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IN-VITRO ANTI-LIPASE AND ANTIOXIDANT ACTIVITY OF POLYHERBAL AYURVEDIC MEDICINE *VARANADI KASHAYAM*

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Keywords:

Varanadi kashayam, Ayurveda, Pancreatic lipase, Antioxidant assay, HPTLC, Phytochemicals

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ABSTRACT: Objective: The purpose of the study was to evaluate the effect of an Ayurvedic formulation *Varanadi kashayam* to inhibit pancreatic lipase activity *in-vitro*. It also focuses on antioxidant activity, total phenol, flavonoid and phenolic acid content of fractions of *Varanadi kashayam* and HPTLC fingerprinting of fractions. **Methods:** Fractionation of *Varanadi kashayam* was done by using five solvents of increasing polarity. Qualitative analysis of phyto-chemicals and quantitative determination of phenol, flavonoids, and phenolic acids were determined by standard protocols. The antioxidant activity of fractions was measured by DPPH and ABTS scavenging assay through spectroscopic analysis. The anti-lipase activity of fractions of *Varanadi kashayam* was determined by using porcine pancreatic lipase as enzyme and p-Nitrophenyl palmitate as substrate. HPTLC analytical method was used for the determination of compounds presents in polyherbal formulation *Varanadi kashayam*. **Results:** Ethyl acetate fraction of *Varanadi kashayam* showed free radical scavenging activity with IC_{50} values close to that of BHT and quercetin standards. The amount of phenol, flavonoid, and phenolic acid was also found to be highest in ethyl acetate fraction. This shows the relation between antioxidant activity and polyphenol content of fractions. The methanol fraction of *Varanadi kashayam* showed highest anti-lipase activity compared to orlistat standard. This may be due to the high phyto-chemical content of methanol fraction. **Conclusion:** From the results, it is evident that *Varanadi kashayam* can effectively inhibit pancreatic lipase enzyme and hence the absorption of dietary lipids. It is a rich source of phytochemicals and antioxidants. Hence, it could be a better remedy to reduce lipid absorption and provide protection against reactive oxygen species.

INTRODUCTION: Ayurveda is one of the ancient and living traditions widely practiced in India. Herbal medicines are therapeutically used all around the world; hence these are the oldest form of healthcare known to the mankind.

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Hence, clinical validation and standardization of Ayurvedic medicines are required [1]. Medicinal plants contain plenty of phytochemical constituents and are helpful for curing human diseases [2] and gained much attention nowadays because of its cost-effectiveness and efficiency.

There is evidence that crude medicinal plant extracts have more biological activity than isolated compounds due to synergistic interaction [3]. Determination of antioxidant activity of herbal drugs is necessary because of its pharmaceutical effects.

Anti-oxidants are secondary phytochemicals found in plants, inhibits the oxidation of the susceptible substrate, or they can terminate the chain reactions initiated by free radicals [4]. A large number of anti-oxidants produced by plants include flavonoids, carotenoids, cinnamic acid, tocopherols, folic acid, benzoic acid *etc*. [5] These anti-oxidants provide protection against unavoidable reactive oxygen species [6]. The rapid production of free radicals cause oxidative damage to biomolecules and can cause diseases like cirrhosis, atherosclerosis, arthritis, cancer, neurological disorders, emphysema *etc*. [7, 8] Available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) cause various side effects [9, 10]. Therefore the interest of finding natural antioxidants with low cytotoxicity increased because of carcinogenicity of synthetic antioxidants [11]. As an alternative to synthetic medicines, Ayurvedic herbal medicines have applications in managing lifestyle disorders [12]. However, there is inadequate scientific validation are required for these medicines.

Pancreatic lipase is an enzyme that hydrolyzes dietary lipids and is an important target to treat obesity disorder [13]. Orlistat is a synthetic drug that inhibits pancreatic lipase enzyme have various side effects like oily stools, diarrhea, abdominal pain, fecal spotting and some hepatotoxicity [14, 15]. Hence, the natural medicines that can inhibit pancreatic lipase enzyme and absorption of dietary fat gained much attention nowadays. In the current study, we evaluated the anti-lipase activity of herbal decoction *Varanadi kashayam*. *Varanadi kashayam* is a polyherbal formulation in Ayurveda contains sixteen plants, widely used to treat obesity [16, 17]. No much scientific standardization and validation approaches have been made for this herbal formulation for its medicinal uses. The present study was carried out to identify the presence or absence of phytochemicals in the herbal combination of *Varanadi kashayam* and to identify its anti-lipase and antioxidant properties. It also focuses on High-Performance Thin Layer Chromatography (HPTLC) analysis of fractions of *Varanadi kashayam*.

