

COMPARATIVE PHARMACEUTICO-ANALYTICAL AND IN VITRO ANTIOXIDANT ACTIVITY OF *GUDUCHI* (TINOSPORA CORDIFOLIA) SWARASA AND *GUDUCHI* GHRITA

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ABSTRACT

Background: *Guduchi* (*Tinospora cordifolia*), known as *Amrita* in *Ayurveda*, is a renowned *Rasayana* drug with diverse therapeutic applications. It is administered in various dosage forms, among which *Guduchi Swarasa* (fresh juice) is used as *Medhya Rasayana* as per *Charakacharya*. However, it has some limitations such as less shelf life, need for fresh preparation. *Guduchi Ghrita* (medicated ghee) is considered highly effective. However, scientific evidence comparing their pharmaceutical characteristics and antioxidant potential remains limited. **Aim:** To prepare and standardize *Guduchi Swarasa* and *Guduchi Ghrita*, evaluate their organoleptic and physicochemical characteristics, determine their total phenolic content, and assess their antioxidant activity using DPPH free radical scavenging assay. **Materials and Methods:** *Guduchi Swarasa* was prepared by extracting fresh juice from *Guduchi* stems following classical methods. *Guduchi Ghrita* was prepared through *Sneha Kalpana* using *Murcchita Go-Ghrita*,

Guduchi Kalka, and water. Both formulations were subjected to organoleptic evaluation and physicochemical analysis. Total phenolic content was estimated by the Folin-Ciocalteu Reagent (FCR) method, and antioxidant activity was evaluated using DPPH assay with

Ascorbic Acid as standard. **Results:** *Guduchi Swarasa* appeared as greenish-brown liquid (pH 6.69, specific gravity 0.99, refractive index 1.344), while *Guduchi Ghrita* was an off-white thick solid mass (pH 6.80, specific gravity 0.89, refractive index 1.47, acid value 3.5, iodine value 42, saponification value 182). Total phenolic content at 100 µg/mL was 74.13 µg GAE/g for *Guduchi Swarasa* and 51.00 µg GAE/g for *Guduchi Ghrita*. DPPH assay showed concentration-dependent antioxidant activity with IC₅₀ values of 72.49 µg/mL for *Guduchi Swarasa* and 77.67 µg/mL for *Guduchi Ghrita*. **Conclusion:** Both formulations demonstrated significant antioxidant potential. *Guduchi Swarasa* exhibited superior activity, likely due to higher phenolic content. However, *Guduchi Ghrita* offers advantages of enhanced stability and palatability through *Sneha Kalpana*.

1. INTRODUCTION

Guduchi (*Tinospora cordifolia* (Willd.) Miers, family Menispermaceae) is one of the most important *Rasayana* drugs described in Ayurveda, extensively used for its immunomodulatory, antioxidant, anti-inflammatory, and rejuvenative properties. Classical texts describe it as *Tikta-Katu-Kashaya* in *Rasa*, *Ushna* in *Virya*, and *Madhura* in *Vipaka*, with *Tridoshahara*, *Jwaraghna*, *Deepana*, and *Rasayana* actions. Modern phytochemical studies have revealed the presence of alkaloids, diterpenoid lactones, glycosides, steroids, phenolic compounds, and polysaccharides contributing to its diverse pharmacological activities.^[1,2,3]

Sneha Kalpana represents one of the most commonly employed pharmaceutical processes in Ayurveda, wherein oleaginous preparations are formulated by combining *Kalka* (fine paste), *Kwatha* (decoction), and other drugs in specified proportions, followed by controlled heating. Among the four types of *Sneha*—*Ghrita*, *Taila*, *Vasa*, and *Majja*—*Ghrita* is considered superior due to its *Yogavahi* property, which facilitates the assimilation of drug properties while retaining its inherent qualities.^[4,5]

As per *Sharangdhara Acharya*, *Guduchi* should always be used in its fresh or moist form.^[6] *Guduchi Swarasa*, being the fresh juice of the plant, represents the most potent dosage form among *Panchavidha Kashaya Kalpanas* due to maximum retention of active phytoconstituents and rapid therapeutic action. Conversely, *Guduchi Ghrita*, prepared through *Sneha Kalpana*, facilitates the incorporation of active constituents into a lipid medium, enhancing palatability, bioavailability, and sustained therapeutic action. These rejuvenative therapies (*Rasayanas*) grant long life, eradicate diseases, and enhance physical

strength, digestive power (Agni), skin complexion, and voice quality.^[7]

Oxidative stress is recognized as a major contributor to numerous chronic, metabolic, inflammatory, and degenerative diseases. Phenolic compounds and natural antioxidants play a crucial role in neutralizing free radicals and protecting tissues from oxidative damage. Therefore, this study was undertaken to prepare, standardize, and comparatively evaluate the antioxidant potential of *Guduchi Swarasa* and *Guduchi Ghrita*.

