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PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITIES AND LIPASE INHIBITORY EFFECT OF TRICHOSANTHES CUCUMERINA

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ABSTRACT

This study was aimed to investigate the chemical composition of Trichosanthes cucumerina ethanolic extract. The antioxidant activities and antilipase activity of crude ethanolic and aqueous extract were determined. **Trichosanthes cucumerina** showed the highest antioxidant activity against DPPH and ABTS radicals with IC_{50} values of 75 and 155 µg/mL, respectively. The extract showed its capability to decrease the lipid peroxidation induced by iron and sodium nitroprusside in egg yolk phospholipid homogenates. The extracts demonstrated chelating effect against iron and showed reducing activities in phosphomolybdenum assay. Trichosanthes cucumerina ethanolic extract showed 53.3 % while aqueous extract 39.1% inhibition against lipase enzyme.The GC-MS analysis of ethanolic extract showed the presence of 27 compounds. The major compounds identified were4-Cyanomethyl-3-pyrrolepropiononitrile (23%),3- Chloromethylsulfunyl-3-dimethylfuran (19%), Henecosanoic acid methyl ester (15.13%), Octadecanoic acid methyl ester (15.13%),Neophytadiene (9.88%), Pentadecanal (9.88%), Tridecanal (9.88%), Phytol acetate (4.16%), Iavandulyl acetate (4.15%), Nonanoicacis, methyl ester (2.65%), Decanoic acid, methyl ester (2.62%) and Neopentylhydroxyacetate (2.09%). On the basis of above results it is concluded that Trichosanthes cucumerina isrich source of bioactive compounds and can be effectively utilized as antioxidant and antiobesity agent due to its diverse biological activities.

Keywords: Trichosanthes Cucumerina, Radical Scavenging Activity, Lipid Peroxidation, Metal Chelating Activity, GC-MS.

I. INTRODUCTION

Since archaic times humans have been utilizing medicinal plants for curing diseases[1]. Antioxidant components play crucial role in control of various health issues and many diseases. Diurnal research progressively shows that the food which have affluent amount of antioxidant have beneficial effect on health. Vegetable, fruits, and leaves are the main origin of antioxidant [2]. The main property of antioxidant is its ability to scavenge the free radicals. Medicinal plants have been found to possess lipase inhibitory activity[3].

Trichosanthes cucumerina is a dioecious aromatic annual creeper, belonging to the family cucurbitaceaea[4]. It is native to Southeastern Asia and Australia that is widely cultivated throughout the worlds for its curved and irregularly shaped fruits that resembles snakes [5]. It's also known as Snake gourd due to its unusual shape, which resembles an eel with a length of 30 -180cm by 5-10cm. Snake gourd is a popular summer vegetable [6]. [7]Habitat of these plants can be found in the plains as well as lower hills, roadsides gulley. Because of its superb keeping qualities, it has a significant export potential.Leaves are scabrous, palmately lobed and reach a length of up to 25cm long. Fruits are narrow, long, cylindrical, scented, pointed at both end and semi-ellipsoid flattened seeds having undulating margin [8]. It has whitish and tuberous roots. Flowers are fragile, lace like white in color and evening blooming[9] flowering season ranges between July to September while fruits ripened during September to October.

Trichosanthes cucumerina is high in functional constituents such as carotenoids, phenolic compounds, flavonoids and essential minerals, in addition to its basic nutrition making the plant pharmacologically [10]and therapeutically active [11].It is rich in carbohydrates, minerals, proteins and vitamins E and A in high levels[12] and is capable of acting as antioxidants. The roots, leaves, fruits and seed of the plants all have medicinal properties[13].The plant has long been used to heal a variety of diseases. Root is used in diabetes, skin problems, seeds are used for the treatment of fever, bronchitis, diarrhea, fruits increases appetite, act as a

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tonic and stomachic and cure biliousness[14].Because it is high in vitamins, minerals and carotenes, snake gourd aids in the formation of new hair and protects follicles against hair loss[15].Main aim of the study was to highlight the anti-lipase and antioxidant potential of Trichosanthes cucumerina as well as determination of its phytochemical composition.

II. MATERIALS AND METHODS

Plant extract Preparation

Trichosanthes cucumerina Leaves were collected from regions of Azad Kashmir and were checked by a Taxonomist at University of Poonch, Rawalakot. The extracts were prepared by using the method [16]. Leaves were crumbled and dried into fine powder. Twenty five gram of fine powder of leaves then mixed with 100 ml of hot water for fifteen minutes, permitted to cool and then filtered by using whatsman filter paper. Five gram of leaves extract were extracted with ethanol (50ml) for three days at room temperature and then filtered by using whatsman filter paper. The residue was extracted twice and finally the solvent was evaporated in rotary evaporator (50 ^{0}C). Serial dilutions were carried out to get the desired concentrations of plant for trails.

