Pharmaceutico-Analytical Study of '*Nagavalli Marit Abhrak Bhasma*' and Evaluation of Its Anticancer Activity using A549 Cell Line Model.

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ABSTRACT

Introduction: "Cancer" is a wide range of illnesses that can impact any region of the body. Other names that are used are neoplasms and malignant tumors. One characteristic of cancer is the quick development of aberrant cells that proliferate beyond their normal limits. These cells can then infect other body parts and move to other organs; this process is known as 'metastasis'. Lung cancer is the leading cause of cancer-related deaths worldwide, accounting for the highest mortality rates among both men and women. Overall, the chance that a man will develop lung cancer in his lifetime is about 1 in 16; for a woman, the risk is about 1 in 17. Lung cancer is the term for the illness that results in uncontrollably dividing lung cells. Ayurvedic practices often include Herbal and Herbo-mineral remedies. *Bhasma* holds significant importance in managing various type of cancer with Ayurvedic medicine. The Herbomineral preparation like *Abhrak Bhasma* possess a properties like *Rasayana* (which promotes life), Sarvarogahara, *Yogavahitwa*(which promotes passage and movements of a drug) and Shigravyapti(fast spreading), Tridosha shamak. In this study, *Nagavalli marit Abhrak Bhasma* was prepared, analysed and investigated the Anticancer activity using A549 cell line model and assessed the results via MTT assay, and calculated the IC50 value of *Nagavalli marit* Abhrak Bhasma. **Aim:** To do Pharmaceutico-analytical Study and evaluate Anticancer activity of "*Nagavalli marit Abhrak Bhasma"* in A549 cell line model. **Objectives**: Primary Objective: 1. To evaluate Anticancer Activity of "*Nagavalli marit Abhrak Bhasma"* using A549 cell line model. **Secondary Objectives:** 1. To study pharmaceutical and analytical process *of "Nagavalli marit Abhrak Bhasma"* as mentioned in *Rasatarangini.* 2. To estimate the IC50 value of "*Nagavalli marit Abhrak Bhasma".* **Materials & Methods:** *"Nagavalli marit Abhrak Bhasma"* was prepared according to the reference given in *Rasatarangini*-10/72-73. Analytical tests of prepared *Abhrak Bhasma* were performed according to methods described in Ayurvedic pharmacopoeia of India. Experimental study was done in lab by inducing lung cancer to A549 cell line model & treating it with 'Nagavalli marit *Abhrak Bhasma'.* **Result:** The "*Nagavalli marit Abhrak Bhasma"* has shown decreased % cell viability as there was increase in "*Nagavalli marit Abhrak Bhasma*" concentration. The considerable decreased % cell viability i.e. 48.48 and 53.12 % was observed at the concentrations of 2000 and 1000 µg/mL that there is a significant Anticancer Activity of "*Nagavalli marit Abhrak Bhasma"* using A549 cell line model with half-maximal inhibitory concentration (IC50) value is 1766.358 µg/mL.

Conclusion: According to this study, "*Nagavalli marit Abhrak Bhasma"* can be considered as a potent medicine for treatment of lung cancer and can be preceded for using as a therapeutic drug.

Keywords: *Abhrak Bhasma*, Cancer, Lung cancer, *Arbuda*, Anticancer activity, Preclinical, A549 cell line model, In vitro study, MTT assay, IC50.

Introduction

Cancer is a phrase used to describe a condition where aberrant cells proliferate uncontrollably and have the ability to move into or spread to other body parts through the lymphatic and circulatory system[(1)]. Lung cancer is the most common type of cancer diagnosed globally and the main cause of deaths related to cancer. Lung cancer makes up 5.9% of all malignancies and 8.1% of deaths from cancer-related causes in India.[(2)] Lung cancer is the term for the illness that results in uncontrollably dividing lung cells. Roughly 90% of instances of lung cancer are brought by using tobacco products and smoking. But additional elements such as asbestos, radon gas, air pollution, exposures, and long-term illnesses can facilitate the development of lung cancer.[(3)] The most common type of lung cancer is a Non-small-cell lung cancer (NSCLC) which accounts 85% and other small-cell lung cancer which accounts 15%.[(4)]Lung cancer manifests symptoms extremely late in the disease's development and only after it has spread widely. The most typical respiratory symptom of lung cancer, according to reports, is a cough, others symptoms of lung cancer include dyspnea, weight loss, clubbing, fever, haemoptysis, chest discomfort, dysphagia, wheezing, and stridor.[(5)] One of the major risk factors and 80% of deaths from lung cancer is caused by tobacco smoking,[(6)]Genetic Factors,[(7)] Gender,[(8)]Age,[(9)] Exposure to Mine Dust,[(10)] Radiation exposure,[(11)] etc. are some risk factors for developing lung cancer.

Rasashashtra is nothing but the "Science of Mercury". *Rasashastra* is a specific branch that works primarily with substances referred to as "*Rasadravyas".*[(12)] The focus is primarily on "Mercury" and its preparations. Products falling under this category have a significant role in Ayurvedic medicine.[(13)] Properly prepared herbomineral/metal/non-metal preparations have properties such as *Rasayana* (immunomodulation and anti-aging property) *and Yogavahi (*ability to target medications to the site), and they are also nontoxic, gently absorbable, adaptable, and digested in the body.[(14)]*Acharya Charaka* has clearly stated that nomenclature of all the diseases cannot be done. Hence such diseases should be treated according to their *Nidan, Dosha, Dushya,*etc. The treatment focused on this entity definitely cure the disease.[(15)] *Avastha of doshas, dushya, bheshaj, desh, kala, bala, sharir, ahara, saatmya, satva, prakruti, and vaya* should be taken into mind when treating *Anukta vyadhi*.[(16)]Numerous classical books in Ayurveda prescribe numerous forms of *Arbuda*. *Vata, Pitta, Kapha, Rakta, Meda and Mamsa Arbuda* are among them. [(17)] In Ayurvedic texts, no direct reference of Lung cancer is found, for taking the above statement into consideration and their signs and symptoms are similar therefore we can correlate *Arbuda* with lung Cancer. Lung cancer shares several characteristics with *Vatarbuda, Kapharbuda, and Mamsarbuda*. The Ayurvedic book provides a precise description of the microenvironment through the *Strotas* and lung cancer is directly associated with the vitiation of *Pranavaha* and *Mamsavaha Strotas*.[(18)]

