VIOLA TRICOLOR LINN.: A COMPREHENSIVE REVIEW ON ITS PHYTOCHEMISTRY AND PHARMACOLOGICAL ACTIVITIES

Abhishek Godara[1*], Nayan Kumar Patel[2], Reecha Madaan[2], Rajni Bala[2], Madhukar Garg[2]

Abstract:

Background: The global burden of health care is increasing with advent of new diseases. The scope of new medicines including phytomedicines is all time high. The herbals drugs as healers are well reported since ancient time and have traditional history of use. The medicinal herbs have enormous commercial potential globally especially due to popularity and alternatives for modern medicines.

Objective: The main objective of this review is to highlight the importance of the plant *V. tricolor* Linn. and its usage globally.

Conclusion: This review compiles details of phyto-constituents which have been isolated from the various parts of *V. tricolor*. Pharmacological activity of various extracts or fractions from the various parts of *V. tricolor* have been discussed. This will help researchers for the development of clinical and evidence-based studies on *V. tricolor.*

Keywords: Flavonoids; Pansy; Phytochemicals; Viola tricolor

[1*]Jaipur College of Pharmacy, Rajasthan University of Health Sciences, Jaipur, Rajasthan, India,
Email Id: garimaabhigodara@gmail.com
[2]Chitkara College of Pharmacy, Chitkara University, Punjab, India

***Corresponding Author:** Abhishek Godara
*Jaipur College of Pharmacy, Rajasthan University of Health Sciences, Jaipur, Rajasthan, India,
Email Id: garimaabhigodara@gmail.com

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1. Introduction:

Health status varies globally from person to person. Different categories of diseases pose a considerable challenge to the prevailing health system globally and with advent of new diseases the challenge is all time high. The Global Burden of Diseases (GBD) study was initiated two decades ago for the assessment of the health of global population. Country-wise GBD data is available and is utilized by governments for healthcare policy interventions in their countries [1]. The GBD hints at the more rigorous approach for treatment of the diseases of burden. Allopathic medicines are very popular and important part of global health care system, however traditional and alternative system of medicines is also necessary to overcome various diseases. In many countries, natural products have been used as alternative system of medicines [2]. Herbal medicines are popular in many countries and are not necessarily reached to the isolation of a single molecule. Due to the absence of reported literature and poor regulation, their acceptability has not become adaptable in modern medicine. Thus, to overcome the safety concern the global regulatory agencies such as FDA, WHO, EU has come up with various polices for the usage of the herbal as medicines [3]. India had been using traditional system of medicines since ages but with advent of new regulatory guidelines for usage, India has also come with regulations for use of herbals as phytopharmaceutical drugs which is equivalent to that of an NCE-based drug [4].

As per WHO reports 25% of the medication contains one or more kind of herbs. The medicinal herbs have enormous commercial potential globally especially due to popularity of organic, sustainable alternatives for modern medicines. Ongoing herbal boom will lead exponential growth in herbal medicines usage and market is being expected with CAGR of 6-7 % growth over the period from 2017-2025. It is estimated that high quality herbal medicines will provide safe and effective medication. India and China have long history for the usage of herbals as medicines in traditional system [5]. Plants and herbs were used and exploited to establish the traditional system of medicines in these countries. *V. tricolor* Linn is one of the popular medicinal plants which were studied for its medicinal values and with a long history in phytomedicine, *V. tricolor* L (family: Violaceae), also known as wild pansy, heartsease, or Gul-e banafsha in Urdu, was first mentioned in the Pharmacopoeia of the Europe and in the Medical and Physical Journal in 1805.

V. tricolor commonly known as wild pansy, heartsease, heart's delight, tickle-my-fancy, Jack-jump-up-and-kiss-me, come-and-cuddle-me, three faces in a hood, love-in-idleness and in Urdu Gul-e banafsha. Widespread distribution of the traditional medicinal herb can be found throughout Europe, Asia, America, and Australia.

V. tricolor L. is annual, biennial, or perennial with a short rhizome. Alternate oval, serrated leaves with a rounded base and noticeable, strongly lobed, pinnate leaf-like stipules are carried by stems which is up to 30 cm in length [6]. Flowers (1–2.5 cm) across, occur in summer, vary in colour and contain white, yellow and violet of varying tones. The petals are longer than the sepals which is a distinguishing feature. It has a weak fragrance [6].

Traditional uses of the plant as phytomedicine are well documented and even plant was included in the pharmacopeia in 2011 [7]. *V. tricolor* contains various phytoconstituents like flavonoids, tetraterpenoids, phenyl propanoic acids, essential oils, cyclotides and amino acids. *V. tricolor* also stated for the cure of skin conditions such as atopic dermatitis, eczema [8], seborrhea, impetigo etc [9]. *V. tricolor* explored for numerous pharmacological actions including anti- epileptic activity [10], sedative [11], cardio protective and hypotensive agent [12], immunosuppressant [13], diuretic [14], antimicrobial [15] and cytotoxic agent [16,17] anti-inflammatory [18], antioxidant [19], antianxiety [20], antiviral [21] etc.

Table 1: Scientific Classification of *V. tricolor* Linn.

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Super division	Embryophyte
Division	Tracheophyte
Subdivision	Spermatophytina
Class	Magnolipsida
Superorder	Rosanae

Order	Malpighiales
Family	*Violaceae*
Genus	Viola L.
Species	*Viola tricolor* L.

