ISSN No:-2456-2165

Physicochemical, Phytochemical, Mineral Analysis and Invitro Anti-Oxidant Activity of Shatavari Ghrita (Polyherbal Formulation)

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Abstract:- Shatavari Ghrita is one of the formulations mentioned for the use of Anti-oxidant, Lubricant, Immunostimulant and anti-inflammatory and they are useful in CNS disorder, indigestion, diarrhea, loose stools, stomach irritation and colic disorder. The herbal medicines Shatavari Ghrita is containing shatavari (*Asparagus recemosus*)root, milk and ghee. To carry out Shatavari Ghrita to analyze the physicochemical properties, phytochemical analysis, metal and mineral analyisis and its quantification. Various chemical invitro assays have been developed to measure antioxidant properties of plant products. The antioxidant activity of plant extracts was determined by DPPH radical scavenging assay and Hydrogen peroxide radical scavenging assay. In present article shows the efficacy of antioxidant activity on invitro methods and complete review on Shatavari ghrita.

Keywords:- *Shatavarighrita; Phytochemical; Asparagus Recemosus; Physicochemical; Antioxidant.*

I. INTRODUCTION

Ghrita is one of the Ayurvedic formulation that contain ghee to dissolve the active ingredients. Shatavari (Asparagus recemosus) root, cow's ghee and cow's milk in 1:4:16 proportions respectively. There are generally three essentials components required for the preparation of Ghrita. A. fine paste of the drug or drugs-1 part, B. ghrita- 4 parts, C. liquid which may be one or more as kashaya 16 parts. The ingredients are used *Asparagus racemosus* (Thaneervittan kilangu) *Phyllanthus embilica* (Nellikaai saru) Cocus nucifera (Ilaneer) *Curcubita maxima* (Pumpkin) *Saccharum officinarum* (karumbu saaru) Cow milk¬ Cow ghee¬

II. PHYSICO CHEMICAL ANALYSIS

➢ *Specific Gravity*

The empty specific gravity bottle was filled with shatavari ghrita, and the weight was recorded. The process was then repeated using distilled water instead of shatavari ghrita. Shatavari ghrita, weighing around 10 grams, was taken and put into the bottle. A new 10ml of distilled water was added to the bottle. Once installed, the stopper was maintained at a steady temperature. After that, the bottle was outdoors, cleaned, dried, and documented. The bottle's weight and its contents were now ascertained. After emptying and cleaning the bottle with cloth and acetone, distilled water was added, and the bottle was weighed.

➢ *Rancidity*

1 ml of shatavari ghrita, 1 ml of con. Hcl, and 1 ml of phloroglucinol in diethyl ether were added, and the mixture of fat acids was then combined. While the ghrita is unquestionably oxidized and produces a red color, it is only slightly oxidized and produces a pink color.

➢ *Acid Value*

Accurately weigh out 10 grams of shatavari ghrita. Transfer 50 milliliters of an acid-free alcohol ether combination (25 + 25 milliliters) into a conical flask. First, neutralize the mixture by adding 1 milliliter of phenolphthalein solution and 0.1 N potassium hydroxide solution, which is used as a titrant. The finish point is when a light pink color appears. Triglycerides undergo oxidation to produce fatty acids and glycerol, which has an impact on acidity. Fatty acid is released as a result of hydrolysis.As a result, rancidity and acid value change linearly.

➢ *Saponification Value*

Weigh precisely After weighing out about 2 grams of shatavari ghrita in a beakaer, 3 milliliters of the ethanol/ether combination was added. Three quantitative transfers of the beaker's contents were made using an additional 7 milliliters of the solvent. Using a reflux condenser, 25 milliliters of 0.5N alcoholic KOH was added and carefully mixed. With the exception of the sample, there was another reflux condenser acting as the blank. For thirty minutes, both flasks are heated in a bath of boiling water. It is then allowed to cool to room temperature. Add 3 drops of phenolphthalein and 0.5N HCL, which is used as a titrant, to each of the flasks now. Make a note of the test's endpoint and blank. The milliliters of the sample that are contained in the difference between the test and blank readings.

➢ *Iodine Value*

Weigh 10 milliliters of shatavari ghrita and add 10 milliliters of chloroform to an iodination flask labeled TEST. Add 20 milliliters of iodine monochloride reagent to the flask and mix well. The flask is then left to stand for thirty minutes. In the meantime, set up a BLANK in another iodination flask by adding 10 milliliters of chloroform to the

flask and adding 20 milliliters of iodine monochloride reagent, mix well, and let stand for thirty minutes. After 30 minutes of incubation, remove the TEST from the flask and add 10 milliliters of potassium iodide solution to the flask. Using sodium thiosulphate as titrant, the solution in the flask begins to appear pale straw color, and the titration is repeated until the color of the flask turns colorless.

➢ *Peroxide Value*

0.3gm of shatavari ghrita, add chloroform, acetic acid and dissolve by shaking. The it was added with 1ml of saturated potassium iodide solution and stand for 5minutes. Titrate the liberated iodine with 0.01 N sodium thiosulphate solution as an titrant it produce light yellow colour. Add 1ml of starch solution as indicator and titrate till colourless as the end point. Carry out blank test in the same procedure without shatavari ghrita.