According to Ayurveda, *Abhrak Bhasma* possess a properties like *Rasayana* (which promotes life)[(19)]according to recent research it can be correlate with concept of immunomodulation,[(20)]Sarvarogahara.[(21)] and its therapeutic properties undergo changes with incineration. Repeated incineration lead to fineness in particle size so show *Yogavahitwa*(which promotes passage and movements of a formulation or drug) and Shigravyapti(fast

spreading),[22] Tridosha shamak. Nanomedicine is emerging as important field for cancer treatment. *Abhrak Bhasma* have unique properties like fast acting and target specific drug delivery and it is mostly acting on *Pranavaha strostas vyadhi* and Lung cancer *(Phuphphusarbuda*)is a *vyadhi* of *Pranavaha strostas.*

As per earlier cited publications, studies, and books, it has been discovered that *Abhrak Bhasma* possess anticancer activity [23-26] and *Shodhan dravyas*, such as '*Triphala'*,[27] it include- *Amalaki* (Emblica officinalis)[28,29] is Strong antioxidant, cytoprotective properties. It have constituents like phenols, tannins, gallic acid, ellagic acid and pyrogallol that show inhibition of proliferation and to induce apoptosis in nonsmall cell lung cancer(NSCL) disease. *Bhibhitaki*(Terminalia belerica)[30]- Fruit contains active principle like Tannins, beta sistersterol, chebulic acid, mannitol, flavones, lignans gallic acid, etc. which have anti-inflammatory, immunomodulatory properties. *Haritaki*(Terminalia chebula)[31-33])- It have phytochemicals such as polyphenols, terpenoids, Tannic acid, chebulagic acid, chebulinic acid, terpenes, flavonoids, sterols and possess anti-oxidant, immunomodulatory, anti-pyretic, anti-cancer properties and *Marana Dravya* such as '*Nagavalli Patra' (*Betel leaf)- It contain Ascorbic acid, sucrose, tannin, carotene, and essential oils. The primary components of essential oils are phenol and eugenol, and they also often have high in terpene and phenolic content that show antioxidant and carcinogenic activity.[34,35] It also contain beta-carotene and alpha-tocopherol(Vit.E) and carotenoids is a effective inhibitors of cancer in humans.[36]This drug shows promising anticancer activities individually, there is no study found in combination of these drug with *Abhrak Bhasma*. Therefore, *Abhrak Bhasma*, incorporated by these drugs is the potential medicine to be studied for anticancer activity using A549 cell line model.

Material & Method:

This Study was conducted under 3 headings:

1. Pharmaceutical study
2. Analytical Study
3. Experimental Study

Pharmaceutical study:-

Collection and Authentication of Raw Drug-

Each and every raw materials were obtained from authenticated vendor and further authenticated from experts of RSBK department of our institute and verified by the Botany department of Rashtrasant tukadoji maharaj, Nagpur university(RTMNU). In this study *Abhrak Bhasma* was prepared by the traditional method according to *"Rasatarangini*" mentioned in *Dashamtarang*- chapter 10.

It includes-

1. *Shodhana* of *Abhrak(mica)* - a) Preparation of *Triphala kwatha* (Decoction)[37]

b) Shodhana of *Vajraabhrak* by *Nirvapa* (quenching)[38]

2. Preparation of *Dhanyabhrak* - a) *Kanji* preparation[39]

b) *Dhanyabhraka Nirmana*[40]

3. Extraction of *Nagavalli Patra swarasa*[41]
4. *Marana* of *Abhrak* (Incineration of Mica)[42]

***Shodhana* of *Abhrak (purification of mica)*[43]-**

Triphala kwath was prepared according to *Sharangdhar Samhita, M.K. 2/1-2* and quenching of *Ashuddha Vajraabhrak* into prepared *triphala kwatha, Shodhan (purification)* of *Abhrak(mica*) was done according to *Rasa Ratna Samucchaya* 2/16-17. Good quality of *Triphala Bharad* was taken and boiling in water until the volume of water was reduced to 1/4th. 1000 g of impure *Abhrak* was taken and heated on gas stove until it gets red hot. Red hot *Abhrak Patra* were quenched into *Triphala kwatha(decoction)* medium. After cooling they were removed and then dried. The above procedure of heating and quenching repeated 7 times with new *Triphala kwatha* medium was taken per round.

