IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

# BIOACTIVE SPECIES OF GENUS *CLEMATIS*-A REPORT

**Rajeev Rattan**

*Associate Professor Chemistry, Government College, Haripur, Kangra, HPU-Shimla, India.*

***ABSTRACT**:* The genus *Clematis* has been a source of various traditionally useful and pharmacologically active species. Many plants of this genus are prominently climbers and woody vines. The species are mosly wild however; few are grown as ornamental plants. The species *Clematis orientalis*, *Clematis mandshurica*, *Clematis heracleifolia*, and *Clematis terniflora* were selected to study on their traditional use, chemical composition and pharmacological effects reported in literature. In folklore these species are used as antispasmodic, carminative, diuretic, anodyne, antidote, diuretic and sedative agents. The triterpenoid saponins are the dominant compounds of these species flavonoids, alkaloids, lignans, coumarins, steroids and volatile oils have also been reported from sister spectes. The pharmacological effects evaluated are antioxidant, cytotoxic, antimicrobial, antidiabetic, hepatoprotectve, and anti-inflammatory activities. As such these species has emerged as good source of traditional medicines. The chemical compounds isolated from these species have been reported for their pharmacological effects. Although, few experimental studies validated their traditional claim, but uncharacterized crude extracts were employed in most of the activities. Such species need to be explored properly for their bioactive principle and exploited as potential drug. The review will help the researchers to select medicinally potential species of *Clematis* for future research.

***Index Terms - Clematis orientalis, Clematis mandshurica, Clematis heracleifolia* and *Clematis terniflora*.**

## INTRODUCTION

*Clematis* L. is a genus of family Ranunculaceae consists of 295 species indigenous in north and south temperate, oceania and tropical African mountains [1]. In India, it is represented by thirty-two species including four sub species and five varieties [2]. The triterpenoids saponins, are the dominant components of this genus. The species are used traditionally for various ailments by the native and nomadic communities. The crude extract and isolated pure compounds possess extensive pharmacological effects such as anti-inflammatory, antitumor, analgesic, anti-inflammatory, arthiritis, antioxidant, antipypretic, antimicrobial, apoptosis, cardioprotective and cytotoxic agents comparable to their traditional claim. The extensive study revealed that monodesmodic saponins, flavonoids and alkaloids components present in these species were mainly responsible for most of the biological effects. As a source of herbal medicines for traditional use, chemical constituents diversity and various biological effects the species *Clematis orientalis*, *Clematis mandshurica*, *Clematis heracleifolia*, and *Clematis terniflora* were selected for the study. In folklore these species are used as antispasmodic, carminative, diuretic, anodyne, antidote, diuretic and sedative agents. The chemical compounds isolated were saponins, flavonoids, alkaloids, lignans, coumarins, steroids and volatile oils. The present study revealed that hederagenin aglycone based new saponins isolated were 1 from *C. mandshurica*, 3 from *C. orientalis*, and 1 from *C. graveolens* and oleanane aglycone based were 6 from *C. mandshurica*, 3 from *C. heracleifolia* and 4 from *C. terniflora*. The pharmacological activities have been antioxidant, cytotoxic, antimicrobial, antidiabetic, hepatoprotectve, and anti-inflammatory activities. The main objectives of the review are as under;

a) to evaluate the diversity of isolated chemical compounds on the basis of their structural and biological activities.
b) to evaluate whether the traditional use of *Clematis* species has validation in scientific methods in clinical studies.
c) to evaluate whether structure-activity relationship carried out from the isolated compounds.

The data has been compiled using various databases like Google Scholar, Scopus-Elsevier, PubMed, AGRICOLA and Shodhganga. The review will help the researchers to select the species for future investigations.

**Traditional uses of clematis species**:

**Clematis orientalis**: **Chinese clematis**, **Oriental virginsbower**, **orange peel**, and **orange peel clematis**. the species is native to Afghanistan, Altay, Buryatiya, China North-Central, East Aegean Is., Inner Mongolia, Iran, Iraq, Kazakhstan, Kirgizstan, Mongolia, Nepal, North Caucasus, Oman, Pakistan, South European Russi, Tadzhikistan, Transcaucasus, Turkey, Turkmenistan, Tuva, Ukraine, Uzbekistan, West Himalaya, Xinjiang [3]. *Clematis orientalis* is a deciduous perennial woody vine. It can climb and grows from 7-26 ft. (2-8 m) long and has longitudinally fissured or ridged gray-brown bark. *Clematis orientalis* is grown as an ornamental plant. It is also used as an antiseptic and refrigerant. This plant has been documented in Ancient Chinese medicine to treat dog bites and to treat ulcerated throats by gargling its infusion [4].

**Clematis mandshurica**: The species is native to Mongolia, Russian Far East, northeast China (Hebei, Heilongjiang, Jilin, Liaoning, Nei Mongol), Korea. *Clematis mandshurica* is a perennial plant with spreading to scrambling stems that can grow up to 1.20 meters tall. The plant is harvested from the wild for local use as a medicine and as an ingredient in commercial cosmetics. The plant is sometimes grown as an ornamental. An extract of the whole plant is used as an ingredient in commercial cosmetic preparations as a skin conditioner. It contains several medically active constituents including clematosides, hederagenin and anemonin. It is used in Korea in the treatment of leucorrhoea, dysentery, neuralgia, menostasis and delayed menstruation. The roots of the species are used to treat inflammation related problems such as gout, arthritis, and tetnus [5,6].

***Clematis heracleifolia***:

*Clematis heracleifolia*, commonly known as Herbaceous Clematis, is native to China North-Central, China South-Central, China Southeast, Inner Mongolia, Korea, Mandchurica [7]. The whole plants play an important role in folk for treating inflammation and tumors. Despite the folk medicinal use of this plant, few chemical constituents and pharmacological assays have been conducted on it. *C. apiifolia*, *C. florida Thunb*. and *C. heracleifolia* DC. (Korea and China) have been traditionally used as analgesic, diuretic, antitumour, and anti-inflammatory agents in Korean traditional system of medicine [8]. *C. heracleifloia* is used as diuretic and antibacterial in China [9].

