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Mosla dianthera (Buch.-Ham. ex Roxb.) Maxim: Chemical compositions, biochemical assay, *in vitro* antioxidant and anti-inflammatory activity of chloroform extract

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Abstract
Mosla dianthera Maxim. is an annual aromatic plant of family Lamiaceae used in traditional medicine and as spice in foods. The present study is on the investigation of chemical compositions, biochemical assay, antioxidant and *in vitro* anti-inflammatory activities of the chloroform extract of *Mosla dianthera.* The GC-MS analysis led to identification of fifty five compounds contributing 84.6% of the total extract of which tetrapentacontane (21.8%), 7-methoxy-5-hydroxyflavone (8.1%) and hexatriacontane (6.6%) were identified as the major components. The extract possessed good quantity of total phenolics (19.01±0.25 mg/g of GAE) flavonoids (41.04±0.13 mg/g of CNE) and orthodihydric phenols (7.43±0.92 mg/g of CLE). The *in vitro* antioxidant activity in term of DPPH radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, superoxide radical scavenging, reducing power and metal chelating activity in term of IC_{50}/RP_{50} values were found to be IC_{50}= 169.56±0 µg/mL, IC_{50}= 124.66±0.79 µg/mL, IC_{50}= 144.78±0.11 µg/mL, IC_{50}= 132.44±0.06 µg/mL, RP_{50}=204.21±0.75 µg/mL and IC_{50}= 231.81 ±0.14 µg/mL respectively. The extract also exhibited a potential source of *in vitro* anti-inflammatory activity with IC_{50}= 120.83±0.73 µg/mL.

Keywords: *Mosla dianthera*, biochemical assay, tetrapentacontane, flavonoid, antioxidant, anti-inflammatory

Introduction
Lamiaceae Martinov (=Labiatae Juss.) is one of the largest plant families among the flowering plants (Angiosperms) and many species of this family are highly aromatic due to the presence of external glandular structures that produce volatile oil [1]. This family contains about 236-240 genera and 6,700-7,200 species [2, 3]. *Mosla* (Benth.) Buch.-Ham. ex Maxim. is one of the important genera of this family consisting about 10-13 species in the world [3,4]. In India particularly from Uttarakhand the only one reported species of this genus is *Mosla dianthera* (Buch.-Ham. ex Roxb.) Maxim [5, 6]. It is one of the important aromatic and medicinal species growing in Caucasus, Himalaya, East and South-East Asia. It has been used as a spice in foods due to its characteristic aroma and as a medicinal plant against colds, headaches, and intestinal and skin diseases [7, 8]. Traditionally this herb is used as the antimicrobial, antiviral, anti-allergic, anti-inflammatory and diaphoretic agent for the treatment of cough, cold, fever, headache, bronchitis, sore throats and scabies [9, 10]. In Vietnam this species is used in traditional medicine for the treatment of some common diseases like as dyspepsia, diarrhoea, and epidermophytosis [11]. There are very few (negligible) reports in the literature which focused on chemical composition of the whole herb of *Mosla dianthera* [12]. The aqueous extract of this plant has been reported to decrease immediate-type allergic reaction and tumour necrosis factor-α production [13] and inhibition of mast cell-mediated allergic reactions [10]. There is scarcely any literature available on the biochemical assay, *in vitro* antioxidant and anti-inflammatory activities on the extract of *Mosla dianthera* till date. Similarly, the literature search did not reveal any report on these aspects of *Mosla dianthera* from India and particularly from Uttarakhand. Therefore, the present study was aimed to investigate the chemical composition and to evaluate biochemical assay, *in vitro* antioxidant potential and anti-inflammatory activity of chloroform extract of the aerial part of *Mosla dianthera*.

Materials and Methods

Plant material: The aerial parts of the plant were collected from forest along Lalkuan road, Nainital, Uttarakhand, India (29°02′50.2″ N, 79°30′50.4″E, ~250 m a.s.l.) in the month of September 2017. The plant material was taxonomically identified by one of the author (DSR) and voucher specimen (Acc. No. -GBPUH-981/25.10.18) has been submitted in the herbarium of Department of Biological Sciences, College of Basic Science and Humanities, Pantnagar (Uttarakhand).

Preparation of extract: Shade dried powder of the aerial part of *Mosla dianthera* (≈865 g) was subjected for extraction in chloroform by Soxhlet apparatus. The resultant extract was concentrated to dryness under reduced pressure using a rotary vacuum evaporator to yield concentrated crude extract. The crude extract collected and preserved until used for further studies.

