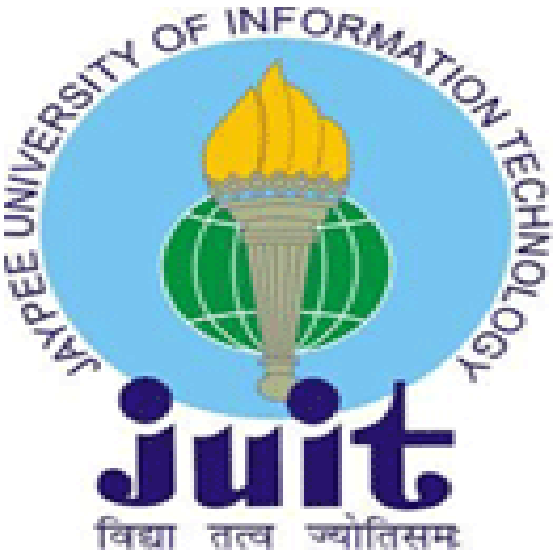
Phytochemical Profiling and Quantification of rare *Arisaema propinquum*

Project Report

Submitted to

Department Of Biotechnology and Bioinformatics



In partial fulfilment for the degree of

M. TECH IN BIOTECHNOLOGY

Project by

Sambhavana

(Enrollment No.: 202552)

Under the Supervision of

Dr Hemant Sood

(Associate Professor, JUIT)

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DECLARATION

I hereby declare that the work presented in this thesis entitled "Phytochemical Profiling and Quantification of rare *Arisaema propinquum*" in partial fulfilment of the requirements for the award of the degree of M.Tech in the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, 173234, India is an original record of my own work carried out over a period from July 2020 to May 2022 under the supervision of Dr. Hemant Sood as guide.

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

(Student's signature)

Sambhavana (202552)

SUPERVISORCERTIFICATE

This is to certify that the work entitled “”Phytochemical Profiling and Quantification of rare *Arisaema propinquum*, submitted by ‘Sambhavana Enrollment no. 202552’ to Jaypee University of Information Technology, Waknaghat, 173234, India; in partial fulfilment for the award of the degree of M. Tech has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institute for the award of any other degree or diploma.

(Supervisor’s signature)

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ABSTRACT

Arisaema propinquum, a member of *Arisaema* has been taken into consideration due to its high pharmacological and commercial significance. This plant is known to be enriched with phytochemicals, that constitute many secondary metabolites such terpenoids, flavonoids, tannins, steroids, reducing sugars and cardiac glycosides, which can cure various diseases such as cancer, diabetes, obesity, ulcers, cardiovascular diseases and many more.

This study concentrates Phytochemical Profiling and Quantification in rare *Arisaema propinquum*. Methods used in extraction is Maceration process was used to find and study the bioactive compounds, in which Phytochemicals including flavonoid and phenolic compound and also the neutraceutical traces including carbohydrates and proteins were found. After that, methanolic extract of the *Arisaema propinquum* was evaluated for the quantitative research of the mentioned compounds which resulted in values. The plant extract contained carbohydrates 1.63% and proteins 12.96%s which were quantified by standardized protocols. In the present study the plant extract contained flavonoid3.27 µg /mg and phenolic compounds 319.98 mg/g. However, till today, this plant is unexplored for the scientific evidences that authenticate the biosynthesis and accumulation of different secondary metabolites and also further exploration for bioactive compounds having pharmacological properties.

