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PHYSICOCHEMICAL ANALYSIS OF SHANKHDRAV RASA AND ITS IN-VITRO CYTOTOXIC ACTIVITY ON HT – 29 HUMAN COLORECTAL ADENOCARCINOMA CELL LINE

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ABSTRACT

Ayurveda emphasizes the maintenance of health through the balance of *Dosha, Agni*, and *Dhatu*, while Rasa Shastra and Bhaishajya Kalpana describe pharmaceutical techniques for preparing safe and effective medicines. Shankhadrav Rasa is a classical distillation-based formulation possessing a unique ***Ksharana*** (dissolving) property, capable of dissolving hard substances such as *Shankha, Varatika, and Dhatu*, which justifies its nomenclature. Classical texts also recommend ***Ghrita-lipta Mukha*** (application of ghee to the oral cavity) during its administration to minimize irritation and ensure safe consumption. Traditionally indicated in conditions such as *Gulma, Granthi, and Vidradhi*, the formulation offers scope for scientific exploration in colorectal cancer. The present study aims to prepare and analytically evaluate Shankhadrav Rasa according to classical principles and assess its in-vitro anticancer activity against HT-29 human colorectal cancer cells using the MTT assay. This study integrates Ayurvedic pharmaceutics with modern analytical and cell-based techniques to generate preliminary scientific evidence regarding its potential anticancer activity.

KEYWORDS: Shankhdrav Rasa, Distillation, HT-29 Cell line, Cytotoxic activity,

Colorectal Cancer, MTT Assay.

INTRODUCTION

Ayurveda is a holistic healthcare system that emphasizes the maintenance of health through equilibrium of Dosha, Agni, Dhatu and environmental factors. Its individualized approach, based on Prakriti, Agni and Dhatu shares conceptual similarities with the modern principle of personalized or precision medicine. The balance of Vata, Pitta and Kapha is considered essential for physiological homeostasis, while their disturbance may contribute to disease development.

Rasa Shastra and Bhaishajya Kalpana are important branches of Ayurvedic pharmaceutics that deal with the preparation and therapeutic application of herbal, mineral, metallic, and herbo-mineral medicines. Pharmaceutical procedures such as Shodhana, Marana, and Amritikarana transform raw substances into therapeutically suitable preparations. Various dosage forms described in Bhaishajya Kalpana further facilitate drug stability, administration, and therapeutic efficacy.

Ayurvedic concepts of Granthi and Arbuda provide a traditional perspective for understanding abnormal tissue growth. Disturbances of Dosha, Dhatu, Agni and Srotas may be considered within the Ayurvedic framework of disease progression. These concepts offer a basis for exploratory correlation with contemporary understanding of carcinogenesis, while recognizing that they are not direct equivalents of modern cancer terminology.

Colorectal cancer (CRC) is a major global health concern, with increasing recognition of early-onset disease. Genetic susceptibility, unhealthy dietary patterns, obesity, physical inactivity, environmental factors, and alterations in the gut microbiome are among the factors associated with its development.

Arka Kalpana is a specialized Ayurvedic pharmaceutical process based on distillation and is traditionally employed for obtaining volatile and subtle constituents of medicinal substances. Shankhadrav Rasa, described in classical texts such as *Rasa Sanket Kalika*, is a distinctive formulation prepared using a distillation-based method and traditionally indicated in conditions including Gulma, Granthi, Vidradhi, Agnimandya, and Udararoga.

The present study focuses on the pharmaceutical preparation and analytical evaluation of Shankhadrav Rasa according to classical principles, followed by assessment of its in-vitro

anticancer activity using the MTT assay in HT-29 human colorectal cancer cells. The study aims to evaluate its effect on cellular viability and proliferation and to generate preliminary scientific evidence regarding its potential against colorectal cancer. This integration of classical Ayurvedic pharmaceutics with contemporary analytical and cell-based techniques may contribute to the systematic scientific evaluation of traditional formulations.

