Clematis- genus of Bioactive Species- a Report

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***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 mostly wild however; few are grown as ornamental plants. The species Clematis lasiandra, Clematis Montana, Clematis stans, and Clematis mandshurica 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 species. The pharmacological effects evaluated are antioxidant, cytotoxic, antimicrobial, antidiabetic, hepatoprotective, 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 lasiandra, Clematis Montana, Clematis stans, and Clematis mandshurica.**

INTRODUCTION

Clematis genus (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, arthritis, antioxidant, antipyretic, antimicrobial, apoptosis, cardioprotective and cytotoxic agents. 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 lasiandra, Clematis montana, Clematis stans, and Clematis mandshurica 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 5 from C. lasiandra, 1 from C. mantana, 4 from C. stans, 1 from C. mandshurica and 1 from C. graveolens and oleanane aglycone based were 6 from C. mantana, 2 from C. stans and 6 from C. mandshurica.

The pharmacological activities evaluated have been antioxidant, cytotoxic, antimicrobial, antidiabetic, hepatoprotectve, and anti-inflammatory. The literature was reviewed and work has been presented in three sections namely- traditional uses, chemical constituents and pharmacological effects of these species. The 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 lasiandra: The species is distributed in Japan, Taiwan, China: W. Hubei, Sichuan, Sikang (E. Tibet, W. Sichuan), Gansu [3]. Clematis lasiandra Maxim is a climbing shrub spreaded on low mountains slopes having biternate compound leaves, leaflets oval with course serration on the margins, and pointed tips. The stems grow to the length of 2-6 meters. The flowers of Clematis lasiandra are bell shaped, campanulate, whitish or more or less strongly tinged in purple-violet, strongest on the inside. The entire plants and rhizome of this species have been used as folk medicines for a long history to treatment of dehygrosis, antitoxic, diuretic, analgesic and antipyretic, etc. [4,5].

Clematis Montana: The **mountain clematis** also **Himalayan clematis** or **anemone clematis**, is a flowering plant a vigorous deciduous climber, in late spring it is covered with a mass of small blooms for a period of about four weeks. The odorous flowers are white or pink, four-petalled, with prominent yellow anthers. It is native to mountain areas of Afghanistan, Assam, Bangladesh, China North-Central, China South-Central, China Southeast, East Himalaya, Inner Mongolia, Myanmar, Nepal, Pakistan, Qinghai, Taiwan, Tibet, west Himalaya [6]. The leaves of Clematis montana have been utilized for the treatment of skin diseases in India [7]. In China, Clematis armandii and C. montana called “mu tong” are also used as traditional Chinese medicines for the treatment of some ailments such as reducing fever to induce urination, stimulating menstrual discharge and

promoting lactation [8]. Clematis montana had many medicinal properties and used for the treatment of a migraine, nervous disorders, skin infections, liver complications, hypertension and diabetes [9,10].

Clematis stans:

C. stans is native to Japan[11]. The species is variable, woody based stem, clump- forming perennial climber with pinnate leaves large, ovate, veined, whorled clusters of fragrant, tubular, bell-shaped pale blue flowers with recurved petals.

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 [12,13].

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 [14].

