WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 12, 945-953. Research Article ISSN 2277–7105

PHARMACEUTICO-ANALYTICAL STUDY OF KHADIRARISHTA AND ITS IN VITRO CYTOTOXIC ACTIVITY ON A431 SKIN CANCER CELL LINE

Dr. Darshana Kulkarni[1]*, Dr. Gangaprasad Asore[2]

[1]MD Ayurveda, Ayurvedic Physician, Mumbai, India.

[2]Professor, Department of Rasashastra and Bhaishajya Kalpana, APM's Ayurveda Mahavidyalaya, Sion, Mumbai, India.

Article Received on 13 May 2026,
Article Revised on 03 June 2026,
Article Published on 16 June 2026,

https://doi.org/10.5281/zenodo.20697290

***Corresponding Author**
Dr. Darshana Kulkarni
MD Ayurveda, Ayurvedic Physician, Mumbai, India.

How to cite this Article: Dr. Darshana Kulkarni[1]*, Dr. Gangaprasad Asore[2] (2026). Pharmaceutico-Analytical Study Of Khadirarishta And Its In Vitro Cytotoxic Activity On A431 Skin Cancer Cell Line. World Journal of Pharmaceutical Research, 15(12), 945–953.

ABSTRACT

Khadirarishta is a classical Ayurvedic fermented formulation traditionally indicated in *Kushtha*, *Granthi*, and *Arbuda*. The present study was undertaken to prepare and standardize Khadirarishta according to classical Ayurvedic references and to evaluate its in vitro cytotoxic activity against A431 skin cancer cell line. The formulation was prepared using authenticated raw materials following standard pharmaceutical procedures. Organoleptic, pharmacognostical, and physicochemical analyses were carried out as per Ayurvedic pharmacopeial standards. In vitro cytotoxic activity was assessed using MTT assay on A431 skin cancer cell line at varying concentrations, and percentage cell viability along with IC50 values were determined. The prepared formulation exhibited acceptable physicochemical characteristics within standard limits. Cytotoxic evaluation demonstrated dose-dependent inhibition of A431 cells with progressive reduction in cell viability at increasing concentrations, indicating appreciable cytotoxic potential of the formulation. The observed activity may be attributed to the presence of phytoconstituents possessing antioxidant, anti-inflammatory, and potential anticancer properties in the constituent drugs of Khadirarishta. The findings of the present study provide preliminary scientific evidence supporting the traditional use of Khadirarishta in conditions comparable to Arbuda and suggest its potential for further experimental and clinical anticancer research.

KEYWORDS: Khadirarishta, A431 cell line, Cytotoxic activity, Skin cancer, MTT assay, Ayurveda.

INTRODUCTION

Skin cancer is one of the most commonly occurring malignancies worldwide, with squamous cell carcinoma being among the major non-melanoma skin cancers associated with significant morbidity. Despite advances in conventional therapies such as surgery, chemotherapy, and radiotherapy, their long-term use is often associated with adverse effects, recurrence, and economical challenges. This has led to increasing interest in traditional and herbal medicines as potential complementary therapeutic approaches with better safety profiles.

Ayurveda describes conditions comparable to abnormal tissue growth and chronic skin disorders under the concepts of *Kushtha*, *Granthi*, and *Arbuda*. Various classical formulations indicated in these conditions possess drugs with documented antioxidant, anti-inflammatory, immunomodulatory, and cytotoxic properties. Khadirarishta is a classical fermented Ayurvedic formulation mentioned in texts such as Bhaishajya Ratnavali and traditionally indicated in *Kushtha* and related disorders. The formulation contains several medicinal plants including Khadira (Acacia catechu), Bakuchi (Psoralea corylifolia), Daruharidra (Berberis aristata), Devadaru (Cedrus deodara), and Triphala, many of which have been reported to possess pharmacologically active phytoconstituents with potential anticancer activity.

Sandhana Kalpana i.e. fermented Ayurvedic formulations such as *Arishta* and *Asava* preparations are known to enhance extraction, preservation, and bioavailability of active constituents due to the process of natural fermentation. Although individual ingredients of Khadirarishta have been studied for various pharmacological activities, limited scientific evidence is available regarding the cytotoxic potential of the compound formulation against skin cancer cell lines. Furthermore, studies evaluating the activity of Khadirarishta on A431 skin cancer cell line are unavailable.

Therefore, the present study was undertaken to prepare and standardize Khadirarishta according to classical Ayurvedic procedures and evaluate its in vitro cytotoxic activity against A431 skin cancer cell line using MTT assay.