AIMS AND OBJECTIVES AIM

To prepare and analyse Shankhdrav Rasa and to study its cytotoxic activity on HT-29 Human Colorectal adenocarcinoma cell line.

OBJECTIVES

Primary Objective

To Prepare Shankhdrav Rasa as mentioned In Ras Sanket Kalika.

Secondary Objective

1. To identify and authenticate raw material.
2. To analyse shankhdrav rasa physicochemically.
3. To evaluate the cytotoxic action of shankhdrav rasa on cancer cell line.

MATERIALS AND METHODS

A) COLLECTION AND AUTHENTICATION OF RAW MATERIALS

All the ingredients required for the preparation of Shankhdrav Rasa were procured from local market. The raw drugs were identified and authenticated in well-known laboratory prior to pharmaceutical processing.

B) PREPARATION

"नृसारं स्फटिका सौरं त्रयमेकत्र चूर्णयेत् ।
तल्क्षिपेन्मृन्मये कूपे शुभे हस्तमिते दृदे ।।
सरन्धोदरकाचोत्थे कूपे तत्सन्नियोजयेत् ।
सत्पधा वेष्टयेत्पश्चात्तत्सन्धिं वस्त्रमृत्स्नया ।।
खपरि बालुकापूरण तिर्यगोषधकूपकम् ।
अर्धं यन्त्र निधायात्र श्रीगुरोः सप्रदायतः ।।
अधोमुखं व्दितीयं तु स्थापत्यों चुल्लेः पराङ्मुखे ।
अतः प्रज्वालयेदग्निं हठाद्भावद्रसः स्त्रवेत् ।।

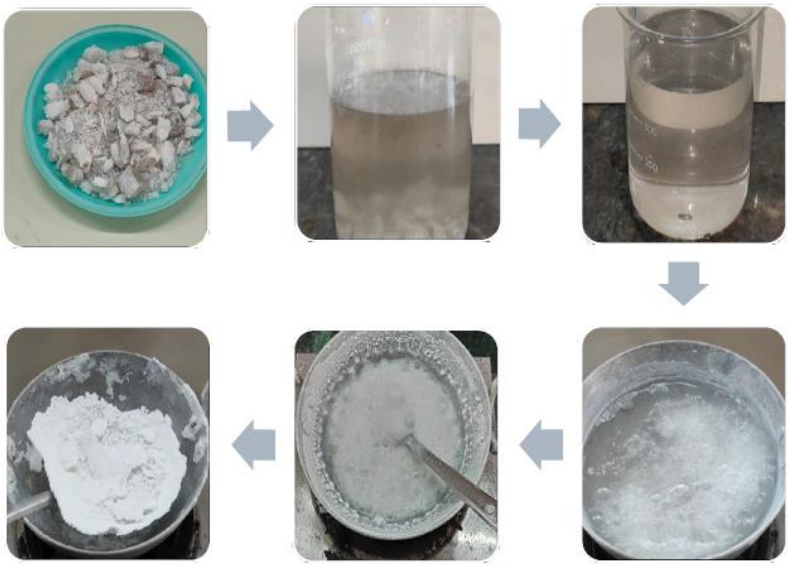
धारयेत्काचजे पात्रके शङ्खद्रावरसं ततः ।
शाणैकं सेवयेत्पश्चाद्दन्तस्पर्शविवर्जितम् ।।
गुल्मोदरयकृत्प्लीहविद्रधिग्रन्थिशूलनुत् ।
बलपुष्टिप्रदो ह्येष भुक्तं जायते क्षणात् ।।
विलोक्यतामहो लोका रसमाहात्म्यमद्भुतम् ।
कपर्दकाम्बुलोहानि यस्मिन् क्षिप्ते गलन्ति हि ।।३
(र. सं. क. चतुर्थोल्लास ८३-८९)

Table - Ingredients Of Shankhdrav Rasa.

