PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACT OF *ARISAEMA PROPINQUUM*

Project report submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

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UNDER THE GUIDANCE OF

Dr. Hemant Sood

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT
DEPT. OF BIOTECHNOLOGY AND BIOINFORMATICS
HP-173234, INDIA, MAY 2023

DECLARATION

We hereby declare that the work presented in this report entitled "**Phytochemical analysis of plant extract of *Arisaema propinquum***" in partial fulfillment of the requirements for the award of the degree of **Bachelor of Technology** in **Biotechnology** submitted in the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of our own work carried out over a period from August 2022 to December 2022 under the supervision of **Dr. Hemant Sood**, Associate Professor, Department of Biotechnology & Bioinformatics.

We also authenticate that we have carried out the above mentioned project work under the proficiency stream Industrial Biotechnology.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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*This is to certify that the thesis on "*PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACT OF *ARISAEMA PROPINQUUM"* submitted by Miss Anchal Guleria, in partial fulfillment of the requirements for the award of the Degree in Bachelor of Technology in Biotechnology is an original work carried out by me under joint guidance. It is certified that the work has not been submitted anywhere else for the award of any degree or diploma of this or any other University. We also certify that he complied with the Plagiarism Guidelines of the University.

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ABSTRACT

The present study is carried out to analyze the phytochemical activity of plant extract of *Arisaema propinquum Schott* (Araceae) rhizomes, leaves and stems. *Arisaema propinquum Schott* is also known as cobra lily. Additionally, this herb exhibits strong cytotoxic effect against some cancer cell lines. Besides, it has an anti-parasitic property that drives internal parasites like worms out of the body and kills them without doing any harm to the host.

The plants were collected and then subjected to standard protocols for phytochemical analysis in herbal plant extract of *Arisaema propinquum.* The extract was used to identify different primary and secondary metabolities using qualitative and quantitative method, in which different saponins, terpenes, carbs, phenols etc different phytochemicals were analyzed. The following results were obtained different parts of the plant and plant used for analysis as a whole. For whole plant flavonoids, phenol, carbohydrates and proteins are 2.05 µg QE/mg, 113.15 µg/mg, 1.63% and 1.157% respectively, in leaves phenol, protein and carbohydrates are present in 279 µg/mg, 18.02% and 7.60% respectively and in rhizomes flavonoids, phenol, carbohydrates and proteins are 537.82 µg QE/mg, 88.54 µg /mg, 28.54% and 26.23% respectively. The antioxidant activity for the leaves and rhizomes are approximately same. So, this plant is having a potential to be further explored for carrying out other biological activities.

CHAPTER 1: INTRODUCTION

1.1 Ethnobotany

Arisaema, sometimes known as cobra lilies, is a genus with around 399 herbaceous species in the Araceae family. The common name for *Arisaema propinquum Schott* Wallich's cobra lily or cobra lily. The people in Kashmir, locally name this plant as Hapatmakei or Hapatmundh. *A. propinquum Schott*, a tuberous plant that grows at a high elevation of 2400–3600 m. This plant is established from temperate regions to tropical regions. Tropical zones are primarily, Himalayas from Kashmir to Southeast Tibet. Plant can reach height of 5-6 feet and has three Leaves which are big lustrous yellowish-green. The hood also known as spathe is broad from the top base and terminates into sharp beak, which measured 8-20 cm, a lengthy tongue which emerges within. There is a white netting effect at the hood's curvature that shows the side edges like a stained window. Brown stains can be seen on the leaf stalk and stem. This plant blooms from the starting months of May and end of June. The dried roots, leaves, rhizomes and fruits have traditionally been used to treat many diseases like skin eruptions, rheumatoid arthritis, boils and diabetic neuropathy. Pastes made from roots of the plant were used to treat scabies, erysipelas and many more diseases which are not known till now.

One of the most common illnesses, Helminthiasis (worm infection), has been affecting many humans across the world. The main cause of disease spreads is poor sanitation and contamination. Helminthiasis, reduces immunological responses to numerous pathogens and causes major clinical consequences such as malnutrition, anaemia, eosinophilia, pneumonia, malaria, TB, and human immunodeficiency virus. As gastrointestinal helminthes develop resistance to numerous anthelminthic medicines, the demand for natural anthelmintics grows exponential. In the current study, adult Indian earthworms (*Pheretima posthuma*) were employed to evaluate the anthelminthic action of herbal medications because they share morphological and physiological similarities with human helminthic parasites. The plant is consumed as food material and also used for the purpose of ethno-medico practice by tribals.

1.2 Medicinal Properties

a) *Arisaema erubescens*: *Arisaema erubescens* extracts have properties that are anti-cancerous in nature. The agents responsible for this plant's anticancer activity are still unknown **(Ducki, 1996)[28].**

b) *Arisaema favum*: The lectin from *Arisaema favum* has anti-proliferative activity against murine cancer cell lines and potent mitogenic activity against human peripheral blood mononuclear cells, measured by lymphoproliferation after lectin incorporation into the cultures. It can effectively synthesize biogenic silver nanoparticles, which have anti-bacterial, and photocatalytic properties **(Rahman, 2019)[28].**

c) *Arisaema tortuosum:* Using affinity chromatography on asialofetuin coupled amino activated silica beads. From the tubers of *Arisaema tortuosum* (Himalayan Cobra lily), a lectin with in-vitro anticancer activity against recognized human cancer cell lines was observed. **(Dhuna, 2005)[28].**

d) *Arisaema intermedium Blume and Arisaema wallichianum Hook*: N-acetyl-D-lactosamine is a marker for cancer cells. *A. intermedium Blume and A. wallichianum Hook*, used for the detection of different types of cancers because of their specificity **(Verma, 2015)[28].**

e) *Blume's Arisaema jacquemontii*: Antioxidant, cytotoxic, anticancer, and kinase inhibitor properties phytochemicals were extracted from solvent extracts of *A. jacquemontii*. *A. jaquemontii* tuber extracts in methanol and ethanol demonstrated significant antioxidant activity and radical scavenging. It also showed strong anticancer activity against cells, such as human leukaemia and prostate cancer cell lines. The roots has two triterpenoids present in them. **(Jeelani et al. 2010)[28].**

f) *Arisaema murrayi Hook*: Tannin, Picric acid, sugar, protein, polyphenols, anthraquinone and other phytochemicals were discovered in the aqueous extract of *Arisaema murrayi Hook*. The extract prepared contains phytochemicals such as favonoids, glycosides and alkaloids.

g) *Arisaema ringens*: The bulbs of *Arisaema ringens* were used to refine a basic lectin. The lectin of *Arisaema ringens* has binding sites for two carbohydrates. Heamagglutination inhibition revealed mono-oligosaccharides and terminal N acetyllactosamine.

h) *Arisaema franchetianum*: *A. franchetianum*, medicinal plant, has long been used to treat snake bites and as an anti-inflammatory agent in Chinese folk medicine. Many different compounds such as bergenin (43) (Miglani et al. 1978), emodin (44) (Ducki et al. 1996), cafeic acid (45) (Zhao et al. 2010), nobiletin (46), (Jung et al. 1996a), 3-O-b-D-galactopyranosyl-hederagenin 28-O-b-D-galactopyranos (Wang et al. 2007) (S)-1-(10-hydroxyethyl)-b-carboline, (He et al. 2002) (He et al. 2002) 1-(b-carboline-1-yl) -3,4,5-trihydroxy-1-mpentanone, **(2004 Huang et al.)**[28]

i) *Arisaema amurense*: Different species of Arisaema has been used in herbal remedies to treat digestive ulcers, rheumatism, and cancer. Because of the presence of the compound 2,3-dihydroxypropyl, 9Z,12Z octadecadienoate, the extracts obtained *from Arisaema amurense Max Var serratum* demonstrated strong phospholipase A2 inhibitory activity **(Chung et al. 1995)**[28]**.**

j) *Apium propinquum*: A large number of different experiments conducted on cobra lilies have revealed that *Arisaema propinquum* rhizomes has a strong anti-helminthic property. This declaration is based on research conducted by different researchers using methanolic or aqueous extracts obtained from *Arisaema propinquum* rhizomes **(Mir, 2020)**[2,28]**.**

