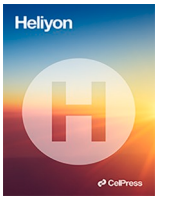
Heliyon 9 (2023) e14644

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Phytochemical characterization, antioxidant activity and antihypertensive evaluation of *Ocimum basilicum* L. in L-NAME induced hypertensive rats and its correlation analysis

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ARTICLE INFO

Keywords:
Ocimum basilicum, GC-MS
ESI-HRMS/MS
Antioxidant
Oxidative hemolysis inhibition assay
Antihypertensive activity
Correlation studies

ABSTRACT

Ocimum basilicum Linn. (basil) is an aromatic culinary herb that has shown a great potential in therapeutic world. It has many promising pharmacological activities that make it centre for investigations for many researchers. Current study has been planned to determine chemical constituents of basil leaves extracts and their *in-vitro* and *ex-vivo* antioxidant and *in-vivo* antihypertensive potential. GC-MS studies of non-polar extracts showed presence of 75 compounds including monoterpenes, hydrocarbons, sesquiterpenes, triterpenes, phyto-sterols and phthalates. Higher percentages of fatty acids were also identified. The major compounds include linalool (7.65%), terpineol (1.42%), *tau*-cadinol (13.55%), methyl palmitate (14.24%), palmitic acid (14.31%), linolenic acid (1.30%) and methyl linolenate (17.72%). Electron spray ionization mass spectrometry ESI-HRMS/MS of the polar extracts revealed the presence of alkaloids, phenolic acid, amino acid, coumarin, lignin, flavanoid and terpene derivative. Total phenolic content and total flavonoid content were determined using spectrophotometric technique and calculated as gallic acid equivalents GAE/g dry weight and rutin equivalent RE/g of dry weight respectively. The highest phenolic content and flavonoid content were found in ethyl acetate extract 9.40 mg GAE/g and 15.9 mg RE/g of dry weight. All the extracts showed significant antioxidant activity in DPPH and ABTS cation decolorization assays. Dichloromethane extract possess the highest DPPH scavenging activity, i.e., 64.12% ± 0.23 at concentration of 4 mg/ml. Moreover in *ex-vivo* studies all the extracts showed prominent effect by inhibiting AAPS induce oxidation in Human erythrocytes being 69.24% ± 0.18 in dichloromethane extract, 64.44% ± 0.04 in ethyl acetate and 53.33% ± 0.09 in acetone extract. The methanol extract of *O. basilicum* exhibited significant decrease in systolic blood pressure in L-Name induced hypertensive rats at the dose of 50 mg/kg for 28 days. Total phenolic content had a higher linear correlation (r = 0.678) with antihypertensive activity, with a level of significance 95% showing that phenolic compounds in the leaves of the plant has important role in inhibiting L –NAME induced hypertension while flavonoid compounds may play a key role in the antioxidant activities of the plant, through synergism. Conclusively, *O. basilicum* leaves with bioactive metabolites are a potential source for the development of antihypertensive drugs.

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https://doi.org/10.1016/j.heliyon.2023.e14644
Received 13 December 2022; Received in revised form 6 March 2023; Accepted 14 March 2023
Available online 23 March 2023

1. Introduction

Spice plants are becoming more popular, with over 80% of the world's population still relying on traditional folk treatments for their primary healthcare needs. Natural plants are low in toxicity and high in phyto constituents, which are responsible for their holistic therapeutic properties [1]. Data is rich enough to justify medicinal herbs as an alternative to traditional and or synthetic drugs [2]. Many Lamiaceae plants have a long history of usage in cuisine as aromatic herbs or spices, as well as in folk medicine. Several of them belong to the *Ocimum* genus, which encompasses more than 30 different species and is collectively known as basil [3]. *Ocimum basilicum*, sometimes known as sweet basil or common basil, is one of the most significant and widely consumed species in the Mediterranean region.

The Latin word basilicum is originated from the greek word Basilikon, that means king, also called as "Herbe Royale" in French. Niazbo is an Urdu/Punjabi term that refers to its pleasant scent. In Persian and Arabic, it is known as reihan and rehan, respectively [4]. *Ocimum basilicum* L. is a well known herbal plant known for its medicinal and culinary use. It is widely cultivated in regions of Central and Southeast Asia such as Iran and Pakistan [5]. In Ayurvedic and Unani system of Medicine the use of sweet basil received a lot of attention for the treatment of numerous diseases [6]. Basil is a well-known source of flavoring components that is also used in traditional medicine [7]. It's popular in American and Mediterranean cuisines [8]. Basil oil is a major component in soaps and shampoos for its antiseptic effects, in lotions, oils and scented products due to its characteristics aroma [9]. Its oil is used in aromatherapy and can be used to alleviate stress, migraines, colds, and hay fever [10]. Basil tea can relief upset gastrointestinal tract such as stomach cramps, constipation, diarrhea, vomiting and improves digestion [11].

O. basilicum leaves and blooming parts have antispasmodic, carminative, digestive, galactogogue, stomachic and tonic qualities. Besides they've been used to treat acne, odour loss, insect stings, snake bites, and skin illnesses [12]. In addition, *O. basilicum* is also cultivated worldwide for its essential oil, with applications in medicine/pharmaceutical, perfumery, cosmetics and as flavoring agent. Secondary metabolites have been demonstrated to have a variety of biological effects, providing a scientific foundation for the use of herbs in traditional medicine in many ancient cultures. Its seeds are used as a source of dietary fibre in Asian drinks and desserts in traditional medicine. Infections of the skin, worms, diarrhea and headaches are also treated with it [10].

Ocimum has marvelous biological properties like anti-allergic, anti-angiogenic, anti-depressant, anti-inflammatory, anti-tumor and anti-microbial [13–15] and have long been known for their healthy effects and have been utilized in traditional folk medicine due to the presence of secondary metabolites such as tannins [16], phenols [17], flavonoids [18], anthocyanins [19,20] and steroids [12]. These metabolites protect against oxidative damage by quenching free radical reactions or absorbing reactive oxygen species (ROS) and preventing them from attacking other biomolecules [21]. These antioxidants can work in tandem with other antioxidants from both natural and synthetic sources. This synergism can increase antioxidant potential and lower high therapeutic doses of diverse medications, minimizing adverse effects from high doses, although the mechanism of the interaction needs to be further investigated [22].

As a result, plant-based natural medications provide excellent disease protection and are deemed safe to use because they include a variety of bioactive chemical ingredients with few or no adverse effects. These chemical elements not only defend against excessive ROS production, but they also boost the body's natural antioxidant defenses [23]. One of the primary variables in the beginning and progression of diseases like diabetes, hypertension, cancer, kidney failure and liver failure is reactive oxygen species (ROS). According to clinical investigations, ROS are essential mediators that have a negative impact on blood flow, arterial wall remodeling, endothelial vascularity and inflammation responses, which compromise significant tissue dysfunction and contribute to atherosclerosis, hypertension and congestive heart failure.

Elevated levels of ROS, impaired endogenous antioxidant system and impaired availability of an endothelium-derived relaxing factor nitric oxide (NO), play a key role in raising the primary blood pressure [24]. According to the Global Burden of Disease study, hypertension is the leading cause of compromised health in the world [25]. According to a report issued by the World Health Organization, the prevalence of hypertension is estimated to reach 1.5 billion people by 2025, with 7 million deaths [26]. Many treatment drugs and complementary therapies, as well as their mechanisms of action, have been explored in light of the global increase in hypertension patients. Antihypertensive medications are categorized as angiotensin-converting enzyme (ACE) inhibitors, beta-blockers and calcium channel blockers, diuretics, and adrenergic receptor antagonists based on these processes. Antihypertensive medications are also used to treat hypertension-related cardiovascular issues, but they have a lot of side effects. Upper respiratory tract obstruction and angioedema have been observed as side effect in children and adults who have used ACE inhibitors and calcium channel blockers. Antihypertensive drugs have also been shown to hasten the progression of malignancy in cancer patients. Alopecia, cough, flushing, headache, edema, and shortness of breath have all been recorded as side effects of these treatments [27]. As a result, a natural herbal medicine is preferred because it is thought to have less adverse effects [28].

Based on its multiple medicinal applications in traditional medicine, *O. basilicum* leaves could be a promising source for novel antihypertensive drugs. It's critical to verify herbalists' and native medical practitioners' assertions about the leaves' potency.