➢ *Refractive Index*

50 grams of shatavari ghrita were weighed into an Erlenmeyer flask, 500 milliliters of hot 100% ethanol were added, and the mixture was vigorously shaken to produce a clear solution. After being cooled to 20°C, the flask was kept there for two hours. After the mixture was filtered, the precipitate was once again cleaned with roughly 50 milliliters of pure ethanol. After being mixed and solvent-freed in a steam bath, the filterate and washing were vacuum-dried. At 40 degrees Celsius, the soluble fraction's refractive index was ascertained. The precipitate was dried in a steam bath before being moved to a vacuum oven to finish drying, and at 40°C, the ghrita's refractive index was measured.

III. PHYTOCHEMICAL ANALYSIS

➢ *Test for Alkaloids*

- *Mayer's Test: When Mayer's reagent (potassium mercuric iodide solution) is added to 1 milliliter of sample, a cream-colored precipitate is produced.*
- *Dragendroff's Test: Dragendroff's reagent (potassium bismuth iodide solution) produced a reddish-brown precipitate when 1 milliliter of the sample was treated with it.*
- *Wagner's Test: When Wagner's reagent (iodine solution in potassium iodide) is added to 1 milliliter of material, a brown precipitate is produced.*
- *Hager's Test: Hager's reagent (a saturated solution of picric acid) produced a yellow-colored precipitation when 1 milliliter of the sample was treated with it.*

➢ *Test for Carbohydrate*

- *Molisch's Test: A purple to violet color ring forms at the intersection of the 0.5 ml sample, 0.2 ml of concentrated sulfuric acid, and a few drops of alcoholic alpha napthol that are gradually poured through the test tube's walls.*
- *Benedict' Test: If reducing sugars are present, a reddish-brown precipitate appears after 1 milliliter of the sample is treated with a few drops of Benedict's reagent (an alkaline solution containing cupric citrate complex) and cooked on a water bath.*
- *Fehling's Test: Brick red precipitate was generated after Fehling's solutions A and B were applied to 1 milliliter of the sample and heated for a few minutes.*
- *Barfoed's Test: After treating 1 ml of the sample with Barfoed's reagent and heating it for a few minutes, a crimson precipitate was produced.*

➢ *Test for Glycosides*

- *Legal's Test: A few drops of pyridine were added to 1 milliliter of the sample, and when an alkaline sodium nitroprusside solution was added, the sample turned blood red.*
- *Balijet Test: When two milliliters of sodium picrate are added to one milliliter of sample, a yellow to orange color is created.*
- *Borntrager Test: Two milliliters of diluted sulfuric acid were added to the sample, and after it boiled for a little while, it was filtered. The organic layer was scraped from the filterate and treated with a few drops of ammonia solution to give it a pink or violet color. The filterate was then treated with one milliliter of ether or chloroform.*

➢ *Test for Cardiac Glycosides*

- *Keller killani Test [Test for Deoxy sugars]: 0.4 milliliters of glacial acetic acid with a tiny amount of ferric chloride were added to the sample. It was then moved to a tiny test tube, and the acetic acid layer turned blue as 0.5 milliliter of condensed sulfuric acid was carefully introduced by the test tube's side.*

➢ *Test for Saponin*

- *Foam froth Test: Following treatment with 10 milliliters of water and a brief boil, the sample was filtered. Well-shaken and recognized for its consistent foam was the filtrate.*

➢ *Test for Sterols*

- Salkowski Test: After the sample was treated with 2 milliliters of chloroform and a few drops of concentrated sulfuric acid, it was agitated and left to stand for a while. The production of a yellow-colored lower layer shows the presence of triterpenoids, while the appearance of red color at the bottom layer indicates the presence of steroids.
- Libermann Burchard Test: 1 ml of con. sulfuric acid, 2 ml of chloroform, and a tiny amount of acetic anhydride are used to treat the sample. Red gives way to bluish green in color.

➢ *Test for Phenolic Compounds*

- *Ferric chloride Test: One milliliter of water was added to the sample, and after it boiled for a few minutes, it was filtered. When ferric chloride solution is added to the filterate, a bluish black color is produced.*

➢ *Test for Tannins*

- Gelatin Test: After adding two milliliters of 1% gelatin and ten percent sodium chloride to the sample, a white precipitate was produced.
- Lead acetate Test: When a few drops of lead acetate solution are added to 2 milliliters of sample, the presence of white precipitate indicates the presence of tannins.
- Potassium dichromate Test: After treating the sample with two milliliters of potassium dichromate solution, a yellow precipitate was produced.
- Potassium ferric cyanide Test: Red color forms on the sample when it is treated with 2 milliliters of potassium ferric cyanide solution and a few drops of ammonia solution.

➢ *Test for Flavonoids*

- Shinoda Test (Magnesium Hydrochloride reduction Test): Two milliliters of the sample, a few pieces of magnesium ribbon, and dropwise addition of concentrated hydrochloric acid caused the sample to turn magenta.
- Alkaline reagent Test: After 1 milliliter of sodium hydroxide was added to the sample, a yellow color emerged.
- Mineral acid Test: The sample was treated with 2ml of con.sulphuric acid, orange colour appears.
- Boric acid Test: The sample was treated with 1ml of boric acid solution , yellow colour appears.