Chemical composition: The GC-MS analyses of the plant extract was performed using GCMS-QP 2010 Ultra instrument in the following conditions: column- DB-5 silica capillary column (30m×0.25mm and film thickness 0.25µm), carrier gas- helium, column flow- 1.21 mL/min, injection temperature- 260 °C, injection mode- split, pressure- 73.3 kPa, split ratio- 20.0, ion source temperature- 210 °C. The oven temperature was initially programmed at 60 °C for 3 minutes then raised to 250 °C at a rate of 7 °C/minutes, held it for 5 minutes then again raised to 280 °C at a rate °of 20 °C/min and held it for 29 minutes. The constituents of extract were identified by matching their mass spectra with those in NIST-MS, FFNSC Wiley Library and comparing the data with literature reports and GC retention indices [14].

Biochemical assay: The biochemical assay of chloroform extract of *Mosla dianthera* was studied quantitatively by spectrophotometer in terms of total phenolics, flavonoids and orthodihydric phenols.

Total Phenolic content: The total phenolic assay was determined using the Folin-Ciocalteu method [15] with slight adjustment. 0.1 mL of the extract sample of 1 mg/mL concentration was mixed with 0.4 mL of 80% methanol, 8 mL of distilled water and 0.5 mL of 1 N Folin-Ciocalteu reagent. After 5 minutes, 1 mL of saturated Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance of test sample was measured at 650 nm in a UV-visible spectrophotometer (Thermo Scientific Evolution 201 series). The same procedure was repeated for the standard solutions of gallic acid and the calibration curve was obtained. The total phenolics in extract were read from the calibration curve and expressed in terms of gallic acid equivalent (mg of gallic acid/g of extract).

Total flavonoid content: Aluminium chloride colorimetric assay [16] was applied for the estimation of flavonoids. 10 mg of extract was dissolved in 10 mL of 80% methanol to prepare stock solution. 0.1 mL of stock solution was mixed with 1.25 mL of distilled water and 750 µL of 5% $NaNO_2$ and incubated for 5 minute followed by the addition of 150 µL of 10% $AlCl_3$ to the mixtures. After 6 minute 500 µL of 1 N NaOH and 275 µL of distilled water was added and the absorbance of the mixture was recorded at 510 nm using UV-visible spectrophotometer. The standard curve for total flavonoids was made using catechin standard solutions under the same procedure. The total flavonoids content were expressed as catechin equivalents in mg per g extract.

Orthodihydric phenols: 0.1 mL of the extract (1000 ppm stock solution) was mixed with 0.4 mL of water, 1 mL of 0.05 N HCl, 1 mL of Arnow's reagent (10 g sodium nitrite and 10 g sodium molybdate were mixed in 100 mL of distilled water), 10 mL of water and 2 mL of 1 N NaOH. The absorbance of mixture was measured at 515 nm using UV-visible spectrophotometer. Standard curve of catechol solution was prepared using the similar procedure. The total orthodihydric phenol were expressed as mg catechol equivalent/g extract sample [17, 18].

***In vitro* antioxidant activity**

DPPH (2, 2-diphenyl-2-picrylhydrazyl) Radical scavenging activity: DPPH radical scavenging activity was evaluated according to the method developed earlier and are being practiced [19-21]. 1 mL of different concentrations of sample (50-250 µg/mL) were mixed with 5 mL of 0.004% methanol solution of DPPH and kept in dark for incubation for 30 minutes. The absorbance was measured at 517 nm in a UV-visible spectrophotometer using BHT (butylated hydroxyl toluene) and catechin as the standard. The % DPPH radical scavenging capacity was calculated in term of IC % by using the equation: IC%= $[(A_0- A_t)/A_0]*100$, where A_0= absorbance of control, A_t = absorbance of test sample or standard and IC= inhibitory concentration**.**

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging capacity was evaluated by the method described by Olabinri *et al.* (2010) [22] and Ramalingam *et al.* (2012) [23]. 60 µL of $FeSO_4$. $7H_2O$ (1 mM) was added to 90 µL of aqueous 1, 10 phenanthroline (1 mM). 2.4 mL of 0.2 M phosphate buffer (pH 7.8) was added to the above mixture, followed by addition of 150 µL of 0.17 mM hydrogen peroxide and 1.5 mL of different concentrations of test sample (50-250 µg/mL). The mixture was incubated for 5 minutes at room temperature. The absorbance was taken at 560 nm in a UV-visible spectrophotometer using ascorbic acid as the standard. The % Hydroxyl radical scavenging capacity was calculated in term of IC % by using the equation: IC%= $[(A_0- A_t)/A_0]*100$, where A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration**.**

