *O. basilicum* leaves, seeds, and stems extracts by GC-MS analysis reveals distinct pharmacological significance and therapeutic potential. On the basis of the nature of bioactive potential of the diverse compounds identified in this spectrophotometer analysis of the seed, stem and leaves extract are depicted in Tables 1, 2, and 3 respectively. These compounds are synthesized in glandular trichomes and exhibit antimicrobial and antioxidant properties with reported in vitro activities (Koziol *et al*. 2014; Hallahan 2000). Recently reported studies from the literature review confirm that linalool is a major monoterpenoid in basil leaves and possesses radical-scavenging activity by donating H atom to free radicals to prevent oxidative chain reactions (Dhama *et al*., 2023). The screening of the extracts in the present work also identified sterols in leaves (20.0%) and seeds (6.7%), including stigmasterol which can enhance membrane stability and photosynthetic efficiency and exhibit anti-inflammatory and antidiabetic effects (Du *et al*., 2022). Stigmasterol is responsible for properties including modulation of inflammatory pathways through inhibition of NF-κB activation, reducing cytokine production, and exhibiting hypoglycemic effects (Bakrim *et al*., 2022). Similarly, presence of phenolic alcohols in leaves (4.2%) (Table 1) revealed in GC-MS analysis is supported by literature to be associated with antioxidant, anti-inflammatory properties (Banik *et al*., 2020). Terpenoid alcohols were present in stem extract (10.7%) (Table 2) associated with antimicrobial potential under stress (Guimarães *et al.,* 2019). Compounds such as γ-Sitosterol (9.5%) are present in higher amounts in seeds than in leaves (Table 3) has been reported with cancer cell proliferation inhibition, restrict the cell cycle, and induce apoptosis (Shah *et al.,* 2018; Evangelina *et al.,* 2021). Stigmasterol exhibits various medicinal activities, including anti-cancer, anti-diabetic, anti-inflammatory, immune modulation potential, anti-parasitic, anti-microbial, anti-oxidant, and neuroprotective activities (Batista *et al.,* 2023). Some compounds like Estragole, found to be highly abundant in the stem extract has also been reported to mediate several biological activities such as anti-microbial, anti-edematogenic, anti-inflammatory activity (Rodrigues *et al.,* 2016; Rodrigues *et al.,* 2017). Other compounds include Benzoic acid, 2-hydroxy, and α-amyrin, found to be present in the leaf extract of the present work. α-amyrin possess significant anti-inflammatory and anti-oxidant activities (Rodrigues *et al.,* 2017; Pal *and* Lal 2023). Stigmasterol present in high amounts in seed extract and in trace amounts in leaves, is reported to help lower cholesterol levels and is also a potential anticancer compound (Batta *et al.,* 2006; Mostafa and Essawy, 2019).

Pharmacological Evaluation DPPH (2,2-diphenyl-1-picrylhydrazyl) radical Scavenging activity

DPPH radical scavenging activity of leaf, seed, and stem extract compared to BHT increased in a dose-dependent manner from 200 to 800 µg/mL. BHT (Butylated Hydroxy Toluene) consistently exhibited the highest percentage inhibition (66.60 ± 0.85% at 800 µg/mL) (Fig. 4). The seed extract has exhibited greater radical scavenging percentage at higher concentrations seed (up to 63.65% at 800 µg/mL), closely followed by leaf extract (61.78 ± 0.72%), While the stem showed the lowest activity (58.56 ± 0.60%). The IC□□ values, calculated via non-linear regression (four-parameter logistic model), indicated BHT with greater value

Table 1. Showing phytocompounds identified in present study from the GC-MS analysis of methanolic extract of seed of *Ocimum basilicum* and their therapeutic properties as discussed in previous studies

