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Chemicals and Pharmacological Effects of Clematis Species-a Review

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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 mosly wild however; few are grown as ornamental plants. The species Clematis vitalba, Clematis stans, Clematis tangutica, and Clematis graveolens 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. 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.

KEYWORDS: Clematis vitalba, Clematis stans, Clematis tangutica, and Clematis graveolens, saponins.

I. INTRODUCTION

Clematis L. 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, arthiritis, antioxidant, antipypretic, antimicrobial, apoptosis, cardio- protective 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 vitalba, Clematis stans, Clematis tangutica, and Clematis graveolens 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 3 from C. vitalba, 4 from C. stans, 12 from C. tengutica and 1 from C. graveolens and oleanane aglycone based were 2 from C. stans, 3 from C. tengutica. 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.

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Traditional uses of clematis species:

Clematis vitalba: Old man's beard is native to northern Africa (i.e. northern Algeria), Europe (i.e. France, Portugal, Spain, UK, Austria, Belgium, Czechoslovakia, Germany, Hungary, the Netherlands, Poland, Switzerland, southern Russia, Ukraine, Albania, Bulgaria, Greece, Italy, Macedonia, Romania and Yugoslavia) and western Asia (i.e. Afghanistan, Cyprus, northern Iran, Lebanon, Syria, Turkey, Armenia, Azerbaijan and Georgia)[3]. As this species is a woody plant, the stem was used in the past to make baskets. It is called traveller's joy because it adorns hedges and banks in the countryside with billows of beautiful feathery seed heads in the grey months leading up to Christmas. C. vitalba has been used in various treatments as it is said to contain anti-inflammatory properties. Traditional recipes used the plant to treat various ailments, including skin irritations and stress [4]. As this species is a woody plant, the stem was used in the past to make baskets.

Clematis stans:

C. stans is native to Japan [5]. 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 tangutica(Maxim.) Korsh. – Yellow Clematis, Golden Clematis, Yellow Bower- is a Central Asian species, and is distributed in southeast Kazachstan, Mongolia, western Chinese provinces Gansu, Qinghai, Shaanxi, Sichuan, Xinjiang, in Tibet and Kashmir[6]. In the whole range there are several varieties. This is a decorative species grown in many regions of the world in gardens and parks unfortunately it easily escapes and can become invasive. In the wild it grows in forests and shrubby slopes, on the banks of streams, at elevations from 300 to 4900 m. It blooms from June to August. The whole plants have been used in traditional Tibetan medicine for the treatment of indigestion and invigorating blood circulation [7]. The plant is the main ingredient of Kang Tai capsules, a compound preparation of Chinese herbs, which has showed significant effect in preventing and treating cardiac disease in clinical practice. Previous chemical studies have shown that triterpenoid saponins are the main components of the plant, and the antifungal activities of several saponins were confirmed [8].

Clematis graveolens occurs as woody climber, generally present along the road banks and fences of agricultural fields. It is distributed from temperate (Asia), Western Asia (Afganistan), Xizang (China), to the tropical (Himachal Pradesh, Jammu Kashmir, Utter Pradesh, Nepal and Pakistan in the altitude range between 900-3000m [9]. The plant is harvested from the wild for local use as a medicine. It is sometimes grown as an ornamental. In traditional medicins the powdered stem, combined with powdered Bistorta milletii, is drunk with warm water as a treatment of coughs and colds [10]. The squeezed seeds are applied to the foreheads in order to relieve headaches [10]. The long, yellow-coloured root has an exceedingly bitter taste. It is considered to be antiseptic and cooling. It is prescribed as a gargle in the treatment of ulcerated throat, as an application in treating dog and serpent bites, and is also used in cases of haemorrhage from the stomach or throat [11]. The leaves of this plant possess distinct character of causing blister in the mouth [12]. The tincture prepared in the spirit is used for treatment of goiters and tumors of the neck [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].