DPPH radical scavenging activity

DPPH radical scavenging activity was estimated by using method [17].25mM solutions of DPPH radical (0.5mL) was added into ethanol, aqueous extract solution (1ml) in concentration from (25-200ug/ml). After shaking the mixture put it into dark for 30 minutes and then absorbance was checked in spectrophotometer at 517 nm .The ability to scavenge DPPH radical determined as;

Scavenging % = $[(A_o - A1)/A_o)] \times 100$

A1 is absorbance of sample and A_o is absorbance of the control. All conclusions were done in triplicate.

ABTS radical scavenging activity

Spectrometric analysis of ABTS radical cation scavenging was determined using the method [18] with some modification. This method is based on the capability of antioxidants to quench the ABTS radical cation, a blue/green chromophore with characteristic absorption. The ABTS solution was prepared by adding 80 mg ABTS in phosphate buffer with 13.2mg potassium per sulphate (K2S2O8), stored in the dark at room temperature for 12 hours. To obtain the absorbance of 0.700 at 734 nm the solution was diluted with Phosphate buffer with pH (7.4). This stock solution was 1ml and 0.1ml of extract was added in working stock solution. After 30min, percentage inhibition at 734nm was calculated for each concentration.

The ABTS scavenging was calculated using the following formula:

ABTS scavenging effect (%) = $(A_o - As)/ A_o \times 100$

Where A_o is the initial concentration of the ABTS radical cation and As is absorbance of the remaining concentration of ABTS radical cation in presence of the extract.

Metal chelation activity

Iron chelating capacity of the extract was monitored by using the method[19]. Briefly, 150L of the freshly formed 2mM FeSO4.7H2O was added in a mixture which have 168µL of the 0.1M Tris HCL (ph 7.4), 218µL of the saline and 25-200µg/mlL concentrations of the plant extracts. The mixture of samples was incubated five minutes before adding of 13µL of 0.25% 1,10-phenanthroline (w/v). Absorbance was checked at 510nm in spectrophotometer.

Antioxidant potential assay

By using the phospho-molybdenum assay total antioxidant potential was measured[20]. This assay depends on the reduction of Mo (VI) to Mo (V) by extracts and results in green phosphate Mo (V) compound at acidic PH. Extracts (25-200 µg/ml) were mixed with 3ml of the reagents mixture (0.6 M H2SO4, 28mM sodium phosphate solutions and then 4mm ammonium molybdate). Then the tubes were finally incubated at 95^{0}C for 90 minutes. The absorbance was checked at 695 nm in a spectrophotometer.

Lipid peroxidation assay

Anti-lipid peroxidative properties of extracts were studied by a method [21]. The assay was carried out with egg yolk approximately 1 gram, diluted to 100 ml at pH 7.4 with 100 mM of Tris-HCl. This homogenate was incubated with Fe (II) or sodium nitroprusside with or without the extract and color reaction was carried out

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by adding TBA (600 µl) and acetic acid (600 µl) for 1 h. The tubes were cooled and finally n-butanol of 2 ml was added and centrifuged. Finally, absorbance was noted at 532 nm using spectrophotometer.

Lipase inhibitory effect of plant

The porcine pancreatic lipase inhibitory of samples was carried out by method [22]. 5mg/ml of lipase solution was dissloved in 0.1M sodium phosphate buffer (PH =8) and 4.5mg of p-nitrophenylpalmitate (p-NPP) in 200µl of dimethylformamide.In a reaction mixture 0.5 ml of lipase was incubated with 0.1 ml of plant extract and incubated for 30 minutes. Finally, 1 mL substrate p-NPP (3 mM in 2-propanol) was added into it and incubated for 5 minutes at 37^0C in water bath and absorbance was read at 405nm using spectrophotometer.

Determination of total phenolic content

The Phenolic content was analyzed using the method [23]. 0.5ml of extract was mixed with 2.5ml of 10% Folin-Ciocalteau'sregeant (v/v) and 2ml of 7.5 percent sodium carbonate. Then this mixture was allowed to stand for 40minutes at 45^0C and then absorbance was checked at 765nm spectrophotometrically. As a reference phenol gallic acid was used. The total phenolic content was determined as mg of gallic acid equivalent/g of extract by taking mean of the three readings.

