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Chemical composition and biological activities of *Nepeta hindostana* (Roth) Haines, *Nepeta graciliflora* Benth. and *Nepeta cataria* L. from India

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ABSTRACT

Background & Aim: In traditional medicine system, *Nepeta* species are widely used to reduce chicken pox, tuberculosis, malaria, pneumonia, influenza, measles, stomach disorders, eye complaints, respiratory disorders, asthma, colds, coughs etc. The aim of the present study was to evaluate the chemical composition and biological activities of the essential oils from three species of genus *Nepeta* viz: *Nepeta hindostana* (Roth) Haines (NHO), *Nepeta graciliflora* Benth (NGO) and *Nepeta cataria* L (NCO).

Experimental: The essential oils were analysed by the combination of GC and GC-MS. Antioxidant activity was tested by using reducing power assay, metal chelating of Fe^{2+} assay, and DPPH radical scavenging assay. *In-vitro* antinflammatory activity was evaluated using albumin denaturation assay and anti-diabetic activity was determined by using α-amylase assay.

Results: The major components present in NCO, NHO and NGO were *cis*-nepetalactone (69.78%), β-farnesene (43.41%) and sesquisabinene (28.75%), respectively. NCO showed the highest percentage inhibition of DPPH radical (IC_{50}=5.89 µl/ml) followed by NHO (IC_{50}=8.63 µl/ml) and then NGO (IC_{50}=13.81 µl/ml). In terms of reducing power assay and metal chelating of Fe^{2+} assay, the highest antioxidant activity was also shown by NCO. Among the tested essential oil, NCO showed highest *in-vitro* anti-inflammatory potential (IC_{50} 18.463±0.14 µg/ml) followed by NGO and NHO with IC_{50} 22.035±0.11 µg/ml and 26.17±0.14 µg/ml, respectively. NHO showed maximum antidiabetic activity with IC_{50} 8.92±0.10 µg/ml of α-amylase.

Recommended applications/industries: On the basis of present research work it is marked that the essential oil of *Nepeta hindostana* (Roth) Haines, *Nepeta graciliflora* Benth. and *Nepeta cataria* L. is a potent antioxidant, anti-inflammatory and anti-diabetic agent indicating their potentiality in the field of food, pharmaceutical and cosmetic industry.

1. Introduction

Reactive oxygen species includes the free radicals like the superoxide anion, singlet oxygen, lipid peroxides and the hydroxyl radical caused the oxidative damage and free radicals also induces severe damage to the biomolecules including lipid, protein, nucleic acids and in severe cases causes coronary diseases like myocardial infarction, neurological disorders, asthma, diabetes, rheumatoid arthritis, cancer aging etc. (Valko *et al.*, 2009; Jeong *et al.*, 2009). Antioxidants are the substances that can prevent or slow damage to cells

caused by free radicals; the most widely used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ). However, there are several published studies mentioning the safety and health issues of using synthetic antioxidants (Botterweck *et al.*, 2000). Similarly, Inflammation is a complex process which is associated with pain and involves occurrences like enhanced protein denaturation, increased vascular permeability and rearrangement of membrane. Chronic pain induced by inflammatory process is a major clinical problem. Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defect in insulin secretion. High blood sugar increases the risk of paralysis, gangrene and several coronary diseases leading to long-term complications. The inhibition of α-amylase is an important therapeutic approach in the management of type-2 diabetes mellitus to reduce the reabsorption of glucose in the intestine (Sim *et al.*, 2010). Consequently, there has been increasing interest in searching for and using natural antioxidant, anti-inflammatory and antidiabetic agents. In present scenario, essential oils and their components are being used for therapeutic conditions as an alternative to synthetic drugs because of their relatively safe status, wide acceptance, efficiency and potential multipurpose functional uses (Oboh *et al*., 2017; Navarra *et al*., 2015; Kumar *et al*., 2019).

Lamiaceae is an important family of flowering plants which is known for its aromatic members, especially the mints and balms with great medicinal value. The family has the cosmopolitan distribution of about 236 genera and 6,900 to 7,200 species (Yuan *et al*., 2010). The genus Nepeta is one of the largest genera of the Lamiaceae family belongs to the subfamily nepetoideae and tribe mentheae. It comprises of about 280 herbaceous, perennial, rarely annual species which are substantially distributed in Himalayas and the Southwestern Asia particularly in Iran, Turkey and Hindu Kush. Iran is considered as the birthplace of the genus Nepeta as about 53% out of 67 species of this genus in Iran are endemic (Asgarpanah *et al*., 2014).