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A B C D

CA IF MC DMC

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)		
Tanguticoside A	R = Glc R^1 = Rha(1→4)Glc(1→6)Glc	C. tangutica	[15]
Tanguticoside B	R = Glc(1→2)Glc R^1 = Rha(1→4)Glc(1→6)Glc	C. tangutica	[15]
Saponin PK	R = Rha(1→2)Glc R^1 = Rha(1→4)Glc(1→6)Glc	C. tangutica	[15]
Clematangoticoside B	R=Rha(1→2)Ara R^1= Rha(1→4)Glc(1→6)[Rha(1→2)]Glc	C. tanguitica	[16]
Clematangoticoside C	R= H R^1= Rha(1→4)Glc(1→6)[Rha(1→2)]Glc	C. tanguitica	[16]
Clematangoticoside D	R=H R^2=Glc R^1= Rha(1→4)Glc(1→6)[Rha(1→2)]Glc	C. tanguitica	[16]
Clematangoticoside E	R= H R^2= Glc R^1 = Rha(1→4)Glc(1→6)Glc	C. tanguitica	[16]
Clematangoticoside F	R= H R^2= Glc R^1 = Rha(1→4)Glc(1→6)Glc	C. tanguitica	[16]
Clematangoticoside G	R=H R^2= Glc R^1 = Rha(1→4)Glc(1→6)Glc	C. tanguitica	[16]
Clematangoticoside H	R= Glc[(2←1)Caffeoyl](1→4)Glc(1→4)Xyl(1→3)Rha (1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. tanguitica	[16]
Clematangoside C	R= Rha(1→2)Ara R^1= Glc(1→6)Glc(1→4)Rha(1→4)Glc(1→6)Glc	C. tanguitica	[17]
Clematangoside D	R= Rha(1→2)Ara R^1= Rha(1→4)Glc(1→4)Rha(1→4)Glc(1→6)Glc	C. tanguitica	[17]
Vitalboside G	R= Rha(1→3)Rha(1→4)Rib(1→2)Glc(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. vitalba	[18]
Vitalboside D	R= Rha(1→4)Rib(1→2)Glc(1→2)Ara R^1 = H	C. vitalba	[18]
Vitalboside H	R= Rha(1→3)Rha((1→4)Rib(1→2)Glc(1→2)Ara	C. vitalba	[18]

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	R^1 = Rha(1→4)Glc(1→6)Glc		
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	[19]
	Hederagenin 18- en-28-oic acid		
Clematangoside A	R= Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. tanguitica	[17]
	Hederagenin 21-OH		
Clematangoside B	R= Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. tanguitica	[17]
Huzhangoside D	R = Rib(1→3)Rha(1→2)Ara R^1= Rha(1→4)Glc(1→6)Glc	C. stans	[20]
Clemastanoside D	R = Rib(1→3)Rha(1→2)Ara R^1 =Glc	C. stans	[21]
Clemastanoside F	R = Ara(1→3)Rha(1→2)Ara R^1= Rha[(3←1)IF](1→4)Glc(1→6)Glc	C. stans	[21]
Clemastanoside G	R = Ara(1→3)Rha(1→2)Ara R^1= Rha[(2←1)IF](1→4)Glc(1→6)Glc	C. stans	[21]
Kizutasaponin K12	R= Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. stans	[21]
	Oleanane Type B		
Sieboldianoside B	R=Xyl(1→3)Rha(1→2)Ara R^1= Rha(1→4)Glc(1→6)Glc	C. Stans	[21]
Huzangoside C	R = Rib(1→3)Rha(1→2)Xyl R^1 = Rha(1→4)Glc(1→6)Glc	C. Stans	[21]
Ciwujianoside A	R = Glc(1→6)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. tangutica	[16]
Saponin Pj3	R= Rha(1→2)Ara R^1 = Rha(1→4)Glc(1→6)Glc	C. tangutica	[16]
Clematangoticoside A	R=Rha(1→2)Ara R^1 = (28←1)Glc[(2←1)Rha](6←1)Glc(4←1)Rha	C. tanguitica	[17]

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 3 from C. vitalba, 4 from C. stans, 12 from C. tengutica and 1 from C. graveolens and oleanane aglycone based were 2 from C. stans, 3 from C. tengutica. 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 identified compounds were 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).