Figure 1: *Viola tricolor* Linn.

2. Geographical distribution:

The plant is indigenous to West Asia, Western Siberia, India, and Europe. It was introduced to countries including the Middle East and Central Asia, Australia, North America, and other temperate and subtropical regions. It was also discovered through the United States, Gaurama in Rio Grande do Sul, Brazil, Tehran (seed was imported from the Netherlands), Poznan in Poland, Nagyrécse in Hungary, Belgrade in Serbia, Mashhad in Iran's northeast, Multan in Egypt, and other places [22].

3. Phyto-chemistry: Plant-derived chemical constituents are of great potential in new drug discovery due to their biodiversity. *V. tricolor* comprise rich sources of chemical constituents like amino acids, anthocyanins, cyclotides, essential oils, flavonoids, tetraterpenoids, polyphenolic compound, polysaccharides, salicylic acid and xanthophyllus [23–25] etc.

Amino Acids: Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, asparagine, and glutamine are among the 19 different types of free amine acids found in *V. tricolor*. And the total free amino acids (TAA) content of *V. tricolor* ranged from 9938.0 to 11393.8 mg/kg of fresh weight [26].

Anthocyanins: The presence of anthocyanin pigments have been reported in *V. tricolor* flowers [27–30] and summarized in Table 2. Saito *et al.* 1983 stated the presence of anthocyanins, violanin, platyconin, and violanin chloride in *V. tricolor* flowers [31].

Table 2: Anthocyanins pigments of *Viola tricolor* flowers.

S.No.	Name
1.	cyanidin-*p*-coumarylglycoside
2.	Delphinidin-3:5-*p*-coumarylglucorharnnoside
3.	Cyanidin-3- glucorhamnoside (keracyanin)
4.	Delphinidin-3-glucorhamnoside (tulipanin)
5.	Cyanidin-3:5-glucoglucorhamnoside
6.	Delphinidin-glucorhamnoside

Cyclotides (Macrocyclic Peptides): Cyclotides are cysteine-rich gene-encoded plant peptides. In cyclotides, a head-to-tail cyclized backbone is knotted by the interlocking arrangement of three disulfide bonds [32]. These peptides are responsible for cytotoxic, anti-HIV, insecticidal or antimicrobial activities and plant defense actions [33]. Cyclotide are widely distributed within flowering plants and members of the angiosperm families such as Cucurbitaceae, Fabaceae, Solanaceae, Poaceae, Rubiaceae and Violaceae [34].

Cyclotides have been reported in many violet species *viz. V. uliginosa, V. biflora, V. arvensis, V. tricolor, V. hederaceae and V. odorata* [35–37]. Svangard *et al.* (2004), isolated the three rich cytotoxic small lipophilic proteins from the crude fractions of *V. tricolor* such as vitri A, varv A and varv E[16]. Tang *et al.* (2010) and isolated the 14 cyclotides from *V. tricolor* flower, which includes seven novel cyclotides [vitri B, C, D, E, F, varv Hm, and He], together with seven known cyclotides [varv A, D, E, F, H, vitri A, and cycloviolacin O2] by using the chromatography-based method. A number of the cyclotides exhibited cytotoxic properties against the U251, MDA-MB-231, A549, DU145, and BEL-7402 cancer cell lines.[38] According to Hellinger *et al.* (2015), *V. tricolor* contains at least 164 distinct cyclotides that were found using peptide and nucleic acid analysis. Mass spectrometry and bottom-up proteomics were used to investigate the cyclotide peptidome while employing the recovered peptide sequences as database search queries.[39]

Essential oils: Essential oil have been reported in many Viola species [40]. The essential oil obtained from dried aerial parts of *V. tricolor* comprises 24 compounds aliphatics (14), monoterpenes (4), sesquiterpenes (2) and shikimic acid derivatives (4). Aliphatics were the major components, followed by monoterpenes**,** sesquiterpenes, and shikimic acid derivatives. The main volatile components found were β-ionone (1.00%), hexahydrofarnesyl acetone (4.06%) and methyl salicylate (1.22%) [41]. Whereas essential oil obtained from fresh aerial parts of *V. tricolor* have been comprise of 35 compounds viz aliphatics (17), monoterpenes (4), sesquiterpenes (8) and shikimic acid derivatives (6). The main constituents were sesquiterpenes, aliphatics, monoterpenes, and derivatives of shikimic acid. The main volatile components found were bisabolol oxide A and B (7.78% and 2.28%), bisabolone oxide (43.25%) and trans-β-farnesene (4.01%) [41].

Phenolic Compounds and Flavonoids: Phenolics are chemicals that can function as antioxidants to prevent diabetes, cancer, heart disease, and inflammation. An aromatic ring with one or more hydroxyl groups is the primary structural component of phenolic substances. Simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids, and flavonoids make up plant phenolics [42]**.** Flavonoids are a group of secondary plant metabolites with a polyphenolic structure that are prevalent in fruits and vegetables[43]. In a wide range of nutraceutical, pharmacological, therapeutic, and cosmetic applications, flavonoids are now seen as an essential component. Chalcones, flavones, flavonols, flavanones, anthocyanidins and isoflavones are a few of the subclasses of flavonoids [44]. The antioxidant activity of viola species is due to presence of phenolic compounds and flavonoids [25]. The indigenous species *V. tricolor* and *Viola arvensis* were found to contain the following phenolic acids: caffeic, protocatechuic, genistic, p-hydroxybenzoic, 4-hydroxyphenylacrtic, p-coumaric (trans and cis forms) vanillic and salicylic acids [24,45]. 10% tinctures of air-dried flowering aerial parts of *V. tricolor* comprise of polyphenolic compounds [23]. Aa per literature**,** flowers of *V. tricolor* comprise of rutin, Caffeic acid, chlorogenic acid [23,46] and *p*-Coumaric acid (trans-*p*-hydroxycinnamic acid) [30]**.** Many flavonoids and their glycosides have been isolated from aerial parts of *V. tricolor* which are summarized in Table 3 and their structures are shown in figure 2.

