WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 12, 945-959. Research Article ISSN 2277–7105

FORMULATION AND EVALUATION OF ANTI-INFLAMMATORY GUTIKA

Sajeena Ch.[1]*, Mohammed Dilshad Kalliyath[2], Mohammed Nihad P.[3], Mohammed Shabeeb[4], Mufeedha K. P.[5], Muhammed Salih K. T.[6], Celestin Baboo R. V.[7] and Sirajudheen M. K.[8]

[1-7]Department of Pharmacognosy, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram, Kerala, India.

[8]Department of Pharmaceutics, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram, Kerala, India.

Article Received on
01 May 2024,

Revised on 21 May 2024,
Accepted on 11 June 2024

DOI: 10.20959/wjpr202412-32871

***Corresponding Author**
Sajeena Ch.
Department of Pharmacognosy, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram, Kerala, India.

ABSTRACT

This research article presents a comprehensive study on the formulation and evaluation of Anti-inflammatory Gutika. Gutika is a traditional ayurvedic pill, using the plants of *Crossandra infundibuliformis* and *Cnidoscolus aconitifolius*.[1] The study outlines the procurement and preparation of plant materials, the process of creating the Gutika, and its subsequent organoleptic, phytochemical, and pharmacological evaluation. The findings demonstrate the Gutika's potential in treating inflammation, supported by in vitro tests for Anti-inflammatory and anti-oxidant activities. The article emphasizes the significance of integrating modern analytical techniques with classical Ayurvedic formulations, ensuring their reliability and efficacy.

KEYWORDS: *Crossandra infundibuliformis, Cnidoscolus aconitifolius*, Gutika, Anti-inflammatory, Evaluation, Anti-oxidant.

INTRODUCTION

Ayurveda is one of the earliest medical systems. In Ayurveda, drug formulation is classified into two types: (i) using a single drug and (ii) using a combination of drugs (polyherbal formulation). Ayurvedic formulations include choornas (fine powders), Kwathas (aqueous extracts), Tailas/Grithas (lipid extracts), Gutikas (tablets), and so on. The methods of

manufacture and protocols for standardization of formulations mentioned in classical Ayurvedic texts are quite premature; thus, in the present era, changing trends necessitate the establishment of standards for Ayurvedic drugs and formulations, as well as the development of reliable quality protocols for Ayurvedic formulations using modern analytical techniques.[1]

Gutika, an ancient and traditional Ayurvrdic dose form, is derived from kalkakalpana, one of the five essential principles of Ayurvedic Ayurveda. They are significantly smaller than vati. Acharya defines Sharangdhargutika as a synonym for vatikalpana, also known as tablets in modern dosages. Gutika is an essential part of the ayurvedic pharmacy. Gutika are pills, while spheroids are fine powders or granules containing bulk medicines and excipients.

Inflammation is a defense mechanism that allows the body to protect itself from infection, burns, toxic chemical allergens, and other potentially hazardous stimuli. Inflammation is a significant reaction to damage, sickness, or destruction, characterized by heat, redness, discomfort, swelling, and impaired physiological activities.[4]

Protein denaturation is a process in which a protein is denatured by external forces such as heat, a strong acid or base, an organic solvent, or a concentrated inorganic salt, causing the proteins tertiary and secondary structures to become confused. Enzymes lose their activity, which causes autoimmune disorders. These medications have a number of side effects, including stomach irritation, which can lead to the formation of gastric ulcers.[5] The substrates can no longer connect to the active site. Nonsteroidal anti-inflammatory medicines (NSAIDS) are widely prescribed treatments around the world due to their proven effectiveness in lowering pain and inflammation. NSAIDS have accounted for inhibition of protein denaturation, which functions as an antigen.[6]

The main objective of present study was to focus on the formulation and evaluation of anti-inflammatory gutika using *Crossandra infundibuliformis* and *Cnidoscolus aconitifolius*. These plants were selected for the formulation of gutika used for the treatment of inflammation.

