



ORIGINAL ARTICLE

Pharmaceutical Preparation and *In Vitro* Evaluation of *Narikela Khanda*: A Classical Ayurvedic Formulation for Gastrointestinal Disorders

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ARTICLE HISTORY

Received: 04 October 2025

Accepted: 03 December 2025

Available online

Version 1.0 : 31 December 2025

Keywords

Narikela Khanda, *Khanda Kalpana*, Cytotoxicity, Antioxidant activity, *Rasayana*

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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CITE THIS ARTICLE

Avanish KSP, Noble TM. Pharmaceutical Preparation and *In Vitro* Evaluation of *Narikela Khanda*: A Classical Ayurvedic Formulation for Gastrointestinal Disorders. Kerala Journal of Ayurveda. 2025;4(4):49-59. <https://doi.org/10.55718/kja.489>



Abstract

Background: *Narikela Khanda*, a classical Ayurvedic formulation mentioned in *Bhaisajya Ratnavali (Śūla Roga Prakaraṇa)*, is indicated in *Amlapitta* and allied gastrointestinal disorders. It is a modification of *Avaleha Kalpana*, prepared with *Narikela (Cocos nucifera)*, *Ghṛita*, *Khanda Sharkara*, and selected *prakshepa dravyas*. Traditionally, it is attributed with *deepana-pachana*, *pitta-shamana*, *rakta-stambhana*, and *rasayana* properties. From a biomedical perspective, gastrointestinal pathologies like peptic ulcers and bleeding disorders involve oxidative stress, necessitating scientific validation of antioxidant and safety potential of classical formulations.

Objectives: To prepare *Narikela Khanda* as per classical guidelines and evaluate its *in vitro* safety (MTT assay) and antioxidant potential (ABTS, DPPH, and FRAP assays).

Methods: Pharmaceutical preparation was carried out using authentic raw materials under controlled conditions. Cytotoxicity was assessed on L929 fibroblast cell lines using the MTT assay at concentrations ranging from 6.25-100 µg/mL. Antioxidant assays including ABTS, DPPH, and FRAP were performed at concentrations from 1.56-1000 µg/mL.

Results: The formulation exhibited no cytotoxicity, with >90% cell viability in all tested concentrations, confirming its safety on normal fibroblast cells. Antioxidant assays demonstrated dose-dependent radical scavenging and reducing potential. IC₅₀ values were found to be 447.50 µg/mL (ABTS) and 735.82 µg/mL (DPPH). FRAP assay confirmed the electron-donating capacity of the formulation.

Conclusion: *Narikela Khanda* was found to be safe *in vitro* and exhibited measurable antioxidant activity, validating its classical claims as a gastroprotective, haemostatic, and *Rasayana* formulation. These findings support its potential therapeutic role in oxidative stress-mediated gastrointestinal disorders like *Amlapitta* and *Raktapitta*. Further *in vivo* and clinical studies are recommended to substantiate its efficacy.

Introduction

Bhaisajya Kalpanā, one of the core branches of *Āyurveda*, deals with the systematic transformation of raw herbs and other natural substances into therapeutically effective dosage forms suitable for clinical administration. The foundational framework of this discipline is based on *Pañcavidha Kaṣāya Kalpanā*, namely *Svarasa* (expressed juice), *Kalka* (paste), *Kvātha* (decoction), *Hima* (cold infusion), and *Phāṇṭa* (hot infusion), which serve as the primary pharmaceutical preparations. Over centuries, with the objective of enhancing stability, palatability, shelf life, and therapeutic potency, more complex and standardized dosage forms were evolved, including *Avaleha*, *Guḍa*, *Khanda*, *Vaṭi*, *Taila*, *Ghṛta*, and others.

Among these, *Khanda Kalpanā* occupies a distinct and significant position in *Āyurvedic pharmaceuticals*. The term *Khanda* denotes “fragment” or “granule,” and the formulation is characterized by its free-flowing, crystalline granular consistency obtained through the processing of sugar syrup with herbal extracts (*Svarasa*, *Kvātha*, *Hima*, or *Phāṇṭa*). It is essentially a modified form of *Avaleha Kalpanā*, wherein the final stage of *Pāka* does not yield a semi-solid mass but instead results in a granular, non-sticky product. This structural modification improves shelf stability while also enhancing *patient palatability* and acceptability, making it more suitable for long-term therapeutic use.

One such formulation, *Nārikelā Khanda*, is described in *Bhaisajya Ratnāvali*, *Śūla Roga Prakaraṇa*, for the management of *Śūla* (colicky abdominal pain) associated with *Amlapitta* and related gastrointestinal disorders [1]. Classical descriptions associate its therapeutic actions with clinical conditions resembling peptic ulcer disease, gastrointestinal bleeding, and dyspepsia, wherein *Pitta* and *Rakta duṣṭi* play a central role in pathogenesis. The formulation contains *Nārikelā* (*Cocos nucifera*) as the chief ingredient, along with *Ghṛta*, *Khanda Śarkarā*, and a group of *Prakṣepa Dravyas* including *Dhānyaka* (*Coriandrum sativum* Linn.), *Pippalī* (*Piper longum* Linn.), *Vaṁśalocana* (siliceous concretion of *Bambusa bambos* (L.) Voss), *Jīraka* (*Cuminum cyminum* Linn.), *Nāgarmothā* (*Cyperus rotundus* Linn.), *Tvak* (*Cinnamomum verum* J. Presl), *Elā* (*Elettaria cardamomum* (L.) Maton), *Patra* (*Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm.), *Nāgakeśara* (*Mesua ferrea* Linn.)

Collectively, these ingredients impart *Dīpana–Pācana* (digestive and metabolic enhancement), *Pitta–śamana* (pacification of aggravated *Pitta*), *Rakta-stambhana* (haemostatic action), and *Rasāyana* (rejuvenative) effects.

From a contemporary biomedical perspective, the pathogenesis of peptic ulcer disease and related

gastrointestinal disorders involves oxidative stress, excessive free radical generation, mucosal inflammation, and impaired cytoprotective mechanisms. In this context, antioxidant-rich *Āyurvedic* formulations may play a crucial role in scavenging free radicals, strengthening mucosal defenses, and mitigating inflammatory damage, thereby offering mechanistic support to their classical indications. Despite its repeated description in classical literature and ongoing use in *Āyurvedic* practice, systematic scientific validation of *Nārikelā Khanda*, particularly in terms of its safety and antioxidant potential, remains limited.