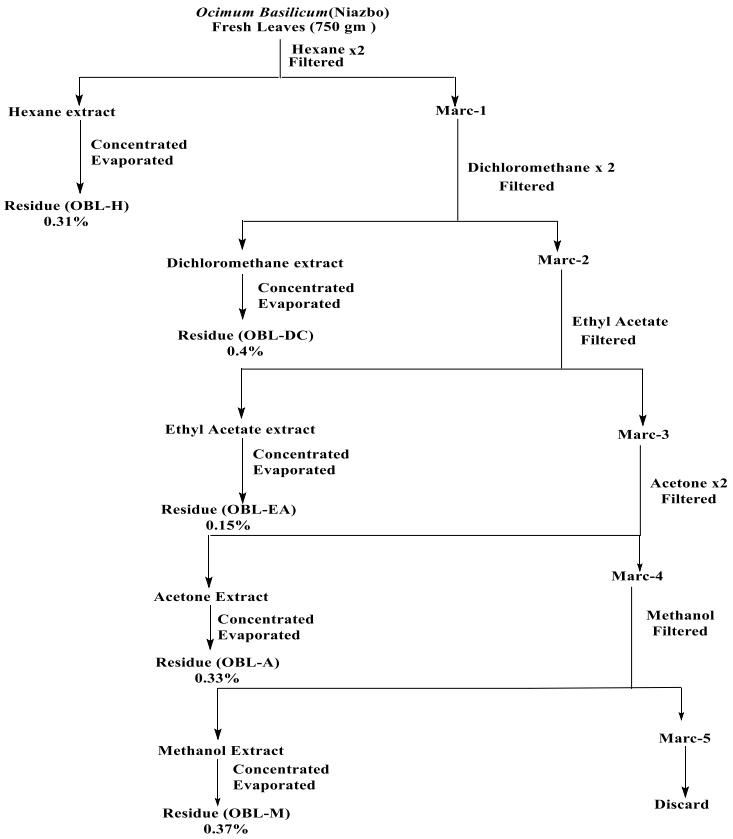
The goal of this study was to confirm the anti-oxidant activity of its hexane, dichloromethane, ethyl acetate, acetone, and methanol extracts using *in vitro* and *ex vivo* assays, as well as to investigate the efficacy of *O. basilicum* leaves methanolic extract as an antihypertensive agent in the L-Name induced hypertensive rat model. It also aims to investigate the phytochemical analyses of *O. basilicum*. GC-FID and GC-MS analyses were employed to analyze bioactive (hexane and dichloromethane) extracts evoking pharmacological activity. This investigation led to the identification of cadinol, linalool and linalool related terpenes along with other constituents. Whereas methanol extract was subjected to ESI-HRMS/MS investigations for qualitative analysis.

2. Results and discussion

2.1. Composition and characterization

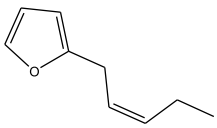
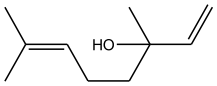
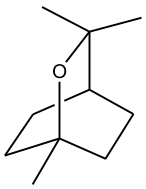
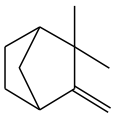
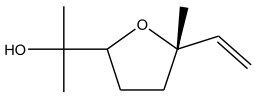
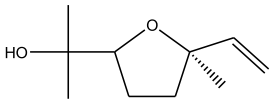
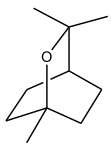
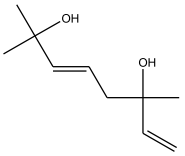
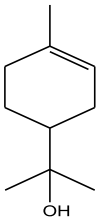
Despite the availability of a number of antihypertensive drugs, most patients' blood pressure remains abnormally high, putting them at risk for cardiovascular problems. Due to the limits of present pharmaceuticals, new antihypertensive medicines with unique mechanisms of action are being researched to provide better blood pressure management, stronger organ damage protection, enhanced tolerability, and more effective cardiovascular disease prevention. Essential hypertension, on the other hand, is a multifactorial and multigenic disorder in which different pathways contribute to blood pressure elevation by involving multiple pathways. *O. basillicum* contains several bioactive chemicals that target a number of pharmacological processes linked to hypertension, making it a promising new therapeutic candidate for blood pressure control and oxidative stress reduction.

Fresh leaves of *O. basilicum* were sequentially extracted with n-hexane, dichloromethane, ethyl acetate, acetone and methanol twice at room temperature for three days (Scheme 1). The extracts were filtered and evaporated under reduce pressure using rotary evaporator to obtain a concentrate masses of OBL-H, OBL-DC, OBL-EA, OBL-A and OBL-M. *O. basilicum* on extraction with hexane and dichloromethane, gave a pale yellow hexane extract OBL-H and a dark brown dichloromethane extract OBL-DC. GC-FID and GC-MS analyses of the extracts showed the presence of total of seventy five (75) compounds (Table 1). GC-MS Chromatograms showing peaks and retention time is represented in Fig. 1. Constituents with significant abundance include fatty acid, monoterpenes, sesquiterpenes, triterpenol, phyto-sterols, and phthalates and hydrocarbons derivatives. Higher percentages of fatty acids were identified and quantified (53.55%) in OBL-H. Hexadecanoic acid (43) (13.73%), methyl (9Z, 12Z, 15Z)-octadeca-9, 12, 15-trienoate (44) (16.74%) and methyl *cis*, *cis* −9, 12- octadecadienoate (45) (18.17%) were found to be most abundant fatty acid found in OBL-H extract. Other fatty acids like methyl 9- oxononanoate (22), octadecanoic acid (48), methyl (6*E*,9*E*,12*E*, 15*E*)- 6,9,12,15-docosatetraenoate (50), methyl 12-hydroxy-9- octadecenoate (40), methyl 16-methyl-heptadecanoate (47) were also quantified in small quantitates, methyl hexadecanoate (42) (14.24%) was the major fatty acid ester observed in OBL- DC. The second major class of the compounds observed in OBL-H was hydrocarbons (17.79%). However lesser quantities of hydrocarbons (2.81%) were observed in OBL-DC. High amount of tetratriacontane (58) (5.25%) and tetratetracontane (60) (7.31%) was observed in OBL- H, while other hydrocarbons (54, 57, 59, 61) were also observed in lesser amount. Heneicosane (64) was the major hydrocarbon (2.81%) detected in OBL-DC. Oxygenated monoterpenes and monoterpenes hydrocarbon were identified in OBL-H and OBL-DC (11.08 and 11.3%) respectively. Linalool (2)



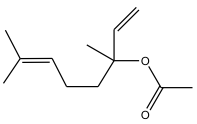
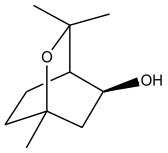
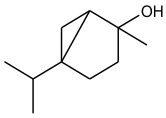
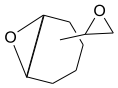
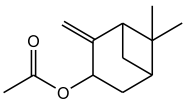
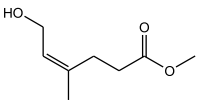
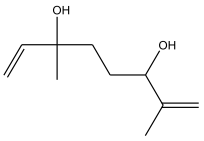
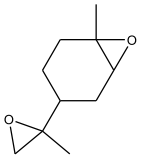
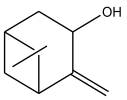
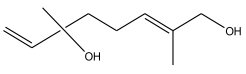
Scheme 1. Scheme followed for the extraction of leaves of *Ocimum basilicum*.

Table 1
Compounds identified by GC-MS in hexane and dichloromethane extracts of *O. basilicum*.leaves.

S#	Compounds	MW/MF	RI[a]	RI[b]	OBL-H[c]	OBL-DC[c]	Identification	Reference
1	 *cis*-2-(2-pentenyl) furan (1)	152 $C_9H_{12}O_2$	1015	1004	0.35	–	MS,RI	[56]
2	 Linalool (2)	154 $C_{10}H_{18}O$	1016	1081	1.8	5.85	MS,RI	[57]
3	 Eucalyptol (3)d	154 $C_{10}H_{18}O$	1027	1023	0.39	0.66	MS,RI	[58]
4	 Camphene (4)	136 $C_{10}H_{16}$	1041	954	–	0.42	MS,RI	[59]
5	 *trans*-linalool oxide (5)d	170 $C_{10}H_{18}O_2$	1068	1074	0.69	–	MS,RI	[60]
6	Unidentified	–	1070		0.69	–	–	
7	 *cis*-Linalool oxide (6)d	170 $C_{10}H_{18}O_2$	1081	1080	0.03	0.07	MS,RI	[60]
8	 Camphor (7)	152 $C_{10}H_{16}O$	1136	1146	0.24	0.54	MS,RI	[59]
9	 3,7-Octadiene-2,6-diol,2,6-dimethyl (8)	170 $C_{10}H_{18}O_2$	1148	1173	2.49	–	MS,RI	[61]
10	 Terpineol (9)	154 $C_{10}H_{18}O$	1166	1172	0.43	0.99	MS,RI	[59]

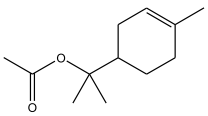
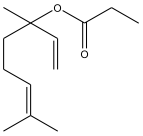
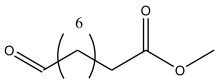
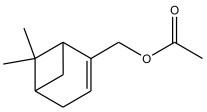
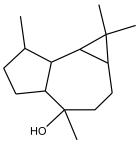
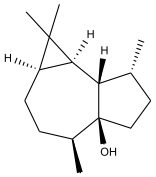
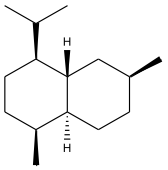
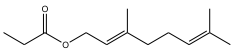
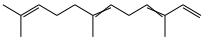
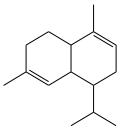
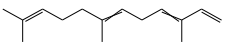
(*continued on next page*)

Table 1 (*continued*)

