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Ethnomedicinal, phytochemical and pharmacological profile of genus *Viola*

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Abstract

The genus *Viola* (Violaceae) consists of approximately 500 species widely distributed throughout the world. In Pakistan, seventeen (17) different species of *viola* are abundantly found. Traditional healers have been patronizing various species of this genus in numerous disorders since time immemorial. Some of them are already validated scientifically such as antifungal antibacterial, antiplasmodial, antihypertensive, antidyslipedimic, anticancer, analgesic, antipyretic, anti-inflammatory, diuretic, anthelmintic, antioxidant, anticancer, anti-HIV and antiasthmatic etc. while many more are still needed to be addressed. Phytochemical-ly, different groups of compounds have been isolated from various species of this genus like cyclotide alkaloids, flavonoids, caffeic acid derivatives, salicylic acid and triterpenoids. Traditional knowledge when tested pharmacologically and phytochemically could provide new effective therapeutic agents.

Keywords: *Viola;* pharmacological; phytochemical

Introduction

The family Violaceae (alternatively known as Alsodeiace or Leoniaceae or Retrosepalaceae) comprise of twenty genera and about 800 species (Mabberley, 1987). In Pakistan it is represented by 1 genus (*Viola*) and 17 species (Perveen and Qaiser, 2009) as given in Table 1. The plants of this family are perennial herbs or shrubs with simple leaves (alternate or opposite), which are palmate or deep dissected shaped, the flowers are bisexual, zygomorphic or actinomorphic, calyx 5, corolla of 5 petals, anterior petal large and spurred. Androecium of 5 stamens. Gynoecium a compound pistil of 3 united carpals, ovules superior, fruit capsule. The family is of little economic importance (Perveen and Qaiser, 2009).

Members of the genus *Viola:* The genus *Viola* is comprised of more than 500 species, while in Pakistan 17 species are distributed in various localities (Qaiser and Omer, 1985).

General description

The plants (*Viola* genus) are annual or perennial herbs with short stem, rhizome is present or absent. Leaves are ovate-triangular or reniform, cordate, serrate, crenate, stalked, sometimes winged. Stipules lanceolate-ovate, entire, dentate or fimbriate. Flowers zygomorphic, peduncle bracteolate, bracteoles 2, oppoiste or subopposite. Sepals 5, persistent. Petals 5, lateral larger than others, forming a spur. Anthers connate, encircling the ovary, filaments short, broad and distinct, the two lateral end up in the corolla spur. Ovary sessile; style mostly curved at base, thickened or attenuate above; stigma lobed, straight or beaked. Fruit a 3-valved capsule, dehiscing loculicidally. Seeds are rounded-ovate, smooth and shiny (Qaiser and Omer, 1985). Recently we have reported the Pharmacognostic profile of the whole plant of *Viola betonicifolia* (Muhammad et al., 2012a). The list of *Viola* species found in Pakistan is presented in Table 1.

Ethnomedicinal uses

A large number of ethnobotanical uses of different species of the *Viola* genus have been documented in literature. After the though literature survey we tabulated the plants name, rout of administration and folk use in Table 2.

Table 1. List of some of the *Viola* species and their worldwide distribution.