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Pharmacological effects of clematis species-
Clematis vitalba:
Antimicrobial activity-A broad activity against pathogenic yeast and yeast-like microorganisms was shown in crude extracts of young shoots of C. vitalba. Antimycotic activity evaluated by using the agar diffusion well bioassay. MICs ranging from 1.4 to 12.3 mg/ml were observed. After fractionating with petroleum ether, ethyl acetate and methanol, antimycotic activity has been observed only in methanol fractions. The extract 100 mg/ml of C. vitalba was used to record antimicrobial activity against microbial strans C. albicans, C. glabrata, S. cerevisiae Cl. lusitaniae Cr. laurentii Fil. neoformans Prototheca zopfii Filobasidiella neoformans. Nystatin (30 mg/ml) was used as positive control. The MIC (mg/ml) recorded from different strains was between 9 to15. The broad antimicrobial activity exhibited by extracts of C. vitalba points to this species as a potential source of antimycotic agents against some Prototheca species particularly Pr. zopfii, which is considered responsible for a number of sub-clinical mastitis in milk cows.[45].

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	25,26,27,28,31, 32,33
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-amyrin 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	41,23,35
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

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Anti-inflammatory, antinociceptive and antipyretic effects-

The effectiveness of extracts, fractions and subfractions from dried C. vitalba L. aerial parts were studied on Male Sprague-Dawley rats and Swiss albino mice. 2 kg of powdered aerial part of C. vitalba was extracted with EtOH and fractioned to yield $CHCl_3$ Fr' (15.06 g), EtOAc Fr. (12.33 g), BuOH Fr. (47.54 g) and remaining H_2O Fr. (74.25 g). Extracts were shown to have a potent effect on carrageenan-induced hind paw edema and acetic acid-induced increased vascular permeability models. Through bioassay-guided fractionation procedures a new C-glycosylflavon, 4′ -O-coumaroyl-isovitexine (vitalboside) was isolated as the main active ingredient of the aerial parts. Vitalboside showed a potent and dose-dependent (in 75 and 150 mg/kg) in vivo anti-inflammatory activity against acute (carrageenan-, serotonin- and PGE_2 -induced hind paw edema model, castor oil-induced diarrhea), subacute (subcutaneous air-pouch) and chronic (Freund's complete adjuvant-induced arthritis) models of inflammation. The same compound was also isolated as the main antinociceptive principle which was assessed by using the models based on the inhibition of p-benzoquinone-induced writhings, as well as antipyretic activity against Freund's complete adjuvant-induced increased body temperature. Acute and subchronic toxicity studies were also performed [45].

Clematis tengutica

Cytotoxic activity- The cytotoxic activities of the isolated saponins (all the purities > 95%, analyzed by HPLC) against SGC-7901 (human gastric carcinoma cell line), HepG2 (human liver hepatoma cancer cell line), HL-60 (human promyelocytic leukemia cancer cell line) and U251MG (human glioblastoma cancer cell line) were determined according to the MTT method. Adriamycin (98%, Sigma, USA) was used as a positive control against SGC-7901, HepG2, and HL-60 cells, and nimustine (98%, Sigma, USA) was used as a positive control against U251MG cell. The tested saponins (0.5, 2, 10, and 50 mM), adriamycin (0.05, 0.2, 0.5, and 1 mM).

Cytotoxic activity- The cytotoxic activities of the isolated saponins (all the purities > 95%, analyzed by HPLC) against SGC-7901 (human gastric carcinoma cell line), HepG2 (human liver hepatoma cancer cell line), HL-60 (human promyelocytic leukemia cancer cell line) and U251MG (human glioblastoma cancer cell line) were determined according to the MTT method. Adriamycin (98%, Sigma, USA) was used as a positive control against SGC-7901, HepG2, and HL-60 cells, and nimustine (98%, Sigma, USA) was used as a positive control against U251MG cell. The tested saponins (0.5, 2, 10, and 50 mM), adriamycin (0.05, 0.2, 0.5, and 1 mM), and nimustine (0.1, 0.5, 1, and 2 mM), were added into triplicate wells, and were incubated for another 72 h. The plates were at 570 nm on a microplate reader. Saponins possessing a free carboxylic group at C-28 exhibited moderate cytotoxicity against all of the test cancer cell lines with the values of IC_{50} in the range of 1.88e27.20 mM, while saponins 4 and 6 showed selective cytotoxicity against PC-3 with the IC_{50} values of 24.14 and 21.35 mM, respectively. The other tested saponins were inactive (IC_{50} > 50 mM) against the test cancer cell lines. All isolated saponins were inactive (IC_{50} > 50 mM) to the normal human hepatocyte cell line (L02). Clematangoticosides D-G found to be unusual 23, 28-bidesmosidic glycosides. Clematoside S, sapindoside B, kalopanax saponin A, and koelreuteria saponin A exhibited cytotoxicity against all of the test cancer cell lines with IC_{50} values in the range of 1.88e27.20 mM, while clematangoticoside D and F showed selective cytotoxicity against SGC-7901 with IC_{50} values of 24.22 and 21.35 mM, respectively [46].

