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-Shikshanmaharshi Dr. Bapuji Salunkhe

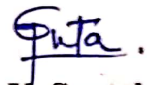
Vivekanand College, Kolhapur (Empowered Autonomous)
Department of Zoology
Academic Year- 2024-25

Date: 09/07/2024

NOTICE

B. Sc. III Students Project

All students of B. Sc. III here by informed that, as per part of practical work it is essential to all students to complete their projects and it is compulsory to all. List of students and guide is given below, follow the list and complete your project up to 20th February 2025


Dr. G. K. Sontakke

HEAD
DEPARTMENT OF ZOOLOGY
VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

Vivekanand College, Kolhapur (Empowered Autonomous)
Department of Zoology
Academic Year- 2024-25

Date: 09/07/2024

B. Sc. III Students Project

List of Students and Project Guide

Sr. No.	Roll No.	Student Name	Name of Project Guide
1.	8341	Abrange Vaishnavi Vinod	Ms. P. R. Gaikwad
2.	8343	Dindayal Prabodhini Raju	
3.	8344	Jadhav Suyash Kuber	Ms. N. A. Jadhav
4.	8345	Kamble Rutuja Vishal	
5.	8358	Singh Priya Gopal	Ms. P. S. Shetfalkar
6.	8347	Kukade Priyanka Sandeep	
7.	8348	Mansuri Sahida Allauddin	Dr. T. C. Patil
8.	8349	Mishra Shambhavi Mukesh Kumar	
9.	8350	Mitake Komal Anil	Mr. G. H. Fadake
10.	8351	Mulla Mahamadkaif Shahanul	
11.	8352	Nayakavadi Ashiya Riyaj	Ms. N. A. Patel
12.	8353	Patil Pradnya Rajendra	
13.	8342	Chougule Shraddha Shivaji	Dr. S. S. Desai
14.	8354	Raje Shruti Sudarshan	
15.	8355	Rathod Sonali Ramesh	Dr. T. C. Gaupale
16.	8356	Sayyad Afroja Aslam	
17.	8357	Shaikh Ayesha Aslam	Dr. G. K. Sontakke
18.	8346	Katkar Siddhi Sanjay	
19.	8359	Ustad Rifat Nasirkhan	


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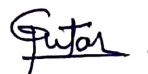
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B. Sc. III Students Project

List of Students and Project Title

Sr. No.	Roll No.	Student Name	Name of Project Guide
1	8341	Abrange Vaishnavi Vinod	Study of Butterfly Diversity in Vivekanand College Campus, Kolhapur
2	8343	Dindayal Prabodhini Raju	
3	8344	Jadhav Suyash Kuber	Studies on Phytochemical Analysis of Ethanolic Extract of Moringa Leaves and its Effect on Angiogenesis in Chick Embryo
4	8345	Kamble Rutuja Vishal	
5	8358	Singh Priya Gopal	Study of physicochemical parameter and biodiversity around the Shirolake, Kolhapur, Maharashtra India
6	8347	Kukade Priyanka Sandeep	
7	8348	Mansuri Sahida Allauddin	Effect of Tobacco and Curry leaves on Angiogenesis in Chick
8	8349	Mishra Shambhavi Mukesh Kumar	
9	8350	Mitake Komal Anil	Insect Fauna and Biodiversity in Mahaveer Garden Kolhapur, Maharashtra, India
10	8351	Mulla Mahamadkaif Shahanul	
11	8352	Nayakavadi Ashiya Riyaj	Isolation of Chitin From Crab Shell
12	8353	Patil Pradnya Rajendra	
13	8342	Chougule Shraddha Shivaji	In Vitro Anthelmintic Activity of Ethanolic Extract of <i>Vitex negundo</i> Linn.; A Phytopharmacological Study
14	8354	Raje Shruti Sudarshan	
15	8355	Rathod Sonali Ramesh	Analysis of Effect of Light on Circadian Rhythm and Lipid Peroxidation in fish <i>Cirrhinus mrigala</i>
16	8356	Sayyad Afroja Aslam	
17	8357	Shaikh Ayesha Aslam	Study of physicochemical parameter and biodiversity around the Rankala lake, Kolhapur, Maharashtra India
18	8346	Katkar Siddhi Sanjay	
19	8359	Ustad Rifat Nasirkhan	