***Clematis terniflora*** (**sweet autumn clematis**, **sweet autumn virginsbower**) is a plant in the buttercup family, Ranunculaceae. It is native to northeastern Asia (China, Japan, Korea, Mongolia, Russia (Siberia), Taiwan) [10]. It was introduced into the United States in the late 1800s as an ornamental garden plant, and has naturalized in many of the eastern states. It is considered a Category II invasive plant in Florida (north and central) and some other eastern states, meaning invading native plant communities but not yet seen as displacing native species [11]. *Clematis terniflora* is a vine with opposite, pinnately compound leaves, on climbing stems. The flowers are white, borne in fall. The whole plant is used as antidote, antiscrofulatic and ophthalmic agent and in the treatment of corneal opacities [12]. In traditional Chinese medicine, it is used to treat tonsillitis, rheumatoid arthritis, and prostatitis [13]. Consistent with these uses, the medicinal properties of *C. terniflora* include anti-nociceptive and anti-inflammatory activities [14].

**Chemical constituents from *Clematis* species**:

The genus *Clematis* is distributed with wide range of chemical constituents such as triterpenic saponins, alkaloids, flavonoids, coumarins, volatile oils, organic acids, macromolecules, polyphenols etc. The triterpenoid saponins constitute the major class of constituents. The aglycone of *Clematis* species is five-ring triterpenoid oleanane structure (B), 23-OH hederagenin (A), 2, 23-OH Arjunolic acid (C) and quinatic acid (D) (Fig-1). These saponins are both monodesmodic and bidesmodic with glycosylation at Agl C←3 and Agl C←28 except in few cases at Agl C←23. The sugar moieties attached are D-Glucose (Glc), L-Rhamnose (Rha), L-Arabinose (Ara), D-xylose (Xyl), D-Ribose (Rib). The tabulation of saponins is attempted to present in order of increasing oligosaccharide chain on either side. In some cases oligosaccharide chains are also substituted with acetyl, caffeoyl (CA), isoferuloyl (IF), p-methoxy cinnamyl (MC), 3,4-dimethoxy cinnamyl(DMC) moieties. Till date more than 120 new saponins are isolated from Clematis, including 70 oleanane, 50 hederagenin and 2 gypsogenin type [15].

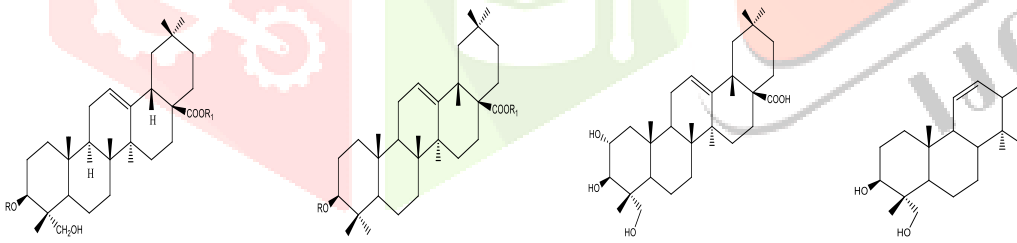


**Fig-1** The aglycones from *Clematis*: A-hederagenin, B-oleanane, C-arjunolic acid, D- quinatic acid; moieties-caffeoyl (CA), isoferuloyl (IF), p-methoxy cinnamyl (MC), 3,4-dimethoxy cinnamyl(DMC).

**Table-1 Saponins from Clematis species.**

| Compound | Structure | Source | Ref. |
|---|---|---|---|
| | **(Hederagenin Type A)** | | |
| Orientaloside H | R= Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. orientalis* | [16] |
| Orientaloside I | R = Rha(1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. orientalis* | [17] |
| Orientaloside K | R = Rha(1→2)Glc $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. orientalis* | [18] |
| Mandshunoside H | R= Glc(1→4)Glc(1→4)Rib(1→3)Rha(1→2)Ara $R^1$= Glc(1→6)Glc | *C. mandshurica* | [19] |
| | **Oleanane Type-B** | | |
| Mandshunoside I | R= Glc(1→4)Glc(1→4)Rib(1→3)Rha(1→2)Ara $R^1$ = Glc(1→6)Glc | *C. mandshurica* | [19] |
| Clematomandshurica E | R=Rha(1→6)Glc(1→4)Glc(1→4)Rib(1→3)Rha (1→2)Ara $R^1$ = Glc(1→6)Glc | *C. mandshurica* | [20] |
| Clematomandshurica C | R = Glc(1→4)Glc(1→4)Rib(1→3)Rha(1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. mandshurica* | [21] |
| Clematomandshurica D | R= Rha(1→6)Glc(1→4)Glc(1→4)Rha (1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. mandshurica* | [21] |
| Clematomandshurica B | R = Glc[(2←1)IF][(6←1)Rha](1→4)Glc(1→4)Rib(1→3) Rha(1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. mandshurica* | [21] |
| Clematomandshurica A | R = Glc[(3←1)IF][(6←1)Rha](1→4)Glc(1→4)Rib(1→3) Rha(1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. mandshurica* | [21] |
| Clematernoside E | R=Glc[(2←1)IF][(3←1)Glc(6←1)Rha][(4←1)Glc] (1→4)Glc(1→4)Rib(1→3)Rha(1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. terniflora* | [22] |
| Clematernoside I | R=Glc[(4←1)Glc][(6←1)Rha(2←1)Glc](1→3)Glc(1→4) Glc(1→4)Rib(1→4)Rha[(2←1)IF](1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. terniflora* | [22] |
| Clematernoside J | R=Glc[(4←1)Glc(2←1)Glc][(6←1)Rha](1→3) Glc(1→4)Glc(1→4)Rib(1→4)Rha[(2←1)IF] (1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. terniflora* | [22] |
| Clematernoside K | R=Glc[(2←1)IF][(3←1)Glc][(6←1)Rha(2←1)Glc] (1→4)Rib(1→3)Rha(1→2)Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. terniflora* | [22] |
| | (18-en-Oleanane) | | |
| Heracleifolianoside A | R= Rha(1→2)Glc $R^1$ = Glc(1→6)Glc | *C. heracleifolia* | [23] |
| Heracleifolianoside B | R = Ara $R^1$ = Rha(1→4)Glc(1→6)Glc | *C. heracleifolia* | [23] |
| Heracleifolianoside C | R= Ara(1→2)Glc $R^1$ =Rha(1→4)Glc(1→6)Glc | *C. heracleifolia* | [23] |
| | | | |