In Indian Himalayas region (Uttarakhand, Jammu & Kashmir, Himachal Pradesh and Ladakh), many cultural groups uses the some Nepata species (N. hindustana (B. Heyne ex Roth) Haines, N. laevigata (D. Don) Hand.- Mazz., *N. leucophylla* Benth., *N. longibracteata* Benth.) as a traditional medicine to reduce fever, cure cold and coughs, relief of pain (ear and tooth pain) and fever, cure dysentery, treat malaria, cure stomach disorders, and various other problems (Bhat *et al*., 2013; Phondani *et al*., 2010; Bist *et al*., 2012; Sharma *et al*., 2021). The enthno-medicinal uses observed in the genus are mainly due to the presence of phytochemical constituents. Several species of the genus in their essential oils have been reported rich in monoterpenes viz; 1,8-cineole, neral, α-citral, α-pinene, linalool etc. (Sharma *et al*., 2021), along with β-caryophyllene (Bist *et al*., 2010; Hassan *et al*., 2011; Joshi and Mathela, 2013), caryophyllene oxide (Rather and Hassan, 2011), germacrene D (Bist *et al*., 1997). The essential oil from aerial part of several Nepeta species are also rich in biologically active compound like nepetalactones that exhibits feline attractant properties as it binds to the olfactory receptor of domestic cats causing temporary euphoria (Herron, 2003; Reichert *et al*., 2010). A diversity of biologically active compounds has been isolated from species of Nepeta, such as phenolic acids and their glycosides (rosmarinic acid, gallic acid, caffeic acid), flavonoids and their glycosides (cirsimaritin, salvigenin, luteolin, apigenin), iridoids (nepetalactones), terpenoids (1,8-cineole, linalool, β-caryophyllene, germacrene D, parnapimaro, β-amyrin, oleanolic acid, ursolic acid), steroids (β-sitosterol, stigmasterol) etc. (Sharma *et al*., 2021). Essential oil of the genus also possesses biological activities like insect repellent, cytotoxic activity, antioxidant activity, analgesic, anti-inflammatory and CNS depressant activity (Peterson *et al*., 2002; Suschke *et al*., 2007; Formisano *et al*., 2011; Hussain *et al*., 2012). The project work is aimed at phytochemical investigation and evaluation of biological activities of Nepeta species: *Nepeta hindostana* (Roth) Haines, *Nepeta graciliflora* Benth. and *Nepeta cataria* L. in their essential oil from two different Indian Himalayas region.

2. Materials and Methods

2.1. Collection of plant material

The plant *Nepeta gracliliflora* Benth. (herbarium no. 05098) was collected in blooming phase from Rajaji national park region, Uttarakhand (30.2385° N, 77.9584° E), while *Nepeta hindostana* (Roth) Haines (herbarium no. 47270) was also collected in blooming phase from near Forest Research Institute region

Dehradun, Uttarakhand (30.3438° N, 77.9996° E). Both the plant material was identified by Dr. H.B Naithani, botanist, Forest Research Institute Dehradun and authenticated by Dr. D. S. Rawat, Assistant Professor (Plant Taxonomist), Department of Biological Sciences, College of Basic Science and Humanities, Pantnagar. The *Nepeta cataria* L. was obtained from Jammu by the courtesy of Dr. N. Mengi, retired Scientist CIMAP, CSIR.

2.2. Isolation of essential oil

Essential oil from aerial parts of *Nepeta graciliflora* Benth, *Nepeta hindostana* (Roth) Haines and *Nepeta cataria* L. were isolated by hydrodistillation in a Clevenger-type apparatus (Clevenger, 1928). The plant material (500 g) was crushed and hydrodistilled for 3 h. Essential oils were collected, extracted with hexane and desiccated over Na_2SO_4. The obtained oils were stored in amber glass vials at 4 °C until use.

2.3. GC-MS analysis of essential oils

The essential oils were analysed on GC/MS-QP2010, Ulta DB-5, with column (30 m×0.25 mm; 0.25 µm). The column temperature was programmed for 60-210 ºC at the rate of 3 ºC/min and then again up to 280 ºC at the rate of 8 ºC/min and then hold upto11min. Helium gas at the rate of 1.21 ml/min was used as the carrier gas. The injector temperature was 210 ºC. MS were recorded under EI condition (70 ev) with injection volume of 0.1 µl with split mode of 1:100. Identification of the constituents of the essential oils done by comparing their mass spectra fragmentation pattern and their retention indices with that of MS library (NIST14.lib, FFNSC2.lib, WILEY8.LIB) and comparing the spectra with literature data (Adams, 2007).