Determination of total Flavonoids content

Total flavonoids content as quercetin equivalents were checked following method [24]. Quercetin was used to prepare a calibration curve (0.04, 0.02, 0.0025 and 0.00125 mg/ml) in 80% ethanol (v/v). Standards solution or (0.5ml) concentrations of extract were mixed with 1.5ml of 95% ethanol (v/v), 10% aluminum chloride, 0.1ml of 1mol/L sodium acetate and then 2.8ml of distill water was added. After keeping mixture at room temperature for 30min, absorbance of the reaction mixture was checked spectrophotometrically at 415nm. Flavonoid content obtained was expressed as milligrams of quercetin equivalents/g of extract.

Gas chromatography mass spectrometry (GC-MS) of plant

10g of powdered sample (Trichosanthes cucumerina) was soaked with 30 mL ethanol over night and filtered through ash less filter paper with sodium sulphate.GC-MS analysis was completed by utilizing GC- Mass spectrometer system DB 35-MS Capillary standard non-polar column (30 mts. X 0.25 mm, 0.25 m film thickness). Oven temperature programmewas from 50-150^0C with increasing rate of 3 ^{o}C/min and holding time of about 10 minutes. The components were alienated using Helium as carrier gas at a flow of 1.0 ml/min. 1ml sample extract injected into the instruments. Injector temperature was 250^0C.The constituent's present in the analyzed samples of Trichosanthes cucumerina are recognized in comparison with their specters of mass with those accumulated in a library search (NIST-MS) results and those are reported in this literature.

Statistical analysis:

Means and standard deviations were calculated and the means were subjected to ONE WAY ANOVA using the software package Statistica. Differences among means were compared with Duncan's multiple range post hoc test.

III. RESULTS AND DISCUSSION

Scavenging activity of extract by DPPH

DPPH assay is widely utilized in vitro method because this is a rapid and reliable method to determine the antioxidants activity of natural materials and plant extracts. Antioxidants have the ability to neutralize DPPH radicals. The extract's scavenging activity was determined by measuring the scavenging activity of DPPH radicals. Concentration and extract have a direct relationship with each other many extract have the ability to denote hydrogen and electrons. When extract/compound is added to DPPH solution, then colour of the solution changes from purple to yellow. Antioxidants which are found in plant extracts help to scavenger free radicals[25]. Here radical scavenging activities of the ethanolic extract was found to be 91.1±0.077 % and aqueous extract was 86±0.12 % (Figure 1). The high radical scavenging activity of Trichosanthes cucumerina justifies its popular use in traditional medicine. The DPPH radical scavenging activity of this plant was found in leaves (90.17±0.67%) at 100 µg/ml concentration [26].

Scavenging activity of extract by ABTS method

ABTS is a protonated radical with a distinctive absorption maxima at 734nm, which lowers when scavenged by

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the plant extracts. In our study ethanol extract showed high scavenging efficacy (80.2 ± 0.071) then aqueous (77.33 ± 0.064) at the same concentration (Figure 2). Our results are in agreement to the [27] where ethanolic extracts from flowers, fruits, and leaves of T. cucumerina showed high ABTS radical scavenging activities.

Metal chelation Activity of extract

When a metal such as iron accumulates in our bodies, it can be poisonous. Free radicals are known to be produced by the ferrous ion (Fe^{2+}).Secondary antioxidant compounds are effective metal chelating agents that also limit the formation of free radicals. These chelating agents are shown to lower the redox potential by stabilizing the oxidized state of the metal ion by forming of Ω- bonds with it. Reactive oxygen species are the most prominent intermediate in metal catalyzed redox reactions. The production of free radicals by pick up or removing electron was observed in metal with partially filled orbital's, such as ferrous ions. Extract of Trichosanthes cucumerina showed the potential for metal chelation. At maximum concentration (300µg/ml) ethanol and aqueous extract showed chelation of 62.23 ± 0.232 and 60.36 ± 0.0765 % (Figure 3).

Antioxidant activity of extract by phosphomolybdenum assay

Phosphomolybdenum assay is used to assess the antioxidant properties of extracts. Trichosanthes cucumerina extract has a total antioxidant activity show in Figure 4. The antioxidant activity was expressed as µg of ascorbic acid equivalent. Antioxidant activity increased with increase in extract concentration. Ethanol and water extract of Trichosanthes cucumerina revealed high antioxidant activities (229.64 ± 0.053 and 220.8 ± 0.39µg of ascorbic acid equivalent) respectively at maximum concentration.

