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STANDARDIZATION AND PHARMACOLOGICAL ACTIVITIES OF MAHARASNADI KASHAYAM (MRK)

Anjali Nair G.*[1], Anjali Murali[1], Devika Suresh[1], Farsena Tabassum[1], Mariya Paulson[1] and Shahin Mohammed K.V.[1]

[1]ELIMS College of Pharmacy, Thrissur.

***Corresponding Author: Anjali Nair G.**
ELIMS College of Pharmacy, Thrissur.

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ABSTRACT

Aim: The present study was to standardize and evaluate antibacterial, antioxidant and *invitro* antiarthritic activity of Maharasnadi Kashayam (MRK). **Methodology:** The formulation was physicochemically and microbiologically standardized. Standardization parameters like organoleptic characters, physicochemical analysis, phytochemical analysis and sterility test were carried out as per Ayush guidelines. Antibacterial studies were carried out by using agar well diffusion method. *In vitro* antioxidant study such as DPPH assay was performed and was compared with standard ascorbic acid. *Invitro* antiarthritic activity of MRK was performed by using egg albumin protein denaturation method. **Results and discussion:** The physicochemical parameters was performed and the result were found to be in permissible limits. Antibacterial studies showed significant zone of inhibition against *Bacillus subtilis, Pseudomonas fluorescens, Staphylococcus aureus* and *Lactic acid bacillus*. Among the bacterial strains, *P. flurescens* shows highest zone of inhibition. Maharasnadi kashayam (MRK) showed comparable antioxidant potential by DPPH assay method when compare to standard ascorbic acid and IC_{50} value found to be as 13.11and 22.81µg/ml for ascorbic acid and Maharasnadi kashayam (MRK) respectively. The activity of MRK was compared with standard Diclofenac sodium. MRK showed considerable inhibition of protein denaturation compared to that of standard. **Conclusion:** The outcome of this study clearly proves the quality, purity, safety and potency of the drug which will help the medicine to survive and succeed in future researches on both clinically and economically.

KEYWORDS: Antioxidant, Antiarthritic activity, Standardization, Maharasnadi Kashayam, Ascorbic acid, Diclofenac sodium.

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Standardization of herbal drugs comprises of total information and controls to guarantee consistent composition of all herbals including analytical operations for identification, marker based estimation and assay of active principles.[1] Rheumatoid arthritis is a progressive autoimmune disorder characterized by chronic inflammation and hypertrophy of synovial membranes that ultimately leads to destruction of the bones, joints and articular cartilages. The pathology of rheumatoid arthritis is very complex and the reason underlying the mechanism also remains unknown. The reactive oxygen species (ROS) such as hydroxyl, hydrogen peroxide, superoxide, nitric oxide radical are continuously being produced during regular physiological processes. These reactive radicals may cause cellular injuries, damage bio-molecules such as carbohydrates, nucleic acids, proteins, poly unsaturated fatty acids and lipids, eventually resulting in cancer, cardiovascular diseases, and diabetes.[2] Maharasnadi kashayam is a popular yoga from Sahasrayoga mentioned in the context of vatavyadhis – disease or morbidity arising from the derangement of vata like arthritis, inflammatory auto-immune disorders, bone degeneration and loss of function..'Kashaya' or herbal decoctions harness the healing properties of herbs and roots in a mild and easily absorbable water base.[3] Hence the present work was focused to standardize and evaluate the antibacterial, antioxidant and *in vitro* anti arthritic activity of Maharasnadi Kashayam.

MATERIALS AND METHODS

Procurement of Maharasnadi Kashayam (MRK)

Maharasnadi kashayam (Batch no. 21KKS1976; Mfg date: 11/2021; Exp date: 10/2024) of Sitaram Ayurveda was purchased from Sitaram Ayurveda pharmacy limited, Thrissur, Kerala. The solvents and chemicals used in the research work were of analytical grade.

Standardization of Maharasnadi Kashayam (MRK)[4],[5]

Organoleptic properties

Organoleptic characters like colour, odor, taste and consistency of the Maharasnadikashayam were evaluated.

pH

The pH was determined using a caliberated pH meter. About 25 ml of test sample filtered and the resultant solution is subjected to pH evaluation.

Specific gravity and density

The specific gravity of 10 ml MRK formulation was determined using 10 ml specific gravity bottles and calculated using the following formula

$$\text{Sp. gravity of MRK} = \frac{\text{Weight of 10 ml of MR/ 10}}{\text{Weight of 10 ml of water/ 10}}$$

The density of MRK is calculated using the following formula

Density of MRK = Specific gravity of the MRK X Density of the distilled water

Total solid content

This was determined by taking 10 ml of the formulation in porcelain evaporating dish and heating it on an electric water bath at 60 – 70˚C and then in an oven at 105˚C until constant weight of residue was obtained.