Sr. No.	INGREDIENTS	QUANTITY
1	Navsagar	100gm
2	Kankshi	100gm
3	Kalmisora	100gm

NAVSADAR SHODHAN

- All the ingredients were accurately weighed using a weighing machine and measuring cylinder.
- Navasagara was finely powdered.
- The powdered Navasagara was dissolved in water, and the resulting solution was filtered.
- The filtered solution was heated until complete evaporation of the water.
- After complete evaporation, purified Navasagara powder remained in the vessel.
- The obtained Navasagara powder was collected on a clean plate.

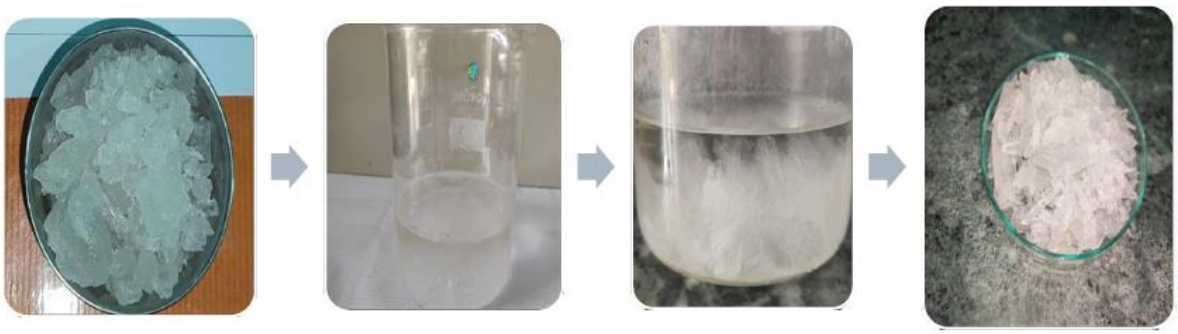


KANKSHI SHODHAN

- Fine powder of Kankshi was made with the help of khalvayantra.
- This powder was then poured into an iron vessel and heated.
- Kankshi melted due to heat and water was evaporated
- After purification, Kankshi was obtained as light, expanded crystals resembling puffed sugar candy (Batasha).

KALMISORA NIRMALIKARAN

- One part of Kalmisora was dissolved in half part of hot water.
- After complete dissolution, the solution was filtered through a clean cloth to remove impurities.
- The filtered solution was transferred to a clean vessel and allowed to cool undisturbed.
- On cooling, long, needle-shaped crystals of purified Kalmisora were formed.
- The crystals were collected, dried and stored in a clean, dry & airtight container for further use.



