ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Preliminary Phytochemical Analysis and *In vitro* Pharmacological Evaluation of Phytosterol rich fraction from *Vetiveria zizanioides* Nash

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Article History:

Received on: 14.01.2018
Revised on: 24.05.2018
Accepted on: 27.05.2018

Keywords:

GC-MS,
ABTS,
HRBC,
α-amylase,
α-glucosidase

ABSTRACT

Synthetic Steroids are well known for their analgesic and anti-inflammatory activity. Adverse effects of steroids include immunosuppression, skin decolouration, weakening of muscle, bone etc. Phytosterols exhibit better potency with less toxicity. In the present study, the synergistic effect of various phytoconstituents present in phytosterol rich fraction (SVZ) prepared from the root of *Vetiveria zizanioides* is evaluated for their pharmacological activities. Qualitative analysis of phytoconstituents and GC-MS analysis of SVZ was carried out. *In vitro* free radical scavenging by ABTS, DPPH scavenging assay, anti-diabetic activity by alpha-amylase and alpha-glucosidase inhibition assay and anti-inflammatory activity by HRBC membrane stabilization effect of SVZ were evaluated. The results reveal that SVZ is a rich source of macromolecules like carbohydrate, fixed oil and fat. Alkaloids, phenols are observed to be absent in SVZ. GC-MS spectra show the presence of 24 phytoconstituents. Most of them are sterols and they are present in association with other bioactive compounds like aromadendrene oxide-1, n-hexadecanoic acid etc., The sterol-rich fraction exhibits scavenging potency of free radicals like ABTS, DPPH and NO with IC_{50} value at 112.34, 171.2, 95.4 µg respectively. SVZ inhibits 50% of α- amylase and α-glucosidase at 425.5 and 453.3 µg. The IC_{50} value of SVZ against HRBC membrane stabilization was calculated as 59.6 µg. A significant difference has not been observed between SVZ and ascorbic acid in free radical scavenging activity. SVZ exhibits HRBC membrane stabilizing activity similar to that of paracetamol. In conclusion, SVZ is a rich source of phytosterol with potent antioxidant, anti-inflammatory activity. Anti-diabetic activity of SVZ should be evaluated further.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i3.1599

Production and Hosted by
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INTRODUCTION

Ancient Americans, Egyptians, Chinese and more specifically Indians used medicinal herbs to treat disease and enhance general health and wellbeing. Some herbs have potent active compounds which serve as the most significant pharmacologically active agent. Active pharmaceutics is synthesized versions of natural products. Many international studies have shown that plants are capable of treating disease and improving health, often without any significant side effects.

Vetiveria zizanoides Nash, belongs to the family *Poaceae* is commonly known as Vetiver, Khas-Khas, Khas or Khus grass in India, Vetiver is the most versatile, multifarious grass with immense potential. Vetiver is a densely tufted grass with the culms arising from an aromatic rhizome, grows up to 2 m tall. The roots are stout, dense and aromatic. Leaves are narrow, erect with scabrid margins. The roots of vetiver grow downwards up to 2 – 4 m in

depth. It is a plant known for its ability to produce essential oil from the roots which are especially used in the perfume industry (Khushmider *et al.,* 2015).

Countries like India, Haiti, and Reunion are the largest producer of Vetiver worldwide. This grass grows throughout the plains of India. It is found throughout the plains and lower hills of India, particularly on the riverbanks and in the rich marshy soil. Vetiver grass is cultivated to retain soil, prevent erosion, to produce commercially important essential oil used in aromatherapy (Jean-Jacques *et al*., 2013).

Various tribes use different parts of this grass for several complications like a mouth ulcer, boil, epilepsy, burn, snakebite, fever, rheumatism, headache etc., It is also used in traditional medicine and as a botanical pesticide. The plant is well known for its oil that is used in medicine and perfumery. Roots are used for preparing soft drink during summer, for cooling purposes, flavouring sharbats, and making mats, hand fans etc. (Balasankar *et al*., 2013).