Table No.1: Ingredients of *Abhrak Shodhana* process

Sr. no.	**Ingredients**	**Quantity**
1.	*Ashu. Abhrak*	1000 g
2.	*Triphala Bharad*	500 g
3.	Water	4000 ml
4.	Prepared *Triphala kwath* for 1 Nirvap	1000 ml
5.	Obtained *Shuddha abhrak*	960 g

Ashuddha Abhrak

Triphala Kwatha

Heating *of Ashuddha Abhrak*

Quenching of *Abhrak To Triphala kwatha*

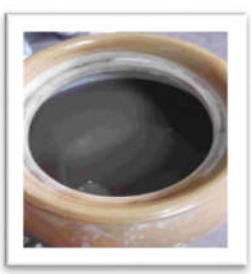
Shuddha Abhrak

Preparation of *Dhanyabhraka* (fine particles of *mica*) -

Kanjii was prepared for *Dhanyabhraka* according to *Sharangadhara Samhita.* It is a fermentative preparation for *shodhana* of metals/minerals. It is prepared from Red variety rice(1000g) and *Masha* (250 g) and fermented for

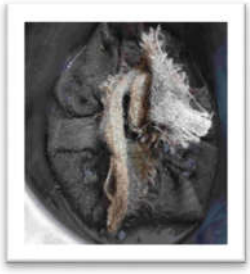
21 days after that used for *Dhanyabhraka Nirmana*. *Dhanyabhraka* was prepared according to reference given in *Rasaratna samuchaya*: 2/21. One part of *Shuddha Abhrak* and quarter part of *Rakta Shalidhanya* were taken and mixed properly. Then tied in jute cloth tightly in the form of *pottali* and immersed in *kanji* and kept undisturbed for 3 days. After 3 days, *Pottali* was rubbed with the help of wooden bat or hand. Fine particles of *Shuddha Abhrak* were gathered in *kanji.* It was left to settle and the *kanji* was drained and *Dhanyabhraka* was prepared.

Ingredients in Dhanyabhraka Nirman:



Kanjii

Shuddh Abhrak+ Dhana+Kanjii



Soaking of Pottali into kanji

Final Dhanyabhrak

Extraction of *Nagavalli Patra swarasa-*

Nagavalli Patra swarasa was prepared according to reference given in *Sharangdhara Samhita, Madhyama Khanda 1/2*. *Nagavalli patra*(700g) were carefully cleaned and divided into tiny pieces. These tiny pieces were put on the grindstone and crushed well until it turned into smooth paste. After that, the paste was spread out on a fresh cotton cloth and squeezed until all of the liquid was extracted from the *Kalka*. This liquid substance was appropriately gathered and preserved as *Nagavalli patra swarasa*(410 ml). *Haritabha*(greenish), *Nagavalli patra Gandhi* (typical smell), *Nagavalli Patra Swarasa* was obtained.

Prepartion of *Abhrak Bhasma-*

The whole procedure of *Abhrak Marana* was divided under 3 headings as below :

1. *Purva karma*: a. Preparation of *Nagavalli Patra swarasa*

b. *Bhavana of Nagavalli patra swarasa* to *shuddha Abhrak*

c. Preparation of *Chakrika*

d. Preparation of *Sharava Samputa*

2. *Pradhana karma*: a. Subjecting *Samputa to Puta*

b. Observation and recording of Temperature

3. *Pashchat karma*: a. Removal of *Sharava-samputa from Puta*

b. Collection of the product

Procedure-

Shuhddha Abhrak was taken in a clean *Khalva yantra*(Mortar & pestle) and triturated with *Nagavalli patra swarasa* properly. Trituration was done until mixture attained paste like semisolid consistency. After proper

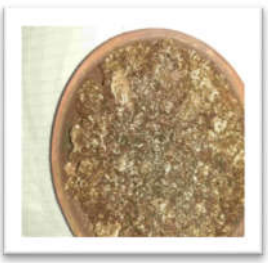
trituration, *Chakrikas*(pellets) were prepared from this paste and allowed them to dry. When the *Chakrikas* dried completely they were placed in earthen saucers facing each other mouth to mouth(*Sharava-samputa*). Then *Sharava-samput*a was sealed properly with the help of mud smeared cotton cloth and dried well. After that, *Sharava* was placed in Gajaputa[44,45] in EMF at 750^0c and incinerated. Then allowed to cool down on its own. After that, the *Sharava-samputa* was opened properly. Same process was repeated until the sample of *Abhrak Bhasma* satisfied all *Bhasmapareekshas.* The change in appearance and weight of observation in *Abhrak Bhasma* after trituration and after incineration was recorded each time. The amount of *Nagavalli patra swarasa* was also measured each time.

Dhanyabhraka

Nagavalli patra swarasa

Bhavana

Sharava-samputa



After 1st *puta*

Abhrak Bhasma

Analytical Study:-

In this study, Analytical evaluation of '*Nagavalli marit Abhrak Bhasma'* was carried out.

A) Organoleptic Characters[46]:- The samples were analyzed for the characters like color, taste, touch and odour.

B) *Bhasma Pariksha*:-

1. Physical parameter of *Bhasma*: *Varitara*[47], *Unama*[48], *Rekhapurnatava*[49], *Sukshma pariksha*[50], *Slakshnatvam* [51], *AnjanaSannibha*[52], *Uttamum, Dantagrakachkachabhav,* etc.

2. Chemical parameter: *Varna, Nirdhumatvam, Nishchandratvam, Niruttha*, etc.

C) Physico-Chemical Analysis:- These also help to decide the standard quality of *Bhasma*

1. Estimation of Ph [53]
2. Loss on Drying105ºC [54]
3. Total ash value [55]
4. Acid insoluble ash [56]
5. Water soluable ash
6. X-Ray Diffraction (XRD)[57]

7. SEM (Scanning Electron Microscopy)[58]

Experimental study:-

In the present study, we evaluated the "Anticancer activity of '*Nagavalli marit Abhrak Bhasma'* using A549 cell line model." We also evaluated the results in terms of morphological changes, the MTT assay, and the IC 50 value.

