Radical scavenging activity of Gulguluthikthakam Kashayam and Prasarinyadi Kashayam

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Abstract: The healthy state of our body is maintained by equilibrium between the oxidative stress and the antioxidant systems of our body. Oxidative stress occurs due to the imbalance between the productions of reactive oxygen species (ROS) and the availability of the antioxidant or radical scavengers. The excess ROS produced can structurally alter proteins/gene. This in turn will lead to the onset and progression of inflammatory diseases. The inflammation triggered by oxidative stress is the cause of many chronic diseases such as heart diseases, AIDS, Rheumatoid arthritis, neurodegenerative diseases, and cancer and in the aging process. Gulguluthikthakam Kashayam and Prasarinyadi Kashayam are well known Ayurvedic formulations usually prescribed for the treatment of many chronic ailments such as arthritis, diabetes, autoimmune diseases. The present study was to evaluate anti-oxidant activity of methanol extract of Gulguluthikthakam Kashayam and Prasarinyadi Kashayam.

The antioxidant studies were conducted using DPPH assay, hydroxyl radical scavenging assay and Ferric iron reducing antioxidant power assay.

Phytochemical screening reveal the presence of alkaloids, flavanoids ,phenolics compounds and steroids as major constituents and amino acids ,sugars, glycosides and proteins as minor constituents. The FRAP assay reveals the dose dependent increase in absorbance. The IC50 value calculated in the DPPH assay and Hydroxyl radical scavenging assay. The IC50 value found to be lowest in the methanol extract of Gulguluthikthakam Kashayam residue (MGP), 317.333µgmL^{-1} in DPPH assay and 525.196µgmL^{-1} in Hydroxyl radical scavenging assay, which is lower than that of positive control used. Low IC50 value indicates high antioxidant property. Highest anti oxidant property shown by MGP compared to all other extracts.

Key words

Gulguluthikthakam Kashayam, Prasarinyadi Kashayam, anti-oxidant, DPPH, FRAP, Free radicals, hydroxyl radical scavenging, Mannitol, Ayurvedic formulation.

INTRODUCTION

Antioxidants are the substances which are used for the prevention and cure of various diseases which are associated with the oxidation stress caused by free radicals[1]. Free radicals are unstable molecules that are made during normal cell metabolism .They can build up in cells and they cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid per- oxidation, with subsequent tissue injury. Oxidative damage/ stress plays a role in inflammatory reactions in human body and may lead to heart disease, neurodegenerative diseases, AIDS, cancer and also play major role in aging processes [2,3].

Many Ayurvedic formulations and modern medicines are available for the treatment of oxidative stress related diseases. The study conducted by WHO revealed that Ayurvedic formulations are highly effective in some of the diseases, e g; rheumatoid arthritis, caused by oxidative stress where the modern medicines failed. Ayurvedic formulations are mainly composed of herbal combinations [4].Radical scavenging activity of Ayurvedic drugs is associated with the amount of secondary metabolites namely poly phenols, alkaloids and terpinoids present in herbs used for the preparation. Plant phenolics are widely distributed in the plant tissues, and play a vital role as highly effective free radical scavengers, and exhibit antioxidant activity and highly effective as free radical scavengers [5]. Natural antioxidants have attracted much interest because of their ability to scavenge free radicals[6].

Gulguluthikthakam Kashayam and Prasarinyadi Kashayam are polyherbal formulations used in the treatment of inflammatory conditions and related diseases. Gulguluthikthakam Kashayam is an excellent medicine for all types of inflammations especially pertaining to connective tissues, bones and joints. Prasarinyadi Kashayam is mainly used in Ayurvedic treatment of joint pain, musculoskeletal problems. It is traditionally used in treating frozen shoulder and cervical spondylosis and headache.

Gulguluthikthakam Kashayam is prepared, from 29 well known plant following the text "Astanga Hridayam" a compendium of the Ayurvedic System by Vagbhata considered as “Heart or Essence of all the Eight Branches of Ayurveda,” is one of the primary ancient root texts of Ayurveda. The Ashtanga Hridayam

continues to serve as a root source for Ayurvedic philosophy and protocol, providing clear guidelines in all aspects of health.