MATERIALS AND METHODS:

Chemicals: *Varanadi kashayam* was purchased from Kottakal Arya Vaidya sala, Kerala, India (Batch No: 512540). DPPH (1,1- Diphenyl -2-Picryl hydrazyl), ABTS (2,2'-azino bis ethyl benzthiazoline 6- sulfonic acid), BHT, quercetin, gallic acid, and orlistat were obtained from Himedia Laboratories, Ltd. India. Para nitro-phenyl palmitate, type II porcine pancreatic lipase was bought from Sigma Aldrich, USA. Aluminium coated silica gel GF_{256} TLC plates were purchased from Sigma Aldrich, USA. All the solvents used were analytical grade obtained from Merck Ltd., India.

***Varanadi kashayam* Fractionation:** *Varanadi kashayam* was fractionated with five solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, methanol and water). Solvents from samples were removed by evaporation using Rotary evaporator. Water samples were lyophilized and percentage of yield was calculated and stored at 4ºC for further analysis.

Qualitative Phytochemical Screening: The preliminary qualitative phytochemical analysis was done to identify the secondary metabolites present in fractions of *Varanadi kashayam* according to standard procedures [18].

Test for Carbohydrates (Molish's Test): The test sample was mixed with a few drops of Molish's reagent. This was followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2-3 min and observed for the formation of red or violet color at the interphase of two layers.

Alkaloids (Wagner's Reagent Test): Sample was treated with few drops of Wagner's reagent and observed for the formation of a reddish precipitate.

Test for Phenols (Ferric Chloride Test): Sample was treated with 5% ferric chloride solution. Formation of deep blue or black color indicates the presence of phenols.

Test for Cardiac Glycosides (Keller Kelliani's Test): Sample was treated with 2 ml of glacial acetic acid and a drop of ferric chloride solution. 1ml of concentrated sulphuric acid was added along the sides of the test tube. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides.

Test for Amino Acids and Proteins (1% Ninhydrin Solution in Acetone): 2 ml of the sample was treated with few drops of minhydrin solution and placed the test tube in a boiling water bath for 1-2 min and observed for the presence of purple color.

Test for Saponins (Foam Test): Sample was mixed with water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Tannins (Braymer's Test): Sample was treated with 10% alcoholic ferric chloride solution and observed for the formation of blue or greenish coloration.

Test for Terpenoids (Bragmer's Test): Sample was mixed with 1 ml chloroform and few drops of concentrated sulphuric acid were added. Formation of reddish brown precipitate indicated the presence of terpenoids.

Test for Quinines: The sample was mixed with concentrated HCl and the formation of yellow precipitate indicates the formation of quinines.

Test for Coumarins: 2 ml of the sample was mixed with few drops of 10% NaOH and observed for the formation of yellow color.

Test for Fatty Acids: Ether was added to sample and allowed to evaporate on filter paper. Dry the filter paper and appearance of formation of transparency on filter paper was observed.

Quantitative Phytochemical Analysis:

Determination of Total Phenol Content: Total phenol content of the extract was determined by using Folin-ciocalteu reagent assay [19]. In brief, 100µl of sample was mixed with 200 µl of 10% folin-ciocalteu reagent and 100 µl of 15% sodium hydroxide solution. The mixture was shaken well and incubated in dark for 2 h.

Absorbance was measured at 765 nm using UV-Vis spectrophotometer against blank. The total phenol content in the extracts was determined from the standard curve of gallic acid and the results were expressed as gallic acid equivalent in mg per gram of dry weight of extracts.

Determination of Total Flavonoid Content: The total flavonoid content of the extract was determined by using aluminium chloride colorimetric method [20]. 500 µl of sample extract dissolved in methanol was mixed with 100 µl of 10% aluminium chloride, 100 µl of 1M potassium acetate and 2 ml water. Samples were incubated at room temperature for 30 min. The absorbance was measured at 415 nm using UV-Vis spectrophotometer. Quercetin was used as standard and total flavonoid was expressed as quercetin equivalent in milligram per gram of dry weight of the extract.

Determination of Total Phenolic Acid: The total phenolic acid content of sample extracts was determined by a colorimetric method using Arnov's reagent [21]. 100 µl of sample dissolved in methanol mixed with 100 µl of 0.5% HCl, 100 µl of 1M NaOH, 100 µl of Arnov's reagent (10 g sodium molybdate and 10 g sodium nitrite) and 600 µl of deionized water. Phenolic acids were determined spectrophotometrically at 490 nm. The caffeic acid standard was used for the calibration curve, and total phenolic acid content in the extracts was expressed as caffeic acid equivalents in micrograms per gram of extract.