Dr. G. K. Sontakke

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DEPARTMENT OF ZOOLOGY
VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

A PROJECT REPORT ON:

“Effect of Tobacco and Curry leaves on Angiogenesis in Chick”

SUBMITTED TO:

**VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)**



**IN THE PARTIAL FULFILLMENT OF BACHELOR OF SCIENCE IN
ZOOLOGY**

IN THE YEAR: 2024-2025

NAME & ROLL NO.-Sahida Allauddin Mansuri (8348)

NAME & ROLL NO.-Shambhavi Mukesh Kumar Mishra (8349)

CLASS B. Sc. III

UNDER THE GUIDANCE OF

Dr. T. C. Patil

Assistant Professor,

Vivekanand College, Kolhapur (An Empowered Autonomous Institute)

DECLARATION

We the undersigned students, declare that the project entitled, "Effect of Tobacco and Curry leaves on Angiogenesis in Chick" is submitted by us under the supervision of Dr. T. C. Patil, Assistant Professor, Vivekanand College, Kolhapur (An Empowered Autonomous Institute).

It is our original work. The empirical findings in this project are based on the data collected by us and it is authenticable to the best of our knowledge. The presented matter is not copied from any other source.

Place *Kolhapur*

Date: *20/02/25*

Student sign

M. Sahida

Sahida Allaiddin Mansuri (8348)

Shambhavi

Shambhavi Mukesh Kumar Mishra (8349)

CERTIFICATE

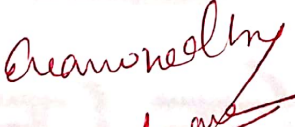
This is to certify that the project entitled, **Effect of Tobacco and Curry leaves on Angiogenesis in Chick** being submitted herewith for the Degree of **Bachelors of Zoology**, Vivekanand college, Kolhapur (An Empowered Autonomous Institute) Affiliated to Shivaji University, Kolhapur, under the faculty of Science is the result of the original work completed by Sahida Allauddin Mansuri and Shambhavi Mukesh Kumar Mishra under my supervision and guidance and to the best of my knowledge and belief, the work embodied in this project has not formed earlier.

Place: Kolhapur

Date: 20/02/2025


Project Supervisor
Dr. T. C. Patil.

10/10


Examiner


Dr. G. K. Sontakke
HEAD
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VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

ACKNOWLEDGEMENT

I wish to express my deepest sense of gratitude to my project supervisor Dr. T. C. Patil, for his/ her continuous help and valuable guidance during the entire period of my Project work. It is a genuine pleasure to express my deep sense of thanks to **Dr. G. K. Sontakke**, Head, Department of Zoology, Vivekanand College Kolhapur (An Empowered Autonomous Institute) for administrative help and encouragement during the Project work. I express my profound thanks to **Dr. R. R. Kumbhar** Principal, Vivekanand College Kolhapur (An Empowered Autonomous Institute) for his constant support and inspiration.

I am also thankful to other faculties of Department of Zoology who have directly or indirectly guided and helped me in the Project work.

I am also thankful to non-teaching staff for their constant co-operation during project work.

Name of Student

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Shambhavi

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INTRODUCTION:

Angiogenesis is the process that forms new blood vessels from existing blood vessels in body. Angiogenesis helps body to heal the wounds and perform other vital function. This process begins before birth and continues throughout life (Adair and Montani, 2010).

It is a vital process that is essential for growth, development, healing and transporting nutrients and proteins. Angiogenesis is controlled by chemical signals in the body, such as vascular endothelial growth factors (VEGF), when these signals bind to receptors on endothelial cells, the cells grow and survive, promoting new blood vessels formation. Other chemical signals called angiogenesis inhibitors, interfere with the formation blood vessels. Angiogenesis can occur abnormally, leading to diseases. For example, tumours, it needs new blood vessels to grow, and this process is called tumour angiogenesis. Abnormal angiogenesis can also cause age-related wet macular degeneration, ischemic vascular disorders, and diabetic retinopathy. (Bamias and Dimopoulos, 2003).