Name	Flowering season	Geographical Distribution
Viola betonicifolia	May-August	Pakistan, India, Nepal, Sri Lanka, Burma, Indo-China, China, S. Japan, Malaysia, Australia
Viola biflora	May-August	Europe, Central Asia, Pakistan and India
Viola canescens	March-June	Pakistan, India, Nepal and Bhutan
Viola cinerea	January-March	Pakistan (Makran), Iran & Oman
Viola falconeri	April-July	Pakistan & India
Viola fedtschenkoana var. fedtschenkoana	March-August	Pakistan, Central Asia - A central Asian element extending up to Nothern Pakistan
Viola fedtschenkoana var. muzaffarabadensis	March-August	Pakistan
Viola kashmiriana	June-August	India, Pakistan and Afghanistan.
Viola kunawurensis	July-September	Afghanistan, Pakistan, Turkestan, India, Nepal, Tibet and W. China
Viola macroceras	March-June	Pakistan, Afghanistan
Viola makranica	Feb.-July	Pakistan
Viola odorata	March-July	Pakistan, India, Iran Afghanistan, Iraq, Mediterranean region and Caucasiav
Viola pilosa/Viola serpens	April-August	Pakistan, India, Ceylon, Nepal, China and Java.
Viola reichenbachiana	May-August	Europe, N.W. Africa, Caucasia and Pakistan
Viola rupestris	May-July	Eurasia. Most of Europe, Asia minor, Central Asia, Afghanistan and Pakistan
Viola stocksii	February-May	Pakistan, India, Afghanistan and Iran
Viola tricolor	November-June	Pakistan, Europe, Asia, America and Australia
Viola turkestanica	May-September	Pakistan and Central Asia

Table 2. Ethnobotanical use of various species of genus *Viola.*

Name	Part used (Route of administration)	Folk medicinal uses
Viola betonicifolia	Leaves, flower and whole plant (oral)	The whole plant is used as astringent, diaphoretic, antipyretic, anticancer and purgative (Shinwari, 2010). It is also used in epilepsy and various nervous disorders. Flower and leaves are used for sinusitis, skin and blood disorders, cough, pharyngitis (Bhatt and Negi, 2006). It is also used as an astringent, diuretic, having cooling effect, laxative and purgative (Husain et al., 2008). Roots and fruits are used for kidney diseases, pneumonia and bronchitis. Leaves are useful for the healing of boils (Husain et al., 2008).
Viola biflora	Flower (oral)	Diaphoretic, antipyretic, febrifuge, cancer, epilepsy and nervous disorders (Hamayun et al., 2006)
Viola canescens	Whole plant (oral)	Whole plant is used as Astringent, demulcent, purgative, diaphoretic, antipyretic, febrifuge and anti cancerous (Hamayun et al., 2006)
Viola cinerea	Whole plant (oral)	Aphrodisiac (Marwat, 2008)
Viola falconeri	Flower and roots (oral)	Flower are used for cough and cold while roots are used in jaundice (Saqib and Sultan, 2005)
Viola hondoensis	Whole plant (oral)	Expectorant, a diuretic, and an anti-inflammatory for bronchitis, rheumatism, skin eruptions, and eczema (Moon et al., 2004)
Viola odorata	Whole plant (oral)	Diaphoretic, febrifuge, infantile disorder, lung troubles (Ahmad et al., 2009).
Viola pilosa/ viola serpens	Whole plant (oral)	Whole plant is crushed and boiled along with zingiber in half liter water and given orally to cure cough.
Viola tricolor	Aerial parts (topical and oral)	The aerial parts are used as anti-inflammatory, expectorant and diuretic also used in skin conditions, bronchitis, cystitis and rheumatism (Anca et al., 2009)
Viola arvensis	Aerial parts (oral)	anti-inflammatory, expectorant and diuretic also used in skin conditions, bronchitis, cystitis and rheumatism (Anca et al., 2009)

Phytochemical studies

Nutritional value

The elemental compositions of the various parts (stem, leaves, petiole and flower) of *Viola odorata* have been investigated for detection of various elements like carbon, oxygen, sodium, calcium, magnesium, aluminum, silicon, chloride and iron. This study proved that *Viola odorata* is a rich source of these elements (Bibi et al., 2006). The *Viola betonicifolia* various parts (leaves, petioles, roots, flower and whole plant powder), crude methanolic extract and its subsequent solvent fraction have been reported for various macro and micro nutrients (lead, cadmium, copper, chromium, zinc, nickel, magnesium, calcium, sodium and potassium). It was proved that the whole plant of *Viola betonicifolia* is a good source of various nutrients, fats, proteins, lipids and carbohydrates (Muhammad et al., 2012d). Recently we have tested *Viola serpens* for its elemental composition and found a rich source of various nutritious elements (unpublished).