CHAPTER 2: LITERATURE SURVEY

2.1 Biology of *Arisaema propinquum*

Arisaema, also known as cobra lilies, is a genus of over 399 herbaceous species in the Araceae family. In Kashmir the plant is also known as Hapat makei or Hapat mundh. *Arisaema propinquum Schott*, a tuberous plant, grows at elevations ranging from approx. 2400 to 3600 metres. The plant is found from temperate to tropical areas, but it is most common in the Himalayas, from Kashmir to Southeast Tibet **(Mubashir S, Shah WA, 2012)[1,2]**. The plant can reach a height of 5-6 feet and have three large yellowish-green leaves which are glossy. The spathe also known as hood, which is dark purple with white strips on it, ends in a pointy beak with an 8-20 cm long tongue emerging from inside and thick at the base. There is a white netting effect at the hood's curvature that presents the side edges like a stained window. There is presence of brown spots on the leaf stalk and stem. The plant blooms during months of starting of May and June. **[Last accessed on 2018 Jul 22][6].**

Fig 2.1 ***Arisaema propinquum***

2.2 Review of literature

Arisaema propinquum Schott, the common name is cobra lily. Rhizomes which is the important part of the plant are traditionally used to treat many health concerns such as, rheumatism, vermifuge, snake bites and stomachaches. The main objective of this study to analyse the anthelminthic activity and pharmacognostic parameters of rhizomes of *Arisaema*. The rhizomes of plant were collected, dried, and powdered before being tested for pharmacognostical parameters such as micro and macroscopical characters, and physicochemical and phytochemical analysis using standard procedures **(Evans WC, Evans *et al* 2009 and Trease GE *et al* 2008)[16]**. The extracts' antihelminthic activity against *Pheretima posthuma* was increased **(Gnaneswari K *et al* ,2013)[27]**. The rhizomes of the plant are light brown in color with a pungent odor and an astringent taste. In the transverse section of the rhizome, the xylem vessels, intercellular schizogenous cavities, phloem vessels, and parenchymatous cells were visible. The phytochemical analysis showed the presence of carbohydrates, alkaloids, proteins, cardiac glycosides, coumarins, amino acids, phenols, tannins, flavonoids, steroids, and terpenoids and saponins.**(Tiwari P, Kumar *et al*, 2011; Menpara D et al, 2014)[23]**. The physiochemical parameters, which included ash values, revealed that there was 6.32% total ash, 1.77% acid insoluble ash, 5.15% water soluble ash, and 8.55% sulfated ash. Other parameters determined included extractive value, foreign matter, moisture content, swelling index, foaming index, pH of different solvents, and fluorescence analysis **(Quality Control Methods for Herbal Materials, 2011; Mukherjee PK *et al*, 2008; Mukherjee KP et al, 2002)[19,20]**.
When compared to standard albendazole, both the aqueous and methanolic extracts of *A. propinquum* showed dose-dependent anthelminthic activity against *P. posthuma*.
Data derived from this study has been used as a standard protocol for quality control of this plant, which is used as a herbal medicine to treat a variety of diseases.
(PRINCE MIR *et al*: 2020)[2].

Rhizomes part of *Arisaema propinquum* Schott were tested in-vitro for the anti-inflammatory and antioxidant activity. Phytochemical screening for the alcoholic and aqueous extracts came first. The anti-oxidant activity was determined using methods like, reducing power method, the hydrogen peroxide scavenging method **(Braca A et al, 2001; Dar MA et al, 2014)[1]** and DPPH (1, 1diphenyl-2-picryl-hydrazyl) scavenging method. In-vitro anti-inflammatory activity was determined using

Human Red Blood Cell membrane stabilization and percentage protein denaturation inhibition methods. **(Sadique J et al, (1989); Obaseki OE et al, (2016))[1]**.

The extracts contain carbohydrates, steroids, cardiac glycosides, proteins, amino acids, phenols, saponins, tannins, flavonoids, alkaloids, terpenoids and coumarins, according to phytochemical analysis. Total phenolic compounds was found in aqueous and alcoholic extracts (mg/g) in Gallic acid equivalent was 425.21 and 554.06 mg/g respectively and total flavonoid content was found in aqueous and alcoholic extracts was 225.56 and 324.71 (mg/g) in Rutin Equivalents. When compared with standards rutin and ascorbic acid, the methanolic extract inhibited DPPH and Hydrogen peroxide scavenging activity by a significant percentage. When compared to standard Indomethacin, both extracts demonstrated good membrane stabilizing and protein denaturing activity (**Mir PA et al, (2019)**)[1].

The study's findings show that methanolic and aqueous extracts of Arisaema propinquum Schott rhizomes have antioxidant and anti-inflammatory properties. To determine the mechanism of action, the active principles responsible for pharmacological activity must be isolated and purified. **(Mir PA, Dar MA, Mohi-ud-din R, Bader GN (2019)[1]**.

Phytochemicals like phenols, alkaloids, flavonoids, terpenes, glycosides, saponins, oxalates, coumarins, triterpenoids, n-alkanes, n-alkanols, tannins etcetera are found in the plant. Furthermore, plants in the genus Arisaema exhibit antifungal, antioxidant, antibacterial, antimicrobial, insecticidal, nematocidal, antiallergic, anticancer, cytotoxic, and antitumor properties. *Arisaema* plants have traditionally been used to treat a variety of ailments, including dampness, phlegm, and bronchitis, asthma, cough, cold, and laryngitis. Through different experiments conducted on these plants it is found, several species are toxic by nature. It has been discovered that several species are toxic by nature. There is a certain delay in the development of clinical applications of arisaematis rhizomes because of the toxic properties such as lingua and mouth pain or sometimes suffocation and respiratory slowing, skin and mucous membrane irritation, and so on. This is because of a component present in the plant known as raphide.

The phytochemistry of the *Arisaema* is insufficient, more research is needed to be done on the phytochemical and medicinal properties of the plant treat various diseases around the world. **(Heena Ali and Ubaid Yaqoob, 2021)[28]**

2.3 Rationale/Gaps

- The family *Arisaema* are valuable plants due to their medicinal significance and are traditionally used by different native cultural and traditional communities.
- However, there is a lack of studies on the phytochemical aspects of the species *Arisaema propinquum* .
- In the present study we would like to focus on the different phytochemical aspects of the plant species *Arisaema propinquum* in order to explore the commercial viability of the plant.