MATERIALS AND METHODS

Collection of plant material

Dried leaves of *Crossandra infundibuliformis* and *Cnidoscolus acountifolius* were procured in the month of March, 2024 from Kottakkal, India. After the procurement, the leaves were ground mechanically into a coarse powder and kept into an air-tight container for use in the study.

1) Crossandra infundibuliformis

Fig. 1: *Crossandra infundibuliformis.*

Biological name: *Crossandra infundibuliformis*

Family: Acanthaceae.

Chemical constituents: Alkaloids, Glycosides, Saponins, Steroids, Tannins.

Uses: Aphrodisiac, Anti-inflammatory, and Analgesic effects.[7]

2) Cnidoscolus aconitifolius

Fig. 2: *Cnidoscolus aconitifolius.*

Biological name: *Cnidoscolus aconitifolius*

Family: Euphorbiaceae.

Chemical constituents: Phenolic acids, Alkaloids, Saponins, Flavonoids.

Uses: Maintain blood sugar levels, acting as an anti-inflammatory, anti anemic, anti microbial, and antioxidant effect.[8]

Preparation of Anti-inflammatory Gutika

Raw materials complying the pharmacopoeial quality and quantity were subjected to the preparation of anti-inflammatory gutika as per the composition (Table:1). All the powders of *Crossandra infundibuliformis* and *Cnidoscolus aconitifolius* and Guda were mixed thoroughly as per the standard protocol and stored in air tight container.

Table 1: Formula for preparation.

Si. No.	Ingredients	Part used	Quantity
1	*Crossandra infundibuliformis*	Leaf Powder	1 Part
2	*Cnidocolus acounitifolius*	Leaf Powder	1Part
3	Sucrose solution		Q.s

- Take the necessary amount of base medication (eg; Sucrose solution) in a clean, wide-mouthed stainless steel container.
- Add water and boil on heat, stirring regularly.
- When syrup is ready, add fine pharmaceutical powder in tiny amounts and stir thoroughly until desired consistency is attained.
- The mass is shaped into pills of the required shape and size.
- Prepared pills are dried in the shade and stored in airtight containers.

Fig. 3: Ingredients.

Fig. 4: Addition of sucrose solution.

Fig. 5: Desired consistency.

Fig. 6: Desired Shape and Size (Gutika).

Evaluation of gutika

1) Organoleptic evaluation

Various characters like colour, odour, taste and touch are evalyated by sensory organs.

2) Preliminary phytochemical evaluation

Preliminary color-based tests were used to screen various secondary metabolites, including flavonoids, tannins, alkaloids, glycosides, terpenoids, steroids, phenolic compounds, and saponins.

3) Physiochemical analysis

- **Hardness test**

To determine the average tablet hardness, three tablet from each batch were selected and measured using a Monsanto hardness tester.

Fig. 7: Monsanto tester.

Fig. 8: Pfizer tester.

- **Friability (% F)**

Twenty tablets from each batch were randomly picked and weighed. These pills were friability tested using a Roche friabilator for 100 revolutions. The tablets were removed, dedusted, and weighed again.

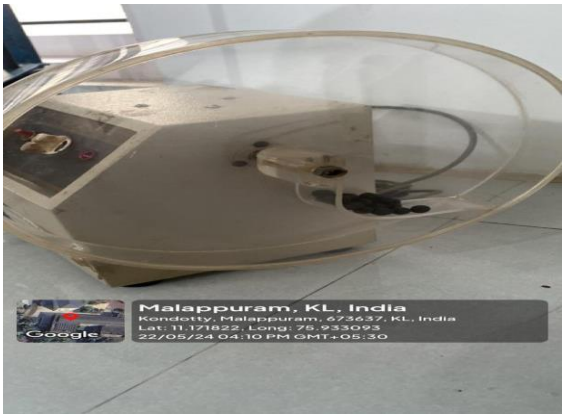


Fig. 9: Roche friabilator.

- **Total ash**

Two grams of coarse powdered air-dried materials were precisely weighed in a previously activated and tarred silica crucible. The medication was progressively ignited by raising the temperature to 450^0C in a muffle furnace until it turned white, indicating the lack of carbon. A desiccator was used to chill the sample, Which was then weighed. The percentage of total ash was estimated in relation to air dried medication. A high ash value indicate contamination, substitution, adulteration, the inclusion of silica, rice husk, or poor preparation of the medicine.