Essential oils

In the literature there is inadequate data on the essential oil composition of *Viola spp*., only few species have been studies so far. The essential oils of the leaves of *Viola odorata*

have been reported with 23 volatile components, most of them were aliphatic or shikimic acid derivative, while the main components of the essential oil of *Viola etrusca* was methyl salicylate (Anca et al., 2009). The essential oils from the fresh aerial parts of *Viola tricolor* were consisted of 35 constituents containing mainly sesquiterpenes, aliphatics, shikimic acid derivatives and monoterpenes. The major constituents of volatile oils of dried aerial parts of *Viola tricolor* were consist of 24 components which were mainly aliphatics, shikimic acid derivatives, sesquiterpenes and monoterpenes. The essential oils of dried aerial parts of *V. arvensis* were mainly aliphatics, shikimic acid derivatives, monoterpenes and sesquiterpenes (Anca et al., 2009).

Preliminary phytochemical profile

The methanolic extract of the leaves of *Viola odorata* was found to contain 15total phenolic (35.4 mg/g) and total flavonoid (22.8 mg/g) contents (Ebrahimzadeh et al., 2010). Various phytochemical constitutes (alkaloids, steroids, tannins, flavonoids, and saponins) has been re-ported in aerial parts of *Viola odorata n*-hexane, butanolic, methanolic and aqueous extracts (Vishal et al., 2009). The Methanolic extract of the whole plant of *Viola betonicifolia* has been reported as rich source of alkaloids, flavonoids, tannins, proteins, phenolic compounds, saponins, sterols and triterpenoids (Muhammad and Saeed, 2011; Muhammad et al., 2012c). The dichloromethane, ethyl acetate and methanolic extract of *Viola tricolor* whole plant has been reported as rich source of terpenoids, phenolic compounds, flavonoids and saponins (Witkowska-Banaszczak et al., 2005). The methanolic extract of *Viola tricolor* has been investigated for their flavonoid contents through LC-MS, HPLC and NMR and five minor flavonoids were identified (Vukics et al., 2008a). Various species of *Viola* has been tested for their cyclotides contents and all were proved a rich source of cyclotides. The leaves of *Viola canescens* reported to have alkaloids, phenolic compounds, tannins, saponins, phytosterols and flavonoids as phytochemical constitutes (Barkatullah et al., 2012).

Isolated compounds

The literature survey of the genus *Viola* proved that a large number of pharmacologically active compounds have been isolated from different species. The genus *Viola* is a rich source of different classes of natural products like cyclotide alkaloids (Chen et al., 2005b),

Table 2. List of secondary metabolites (compounds) isolated from various species of genus *Viola.*

S. No.	Name	Source	Molecular formula
1	6-Arabinopyranosyl-4',5,7-trihydroxy-8-xylopyranosylflavone (Chopin et al., 1982)	*Viola yedoensis*	$C_{25}H_{26}O_{13}$
2	Auroxanthin (Goodwin, 1956)	*Viola tricolor*	$C_{40}H_{56}O_4$
3	Cyanidin 3-glycosides, 3-O-[-L-Rhamnopyranosyl-(1→6)-β-D-glucopyranoside] (Harborne, 1963; 1964; Karioti et al., 2011)	*Viola odorata*	$C_{27}H_{31}O_{15}^{+}$
4	*Viola cotyledon* Cyclic peptides (Göransson and Craik, 2003)	*Viola cotyledon,*	-
5	CycloViolacin H_1 (Craik et al., 1999)	*Viola hederaceae*	$C_{132}H_{208}N_{36}O_{40}S_6$
6	CycloViolacin H_2 (Chen et al., 2005a)	*Viola hederaceae*	$C_{133}H_{201}N_{37}O_{38}S_6$
7	CycloViolacin H_3 (Chen et al., 2005a)	*Viola hederaceae*	$C_{129}H_{190}N_{36}O_{40}S_6$
8	CycloViolacin H_4 (Chen et al., 2006)	*Viola hederaceae*	$C_{133}H_{206}N_{34}O_{41}S_6$
10	CycloViolacin O_1 (Craik et al., 1999; Rosengren et al., 2003)	*Viola odorata*	$C_{131}H_{203}N_{35}O_{41}S_6$
11	CycloViolacin O_{10} (Craik et al., 1999)	*Viola odorata*	$C_{131}H_{206}N_{36}O_{40}S_6$