2.3. In-vitro antioxidant activity

2.3.1. DPPH (2,2-diphenyl-2-picrylhydrazyl) scavenging activity

The free radical scavenging activity of the essential oils were performed according to the method developed earlier and are being practiced (Kumar *et al*., 2012; Goswami *et al*., 2019; Lu *et al*., 2011). The assay mixture contained 5 ml of 0.004% methanolic solution of DPPH and different amount of test sample solution of different concentrations (5-25 µl) and kept in dark for half an hour for incubation and absorbance was taken at 517 nm (Thermo fischer, evolution 101). The % inhibition of DPPH free radical was calculated by using the formula:

$$IC\% = (A_0 - A_t)/A_0 \times 100$$

Where, A_0 = absorbance value of control sample, A_t = absorbance value of test sample, IC = inhibitory concentration.

The standard curve was drawn using catechin as standard antioxidant to calculate the IC_{50} values for different concentrations of essential oils.

2.3.2. Metal chelating activity

The metal chelating activity of Fe^{2+} of essential oils was measured as per the prescribed protocol by Gairola *et al*. (2021). Reaction mixture consisting of 0.1 ml (2 mM) $FeCl_2.4H_2O$, 0.2 ml (5 mM) ferrozine and 4.7 ml of methanol was added to different concentration of essential oil (5-25 µL) and was incubated for half an hour. The absorbance was taken at 562 nm. Na_2EDTA was used as the standard antioxidant. The metal chelating activity was evaluated by using the formula:

$$IC\% = (A_0 - A_t)/A_0 \times 100$$

Where, A_0 = absorbance value of control sample, A_t = absorbance value of test sample, IC = inhibitory concentration.

2.3.3. Reducing power activity

The reducing power of oils was evaluated by the method developed earlier and is being practiced (Parki *et al*., 2017; Palariya *et al*., 2019).Varying concentrations of essential oil (5-25 µl) were mixed with 2.5 ml of phosphate buffer (200 mM, pH= 6.6) and 2.5 ml of 1% potassium ferricyanide, K3[FeCN6]. After 20 minute incubation at 50±1ºC, 2.5 ml of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 RPM for 10 min. The upper layer (1 ml) was mixed with 5ml distilled water and 1 ml of 0.1% ferric chloride and absorbance of the resultant solution were measured at 700 nm. All the readings were taken as triplicate and BHT was used as the standard. The reducing power of samples was calculated using the following formula:

$$RP\% = (A_0 - A_t)/A_0 \times 100$$

where, A_0 = absorbance value of control sample, A_t = absorbance value of test sample, RP%= Reducing power.

2.4. In-vitro antinflammatory activity

In-vitro anti-inflammatory activity was screened as per the developed protocol along with minor adjustments in the protocol being practiced (Kar *et al*., 2012; Dhami *et al*., 2019). The reaction mixture consisting of essential oil (10-50 µl), 100 ppm (200 µl) fresh albumin protein, 2.8 ml of freshly prepared phosphate buffered saline (PBS) of pH 6.4 and make up the final volume to 5 ml. The solution was kept in incubation at 37 ºC for 15 min and then at 70 ºC for 5min. After cooling the absorbance was measured at 660 nm. Diclofenac sodium of various concentrations was used as standard. The percent inhibition was calculated by the formula:

$$IB\% = (A_0 - A_t)/A_0 \times 100$$

Where, A_0 = absorbance value of control sample, A_t = absorbance value of test sample, IB = inhibitory concentration. The oil/drug concentration for 50% inhibition (IB_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

2.5 Anti-diabetic activity

The antidiabetic activity of NHO, NGO and NCO was determined by using α-amylase assay as described previously with slight modifications (Narkhede *et al*., 2011; Nazir *et al*., 2021). Different concentration of NHO, NGO, NCO and standard drug, Acarbose (10-50 µg/ml)were added to a reaction mixture containing 500 µl of 0.20 mM phosphate buffer (pH 6.9) and α-amylase (0.5 mg/ml) and then incubated at 25ºC for 10 min. 500µl of starch solution was added to the above solution and then incubated at room temperature for 10 min. Then 1 ml of 3,5- Dinitrosalicylic acid (DNSA) solution was added to the mixture and placed in a boiling water bath for 5 min and cooled at room temperature. The reaction mixture was diluted by adding 10 ml of distilled water and absorbance was taken at 540nm wavelength in a UV spectrophotometer. The percentage inhibition was calculated using the following formula:

$$\text{Inhibition of α-amylase activity (\%)} = (A_0 - A_t)/A_0 \times 100$$

Where, A_0 = absorbance value of control sample, A_t= absorbance value of test sample.

