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PHARMACEUTICO-ANALYTICAL STUDY OF ABHAYARISHTA AND ITS IN VITRO CYTOTOXIC ACTIVITY ON HT-29 HUMAN COLORECTAL ADENOCARCINOMA CELL LINE

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ABSTRACT

Ayurveda, the ancient system of Indian medicine, encompasses a wide range of pharmaceutical preparations described under the discipline of Bhaishajya Kalpana. Among these, *Sandhana Kalpana* represents a unique class of formulations prepared through controlled fermentation processes. Abhayarishta is one such Ayurvedic fermented formulation indicated in various Gastrointestinal diseases like *Arsha, Grahani, Udara, Aruchi. Abhayarishta* is also useful in *Pliharoga, Hridroga, Shotha, Pandu, Kamala, Gulma, Kushtha, Shwitra, Vyanga, Rajayakshma, Jwara, Krumi, Granthi, Arbuda.* Abhayarishta is discussed in various Ayurvedic Granths, but in this particular study, it has been cited from Charak Samhita. The current research was conducted for the preparation and standardization of Abhayarishta based on the classical references of Charak Samhita and to evaluate its in vitro cytotoxic activity against HT29 Colorectal adenocarcinoma cell line. The formulation was prepared using authenticated raw materials following standard pharmaceutical procedures. Organoleptic and physicochemical analyses were carried out as per Ayurvedic pharmacopeial standards. In vitro cytotoxic activity was assessed using MTT assay on HT29 Colorectal adenocarcinoma 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. Evaluation through cytotoxic studies revealed the dose-dependent cytotoxicity with increased inhibition in cell viability with increase in concentration, suggesting considerable cytotoxic property of the formulation. The results of the present study offer a preliminary scientific validation for the traditional use of Abhayarishta in ailments related to gastrointestinal diseases and Arbuda suggesting its potential for further in-vivo and clinical anticancer research.

KEYWORDS: Ayurveda, Abhayarishta, Sandhan kalpana, Arishta, Arsha.

INTRODUCTION

Ayurveda, the traditional system of medicine of India, aims to maintain health and cure diseases through a holistic approach. Along with diagnosis and treatment, the preparation of medicines plays a vital role in Ayurvedic therapeutics. The science that deals with the preparation, processing, preservation, and administration of medicines is known as Bhaishajya Kalpana.

Sandhana Kalpana is one of the important pharmaceutical preparations described in Ayurvedic classics. The word "Sandhana" refers to the process of fermentation. Among the various formulations of Sandhana Kalpana, Asava and Arishta are the most commonly used and clinically important preparations. Asava is prepared by fermenting fresh herbal juices, cold infusions, or other liquid extracts without boiling, whereas Arishta is prepared by fermenting herbal decoctions after boiling the drugs in water.

The therapeutic importance of Asava-Arishta lies in their rapid absorption, longer shelf life, improved palatability and enhanced bioavailability. Due to the presence of self-generated alcohol, these formulations can efficiently carry the active principles of herbs into the body and produce quicker therapeutic action.

Modern studies suggest that fermentation may improve the extraction of phytochemicals ensuring extraction of both water and alcohol soluble constituents, increase the concentration of bioactive compounds, and enhance therapeutic efficacy. As a result, Asava and Arishta continue to hold an important place in both traditional and contemporary Ayurvedic medicine.

Abhayarishta is discussed in various Ayurvedic Granths, In the present study the reference is taken from Charak Samhita Arsha Rogadhikar. Abhayarishta is one such classical Ayurvedic

formulation, which has been traditionally employed to manage all kinds of gastrointestinal ailments like *Arsha, Grahani, Udara, Aruchi, Pliharoga, Shotha, Pandu, Kamala, Gulma, Kushtha, Shwitra, Vyanga, Rajayakshma, Jwara, Krumi, Granthi, Arbuda* etc and heart conditions.

Abhayarishta contains various medicinal plants including *Haritaki, Amalaki, Kapittha, Indravaruni, Pippali* many of which have been reported to possess Cytotoxic, Chemopreventive, Radioprotective, anti-ulcer, anti-tumor, anti-inflammatory, anti-oxidant and anti-proliferative activity.