S. No.	Name	Source	Molecular formula
12	CycloViolacin O_{11} (Craik et al., 1999)	*Viola odorata*	$C_{138}H_{216}N_{36}O_{40}S_6$
13	CycloViolacin O_3 (Craik et al., 1999)	*Viola odorata*	$C_{134}H_{208}N_{38}O_{39}S_6$
14	CycloViolacin O_4 (Craik et al., 1999)	*Viola odorata*	$C_{134}H_{209}N_{37}O_{39}S_6$
15	CycloViolacin O_6 (Craik et al., 1999)	*Viola odorata*	$C_{136}H_{212}N_{36}O_{40}S_6$
16	CycloViolacin O_7 (Craik et al., 1999)	*Viola odorata*	$C_{136}H_{213}N_{35}O_{39}S_6$
17	CycloViolacin O_8 (Craik et al., 1999)	*Viola odorata*	$C_{138}H_{216}N_{36}O_{41}S_6$
18	CycloViolacin O_9 (Craik et al., 1999)	*Viola odorata*	$C_{133}H_{207}N_{37}O_{39}S_6$
19	Prionanthoside (Jakupovic et al., 1988)	*Viola prionantha*	$C_{17}H_{18}O_{10}$
20	6,8-Di-a-L-arabinopyranosyl-4',5,7-trihydroxyflavone(Theodor et al., 1981)	*Viola yedoensis*	$C_{25}H_{26}O_{13}$
21	2,4-Dimethyldodecane (Cu et al., 1992)	*Viola odorata*	$C_{14}H_{30}$
22	3,4-Dimethylheptane(Beierbeck and Saunders, 1980)	*Viola odorata*	C_9H_{20}
23	2,5-Heptadien-1-ol(Cu et al., 1992)	*Viola odorata*	$C_7H_{12}O$
24	3,3',4',5,5',7-Hexahydroxyflavone; 3-*O*-[a-L-Rhamnopyranosyl-(1→6)-b-D-glucopyranoside], 7-*O*-a-L-rhamnopyranoside (Henrick and Jefferies, 1964)	*Viola spp.*	$C_{33}H_{40}O_{21}$
25	3,3',4',5,5',7-Hexahydroxyflavone; 3-*O*-[a-L-Rhamnopyranosyl-(1→6)-b-D-glucopyranoside], 7-*O*-b-D-glucopyranoside(Henrick and Jefferies, 1964)	*Viola spp.*	$C_{33}H_{40}O_{22}$
26	2-Hydroxybenzoic acid; *O*-[a-L-Arabinopyranosyl-(1®6)-b-D-glucopyranoside], Me ester(Kanchanapoom, 2007)	*Viola cornuta*	$C_{19}H_{26}O_{12}$
27	*N*-Tetracosanoyl	*Viola yedoensis*	$C_{32}H_{57}NO_2$
28	Isorhamnetin 3,4'-diglycosides, 3-O-[α-L-Rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside], 4' -O-α-L-rhamnopyranoside (Flamini, 2007)	*Viola etrusca*	$C_{40}H_{52}O_{24}$
29	IsoViolanthin (Flamini, 2007)	*Viola etrusca*	$C_{27}H_{30}O_{14}$
30	1-Octadecene	*Viola odarata*	$C_{18}H_{36}$
31	5,10-Pentadecadien-1-ol(Cu et al., 1992)	*Viola odarata*	$C_{15}H_{28}O$
32	3-Pentadecenal(Cu et al., 1992)	*Viola odarata*	$C_{15}H_{28}O$