GC-MS analysis of seed (Present study)						Therapeutic uses reported earlier	References
Sl. No.	Retention time	Area%	Name	Molecular formula	GC-MS Analysis of seed		
1	14.264	2.04	τ-Cadinol	$C_{15}H_{26}O$	2.04	Anti-inflammatory; antioxidant; antimicrobial	(Kilonzo *et al*. 2019)
2	17.577	2.9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.9	Anti-inflammatory; antimicrobial	(Umoh *et al*. 2024)
3	30.24	3.12	β-Amyrin	$C_{30}H_{50}O$	3.12	Anti-inflammatory; potential antidiabetic	(Viet *and* La Hoang, 2025)
4	28.475	3.44	Ergost-5-en-3-ol, (3β,24R)-	$C_{28}H_{48}O$	3.44	Anti-inflammatory; antidiabetic	(Yusnaini and Ikhsan 2023)
5	8.303	3.46	Estragole	$C_{10}H_{12}O$	3.46	Anti-inflammatory; antioxidant; antimicrobial	
6	31.488	3.68	γ-Sitostenone	$C_{29}H_{48}O$	3.68	Potential anti-inflammatory	
7	8.845	4.74	2-Furancarboxaldehyde, 5-(hydroxymethyl)	$C_6H_6O_3$	4.74	Anti-oxidant; anti-inflammatory	(Madouh, and Davidson 2024)
8	30.946	5.41	α-Amyrin	$C_{30}H_{50}O$	5.41	Anti-inflammatory; anti-diabetic	(Viet and La Hoang Anh 2025)
9	7.543	5.63	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	5.63	Antioxidant; free radical scavenging	(Boora *et al*. 2023)
10	28.779	7.72	Stigmasterol	$C_{29}H_{48}O$	7.72	Anti-inflammatory; anti-diabetic; anti-oxidant	(Yusnaini and Ikhsan 2023)
11	29.563	9.5	γ-Sitosterol	$C_{29}H_{50}O$	9.5	Anti-diabetic; anti-inflammatory	

(120.23 µg/mL), followed by seed extract (391.74 µg/mL). Statistical comparisons using one-way ANOVA and Tukey's post hoc test showed significant differences ($p < 0.05$). Detailed fitting parameters are provided in Supplementary Table S5 and Fig. S1. Gülçin *et al*. (2007) reported significant DPPH radical scavenging (50-60%) in water and ethanolic extracts of the basil leaves. Jayasinghe *et al*. (2003) also observes a high DPPH inhibition which correlates with the greater phenolic content. Aglycones in ethanolic extracts were generally responsible in enhancing antioxidant properties (Politeo *et al.,* 2007). The presence of high levels of aliphatic alcohols and sterols in seeds which act as hydrogen donors (Felhi *et al.,* 2017). Comparative studies on basil species confirm moderate antioxidant effects in linalool-rich leaf extracts (Qamar *et al.,* 2023). Whereas radical scavenging activity of the stem extract may be aligns with reduced phenolic content and a dominance of aliphatic amines and terpenoid alcohols (Kwee and Niemeyer, 2011; Kaurinovic *et al.,*, 2011). Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one is one of the compounds found at the highest levels in stem extracts and has potential antioxidant effects (Pahlavan and Mohammadi 2017).

Protein denaturation activity

The protein denaturation inhibition activity of leaf, seed, and stem extracts from *O. basilicum,* compared to salicylic acid, increased dose-dependently from 200 to 800 µg/mL (Fig. 5). Salicylic acid showed the highest inhibition at higher concentrations (75.96 ± 0.87%). The leaf extract exhibited the strongest anti-inflammatory potential, particularly at lower concentrations, whereas the seed extract possessed the weakest inhibition percentage at higher doses. The IC_{50} values from non-linear regression shows seed extract with low IC_{50} value (102.89 µg/mL), than salicylic acid (149.55 µg/mL). Differences were significant (one-way ANOVA with Tukey's test, $p < 0.05$). The control sample exhibited negligible activity (<5% inhibition/scavenging) confirming that the observed effects were due to the plant extracts and not the solvent. See Supplementary Table S5 for

Table 2. Showing phytocompounds identified in present study from the GC-MS analysis of methanolic extract of stem of *Ocimum basilicum* and their therapeutic properties as discussed in previous studies