The plants used are Cuminum cyminum(Fruit) , Cedrus deodara (Wood), Celestrus paniculatus(Seed) ,Scindapsus officinalis (fruit) ,Picrorhiza kurroa (Root) , saussura lappa (Root) Piper nigrum (Fruit),Rubia cordifolia(Stem) Cyprus rotundus(Rhizome) ,Curcuma longa(Rhizome) ,Adhatoda vasica(Root) ,Tinospora cordifolia(Stem) ,Solanum indicum(Root) , Azadirachta indica(Stem **)** , Semicarpus anacardium(Seed) , Commiphora mukul(Resin) ,Plumbago zeylanica(Rhizome) ,Piper brachystachyum (Root), Embelia ribes(Fruit) ,Acorus calamus(Rhizome) ,Tricosanthes dioca(Whole Plant) ,Zingiber officianali(Rhizome) ,Alpinia calcarata(Rhizome) ,Aconitum heterophyllum(Tube root) ,Holarrhena antidysentrica(Seed) ,Anetham graveolens (Fruit), Cyclea peltata(Tube root) ,Trachyspermum roxberghanam(Fruit) ,Piper longum (Root) and Celestrus paniculatus(Seed).

Sahasrayogam, is an important classics in Ayurveda system compiled by Kerala tradition of Ayurveda practitioners .This is a compendium of more than thousand Ayurvedic medicinal preparations involving all Ayurveda systems procedures. A good number of formulations of this book find place in the Ayurvedic formulary of India and considered as one of the authentic book of Ayurveda for all practical purposes Prasarinyadi Kashayam is prepared as per Sahasrayogam using 6 medicinal plants. They are Zingiber officianali (rhizome) , Alpinia calcarata (rhizome), Merremia tridentate(whole plant) ,Allium sativum(bulb), Vigna mungo(seeds) and Sida rhombifolia(root).

In search of natural antioxidants and the popularity and wide use of Gulguluthikthakam Kashayam and Prasarinyadi Kashayam as Ayurvedic medicine in the treatment of different types of inflammatory conditions necessitates the investigation of the antioxidant potential of the two formulations.

EXPERIMENTAL PART

Chemiicals:DPPH purchased from Sima-Adrich Germany.2 -deoxy-D-ribose extra pure, ascorbic acid and mannitol were obtained from Loba chemie Pvt Ltd, ethanol from Heyman group Ltd UK, Methanol from Spectrochem Mumbai India. All other chemicals are of analytical grade were purchased from Merck.

Instruments: Soxhlet apparatus used for the extraction and Elico UV-Vis spectrophotometer used for measuring the absorbance in antioxidant study.

Preparation of Methanol extract: Gulguluthikthakam Kashayam and Prasarinyadi Kashayam are procured from an Ayurveda Institute The Arya Vaidya pharmacy, Coimbatore. The kashayam is a water extract and comes as a suspension. This suspension is administered to the patients and advised them to take 30 ml aliquots two or three times daily. The water dissolved compounds in the suspension will get absorbed to the system easily whereas the undissolved residue may /may not. To ascertain whether the residue also gives some amount of antioxidant activity to facilitate the therapeutic value, the decoction is separated into the soluble portion and the residue and studied separately.

About 1500mL kashayam was centrifuged and the residue obtained dried in oven at 50^0C.The filtrate was lyophilised to get the semi solid dark brown colure substance. Gulguluthikthakam Kashayam residue and the filtrate are weighed 48 and 90 gm respectively and that of Prasarinyadi Kashayam are 29 and 76gm respectively the residue and the lyophilised filtrate were extracted with methanol using Soxhlet extractor. The % yield of methanol extract from Gulguluthikthakam Kashayam residue and filtrate are 57.88 and 5.56 and that of Prasarinyadi Kashayam are 6.84 and 22.10. The methanol extracts were shaken with petroleum ether to remove pet ether soluble fractions (fatty substances). Methanol extract of residue and the filtrate of both the kashayams were analysed for phytochemical contents and anti-oxidant activity using standard procedures.