2. Need of study

Nowadays, people prefer products that are easy to administer & having good taste. There is also a need for formulations that remain stable for a longer time & maintain consistent quality. *Guduchi Swarasa*, although effective, does not always meet these requirements, because of its short shelf life and inconvenience, its wider use becomes limited.

To overcome these issues there is need to convert *Guduchi Swarasa* into *Ghrita*. Such modifications can help in improving convenience & long shelf life.

3. MATERIALS AND METHODS

3.1 Study Design

The study was conducted in three phases:

1. **Conceptual Study:** Review of *Sneha Kalpana*, *Guduchi*, and related concepts from Ayurvedic texts.
2. **Pharmaceutical Study:** Preparation of *Guduchi Swarasa* and *Guduchi Ghrita*.
3. **Analytical and Experimental Study:** Organoleptic, physicochemical, and antioxidant evaluation.

3.2 Pharmaceutical Study

3.2.1 Preparation of *Murcchita Go-Ghrita*^[8]

Ingredients for *Murcchana*

Ingredient	Quantity
<i>Haritaki</i>	15.63 gm
<i>Bibhitaki</i>	15.63 gm
<i>Amalaki</i>	15.63 gm
<i>Musta</i>	15.63 gm
<i>Haridra</i>	15.63 gm
<i>Matulunga Swarasa</i>	15.63 ml
Water	1 Litre
<i>Go-Ghrita</i>	250 ml

Procedure

All *churna* were mixed thoroughly, and *Matulunga Swarasa* was added to prepare *Kalka*. *Ghrita* was heated gently over *Mandagni*, *Kalka* was added along with water, and heating continued until *Siddhi Lakshana* appeared. The total time required was 2 hours 15 minutes, with a loss of 10 ml from 250ml initial quantity.



3.2.2 Preparation of *Guduchi Ghrita*.^[9]

Ingredient	Quantity
<i>Guduchi Kalka</i>	50 gm
<i>Murcchita Ghrita</i>	200 ml
Water	800 ml

Ingredients

Procedure

Fresh *Guduchi* stems were collected, washed, cut into small pieces, and crushed to form a homogenous pulp. *Murcchita Ghrita* was heated with *Guduchi Kalka* and water until *Siddhi Lakshana* appeared. Total time required was 2 hours 25 minutes.

Shelf life of *sneha* – According to *Acharya Sharangdhara*, the shelf life of *sneha* is 16 months.^[10]

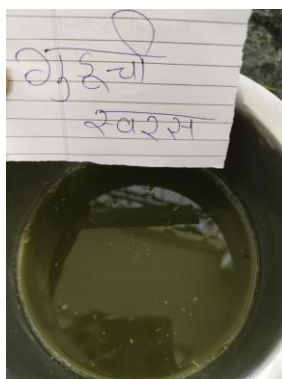
Dose(matra)

Bhaisajya ratnavali^[11]- *Uttama matra* 1 pala, *Madhyama matra* 3 tola, *Heena matra* 2 tola
Sharangadhara^[12]- 1 pala



3.2.3 Preparation of *Guduchi Swarasa*^[13]

Fresh *Guduchi* stems were collected, thoroughly washed, cut into small pieces, and crushed to form a homogeneous pulp. The pulp was expressed through clean muslin cloth, and the extracted juice was filtered to obtain *Guduchi Swarasa*.



3.3 Analytical Study

3.3.1 Organoleptic Evaluation

Both formulations were evaluated for colour, odour, taste, appearance, and consistency.