Total bacterial count

This was determined by taking mixture of 1 ml of MR and about 15 ml of Muller Hinton agar medium were added to a petri dish at a temperature not exceeding 45ºC. Plates were prepared using the same dilution and were incubated at 30-35ºC for 24 hrs.

Total fungal count

1 ml of MR is mixed with 15 ml of liquefied Sabouraud dextrose agar medium at temperature not exceeding 45ºC to a petri dish. Plate was prepared using the same dilution and was incubated at 20-25ºC for 24 hrs.

Phytochemical screening[6]

The MRK was subjected to standard phytochemical screening tests for establishing different constituents present in it.

Test for alkaloid

A small amount of MRK was mixed with few ml of dilute Hydrochloric acid. Shaken well and filtered.

- **Mayer's test:** A few drops of Mayer's reagent were added to 2-3 ml of filtrate. Cream (dull white) precipitate indicates the presence of alkaloids.
- **Dragendorff's test:** A few drops of Dragendorff's reagent were added to 2-3 ml of filtrate. Orange red precipitate indicates the presence of alkaloids.
- **Hager's test:** A few drops of Hager's reagent were added to 2-3 ml of filtrate. Yellow precipitate indicates the presence of alkaloids.
- **Wagner's test:** A few drops of Wagner's reagent were added to 2-3 ml of filtrate. Reddish brown precipitate indicates the presence of alkaloids.

Test for carbohydrates

- **Molisch's test:** Few drops of Molisch's reagent were added to 2-3 ml of filtrate, followed by addition of concentrated Sulphuric acid along the sides of the test tube. Formation of violet colour at the junction of two liquids indicates the presence of carbohydrates.
- **Benedict's test:** Few ml of filtrate was mixed with equal volume of Benedict's reagent and heated in boiling water bath for 5 minutes. Formation of reddish brown precipitate infers the presence of reducing sugars.
- **Fehling's test:** 1 ml Fehling's-A was added to 1 ml of Fehling's-B solution, boiled for one minute. To this added 1 ml of filtrate and heated gently. Formation of brick red precipitate indicates the presence of reducing sugars.

Test for steroids and sterols

- **Salkowski reaction:** A small quantity of the MRK was mixed with 2 ml Chloroform and 2 ml concentrated Sulphuric acid. Shake it well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
- **Liebermann- Burchard reaction:**A small quantity of the MR was mixed with Chloroform. To that mixture added 1-2 ml of acetic anhydride and 2 drops of concentrated Sulphuric acid along the sides of the test tube. The solution becomes red, then blue and finally bluish green color.

Test for saponins

- **Froth test:** 5 ml of test sample was added to Sodium bicarbonate solution. After vigorous shaking the mixture, kept it for 3 minutes. A honey comb like froth formation indicates the presence of saponins.
- **Foam Test:** A small quantity of the extract was diluted with 20 ml of distilled water and shaken it in a graduated cylinder for 3 minutes. Foam of 1cm after 10 minutes indicates the presence of saponins.

Test for glycosides

- **Keller-Killiani test:** Glacial acetic acid was added to 2 ml extract, followed by the addition of trace quantity of Ferric chloride and 2 to 3 drops of concentrated Sulphuric acid. Reddish brown color appears at the junction of two liquid indicates the presence of cardiac glycosides.
- **Legal's test:** 1 ml of Pyridine and 1 ml of Sodium nitroprusside was added to a small quantity of the extract. Pink to red color indicates the presence of glycosides.
- **Baljet test:** A small quantity of the extract was added to Sodium picrate solution. Yellow to orange color formation indicates the presence of glycosides.

Test for flavonoids

- **Alkaline reagent test:** A few drop of Sodium hydroxide solution was supplemented to the extract. Development of an intense yellow color, which turns to colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Test for proteins and aminoacids

- **Ninhydrin test:** A mixture of 3 ml test solution and 3 drops of 5% Ninhydrin solution was heated in a boiling water bath for 10 minutes. Formation of purple or bluish color indicates the presence of free amino acids.
- **Biuret test:** 3 ml of test solution was added to 4% Sodium hydroxide and few drops of 1% Copper sulphate solution. Formation of violet color indicates the presence of proteins.

Test for tannins

- **Lead acetate test:** A few drop of Lead acetate was added to 5 ml of aqueous extract. Formation of yellow or red color precipitate indicates the presence of tannins.

Estimation of total phenolic content[7]

Total phenolic assay was conducted by mixing 2.7ml of de-ionized water 0.3ml of sample (MRK) and standard (gallic acid), 0.3ml of 7% of Na_2CO_3 and 0.15ml Folin-ciocalteu reagent.Absorbance of mixture was measured at 725nm.The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in sample was expressed in terms of gallic acid equivalent (mg of GA/g of sample).

