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Antioxidant Potential and Phytochemical Screening of *Mrudweekadi Kashayam* – An analytical study

Research Article

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Abstract

Introduction: The antioxidant potential of herbal formulations is gaining significant attention due to their potential therapeutic benefits in combating oxidative stress-related diseases. *Mrudweekadi Kashayam*, a traditional polyherbal formulation, is used in Ayurvedic medicine for various health benefits. This study aims to evaluate the antioxidant potential and phytochemical composition of *Mrudweekadi Kashayam*. Methods: The formulation was procured from GMP certified pharmacy. The antioxidant potential was assessed using standard in vitro assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, FRAP (ferric reducing antioxidant power) assays. The phytochemical screening was performed in the Central Research Facility of KAHER's Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belagavi.to identify the presence of bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and saponins. Results: *Mrudweekadi Kashayam* demonstrated significant antioxidant activity in all the assays, with the highest activity observed in the DPPH assay. The phytochemical screening revealed the presence of a rich profile of bioactive compounds, including alkaloids, flavonoids, tannins, phenols, and saponins. These compounds are known for their antioxidant properties and contribute to the overall antioxidant potential of the formulation. Discussion: The findings indicate that *Mrudweekadi Kashayam* possesses potent antioxidant activity, which may be attributed to its diverse phytochemical composition. The presence of multiple bioactive compounds suggests a synergistic effect, enhancing the formulation's ability to neutralize free radicals and mitigate oxidative stress. Conclusion: *Mrudweekadi Kashayam* exhibits significant antioxidant potential and contains a variety of bioactive phytochemicals. This formulation may serve as a promising candidate for further research and development in the treatment and prevention of oxidative stress-related diseases.

Keywords: *Mrudweekadi Kashayam,* Antioxidants, DPPH, FRAP, Phytochemicals, Analytical study.

Introduction

Free radicals can damage nucleic acids, lipids, proteins, and carbohydrates, they are important players in the pathophysiology of many diseases, including atherosclerosis, cancer, cardiovascular diseases, diabetes, inflammation, and neurological disorders (1). One such disease is hydrogen peroxide, a type of reactive oxygen species (ROS). Studies show a beneficial correlation between antioxidant consumption and a lower risk of aging- and chronic-related disorders (2).

Bioactive chemicals with notable pharmacological activity, low toxicity, and negligible adverse effects are abundant in plants (3). Herbal mixtures have been used millennia to treat various illnesses in traditional medical systems such as Ayurveda, Chinese medicine, Unani, and Peruvian customs.

For millennia, herbal remedies have been used by traditional medical systems such as Ayurveda, Chinese medicine, Unani, and Peruvian customs to treat a variety of illnesses. The idea of polyherbalism is well-known in Ayurveda and proposes that mixing many herbs in particular ratios might improve therapeutic benefits and decrease toxicity (4).

However, because of component interactions and reaction environment, the antioxidant activity of herbal combinations may not always be additive (5). To determine if different bioactive chemicals are present in plant materials, preliminary phytochemical screening is a crucial step in the analytical process.

This screening offers insightful data on the plant's chemical components, which may be connected to some of its possible medicinal uses. Through redox adaption, reactive oxygen species—most notably hydrogen peroxide—function as second messengers in cellular signaling, aiding in the malignant transformation of cancer cells and fostering the growth of tumors (6). By giving electrons to free radicals, antioxidants counteract their effects and stop oxidative damage to biological

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components including proteins, DNA, and cell membranes (7).

The synergistic properties of polyherbal formulations, which are renowned for their wide therapeutic spectrum, low side effects, and environmental friendliness, are historically highlighted in Ayurvedic literature such as the "*Sarangdhar Samhita*" (8). The World Health Organisation reports that 75% of people worldwide get their main healthcare from herbal medicines (9).

The potential of herbal supplements in the prevention and treatment of disorders related to oxidative stress is highlighted by the increased interest in natural antioxidants due to concerns about the toxicity of synthetic antioxidants (10). Using FRAP and DPPH tests, the antioxidant characteristics of *Mrudweekadi Kashayam* are the main objective of this investigation. Consequently, the purpose of this study is to investigate the phytochemical screening and quality parameters such as pH, specific gravity, total solids, and microbial load to determine the antioxidant potential of *Mrudweekadi Kashayam*, a polyherbal formulation.