➢ *Test for Proteins and Amino acids*

- *Millon's Test: The sample was treated with 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating.*
- *Ninhydrin Test: Few amount of sample was boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate), violet colour appears indicated the presence of Amino acids and proteins.*
- *Biuret Test: After being treated with 1 milliliter of 10% sodium hydroxide and 1 milliliter of 1% copper sulfate solution, the sample took on a violet hue.*
- *Xanthoprotein Test: When two milliliters of concentrated citric acid were added to the sample, it became orange.*
- *Tannic acid Test: After the sample was treated with 1 milliliter of tannic acid solution, it turned white.*

➢ *Test for Fats and Fixed Oils*

- Stain Test: A tiny amount of the sample was sandwiched between two filter sheets; the presence of fixed oils is indicated by a stain on one of the filter papers.
- Saponification Test: After treating the sample for one to two hours with 0.5N alcoholic potassium hydroxide and phenolphthalein, the presence of fixed oils is indicated by the formation of soap or the removal of pink color.

IV. QUANTIFICATION OF PHYTOCHEMICALS

➢ *Quantification of Carbohydrates (Anthrone Method)*

- *Principle*

Carbohydrates are dehydrated with concentrated H2SO4 to form "Furfural", which condenses with anthrone to form a green color complex which can be measured by using colorimetrically at 578nm. Anthrone reacts with dextrins, monosaccharides, disaccharides, polysaccharides, starch, gums, and glycosides. But they yields of color where is to form carbohydrate to carbohydrate.

- *Procedure*

Prepare 75% of sulphuric acid and added 0.05 gm of Anthrone in 1ml of absolute alcohol and make upto 25 ml using 75% H2So4. Weigh 0.05 gm of Glucose and prepare standard from S1 to S8 (5mg/ml onwards) .Take 100 µl of standard and mix with 200 µl of 75% H2So4 . Add 400 µl Anthrone reagent and vortex well . Boil the tubes at 100oC for 15 min. Read OD at 578 nm using microplate reader.

➢ *Quantification of Flavanoid Content*

- *Principle*

Formation of acid stable complexes with aluminum chloride in addition to the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Additionally, aluminum chloride and the ortho-dihydroxyl groups in the flavonoid A- or B-rings combine to produce acid-labile complexes. As standard material, quercetin is utilized to generate the calibration curve. A standard calibration curve was created using different concentrations of the standard quercetin solution.

- *Procedure*

The aluminum chloride colorimetric assay was used to determine the total flavonoid concentration. Test tubes were filled with 1 ml of aliquots, 1 ml of standard quercetin solution (50 mg/ml), 4 ml of distilled water, and 0.3 ml of 5% sodium nitrite solution. 0.3 cc of 10% aluminum chloride was added after 5 minutes. 2 ml of 1 M sodium hydroxide was introduced at the sixth minute. Finally, add distilled water to make up to 10 ml and thoroughly mix. A yellowish-orange hue emerged. Using a UV-visible instrument, the absorbance was measured at 510 nm using a spectrophotometer. Distilled water was used for the blank. The standard was quercetin. Three copies of each sample were used for the test. The standard quercetin was used to plot the calibration curve.

➢ *Quantification of Phenol Content*

- *Principle*

Basis for the Phenolic Quantification Assay is the Folin-Ciocalteu technique. Phosphomolybdic phosphotungstic acid complexes are present in the FC reagent. The technique is based on the transfer of electrons from phenolic compounds to an alkaline media, where the phosphotungstic/ phosphomolybdenum complex forms a blue chromophore. The maximum absorption of this complex is dependent on the concentration of phenolic compounds. A

spectrophotometer can identify the decreased Folin-Ciocalteu reagent between 690 and 750 nm. The reaction temperature (T=37ºC) has been utilized to shorten the time required to get the maximum color. Gallic acid is typically utilized as the reference standard compound, and the results are reported as mg/ml of gallic acid equivalents.

- *Procedure*

Folin Ciocalteu's technique was used to estimate the total phenolic content. Test tubes containing 1 milliliter of aliquots and standard gallic acid (0.007 mg/ml to 1 mg/ml) were filled, and then 5 milliliters of distilled water and 0.5 milliliters of Folin Ciocalteu's reagent were combined and shaken. Five minutes later, 1.5 ml of 20% sodium carbonate was added, and distilled water was added to bring the volume up to 10 ml. It was incubated at room temperature for two hours. A deep shade of blue emerged. Following incubation, absorbance was determined using a UV visible spectrophotometer at 750 nm. Three separate extractions were made. Reagent blank with solvent was used for the blank. The standard was gallic acid. The standard gallic acid was used to plot the calibration curve.

➢ *Quantification of Tannin Content*

- *Procedure*

We used the Folin-Ciocalteu technique to determine the tannin content. A volumetric flask (10 ml) was filled with 7.5 ml of distilled water, 0.5 ml of Folin Ciocalteu phenol reagent, 1 mi of 35% sodium carbonate solution, and approximately 0.1 ml of the sample extract. The flask was then diluted to 10 ml with distilled water. I gave the mixture a good shake and let it sit at room temperature for half an hour. Tannic acid reference standard solutions (0.07 to 1 mg/ml) were made using the same technique as previously mentioned. Using a UV/visible spectrophotometer, the absorbance of the test and standard solutions was measured at 700 nm in relation to the blank. Three separate measurements of the tannin content were made.