2.4 Objectives

- To carry out phytochemical analysis of extracts of different parts of the plant.
- To carry out Phytochemical profiling of whole plant *Arisaema propinquum.*

CHAPTER 3: MATERIALS AND METHODS

3.1 Plant Material

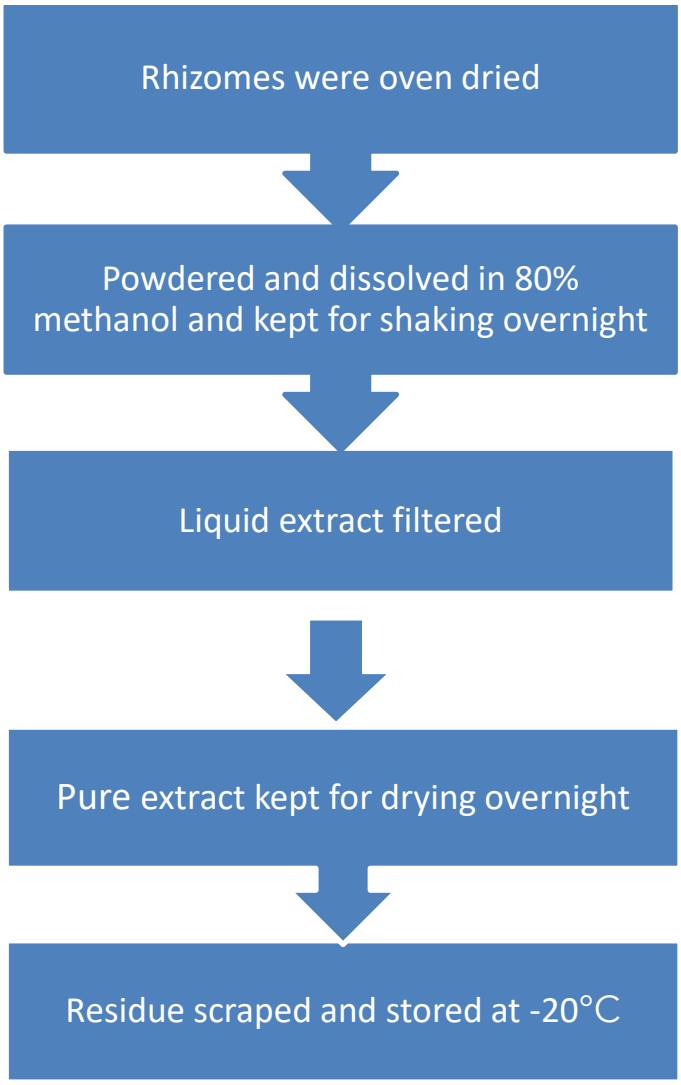
The plant has been collected from wild areas of district of Mandi and sorted in lab and stored at -80°C for further experimentation and analysis.

(a) (b)

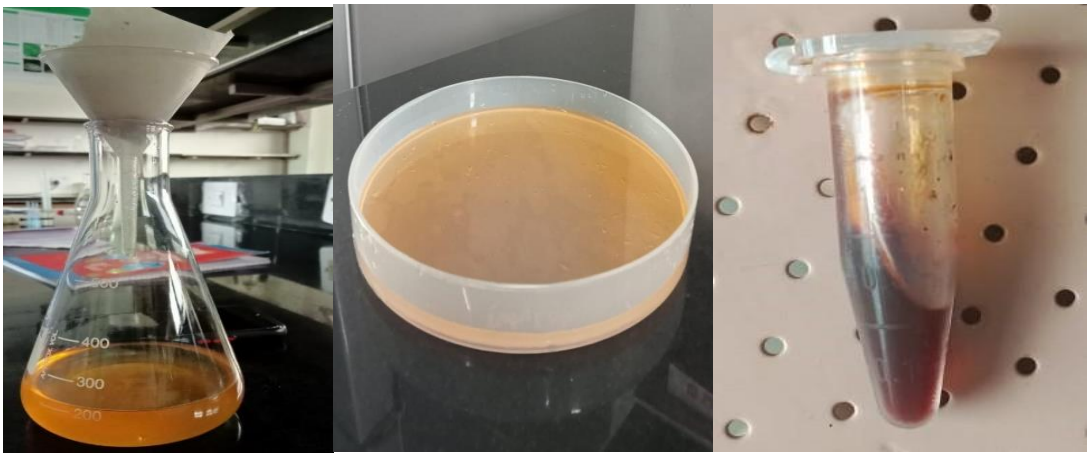
Fig 3.1 (a) Arisaema propinquum plant material (b) Rhizomes of Arisaema propinquum

3.2 Extraction Process:

1. Shade drying and oven drying of *A. propinquum* plant rhizomes for 1-2 days and powdered.
2. Powder form of extract (10 gm) dissolved in 80% methanol and kept for shaking for at least 48 hrs.
3. Liquid extract was filtered using blotting sheet followed by Whatman filter paper.
4. Pure extract was kept for drying in petri plate overnight.
5. After 2 days, the residue is scraped and stored at -20°C.



(a) (b) (c)



(d) (e) (f)

Fig 3.2 (a) Oven-dried rhizomes of *Arisaema propinquum* (b) Rhizomes crushed into powder form (c) Powdered extract mixed into 80% methanol and shaken overnight (d) Liquid extract filtered using Whatman filter paper (e) Pure extract kept for drying (f) Plant Extract

3.3 Qualitative Phytochemical Analysis

Following tests were performed for the plant *Arisaema Propinquum* to analyse the presence of some common phytochemicals. The tests were performed in triplicates to ensure credibility.

1. **Test for carbohydrates**

   Molish Test:

   - Add a few drops of alpha-naphthol solution to 3 ml of test extract.
   - When a few drops of conc. H_2SO_4 is added to the mixture, we can observe a violet ring at the junction of two liquids.

   ➢ 3ml PE + few drops of Molish reagent + few drops of H_2SO_4 ⟶Violet ring formation

2. **Test for alkaloids**

   - 2 ml of 2% HCl was mixed with 2 ml of liquid extract.
   - Few drops of Mayer's reagent were added to the solution.
   - Occurrence of reddish brown precipitate confirms the presence of alkaloids

     ➢ 2ml HCl + 2ml liquid extract + a few drops of Mayer's reagent ⟶ Reddish brown precipitate.

3. **Test for flavonoids**

   - To the 2 ml of the extract add a few drops of the NaOH.
   - There will be appearance of intense yellow color.
   - Then a few drops of dil. HCl to be added to the solution.
   - The solution turns colourless as indicator of presence of flavonoids.

     ➢ 2ml PE + few drops of NaOH ⟶ yellow color + few drops of HCl ⟶ soln. turns colorless.

4. **Test for saponins**

   - To 1 ml of the extract a few drops of (1%) lead acetate solution was added.
   - There will be appearance of intense white ppt. due to presence of Saponins.

     ➢ 1 ml + few drops of (1%) Lead acetate ⟶ intense white ppt.

5. **Test for sterols**
   - Add 2 ml conc. H_2SO_4 to 2 ml of the extract.
   - The formation of red ppt. indicates presence of sterols.
     - 2ml + 2ml H_2SO_4 ⟶ Red color
6. **Test for tannins**
   - 1 ml of 3% of ferric chloride to be added to 1 ml of the extract.
   - Brownish green color development shows presence of tannins.
     - 1ml + 3% $FeCl_2$ ⟶ Brownish green color
7. **Test for terpenes**

   Salkowski test:
   - To 1 ml of liquid extract 1 ml of conc. H_2SO_4 was added.
   - If the solution turns blue-green colour/brick red, then terpene is present.
     - 1 ml + 1 ml H_2SO_4 ⟶ blue-green colour/brick red
8. **Test for proteins**

   Millions test:
   - Take 2ml of extract in a test tube.
   - Add a few drops of millions reagent and observe the change.
   - If there is the formation of white ppt. or if the sample changes to brick red on heating then presence of protein is confirmed.

9. **Test for phenolic compound**
   - 2 ml of ferric chloride 3% was mixed with 2 ml of liquid extract.
   - The solution will turn to blur green colour/brisk red.
     - 2ml + 2ml $FeCl_2$ ⟶ Blur green color/brisk red.

3.4 Quantitative Phytochemical Analysis

Following tests were performed for the plant *Arisaema propinquum* to analyze the presence of some common phytochemicals.