Inhibitory effect of extract against lipid peroxidation

Lipid per-oxidation was induced when iron and sodium nitroprusside was used as prooxidant in phospholipid homogenate. High concentrations of iron cause different diseases like cancer, liver diseases, heart disease and neurological disorders[28]. Trichosanthes cucumerina extract has antioxidant activity and can be effectively utilized in accidental toxicities caused by iron overload. Sodium nitroprusside causes smooth muscle relaxation which includes dilation of blood vessels and serves as anti-hypertensive agent. It also causes photo-degradation which includes in the formation of ($[(CN)5\text{-}Fe]^{3+}$, $[(CN)4\text{-}Fe]^{2+}$. Figures 5&6 depicted the changes in TBARS generation as a result of lipid per-oxidation with various concentrations of Trichosanthes cucumerina. Overall extract showed high capability in reducing TBARS compared to the control. The antilipidperoxidative properties of Trichosanthes cucumerina are in agreement to the report of [29].

Total phenolic and flavonoids content of Trichosanthes cucumerina

Total phenolic and flavonoid content of Trichosanthes cucumerina is shown in Table 2. Generally the ethanolic extract contained high content of phenolic and flavonoid contents compared to the aqueous extract which explains the higher biological activities of ethanolic extract.The total level of phenolic contents (32.2±0.49mg GAE/gm DW) and flavonoids (7.82±0.67mg QE/gm DW) were found higher in leaves than other plant parts [30].

Lipase inhibitory effect of extract

Most of the medicinal plants have been known for Lipase inhibitory activity that has increased the spectrum of the potential of the compounds present. More analysis has been centered on discovering newer pancreatic lipase inhibitors with less unpleasant adverse effects owing to the tremendous success of natural product for management. So far, due to their lipase inhibition properties, several natural products (plant extracts and isolated compounds) have been reported. Ethanolic extract of Trichosanthes cucumerina showed 53 % inhibition of lipase and aqueous extract inhibition is 39.1%.Orlistat at similar concentration showed 92.3 % lipase inhibitory effect (data not shown).

GC-MS Analysis

GC-MS is an integrated composite analysis tool that combines gas chromatography GC, which is excellent in its ability for separation, with mass spectrometry, which is ideal for identifying and elucidating the structure of separated components. The chemical makeup of medicinal plants can be identified using Gas Chromatography Mass Spectrometry (GC-MS). GC-MS is the most effective method for identifying volatile matter constituents such as long -chain, branched chain hydrocarbons, alcohols acids and esters, etc. The chemical constituent of Trichosanthes cucumerina was disclosed by GC-MS analysis, and the results were tabulated in Table 1. The GC-

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MS analysis of crude ethanolic extract showed the presence of 27 compounds. The major compounds identified were 4- Cyanomethyl-3-pyrrolepropiononitrile (23%),3- Chloromethylsulfunyl-3-dimethylfuran (19%), Henecosanoic acid methyl ester (15.13%), Octadecanoic acid methyl ester (15.13%),Neophytadiene (9.88%), Pentadecanal (9.88%), Tridecanal (9.88%), Phytol acetate (4.16%), Iavandulyl acetate (4.15%), Nonanoicacis, methyl ester (2.65%), Decanoic acid, methyl ester (2.62%) and Neopentylhydroxyacetate (2.09%). The screening of leaves extract showed high presence of heterocyclic compounds and their derivatives, esters and aldehydes. Based on studies some of the constituents revealed by GC-MS analysis are bioactive compounds. Many volatile compounds such as Nonanoicacis, methyl and octadecanoic acid methyl ester were detected in the Trichosanthes cucumerina. Phytol detected in leave extract has shown anti conceptive and antioxidant effects [31]. The main compound lavandulyl acetate (acetate ester of lavandulol, a known component of lavender oil),the biological potentiality of this compound was demonstrated through the larvicidal activity against three important mosquito vectors[32]. The report is the first of its kind to analyze the chemical constituents from the ethanolic extract of Trichosanthes cucumerinaleaves using GC-MS.

FIGURE LEGENDS

Figure 1: DPPH radical scavenging activities (percent inhibition) of extract Trichosanthes cucumerina extract.Values are means±SD (n=3).Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.

Figure 2: ABTS radical scavenging activities (Percent inhibition) of Trichosanthes cucumerina extracts. Values are means±SD (n=3).Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.

Figure 3: The Metal chelating activity of Trichosanthes Cucumerina extract by iron.Values are means±SD (n=3).Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.

