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PHYTOCHEMICALS AND PHARMACOLOGICAL EFFECTS OF CLEMATIS SPECIES-A REPORT

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

ABSTRACT*: Clematis* species have been a source of various traditionally useful and pharmacologically effects. The plants are prominently climbers and woody vines. The species are mosly wild. The species *Clematis argentilucida, Clematis montana*, *Clematis grata*, and *Clematis ganpiniana* were selected to study on their traditional use, chemical composition and pharmacological effects reported in literature. In folklore these species are used as for the treatment of wound, rheumatism, arthralgia, aphonia hoarseness, limb numbness, migraine, nervous disorders, skin infections, liver complications, hypertension and diabetes. The triterpenoid saponins are the dominant compounds of these species. However, flavonoids, alkaloids, lignans, coumarins, steroids and volatile oils have also been reported. The chemical constituents isolated were hederagenin aglycone based new saponins- 1 from *Clematis argentilucida*, 1 from *Clematis montana*, *1* from *Clematis grata*, and 3 from *Clematis ganpiniana* and 6 oleanane aglycone based saponins from *Clematis montana*. The pharmacological activities have been antioxidant, cytotoxic, antimicrobial, antidiabetic, hepatoprotectve, and anti-inflammatory activities. As such these species has emerged as good source of traditional medicines, chemical diversity and pharmacological effects. Though the isolated compounds from these species have been reported for their pharmacological effects but uncharacterized crude extracts were employed in most of the activities. Such species need to be explored scientifically 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.

IndexTerms - Clematis argentilucida, Clematis montana, Clematis grata, Clematis ganpiniana,

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. In China, *Clematis armandii* and *C. montana* called "mu tong" are also used for the treatment of reducing fever to induce urination, stimulating menstrual discharge and promoting lactation. *Clematis grata* is used as a remedy to treat conditions such as leprosy, blood diseases, and fevers. *Clematis argentilucida* have been used for the treatment of wound, rheumatism, arthralgia, aphonia hoarseness as well as limb numbness. 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. argentilucida*, 1 from *C. montana*, , 1 from *C. grata*,3 from *C. ganpiniana* and 1 from C. graveolens and oleanane aglycone based were 6 from *C. montana*. The pharmacological activities have been antioxidant, cytotoxic, antimicrobial, antidiabetic, hepatoprotectve, and anti-inflammatory activities. As a source of herbal medicines for traditional use, chemical constituents diversity and various biological effects the species *Clematis argentilucida*, *Clematis montana*, *Clematis grata*, and *Clematis ganpiniana* were selected for the study. 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 argentilucida: This species is native to China North-Central, China South-Central, China Southeast [3]. *Clematis argentilucida* is a perennial woody climber, having the effect of analgesic, detoxification, and activating blood circulation. The rhizome of the plant has been used as traditional folk medicines for the treatment of wound, rheumatism, arthralgia, aphonia hoarseness as well as limb numbness [4].

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 [5]. The leaves of *Clematis montana* have been utilized for the treatment

of skin diseases in India [6]. 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 [7]. *Clematis montana* had many medicinal properties and used for the treatment of a migraine, nervous disorders, skin infections, liver complications, hypertension and diabetes [8, 9].

Clematis grata: **Charming Clematis-** the species is native to Afghanistan, East Himalaya, Myanmar, Nansei-shoto, Nepal, Pakistan, Taiwan, Tibet, West Himalaya, India [10]. The plant is grown as an ornamental, often being allowed to grow into trees and large shrubs. It flowers on the current season's new growth and generally does not need pruning unless it is growing too large, in which case simply thinning out some of the stems in late winter or early spring before now growth commences is usually all that is needed. The plant is used as a remedy to treat conditions such as leprosy, blood diseases, and fevers [11]. The stems, or the fibres obtained from them, are used as a tying material.

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 [12].