S#	Compounds	MW/MF	RI[a]	RI[b]	OBL-H[c]	OBL-DC[c]	Identification	Reference
11	 Linalyl acetate (10)	174 $C_{12}H_{22}O_2$	1216	1236	–	5.85	MS,RI	[62]
12	 2-hydroxy-1,8-cineol (11)	170 $C_{10}H_{18}O_2$	1225	1227	0.75	–	MS,RI	–
13	Unidentified	–	1229	–	0.75	–	–	
14	 *cis*-Sabinene hydrate (12)	154 $C_{10}H_{18}O$	1241	–	0.01	0.02	MS	[63]
15	 7-Oxabicyclo [4.1.0] heptane, 3-oxiranyl- (13)	140 $C_8H_{12}O_2$	1242	–		0.01	MS	–
16	Unidentified	–	1270	–		0.02	–	
17	 (−)-*trans*-Pinocarvyl acetate (14)	170 $C_{12}H_{18}O_2$	1274	1298		0.16	MS,RI	[64]
18	 Methyl (4Z)-6-hydroxy-4-methyl-4-hexenoate (15)	158 $C_8H_{14}O_3$	1276	–		0.01	MS	–
19	 1,7-Octadiene-3,6-diol,2,6-dimethyl (16)	170 $C_{12}H_{18}O_2$	1276	1277	2.40	–	MS,RI	[65]
20	 Limonene dioxide (17)d	168 $C_{10}H_{16}O_2$	1307	1294	0.23	0.51	MS, RI	–
21	Un Identified	–	1309	–	0.03	0.06	–	
22	 Isopinocarveol (18)	152 $C_{10}H_{16}O$	1315	–	0.19	0.42	MS	[66]
23	 *trans*-8-hydroxylinalool (19)d	170 $C_{12}H_{18}O_2$	1359	1355	0.84	0.64	MS,RI	[67]

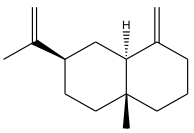
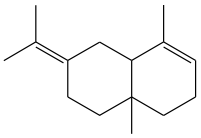
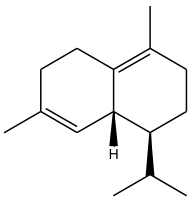
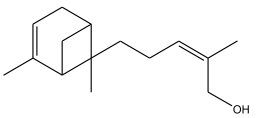
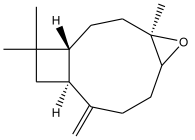
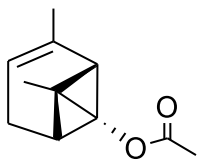
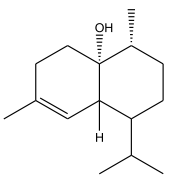
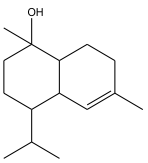
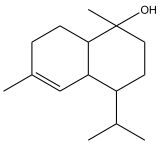
(*continued on next page*)

Table 1 (*continued*)

S#	Compounds	MW/MF	RI[a]	RI[b]	OBL-H[c]	OBL-DC[c]	Identification	Reference
24	 Terpinol acetate (20)	172 $C_{10}H_{20}O_2$	1364	1351	–	0.12	MS,RI	[68]
25	 Linalyl propanoate (21)	210 $C_{13}H_{22}O_2$	1372	1340	–	0.04	MS,RI	[69]
26	 Methyl 9-oxononanoate (22)	186 $C_{10}H_{18}O_3$	1423	1439	0.71	–	MS,RI	[70]
27	 Myrtenyl acetate (23)	170 $C_{12}H_{18}O_2$	1474	–	–	0.30	MS	[71]
28	 Epiglobulol (24)	222 $C_{15}H_{26}O$	1477	1532	0.66	–	MS,RI	[72]
29	 Palustrol (25)	222 $C_{15}H_{26}O$	1486	1550	0.66	1.50	MS.RI	[73]
30	 Cadinene (26)	204 $C_{15}H_{24}$	1503	1505	0.32	4.78	MS,RI	[74]
31	 Geranylpropanoate (27)	196 $C_{12}H_{20}O_2$	1508	1475	0.21	0.47	MS,RI	[75]
32	 α-Farnesene (28)	204 $C_{15}H_{24}$	1532	1509	–	3.76	MS,RI	[76]
33	 α-Muurolene (29)	204 $C_{15}H_{24}$	1550	1523	–	0.26	MS,RI	[77]
34	 Farnasene isomer (30)	204 $C_{15}H_{24}$	1554	1522	–	0.44	MS,RI	[76]

(*continued on next page*)

Table 1 (*continued*)

S#	Compounds	MW/MF	RI[a]	RI[b]	OBL-H[c]	OBL-DC[c]	Identification	Reference
35	 β Selinine (31)	204 $C_{15}H_{24}$	1555	1509	–	0.60	MS,RI	[78]
36	 3,7 (11)-Selinadiene (32)	204 $C_{15}H_{24}$	1560	1542	–	0.40	MS,RI	[79]
37	 σ- Cadinene (33)	204 $C_{15}H_{24}$	1570	1562	–	1.06	MS,RI	[80]
38	 Bergamotol (34)	220 $C_{15}H_{24}O$	1572	–	–	0.25	MS	[81]
39	 Caryophyllene oxide (35)d	220 $C_{15}H_{24}O$	1585	1576	1.13	–	MS,RI	[82]
40	 *trans*-Chrysanthenyl acetate (36)	170 $C_{12}H_{18}O_2$	1606	–	–	0.42	MS	[83]
41	 Cubenol (37)	222 $C_{15}H_{26}O$	1618	1618	0.62	1.85	MS,RI	[84]
42	 *tau*-Cadinol (38)	222 $C_{15}H_{26}O$	1643	1628	3.56	9.99	MS,RI	[39]
43	 *tau*-Muurolol (39)	222 $C_{15}H_{26}O$	1662	1642	–	0.48	MS,RI	[85]

(*continued on next page*)

Table 1 (*continued*)

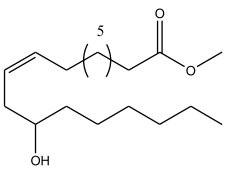
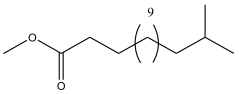
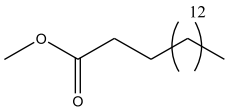
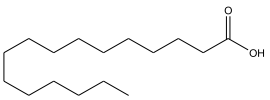
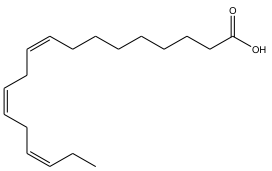
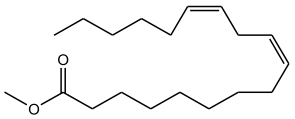
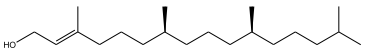
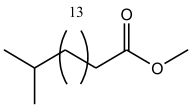
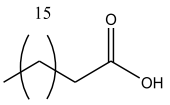
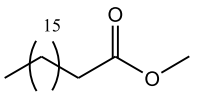
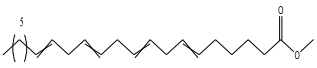
S#	Compounds	MW/MF	RI[a]	RI[b]	OBL-H[c]	OBL-DC[c]	Identification	Reference
44	 Methyl 12-hydroxy-9-octadecenoate (40)	312 $C_{19}H_{36}O_3$	1781	–	1.46	–	MS	[86]
45	 Methyl 14-methylpentadecanoate (41)	272 $C_{17}H_{34}O_2$	1850	1884	0.26	0.59	MS,RI	[87]
46	 Methyl hexadecanoate (42)	272 $C_{17}H_{34}O_2$	1910	1908	–	14.24	MS,RI	[88]
47	 Hexadecanoic acid (43)	256 $C_{16}H_{32}O_2$	1948	1950	13.73	0.58	MS,RI	[88]
48	 Methyl (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate (44)	292 $C_{19}H_{32}O_2$	2055	2077	16.74	0.98	MS,RI	[89]
49	 Methyl *cis*,*cis*-9,12-octadecadienoate (45)	294 $C_{19}H_{34}O_2$	2088	2071	18.17	–	MS,RI	[88]
50	 Phytol (46)	276 $C_{20}H_{40}O$	2109	2104	0.73	0.42	MS,RI	[46]
51	 Methyl 16-methyl-heptadecanoate (47) (Methyl isostearate)	298 $C_{19}H_{38}O_2$	2121	–	0.74	0.42	MS	[90]
52	 Octadecanoic acid (48)	284 $C_{18}H_{36}O_2$	2178	2187	1.30	–	MS,RI	[88]
53	 Methyl octadecanoate (49)	298 $C_{19}H_{38}O_2$	2125	2128	–	0.05	MS,RI	[91]
54	Unidentified	–	2352	–	0.85	–	–	
55	 Methyl (6*E*,9*E*,12*E*,15*E*)-6,9,12,15-docosatetraenoate (50)	346 $C_{23}H_{38}O_2$	2375	–	0.44	–	MS	–

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Table 1 (*continued*)