Estimation of total flavanoid content[8]

Total flavonoid content was estimated using the method of Barros *et al*. 400 µl of MRK was taken in a test tube and to this added 1600µl of deionized water and 120 µl of sodium nitrite solution (5% w/v). After that, the mixture was incubated for 6 min at room temperature. After incubation, 120 µl of aluminium nitrate solution (10% w/v) was added and allowed to stand for another 6 min. Then added 800µl of NaOH solution (4% w/v) and made up the volume to 960µl with deionized water. The reaction mixture was kept in dark at room temperature for 15 min. The intensity of the yellow colour developed indicated the concentration of the flavonoid content in the MRK. The intensity of the developed pink colour was measured at 510 nm using a spectrophotometer. Total flavonoid content was calculated with the help of a standard curve with quercetin and the flavonoid content was expressed as mg quercetin equivalents (QE)/g.

Antibacterial studies of MRK[9]

Microorganisms

The selected microorganisms for this study were obtained from American Type Culture Collection (ATCC) from Ron Labs, Ernakulam. The bacterial strains *Bacillus subtilis* (ATCC 19659), *Pseudomonas fluorescens* (ATCC 13525), *Staphylococcus aureus* (ATCC 33592) and *Lactic acid bacillus* (ATCC 15578) were used in this study. MRK was tested for anti-bacterial activity in the well diffusion method by using standard procedure. *Bacillus subtilis*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Lactic acid bacillus*. All the stock cultures were obtained from ATCC lab. The microorganisms were grown overnight at 37˚C in nutrient broth (pH 7.4).

Well diffusion method

The well diffusion method was used to screen the antimicrobial activity. The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min, inoculum suspension was spread uniformly by using sterile swab, and the inoculums were allowed to dry for 5 min. The concentration of MRK used is 100µg/ml. From this, 50, 100, 150 and 200 µl of the extracts containing 5, 10,15 and 20 µg, respectively of MRK were loaded on 6 mm well, and then allowed to diffuse for 5 min, and the plates were kept for incubation at 37^{o}c for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured in millimetres.

Determination of minimum inhibitory concentrations (MIC)

MIC is defined as the lowest concentration of the antimicrobialagent that inhibits the microbial growth after 24 h. of incubation. Among the bacterial strains, the one which shows highest zone of inhibition was selected and the MIC value was determined. Different concentrations of MRK (1, 2, 3, 4and 5µg/ml)were loaded on 6 mm well, and then allowed to diffuse for 5 min, and the plates were kept for incubation at 37^{o}c for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured in millimetres. The concentration which shows less than 8 mm of zone of inhibition was noted as MIC values. The experiment was peformed in triplicates.

Determination of antioxidant activity[10]

The antioxidant activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical. 2.5 ml solution of DPPH in methanol was added to 1.5ml of various concentrations (10, 20, 30, 40, 50 µg/ml) of test (MRK) and the standard (Ascorbic acid), mixed and left to stand in the dark at room temperature for 30 min and then absorbance was measured at 517 nm against a blank. A control reaction was carried out without the test sample. All the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the

control and test samples. Antiradical activity was expressed as percentage inhibition (I %) and calculated using the following equation.

$$\textbf{Percentage inhibition (I \%)} = \frac{\textbf{Abs}_{\textbf{control}} - \textbf{Abs}_{\textbf{sample}}}{\textbf{Abs}_{\textbf{control}}}$$

Where '$Abs_{control}$' was the absorbance of the control reaction and 'Abs_{sample}' was the absorbance in the presence of the test/ standard. The antioxidant activity of the extract was expressed as $IC_{50.}$ (IC_{50} - concentration required to obtain a 50% radical scavenging activity).

***Invitro* anti arthritic activity**

Egg albumin protein denaturation method[11]

The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin (from hens egg), 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration (1.25, 2.5, 5, 12.5, 25 µg/ml) of extracts. Similar volume of double distilled water served a control. Then the mixture was incubated at 37^{O} C in BOD incubator for about 15 mins and then heated at 70^{o} C for 5 mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (DFS) was used as reference drug and treated as such for determination of absorbance.

$$\text{Percentage inhibition} = \frac{(\text{Absorbance(control)} - \text{Absorbance(sample)})}{\text{Absorbance(control)}} \times 100$$

RESULTS AND DISCUSSION

Standardization of Maharasnadi Kashayam (MRK)

Values are expressed in mean±SD

Table 1: Physicochemical parameters of MRK.

Sl.No.	Parameters	Observation
1	Organoleptic properties	Brown, muddy coloured liquid, bitter in taste
2	pH	4.903±0.030
3	Specific gravity	1.071±0.009
4	Density	1.068±0.009 g/ml
5	Total solid content	32.06 ±0.901 % w/v

Table 2: Microbial contamination of MRK.