Nearly, 30 species have been characterized through isolation and structure determination of saponins from *Clematis.* In the present study the hederagenin aglycone based (OH group at C-23 position) new saponins identified from species are 11 from *C. chinensis*, 5 from *C. lasiandra*, 4 from *C. tibtana*, 1 from *C. apiifolia* and 1 from C. graveolens. The oleanane aglycone based (H at C-23 position) (Fig.-1) 11 from *C. chinensis* and 1 from *C. apiifolia* new saponins have been identified (Table-1). Furhtermore, from *C.tibtana* a saponin Clematibtoside B (CHO group at C-23 position) have also been isolated. The sugars and their point of attachment with the sugar chain saponins have large structural diversity. Out of 56 reported saponins, 45 are bidesmodic and 11 are from monodesmodic class. In monodesmodic saponins glycosylation of sugars at (C-3-O←1)Ara(2←1)Rha(3←1)Rib in mostly present however, substitution and further enlargement of chain with glucose, rhamnose and xylose, galactose sugars have also been encountered. Among bidesmodic saponins glycosylation at (C-3-O←1)Ara(2←1)Rha(3←1)Rib and (C-28-O←1)Glc (6←1)Glc(4←1)Rha are commonly observed (Table-1). However, the sugar chains on either side are further enlarged with glucose, rhamnose, galactose and xylose moities.

**Table-2 Steroids, Lignans, Coumarins, Macrocyclic, Volite oils from Clematis species.**

| Compound | Source | Ref. |
|---|---|---|
| **Alkaloids** | | |
| Corytuberine, b-magnoflorine, a-magnoflorine, Me-7-methoxy-3-indolecarbonate, Clemaine | C. erecta, C. mandshurica, C. purpurea | 24, 25,26 |
| **Flavonoids** | | |
| Apigenin, Vitaboside, Kaempferol, Clematine, Hesperetin, Daidzein, Genistein, Luteolin, Quercetin, Rutin, Tangeritin, Isovitexin-6-O-e-p-coumarate, 3,5,7,3' tetrahydroxy flavone | C. viornae L., C. vitalba, C. purpurea , C. armandii, C. hexepetala, C. intricate, | 27,28,29,30,31, 32,33 |

| | | |
|---|---|---|
| | C. stans, C. terniflora | |
| **Lignans** | | |
| Armandiside, Clemastanin B, (þ )-lariciresino-4-O-β-D-glucopyranoside, Salvadoraside, episyringaresinol, Clemaphenol A, (þ )-pinoresinol , Clemastanin A, Isolariciresinol | C. armandii C. stans, C. parviloba, C. chinensis C. hexapetala | 34,32,36,35,30 |
| **Steroids** | | |
| Stigmasterol, Daucosterol, β-sitosterol, β-amyrin, α-amyrin and their glycosides | C. apiifolia, C. hexapetala, C. montana, C. purpurea | 37,38,39,26 |
| **Coumarins** | | |
| 4,7-dimethoxy-5-methyl-coumarin, Siderin, Scopoletin | C. delavayi, C. ligusticifolia, C. intricate | 40,41,42 |
| **Macrocyclic compounds** | | |
| Clemoarmanosides A, B, Bercholine, Clemahexapetoside A, B, Clemochinenoside A, B, Ibotanolide B | C. armandii, C. hexapetala, C. chinensis, C. crassifolia | 29,38,35,25 |
| **Phenolic compounds** | | |
| Ibotanolide B, Calceolarioside B, Clemomandshuricoside A, B, C, Tricosanol, Heptacosanoic acid | C. crassifolia, C. mandshurica, C. terniflora | 43,25,33 |
| Anemonin, Protoanemonin, Ranunculin | C. angustifolia, C. apiifolia, C. flammula | 45,37,44 |
| **Volatile oils** | | |
| Palmitic acid, Myristic acid, Decasanoic acid, Para-coumatic acid, Caffeic acid, Ferulic acid, 3-hydroxy-4-methoxy benzaldehyde, Inositol, Coniferaldehyde, Vanillin, Pluchoic acid, Protocatechualdehyde, Caffeic acid | C. angustifolia, C. armandii, C. delavayi, C. crassifolia, C. hexepetala, C. montana | 45,33,40,43,38,46 |

The clematis species has been subjected to isolate various biologically active compounds other than saponins. The alkaloids - phenanthrene, indolecarbonate and clemaine from *C.erecta, C. mandshurica* and *C. parviloba*. The flavonoids from Clematis species are mainly flavonols, flavones, isoflavones, flavanones, xanthones and their glucosides, the aglycones of which are mainly apigenin, kaempferol, luteolin and quercetin. The lignans from *Clematis* are mainly eupomatene lignans, cyclolignans, monoepoxylignans, bisepoxylignans and lignanolides from *C. viornae L., C. vitalba, C. purpurea, C. armandii, C. hexepetala, C. intricate, C. stans, C. terniflora*. Steroids - stigmasterol, β-sitosterol, α, β-amyrin and their glycosides. Macrocyclic compounds- clemoarmanosides, bercholine, clemahexapetoside Clemochinenoside, Ibotanolide from *C. armandii, C. hexapetala*. The volatile oils- palmitic acid, myristic acid, caffeic acid, ferulic acid, inositol, coniferaldehyde, vanillin, pluchoic acid, protocatechualdehyde, caffeic acid mainly from *C. armandii, C. delavayi, C. crassifolia, C. hexepetala* and *C. montana* (Table-2).