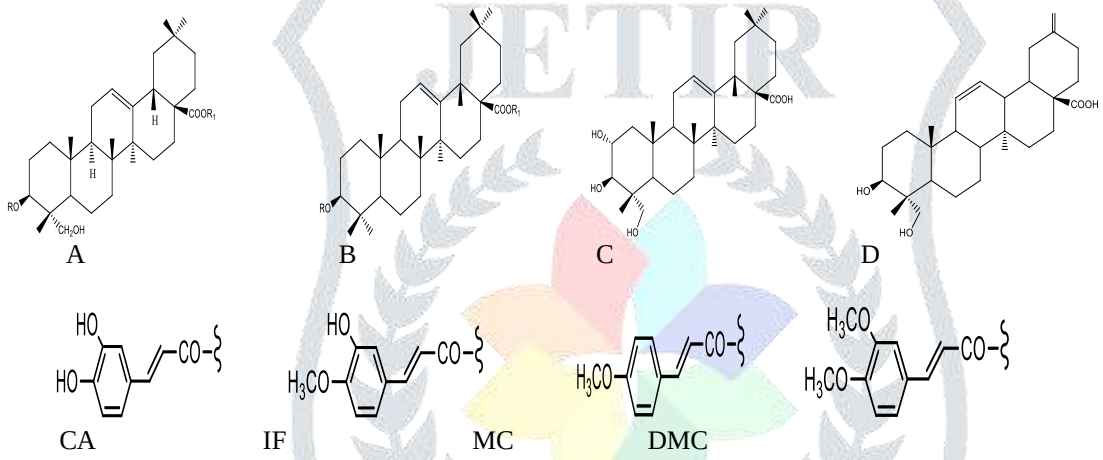

Fig-1 The aglycones from *Clematis*: A-hederagenin, B-oleanane, C-arjunolic acid, D- quinatic acid; moieties- caffeoyl (CA), isoferuloyl (IF), p-methoxy cinnamyl (MC), 3,4-dimethoxy cinnamyl(DMC).

Table-1 Saponins from Clematis species.

Compound	Structure	Source	Ref.
	Hederagenin Type-A		
Clemontanoside C	R = Ara(1→3)Rha(1→2)Ara R^1 = H	*C. montana*	[13]
Clematiganoside A	R= Rib(1→3)Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	*C. ganpiniana*	[14]
Hederacholichiside F	R= Glc(1→4)Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	*C. ganpiniana*	[14]
Prosapogenin CP11	R= Glc(1→4)Glc(1→4)Rib(1→3)Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	*C. ganpiniana*	[14]
Clematoside S	R = Rib(1→3)Rha(1→2)Ara R^1 = H	*C. grata*	[15]
	Hederagenin 11,13- dien-28-oic acid		
5.*	R= Rib(1→3)Rha(1→2)Ara R^1 = H	*C. argentilucida*	[16]
Clematograveo-lenoside A	R= Rib(1→3)Rha(1→2)[Glc1→4)Glc(1→4)]Ara R^1= Rha(1→4)Glc(1→6)Glc	*C. graveolens*	[17]
	Oleanane Type-B		
Clemontanoside A	R= Glc R^1 = Glc(1→6)Glc(1→6)Glc	*C. montana*	[18]
Clemontanoside B	R= Glc R^1= Rha(1→6)Glc[(2←1)Glc]	*C. montana*	[19]
Clemontanoside C	R= Glc R^1= Glc(1→6)Glc[(4←1)Rha]	*C. montana*	[20]
Clemontanoside E	R= Glc R^1 = Gla(1→6)Glc	*C. montana*	[20]
Clemontanoside A	R= Glc R^1 = Glc(1→6)Glc(1→6)Glc	*C. montana*	[18]
	(C- Arjunolic acid)		
Arjunolic acid	2,3,23 trihydroxyolean-12-ene-28-oic acid	*C. montana*	[21]