2.6. Statistical analysis

All the experiments were conducted in three replicates and the data were expressed in terms of mean± standard deviation. Data illustrated in the tables and the graphs were subjected to ANOVA at 1% level of significance ($P<0.01$) for *in-vitro* antioxidant activity, anti-inflammatory and antidiabetic activity. Data analyzed were found to be significantly different at the respective level of significance.

3. Results and discussion

3.1. Chemical composition (%) of essential oil NCO, NHO and NGO collected from two different regions (Uttarakhand and Jammu) of India

The yields (v/w) of essential oils were found 0.3% (NHO), 0.1% (NGO) and 0.3% (NCO), respectively. The GC-MS analysis NCO (collected from Jammu) revealed the presence of over thirteen compounds. The major constituent found was *cis*-nepetalactone accounting 69.7 % of the total oil composition. The other main constituents identified were bicyclo [3.1.0] hexane-2-undecanoic acid, methyl ester (14.0%), (*cis*-,*trans*) nepetalactone (10.4%), 1-methyl-1-(2-methyl-1-propenyl)cyclopentane (0.84%), β-pinene oxide (0.5%), α-pinene (0.4%). Dehydronepetalactone was identified to be present very low (0.7%) in amount. Gilani *et al*. (2009) investigated the essential oil of *Nepeta cataria* L. from North Western Pakistan and reported 27 identified compounds. The major components reported were 1,8-cineol (21.0%), humulene (14.4%), α-pinene (10.4%), geranyl acetate (8.2%) and β-caryophyllene (6.3%). The major difference was the absence of 1,8-cineol, geranyl acetate, β-caryophyllene in NCO, which were the major compounds as reported by Gilani *et al*. (2009).

The GC-MS analysis of NHO revealed the presence of over forty three compounds from Garhwal region of Uttarakhand. The essential oil was found to be rich in sesquiterpenoids with β-farnesene (43.4%) as major identified component of the essential oil. The other major constituents (>1.0%) present were (*E*)-caryophyllene (19.5%), α-bergamotene (5.7%), β-caryophyllene epoxide (4.9%), β-bisabolene (3.3%), α-bisabolene (3.0%), nerolidol (2.6%), β-sesquiphellandrene (2.4%) *cis*-limonene oxide (2.0%), *cis*-α-bisabolene (2.0%), *trans*-β-bergamotene (1.5%), and limonene dioxide (1.2%). Kumar *et al*. (2019)

reported the presence of β-caryophyllene (15.6%), caryophyllene oxide (6.9%), δ-cadinene (39.8%) and 2,3-dihydrofarnesol (6.4%) as the major components in the essential oil from aerial part of *Nepeta hindostana* from Kumaun region of Uttarakhand. Uniquely it was observed that the essential oil composition of *N. hindostana* collected from Garhwal region has different chemical makeup, than that of Kumaon region Kumar *et al*. (2019).

Table 1. Chemical composition (%) of essential oil NCO, NHO and NGO collected from two different regions (Uttarakhand and Jammu) of India**.**