ABTS Assay: ABTS assay was based on the activity of different samples to scavenge ABTS (2,2'-azino bis ethyl benzthiazoline 6- sulfonic acid) free radical [22]. 7.4 mM ABTS and 2.6 mM potassium persulphate dissolved in distilled water. The two solutions were mixed in equal ratio and the mixture was allowed to stand for 16 h in dark before use to produce ABTS radical.

ABTS reagent was diluted with methanol to an absorbance of 0.7 ± 0.05 OD. For the assay 900 µl of ABTS reagent was mixed with 100 µl of sample extracts dissolved in methanol and absorbance was measured at 734 nm colourimetrically. Quercetin was used as reference standard. Antioxidant activity was expressed as IC_{50}, which is the concentration of extract required to inhibit 50% free radical.

$$\% \text{ scavenging activity} = A_C - A_T / A_C \times 100$$

Where, A_C = Absorbance of control, A_T = Absorbance of the test sample.

DPPH Assay: Free radical scavenging activity of fractions was determined by DPPH (1,1- Diphenyl -2- Picryl hydrazyl) assay. 100 µl of 0.5 mM DPPH dissolved in methanol was added to 100 µl of different extracts dissolved in methanol at different concentrations. This mixture was incubated at dark for 30 min. Absorbance was measured at 520 nm using a spectrophotometer. BHT was used as a reference standard and the antioxidant activity was expressed as IC_{50}. Percent DPPH scavenging activity was determined by the equation,

$$\% \text{ scavenging effect} = A_C\text{-}A_T / A_C \times 100$$

Where, A_C = Absorbance of control, A_T = Absorbance of the test sample.

Pancreatic Lipase Inhibition Assay: Lipase activity was assayed by the colorimetric method [23], by measuring the amount of 4-nitrophenol released from 4-nitrophenyl palmitate (p-NPP) substrate. A stock solution of p-nitrophenyl palmitate was prepared in isopropanol and sonicated until complete dissolution of p-NPP. Stock diluted with isopropanol to a concentration of 2 mM. The porcine pancreatic lipase type II was dissolved in tris-HCl buffer (pH 8.5) at the concentration of 2 mg/mL. This suspension was centrifuged at 2000 rpm for five minutes and the supernatant used as the source of enzyme. The reaction mixture containing the enzyme and test sample or orlistat standard in tris HCl buffer was pre-incubated at 37°C for 10 min. The reaction was started by adding 400 µl of the p-NPP substrate and the final volume will be 1 ml.

After incubation at 37 °C for 20 min, the amount of 2,4-dinitrophenol released in the reaction was measured at 360 nm using a UV-Visible spectro-photometer (Shimadzu). A standard graph of the reaction product p-nitrophenol was drawn and using the equation obtained from the graph, the unknown concentration of product formed was calculated. The inhibitory activity was calculated by the formula,

$$\% \text{ of Inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

The IC_{50} values were calculated by logarithmic regression analysis using GraphPad Prism® (version 5.03).

High-Performance Thin Layer Chromatography: HPTLC fingerprinting analysis of *Varanadi kashayam* fractions was done according to the protocol described in Mohind *et al.,* with modifications [24]. CAMAG HPTLC system (Switzerland) with a Linomat 5 sample applicator was used for the analysis. 15µl of fractions were spotted as bands into pre-coated silica gel GF_{256} (Sigma Aldrich) aluminium plates with the help of Linomat 5 applicator attached to a CAMAG HPTLC system. Loaded plates were kept in a developing chamber with the respective solvent system, previously saturated with solvent vapour for 30 min. After development, the plates were dried in hot air to evaporate the solvents. The plate was kept in a photodocumentation chamber (CAMAG TLC Scanner 3) and the images were captured at UV 254 nm and UV 366 nm, scanning was then performed with a CAMAG TLC Scanner 3 equipped with the win CATS Software.

Statistical Analysis: All the samples were analyzed in triplicate and results were expressed as mean ± SD. One-way analysis of variance and the Dunnett's multiple comparison tests (GraphPad Prism® version 5.03) was used to determine the significance of test samples compared to control. A p-value of less than 0.05 ($p<0.05$) was considered to be statistically significant.