Total microbial count	Colonies obtained
Bacteria /NMT 300 CFU	8 CFU
Fungi /NMT 100 CFU	3 CFU

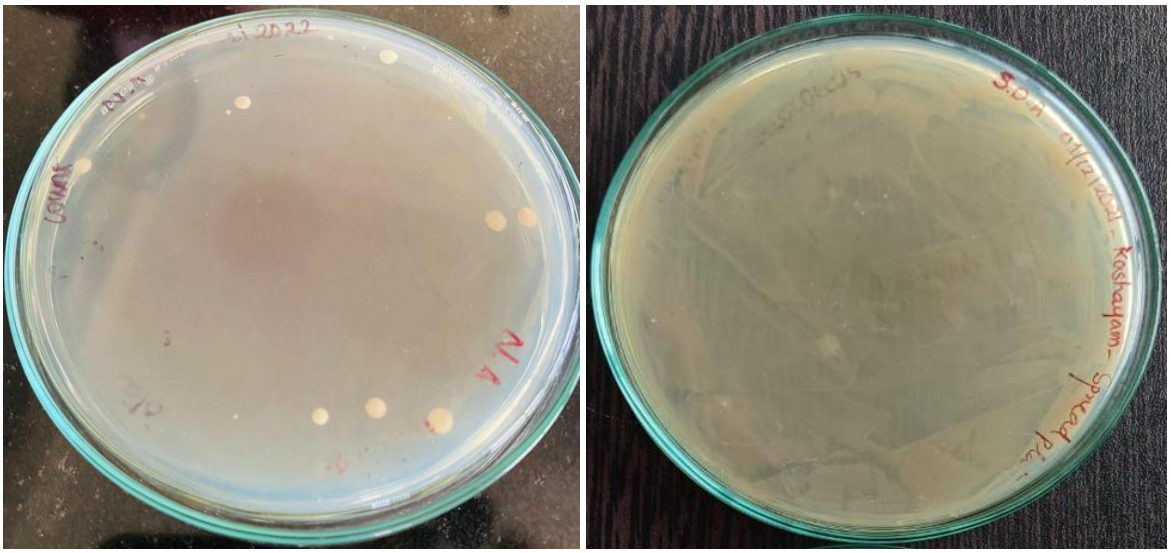

Fig. 1: Photodocumentation of petriplates of total bacterial count and total fungal count.

The physicochemical parameters like organoleptic properties, pH, specific gravity, density, total solid content was performed and the result were found to be in permissible limits as given in **Table 1**. Total bacterial count and total fungal count were performed. Bacteria and fungi were within permissible WHO limits. This formulation was found to comply with the specification limit for total bacterial count i.e. NMT 300 CFU/ml and total fungal count i.e. NMT 100 CFU/ml as given in the **Table 2.**

Phytochemical screening

Table 3: Phytochemical screening in MRK.

Sl.No	Phytochemical constituents	Test done for identification	Result
1	Alkaloids	Mayer's test	+
		Wagner's test	
		Dragandroff's test	
		Hager's test	
2	Carbohydrates	Molisch's test	+
		Benedicts test	
		Felhing's test	
3	Steroids & Sterols	Salkowaski test	-
		Libermannbutchards test	
4	Saponins	Froth test	+
		Foam test	
5	Glycosides	Killer kalyani test	+
		Legal test	
		Baljet test	
6	Flavanoids	Alkaline test	+
7	Proteins &Aminoacids	Ninhydrin test	-
		Biuret test	
8	Tannins	Lead acetate test	+

Phenolic compounds are very important plant constituents because their hydroxyl groups confer scavenging ability. Phenolics present in leaves have received considerable attention because of their potential antioxidant activities. Higher phenolic contents such as flavonoids, polyphenols and mono-phenols are some features of medicinal plants.[7] The results of phytochemical screening was showed in **Table 3**.

Estimation of Total Phenolic content

Table 4: Estimation of total phenolic content.

Sample	Concentration (µg/ml)	Absorbance
Standard. (gallic acid)	10	0.103±0.011
	20	0.233±0.089
	30	0.429± 0.079
	40	0.585 ±0.161
	50	0.899± 0.041
MRK	30	0.119 ± 0.019