Figure 4: Total antioxidant activity of Trichosanthes cucumerinaextract measured on basis of Phosphomolybdenum reduction assay. Values are means±SD (n=3).Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.

Figure 5: TBARS inhibition in phospholipid by leave extract of Trichosanthes cucumerina with FeSO4**.** Values are means±SD (n=3).

Figure 6: TBARS inhibition in phospholipid by leaves extract of Trichosanthes cucumerina with Sodium nitroprusside**.** Values are means±SD (n=3).

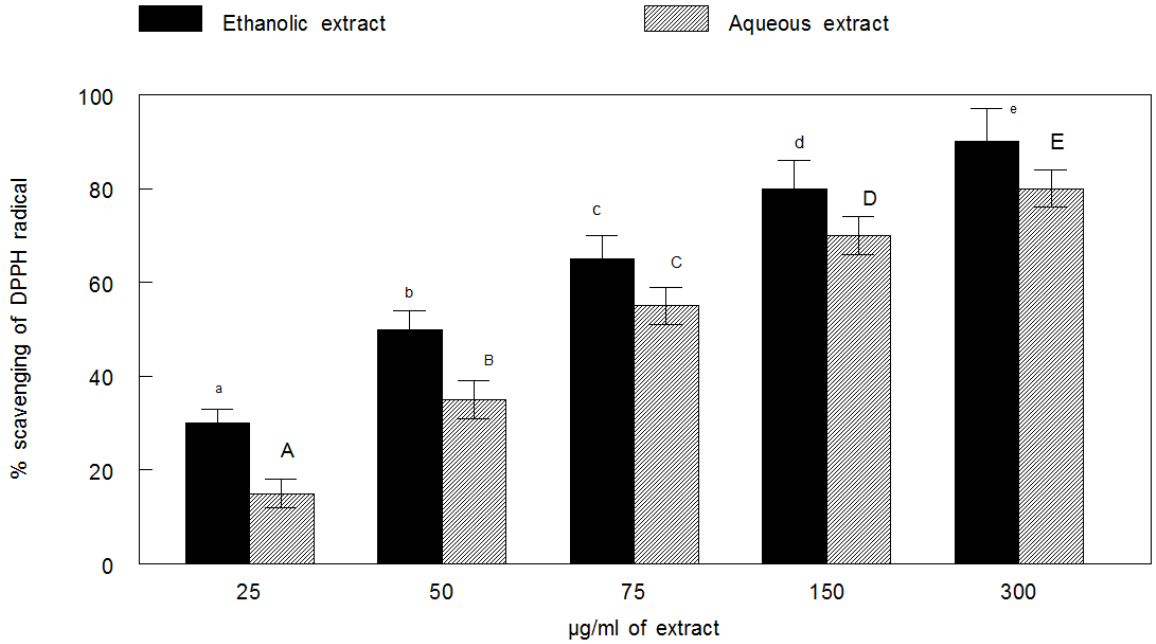

Figure (1)

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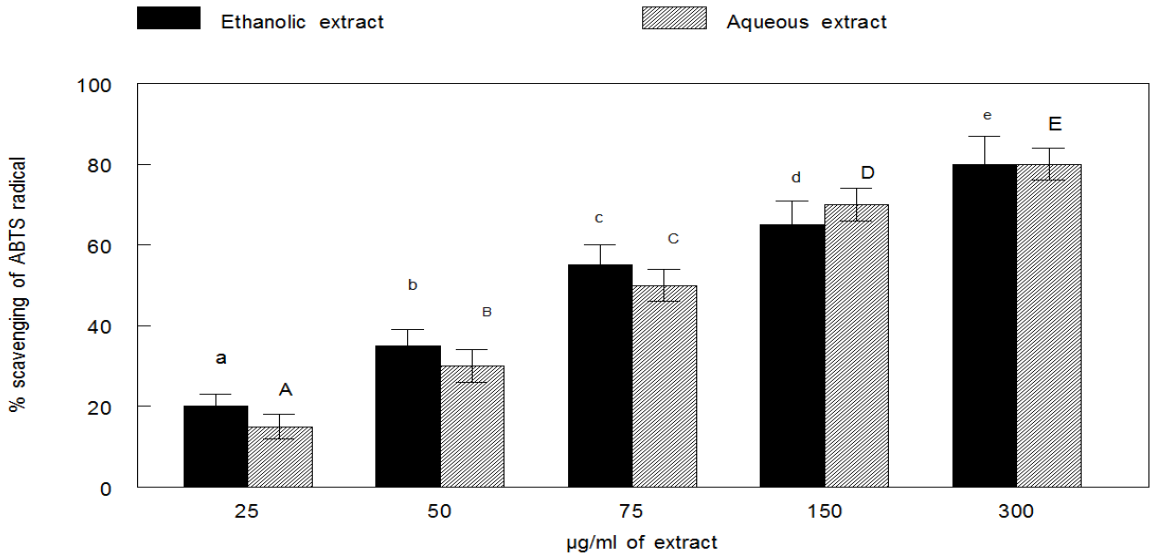