- **Acid insoluble ash**

The ash was heated with 25 ml of 2 M hydrochloric acid for 5 minutes, then the insoluble matter was collected on ash-free filter paper, washed with hot water until neutral pH, ignited, cooled in a desiccator, and weighed. The proportion of acid insoluble ash was estimated in relation to the air-dried medication. A low acid insoluble ash value influences the amount of pharmacological component absorbed in the gastrointestinal canal when given orally.

- **Determination of water-soluble extractive value**

In a conical flask, 5 g of the medication was macerated with 100 ml of water for 1 day. stirring every 6 hours and filtering. Evaporated 25 ml of filtrate in a petri plate at 105˚C and weighed the solid matter.

- **Determination of alcohol-soluble extractive value**

In a conical flask, 5 g of the substance was macerated with 100 ml of alcohol for 1 day, stirring every 6 hours and filtering. Weighed the solid stuff after evaporating 25 ml of filtrate in a petri plate at 105^0C.

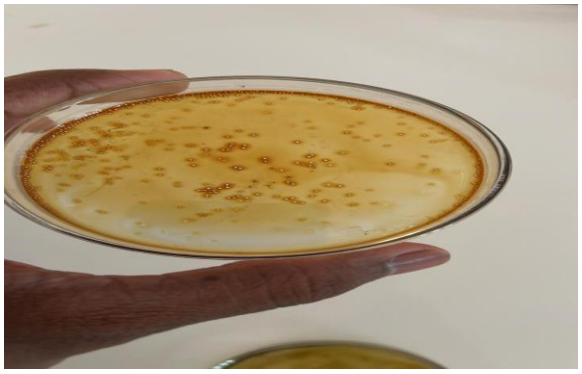


Fig. 10: Alcohol soluble extractive value.

- **Loss on drying**

Place 1-2 g of the powdered medication in a petri plate and spread it evenly. For 2 hours. Place the dish in an oven set to 100-105˚C. Cooled the sample in the desiccator, weighed it, and computed the percentage of loss from drying.

- **Weight variation test**

Determine the average weight of twenty pills. Examine each tablet's weight individually. Calculate and represent the departure from the average weight as a percentage.

Fig. 11: Weighing machine.

4) Pharmacological evaluation

Anti-inflammatory activity

In vitro testing can establish the anti- inflammatory effectiveness of unknown crude extracts by inhibiting the denaturation of egg albumin.

- Combine 0.2 ml of 1-2% egg albumin solution (from hen's egg or commercially available of egg albumin powder), 2 ml of sample extract or standard (diclofenac sodium) at varying concentration and 2.8 ml of phosphate-buffered saline (pH 7.4) to create a 5 ml reaction mixture.
- A 5 ml control solution was made by blending 2 ml of triple-distilled water , 0.2 ml of 1-2% egg albumin solution, and 2.8 ml of phosphate buffered saline.
- The reaction mixture was incubated at 37± 2°C for 30 minutes before being heated in a water bath to 70±2°C for 15 minutes.
- After cooling absorbance was measured at the 280 nm using UV/Vis spectrophotometer using triple distilled water as the blank.[1]
- The percentage inhibition of protein denaturation was calculated using the following equation:

$$\% \text{ inhibition} = \frac{AC - AT}{AC} \times 100$$

Where, AC is absorbance of control and AT is absorbance of test.

Fig. 12: Anti-inflammatory test.

Anti- oxidant activity

DPPH Technique to Determine Antioxidant Activity of Gutika

Using the stable DPPH radical, which has an absorption maximum at 515 nm, the total free radical scavenging capability of the Gutika was calculated using the previously reported method with a minor modification. To make a solution of the radical, dissolve 2.4 mg of DPPH in 100 ml of methanol. 3.995 ml of methanolic DPPH was mixed with 5 µl of the test solution. The mixture was vigorously mixed and left at room temperature in the dark for 30 minutes. The reaction mixture's absorbance was spectrophotometrically measured at 515 nm. DPPH radical absorption in the absence of an antioxidant.