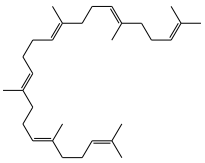
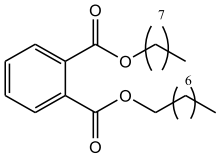
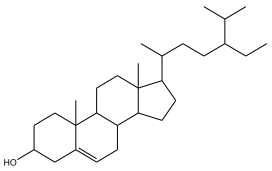
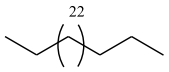
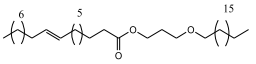
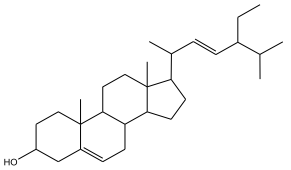
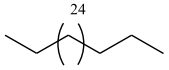
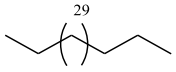
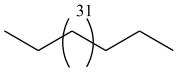
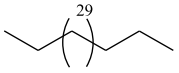
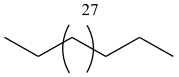
S#	Compounds	MW/MF	RI[a]	RI[b]	OBL-H[c]	OBL-DC[c]	Identification	Reference
57	 Squalene (51)	410 $C_{30}H_{50}$	2486	–	–	1.03	MS	[92]
58	 Isooctyl Phthalate (52)	390 $C_{24}H_{38}O_4$	2551	–	1.05	–	MS	[88]
59	Unidentified	–	2555	–	–	32.12	–	
60	Unidentified	–	2571	–	–	0.93	–	
61	Unidentified	–	2576	–	–	0.94	–	
62	 β-Sitosterol (53)	414 $C_{29}H_{50}O$	2633	–	–	0.77	MS,RI	[88]
63	 Heptacosane (54)d	380 $C_{27}H_{56}$	2651	2700	0.55	–	MS,RI	[93]
64	 3-octadecoxypropyl (Z)-octadec-9-enoate (55)	592 $C_{39}H_{76}O_3$	2655	–	–	0.72	MS	
65	 Stigmasterol (56)	412 $C_{29}H_{48}O$	2656	–	–	0.45	MS	[88]
66	 Nonacosane (57) d	408 $C_{29}H_{60}$	2844	2900	1.61	–	MS,RI	[93]
67	 Tetratriacontane (58) d	478 $C_{34}H_{70}$	3027	–	5.25	–	MS	[94]
68	Unidentified	–	3450	–	1.22	–	–	
69	 Hexatriacontane (59)d	506 $C_{36}H_{74}$	3460	–	1.06	–	MS	[95]
70	 Tetratetracontane (60)d	478 $C_{34}H_{70}$	–	–	7.31	–	MS	[95]
71	 Dotriacontane (61)d	450 $C_{32}H_{66}$	–	–	2.01	–	MS	[96]

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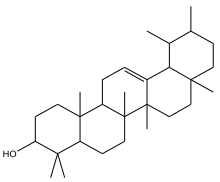
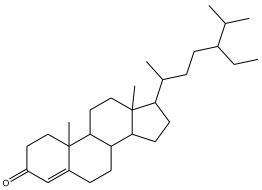
Table 1 (*continued*)

S#	Compounds	MW/MF	RI[a]	RI[b]	OBL-H[c]	OBL-DC[c]	Identification	Reference
72	 Amyrin (62)	426 $C_{30}H_{50}O$	–	–	2.47	–	MS	[97]
73	 Sitostenone (63)	412 $C_{29}H_{48}O$	–	–	0.89	–	MS	–
74	Unidentified	–	–	–	1.97	–	–	
75	 Heneicosane (64) d	296 $C_{21}H_{44}$	–	–	–	2.81	MS	
	Monoterpenes				11.24	16.97		
	Sesquiterpenes				6.95	25.37		
	Diterpene alcohol				0.73	0.42		
	Triterpenes				2.47	1.03		
	Oxygenated hydrocarbons			–	0.58	0.52		
	Fatty acids				53.55	17.59		
	Hydrocarbons				17.79	2.82		
	Phthalates				1.05			
	Phytostrerol				0.89	0.77		
	Unidentified				5.51	34.08		
	Total %				100.76	99.05		

Retention indices (Optima-5 Column),b; Retention indices compared with NIST database Mass Spectral Library. Wiley Registry of Mass Spectral Data 8th edition and literature,; cPercentage calculated by their relative percentages of total chromatogram area.d Identification was based on comparison of the compounds mass spectral data (MS) and retention indices (RI) with those standards.

(1.8%), 3,7-octadiene-2,6-diol, 2,6-dimethyl (8) and its isomer 1,7-octadiene-3,6-diol, 2,6-dimethyl (16) were found to be the most abundant oxygenated monoterpenes in OBL-H. while linalool (2) (5.85%) and linalool acetate (10) (5.85%) were observed in 1: 1 ratio are the major monoterpenes in OBL- DC along with other linalool derivatives (6,19,21). However, these derivatives were detected in minor quantities. The concentration of oxygenated sesquiterpenes in this study were found to be around (6.95%) in OBL- H with tau-cadinol (38) (3.56%) was the dominating one. Oxygenated sesquiterpenes appeared as major class of compound in OBL-DC around (25.37%) w/w of the extract. Cadinene (26) (1.06%), farnesene (28) (3.76%), σ-cadinene (33) (1.06%), cubenol (37) (1.85%) and tau cadinol (9.9%) were the dominating oxygenated sesquiterpenes. Other compounds observed in OBL-H was triterpenes, amyrins (62) (2.47%), iso octyl phthalate (52) (1.05%), *cis*-2-(2-pentenyl) furan (1) which was oxygenated hydrocarbon (0.35%) and phytostrerol sitostenone (63) (0.89%), while five compounds remained unidentified. In case of OBL-DC triterpenes squalene (51) (1.03%) and phytosterol stigmasterol (56) (0.45%) were also detected. These GC-MS profiles revealed that oxygenated mono terpenoids and sesqui terpenoids are the most dominant compounds of the two extracts. It is important to note that terpene acetates are abundantly observed in *O. basilicum* that was not reported so far. Linalyl acetate (10), (−)- *trans*-Pinocarvyl acetate (14) terpineol acetate (20), Myrtenyl acetate (23), *trans*-Chrysanthenyl acetate (36) although in minor quantities. Polar methanol extract (OBL-M) was analyzed through electron spray ionization-high-resolution mass spectrometric detection (ESI-HRMS/MS). Qualitative determination tentatively was done by comparing the EI-HRMS chemical formula where available and by correlation with previous literature report and data in Dictionary of Natural products. ESI-HRMS/MS spectrum showed the presence of nine constituents (Table 2) which included compounds, an indole alkaloid; Pentacyclindole (65),a coumarin; calanone (66), flavonol glycosides, Catechin 3′-glucoside (67), phenolic acid; rosmarinic acid (68), amino acid; theanine (69), terpene ketone; (5*Z*, 9*E*)-farnesyl acetone (70), polyamine alkaloid; (*N*-Acetyl-*N*-deoxymayfoline) (71), lignin; eudesmin (72) and Quercetin (73) a flavonoid.

All the compounds were observed keenly for the characteristic peak and the fragmentation pattern of particular functional groups that helped greatly to identify the compounds. Pentacyclindole (65) showed peak at *m/z* 336 [M+H] and fragmentations at *m/z* 319, 302, 242, 218. Calanone (66) displayed peak at *m/z* 424 [M+H] and showed fragments at *m/z* 408, 391, 320, 334, 306, 274. Molecular ion peak of Catechin 3′-glucoside (67) was observed at *m/z* 452 [M+H] and fragmentation at *m/z* 436, 418, 402, 344, 320. Rosmarinic acid. (68) showed its peak at m/z 361 [M+H] and fragmentations at *m/z*, 344, 329, 300, 270, 256, 238, 224, 168. Theanine (69) has a characteristic peak at *m/z* 174 [M+H], and showed fragments at *m/z* 160, 156,145,143, 129, 126, 114, 104. (5Z, 9 E)-farnesyl acetone