Figure (2)

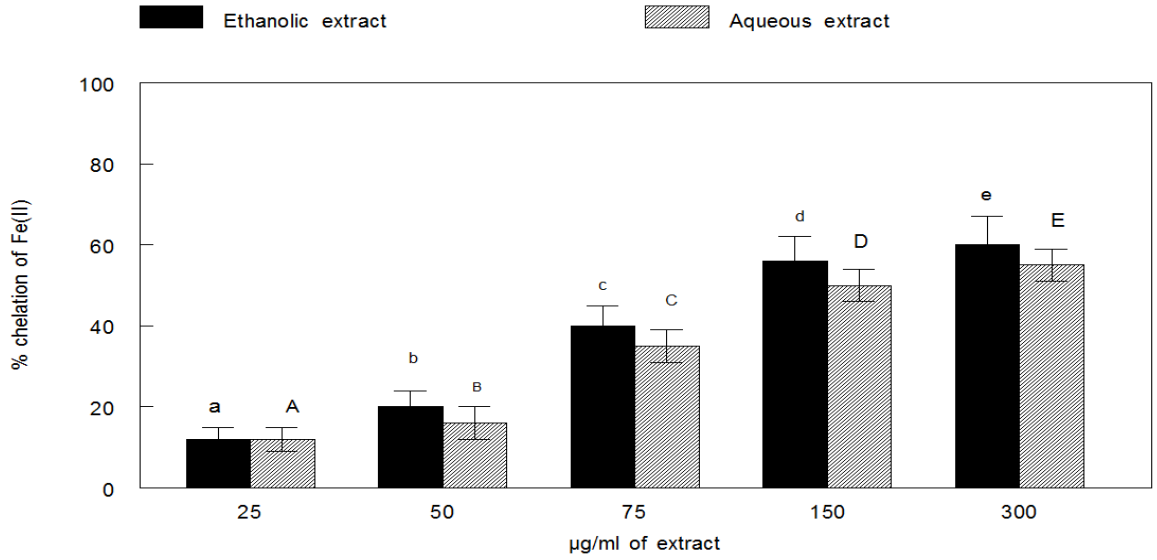

Figure (3)

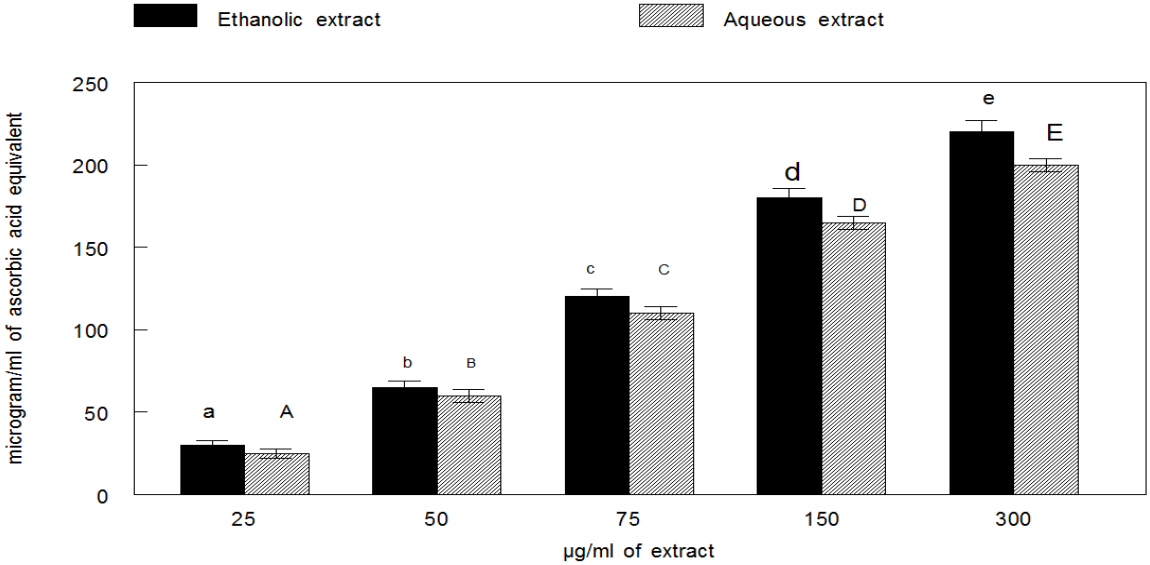

Figure (4)