3.3.2 Physicochemical Analysis^[14,15,16]

Guduchi Swarasa

- pH Value
- Specific Gravity
- Refractive Index

Guduchi Ghrita

- pH Value
- Specific Gravity
- Refractive Index
- Acid Value

- Iodine Value
- Saponification Value
- Viscosity

3.3.3 Estimation of Total Phenolic Content (FCR Method)^[17]

Total phenolic content was determined using the Folin-Ciocalteu Reagent method. Sample concentrations of 20, 40, 60, 80, and 100 µg/mL were used. Gallic acid was employed as the standard reference. Absorbance was measured at 550 nm after 90 minutes of incubation.

3.4 Experimental Study - DPPH Antioxidant Assay^[18]

Antioxidant activity was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Different concentrations (20, 40, 60, 80, 100 µg/mL) of samples were prepared. DPPH solution (0.1% in methanol) was added to each concentration, and after 30 minutes incubation at room temperature, absorbance was measured at 520 nm. Ascorbic Acid served as the standard reference. Percentage inhibition was calculated using the formula:

DPPH radical scavenging activity (%) = [(Absorbance of control – Absorbance of test sample) / Absorbance of control] × 100

IC₅₀ values were determined from the inhibition curves.

4. OBSERVATION AND RESULTS

4.1 Organoleptic Evaluation

Parameter	<i>Guduchi Swarasa</i>	<i>Guduchi Ghrita</i>
Colour	Greenish-brown liquid	Greenish Yellow
Odour	Characteristic	Characteristic
Taste	Bitter (<i>Tikta</i>)	Predominantly bitter with unctuous taste
Consistency	Liquid	Thick solid/semi-solid mass

4.2 Physicochemical Analysis

4.2.1 *Guduchi Swarasa*

Parameter	Result
pH Value	6.69
Specific Gravity	0.99
Refractive Index	1.344

4.2.2 *Guduchi Ghrita*

Parameter	Result
pH Value	6.80
Specific Gravity	0.89
Refractive Index	1.47

Acid Value	3.5
Iodine Value	42
Saponification Value	182

4.3 Total Phenolic Content (FCR Method)

Table 1: Total Phenolic Content of Gallic acid.

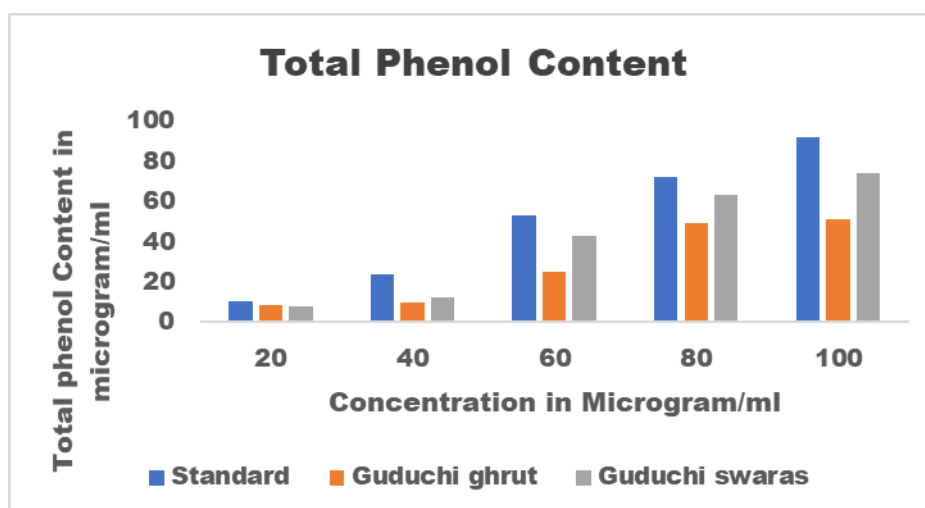
Sr. no.	Gallic acid Concentration (µg/mL)	Absorbance at 550 nm	Concentration of Total gallic acid contents (µg GA/g) /Total Phenol Content
1	20	0.102	10.2
2	40	0.241	24.1
3	60	0.532	53.2
4	80	0.721	72.1
5	100	0.920	92.0

Table 2: Total Phenolic Content of *Guduchi Ghrita*.

Concentration (µg/mL)	Absorbance (Mean)	Total Phenolic Content (µg GAE/g)
20	0.084	8.40
40	0.103	10.03
60	0.253	25.30
80	0.495	49.50
100	0.510	51.00

Table 3: Total Phenolic Content of *Guduchi Swarasa*.