Variously reported phytoconstituents of selected plants are Vetiverol, Vetivone, Khusimone, Khusimol, Vetivene, Khositone, Terpenes, Benzoic acid, Terpene-4-ol, ß- Humulene, Epizizianal, vetivenyl vetivenate, iso khusimol, β-vetivone, vetivazulene. In the roots, the main component was valencene (30.36%), while in the shoots and leaves, they were 9-octadecenamide (33.50%), 2,6,10,15,19,23- hexamethyl-2,6,10,14,18,22-tetracosahexaene (27.46 %), and 1,2-benzene dicarboxylic acid, isooctyl ester (18.29 %) (Saravana Kumar and Gayathri, 2017).

The antioxidant activity of vetiver root has been reported in Invitro condition (Varadharajan *et al*., 2010). Recently, the antioxidant activity of vetiver leaves has been attributed to β-feminine, β-vetinone and α-vetinone. Presence of Pb, Ni, Zn and other elements of Vetiver leaves has been reported (Gaarima *et al*., 2017). Methanolic extract of Vetiver shows hepatoprotective activity at the dose of 300-500 mg/kg p o damage induced by ethanol 20 % at the dose of 3.76 gm/kg p.o. (Mihir *et al*., 2008).

With the aim of screening the phytoconstituents and pharmacological activities of a sterol-rich fraction of vetiver (SVZ), the study was initiated. The study may guide the traditional and Siddha practitioners to develop a drug against diabetic and inflammation.

MATERIALS AND METHODS

Collection and preparation of phytosterol rich fraction

The root of vetiver was purchased from local Siddha medicals. The root was washed in water and dried at room temperature for 15 days. The root was extracted with chloroform by cold percolation method. 100.0 g of root was soaked in 1.0 l of chloroform for 72 hrs (SVZ). The extract was filtered and concentrated under reduced pressure. The yield was calculated as 4.67 g/100gm.

Qualitative analysis of various phytoconstituents in SVZ

Test for carbohydrate

A small quantity of extract was dissolved separately in 5.0 ml of chloroform and filtered. The filtrate was used to confirm the presence of carbohydrate by subjecting to Molisch's test and Fehling's test.

Molisch's test: Filtrate was treated with 2–3 drops of 1 % alcoholic α - naphthol solution and 2.0 ml of concentrated sulphuric acid. The reagents were added along the sides of the test tube. The formation of purple colour showed the presence of carbohydrates.

Fehling's test: The filtrate was treated with 1.0 ml of Fehling's solution (prepared by mixing an equal volume of Fehling's A and B solution and heated. The formation of orange precipitate shows the presence of carbohydrates.

Test for Proteins and Amino acids

Small quantities of the extract were dissolved in a few ml of chloroform and treated with different reagents like Ninhydrin reagent and Millon's reagent. The formation of red and purple precipitate indicated the presence of proteins and amino acids respectively.

Test for fixed oil and fat

Few drops of 0.5 N alcoholic potassium hydroxide was added to a small number of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for alkaloids

A small portion of the extracts was stirred with a few drops of dilute hydrochloric acid and ammonium hydroxide and filtered. The filtrate was used to react with the following reagents; Dragendroff's reagent, Hager's reagent, Wagner's reagent, Mayer's reagent. The presence of alkaloid was confirmed by the formation of precipitate in different colours like orange-brown, yellow, reddish brown and cream respectively.

Test for saponin

In determining the presence or absence of saponins, 0.5 g of the dried extract was placed in a test tube and 3.0 ml of distilled water was added and boiled for fifteen minutes. The content was filtered and shaken vigorously.

Test for flavonoids

The aqueous extracts were tested for the presence of flavonoids using 5% aqueous sodium hydroxide solution. An increase in the intensity of yellow colour indicates the presence of flavonoids.

Test for phenols

A portion of the above said preparation was mixed with 0.5 ml of Folin phenol reagent. The tubes were allowed to stand for 5 minutes in room temperature36 and 2.0 ml of 20 % sodium carbonate. The tubes were then kept in a boiling water bath for5 minutes. Formation of a blue colour indicates the presence of phenolic compounds.