Keywords: *Arisaema propinquum*, phytochemicals, bioactive compounds, maceration, secondary metabolites,

CHAPTER 1: INTRODUCTION

Arisaema martius (1831: 449) is represented by 215 taxa worldwide, with two recent disclosures from the Western Ghats provinces of Kerala and Tamil Nadu, namely A.peerumedense (J. Mathew in Mathew and George (2017: 29), A. madhuanum Nampy and Manudev in Manudev and Nampy(2014: 269) and A. graci (J. Mathew in Mathew). The designers discovered two fascinating Arisaema species during long investigations in the Nilgiri Biosphere Reserve in Tamil Nadu, one from Thia Shola and the other from the Pennant's Valley woodlands sections. The primary species is appealing because to its translucent spathe, but the second option is appealing due to its long caudate appendage with a filiform string. Their identities as *A. translucens* Fischer (1933: 344) and *A. tuberculatum* Fischer (1933: 344) were confirmed by a thorough analysis of the literature (Fischer 1933, Fischer 1934, Gusman and Gusman 2002). (1934: 167). Fischer (1933) based his depiction of *Arisaema translucens* on an assortment obtained by E. Barnes in the Thia Shola woodlands in 1932 at a height of 6000 feet. This taxon had not been collected since around 1932, according to a thorough literature research and herbarium interviews. After an 84-year hiatus from its original site, our discovery is now a memory of this species essential depictions, colour pictures, and distribution information are provided in accordance with the new assortments. More work on phytochemical and therapeutic premise is required, as the quantity of information known on the Arisaema family's phytochemistry is insufficient [1]. The Arisaema class contains a huge number of plant species that are useful as pharmaceuticals, but only a handful have been examined, and the rest have been ignored. The huge quantity of information available on the phytochemistry and restorative capabilities of plants in the genus Arisaema reveals several types of Arisaema that are restoratively significant and have been used to treat a variety of ailments in both ancient and modern times.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction to *Arisaema propinquum*

Arisaema propinquum is a flowering plant in the Araceae family. *Arisaema propinquum* is a Himalayan species. The Wallach's cobra lily is a plant that looks a lot like a cobra hood. The plant can grow up to 30 cm tall and has 1-2 very large trilobed yellowish green leaves. The spathe, or cobra hood, is a dark purple elongated praise with a plain white or purple upper portion and a worried example in the upper part, with a limited tail-like tip, 1-4 cm long. Spadix has a thick base and a long string-like component that is 8-20 cm long. Brown spots can be found on the leaf tail and stem in many situations. The Wallach's cobra lily can be found in the Himalayas at elevations of 2400-3600 metres, from Kashmir to SE Tibet. May-June is the months when the flowers bloom [2].

Figure: complete field grown plant.

In 1932, This plant was discovered in the Nilgiri Mountains of southern India by botanist Edward Barnes. The plant, sometimes known as a cobra lily, was deductively shown in 1933 and is notable for its translucent spathe (a big leaf-like plant component that encases a group of roses). There have been no confirmed sightings of this species in the area since then. Tarun Chhabra, a dental specialist and naturalist, began looking for *Arisaema translucens* in the Nilgiris after being intrigued by the plant's history [3]. In 2009, he came uncovered a few blossoming *Arisaema translucens* inside a small patch of shola forest after what seemed like an eternity of searching. It felt weird to see this plant for the first time. K.M. Prabhukumar gathered numerous specimens of these plants in 2016, noting that none of the other Arisaema species had translucent spathe. According to K.M. Prabhukumar, a botanist and co-creator from Kerala's Center for Medicinal Plants Research, who gathered examples of these plants in 2016, no other Arisaema species has such a clear spathe. *Arisaema tuberculatum*, a rare cobra lily species from the Nilgiris, was also collected. Chhabra then co-authored a review