Concentration (µg/mL)	Absorbance (Mean)	Total Phenolic Content (µg GAE/g)
20	0.078	7.80
40	0.125	12.50
60	0.427	42.79
80	0.636	63.61
100	0.741	74.13



Graph 1: Total Phenolic Content - Comparative Analysis.

The FCR analysis demonstrated a concentration-dependent increase in total phenolic content in both formulations. *Guduchi Swarasa* exhibited significantly higher total phenolic content compared to *Guduchi Ghrita* at all tested concentrations. At the highest concentration (100 µg/mL), *Guduchi Swarasa* showed 74.13 µg GAE/g compared to 51.00 µg GAE/g for *Guduchi Ghrita*, indicating approximately 45% higher phenolic content in the *Swarasa* preparation.

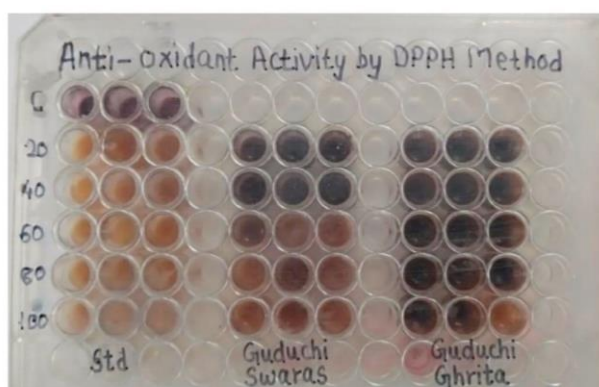
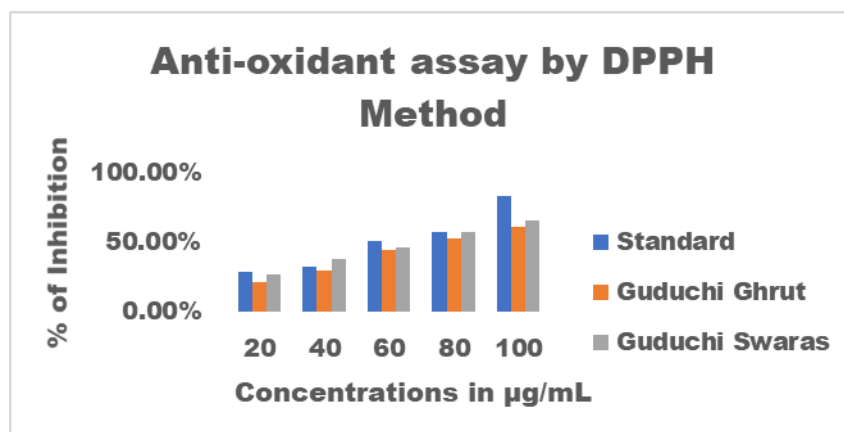


4.4 Antioxidant Activity (DPPH Assay)

Table 3: DPPH Radical Scavenging Activity.

SR.NO	Sample Code	Concentration (µg/mL)	Absorbance at 510 nm				% Inhibition	IC ₅₀ (µg/mL)
			Test 1	Test 2	Test 3	Mean		
1	Control	-	1.93	1.93	1.93	1.93	-	
2	Standard	20	1.34	1.37	1.39	1.37	29.02%	72.49
	(Ascorbic Acid)	40	1.31	1.29	1.33	1.31	32.12%	
		60	0.95	0.97	0.93	0.95	50.77%	
		80	0.82	0.85	0.79	0.82	57.51%	
		100	0.35	0.32	0.32	0.33	82.90%	
3	<i>Guduchi Ghrita</i>	20	1.48	1.52	1.54	1.51	21.76%	77.67
		40	1.39	1.33	1.37	1.36	29.53%	
		60	1.05	1.08	1.12	1.08	44.04%	
		80	0.92	0.89	0.95	0.92	52.33%	
		100	0.75	0.79	0.72	0.75	61.14%	
4	<i>Guduchi Swarasa</i>	20	1.38	1.41	1.44	1.41	26.94%	72.49

		40	1.17	1.21	1.23	1.20	37.82%
		60	1.01	1.03	1.05	1.03	46.63%
		80	0.78	0.81	0.86	0.82	57.51%
		100	0.66	0.65	0.7	0.67	65.28%



Graph 2: DPPH Radical Scavenging Activity - Comparative Analysis.