➢ *Quantification of Terpenoids Content*

- *Procedure*

1g of powder was soaked in ethanol for 24h The solution was later filtered and the filtrate was extracted with petroleum ether. The ether extract was collected and measured. The value quantitatively gives the amount of terpenoids present in samples.

Total Terpenoids = Initial weight – Final weight / Initial weight × 100

➢ *Quantification of Alkaloids*

- *Procedure*

Along with 0.63g of sample (SG), 200mL of 10% acetic acid in ethanol was added. After being left undisturbed for four hours, the solution was boiled to a quarter of its initial volume. After filtering the mixture, 15 drops of conc were added. The addition of ammonium hydroxide was followed by a three-hour break. After three hours of incubation, the precipitate was collected, and the supernatant was thrown away. Afterwards, 20 mL of 0.1M ammonium hydroxide was used to wash the precipitates, and filtration was done to gather the residue. After that, the residue was weighed, providing a quantitative indication of the alkaloid content of the sample.

➢ *Quantification of Glycosides*

- *Procedure*

The procedure involved transferring 8 milliliters of sample (SG) to a 100 milliliter volumetric flask, adding 60 milliliters of water and 8 milliliters of 12.5% lead acetate, mixing, and filtering. Next, 50 milliliters of the filtrate were transferred to another 100 milliliter flask, where 8 milliliters of 47% disodium hydrogen phosphate were added to precipitate excess pb2+ ion. This mixture was mixed and completed to volume with water, and the mixture was filtered twice through the same filter paper to remove excess lead phosphate. Finally, 10 milliliters of the purified filtrate were transferred into a clean Erlyn-Meyer flask and treated with 10 milliliters of Balijet reagent. This was left to stand for an hour to allow for complete color development. The color intensity was measured colorimetrically at 495 nm.

➢ *Quantification of Saponins*

- *Procedure*

They took 100mL of 20% aqueous ethanol and added 5g of sample (SG). The mixture was stirred for four hours at 55°C in a hot water bath. After filtering, the residue was boiled in a water bath for four hours at 55°C to extract it again using another 100mL of 20% aqueous ethanol. At 90°C, the mixed extract evaporated to 40 mL. Diethyl ether (20 mL) was added, and the mixture was vigorously stirred to concentrate it in a separating funnel. It was done twice, recovering the aqueous layer and discarding the ether layer. After adding 60 mL of n-butanol and extracting twice with 10 mL of 5% NaCl, the NaCl layer was disposed of. After 30 minutes of heating in a water bath, the residual solution was dried in a crucible.

V. ANALYSIS OF HEAVY METALS AND MINERALS

A. Test for Iron

Add 1 milliliter of potassium ferrocyanide solution to 1 milliliter of the sample; the presence of iron is indicated by a strong blue precipitate that is insoluble in diluted hydrochloric acid.

Add 1 milliliter of 2M hydrochloric acid and 1 milliliter of ammonium thiocyanate solution to 3 milliliters of sample; the solution turns blood red, indicating the presence of iron.

➢ *Test for Calcium*

Add 2 milliliters of ammonium oxalate solution to 2 milliliters of sample; the murky look and white precipitate suggest the presence of calcium.

➢ *Test for Potassium*

Two milliliters of sodium nitrite solution and two milliliters of cobalt nitrate in 30% glacial acetic acid were used to treat the sample. Potassium is shown by yellow precipitate.

➢ *Test for Zinc*

After treating the sample with two milliliters of potassium ferrocyanide solution, zinc was found in the form of a white precipitate that was insoluble in diluted hydrochloric acid.

➢ *Test for Magnesium*

A white precipitate that forms after the sample is treated with 1 milliliter of diluted ammonia solution suggests the presence of magnesium.

➢ *Test for Manganese*

When heated over a flame, the sample turns a yellowish green color.

➢ *Test for Copper*

Brick red color indicates the presence of copper in the sample, which was treated with acetic acid and a little amount of potassium ferricyanide solution.

➢ *Test for Cobalt*

After the material was treated with a con. hydrochloric acid solution, cobalt was found because a bluish purple color was formed.

➢ *Test for Selenium*

Following treatment with hydrazine and perchloric acid to yield selenic acid, the sample's red color shows the presence of selenium.

B. Invitro Anti-Oxidant Activity

➢ *DPPH Radical Scavenging Assay*

- *Principle*

The idea behind this assay is that an antioxidant is a hydrogen donor. It gauges substances that scavenge radicals. the process by which hydrogen is taken up by DPPH from an antioxidant. One of the few stable organic nitrogen radicals that is accessible for purchase is DPPH. The rate at which DPPH vanishes in test samples indicates the strength of the antioxidant effect. Due to its precision and ease of use, UV spectrometer monitoring of DPPH has been the most popular approach. At 517 nm (purple), DPPH has a significant absorption maximum. When hydrogen from an antioxidant is absorbed, the color changes from purple to yellow and DPPH is formed. In terms of the quantity of hydrogen atoms absorbed, this reaction is stoichiometric. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.