To address this research gap, the present study was designed with the following objectives:

1. To prepare *Nārikelā Khanda* pharmaceutically in accordance with classical *Āyurvedic* guidelines.
2. To evaluate its safety profile through an *in vitro* MTT cytotoxicity assay using normal fibroblast (L929) cell lines.
3. To assess its antioxidant activity using ABTS, DPPH, and FRAP assays for determining free radical scavenging and reducing potential.

By integrating traditional *Āyurvedic* pharmaceutical principles with contemporary analytical methodologies, this study aims to provide scientific substantiation for the classical claims attributed to *Nārikelā Khanda* as a gastroprotective, haemostatic, and antioxidant-rich *Rasāyana* formulation, thereby supporting its potential relevance in present-day clinical practice.

Methodology of Study:

Pharmaceutical Preparation of *Nārikelā Khanda*

For the preparation of *Nārikelā Khanda*, all raw materials of assured quality were procured from the Pharmacy of Government Ayurveda College, Tripunithura. All ingredients were authenticated and weighed accurately as per classical references before processing. Initially, the dry herbal drugs were thoroughly washed with clean tap water to remove surface impurities. They were then shade-dried to preserve their phytoconstituents. After complete drying, each drug was powdered individually using a mechanical pulverizer and stored in separate, labeled airtight containers.

Dried coconuts were procured from the Puthiyakavu market. The fibrous husk and hard shell were carefully removed to obtain the coconut kernel. The kernels were washed with potable water, grated using a stainless-steel grater, and measured accurately in the required quantity. Fresh coconut water was extracted from mature coconuts. It was filtered through a clean muslin cloth to remove suspended impurities and kept aside for syrup preparation.



Figure 1
Raw *Prakshepa Dravya*



Figure 2
Grated Coconut Pulp



Figure 3
Pure Cows Ghee



Figure 4
Frying of Pulp with Ghrita



Figure 5
Fried Reddish Brown Pulp



Figure 6
Preparation of 1 Thread
Consistency of Paka



Figure 7
Mixing of Pulp with Paka
of KhandaSharkara



Figure 8
Final Product, Narikela
Khanda

Table 1. *Rasa Pañchaka* of the Ingredients of *Narikela Khanda*

Name of the Drug	Reference	Latin Name	Quantity	Rasa	Guna	Vīrya	Vipāka	Karma
<i>Narikela</i> Pulp (Grated Coconut) [2]	Bhāvaprakāśa Amradiphala Varga 9/38-39	<i>Cocos nucifera</i>	1 <i>Kudava</i> (192 g)	<i>Kaṣāya</i> , <i>Mādhura</i>	<i>Snigdha</i> , <i>Guru</i>	<i>Śīta</i>	<i>Mādhura</i>	<i>Bālya</i> , <i>Br̥mhaṇa</i> , <i>Daha-śamana</i> , <i>Basti-śodhana</i>
<i>Narikela</i> Jala (Coconut Water) [3]	Bhāvaprakāśa Amradiphala Varga 9/41	<i>Cocos nucifera</i>	1 <i>Prastha</i> (768 g)	<i>Mādhura</i>	<i>Laghu</i> , <i>Śīta</i>	<i>Śīta</i>	<i>Mādhura</i>	<i>Dhātu-varadhana</i> , <i>Pipāsā-jita</i> , <i>Agnidīpana</i> , <i>Hṛdya</i> , <i>Śukrala</i>
<i>Ghṛita</i> (Cow's Ghee)	—	—	1 <i>Pala</i> (48 g)	<i>Mādhura</i>	<i>Snigdha</i>	<i>Śīta</i>	<i>Mādhura</i>	<i>Yogavāhī</i> , <i>Bālya</i>
<i>Khanda Śarkarā</i> (Sugar Candy)	—	—	1 <i>Kudava</i> (192 g)	<i>Mādhura</i>	<i>Guru</i> , <i>Śīta</i>	<i>Śīta</i>	<i>Mādhura</i>	<i>Bālya</i> , <i>Pittahara</i>
<i>Dhānyak</i> ^[4]	Bhāvaprakāśa Haritakyādi Varga 22/86-88	<i>Coriandrum sativum</i>	1 <i>Śana</i> (3 g)	<i>Kaṣāya</i> , <i>Tikta</i> , <i>Kaṭu</i>	<i>Laghu</i> , <i>Snigdha</i>	<i>Uṣṇa</i>	<i>Mādhura</i>	<i>Dīpana</i> , <i>Pācana</i> , <i>Pittāśamana</i> , <i>Grahi</i>
<i>Pippali</i> ^[5]	Bhāvaprakāśa Haritakyādi Varga 6/53-58	<i>Piper longum</i>	1 <i>Śana</i> (3 g)	<i>Kaṭu</i>	<i>Laghu</i> , <i>Snigdha</i>	<i>Anuṣṇa</i>	<i>Mādhura</i>	<i>Agni-varadhana</i> , <i>Vṛṣya</i> , <i>Śvāsa-Kāsa-hara</i>
<i>Nāgaramotha</i> ^[6]	Bhāvaprakāśa Karpoorādi Varga 37/92-94	<i>Cyperus rotundus</i>	1 <i>Śana</i> (3 g)	<i>Kaṭu</i> , <i>Tikta</i> , <i>Kaṣāya</i>	<i>Laghu</i> , <i>Grahi</i>	<i>Śīta</i>	<i>Kaṭu</i>	<i>Dīpana</i> , <i>Kapha-Rakta-śamana</i> , <i>Krumighna</i>
<i>Vaṃśalocheda</i> ^[7]	Bhāvaprakāśa Haritakyādi Varga 40/116-117	<i>Bambusa arundinacea</i>	1 <i>Śana</i> (3 g)	<i>Mādhura</i> , <i>Kaṣāya</i>	<i>Laghu</i>	<i>Śīta</i>	<i>Mādhura</i>	<i>Kāsa-śvāsa-hara</i> , <i>Raktapitta-śamana</i> , <i>Mutrakṛcchra-nāśaka</i>
<i>Śveta Jīraka</i> ^[8]	Bhāvaprakāśa Haritakyādi Varga 19/81-83	<i>Cuminum cyminum</i>	1 <i>Śana</i> (3 g)	<i>Kaṭu</i>	<i>Laghu</i> , <i>Rūkṣa</i>	<i>Uṣṇa</i>	<i>Kaṭu</i>	<i>Medhya</i> , <i>Pācana</i> , <i>Kapha-nāśaka</i>
<i>Śyāma Jīraka</i> ^[9]	Bhāvaprakāśa Haritakyādi Varga 19/84-85	<i>Cuminum carvi</i>	1 <i>Śana</i> (3 g)	<i>Kaṭu</i>	<i>Laghu</i> , <i>Rūkṣa</i>	<i>Uṣṇa</i>	<i>Kaṭu</i>	<i>Stanya-janana</i> , <i>Anulomana</i> , <i>Pācana</i>
<i>Tvak (Dalchini)</i> ^[10]	Bhāvaprakāśa Karpoorādi Varga 24/66-67	<i>Cinnamomum zeylanicum</i>	1 <i>Śana</i> (3 g)	<i>Mādhura</i> , <i>Tikta</i>	<i>Laghu</i> , <i>Rūkṣa</i>	<i>Uṣṇa</i>	<i>Mādhura</i>	<i>Vāta-Pitta-śamana</i> , <i>Śukra-balya</i>
<i>Elā (Elaichi)</i> ^[11]	Bhāvaprakāśa Karpoorādi Varga 24/63	<i>Elettaria cardamomum</i>	1 <i>Śana</i> (3 g)	<i>Kaṭu</i>	<i>Sūkṣma</i>	<i>Śīta</i>	<i>Mādhura</i>	<i>Kapha-śamana</i> , <i>Kāsa-śvāsa-hara</i>
<i>Patra (Tejpatra)</i> [12]	Bhāvaprakāśa Karpoorādi Varga 27/68	<i>Cinnamomum tamala</i>	1 <i>Śana</i> (3 g)	<i>Mādhura</i>	<i>Tikṣṇa</i> , <i>Laghu</i> , <i>Picchila</i>	<i>Uṣṇa</i>	<i>Kaṭu</i>	<i>Kapha-Vāta-hara</i> , <i>Aruci-hara</i>
<i>Nāgakeśara</i> ^[13]	Bhāvaprakāśa Karpoorādi Varga 28/69-71	<i>Mesua ferrea</i>	1 <i>Śana</i> (3 g)	<i>Kaṣāya</i>	<i>Laghu</i> , <i>Rūkṣa</i>	<i>Uṣṇa</i>	<i>Kaṭu</i>	<i>Jvara-hara</i> , <i>Raktapitta-śamana</i> , <i>Viśapraśamana</i>