1. Total Flavonoid Content

- Quercetin is used for the standard with conc. 1mg/ml of methanol and five controls were made.
- Distilled water was then added to extract.
- 5% sodium nitrite was allowed to rest for 5 minutes.
- Then Aluminium Chloride was allowed to rest for 6 minutes.
- NaOH 1M were added and kept for 15 minutes.
- Absorbance at 510 nm was taken.

S.No.	**Conc.(µl)**	**Methanol (µl)**	**dH_2O (ml)**	**$NaNO_2$ (µl)**		**$AlCl_3$ (µl)**		**NaOH (µl)**		
C1	100	900	2	150	**Stand for**	150	**Stand for**	1000	**Stand for**	**Absorbance at**
C2	200	800	2	150	**5 minutes**	150	**6 minutes**	1000	**15 minutes**	**510 nm**
C3	300	700	2	150		150		1000		
C4	400	600	2	150		150		1000		
C5	500	500	2	150		150		1000		
Blank	0	1000	2	150		150		1000		
Extract Sample	500	500	2	150		150		1000		

2. Total Phenolic Content

- Gallic acid is used as a standard with conc. 1mg/ml and five control was made.
- Then distilled water was added.
- FC reagent was added and allowed to rest for 6 minutes.
- 15% sodium carbonate was added and allowed to rest for 20 minutes in dark.
- Absorbance taken at 760nm.

S.No.	**Conc. (µl)**	**DH_2O (µl)**	**DH_2O (ml)**	**FC-reagent (µl)**	**Na_2CO_3 (ml)**		
C1	40	160	8.3	500	1	**20 minutes**	**Absorbance at**
C2	80	120	8.3	500	1	**wait in dark.**	**760 nm**
C3	120	80	8.3	500	1		
C4	160	40	8.3	500	1		
C5	200	0	8.3	500	1		
Blank	0	0	8.5	500	1		
Extract Sample	200	0	8.3	500	1		

3. Anthrone Method for Carbohydrate Quantification

- Glucose was used as a standard with conc. 1mg/ml.
- 200mg of Anthrone was mixed with 100ml of chilled 95% Sulphuric acid and added to each test tube.
- Then incubation in water bath for 15 minutes at 90^{o}C.
- Absorbance at 620nm.

Glu. Conc. (mg/ml)	**Vol. (µl)**	**DH_2O (µl)**	**Anthrone reagent (ml)**		
0.2	20	980	4	**Incubation in**	**Absorbance at**
0.4	40	960	4	**Water bath**	**620 nm**
0.6	60	940	4	**for 15 minutes**	
0.8	80	920	4		
1	100	900	4		
Blank	0	1000	4		
Extract Sample	100	900	4		

4. Lowry's Method for Protein Quantification

- BSA is used for standard with conc. 1mg/ml and five control was made.
- Reagent A was made by 2% sodium carbonate dissolved in (0.04%) 1N NaOH.
- In reagent B, 1% sodium potassium tartrate dissolved in distilled water and 0.5% copper sulphate dissolved in distilled water then both were mixed together.
- 1 ml of reagent B and 50 ml of reagent A was mixed and reagent C was made
- FC reagent was made by 1:1 with distilled water.

BSA (µl)	**DH_2O (µl)**	**Reagent C (ml)**		**FC reagent (ml)**		
200	800	4.5	**Incubation for**	0.5	**Incubation for**	**Absorbance at**
400	600	4.5	**15 minutes.**	0.5	**40 minutes**	**660nm.**
600	400	4.5		0.5	**in dark.**	
800	200	4.5		0.5		
1000	0	4.5		0.5		
Blank	1000	4.5		0.5		
Extract Sample	0	4.5		0.5		

5. **Antioxidant assay using DPPH(2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity method**
    - Four conc. of control were made and gallic acid was used as a standard with conc. 1mg/ml GA.
    - 2mg of 0.002% of DPPH was mixed with 100% methanol to form DPPH solution.
    - Then kept for incubation for 30 minutes.
    - Absorbance was taken at 517nm.

Conc. GA (µl)	**Methanol (µl)**	**DPPH(0.002%)(ml)**		
50	350	3.6	**Incubate for**	**Absorbance**
100	300	3.6	**30 minutes**	**At 517nm.**
200	200	3.6	**In dark.**	
400	0	3.6		
Blank	400	3.6		
Extract sample	0	3.6		

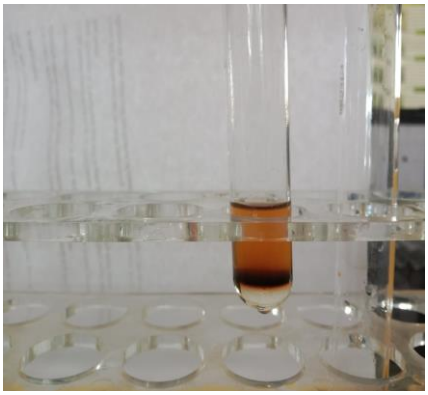
CHAPTER 4: RESULTS

4.1 Results for Qualitative Phytochemical Analysis

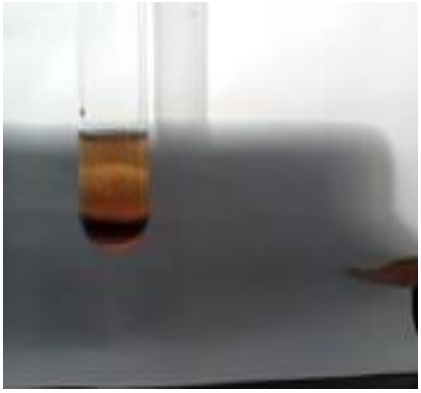
Results obtained from qualitative test of different phytochemicals in the plant *Arisaema propinquum.*

1. **Carbohydrates**

   Presence of carbohydrates is seen if violet ring is formed at the junction of two liquid.



(a)

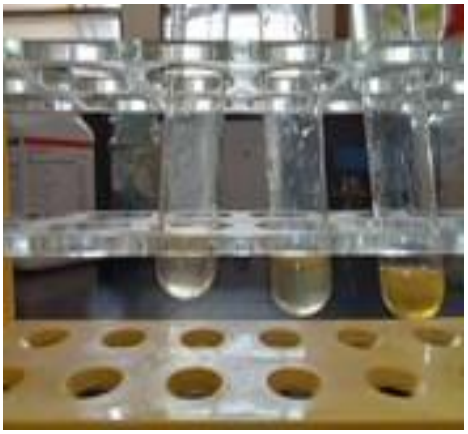


(b)

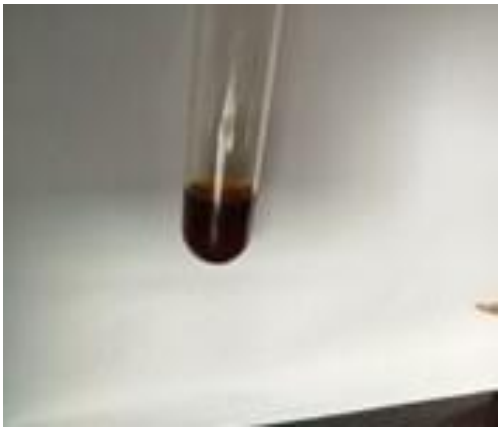
Fig 4.1.1 (a) Rhizome (b) Leaf

2. **Flavonoids**

   First there will be appearance of the intense yellow color and then when we add a few drops of dil. HCl, the solution turns colorless. This shows presence of flavonoids.