**Pharmacological effects of clematis species-**

***Clematis mandshurica*:**

**Cytotoxic activity-**

The dried roots and rhizomes of C.mandshurica were extracted with 50%EtOH. (1262 g) was suspended in $H_2O$ and extracted with EtOAc and n-BuOH. The n-BuOH extract (120 g) was subjected to chromatography and to afford compounds Mandshunosides A 29g and Mandshunosides B 31g respectively. Both compounds showed inhibitory activities against two colorectal human cancer cells HCT 116 ($IC_{50}$ 2.1 mM for 1 and 2.5 mM for 2) and HT-29 ($IC_{50}$ 3.7 mM for 1 and 3.3 mM for 2) [47].

**Anticancer activity**- The dried roots and rhizomes of *C. mandshurica* were extracted with 50% EtOH (822 g) further extracted with n-BuOH. The n-BuOH extract (93 g) was subjected to chromatography to isolate compounds namely- mandshunoside C, mandshunoside D, mandshunoside E, clematichinenoside C, clematochinenoside A, huzhongoside B and clematiganoside A. All of these compounds were screened for inhibitory activities against human colorectal cancer lines HCT-116 and HT-29 cells. Paclitaxel was used as positive control. Both monodesmosidic and bidesmosidic saponins showed cytotoxicity, and have use in anti-tumor. The compound- mandshunoside D expressed highest inhibitory effects $IC_{50}$ value (mM) 0.6 and 0.9 followed by mandshunoside E- 2.7, 4.2 and clematiganoside A- 16.1, 12.5 was least effective against two colorectal human cancer cell lines HCT-116 and HT-29. The control Paclitaxel has $IC_{50}$ 0.0035, 0.0034 [48].

**Anti-inflammatory effects-**

*Clematis mandshurica* Rupr roots are used investigate their inhibitory effect on inflammation under non-cytotoxic conditions. The air-dried roots of *C. mandshurica* were powdered. 100 g powder was then extracted with 50% ethanol (500 ml) to yield 35.9 g of *C. mandshurica* ethanol extract. CME was then disolved in DMSO for in vitro experiments or in 0.5% carboxylmethl cellulose (CMC) for in vivo p.o.experiments in female BALB/c mice. The ethanolic extract of Clematis mandshurica at 100 g/ml was found to significantly block the production of the pro-inflammatory mediators, nitric oxide (NO) and prostaglandin $E_2$ ($PGE_2$ ), in lipopolysaccharide (LPS)/interferon(IFN)--stimulated mouse peritoneal macrophages, by up to 77% and 59%, respectively. In

addition, it significantly inhibited cell proliferation and cytokine production (interleukin (IL)-2 and IFN-) in splenocytes stimulated with Con A (concanavalin A; 5g/ml). Furthermore, when splenocytes from extract fed mice (200 mg/kg for 2 weeks) were activated with Con A, cell proliferation and the production of IL-2 and IFN- were significantly inhibited. In addition, the extract reduced in vivo inflammation in oxazolone-induced delayed type hypersensitivity (DTH) model mice. Taken together, these data suggest that *C. mandshurica* is able to ameliorate inflammatory disease by exerting an anti-inflammatory effect in cases of proinflammatory and cell-mediated inflammation [49].

**Arthritis activity**-

Effect and mechanism of *Clematis mandshurica* water extract (CMA), a dual inhibitor of interleukin-1 (IL-1) and tumor necrosis factor-(TNF-), on Male Sprague-Dawley (SD) rats adjuvant arthritis (AA) were investigated. Complete Freund's adjuvant (CFA) was used to induce AA in rats. All the animals which received CFA, developed severe inflammation, and typically hind limb became severely red and edematous within 16-24 h period such that inflammation score was 6.8 ± 0.5 mm (mean ± S.E.M.) for a group of eight rats evaluated on the day of onset of arthritis from age matched untreated, treated and normal control. The extents of inflammation and treatment response were evaluated with regard to lymphocyte proliferation. Serial evaluation was carried out on days 1, 7, 14, 21 and 28 after creation of inflammation. The lymphocyte proliferation study revealed cellular immune suppression during the early phase of the disease. Administration of CMA on the same day or 5 days prior to inflammatory insult into the joint significantly reduced the inflammation as compared to the untreated animals in a dose dependent manner. The administration of CMA (2, 5 and 10 mg/kg, subcutaneously (s.c.)) inhibited the inflammatory response and restored the weight of body and immune organs of AA rats. Synoviocytes proliferation of AA rats significantly increased, and the levels of TNF- and IL-1 in supernatants of syno viocytes in AA rats were also elevated compared with the non immunized rats group. The administration of CMA (2, 5 and 10 mg/kg, s.c.) reduced the above changes significantly. In contrast to TNF- and IL-1, IL-10 production and the level of its mRNA of synoviocytes in AA rats were apparently decreased. CMA (2, 5 and 10 mg/kg, s.c.) markedly increased IL-10 in synoviocytes at protein and transcription level. In CMA- and DEX-treated animals, edema began to subside gradually and showed a significant reduction ($P < 0.05$) in swelling as compared to the untreated animals. The reduction in swelling in CMA-treated groups was at par with DEX-treated animals. After 20 days of treatment, the swelling in treated animals was reduced almost equal to normal controls, whereas it persisted longer in untreated animals. The results indicated that CMA had a beneficial effect on rats AA due to modulating inflammatory cytokines production of synoviocytes, which played a crucial role in pathogenesis of this disease [50].