RESULTS:

Yield of Fractions: *Varanadi kashayam* was fractionated by liquid-liquid extraction protocol using five solvents hexane, dichloromethane, ethyl acetate, methanol and water. The yield of each fraction was calculated by measuring the dry weight of solvent removed fractions. The yield was found to be more in methanol fraction and low in hexane fraction due to its low polarity. The yield of each extract was given in **Table 1**.

TABLE 1: PERCENTAGE YIELD OF FRACTIONS

Type of Fractions	Yield (% w/w)
Hexane fraction	3.10%
Dichloromethane fraction	8.54%
Ethyl acetate fraction	10.25%
Methanol fraction	52.8%
Water fraction	25.31%

Qualitative Phytochemical Analysis: Qualitative analysis of phytochemicals present in five fractions was analyzed and a wide range of phytochemicals

was found to present in all fractions shown in **Table 2**. Due to nonpolar nature hexane is able to extract very low compounds like steroids, saponins, and tannins. The dichloromethane fractionwas found to positive for flavonoids, phenols, tannins carbohydrates and coumarins. Ethyl acetate and methanol extract contain a large number of phyto compounds like alkaloids, phenols, flavonoids, terpenoids, tannins, carbohydrates, amino acids and proteins, and coumarins. The aqueous extract contains phenols, alkaloids, saponins, and carbohydrates. The presence of these large varieties of bioactive compounds in *Varanadi kashayam* shows that it can be beneficiary for a number of ways.

TABLE 2: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF FRACTIONS OF *VARANADI KASHAYAM*

Phytochemicals	Samples				
	Hexane fraction	Dichloromethane fraction	Ethyl acetate fraction	Methanol fraction	Water fraction
Flavonoids	-	+	+++	+ +	++
Phenols	-	+ +	+ ++	+ ++	+
Alkaloids	-	-	+++	+ +	+
Saponins	++	-	-	-	+
Terpenoids	-	-	+++	+ ++	-
Tannins	+	+ +	+ ++	+ ++	-
Carbohydrates	-	+ +	+ +	+ ++	+ +
Amino acids and proteins	-	-	++	+ +	-
Coumarins	-	+ +	+ ++	+ ++	-
Steroids	+ +	-	-	-	-
Fatty acids	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-

+++: highly present, ++: moderately present, +: low, -: absent

Quantitative Determination of Phenol: The total phenol content in all the fractions was determined and the results were expressed as gallic acid equivalents. Among the five fractions, ethyl acetate fraction contains the highest phenolic content (195.8 ± 0.07 mg GAE/g) followed by methanolic fraction (181.7 ± 0.076 mg GAE/g), water fraction (96.5 ± 0. 052 mg GAE/g) and dichloromethane fraction (67.2 ± 0.072 mg GAE/g). Phenol content was found to be absent in the hexane fraction.

Quantitative Determination of Flavonoids: Total flavonoid content of fractions, determined from the calibration curve of quercetin (R^2: 0.998). Flavonoid content is significantly high in ethyl acetate fraction (26.242 ± 0.104 mg QUE/g) of *V. kashayam.* Methanol fraction, dichloromethane fraction and water fraction contains 20.09 ± 0.039, 17.2 ± 0.05, 13.57 ± 0.05 mg QUE/g of flavonoids respectively. Flavonoid content was found to be absent in hexane fraction.

Quantitative Determination of Phenolic Acids: The total phenolic acid content of fractions analyzed by Arnov's reagent and calculated as caffeic acid equivalents (mg CAE/gm). Among the five fractions, the phenolic acid content was found to be higher in ethyl acetate fraction (32.83 ± 0.144) followed by methanol fraction (143.3 ± 0.803), dichloromethane fraction (70.8 ± 0.144) and water fraction (54.16 ± 0.211). Hexane fraction showed the absence of phenolic acid.

ABTS Assay: As shown in figure all the fractions exhibited a dose-dependent increase in free radical scavenging activity. The radical scavenging activity of fractions was found to be increased with increased concentrations. Among the five fractions tested, ethyl acetate fraction showed better antioxidant activity than quecertin standard. IC_{50} value, the inhibition of free radicals by 50% was used as a parameter to test antioxidant activity. IC_{50} values of quercetin standard and sample extracts were presented in **Table 4**.

DPPH Assay: The free radical scavenging activity of five fractions of *Varanadi kashayam* was determined by its ability to reduce DPPH radical. DPPH is a purple dye, upon reaction with a hydrogen donor the purple colour disappears. DPPH radical scavenging activity of BHT standard and extracts were presented in **Table 3**. Ethyl acetate fraction of *Varanadi kashayam* showed a strong scavenging of DPPH radical when compared

to BHT standard. The IC_{50} value obtained for ethyl acetate fraction and BHT standard was 0.022 µg/µL and 0.024 µg/µL. Antioxidant activity of remaining dichloromethane, methanol, water and hexane fraction were 0.236 µg/µL, 0.147 µg/µL, 0.257 µg/µL and 34.35 µg/µL respectively.