(a)

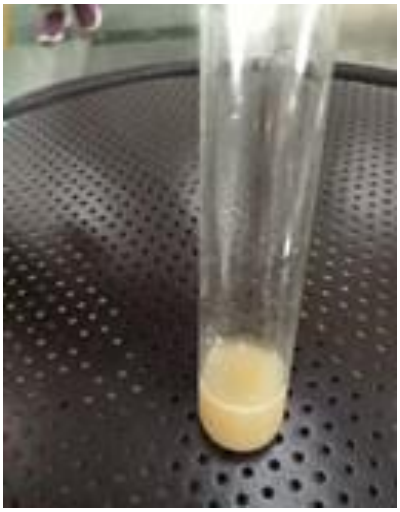


(b)

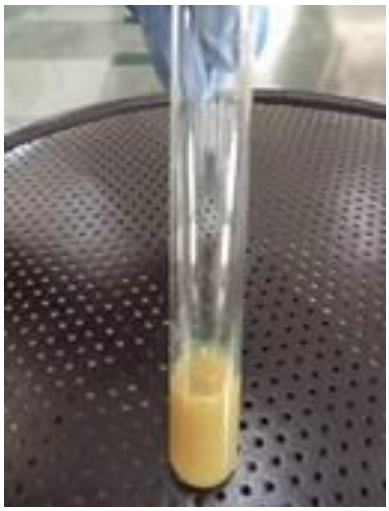
Fig 4.2.1 (a) Rhizome (b) Leaf

3. Saponins

Appearance of intense white ppt. due to presence of Saponins.



(a)



(b)

<u>Fig 4.3.1</u> (a) Rhizome (b) Leaf

4. Sterols

Formation of red ppt. indicates presence of sterols.

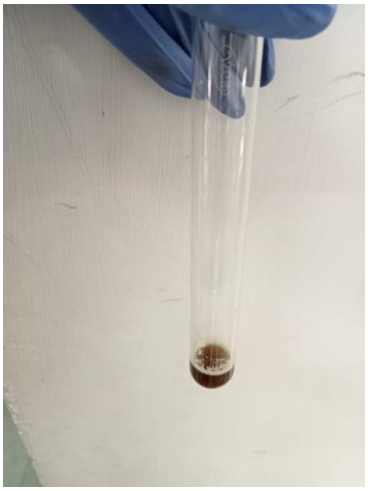
(a)

(b)

<u>Fig 4.4.1</u> (a) Rhizome (b) Leaf

5. Tannins

Brownish green colour development indicates presence of tannins.



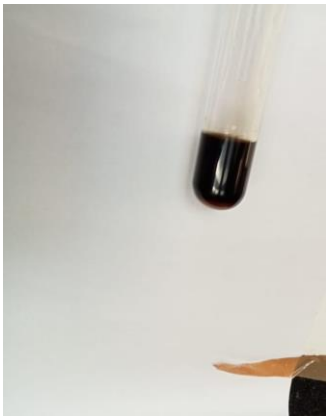
(a)

(b)

<u>Fig 4.5.1</u> (a) Rhizome (b) Leaf

6. Terpenes

If the solution turns blue-green colour/brick red, then terpene is present.



(a)

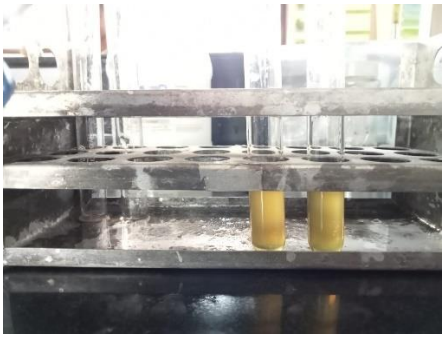
(b)

<u>Fig 4.6.1</u> (a) Leaf (b) Rhizomes

7. Alkaloids

Occurrence of reddish brown precipitate confirms the presence of alkaloids.

(a)



(b)

Fig 4.7.1 (a) Leaf (b) Rhizomes

8. Proteins

White ppt. formation or if the sample changes to brick red on heating then presence of protein is confirmed.

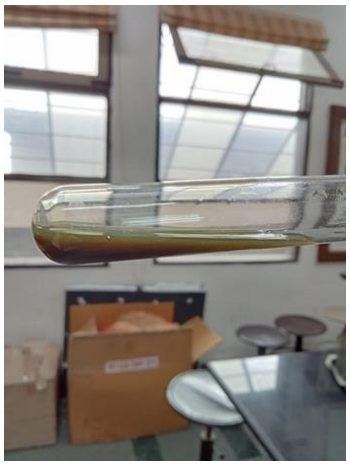
(a)

(b)

Fig 4.8.1 (a) Leaf (b) Rhizomes

9. Phenols

Blur green colour/brisk red.



(a)

(b)

Fig 4.9.1 (a) Leaf (b)Rhizomes

Table 4.1 Qualitative Analysis Report

Tests	**Rhizome**	**Leaf**	**Whole Plant**	**Colour/Indicator**
1. Test for Terpenes	Present	Absent	Absent	Blue-green color/ brick red
2. Test for Flavonoids	Present	Absent	Present	Colorless
3. Test for Phenols	Present	Present	Present	Blue-green color/ brick red
4. Test for Sterols	Present	Present	Absent	Red precipitates
5. Test for Tannins	Present	Present	Absent	Brownish-green
6. Test for Carbohydrates	Present	Present	Present	Violet ring at junction.
7. Test for Proteins	Present	Present	Present	Violet color
8. Test for Alkaloids	Absent	Present	Absent	Reddish-brown precipitates
9. Test for Saponins	Present	Present	Present	Intense white precipitates

4.2 Results for Quantitative Phytochemical Analysis

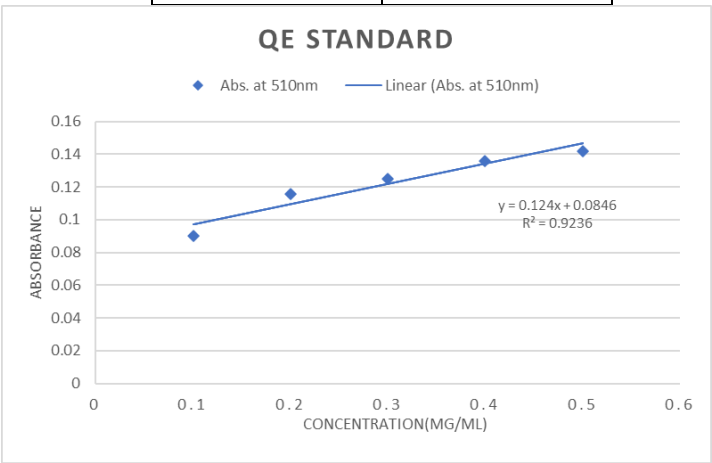
Following are the results obtained for the quantitative analysis of different parts of the plant extract and extract obtained from whole plant.

Table 4.2. Quantitative Analysis Report

4.2.1. Whole Plant:- 1mg of sample contains 2.05 µg flavonoid, 113.15 µg phenols, 1.63% carbohydrates and 1.157% protein are analyzed using following methods used below.