Table 3: Flavonoids isolated from the various parts of *Viola tricolor Linn*.

S.No.	Compound Name	Parts of Plant	Ref.
Flavonols			
1	3,4',5,7-Tetrahydroxyflavone (kaempferol) **(1)**	Aerial	[18]
2	3,3',4',5,7-Pentahydroxflavone (quercetin) **(2)**		
3	Myricetol **(3)**		
4	Patuletin **(4)**		
Anthocyanidins			
5	2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-1-benzopyrylium chloride (cyanidin) **(5)**	Flowers	[27,30]
6	Delphinidin **(6)**		
7	Keracyanin **(7)**	Whole plants	[28]
Flavone C-Glycosides			
8	Luteolin 6-C-*β*-D-glucopyranoside (isoorientin) **(8)**	Whole plants	[47–49]
9	Luteolin 8-C-*β*-D-glucopyranoside (orientin) **(9)**		
10	Vitexin **(10)**		
11	Isoviolanthin **(11)**		
12	Apigenin 6,8-di-C-*β*-D- glucopyranoside (vicenin 2) **(12)**		
13	6-C-glucosyl-8-C-rhamnosyl apigenin (Violanthin) **(13)**		
14	Saponaretin **(14)**		
Flavone O-glycosides:			
15	Quercetin 3-O- rutinoside (rutin, rutoside) **(15)**	Aerial parts	[18,47,49,50]
16	Quercitrin **(16)**	Aerial parts	[18]
17	Quercetin 3-O-*β*-D-glucopyranoside (isoquercitrin) **(17)**	Aerial parts	[18]
18	Quercetin *3-β-D*-glactopyranoside (Hyperoside) **(18)**	Whole plants	[50]
19	Luteolin 7- O-*β*-D-glucopyranoside **(19)**	Aerial parts	
20	Violanin **(20)**	Whole plants and Flowers	[28]

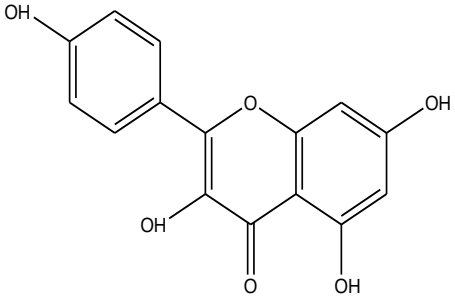
Additionally, sixteen flavonoids have been separated from the methanolic extract of *V. tricolor* which are characterized by micro-liquid chromatography linked to multistage MS and are summarized in table 4 [19].

Table 4: Flavonoid glycoside in methanol extract of *Viola tricolor* characterized by LC-MS.

Type	Name	MW
O-Glycosides	Kaempferol-3-O-deoxyhexosyl(1→6)hexoside	594
	Quercetin-3-O-deoxyhexosyl(1→6)hexoside	610
	Isorhamnetin-3-O-deoxyhexosyl(1→6)hexoside	624
	Quercention-3-O-deoxyhexosylhexoside-7-O-deoxyhexoside	756
C-Glycosides	Luteolin-6-C-hexoside	448
	Chrysoeriol-6-C-hexoside	462
	Apigenin-6-C-pentoside-8-C-hexoside	564
	Apigenin-6-C-hexoside-8-C-pentoside	564
	Apigenin-6-C-deoxyhexoside-8-C-deoxyhexoside	578
	Apigenin-6-C-hexoside-8-C-deoxyhexoside	578
	Apigenin-6,8-di-C-hexoside	594
	Luteolin-6-Cdeoxyhexoside-8-C-hexoside	594
	Luteolin-6-C-hexoside-8-C-deoxyhexoside	594
C and O-Glycosides	Apigenin-X-O-hexoside-Y-C-deoxyhexoside-Z-C-hexoside	740
	Apigenin-6-C-deoxyhexoside-(6-O-hexosyl-8-C-hexoside)	740
	Apigenin-(6-O-hexosyl-6-C-hexoside)-8-C-deoxyhexoside	740

Tetraterpenoids (Carotenoids): Many carotenoids have been isolated from the flowers and aerial parts of *V. tricolor* viz. zeaxanthin **(21)**, violaxanthin **(22)**, auroxanthin **(23)**, flavoxanthin **(24)**, (15Z)-violaxanthin **(25)**, (13Z)- violaxanthin **(26)** [51–53] and their structures are depicted in figure 2.

Molnár *et al.* (1985) reported to contain four geometrical isomers of violaxanthin (5,6,5',6'-diepoxy- 5,6,5',6'-tetrahydro- β, β-carotene-3,3'-diol) in *V. tricolor* blossoms. The new pigments were found to be 9, 9'-, 9, 13'-, 9,15-. and 9,13-di-cis violaxanthins [54].