Although individual ingredients of Abhayarishta have been studied for various pharmacological activities, limited scientific evidence is available regarding the cytotoxic potential of the compound formulation against colorectal cancer cell lines. No studies have been conducted so far to examine the cytotoxic effect of Abhayarishta on colorectal cancer.

Therefore, the present study was undertaken to prepare and standardize Abhayarishta according to classical Ayurvedic procedures and evaluate its in vitro cytotoxic activity against HT29 Colorectal adenocarcinoma cell line using MTT assay.

AIM: The study of in-vitro cytotoxic action of Abhayarishta on colorectal adenocarcinoma using HT-29 cell line.

OBJECTIVES

PRIMARY OBJECTIVE

To prepare Abhayarishta as mentioned in the Charak Samhita.

SECONDARY OBJECTIVES

1. To analyse pharmaceutical characteristics of Abhayarishta.
2. To evaluate the in vitro cytotoxic action of Abhayarishta on colorectal adenocarcinoma cell line.

MATERIALS AND METHODS

The entire study is divided into three stages

- Pharmaceutical work i.e. Preparation of Abhayarishta
- Analytical study
- Experimental study i.e. In vitro cell line study

1. PHARMACEUTICAL STUDY

a. Collection, identification, authentication of herbal drug

Raw materials like *Haritaki, Amalaki, Kapittha, Indravaruni, Vidanga, Pippali, Lodhra, Maricha, Elavaluka* etc. were procured in whole dry form. They were collected from well-known authentic source and authenticated from renowned pharmacy of Mumbai. Jaggery was also procured from authentic source.

b. Preparation before fermentation

All the dry whole drugs were pounded using mortar pestle, *Amalaki* and *Kapittha* fruit pulp was deseeded and sun-dried. Jaggery was cut into small pieces. The fermentation jars and other equipment's were rinsed using hot water, dried in sunlight. Then Lepana was done by *Ghrit* and it was fumigated using *Dhoopana* drugs (*Guggulu, Vacha, Jatamansi churna, Raal, Nimba churna*).

c. Preparation of Abhayarishta

1. For the preparation of Abhayarishta kwath, all the pounded dry ingredients were taken in steel vessel and soaked in 2.5 litres of water overnight.
2. Next morning potable water was added around 3.8 litres and the level of water was marked in the vessel. The remaining 11.5 litres of water was added along with the soaked kwath dravyas.
3. This mixture was allowed to heat on medium flame. The kwath was reduced until 1/4th part was obtained i.e. 3.8 litres of kwath was obtained and filtered using a clean muslin cloth in a sterile fumigated stainless-steel vessel under aseptic conditions.
4. Once the kwath got cooled down, jaggery pieces were added and stirred till it dissolved completely. This mixture was again filtered to remove any undissolved physical impurity and thereafter was filled in the fumigated Sandhan patra.
5. The fermentation jar (sandhan patra) was sealed using cotton cloth smeared with multani mitti (fuller's earth clay) and kept in a clean, dry, dark place for the duration of fifteen days.
6. After 15 days, the jar was opened to observe the progress of the fermentation process. Confirmatory tests such as lime water test, sound test, and matchstick test were conducted to verify that the fermentation process is completed successfully.
7. The fermented mixture was then filtered by using clean cotton cloth in different containers. The final product was kept separately in amber colored glass jars.

Table 1: Ingredients of Abhayarishta.

SR.NO	INGREDIENTS	LATIN NAME	PART USED	QUANTITY
1.	Haritaki	*Terminalia chebula*	Pericarp	120g
2.	Amalaki	*Emblica officinalis*	Fruit	240g
3.	Kapittha	*Feronia limonia*	Fruit pulp	150g
4.	Indravaruni	*Citrullus colocynthis*	Root	75g
5.	Vidanga	*Embelia ribes*	Fruit	30g
6.	Pippali	*Piper longum*	Fruit	30g
7.	Lodhra	*Symplocos racemosa*	Stem bark	30g
8.	Maricha	*Piper nigrum*	Fruit	30g
9.	Elavaluka	*Prunus avium*	Stem bark	30g
10.	Guda	Jaggery	------	3000g
11.	Jal	Water	-------	15.36L

2. ANALYTICAL PARAMETERS

- **Organoleptic and Physicochemical Analysis**

The prepared Abhayarishta sample was subjected to analysis for organoleptic studies such as colour, odour, taste and texture, followed by physicochemical analysis such as pH, alcohol percentage, specific gravity, total solids and total sugar content according to standard Ayurvedic pharmacopeial procedures.