Test System and Management:

1. **Cell line Name**: A549
2. **Source:** National Centre for Cell Science
3. **Media used:** Hams F12 +10% Fetal Bovine Serum
4. **No of cells used per plate:** 105 cells/ mL

Materials and Methods:

Table No.2: Test item-*Nagavalli marit Abhrak Bhasma*

Name of Test Item	Test item code	Physical State	Vehicle	Quantity Receive
Nagavalli marit	*Abhrak Bhasma* TI-13	Powder	Hams F12 media	10.20 g

Table No.3: Reference item-Capecitabine

Name of Positive control	Make	Batch No.	Expiry Date
Capecitabine	Cipla	GJ30444	Jun. 2025

Study design:

The "*Nagavalli marit Abhrak Bhasma"* was treated at the concentrations of 2000, 1000, 500, 250, 125, 62.5 and 31.25 µg/ mL along with vehicle control, Hams F12 media. Positive control is a capecitabine drug (standard chemotherapeutic agent) at the concentrations of 2000, 1000, 500, 250 and 125 µg/mL in same media. Study was performed by treatment in 96 well plate. Assay was performed in triplicate in each of the test groups. The IC50 value was calculated using GraphPad Prism 10.3.1.

Procedure:

Isolation of A549 cell line (Lung cancer cell):-

A549 is an epithelial cell that was isolated from the lung of a 58-year-old, male with lung carcinoma. This cell line is widely used in cancer research. Growth properties of the cell line is adherent type**.**

MTT assay:-

Principle: Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5- dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular **purple formazan** can be solubilized and quantified by spectrophotometric means.

Preparation of media: A549 cells were maintained in T-75/T-25 cm^2 filter cap flasks in Hams F12 medium + 1% Penicillin Streptomycin / L-glutamine + 10% heat inactivated Fetal Bovine Serum.

Table No.4: Details of media components are given in following table:

Sr. No.	Name of component	Make	Batch No.
1.	F12 media	Himedia	0000650624
2.	Fetal bovine serum	Himedia	0000608853
3.	Antimycotic solution	Himedia	0000216918
4.	Thiazolylblue tetrazolium bromide	Sigma aldrich	LRAD4843
5.	Dimethyl Sulphoxide	Oxford	4868

Table No.5: *Nagavalli marit Abhrak Bhasma* formulation at different concentation:

Weight (mg)/ Vol. (mL)	Total Vol. (mL)	Vehicle Vol. (mL)	Conc. (mg/mL)	Final Conc. in µg/mL	Formulation ID
40 mg	2	2	20	2000	F1
0.5 mL of F1	1.0	0.5	10	1000	F2
0.5 mL of F2	1.0	0.5	5	500	F3
0.5 mL of F3	1.0	0.5	2.5	250	F4
0.5 mL of F4	1.0	0.5	1.25	125	F5
0.5 mL of F5	1.0	0.5	0.625	62.5	F6
0.5 mL of F6	1.0	0.5	0.1325	31.25	F7

Experimental Procedure:

A549 cell was received from NCCS (National centre for cell science), Pune. After receiving of cell line, cells were passaged for two times before using in the test. Cell cultures were removed from culture flask by enzymatic digestion (trypsin/EDTA) and the cell suspension was centrifuged for 1000 RPM and for 3 min. After that cells were resuspended in culture medium and the cell suspension was adjusted at a density of 1 × 105 cells/mL. 100 µL of a cell suspension of 1 × 105 cells/mL (1 × 104 cells/well) was dispensed in each cell. Culture plates were incubated for 24hrs (5 % CO2, 37 ± 1°C, > 95 % humidity) so that cells could form a half-confluent monolayer. This incubation period ensured cell recovery, and adherence and progression to exponential growth phase. Plates were examined under a phase contrast microscope to ensure that cell growth is relatively even across the microtitre plate. 20 µL of treatment medium containing either the appropriate concentration of sample, or the negative

control, or the positive control was added per well in triplicate. Seven different concentrations of the Nagavalli marit Abhrak Bhasma and five concentrations of positive controls capecitabine were used in the assay. Again plate was incubated for 24hrs (5 % CO2, 37 °C, > 95 % humidity). After 24 hrs incubation, treated culture was carefully removed. 10 µL of MTT reagent was added and plate were incubated for 3 hrs. After incubation period MTT reagent was removed completely and 100 µL of dimethyl sulphoxide was added to dissolve the crystals of formazon. Absorbance was measured at 570 nm on ELISA plate reader.

Table No.6: Observation & Result:

Showing observation during *Shodhana of Abhrak*-

Parameters	**1st Nirvap**	**2nd Nirvap**	**3rd Nirvap**	**4th Nirvap**	**5th Nirvap**	**6th Nirvap**	**7th Nirvap**
Wt of Raw Abhrak (g)	1000 (g)	994(g)	984(g)	976(g)	970(g)	966(g)	962(g)
Triphala kwatha (litre)	1 litre	1 litre	1 litre	1 litre	1 litre	1 litre	1 litre
Duration of heat for Abhrak getting red hot (min)	45 min	55 min	55 min	45 min	50 min	50 min	50 min
Initial temp of Triphala Kwatha (ºC)	36.5	36	35	35	36	36	35
Triphala Kwatha remaining after quenching of Abhrak (ml)	600 ml	620 ml	600 ml	600 ml	610 ml	620 ml	620 ml
Final temp of Triphala Kwatha (ºC)	78	80	82	86	80	86	88
Wt of Abhrak a Just after Nirvap (g)	994(g)	984(g)	976(g)	970(g)	966(g)	962(g)	960(g)
Total duration required for complete Nirvapa process (Min)	60 min	55 min	55 min	66 min	60 min	60 min	60 min

Results:

Total amount of *triphala kwatha* used- 7000 ml

Total amount of *triphala kwatha* remained after quenching- 4270 ml

Initial weight of *Abhrak*- 1000 g

Final weight of *Abhrak shodhana*- 960 g

Total amount of *Abhrak* lost- 40 g

Percentage loss after *shodhana*- 4%

Reason for weight loss: Due to *Nirvapa p*rocess, particles size of *Abhrak* becomes small so unable to collect small particles of *Abhrak* and some particles washed out with *Triphala kwatha.*

Table No.7: Showing Observation during *Dhanyabhraka nirman*

Shodhit Abhrak	Yava shali (g)	Kanjii (litre)	Duration (days)	Yield of Dhanyabhraka (g)	Loss of Abhrak (g)
960	240 g	10 lit.	4 days	920 g	40 g

Result-

Initial weight of *Shuddha Abhrak*- 960 g

Final Weight of *Dhanyabhrak*-920 g

Loss of *Abhrak*- 40 g

% loss of after *Dhanyabhraka nirman*- 4.1%

Reason for loss weight loss: The particles of the *Dhanyabhraka* were so fine or light that it moves with water and washed out during draining and some particles with *Dhana* were trapped in the *Pottali.*

Table No.8: Observations & results after every *Puta* (incineration):

After each *Puta,* the color of *Chakrikas* was changed from blackish to light brown, lusterless, soft in texture.