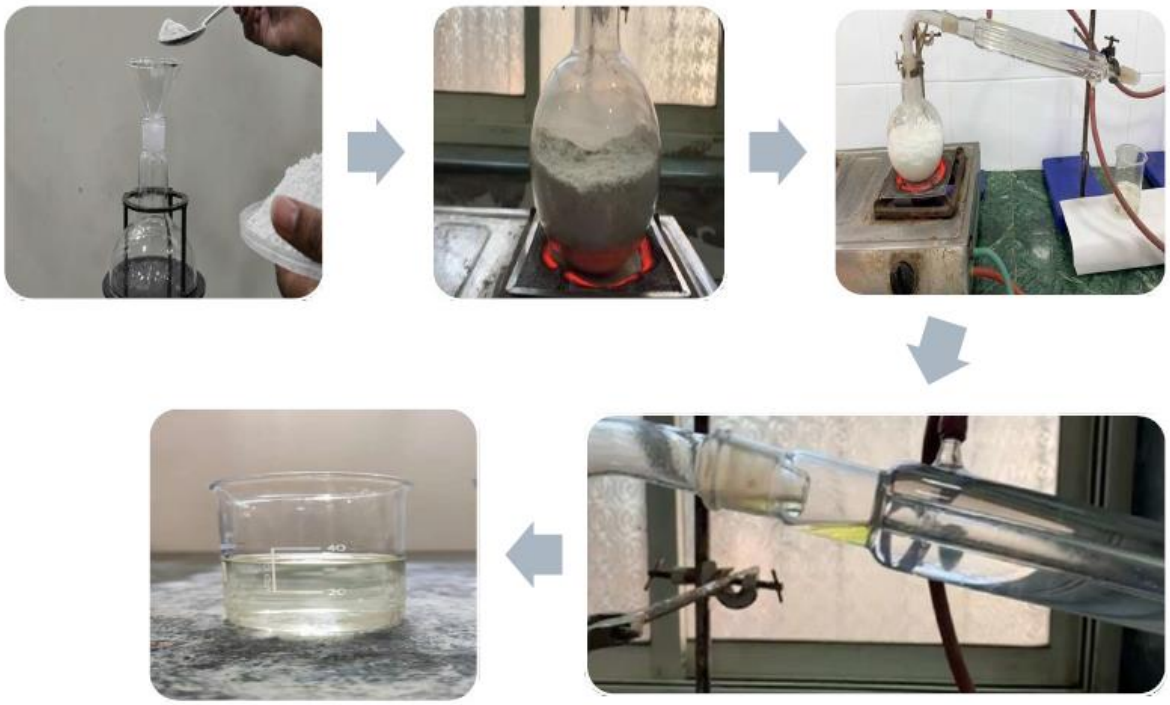
KALMISORA SHODHAN

- Nirmalikrut Kalmisora crystals was taken in a mortar and finely powdered.
- Ela Hima was then added, and the mixture was thoroughly triturated.
- This process was repeated three times. After repeated trituration with Ela Hima, the Kalmisora was considered purified.

PREPARATION OF SHANKHDRAV RASA

1. All ingredients of Shankhadrav Rasa were purchased from the market, shodhana of required drugs were done.
2. All the ingredients were taken one by one in a clean khalva yantra and are triturated well to obtain a homogenous mixture.
3. This mixture was meticulously transferred to the still (round bottom flask) of a clean glass bodied distillation apparatus.
4. The apparatus was all set, mild heat was applied to the still with the help of Gas burner.
5. The vapors were condensed in a condenser (condensation is the reverse process of vaporization)
6. After heating for half an hour, the distillate gradually started collecting in the receiver (round bottom flask).
7. Temperature control was an important factor in Drav preparation, hence it was heated upto 50^0C – 80^0C for the formation of vapor. Temperature less than 50^0C is not enough to get Drav while temperature more than 80^oC may burn the flask.

8. Initially the distillation was quick and later on after 2 hours there were no features of distillation.
9. Even after the completion of the procedure, the smoke-like appearance remained in the round bottom flask for another half an hour.
10. The yield of Drav obtained out of 300gm of raw material was 78 ml.



C) ORGANOLEPTIC & PHYSICOCHEMICAL EVALUATION

The prepared Shankhdrav Rasa was subjected to organoleptic evaluation including color, odor, and appearance. Physicochemical parameters such as pH, specific gravity, total solids, etc were analyzed according to standard Ayurvedic pharmacopeia procedures.

D) IN VITRO CYTOTOXIC ACTIVITY

The cytotoxic activity of Shankhdrav Rasa was evaluated against HT–29 Colorectal adenocarcinoma cell line using MTT assay. The HT – 29 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 Shankhdrav Rasa 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.

OBSERVATION

TABLE – OBSERVATION AFTER SHODHAN.

	NAVSADAR	KANKSHI	KALMISORA
BEFORE WEIGHT	175gm	270gm	200gm After nirmalikaran 140gm
AFTER WEIGHT	160gm	160gm	After shodhan 120gm
SPARSH	Soft	Smooth	Smooth
RUPA	White flakes	White	Greyish
GANDHA	Ugra gandha (Ammonia like)	Non specific	Ugra gandha

TABLE – OBSERVATION REGARDING SHANKHDRAV RASA.