- **Thin Layer Chromatographic Analysis**

Abhayarishta consists of both active and inactive components, thus, TLC was employed using suitable solvent systems to detect these phytoconstituents components using toluene: ethyl acetate: formic acid: methanol (3:3:0.8: 0.2) as mobile phase.

- **Microbial contamination**

Total Bacterial Count and Total Yeast and Mould Count are used to determine microbial contamination of the Abhayarishta sample.

3. IN VITRO CYTOTOXIC ACTIVITY

The cytotoxic effect of Abhayarishta on the HT29 Colorectal adenocarcinoma cell line was assessed by employing the MTT assay method.

1. The HT29 cells were grown in a proper culture medium enriched with fetal bovine serum under standardized incubating conditions at 37°C in 5% CO_2 environment.
2. The cells were seeded in 96 well microplates containing DMEM medium with 10% FBS at the required cell density (2×10^3 cells per well) and left for a suitable time period to attach properly.
3. Various concentrations of Abhayarishta (10 µL/mL, 5 µL/mL, 2.5 µL/mL, 1.25 µL/mL,

0.62 µL/mL, 0.31 µL/mL) were administered to the cultured cells and allowed to incubate for 48 hrs.

4. After the incubation, the cells were treated with the MTT reagent and incubated further to allow the production of formazan crystals from the living cells.
5. The crystals were dissolved in a DMSO solvent and absorbance was measured by a microplate reader at appropriate wavelength.
6. Percentage cell viability and IC50 value were calculated from the obtained absorbance values.

RESULTS

Table 2: Organoleptic and Physicochemical values.

TEST	RESULT
Appearance	Fermented Liquid
Colour	Dark blackish brown colored
Odour	Mild alcoholic
Taste	Astringent
pH	4.10
Specific Gravity	1.09
Loss on Drying	0.57%w/w
Total Solid Content	16.54%w/w
Alcohol Content	6.78%w/w
Total Sugar	20.10%w/w
Reducing Sugar	19.71%w/w
Non-Reducing Sugar	0.39%w/w
Microbial Contamination Total Bacterial Count Total Yeast and Mold Count	68 CFU/ml 88 CFU/ml

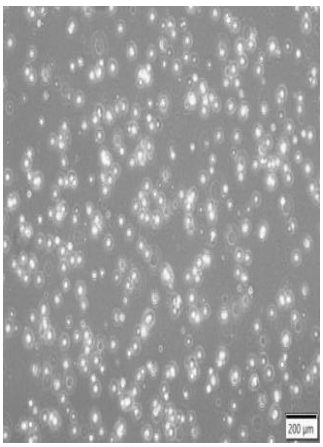
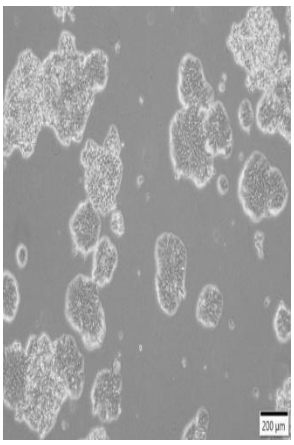
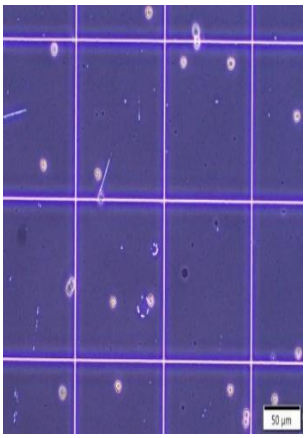
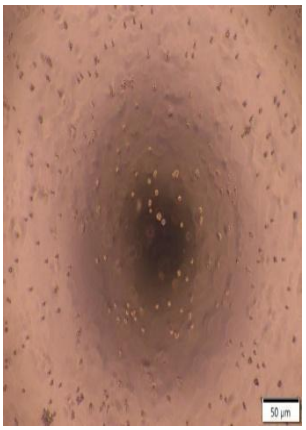
Thin Layer Chromatographic Analysis

Two distinct active phytochemical spots visible at Rf, 0.44 (yellow fluorescent) and Rf, 0.64 (light violet), confirming the successful extraction of key herbal marker compounds.