Nitric oxide radical scavenging activity: Nitric oxide radical scavenging activity was evaluated by the method used by Naskar *et al*. (2010) [24]. 1mL of 10 mM sodium nitroprusside in phosphate buffer (pH 7.4) was mixed with 1 mL of different concentrations of test sample (50-250 µg/mL) and the mixture was incubated at 25 °C for 150 minutes. From the incubated mixture, 1 mL was taken out and 1 mL of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added to it. Absorbance was read at 546 nm in a UV-visible spectrophotometer using ascorbic acid as a standard. The % nitric oxide radical scavenging capacity was calculated in term of IC % by using the equation: IC%= $[(A_0- A_t)/A_0]*100$, where A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration**.**

Superoxide radical scavenging activity: Superoxide radical scavenging activity was determined by the nitroblue tetrazolium reduction method [25]. 1 mL of 156 µM NBT

nitroblue tetrazolium (NBT) and 1 mL of 468 µM NADH (Nicotinamide adenine dinucleotide) in 100 mM phosphate buffer (pH 7.4) were mixed with 0.1 mL of different concentrations of test sample (50-250 µg/mL). The reaction was started by adding 0.1 mL of 60 µM phenazine methosulphate (PMS) solution in 100 mM phosphate buffer (pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 minutes and absorbance was recorded at 560 nm in a UV-visible spectrophotometer using ascorbic acid as the standard. The % superoxide radical scavenging capacity was calculated in term of IC % by using the equation: IC%= $[(A_0- A_t)/A_0]*100$, where A_0= absorbance of control, A_t = absorbance of test sample or standard and IC= inhibitory concentration**.**

Reducing power: The reducing power was performed by the method reported by Yen and Duh (1993) [26] and are being practiced [20, 21] with a slight modification in quantity of solution. Varying concentrations of test sample (50-250 µg/mL) were mixed with 2.5 mL of 200 mM phosphate buffer (pH= 6.6) and 2.5 mL of 1% potassium ferricyanide. After 20 minutes incubation at 50 °C, 2.5 mL of trichloroacetic acid was added to the mixture, followed by centrifugation at 650 RPM for 10 minutes. Then the upper layer (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the resultant solution was measured at 700 nm in a UV-visible spectrophotometer using BHT (butylated hydroxytoluene) and ascorbic acid as the standard. The % reducing power was calculated in term of RP % by using the equation: RP%= $[(A_0- A_t)/A_0]*100$, where A_0 = absorbance of control, A_t = absorbance of test sample or standard and RP= reducing power.

Metal chelating activity: The metal chelating activity was examined by the method reported by Hsu *et al.* (2003) [27] and are being practiced [20, 21]. 1 mL of different concentrations of test sample (50-250 µg/mL), 0.1 mL of 2mM $FeCl_2$. $4H_2O$, 0.2 mL of 5mM ferrozine and 3.7 mL of methanol were mixed together and incubated for 10 minutes. The absorbance of test sample was measured at 562 nm in a UV-visible spectrophotometer using EDTA (Ethylene diamine tetraacetic acid) as a standard. The % metal chelating capacity was calculated in term of IC % by using the equation: IC%= $[(A_0- A_t)/A_0]*100$, where A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration.

***In vitro* anti-inflammatory activity:** *In vitro* anti-inflammatory activity was carried out by using inhibition of albumin denaturation technique as reported by Ullah *et al.* (2014) [28] and being followed [21] with slight modification. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of test sample (100-500 µg/mL). This mixture was incubated at 37±2 °C in a BOD incubator for 15 minutes and then heated at 70 °C for 5 minutes in a water bath. After cooling, the absorbance was measured at 660 nm in a UV visible spectrophotometer using diclofenac sodium as a standard. The % inhibition of protein denaturation was calculated in term of IC % by using the equation: IC% = $[(A_0- A_t)/A_0]*100$, where A_0 = absorbance of control, A_t = absorbance of test sample or standard and IC = inhibitory concentration.

Statistical analysis: All the experiments were performed in triplicate and the results were expressed as mean ± SD. The recorded data were analysed by Tukey's test in conjunction with ANOVA (post-hoc) analysis with the help of SPSS software (Statistical Package for the Social Sciences). Differences were considered significant at $p<0.05$.