**Anti-inflammatory effects**-

The dried roots (40 kg) extracted of *Clematis mandshurica* with methanol to yield 2.73 kg of extracts. The extracts partitioned with n-hexane, chloroform ($CHCl_3$), and ethyl acetate saturated with water. $CHCl_3$-soluble layer (CRC, 14.9 g) was chromatographed to yield fraction 5-DRL (6.1 mg). The isolated compound was characterized a novel compound, 5-O-isoferuloyl-2-deoxy-D-ribono-γ-lacton (5-DRL) from *C. mandshurica*, and evaluated its anti-inflammatory effect in lipopolysaccharide (LPS)-treated BV2 microglial cells. 5-DRL inhibited the expression of LPS-stimulated proinflammatory mediators such as nitric oxide (NO) and prostaglandin E2 (PGE2), as well as their regulatory genes inducible NO syntheses (iNOS) and cyclooxygenase-2 (COX-2). 5-DRL also down regulated the LPS-induced DNA-binding activity of nuclear factor-κB (NF-κB) through suppression of the nuclear trans-location of the NF-κB subunits, p65 and p50. Consistent with the inhibition of iNOS and COX-2 via NF-κB activity with 5-DRL, an inhibitor of NF-κB, pyrrolidine dithiocarbamate (PDTC), also led to the suppression of LPS-induced iNOS and COX-2 expression. Additionally, 5-DRL corresponding with antioxidants, N-acetylcysteine (NAC) and glutathione (GSH), remarkably inhibited reactive oxygen species (ROS) generation. Both NAC and GSH, thus attenuated the expression of iNOS and COX-2 by suppressing NF-κB activation, indicating that 5-DRL suppresses LPS-induced iNOS and COX-2 expression through down regulation of the ROS-dependent NF-κB signaling pathway. The present study also indicated that 5-DRL suppresses NO and $PGE_2$ production by inducing heme oxygenase-1 (HO-1) via nuclear factor erythroid 2-related factor 2 (Nrf2). Taken together, the present data indicate that 5-DRL attenuates the production of pro inflammatory mediators such as NO and $PGE_2$ as well as their regulatory genes in LPS-stimulated BV2 microglial cells by inhibiting ROS-dependent NF-κB activation and stimulating the Nrf2/HO-1 signal pathway. These data may be implicated in the application of 5-DRL in LPS-stimulated inflammatory disease [51].

**Clematis heracleifolia**:

**HIV-1 protease inhibitor activity**-

The methanolic extract of whole plant of C. heracleifolia (20mg/100ml) was subjected for the screening of anti-HIV-1 activity using MT-4 cell line. The extract aws active for inhibitory activities on essensial viral enzymes reverse transcriptates (RT and RNase H activities). Reverse transcriptates and PR heve been important for viral replication and promising targets for finding anti-HIV agents. At the contration of 100 µg/ml extract had inhibitory effects of RT= 14.6 ± 9.1, RNase = 16.8 ± 6.3 and protease = 45.3 ± 2.7. The clematis species contain oleanolic acid responsible for significant inhibitory effects of HIV protease [52].

The cytotoxicity of eight saponins of C. heracleifolia and five prosapogenins against human breast tumor MDA-MB-231 and gastric carcinoma SGC- 7901 cell lines were evaluated by MTT method. Prosapogenins monodesmodic showed significant cytotoxic activities against MDA-MB-231 and gastric carcinoma SGC- 7901 cell lines with $IC_{50}$ value in the range of 6.05-6.32 µmol/L. The cytotoxic activity might be feasible due to change of inactive bidesmodic saponin to active monodesmodic saponin by alkaline hydrolysis [53].

***Clematis terniflora***

**Apoptosis activity**- The aqueous extract of *Clematis terniflora* at the concentration of 300 and 500 µg/ml aws used to evaluate the neuroprotective effects against corticosterone- induced apoptosis in rat pheochromocytoma (PC 12) cells. The extract at respective concentration decreased apoptotic cell death and mitochondrial damage induced by 200 µM corticosterone. The extract decreased

expression of andoplasmic reticulum (ER) stress proteins GRP78, GADD153 and mitochondrial damage- related protein BAD. These protective effects were mediated by upregulation of p-AKT and p-ERK1/2, which are involved in cell survival signaling [54].

**Antinociceptive activity and anti-inflammatory activity**- these activities were evaluated from water, 70% ethanolic extratc and fractions using mice writhing test with different doses. The anti-inflammatory activity was tested on rat models of carrageenan-induced chronic non-bacterial prostatis (CNP). Significant writhing inhibitory effect was found with EE at small (7.5 g/kg body wt.), moderate (15 g/kg body wt.) and large (30 g/kg body wt.) doses as well as EEPMR at moderate and large doses by oral administration (OA) ($p \leq 0.01$). Data from prostatic index, lecithin microsome density and white blood cell level showed that moderate dose of EE and EEPMR both had significant ($p \leq 0.05$ or $p \leq 0.01$) inhibition effect on carrageenan-induced inflammation in rat prostate. The results showed that flavonoids were the main active compounds in the extracts. . And most flavonoids were accumulated into the part of EEPMR by AB-8 macroporous resin leaving only few compounds in WEPMR [55].

**Antibacterial activity and antifungal activity**- these activities were recorded in methanolic leaf extract of *C. terniflora*. Agar well diffusion method for antibacterial and well diffusion assay to analyse the anti-fungal activity of the plant extract. The extract (40 and 80 µL) from 100 mg/mL stock was added to the wells. One well with cotrimazole was taken as the positive control and solvent without the compound as negative control. Eight important strains of micro organisms were selected for the present study which included bacteria (*Bacillus substilis, Pseudomonas fluorescence, Staphylococcus aureus, Escherichia coli*) and fungi (*Candida albicans, spergillus niger, Fusarium solani, Pencillium notatum*). At the concentrations (40µl & 80µl) of methanolic extracts of leaf and stem used for the analysis. Anti bacterial activity is less compared to the standard antibiotic available in the market. Methanolic leaf extraction (80 µl) showed greater inhibitory zone against *E. coli* compared to other organism. The stem extraction showed less anti fungal activity. The leaf extraction have high inhibition against *Fusarium solani, Pencillium* and *Candida albicans*[56].