No. of puta	Max Temp	Accel Eration in EMF	Time taken to reach temp (hour)	Wt of Abhrak Chakrika Before puta(g)	Wt of Abhrak Chakrika after puta (g)	Colour of Chakrika after puta	Wt.loss after puta (g)	Observation in Chakrika after puta
1st puta	800^0c	45	4 hour Mainted for 1 hour	920	914	Blackish	6	Hard to break and Rough
2nd puta	800c	45	4 hour Mainted for 1 hour	914	910	Blackish and Slightly Golden	4	Hard to break, Rough.

5th puta	800	45	4 hour Mainted for 1 hour	910	910	Yellowish Golden	0	Hard and slightly rough
10th puta	800	45	4 hour Mainted for 1/2 hour	910	908	Yellowish golden	2	Lustrous, Hard and slightly rough.
15th puta	750	45	3.5 hour Mainted for 1 hour	908	908	Dark golden	0	Same as above
20th puta	750	45	3.5 hour Mainted for 1 hour	908	908	Dark golden	0	Same as above
25th puta	750	45	3.5 hour Mainted for 1/2 hour	908	906	Slightly redish	2	Bhasma entered the furrows of fingers and lustrous.
30th puta	750	45	3 hour Mainted for 1 hour	906	904	Light brick red	2	Same as above
35th puta	750	45	3 hour Mainted for 1 hour	904	902	Dark brick red	2	Chakrika were easily break ,Lusture reduced, 70 % Varitarva was seen
40th pua	750	45	3 hour Mainted for 1 hour	902	902	Brick red	0	Lusterless, Nischandratva was seen

Result-

Weight after *Dhanyabhraka*- 920g

Final weight after *marana*- 902 g

Loss of *Abhrak* after *marana*- 18 g

% loss of *Abhrak*- 1.9%

Table No.9: Results of *Bhasma Pariksha* after each *Puta*:

No. of puta	1st	5th	10th	15th	20th	30th	35th	40th
Rekhapurnatva	-	-	+	+	+	+	+	+
Shlakshnatva	-	-	-	-	+	+	+	+
Sukshamatva	-	-	-	-	-	+	+	+
Varitarva	-	-	-	-	-	-	-	+
Unnam	-	-	-	-	-	-	-	+
Nischandratva	-	-	-	-	-	-	-	+

Table No.10: Total weight loss during *Abhrak Bhasma* preparation:

Final result:

Initial weight	**1000 g**
Final weight	**902 g**
Total weight loss	**98 g**
% of Total weight loss	**9.8%**

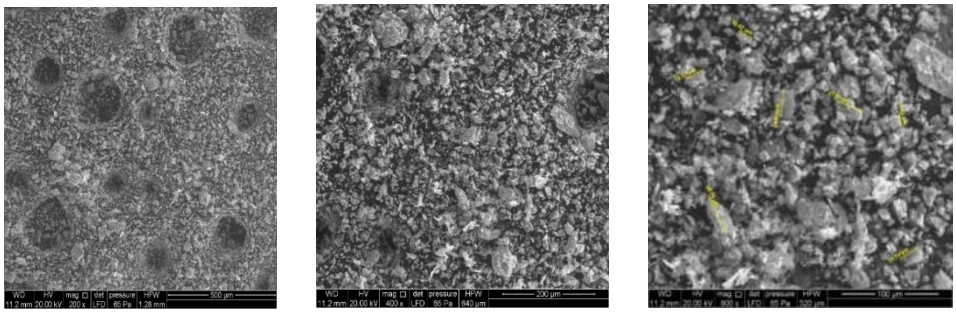
Observations and Result of Analytical study:

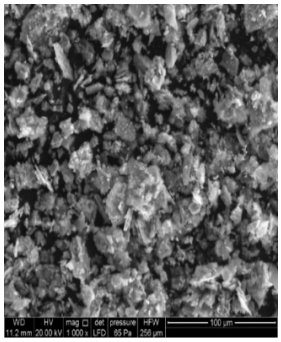
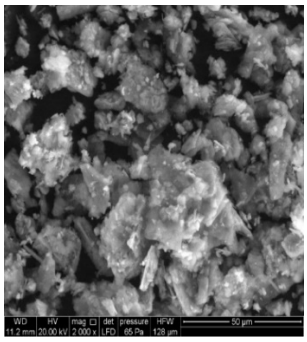
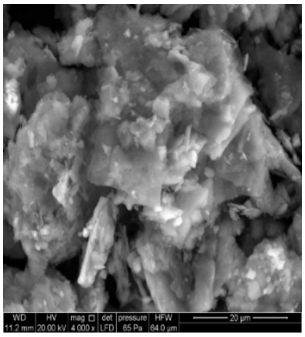
Table No.11:

PH	**7.56**
Total ash value	57.45 %w/w
Acid insoluble ash	33.57 %w/w
Water soluble ash	1.8 %
Loss on drying at 105°c	7.77%w/w

XRD Results- The XRD study of *Abhrak Bhasma* showed it contains ferrous compound like $Feso_4$, Fe_2o_3, Magnesium Iron Aluminium Tetraoxide($Al_1\ Fe_1\ Mg_1\ O_4$), Tripotassium Aluminium Hexafluoride– Gamma($Al_1\ F_6\ K_3$), Magnesioferrite($Fe_2\ Mg_1\ O_4$), Magnesium(Mg_1). Hence, Fe, Mg, Al & O in enormous amount.