Phytochemical analysis of methanol extract of Gulguluthikthakam Kashayam and Prasarinyadi Kashayam: Identification of phytochemical constituents is carried out using standard procedure as described by Rahman et al [7] and Shanmugam et al [8]

Test for Carbohydrate

Molisch's Test: 2 ml of the extract taken in test tube and 2 ml of the Molisch's reagent is added and shaken carefully, then about 1 ml. of conc. H_2SO_4 is poured through side of the test tube and allowed to stand for one 1 minutes. A violet colour ring appear at the junction of the two layers indicates the presence of Carbohydrate.

Fehling's test: Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed and few drops of sample is added and Boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present .

Test for Alkaloids

The following Colour tests are used to detect the presence of an Alkaloid in the sample.

(1) Wagner's test: Alkaloids give a reddish brown precipitate with Wagner's reagent [Solution of iodine in potassium iodide].

(2) Hager's test: Alkaloids give yellow colour precipitate with Hager's reagent [saturated solution of Picric acid].

(3) Mayers test: Alkaloids give cream colour precipitate with Mayer's reagent (potassium mercuric iodide solution).

Test for amino acids and proteins

Ninhydrin test: To an aqueous solution of extract, alcoholic solution of Ninhydrin is added and then heated. Formation of blue to Violet colour suggests the presence of amino acids.

Biuret test : The extract mixed with 4% NaOH solution and few drops of 1% CuSO4 solution was added .The solution turn violet in colour.

Test for Tannin

(1)Ferric chloride test: In a test tube mixed the extract and a few drops of ferric chloride reagent and shake well. An intense green or blue color developed indicates the presence of tannin.

(2)Lead acetate test: In to the extract a few drops of 10% lead acetate were added. Precipitate was formed indicate the presence of tannin

Test for Glycoside

Killer – Killiani test: To an extract in Glacial Acetic Acid, a few drops of $FeCl_3$ and conc. H_2SO_4 are added. Formation of reddish brown colour at the junction of two layers and changing of the upper layer into bluish green indicates presence of Glycoside.

Test for Saponins

About 1 ml of extract is diluted to 10 ml by distilled water and shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of froth indicates presence of Saponin .

Test for Phenols

In a test tube 2ml of extract mixed with 2 ml of $FeCl_3$ solution. Blue or deep green colour of the solution is suggestive to presence of Phenols.

Test for Flavonoids

(1)Ferric chloride test: Test solution when treated with few drops of ferric chloride solution would result in the formation of green colour indicate the presence of flavanoids

(2)Alkaline regent test: When the extract treated with sodium hydroxide solution, increase the intensity of yellow colour and decolourise by the addition of dilute hydrochloric acid.

(3)Lead acetate solution test. When the test solution treated with 10% lead acetate solution yellow precipitate formed indicates the presence of flavanoids.

Test for Sterols and Triterpenoids

Salkowskis' test: Take the extract in chloroform with few drops of concentrated sulphuric acid ,shaken well and allow to stand for some time, red colour appears in the lower layer indicate the presence of of sterols and the formation of yellow colour indicate the presence of triterpinoids.

Antioxidant Property of Gulguluthikthakam Kashayam and Prasarinyadi Kashayam:

Ferric ion reducing anti-oxidant power: Ferric reducing property or antioxidant power is determined as per the standard procedure of (Qyaizu M)with slight modification[9].To 1ml of the extract(100-350µg/ mL) 2.5ml phosphate buffer and 2.5ml of 1% potassium ferricyanide are added. Then the mixture heated on a water bath at 50^0C for 20 minutes, then rapidly cooled and mixed with 2.5ml of 2.8% trichloroacetic acid. From this solution 2.5 ml is pipetted out and mixed with 2.5ml distilled water and 0.5ml of 1% ferric chloride .The resulting solution mixed well and kept aside for 10 minutes .Absorbance of resulting solution measured using UV-Vis spectrometer at λ 700nm.Ascorbic acid used as a positive control.