In Vitro Cytotoxic Activity

The cytotoxic effect of Abhayarishta against the HT29 Colorectal adenocarcinoma cell line was determined by using MTT assay. The formulation showed dose-dependent cytotoxic effect, maximum decrease in percentage cell viability was recorded at high doses. At low doses, mild inhibition in growth of cells was recorded, while at high doses, more cytotoxic effects were observed in HT29 cells.

Fig 1-8. - OBSERVATION OF MICROSCOPIC IMAGES OF CELL LINE

1	2	3	4
			
Cells after revival from liquid nitrogen	Before splitting cancer cells	Before seeding cancer cells	Cancer Cells seeded

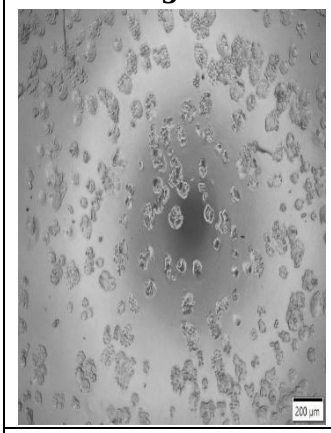
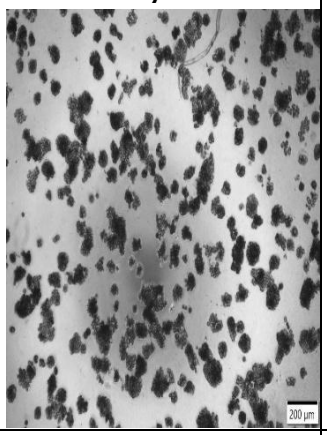
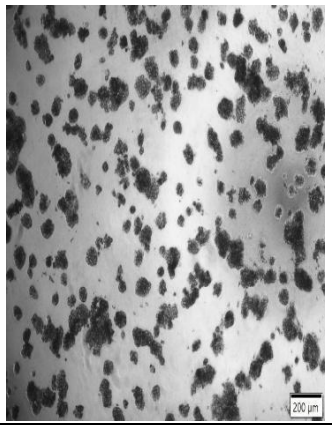
5	6	7	8
			
After treatment control	After treatment with Abhayarishta	After treatment with MTT- control	After treatment with MTT - Abhayarishta

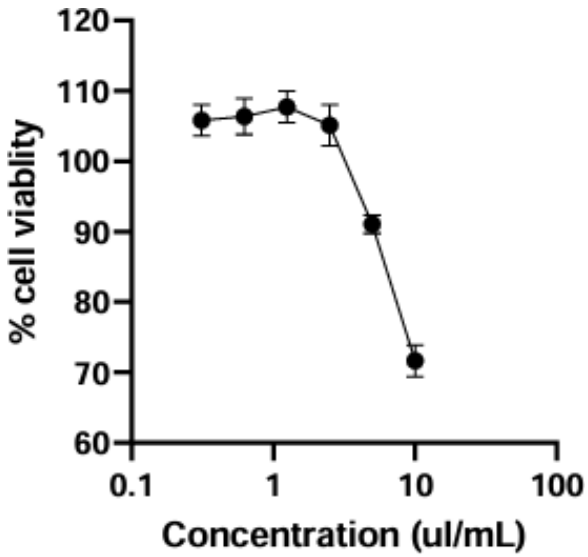
Table 3: The percentage cell viability at different concentrations.

Treatment	Conc. (µL/mL)	% Cell survival (n1)	% Cell survival (n2)	% Cell survival (n3)
Test Formulation	10	75.7576	67.9121	71.3137
Test Formulation	5	92.6407	92.0879	88.4718
Test Formulation	2.5	100.866	103.736	110.724
Test Formulation	1.25	103.247	110.769	109.115
Test Formulation	0.62	105.195	102.637	111.26
Test Formulation	0.31	106.277	101.758	109.383
DMEM + Cell Control	0	100.00	100.00	100.00

IC50 Determination

Table No. 4: Inhibitor vs. response – variable slope.