- *Procedure*

In a nutshell, make a 0.1 mM DPPH solution in methanol and mix 100 µl of it with 300 µl of the sample (SG-ethanol) solution at several concentrations (500, 250, 100, 50, and 10 µg/ml). After giving the combinations a good shake, they must stand at room temperature for half an hour. The absorbance then needs to be determined with a UV-VIS spectrophotometer at 517 nm. You can use ascorbic acid as a reference. Higher free radical scavenging activity is shown by reaction mixture absorbance values that are lower. The capability of scavenging the DPPH radical can be calculated by using the following formula.

DPPH scavenging effect (% inhibition) = [(absorbance of control- absorbance of reaction mixture)/absorbance of control] X 100.

➢ *Hydrogen Peroxide Scavenging Assay*

- *Principle*

Being a mild oxidizing agent, hydrogen peroxide can directly inactivate a small number of enzymes, mainly by the oxidation of crucial (-SH) groups. Rapid cell membrane penetration allows hydrogen peroxide (H2O2) to likely react with Fe2+ and potentially Cu2+ ions inside the cell to create hydroxyl radicals, which may be the source of many of the H2O2's harmful effects. Therefore, it is advantageous for cells biologically to regulate the amount of hydrogen peroxide that is permitted to build up.

- *Procedure*

A 43 mM hydrogen peroxide solution is made in 1 M pH 7.4 phosphate buffer. In a hydrogen peroxide solution (0.6 ml, 43 mM), various concentrations of sample SG (500, 250, 100, 50, and 10 µg/ml) were administered. After ten minutes, the absorbance of hydrogen peroxide at 230 nm was measured in comparison to a blank solution that contained phosphate buffer but no hydrogen peroxide. The standard was ascorbic acid. The free radical scavenging activity was determined by evaluating % inhibition as above. % inhibition = [(Control- Test)/control] ×100.

VI. RESULTS

- *Organoleptic Characters*

The Shatavari ghrita was subjected to various organoleptic tests and the results were determined.

Table 1 Organoleptic Properties of SG

S. No	CHARACTER	OBSERVATION
1.	Colour	Yellow
2.	Odour	Characteristic odour
3.	Texture	Oily texture
4.	Taste	Pungent taste

- *Physicochemical Analysis*

The Shatavari ghrita was evaluated for physicochemical analysis and the results were determined.

Table 2 Physico Chemical Properties of SG

S. No	PROPERTY	VALUE
1.	Rancidity test	Negative
2.	Acid value	6.638
3.	Saponification value	200.96
4.	Iodine value	26.115
5.	Peroxide value	34.7
6.	Specific gravity	0.9845
7.	Refractive index	1.457

- *Phytochemical Analysis*

Table 3 Phytochemical analysis of SG

S. No	Phytochemical	Observation
1.	Alkaloids	Positive
2.	Carbohydrates	Positive
3.	Flavanoids	Positive
4.	Cardiac glycosides	Positive
5.	Saponins	Positive
6.	Sterols	Positive
7.	Phenolic coumpounds	Positive
8.	Tannins	Positive
9.	Proteins and amino acids	Positive
10.	Fats and fixed oils	Positive

- *Mineral and Metal Analysis*

Table 4 Minerals and Metals Present in SG

S. No	Experiment	Observation	Inference
1.	Test for Iron	Red colour	Presence of Iron
2.	Test for Lead	No yellow precipitate	Absence of Lead
3.	Test for Potassium	Yellow precipitate	Presence of Potassium
4.	Test for Sodium	No yellow flame	Absence of sodium
5.	Test for Cobalt	Blue colour	Presence of Cobalt
6.	Test for Selenium	Red colour	Presence of Selenium
7.	Test for Magnesium	White precipitate	Presence of Magnesium
8.	Test for Manganese	Yellow green flame	Presence of Manganese
9.	Test for Copper	Brick red colour	Presence of Copper
10.	Test for Zinc	Blue precipitate	Presence of Zinc
11.	Test for Calcium	White precipitate	Presence of Calcium

➢ *Quantification of Phytochemicals*

Table 5 Quantitative Estimation of SG

S. No	Phytochemical	Amount present
1.	Carbohydrate	2.1 mg/ml
2.	Flavanoid	0.6 mg/g
3.	Phenol content	0.1mg/g.
4.	Tannin content	0.7 mg/ml.
5.	Terpenoids	1.32g/ml
6.	Glycosides	3.22 mg/100g.
7.	Alkaloids	0.29g/ml
8.	Saponins	0.37 mg/ml

➢ *Quantification of Metals*

Table 6 Quantitative Estimation of minerals

Elements	Shatavari ghrita (parts/ million)
As	BDL
Cd	BDL
Cu	8.33
Fe	22.19
K	BDL
Mg	BDL
Mn	0.31
Mo	BDL
Pb	BDL
Zn	16.19
Ca	BDL
Na	BDL
Hg	BDL

➢ *In-Vitro Anti-Oxidant Activity*

- *DPPH Radical Scavenging Assay*

✓ *OD Value at 517 nm*
✓ *Control Mean OD value: 0.98*

Table 7 OD Value of Shatavari Ghrita and Ascorbic Acid

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	0.998	0.974	0.968
2.	500 µg/ml	0.217	0.219	0.217
3.	250 µg/ml	0.316	0.3	0.367
4.	100 µg/ml	0.528	0.521	0.543
5.	50 µg/ml	0.771	0.797	0.767
6.	10 µg/ml	0.83	0.841	0.834
7.	Ascorbic acid	0.118	0.111	0.105