Modified hydroxyl radical scavenging assay: The hydroxyl radical scavenging activity of the methanol extract of Gulguluthikthakam Kashayam and Prasarinyadi Kashayam was determined by the procedure described by Shanmugam et al with slight modification[8] .In a test tube , 1.5mL of phosphate buffer mixed with 1mL of 10mM 2-deoxy-D-ribose,.2.5mL of 20mM Na_2-EDTA ,.2.5mL of 20mM $FeCl_2$ solution,0.1mL of sample solution or standard (100-900µg/mL),1.9mL distilled water and .5mL of 10mM H_2O_2 rapidly in this order. The resulting mixture was incubated for 1Hr at 37^0C in a water bath. After that the reaction arrested by adding 2.5mL of 2.8% tri chloro acetic acid then added 2.5mL of 1% thio barbituricacid and kept the reaction mixture at 100^0C in a boiling water bath for 10 minutes. Cool the mixture under running tape water and recorded the absorbance at 415nm.Mannitol was the positive control used .Hydroxyl radical scavenging activity expressed in percentage. The percent antioxidant or radical scavenging activity was calculated using the following equation

% Hydroxyl radical scavenging activity = [1-(test sample absorbance/blank sample absorbance)] x 100

DPPH scavenging assay: In a test tube 0.250mL of 0.5mM DPPH radical solution mixed with 2mL of the extract or the standard solution and the reaction mixture was vortexes for 10 s and incubated in the dark at room temperature for 30 minutes. The absorbance is recorded at 517nm by UV-Vis spectrometer. Ascorbic acid was used as the standard antioxidant compound[10].The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (no sample).The ability to scavenge the DPPH radical was expressed in percentage. The percentage antioxidant activity was calculated using the equation given

% Antioxidant activity= (A0-A1)/A0 x 100.

Where A0 –absorbance of blank solution and A1-absorbance of sample.

Statistical analysis: The recorded values were expressed as Mean ± SD of three replicate determinations using MS Excel software.

RESULT AND DISCUSSION

Phytochemical analysis of methanol extract of Gulguluthikthakam Kashayam and Prasarinyadi Kashayam: The results of the phytochemical analysis of methanol extracts of kashayams revealed the

presence of different biochemical constituents, such as alkaloids, flavanoids ,phenolics , tannins, steroids, amino acids, carbohydrates, terpinoids ,saponins and glycosides in different concentration. The result is depicted in Table 1. The presence of all these class of compounds are quite natural because Gulguluthikthakam kashayam and Prasarinyadi kashayam are prepared using, the fruit, rhizome, stem ,leaves ,root ,seed and whole plant, of 29 and 6 number of medicinal plants respectively.

Antioxidant Property of Gulguluthikthakam Kashayam and Prasarinyadi Kashayam: All analysis were done in triplicate and these values were then presented as average values along with their standard deviation with the help of MS Excel software**.**

Antioxidants are vital substances that possess the ability to protect the body from the damage caused by free radical induced oxidative stress [11] . Natural antioxidants have benefits over synthetic antioxidants due its side effects [12]. Ayurvedic formulations are widely used for the treatment of various oxidative stress related diseases such as heart diseases, AIDS, neurodegenerative diseases, cancer and in the aging process because of its property to eliminate the root cause of the disease by restoring balance and create a healthy life style to prevent the recurrence of imbalance [12][13] .

The methanol extract of Gulguluthikthakam kashayam and Prasarinyadi kashayam were used for antioxidant study. Antioxidant property of extracts determined by FRAP, DPPH and modified hydroxyl radical scavenging assays.

Ferric ion reducing anti-oxidant power assay: Antioxidant property of extracts was determined using FRAP assay. In this method, the anti-oxidants reduced the $Fe^{3+}$to Fe^{2+} .This ion then conjugated the ferricyanide ion to form a Prussian blue colour product, which is spectrophotometrically measured at λ 700nm. Higher absorbance indicates higher reducing power. Then, reducing power of the compound can be contributed to its antioxidant potency. The reducing power values of the plant extracts tested in this study are illustrated in Figure 1 .All the extracts showed increasing trend in activity with increasing extract concentration. Absorbance values are given in Table 2. Antioxidant study by FRAP assay shows MGP and MPF has higher activity than ascorbic acid (positive control) and MPP and MGF got approximately same activity. It is one of the most rapid tests and very useful for routine analysis

Modified hydroxyl radical scavenging assay: The result of hydroxyl radial scavenging assay were presented, in the Figure (2 and 3) and Table(3 and 4) highest inhibition of hydroxyl radical shown by MGP(525.196 µgmL^{-1}) with the equation y = 0.102x - 3.570 ,R^2 = 0.997 and lowest for MGF (1421.40 µgmL^{-1}) with the equation y=0.032x+4.515, R^2=0.978. Positive control, Mannitol, have IC50 value 481.639 µgmL^{-1} with the equation y=0.0978x+3.281, R^2=0.991.The IC50 value calculated in the case of MPP and MPF are found to be 754.935 µgmL^{-1} with the equation y=0.062x+3.194, R^2=0.986 and 1359.2 µgmL^{-1} with the equation y=0.030x+9.224, R^2=0.951 respectively.