Test for tannin

A portion of the above said preparation was mixed with 0.5 ml of Folin Danni's reagent. The tubes were allowed to stand for 5 minutes at room temperature and 2.0 ml of 20.0 % sodium carbonate. The tubes were then, kept in a boiling water bath for 5 minutes. Formation of a blue colour indicates the presence of tannins.

GC-MS analysis of SVZ

10 mg of sample was dissolved in chloroform and analyzed by GC Clarus 500 Perkin Elmer using the following experimental conditions: Column type - Elite -5 (5 % diphenyl 95 % dimethyl polysiloxane), Column dimension 30 m X 0.32 mm), carrier gas – Helium 1 ml/min, column temperature from 50 °C up to 285°C at the rate of 10 °C/min and 5 min hold, at 285 °C, injector and detector temperature - 290°C, injection mode split, volume injected: 0.5 µl of a solution prepared from 2 mg/100 ml in methanol. Total run time was 30 minutes. Mass spectrum was taken using Mass detector – Turbo Mass gold – Perkin Elmer. Transfer line temperature – 230 °C, Source temperature – 230 °C, scan range is from 40 – 450 amu, ionization technique – Electron ionization technique.

***The in-vitro* antioxidant activity of SVZ**

ABTS radical scavenging assay

ABTS radical scavenging activity was performed as described by Re *et al.,* (1999). 7.0 mM ABTS in 14.7 mM ammonium peroxo-disulphate was prepared in 5.0 ml of distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue-green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.020 at 734 nm. Various concentrations of the sample solution dissolved in ethanol (20.0 µl) were added to 980.0 µl of ABTS radical solution and the mixture was incubated in darkness for 10 min. The decrease in absorbance was read at 734 nm. A test tube containing 20.0 µl of ethanol and 980.0 µl of ABTS served as control tube. Different concentrations of ascorbic acid were used as a reference compound. Percentage of inhibition was calculated as follows.

% inhibition = {(Control – Test) Control} X 100

DPPH radical scavenging assay

DPPH radical scavenging activity was carried out by the method of Bloir, 1958. To 1.0 ml of 100.0 µM DPPH solution in methanol, an equal volume of the test sample in methanol of different concentration was added and incubated in the dark for 30 min. The change in colouration was observed in terms of absorbance using a UV spectrophotometer at 514 nm. 1.0 ml of methanol instead of the test sample was added to the control tube. Different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated using the same formula as mentioned in ABTS radical scavenging assay.

Nitric oxide radical scavenging assay

Sodium nitroprusside (10.0 mM) in phosphate buffered saline was mixed with different concentrations of the test sample and incubated at room temperature for 180 min. The same reaction mixture without the extract but with an equivalent amount of phosphate buffered saline served as control. After incubation period, 0.5 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1 % N-(naphthyl) ethylenediamine hydrochloride) was added. The absorbance of chromophore formed during the diazotization of nitrite with sulphanilamide was measured at 546 nm using UV Spectrometer (Shimadzu 1700). Percentage of inhibition was calculated using the same formula as mentioned in ABTS radical scavenging assay.

***The in-vitro* antidiabetic activity of SVZ**

Alpha-amylase inhibitory activity

The effect of the sample on α-amylase activity was determined by following the method of Ali *et al*., (2006). Different concentrations of the sample were mixed by stirring with 25 mL of 4% potato starch in a beaker; 100 mg of α-amylase was added to the starch solution stirred vigorously and incubated at 37°C for 60 min. After the incubation period 0.1 M NaOH was added, to terminate enzyme activity. The mixture was centrifuged (3000 X g; 15 min) and the glucose content in the