DPPH Scavenged (%) = $((A_B - A_A)/A_B) \times 100$

A_B is absorbance of blank

A_A is absorbance of the antioxidant.

A calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidant (Ascorbic acid).

Anti microbial activity

One popular approach for determining a substance's antibacterial activity is the agar well diffusion method. Here is a detailed process:

1. **Nutrient agar plate preparation:** 1.52 g of dehydrated Muller Hinton agar medium were suspended in 40 milliliters of distilled water to create nutrient agar (Muller- Hinton) plates. Let to come to a boil, completely dissolving the medium, and then autoclaved for 15 minutes at 121^{o}C to sanitize. Fill a clean petri dish with the medium, then let it cool so it solidifies.
2. **Bacterial culture inoculation:** Inoculate the agar plates with a gram-positive (Pseudomonas aeruginosa) or gram- negative (Enterobacter) bacterial culture. Using a sterile cotton swab, uniformly distribute the culture across the agar's surface.
3. **Forming wells:** Make wells in the agar by using a sterile pipette tip or cork borer. The compounds under test will be kept in these wells.
4. **Test substance preparation:** Get ready the materials that will be examined for their ability to fight bacteria. Using Gutika as the test subject and ciprofloxacin (200 mg/100ml) as the reference.
5. **Adding test substances:** Using a micropipette, carefully add a measured amount of each test substance into a different well.

6. **Incubation:** Incubate the plates for a suitable amount of time (generally 24 hours) at the proper temperature for the bacteria being tested (37^0C for most bacteria).
7. **Zones of inhibition measurement:** Following incubation, check the plates to see if there are any zone of onhibition surrounding the wells. Using a ruler, find these Zone's diameters.
8. **Data analysis:** To ascertain the relative antibacterial activity of the test drug, compare the diameters of the zones of inhibition it created.

RESULTS

Phytochemical screening

Chemical test	Aqueous extracts
Carbohydrates	+
Alkaloids	+
Flavonoids	+
Glycosides	+
Steroids	-
Saponin	-
Tannins	+

Evaluation of gutika

Organoleptic characters

Sr. No	Characters	Antiinflammatory Gutika
1	colour	Dark Brown
2	Taste	Spicy Bitter
3	Texture	Hard Solid
4	Odour	No Specific
5	Appearance	Spherical

Physicochemical analysis

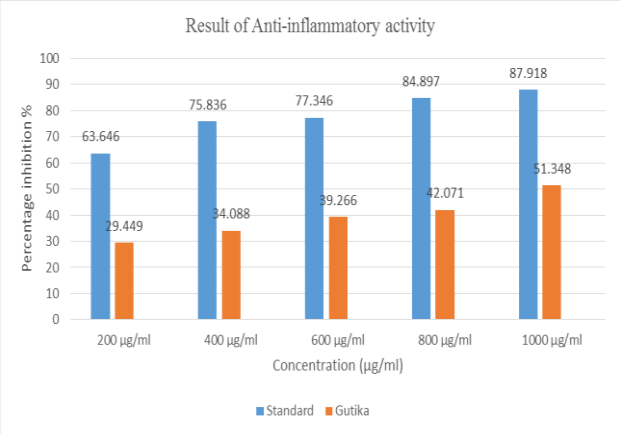
Parameter	Observation
Hardness	Monsanto test : 7.06 kg Pfizer test : 6.8 kg
Friability	0.79%
Loss on drying	3% w/w
Weight variation	No Gutika show percentage with variation more than 10 %
Total ash	10.5% w/w
Acid insoluble ash	7.86% w/w
Water soluble extractive value	5.16% w/w
Alcohol soluble extractive value	13.98% w/w