The circulatory system is the first organ system to develop in the embryo. Hemangioblasts are the cells derived from mesodermal cells which give rise to hematopoietic stem cells and angioblasts. The Vasculogenesis is the process of de novo formation of blood vessels from angioblasts (Risau, W., 1997). It occurs in the extraembryonic and intraembryonic tissues of embryos. Blood vessel formation is a dynamic process that involves cell-cell and cell-extracellular matrix interactions directed spatially and temporally by-growth factors and morphogens. This process includes differentiation of mesodermal stem cells into angioblasts, growth factor directed migration of angioblasts to form blood islands where angioblasts give rise to endothelial cells (Schmidt et al; 2007).

The chick chorion allantoic membrane (CAM) is the extraembryonic membrane. The CAM is a membrane that supports for gas exchange, nutrient exchange and waste removal. It is highly vascularized, and rich in capillary network. It is easy to manipulate and also it helps in rapid growth. CAM is also used in research as model for studying a variety of biological processes, tumour growth, angiogenesis, tissue grafts, drug toxicity, Biocompatibility, wound healing, drug screening. The CAM assay is a low cost effective and quick model that bridges the gap between in vitro cell culture studies and in vivo animal experimentation. (Nowak-Sliwinska, et al; 2014).

Curry leaves: Curry leaves are a popular spice and condiment in India and are also used in Ayurvedic medicine. The small pods of curry leaves are also edible but should be avoided as they are suspected to be toxic

Curry leaves have some medicinal uses such as:

- ❖ Anti-oxidant: Curry leaves are rich in anti-oxidant which can help wash out toxins from the body.
- ❖ Anti-diabetic: Curry leaves can help manage diabetes by regulating blood sugar level.
- ❖ Anti-inflammatory, curry leaves have anti-inflammatory properties.
- ❖ Anti-microbial: Curry leaves have anti-microbial properties.
- ❖ Anti-ulcer, curry leaves have anti-ulcer properties. Anti-anaemic: Curry leaves are high in iron, which can help increase haemoglobin and red blood cell count.
- ❖ Anti-osteoporotic: Curry leaves can be a natural calcium supplement for people with osteoporosis or calcium deficiency. Curry leaves are rich in Vitamin A.

Tobacco Leaves:

Scientific name of the Tobacco plant is *Nicotiana tabacum*. The dried leaf of this plant is used after processing in various ways, such as for smoking, chewing, snuffing, and extraction of nicotine. Various other species of the genus *Nicotiana* are grown as ornamental plants called as flowering tobaccos. The leaves of tobacco plant, *Nicotiana*, has perhaps been responsible for more deaths than any other herb. At present, tobacco smoking is causing over 3 million deaths a year worldwide, and if current smoking trends continue the annual mortality will exceed 10 million by around 2030. Here are some harmful effects of tobacco leaves: Tobacco leaves promote endothelial cell migration, proliferation, and nicotine acts as the growth suppressor, it reduces bone development, lower survival rate, embryonic movements and malformations, abnormal angiogenesis, cardiovascular defects, irregular heart rate, reduced cardiac autonomic nerve reactivity, abnormal lung development and function, brain growth inhibited, neural tube defects, imprinting impairment, abnormal cerebellar cortex development, cognition/learning deficiencies and neural circuit formation etc.

Objective:

1. To extract the components from curry leaves *Murraya koenigii* and Tobacco leaves *Nicotiana tabacum*.
2. To study the angiogenesis in chick embryo.
3. To study the effect of curry leaves *Murraya koenigii* and Tobacco leaves *Nicotiana tabacum* on the growth of blood vessel in chick embryo.

Literature review:

Augustin *et al.*, (2009) has studied angiogenesis in animals as the fundamental process. Decker, (2003) have reviewed on angiogenesis. The angiogenesis process is controlled by a balance of various growth factor and inhibitors given by William, (2003). Folkman, (2007) has studied the drugs that promote or inhibit angiogenesis are currently being developed, especially those that can be used in cancer treatment. Steven *et al.*, (2014) studied angiogenesis expansion and remodeling of micro-vascular networks. Ajay, (2011) given Comprehensive review on *Murraya koenigii* Linn. Schmidt et al; (2007) has reviewed on the de novo process of formation of blood vessels

Material:

Eggs, leaves of curry and tobacco, tray, conical flask, beaker, measuring cylinder, Whatman filter, paper, cotton, glows, syringes, needles, forceps, Petri plate, funnel etc.