ISSN No:-2456-2165

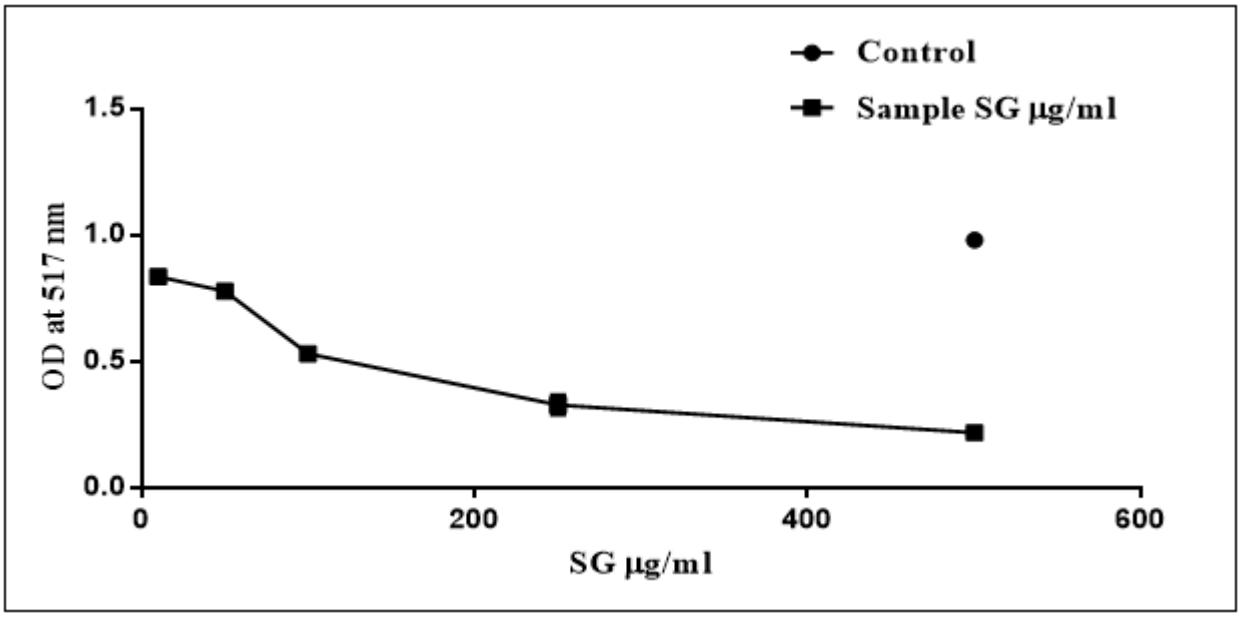

Fig 1 Concentration vs OD value

➢ *Percentage of Inhibition*

Table 8 OD % Inhibition of Shatavari Ghrita and Ascorbic Acid

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Ascorbic acid	87.9592	88.6735	89.2857	88.6395
2.	500 µg/ml	77.8571	77.6531	77.8571	77.7891
3.	250 µg/ml	67.7551	69.3878	62.551	66.5646
4.	100 µg/ml	46.1224	46.8367	44.5918	45.8503
5.	50 µg/ml	21.3265	18.6735	21.7347	20.5782
6.	10 µg/ml	15.3061	14.1837	14.898	14.7959