Antifungal activity- The triterpene saponins isolated ethanol extract of aerial part of C. tangutica showed antifungal activities against seven fungal strains- Candida albicans, Candida glabrata, Saccharomtces cerevisiae, Cryptococcus neoformans,and Trichosporon beigelii and plant pathogenic fungus Pyricularia oryzae using Agar Diffusion Assay. The amphotericin B was used as positive control. The compounds 1 and 2 showed antifungal activitie with MIA= 2.5 µg/disc against S. cerevisiae simillar to control (amphotericin) and ordinary activity MIA= 10 µg/disc against P. avellaneum, C. glabrata, T. beigelii and P. oryzae. The

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activity observed for the compounds showed that with increasing sugar moiety compound 2 was better antifungal than1 [47].

Anti-Myocardial Ischemia Activity- The isolated compounds of C. tangutica showed anti-ischemic activities were evaluated by measuring the serum levels of LDH and CK‑MB in hypoxia-treated cardiomyocytes, using diltiazem hydrochloride injection (10 mg/vial, > 98 %) as positive control. Cells were pretreated with various concentrations of saponins 1-10 and diltiazem hydrochloride injection for 24 h and then subjected to hypoxia condition for 3 h. The CK−MB and LDH levels were determined spectrophotometrically at 660 nm and 340 nm, respectively, using diagnostic kits. All saponins were evaluated for their protective effects in hypoxia-induced myo- cardial injury model. CompoundsClematangosideB, Clematangoside C, Clematangoside D, cauloside D, and asperosaponin VI exhibited anti-myocardial ischemia activities with ED_{50} values in the range of 75.77-127.22 μM [48].

Cardioprotective activity- From air-dried powders of the whole plants of C. tangutica were extracted with 70% ethanlnol. The cardioprotective activities of compounds were evaluated by measuring the levels of CK-MB and LDH in A/R treated cardiomyocytes, using diltiazem hydrochloride injection (10 mg/vial,) as positive control. Then cells were incubated in normal culture medium for 6 h in CO_2 incubator. The CK-MB and LDH levelswere determined spectrophotometrically at 660 nm and340 nm, respectively; The results showed that all of these compounds at a concentration of 0.05 mM displayed decreasing effects of the serum CK-MB and LDH levelscompared with the A/R group. The presence of a free hydroxyl group at C-3 played an important role in terms of cardioprotective activities. The results showed that those saponins exhibited cardioprotective effects by decreasing the levels of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) [49].

Clematis graveolens:

Antioxidant activity - Different assays were used to analyse antioxidant potential of the essential and fixed oils of C. graveolens. The linoleic acid system was used to determine the antioxidant activity of essential oil and fixed oil of the plant in terms of percentage inhibition per oxidation in the linoleic acid system. DPPH radical scavenging activity of essential oil and that of fixed oil sample. The percentage inhibition in linoleic acid system of essential oil ranges from 69.69-71.61% and that of fixed oil from 59.59-79.92. The fixed oil of stem showed lower free radical scavenging activity and highest IC_{50} values (47.82 μg/ml) and fixed oil of leaves showed lower IC_{50} values (14.06 μg/ml) and highest free radical scavenging activity. On comparing the fixed and essential oil of stem and leaves maximum IC_{50} values were shown by fixed oil of stem (47.82μg/ml) [50].