Subsequently, *Khanda Śarkarā* (sugar candy, 192 g) was powdered using a grinder. The powdered sugar was examined organoleptically to ensure purity and absence of foreign matter.

In the next step, the grated coconut kernel was fried in cow's *Ghṛta* over mild heat. The material was stirred continuously until it attained a uniform brownish-red color, indicating proper *bhāvanā* and reduction of moisture content.

Parallely, sugar syrup was prepared using measured coconut water and powdered *Khanda Śarkarā*. The mixture was heated gently and boiled until it attained 2–3 thread consistency (*pāka lakṣaṇa*). Once the desired consistency was achieved, the fried coconut kernel was gradually incorporated into the syrup with continuous stirring.

The heating was continued over a gentle flame. The mixture gradually thickened and reached a homogenous paste-like consistency. At this stage, the finely powdered *prakṣepa dravyas* were added slowly through sieve number 85 to ensure uniform dispersion. The *prakṣepa dravyas* included *Dhānyaka* (*Coriandrum sativum* Linn.), *Pippalī* (*Piper longum* Linn.), *Vaṃśalocana* (siliceous concretion of *Bambusa bambos* (L.) Voss), *Jiraka* (*Cuminum cyminum* Linn.), *Nāgarmothā* (*Cyperus rotundus* Linn.), *Tvak* (*Cinnamomum verum* J. Presl), *Elā* (*Elettaria cardamomum* (L.) Maton), *Patra* (*Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm.), *Nāgakeśara* (*Mesua ferrea* Linn.)

After the addition of *prakṣepa dravyas*, the formulation was mixed thoroughly to achieve uniformity. Heating was discontinued, and the formulation was allowed to cool naturally.

Finally, the cooled *Nārikelā Khanda* was transferred into clean, dry, airtight glass containers. The containers were sealed with aluminum foil to prevent moisture ingress and contamination. Each container was labeled with the name of the formulation, list of ingredients, date of manufacture, dosage (6 *māṣa*-1 *tola*; 6-12 g), indications (*Amlapitta*, *Aruci*, *Kṣayaroga*, *Raktapitta*, *Śūla*, *Vamī*, *Pauruṣa-karī*), and shelf life (3 years), in accordance with the *Drugs & Cosmetics Act, 1945* and the *Drugs and Standards for Ayurvedic Medicines (DSR)*.

Cytotoxicity Assay (MTT)^{[14][15]}

The cytotoxic potential of *Nārikelā Khanda* was evaluated using the MTT assay on L929 mouse fibroblast cell lines procured from the National Centre for Cell Sciences (NCCS), Pune. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. Cultures were maintained

under standard incubation conditions of 37 °C with 5% CO₂ in a humidified atmosphere.

For the assay, cells were harvested in the logarithmic growth phase and seeded in 96-well culture plates at a density of 2.5 x 10³ cells per well. After allowing 24 h for cell attachment, the cells were treated with different concentrations of the test formulation (*Nārikelā Khanda*, sample code CMO3) ranging from 6.25 to 100 µg/mL. Untreated cells served as the control group. Following treatment, the plates were incubated for 24 h under the same standardized conditions. Subsequently, the culture medium was aspirated, and MTT solution (0.5 mg/mL prepared in phosphate-buffered saline, PBS) was added to each well. The plates were then incubated for 4 h, allowing viable cells to reduce the MTT reagent into insoluble formazan crystals. After incubation, the MTT-containing medium was carefully removed, and the formazan crystals were solubilized using dimethyl sulfoxide (DMSO) to obtain a homogenous colored solution.

The absorbance was measured at 570 nm using a microplate reader. Percentage cell viability was calculated by comparing the absorbance of treated wells with that of the control wells, which were considered to represent 100% viability. The results demonstrated that *Nārikelā Khanda* (sample CMO3) did not produce a significant reduction in cell viability at the tested concentrations, indicating non-cytotoxic behavior toward normal fibroblast cells. Representative cellular morphology of control and treated cells was further examined using an inverted phase-contrast microscope, confirming the preservation of normal cell architecture.