Reactive oxygen species (ROS), in particular, are free radicals that are essential to the pathophysiology of alcohol-related liver damage. ROS produced by alcohol metabolism, such as hydroxyl and hydrogen peroxide, greatly increase oxidative stress in the liver. Damage to cellular macromolecules including lipids, proteins, and nucleic acids as a result of this oxidative stress causes inflammation, fibrosis, and eventually cirrhosis or hepatocellular cancer in the liver (11).

Alcohol dehydrogenase (ADH) is the first enzyme to convert ethanol to acetaldehyde during liver metabolism. Aldehyde dehydrogenase (ALDH) then proceeds with the further metabolism of acetaldehyde. ROS generation rises throughout these metabolic activities, surpassing the liver's antioxidant defenses. Lipid peroxidation, protein oxidation, and DNA damage are the outcomes of this oxidative stress, which impairs normal liver function and encourages liver injury (12).

When there are too many ROS present, the liver's capacity to cleanse and heal itself is severely hampered. Alcoholic liver disease (ALD) can occur as a result of long-term oxidative damage brought on by chronic alcohol use. The build-up of ROS in ALD worsens fibrosis and inflammation, further compromising liver function and hastening the development of more severe liver diseases (13).

Antioxidants are essential for reducing the harm that free radicals may do. By giving electrons to neutralize ROS, they lessen oxidative stress and stop more cellular damage. According to studies, antioxidants can lessen the harm that alcohol does to the liver by scavenging reactive oxygen species (ROS) and improving the liver's ability to cleanse and heal itself(14). Natural antioxidants with the potential to be therapeutically beneficial in shielding the liver from oxidative stress and damage caused by alcohol include those found in polyherbal preparations(15). This study focuses on assessing the antioxidant properties and phytochemical profile of *Mrudweekadi Kashayam*, exploring the link between its bioactive compounds and antioxidant activity to establish scientific validation of its traditional applications. The results will contribute to evidence-based Ayurveda, facilitate standardization and quality control, and promote further pharmacological exploration of polyherbal formulations.

Materials and Methods

The formulation was procured from GMP certified pharmacy. The antioxidant potential was assessed using standard in vitro assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, FRAP (ferric reducing antioxidant power) assays. The phytochemical screening was performed in the Central Research Facility of KAHER's Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belagavi.to identify the presence of bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and saponins.

Sample

Mrudweekadi kashayam a well-known *ayurvedic* formulation mentioned under *Madatyaya - hara kashayam* indicated in *Trishna, Murcha, Mada* and *Mati vibhrama.*

The formulation *Mrudweekadi Kashayam*(16) was procured from a GMP certified ayurveda pharmacy. It is available in the form of decoction (*Kashyam*) with 200 ml bottle packing. The ratios of each component of the formulation are shown in Table 1.

Table 1: Ratios of each component of the formulation

S.No.	Ingredient (Sanskrit Name)	Botanical Name (Author)	Family	Part Used	Quantity per 15 ml
1	Draksha	Vitis vinifera L.	Vitaceae	Dried fruit	3 g
2	Madhuka	Glycyrrhiza glabra L.	Fabaceae	Root	3 g
3	Madhooka	Madhuca indica J.F. Gmel.	Sapotaceae	Heartwood	3 g
4	Kharjura	Phoenix dactylifera L.	Arecaceae	Fruit	3 g
5	Pippali	Piper longum L.	Piperaceae	Fruit	3 g
6	Chandana	Santalum album L.	Santalaceae	Heartwood	3 g
7	Sariva	Hemidesmus indicus (L.) R. Br.	Apocynaceae	Root	3 g
8	Mustaka	Cyperus rotundus L.	Cyperaceae	Rhizome	3 g
9	Laja	Oryza sativa L.	Poaceae	Fried grain	3 g
10	Usheera	Vetiveria zizanioides (L.) Nash	Poaceae	Root	3 g

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Results

Table 2: Quality assessment parameters: Physicochemical standards(17)

S.no.	Parameters	Results	Reference values	Unit	Test Method
1	pH	4.63	4.6-5.2	-	API
2	Brix	22	22-26	%	API
3	Specific gravity	1.094	1.08-1.11	-	API
4	Total solids	23.05	22-26	w/w%	API

The table presents the physicochemical quality assessment of a sample, evaluated against reference values using the API test method.