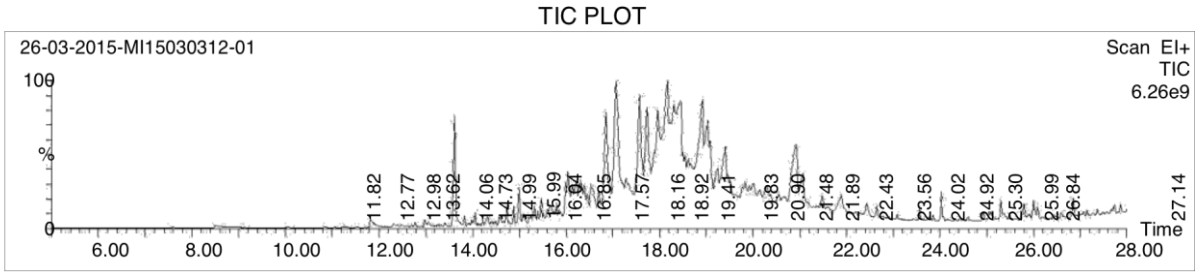

Figure 1: Gas Chromatogram of Phytosterol rich fraction from *Vetiveria zizanoides* root extract (SVZ)

Table 1: Qualitative analysis of Phytosterol rich fraction from *Vetiveria zizanoides* root extract (SVZ)

Parameters	Present /Absent
Carbohydrate	Present
Protein	Absent
Fixed oil and fat	Present
Phenol	Absent
Alkaloids	Absent
Saponins	Present
Tannin	Absent
Flavonoids	Absent

supernatant was determined. Percentage of inhibition was calculated using the same formula as mentioned in ABTS radical scavenging assay.

Alpha-glucosidase inhibitory activity

α-glucosidase inhibitory activity was determined according to the method of Doss *et al*., (2016). 10.0 ml of enzyme solution and varying concentrations of the sample was incubated together for 10 min, at 37°C, and the volume was made up to 210 µL with maleate buffer, pH 6.0. The enzyme reaction was started by adding 200 µl of 2 mM p-nitrophenyl-α-D-glucopyranoside solution and further incubated at 37°C for 30 minutes. The reaction was terminated by treating the mixture in a boiling water bath for 5 min. After the addition of 1.0 ml of 0.1 M disodium hydrogen phosphate solution, the absorption of liberated p-nitrophenol was read at 400 nm. Percentage of inhibition was calculated using the same formula as mentioned in ABTS radical scavenging assay.

***The in-vitro* anti-inflammatory activity of SVZ**

HRBC membrane stabilizing the effect of SVZ

The in-vitro anti-inflammatory activity of extracts of SVZ was assessed by Human Red Blood Corpuscles (HRBC) membrane stabilizing method with a slight modification of Parvin *et al*., (2015). The blood was collected from a healthy human volunteer who had not taken any anti-inflammatory drugs for 2 weeks before the experiment and transferred to the heparinized centrifuge tubes and centrifuged at 3,000 rpm. The packed cells were washed with is saline and a 10% suspension in normal saline was made. Diclofenac potassium (50 mcg/ml) was used as a standard. The reaction mixture (4-5 ml) consisted 2 ml of hypotonic saline (0.25% w/v NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4), 1 ml of test solution (1000 µg/ml) in normal saline and 0.5 ml of 10% HRBC in normal saline. For control, 1 ml of isotonic saline was used instead of the test solution. The mixtures were incubated at 56°C for 30 min. and cooled at running tap water, centrifuge at 3000 rpm for 20 min. The absorbance of the supernatant was read at 560 nm. The experiment was performed in triplicates. The control represents100 % lyses. Percentage of inhibition was calculated using the same formula as mentioned in ABTS radical scavenging assay.

Statistical Analysis

All assays were carried out in triplicate and values are mentioned as Mean ± SD.

RESULTS AND DISCUSSION

Table 1 depicts the presence or absence of phytoconstituents in phytosterol rich fraction of *Vetiveria zizanoides* root (SVZ). Among the primary metabolites carbohydrates, saponins fixed oils and fat has been reported positively in SVZ. Other primary metabolites like protein, amino acids and secondary metabolites have not been reported in SVZ.

Table 2 depicts the presence of various compounds in SVZ. 24 compounds have been reported in MS library matching. Among them, most of them are derivatives of sterol.