TABLE 3: IC_{50} VALUES OBTAINED FOR *VARANADI KASHAYAM* FRACTIONS AND STANDARDS FROM DPPH AND ABTS ASSAY

Sample	IC_{50} value (µg/µL) ± SE	
	DPPH assay	ABTS assay
Quecertin/BHT standard	0.024 ± 0.021	0.0129 ± 0.019
Ethyl acetate fraction	0.022 ± 0.021	0.011 ± 0.027
Dichloromethane fraction	0.236 ± 0.024	0.342 ± 0.026
Methanol fraction	0.147 ± 0.003	0.103 ± 0.028
Water fraction	0.257 ± 0.017	0.168 ± 0.024
Hexane fraction	34.35 ± 0.141	32.26 ± 0.218

All the values are expressed as mean ± SD of three independent experiments.

TABLE 4: INHIBITORY EFFECT OF *VARANADI KASHAYAM* ON PORCINE PANCREATIC LIPASE

Sample	IC_{50} (µg/mL) ± SE of Pancreatic lipase enzyme
Orlistat	0.328 ± 0.018
Hexane fraction	1.069 ± 0.02
Dichloromethane fraction	0.643 ± 0.002
Ethyl acetate fraction	0.742 ± 0.006
Methanol fraction	0.410 ± 0.011
Water fraction	No Inhibition

Orlistat was used as positive control. All the values are expressed as mean ± SE (N=3). IC_{50} values calculated by non-linear regression analysis using Graph Pad Prism software.

Effect of *Varanadi kashayam* Fractions on Pancreatic Lipase Inhibition: Pancreatic lipase inhibition of all fractions was calculated as a percent of inhibition at five different concentrations (0.2-1 mg/mL). IC_{50} values calculated by statistical analysis using Graph Pad Prism software. Among the five fractions tested for PL inhibition, methanol fraction of *Varanadi kashayam* exhibited a strong inhibition on PL with IC_{50} value 0.410 ± 0.011µg/mL compared with orlistat standard with IC_{50} value 0.328 ± 0.018µg/mL. Hexane, dichloromethane and ethyl acetate fractions inhibited PL enzyme with IC_{50} values 1.069 ± 0.02 µg/mL, 0.643 ± 0.002 µg/mL, 0.742 ± 0.006 µg/mL, 0 respectively **Table 4**. Water fraction has no inhibitory effect on lipase enzyme.

HPTLC Analysis: Various mobile phase combinations were tested in order to get better resolution of peaks. The solvent ratio used for each fraction of *V. kashayam* was given in **Table 5**. The developed HPTLC plates exposed to 254 nm showed the presence of various spots. The chromatograms of all the five fractions were given in **Fig. 1**. The results from HPTLC fingerprint of methanol fraction scanned at 254 nm gave a maximum of twelve peaks with R_f values ranging from 0.01 to 0.95 in which the highest concentration was found to be 40.14% and its corresponding R_f value is 0.03 **Table 5**.

HPTLC fingerprint scanned at 254 nm of ethyl acetate fraction showed the presence of 9 compounds with R_f values 0.02 to 0.76. Similarly, dichloromethane, hexane and water fractions indicated the occurrence of 10, 8 and 7 compounds respectively. R_f value and serve the better tool for standardization of the drug. Out of the 11 components in DCM fraction, R_f values 0.26, 0.67, and 0.82 are found to be more predominant with an area of 10.94%, 35.79% and 11.56% respectively. Hexane fraction exhibited 8 bands indicating the occurrence of at least 8 compounds with the highest area was found to be 68.86% and its corresponding R_f value is 0.43. The water fraction revealed 7 compounds with R_f values ranging from 0.03 to 1.04 indicated the occurrence of 7 different compounds in the fractions.