Antioxidant Activity - ABTS Assay^[16]

The antioxidant potential of *Nārikelā Khanda* was evaluated using the ABTS radical cation decolorization assay, which measures the free radical scavenging ability of antioxidant compounds. The ABTS•⁺ radical cation was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate, and the reaction mixture was kept in the dark at room temperature for 12-16 h to allow complete radical formation. Prior to use, the ABTS•⁺ solution was diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm.

Varying concentrations of *Nārikelā Khanda* extract (1.56 -1000 µg/mL) were prepared in methanol. An aliquot of each concentration was mixed with the diluted ABTS•⁺ solution and incubated for 6 min at room temperature to allow the reaction to occur. Methanol served as the blank, while the ABTS•⁺ solution without sample served as the control. Trolox was used as the standard reference antioxidant for comparison.

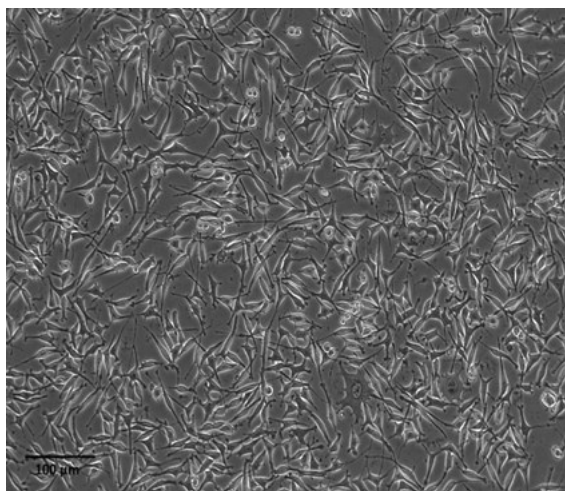


Figure 9. MTT assay of Control Group

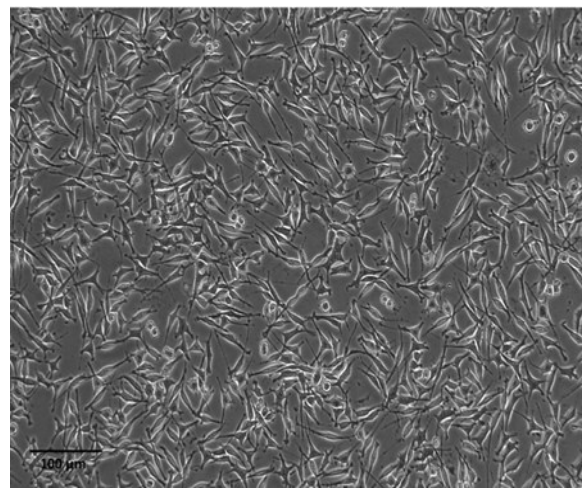


Figure 10. MTT assay at 6.25 conc

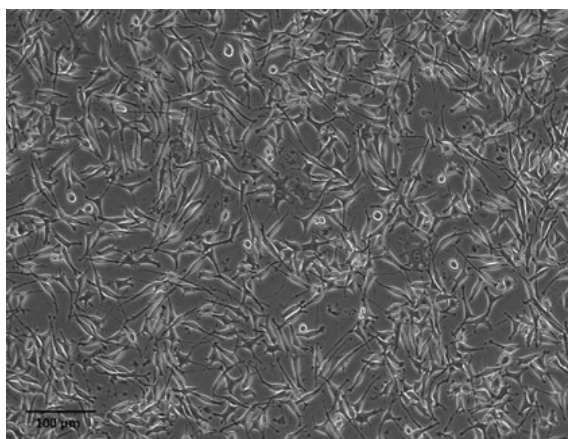


Figure 11. MTT assay at 12.5 conc.

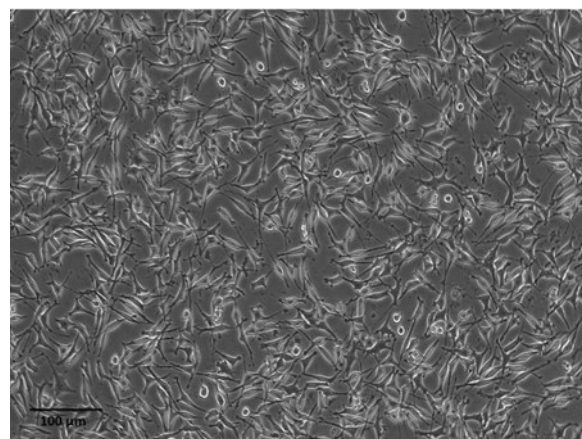


Figure 12. MTT assay at 25 conc.

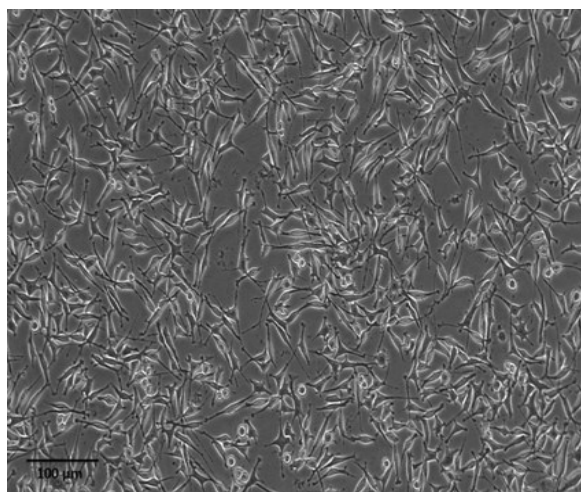


Figure 13. MTT assay at 50 conc.

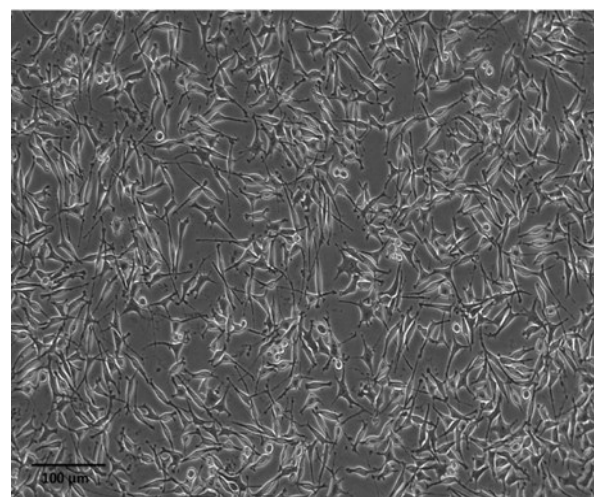


Figure 14. MTT assay at 100 conc.