Table 2: List of compounds present in Phytosterol rich fraction from *Vetiveria zizanoides* root extract (SVZ) reported by MS Library matching

Name of the compound	retention time	% peak area
Trans–isoeugenol	13.62	3.005
Cyclohexanemethanol, 4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-,	16.041	1.455
7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-	16.221	1.214
2-Naphthalenemethanol, 8-ethenyl-3,4,4a,5,6,7,8,8aoctahydro-5-methylene-	16.321	1.294
1H-3a,7-Methanoazulene-6-methanol, 2,3,4,7,8,8ahexahydro-3,8,8-trimethyl-,(3R-(3à,3aá,7á,8aà))-	16.531	1.051
ç-Gurjunenepoxide-(2)	16.851	3.715
Aromadendrene oxide-(1)	17.061	6.129
ç-Gurjunenepoxide-(2)	17.571	3.665
1H-Cyclopropa(a)naphthalene, 1a,2,3,5,6,7,7a,7boctahydro-1,1,7,7a-tetramethyl-, (1aR-(1aà,7à,7aà,7bà))-	17.731	2.466
6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8ahexahydro-1H-naphthalen-2-one	17.961	3.574
6-(1,3-Dimethyl-buta-1,3-dienyl)-1,5,5-trimethyl-7-oxabicyclo(4.1.0)hept-2-ene	18.161	6.512
3-Buten-2-ol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	18.301	3.489
2-Naphthalenecarboxylic acid, 8-ethenyl-3,4,4a,5,6,7, 8,8a-octahydro-5-methylene-	18.451	4.503
12-Oxatetracyclo(4.3.1.1(2,5).1(4,10))dodecane, 11-isopropylidene-	18.922	4.216
6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8-hexahydro-1H-naphthalen-2-one	19.032	3.927
n-Hexadecanoic acid	19.412	3.084
9,12-Octadecadienoic acid (Z,Z)-	20.902	3.947
Octadecanoic acid	21.062	1.500
Deoxynivalenol	21.892	1.124
ç-HIMACHALENE	28.643	1.361
Campesterol	28.803	2.068
Stigmasterol	29.044	2.372
á-Sitosterol	29.534	2.606
Stigmast-4-en-3-one	30.774	1.018

The % peak area of 8 compounds is more than 3. 2 compounds have % peak area of more than 4 and another 2 have more than 6. Remaining compounds exhibits % peak area > 1.0. Aromadendrene oxide-1, 6-(1,3-Dimethyl –buta -1,3-dienyl) -1,5,5-trimethyl -7-oxabicyclo (4.1.) hept-2-ene are two major compounds present in SVZ with % peak area > 6.

Table 3 depicts the free radical scavenging potency of SVZ and compared against Vitamin C. Though significant di fference has been observed in DPPH IC_{50} value ($p<0.05$, Table 3), significant difference has not been observed in nitric oxide radical and ABTS radical scavenging assay, These results reveal that Vitamin C and SVZ exhibits similar free radical scavenging activity.

Table 4 depicts the anti-diabetic and HRBC membrane stabilizing the effect of the reference compound and SVZ. Results of the present study reveal that SVZ exhibits significantly less activity than that of agarose in both α-amylase and α-glucosidase inhibition activity ($p<0.05$). This result also reveals that SVZ exhibits membrane stabilizing and anti-inflammatory activity similar to that of paracetamol. A significant difference has not been observed in IC_{50} value of SVZ against paracetamol (Table 4).

Medicinal plants play an important role in pharmaceutical industries. Increasing evidence reveals that secondary metabolites play crucial role nutraceuticals. Secondary metabolites exhibit free radical scavenging, anti-inflammatory, cardioprotective, hypolipidemic activities etc., (Compean and Ynalvez, 2014).