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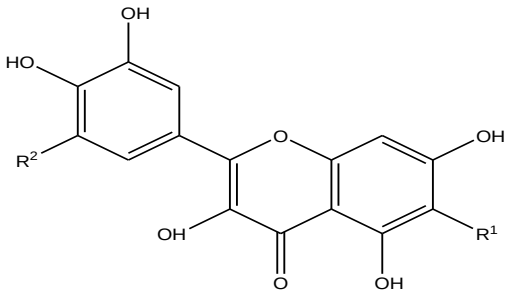
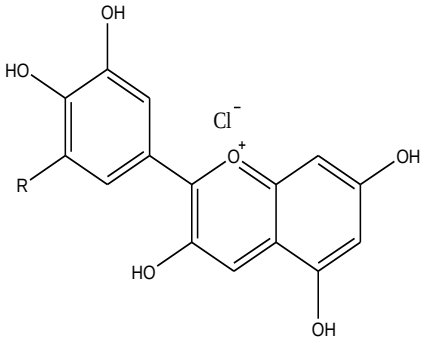
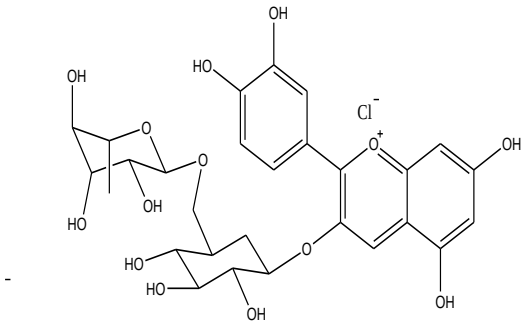


2 $R^1=R^2=H$
3 $R^1=H$ $R^2=$ OH
4 $R^1=MeO$ $R^2=H$



5 R=H
6 R=OH



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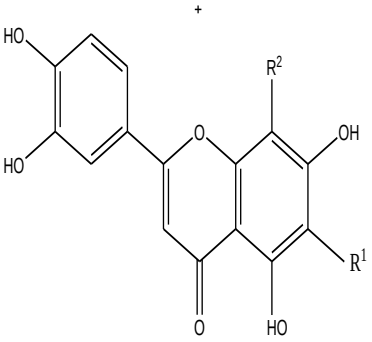
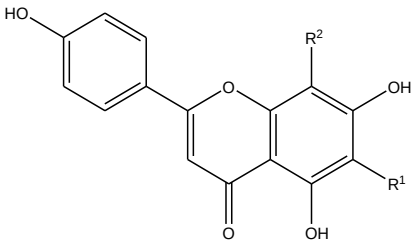
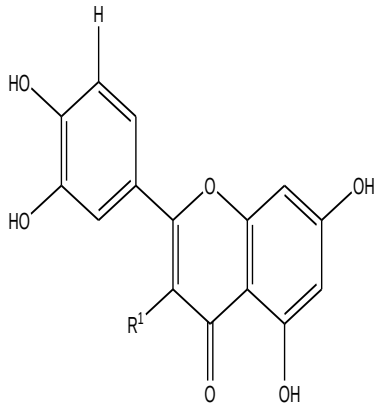


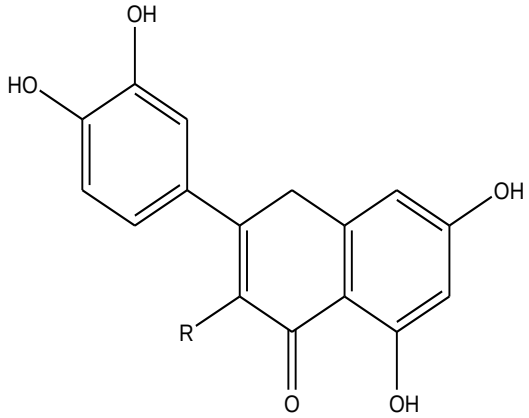
8 R^1=Glc, R^2= H
9 R^1=H, R^2=Glc



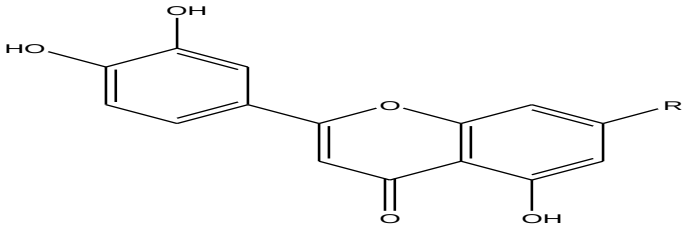
10 R^1=H R^2= Glc
11 R^1=Rha R^2= Glc
12 R^1=Glc R^2= Glc
13 R^1=Glc R^2= Rha
14 R^1=Glc R^2= H