A) DURING DISTILLATION	B) AFTER DISTILLATION
After 1 hour of heat, vapour's got condensed.	The smell of ammonium chloride was present.
Vapors were seen at the neck of still.	Colour of Shankhdrav Rasa was pale yellow transparent liquid
Vapors got condensed and transformed into liquid form when they pass through the condensing tube.	

RESULTS

A) ORGANOLEPTIC EVALUATION

The prepared Shankhdrav Rasa was observed to be a Pale yellow colored liquid with characteristic ammonia odour. The formulation exhibited to be transparent and homogenous appearance.

TABLE - ORGANOLEPTIC PROPERTIES OF SHANKHDRAV RASA.

ORGANOLEPTIC CHARACTERS	PROPERTIES
Colour	Transparent , Pale yellow
Odour	Ammonia like
Taste	Not Specific
Taste & Form	Liquid

B) PHYSICOCHEMICAL ANALYSIS

The physicochemical parameters of the prepared Shankhdrav Rasa were found to be within acceptable limits as per Ayurvedic pharmacopeia standards. The formulation showed acidic

pH, Specific gravity, total solids, Refractive Index and Solubility.

TABLE – ANALYTICAL TEST REGARDING SHANKHDRAV RASA.

Sr. No.	SPECIFICATIONS	RESULT
1	pH	1.7
2	Specific Gravity	1.0955
3	Refractive Index	1.352
4	Total Solid Content	4.18%
5	Solubilty	Test Complies

C) IN VITRO CYTOTOXIC ACTIVITY

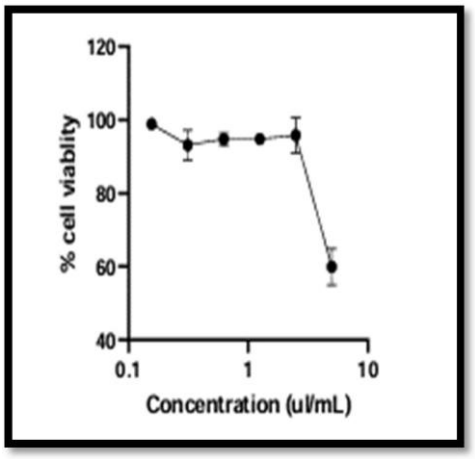
The cytotoxic activity of Shankhdrav Rasa against HT–29 Colorectal Adenocarcinoma cell line was evaluated using MTT assay. The formulation exhibited dose-dependent cytotoxic activity with reduction in percentage cell viability at highest concentrations. At lower concentrations, mild inhibition of cell growth was observed, whereas higher concentrations demonstrated greater cytotoxic effect on HT – 29 cells.

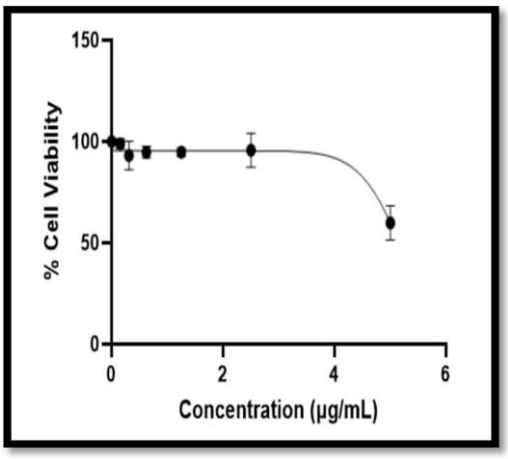
TABLE – PERCENTAGE OF CELL VIABILITY.