The sample analysis reveals a pH of 4.63, which is within the acceptable range of 4.6 to 5.2, indicating appropriate acidity for stability and microbial control. The Brix value is 22%, meeting the standard range of 22 to 26%, reflecting the sample's sugar content essential for flavor and preservation. The specific gravity is 1.094, within the acceptable range of 1.08 to 1.11, indicating the concentration of dissolved substances. The total solids content is 23.05% (w/w), consistent with the reference range of 22 to 26%, indicating the sample's solid material content, which affects texture, nutritional value, and consistency.

The table 3 presents a microbiological analysis of MK, against reference values and using the API test method, reveals no detectable aerobic microbes, yeast, or mould, with results within the acceptable limits of NMT 10510^5105 cfu per 10 ml and NMT 10310^3103 cfu per 10 ml, respectively. The sample also tested negative for harmful bacteria, including *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, all of which align with the required absence for safe consumption.

Table 3: Microbial analysis(18)

S.no.	Parameters	Results	Reference values	Unit	Test Method
1	Total aerobic microbial plate	Nil	NMT 10^5	cfu/10ml	API
2	Total Yeast and Mould count	Nil	NMT 10^3	cfu/10ml	API
3	Escherichia coli	Absent	Absent	cfu/10ml	API
4	Salmonella sp.	Absent	Absent	cfu/10ml	API
5	Pseudomonas aeruginosa	Absent	Absent	cfu/10ml	API
6	Staphylococcus aureus	Absent	Absent	cfu/10ml	API

(NMT: Not More Than API: Ayurvedic Pharmacopocia of India, cfu: Colony forming unit)

Table 4: Organoleptic characteristics: Macroscopic description of formulation

S.no.	Tests	Results
1	Form	Decoction (*Kashayam*)
2	Colour	Dark brown
3	Taste	Sweetish bitter
4	Odour	Distinct

The organoleptic characteristics of the formulation macroscopic description, encompassing form, color, taste, and odor. The form of the formulation is a decoction, The colour is a dark brown, indicative of the concentrated herbal components used in its preparation. This deep hue is typical for decoctions, reflecting the presence of various phytochemicals. The taste is described as sweetish bitter, a common profile for many Ayurvedic formulations that balance different flavors to promote therapeutic benefits. This taste profile can enhance the digestive process and stimulate various bodily functions. The odour is characterized as distinct, which is expected from a rich mixture of herbs, each contributing to a unique aromatic profile.

Table 5: Preliminary phytochemical screening:(19)

S.no.	Phytochemical constituents	Present (+) / Absent (-)	Changes Observed
1	Tannins	+	Presence of Deep blue-black coloured precipitate
2	Flavonoids	+	Presence of Yellow coloured precipitate
3	Steroids	-	Absence of red and greenish yellow colour fluorescence
4	Alkaloids	-	Absence of brown precipitate
	Glycosides		
5	C-Saponin glycosides	+	Presence of frothing

Tannins

3 mL of MK was taken in a test tube and ferric chloride was added. The presence of deep blue-black coloured precipitate reveals the presence of tannins.

Flavonoids

2 mL of MK was taken in a test tube and lead acetate solution was added. The presence of yellow coloured precipitate reveals the presence of flavonoids.

Steroids

Salkowski reaction: A sample of 2 ml of MK was taken then 2ml chloroform and 2ml concentrated hydrosulphuric acid was added. The whole mixture was then, shaken well. Absence of red and greenish yellow colour fluorescence showed the absence of steroids in sample.