SEM of *Nagvalli marit Abhrak Bhasma*-



SEM Result:

SEM Result of Nagavalli marit *Abhrak Bhasma* were captured at different magnification ranging from 200X upto 4000x. Smallest particle size was found to be ranging between 100 µm at 1000X magnification and 20µm at 4000X magnification.

Experimental Study:

Absorbance, % Viability for vehicle, positive control Capecitabine and Nagavalli marit *Abhrak Bhasma* are tabulated below for 24 hrs timeline.

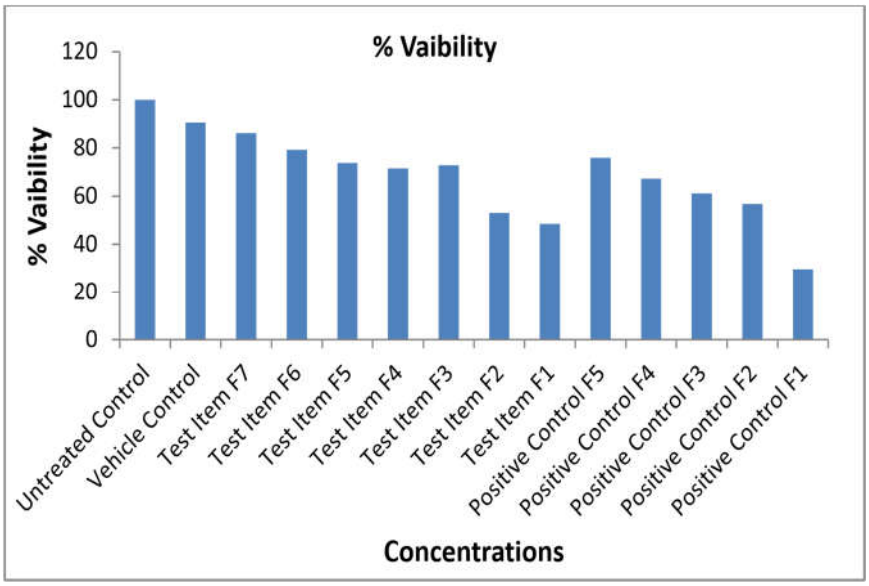
Table no.12:- Tabular presentation of data

		Absorbance at 570 nm						
Formulations		**Concentration (µg/mL)**	**Ab. 1**	**Ab. 2**	**Ab. 3**	**Mean**	**SD**	**% Viability**
Blank		-	0.000	0.000	0.000	0.000	0.000	-
Untreated Control		-	0.404	0.491	0.485	0.460	0.049	100
Vehicle Control		100 µL	0.484	0.417	0.348	0.416	0.068	90.51
Nagavalli marit Abhrak Bhasma	F7	31.25	0.422	0.405	0.362	0.396	0.031	86.16
	F6	62.5	0.367	0.397	0.329	0.364	0.034	79.20
	F5	125	0.363	0.318	0.337	0.339	0.023	73.77
	F4	250	0.315	0.347	0.325	0.329	0.016	71.52
	F3	500	0.338	0.35	0.317	0.335	0.017	72.83
	F2	1000	0.256	0.253	0.224	0.244	0.018	53.12
	F1	2000	0.228	0.224	0.217	0.223	0.006	48.48
Positive Control Capecitabine	F5	125	0.346	0.339	0.363	0.349	0.012	75.94
	F4	250	0.303	0.307	0.318	0.309	0.008	67.25
	F3	500	0.286	0.267	0.291	0.281	0.013	61.16