TREATMENTS	Conc.(µL/mL)	%Cell Viability(n1)	%Cell Viability(n1)	%Cell Viability(n1)
TEST FORMULATION	5	66.29	63.38	50.25
TEST FORMULATION	2.5	99.55	101.60	86.26
TEST FORMULATION	1.25	95..5	96.79	92.74
TEST FORMULATION	0.625	91.46	96.33	96.63
TEST FORMULATION	0.31	86.96	91.76	100.77
TEST FORMULATION	0.15	96.40	99.08	101.2
DMEM + Cell	-	100	100	100

D) IC50 DETERMINATION

The IC50 value of Shankhdrav Rasa against HT - 29 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 5.489. The results indicate that Shankhdrav Rasa possesses in-vitro cytotoxic activity against on HT – 29 Colorectal Adenocarcinoma cell line.





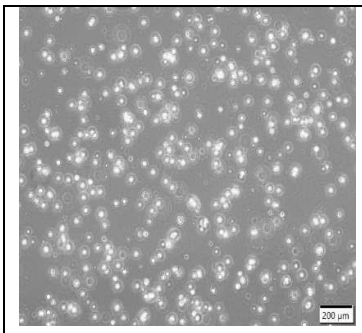
IC50 VALUE GRAPH

IC50 – 5.489 µL/mL

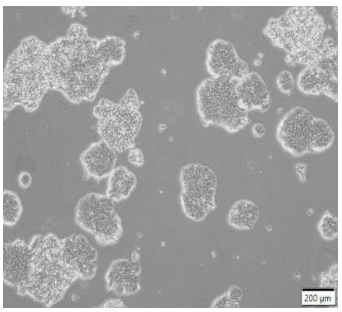
TABLE - INHIBITOR VS RESPONSE-VARIABLE SLOPE.

Best Fit Values	
IC50	5.401
HillSlope	-14.22
LogIC50	0.7325
Span	Unstable
Goodness of fit	
Degrees of Freedom	14
R Squared	0.8694
Sum of squares	474.5
Sy.x	5.821
Constraints	
IC50	IC50>0
# of X values	18
# of Y values analysed	18

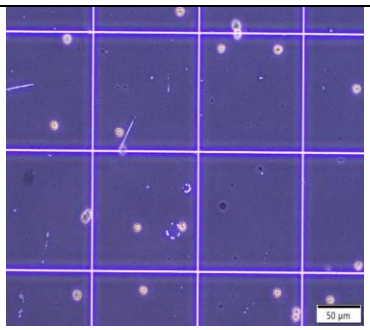
MICROSCOPIC IMAGES OF CELL LINE



1. After Revival of cancer cells



2. Before splitting of cancer cells



3. Before seeding cancer cells

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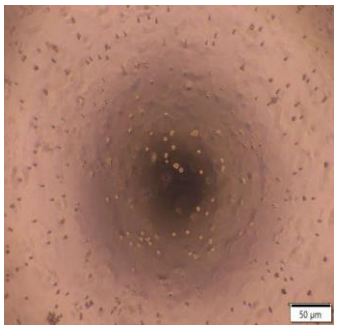
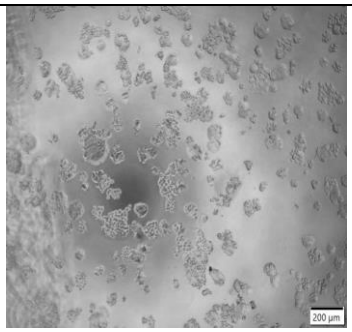
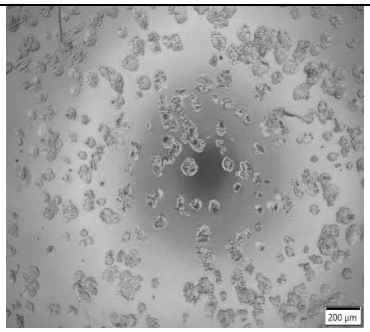
<td> 
4. Seeded cancer cells</td>
<td> 
5.After treatment control</td>
<td> 
6. After treatment with Shankhdrav Rasa</td>
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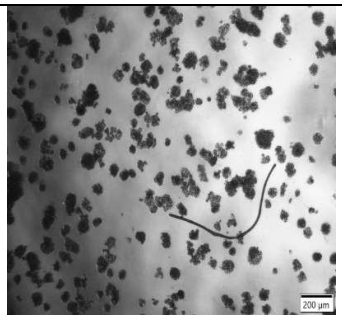
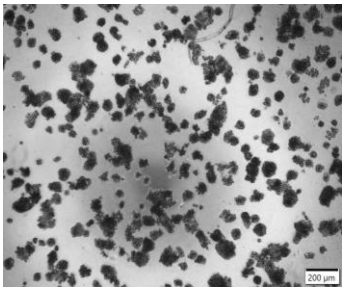
<td> 
7. After treatment with MTT Shankhdrav Rasa</td>
<td> 
8. After Treatment with MTT Control Reagant</td>
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DISCUSSION