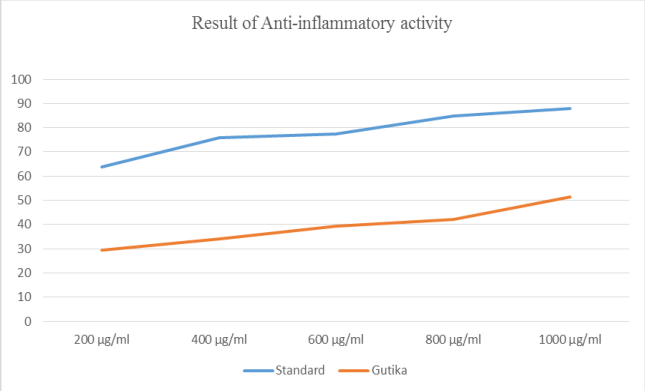
Pharmacological evaluation

Anti-inflammatory activity

Table: Percentage inhibition of Diclofenac and Gutika.

Sl. No.	Concentration (µg/ml)	% Inhibition of Diclofenac	%Inhibition of Gutika
1	200 µg/ml	63.64	29.44
2	400 µg/ml	75.83	34.08
3	600 µg/ml	77.34	39.26
4	800 µg/ml	84.89	42.07
5	1000 µg/ml	87.91	51.34





Graph showing the Anti-inflammatory activity of gutika.

Anti-oxidant activity

Anti-oxidant activity shown in the performed experiment For determination of antioxidant activity of anti-inflammatory gutika by method DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

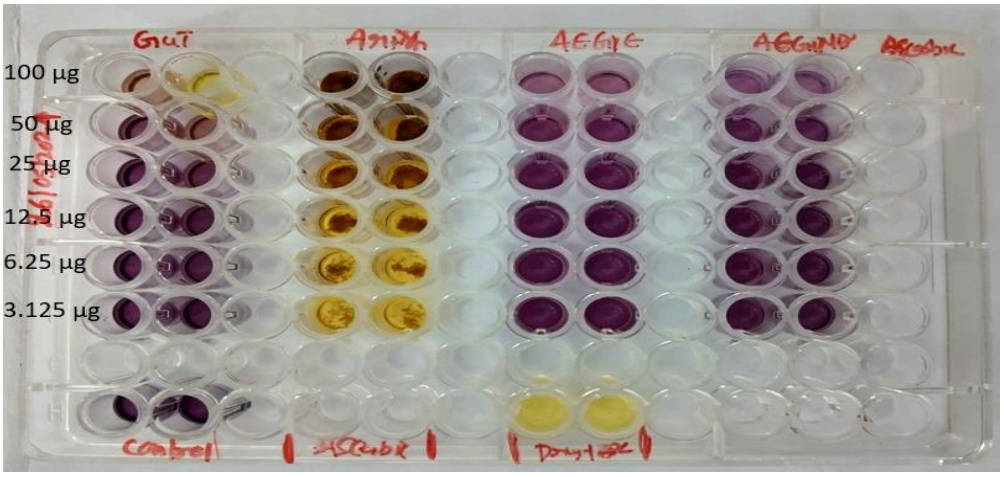
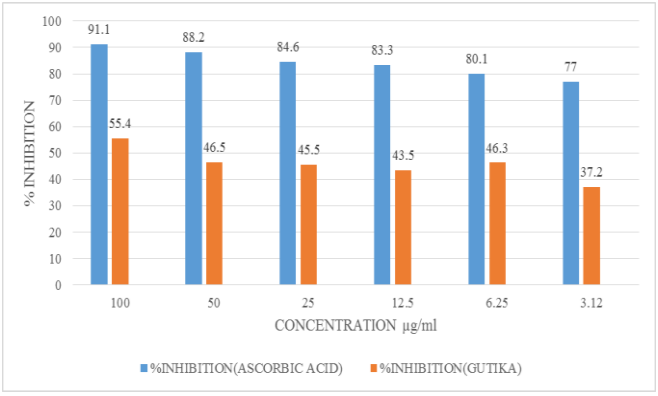
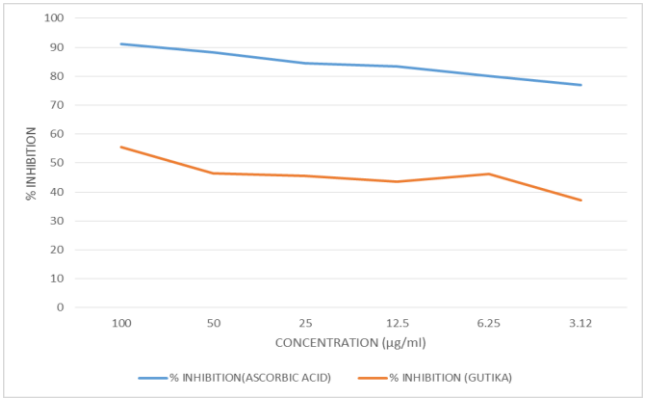