DPPH scavenging assay: DPPH assay method is very simple and is also quick for manual analysis of antioxidant contents. This method is not only specific to any particular antioxidant but also applies to the overall antioxidant capacity of the sample. The DPPH is reacted with absolute ethanol to yield a purple colour DPPH radical. The presence of antioxidants which include polyphenols and flavanoids will scavenge the formed DPPH radical and there by a decreased colour will be observed which is spectrophotometrically measured at 517nm. In this study Figure 4 and Table 5, showed that all samples had significant levels of radical scavenging activity in a dose dependent manner .All samples inhibited DPPH radical formed in different extent. IC50 value also calculated, Figure 5 and Table 6, found to be small for MGP (317.33 µgmL^{-1}) calculated from the equation y=0.075x+26.20, R^2=0.874 and highest for MPF(652.5925 µgmL^{-1}) calculated from the equation y=0.054x+14.76, R^2=0.990 .While that for L-Ascorbic acid was found to be 216.5137 µgmL^{-1}and calculated from the equation y=0.092x+26.4,R^2=0.961.MGF and MPP got intermediate inhibition values as 483.0769 µgmL^{-1} (y=0.052x+24.88,R^2=0.862) and 495.3406 µgmL^{-1} (y=0.091x+4.924, R^2=0.956 respectively Low value of IC50 indicates high anti-oxidant nature.

CONCLUSION

Preliminary phytochemical analysis of methanol extract of both the kashayam answered the most of the phytochemical tests. Antioxidant study of Gulguluthikthakam kashayam and Prasarinyadi kashayam were carried out using methanol extract of its residue and filtrate as per standard procedure with slight modification and found that methanol extract of Gulguluthikthakam kashayam residue has highest antioxidant property than other extracts in all methods (FRAP, hydroxyl radical scavenging and DPPH assay). Finally, the results in this study prove that both the kashayam are perfect sources of antioxidants. The diversity in the results obtained under each method depend on the chemical compounds present in each extract. The isolation and the

identification of each compound will give insight towards the mechanism of the kashayam performed in treating different types of oxidative stress related diseases.

REFERENCES

[1] Lather A. , Gupta V. , Bansal P. , Sahu M. , Sachdeva K. , and Ghaiye P., "An Ayurvedic Polyherbal Formulation Kaishore Guggulu: A Review," *Int. J. Pharm. Biol. Arch.*, vol. 2, no. 1, pp. 497–503, 2011.

[2] Halliwell B. And Gutteridge J.M.C.(1985b) Protection against oxygen radicals in biological systems; the superoxide theory of oxygen oxicity.In:Free Radicals in Biology and Medicine.pp.100-103,Clarendon Press,Oxford.

[3] May A.El-Syed,Amal.A.Al-Gendy,Dalia.I.Hamdan,Assem M El-Shazly Phytoconstituens , LC-ESI- MS profile,Antioxidant and Antimicrobial Activities of Citrus x limon ,L.Burm.f.Cultivar VariegatedPink Lemon.Journal of Pharmaceutical Science and Research pp.375-390.

[4] Syal Kumar MD,Gustav J.Dobos MD and Thomas Rampp MD The significance of Ayurvedic , Medicinal Plants.Journal of Evidence Based Complementary & Alternative Medicine 2017,vol.22(3)494-501

[5] Bhoyar M. S. , Mishra G. P. , Naik P. K. , and Srivastava R. B. , "Estimation of antioxidant activity and total phenolics among natural populations of Caper (Capparis spinosa) leaves collected from cold arid desert of trans-Himalayas," Aust. J. Crop Sci., vol. 5, no. 7, pp. 912– 919, 2011.