4.2.1.1 Total Flavonoid Content

Conc. (mg/ml)	Abs. at 510nm
100	0.09
200	0.116
300	0.125
400	0.136
500	0.142
Blank	0.055
Extract sample	0.331



Y= absorbance

X= concentration of extract

Y= mx + c

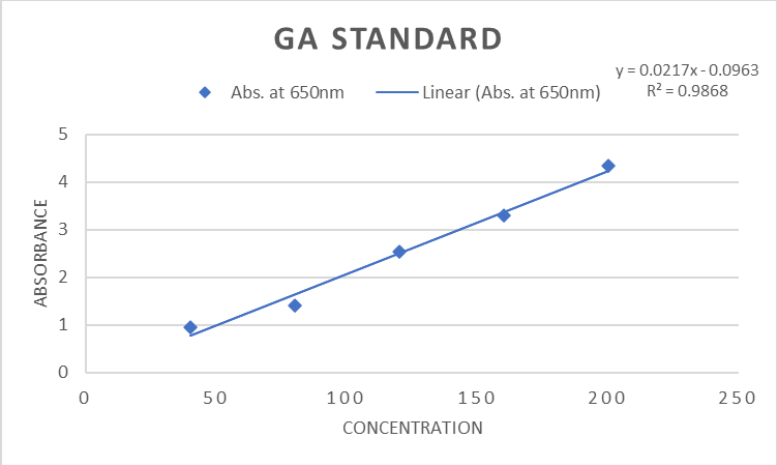
0.331= 0.124x + 0.0846

X= 2.05 µg QE/mg

Result:- 1mg of sample contain 2.05 µg flavonoid in context of Quercetin Equivalence (standard).

4.2.1.2 Total Phenolic Content

Conc. (mg/ml)	Abs. at 650nm
40	0.945
80	1.411
120	2.544
160	3.29
200	4.344
Blank	0.063
Extract Sample	2.359



Y= mx + c

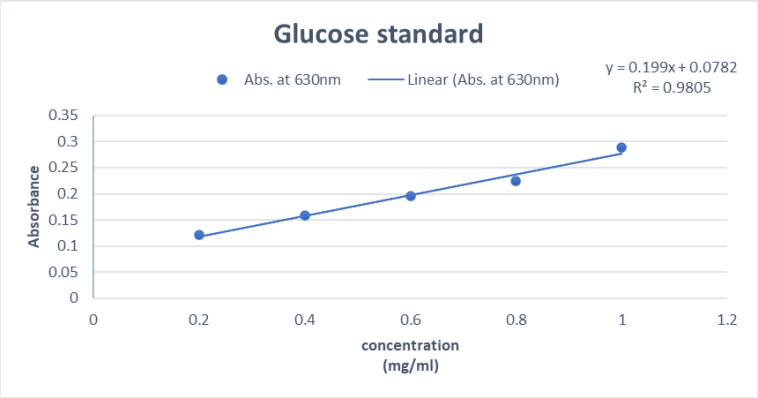
2.359 = 0.0217x – 0.0963

X= 113.15 µg/mg

Result:- 1mg of sample contain 113.15 µg phenolic content in context of gallic acid Equivalence (standard).

4.2.1.3 Anthrone Method for Carbohydrate

Conc.(mg/ml)	Abs. at 630nm
200	0.122
400	0.158
600	0.196
800	0.224
1000	0.288
Blank	0.085
Extract sample	0.403



Y= mx + c

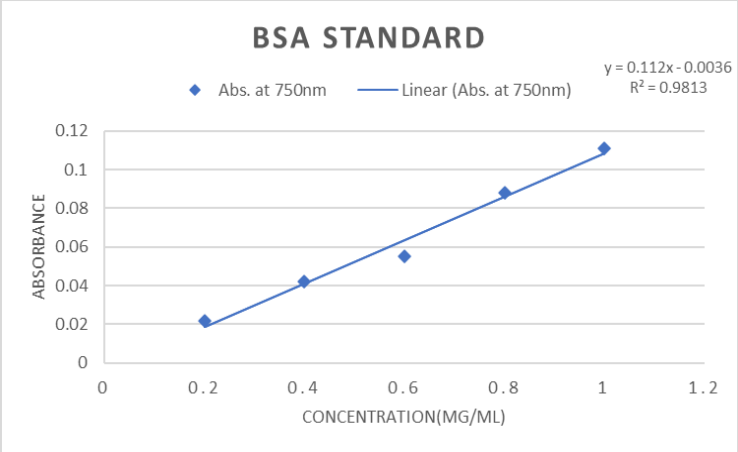
0.403= 0.199x + 0.0782

X= 1.63%

Result:- 1mg of sample contain 1.63% carbohydrates in context of glucose Equivalence (standard).

4.2.1.4 Lowry Method for Protein

Conc. (mg/ml)	Abs. at 750nm
200	0.022
400	0.042
600	0.055
800	0.088
1000	0.111
Blank	0.019
Extract Sample	0.126



Y= mx + c

0.126 = 0.112x – 0.0036

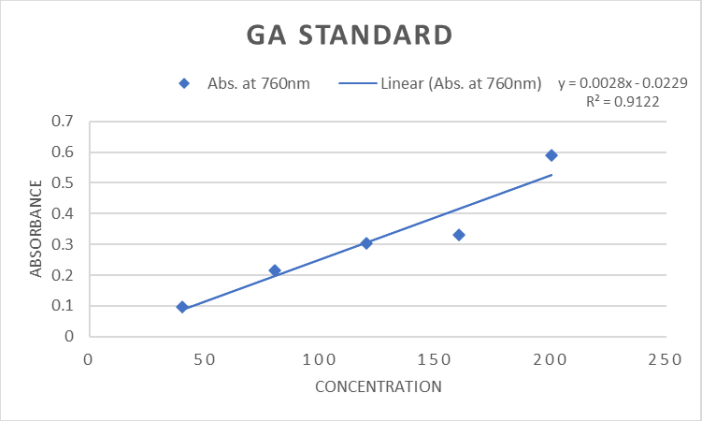
X= 1.157%

Result:- 1mg of sample contain 1.157% protein in context of BSA Equivalence (standard).

4.2.2 Leaves:- 1mg of extract sample contains 279µg phenols, 18.02% protein and 7.60% carbohydrates are present but secondary metabolite was absent seen in qualitative test.

4.2.2.1 Total Phenolic Content

Conc. (mg/ml)	Abs. at 760nm
40	0.0963
80	0.2155
120	0.3040
160	0.3309
200	0.5886
Blank	0.065
Extract Sample	0.5351



Y= mx + c

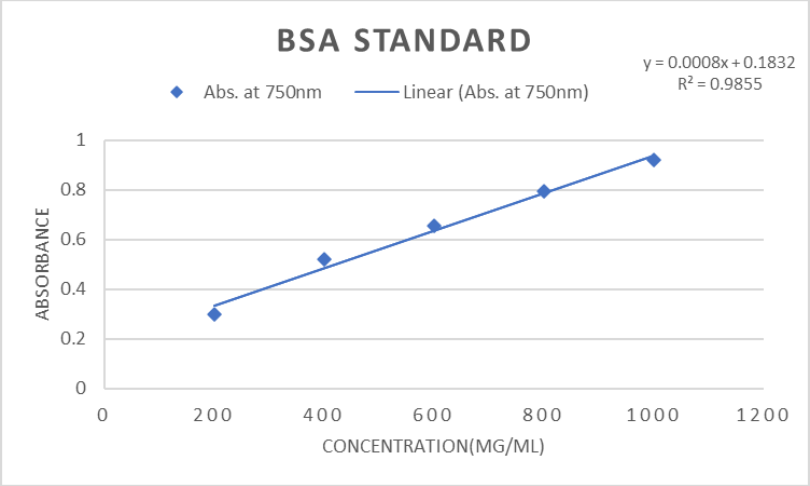
0.5351= 0.0028x – 0.0229

X= 279 µg/mg

Result:- 1mg of sample contain 279µg phenolic content in context of gallic acid Equivalence (standard).