Results and discussion

Chemical composition: The yield of chloroform extract from the aerial part of the plant was found to be 1.32% (w/w). Fifty five components were identified by GC-MS analysis which represented about 84.6% of the total extract of which tetrapentacontane (21.8%), 7-methoxy-5-hydroxyflavone (8.1%), hexatriacontane (6.6%), *trans*-coniferyl alcohol (6.2%), *(E)*-sinapyl alcohol (3.7%), palmitic acid (3.6%), 3-pyrroline (3.4%), bayogenin methyl ester (2.5%) and mosloflavone (2.1%), were identified as major constituents. The detailed chemical composition has been presented in Table-1**.** Palmitic acid was also found to contain in the previous literature [12] but the major chemical constituents have been reported in our investigation were completely absent in previous study [12]. Whereas fatty alcohols, arjunolic acid, 2, 4, 5-trimethoxybenzaldehyde, mixture of *β*-sitosterol & stigmasterol, betulinic acid, oleanolic acid, ursolic acid, bsitosteryl glucopyranoside, myo-inositol, luteolin and rosmarinic acid have been reported in the literature [12] could not identified in present study. The flavonoids constituents were present in good amount in the chloroform extract of *Mosla dianthera*. It has been reported that flavonoids, exhibit a wide range of biological activity, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions [29]. To the best of our knowledge there is no literature is available on the chemical composition of *Mosla dianthera* from India. Thus this study could be considered as the first report on this topic from India.

Table 1: Chemical composition of the chloroform extract of the aerial part of *Mosla dianthera*

S. No.	Compound	Retention Index	Area %
1	1, 2-dichloroisobutane	717	0.1
2	*p*-cymene	1024	0.5
3	dimethyl succinate	1034	0.2
4	*m*-cymenene	1085	0.1
5	*trans*-linalool oxide (furanoid)	1086	0.3
6	3-pyrroline	-	3.4
7	isothymol methyl ether	1231	0.2
8	methyl thymol ether	1234	0.4
9	cumic aldehyde	1241	t
10	thymoquinon	1252	1.4
11	barosma camphor	1288	0.3
12	*m*-thymol	1290	0.7

13	*p*-vinylguaiacol	1309	0.8
14	syringol	1349	0.7
15	n-acetylproline	1366	0.2
16	dihydroactinidiolide	1426	0.4
17	γ-thujaplicin	1484	1.3
18	vitispirane	1489	0.6
19	thymohydroquinone	1555	1.6
20	2-(1-methylheptyl)cyclohexanone	1584	0.6
21	coniferyl aldehyde	1599	0.2
22	4-oxo-β-damascone	1629	0.4
23	3-hydroxy-β-damascone	1646	0.5
24	syringic aldehyde	1657	0.4
25	methyl dihydroferulate	1684	0.4
26	*trans*-4-propenylsyringol	1704	0.5
27	*trans*-coniferyl alcohol	1734	6.2
28	neophytadiene	1837	0.9
29	pluchidiol	-	1.3
30	diisobutyl phthalate	1908	1.3
31	palmitic acid, methyl ester	1924	0.9
32	*(E)*-phytol	1943	0.3
33	palmitic acid	1960	3.6
34	dimethylglycol phthalate	1986	0.3
35	n-eicosane	2000	0.1
36	*(E)*-sinapyl alcohol	2034	3.7
37	syringic acid hydrazide	2036	0.2
38	methyl stearate	2077	0.2
39	methyl linolenate	2079	1.2
40	linoleic acid, methyl ester	2091	0.5
41	2-carboxymethyl-3-n-hexylmaleic acid anhydride	2110	0.6
42	*cis*-oleic acid	2142	0.2
43	stearic acid	2172	1.1
44	n-butyl benzyl phthalate	2341	0.4
45	7-methoxy-5-hydroxyflavone	2382	8.1
46	dicyclohexyl phthalate	2561	1.3
47	Bis (2-Ethylhexyl) Phthalate	2704	0.3
48	γ-sitosterol	2731	0.6
49	Bis (2-ethylhexyl) phthalate	2846	0.3
50	hexatriacontane	3600	6.6
51	n-tetracontane	3997	1.2
52	moslosooflavone	-	0.6
53	mosloflavone	-	2.1
54	tetrapentacontane	5389	21.8
55	bayogenin methyl ester	-	2.5
Total			84.6