Chemicals:

Methanol, Ammonium Hydroxide, Acetic Acid, Glacial Acetic Acid, N –Butane, Sodium Chloride, 80% Aqueous Methanol, Ascorbic Acid, concentrated sulphuric acid, Chloroform, Sodium hydroxide, Wagner's Reagent, Acetic Anhydride, Ammonia, Potassium Ferricyanide, Ferric Chloride, Hydrochloric Acid, Olive Oil, Tannic Acid, Folin- Cioaltea Reagent, Sodium Carbonate Aqueous Solution, Diethyl Ether, mercuric chloride, Iodine etc.

Method:**Plant Extraction:**

The leaves of curry and tobacco were collected from different location. Curry leaves was collected from Shirol, taluka Hatkanangale. The tobacco leaves collected from Bhoj, Tal chikurdi, Dist.- Belgaum farm. The collected leaves of curry and tobacco were washed with water to remove the dust. The leaves were spread on cloth and shade dried for 2-4 days. After drying the leaves, the leaves ground and powder prepared this powder sieved with muslin cloth, this powder used for extraction.

For the extraction of plant extract the maceration techniques used, in this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.



Curry Leaves



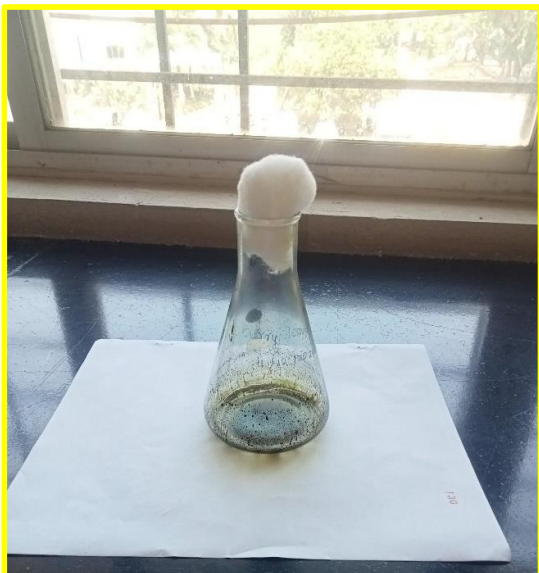
Tobacco-Leaves



Tobacco Leaves Powder



Curry Leaves Powder



Curry Leaves Extract



Tobacco Leaves Extract

Drying and extraction of plant Extract

The powder of curry and tobacco leaves were placed in methanol and allowed to stand at room temperature, For the preparation of the extract, we add 5gm of curry leaves powder and 5gm tobacco leaves powder in 50ml of methanol in separate sterilized container and allowed to stand for 7 days in room temperature with frequent agitation. The mixture then strained with Whatman's filter paper, the marc pressed, and the combined liquids are clarified by filtration.

Evaporation of solvent is the next step in this filtrate placed in a glass flask, and stirred it with a magnetic stir bar with gentle heating on a hot plate. The solvent gradually evaporated and left the extract behind. This plant extract used for phytochemical analysis and to study the effect of angiogenesis.

Phytochemical Analysis:

Test for Alkaloids (Mayers test):

1.36 gm of mercuric chloride dissolved in 60ml and 5gm of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100ml using distilled water. To 1ml of acidic aqueous solution of samples few drops of reagent was added. Formation of white orpale precipitate showed the presence of alkaloids.

Test for Flavonoids:

In a test tube containing 0.5ml of alcoholic extract of the samples, 5 to 10 drops of diluted HCl and small amount of Zn or Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

Test for Glycosides:

A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicate the presence of glycosides.