Antibacterial activity- The antimicrobial activity of C. graveolens was evaluated against selected bacterial strains Escherichia coli, Staphylococcus aureus, S. epidermidis, Nitrosapira and fungal strains Aspergillus niger, C. albicans and Aspergillus flavus by disc diffusion and minimum inhibitory concentration method. Results showed that essential oil of leaves showed good inhibitory activity against E. coli (15.66 mm) and minimum activity against B. cereus (9.44 mm) and for fungal strains good activity was shown against C. albicans (11.44 mm) and minimum activity was shown against A. niger (9.86 mm). The fixed oil of stem was maximum against B. cereus (13.87 mm) and minimum activity was shown against E. coli (8.91 mm) Fungone and Novidate standards were used [50].

Antifungal Activity: The crude ethanolic extract of flowers of C. graveolens showed antifungal activity. Food poisoned technique was used to determine the growth inhibition of fungi by the plant extracts. Terbinafine, was used as positive control. Antifungal assay of C. graveolens was carried out in five solvents. The flowers extract in methanol: The extract was tested at three different doses 500, 1000 and 2000 ppm. At 500 ppm, the percent inhibition in the growth of A. fumigatus was 41.10%±9.21, followed by A. flavus 39.52%±5.51 and A. niger 32.82%±1.85. Ethanol: The inhibition in at 2000 ppm, the percent inhibition in the linear growth of F. oxysporum (92.22%±1.33) was maximum, followed by A. fumigatus (86.13%±1.20), A. flavus (85.83%±1.15), A. niger (83.46%±1.76), S. cerevisiae (80.69%±2.60) and P. notatum (73.59%±1.67). Chloroform: At 2000 ppm, the percent inhibition in the growth of F. oxysporum (95.37%±1.53) was maximum, followed by S. cerevisiae (93.22%±1.76), A. niger (90.46%±2.03), A. fumigatus (86.99%±0.67), A.

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flavus (83.95%±5.69) and P. notatum (59.04%±3.51). DW: 2000 ppm the inhibition was up to 97.92%±0.58 in A. niger, followed by 90.94%±1.45 in F. oxysporum, 80.28%±1.53 in A. fumigatus, 69.02%±4.81 in S. cerevisiae, 77.50%±6.08 in A. flavus and 65.92%±0.67 in P. notatum. Acetone: In lower dose (500 ppm), inhibition in the fungal growth was found maximum in case of A. niger 69.33%±1.76, followed by S. cerevisiae 58.45%±0.88, F. oxysporum 55.74%±1.45, A. fumigatus 50.50%±0.88 and minimum in case of P. notatum 19.67%±1.67 and A. flavus 8.55%±1.45 [51].

Insecticidal activity- Insecticidal activity of compounds clematograveolenoside A, tomentoside A, huzhangoside D, clematoside S was evaluated against aphid, Aphis craccivora and termite, Coptotermis homii: Toxicity of pure compounds was tested following Potters spray tower method against A. craccivora and force-feeding method against C. homii. For control, leaf disks were sprayed with distilled water containing 0.05 percent Tritone. Mortality was determined after 72 and 96h of treatment. The synthetic insecticide dimethoate at recommended dose (1-25 ppm) was used as a positive control for comparison. For C. homii: The test solutions of compounds and the imidacloprid (chemical insecticide) were prepared at different concentrations (50, 100, 500, 1000 ppm/mL). A. craccivora: Among the compounds tested, tomentoside A was more effective against A. craccivora with an LC_{50} of 1.2 and 0.5 mg/mL after treatment for 72 and 96 h, respectively and was followed by compound clematoside S (LC_{50} = 2.3 and 1.9 mg/mL) and clematograveolenoside A (LC_{50} = 3.2 and 2.6 mg/mL). The huzhangoside D was not effective and its mortality was < 50%. C. homii: The insecticidal activity of compounds has been investigated against the C. homi showed encouraging results. Among the compounds tested, compound clematograveolenoside A was more effective against C. homi with an LC_{50} of 0.1 mg/mL after 24 h of treatment and was followed by compounds tomentoside A, huzhangoside D, and clematoside S (LC_{50} = 0.1, 0.2 and 0.2 mg/mL respectively). All these compounds are comparable with positive control, imidacloprid (LC_{50} = 0.1 mg/mL) and can be used as effective biotermiticide and an efficient alternative to synthetic insecticides [52].

II. 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 3 from C. vitalba, 4 from C. stans, 12 from C. tengutica and 1 from C. graveolens and oleanane aglycone based were 2 from C. stans, 3 from C. tenguticaThe 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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