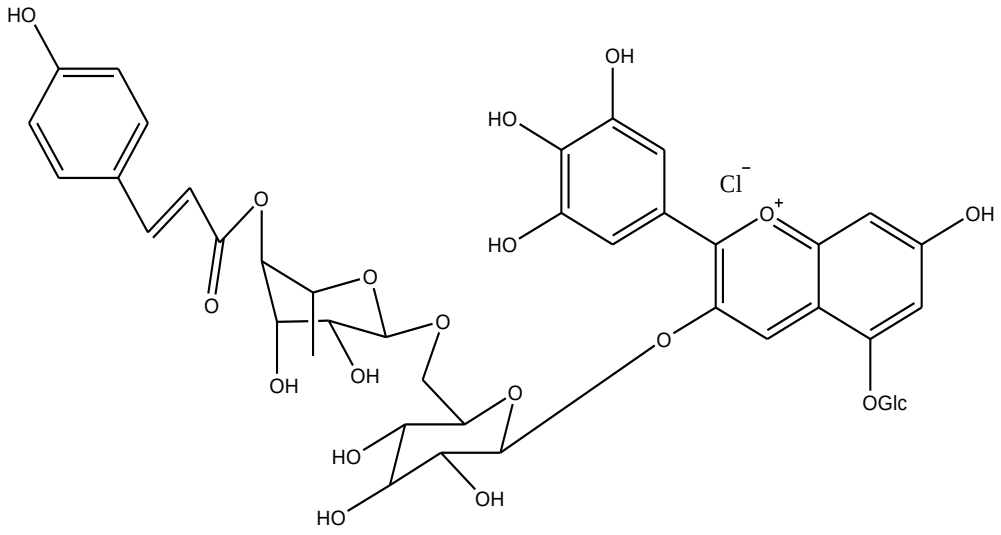
15 R^1=Rha(1–6)-GlcO-



16 R=RhaO-
17 R=GlcO
18 R= GalO



19 R=GlcO



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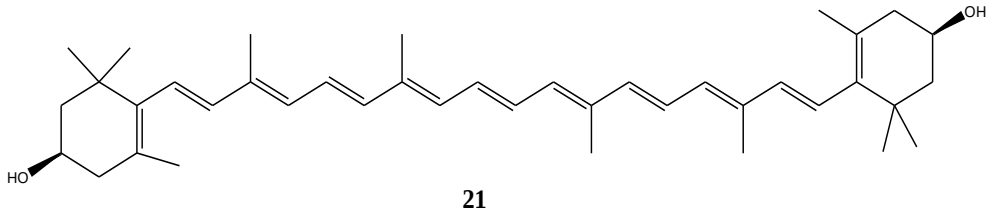

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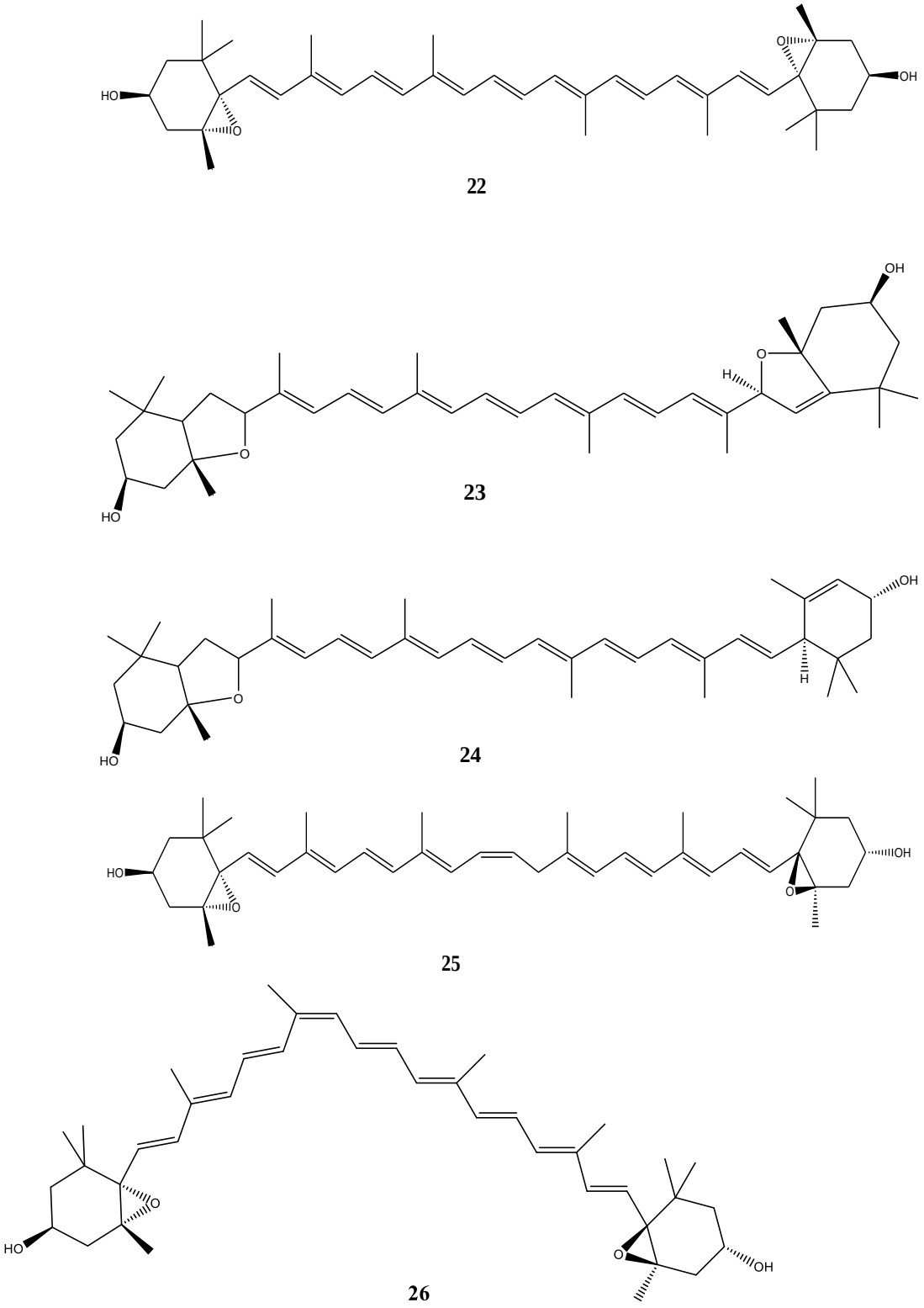

Figure 2: Chemical structures of Flavonoids and tetraterpenoids (carotenoids) compounds identified in *Viola tricolor* L.