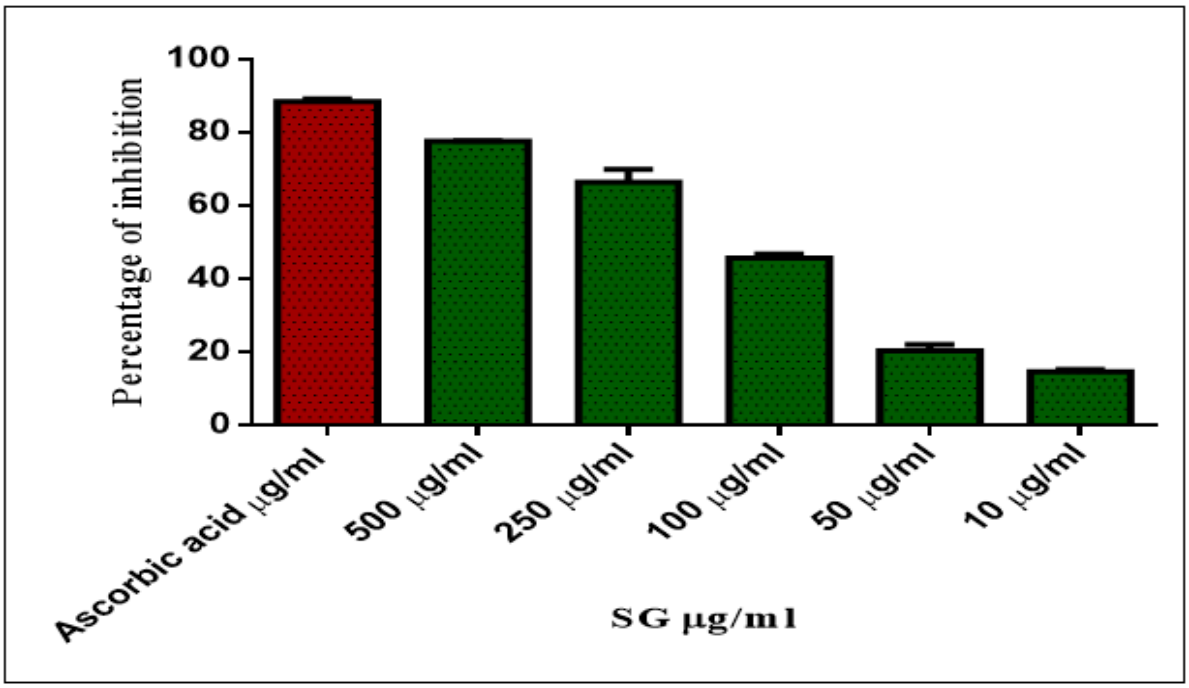

Fig 2 % Inhibition vs Concentration

IC 50 value of standard Ascorbic acid was found to be 99.74 µg/ml and IC50 value of SG sample was found to be 109.1 µg/ml As compared with the standard SG sample has good antioxidant activity.

➢ *Hydrogen Peroxide Radical Scavenging Assay*

- *OD Value at 230 nm*

Table 9 OD value of Shatavari Ghrita and Ascorbic acid

S. No	Tested sample concentration (µg/ml)	OD Value at 230 nm (in triplicates)		
1.	Control	0.745	0.753	0.725
2.	500 µg/ml	0.141	0.136	0.134
3.	250µg/ml	0.208	0.205	0.207
4.	100µg/ml	0.255	0.262	0.261
5.	50µg/ml	0.433	0.436	0.438
6.	10µg/ml	0.516	0.518	0.521
7.	Ascorbic acid	0.121	0.127	0.132

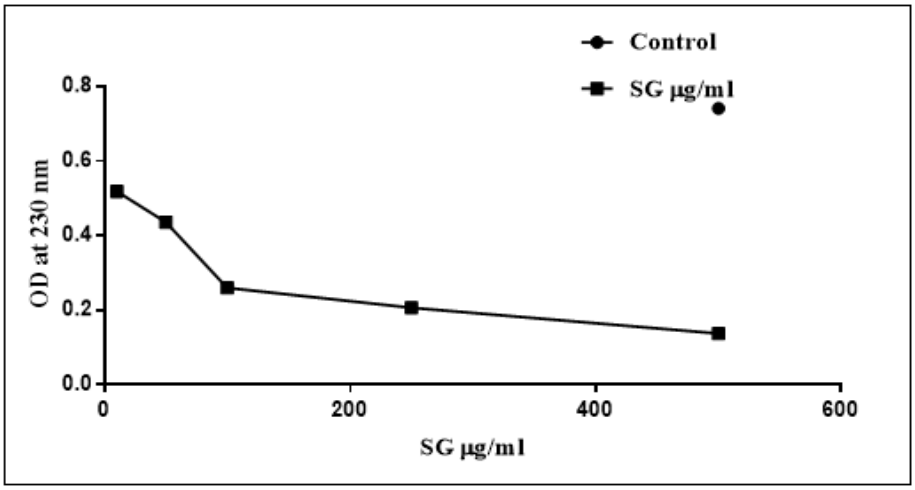

Fig 3 Concentration vs OD value

➢ *Percentage of Inhibition*

Table 10 OD % Inhibition of Shatavari Ghrita and Ascorbic acid

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Ascorbic acid	83.67	82.86	82.18	82.90
2.	500 µg/ml	80.97	81.64	81.91	81.51
3.	250 µg/ml	71.92	72.33	72.06	72.10
4.	100 µg/ml	65.58	64.64	64.77	65.00
5.	50 µg/ml	41.56	41.16	40.89	41.20
6.	10 µg/ml	30.36	30.09	29.68	30.04

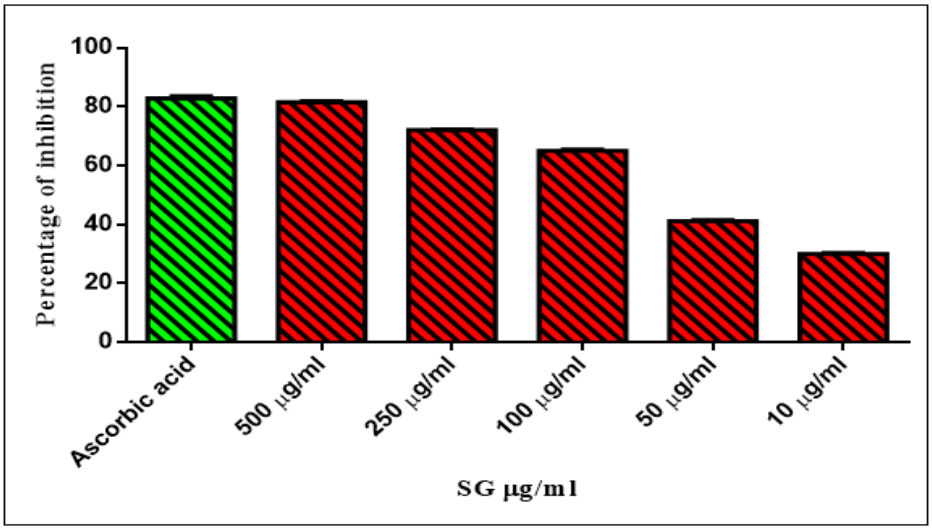

Fig 4 % Inhibition vs Concentration

IC 50 value of standard Ascorbic acid was found to be 72.02 µg/ml and IC50 value of SG sample was found to be 80.96 µg/ml. As compared with the standard SG sample has good anti-oxidant activity.