TABLE 5: HPTLC FINGERPRINT OF *VARANADI KASHAYAM* FRACTIONS AT 254 nm

Sample	Solvent system	No of Peaks	R_f values at 254nm	Percentage Area
Hexane fraction	Acetic acid: Chloroform: Diethyl ether (1:7:2)	8	0.01, 0.18, 0.31, 0.37, 0.43, 0.55, 0.73, 0.78	1.30, 0.67, 1.30, 2.10, 3.47, 68.86, 6.77, 15.54
Dichloromethane fraction	Acetic acid: Chloroform: Diethylether (1:6:3)	10	0.11, 0.15, 0.20, 0.26, 0.36, 0.41, 0.48, 0.53, 0.67, 0.82	2.80, 3.57, 2.30, 10.67, 4.39, 1.97, 5.51, 8.30, 35.79, 11.56
Ethyl acetate fraction	Acetic acid: Chloroform: Diethylether (1:6:3)	9	0.02, 0.04, 0.09, 0.16, 0.25, 0.32, 0.45, 0.62, 0.76	5.36, 9.50, 13.37, 3.28, 11.99, 4.46, 16.95, 22.61, 8.55
Methanol fraction	Acetic acid: Ethyl acetate: Chloroform (1:1:8)	12	0.01, 0.03, 0.30, 0.34, 0.42, 0.47, 0.54, 0.56, 0.62, 0.70, 0.80, 0.95	15.38, 40.14, 1.08, 1.46, 4.06, 3.01, 8.05, 7.25, 2.48, 2.20, 11.50, 3.38
Water fraction	Acetic acid: Methanol: Chloroform (1:1:8)	6	0.03, 0.07, 0.08, 0.12, 0.18, 0.74, 1.04	2.64, 7.35, 3.87, 5.22, 2.73, 7.62, 70.57

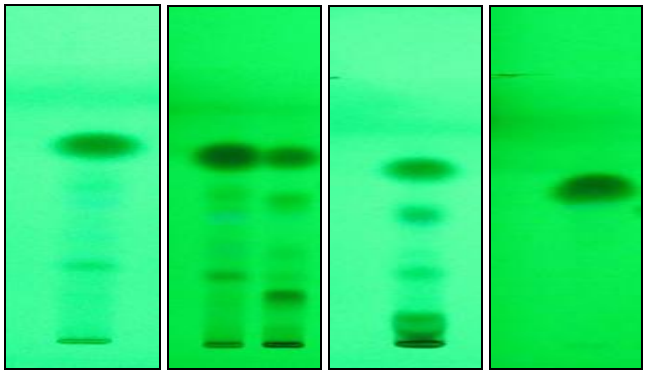

FIG. 1: HPTLC PLATE SEEN AT 254 nm

Track 1: Hexane fraction, Track 2: Dichloromethane fraction, Track 3: Ethyl acetate fraction, Track 4: Methanol fraction, Track 5: Water fraction

DISCUSSION: *Varanadi kashayam* is one of the most known Ayurvedic decoction used to treat, hyperlipidemia, abscess, tumor, chronic arthritis *etc*. It contains 16 plants, which include Varana (*Crataeva magna*), Sairyakayugma (*Barleria strigosa*), Satavari (*Asparagu sracemosus*), Dahana (*Plumbago zeylanica*), Morata (*Chonemorpha fragrans*), Vilwa (*Aegle marmelos*), Vishanika (*Aristolochia bracteolate*), Brihati (*Solanum anguivi*), Nidigdhika (*Solanum surattense*), Karanja (*Pongamia pinnata*), Putikaranja (*Holoptelea integrifolia*), Jaya (*Premna corymbosa*), Hritaki (*Terminalia chebula*), Bahalapallava (*Moringa concanensis*), Darbha (*Desmostachya bipinnata*), Rujakara (*Semecarpus anacardium*). Many of these traditional medicines need proper scientific validation for development and documentation.

In the current study, anti-lipase and antioxidant activities of *Varanadi kashayam* fractions were evaluated. Antioxidants present in medicinal plants are widely studied nowadays for developing natural antioxidant formulations for food, cosmetics and other applications [25]. There are mainly three groups of phytochemicals present: alkaloids, terpenoids and phenolic compounds [26]. Among them, phenolic compounds including phenolic acids, polyphenols, and flavonoids are reported to have a protective role against oxidative damage. Flavonoids are very effective scavengers of oxidizing molecules and can suppress the formation of reactive oxygen formation [27]. Phenolic compounds are the most abundant source of natural antioxidants and are found on a variety of medicinal plants [28]. Studies have reported that there is a higher correlation between the amount of phenolic content and antioxidant property. Therefore, the natural sources of higher supplements of phenolic content can reduce the risk of various antioxidant related disorders [29].