GC-MS analysis of stem (Present study)							
Sl. No.	**Retention time**	**Area%**	**Name**	**Molecular formula**	**GC-MS Analysis of stem**	**Therapeutic uses reported earlier**	**References**
1	30.24	0.62	β-Amyrin	$C_{30}H_{50}O$	0.62	Anti-inflammatory; potential antidiabetic	(Viet and La Hoang Anh 2025)
2	30.949	0.69	α-Amyrin		0.69	Anti-inflammatory; antidiabetic	
3	6.744	0.82	Linalool	$C_{10}H_{18}O$	0.82	Anti-inflammatory; antioxidant; anxiolytic	(Al-Harrasi *et al*. 2022)
4	28.769	1.42	Stigmasta-5,23-dien-3-ol, (3β)-	$C_{29}H_{48}O$	1.42	Anti-inflammatory; antidiabetic	(Yusnaini and Ikhsan 2023)
5	17.596	5.61	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	5.61	Anti-inflammatory; antimicrobial	(Umoh *et al*. 2024)
6	29.563	4.89	γ-Sitosterol	$C_{29}H_{50}O$	4.89	Anti-diabetic; anti-inflammatory	(Yusnaini and Ikhsan 2023)
7	7.584	9.44	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	9.44	Antioxidant; free radical scavenging	(Boora *et al*. 2023)
8	14.276	9.8	τ-Cadinol	$C_{15}H_{26}O$	9.8	Anti-inflammatory; antioxidant; antimicrobial	(Kilonzo *et al*. 2019)
9	8.32	18.19	Estragole	$C_{10}H_{12}O$	18.19	Anti-inflammatory; antioxidant; antimicrobial	(Yusnaini and Ikhsan 2023)

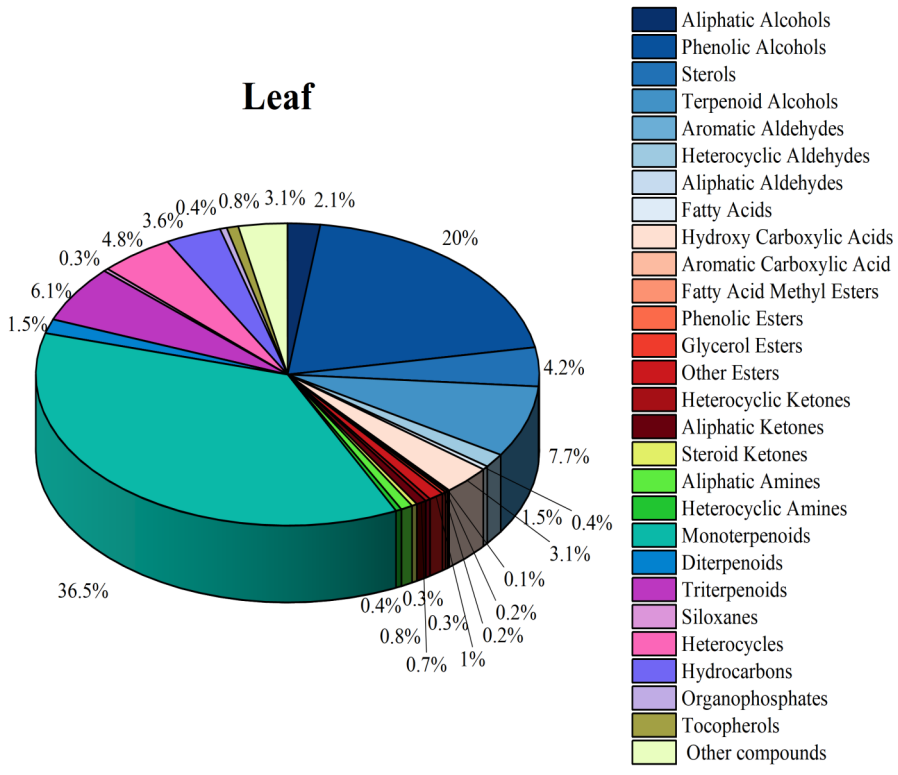

Fig. 1. *GC-MS phytochemical profile of Ocimum basilicum leaf extract*

Table 3. Showing phytocompounds identified in present study from the GC-MS analysis of methanolic extract of leaf of *O. basilicum* and their therapeutic properties as discussed in previous studies