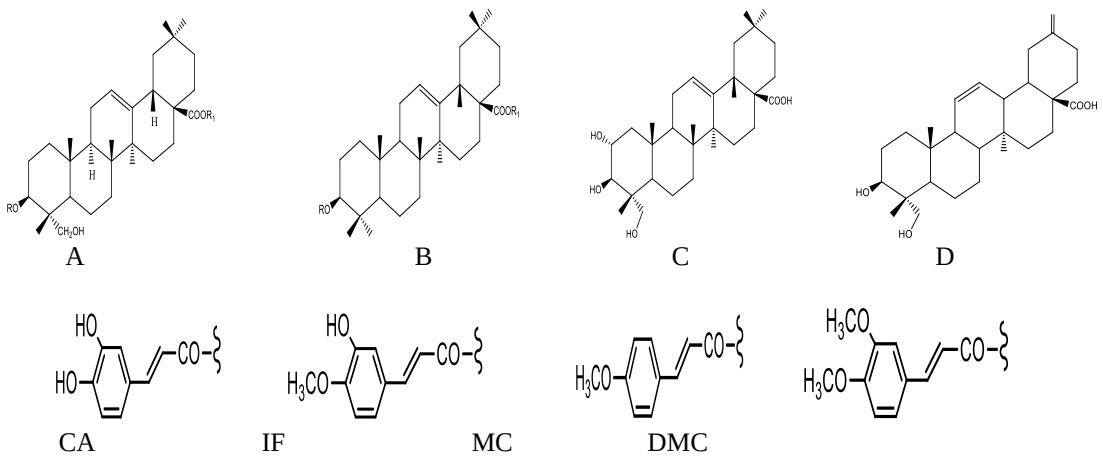

Fig-1 The aglycones from Clematis: A-hederagenin, B-oleanane, C-arjunolic acid, D- quinatic acid; moieties-caffeoyl (CA), isoferuloyl (IF), p-methoxy cinnamyl (MC), 3,4-dimethoxy cinnamyl(DMC).

Table-1 Saponins from Clematis species.

Compound	Structure	Source	Ref.
Neolasiandroside C	R=H R^2=Ara R^1= Rha(1→4)Xyl[(6←1)Glc](1→2)Glc	C. lasiadra	[15]
1.*	R= Rib(1→3)Rha(1→2)[Glc(1→4)]Xyl R^1 = H	C. lasiadra	[16]
2.*	R= Rib(1→3)Rha(1→2)Xyl R^1 = H	C. lasiadra	[16]
3.*	R= Rib(1→3)Rha(1→2)[Glc(1→4)]Ara R^1 = H	C. lasiadra	[16]
4.*	R= Rib(1→3)Rha(1→2)Xyl R^1 = Glc	C. lasiadra	[16]
Clemontanoside C	R = Ara(1→3)Rha(1→2)Ara R^1 = H	C. montana	[17]
Huzhangoside D	R = Rib(1→3)Rha(1→2)Ara R^1= Rha(1→4)Glc(1→6)Glc	C. stans	[18]
Clemastanoside D	R = Rib(1→3)Rha(1→2)Ara R^1 =Glc	C. stans	[19]
Clemastanoside F	R = Ara(1→3)Rha(1→2)Ara R^1= Rha[(3←1)IF](1→4)Glc(1→6)Glc	C. stans	[19]
Clemastanoside G	R = Ara(1→3)Rha(1→2)Ara R^1= Rha[(2←1)IF](1→4)Glc(1→6)Glc	C. stans	[19]
Kizutasaponin K12	R= Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. stans	[19]
Mandshunoside H	R= Glc(1→4)Glc(1→4)Rib(1→3)Rha(1→2)Ara R^1= Glc(1→6)Glc	C. mandshurica	[20]
Clematograveo-	R= Rib(1→3)Rha(1→2)[Glc1→4)Glc(1→4)]Ara	C. graveolens	[21]

lenoside A	R^1= Rha(1→4)Glc(1→6)Glc		
	Oleanane		
Clemontanoside B	R= Glc R^1= Rha(1→6)Glc[(2←1)Glc]	C. montana	[22]
Clemontanoside C	R= Glc R^1= Glc(1→6)Glc[(4←1)Rha]	C. montana	[23]
Clemontanoside E	R= Glc R^1 = Gla(1→6)Glc	C. montana	[23]
Clemontanoside A	R= Glc R^1 = Glc(1→6)Glc(1→6)Glc	C. montana	[24]
Clemontanoside B	R= Glc R^1= Rha(1→6)Glc[(2←1)Glc]	C. montana	[25]
	(C- Arjunolic acid)		
Arjunolic acid	2,3,23 trihydroxyolean-12-ene-28-oic acid	C. montana	[26]
Huzhangoside D	R = Rib(1→3)Rha(1→2)Ara R^1= Rha(1→4)Glc(1→6)Glc	C. stans	[18
Clemastanoside D	R = Rib(1→3)Rha(1→2)Ara R^1 =Glc	C. stans	[19]
Clemastanoside F	R = Ara(1→3)Rha(1→2)Ara R^1= Rha[(3←1)IF](1→4)Glc(1→6)Glc	C. stans	[19]
Clemastanoside G	R = Ara(1→3)Rha(1→2)Ara R^1= Rha[(2←1)IF](1→4)Glc(1→6)Glc	C. stans	[19]
Kizutasaponin K12	R= Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. stans	[19]
Mandshunoside I	R= Glc(1→4)Glc(1→4)Rib(1→3)Rha(1→2)Ara R^1 = Glc(1→6)Glc	C. mandshurica	[20]
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	[27]
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	[28]
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	[28]
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	[28]
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	[28]

1.*=3-O-β-D-ribopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-xylopyranosyl hederagenin.2.*=3-O-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosylhederagenin.3.*=3-O-β-D-ribopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-α-L-arabinopyranosyl hederagenin. 4.*=3-O-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosylhed-28-O-β glucopyranoside.