33	2,2,6,6-Tetramethyl-4-piperidinone(Rodrigues et al., 2007)	*Viola odarata*	$C_9H_{17}NO$
34	4',5,7-Trihydroxy-6-methoxyisoflavone; 4',7-Di-*O*-b-D-glucopyranoside(Moon et al., 2005)	*Viola hondoensis*	$C_{28}H_{32}O_{16}$
35	Varv peptide A(Svangård et al., 2004)	*Viola arvensis and Viola tricolor*	$C_{116}H_{183}N_{35}O_{39}S_6$
36	Varv peptide B(Göransson et al., 1999)	*Viola arvensis*	$C_{125}H_{182}N_{36}O_{41}S_7$
37	Varv peptide C(Göransson et al., 1999)	*Viola arvensis*	$C_{116}H_{177}N_{35}O_{39}S_6$
38	Varv peptide D(Göransson et al., 1999)	*Viola arvensis*	$C_{116}H_{177}N_{35}O_{39}S_6$
39	Varv peptide E(Göransson et al., 1999)	*Viola arvensis and Viola tricolor*	$C_{117}H_{179}N_{35}O_{39}S_6$
40	Varv peptide F(Göransson et al., 1999)	*Viola arvensis*	$C_{122}H_{184}N_{34}O_{40}S_6$
41	Varv peptide G(Göransson et al., 1999)	*Viola arvensis*	$C_{124}H_{180}N_{36}O_{41}S_6$
42	Varv peptide H(Göransson et al., 1999)	*Viola arvensis*	$C_{125}H_{184}N_{36}O_{42}S_6$
43	Vhl 1(Chen et al., 2005a)	*Viola hederaceae*	$C_{140}H_{215}N_{35}O_{44}S_7$
44	Vhl 2(Chen et al., 2005a)	*Viola hederaceae*	$C_{133}H_{192}N_{36}O_{43}S_6$
45	Viola peptide I(Schöpke et al., 1993)	*Viola arvensis*	
46	Violacin A(Ireland et al., 2006)	*Viola odorata*	$C_{129}H_{193}N_{33}O_{38}S_6$
47	Violanthin(Carnat et al., 1998)	*Viola tricolor*	$C_{27}H_{30}O_{14}$
48	Violanthin; 5"-Epimer (Carnat et al., 1998)	*Viola tricolor*	$C_{27}H_{30}O_{14}$
49	Violaxanthin; (all-*E*)-form (Molnár et al., 2004)	*Viola tricolor*	$C_{40}H_{56}O_4$
50	Vitri peptide A(Craik et al., 1999; Svangård et al., 2004)	*Viola tricolor*	$C_{134}H_{215}N_{37}O_{39}S_6$
51	Vitri peptide A; 11-L-Serine analogue(Craik et al., 1999; Svangård et al., 2004)	*Viola odorata*	$C_{133}H_{213}N_{37}O_{39}S$
52	Vodo peptide M(Svangård et al., 2003)	*Viola odorata*	
53	Vodo peptide N (Svangård et al., 2003)	*Viola odorata*	
54	Violasterol A, Lee, S.-W. et al., J. Chin. Chem. Soc. (Taipei), 1993, 40, 305 (isol, pmr, cmr)	*Viola formosana*	$C_{31}H_{50}O$
55	Tectorigenin 4',7-diglucoside	*Viola hondoensis*	$C_{28}H_{32}O_{16}$