Nearly, 30 species have been characterized through isolation and structure determination of saponins from *Clematis.* The present study revealed that hederagenin aglycone based new saponins isolated were 1 from *C. argentilucida*, 1 from *C. montana*, , 1 from *C. grata*,3 from *C. ganpiniana* and 1 from C. graveolens and oleanane aglycone based were 6 from *C. montana*. 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	22,23,24
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	25,26,27,28, 29,30, 31,
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	32,30,34,33, 28
Steroids		
Stigmasterol, Daucosterol, β-sitosterol, β-amyrin, α-amyrinb and their glycosides	C. apiifolia, C. hexapetala, C. montana, C. purpurea	35,36,37,24
Coumarins		
4,7-dimethoxy-5-methyl-coumarin, Siderin, Scopoletin	C. delavayi, C. ligusticifolia, C. intricate	38,39,40
Macrocyclic compounds		
Clemoarmanosides A, B, Bercholine, Clemahexapetoside A, B, Clemochinenoside A, B, Ibotanolide B	C. armandii, C. hexapetala, C. chinensis, C. crassifolia	27,36,33,23
Phenolic compounds		
Ibotanolide B, Calceolarioside B, Clemomandshuricoside A, B, C, Tricosanol, Heptacosanoic acid	C. crassifolia, C. mandshurica, C. terniflora	41,23,31
Anemonin, Protoanemonin, Ranunculin	C. angustifolia, C. apiifolia, C. flammula	43,35,42
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	43,31,38,41,36,44

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

The clematis species has been subjected to isolate various biologically active compounds other than saponins. 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, 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 argentilucida:

Cytotoxic Activity-
The air-dried and powdered roots of *C. argentilucida* extracted with 70 % EtOH and partitioned with petroleum ether and n-BuOH to give seven fractions (Fr. A-G). The cytotoxicities of saponins 1-18 (all the purities > 95 %, analyzed by HPLC) against human leukemia HL-60 cells (ATCC), human hepatocellular carcinoma Hep-G2 cells (ATCC) and humangastric carcinoma SGC-7901 cells (ATCC) were evaluated by the MTT method, with adriamycin (Sigma, ≥ 98 %) as the positive control. The cytotoxicities of saponins 1-18 against human leukemia HL-60 cells, human hepatocellular carcinoma Hep-G2 cells, and human gastric carcinoma SGC-7901 cells were evaluated by the MTT colorimetric assay. The IC_{50} value of each compound was measured on the basis of cell viability after 72 hrs. The results showed that the monodesmosidic saponins 4, 7, 8, and 14-18, with only one oligosaccharide chain attached at C-3 or C-28, displayed cytotoxicity against the three cell lines with IC_{50} values ranging from 0.87 to 19.48 μM, whereas the bisdesmosidic saponins 1, 2, 5, 6 and 9-13 were all inactive. This suggested that the presence of a free carboxylic group at C-28 or a free hydroxy group at C-3 is important for the cytotoxic activity. The monodesmosidic saponin 3 with 21α-OH oleanolic acid as the aglycone showed no cytotoxicity. Therefore, we postulated that the hydroxyl group at C-21 had a strong negative effect on the cytotoxic activity. Saponins 14 and 16 possessed the same oligosaccharide chain, but 14 with hederagenin (C-23 hydroxylated) as the aglycone exhibited weaker cytotoxicity than 16 with oleanolic acid as the aglycone [45].