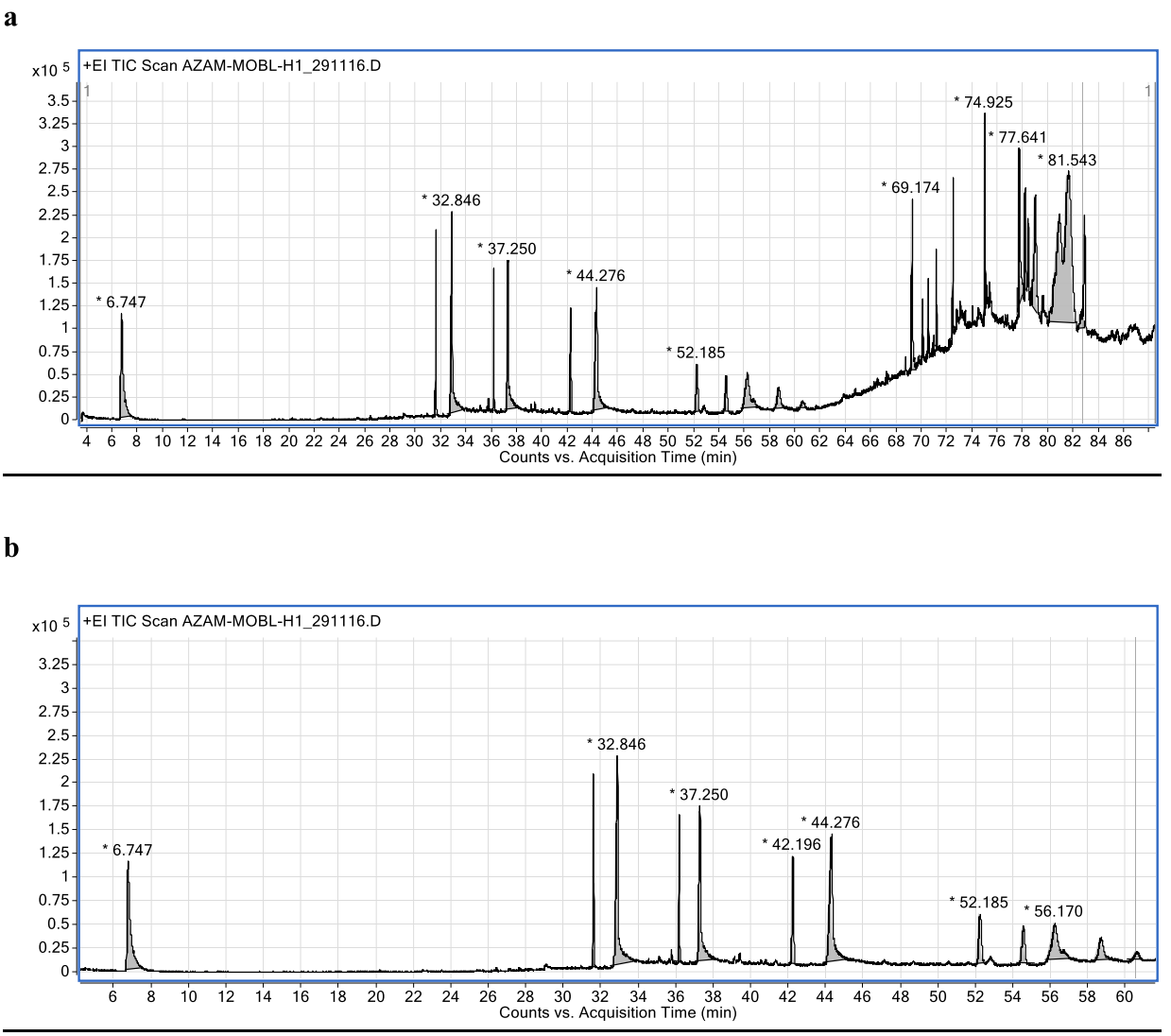


Fig. 1. GC-MS Chromatograms showing peaks and retention time a: GC-MS Chromatograms of n-hexane extract, b: GC-MS Chromatograms of n-dichloromethane extract.

(70) showed molecular ion peak at *m/z* 262 [M+H] and fragmentation at *m/z* 248, 204, 194, 176. (*N*-Acetyl-*N*- deoxymayfoline) (71), showed peak at *m/z* 317 [M+H] and fragmentations at *m/z* 300, 256. Eudesmin (72) showed peak at *m/z* 386 [M+H] and fragmentations at *m/z* 328, 235, 217, 113 and Quercetin (73) showed peak at *m/z* 303 [M+H] and fragmentations at *m/z* 289, 227, 218, 211, 190, 161.

2.2. Total phenolic and flavanoids content

The total phenolic content of the *O. basilicum* extracts were mentioned in Table 3. The highest phenolic content were found in ethyl acetate extract in OBL-EA i.e. 9.40 mg GAE/g of dry weight and lowest was observed in acetone extract i.e. 2.96 mg GAE/g of dry weight while hexane, dichloromethane and methanol extract showed moderate content of 4.13, 5.05 and 4.2 mg GAE/g of dry weight respectively. In the current study ethyl acetate extract has highest flavonoid content as compared to other *Ocimum* extracts 15.9 mg RE/g. OBL- Dc and OBL-H showed lowest flavonoid content of all the extracts 0.4 and 0.87 mg RE/g whereas OBL-A and OBL-M showed moderate content 8.4, and 3.2 mg RE/g.

Nadeem et al., 2022 reported notable concentrations of total phenolic content in ethanol, dichloromethane and aqueous extract of *O. basilicum*. The study also reported the least phenolic content (29.7 mg GAE/g) in hexane extract of *O. basilicum*. In terms of total flavonoids, the current findings are consistent with the above-mentioned study, which found the least TFC level in hexane extract [29]. Literature survey revealed that the total phenolic and flavonoid content of *O. basillicum* varied and various factors like solvents, extraction techniques, drying methods, pretreatment of sample with enzymes, UV-B irradiation etc. are responsible for this variation

Table 2
Compounds identified using ESI-HRMS/MS in OBL-M.

S#	Tentative identification[a]	HRM [M + H]m/z	M. Formula M. Weight	Δ[b] [ppm]	RDBdequiv[c]	MS2[d]	Class of compound
1.	Pentacyclindole (65)	$C_{23}H_{30}NO$ 336.2322	$C_{23}H_{29}NO$ 335	7	10	319, 302, 242, 218	Indole alkaloid [98]
2.	Calanone (66)	$C_{27}H_{21}O_5$ 425.1367	$C_{27}H_{20}O_5$ 424	3.8	18	408, 391, 320, 334, 306, 274	Coumarin [99]
3.	Catechin 3′-glucoside (67)	$C_{21}H_{25}O_{11}$ 453.1677	$C_{21}H_{24}O_{11}$ 452	–	–	436, 418, 402, 344, 320	Flavan-3-ol glycoside{98]
4.	Rosmarinic acid (68)	$C_{18}H_{18}O_8$ 362.3264	$C_{18}H_{16}O_8$ 360	–	–	344, 329, 300, 270, 256, 238, 224, 168	Phenolic acid [13]
5.	Theanine (69)	$C_7H_{15}N_2O_3$ 175.1075	$C_7H_{14}N_2O_3$ 174	1	2	160,156,145,143, 129, 126, 114, 104	Amino acid [1]
6.	(5*Z*,9*E*)-farnesyl acetone (70)	$C_{18}H_{31}O$ 263.2369	$C_{18}H_{30}O$ 262	0.1	4	248, 204, 194, 176	Terpene ketone [100]
7.	(*N*-Acetyl-*N*-deoxymayfoline) (71)	$C_{18}H_{28}N_3O_2$ 318.2193	$C_{18}H_{28}N_3O_2$ 317	5.2	7	300,256	Polyamine alkaloid [101]
8.	Eudesmin (72)	$C_{22}H_{27}O_6$ 387.1809	$C_{22}H_{26}O_6$ 386	1.7	10	328, 235, 217, 113	Lignin [102]
9.	Quercitin (73)	$C_{15}H_{12}O_7$ 304.0609	$C_{15}H_{10}O_7$ 302	–	–	289, 227, 218, 211, 190, 161	Flavonoid [103]

Compounds were tentatively identified by comparison with ESI-HRMS/MS chemical formula where available and by correlation with previous literature reports and data in Dictionary of Natural Product (DNP). [b] Error (•• in ppm.[c]Ring and Double-Bond (*RDB*) *equivalents.* [d]*MS/MS Fragmentations*.

Table 3
Total phenolic Content and Total flavonoid content of *O. basilicum* L. extracts.

Extracts	TPC values mg GAE/g of DW	TFC values mg RE/g of DW
OBL-H	4.13 ± 0.0025	0.87 ± 0.001667
OBL-DC	5.05 ± 0.003125	0.4 ± 0.003005
OBL-EA	9.40 ± 0.0011	15.9 ± 0.023333
OBL-A	2.96 ± 0.022	8.4 ± 0.018559
OBL-M	4.2 ± 0.002708	3.2 ± 0.017638

Table 4
DPPH colorimetric assay of extracts of *Ocimum basilicum* L.

Extract	Conc. mg/ml	RSA %	IC_{50}(mg/ml)	AAE (mM/100 g)
OBL-H	5	18.41 ± 0.03	ND	0.23 ± 0.03
	4	23.85 ± 0.09		0.18 ± 0.05
	3	21.52 ± 0.10		0.22 ± 0.01
	2	19.97 ± 0.05		0.2 ± 0.05
	1	12.12 ± 0.18		0.29 ± 0.01
OBL-DC	5	56.02 ± 0.06	1.27 ± 0.02	0.17 ± 0.02
	4	64.12 ± 0.23		0.24 ± 0.05
	3	65 ± 0.29		0.24 ± 0.02
	2	55.67 ± 0.01		0.16 ± 0.03
	1	42.44 ± 0.43		0.03 ± 0.01
OBL-EA	5	41.51 ± 0.01	4.2 ± 0.01	0.17 ± 0.01
	4	53.37 ± 0.01		0.24 ± 0.02
	3	57.09 ± 0.04		0.27 ± 0.02
	2	48.14 ± 0.01		0.21 ± 0.05
	1	26.51 ± 0.71		0.06 ± 0.01
OBL-A	5	65.9 ± 0.29	3.1 ± 0.02	0.3 ± 0.01
	4	57.08 ± 0.30		0.24 ± 0.02
	3	55.4 ± 0.01		0.23 ± 0.05
	2	39.98 ± 0.01		0.12 ± 0.01
	1	26.76 ± 0.74		0.03 ± 0.01
OBL-M	5	47.49 ± 0.02	ND	0.17 ± 0.05
	4	39.41 ± 0.01		0.1 ± 0.01
	3	38.49 ± 0.02		0.09 ± 0.02
	2	20.88 ± 0.01		0.03 ± 0.01
	1	9.83 ± 0.01		0.11 ± 0.02

[a]RSA %; Percent radical scavenging, AAE; Ascorbic acid equivalent, ND not determined, All values are presented as Mean ± SEM.