Best fit value	
IC50	**5.348**
Hillslope	**-4.205**
Logic50	**0.7282**
Span	**36.88**
Goodness of fit	
Degree of freedom	**17**
R Squared	**0.8989**
Sum of squares	**329.0**
Sy.x	**4.399**
Constraints	
IC50	**IC50 > 0**
Number of points	
# of X value	**21**
# of Y values analyzed	**21**



IC50 value for Abhayarishta on HT29 Colorectal adenocarcinoma cell line was determined based on the dose response curve using MTT assay. The formulation showed significant cytotoxic potential against the tested cell line with an IC50 value of 5.348. This implies that the drug has in vitro cytotoxic efficacy against HT29 Colorectal adenocarcinoma cell line.

DISCUSSION

Abhayarishta as mentioned in Charak Samhita Arsha Rogadhikar is explained in the current research paper. Abhayarishta is useful in treating different gastrointestinal disorders and has a unique action on account of which it is helpful in treating colorectal cancer. For this current research work, the preparation of the formulation was done using classic Ayurvedic methods,

and evaluated through analytical and in vitro cytotoxic assessment. This research aimed to scientifically justify the traditional use of Abhayarishta in conditions similar to those caused by Arbuda.

Abhayarishta in this study is prepared according to the reference of Charak Samhita, production of Abhayarishta is done according to the principles of Arishta Kalpana in which Kwatha (decoction) and Madhura dravya (Jaggery) is mixed together and allowed to ferment in a sealed container known as Sandhana Patra.

Decoction made using *Haritaki, Amalaki, Kapittha, Indravaruni, Vidanga, Pippali, Lodhra, Maricha and Elavaluka* as per the required formula led to the extraction and preservation of bioactive phytoconstituents that provide the therapeutic basis for Abhayarishta. Jaggery added as Madhur Dravya provides the substrate required for fermentation, which is essential for natural fermentation and production of self-generated alcohol. This self-generated alcohol serves as a natural preservative and aids in the extraction of both the water-soluble and alcohol-soluble active components. This mechanism may account for enhanced bioavailability and increased effectiveness of the preparation. The fermentation process occurred under warm and shaded conditions for 15 days.

The analytical studies of Abhayarishta have further confirmed the quality and stability of the formulation, with pH 4.1, meaning it is acidic in nature, stabilizes the preparation and inhibits pathogenic growth. Specific gravity 1.09, moisture content 0.57 %w/w, solid content value 16.54%w/w can be attributed to the decoction process, which enhances the extraction of tannins and alkaloids. Alcohol content is 6.78%w/w, Total sugar is 20.10%w/w, reducing sugar is 19.71 %w/w and non-reducing sugar is 0.39%w/w, Total Bacterial Count of Abhayarishta is 68 cfu/ml. Total Yeast and Mold Count of Abhayarishta is 88 cfu/ml, which confirms that the formulation was prepared and stored under hygienic conditions. Thin Layer Chromatography (TLC) profiling showed a prominent yellow fluorescent spot Rf at 0.44 light violet spot at Rf 0.64, verifying the successful extraction of key herbal marker compounds.

The cytotoxicity evaluation of Abhayarishta against the HT29 Colorectal adenocarcinoma cell line demonstrated significant anticancer potential. The MTT assay showed a clear dose-dependent response in cell viability, with higher concentrations producing greater cytotoxic effects.

Such observed effects might have been due to the presence of phytoconstituents with antioxidant, anti-inflammatory, and possibly anticancer potentials in the component drugs of Abhayarishta like Haritaki which is Dipaneeya, Anuloman, Pachaniya, Arshoghna, Vranaropaka, Shulahara, Rasayana. Modern research shows ethanolic fruit extract of Terminalia chebula, chebulagic acid, chebulinic acid showed potent anti-proliferative activity against HT29 colon cancer cells. Its aqueous extract showed Chemopreventive and Radioprotective activity.

Modern cancer research shows that Indravaruni (Citrullus colocynthis) leaf extracts have been shown to reduce the growth of HT-29 cancer cells in a dose dependent manner. This effect is likely due to the presence of flavonoids and phenolic compounds, cucurbitacin glycosides Piper nigrum Extract Inhibits the Growth of Human Colorectal Cancer HT-29 Cells by Inducing p53-Mediated Apoptosis.

Phenolic compounds from Emblica officinalis (Amalaki) extract identified by HPLC having anticancer properties like Ellagic acid (tannin), Chebulagic acid in Colon.