Values are expressed in mean±SD

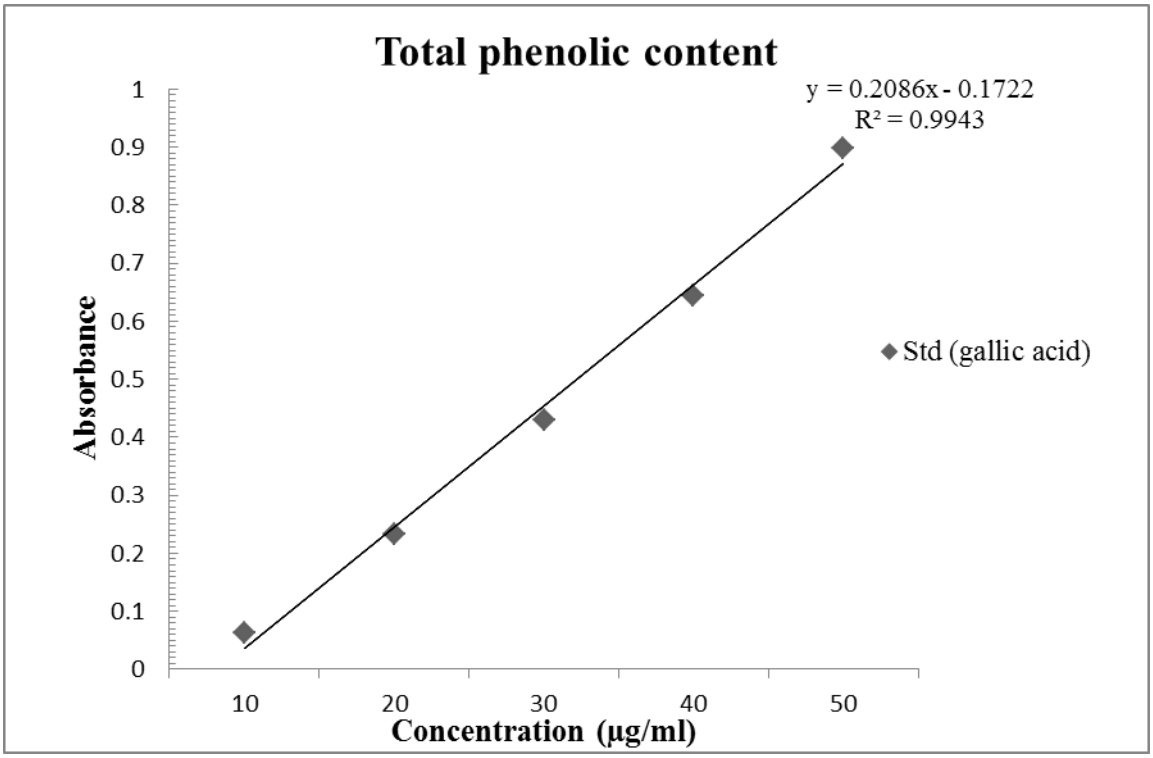

Fig. 2: Standard graph for gallic acid for the estimation total phenolic content.

Antioxidants, derived from plant origin, especially flavonoids and polyphenols have been used to treat various disease such as aging, diabetic, cancer and prevention of cardiovascular diseases.[8] The Folin-Ciocalteu method is a routine assay in studying phenolic antioxidants as it is rapid, convenient, simple and reproducible. A calibration curve was prepared as shown in **Figure 2** The total phenolic content of Maharasnadi kashayam was found to be 13.99µg of GAE/ mg of sample.

Total Flavanoid content

Table 5: Estimation of Total flavanoid content.

Sample	Concentration (µg/ml)	Absorbance
Standard. (quercetin)	7.5	0.265±0.008
	15	0.319±0.004
	22.5	0.475±0.003
	30	0.537±0.0005
	37.5	0.689±0.0005
MRK	40	0.417±0.0135

Values are expressed in mean±SD

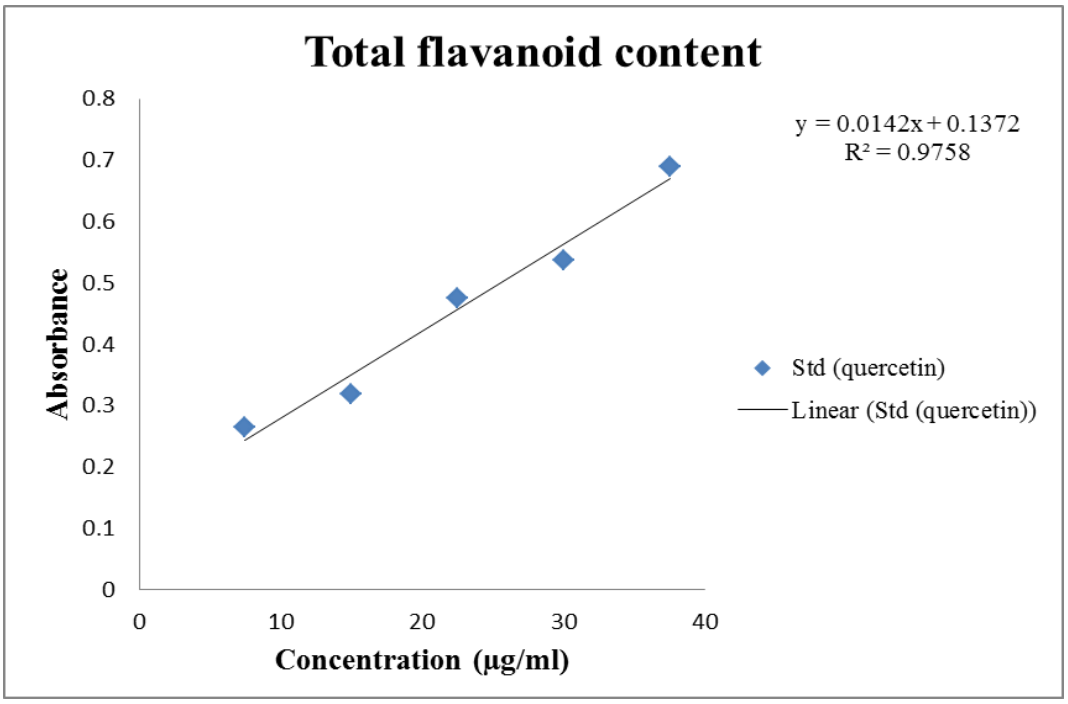

Fig. 3: Standard graph of quercetin for the estimation total flavonoid content.