with academics K.M. Prabhukumar, Indira Balachandran, and others in the journal Phytotaxa that presented the two species. Only southern India is known to be home to the two species. Many *Arisaema* species are admired for their beauty and are threatened by pollution, such as the Japanese *A. heterocephalum.* [3]. *A. translucens*, according to specialists, could suffer a similar threat and require prompt protection. Furthermore, the species' discovery site, a small patch of shola, is bordered by increasing tea domains, which are removing the plant's habitat. "These shola trees are being chopped away by enormous tea bequests, and no one knows about it," Chhabra remarked. "Every day, small patches of sholas disappear, and these plants may perish as well. In this regard, we must exercise caution. People running after these plants is unnecessary, and we want to keep them safe." Chhabra wants to work with tea bequests to help safeguard the rural regions where these endangered animals can be found. Despite the fact that experts have yet to assess A. translucens_preservation status, the species is likely to face extinction, “I think the plant is threatened, but in order to know if it is critically endangered or not, somebody has to study the species in greater detail,” Chhabra said. “We found the species in one patchyet it is conceivable that there are fixes close by that have this plant, and haven't been investigated at this point. All things considered the species may be safer.".*Arisaema translucens* also called as the translucent cobra Lily, cobra Lily, or cobra plant. The cobra lily (Translucent Cobra Lily) is a unique and eye- catching plant,leaves that resemble the heads of cobra snakes[4] .This plant is designated as uncommon due to its rarity in the field. Its Botanical name is *Arisaema translucens* ., It is a member of *Araceae* family and genus Arisaema. . It is a species of carnivorous plant, have a mature size upto 1 feet .It grows in 6-8 ph and blooms in spring.. The colossal work in spite of the fact that have been done on the phytochemistry of *Arisaema* in any case, there is as yet a need to investigate a greater amount of its species on phytochemical and restorative premise so it can be utilized an amazing medication against huge number of diseases[5]. A portion of its animal categories have additionally been utilized as insect poisons, for example against bug bother, Bacterosacurcurbitae.The sort's healing capabilities have revealed that it could play an important role in the existing restorative business, as well as in pharmaceuticals*[6]*.

2.2 Arisaema (Areaceae): traditional applications, phytochemistry, pharmacology, and toxicity

Plant phytochemicals include alkaloids, phenols, terpenes, flavonoids, lectins, saponins, glycosides, triterpenoids, stigmasterols, n-alkanes, n-alkanolssitosterols, campesterol, oxalates, coumarins, tannins, and other phytochemicals. Arisaema plants also have antifungal, antibacterial, insecticidal, antimicrobial, cytotoxic, nematocidal, antiallergicantitumour, and anticancer properties. *Arisaema* plants have long been used to cure a variety of ailments, including settling mucus, soddenness, and asthma, bronchitis, colds, hacks, and laryngitis, among others. It has been discovered that a few animal groups are poisonous on a regular basis. Because of its poisonous properties, such as mouth and lingua torment, even breath easing back and suffocation, mucous film and skin bothering, and so on, the advancement of clinical uses of *Arisaematis rhizomes* has been compelled, and this poisonousness of *Arisaematis rhizomes i*s expected to raphide parts. [7].

The amount of information available on the phytochemistry of the Arisaema class is insufficient, and more research on phytochemical and therapeutic premise is needed. The knowledge available on the phytochemistry and therapeutic capabilities of plants belonging to the Arisaema family illuminates numerous forms of Arisaema that are restoratively significant and have been used to treat a variety of infections around the world [8].

2.3Traditional Uses

This plant, which is native to Sri Lanka and southern India, is used to treat skin conditions and piles. The corm of this plant contains cyanogenic glycosides and high calcium levels due to the presence of calcium oxalate precious stones and free calcium.

(a) *Arisaema tortuosum* is a tortoise-like plant. *Arisaema* has been used traditionally to treat rheumatism and stomachache, snake bite, heaps, intestinal system diseases such as blockage, acid reflux, stomach torment, loose bowels, and contraception [8]. It has also been used to treat nematodal infections, dog bites, and liver grumbling. The tubers are used as a nematodicide and wound healer, while the rhizomes are utilised as an antihelmentic[9].

(b)Blume's*Arisaema leschenaulti* Dhei or cobra is the common name for the Asiatic species *Arisaema leschenaulti*_Blume. This is mainly used in Ayurvedic medicine to treat urinary tract infections, colitis, dermatitis, cleaning, roundworm, fistula, and sinusitis [10].

(c)*Arisaema erubescens* is a species of erubescens. This plant has been utilised to treat a range of organic ailments in Chinese traditional medicine. In Chinese traditional medicine, Arisaema erubescens Wall is said to help with the removal of soggy mucus, the avoidance of convulsions, and the relief of induration and swelling [10].