VII. DISCUSSION

➢ *Classical Characterization*

Shatavari ghrita was compliant with every traditional analysis technique. The mixture was discovered to have a distinct flavor, odor, and yellowish color. It is texturally greasy and it is represented in Table no:1

➢ *Physicochemical Analysis*

The physiochemical analysis of Shatavari ghrita are presented as Table no:2. In addition to structural research, quantitative and qualitative analysis can be conducted using the refractive index measuring method. It is a material's intrinsic quality. Because of this, it's employed to identify and assess the purity of chemicals. The elements that cause light to refract through the ghrita sample are indicated by the increase in refractive index value.

The likelihood that dissolved materials may alter its specific gravity. This facilitates non-invasive access to the molecular information. The shatavari ghrita's specific gravity revealed the active ingredients it contained.

The acid value is a measure of the presence of free acids and the degree of rancidity. Free acid is released as a result of rancidity. The compound's rancidity is caused by the free fatty acids. The greater rancidity in ghrita, the higher the free fatty acid content. The acid number of a fat has an inverse relationship with its edibility.

Higher saponification values of shatavari ghrita result in improved absorption. This method can be used to determine the molecular size. It is inversely correlated with fat's molecular weight. A high saponification value suggests the existence of low molecular weight fatty acids. Short-chain fatty acid-containing fats have a high saponification value.

The degree of fat unsaturation is gauged by the iodine value. The amount of unsaturated fatty acid linkages increases with iodine levels. The iodine value increases and becomes more reactive, less stable, and oxidation-prone when additional iodine is connected.The value of iodine increases the susceptibility to rancidity.

The degree of oxidation and the duration of shtavari ghrita's shelf life are both determined by rancidity.

A measurement of the peroxides in the Shatavari ghrita is called the peroxide value. The percentage of oxidation of shatavari ghrita is indicated by the peroxide value. It aids in determining the sample's stability. A higher peroxide number indicates greater oxidation and increases the likelihood of rancidity.

➢ *Phytochemical Analysis*

Bioactive compounds (Table no:3) such as alkaloids, carbohydrates, glycosides, saponins, sterols phenolic compounds, tannins, flavonoids, proteins and amino acids, fats and fixed oils are present in the Shatavari ghrita and also quantify it.

➢ *Heavy Metal and Mineral Analysis*

From the metal analysis, it was revealed that Shatavari ghrita contains iron, calcium, potassium, zinc, magnesium, manganese, copper, cobalt and selenium and quantification of metals presented in Table no: 4

➢ *Anti-Oxidant Activity*

Based on the findings of the DPPH free radical scavenging assay using Shatavari ghrita and standard ascorbic acid, as well as the in vitro antioxidant activity, these results are shown in Table No. 22 and Figure 36. Standard ascorbic acid (IC50 value: 72.02 µg/ml) and Shatavari ghrita (IC50 value: 109.1 µg/ml) both demonstrated strong antioxidant activity. At 500 µg/ml, the maximum percentage inhibition (77.78 ± 0.60 %) was observed. Table No. 24 and Figure 39 provide the findings of the H2O2 scavenging assay employing Shatavari ghrita and ascorbic acid as standards. The concentration of 500 µg/ml yielded the maximum percentage inhibition of 82.90 ± 0.26 %, while the IC50 values of conventional Ascorbic acid (72.02 µg/ml) and Shatavari ghrita (80.96 µg/ml) demonstrated their strong antioxidant activity.

VIII. SUMMARY AND CONCLUSION

Ayurvedic preparation that uses ghee as a foundation to dissolve the active components is ghrita. Shatavari ghrita (SG) is a herbal remedy that has 1:4:16 ratios of cow's milk, cow ghee, and ash from Shatavari (Asparagus recemosus) root.

This study found that Shatavari ghrita complied with all of the classical features of ghrita (i.e., color, aroma, taste, and appearance) based on a preliminary classical organoleptic analysis

Shatavari ghrita was found to be alkaline in nature and to possess traditional qualities including rancidity, specific gravity, acid value, saponification value, iodine value, and peroxide value, according to the study's preliminary physicochemical examination.

The phytochemical analysis of Shatavari ghrita indicated the presence of bioactive organic compounds such as carbohydrates, phenols, terpenoids, alkaloids, sterols, saponins, glycosides and the chemical analysis revealed that it contains zinc, iron, potassium, sodium, cobalt, magnesium, manganese and copper.

The DPPH free radical scavenging assay and the hydrogen peroxide radical scavenging assay were used to assess the in vitro antioxidant activity of Shatavari ghrita. The findings indicated that Shatavari ghrita possessed strong antioxidant properties.

IX. ACKNOWLEDGMENT

I acknowledge my sincere thanks to Adhiparasakthi College of pharmacy, Melmaruvathur, Tamilnadu and our beloved Principal for his support in carrying out my research work in a well-disciplined manner.

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