The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity.[9] A calibration curve was prepared using quercetin as standard as shown in

Figure 3. Total flavonoids content (TFC) is one of the most important quality indexes of Maharasnadikashayam, and it is concerned with total antioxidant activity. The total flavanoid content of Maharasnadi kashayam was found to be 197.18µg of QE/ mg of sample.

Anti bacterial studies of MRK

Table 6: Zone of inhibition in diameter on human pathogens on agar well diffusion method.

Sl no.	Concentration of MRK	Zone of inhibition (mm)			
		S.aureus (gram +ve)	*B.subtilus* (gram +ve)	*L.bacillus* (gram +ve)	*P.florescence* (gram -ve)
1	5	11	11	11	13
2	10	14	14	16	15
3	15	17	16	18	19
4	20	19	16	19	22

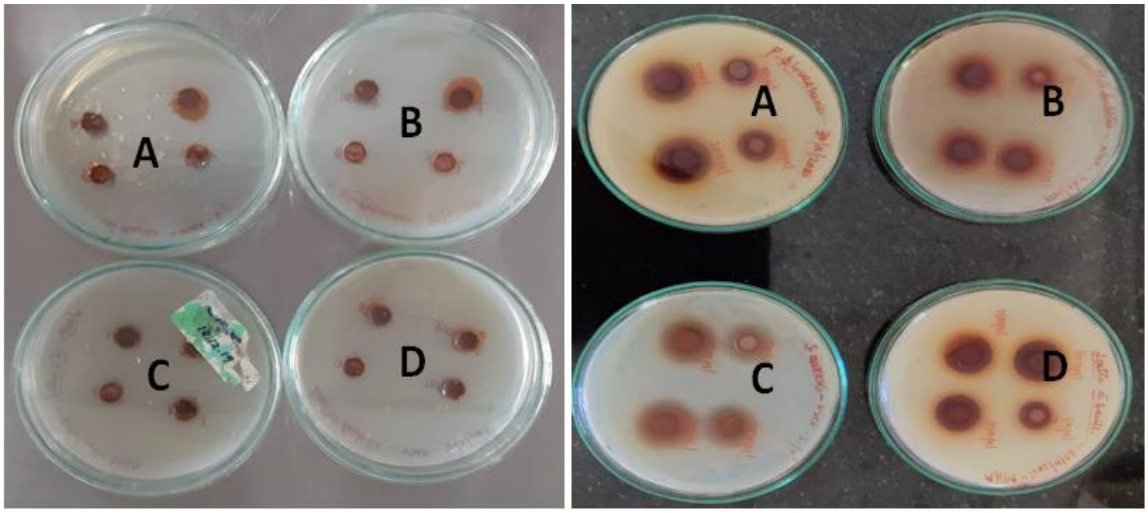

Fig.4A: Images showing petriplates inoculated with various bacterial strains before incubation(A- *P. flurescence*; B-*B.subtilus*; C-*S. aureus*, D-*L. bacillus*).
Fig 4B: Images showing the zone of inhibition in petri plates after incubation of 24 hrs (A- *P. flurescence*; B-*B.subtilus*; C-*S. aureus*, D-*L. bacillus*).

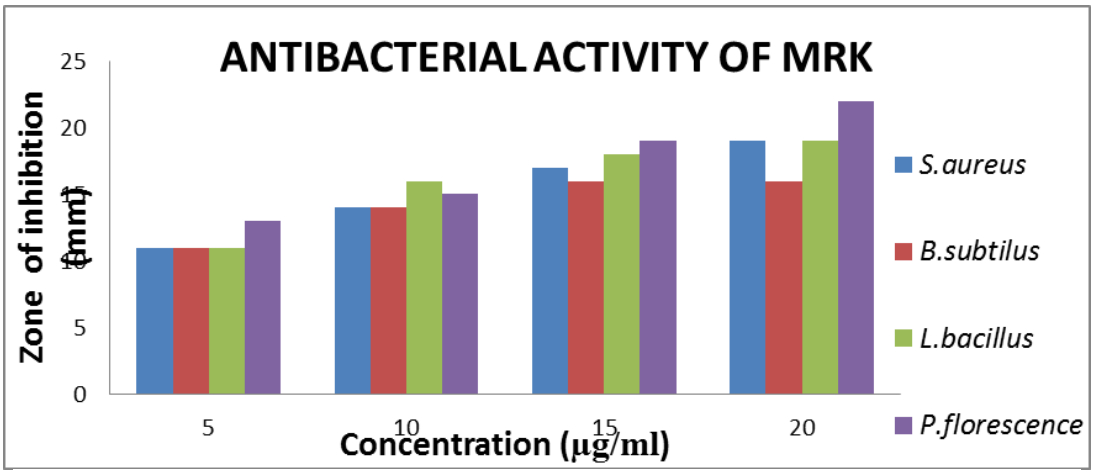

Fig. 5: Graph representing the zone of inhibition formed in various bacterial strains by MRK.