**Anti-inflammatory and Antinociceptive effects activities**– The anti-inflammatory properties of the 70% ethanol eluted fraction of the 70% ethanol extract of C. terniflora DC. (EECTD) were evaluated using the xylene-induced ear swelling test, the carrageenan-induced edema model and the cotton pellet granuloma method. Its antinociceptive activities were determined using both the acetic acid-induced writhing test and the hot plate assay. In parallel, in vitro assay in LPS-induced RAW264.7 cells to examine the anti-inflammatory effects of EECTD and its purified form, aurantiamide acetate (AA) on inhibition of nitric oxide (NO) and prostaglandin $E_2$ ($PGE_2$) release.

**Anti-inflammatory activities**- In vehicle treated controls, the right ears exhibited obvious inflammatory symptoms including redness and swelling with a calculated percentage swelling of 106.99%. However, mice pretreated with either extract or the positive drug control aspirin had significantly reduced swelling. In acute xylene-induced inflammation the dose of 300mg/kg had swelling rate 49.08% and aspirin 39.92%. In the carrageenan-induced model, the paws of vehicle-treated rats began to exhibit swelling one hour after carrageenan injection and had a maximum edema of 48.99% at 6 h. Rats pre-treated with the EE (300 mg/kg) showed significantly reduced paw edema from 2 h until 6 h. This reduced in edema resembled that of the positive control, reference drug aspirin. The lower doses of EE (75 mg/kg and 150 mg/kg) also had a reduction in edema, but from 3 h to 6 h. Six hours after carrageenan injection, vehicle-treated animals showed obvious symptoms of acute inflammation upon histopathological examination, including a large number of neutrophils, lymphocytes,and other inflammatory cells. However, the infiltration of inflammatory cells in rats pre-treated with EE was significantly reduced, and the tissue damage was markedly improved. Compared to vehicle control group, rats treated with EECTD for six days showed an obvious inhibition of granuloma formation. This inhibitory effect was closest to that of the positive drug control, aspirin. Furthermore, the dry weight of the cotton pellet granuloma in two of the EE-treatment conditions (300 mg/kg and 150 mg/kg) was significantly decreased when compared to controls.

**Antinociceptive effects**-The result of the acetic acid-induced writhing test showed that EE derived from C. terniflora DC. decreased the number of writhing. Compared to the vehicle group, a high dose of EE (300 mg/kg) had a significant inhibitory effect (81.78%) that was comparable to the positive, drug control (aspirin, 88.48%). The writhing test was used to investigate peripheral analgesic activity. However, the hot-plate assay was used to evaluate the central antinociceptive effects of the extract in mice,The results indicated that the extract extended the latency to reaction against hyperthermic stimulation. Moreover, those two doses of EECTD (300 mg/kg body and 150 mg/kg) caused a significant extension to the time of paw licking when compared to the vehicle group[57].

**Conclusion**

Out of 355 species of genus *Clematis (Ranunculaceae)* 30 species have been systematically characterized for their chemical constituents. The constituents identified from *Clematis* species are flavonoids, triterpenoid saponins, lignans, steroids, polyphenols, and coumarins. Few compounds, especially flavonoids and alkaloids also possess strong evidence of biological importance but no systematic work has been carried out to validate pharmacological activities responsible for bioactive principles. The triterpenoid saponins are mainly of interest of this genus as these are most potent compounds responsible of most of activities. In literature, 26 species are reported in traditional use for the treatment of various ailments like gout, dysentery, rheumatism, analgesic, antitumor, antibacterial, diuretic, anticancer, antimicrobial, anti-inflammatory, arithritis, hepatoprotective, osteoarthritis and HIV-1 protease inhibitors activities. The chemical constituents isolated were hederagenin and oleanane aglycone based saponuns, flavonoids, alkaloids, lignans, coumarins, steroids and volatile oils. The present study revealed that hederagenin aglycone based new saponins isolated were 1 from *C. mandshurica*, 3 from *C. orientalis*, and 1 from *C. graveolens* and oleanane aglycone based were 6 from *C. mandshurica*, 3 from *C. heracleifolia* and 4 from *C. terniflora*. The pharmacological effects reported have been antioxidant, cytotoxic, antimicrobial, antidiabetic, hepatoprotectve, and anti-inflammatory. In most of activities crude extract was used to evaluate these activities. Being a

potential folklore medicine and pharmacologically active species clinical studies are needed to establish biological alternatives to synthetic drugs. In lieu of these observations, it is suggested that the research is needed:

(i) to validate more *Clematis* species of traditional uses with pharmacological effects.

(ii) to characterize and isolate bioactive constituents as per market need.

(iii) to investigate more *Clematis* species for isolation of compounds and their mode of actions.

(iv) more clinical studies to establish structure -biological activity relationship.

**References**:


[1] Mabberley DJ. 2005. The Plant-book, A Portable Dictionary of Vascular Plants. Cambridge University Press, Cambridge, 163.

[2] Sharma BD, Balakrishnan NP, Rao RR, Hajra PK. (1993) Flora of India, Botanical Survey of India. Calcutta, 1, 52-80.

[3] . "Clematis orientalis – L.." Plants For A Future. N.p., n.d. Web. 28 Apr 2012.

[4] Francis, Frank K. United States. U.S. Department of Agriculture, Forest Service. Clematis orientalis L..

[5] Huang, K.C., The Pharmacology of Chinese Herbs. CRC press, USA, 1993.;161.

[6] Jung, Y.B., Roh, K.J., Jung, J.A., Jung, K., Yoo, H., Cho, Y.B., Kwak, W.J., Kim, D.K., Kim, K.H., Han, C.K., 2001. Effect of SKI 306X, a new herbal antiarthritic agent, in patients with osteoarthritis of the knee: a double-blind placebo controlled study. American Journal of Medicine 29, 485-491.