[6] Hasbal G., Yilmaz-Ozden T., and Can A. , "Antioxidant and antiacetylcholinesterase activities of Sorbus torminalis (L.) Crantz (wild service tree) fruits," *J. Food Drug Anal.*, vol. 23, no. 1, pp. 57–62, 2015.

[7] Rahman Gul,Syed Umer Jan,SyednFaridullah,Samiullah Sherani,and usrat Iahan. Preliminary Screening, Qualitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts. From Ephedrine intermediate Indigenous to Baluchistan. The Scientific World Journal Vol .2017

[8] Qyaizu M.Studies on product of browning reaction: antioxidative activities of products of browning reaction prepared from glucose amine.Jpn.J.Nutr 1986;44 ,307-315.

[9] Shanmugam S.,Satish Learning Kumar T.,Paneer Selvam K.Laboratory Handbook on Biochemistry.PHI Learning Private Limited New Delhi

[10] Blois MS.Antioxidant determinations by the use of a stable free radical . Nature 1958,181,1199-1200.

[11] Sunitha Dontha A review on antioxidant methods Asian Journal of pharmaceutical and clinical research Vol9 Suppl.2 2016

[12] Humber JM ,The role of complementary and alternative medicine: Accommodating pluralism.J Am Med Assoc.2002;288:1655-6

[13]Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 1999;299:15-27.

Table 1 Preliminary phytochemical profile of methanol extract of kashayam

SI No.	Phytochemical test	Name of the test	MGP	MPF	MPP	MPF
1	Tannins	Lead acetate,$FeCl_3$	+	+	+	+
2	Steroids	Salkowski test	+++	+++	+++	+++
3	Flavanoids &phenolics	Ferric chloride test	+++	+++	+++	+++
		Lead acetate solution test	+++	+++	+++	+++
		Alkaline reagent test	+++	+++	+++	+++
4	Saponins	Frothing test	+	-	-	-
5	Amino acids & Proteins	Ninhydrin test Biuret test	-	+	+	+
6	Alkaloids	Wagner's Hager's &Mayer's test	+++	+++	+++	+++
7	Carbohydrates	Molisch's test	+	+	+	+
8	Cardiac glycosides	Keller killiani test	+	+	-	+
9	Terpinoids	Salkowski test	++	++	++	++

+Present, ++ Moderately present ,+++ Highly present,- Absent

MGP-Methanol extract of Gulgulthikthakam Kashayam residue

MGF- Methanol extract of Gulgulthikthakam Kashayam filtrate

MPP- Methanol extract of Prasrinyadi Kashayam residue

MPF- Methanol extract of Prasrinyadi Kashayam filtrate

Table 2 Antioxidant study by FRAP Assay

Extracts	Concentration (µgmL^{-1})	Absorbance
Ascorbic acid (STD)	100	0.2253±0.0031
	150	0.2567±0.0062
	200	0.2817±0.0043
	250	0.3045±0.002
	300	0.3225±0.0016
	350	0.3425±0.0017
MGP	100	0.157±0.002
	150	0.2145±0.0567
	200	0.264±0.004
	250	0.3356±0.0607
	300	0.4092±0.0593
	350	0.519±0.0076
MGF	100	0.1456±0.0423
	150	0.2052±0.0165
	200	0.2464±0.0126
	250	0.2691±0.0119
	300	0.3189±0.0123
MPP	100	0.1789±0.0136
	150	0.1931±0.007
	200	0.2317±0.007
	250	0.2778±0.0108
	300	0.2969±0.0112
MPF	100	0.1982±0.003
	150	0.2738±0.012
	200	0.325±0.0035
	250	0.3789±0.0196
	300	0.4556±0.0069