Test for Steroids:

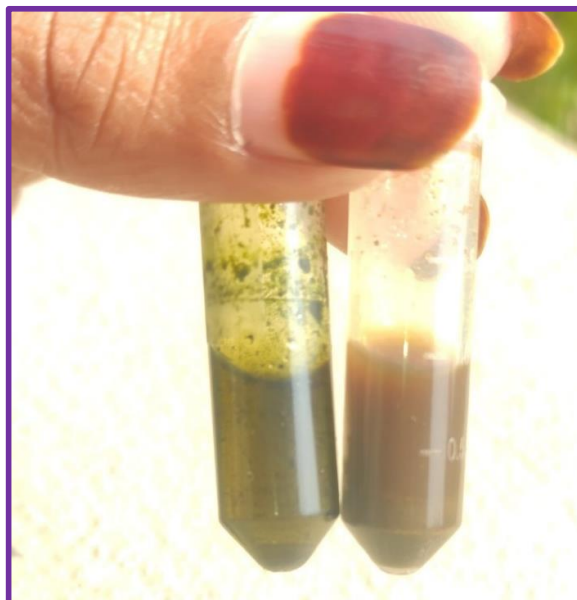
About 100mg of dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interface was an indicative of the presence of steroidal ring.

Test for Cardiac glycosides [Keller killiani's test]:

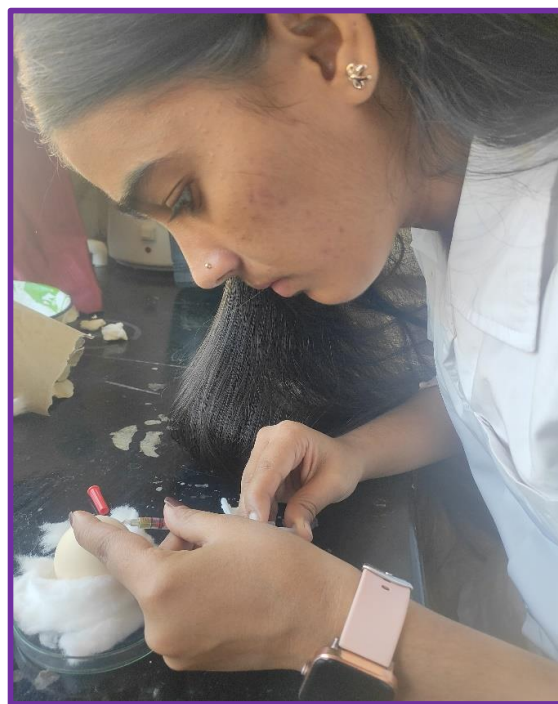
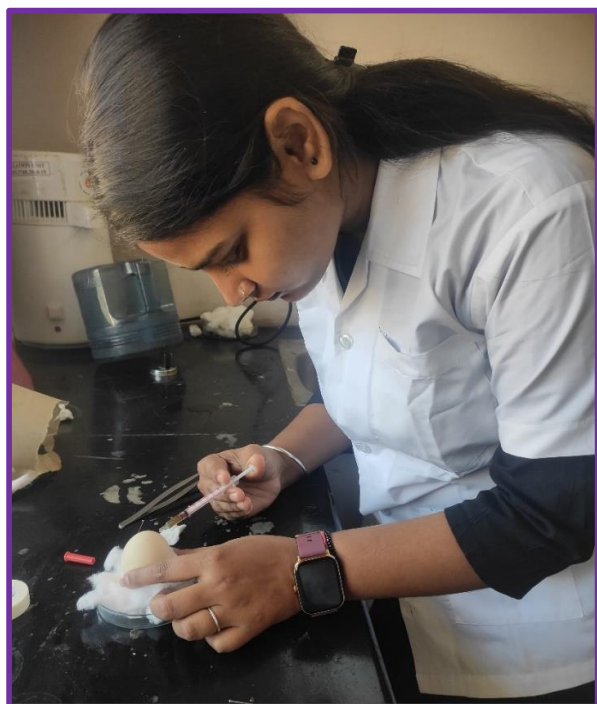
100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid was added. A brown ring obtained at the interface shows the presence of a deoxy sugar characteristic of cardenolides.