GC-MS analysis of leaf (Present study)						Therapeutic uses reported earlier	References
Sl. No.	Retention time	Area%	Name	Molecular formula	GC-MS Analysis of leaf		
1	18.968	2.6	2-Hexadecen-1-ol, 3,7,11,15-	$C_{20}H_{40}O$	2.6	Anti-inflammatory, antioxidant, antimicrobial	(Thejashree and Naika
2	17.577	2.9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.9	Anti-inflammatory, antioxidant	(Umoh *et al*. 2024)
3	30.24	3.12	β-Amyrin	$C_{30}H_{50}O$	3.12	Anti-inflammatory, antioxidant	(Viet and La Hoang Anh 2025)
4	28.475	3.44	Ergost-5-en-3-ol, (3β,24R)-	$C_{29}H_{48}O$	3.44	Anti-inflammatory, antidiabetic, antioxidant, cholesterol-lowering, anticancer	(Joshi *et al*. 2023)
5	8.303	3.46	Estragole	$C_{10}H_{12}O$	3.46	Anti-inflammatory, antioxidant, antimicrobial	(Mahendra *et al*. 2023)
6	12.059	3.67	2,4-Cresotaldehyde	$C_8H_8O_2$	3.67	Antibacterial, antioxidant	(Hebballi *et al*. 2025)
7	31.488	3.68	γ-Sitostenone	$C_{29}H_{48}O$	3.68	Antioxidant activity	(Chouni *et al*. 2021)
8	8.845	4.74	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	4.74	Antioxidant	(Amrita *et al*. 2025)
9	30.946	5.41	α-Amyrin	$C_{30}H_{50}O$	5.41	Anti-inflammatory, antioxidant	(Viet and La Hoang Anh 2025)
10	7.543	5.63	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	5.63	Antioxidant; free radical scavenging	(Boora *et al*. 2023)
11	28.779	7.72	Stigmasterol	$C_{29}H_{48}O$	7.72	Anti-inflammatory, antioxidant, cholesterol-lowering, anticancer	(Li *et al*. 2025)
12	29.563	9.5	γ-Sitosterol	$C_{29}H_{50}O$	9.5	Antidiabetic, anti-inflammatory, antioxidant, cholesterol-lowering, anticancer	(Vasudevan *et al*. 2025)

parameters. The data show significant differences in anti-inflammatory effects across plant parts and standard (Fig. S2). GC-MS analysis also showed that the plant's leaf extracts contain anti-inflammatory agents, including rosmarinic acid, caryophyllene oxide, neophytadiene, and 2-pentadecanone (Kamelnia *et al.,* 2023). The high sterol content, including stigmasterol and γ-sitosterol, which stabilise protein structures by interacting with hydrophobic regions and preventing heat-induced unfolding (Febrina *et al*., 2021).Stem extracts, dominated by terpenoid alcohols such as taucadinol, exhibited moderate activity (67.95 ± 0.76% at 800 µg/mL), consistent with their role in stress defence (Guimarães *et al*., 2019). The present findings extend this by highlighting seed superiority, possibly due to synergistic effects of α- and β-amyrin, known to suppress TNF-α and IL-6 in inflammation models (Rodrigues *et al*., 2017). When comparing the present results with those reported by Okoye *et al*. (2014), we found that hexane leaf extracts inhibited oedema in mice. Aye *et al*. (2019) also discussed that ethanolic leaf extracts reduce pro-inflammatory cytokines in RAW cells, which supports our in vitro results. 2,4-Phytol acetate was found to be present in trace amounts in stem and leaf extracts and has been reported to be associated with anti-inflammatory effects, involving suppression of prostaglandin E2 production and nitric oxide synthase expression, as observed in polysaccharide-stimulated macrophages (Lee *et al*., 2017).