Fig: Result of Anti-oxidant activity by DPPH scavenging assay.

Table: Percentage inhibition effect of anti-oxidant activity of gutika.

Concentration (µ*g/mL*)	% Inhibition (Ascorbic acid)	% Inhibition of Gutika
100	91.1	55.4
50	88.2	46.5
25	84.6	45.5
12.5	83.3	43.5
6.25	80.1	46.3
3.12	77	37.2

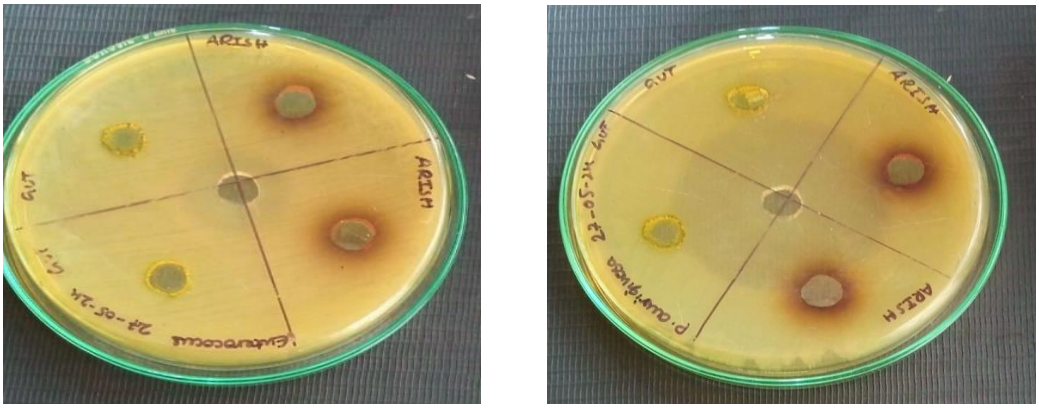




Graph showing the Anti-oxidant activity of gutika.

Antimicrobial activity

There is no zone of inhibition for bacterial growth shown in performed experiement for determination of antimicrobial activity of gutika by agar well diffusion method.



Gram –ve: Enterobacter **Gram +ve: Pseudomonas aeruginosa**

Fig: Anti-microbial activity of Standard and Gutika.

DISCUSSION AND CONCLUSION

Despite the fact that there are many different kinds of formulation in use today, gutika is crucial to ayurvedic pharmaceutics. This is because of it's many benefits, which includes it's ability to keep the medications effectiveness for a long time, quick actions, palatability, ease of administration, ease of dispensing, and ease of transportation. The number of gutika products became known to the pharmaceutical industry due to their enormous potential, excellent patient compliance, and availability of numerous formulation procedures. Additionally, it is emphasized that in order to create a feasible and adaptable dosage form with novel performance and features, more recent scientific and technological advancements are required.

The gutika is standardized by various parameters like physicochemical parameters include Ash value, extractive value, physical parameters include: Hardness, Friability, loss on drying and weight variation.

Biological evaluation like Anti-microbial, Anti-oxidant and Anti-inflammatory tests are performed. In this study Anti-oxidant activity shown in the performed experiment for determination of Anti-oxidant activity of gutika by DPPH and Anti-inflammatory activity by i.n vitro protein denaturation egg albumin method.