(d)*Arisaema calcareum* this is a calcareous fungus. The rhizomes or tubers of A. calcareum are used to treat cancer, annoyances, and discomfort.

(e) Serratum Arisaema In traditional Chinese medicine, the rhizomes or tubers of this plant are used to treat a range of diseases and annoyances, as well as to relieve pain [11].

(f) Arisaema asperatum Arisaema asperatum Arisaema as Traditional Chinese medicine uses the rhizomes or tubers of this plant to treat cancer and irritations, as well as to relieve pain. [12].

(g)*Arisaema heterophyllum* the rhizomes or tubers of this plant are used in traditional Chinese medicine to treat cancer and annoyances, as well as to reduce pain. [12].

(h)*Arisaema amurense this plant's rhizomes or tubers are used in traditional Chinese medicine to cure cancer, annoyances, and discomfort.* [12].

(I) *Arisaema* yunnanense is an Arisaema-type plant that rots the tubers of a plant, most commonly Pinelliaternata, which have been used in Chinese medicine for centuries. [13].

(j)*Arisaema tortuosum* is a tortoise-like plant; Whipcord Cobra Lily is another name for it. It is used in Indian medicine to treat a variety of infections related to pressure and inflammation. The tuber juice and powder were used to treat snake bites and to kill parasites in dairy cattle injuries. The plant was also used by Indian ancestors to treat a variety of intestinal ailments such as constipation, acid reflux, stomachaches, and dysentery [14].

2.4 Medicinal uses and phytochemical description

Different synthetic mixtures got from various types of *Arisaema*

(a) *Arisaema erubescens* is a species of erubescens. *Arisaema erubescens* concentrations have been demonstrated to have anticancer effects. At this time, the professionals who attribute anticancer efficacy to this herb are unknown. Paeonol and a glasslike strong that has been identified as aurantiamide acetic acid derivation have been obtained from dried *Aisaemaerubescens* methanol concentration. In vitro, these concentrates demonstrated anticancer effects against Si80, strong hepatoma, and U14 cervical diseases, while in vivo, they showed antitumor effects against Si80, strong hepatoma, and U14 cervical disorders. Antitumor benefits were seen in Si80, strong hepatoma, and U14 cervical disorders using these equal concentrations. The plant extract from *Arisaema* erubescens was found to be useful in treating cervical cancer patients in clinical trials (fix rate: 78 present).This plant has also been used to treat gastric, oesophageal, pituitary, lung, and cerebrum problems. Alkaloids and saponins are found in *A. erubescens*, however the portions that have antitumor effects are unknown at this time .*Arisaema* rhizome is high in alkaloids, saponins, triterpenoids, and lectins, indicating that it can reduce sogginess, settle mucus, expel wind, soothe spasms, and eliminate swelling and irregularities, according to several researches. The ethanolic concentrate of *Arisaema*erubescens tubers yielded two nematocidal flavone-C-glycosides, Schaftoside and isoschaftoside, with extraordinary nematocidal efficacy against the root-tie nematode (Meloidogyne incognita), which is responsible for massive harvest losses..Various substance compounds have been obtained from this

A. erubescens, including aurantiamide acetic acid derivation, calcium oxalate, paeonol, monoterpenoids, unsaturated fats, flavonoids, and alkaloids, and ethanolic concentrates of its tubers have insecticidal action against house flies. The molluscicidal activity of n-butanol obtained from ethanolic extracts of Anerubescens tubers was confirmed against Oncomlaniahupensis.Break in energy digestion, perturbation of stomach microflora environment, vacuole development in glomerular grid, irritation of renal cylindrical epithelial cells in kidney, film harm, and folate deficiency and injury were all revealed by the organisation of *Arisaematitis rhizomes* in mice[15]. *Arisaema erubescens* Schott has anticoagulant, stomachic pain relief, narcotic, antiemetic, mitigating, and antitumor exercises in its rhizomes. *Arisaema rhizomes* are critical in the management of stroke, hemiparalysis, fever, epilepsy, disease dizziness, and lockjaw. Beta-sitosterol, lectins, dipeptides, saponins, triterpenoids, and alkaloids are phytochemicals found in *Arisaema rhizomes* [16].