[30–32].

2.3. In-vitro anti oxidant assay

Because phytochemicals are so complex, there is no one method for evaluating antioxidant activity [33–35]. Many known methodologies were used to confirm the antioxidant capabilities of the *Ocimum* extracts. Although, all of the extracts showed significant activity at the concentration of 4 mg/ml, dichloromethane extract (OBL-DC) possess the highest scavenging activity, i.e., 64.12% ± 0.23 at concentration of 4 mg/ml amongst all of the extracts. While, hexane extract (OBL-H) showed the lowest scavenging activity, i.e., 23.85% ± 0.09 at a concentration of 4 mg/ml. A concentration dependent increase till a concentration of 4 mg/ml of OBL-H has attained and then a decrease at 5 mg/ml was observed in the percent scavenging activity. Same pattern was observed with OBL-DC and OBL-EA showed increase from 1 to 3 mg/ml and a decrease from 4 to 5 mg/ml. A concentration dependent increase was observed in the percent scavenging activity OBL-A and OBL-M also. OBL-DC showed IC_{50} of 1.27 ± 0.02 mg/ml, OBL_EA-showed IC_{50} of 4.2 ± 0.01 mg/ml and OBL-A showed IC_{50} of 3.1 ± 0.02 mg/ml as shown in Table 4 whereas IC_{50} of OBL-H and OBL-M was not determined. ABTS Cation de-Colorization assay *O. basilicum* extracts showed that all the extracts possessed good antioxidant activity at concentration of 100 μg/ml (Table 5). The calibration curve of Trolox was plotted with a value of r2 = 0.9971 and used for the estimation of antioxidant content as TEAC mM/100 g. Higher antioxidant activity in all the extracts was observed as compared to DPPH assay, i.e., 68% ± 2.6, 85% ± 2.3,97% ± 0.56, 96% ± 0.83 and 90% ± 0.19 for OBL-H, OBL-DC, OBL-EA, OBL-A and OBL-M, respectively.

2.4. Oxidative hemolysis inhibition assay (OxHLIA) of extracts of O. basilicum L

AAPH (hemolysis inducing agent) Oxidative Hemolysis Inhibition Assay was carried out on hemolysed. Human RBC. Remarkable inhibition of oxidative hemolysis was found in all the *O. basilicum* extracts, being 69.24 ± 0.18 in OBL-DC, 64.44 ± 0.04 in OBL-EA and 53.33 ± 0.09 in OBL-A. Lowest inhibition of oxidative hemolysis was observed being 38.02% ± 0.40 in OBL-M, whereas OBL-H showed moderate activity of 42.48% ± 0.02 (Table 5). Very Limited literature is available reporting the Oxidative Hemolysis Inhibition Assay of this plant. One study reported by Pereira C et al., 2020 demonstrate the membrane stabilizing effect of *O. basilicum* extracts on sheep RBCs [3], but this activity was much lower as compared to trolox (positive control). *Ocimum* extract had significant anti hemolytic action at various concentrations; however this activity reduced as the extract concentration increased, presumably due to the presence of the above-mentioned phytochemicals in concentrated form of leaf extract. The extract contains chemicals that interacted with a class of lipids prevalent in the outer monolayer of the human erythrocyte membrane, resulting in a protective effect.

2.5. Antihypertensive activity of OBL-M

Antihypertensive activity of methanol extract of the plant OBL-M was determined in L- Name induces hypertensive rats. Blood pressure was measured from the tails of rats using tail cuff non-invasive blood pressure (NIBP) apparatus. Systolic blood pressure (SBP), Mean blood pressure (MBP) and heart rate were measured directly using pulse tracing. Record of the animal's body weight was maintained throughout the study. Animals were sacrificed at the end of the study to observe the organ weights and histopathological studies. All the experimental results were analyzed using SPSS (Tukey's and dunnet's multiple comparison tests using SPSS statistical software version.20). No significant difference was observed while observing the body weight and organ weights of the animals from the control group $p > 0.005$.

In this study we reported, the hematological parameters while observing the antihypertensive effect of the extract OBL-M (dose of 50 mg/kg). All the Hematological parameters observed were found to be within the reference ranges for animal. The numbers of WBC, RBC and platelet, and the levels of Hb (hemoglobin) were slightly increased in the extract treated group but the values in the groups were not significantly different from the control group and the group receiving standard drug lisinopril $p > 0.005$.

Antihypertensive evaluation of methanol extract OBL-M, showed significant ($p < 0.05$) fall in MABP (96 ± 3.33) as compared to Group 2 at day 0 and day 7. Highly significant fall was observed ($p < 0.01$) at day 14, 21 and 28 when compared with Group 2 diseased model receiving L-NAME. However, comparable results were observed with Group 1 receiving standard drug Lisinopril (Table 6).

Table 5
ABTS cation decolorization and OxHLIA assay of extracts of *O. basilicum* L.

Extracts	AA % 100 μg/ml	TEAC (mM/100 g)	% OxHLIA assay (4 mg/ml)
OBL-H	68 ± 2.6	0.21 ± 0.003	*42.48 ± 0.02
OBL-DC	85 ± 2.3	0.25 ± 0.003	*69.24 ± 0.18
OBL-EA	97 ± 0.56	0.30 ± 0.006	*64.44 ± 0.04
OBL-A	96 ± 0.83	0.29 ± 0.001	*53.33 ± 0.09
OBL-M	90 ± 0.19	0.28 ± 0.001	*38.02 ± 0.40

[a]AA%; Antioxidant activity, TEAC; Trolox equivalent, *Ascorbic acid (Control 0.2 mg/ml)6.2 ± 0.07in OxHLIA assay, All values are presented as Mean ± SEM.

2.6. Acute toxicity

Acute toxicity showed that the extracts OBL-H and OBL - M were safe at the dose of 2000 mg/kg while OBL-DC was found to be safe at the dose of 5000 mg/kg.

OBL-DC at a dose of 5000 mg/kg had no adverse effect on the behavioral responses of the tested animals up to 14 days of observation. Physical observations indicated no signs of changes in the skin, fur, eyes mucous membrane, behavior patterns, tremors, salivation, and diarrhea of the mice. There was no mortality observed at the tested dose nor was the weight loss in the mice affected. There were generally no significant differences observed in the relative organ weights in this study. The LD_{50} of OBL-DC was therefore estimated to be more than 5000 mg/kg. With the extract OBL-H and OBL- M mortality observed at a dose of 5000 mg/kg while no adverse effect on the behavioral responses and no mortality observed at 2000 mg/kg. The LD_{50} of OBL-H and OBL-M was therefore estimated to be more than 2000 mg/kg.

The terpene composition of *Ocimum* species is highly variable; its physiology/morphology is going to be change in response to changes in environmental factors. Some researcher classified essential oil chemo types in four major of basil: as methyl chavicol-rich, linalool-rich, methyl eugenol-rich and methyl cinnamate dominating species [36]. Methyl chavicol, a phenyl propanoid having insecticidal and antimicrobial activity is produced by shikimic acid pathway. The concentration of linalool in *O. basilicum* varies with species and region. Linalool is a terpenoid produced by mevalonic acid pathway and has been reported to possess anti-inflammatory, anticancer, *anti*-hyperlipidemic, antimicrobial, antinoceptive, analgesic and anxiolytic, anti-depressive and neuro-protective properties. Methyl cinnamate is the methyl ester of cinnamic acid, naturally occurring in many aromatic plants. It attracts pollinators [37]. Some studies also reported that tau cadinol dominating species also exist [38]. Current study also revealed the presence of high percentages of tau-cadinol in both hexane and DC extracts indicating the significance of plant in treating cardiovascular diseases. Tau cadinol has been reported to possess calcium antagonistic activity [39]. Many Compounds observed by GC-MS analysis in different extracts of *O. basilicum* had proved to possess antioxidant effects. Linalool (2) [40], eucalyptol (3) [41], camphene (4) [42], terpineol (9) [43], linalyl acetate (10) [44], α-farnesene (28) [45], phytol (46) [46], squalene (51) [44,47], β-sitosterol (53) [48], stigmasterol (56) [49] and α- Amyrins (62) are also known to have antioxidant effects. Linalool (2) [50], eucalyptol (3) [51], camphor (7) [52], terpineol (9) [53], linalyl acetate (10) [54] and tau-cadinol (38) [39] are the major compounds that had showed good antioxidant as well as antihypertensive effects.