4.2.2.2 Total Protein Content

Conc.(mg/ml)	Abs. at 750nm
200	0.298
400	0.521
600	0.656
800	0.793
1000	0.919
Blank	0.078
Extract sample	1.625



Y= mx + c

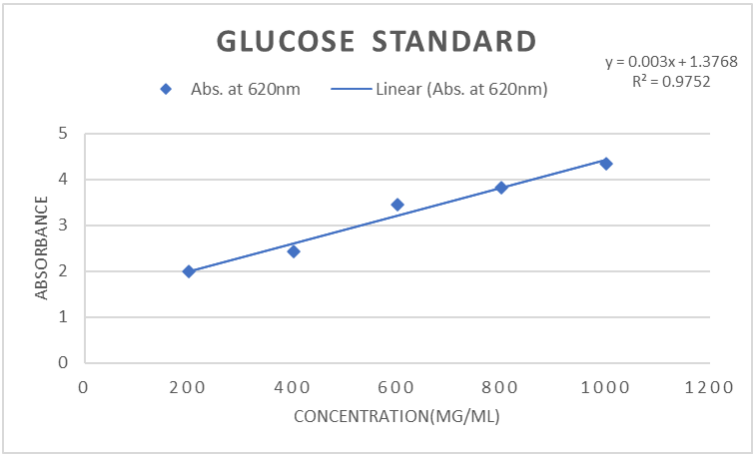
1.625= 0.0008x + 0.1832

X= 18.02%

Result:- 1mg of sample contain 18.02% protein in context of BSA Equivalence (standard).

4.2.2.3 Total carbohydrates content

Conc.(mg/ml)	Abs. at 620nm
200	1.996
400	2.421
600	3.445
800	3.815
1000	4.345
Blank	0.095
Extract sample	3.658



Y= mx + c

1.658 = 0.003x + 1.3768

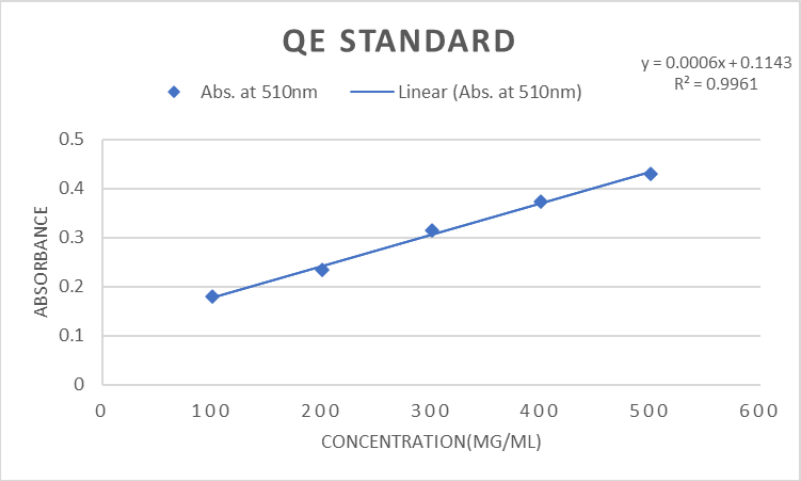
X = 7.60%

Result:- 1mg of sample contain 7.60% carbohydrates in context of glucose Equivalence (standard).

4.2.3 Rhizomes:- 1mg of extract sample contains secondary metabolites as 537.83 µg flavonoids and 88.54µg phenols and primary as 26.23% protein and 28.54% carbohydrates .

4.2.3.1 Total Flavonoids Content

Conc. (mg/ml)	Abs. at 510nm
100	0.179
200	0.235
300	0.315
400	0.374
500	0.430
Blank	0.053
Extract Sample	0.437



Y= mx + c

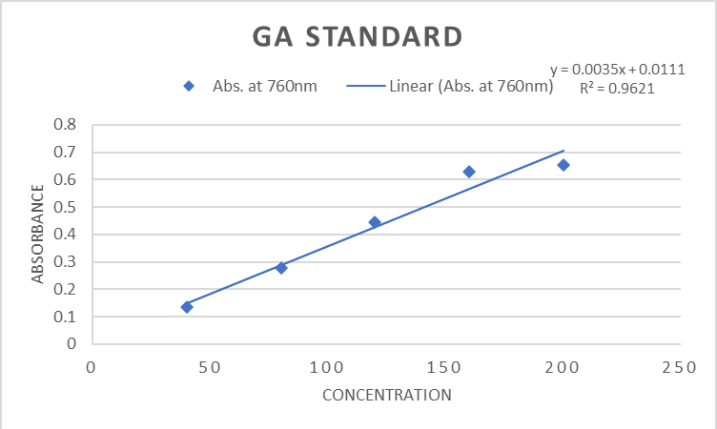
0.437 = 0.0006x + 0.1143

X= 537.83 µg QE/mg

Result:- 1mg of sample contain 537.83µg flavonoid in context of Quercetin Equivalence (standard).

4.2.3.2 Total Phenol Content

Conc. (mg/ml)	Abs. at 760nm
40	0.134
80	0.277
120	0.446
160	0.630
200	0.652
Blank	0.066
Extract sample	0.321



Y= mx + c

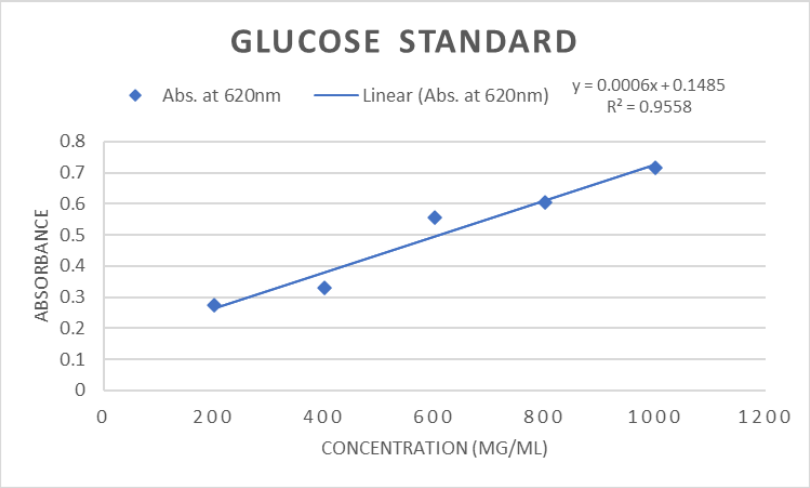
0.321 = 0.0035x + 0.0111

X = 88.54 µg/mg

Result:- 1mg of sample contain 88.54µg phenolic content in context of gallic acid Equivalence (standard).

4.2.3.3 Total carbohydrate content

Conc.(mg/ml)	Abs. at 620nm
200	0.273
400	0.331
600	0.555
800	0.604
1000	0.715
Blank	0.069
Extract sample	1.861



Y= mx + c

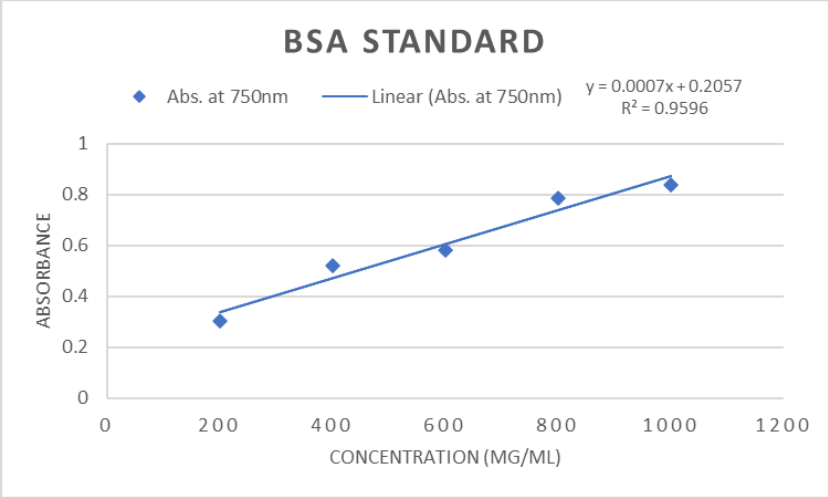
1.861 = 0.0006x + 0.1485

X= 28.54%

Result:- 1mg of sample contain 28.54% carbohydrates in context of glucose Equivalence (standard).