α-Amylase inhibition activity

The α-amylase inhibition activity of leaf, seed, and stem extracts from *O. basilicum*, compared to acarbose, increased in a dose-dependent manner from 200 to 800

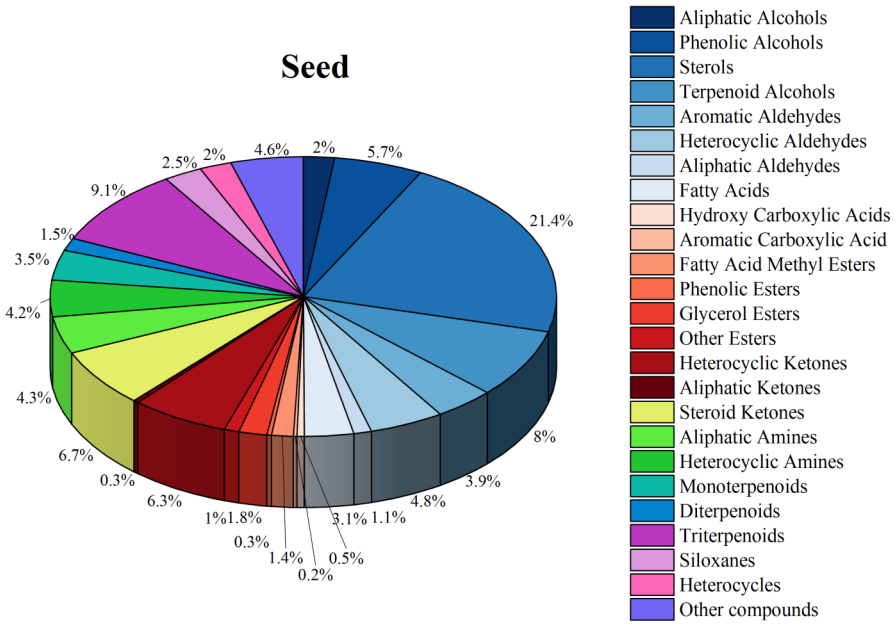

Fig. 2. *GC-MS analysis of Ocimum basilicum seed extract*

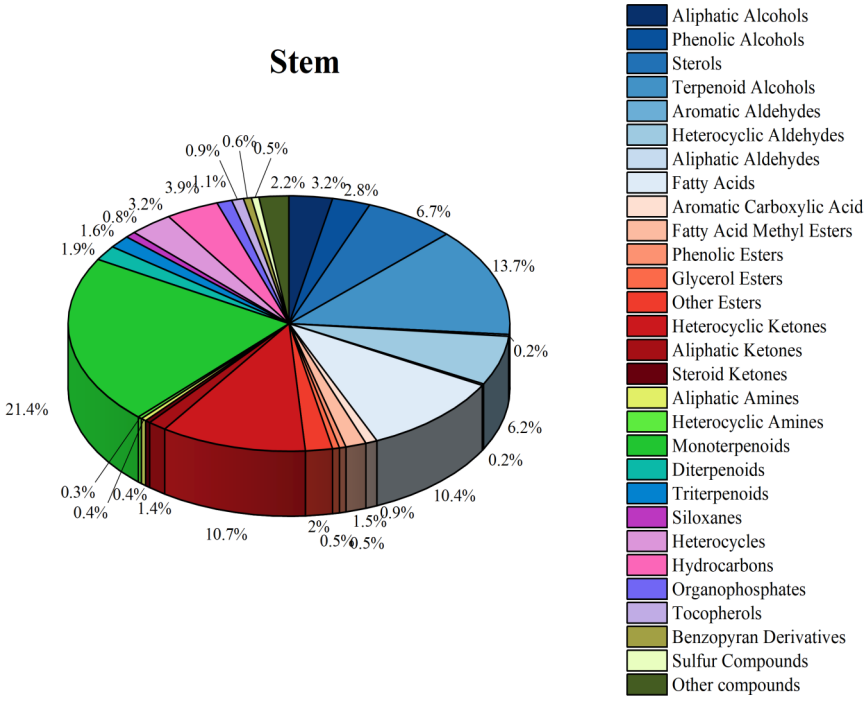

Fig. 3. *GC-MS phytochemical composition of Ocimum basilicum stem extract*

µg/mL, with no single standard consistently leading across all doses (Fig. 6). The seed extract demonstrated the strongest antidiabetic potential (57.18 ± 0.70%), followed by the stem and leaf, which showed variable but generally lower activity among plant parts. Non-linear regression yielded lowest IC_{50} values of 569.71 µg/mL for seed extract than of 703.13 µg/mL for acarbose. Significant differences were observed (one-way ANOVA, Tukey's post-hoc, $p < 0.05$). Parameters are detailed in Supplementary Table S5, Fig. S1. Phyto-compounds