Polysaccharides: Franz (1969) reported that *V. tricolor* contains 10% mucilage content. In addition, hydrolysis of polysaccharides produces arabinose (18.1%), galactose (33.3%), glucose (35.1%), rhamnose (8.4%), uronic acid (6.2%) and xylose (5.1%) [55]. Zabaznaya (1985) isolated the glucose, galactose, and arabinose in ratio (2:1.8:1.1) and galacturonic acid, rhamnose and xylose from water soluble fraction of herbage of *V. tricolor*. While hydrolysates of pectin substances comprise of galacturonic acid, glucose, and galactose [56]. Deters *et al.* 2005 extracted polysaccharides from *V. tricolor* and have been composed of arabinose (2%), rhamnose (7%),

mannose (1%), galactose (34%), glucose (29%) and total uronic acid (27%) [57].

Miscellaneous constituents: The other components detected in various parts of *V. tricolor* are tocopherols 30.2 mg% dry weight [58]; α & β –amyrin, β-sitosterol, 2.4 - 4.5 % tannins and magnesium salts [59]; umbellifeone [60,61] and xanthine derivatives [62].

4. Pharmacological Actions of Viola tricolor:

V. tricolor is prevalent for its wide variety of pharmacological activities (Figure 3) including its traditional usage. Additionally, *V. tricolor* is listed in official Russian medication; in fact, the Russian Pharmacopoeia has a monograph on this plant [63–65]. Some of the major pharmacological actions of *V. tricolor* have been discussed.

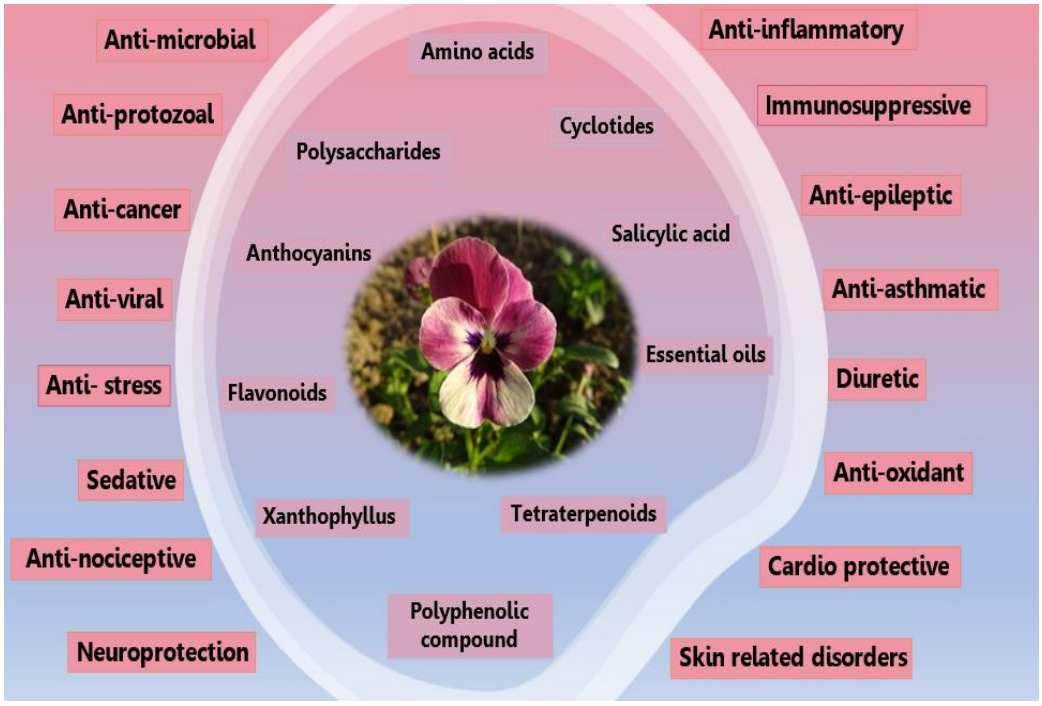

Figure 3: Pharmacological Activity of *Viola tricolor* Linn.