[7] Chang, C.S., Kim, H. & Chang, K.S. (2014). Provisional checklist of vascular plants for the Korea peninsula flora (KPF): 1-660.

[8] Bae, K.H., 2000. The Medicinal Plants of Korea. Kyo-Hak Publishing Co., Seoul, Korea, p. 155.

[9] Lee, Y., 1996. Flora of Korea. Kyo-Hak Publishing, Seoul, Korea, pp. 444-446.

[10] "Clematis terniflora". Flora of China. Retrieved 12 May 2013.

[11] ^ Sweet Autumn Virginsbower (Clematis terniflora) Invasive Plant Atlas of the United States ^ Sweet autumn clematis The Morton Arboritum, Lisle, IL

[12] Stuart. Rev. G. A. Chinese Materia Medica. Taipei. Southern Materials Centre.

[13] Chen, K.C., Sun, M.F., Yang, S.C., Chang, S.S., Chen, H.Y., Tsai, F.J., Chen, C.Y Investigation into potent inflammation inhibitors from traditional Chinese medicine Chem. Biol. Drug Des, 2011; 78, 679-688.

[14] Chen, R.Z., Cui, L., Guo, Y.G., Rong, Y.M., Lu, X.H., Zhang, L., Tian, J.K., 2011b. In vivo study of four preparative extracts of Clematis terniflora DC. for antinociceptive activity and anti-inflammatory activity in rat model of carrageenan-induced chronic non-bacterial prostatitis. J. Ethnopharmacol. 134, 1018-1023.

[15] Lin, T. F., Wang, L., Zhang, Y., Zhou, D.Y. and Liu, B. 2021. Uses, chemical compositions, pharmacological activities and toxicology of *Clematidis Radix* et Rhizome- a Review, Jouranal of Ethnopharmacology 270, 113831.

[16] Thapliyal, R.P., Bahuguna, R.P., 1993a. Clemontanoside-C, A saponin from *Clematis montana*. Phytochemistry 33, 671-673.

[17] Kawata, Y., Kizu, H., Miyaichi, Y., Tomimori, T., 2001. Studies on the constituents of *Clematis* species. VIII. Triterpenoid saponins from the aerial part of *Clematis tibetana* Kuntz. Chemical and Pharmaceutical Bulletin 49, 635-638.

[18] Kizu, H., Shimana, H., Tomimori, T., 1995. Studies on the constituents of *Clematis* species. VI. The constituents of *Clematis stans* Sieb. et Zucc. Chemical and Pharmaceutical Bulletin 43 (12), 2187-2194.

[19] Fu Qiang, Yang Min, Ma Yu, Chen Jiang, Yuan Hei- Mei, (2018) Chinese Journal of Natural Medicines, 16 (2) 131-138.

[20] Shao, B., Qin, G., Xu, R., Wu, H., Ma, K., 1996. Saponins from *Clematis chinensis*. Phytochemistry 42, 821-825.

[49] Shi, S.P., Jiang, D., Dong, C.X., Tu, P.F., 2006a. Triterpene saponins from *Clematis mandshurica*. Journal of Natural Product 69, 1591-1595.

[22] Kawata, Y., Kizu, H., Tomimori, T., 1998. Studies on the constituents of *Clematis* species. VII. Triterpenoid saponins from the roots of *Clematis terniflora* DC. var. robusta Tamura. Chemical and Pharmaceutical Bulletin 46, 1891-1900.

[23] Zhang Quin., Lu Yun-Yang., Yang Liu., Tang Hai-Feng. (2022) New triterpenoid saponin from whole plant of *Clematis haracleifolia*. Fitotrepia, 159, 105179.

[24] Slavik, J., Slavikova, L., 1995. Quaternary isoquinoline alkaloids and some diterpenoid alkaloids in plants of the Czech Republic. Collection of Czechoslovak Chemical Communications 60 (6), 1034-1041.

[25] Shi, S.P., Tu, P.F., Dong, C.X., Jiang, D., 2006b. Alkaloids from Clematis manshurica Rupr. Journal of Asian Natural Products Research 8, 73-78.

[26] Sayed, H.M., El-Moghazy, S.A., Kamel, M.S., 1995. Chemical constituents of stems and leaves of Clematis purpurea hybrida cultivated in Egypt. Indian Journal of Chemistry 34B, 111-1113

[27] Dennis, W.M., Bierner, M.W., 1980. Distribution of flavonoids and their systematic significance in Clematis subsection Viornae. Biochemical Systematics and Ecology 8, 65-67.

[28] Yesilada, E., Kupeli, E., 2007. Clematis vitalba L. aerial par exhibits potent anti inflammatory, antinociceptive and antipyretic effects. Journal of Ethnopharmacology 110, 504-515.

[29] Chen, Y., Liu, J., Davidson, R.S., Howarth, O.W., 1993.Isolation and structure of clematine, a new flavanone glycoside from Clematis armandii Franch. Tetra-hedron 49 (23), 5169-5176.

[30] Dong, C.X., Wu, K.S., Shi, S.P., Tu, P.F., 2006b. Flavonoids from Clematis hexapetala. Journal of Chinese Pharmaceutical Sciences 15 (1), 15-20.

[31] Hung, T.M., Thuong, P.T., Bae, K.H., 2005. Antioxidant effect of flavonoids isolated from the roots of Clematis trichotoma Nakai. Korean Journal of Medicinal Crop Science 13, 227-232.

[32] Kizu, H., Shimana, H., Tomimori, T., 1995. Studies on the constituents of Clematis species. VI. The constituents of Clematis stans Sieb. et Zucc. Chemical and Pharmaceutical Bulletin 43 (12), 2187-2194.

[33] Sun, F., Zhang, L., Tian, J., Cheng, Y., Xiao, P., 2007b. Chemical constituents of Clematis terniflora. Chinese Pharmaceutical Journal 42, 102-103.