Following incubation, the decrease in absorbance was measured at 734 nm using a UV-visible spectrophotometer. The percentage of ABTS radical scavenging activity was calculated relative to the control using the standard formula.

The results demonstrated a dose-dependent increase in ABTS radical scavenging activity of *Nārikelā Khanda*, indicating its effective antioxidant potential and capacity to neutralize free radicals.

Antioxidant Activity - DPPH Assay^{[17][18]}

The free radical scavenging activity of *Nārikelā Khanda* was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay following the method described by *Brand-Williams et al.*, with minor modifications. Different concentrations of the extract (1.56-1000 µg/mL) were prepared in methanol and mixed with an equal volume of freshly prepared DPPH solution (60 µM in methanol). Ascorbic acid was used as the reference standard, while methanol served as the blank. A control

solution containing DPPH reagent without the test sample was also maintained. The reaction mixtures were incubated for 30 minutes in the dark at room temperature to allow complete interaction between the DPPH radical and antioxidant constituents. After incubation, the reduction in absorbance was measured at 515 nm using a UV-visible spectrophotometer. The percentage of DPPH radical scavenging activity was calculated in comparison with the control. The results revealed a dose-dependent increase in DPPH radical scavenging activity of *Nārikelā Khanda*, indicating its effective free radical neutralizing potential

Antioxidant Activity - FRAP Assay^[19]

The ferric reducing antioxidant power (FRAP) of *Nārikelā Khanda* was assessed based on its ability to reduce the ferric (Fe³⁺)-TPTZ complex to the ferrous (Fe²⁺)-TPTZ complex under acidic conditions, as described in standard protocols. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid, and 20 mM ferric chloride (FeCl₃·6H₂O) in the ratio of 10:1:1 (v/v/v).

Different concentrations of *Nārikelā Khanda* extract were prepared and mixed with the freshly prepared FRAP reagent. The reaction mixtures were incubated at 37 °C for 30 minutes to facilitate reduction of the ferric complex. Following incubation, the increase in absorbance was measured at 593 nm using a UV-visible spectrophotometer. A standard calibration curve was constructed using ferrous sulfate (FeSO₄) at known concentrations. The antioxidant capacity of the test sample was calculated from the standard curve and expressed as μmol FeSO₄ equivalents per gram of extract.

The assay results demonstrated a dose-dependent increase in ferric reducing capacity of *Nārikelā Khanda*, confirming its significant electron-donating and antioxidant potential.

Results

MTT Assay (Cytotoxicity Analysis)

The *Narikela Khanda* sample (CMO3) did not show significant cytotoxicity on L929 fibroblast cells at concentrations ranging from 6.25 to 100 μg/mL. Cell viability remained above 90% at all tested concentrations, indicating safety toward normal cell lines. Morphological observations under an inverted microscope also confirmed intact cell membranes and normal cellular morphology.

Table 2. MTT assay results for varying concentrations of *Narikela Khanda* (Sample CMO3)

Concentration (μg/mL)	OD1	OD2	OD3	Average OD
Control	0.788	0.786	0.789	0.788
6.25	0.784	0.782	0.781	0.782
12.5	0.776	0.779	0.778	0.778
25	0.768	0.770	0.772	0.770
50	0.761	0.763	0.762	0.762
100	0.757	0.759	0.755	0.757

Table 3. Percentage cell viability of L929 cells after treatment with *Narikela Khanda*

Concentration (μg/mL)	Percentage Viability (%)
6.25	99.32
12.5	98.73
25	97.76
50	96.74
100	96.11