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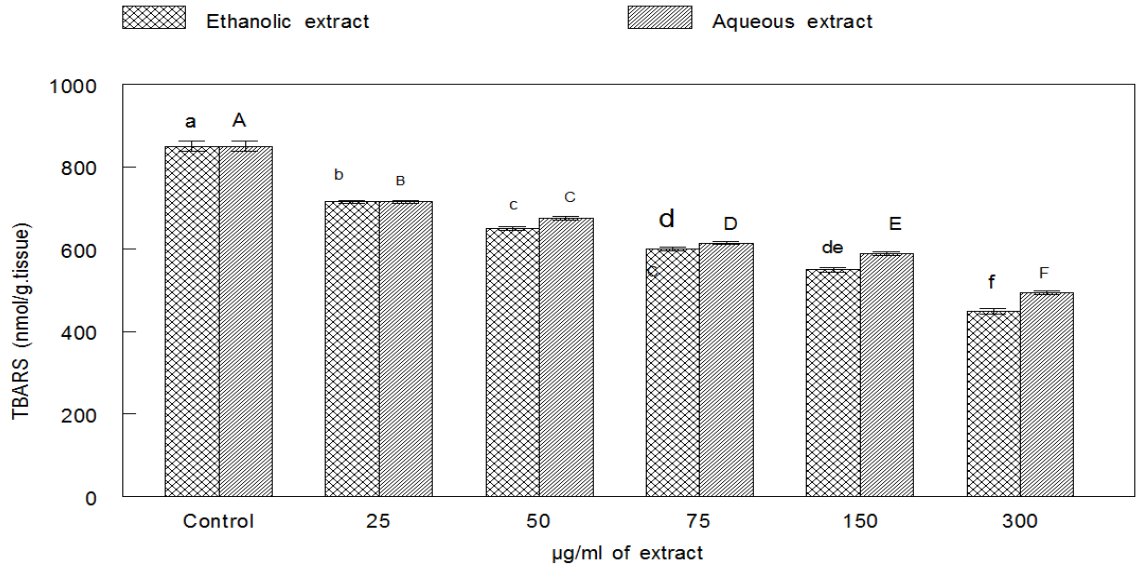

Figure (5)

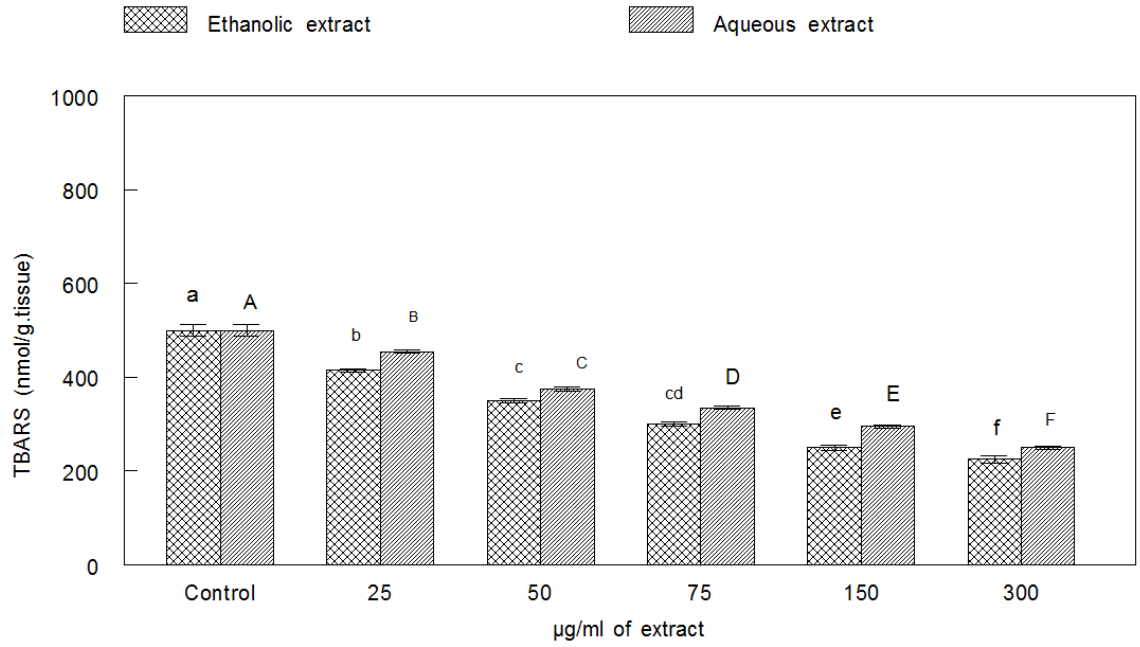

Figure (6)