Biochemical assay: Total phenolic content of *Mosla dianthera* chloroform extract (MDCE) was quantified by gallic acid calibration curve and expressed as gallic acid equivalent per g extract. A good quantity of total phenolics (19.01±0.25 mg/g of GAE) were found in the extract. Total flavonoid content was determined by catechin calibration curve and expressed as mg of catechin equivalent per g extract. Significantly higher quantity of total flavonoids (41.04±0.13 mg/g of CNE) were found in MDCE. Orthodihydric phenol were estimated by catechol calibration curve and expressed as mg of catechol equivalent per g extract. The MDCE was found to contain a significant quantity of orthodihydric phenol (7.43±0.92 mg/g of CLE). The biochemical assay of MDCE has been displayed in table-2.

Table 2: Biochemical assay of the chloroform extract of *Mosla dianthera*

Biochemical assay	MDCE
Total phenolic	19.01±0.25 mg/g of GAE
Total flavonoids	41.04±0.13 mg/g of CNE
Orthodihydric phenol	7.43±0.92 mg/g of CLE

Where, MDCE- *Mosla dianthera* chloroform extract, GAE-gallic acid equivalent, CNE- Catechin equivalent, CLE-Catechol equivalent.

***In vitro* antioxidant activity**

DPPH radical scavenging activity: The reduction capability of DPPH radical depends on the hydrogen donating ability of the antioxidant and was determined by the decrease in absorbance induced by antioxidants [30].

$DPPH^{\bullet} + AH = DPPH + H + A^{\bullet}$

This radical species provides an easy and simple method to evaluate antioxidant potential of all antioxidants. Lower IC_{50} value reflects better antioxidant activity. The DPPH radical scavenging activity of the MDCE was found to be IC_{50}= 169.56±0.29 µg/mL in comparison to BHT (IC_{50}= 52.28±0.74 µg/mL) and catechin (IC_{50}= 38.35 ± 0.72 µg/mL) that represented in Table-3.

Hydroxyl radical scavenging activity: The extract showed dose dependent free radical scavenging activity. The hydroxyl

radical scavenging capacity was found to be IC_{50}= 124.66± 0.79 µg/mL as compare to standard ascorbic acid which had IC_{50}= 58.33± 1.41 µg/mL) (Table-3).

Nitric oxide radical scavenging activity: Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [24]. The nitric oxide radical scavenging capacity was found to be IC_{50}= 144.78± 0.11 µg/mL in comparison to standard ascorbic acid (IC_{50}= 73.89±0.96µg/mL) (Table- 3).

Superoxide radical scavenging activity: Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non enzymatic reactions such as auto oxidation by catecholamines [31]. The superoxide radical scavenging activity was found to be IC_{50}= 132.44±0.06 µg/mL in comparison to standard ascorbic acid (IC_{50}= 72.21±0.75 µg/mL) (Table- 3).

Reducing power activity: Reducing power assay is used to evaluate the ability of antioxidant to donate electron. Presence of reductants causes the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} form. This Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [24]. The MDCE was found to have reducing power activity with RP_{50}=204.21± 0.75 µg/mL in comparison to BHT (RP_{50}= 74.35± 0.45 µg/mL) and ascorbic acid (RP_{50}= 85.52± 0.24 µg/mL) (Table- 3).

Metal chelating activity: Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions [32]. The chelating power increased with increasing the concentration of extract. The metal chelating activity of extract was recorded with IC_{50}= 231.81 ± 0.14 µg/mL in comparison to EDTA having IC_{50}= 115.15 ± 0.30 µg/mL (Table- 3).

The *in vitro* antioxidant activity of MDCE might be possibly due to the significant amount of phenolics, flavonoids and orthodihydric phenols [33-35]. The major and minor constituents present in the extract may also be responsible for the antioxidant activity. The literature search reveals no report on antioxidant activity in chloroform extract of *Mosla dianthera*. Therefore, this study could be assumed as the first report on this topic.