[34] Haung, W.W., Kong, D.Y., Yang, P.M., 2003. Studies on lignan constituents of Clematis armandii Franch. Chinese Journal of Natural Medicine 1 (4), 199-203.

[35] He, M., Zhang, J.H., Hu, C.Q., 2001. Studies on the chemical components of Clematis chinensis. Yao Xue Xue Bao 36 (4), 278-280.

[36] Yan, L., Xu, L., Lin, J., Yang, S., Feng, Y., 2009. Triterpenoids saponins from the stems of Clematis parviloba. Journal of Asian Natural Products Research 11, 332-338.

[37] Woo, W.S., Kang, S.S., Yoon, M.H., 1976. Phytochemical study on Clematis apiifolia. Soul Taehakkyo Saengyak Yonguso Opjukjip 15, 1-4.
[38] Dong, C.X., Shi, S.P., Wu, K.S., Tu, P.F., 2006a. Studies on chemical constituent from root of Clematis hexapetala. Zhongguo Zhongyao Zazhi 31 (20), 1696-1699.
[39] Jangwan, J.S., Bahuguna, R.P., 1990. Clemontanoside B, a new saponin from Clematis montana. International Journal of Crude Drug Research 28, 39-42.
[40] Li, Y., Wang, S.F., Zhao, Y.L., Liu, K.H., Wang, X.M., Yang, Y.P., Li, X.L., 2009. Chemical constituents from Clematis delavayi var. spinescens. Molecules 14, 4433-4439.
[41] Ayer, W.A., Browne, L.M., 1975. Siderin from Clematis ligusticifolia. Phytochemistry 14 (5-6), 1457-1458.
[42] Song, Z., Zhao, Y., Duan, J., Wang, X., 1995. Studies on the chemical constituents of Clematis intricata Bunge. China Journal of Chinese Materia Medica 20 (10), 613-614.
[43] Lee, T.H., Huang, N.K., Lai, T.C., Yang, A.T.Y., Wang, G.J., 2008. Anemonin from Clematis crassifolia, potentand selective inducible nitric oxide synthase inhibitors. Journal of Ethnopharmacology 116, 518-527.
[44] Jose, F., Jose, P., 1966. Ranunculin, protoanemonin and anemonin. I. Isolation of ranunculin from Clematis flammula. Anales de la Real Sociedad Espanola De Fisica y Quimica, Serie B: Quimica 62 (6), 705-707.
[45] Ting, T.H., Chao, Y.S., 1940. Chemical studies on the roots of Clematis angustifolia Jacquin. Pharmaceutical Archives 11, 60-64.
[46] Song, C.Z., Wang, Y.H., Hua, Y., Wu, Z.K., Du, Z.Z., 2008. Chemical constituents of Clematis montana. Chinese Journal of Natural Medicines 6, 116-119.
[47] He Y., Li L., Zhang K., Liu Z. 2011.Cytotoxic triterpene saponins from Clematis mandshurica. Journal of Asian Natural Products Research, 13, 12, 1104-1109.
[48] Li L., Gou M., He Y. 2013. Mandshunosides C-E from the roots and rhizomes of Clematis mandshurica. Phytochemistry Letters. 6, 570-574.
[49] Park E., Ryu M., Kim Y., Lee Y., Lee Sh., Woo DH., Hong SJ., Han JS., Yoo MC., Yang H., Kim KS. 2006. Anti-inflammatory effects of an ethanolic extract from Clematis mandshurica Rupr. Journal of Ethnopharmacology., 108, 142-147.
[50] Suh SJ., Kim KS., Lee SD., Lee CH., Choi HS., Jin UH., Chang YC., Kim CH. 2006.Effects and mechanisms of Clematis mandshurica Maxim. as a dual inhibitor of proinflammatory cytokines on adjuvant arthritis in rats. Environmental Toxicology and Pharmacology. 22,205-212.
[51] Dilshara MG., Lee KT., Lee CM., Choi H., Lee HJ., Choi IW., Kim GY. 2015. New compound, 5-O-isoferuloyl-2-deoxy-D-ribono-γ-lacton from Clematis mandshurica: Antiinflammatory effects in lipopolysaccharide-stimulated BV2 microglial cells. International Immunopharmacology. 24, 14-23.
[52] Min, B.S., Kim, Y.H., Tomiyama, M., Nakamura, N., Miyashiro, H., Otake, T., Hattori, M. 2001. Inhibitory effects of Korean plants on HIV-1 activities. Phytotherapy Research, 15, 481-486.
[53] Lu Y., Yang L., Tang H. 2022. New triterpenoid saponins from the whole plants of clematis heracleifolia. Fitotrepia, 159, 105179.
[54] Noh Y., Cheon S., Kim IH., Kim I., Lee S., Jeong Y. 2018. The protective effects of ethanolic extract of Clematis terniflora against corticosterone-induced neuronal damage via the AKT and ERK1/2 pathway. BMB Rep. 51, 8, 400–405.
[55] Chen, R.Z., Cui, L., Guo, Y.G., Rong, Y.M., Lu, X.H., Zhang, L., Tian, J.K., 2011b. In vivo study of four preparative extracts of Clematis terniflora DC. for antinociceptive activity and anti-inflammatory activity in rat model of carrageenan-induced chronic non-bacterial prostatitis. J. Ethnopharmacol. 134, 1018-1023
[56] Rajeswari S. , Sumitha V. R. 2019. Methanolic extracts of Clematis terniflora leaf and stem as potential antimicrobial agents. Journal of Advances in Biological Science. 6, 1, 33-35.
[57] Liu XB., Yang BX., Zhang YZ., Gong MH., Tian JK. 2015. An in vivo and in vitro assessment of the anti-inflammatory, antinociceptive, and immunomodulatory activities of Clematis terniflora DC. extract, participation of aurantiamide acetate. Journalof Ethnopharmacology.