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.

Steroids, Lignans, Coumarins, Macrocyclic, Volite oils from Clematis species.

The clematis species has been subjected to isolate various biologically active compounds other than saponins. The compounds identified were 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).

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	29, 30,31
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, C. stans, C. terniflora	32,33,34,35,36, 37,38
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	39,37,41,40,35
Steroids		
Stigmasterol, Daucosterol, β-sitosterol, β-amyrin, α-amyrin and their glycosides	C. apiifolia, C. hexapetala, C. montana, C. purpurea	42,43,44,31
Coumarins		
4,7-dimethoxy-5-methyl-coumarin, Siderin, Scopoletin	C. delavayi, C. ligusticifolia, C. intricate	45,46,47
Macrocyclic compounds		
Clemoarmanosides A, B, Bercholine, Clemahexapetoside A, B, Clemochinenoside A, B, Ibotanolide B	C. armandii, C. hexapetala, C. chinensis, C. crassifolia	34,43,40,30
Phenolic compounds		
Ibotanolide B, Calceolarioside B, Clemomandshuricoside A, B, C, Tricosanol, Heptacosanoic acid	C. crassifolia, C. mandshurica, C. terniflora	48,30,38
Anemonin, Protoanemonin, Ranunculin	C. angustifolia, C. apiifolia, C. flammula	50,42,49
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	40,38,45,49,43,51

Pharmacological effects of clematis species-

Clematis lasiandra-

Cytotoxic activity- The air-dried whole plant of C. lasiandra was extracted with 70% EtOH. The cytotox effects of the isolated compounds 1-7 against human leukemia HL-60 cells, human hepatocellular carcinoma Hep-G2 cells, and human gastric carcinoma SGC-7901 cells were evaluated by MTT assay. Streptomycin with Adriamycin was used as positive control. Saponins 1, 3-5 and 7 exhibited significant cytotoxicities against the test tumor cells with IC_{50} values range from 1.40 to 19.50 μmol/L, while saponins 2 and 6 displayed no activity (IC_{50} N 100 μmol/L). The structure-activity relationships (SAR) about the antitumor activity of saponins involved the type, position, and number of sugar moieties attached by a glycosidic bond at different positions. Compounds 2 and 6 structurally related to the active saponins 1, 3-5 and 7 without the free carboxylic acid group at position C-28, indicated it played an important role in the expression of cytotoxicities for triterpenoid saponins. Compared to the oligosaccharide chain at C-3 for the active saponins, compounds 4 and 7 possessed common 3-O-β-D-Rib(1 → 3)-α-L-Rha(1 → 2)-α-L-Ara chain displayed relatively stronger cytotoxicities than 1, 3 and 5. However, saponin 5 with a β-D-Xyl replaced α-L-Ara at C-3 displayed strongest cytotoxicities against HL-60 with IC_{50} value of 1.40 ± 0.52 μmol/L, while showed relatively lower activity against Hep-G2 (IC_{50} = 19.50 ± 5.20 μmol/L). It appears that the cytotoxicities of those triterpenoid saponins not only related with their precious structural features, but also with the selectivity of the tumor cell lines [52].

Insecticidal Activity- Compounds isolated from C. lasiandra exhibited significant aphicidal activities against A. pisum through oral toxicity (LC50 = 0.13–0.98 mg/mL, 72 h) and deterrent effect with deterrence index (DI) of 0.33–0.90 at 0.125 mg/mL after 24 h, while showed no contact toxicity. Compounds 1 and 6 showed potent inhibitory effects

on digestive enzymes of pepsin and α-amylase with the inhibition rate of 84.0 % and 85.7 %, 55.3 %, and 51.5 %, respectively, at the dose of LC80, while appeared inactive to acetyl cholinesterase (AChE) and chitinase in A. pisum. The toxic symptoms of A. pisum caused by 6 involved body-color changes from light green to dark green, and brown until death. Transmission electron microscope (TEM) analysis demonstrated that the organelles including apical microvilli, nuclei, and mitochondria in the midgut tissues were the targeting sites for 6 exerting its aphicidal activity. The results provided new light for the industrial application of triterpenoid saponins from Clematis as novel biopesticides[53].