S.N.	Compounds	KI	Contribution %		
			Jammu	Uttarakhand	
			NCO	NGO	NHO
1	1-octene-3-ol	910	-	0.1	0.1
2	α-pinene (MT)	936	0.4	-	-
3	β-pinene oxide (OM)	943	0.5	-	-
4	2-methyl oct-3-yne	981	-	t	-
5	β-myrcene (MT)	991	-	-	t
6	1-(1-cyclohexen-1-yl)-ethanone	1021	-	-	t
7	1-acetylcyclohexene	1023	-	0.6	-
8	*p*-cymene (MT)	1025	0.1	-	-
9	(*Z*)-β-ocimene (MT)	1037	-	-	0.2
10	α-ocimene (MT)	1050	-	-	0.4
11	α-cumyl alcohol (OM)	1080	-	t	-
12	α-terpinolen (OM)	1088	-	-	0.3
13	3-thujanol	1100	-	0.8	-
14	linalool (OM)	1101	-	t	-
15	nonanal	1103	-	0.1	-
16	1-octen-3-yl acetate	1112	-	0.1	0.1
17	myrcenol (OM)	1124	-	0.2	-
18	1,2 dimethyl-2-norbornanol	1125	-	0.9	-
19	limona ketone	1131	-	t	-
20	dehydrolinalool (OM)	1133	-	0.4	0.1
21	*cis*-limonene oxide (OM)	1144	-	1.2	2.0
22	α-terpineol (OM)	1175	-	-	t
23	1-terpinen-4-ol (OM)	1177	t	-	-
24	neodihydrocarveol (OM)	1198	0.1	-	-
25	β-cyclocitral (OM)	1223	-	0.2	0.7
26	thymol methyl ether (OM)	1235	0.8	-	-
27	6-camphanol acetate	1242	-	-	t
28	hexadecadien-(11z,13z)-al	1243	-	-	0.3
29	2-norbornanol, 1,2-dimethyl-	1246	-	-	0.1
30	bornyl acetate (OM)	1287	-	0.1	-
31	menthyl acetate (OM)	1297	-	t	-
32	2-methyl-10-undecenal	1328		0.2	
33	*cis*-, *trans*-nepetalactone (4aα,7β,7aα –nepetalactone) (OM)	1337	10.4	-	-
34	α-longipinene (ST)	1352	-	-	0.2
35	*epoxy*-α-terpenyl acetate (OM)	1352	-	-	0.2
36	***cis*-nepetalactone (4a-α,7-α,7a-α nepetalactone)** (OM)	**1360**	**69.7**	-	-
37	α- copaene (ST)	1374	-	0.9	0.2
38	β-elemene (ST)	1390	-	-	0.3
39	β-longipinene (ST)	1400	-	-	0.1
40	*Z*-caryophyllene (ST)	1404	-	0.1	-
41	α.-bergamotene (ST)	1412	-	0.1	5.7
42	damascenone A (OM)	1414	-	t	-
43	β-caryophyllene (ST)	1418	-	2.5	-
44	*(E)*-caryophyllene (ST)	1430	0.5	-	19.5
45	β-copaene (ST)	1432	-	0.3	-
46	*trans*-β-bergamotene (ST)	1434	-	1.8	1.5
47	α-farnesene (ST)	1445	-	6.4	-
48	**β-farnesene** (ST)	**1453**	-	-	**43.4**
49	α-humulene (ST)	1454	t	2.4	-
50	germacrene D (ST)	1485	-	t	0.4
51	β-bisabolene (ST)	1505	-	3.7	3.3
52	*cis*-α-bisabolene (ST)	1506	-	-	2.0
53	α-bisabolene (ST)	1507	-	-	3.0
54	sesquicineole (OS)	1515	-	0.1	-

55	β-sesquiphellandrene (ST)	1522	-	1.7	2.4
56	β-sesquisabinene hydrate (ST)	1524	-	-	0.1
57	limonene dioxide 1 (OM)	1526	-	-	1.3
58	dehydronerolidol (OS)	1562	-	0.2	-
59	nerolidol (OS)	1563	-	0.4	2.7
60	tridec-(2e)-en-1-ol	1570	-	-	0.1
61	*epi*-longipinanol (OS)	1575	-	0.4	-
62	caryophyllene oxide (OS)	1579	0.1	10.2	0.1
63	β-caryophyllene epoxide (OS)	1580	-	-	5.0
64	(*Z*,*E*)-farnesol (OS)	1584	-	9.4	-
65	**sesquisabinene (ST)**	**1590**	**-**	**28.7**	**-**
66	(*Z*)-sesquilavandulol (OS)	1607	-	0.2	0.2
67	humulene epoxide (OS)	1608	-	4.7	-
68	(*E*)-sesquilavandulol (OS)	1632	-	-	0.1
69	dodecatrien-1-ol 6,11-dimethyl-2,6,10-	1634	-	0.3	-
70	α-cadinol (OS)	1640	-	-	t
71	cubenol (OS)	1645	-	0.1	-
72	α-bisalolol oxide (OS)	1658	-	0.6	-
73	turmerone (OS)	1669	-	0.2	-
74	farenal	1670	-	0.1	-
75	*trans*-*Z*-α -bisbolene epoxide (OS)	1675	-	0.3	-
76	guaia-3,10(14)-dien-11-ol (OS)	1680	-	t	-
77	*E*,*E*-farnesol (OS)	1692	-	0.1	-
78	neral (OS)	1694	-	0.1	-
79	β-sinensal (OS)	1699	-	t	-
80	*E*-apritone (OS)	1708	-	0.2	-
81	2*Z*,6*E*-farnesol (OS)	1715	-	0.1	-
82	1-hexadecanal	1734	-	0.1	-
83	farnesene epoxide (OS)	1738	-	0.5	-
84	8- α-11-elimodiol	1741	-	2.1	-
85	β-acoradienol (OS)	1763	-	0.1	-
86	curcumen-15-al (OS)	1768	-	0.9	-
87	β-bisabolenal (OS)	1769	-	0.1	-
88	γ-curcumen-15-al (OS)	1775	-	0.6	t
89	β-bisabolenol (OS)	1789	-	1.1	0.2
90	lanceol acetate (OS)	1795	-	0.9	-
91	α-bisabolol acetate (OS)	1798	-	-	0.4
92	(*E*)-phytol (OD)	1949	-	1.0	0.6
93	ledane	1958	-	-	t
94	palmitic acid	1970	-	-	0.1
95	heptadecanoic acid	2074	-	2.5	-
96	phytol acetate (OD)	2218	-	0.1	t
97	tricosane	2300	-	1.1	-
98	(*Z*)-lanceol (OS)	2518	-	2.0	-
99	dehydronepetalactone (OM)	-	0.7	-	-
100	bicyclo[3.1.0]hexane 2 undecanoic acid, methyl ester	-	14.0	-	-
	Monoterpene hydrocarbon (MT)		**0.5**	**0.0**	**0.6**
	Oxygenated monoterpene (OM)		**82.2**	**2.4**	**4.7**
	Sesquiterpene hydrocarbon (ST)		**0.6**	**48.7**	**82.2**
	Oxygenated sesquiterpene (OS)		**0.1**	**33.6**	**8.7**
	Oxygenated diterpene (OD)		**-**	**1.1**	**0.6**
	Others		**14.0**	**9.1**	**0.8**
	Total		**97.4**	**94.9**	**97.6**