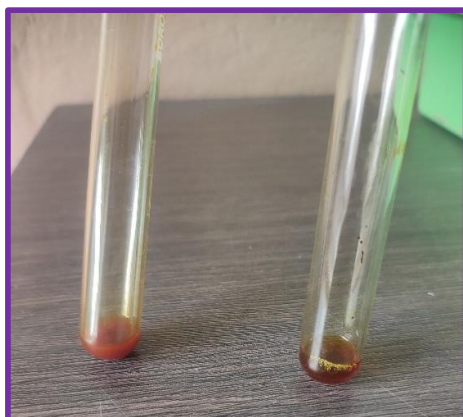
Incubation of Egg



Plant Extract



Dose administration



Phytochemical Analysis

Test for saponins:

A drop of sodium Bicarbonate was added in the test tube containing about 50ml of an aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like froth formed at base of test tube indicates the presence of saponins.

Test for Resins:

To 2ml of chloroform or ethanolic extract 5 to 10 ml of acetic anhydride was added and dissolved by gentle heating. After cooling, 0.5ml of H₂SO₄ was added. Bright purple colour was produced in test tube. The presence of colour indicates the presence of resins.

Test for Phenols [Ferric Chloride Test]:

- To test the presence of phenol, take 1 ml aqueous solution of sample in test tube then add 2 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution. The formation of blue or green colour indicates the presence of phenols in the sample.

Test for Tannins [Lead acetate test]:

- In a test tube containing about 5ml of an aqueous extract, a few drops of 1% solution of lead acetate were added. Formation of a yellow or red precipitate indicated the presence of tannins.

Test for Terpenoid:

2ml of chloroform and 1ml of conc. H₂SO₄ was added to 1ml of plant extract in test tube and observe for reddish brown colour that indicates the presence of terpenoid.

Test for Quinone:

Take 1ml of extract, a few drops of concentrated hydrochloric acid (HCl) were added. A yellowish-brown colour was observed that showed the presence of quinone in sample.

Test for Protein: Ninhydrin test: Ninhydrin dissolved in acetone. The leaf extract with ninhydrin and observed the purple colour formation

Test for phlobatannins: Leaf extract boiled with 2% aqueous HCL. The deposition of red precipitate shows presence of phlobatannins

Test for Coumarin: 10 % NaoH was added to the extract and chloroform added observed yellow colour shows presence of coumarin

Experimental design and administration of dose:

The required numbers of eggs were collected from back Shiroli, taluka Hatkanangale. After collection, healthy and almost same sized eggs of *Gallus domesticus* were selected for experiment. The colour of egg is light brown, shape is small and oval. The eggs were placed in incubator for incubation as per experimental design. The curry leaves and tobacco extract injection to developing embryo in eggs which were incubated at

37°C for different exposure period.

Eggs were selected to start of experiment. For the present experiment two groups eggs were prepared first group kept as control group and second group as an experiment group injected with curry leaves solution after 48 hours of incubation of eggs. In experimental group the eggs were numbered 1, 2, 3 etc. Egg1 was incubated up to 72 h. Eggs 2 was incubated up to 96 hours and Egg 3 in incubated up to 120 hours. All eggs were kept in incubator which was sterilized by using 70% alcohol to maintain the aseptic condition and made it free from germ and microorganisms.

The incubator was pre- started to maintain 37°C temperature which is essential for the development of chick embryo. After 48 hour of incubation eggs were again cleaned with 70% alcohol and under sterilized and aseptic condition the eggs were treated with 0.3 ml of curry leaves extract solution. After injection eggs with developing embryo resealed with adhesive sterile tapes. Again, experimental eggs were kept in incubator for further embryonic development. The eggs were dissected and embryo was observed at 72 hour (24 hour after micro injection) 96 hour (48 hours of injection). After observation of chick embryo body weight of embryo was taken, and comparison done between control group and experimental group

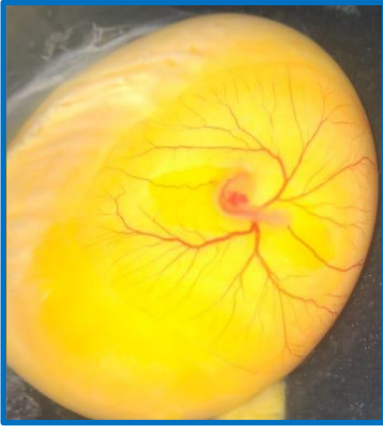
Observation and Result:

Observation of Phytochemical Analysis of leaf extract:

Phytochemical analysis of curry leaves and tobacco leaves extract were explored screening for Alkaloids, Flavonoids, glycosides, steroids, cardiac glycosides, Saponins Tannins, Terpenoid, acids and pro-out Phenols, Quinone, Amino proteins are shown in table.