(b)*Arisaema_tortuosum*a plant that looks like a tortoise. A lectin with in-vitro anticancer action against put out human illness cell lines was decontaminated utilising affinity chromatography on asialofetuin-connected amino actuated silica dabs from the tubers of Arisaema tortuosum, also known as Himalayan Cobra lily. The serum glycoprotein asialofetuin, as well as Nacetyl-D-lactosamine, have a complicated association with *Arisaema_tortuosum* (LacNAc). Its tubers are also aesthetically pleasing, antihepatotoxic, anticancer, antimicrobial, and cell-reinforcing.The tuber juice and powder were used to snake chomps and to the injuries of dairy calves to eradicate parasites. Its tubers have flavorful, antihepatotoxic, anticancer, antibacterial, and cancer-prevention effects. *A. tortousum* contains flavonoids, alkaloids, saponins, triterpenoids, and lectins, with lectin being the most prominent incendiary component, showing anticancer activity against human malignant growth cell lines . Its tubers were used to treat gout, hyperuricemia, and illness in traditional Indian medicine and Ayurveda. The presence of quercetin, rutin, luteolin, and lectin in A. tortuosum was discovered through phytochemical perception and analysis. *Arisaematortuosum* is used to treat ulcers and has antinematodal properties[17]. Its tuber decoction is given to animals to help them recover from broken bones quickly, and it is also used to reduce piles. It was used by the tribals to treat stomachaches, liver problems, and gastrointestinal system problems such as blockage, stomach pain, loose stools, acid reflux, and canine nibbling. Its tubers possessed excellent antihepatotoxic, anticancer,

antibacterial, and cell-reinforcing qualities. It contains starch, glycosides, and steroids, among other phytochemicals. Because of the existence of different dynamic elements being solubilized in promising capacity, the leaf and tuber concentrates of *Arisaema tortousum* divider determined to be key areas of strength for display movement. The evaluation of bacterial resistance to anti-infection drugs aids in the treatment of a variety of infectious disorders. The questionable components discovered in varied *Arisaema tortousum* divider concentrations could be useful in future bacterial disease studies. [18].

(c)Blume, *Arisaema_jacquemontii* the main phytochemicals were extracted from *A. jacquemontii* polar dissolvable concentrates, which show strong cytotoxic, anticancer, and cancer preventive characteristics, as well as significant inhibitory potential. The methanolic and ethanolic tubers of *A. jaquemontii* extract main regions of strength for cancer prevention agent activity and exhibited searching. It also has a powerful anti-cancer effect on HL-60 human leukaemia disease cell lines and DU-145 prostate cancer cell lines[18]. Its foundations have two triterpenoids discovered. A considerable number of auxiliary metabolites are found in *A. jaquemontii* concentrates, paving the way for their use in pharmaceutical enterprises. A monocot lectin known as AJL (A. jacquemontiilectin) was purified from the tubers of a wild Himalayan cobra lily *Arisaema jacquemontii* Blume, which exhibits potent anti-bug and anti-proliferative properties. The presence of a high lectin content of absolute extractable proteins in A. jaquemontii capacity tissues revealed a disulphide linkage shortfall, as evidenced by the component of missing cysteine deposits in the amino corrosive structure of lectins from araceous plants.

(d)Hook, *Arisaema*murrayi Picric corrosive, tannin, protein, sugar, anthraquinone, polyphenols, and other phytochemicals were found in the fluid concentrate of *Arisaema*murrayi Hook, whereas its alcoholic concentration contained phytochemicals such as flavonoids, alkaloids, and glycosides[20].