KI: Kovat index, t: trace amount (less than 0.1%), NCO= *Nepeta cataria* L. essential oil, NGO= *Nepeta graciliflora* Benth. essential oil, NHO= *Nepeta hindostana* (Roth) Haines essential oil

The GC-MS analysis of NGO (collected from Uttarakhand) revealed the presence of sixty four compounds The major constituents identified were sesquisabinene (28.7%), caryophyllene oxide (10.1%), (*Z*, *E*) farnesol (9.4%), α-farnesene (6.4%), humulene epoxide (4.7%), β-bisbolene (3.6%), heptadecanoic acid (2.5%), α-humulene (2.4%), 8-α11-elimodiol (2.1%), β-sesquiphellandrene (1.6%), limonene oxide (1.2%), *trans*-β-bergamotene (1.8%), tricosane (1.1%) and β-bisabonelol (1.1%). The other compounds identified were present in low quantities (<1%). Sharma *et al.* (2015) reported the presence of twenty seven components in *Nepeta graciliflora* Benth. accounting 91.44% of the total oil composition from Himanchal Pradesh. Octenyl acetate, 1- acetyl cyclohexane, limonene oxide, α-copaene, α-bergamotene, β-caryophyllene, germacrene-D, sesquicineole, α-zingiberene, β-sesquiphellandrene,

caryophyllene oxide and humulene epoxide were reported in the sample. Comparing the results it was observed that β-sesquiphellandrene identified in our sample from Garhwal region (Uttrakahand) was lower (1.6%) in quantity than that of reported (28.5%) by Sharma *et al.* (2015) from Himanchal Pradesh.

NCO was found to be possess high content of oxygenated monoterpenoid (81.4%) whereas, NGO and NHO were potentially rich in sesquiterpenoid (49.2%) and (82.2%), respectively. The major compound present in NCO was *cis*-nepetalactone contributing about 69.7% of the total oil composition while in NGO and NHO the major compounds were sesquisabinene (28.7%) and β-farnesene (43.4%), respectively. These chemical differences and similarities might be due to chemotaxonomic differences among plant species, their intra-specific origin and different regions of origin. It has also been reported that the essential oil of *Nepeta* species have complex compositions which varies due to genotypic/chemotypic variations, climatic and ecological conditions.The plant parts and vegetative cycle state also plays significant role (Asgarpanah *et al.*, 2014). It has been observed that within the Indian subcontinent itself there is significant variations in chemical compositions of essential oils of *Nepeta* species growing from Kashmir to North west regions (Bist *et al.*, 2010; Hassan *et al.*, 2011; Joshi and Mathela, 2013; Rather and Hassan, 2011; Thappa *et al.*, 2001) (Table 2). Thus, the results are of chemotaxonomic significance and indicative of rich chemodiversity among *Nepeta* species.

Table 2. Chemotypic difference in essential oils of some *Nepeta* species growing in Uttarakhand and Kashmir regions of India