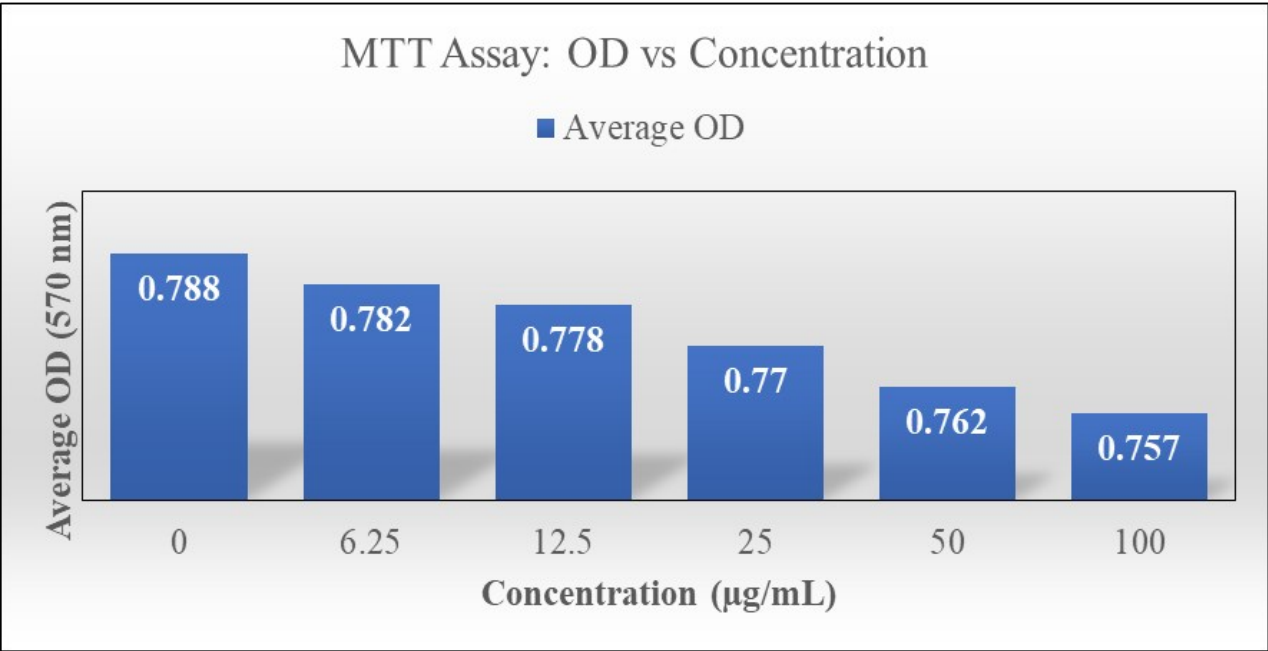


Figure 15. MTT Assay: OD vs Concentration

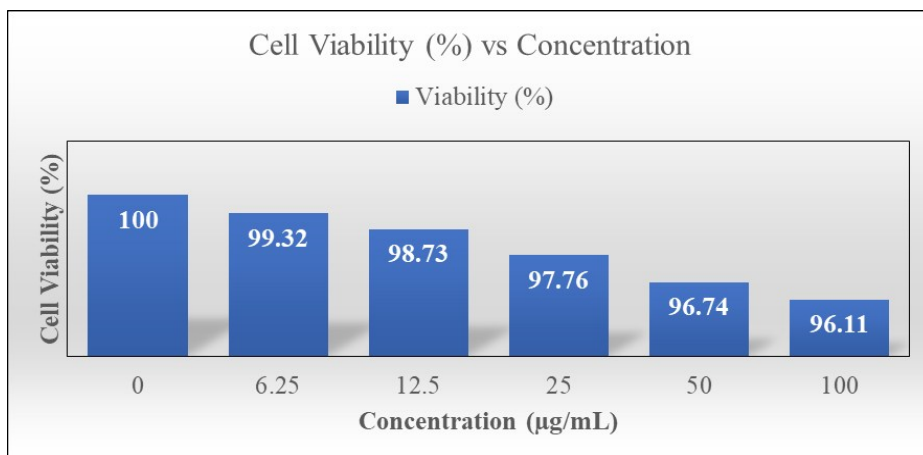


Figure 16. Cell Viability (%) vs Concentration

ABTS Radical Scavenging Assay

The formulation exhibited a dose-dependent antioxidant response in the ABTS assay. Maximum scavenging activity was observed at higher concentrations (500-1000 µg/mL), suggesting that the phytoconstituents in *Narikela Khanda* can effectively quench free radicals. The IC₅₀ value was moderately comparable to standard ascorbic acid, indicating a fair antioxidant potential.

Table 4. ABTS Radical Scavenging Activity of *Narikela Khanda* (Sample CMO3)

Concentration (µg/mL)	Average OD (734 nm)	% Inhibition
Control	0.722	-
1.56	0.683	5.36
3.12	0.665	7.89
6.25	0.633	12.33
12.5	0.594	17.73
25	0.541	25.07
50	0.485	32.83
100	0.436	39.61
200	0.393	45.61
400	0.363	49.68
800	0.327	54.75
1000	0.283	60.76

IC₅₀ = 447.50 µg/mL

DPPH Radical Scavenging Assay

Narikela Khanda showed moderate DPPH radical scavenging capacity, with increasing inhibition percentages at higher concentrations. The change in absorbance at 515 nm suggested gradual reduction of DPPH radicals. Although the IC₅₀ value was higher compared to ascorbic acid, the results confirm that the formulation has hydrogen-donating antioxidant activity.

Table 5. DPPH Radical Scavenging Activity of *Narikela Khanda* (Sample CMO3)

Concentration (µg/mL)	Average OD (515 nm)	% Inhibition
Control	0.893	-
1.56	0.856	4.18
3.12	0.808	9.47
6.25	0.778	12.88
12.5	0.742	16.95
25	0.698	21.84
50	0.627	29.82
100	0.578	35.31
200	0.542	39.31
400	0.498	44.20
800	0.445	50.13
1000	0.396	55.62