flavonoids (Vukics et al., 2008a; Vukics et al., 2008b), caffeic acid derivatives, salicylic acid (Toiu et al., 2008) and triterpenoids (Tabba et al., 1989) etc. the list of secondary metabolites is presented in Table 3.

Pharmacological studies

A large number of *iv-vitro* and *in-vivo* pharmacological studies of *Viola spp*. Crude extract, its subsequent solvent fraction and isolated compounds have been reported by various researchers. The review of these reported studies are as follow.

In-vitro pharmacological studies

Antibacterial activity

The significant antibacterial activity of the aqueous extract of *Viola odorata* against *S. typhi* and *E. coli* has been reported, the crude ethanolic extract and its subsequent solvent fractions (petroleum ether, dichloromethane and ethyl acetate) were proved significant antibacterial against *E. coli* and *K. pneumonia* (Ziad et al., 2012). The antibacterial potential of the methanolic extract and its solvent fraction of *Viola tricolor* have been published in 2005 (Witkowska-Banaszczak et al., 2005). The methanolic and aqueous extract of the flower of *Viola odorata* showed moderate activity against *salmonella typhi*, *salmonella typhi murium* and *salmonella paratyphi* A. the aqueous extract was more bactericidal than that of methanolic extract (Aktivitesi, 2009). The methanolic and chloroform extract of the leaves of Iranian *Viola odorata* and its essential oils have been tested for their antibacterial potential against various bacteria. The methanolic extract showed antibacterial activity against *P. aeruginosa*, *E. coli*, *S. epidermidis* and *P. vulgaris*, the chloroform fraction showed activity against *S. epidermidis* and *P. vulgaris*, while essential oils showed activity against *B. subtilis, K. pneumonia* and *S. epidermidis* (Akhbari et al., 2012). The cyclotide (vhl-1) isolated from *Viola hederaceae* was tested against *E. coli* and *S. aureus* but the growth of tested bacteria was not inhibited (Chen et al., 2005b).

Antifungal activity

The crude ethanolic extract and its solvent fraction (dichloromethane ethyl acetate, methanolic) of *Viola tricolor* whole plant was responsible for low to moderate antifungal activity against *C. albicans* (Witkowska-Banaszczak et al., 2005). The methanolic and chloroform extract of Iranian *viola odorata* and the essential oils of this plant showed no antifungal activity against *C. albicans* (Akhbari et al., 2012). The essential oils of *Viola odorata* have exhibited moderate activity against the hyphea and spores of *Aspergillus niger* (Pawar and Thaker, 2006). The cyclotide (vhl-1) isolated from *Viola hederaceae* was tested against *C. albicans* but the growth of the tested fungus was not inhibited (Chen et al., 2005b).

Antioxidant activity

The antioxidant potential of various flavonoid fractions of *Viola tricolor* were investigated for antioxidant capacity using DPPH protocols and were proved excellent antioxidant agents (Vukics et al., 2008a). The crude methanolic extract of *Viola odorata* has been tested

for their antioxidant potential at DPPH, reducing power assay, ferric thiocyanate, niotrious, hydrogen peroxide scavenging protocols (Ebrahimzadeh et al., 2010). The crude methanolic extract and its various solvent fractions (chloroform and ethyl acetate) of the whole plant of *Viola betonicifolia* has reported as potent antioxidant agents due to the presence of phenolic compounds (Muhammad and Saeed, 2011).

Anthelmintic activity

The isolated compounds cycloviolacin O_2, cycloviolacin O_3, cycloviolacin O_8, cycloviolacin O_{13}, cycloviolacin O_{14}, cycloviolacin O_{15}, and cycloviolacin O_{16} isolated from *Viola odorata* showed highly significant activity against *H. contortus and T.* colubriformis (Colgrave et al., 2008). Recently we have reported the nemiticidal potential of the methanolic extract and its subsequent solvent fractions of whole plant of *Viola betonicifolia* against *Pheretima posthuma, Meloidogyne incognita, Meloidogyne javanica, Cephalobus litoralis* and *Helicotylenchus indicus*. The chloroform, ethyl acetate and methanolic extract showed low to moderate activity against these worm/nematodes (Muhammad and Saeed, 2011; Muhammad et al., 2012f).