Table 3: Effect of Reference compound and Phytosterol rich fraction from *Vetiveria zizanoides* root extract SVZ on free radicals

S.No	Parameters	Concentration of Test sample (µg/ml)	%Inhibition (Vitamin C)	%Inhibition (SVZ)
1.	ABTS	10	10.4 ± 0.12	2.86 ± 0.2
		25	21.0 ± 1.2	15.2 ± 1.2
		50	60.0 ± 2.4	52.7 ± 2.3
		100	69.5 ± 3.4	67.1 ± 3.5
		200	86.9 ± 5.4	70.9 ± 5.4
		IC_{50}	91.6 ± 3.7	112.3 ± 4.3
2.	DPPH	10	13.3 ± 0.98	5.7 ± 0.18
		25	34.2 ± 2.3	13.2 ± 3.2
		50	55.7 ± 3.4	37.4 ± 2.8
		100	71.7 ± 4.3	50.5 ± 4.3
		200	85.7 ± 3.3	64.8 ± 4.4
		IC_{50}	85.7 ± 4.3	171.2 ± 3.4*
3.	Nitric oxide radical	10	2.9 ± 0.12	7.4 ± 0.98
		25	14.8 ± 1.2	28.8 ± 1.2
		50	55.0 ± 2.3	43.6 ± 2.5
		100	72.1 ± 3.4	58.1 ± 3.4
		200	72.7 ± 4.3	59.5 ± 4.5
		IC_{50}	86.6 ± 3.2	95.4 ± 3.4

Note - * - Values differ significantly at $p<0.05$. A significant difference has been observed between SVZ and Vitamin C using Students t-test.

The yield of phytosterol rich fraction from *Vetiveria zizanoides* (SVZ) has been calculated as 4.67 ± 0.7 g/100 g. Amrita and Praveen (2015), demonstrated that ethanolic extract of Vetiver is a rich source of phytoconstituents like cardiac glycosides, saponins etc. In the present study, the phytosterol rich fraction is a rich source of only carbohydrate, saponins and fixed oils in primary and secondary metabolites (Table 1). Chloroform and low polar solvent can extract oils. This might be the reason for a positive result of fixed oils and fats alone. Phytoconstituents solubilized in high polar solvents (phenol, flavonoid, tannins) has not been reported in the present study. Plant steroids exhibit various pharmacological activities like hypocholesterolemic, anti-diabetic, antioxidant and anti-cancer activity (Albert Ferrer *et al*., 2017).

Presence of Aromadendrene oxide – 1 has been reported in essential oil separated from *Senecio sandrasicus* (Ugur *et al*., 2009). They have also reported the antimicrobial activity of the essential oil extracted might be due to the presence of active compound Aromadendrene oxide. Phytosterol rich fraction contains 3.088 % of n-hexadecanoic acid. 34.65 % of n-hexadecanoic acid has been reported in the heartwood of *Albizia adiamthifolia* (Mustapha and Runner, 2016). Anti-inflammatory activity of n-hexadecanoic acid has been reported earlier (Aparna *et al*., 2012)

Increase the content of ROS attack cell protein, lipid, carbohydrate and causes physiological disorders like diabetes, inflammation, cancer etc. (Elisa *et al*., 2000). Antioxidants donate/share an electron with ROS and protect cells from damages and related disorders. Different methods in In vitro condition determined the free radical scavenging potency of phytosterol rich fraction and the results be reported in Table 3.

In ABTS, DPPH and NO radical scavenging method, the absorbance was measured at 720, 520 and 517 nm, respectively. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The free radical scavenging activity of phytosterol rich fraction might be due to the presence of carbohydrate, essential oil and fats. The free radical scavenging activity of various carbohydrates have been reported earlier by (Elizabeth and Ana, 2008). Riccardo *et al*., (2013) have reported that most of the essential oils separated from aromatic plants are edible. These essential oils are added externally to avoid autoxidation.