Cytotoxic Activity
The air-dried and powdered roots of *C. argentilucida* extracted with 70% EtOH partitioned successively with petroleum ether (5 L × 2) and n-BuOH to give seven fractions (Fr.s A-G). The Fr. F exhibited more cytotoxic activity against U251MG cells withsix IC_{50} value 46 μg/mL than the remaining fractions. The cytotoxicity of saponins 1-13 against human glioblastoma U251MG cells was carried out by the MTT colorimetric method, with the anticancer agent nimustine hydrochloride as positive control. The optical density of each well was measured with a Bio-Rad 680 microplate reader at 570 nm, respectively. The cytotoxicity activities of the saponins and positive control were determined at 80, 40, 20, 10, 5 and 2.5 μM. The IC_{50} value of each compound was measured on the basis of cell viability after 48 h treatment. The results showed that the monodesmosidicsaponins 1, 2 and 4-8 with only one oligosaccharide chain attached at C-3 exhibited cytotoxicity against U251MG cells with IC_{50} values ranging from 6.95 to 38.51 μM, whereas the bisdesmosidic saponins 3 and 9-13 were all inactive. This suggested that the presence of a free carboxylic group or a hydroxy group at C-28 played an important role in cytotoxicity activity against tumor cells, which was in agreement with the characteristic structural features of cytotoxic saponins Saponins 1, 2 and 7 shared the same trisaccharid chain, but 2 and 7 with the taraxerane-type (iso-oleanane) or oleanane-type aglycones were more potent than 1 with the ursane-type aglycone, and the positions of the double bond and the 27-CH_3 in the aglycones of 2 and 7 seemed to have little impact on the cytotoxicity. Saponin 4 with the oleanolic acid aglycone was the most cytotoxic among all the tested saponins, which suggested that the presence of the carboxylic group at C-28 is important for the potent cytotoxic activity. Saponin 6 with hederagenin (C-23 hydroxylated) as the aglycone exhibited weaker cytotoxicity than 5 with oleanolic acid as the aglycone. This was in agreement with those reported that the hydroxyl group at C-23 of the aglycone had a negative effect on the cytotoxicity of the oleanane-type saponins. However, saponin 5 possessing a disaccharide chain exhibited weaker cytotoxicity than 4 with a trisaccharide chain, suggesting that the number and type of the sugar units also affected the resultant cytotoxicity. These results demonstrated that the cytotoxic activity of such saponins was influenced by the structures of both the aglycones and the sugar parts, and more extensive studies are required before a clear structure-activity relationship can be reached [46].

Cytotoxic Activity
The air-dried roots of C. argentilucida extracted with 70% EtOH partitioned with petroleum ether and n-BuOH. The n-BuOH phase was evaporated under reduced pressure to give a dark gummy residue (250 g) which was shown to be cytotoxicity against U251MG cells (IC_{50} = 39 μg/mL). 100 g gave fourteen major fractions (Fr. 1-Fr. 14). As Fr. 8, 10 and 11 showed more cytotoxic activities against U251MG cells (IC_{50} = 19 μg/mL, 27 μg/mL, 22 μg/mL, respectively) than the remaining eleven fractions.The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay was used for in vitro evaluation of the cytotoxic potential of the isolated compounds against three cultured human tumor cell lines HL-60 (human leukemia), Hep-G2 (human hepatocellular carcinoma) and U251MG (humanglioblastoma). All drug doses were tested with Doxorubicin (Sigma, ≥ 98%) as the positive control against HL-60cells and Hep-G2 cells, and nimustine (ACNU Sigma, ≥ 98%) as the positive control against U251MG cells. The cytotoxicities of saponins 1-3 against human leukemia HL-60 cell lines, human hepatocellular carcinoma Hep-G2 cell lines and human glioblastoma U251MG cell lines were determined using the MTT colorimetric assay. Among the testedcompounds, 1 and 2 possessed a free carboxylic group at C-28 exhibiting significant cytotoxicity against all of the test cancer cell lines, earlier studies on the cytotoxicity of similar compounds against HL-60 and U251MG cell lines have reached the same result. However, 3 was a monodesmoside without the free carboxylic group at C-28 and no cytotoxic activity was observed which differed from the reported result of another literature in terms of the cytotoxicity against HL-60 cell lines. Hence, a more comprehensive cytotoxicity study covering more potential impact factors, like cell culturing environment, district differentiation of HL-60 cell lines etc., is necessary to understand the difference. As two new saponins(1 and 2) possessed different oleanolic acid aglycones and ligosaccharide chains, the fact of saponin 2 exhibiting more activity than 1 in the cytotoxic experiment suggested that the cytotoxicity of these class of saponins were related to their aglycones and oligosaccharide chains, and further studies on the cytotoxicity of the eleven saponins previously isolated from *C. argentilucida* are necessary to clarify their clear structure activity relationship [47].