MATERIALS AND METHODS

Collection and Authentication of Raw Materials

All the herbal ingredients required for the preparation of Khadirarishta were procured in raw dried form from authentic sources. The raw drugs were identified and authenticated in well-known Ayurvedic laboratory prior to pharmaceutical processing. Honey and sugar used in the formulation were also obtained from standard commercial sources.

Preparation Before Fermentation

The raw drugs used for *Kwatha* preparation were cleaned and coarsely powdered using mortar and pestle. The *Prakshepa Dravyas* were finely powdered and sieved through mesh. Fermentation vessels, instruments, and other equipment were thoroughly cleaned using hot water and dried properly before use. The fermentation container was subjected to *Ghrita Lepana* and fumigation using *Dhoopana Dravyas* to maintain aseptic conditions.

Preparation of Khadirarishta

Khadirarishta was prepared according to the classical reference of Bhaishajya Ratnavali under *Kushtha* Rogadhikara. The coarsely powdered Kwatha Dravyas including Khadira, Devadaru, Daruharidra, Bakuchi, and Triphala were soaked in water overnight. The mixture with addition of 32 Litres was heated on mild flame and reduced to one-eighth of its original volume to prepare *Kwatha* of 4 litres. The prepared decoction was filtered using clean cotton cloth.

Powdered sugar was dissolved in the warm *Kwatha* followed by addition of honey after cooling. The mixture was transferred into the *Sandhana* vessel, and *Prakshepa Dravyas* along with Dhataki Pushpa were added. The container was sealed properly and kept in a clean, dry place for natural fermentation for thirty days.

After signs completion of fermentation were observed, the formulation was filtered and stored in amber-colored airtight containers for further analytical and experimental studies.

Organoleptic and Physicochemical Evaluation

The prepared Khadirarishta was subjected to organoleptic evaluation including color, odor, taste, and appearance. Physicochemical parameters such as pH, specific gravity, alcohol content, total solids, total sugar, reducing sugar, and non-reducing sugar were analyzed according to standard Ayurvedic pharmacopeial procedures.

Thin Layer Chromatography

Thin layer chromatographic analysis of the prepared Khadirarishta was carried out using suitable solvent systems for identification of phytoconstituents. The chromatographic profile was observed and compared with standard reference compounds.

In Vitro Cytotoxic Activity

The cytotoxic activity of Khadirarishta was evaluated against A431 skin cancer cell line using MTT assay. The A431 cells were cultured in suitable growth medium supplemented with fetal bovine serum and antibiotics under standard incubation conditions at 37°C in 5% CO_2 atmosphere. Cells were seeded into 96-well microplates and incubated for adequate cell attachment.

Different concentrations of Khadirarishta were administered to the cultured cells and incubated for a specified duration of 24hrs. Following treatment, MTT reagent was added and further incubated to allow formation of formazan crystals by viable cells. The crystals formed were dissolved using suitable solvent, and absorbance was measured using microplate reader at appropriate wavelength. Percentage cell viability and IC50 value were calculated from the obtained absorbance values.

RESULTS

Organoleptic Evaluation

The prepared Khadirarishta was observed to be a dark brown colored liquid with characteristic fermented odor and sweet-astringent taste. The formulation exhibited a slightly viscous and homogenous appearance.

Physicochemical Analysis

The physicochemical parameters of the prepared Khadirarishta were found to be within acceptable limits as per Ayurvedic pharmacopeial standards. The formulation showed acidic pH, indicating successful completion of fermentation. Specific gravity, alcohol content, total solids, and sugar profile confirmed proper fermentation and standardization of the formulation.

The observed physicochemical parameters are summarized in Table 2.

Thin Layer Chromatographic Analysis

Thin layer chromatographic analysis of Khadirarishta demonstrated the presence of characteristic phytoconstituents. The chromatographic profile confirmed the presence of phenolic

compounds and other bioactive constituents in the formulation. Gallic acid was identified as one of the marker compounds.

In Vitro Cytotoxic Activity

The cytotoxic activity of Khadirarishta against A431 skin cancer cell line was evaluated using MTT assay. The formulation exhibited mild dose-dependent cytotoxic activity with largest reduction in percentage cell viability at highest concentrations.

At lower concentrations, mild inhibition of cell growth was observed, whereas higher concentrations demonstrated greater cytotoxic effect on A431 cells.

The percentage cell viability at different concentrations is summarized in Table 3.

IC50 Determination

The IC50 value of Khadirarishta against A431 skin cancer cell line was calculated from the dose-response curve obtained through MTT assay. The formulation demonstrated appreciable cytotoxic potential against the tested cell line with an IC50 value of 146.3.

The results indicate that Khadirarishta possesses mild in-vitro cytotoxic activity against A431 skin cancer cell line.