IC₅₀ = 735.82 µg/mL

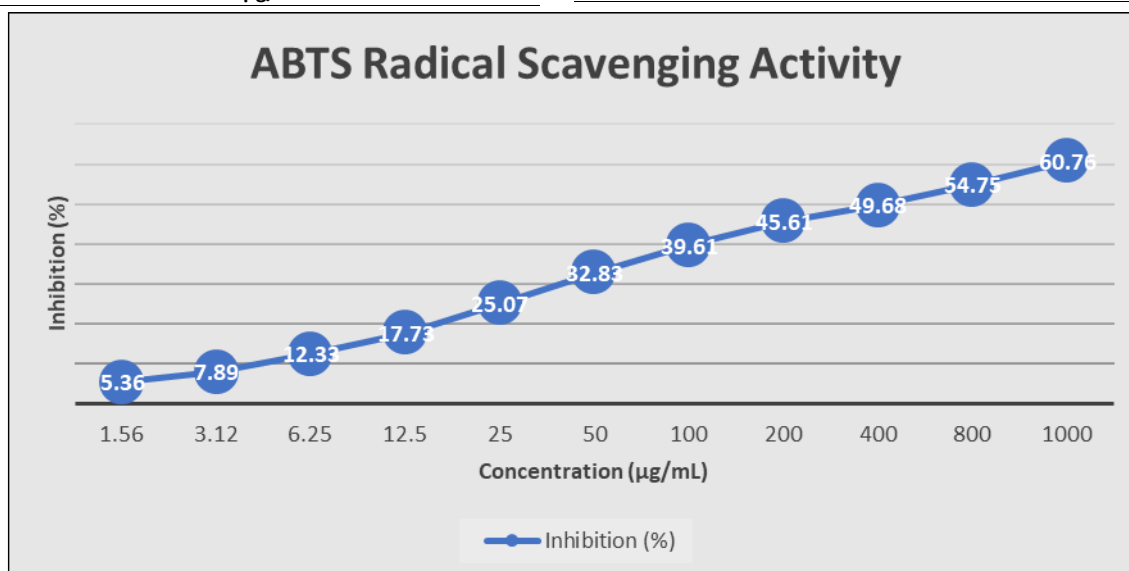


Figure 17. ABTS Radical Scavenging Activity

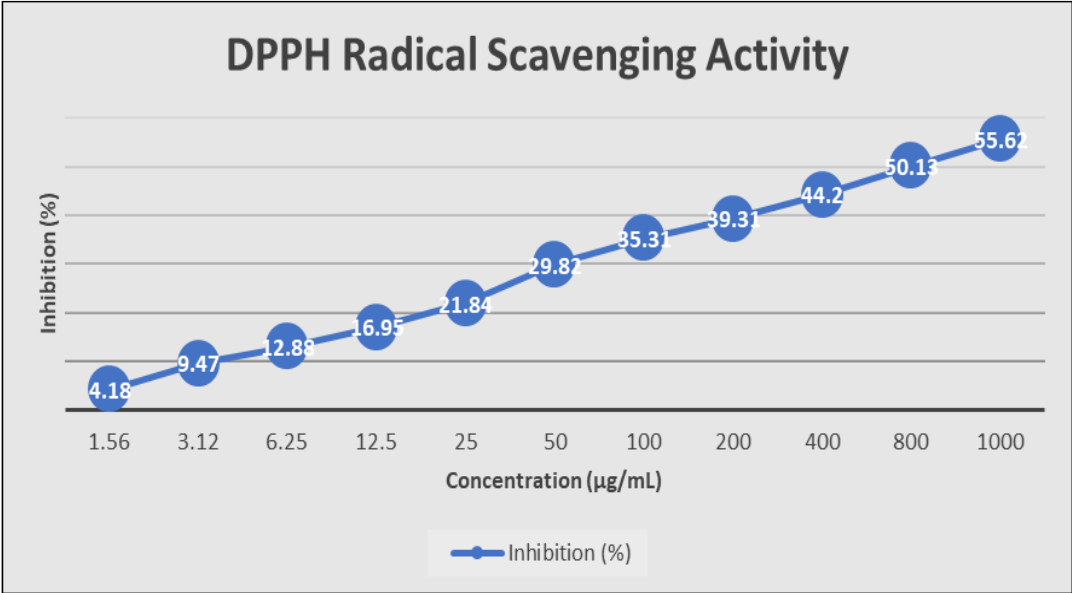


Figure 18. DPPH Radical Scavenging Activity

Ferric Reducing Antioxidant Power (FRAP) Assay

The extract demonstrated a clear reducing ability, with absorbance values proportionally increasing with concentration at 593 nm. This indicates that *Narikela Khanda* possesses electron-donating phytochemicals capable of reducing Fe3+ to Fe2+, thereby contributing to its overall antioxidant potential.

Table 6. Ferric Reducing Antioxidant Power (FRAP) of *Narikela Khanda* (Sample CMO3)

Concentration (µg/mL)	Average OD (593 nm)	Fe ²⁺ Equivalent (ng/mL)
6.25	0.01	0.24
12.5	0.021	0.74
25	0.127	5.58
50	0.223	9.97
100	0.287	12.89