The zone of inhibition of varying concentration MRK was obtained for the selected strains of bacteria and was showed in **Figure 5**. The strains selected were *Bacillus subtilis*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Lactic acid*. The table shows that MRK possess significant antibacterial activity. Among the bacterial strains, *P. flurescens* shows highest zone of inhibition. So it has been selected for obtaining the MIC values.

Table 7. Zone of inhibition in diameter on *P.florescens*.

Concentration (µg/ml)	Zone of inhibition (mm) for *P.florescens*
1	8.33±0.577
2	10.33±1.154
3	10.66±0.577
4	11.33±1.154
5	12.66±0.577

Values are expressed in mean±SD

P. fuorescens is signifcantly less virulent than P. aeruginosa and is a rare cause of invasive hospital-acquired infections, with most common site of infection being the bloodstream. It has been isolated in respiratory samples from patients with lung transplants, ventilator-associated pneumonia (VAP), cystic fibrosis (CF) and rice-feld drowning-associated pneumonia. While P. fuorescens has been identifed in human bronchoalveolar lavage fuid (BALF), sputum specimens or throat swabs, its role in pneumonia pathogenesis is unclear. It has been previously suspected of being an aetiologic agent of pneumonia in several reports, however, the clinical characteristics and drug susceptibility pattern of P. fuorescens pneumonia have rarely been reported.[12]

The zone of inhibition formed for *P.flurescens* was showed in **Table 7**. The Minimum Inhibitory Concentration (MIC) for MRK against *P.flurescens* is found to be 1 µg/ml.

Determination of antioxidant activity

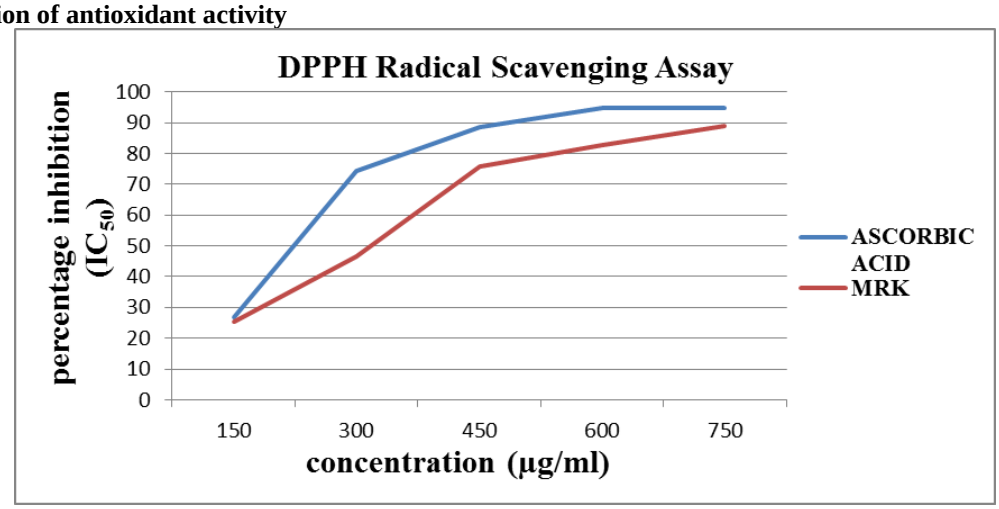

Fig 6: DPPH Radical scavenging assay of Maharasnadhi kashayam.

Table 8: Percentage inhibition and IC $_{50}$ values of DPPH radical by Ascorbic acid and MRK.

Sample	Concentration (µg/ml)	Absorbance	Percentage inhibition (%)	IC $_{50}$
Standard (Ascorbic acid)	10	1.432±0.12	26.75	13.11
	20	0.502±0.08	74.32	
	30	0.226±0.05	88.43	
	40	0.099±0.01	94.93	
	50	0.102±0.01	94.78	
Test (MR)	10	0.984±0.07	25.22	22.81
	20	0.701±0.07	46.73	
	30	0.319±0.05	75.75	
	40	0.226±0.04	82.82	
	50	0.144±0.06	89.05	

DPPH-induced FRS activity has been proposed to be the prime method for assessing the antioxidant potential of extracts or compounds. It is a rapid, simple, cheap, and widely used assay to evaluate the biological action of free radical scavengers as hydrogen donors. The reducing power (RA) of a compound is related to the presence of electron or hydrogen donating groups; free radicals are trapped or stabilised by an electron-donating group of the antioxidant.[10] The DPPH radical scavenging activity of MRK is presented in **Figure 6**. This study determined that Maharasnadi kashayam (MRK) showed comparable antioxidant potential by DPPH assay method when compare to standard ascorbic acid and IC_{50} value found to be as 13.11and 22.81µg/ml for ascorbic acid and Maharasnadi kashayam (MRK) respectively.