Anticancer activity: In cytotoxic study**,** *V. tricolor* methylene chloride extract and methanolic extract induced 79 % and 49% inhibition on the growth of mouse leukemia cells (ATCC L1210) [66]. Svangard *et al.* (2004) highlighted the use of cyclotides from *V. tricolor* as cytotoxic agents with vitri A having IC50 value of 0.6 µM as most potent cyclotide in human lymphoma and myeloma cell lines i.e., U-937 GTB and RPMI-8226/s [16]. Polysaccharide of Heart sease herb significantly reduced the proliferation in keratinocyte cultures [57]. Tang *et al.* (2010) isolated 14 novel cyclotides and reported the remarkable cytotoxic potential of vitri A, vitri F, and cycloviolacin O2 in BEL-7402, A549, U251, DU145, MDA-MB-231 cancer cell lines[38]. Aqueous-ethanol extracts of plant mixture containing one of the components i.e *V. tricolor* exhibited antiproliferative action in human cervix carcinoma cell line (HeLa) and breast cancer (MDA-MB-453 and MDA-MB_361) cell lines [67]. *V. tricolor* exhibited antiproliferative action in neuroblastoma N2a cells [68]. Ethyl acetate fraction of hydroalcoholic extract of *V. tricolor* showed remarkable inhibitory action on the proliferation of uterine cervix carcinoma cells [69]. Ethyl acetate fraction of *V. tricolor* hydroalcoholic extract exhibited anticancer effect by inducing apoptosis in Neuro2a mouse neuroblastoma and MCF-7 human breast cancer cell lines. And also showed antiangiogenic effect in chicken chorioallantoic membrane [70].

Neuroprotection: Hydroalcoholic extracts of *V. tricolor* and *V. odorata* defend neuronal cells against serum / glucose deprivation -induced cell death in an *in vitro* model of ischemia. There seems to be a need for further research on the potential use of these plants in the prevention and/or treatment of cerebral ischemia and neurodegenerative disorders [71].

Antiepileptic/ Anticonvulsant Activity: Ghorbali *et al.* (2018) reported the evaluation of *V. tricolor* L. for anti-epileptic activity and recommended that hydroalcoholic extract dose of 200 mg/kg dose medication for preventing convulsion in an

animal model [72]. Rahimi *et al.* (2019) also investigated the hydro alcoholic herb extract for anticonvulsant activity and confirmed the prolongation of latency to the first generalized tonic-clonic seizures (GTCs) in pentylenetetrazol (PTZ) induced seizure models and decrease in incidence of hind-limb tonic extension (HLTE) induced maximal electroshock stimulation (MES) [10].

Antioxidant Activity: The antioxidant activity of *Viola species* is related due to the presence of phenolic and flavonoid compounds [73]. *V. tricolor* exhibited antioxidant potential by competitive scavenging of the $ABTS^{+}$ radical [74]. Vukics *et al.* (2008a) had carried out detailed study regarding the antioxidant capacity of heartsease herb in Trolox equivalent antioxidant capacity (TEAC) assay and confirmed that heartsease herb contains significant antioxidants[75]. Antioxidant potential of plant was due to the presence of flavonoid mainly rutin as confirmed using TEAC and DPPH in vitro antioxidant methods [47]. Słomka *et al.* (2008) subjected the *V. tricolor* herb to heavy metal contamination stress and proves that herb had well adapted the stress conditions with balanced and tightly regulated state between activities of their ROS and anti-oxidative enzymes[76]. The ethanolic extracts of *V. tricolor* showed lipid peroxidation inhibition in rat brain as well as Mo (VI) reducing power and Fe (II) chelation ability [77]. Gonçalves *et al.* 2012 reported that flavonoid and phenols are responsible for the antioxidant activity for *V. tricolor* herb with high presence of rutin in flowers. DPPH scavenging method investigations had shown antioxidant activity with IC50 values of 13.40 to 14.18 mg/mL in flower and 32.84-284.87 µg/mL for the leaves/roots[46]. Koike *et al.,* 2015 reported the new functional food use of edible flowers of *V. tricolor* due to its antioxidant potential [78]. Piana *et al.* (2013) demonstrated antioxidant activity of *V. tricolor* flower extract much better than that of standard ascorbic[79]. Shahzadi *et al.* (2021) utilized the antioxidant potential of *V. tricolor* for synthesis of silver nanoparticles[80].

Sedative Property: Ghorbani *et al.* (2012) validated the traditional known sedative effect of the *V. tricolor* on pentobarbital-induced sleep model in mice. The duration of sleep was prolonged by Hydroalcoholic extract of *V. tricolor* and its ethyl acetate fraction without significant change in sleep latency time [11].

Anti-nociceptive activity: *V. tricolor* L. extract exhibited an antinociceptive effect in tail-flick and hot-plate tests when examined in ICR mice at oral dose of 200mg/kg. Further it also diminished the writhing numbers in the acetic acid-induced writhing test [81]. The work revealed that the action may be mediated by adrenergic receptor instead of opioidergic and serotonergic receptors [81].

Anti-inflammatory Activity: The aerial part of *V. tricolor* was studied for acute inflammation in male wistar rats induced by oil of turpentine against diclofenac as standard drug. *V. tricolor* extract (50 mg tincture/100g b.w.) significantly lowered percentage of polymorphonuclear leukocytes and monocytes and the activation of circulating phagocytes with a slight decrease in the nitric oxide synthesis as well thus validating the anti-inflammatory effect of the plant [82]. Methanol extract of the plant *V. tricolor* at the dose of 400mg/kg significantly reduced the rat paw oedema in carrageenan-induced edema test [83]. Gel containing *V. tricolor* exhibited anti-inflammatory and antinociceptive effect in static and dynamic mechanical allodynia model, paw edema, and neutrophilic cell infiltration investigations [79].