Clematis montana:

Antioxidant activity-25 mg of ethanolic extract of leaves was dissolved in 25 ml of methanol to get 1000µg/ml stock solution. Lower concentrations (100, 200, 300, 400 µg/ml) was prepared by diluting serially with methanol. The inhibition percentage of extract in 400 (µg/ml) was 80.66 in DPPH and 59.06 in ascorbic acid. The DPPH free radical scavenging activity has been evaluated with IC 50 value was found to be 151.50 µg/ml for extract and 6.727µg/ml for ascorbic acid. In the presence of antioxidant molecules, DPPH dark purple colour change to a colourless solution Discoloration of DPPH solution directly proportional to antioxidant property of the sample [54].

Antimicrobial activity-The ethanolic extract of leaves was examined for antibacterial activity against Gram-positive bacteria S. aureus, B. subtilis, Gram-negative bacteria E. coli, P. aeruginosa and antifungal activity against C. albicans. The antimicrobial screening was performed by agar well diffusion method. Mulle Hinton Agar medium (Hi-media) and Sabouraud agar medium were used for bacterial and fungal strains respectively. Different dilutions of the extract were made having concentration of 100µg/ml, 250 µg/ml, 500µg/ml and 1000µg/ml in DMSO (dimethyl sulphoxide). 0.1 ml of each test solution and control were placed in 6 mm diameter wells. One well was filled with 0.1 ml of standard drug Amoxycillin (10 µg/ml) in the case of antibacterial activity whereas standard drug Fluconazole (10 µg/ml) in antifungal activity. The diameter of the zone of inhibition (mm) for antibacterial activity at the concentration of extract 1000 µg/ml was 21, 24 mm for Gram-positive (S. aureus,B. subtilis) and 28, 25 mm for Gram-negative (E. coli, P.aeruginosa) and 22 mm for antifungal (C. albicans) strains. The diameter obtained for the test samples were compared with diameter produced by the standard Amoxycillin and fluconazole in antibacterial and antifungal activity [54].

Apoptosis-inducing activities - A novel mannose-binding lectin (designated CML) was isolated from Clematis montana Buch.- The purified C. montana lectin was a homodimer of 11,968.9 Da subunits as determined by gel filtration and MS. The hemagglutinating activity of CML was inhibited by branched oligomannosides. Subsequently, CML was also found to exhibit remarkable inhibitory effect on L929, HeLa, MCF7 and HepG2 cells. Furthermore, CML specially induced L929 cell apoptosis in dose-dependent manner as evidenced by MTT, fluorescent microscopy, LDH activity-based cytotoxicity assays and DNA ladder. Moreover, due to both caspase inhibitors and Western blot analyses, caspase was also found to play the important role in the potential apoptotic mechanism of CML. When the carbohydrate-binding site was fully inhibited by sugars, cytotoxicity was abruptly decreased and apoptotic phenomenon in L929 cells was not observed, suggesting a significant correlation between mannose-binding-specific activity and the antineoplastic mechanism [55].

Cytotoxic activity – The triterpenoid saponins montanoside A and B were isolated from the methanol extrat of C. montana. The 50 and 100 µg/ml of the extract was evaluated for cytotoxic activity in vitro in the human tumor cells squmaus carcinoma cells (HSC-2) and Human gingival fibroblast cells (HGF). Both the compounds showed potent activity against the test cells [56.

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) [57].

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 [58].

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 extrac. 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 [59].

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 synoviocytes in AA rats were also elevated compared with the nonimmunized 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 [60].

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 translocation 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 [61].

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 5 from C. lasiandra, 1 from C. mantana, 4 from C. stans, 1 from C. mandshurica and 1 from C. graveolens and oleanane aglycone based were 6 from C. mantana, 2 from C. stans and 6 from C. mandshurica. 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.

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