People all over the world are considering medicinal plants as an alternative to traditional treatment due to the high cost of treating hypertension and cardiovascular problems. Methanolic extract of *Ocimum* revealed the existence of bioactive scaffolds that may have played a role in fighting essential hypertension. Compounds like Theanine, a non-proteinogenic amino acid, have been demonstrated in multiple studies to have a variety of therapeutic effects, including improved brain and gastrointestinal function, cancer therapy therapeutic efficacies, antihypertensive effects and improved immunological function. It was also shown that the terpene ketone (5Z, 9 E)-farnesyl acetone had antioxidant properties. The phenolic acid derivative rosmarinic acid (RA) possesses antihypertensive and cardio protective effects [26]. RA acts as a vasoactive agent and a cardioprotector due to its antioxidant capabilities. When given, the flavonoid Quercetin has been demonstrated to cause a gradual, dose-dependent and long-lasting drop in blood pressure. The flavonoid Quercetin has been shown to elicit a progressive, dose-dependent and persistent reduction in blood pressure when administered chronically to various hypertensive animals. Quercetin also improved the formation of reactive oxygen species, which are linked to hypertension, while reducing morphological and functional abnormalities in the heart, arteries, and kidneys. The synergistic activity of these numerous components may be responsible for the overall blood pressure reducing impact of *Ocimum basillicum* methanolic extract at 50 mg/kg, according to these data. It was also established that the extract might be safe for used as a medication. Correlation between TPC and various antioxidant activities and antihypertensive activity of *Ocimum basilicum* L. Extracts the relationship between TPC and various antioxidant activities and antihypertensive activity was determined (Table 7). TPC had a higher linear correlation ($r = 0.678$) with antihypertensive activity values with a level of significance 95% showing that phenolic compounds present in *O. basilicum* is major contributing factor for antihypertensive effect. Moreover, there was a strong linear orrelation between TFC and DPPH, $r = 0.740$. The results indicate that DPPH radical scavenging activity of the plant is dependent of flavonoids present in the plant composition. The TPC of *Ocimum basilicum* plant extracts showed a strong positive correlation with ABTS radical scavenging activity $r = 0.363$ than with DPPH radical scavenging activity. DPPH radical scavenging activity is negatively correlated with TPC $r = -0.185$. With the decrease in Phenolic content increase in DPPH activity is observed. Thus phenolic content has a key role in contributing DPPH radical scavenging assay. A study has reported ABTS assay is appropriate for analysis of the hydrophilic and lipophilic compounds,

Table 6
Effect of OBL-M on MABP Blood pressure in L-Name induce Hypertensive rats.

Days	MABP(Mean arterilal blood pressure)			
	Control	Group 1 ± S.E.M	Group 2 ± S.E.M	Group 3 ± S.E.M
At Day 7	108 ± 5.49	88 ± 3.55	122 ± 8.52	96 ± 3.24*
At Day 14	110 ± 5.48	87 ± 4.36	148 ± 4.24	98 ± 2.70**
At Day 21	110 ± 5.41	96 ± 2.98	135 ± 5.61	85 ± 2.74**
At Day 28	109 ± 5.53	105 ± 2.82	146 ± 1.48	86 ± 2.05**##

[a]MABP mean arterial blood pressure; Control only receiving normal saline; Group 1: receiving drug lisinopril + L-NAME; Group 2: receiving L-NAME only; Group 3: receiving L-NAME + OBL M; All values are presented as Mean ± SEM n = 5 of five determinations of MABP at the dose of 50 mg/kg; *p < 0.01; **p < 0.05 as compared to Group 1 receiving L-NAME only,#p < 0.01; ##p < 0.05as compared to Standard. …

Table 7
Correlation between various antioxidant and antihypertensive activities of *O. basilicum* L. extracts.

	TPC	TFC	DPPH	ABTS	Hemolysis assay	Antihypertensive activity
TPC	1					
TFC	0.696	1				
DPPH	−0.185	0.205	1			
ABTS	0.363	0.740	0.762	1		
Hemolysis assay	0.523	0.338	0.412	0.385	1	
Antihypertensive activity	0.678	0.099	−0.544		0.535	1

[a]TPC; total phenolic content, TFC; total flavonoid content, DPPH; 2,2-diphenyl-1-picrylhydrazyl, ABTS; 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid).

whereas the DPPH radical scavenging assay is appropriate for analysis of hydrophobic compounds [55]. Thus, in this study ABTS assay results showed a stronger correlation with TPC content than DPPH assay.

TPC also exhibited a strong positive correlation with hemolysis assay r = 0.523 with a level of significance 95% than TPC r = 0.338. DPPH radical scavenging activity showed a strong correlation with ABTS radical scavenging activity r = 0.740. Negative correlation was observed between TPC and DPPH r = −0.185.

3. Conclusion

In this study, we examined the polyphenols and flavonoids contents as well as the anti-oxidant and antihypertensive effects of extracts from the leaves of *O. basilicum*. We observed that leaves of this plant are rich in oxygenated monoterpenes linalool and sesquiterpenes (tau cadinol) that are present in hexane and dichloromethane extracts. The extract containing these terpenes showed strong *in-vitro* and *in-vivo* antioxidant and membrane stabilizing activity, Although multifactors, including genetic and secondary etiology, play important roles in the development of hypertension, roughly 60% of the risk factors for hypertension are associated with metabolic disorder while alkaloids, amino fatty alcohols, amino acid, coumarin, lignin and sesquiterpene lactones were found to be present in methanol extract of *Ocimum* leaves. It can be concluded these constituents might play synergistic mechanism in controlling ROS related damages and thus possess significant role in controlling hypertension in L- Name induced hypertensive rats even at low dose i.e. 50 mg/kg. The extract (hexane, dichloromethane and methanol) were also found to be safe up to 2000 mg/kg with no effect on bloodparameters WBC, RBC and platelet, and the levels of hemoglobin. flavonoids are the major contributing factors for antioxidant activity. Although the relationship with terpenes has not been calculated but their antioxidant activities are well established. Therefore it has been concluded that these phytochemicals act in synergism for the antioxidant and antihypertensive activity of *Ocimum basilicum*.

4. Experimental section

4.1. Materials and methods

All the chemicals and solvents used were of analytical grade. BDH, England; gallic acid (3, 4, 5-trihydroxybenzoic acid), L-ascorbic acid, tween 80 (*polysorbate 80*, poly oxy ethylene sorbitan mono oleate), and potassium persulfate, (Duksan Pure Chemicals, Korea); aluminum chloride anhydrous, Scharlau (Spain); sodium carbonate, folin-ciocalteu'sreagent, (Alpha Aesar, Germany); (+)-rutin-trihydrate, (Daejung Chemicals & Metals CO, LTD, Korea); potassium acetate, (Bioworld, USA); ABTS (2,2′-azinobis (3-ethyl-benzothiazoline-6- sulfonic acid), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid)and phosphate buffer saline tablets (7.4), AAPH (2,2- azobis 2-methyl-propionamidine) dihydrochloride, DPPH (1,1- diphenyl-2-picrylhydrazyl) Trolox (±)-6-hydroxy2,5,7,8- tetramethylchromane-2-carboxylic acid, n-alkanes (C9– C33)and standards used for GC-MS analysis, were purchased from Sigma Aldrich, Chemical CHEME Gmbh, USA. ESI-HRMS/MS (positive ion mode) spectrum was measured on QSTAR XL Applied Biosystem USA. ESI-HRMS/MS (positive ion mode) was measured on QSTAR XL Applied Bio-system USA. GC/MS: JMS 600H (JEOL, Japan) with Agilent 6890 N; EI mode, ionizing potential (70 eV), Agilent column Optima-5MS, (30 m × 250 μm × 0.25 μm), column temperature, 50–250 °C (rate of temperature increase 5 °C/min), carrier gas washelium with flow rate, 1.8 ml/min, split ratio, 1:30 and run time of 90 min. Compounds identified were based on GC/MS studies using two different analytical methods, i.e., retention indices (RI) and mass spectrum. RI was calculated in reference to n-alkanes (C9– C33) (Sigma Aldrich, Germany) using the Kovats retention Index formula [104]. Mass spectra were compared with spectra of authentic compounds and mass spectra literature in National Institute of Standards and Technology (NIST) database (http://webbook.nist.gov/chemistry). Average peak areas were compared to total peak area to obtain the relative percentage amount of each compound.

4.2. Collection of plant material and extraction

Fresh leaves of *O. basilicum* (2 Kg) were collected from Karachi, Pakistan in the month of august 2018. A voucher specimen (GH NO 942382) was deposited in herbarium of department of Botany University of Karachi herbarium of department of Botany University of Karachi which was verified by Taxonomist Dr. Muneeba Khan. Fresh leaves of *O. basilicum* leaves (2 kg) were washed, air dried and sequentially extracted with hexane, dichloromethane, ethyl acetate, acetone and methanol, twice at room temperature for three days.

The extracts were filtered and evaporated under reduce pressure using rotary evaporator at a temperature of 50 °C to obtain a concentrate masses of OBL-H, OBL-DC, OBL-EA, OBL-A and OBL-M. OBL-H and OBL-DC were analyzed through GC-FID and GC-MS spectra. OBL-M was analyzed through ESI-HRMS/MS.