4.2.3.4 Total Protein content

Conc.(mg/ml)	Abs. at 750nm
200	0.305
400	0.520
600	0.580
800	0.787
1000	0.839
Blank	0.075
Extract sample	2.042



Y= mx + c

2.042 = 0.0007x + 0.2057

X= 26.23%

Result:- 1mg of sample contain 26.23% protein in context of BSA Equivalence (standard).

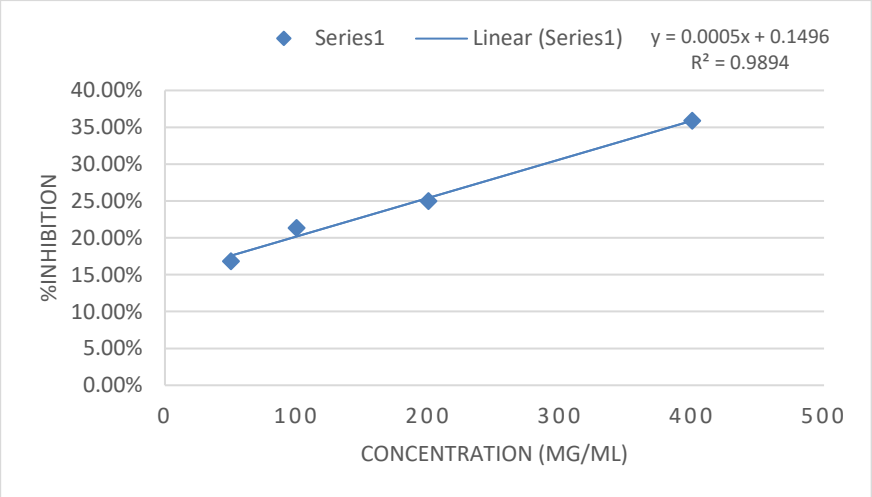
4.3 Results for Antioxidants using DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity method

Results obtained for different parts of the plant extract like Leaves and Rhizomes contain almost similar concentration i.e., 9. 9 µg concentration of extract of leaves is required for the 50% inhibition.

4.3.1 Leaves:

Conc. (mg/ml)	Abs. at 517nm	% inhibition
50	0.161	14.36
100	0.157	16.49
200	0.149	20.74
400	0.128	31.91
Blank	0.188	0
sample	0.181	37.23

% inhibition= Abs. of control- Abs. of sample/ Abs. of control * 100



IC_{50}= y= mx + c

50= 0.0005x + 0.1142

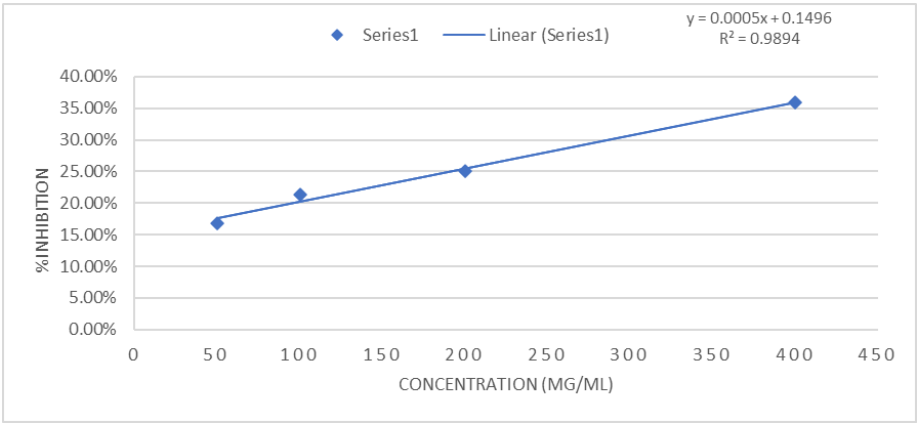
X= 9.9 µg

Result:- 9.9 µg concentration of extract of leaves is required for the 50% inhibition.

4.3.2 Rhizomes:

Conc. (mg/ml)	Abs. at 517nm	% inhibition
50	0.183	16.82
100	0.173	21.36
200	0.165	25
400	0.141	35.91
Blank	0.220	0
sample	0.192	12.73

% inhibition= Abs. of control- Abs. of sample/ Abs. of control * 100



IC_{50}= y= mx + c

50= 0.0005x + 0.1496

X= 9.97µg

Result:- 9.97 µg concentration of extract of Rhizomes is required for the 50% inhibition

4.4 Discussion

Arisaema has been considered because of its high pharmacological and commercial significance. This plant is known to be high in phytochemicals, which are secondary metabolites that include flavonoids, terpenoids, steroids, tannins, reducing sugars, and cardiac glycosides, and can aid in the treatment of diseases including diabetes, cancer, obesity, cardiovascular disease, ulcers, and many others. This research focuses on phytochemical profiling and quantification in the rare *Arisaema propinquum*. The extraction methods are as follows: The maceration process was used to find and study bioactive compounds, including phytochemicals such as phenol and flavonoid compounds, as well as traces such as carbohydrates and proteins. In terms of gallic acid equivalent, the total phenolic content in alcoholic and aqueous extracts was 554.06 and 425.21 mg/g, respectively, while the total flavonoid content was 324.71 and 225.56 mg/g in rutin equivalents. The percentage of inhibition used to measure the ability of the alcoholic and aqueous extracts of *A. propinquum* to scavenge DPPH free radicals was 92.07% and 52.42%, respectively, at concentrations of 100 mg/ml as given in "Evaluation of Antioxidant and Anti-Inflammatory Activity of Methanolic and Aqueous Extract of *Arisaema propinquum Schott* Rhizomes" Dar MA et al (2019) paper.

Whereas the quantification values in alcoholic extract done for different parts of the plant and plant used as a whole was calculated, as flavonoids, phenol, carbohydrates and proteins are 2.05 µg QE/mg, 113.15 µg/mg, 1.63%, and 1.157% respectively, in leaves phenol, protein and carbohydrates are present in 279 µg/mg, 18.02% and 7.60% respectively and in rhizomes flavonoids, phenol, carbohydrates and proteins are 537.82 µg QE/mg, 88.54 µg /mg, 28.54% and 26.23% respectively. The antioxidant activity for the leaves was 37.23% and rhizomes was 12.73% at concentration of 400 mg/ml.

CHAPTER 5: CONCLUSION

1. Conclusion

We have taken the plant *Arisaema propinquum* due to its high pharmacological and commercial significance. In this study, we have carried out the qualitative, quantitative and antioxidant activity on the different parts (rhizome, leaf and whole plant) of the above-mentioned plant. The saponins and flavonoids and many more phytochemicals are present as seen in the test results and also significant amounts of nutraceutical traces such as carbohydrates and proteins. These studies helps us to infer or to elucidate effect of geographical location on biosynthesis and accumulation of primary and secondary metabolites in these plants. This study help in quantifying the amount of these metabolites which will be utilized for further experimentation. The plant is having good accumulation of primary and secondary metabolities whereas the leaves and root portion of the plant is also explored for its biological activities like antioxidant activity whereas it hold the potential for its further exploration on its basis of these studies to be explored for other biological activities which may include anti-cancerous, anti-bacterial etc. The studies on tissue culture parameters will help in providing quality rich herbal material throughout the year for large scale commercialization.

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