Guduchi Swaras exhibited superior antioxidant activity over *Guduchi Ghrita*, with higher inhibition rates and a lower IC_{50} value (72.49 µg/mL vs. 77.67 µg/mL), suggesting the fresh aqueous juice retains active antioxidants more effectively. Standard Ascorbic Acid showed the overall highest radical scavenging efficiency.

5. Statistical Analysis

5.1 DPPH Assay - Two-way ANOVA

Source of Variation	SS	df	MS	F value	p value	Significance
Treatment (Sample)	1265.84	2	632.92	47.63	<0.0001	Highly significant
Concentration	5144.72	4	1286.18	96.82	<0.0001	Highly significant
Treatment × Concentration Interaction	121.54	8	15.19	1.14	0.36	NS
Error (Residual)	398.91	30	13.30			
Total	6931.01	44				

Interpretation: Two-way ANOVA revealed that both treatment type and concentration had highly significant effects on DPPH radical scavenging activity ($p < 0.0001$). The interaction between treatment and concentration was not significant ($p = 0.36$), indicating that all samples followed a similar concentration-dependent pattern of antioxidant response.

5.2 Tukey's Multiple Comparison Test

Comparison of Treatment Groups

Comparison	Mean Difference	p value	Result
Ascorbic acid vs <i>Guduchi Ghrita</i>	10.73	<0.001	Significant
Ascorbic acid vs <i>Guduchi Swarasa</i>	7.32	<0.01	Significant
<i>Guduchi Swarasa</i> vs <i>Guduchi Ghrita</i>	3.41	<0.05	Significant

Interpretation: Tukey's post-hoc analysis confirmed significant differences among all treatment groups. The antioxidant activity order was: **Ascorbic Acid** > *Guduchi Swarasa* > *Guduchi Ghrita*.

5.3 Total Phenolic Content - Two-way ANOVA

Source of Variation	SS	df	MS	F value	p value	Significance
Formulation (Sample)	1074.12	1	1074.12	12.64	0.004	Significant
Concentration	6674.87	4	1668.72	19.63	<0.001	Highly significant
Formulation × Concentration Interaction	263.45	4	65.86	0.77	0.56	NS
Error (Residual)	850.20	10	85.02			
Total	8862.64	19				

Interpretation: Two-way ANOVA showed that formulation type had a significant effect on phenolic content ($p = 0.004$), while concentration had a highly significant effect ($p < 0.001$). The overall mean phenolic content of *Guduchi Swarasa* (40.17 μg GAE/g) was approximately 39% higher than *Guduchi Ghrita* (28.85 μg GAE/g). The interaction between formulation and concentration was non-significant ($p = 0.56$).

Formulation-wise Comparison

Comparison	Mean Difference	p value	Result
<i>Guduchi Swarasa</i> vs <i>Guduchi Ghrita</i>	11.32	0.004	Significant

6. DISCUSSION

6.1 Pharmaceutical Considerations

The successful preparation of both *Guduchi Swarasa* and *Guduchi Ghrita* according to classical references demonstrates the reproducibility of traditional Ayurvedic pharmaceutical

techniques. The preparation of *Murcchita Go-Ghrita* involved meticulous attention to the *Siddhi Lakshana*, including the absence of crackling sound, wick-like formation of *Kalka*, absence of moisture, characteristic colour, and pleasant aroma of *Murcchana* drugs.

The organoleptic characteristics of both formulations were consistent with their expected properties. *Guduchi Swarasa* exhibited the characteristic greenish-brown colour, which can be attributed to the presence of chlorophyll, alkaloids, glycosides, and other water-soluble compounds. The pronounced bitter taste (*Tikta Rasa*) is consistent with the classical description of *Guduchi* and is associated with its *Deepana*, *Pachana*, *Jwaraghna*, and *Tridosahara* properties.

In contrast, *Guduchi Ghrita* appeared as a greenish yellow thick solid mass with characteristic odour. The transformation in colour and consistency reflects the successful incorporation of *Guduchi* constituents into the *Ghrita* medium through *Sneha Kalpana*. The unctuous nature of *Ghrita* moderated the intense bitterness, improving palatability while retaining therapeutic properties.