Alkaloids

Dragendorff's test: By adding few drops of Dragendoff's reagent to the 3 ml of MK, absence of brown precipitate indicated there were no alkaloids present in the sample.

Hager's test: 3ml of MK was taken then few drops of Hager's reagent was added. Absence of yellow precipitate indicated there were no alkaloids present in the sample.

Saponin Glycosides:

Foam test: 5ml MK sample was taken and shaken vigorously. The presence of frothing that for 5 min indicates that saponin glycosides were present.

Antioxidant assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay(20)

Principle and procedure

DPPH is an organic nitrogen radical capable of accepting the hydrogen from the sample(s). DPPH is purple in color and converts to yellow by the formation of the DPPH (upon conversion from radical compound). The anti-oxidant is measured by the disappearance of UV absorption in the DPPH in the test sample and the control samples.

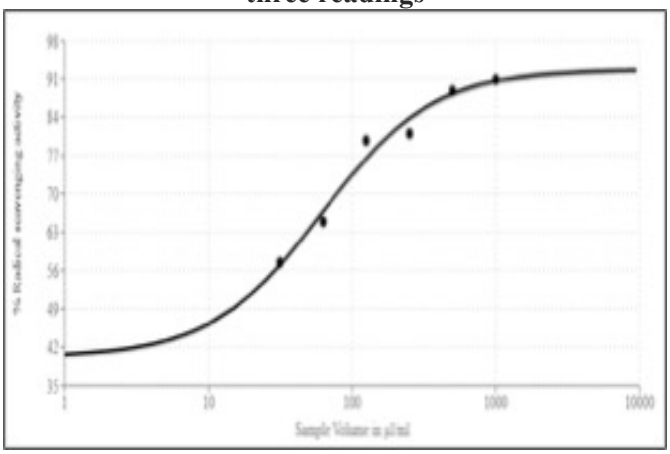
The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay is a method used to evaluate the antioxidant activity of Mrudweekadi Kashayam. The process involves preparing a 0.1 mM DPPH solution in methanol and mixing it with various concentrations of the sample (31.25 µl, 62.5 µl, 125 µl, 250 µl, 500 µl, and 1000 µl). The reaction mixtures are incubated at room temperature in the dark for about 30 minutes to prevent light-induced degradation of DPPH. The change in color from purple to yellow, indicating the reduction of DPPH by the antioxidant, is measured spectrophotometrically at 517 nm. The radical scavenging activity is calculated as a percentage decrease in absorbance, with the results showing 57.44% for 31.25 µl, 64.89% for 62.5 µl, 79.74% for 125 µl, 81.01% for 250 µl, 88.81% for 500 µl, and 90.88% for 1000 µl. The data represent the mean of three readings. The IC50 value = 61.2032, which indicates the concentration needed to inhibit 50% of the DPPH radicals, was obtained by plotting the concentration against the radical scavenging activity in Graph 1. The assay uses a 1:50 dilution of the filtered solution. The radical scavenging activity is calculated as a percentage was calculated by using the equation.

Percentage (%) of radical scavenging activity = (Absorption of the blank-Absorption of the sample/Abs of the blank)*100

Table 6: Percentage of radical scavenging activity

Diluted Sample Solution	
Sample vol l/ml	**% Radical scavenging**
31.25	57.44
62.5	64.89
125	79.74
250	81.01
500	88.81
1000	90.88

Graph 1: Antioxidant properties of Mrudweekadi Kashayam and IC50 value; Results are mean of three readings



FRAP(21)

The Ferrous Reducing Antioxidant Power (FRAP) assay is a widely used method to assess the antioxidant potential of various substances. The assay is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form, which can be monitored spectrophotometrically.