(e)*Arisaemaringens* is a species of ringworm. *Arisaema_ringens* bulbs were used to improve an important lectin. Heamagglutination restraint reveals monooligosacharides and terminal N acetyllactosamine as two carbohydrate-restricting destinations for this lectin[21].

(f) *Amurense Arisaema* In society, numerous types of *Arisaema* are utilised to treat gastrointestinal ulcers, stiffness, and illness [27]. The presence of the chemical 2,3-dihydroxypropyl, 9Z,12Z octadecadienoate in Arisaema amurense Max Var serratum concentrates demonstrated a strong phospholipase A2 inhibitory action. [28].

(h) Arisaema lecshnaultii is a plant that grows in Sri Lanka and southern India and is used to treat skin conditions and piles. The corm of this plant contains cyanogenic glycosides and high calcium levels due to the presence of calcium oxalate precious stones and free calcium. [28][29].

(i)*Arisaema propinquum* exhibits considerable regions of strength for a helminthic property, as revealed by a large number of exploratory confirmations. This exposure were based on research done on watery or methanolic separates obtained from *Arisaema propinquum* rhizomes [31][32].

CHAPTER 3: RATIONALE AND OBJECTIVE OF STUDY

3.1 Rationale of Study

- *Arisaema* family is a very valuable plant of medicinal significance and therefore, it is widely used in tribal, and ayurvedic medicinal systems.
- As no reports on *Arisaema propinquum*, is mentioned due to lack of scientific evidences for its pharmacological importance.
- Since, we are focusing on identification of significant phytochemicals from this plant that can potentially interact withmany diseases, As this plant known for having different phytochemicalsthere is very less exploration carried out so, the present studies would like to focus on different aspects.

3.2 Objective of Study

1. Identification and Quantification of Phytochemicals in *Arisaema propinquum*..

CHAPTER 4: MATERIALS AND METHODS

QUALITATIVE ANALYSIS

Qualitative test carried out in plant extracts of *Arisaema propinquum*

PLANT COLLECTION

The field grown plant is collected from District Mandi and separated and segregated in plant tissue culture lab at JUIT in which the details are mentioned in figure 1, 2, 3 and 4.

Fig 1: complete field grown plant

Fig 2: Separated shoot portion.

Fig 3: separated flower

Fig. 4: separated Rhizome and roots

4.1Solvent selection

Methanol solvent was used.

4.2 Extraction selection and preparation

Maceration Process:

Steps involved are:

I. Washing, shade drying of *Arisaema propinquum* plant for 1-2 days and powdered.
II. Powdered form of extract dissolved in methanol and kept for shaking for atleast 48 hrs.
III. Liquid extract was filtered using syringe filter and left foe evaporation of methanol for 48 hrs.
IV. After complete evaporation of the methanol the powdered extract was stored at 4C for further analysis.

Analysis Test:

1. **Test for Terpenes**

Salkowski test: 1 ml of conc. Sulphuric acid mixed with 1 ml of liquid extract

If the solution turns to blue green colour/ brisk red, then terpene is present.

2. **Test for Flavonoid**

Few drops of sodium hydroxide were mixed with 2 ml of liquid extract.

The appearance of bright yellow colour.

Few drops of dilute hydrochloric acid were added.

The solution will turn to colourless as indicator of presence of flavonoids.

3. **Test for Phenolic Compound**

5 g of ferric chloride were mixed with 80 ml of distilled water.

2 ml of ferric chloride were mixed with 2 ml of liquid extract.

The solution will turn to blur green colour/ brisk red.

4. **Test for Sterols**

2 ml of conc. Sulphuric acid were added to 2 ml of extract.

Red precipitate indicated the formation of sterols.

5. Test for Tannins

2 ml of ferric chloride were mixed with 2 ml of liquid extract.

Brownish green colour development indicates presence of tannins.

6. Test for Alkaloids

2 ml of 2% HCl were 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.

7. Test for Carbohydrates

Few drops of alcoholic naphthol were added to 2 ml of liquid extract.

Concentrated sulphuric acid were added to the mixture.