Phytochemical analysis conducted on extracts revealed the presence of a large variety of phytoconstituents, which have various medicinal properties [30, 31]. The total phenol, flavonoid and phenolic acid content in the fractions of kashayam were estimated and the higher amount of phenol, flavonoid and phenolic acid was found in ethyl acetate fraction. Free radical scavenging activity of fractions was determined by ABTS and DPPH assays. All the fractions exhibited a concentration-dependent antioxidant activity.

In the entire fractions, ethyl acetate fraction showed a higher DPPH radical scavenging activity compared to BHT standard. The remaining fractions dichloromethane, methanol, and water possessed a comparatively better radical scavenging activity. Hexane fraction showed the least antioxidant activity compared to standard. The results of ABTS scavenging activity indicated the higher radical scavenging activity in ethyl acetate fraction. The higher scavenging activity of ethyl acetate fraction might be due to the presence of high amounts of polyphenolic compounds.

One of the most important strategies in the development of anti-obesity drugs involves inhibition of digestion and absorption of dietary lipids since these are the major source of unwanted calories [32, 33]. Pancreatic lipase is the principle lipolytic enzyme secreted by the pancreas, plays an important role in the digestion of dietary triglycerides. It is responsible for the digestion of 50-70% of total dietary fats [33]. Therefore, inhibition of PL suggested as an effective approach for the treatment of obesity and is one of the widely studied mechanisms for the identification of natural products as anti-obesity agents [34]. Orlistat is a potent irreversible inhibitor of pancreatic lipase, was used as positive control in our study, to ensure the reliability of our results. On the basis of results obtained from *in-vitro* pancreatic lipase inhibitory assay, all fraction of *Varanadi kashayam* except water fraction exhibited strong anti-lipase activity with better IC_{50} values compared with orlistat standard.

This suggests that the herbal formulation *Varanadi kashayam* seems to be a potent inhibitor of pancreatic lipase enzyme. The HPTLC analysis was performed for all fractions and the results indicated the presence of many compounds. The antioxidant property of the fractions might be due to the presence of these many compounds.

CONCLUSION: From this study, it is observed that the ethyl acetate fraction of *Varanadi kashayam* contains the highest amount of phenolic compounds and showed better antioxidant activity compared to BHT and quercetin standards. This may be due to the presence of high amount of phenolic compounds. Further, *Varanadi kashayam* fractions could inhibit the activity of pancreatic lipase enzyme and hence we could conclude that *Varanadi kashayam* is a better herbal medicine to reduce lipid disorders and also provide protection against reactive oxygen species.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. Patwardhan B, Vaidya ADB and Chorghade M: Ayurveda and natural products drug discovery. Current Science 2004; 86(6): 789-99.
2. Leitzmann C: Characteristics and health benefits of phytochemicals. Complementary Medicine Research 2016; 23: 69-74.
3. Yarnell E: Synergy in Herbal medicines: Part 1. Journal of Restorative Medicine 2015; 4: 60.
4. Shebis Y, Iluz D, Kinel-Tahan Y, Dubinsky Z and Yehoshua Y: Natural antioxidants: Function and sources. Food and Nutrition Sciences 2013; 4: 643-649.
5. Kasote DM, Katyare SS, Hegde MV and Bae H: Significance of antioxidant potential of plants and its relevance to therapeutic applications. International Journal of Biological Sciences 2015; 11(8): 982-991.
6. Nita M and Andrzej: The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxidative medicine and cellular Longevity 2016; 3164734.
7. Sivanandham V: Free Radicals in health and diseases. Pharmacologyonline 2011; 1: 1062-77.
8. Sharma N: Free radicals, antioxidants and disease. Biology and Medicine 2014; 6(3).
9. Silva MM and Lidon FC: An overview on applications and side effects of antioxidant food additives. Emirates Journal of Food and Agriculture 2016; 28(12):823-832.
10. Murakami Y, Kawata A, Katayama T and Fujisawa S: Anti-inflammatory activity of the artificial antioxidants 2-Tert-butyl-4-methoxyphenol (BHA), 2,6-Di-tert-butyl-4-methylphenol (BHT) and 2,4,6-Tri-tert-butylphenol (TBP), and their various combinations. International Journal of Experimental and Clinical Pathophysiology and Drug Research 2015; 29: 197-206.
11. Anand SP and Sati N: Artificial preservatives and their harmful effects: Looking toward nature for safer alternatives. International Journal of Pharmaceutical Sciences and Research 2013; 4(7): 2496-2501.
12. Mohan MC, Abhimannue AP and Kumar BP: Modulation of proinflammatory cytokines and enzymes by polyherbal formulation Guggulutiktaka ghritam. Journal of Ayurveda and Integrative Medicine 2018.
13. Rahman MJ, Ambigaipalan P and Shahidi F: Biological activities of camelina and sophia seeds phenolics: inhibition of LDL oxidation, dna damage and pancreatic lipase and α-glucosidase activities. Journ 2017; 83: 237-245.
14. Wang SM, Han C, Bahk WM, Lee SJ, Patkar AA, Masand PS and Pae CU: Addressing the side effects of contemporary antidepressant drugs: A comprehensive review. Chonnam Medical Journal 2018; 54: 101-112.
15. Kiortsis DN, Filippatos TD and Elisaf MS: The effects of orlistat on metabolic parameters and other cardiovascular risk factors. Diabetes Metabolism 2005; 31(1): 15-22.
16. Anonymous: The Ayurvedic Pharmacopoeia of India. Government of India, Department of AYUSH, Edition 3, Volume II, 2008.
17. Vidyanath R: Astanga hrdaya of vagbhata. Chaukhamba Surbharati Prakashan, Varanasi 2012.
18. Yadav RNS and Agarwala M: Phytochemical analysis of some medicinal plants. Journal of Phytology 2011; 3(12): 10-4.
19. Kumaran A and Karunakaran RJ: *In-vitro* antioxidant activities of methanol extracts of five *Phyllanthus species* from India. LWT - Food Science and Technology 2007; 40(2): 344-52.
20. Mihai CM, Marghitaş LA, Bobiş O, Dezmirean D and Tămaş M: Estimation of flavonoid content in propolis by two different colorimetric methods. Sci Pap Animal Science and Biotechnologies 2010; 43(1): 407-410.
21. Gawron-Gzella A, Dudek-Makuch M and Matławska I: DPPH radical scavenging activity and phenolic compound content in different leaf extracts from selected blackberry species. Acta Biologica Cracoviensia Series Botanica 2012; 54(2): 32-8.
22. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Hawkins Byrne D: Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 2006; 19(6-7): 669-75.
23. Pai SA, Martis EAF, Joshi SG, Munshi RP and Juvekar AR: Plumbagin exerts antiobesity effects through inhibition of pancreatic lipase and adipocyte differentiation. Phytotherapy Research 2018; 32(8): 1631-1635.
24. Mohan MC, Abhimannue AP and Kumar BP: Identification and Characterization of berberine in *Tinospora cordifolia* by Liquid Chromatography