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 151.50µg/ml for extract and 6.727µg/ml for ascorbic acid. In the presence of DPPH dark purple colour change to a colourless solution Discoloration of DPPH solution directly proportional to antioxidant property of the sample [48].

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 [49].

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 [50].

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.[51]

Clematis grata:

Antibacterial activity- The acetone, distilled water and methanol extract of C. grata were examined using Agar-well diffusion method. The methanol extract of leaf showed remarkable antibacterial activity against all the tested bacteria with maximum zone of inhibition (ZOI) against Staphylococcus aureus (19.33±1.18 mm) and minimum ZOI against Salmonella typhi (12.33±0.26 mm). Also, acetone extract of leaf of this plant was found to be most active against same bacterium S. aureus (ZOI=13.66±0.26 mm). Distilled water extract was not active against S. typhi and Escherichia coli. The methanol extract of root displayed remarkable antibacterial activity against all the tested bacteria with ZOI of 17.00±0.47, 16.00±0.82, 13.00±0.46 and 10.66±0.26 mm against Bacillus cereus, S . aureus , E. coli and S . typhi , respectively. The rest of the two extracts namely acetone and distilled water displayed no appreciable antibacterial activity against the tested bacteria. Acetone extract of stem showed ZOI of 8.66±0.27, 9.33±0.27, 13.66±1.44 and 16.66±15.66 mm from concentrations 25, 50, 75 and 100% respectively in case of B. cereus[51].

Antioxidant activity: The antioxidant activity for acetone, distilled water and methanol extracts of different parts (leaf, root and stem)of C. grata. Ascorbic acid was used as a standard exhibiting IC_{50} value of 28.12µg/mL. In case of leaf, out of all the extracts, methanol extract exhibited maximum percent inhibition with lowest IC_{50} value of 11.55µg/mL followed by acetone extract with IC_{50} value of 22.59 µg/mL and distilled water extract with IC_{50} value of 56.85 µg/mL table 2.2. In case of root, methanol extract had IC_{50} value of 38.73µg/mL while IC_{50} value of root extract prepared in acetone was 49.01µg/mL. Stem methanol extract had IC_{50} value of 11.39 µg/mL whereas acetone extract had IC_{50} value of 15.75 µg/mL and distilled water extract had IC_{50} value of 34.48 µg/mL[51].

***Clematis ganpiniana**:*

Apoptosis activity- The methanol extract of saponins of C. ganpiniana was uesd in the concentration of 0.08, 0.4, 2 and 10µg/ml. Cells treated with only DMSO were used as the control. MCF-7 and MDA-MB-231 breast cancer cell lines were tested using Cytotoxic effect assay (MTT assay). The The clematis hederagenin saponins (CHS) showed cytotoxic effect on breast cancer cells. In this study, MCF-7 and MDA-MB-231 breast cancer cells were used to evaluate the anticancer effect of CHS. Compared to the negative control group, CHS showed cytotoxic effect on both types of breast cancer cells after 12, 24 and 48 h of treatment, in a time- and dose-dependent manner ($P<0.05$). The CHS induced apoptosis in breast cancer cells. After 6, 12, and 24 h of treatment with 2.0 µg/ml CHS, MCF-7 and MDA-MB-231 cells were evaluated using flow cytometry to determine the apoptosis rate. The results showed that CHS induced apoptosis in breast cancer cells and the apoptosis rate increased over time. MCF-7 and MDA-MB-231 cells treated with 2.0 µg/ml CHS for 24 h showed an early apoptosis rate of 29.3 and 19.8%, respectively. CHS regulated the mitochondrial Apaf-1 and Cyto c level.MCF-7 and MDA-MB-231 cells were treated for 2, 6, 12 or 24 h with CHS (2 µg/ml) and both mitochondrial Apaf-1 and Cyto C level were detected by western blotting. CHS significantly reduced mitochondrial Apaf-1 and Cyto C proteins in breast cancer cells, indicating the enhanced release of Apaf-1 and Cyto C