REFERENCE

1. Keerthy S Namboodiri, Prathviraj Puranik, Nagaratna, J Formulation and Standardization of Trivrutadi Gutika – An Ayurvedic Formulation International Journal of Ayurvedic Medicine, 2018; 78: 147-52.
2. Anjitha A.A, Dr. Shebina P Rasheed, Dr. Prasanth S. S, Sujith Unnikrishnan, Anziya P.R, Riya Babu. An Ayurvedic Dosage Form "Gutika". International Journal of Pharmaceutical Sciences Review and Research, 2023; 79: 36-41.
3. Ukharde Rohan Sandu, Pro.S.A.Sul, Dr.Santosh Jain. Formulation and Evaluation of Gutika for PCOD Treatment. International Journal of Novel Research and Development, 2023; 8: 159-163.
4. Sharmila Dharmadeva, Lahiru Sandaruwan, Galgamuwa C, Prasadinie, Nishantha Kumarasinghe. In vitro anti inflammatory activity of Ficus racemose L.bark using albumin denaturation Method. An International Quarterly Journal of Research in Ayurveda, 2019; 39: 239-242.
5. Madhuranga HDT and Samarakoon DNAW. In Vitro Anti-inflammatory Egg Albumin Denaturation Assay: An Enhanced Approach. Journal of Natural & Ayurvedic Medicine, 2023; 7: 1-6.
6. Sangita Chandra, Priyanka Chatterjee, Protapaditya Dey, Sanjib Bhattacharya. Evaluation of In vitro anti inflammatory activity of coffee against the denaturation of proteins. Asian Pacific Journal of Tropical Biomedicine, 2012; S178-S180.
7. Remya SB, P SCM, Aparna P. A Review on Cultivation, Propagation and Harvesting of *Crossandra infundibuliformis*. International Journal of Research Publications and Reviews, 2022; 3: 260-286.
8. Anil Panghal1, Anjali Onakkaramadom Shaji, Kiran Nain, Mukesh Kumar Garg1, NavnidhiChhikara. *Cnidoscolus aconitifolius*: Nutritional, Phytochemical composition and health benefits. Bioactive Compounds in Health and Diseases, 2021; 4: 260-286.
9. Anjitha A.A, Dr. Shebina P Raseed, Dr.Prasanth S.S, Sujith Unnikrishnan, Anziya P. R Riya Babu. An Ayurvedic Dosage Form "Gutika".An Overview. Int. J. Pharm. Sci. Res, 2023; 79: 36-41.
10. Vijay Gupta, Karthik Iyer, Shruti Shah and Sonali Patil. Standardization and detailed aspects of Marichadi Gutika. Journal of Medicinal Plants Studies, 2019; 7: 274-277.
11. Ukharde Rohan Sandu, Pro.S.A.Sul, Dr.Santosh Jain. Formulation and Evaluation of Gutika For PCOD Treatment. International Journal of Novel Research and Development, 2023; 8: 159-163.

12. Keerthy S Namboodiri, Prathviraj, Puranik Nagaratna. J Formulation and Standardization of Trivrutadi Gutika – An Ayurvedic Formulation International Journal of Ayurvedic Medicine, 2020; 11: 50-54.
13. Sonia Paliwal. Standardisation and Evaluation of Two Marketed Polyherbal Formulation (Gutikas). Asian J Phrm Clin Res, 2020; 13: 81-83.
14. Madhuranga HDT and Samarakoon DNAW in Vitro Anti-inflammatory Egg Albumin Denaturation Assay: An Enhanced Approach. Journal of Natural & Ayurvedic Medicine, 2023; 7: 1-6.
15. Mounyr Balouiri N, Moulay Sadiki, Saad Koraichi Ibnsouda. Methods for in vitro evaluating anti microbial activity: A Review, Journal of Pharmaceutical Analysis, 2016; 1: 71-79.
16. Rahul Manmode, Jagdish, Manwar, Mustafa Vohra, Satish Padgilwar, Nitin Bhajipale, Effect of Preparation Method on Antioxidant Activity of Ayurvedic Formulation Kumaryasava. Journal of Homeopathy & Ayurvedic Medicine, 2012; 1(4): 1-5.