Table 3 Antioxidant study by Modified hydroxyl radical scavenging Assay

Extracts	Concentration(µg/mL)	Absorbance (415 nm)	Hydroxyl radicals scavenged (%)
MGP	100	0.4282±0.0003	6.0067±0.0552
	200	0.4082±0.0002	10.4111±0.0335
	300	0.3764±0.0002	17.3763±0.0335
	500	0.3548±0.0002	22.1173±0.0335
	700	0.3337±0.0002	26.7632±0.0456
	900	0.3175±0.0002	30.3189±0.0335
MGF	100	0.4358±0.0003	5.3532±0..0552
	200	0.424±0.0001	6.9285±0.0335
	300	0.3925±0.0005	13.8498±0.1097
	500	0.3773±0.0003	17.1934±0.0552
	700	0.3628±0.0003	20.376±0.0552
	900	0.3423±0.0003	24.8756±0.0552
MPP	100	0.4221±0.002	7.3456±0.4483
	200	0.4172±0.0003	8.4211±0.067
	300	0.3896±0.0007	14.4863±0.1536
	500	0.3787±0.0001	16.8715±0.0335
	700	0.3474±0.0005	23.7489±0.1005
	900	0.3353±0.0003	26.412±0.0552
MPF	100	0.4124±0.0002	9.4746±0.0456
	200	0.4053±0.0004	11.03307±0.077
	300	0.3926±0.0002	13.828±0.0438
	500	0.3793±0.0004	16.7398±0.083
	700	0.3582±0.0004	21.3784±0.0956
	900	0.3393±0.0003	25.5194±0.067
Mannitol (STD)	100	0.3853±0.0003	15.4375±0.0552
	200	0.3582±0.0004	21.3784±0.0956
	300	0.3175±0.0002	30.319±0.0336
	400	0.2616±0.0051	42.5812±1.1098
	500	0.2169±0.0086	52.3924±1.878
	600	0.1696±0.0089	62.7745±1.9500

Table 4 IC50value of extracts in Modified hydroxyl radical scavenging Assay

Sample	IC50value(µg/mL)
STD	481.639
MPF	1359.2
MGP	525.196
MPP	754.935
MGF	1421

Table 5 Antioxidant study by DP PH radical scavenging Assay

Extracts	**Concentration (µg/mL)**	**Absorbance (517 nm)**	**DPPH radicals scavenged (%)**
MGP	100	0.3747±0.0009	26.6588 ± 0.186
	200	0.2882±0.0014	43.5897 ± 0.274
	300	0.2364±0.0011	53.7222 ± 0.2165
	400	0.1962±0.0011	61.6167 ± 0.2055
	500	0.1863±0.0012	63.5349 ± 0.2257
	600	0.1744±0.001	65.8576 ± 0.196
MGF	100	0.3976±0.0009	22.1831 ± 0.1664
	200	0.309±0.0005	39.5185 ± 0.1036
	300	0.2829±0.0002	44.6206 ± 0.0452
	500	0.2255±.0005	55.9535 ± 0.0882
	800	0.1898±.001	62..8498 ± 0.188
MPP	100	0.4578±0.0008	10.3934 ± 0.1494
	200	0.3965±0.0007	22.3918 ± 0.1356
	300	0.3178±0.002	37.7960 ±0 .3625
	400	0.2879±.0011	43.6484 ± 0.2074
	500	0.2466±.0008	51.7518 ± 0.1569
	600	0.2267±0.0002	55.6273 ± 0.0452
MPF	100	0.4179±0.001	18.2031 ± 0.1975
	300	0.3449±0.0006	32.4916 ± 0.1222
	500	0.2877±0.0007	43.6941 ± 0 .1374
	700	0.2364±0.0005	53.7352 ± 0.0883
	800	0.2095±0.0003	58.9939 ± 0.0492
	900	0.1943±0.0003	61.9625 ± 0.0597
Ascorbic acid (STD)	100	0.3559±0.0073	30.3386±1.4248
	200	0.2470±.0104	51.6604±2.0366
	300	0.1873±.0111	63.3392±2.175
	400	0.135±.0058	73.5825±1.1306
	500	0.0882±0.01	82.7363±1.964
	600	0.0554±0.0087	89.1629±1.6943

Table 6 IC 50 value of extracts in DPPH radical scavenging Assay

Sample	IC50value(µg/mL)
STD	216.514
MGP	317.333
MGF	483.077
MPP	495.341
MPF	652.593

Figure 1 Antioxidant study by FRAP Assay

Figure 2 Inhibition (%) of hydroxyl radical by Methanol extract of Gulgulthikthakam Kashayam residue (MGP), Gulgulthikthakam Kashayam filtrate (MGF) , Prasrinyadi Kashayam residue(MPP) , Prasrinyadi Kashayam filtrate(MPF) and Mannitol as STD.