At the junctions of the two liquids a formation of violet ring confirmed presence of various carbohydrates.

8. Test for Proteins

Few drops of Biuret's reagent were added to 2 ml of liquid extract.

The presence of proteins was hence confirmed with a violet colour.

QUANTITATIVE ANALYSIS

Quantitative test carried out in plant extracts of *Arisaema propinquum*

Quantification of Total Flavonoid Content

- Five control concentrations were made and Quercetin was used as a standard with concentrations 1mg/ml of methanol.
- 500 µl of extract was taken for test.
- 2 ml of the distilled water was then diluted with the extract.
- 150 µl of 5% sodium nitrite were allowed to rest for 5 minutes.
- For 6 minutes 150 µl of aluminium chloride were allowed to rest.
- 1 ml of 1M NaOH were added and kept for 15 minutes.
- Absorbance was taken at 510 nm.

Table 1: The standard protocol for Quantification of Total Flavonoid Content

Quercetin	Conc (µl)	Methanol (µl)	Distilled water (ml)	Sodium Nitrite (µl)	Stand for 5 mins	Aluminium Chloride (µl)	Stand for 6 mins	NaOH (µl)	Stand for 15 mins	Absorbance (nm)
Control 1	100	900	2	150		150		1000		0.09
Control 2	200	800	2	150		150		1000		0.116
Control 3	300	700	2	150		150		1000		0.125
Control 4	400	600	2	150		150		1000		0.136
Control 5	500	500	2	150		150		1000		0.142
Blank	0	1000	2	150		150		1000		0.055
Sample	500	500	2	150		150		1000		0.331

Quantification of Total Phenolic Content

- Five concentrations of control were made and gallic acid was used as a standard with concentration 1mg/ml.
- Then distilled water was added.
- 150 µl of FC reagent was added and allowed to stand for 6 minutes.
- 500 µl of 7% sodium carbonate was added.
- Absorbance was taken at 650 nm.

Table 2: The standard protocol for quantification of Total Phenolic Content

Test	Gallic Acid (µl)	Distilled water(µl)	FC Reagent (µl)	Sodium Carbonate (µl)	Absorbance (nm)
Control 1	100	900	150	500	0.945
Control 2	200	800	150	500	1.411
Control 3	300	700	150	500	2.544
Control 4	400	600	150	500	3.29
Control 5	500	500	150	500	4.344
Blank	0	1000	150	500	0.063
Sample	100	400	150	500	2.359

Anthrone Method for Carbohydrate Quantification

- Five control concentrations were made and glucose was taken as standard in concentration 1mg/ml.
- Anthrone reagent with concentration 1mg/ml and volume 4ml was added to each test tube of controls, blank and test.
- OD was taken at 630 nm after incubation in water bath for 8 minutes.

Table 3: The standard protocol for Anthrone method to carry out the Quantification of Carbohydrate

Glucose conc. (mg/ml)	Volume (µl)	Distilled water (µl)	Anthrone reagent (1mg/ml)	Incubation in water bath for 8 mins	Absorbance (nm)
0.2	20	980	4		0.122
0.4	40	960	4		0.158
0.6	60	940	4		0.196
0.8	80	920	4		0.224
1	100	900	4		0.288
Blank	0	1000	4		0.085
Sample	0	0	4		0.403

Lowry's Method for Protein Quantification

- Five control concentrations were made for BSA as a standard with concentrations 1mg/ml.
- Distilled water was added and reagent A was used which included 2% Sodium Carbonate in dissolved 1N NaOH, 1% sodium potassium tartrate dissolved in water and 0.5% Copper Sulphate dissolved in distilled water all mixed together.
- FC reagent was used as a reagent B in 1:1 with distilled water.