Table 1: Compounds present in the ethanolic extract of Trichosanthes Cucumerina extract

Peak no	Retention Time	Area (%)	M. F	M. W	Compound Name
1	3.27	0.51	$C_5H_{13}NO_2$	119	3-(2-Hydroxy ethoxy) propylamine
2	3.27	0.51	$C_7H_{15}DS$	132	1,1-Dimethylethyl-3-dueteriopropyl thioether
3	3.27	0.51	$C_9H_{18}O_2$	158	Hexyl propanoate
4	3.27	0.51	$C_5H_{10}D_2O$	88	Neopentyl alcohol
5	10.62	0.72	$C_{20}H_{29}NO_4$	347	Ethyl 2-(benzyloxycarbonylamino)dec-2-enoate
6	10.62	0.72	$C_{27}H_{56}O$	396	1-Heptacosanol
7	13.74	2.65	$C_{10}H_{20}O_2$	172	Nonanoicacis, methyl ester

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8	13.74	2.62	$C_{13}H_{26}O_2$	214	Decanoic acid, methyl ester
9	14.08	0.56	$C_{11}H_{12}O_2$	176	2-(1, 3- Dioxolan-2-yl)-1-phenylethene
10	14.08	0.56	$C_{15}H_{11}NO_3$	253	2-(E-Cinnamido)-1,4-benzoquinone
11	18.18	9.88	$C_{13}H_{26}O$	198	Tridecanal
12	18.18	9.88	$C_{15}H_{30}O$	226	Pentadecanal
13	18.18	9.88	$C_{20}H_{38}$	278	Neophytadiene
14	19.13	4.15	$C_{12}H_{20}O_2$	196	Iavandulyl acetate
15	19.14	4.16	$C_{22}H_{42}O_2$	338	Phytol acetate
16	20.97	15.13	$C_{19}H_{38}O_2$	298	Octadecanoic acid methyl ester
17	20.97	15.13	$C_{22}H_{44}O_2$	340	Henecosanoic acid methyl ester
18	22.07	0.41	$C_{16}H_{14}OS$	254	4,4- Dimethylmonothiobenzil
19	23.91	1.06	$C_9H_9N_3$	159	4- Cyanomethyl-3-pyrrolepropiononitrile
20	24.19	19.67	C_5H_5ClO	116	3- Chloromethylsulfunyl-3-dimethylfuran
21	26.25	1.23	$C_8H_{18}O$	130	Isooctanol
22	27.78	1.17	$C_{13}H_{19}NO$	205	1-[2-(2-Dimethylamino)ethoxy]ethenyl]-4-methylbenzene
23	27.78	1.17	$C_9H_{16}O_2$	156	Hept-enyl-2-acetate
24	29.96	1.35	$C_6H_{10}N_2O_4$	174	Z-3,4-Dinitro-3-hexane
25	29.96	1.35	$C_6H_{10}N_2O_4$	174	E-3,4-Dinitro-3-hexane
26	31.51	2.09	$C_7H_{14}O_3$	146	Neopentylhydroxyacetate
27	37.50	0.61	$C_9H_{13}N_3O_5$	243	Cytidine

Table 2: Total Phenolic and flavonoid contents in extracts of Trichosanthes Cucumerina

Sample	Phenolic (mg/g)	Flavonoid (mg/g)
Ethanol extract	1.61 ± 0.11	1.46 ± 0.31
Water extract	0.93 ± 0.51	0.72 ± 0.09

IV. CONCLUSION

From the present study it is concluded that the ethanolic and water extract of Trichosanthes cucumerina, leaves possessed significant amounts of beneficial compounds. The highest concentration of phenolic compounds, flavonoids, and antioxidant capacity was found in the leaves. The Lipase inhibitory activity of the plant was high which justifies the use of the plant in obesity and other degenerative diseases.

Conflict of interest statement: The authors declare that no conflict of interest is involved in this study.

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