***Nepeta* species**	**Uttarakhand Region, (Major constituent %)**	**[Ref.]**	**Jammu & Kashmir Region, (Major Constituent %)**	**[Ref.]**
N. discolor	1-8 cineole (25.5), β-caryophyllene (18.6), *p*-cymene (9.8)	Bisht *et al.*, 2010	α-pinene (15.5), β-Pinene (12.6), linalyl acetate (12.3)	Thappa *et al.*, 2001
N. govaniana	isoiridomyrmecin (35.2), pregeijerene (20.7)	Bisht *et al.*, 2010	4aα, 7α, 7aα nepetalactone (25.9), germacrene D (20.5), β-lemene (17.5)	Thappa *et al.*, 2001
N. clarkei	iridodial β-monoenol acetate diastereomers (25.3), β-sesquiphallandrene (22.0), germacrene D (13.0), α-guaine (10.0)	Bisht *et al.*, 2010	caryophyllene oxide (14.1), kaur-16-ene (36.6), pimara 7, 15 -diene -3-one (19.7), pimara-7, 15 -diene -3-ol (4.0)	Rather and Hassan, 2011
N. elliptica	(7*R*)-*trans*,*trans*-nepetalactone (83.4)	Bisht *et al.*, 2010	β-lemene (23.4), α-Humulene (11.8), bicyclogermacrene (13.1)	Hassan *et al.*, 2011
N. laevigata	1,8-cineole (11.1), β-caryophyllene (5.7), caryophyllene oxide (15.2), pimaradiene (4.7)	Joshi *et al.*, 2010	4aα,7α, 7aαNepeta lactone (2.0), β-citronellol (16.5), β-bourbonene (4.5), β-caryophyllene (10.8), germacrene D (19.4), α-bisabolol oxide (12.4), α-bisabolol (5.3)	Hassan *et al.*, 2011

3.2 In-vitro antioxidant activity

3.2.1. DPPH radical scavenging activity

DPPH free radical assay is based on electron-transfer that produces a violet solution in ethanol. This free radical is stable at room temperature and when it comes in contact with an antioxidant molecule, it is reduced and changes its colour. Hence DPPH assay is an easy way to determine antioxidant activity by using spectrophotometer. In the present study DPPH assay was carried out to determine the antioxidant properties of essential oils. The highest antioxidant activity was possessed by NCO (IC_{50}=5.89±0.00 µg/ml) followed by NHO (IC_{50}=8.63±0.02 µg/ml) and then NGO (IC_{50}=13.81±0.1 µg/ml) (Table 3). However, no essential oils showed maximum antioxidant potential than the synthetic antioxidant catechin (IC_{50}=2.07±0.01 µl/ml).

3.2.2. Metal chelating activity of Fe^{2+}

The chelating reagent ferrozine used as chelator in the given reaction system. The ferrozine forms complexes with the free Fe^{2+} ions. In the presence of chelating agent, the complex formation between the ferrozine and Fe^{2+} ions is disrupted, which results the change in the colour of the complex. By measuring the colour reduction, it allows us to estimate the metal chelating activity of the chelator (Pal *et al.*, 2011). NCO (9.80±0.09 µg/ml) showed maximum chelating power and NGO (25.28±0.05 µg/ml) possessed least chelating power. The decreased chelating power in different oils was observed in the order: NCO (9.80±0.09 µg/ml) > NHO (14.72±0.02 µg/ml) > NGO (25.28±0.05 µg/ml), compared to the standard antioxidant EDTA (IC_{50}=5.56±0.07 µg/ml). The observed dose dependant chelating powers in oils and standard antioxidant has been recorded in Table 3**.**

Table 3. Antioxidant activity in terms of IC_{50} values for essential oil of Nepeta species**.**

Sample and standards	DPPH radical scavenging activity	Metal chelating activity of Fe^{2+}	Reducing power Activity
NHO	8.63±0.02	14.72±0.02	10.18±0.07
NCO	5.89±0.00	9.80±0.09	9.58±0.01
NGO	13.81±0.11	25.28±0.05	12.02±0.01
Catechin	2.07±0.01	-	-
Na_2-EDTA	-	5.56±0.07	-
BHT	-	-	3.41±0.10

NCO= *Nepeta cataria* L. essential oil, NGO= *Nepeta graciliflora* Benth. essential oil, NHO= *Nepeta hindostana* (Roth) Haines essential oil, Na_2-EDTA= Disodium salt of ethylenediaminetetraacetic acid, BHT= Butylated hydroxyl toluene

3.2.3. Reducing power Activity

The ferric reducing antioxidant power (FRAP) method was used for assessing "antioxidant power." *In* this method reduction of yellow colored ferric ions (Fe^{3+}) to bluish green ferrous ions (Fe^{2+}) complex took place at low pH (acidic medium). NCO showed highest antioxidant activity (RP_{50}= 9.58±0.01 µg/ml) followed by NHO (RP_{50}=10.18±0.07 µg/ml). Reducing power of NCO, NHO and NGO was found in the order: NCO> NHO> NGO. However, none of the oils exhibited higher reducing power activity than the synthetic antioxidant that is used as a standard, BHT (RP_{50}= 3.41±0.10 µg/ml) (Table 4).