- Principle: The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe3±TPTZ) to its ferrous, coloured form(Fe2+-TPTZ) in the presence of antioxidants (sample and control).
- Standard: FeSO4.7H20
- Procedure of the FRAP Assay: The antioxidant activity of Mrudweekadi Kashayam was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay, as described by Benzie and Strain (1996). In this method, 150 µL of the sample was mixed with 2850 µL of FRAP reagent, consisting of TPTZ, $FeCl_3$, and acetate buffer. The mixture was incubated for 10 minutes, during which the antioxidants in the sample reduced the ferric-tripyridyltriazine complex to its ferrous form, resulting in a blue color. The absorbance was measured at 593 nm using a spectrophotometer. The assay was conducted in triplicate, and the mean absorbance values were calculated. The antioxidant activity was determined by comparing the absorbance readings to a standard curve of $FeSO_4.7H_2O$, with the results expressed in mM Fe^{2+} equivalents. The specific FRAP value obtained for the sample was 37.70 ± 0.34 mM Fe^{2+}/ml sample. Slope obtained from the graph 2 of; $FeSO_4.7H_2O$ standard concentrations: (y=1.2191x + 0.0015, R_2 = 0.9902.

Table 7: Ferrous Reducing Antioxidant Power

Sample code	Sample Name	Type of Sample	Results
FG/188	*Meudweekadi Kashyam*	Liquide	37.70+_ 0.34mMFe(II) /ml sample

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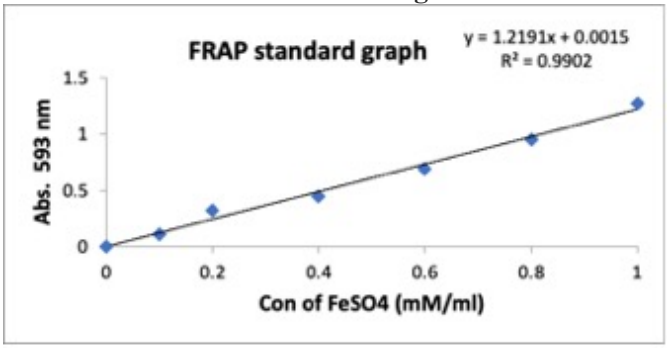
Graph 2: FRAP Standard Graph; Results are mean of three readings



Discussion

Quality assessment parameters: Physicochemical standards

These physicochemical characteristics are essential for guaranteeing the sample's uniformity, safety, and quality. Stabilizing the product and preventing microbial development are achieved by keeping the pH(22) within the prescribed range. The right amount of Brix(23) guarantees the right sweetness and preservation properties. The specific gravity(24) of a sample can reveal its concentration, which in turn influences its physical characteristics and overall quality. Determining the nutritional value and making sure the sample satisfies the required standards for texture and consistency depends on the total solids content.

Microbiological Analysis

Through the verification of the lack of pathogenic microorganisms and maintenance of the total microbial count below acceptable limits, guarantees the sample's safety and quality. Preventing diseases and guaranteeing consumer safety necessitates the removal of organisms such as Salmonella, E. Coli, Pseudomonas aeruginosa, and Staphylococcus aureus(25). Sustaining reduced concentrations of yeast, mold, and total aerobic microorganisms contribute to the integrity of the product and increases its shelf life.

Phytochemical Constituents

Tannins(26)

Because of their antioxidant qualities, tannins aid in scavenging the damaging free radicals produced by the metabolism of alcohol. One of the main causes of liver damage, oxidative stress, can be warded off by the antioxidant activity of liver cells. Additionally, because tannins have anti-inflammatory qualities, they may help lessen liver inflammation brought on by heavy alcohol use. Due to these properties, it was indicated in Madatyaya as per classics also. Mrudweeka seeds and skins are rich in tannins, which have antioxidant and astringent properties. They contribute to the bitterness and astringency of grapes.(27)

Flavonoids (28)

Strong antioxidants, and flavonoids aid in the scavenging of free radicals. By strengthening the body's antioxidant defense systems, they can prevent harm to liver cells. Additionally, flavonoids present in *Khajura* (Phoenix dactylifera) in Mrudweekadi formulation have anti-inflammatory qualities that aid in reducing liver inflammation and fostering the regeneration of liver cells.(29)

Steroids (30)

While they aren't in your sample, certain natural steroids have hepatoprotective and anti-inflammatory qualities. On the other hand, their lack in this instance implies that steroids in the MK sample are not responsible for these effects.