quadrupole time of Flight Mass Spectrometry (LC MS/MS Q-tof) and evaluation of its anti inflammatory potential. Pharmacognosy Journal 2017; 9(3): 350-355. Available from: http://phcogj.com/article/327

25. Miliauskas G, Venskutonis PR and Van Beek TA: Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 2004; 85(2): 231-7.
26. Tiwari R and Rana CS: Plant secondary metabolites: a review. International Journal of Engineering Research and General Science 2015; 3(5): 661-670.
27. Agati G, Azzarello E, Pollastri S and Tattini M: Flavonoids as antioxidants in plants: Location and functional significance. Plant Science 2012; 196: 67-76.
28. Farhadi K, Esmaeilzadeh F, Hatami M, Forough M and Molaie R: Determination of phenolic compounds content and antioxidant activity in skin, pulp, seed, cane and leaf of five native grape cultivars in West Azerbaijan province, Iran. Food Chemistry 2016; 199: 847-55.
29. Gonçalves S, Moreira E, Grosso C, Andrade PB, Valentão P and Romano A: Phenolic profile, antioxidant activity and enzyme inhibitory activities of extracts from aromatic plants used in Mediterranean diet. Journal of Food Science and Technology 2017; 54(1): 219-227.
30. Wink M: Modes of action of Herbal medicines and plant secondary metabolites. Medicines 2015; 2(3): 251-86.
31. Kabera JN, Semana E, Mussa AR and He X: Plant Secondary metabolites: Biosynthesis, classification, function and pharmacological properties. Journal of Pharmacy and Pharmacology 2014; 2: 377-92.
32. Mukherjee M: Human digestive and metabolic lipases - A brief review. Journal of Molecular Catalysis B: Enzymatic 2003; 22(5-6): 369-76.
33. Glisan SL, Grove KA, Yennawar NH and Lambert JD: Inhibition of pancreatic lipase by balck tea theaflavins: Comparative enzymology and *in-silico* modeling studies. Food Chemistry 2017; 216: 296-300.
34. Lunagariya NA, Patel NK, Jagtap SC and Bhutani KK: Inhibitors of pancreatic lipase: State of the art and clinical perspectives. EXCLI Journal 2014; 13: 897-921.

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