6.2 Physicochemical Evaluation

The near-neutral pH values of both formulations (6.69 for *Swarasa* and 6.80 for *Ghrita*) indicate suitability for internal administration without gastrointestinal irritation. The specific gravity of *Guduchi Swarasa* (0.99) confirms its aqueous nature, while the lower specific gravity of *Guduchi Ghrita* (0.89) reflects its lipid-based characteristics. The higher refractive index of *Guduchi Ghrita* (1.47) compared to *Swarasa* (1.344) indicates the presence of fatty acids and lipid-soluble phytoconstituents incorporated during processing.

The acid value of 3.5 for *Guduchi Ghrita* indicates minimal hydrolytic degradation and good stability. The iodine value of 42 reflects the degree of unsaturation in fatty acids, while the saponification value of 182 confirms appropriate fatty acid molecular characteristics. These values are consistent with standard medicated *Ghrita* preparations and confirm the quality of the lipid base.

6.3 Total Phenolic Content

The Folin-Ciocalteu assay revealed significant phenolic content in both formulations. *Guduchi Swarasa* demonstrated consistently higher total phenolic content than *Guduchi Ghrita* at all tested concentrations. At 100 µg/mL, *Guduchi Swarasa* showed 74.13 µg GAE/g

compared to 51.00 µg GAE/g for *Guduchi Ghrita*, indicating approximately 45% higher phenolic content in the fresh juice preparation.

This finding is consistent with the Ayurvedic principle that *Swarasa*, being freshly expressed juice, retains maximum water-soluble phytoconstituents including phenolic compounds. Phenolic compounds are important secondary metabolites known for their antioxidant, anti-inflammatory, and free radical scavenging properties. The presence of substantial phenolic content in *Guduchi Ghrita*, despite being a lipid-based formulation, confirms the successful transfer of phenolic compounds from *Guduchi* into the *Ghrita* medium during *Sneha Kalpana*.

The concentration-dependent increase in phenolic content observed in both formulations validates the reliability of the FCR method and supports the quantitative relationship between sample concentration and phenolic compound availability.

6.4 Antioxidant Activity

The DPPH radical scavenging assay demonstrated significant antioxidant activity in both formulations, with a clear concentration-dependent response. *Guduchi Swarasa* exhibited superior antioxidant activity compared to *Guduchi Ghrita*, with an IC₅₀ value of 72.49 µg/mL, comparable to that of Ascorbic Acid (72.49 µg/mL). *Guduchi Ghrita* showed an IC₅₀ value of 77.67 µg/mL, indicating slightly lower but appreciable free radical scavenging potential.

The statistically significant difference between *Guduchi Swarasa* and *Guduchi Ghrita* (mean difference = 3.41, $p < 0.05$) in Tukey's post-hoc analysis confirms the comparative superiority of *Guduchi Swarasa* in antioxidant activity. This observation correlates well with the higher total phenolic content found in *Guduchi Swarasa*, supporting the established relationship between phenolic constituents and antioxidant capacity.

The antioxidant activity demonstrated by both formulations may be attributed to the presence of various phytoconstituents including alkaloids, diterpenoid lactones, flavonoids, glycosides, and phenolic compounds identified in *Guduchi*. These constituents are known to neutralize reactive oxygen species, donate hydrogen atoms or electrons to stabilize free radicals, and protect cells from oxidative damage.

From an Ayurvedic perspective, the antioxidant activity demonstrated by both formulations

supports the classical description of *Guduchi* as a *Rasayana* drug. *Rasayana* therapy is known to promote longevity, enhance immunity, protect tissues from degeneration, and prevent premature aging. Modern scientific understanding correlates these effects with antioxidant mechanisms that reduce oxidative stress and cellular damage.

6.5 Comparative Analysis

The comparative analysis reveals distinct advantages and disadvantages of each formulation:

Guduchi Swarasa

- **Advantages:** Higher phenolic content, superior antioxidant activity, rapid therapeutic action, retention of heat-sensitive constituents
- **Disadvantages:** Shorter shelf life due to high moisture content, requires fresh preparation, limited stability

Guduchi Ghrita

- **Advantages:** Enhanced stability and shelf life, improved palatability, sustained therapeutic action, better bioavailability of lipid-soluble constituents through *Sneha Kalpana*
- **Disadvantages:** Lower phenolic content, more complex preparation process, longer duration of action

The higher antioxidant activity of *Guduchi Swarasa* may be attributed to the presence of a greater concentration of water-soluble phytoconstituents that are retained in the fresh juice. However, the process of *Sneha Kalpana* offers the advantage of converting these active principles into a more stable, palatable, and therapeutically sustained dosage form while still retaining significant antioxidant activity.