Table 1: Ingredients of Khadirarishta.

Sr. No.	Ingredient	Latin Name	Part Used	Quantity
1	Khadira	*Acacia catechu*	Heartwood	800 g
2	Devadaru	*Cedrus deodara*	Heartwood	800 g
3	Bakuchi	*Psoralea corylifolia*	Seeds	192 g
4	Daruharidra	*Berberis aristata*	Root and stem	320 g
5	Haritaki	*Terminalia chebula*	Fruit	106 g
6	Bibhitaki	*Terminalia bellirica*	Fruit	106 g
7	Amalaki	*Emblica officinalis*	Fruit	106 g
8	Dhataki	*Woodfordia fruticosa*	Flowers	320 g
9	Kankola	*Piper cubeba*	Raw fruit	16 g
10	Nagakeshara	*Mesua ferrea*	Stamens	16 g
11	Jatiphala	*Myristica fragrans*	Fruit	16 g
12	Lavanga	*Syzygium aromaticum*	Flower bud	16 g
13	Elaichi	*Elettaria cardamomum*	Fruits and seeds	16 g
14	Dalchini	*Cinnamomum zeylanicum*	Bark	16 g
15	Tejpatta	*Cinnamomum tamala*	Leaf	16 g
16	Pippali	*Piper longum*	Fruit	64 g
17	Madhu	Honey	—	3.2 kg
18	Sharkara	Sugar	—	1.6 kg
19	Jala	Water	—	32 L

Table 2: Analytical test.

Parameter	Observation
Appearance	Sticky liquid
Color	Dark brown
Odor	Fermented
Taste	Sweet and astringent
pH	4.4
Specific gravity	1.081
Alcohol content	6.25%
Total solids	9.21%
Total sugar	32%
Reducing sugar	22.5%
Non-reducing sugar	7.01%

Table 3: Percentage Cell Viability.

Treatment	Conc. (µg/mL)	% Cell Viability (n1)	% Cell Viability (n2)	% Cell Viability (n3)
Test Formulation	1000	152.25	85.62	128.39
Test Formulation	500	163.85	92.29	124.40
Test Formulation	250	93.08	108.89	117.41
Test Formulation	125	166.10	106.54	101.47
Test Formulation	62.5	97.02	111.96	94.18
DMEM + Cell Control	-	100.00	100.00	100.00

DISCUSSION

Khadirarishta is a classical Ayurvedic fermented formulation widely indicated in Kushtha and related disorders. In the present study, the formulation was prepared according to classical Ayurvedic procedures and evaluated through analytical and in vitro cytotoxic assessment. The study attempted to establish a scientific basis for the traditional use of Khadirarishta in disorders comparable to *Arbuda*.

Fermented Ayurvedic preparations are considered pharmaceutically important due to enhanced extraction and preservation of phytoconstituents. During *Sandhana Kalpana*, self-generated alcohol acts as a natural preservative and facilitates extraction of both water-soluble and alcohol-soluble active compounds. This process may contribute to improved bioavailability and therapeutic efficacy of the formulation.

The analytical profile obtained in the present study indicates proper completion of fermentation and acceptable quality of the prepared formulation. The acidic nature of Khadirarishta may be attributed to the formation of organic acids during fermentation. Thin layer chromatographic analysis confirmed the presence of important phytoconstituents including

phenolic compounds such as gallic acid, which are known for antioxidant activity.

The cytotoxic activity observed against A431 skin cancer cell line may be attributed to the combined action of multiple herbal ingredients present in Khadirarishta. Khadira (Acacia catechu) contains catechins and flavonoids with antioxidant and anti-inflammatory properties. Bakuchi (Psoralea corylifolia) is reported to possess antiproliferative activity due to phytoconstituents such as psoralen and bakuchiol. Daruharidra (Berberis aristata) contains berberine, which has demonstrated apoptotic and anticancer activity in various experimental studies. Similarly, Triphala constituents are reported to exhibit antioxidant, immunomodulatory, and cytoprotective activities.

The observed cytotoxic potential of Khadirarishta may involve mechanisms such as induction of apoptosis, modulation of oxidative stress, inhibition of cellular proliferation, and interference with tumor progression pathways. The synergistic interaction of multiple phytoconstituents present in the formulation may also contribute to the overall activity observed in the study.

The findings of the present study provide preliminary scientific support for the classical indication of Khadirarishta in conditions comparable to *Arbuda*. However, the study is limited to in vitro evaluation, and further molecular, experimental, and clinical studies are required to establish its safety, efficacy, and exact mechanism of action.