- *Shankhdrav Rasa* is a pale yellowish - Colored liquid, which is having ammonia like odour.
- pH - 1.76, acidic nature.
- Specific gravity - 1.0955, indicating that formulation possesses higher density than water due to dissolved minerals and acidic constituents.
- This implies proper extraction of components present in mineral. The denser liquid remains in contact with gastric mucosa slightly longer, allowing better interaction in stomach.
- The medicine has an amazing *Ksharana* property, which enables it to dissolve tough materials such as *Dhatu, Varatika and Shankha*. The dissolution of these substances makes the name "*Shankhadrav"* more appropriate for this formulation.
- The practice of *Ghrita-lipta Mukha* has been referred in *Rasayoga Sagara* as a safety measure while consuming this medicine.
- R.I - 1.352, indicating presence of dissolved mineral salts.
- This indirectly supports consistent bioavailability and therapeutic efficacy.
- The cytotoxicity study of the test formulation against the HT 29 human colorectal adenocarcinoma cell line provided valuable insights into its anti-cancer potential.
- The MTT assay demonstrated a clear dose-dependent response, where higher

concentrations of the drug markedly reduced cell viability, while lower concentrations allowed negligible inhibition. This trend is consistent with the behaviour expected from an effective cytotoxic agent.

- The IC_{50} value of 5.498 µL/mL highlights the potency of the test drug, as compounds with IC_{50} values in the low range are generally considered pharmacologically significant.
- Overall, the results establish that the formulation exhibits cytotoxic and anti-proliferative activity against HT 29 carcinoma cells.
- The clear dose-response relationship suggests that its activity is not random but linked to a consistent mechanism of action
- Thus, this experiment provides scientific evidence that the traditional formulation may hold promise as an anticancer agent, especially in the context of colorectal cancers, and warrants further investigation through in vivo studies and mechanistic explorations.

CONCLUSION

- Identification and authentication of Raw material was done from reliable laboratories.
- Shankhadrav Rasa was prepared by the method mentioned is Ras Sanket Kalika (4/83-89).
- Shankhdrav Rasa was prepared by distillation method and analytical study was done according to given guidelines
- Shankhadrav Rasa prepared was transaparent liquid and having ammonia like smell, alkaline in nature.
- Results of Organoleptic and Analytical study of Shankhadrav Rasa are within permissible limits. Following conclusions can be drawn from analytical study-Ph is 1.7 i.e Highly Acidic in nature

  Specific Gravity is 1.0955 Refractive Index is 1.352
- The IC50 value obtained from the in vitro HT-29 colorectal adenocarcinoma cell line study is 5.498 µg/mL, highlights the potency of the drug and this range is generally considered pharmacologically significant.
- Shankhdrav Rasa in concentration of 5 µL/ml showed cytotoxic activity on HT-29 Cancer cells.
- The clear dose response relationship suggests that its activity is not random but linked to a consistent mechanism of action. Importantly, the relatively low IC_{50} indicates that effective activity is achieved with small amounts of the drug, which is desirable from a

therapeutic perspective.

FUNDING

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