Table 3: *In vitro* antioxidant activity of the chloroform extract of *Mosla dianthera*

Sample/ Standard	Antioxidant activity in terms of IC_{50}/RP_{50} (µg/mL ± SD)					
	DPPH radical scavenging activity	Hydroxyl radical scavenging activity	Nitric oxide radical scavenging activity	Superoxide radical scavenging activity	Reducing power activity	Metal chelating activity
MDCE	169.56±0.29	124.66±0.79	144.78±0.11	132.44±0.06	204.21±0.75	231.81±0.14
BHT	52.28±0.74	-	-	-	74.35±0.45	-
Catechin	38.35 ± 0.72	-	-	-	-	-
Ascorbic acid	-	58.33±1.41	73.89±0.96	72.21±0.75	85.52±0.24	-
EDTA	-	-	-	-	-	115.15±0.30

Where, MDCE= *Mosla dianthera* chloroform extract, BHT= Butylated hydroxytoluene, EDTA= Ethylene diamine tetraacetic acid, IC_{50}= Half maximal inhibitory concentration, RP_{50}= Half maximal reducing power, SD= Standard deviation

***In vitro* anti-inflammatory activity:** Protein denaturation is a process in which proteins lose their tertiary and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation [36]. In the present study for *in vitro* anti-inflammatory activity, MDCE showed mean inhibition of protein denaturation of 35.03, 45.24, 54.57, 65.35 and 74.00% for doses of 100, 200, 300, 400 and 500 µg/mL respectively, whereas, for diclofenac sodium, the standard it was found to be 45.21, 53.49, 65.34, 72.40 and 80.65% for the same doses respectively (Figure-1). The extract showed good anti-inflammatory activity with a linear response. Maximum inhibition of 88.03±0.08% was observed at 500 µg/mL and standard anti-inflammatory drug diclofenac sodium showed the maximum inhibition, 94.32±0.04% at the concentration of 500 µg/mL. The ability of MDCE to inhibit protein denaturation was recorded with IC_{50}= 120.83±0.73 µg/mL in comparison to diclofenac sodium having IC_{50}=101.50±0.97 µg/mL that represented in the table-4. The *in vitro* anti-inflammation capacity may be attributed to the higher concentration of flavonoid present in the extract as mosloflavone has been reported to show analgesic and anti-inflammation effect [37]. The other constituents of the extract may also be synergistically responsible for good *in vitro* anti- inflammatory activity.

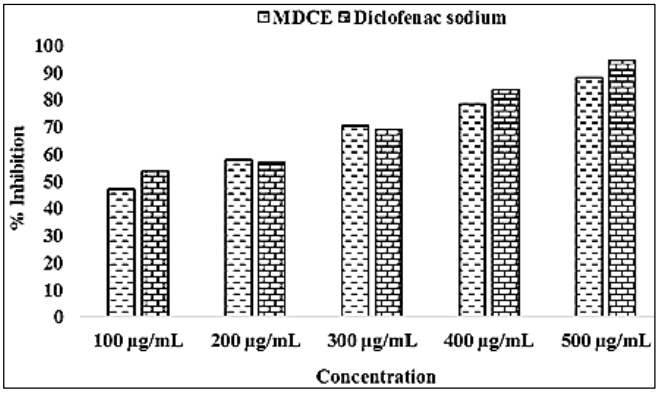

Fig 1: Percentage inhibition of protein denaturation by MDCE and diclofenac sodium at different concentrations

Table 4: *In vitro* anti-inflammatory activity of the chloroform extract of *Mosla dianthera*

Sample/Standard	IC_{50} value (µg/mL ± SD
MDCE	120.83±0.73
Diclofenac sodium	101.50±0.97

Where,
MDCE- *Mosla dianthera* chloroform extract, IC_{50}- Half maximal inhibitory concentration, SD- Standard deviation.

Conclusion

This is the first investigation on *Mosla dianthera* chloroform extract for its biochemical assay, *in vitro* antioxidant and anti-inflammatory activity. The extract constituted significant quantity of phenolics, orthodihydric phenols and a good quantity of flavonoids that are assumed to be attributed for antioxidant property. The extract showed effective DPPH radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, superoxide radical scavenging, reducing power, and metal chelating activities when compared with different standards such as BHT, catechin, ascorbic acid and EDTA. On the basis of these finding, the plant could be considered as a potential source of natural antioxidant that impart a major role for the treatment of radical related diseases. It showed excellent anti-inflammatory activity which may be mediated by the higher quantity of flavonoids and synergetic action of other secondary metabolites such as tannins, saponins, steroids, alkaloids and terpenoids. The components responsible for the *in vitro* antioxidative and anti-inflammatory activity of chloroform extract of *Mosla dianthera* are currently unclear. Therefore, further investigations are required to isolate the active constituents responsible for the observed effect, and to elucidate the possible mechanisms involve in the *in vitro* antioxidant and anti-inflammatory activities of the plant extract.

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