7. CONCLUSION

The present study successfully achieved its objectives of preparing, standardizing, and evaluating *Guduchi Swarasa* and *Guduchi Ghrita*, along with the comparative assessment of their antioxidant activity.

The key conclusions are

1. **Pharmaceutical Preparation:** Both *Guduchi Swarasa* and *Guduchi Ghrita* were successfully prepared according to classical references, demonstrating the reproducibility of traditional Ayurvedic pharmaceutical techniques.

2. **Organoleptic Characteristics:** *Guduchi Swarasa* possessed a characteristic greenish-brown colour, characteristic odour, and bitter taste, while *Guduchi Ghrita* appeared as a greenish yellow thick solid mass with characteristic odour and properties of a properly processed medicated *Ghrita*.
3. **Physicochemical Quality:** Both formulations exhibited acceptable physicochemical parameters. *Guduchi Swarasa* showed pH 6.69, specific gravity 0.99, and refractive index 1.344. *Guduchi Ghrita* showed pH 6.80, specific gravity 0.89, refractive index 1.47, acid value 3.5, iodine value 42, and saponification value 182, all within acceptable limits for quality medicated *Ghrita* preparations.
4. **Total Phenolic Content:** The FCR analysis confirmed significant phenolic content in both formulations, with a concentration-dependent increase. *Guduchi Swarasa* exhibited higher total phenolic content (74.13 µg GAE/g at 100 µg/mL) compared to *Guduchi Ghrita* (51.00 µg GAE/g at 100 µg/mL), indicating approximately 45% higher phenolic content in the fresh juice preparation.
5. **Antioxidant Activity:** The DPPH assay revealed significant antioxidant activity in both formulations. *Guduchi Swarasa* showed superior activity with an IC₅₀ value of 72.49 µg/mL, comparable to the standard Ascorbic Acid, while *Guduchi Ghrita* showed an IC₅₀ value of 77.67 µg/mL. Both formulations demonstrated concentration-dependent free radical scavenging activity.
6. **Statistical Validation:** Two-way ANOVA revealed highly significant effects of treatment type and concentration on antioxidant activity ($p < 0.0001$). Tukey's multiple comparison test confirmed significant differences among all treatment groups, with the activity order: Ascorbic Acid > *Guduchi Swarasa* > *Guduchi Ghrita*.
7. **Therapeutic Significance:** Both *Guduchi Swarasa* and *Guduchi Ghrita* demonstrated antioxidant potential, supporting their traditional therapeutic significance as *Rasayana* drugs. The higher antioxidant activity of *Guduchi Swarasa* suggests its suitability for conditions requiring rapid action, while *Guduchi Ghrita* offers enhanced stability and palatability for sustained therapeutic use.
8. **Pharmacological Rationale:** The higher phenolic content of *Guduchi Swarasa* correlates with its superior antioxidant activity, providing scientific evidence for the relationship between phenolic constituents and antioxidant potential.

In conclusion, this study provides scientific evidence for the pharmaceutical quality and antioxidant potential of both *Guduchi Swarasa* and *Guduchi Ghrita*. The findings validate

their traditional therapeutic use and support their utility in the management of oxidative stress-related disorders. The study also highlights the importance of pharmaceutical processing in determining the therapeutic characteristics of Ayurvedic formulations.

8. Scope for Further Study

1. **Quantification of Marker Compounds:** HPTLC/HPLC analysis to quantify lipid-soluble versus water-soluble constituents extracted into both formulations
2. **Fatty Acid Profile Analysis:** Evaluation of *Ghrita* formulations before and after processing to understand changes in fatty acid composition
3. **Thermal Degradation Kinetics:** Comparative study of thermolabile antioxidants during boiling phase of *Ghrita* preparation
4. **Comparative Clinical Studies:** Evaluation of therapeutic efficacy in specific disease conditions
5. **Stability Studies:** Assessment of shelf life and stability of both formulations under various storage conditions

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