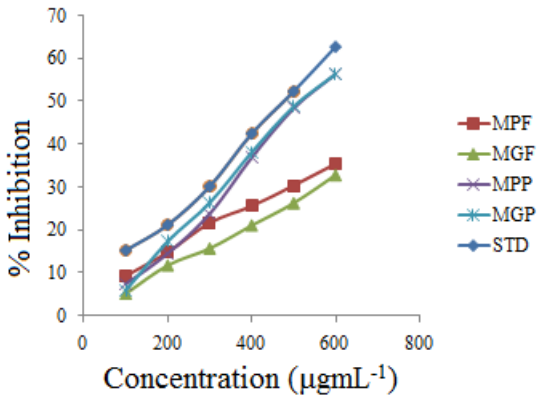


Figure 3 IC 50 value of Methanol extract of Gulgulthikthakam Kashayam residue (MGP), Gulgulthikthakam Kashayam filtrate (MGF) , Prasrinyadi Kashayam residue(MPP) , Prasrinyadi Kashayam filtrate(MPF) and Mannitol as STD in hydroxyl radical scavenging assay.

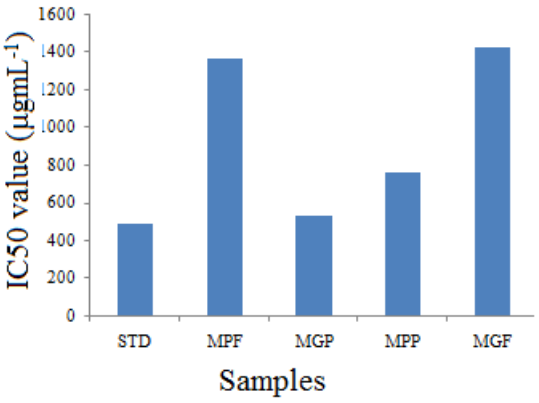


Figure 4 Graph of inhibition (%) of DPPH free radical by Methanol extract of Gulgulthikthakam Kashayam residue (MGP), Gulgulthikthakam Kashayam filtrate (MGF) , Prasrinyadi Kashayam residue(MPP) , Prasrinyadi Kashayam filtrate(MPF) and Ascorbic acid as STD.

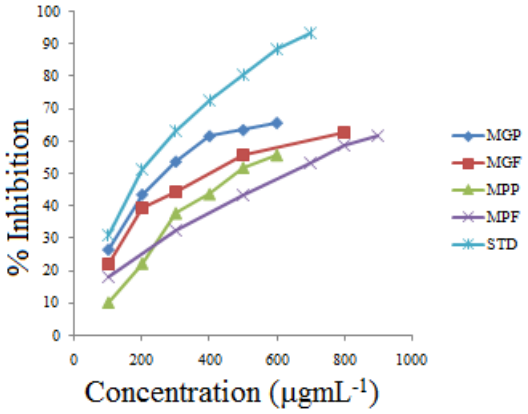
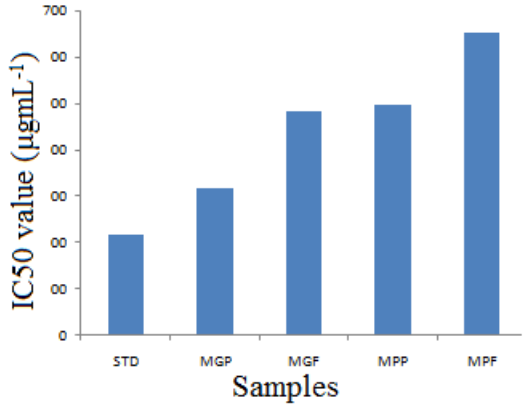


Figure 5 IC 50 value of Methanol extract of Gulgulthikthakam Kashayam residue (MGP), Gulgulthikthakam Kashayam filtrate (MGF) , Prasrinyadi Kashayam residue(MPP) ,Prasrinyadi Kashayam filtrate(MPF)and Ascorbic acid as STD in DPPH assay**.**



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