Anti-Asthmatic Activity: Harati *et al.* (2018) investigated the anti-asthmatic effect of *V. tricolor* flower hydroalcoholic extract in ovalbumin sensitized mice and revealed that mice exposed to ovalbumin exhibit less anxiety-like behavior in contrast to corticosteroid medication, which cannot reduce anxiety.[20] The management of anxiety related to asthma seems to be alleviated by *V. tricolor* [20]. *V. tricolor* treatment at 200 mg/kg significantly reduced IL-4 without affecting the IFN-γ level. There was decrease in infiltration of leukocytes particularly eosinophil and peribronchial inflammation when compared to standard drug dexamethasone thereby recommending the use in asthma remedy [84].

Anti-microbial Activity: Decoction, infusion and ethanolic extract of *V. tricolor* exhibited significantly inhibitory effect against tested microorganisms i.e., *B. cereus, C. albicans, S. aureus* and *S. epidermidis,* while moderate anti-microbial effect against *E. faeczlis, E. coli, K. pneumoniae and P. aeruginosa* [15]. Khoshkam *et al.* 2016 investigated the partially purified cyclotides and extracts from *V. tricolor* for antimicrobial potential and found that cyclotides possess good antimicrobial activity against gram

negative bacteria such as *E. coli* and *P. aeruginosa* with MIC of 25 mg/ml in blank disc method and MIC of 0.1 mg/ml in broth microdilution method.[85]

Antiprotozoal effect: Methanolic extract of *V. tricolor* showed antiprotozoal effect against five *Babesia* parasites like *B. bovis, B. divergens, B. bigemina, B. caballi* and *B. microti* [86]. Further, oral administration of the extract prohibited *B. microti* multiplication in mice by 35.1% when tested on in vivo studies [86].

Pediculicidal Activity: Iryna *et al.* (2009) reported *V. tricolor* for pediculicidal properties which amounted 84.00–100 % death rate of lice *P. humanus*.[87]

Diuretic Activity: Toiu *et. al.,* (2009) evaluated the aerial parts of *V. tricolor* tincture for diuretic activity in male wistar-bratislava rats and reported the modest diuretic effect with index of 1.103 and saluretic index of 1.181 1.365 for Na^+, K^+ respectively[88].

Hypoglycemic Effect: Herbal extract comprising *V. tricolor* under different conditions in small intestine of rat for the hydrolysis and transport of sugars. *V. tricolor* reduces the hydrolysis rate of sucrose and the absorption of produced hexoses and water [89].

Cardio protective and Hypotensive Activity: Saqib *et al.* (2020) executed study on the traditional claims of the herb *V. tricolor* in cardiovascular disorders on rabbit atria and aorta.[12] Crude extract and its fractions decreased heart rate and contractile force in paired atria and relaxed phenylephrine stimulated contractions in aorta possibly via voltage dependent L-type calcium channel blockage supported by in vivo hypotensive action [12].

Immunosuppressive activity: Hellinger *et al.* (2014) investigated the immunosuppressant effect of the *V. tricolor* aqueous extract and revealed that the extract inhibited proliferation of activated lymphocytes by reducing the interleukin-2 cytokine secretion without disturbing expression of interleukin -2 receptor.[13] Cyclotides of aqueous extract were identified for the plausible immunosuppressive activity. The immunosuppressive effect was also investigated on the effector function of T-lymphocytes which confirms the lessening of IFN-γ and TNF-α production [13].

Skin Related disorders: *V. tricolor is* widely used internally and externally. *V. tricolor* traditionally had been known for treatment of several skin disorder, which includes pain, burn injuries and inflammation [90, 91], eczema, seborrhea, impetigo, acne *etc* [8]. European Medical Agency in 2010 approved the herbal tea traditional product of *V. tricolor* for mild seborrheic skin conditions. In a randomized, vehicle-controlled, double-blind, half-side comparison clinical investigation, patients between the ages of 18 and 65 were treated for 4 weeks with an ointment containing *Mahonia aquifolium, V. tricolor, and Centella asiatica* for atopic dermatitis [92]. Although the changes were not statistically significant, the ointment modestly reduced the primary and secondary endpoints more than the basic cream that was utilized as the vehicle [92]. Khazaeli *et al.* (2008) investigated the sun protection effect of the *V. tricolor* ethyl acetate extract fraction during the study of various medicinal plants and found that *V. tricolor* flower extract showed sun protection factor (SPF) of 25.69 which is second among the plants under investigation. Additionally, a high SPF is attributed by the presence of flavonoids and phenolic substances.[93]

Antiviral Activity: The *V. tricolor* cyclotides-enriched fraction demonstrated antiviral activity by preventing HIV-1 infection with IC50 values ranging from 0.6 to 11.2 g/ml and selectivity indices as high as 8.1 [21].

Antistress: Mohammadi *et al.* (2022) investigated the effect of hydro alcoholic extract of *V. tricolor* in rats subjected to chronic immobilization stress on hippocampal neuronal death, interleukin (IL)-6 and IL-10 expression, spatial memory, anxiety, and depression [94].

V. tricolor ameliorates memory loss, diminishes anxiety- and depression-like behaviors in immobilized rats. Additionally, it lessens the hippocampus of immobilized rats' expression of IL-6 and the neuronal death brought on by continuous immobilization [94].

Conclusion: The literature study revealed that *V. tricolor* Linn has many phyto-constituents with therapeutic values for different pharmacological activities. This review will open an avenue to undertake more research on *V. tricolor* Linn and find new phytochemicals with promising therapeutic activities.

Authors Contribution
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