from mitochondria in breast cancer cells. After the cells were treated with the compound for 2 to 24 h, there was gradual reduction of mitochondria Apaf-1 and Cyto C proteins [52].

Cytotoxic and antibacterial activity-

The dried plant of *Clematis ganpiniana* was extracted with 70% EtOH. The estrogen independent human breast cancer cell line MDA-MB-231 and the estrogen dependent human breast cancer cell line MCF-7 adriamycin (2 g/ml) was used as positive control.The MTT assay was used to measure the cytotoxicity of compounds. Antibacterial activities of compounds were determined against Micrococcus luteus, Escherichia coli, Bacillus subtilis, Salmonella paratyphi B, Candida albicans, Staphylococcus aureus, and Bacillus pumilus by the paper diffusion method. Rifampicin was used as a positive control against pathogenic bacteria with ten concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 g/disk [53] .

Cytotoxic activity- The IC_{50} value and apoptosis rates of four compounds were evaluated and showed in the range of MCF-7 (0.7 to 2.3) and MDA-MB-23 (0.9- 3.7) as in comparison to the adriamycin 2.4 and 7.2 respectively. The apoptosis % of MCF-7 (25.5 to 38.9) and MDA-MB-23 (17.3- 28.9) as in comparison to the adriamycin 27.3 and 16.3%. The compound 1, 2, 4 showed lower IC50 comparing with adriamycin, compounds 1 and 4 showed higher apoptosis rates than that of adriamycin, and the apoptosis rate of compound 2 was similar with positive control, so all of these three compounds are potential anti-tumor agents. Especially, compound 4 showed the strongest activity against cancer cells and highest apoptosis rates among these four com-pounds. Comparing the chemical structures of these compounds, it suggested that the synergistic effect of two groups, 3-O--l-rhamnopyranosyl-(1→ 2)--l-arabinopyranoside and 23-hydroxy methyl group, to improve the anti-tumor bioactivity. So 3-glycosyl and 23-hydroxy group substituent will be important in these activities.

Antibacterial Activity- The antibacterial activities of compounds 1-4 were tested against Micrococcus luteus, Escherichia coli, Bacillus subtilis, Salmonella paratyphi B, Candida albicans, Staphylococcus aureus, and Bacillus pumilus, compound 4 showed weak active against almost all tested bacterium besides Salmonella paratyphi B. while almost other three compounds showed no active, it seemed that compound 4 maybe one potential wide-spectrum compound. All of these data give reason to ethnobotanical field survey and speculate that triterpenoid saponin from clematis might behelpful to assay their anticancer activity in vitro[53].

Conclusion

The traditional uses from the species *Clematis argentilucida, Clematis montana, Clematis grata*, and *Clematis ganpiniana* were for the treatment of wound, rheumatism, arthralgia, aphonia hoarseness as well as limb numbness. *Clematis montana* had many medicinal properties and used for the treatment of a migraine, nervous disorders, skin infections, liver complications, hypertension and diabetes. The *Clematis grata* has been used as a remedy to treat conditions such as leprosy, blood diseases, and fevers. 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. The 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 aglycone based new saponins- 1 from *Clematis argentilucida,* 1 from *Clematis montana, 1* from *Clematis grata*, and 3 from *Clematis ganpiniana* and 6 oleanane aglycone based saponins from *Clematis montana.* 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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