with reported antidiabetic activity, such as alpha and beta amyrin, rosmarinic acid, apigenin, ferulic, catechin, were also found to be present in *O. basilicum* (Dini and Laneri 2021). This inhibition was dose-dependent, peaking at 57.18 ± 0.70% at 800 µg/mL for seed, likely due to the presence of the sterols and aliphatic alcohols that competitively bind the enzyme's active site, thereby reducing starch hydrolysis (He *et al.,* 2018). Leaf extracts, rich in monoterpenoids and phenolics such as benzoic acid derivatives and apig-

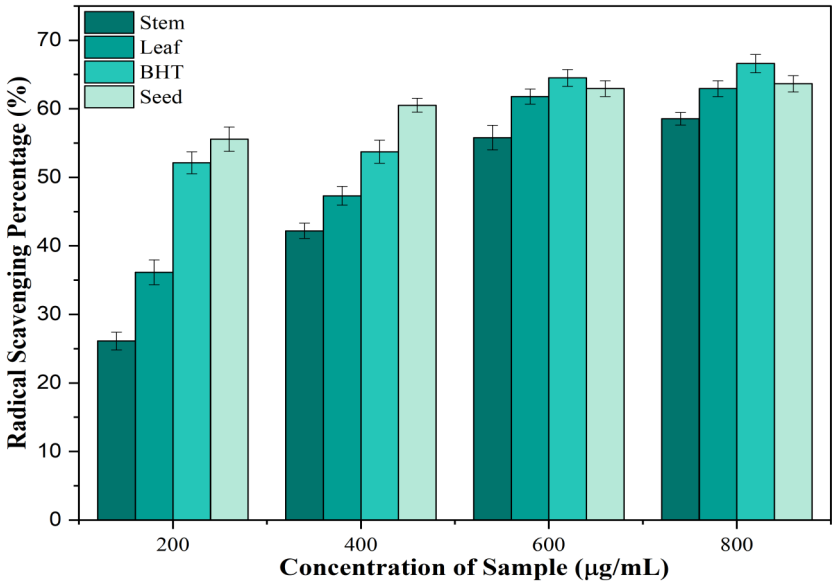

Fig. 4. *DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of stem, leaf and seed extracts of Ocimum basilicum and standard compound BHT (Butylated hydroxytoluene)p value < 0.05*

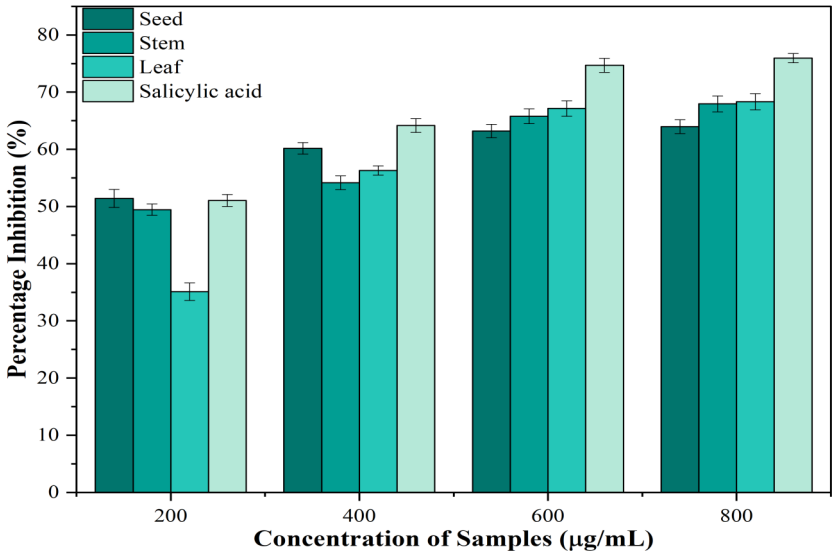

Fig. 5. *Protein denaturation activity of stem, leaf and seed extracts of Ocimum basilicum and standard compound salicylic acid p value < 0.05*