4.3. Total phenolic content

Total phenolic content of hexane, dichloromethane and methanol extracts was determined by slight variations in folin–ciocalteau spectrophotometric method [106]. Gallic acid was used as the standard and the standard curve was plotted using concentration range of 4–40 μg/ml. One ml of extract sample was taken, to which 2.5 ml of folin-Ciocalteau reagent and 2.5 ml of 7.5% Na2CO3 were added. After half an hour of incubation in dark at room temperature, the absorbance was measured at 740 nm against reagent blank. The results were expressed as Gallic acid equivalents.

4.4. Total flavonoid content

An aluminum chloride colorimetric method was used for flavonoids determination [107]. The standard curve was prepared using concentration range of rutin (10–100 μg/ml) in methanol. 0.1 ml of aluminum chloride 10% and 0.1 ml 1 M potassium acetate were added to 1 ml of sample and kept for 5 min and distilled water was used to make the final volume up to 5 ml. The mixture was allowed to stand in dark at room temperature for 5 min and the absorbance of the reaction mixture was measured at 415 nm. The total flavonoid contents were calculated as rutin equivalent.

4.5. DPPH colorimetric assay of extracts of O. basilicum L

To determine DPPH radical scavenging activity method of Meda et al. (2005) was followed with slight variations [105]. Extract samples (0.1–4 mg/ml) were taken in separate test tubes and then mixed with 3.5 ml of DPPH solution. The reaction mixtures were incubated in dark for 30 min. Absorbance was recorded for each sample at 517 nm using methanol as a blank. Ascorbic acid Calibration curve was obtained in the concentration ranges of 0.03mM–0.27 mM, all the observations were obtained in triplicates. Results were expressed as Ascorbic Acid Equivalent Antioxidant Capacity (AAE) g/100 g of dry extract.

The antioxidant activity of the extracts were performed Percentage inhibition was calculated and results were comprehended as IC_{50}.

$$\%\ inhibition = \frac{(Absorbance\ Control - Absorbance\ tect)}{Absorbance\ Control} \times 100$$

4.6. ABTS cation de-colorization assay of extracts of O. basilicum L

Antioxidant activity was determined using ABTS (2,2′-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid)cation de-colorization assay method, described by Alzoreky et al., 2001 with slight variations [108]. A stock solution was prepared by mixing 7 mM of ABTS solution and 2.45 mM of potassium per sulfate at room temperature and the mixture was incubated in dark for 16 h at room temperature to produce a dark-green solution which was then diluted using distilled water to get the absorbance of 0.700 ± 0.02 at room temperature. Freshly prepared working solution was used for the estimation. Extract solutions were prepared in different concentrations and3.5 ml of the extract solution was mixed with 3.5 ml of ABTS solution and incubated for 6 min. Absorbance was then noted at 743 nm, and readings were observed in triplicates. Trolox was used as a standard and results expressed in Trolox Equivalent Antioxidant Capacity (TEAC) g/100 g of dry extract.

Percentage inhibition was calculated as

$$\%\ inhibition = \frac{(Absorbance\ Control - Absorbance\ tect)}{Absorbance\ Control} \times 100$$

Using above mentioned method antioxidant activity of some standards was also determined.

4.7. Oxidative hemolysis inhibition assay (OxHLIA) of extracts of O. basilicum L

The oxidative hemolysis inhibition assay was carried out according to the procedure reported by Takebayashi et al. [109], with some modifications. EDTA was used as anticoagulant and mixed with 3 ml of human blood. The blood sample was then centrifuged at 1500 rpm to separate RBCs (red blood cells) from the plasma using graduated centrifuge tube. The separated RBCs were then washed thrice with phosphate buffer saline (PBS; pH 7.4) and centrifuged each time. Using PBS 7.4, 20% suspension of RBCs was prepared. The reaction mixture was prepared by mixing 1 ml of RBCs suspension, 2 ml of AAPH (200 mM) and 1 ml of each extract. After fixed interval of time, the reaction mixture was then gently shaken during incubation for 3 h at 37 °C. After incubation, the reaction mixture was centrifuged with 8 ml at 1500 rpm for 5 min s. Supernatant was then withdrawn and absorbance was recorded at 540 nm against the blank (PBS 7.4). The percent inhibition was calculated by using the following formula:

$$\%\ \text{Hemolysis inhibition} = \frac{\text{Abs(control)} - \text{Abs (Sample)}}{\text{Abs (control) x 100}}$$

Where, Abs control is the absorbance of control without extract sample. While, Abs Sample is the absorbance of sample containing the fraction of extract. Ascorbic acid was used as standard.

4.8. Anti-hypertensive activity of OBL-M in L-Name induces hypertensive rats

Antihypertensive activity of methanol extract of OBL-M was determined. Fifteen male Sprague-Dawley adult rats (225 g–250 g) were housed in cages and were randomly distributed into four groups: one group was kept as control and received saline water orally; Group 1 received an oral dose of freshly prepared L-NAME (50 mg/kg) and a standard drug Lisinopril (10 mg/kg). Group 2 received L-NAME (50 mg/kg) only and Group 3 received L-NAME. (50 mg/kg) and freshly prepared extract (50 mg/kg). For acclimation, animals were restrained in the restrainers for 10–20 min/day for 7 days prior to recording BP in the tail-cuff method. Animals were maintained at a temperature of 28 °C. Tail was passed through cuff equipped with sensor coupled with an amplifier to amplify pulse signals. The data were recorded using Lab Chart during automatic inflation and deflation of the tail cuff. Record of the animal's body weight was maintained throughout the study. Animals were sacrificed at the end of the study to observe the organ weights. Blood was also collected to record any hematological changes. All the experimental results were analyzed using SPSS20.

5. Experimental animals

Male and female mice weighing 25–30 g were used for the acute toxicology studies. The mice were obtained from the Animal Research Centre, Jinnah University for Women Karachi. The animals were familiarized to laboratory conditions earlier to the experiments for 7 days. The mice were maintained at a room temperature of 28 °C, with a 12 h light/dark cycle. The animals were housed in cages with a standard diet and water. All procedures in this study were performed according to the Animal Ethics Committee, Jinnah University for Women Karachi Pakistan, (JUW/Animal Ethics Approval/2017).

6. Acute oral toxicity study

An acute toxicity test was performed according to the Organization of Economic Co-operation and Development (OECD) guideline 420 for testing of chemicals. Mice of both sexes were used. Extracts were dissolved in 10% Tween 80 and administered orally 5000 mg/kg only once as a single dose. The control group received only 10% Tween 80 as a vehicle. After administration of extracts, mice were observed for 24 h, with special attention given to the first 4 h and once daily further for a period of 14 days. The mice were weighed and visual observations for mortality, behavioral pattern (salivation, fur, lethargy, and sleep), changes in physical appearance, injury, pain, and signs of illness were conducted once daily during the period. At the end of the experiment, the mice were anesthetized through intra-peritoneal injection of ketamine. The organs were removed, weighed, and observed macroscopically. The relative organ weight was calculated. Principal vital organs (liver, kidney, lung, heart, and spleen) were preserved in a fixation medium of 10% solution of buffered formalin for histopathological study.

7. Statistical analysis

All the experimental results were analyzed using SPSS statistical software version.20. All results were expressed as mean ± standard deviation (SD) in at least triplicate and were analyzed using Tukey's and dunnet's multiple comparison tests using one way analysis of variance (ANOVA). The correlation between TPC, TFC, and other activities is presented by Pearson correlation coefficient. Results were considered statistically significant when *P*-values were below 0.05.

Author contribution statement

Fatima Qamar: Performed the experiments; Wrote the paper.
Safila Naveed: Analyzed and interpreted the data.
Aisha Sana: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Shaheen Faizi: Conceived and designed the experiments.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Acknowledgements

Fatima Qamar thankfully acknowledges Jinnah University for women, University of Karachi, Pakistan for providing research opportunity, and H.E.J. Research Institute of Chemistry, ICCBS, University of Karachi, Pakistan for spectroscopic facilities.

Abbriviations

O. basilicum	Ocimum basilicum
GC-MS	Gas chromatography mass spectrometry
ESI-HRMS/MS	Electrospray ionization high-resolution tandem mass spectrometry
OBL-H	Hexane extract of Ocimum basilicum leaves
OBL-DC	Dichloromethane extract of Ocimum basilicum leaves
OBL-EA	Ethyl acetate extract of Ocimum basilicum leaves
OBL-A	Acetone extract of Ocimum basilicum leaves
OBL-M	Methanolic extract of Ocimum basilicum leaves
TPC	Total phenolic contents
TFC	Total flavonoid content
GAE	Gallic acid equivalents
RE	Rutin equivalent
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
L–NAME	NG-Nitro-L-arginine-methyl ester
MABP	Mean arterial blood pressure
ROS	Reactive oxygen species
ACE	Angiotensin-converting enzyme
LD50	Median lethal dose
IC50	Half maximal inhibitory concentration
AAE	Ascorbic Acid Equivalent Antioxidant Capacity
TEAC	Trolox Equivalent Antioxidant Capacity
OxHLIA	Oxidative hemolysis inhibition assay
EDTA	Ethylenediaminetetraacetic acid
RBCs	Red blood cells
PBS	Phosphate buffer saline
OECD	Organization of Economic Co-operation and Development
mg/mL	Milligrams per milliliter
ug/mL	Micrograms per milliliter
mg/kg	Milligrams Per Kilogram

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