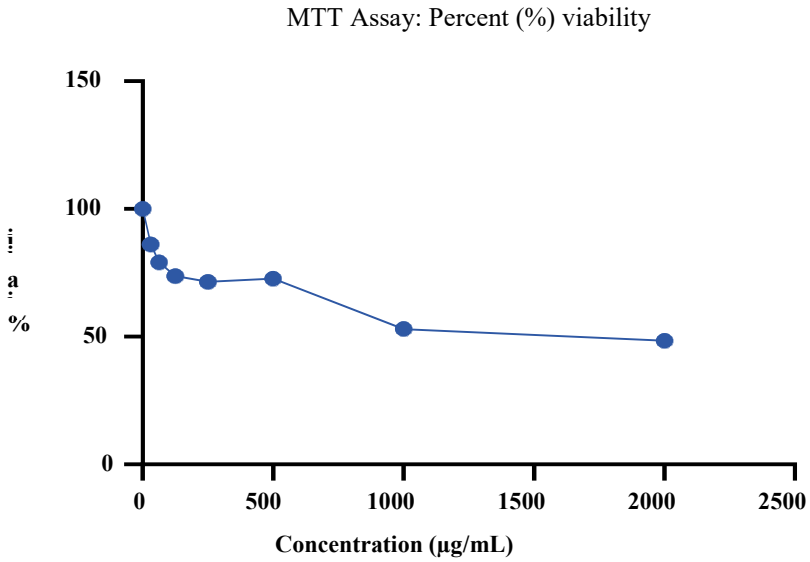
Graphical presentation of data

	F2	1000	0.290	0.202	0.292	0.261	0.051	56.81
	F1	2000	0.139	0.169	0.102	0.137	0.034	29.71

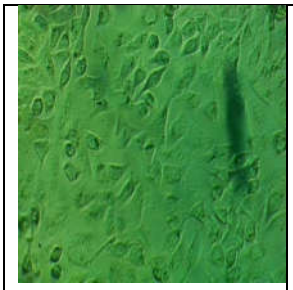
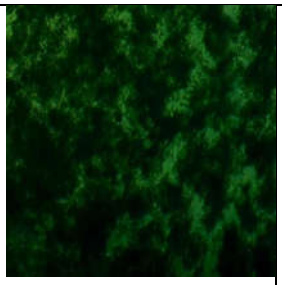
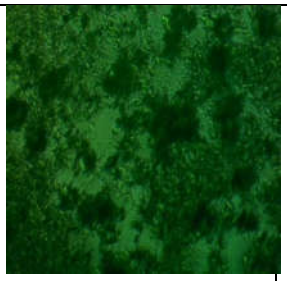
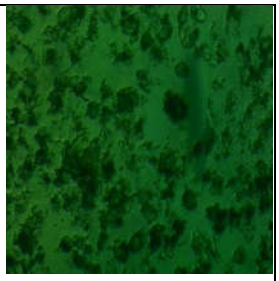
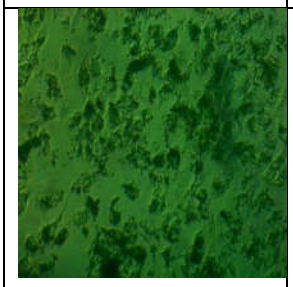
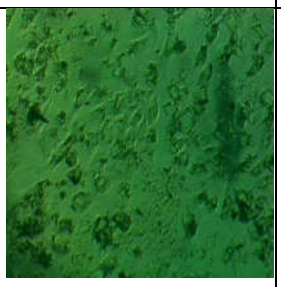
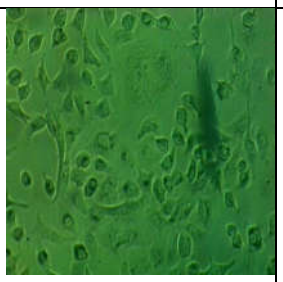
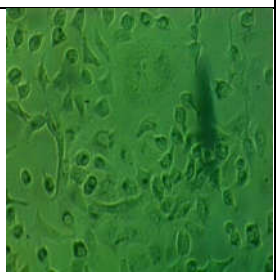
Graphical presentation of Absorbance, % Viability for vehicle, positive control and Nagavalli marit *Abhrak Bhasma*.

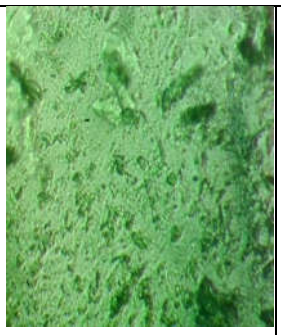
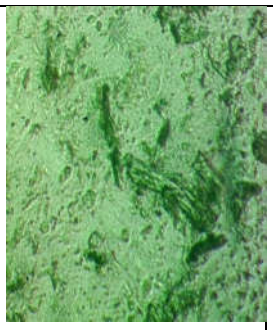
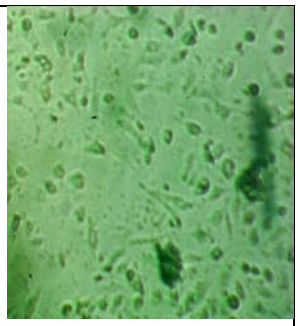


Graphical presentation of MTT Assay (% viability) of - Nagavalli marit *Abhrak Bhasma*:



Photographs-

			
Vehicle Control 24 hour	**Nagavalli marit *Abhrak Bhasma* F1- 2000 µg/mL 24 hour**	**Nagavalli marit *Abhrak Bhasma* F2- 1000 µg/mL 24 hour**	**Nagavalli marit *Abhrak Bhasma* F3- 500 µg/mL 24 hour**
			
Nagavalli marit *Abhrak Bhasma* F4- 250 µg/mL 24 hour	***Nagavalli marit* *Abhrak Bhasma* F5- 125 µg/mL 24 hour**	**Nagavalli marit *Abhrak Bhasma* F6- 62.5 µg/mL 24 hour**	**Nagavalli marit *Abhrak Bhasma* F7- 31.25 µg/mL 24 hour**

			
Untreated control - 24hour	**Positive Control F1- 2000 µg/mL 24 Hour**	**Positive Control F2- 1000 µg/mL 24 Hour**	**Positive Control F3-500 µg/mL 24 Hour**

	
Positive Control F4- 250 µg/mL 24 Hour	**Positive Control F5- 125 µg/mL 24 Hour**

DISCUSSION:

Rasa-aushadhi are supposed to be superior to other drugs on an account of their effectiveness of low dosages, easy palatability & quick action. These merits of Rasa-aushadhi helps to cure the diseases which are difficult to cure. *Abhrak Bhasma* is a herbomineral preparation prepared according to reference given in Rastarangini. *Abhrak Bhasma* possesses the qualities of Sarvarogahara, Cumulative, Rejuvenating, Tridosha shamak, and Rasayana properties(which promotes life). Repeated incineration lead to fineness in particle size and show *Yogavahitwa*(which promotes passage and movements of a formulation or drug) and Shigravyapti(fast spreading), *Abhrak Bhasma* have unique properties like fast acting and target specific drug delivery. In pharmaceutical study, It was prepared using standard operating procedure in a number of pharmaceutical processes, including *Shodhana, Bhavana, and Marana*. Shodhan of Abhrak was done by Triphala kwatha and *Marana dravya* as *Nagavalli Patra swarasa* was used for study. The Shodhana (heating and quenching) procedure separates the *Abhrak* flakes and removes physical and chemical impurities. As the temperature increases in *Nirvap*, particles gather energy and vibrate more vigorously, taking up more space so, the solid material swells. Along the parallel cleavage planes of *Abhrak's* mineral separation, where water media enters it immediately and water soluable impurities dissolve in it as a result of the residual ionic bonds dissolving, water molecules evaporate immediately and emerge in different layers. Fast variation in temperature can also break other strong connections, which decreases the material's flexibility and makes it more brittle. Even if Shodhana divides the Abhrak sheets, the finer sheets are still intact and have physical impurities between them. Sheets are separated and made fine for additional processing by *Dhanyabharka Nirmana.* This procedure is exclusive to *Abhrak Marana*. In this phase, interaction between *Shuddha Abhrak, Dhanya, Kanjii* occurred. Due to sharp edges of *Dhanya*, it turns *Abhrak* flakes into a coarse powder. The brittleness of *Abhrak* is attributed to certain characteristics of k*anji*, particularly its Tikshnatva and amla rasa which are strengthened by its acidic nature. Hand pressure enhances this process by raising friction. Consequently, *Abhrak* gains granular form during each of these phases and is prepared for the *marana* process. When the *Abhrak* comes into contact with the blunt edges of the paddy, it turns into a fine powder. An inorganic mineral may transform into active organic compounds after this process. This would make possible the mineral's quick absorption by the body. Marana of the metals is done to convert the metal into a form which is fit for therapeutic administration. Temperature of 750ºc is best observed for preparation of fine & smooth *Abhrak*

Bhasma. During *marana* procedure, constituents of *Nagavalli patra* interact with *Dhanyabhraka* and carbonates, hydroxides, silicates and other substance may occurred. After that during calcination, these hydroxides or carbonates can be changed into oxides forms changing *Bhasma* colour. *Abhrak Bhasma* was **Ishtikabha (Brick red)** in colour, denotes the development of a unique mineral compound. After 7th puta was *Rekhapurnatva*, after 25th puta was *Slakshnatva*, after 40th puta *was Nichandratva and Varitartva*. The crystalline form of silicon dioxide have lustre turns into lustreless amorphous form.

In Analytical Study, The L.O.D. value of *Abhrak Bhasma* was 7.77 % w/w, because it also contains organic substances as its ingredients which absorb moisture. The Acid insoluble Ash value of *Abhrak Bhasma* was 33.57 % w/w, which facilitates the easy absorption of drug. The PH value of *Abhrak Bhasma* was 7.56 which shows alkaline in nature.*Abhrak Bhasma* was evaluated for ash value and it was found 57.45% w/w. Water soluble ash value of *Abhrak Bhasma* was 1.8 %.Particle size of *Abhrak Bhasma* with help of sieves 85 is 100 % satisfying, this was indicated the fineness of Bhasma. The XRD study of *Abhrak Bhasma* showed it contains ferrous compound like $Feso_4$, Fe_2o_3, Magnesium Iron Aluminium Tetraoxide(Al_1 Fe_1 Mg_1 O_4), Tripotassium Aluminium Hexafluoride– Gamma(Al_1 F_6 K_3), Magnesioferrite(Fe_2 Mg_1 O_4), Magnesium(Mg_1). Hence, Fe, Mg, Al & O in enormous amount. Scanning Electron Microscopy(SEM) analysis of *Nagavalli marit Abhrak Bhasma* were captured at different magnification ranging from 200X upto 4000x. Smallest particle size was found to be ranging between 100 µm at 1000X magnification and 20µm at 4000X magnification. Particle size is one of the factors which will affect the dissolution and absorption of the drug particle size and surface area are inversely proportional to each other, as particle size decreases surface area increases. This leads to an increase in the dissolution and rapid absorption.

In Experimental study, the anticancer activity of the "Nagavalli marit *Abhrak Bhasma"*, evaluated by MTT assay on A549 cell line. After 24 hours of treatment at the concentrations of 2000, 1000, 500, 250, 125, 62.5 and 31.25 µg/mL. The "Nagavalli marit *Abhrak Bhasma"*, has shown decreased % cell viability as there was increase in "*Nagavalli marit Abhrak Bhasma"* concentration. The considerable decreased % cell viability i.e. 48.48 and 53.12 % was observed at the concentrations of 2000 and 1000 µg/mL with an IC 50 value of 1766.358 µg/mL. The % cell viability of the standard drug Capecitabine was evaluated using five concentrations at 2000, 1000, 500, 250 and 125 µg/mL. Considerable decrease in cell viability was observed at the concentrations of 2000 and 1000 µg/mL, further slight reduction was observed at the concentrations of 500, 250 and 125 µg/mL when compared with untreated control.

CONCLUSION:

"Nagavalli marit Abhrak Bhasma" was prepared and analyzed according to Standard Operating Procedure. In-vitro model of A549 cell was developed in authentic laboratory conditions & Anticancer activity of "*Nagavalli marit Abhrak Bhasma"* was studied. From the above Experimental results, the "*Nagavalli marit Abhrak Bhasma"* has shown decreased % cell viability as there was increase in concentration of "*Nagavalli marit* Abhrak bhamsa". The *"Nagavalli marit Abhrak Bhasma", %* cell viability observed were 48.48, 53.12, 72.83, 71.52, 73.77, 79.20 and 86.16 % at the concentrations of 2000, 1000, 500, 250, 125, 62.5 and 31.25 µg/ mL respectively. IC50 was observed at the concentration of 1766.358 µg/mL. According to this study, It can be conclude that there is a

significant Anticancer Activity of "*Nagavalli marit Abhrak Bhasma"* using A549 cell line model with half-maximal inhibitory concentration (IC 50) value of 1766.358 µg/mL and Nagavalli marit abhraka Bhasma can be considered as a potent medicine for treatment of lung cancer.

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