Antiplasmodial activity

In 2007 Moon *et al* tested the crude petroleum ether extract of fifteen species of *Viola* (*Viola tokubuchiana, Viola japonica, Viola dissecta, Viola acuminate, Viola verecunda, Viola albida, Viola keiskei, Viola grypoceras, Viola mandshurica, Viola lactiflora, Viola takeshimana, Viola variegate, Viola websteri, Viola hondoensis* and *Viola ibukiana*) found in southern Korea, for its antiplasmodial action (Moon et al., 2007) epi-oleanolic acid, isolated from the petroleum ether extract was proved a significant antiplasmodial with IC_{50} value 0.18 µg/ml (Moon et al., 2007). Lee et al has also reported the crude petroleum ether extract of *Viola websteri* for it antiplasmodial potential against *plasmodium falciparum* (Lee et al., 2009). Two isolated compounds (6-(8′Z-pentadecenyl)-salicylic acid and 6-(8′Z, 11′Z, 14′Z-heptadecatrienyl)-salicylic acid) isolated from petroleum ether extract of *Viola websteri* showed good antiplasmodial potential against chloroquine-sensitive *P. falciparum* strain (Lee et al., 2009).

The essential oils of *Viola odorata* in combination with essential oils of other plants have been reported with significant repellant activity against various mosquito *Aedes aegypti Anopheles stephensi* and *Culex quinquefasciatus (Amer and Mehlhorn, 2006)*. In some of combinations 100 % activity was observed against all the test species. The crude methanolic extract and its various solvent fractions of *Viola betonicifolia* have been reported with significant larvecidal activity against the larvae of *Aedes aegypti* (Muhammad and Saeed, 2011).

Cytotoxic activity

Three isolated compounds (vitri A, varv A, varv E) from *Viola tricolor* were tested for cytotoxic activities against two human cancer cell lines, U-937 GTB (lymphoma) and RPMI-8226/s (myeloma). All of these were potent cytotoxic having IC_{50} 1 µM 3 µM and 4 µM, respectively (Svangård et al., 2004). Two isolated compounds (6-(8′Z-pentadecenyl)-salicylic acid and 6-(8′Z, 11′Z, 14′Z-heptadecatrienyl)- salicylic acid) from petroleum ether

extract of *Viola websteri* were tested against adenocarcinoma (SK-OV-3) cell line, both of test compounds were failed to show cytotoxic effect (Lee et al., 2009). Cyclotides (vibi E, G, H and D) isolated from *Viola biflora* has been tested against human lymphoma cell lines (U-937 GTB), the vibi E, G and H were cytotoxic and vibi D was not cytotoxic (Herrmann et al., 2008).

Anti- HIV activity

The cyclotide (vhl-1) isolated from *Viola hederaceae* has been tested for anti-HIV potential and showed significant activity with EC_{50} value 0.87μM (Chen et al., 2005b). Cyclotides (kalata B_1, Varv E, cycloviolacin Y_1, cycloviolacin Y_4, cycloviolacin Y_5) from *Viola yedoensis* has been tested as anti-HIV agents. Among these cyclotides the cycloviolacin Y_5 was the most potent anti-HIV (Chen et al., 2006).

Antihypertensive activity

Recently, the antihypertensive (Siddiqi et al., 2012) activity of *Viola odorata* has been reported. The crude extract of the leaves of *Viola odorata* exhibited a potent antihypertensive activity in animal models.

Antidyslipidemic activity

The crude extract of the leaves of *Viola odorata* has been recently reported with recently with antidyslipidemic activity (Siddiqi et al., 2012).