enin helps in modulating carbohydrate metabolism (Dini and Laneri, 2021). Recent studies reported that basil leaf extracts inhibit α-amylase by 60-70% through hydrogen bonding of rosmarinic acid to catalytic residues (Elgindi *et al*., 2021). Ezeani *et al*. (2020) reported 50% inhibition with aqueous basil extracts and the methanolic seed extract, suggesting solvent-specific enhancement. Leaves play a significant role in photosynthesis and mono terpenoids help them in defence; seeds serve as storage organs with sterols and alcohols; and stems provide structural support with terpenoids (Mabou and Yossa 2021). Gamma-sitosterol present in the extracts of *O. basilicum* possess antidiabetic potential by delaying carbohydrate absorption, and improving glucose tolerance (Su *et al*., 2023).

One-way analysis of variance analysis (ANOVA)

Concentration-dependent effects were evaluated separately for each extract and standard using ANOVA on concentration (200, 400, 600, and 800 µg/mL) as the independent factor and percentage inhibition/ scavenging as the dependent variable has illustrated in Table 4. In the DPPH radical scavenging assay, all components exhibited significant differences across concentrations (Stem: $p = 1.49*10^{-13}$, Leaf: $p = 0.03272$, BHT: $p = 6.96*10^{-11}$, Seed: $p = 4.06*10^{-06}$). Activity by leaf parts had the highest p-value, indicating a relatively weaker concentration effect than for stem, seed, and BHT. Similarly, the albumin inhibition assay revealed significant differences for all components (Seed: $p = 6.32*10^{-12}$, Stem: $p = 5.43*10^{-11}$, Leaf: p =

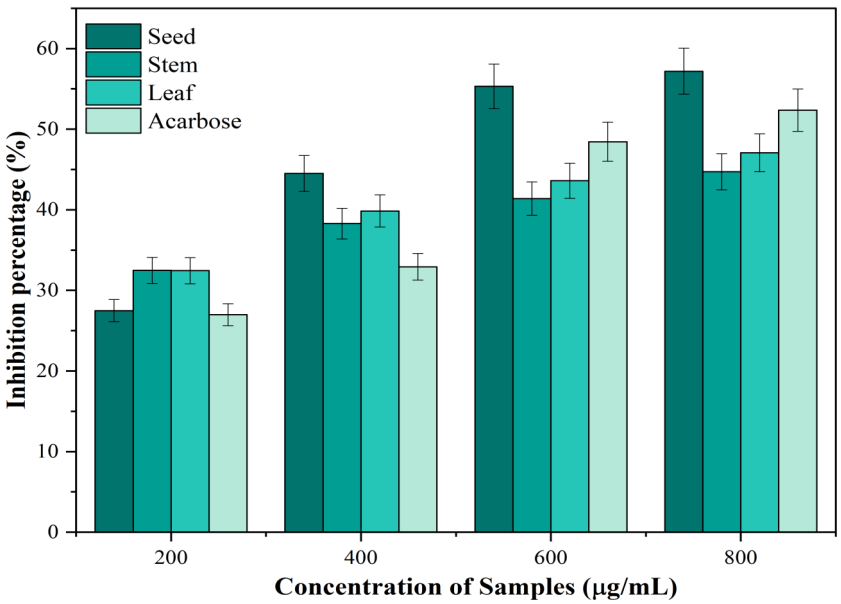

Fig. 6. *α- amylase inhibitory activity of stem, leaf and seed extracts of Ocimum basilicum and standard compound Acarbose p value < 0.05*

Table 4. One-way ANOVA analysis table for concentration-dependent effects in DPPH radical scavenging, albumin inhibition, and α-amylase inhibition assays