Ethanolic extract of Symplocos racemosa (Lodhra) and extract of Prunus avium (Elavaluka) exhibited invitro cytotoxic activity against HT29 Colorectal adenocarcinoma cell line.

Altoghether synergistic interaction of these multiple phytoconstituents present in the formulation may contribute to the overall activity observed in the study.

CONCLUSION

In the current study, preparation of Abhayarishta was performed on the basis of classical Ayurveda principles. The prepared formulation exhibited satisfactory organoleptic and physicochemical properties, suggesting proper fermentation and quality of the formulation.

In vitro cytotoxic evaluation against the HT29 Colorectal cancer cell line has shown considerable cytotoxic activity of Abhayarishta. The above-mentioned effect may be due to the interaction between different plant phytoconstituents available in the drugs used for the development of this preparation.

The results of this study may serve as scientific evidence in favor of the traditional Ayurvedic usage of Abhayarishta in diseases that may be associated with Arbuda. However, further research studies involving in vitro, in vivo, and clinical trials are required to ascertain the

mechanism of action, therapeutic potential, efficacy, and safety of the formulation in managing cancer.

Overall, the study illustrates the importance of Abhayarishta as a potentially valuable Ayurvedic preparation for future anticancer research.

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REFERENCES

1. Kashinatha Sastri PL, Gangasahaya Pandeya. The Caraka Samhita of Agnivesa: Revised by Caraka and Drdhabala with the Ayurveda-Dipika Commentary of Cakrapanidatta and Vidyotini Hindi Translation, Arshachikitsaadhyaya14. 4th ed. Varanasi: Chaukhambha Sanskrit Sansthan, 1994.
2. Acharya Paksadhara. AsavaristaVijñāna. Varanasi: Chaukhamba Bharati Academy, 2008.
3. Sharma PV. Dravyaguna Vigyan. Vol 2. Varanasi: Chaukhamba Bharati Academy, 2013.
4. Ranade M. Textbook of Rasashastra evam Bhaishajya Kalpana: Ayurvediya Aushadhi Nirmana Vigyana. Paper 1. Varanasi: Chaukhambha Classica, 2025.
5. Shastri D. Ayurvedeeya Aushadhikaran. 4th edition. Shree Dhootpapeshwar Ltd, 1964, Government of India, Ministry of Health and Family Welfare, Department of AYUSH. The Ayurvedic Pharmacopoeia of India.
6. Mohan H. Textbook of Pathology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers, 2010.
7. Davidson's Principles and practice of Medicine: Edited by Christopher haslett, published by, Churchill Livingstone, London, New York, 1999 (18th 150 edition).
8. MTT Cell Proliferation Assay Instruction Guide – ATCC, VA, USA www.atcc.org
9. Denis Gerlier, Nicole Thomasset, Use of MTT colorimetric assay to measure cell activation, Journal of Immunological Methods, 2, 1986; 94(1): 57-63, ISSN0022-1759, https://doi.org/10.1016/00221759(86)90215 2. https://www.sciencedirect.com/science/article/pii/0022175986902152
10. Tim Mosmann, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, Journal of Immunological Methods, 1983; 65(1-2): 55-63, ISSN 0022-1759, https://doi.org/10.1016/0022 1759(83)903034. https://www.sciencedirect.com/science/article/pii/0022175983903034.
11. Alley, M. C., Scudiere, D. A., Monks, A., Czerwinski, M., Shoemaker, R. II., and Boyd,

M. R. Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. Proc. Am. Assoc. Cancer Res., 1986; 27: 389. https://pubmed.ncbi.nlm.nih.gov/3335022/

12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods, 1983; 65: 55-63. https://www.nccs.res.in/cellrepository
13. Bhati K, Wagh K. Cancer: An Ayurvedic outlook. Int Ayurvedic Med J., 2015; 3(3): 958-965.
14. Sastry JLN. Introduction to oncology, cancer in Ayurveda. Varanasi: Chaukhambha Orientalia, 2001; p. 1–24.
15. Rosenberg R, Keerl A, Kocher T, Bader F, Schmid R, Friess H, et al. The informed patient: Colorectal cancer, colon carcinoma, rectal carcinoma. 1st ed. Freiburg (Germany): Falk Foundation e.V., 2013.
16. 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