In-vivo pharmacological studies

Analgesic activities

The crude methanolic extract at the dose of 400 mg/kg of *Viola odorata* was proved analgesic in acetic acid induced writhing and tail immersion animal models (Antil et al., 2011). We have tested the methanolic extract and its *n*-hexane fraction for analgesic potential. The methanolic extract (300 mg/kg) was significant central and peripheral analgesic (Muha-mmad et al., 2012c) while the *n*-hexane fraction was significant peripheral analgesic (Muhammad et al., 2012b). The crude ethanolic extract of *Viola canescens* reported as significant analgesic in a dose dependant manner (Barkatullah et al., 2012).

Antipyretic activities

A very limited study is reported on the antipyretic effect of genus *Viola.* The antipyretic effect of *n*-hexane fraction of *Viola odorata* was reported in 1985 (Khattak et al., 1985). A significant antipyretic activity of *Viola betonicifolia* has been reported by our research group. The crude methanolic extract of *Viola betonicifolia* exhibited a dose dependent (100, 200 and 300 mg/kg) antipyretic activity (Muhammad et al., 2012c).

Anti-inflammatory activities

The aqueous extract of *Viola odorata* has been proved a good remedy for inflammatory lungs (Koochek et al., 2003) The anti-inflammatory effect of the crude ethanolic extract of *Viola tricolor* (500 mg/kg) aerial part has been explored (Toiu et al., 2007). The antiinflammatory and anti-asthmatic effects of *Viola mandshurica* ethanolic extract has been reported in allergic asthmatic mice (Lee et al., 2010). Our research group recently reported the anti-inflammatory effect in carrageenan and histamine induced animal models, the methanolic extract of *Viola betonicifolia* has reported as anti-inflammatory against carrageenan and histamine induced inflammation in animals (Muhammad et al., 2012c), while the *n*-hexane fraction exhibit activity against only carrageenan induced model (Muhammad et al., 2012b).

Diuretic activities

The well known specie of this genus, *Viola odorata* has been screened for various biological activities. The butanolic, aqueous and *n*-hexane fraction of the arieal parts of *Viola odorata* exhibited good diuretic effect (Vishal et al., 2009). Recently we have tested (unpublished) the methanolic and *n*-hexane fraction for its diuretic activity but the dieresis was not induced by any of the tested samples.

Laxative effect

The well known specie of this genus, *Viola odorata* has been screened for various biological activities. The butanolic, aqueous and *n*-hexane fraction of the arieal parts *of Viola odorata* aerial parts exhibited good purgative effect (Vishal et al., 2009). Recently we have reported the eth-anolic crude extract of the leaves of *Viola canescens* with significant activity. A dose depen-dant purgative effect was observed in BALB/c mice using charcoal meal paradigm (Barkat-ullah et al., 2012). The crude methanolic extract of *Viola serpense* was also good laxative in a dose dependant manner (unpublished), tested in our research laboratory.

Neuropharmacological activities

The used of *Viola spp.* in epilepsy and other nervous disorder in traditional medicines system needs rationalization. Aftnimoni, a herbal branded formulation (having *Viola odorata as* one of its active ingredients) has been tested for its analgesic, anxiolytic, hypnotic and explor-atory effects (Sadaf and Ahmad, 2009). The *n*-hexane fraction of whole plant of *Viola beton-icifolia* has been reported as significant anxiolytic, muscle relaxant and sleep inducer (Muha-mmad et al., 2012e).

Acute toxicity studies

In acute toxicity of the aerial parts of *Viola odorata* bioassay the crude methanolic as well as it subsequent fractions were found safe upto 2000 mg/kg (Vishal et al., 2009). The crude methanolic extract and its *n*-hexane fraction has been proved safe at the test doses of 500, 1000 and 2000 mg/kg in BALAB/c mice (Muhammad et al., 2012c; Muhammad et al., 2012e). It can be concluded that the genus may be safe at higher doses for clinical uses. The crude methanolic extract of *Viola serpense* has been tested for its acute toxicity in mice at the

tested doses (500, 1000 and 2000 mg/kg) previous doses and was found very safe (unpublished).

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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