CONCLUSION

The present study successfully demonstrated the pharmaceutical preparation and analytical standardization of Khadirarishta according to classical Ayurvedic principles. The prepared formulation exhibited acceptable organoleptic and physicochemical characteristics, confirming proper fermentation and quality of the formulation.

The in vitro cytotoxic evaluation against A431 skin cancer cell line revealed Mild cytotoxic activity of Khadirarishta. The observed activity may be due to the action of various phytoconstituents present in the drugs of the formulation.

The findings of the present study provide preliminary scientific evidence supporting the traditional use of Khadirarishta in conditions comparable to Arbuda described in Ayurveda. However, further experimental studies, in vivo evaluation, and clinical trials are required to establish its exact mechanism of action, therapeutic efficacy, and safety profile in cancer management.

Overall, the study highlights the potential of Khadirarishta as a promising Ayurvedic

formulation for future anticancer research.

REFERENCES

1. Bhaishajya Ratnavali, edited by Govind Das Sen (Mishra), in Kushtha Roga Adhikaraṇam (Chapter 54), verses 365–370; Chaukhamba Surbharati Prakashan, Varanasi (Reprint ed., 2013).
2. Sharangdhara, Sharangdhara Saṃhita, Madhyam Khanda, 10/60- 65 Edited with Hindi commentary by Pandit Parasurama Sastri Vidyasagar. Varanasi: Chaukhambha Orientalia; Reprint edition, 2018.
3. Vaidya Sodhāla. Gadanigraha. Vol. 1, Prayoga Khaṇḍa. Hindi commentary by Tripāṭhī I. Edited by Pāṇḍeya GS. Reprint ed. Varanasi: Chaukhambha Sanskrit Sansthan, 2018
4. Dr Chunekar KC, Bhavaprakash Nighantu,edited by DR.G.S.Pandey,Chaukambha bharati academy 12. Kamrun Nahar, Most. Shammi Rahman, Shahana Jahan, Md. Zakir Sultan, Md. Musfaqur Rahman Sajjad, et al. (2015). Evaluation of Some Physicochemical Properties as Quality Control Parameters of an Ayurvedic Preparation "Khadirarishta". European Journal of Preventive Medicine, 3(2): 17-21. https://doi.org/10.11648/j.ejpm.20150302.11
5. Shastri D. Ayurvedeeya Aushadhikaran. 4th edition. Shree Dhootpapeshwar Ltd, 1964; Part 2, 34.
6. Koyande, Aishwarya C Gadgil, Swati. (2020). PHARMACEUTICAL PREPARATION AND DETERMINATION OF QUALITY STANDARDS OF SARASWATARISHTA: A HERBO-MINERAL ALCOHOLIC FORMULATION. International Journal of Research in Ayurveda and Pharmacy, 11: 88-93 10.7897/2277-4343.1105147.
7. Das PK, Goswami S, Chinniah A, Panda N, Banerjee S, Sahu NP, et al. Woodfordia fruticosa : Traditional uses and recent findings. J Ethnopharmacol, 2007; 110: 189–99.
8. Dhruve K. A Comparative-Pharmaceutical Study on Khadirarishta wsrto Fungal growth in Arishta and its effect on Kushtha. [Jamnagar]: Institute of Teaching CResearch in Ayurveda, 2007.
9. Shinde RR, Bhangale K. KHADIRARISHTA: A MEDICAL REVIEW. Int J Res Granthaalayah [Internet], 2017; 5(10): 72–5. Available from: http://dx.doi.org/10.29121/granthaalayah.v5.i10.2017.2270
10. Katare S. sandhan kalpana antargat khadirarishta nirman uska bhuktik ev rasayanik parikshan kar pama vyadhi par aturalayin adhyan. [Nagpur]: Government Ayurveda College, 2003.
11. Das C, Ghosh G, Das D. Ayurvedic Liquid Dosage form Asava and Arista: An Overview. Indian J Pharm Educational Research, 2017; 51(2): 169–76.

12. Ayush Government of India, Ayurvedic Pharmacopoeia of India.
13. Gaurav, Anoop Kumar, Rachana Thakur. An Insightful Review on Sandhana Kalpana. J Ayurveda Integr Med Sci, 2024; 10: 206- 210. http://dx.doi.org/10.21760/jaims.9.10.34
14. Mohammad H, Prabhu K, Rao MRK, Sundaram RL, Shil S, Vijayalakshmi N. The GC MS study of one ayurvedic medicine, khadirarishtam. Res J Pharm Technol [Internet], 2019; 12(2): 535. Available from: http://dx.doi.org/10.5958/0974-360x.2019.00094.5
15. Dr G, Parulkar KA. KHADIRARISHTA: A MEDICO STUDY. World Journal of Pharmaceutical Research, 5(11): 568–72.