Table 2: Phytochemical Analysis on leaf extract: (++ Present, -- Absent)

Sr. No.	Phytochemical constituents	Curry	Tobacco
1.	Alkaloids	++	++
2.	Flavonoids	++	++
3.	Glycosides	++	+
4.	Steroids	++	++
5.	Cardiac glycosides	++	--
6.	Saponins	++	++
7.	Phenols	++	++
8.	Tannins	++	++
9.	Terpenoid	++	++
10.	Quinone	--	--
11.	Amino acids & protein	--	--
12.	Coumarin	++	++
13.	Resins	--	--
14.	Phlobatannins	--	--



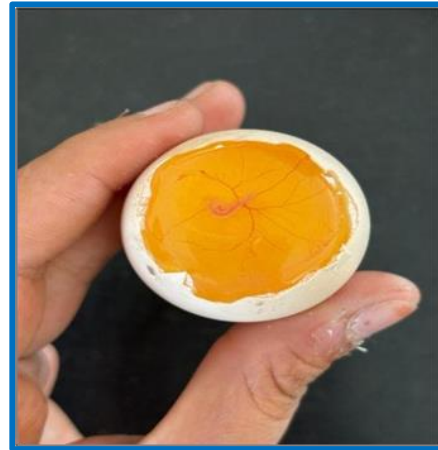
72 Hours Normal



96 Hours Normal



72 Hours treated (Curry)



96 Hours treated (Curry)



72 Hours treated (Tobacco)



96 Hours treated (Tobacco)

Effect of Plant Extract on Angiogenesis in Chick

Observation of chick embryo:

After the incubation control and experimental groups of eggs were observed and compared. It is observed in the experimental group that there is an insignificant reduction in the weight of chick embryo at 72 hours and 96 hours after treatment with curry leaves and tobacco leaves extract as compared to weight of chick embryo in control group. The difference between weight of control egg embryo and experimental egg embryo are given below:

Table 3: Weight of chick embryo:

Sr. No.	Control		Treated (Curry Leaves)	
	Incubation hours	Weight of Embryo	Incubation hours	Weight of Embryo
1	72	0.128 gm	72	0.100 gm
2	96	0.239 gm	96	0.130 gm

Table 4: Weight of chick embryo:

Sr. No.	Control		Treated (Tobacco Leaves)	
	Incubation hours	Weight of Embryo	Incubation hours	Weight of Embryo
1	72	0.126 gm	72	0.99 gm
2	96	0.240 gm	96	0.129 gm

Changes in angiogenesis:

After the incubation control and experimental group eggs were observed and compared. It is observed that in the experimental group that there is an insignificant reduction in the weight of chick embryo at 72 hours and 96 hours after treatment with curry leaves extract as compared to weight of chick embryo in control group. 72 hours (i.e. 24 hours after leaves extract exposure) and 96 hours (i.e. 48 after leaves extract exposure) of incubation the treated embryo showed reduced number of blood vessels as compared to the control embryo also reduction in size of blood vessel. Reduced in size of heart and reduced in movement of heart the weight of embryo is also reduced. In the case of tobacco leaves extract increase in rate of heart beat observed.

Conclusion:

In present to study the effect of curry and tobacco leaves extract against development of chick embryo were observed. During the study the embryo showed some changes. The treated embryo after intoxication with 0.3 ml leaves extract showed reduced the number of

blood vessels, reduction in the body weight of embryo. As well as the angiogenesis pattern get disturbed by extraction of curry leaves treatment. It can be concluded that curry leaves extract can induce toxic interaction which can highly reduce the viability of the embryo.

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