***Invitro* anti arthritic activity by egg albumin denaturation method**

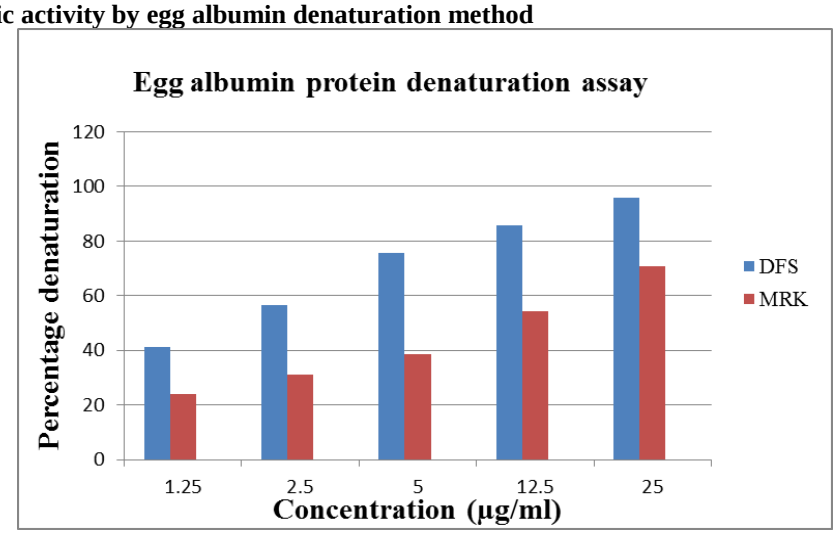

Figure 7: Percentage denaturation of egg albumin by Diclofenac sodium and MRK.

Table 9: Percentage denaturation of egg albumin by Diclofenac sodium and MRK.

Sample	Concentration (µg/ml)	Absorbance	Percentage denaturation (%)
Standard (DFS)	1.25	0.255±0.05	41.16
	2.5	0.189±0.02	56.45
	5	0.105±0.06	75.65
	12.5	0.061±0.07	85.86
	25	0.017±0.03	96.02
Test (MRK)	1.25	0.33±0.63	23.88
	2.5	0.298±0.02	31.18
	5	0.266±0.06	38.70
	12.5	0.198 ±0.07	54.22
	25	0.126±0.03	70.96

Values are expressed in mean±SD

Denaturation of tissue proteins may be the cause behind the production of auto-antigens in certain arthritic diseases. So it may be said that tissue protein denaturation is a marker for inflammatory and arthritic diseases. Agents that can prevent protein denaturation, therefore, would be possible candidate for anti-inflammatory drug development.[11] The formulation at dissimilar concentrations offers significant fortification against protein denaturation which is presented in **Table 9** and **Figure 7**. The maximum percentage inhibition of protein denaturation was observed at a concentration of 25µg/ml for MRK and DFS about 70.96% and 96.02% inhibition respectively using egg albumin denaturation method. The formulation possesses substantial anti-arthritic activity equivalent to that of Diclofenac sodium.

CONCLUSION

According to the kashaya sangrahaya and ayurveda pharmacopoeia, Maharasnadi Kashayam is a polyherbal formulation proved to be safe and nontoxic has the potential for providing relief to RA patients. This formulation is prepared from parts of 26 different plants that are used in traditional medicine for a variety of purposes such as reduction of pain, reduction of inflammation, antipyrectic and antioxidant activity. Although MRK is used for its antiarthritic, antioxidant properties, there is no scientific validation till the date. Hence, in the present study, we have analysed the phytochemical profile, total phenolic content, total flavanoid content, antioxidant and antibacterial properties of Maharasnadi kashayam of Sitaram Ayurveda. MRK was standardized with respect to physicochemical and microbial based parameters. The physicochemical parameters like organoleptic properties, pH, specific gravity, density, total solid content was performed and the result were found to be in permissible limit. Bacteria and fungi were within permissible WHO limits. In summary, this contemporary research lends pharmacological support to preliminary phytochemical screening of MRK showed the presence of carbohydrates, glycosides, flavonoids, phenols, tannins and amino acids in the crude drug. MRK shows maximum phenolic and flavanoid content. usage in the treatment and management of painful arthritic inflammatory conditions. The findings of present investigation clearly indicates that MRK possess significant antioxidant and antibacterial capacity and a good source of various phytoconstituents which recommends further research needed for isolation of bioactive principles.

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