The antioxidant activity of two different genotypes like ev KS1 and ev gulabi of *Vetiveria Zizaoides* were compared and observed the result that the former is more potent free radical scavenger than that of later (Suaib *et al*., 2009). DPPH radical scavenging assay and Iron metal chelating activity of essential oil from Vetiver has been reported

Table 4: *In vitro* Anti-diabetic and HRBC membrane stabilizing the activity of SVZ against reference compound

S.No	Parameters	Concentration of Test sample (µg/ml)	% Inhibition (Agarose)	% Inhibition (SVZ)
1.	Alpha-amylase	50	27.8 ± 2.3	9.3 ± 1.2
		100	33.8 ± 3.2	22.1 ± 3.2
		200	65.3 ± 4.3	44.9 ± 4.3
		400	88.6 ± 3.4	55.8 ± 5.4
		800	88.8 ± 4.5	88.7 ± 3.5
	IC_{50}		215.5 ± 12.5	425.5 ± 14.5 *
2.	Alpha Glucosidase	50	20.1 ± 1.2	6.3 ± 0.9
		100	38.0 ± 2.3	12.6 ± 2.3
		200	47.8 ± 3.4	25.3 ± 3.4
		400	73.5 ± 5.4	50.7 ± 4.5
		800	87.3 ± 5.6	73.2 ± 4.3
	IC_{50}		312.5 ± 21.3	453.3 ± 21.5*
3.	HRBC Membrane stabilisation	10	0.0	0.0
		25	2.8 ± 0.1	1.8 ± 0.1
		50	14.5 ± 1.2	29.0 ± 1.2
		100	56.8 ± 3.4	46.0 ± 2.3
		200	72.5 ± 4.5	59.6 3.4
	IC_{50}		112.4 ± 10.4	198.6 ± 12.5

Note - * - Values differ significantly at $p<0.05$. A significant difference has been observed between SVZ and Vitamin C using Students t-test.

Earlier by Hyun *et al*., (2005). Varadharajan *et al*., (2010) stated that ethanolic extract of V. zizanioides scavenges free radicals, ameliorating damage imposed by oxidative stress in different disease conditions and serve as a potential source of natural antioxidant.

Antidiabetic activity of any drug can be evaluated both in *In vitro* and *In vivo* study. *In vitro* antidiabetic studies are used by many researchers for screening the potency of new drugs and evaluating its mechanism of action. A large number of In-vitro antidiabetic activities are available. Two different methods are selected in the current study and the results are furnished in Table 4.

The antidiabetic activity has been evaluated by α-amylase, α- glucosidase inhibitory activity. Diabetes mellitus is one amongst the main diseases prevailing worldwide. α-amylase is responsible for postprandial glucose levels. *In vitro* antioxidant activity of numerous herbs has been reported earlier (Sudha *et al*., 2011). In the present α-amylase and α-glucosidase has been inhibited by SVZ with IC_{50} value > 400 µg/ ml. Agarose exhibits potent activity than that of SVZ. Though carbohydrates are observed to be present in SVZ, the presence of other phytoconstituents may prevent SVZ from inhibition of α amylase and α-glucosidase. The absence of phenols, tannins and flavonoids might be the reason for the least anti-diabetic activity of SVZ. The antidiabetic activity of SVZ should be evaluated further.

Membrane stabilization has been considered as one of the mechanisms for the treatment of inflammation. HRBC membrane stabilization of numerous herbs has been reported earlier. SVZ exhibits potent HRBC. This membrane stabilizing activity than that of paracetamol (Table 4). HRBC membrane stabilizing the effect of SVZ might be due to the presence of sterols (Ramzi *et al*., 2016).

CONCLUSION

In conclusion, Phytosterol rich fraction (SVZ) is a rich source of various sterols, carbohydrate, fixed oil and fats. SVZ scavenges ABTS and NO radicals potentially similar to that of ascorbic acid. SVZ of agarose. Does not inhibit α- amylase and α-glucosidases significantly potent than that of agarose. SVZ exhibits HRBC membrane stabilizing activity similar to that of paracetamol. The results of the present study reveal that SVZ acts as a potent antioxidant and anti-inflammatory test sample. The anti-diabetic activity of SVZ should be evaluated further.

Acknowledgement

Authors are grateful to Dr. Ishari Ganesh, Chairman, Vels University, his able administrative colleagues for allowing us to complete this work. The authors would like to thank the Faculty of the Department of Biochemistry, Vels University, for

their valuable suggestions and assistance to complete this work successfully.

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