Bioactivities	Part	DF	Sum of squares	Mean square	F value	Prob > F
DPPH scavenging assay	Stem	3	3671.31083	1223.77028	5369.17966	1.49E-13
	Leaf	3	529.1358	176.3786	4.86473	0.03272
	BHT	3	488.67503	162.89168	1153.2154	6.96E-11
	Seed	3	48.81863	16.27288	71.48199	4.06E-06
Protein denaturation assay	Seed	3	3076.54763	1025.51588	2103.62231	6.32E-12
	Stem	3	635.11103	211.70368	1227.26768	5.43E-11
	Leaf	3	16.662	5.554	18.82712	5.33E-04
	Salicylic Acid	3	6.8987	2.2998	20.43467	4.16E-04
α-amylase inhibition assay	Seed	3	3888.4583	1296.4861	4312.21536	3.59E-13
	Stem	3	2088.0688	696.0229	1489.4422	5.21E-12
	Leaf	3	197.27483	65.75828	783.51841	3.30E-14
	Acarbose	3	1687.6187	562.5396	4390.45354	3.44E-13

$5.33*10^{-04}$, Salicylic acid: $p = 4.16*10^{-04}$), though Leaf and Salicylic acid displayed higher p-values, suggesting greater variability or smaller effect sizes at different concentrations. The alpha-amylase inhibition assay showed the most pronounced concentration effects, with extremely low p-values (Seed: $p = 3.59*10^{-13}$, Stem: $p = 5.21*10^{-12}$, Leaf: $p = 3.30*10^{-14}$, Acarbose: $p = 3.44*10^{-13}$), particularly for Leaf, indicating strong concentration-dependent inhibition. These findings underscore those increasing concentrations significantly enhanced bioactivity, with Stem, Seed, BHT, and Acarbose consistently showing differences ($p < 10*10^{-10}$) across assays. At the same time, Leaf exhibits more variable responses in DPPH radical scavenging assay and protein denaturation assays. The results suggest that plant parts, particularly the Seed and Stem, and the standard Acarbose possess significant potential for applications requiring potent antioxidant and enzyme inhibitory activities, with optimal efficacy at higher concentrations. The comparative GC-MS analysis of *O. basilicum* revealed distinct phytochemical profiles across plant parts: leaves were dominated by mono-

terpenoids such as linalool and estragole, seeds showed the highest abundance of sterols (including γ-sitosterol and stigmasterol) and aliphatic alcohols, while stems were richer in terpenoid alcohols and estragole. On the basis of the present study, seed extracts emerged as the most pharmacologically significant, exhibiting the strongest antioxidant (lowest IC□□ in DPPH assay), and α-amylase inhibitory activities with constitutes to high abundance of sterol and aliphatic alcohol content. The leaf extract exhibit high anti-inflammatory (lowest IC_{50} in protein denaturation activity rich in monoterpenoids, These findings suggest that seeds represent the most promising part of *O. basilicum* for developing natural therapeutic agents targeting oxidative stress, inflammation, and hyperglycemia.

Conclusion

The methanolic extracts of *O. basilicum* exhibit distinct phytochemical profiles across the leaves, seeds, and stems as revealed by GC-MS analysis. Leaves were predominantly rich in mono-terpenoids such as linalool and estragole, which possess high anti-inflammatory potential. Seed extract contains characteristic sterols, including stigmasterol and γ-sitosterol, along with abundant aliphatic alcohols, while stems extracts were particularly enriched with estragole and terpenoid alcohols compared to other plant parts. Seeds exhibited the strongest antioxidant activity, with the lowest IC□□ of 391.74 µg/mL in the DPPH assay, compared to the leaf and stem extracts. Leaf extracts exhibit superior anti-inflammatory potential in the protein denaturation assay, with an IC□□ of 102.89 µg/mL. In both anti-oxidant and anti-inflammatory activities, the reference standards BHT and salicylic acid exhibit greater activity, respectively. But in the case of anti-diabetic assay, the seed extract exhibits the highest α-amylase inhibitory activity, reaching 57.18% inhibition at 800 µg/mL, indicating greater anti-diabetic potential than that of acarbose. These enhanced bioactivity of seeds are primarily attributed to their high sterol and aliphatic alcohol content, which highlights significant pharmacological effects. The findings of the present study clearly depict the *O. basilicum* to be a natural source of bioactive compounds presence in different plant parts with profound biological activity which support further exploration of the plant to be utilized for diverse applications in healthcare, food and allied industries.

Supplementary information

The author(s) are responsible for the content or functionality of any supplementary information. Any queries regarding the same should be directed to the corresponding author. The supplementary information is available for download from the article's webpage and will not be included in the print copy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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