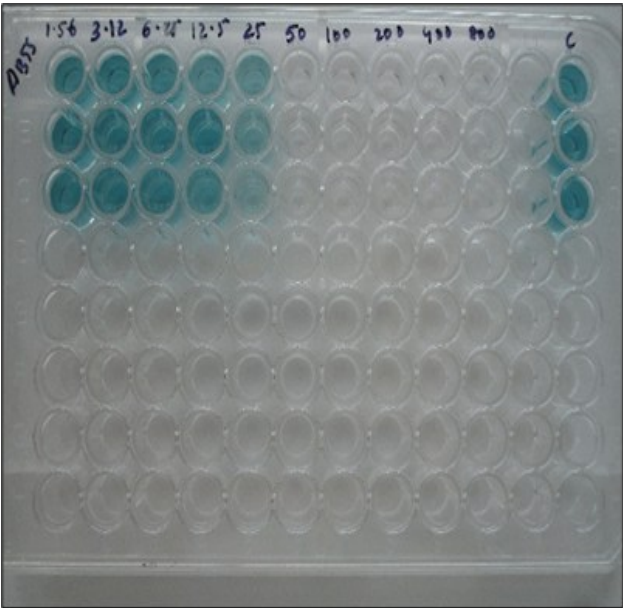


Figure 20. ABTS assay - Standard

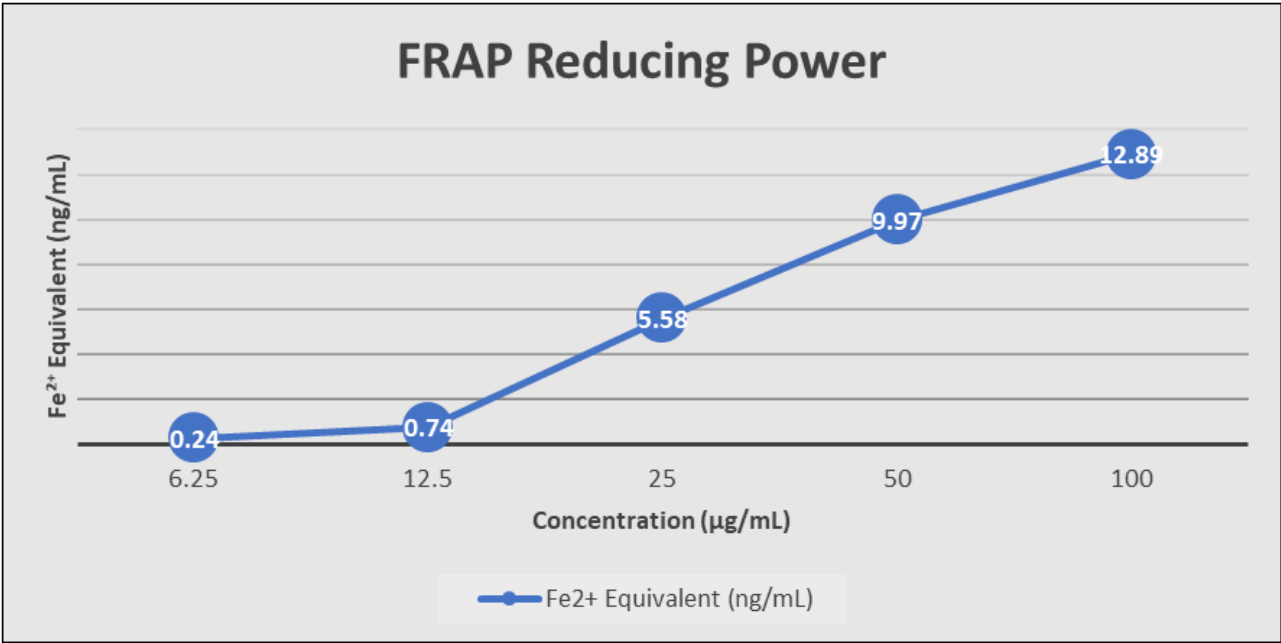


Figure 19. FRAP Reducing Power

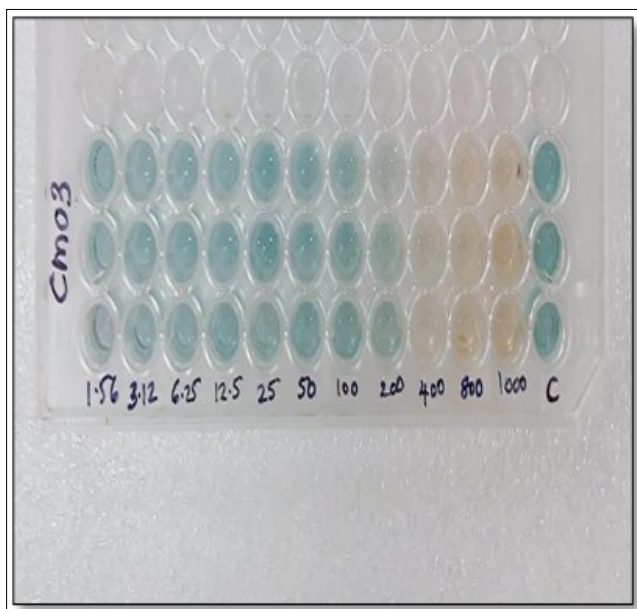


Figure 21. ABTS assay of Narikela Khanda



Figure 24. FRAP assay of Narikela Khanda



Figure 22. DPPH assay - Standard

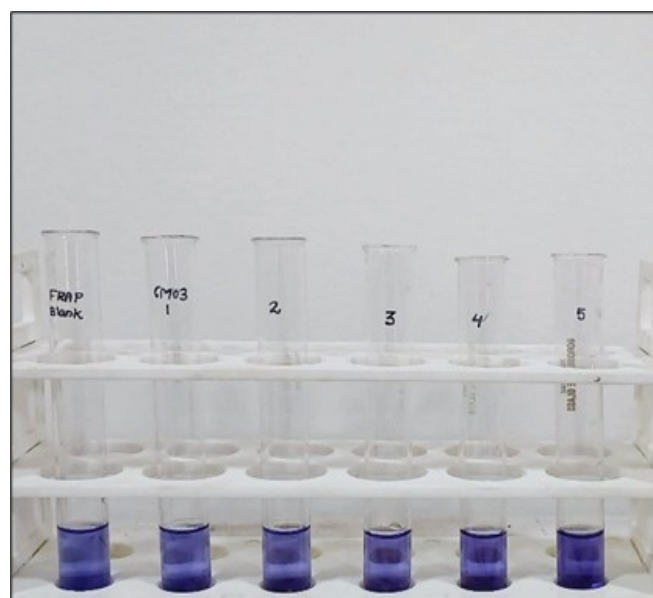


Figure 25. FRAP assay - Standard solution series

Discussion

The present investigation offers the first systematic scientific validation of the *in vitro* safety and antioxidant potential of *Nārikelā Khanda*, a classical *Āyurvedic Rasāyana* formulation. Demonstrating both safety and bioactivity is essential for substantiating traditional medicinal preparations within modern pharmacological paradigms, especially those intended for prolonged therapeutic use.

Evaluation using the MTT cytotoxicity assay revealed that *Nārikelā Khanda* did not induce any significant decrease in cell viability of L929 normal fibroblast cells over the tested concentration range, thereby confirming its non-cytotoxic nature under *in vitro* conditions. This favorable safety profile lends experimental support to its classical classification as a *Rasāyana* and reinforces its suitability for long-term administration without adversely affecting cellular integrity.



Figure 23. DPPH assay of Narikela Khanda

Assessment of antioxidant activity through ABTS, DPPH, and FRAP assays consistently demonstrated dose-dependent radical scavenging and ferric reducing capacities. The IC₅₀ values obtained from ABTS and DPPH assays, as presented in the Results section, reflect moderate yet reproducible antioxidant potency. Although the scavenging activity was comparatively lower than that of pure reference standards such as ascorbic acid, this finding remains pharmacologically relevant rather than disadvantageous.

Unlike isolated antioxidants, *Nārikelā Khanda* constitutes a complex, multi-component phytochemical matrix, wherein phenolics, flavonoids, essential oils, and other bioactive constituents function collectively. Ingredients such as *Dhānyaka*, *Jiraka*, *Tvak*, *Elā*, and *Nāgakeśara* are recognized for their antioxidant, anti-inflammatory, and cytoprotective properties. The comparatively lower activity observed in relation to pure standards may represent gradual and sustained free radical neutralization, a property that could be therapeutically beneficial by reducing the risk of pro-oxidant rebound effects associated with high doses of single-molecule antioxidants. Consequently, the antioxidant action of *Nārikelā Khanda* may be better understood as synergistic and physiologically harmonious, rather than immediately maximal.

The antioxidant efficacy demonstrated in the present study bears considerable clinical relevance in conditions such as *Amlapitta*, *Raktapitta*, *Kṣayaroga*, and peptic ulcer disease, where oxidative stress, free radical-induced mucosal injury, and microvascular damage contribute significantly to disease pathogenesis. By mitigating oxidative stress and augmenting endogenous defense mechanisms, *Nārikelā Khanda* may confer gastroprotective and haemostatic benefits, in consonance with its classical therapeutic indications.

Moreover, earlier experimental studies on comparable *Khanda Kalpanā* and *Avaleha* formulations have reported similar antioxidant and cytoprotective effects, thereby corroborating the present findings and further strengthening the scientific foundation of traditional *Āyurvedic* pharmaceuticals. Collectively, these observations suggest that *Nārikelā Khanda* functions not only as a digestive and haemostatic medicine but also as a functional, antioxidant-rich *Rasāyana*, effectively integrating classical *Āyurvedic* principles with contemporary pharmacological understanding.

Conclusion

This study standardized the classical pharmaceutical preparation of *Nārikelā Khanda* and confirmed its *in vitro* safety and antioxidant potential through systematic experimental evaluation. The formulation was prepared strictly

according to classical *Āyurvedic* principles, yielding a stable, palatable *Khanda Kalpanā*, and its safety was established by the MTT assay, which demonstrated the absence of cytotoxic effects on L929 normal fibroblast cells across the tested concentration range.

The antioxidant profiling using ABTS, DPPH, and FRAP assays consistently confirmed dose-dependent free radical scavenging and ferric reducing activities, validating the presence of functionally active antioxidant constituents within the formulation. Although the antioxidant potency was lower than that of pure reference standards, the observed activity reflects a synergistic, sustained antioxidant effect arising from the complex phytochemical matrix of the formulation rather than isolated, single-molecule action.

Collectively, these findings establish *Nārikelā Khanda* as a non-toxic, antioxidant-rich *Rasāyana*, providing scientific support to its traditional use in *Amlapitta*, *Raktapitta*, *Kṣayaroga*, and other oxidative stress-mediated gastrointestinal conditions. Future work should focus on *in vivo* experimental studies to delineate gastroprotective and haemostatic efficacy, followed by controlled clinical trials to substantiate its therapeutic effectiveness and translational relevance in human subjects.

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