Alkaloids (31)

Although certain alkaloids may have hepatoprotective properties, the MK sample's potential therapeutic benefits are not impacted by their absence.

C-Saponin Glycosides (32)

Due to their antioxidant qualities, saponin glycosides help lessen oxidative stress. Additionally, they may have anti-inflammatory properties that aid in lowering liver inflammation. Furthermore, saponins can interact with cholesterol and bile acids, which may help prevent fatty liver disease, a prevalent disorder linked to alcohol addiction.

DPPH assay

Results for Mrudweekadi Kashayam, which show radical scavenging activity ranging from 57.44% to 90.88% and an IC50 value of 61.2032 µl,(20)indicate strong antioxidant potential. This suggests that the herbal formulation contains potent antioxidant compounds capable of neutralizing free radicals. These findings imply potential health benefits, such as protection against diseases linked to oxidative stress, including cardiovascular diseases, cancer, and neurodegenerative disorders. Piperine is the major bioactive compound in Piper longum. It has anti-inflammatory, antioxidant, and hepatoprotective properties. Piperine helps reduce oxidative stress in the liver, potentially protecting against liver damage.(33)

FRAP Analysis

The formula used to calculate the antioxidant activity typically involves determining the concentration of Fe^{2+} equivalent in the sample based on the standard curve of $FeSO_4.7H_2O$. The absorbance readings from the sample are compared against this standard curve, which is described by the linear equation, $y = 1.2191x + 0.0015$ (where y is the absorbance and x is the concentration of Fe^{2+}), with a correlation coefficient (R^2) of 0.9902, indicating a strong linear relationship.

The results reported in the document indicate that the *Mrudweekadi Kashayam* sample has a reducing power equivalent to 37.70 ± 0.34 mM Fe^{2+} per ml of the sample. The FRAP assay provides a quantitative measure of the antioxidant capacity of the sample by assessing its ability to reduce Fe^{3+} to Fe^{2+}. A higher FRAP value indicates greater antioxidant potential. The

FRAP test can be used to examine how dietary supplements or herbal therapies impact oxidative stress in studies evaluating their effectiveness for liver health. (34) Because of its antioxidant qualities, a specific herbal formulation may have a preventive effect against liver damage if research demonstrates that it has a high FRAP value.(35)

As per *Aacharyas Mrudweekadi kashayam* has Madhura, Tikta, Katu Rasa, Sheeta Virya, Madhura Vipaka, Guru, Snigdha Guna, and Tridoshashamaka properties. The ingredients also have properties such as *Rasayana, Balya, Medhya, Manasdoshahara*, and *Vedanasthapana*(16). *Rasayana* herbs are rich in bioactive compounds, such as polyphenols, flavonoids, and tannins, which have potent antioxidant properties. By scavenging free radicals, these herbs help protect the body from cellular damage. They not only help in detoxifying the body but also enhance the immune system, improve cognitive function, and promote longevity.

Conclusion

The evaluation of the antioxidant potential and phytochemical screening of the polyherbal formulation *Mrudweekadi Kashayam* has revealed significant findings. The presence of tannins, flavonoids, and saponin glycosides in the MK sample suggests that this formulation may have potential hepatoprotective effects, particularly in protecting the liver from alcohol-induced damage. These phytochemicals can work synergistically to reduce oxidative stress, and inflammation, and promote liver cell regeneration, making the MK sample a potentially valuable formulation for supporting liver health in the context of alcohol-induced liver damage.

The formulation demonstrated strong antioxidant activity, as evidenced by the results of the FRAP and DPPH assays. The presence of potent antioxidant compounds suggests its potential for neutralizing free radicals and providing protective benefits against oxidative stress-related diseases. Additionally, the phytochemical screening highlights the diverse bioactive components within the formulation, contributing to its therapeutic efficacy. These findings support the traditional use of *Mrudweekadi Kashayam* in managing various health conditions and provide a scientific basis for its continued exploration as a natural antioxidant source. The significant antioxidant activity also supports the therapeutic use of *Mrudweekadi Kashayam* in traditional medicine and offers a basis for further research into its bioactive components.

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Conflict of Interest

NIL

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