Table4. *In-vitro* anti-inflammatory activity in terms of IB_{50} values for essential oil of *Nepeta* species**.**

Sample and standards	IB_{50} value
NHO	26.17±0.14
NCO	18.46±0.10
NGO	22.03±0.10
Diclofenac sodium	18.91±0.2

NCO= *Nepeta cataria* L. essential oil, NGO= *Nepeta graciliflora* Benth. essential oil, NHO= *Nepeta hindostana* (Roth) Haines essential oil.

Literature survey revealed that essential oils of genus *Nepeta* viz: *N. nuda, Nepeta faassenii, N. cataria, N. clarkei, N.hindostana, N. erecta, N. govaniana, N. laevigata, N. bulgaricum, N. transcaucasica, N. menthoides* etc. and constituents (nepatolactones, caryophyllene, caryophyllene oxide, α-pinene, β-farnesene, sesquisabinene hydrate etc.) presents in the essential oils of *Nepeta* species and other plants possess good antioxidant potential (Bist *et al*., 2012; Sharma *et al*., 2021; Jianu *et al*., 2021; Panday *et al*., 2015). The highest antioxidant potential of NCO may be due to the richness of biologically active secondary metabolite nepetalactone (69.7%) in the essential oil. Previous study has also revealed the highest percentage of nepetalactone in the essential oil possessing good antioxidant activity (Ashrafi *et al*., 2019; Adiguzel *et al*., 2009). The good antioxidant activity of NCO essential oil might also be due to the synergistic effect of major, minor constituents of the essential oil.

3.3 In-vitro Anti-inflammatory activity

NCO, NGO and NHO exhibited potential to inhibit protein denaturation at all concentrations. NGO showed highest inhibitory activity with IB_{50} 22.033±0.11 µg/ml followed by NHO and NCO with IB_{50} 27.07± 0.18 µg/ml and 31.22 ± 0.84 µg/ml, respectively (Table 4). Essential oils/extracts/ bioactive components (phenolics, glycosides, flavonoids, terpenoids, fatty acids & steroids) of various species of *Nepeta* (*N. atlantica, N. tuberosa, N. caesarea, N. cataria, N. clarkei, N. crispa, N. granatensis, N. italic, N. parmiriensis, N. sibthorpii, N. pogonosperma, N. tuberose, N. asterotricha, N. angustifolia*) exhibited analgesic, antipyretic and anti-inflammatrory activity (Sharma *et al*., 2021). Compounds like α-pinene and β-caryophyllene has been reported to possess anti-inflammatory activity (Kim *et al*., 2015; Öztürk and Özbek, 2011). These compounds are also reported in

the chemical composition of *Nepeta sp*. essential oil. In *N. cataria* essential oil, the anti-inflammatory activity may be due to the presence of nepetalactone which has been also reported in the previous studies (Ricci *et al.*, 2010).

3.4. Anti-diabetic activity

Acarbose (standard drug) at a concentration of 50 µg/ml showed 85.72% inhibitory effects on the α-amylase activity with an IC_{50} value 13.72±0.21 µg/ml. Essential oil of NHO exhibited highest inhibitory effects with an IC_{50} 8.92±0.10 µg/ml followed by NCO and NGO with IC_{50} 14.61±0.08 µg/ml and 15.60±0.29 µg/ml, respectively (Table 5). Methanolic extract of *Nepeta hindostana* has been also reported to possess anti diabetic activity against streptozotocin-induced diabetes model (Devi and Singh, 2016). The *in vitro* anti-diabetic activity of essential oil of *Nepeta hindostana* (Roth) Haines, *Nepeta graciliflora* Benth. and *Nepeta cataria* L. has not been reported previously. Anti-diabetic activities of essential oils from three *Nepeta* species can mainly be attributed to the active major compounds present in them. However, minor constituents as well can have a synergic role in the total activity of the oil.

Table 5. Anti-diabetic activity of essential oil of *Nepeta* species in terms of IC_{50}.

Sample and standards	IC_{50} value
NHO	8.92±0.10
NCO	14.61±0.08
NGO	15.60±0.29
Acarbose	13.72±0.21

NCO= *Nepeta cataria* L. essential oil, NGO= *Nepeta graciliflora* Benth. essential oil, NHO= *Nepeta hindostana* (Roth) Haines essential oil.

4. Conclusion

The *Nepeta* species are aromatic and medicinal herbs which provide us a rich source of bioactive essential oils and biological active secondary metabolites. On the basis of present research work it is marked that the essential oil of *Nepeta hindostana* (Roth) Haines, *Nepeta graciliflora* Benth. and *Nepeta cataria* L. is a potent antioxidant, anti-inflammatory and anti-diabetic agent indicating their potentiality in the field of food, pharmaceutical and cosmetic industry.

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