Table 4: The standard protocol for Lowry's method to carry out the Quantification of Proteins

BSA (µl)	Distilled water (µl)	Reagent A (ml)	Incubation for 15 mins	FC reagent (ml)	Incubation for 40 mins in dark	Absorbance (nm)
200	800	4.5		0.5		0.022
400	600	4.5		0.5		0.042
600	400	4.5		0.5		0.055
800	200	4.5		0.5		0.088
1000	0	4.5		0.5		0.111
Blank	1000	4.5		0.5		0.019
Sample	0	4.5		0.5		0.126

CHAPTER 5: RESULTS AND DISCUSSION

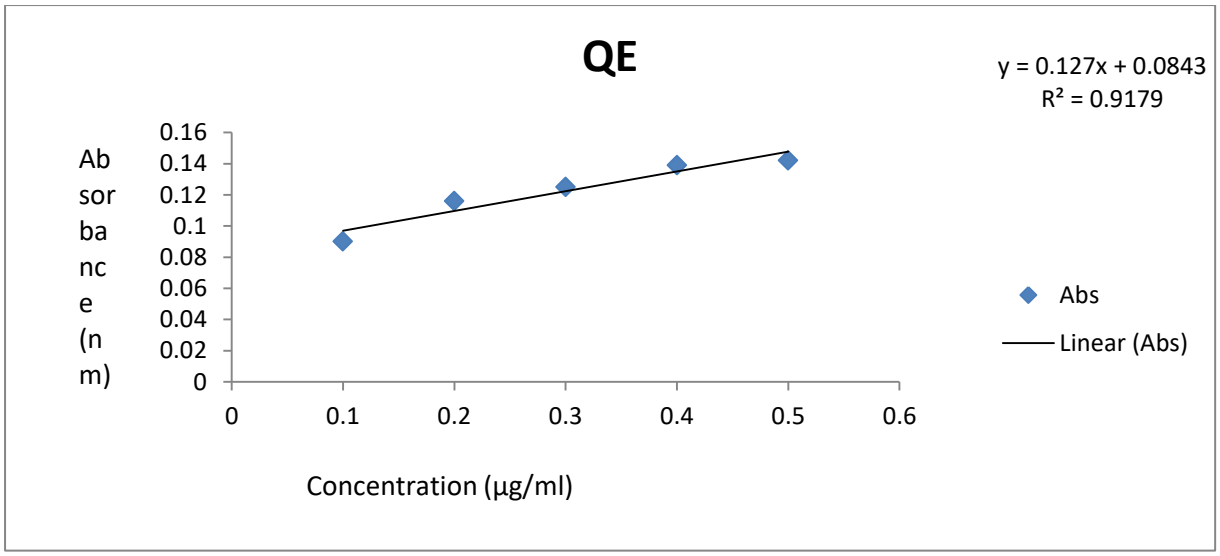
Qualitative test carried out in plant extracts of *Arisaema propinquum*, in which Phytochemicals including flavonoid and phenolic compound and also the neutraceutical traces including carbohydrates and proteins were found. After that, methanolic extract of the *Arisaema propinquum* was evaluated for the quantitative research of the mentioned compounds which resulted in values. The plant extract contained carbohydrates 1.6%32 and proteins 12.96%s which were quantified by standardized protocols. In the present study the plant extract contained flavonoid 3.27 µg /mg and phenolic compounds 319.98 mg/g. However, till today, this plant is unexplored for the scientific evidences that authenticate the biosynthesis and accumulation of different secondary metabolites and also further exploration for bioactive compounds having pharmacological properties.

Table 5: Results of Qualitative test carried out in plant extracts of Arisaema propinquum

Name of Test	Colour	Present/Absent
Terpenes	Colourless	Absent
Flavonoid	Colourless	Present
Phenol	Yellow	Present
Sterols	Colourless	Absent
Tannins	Yellow	Absent
Alkaloids	Reddish brown precipitate	Absent
Carbohydrates	Violet ring at the junction of two liquids	Present
Protein	Violet colour	Present

Quantitative Analysis of Flavonoid

Fig 1: Standard curve for Quantification of Total Flavonoid Content of Quercetin



Calculation

Y= absorbance of extract

X= Concentration of extract

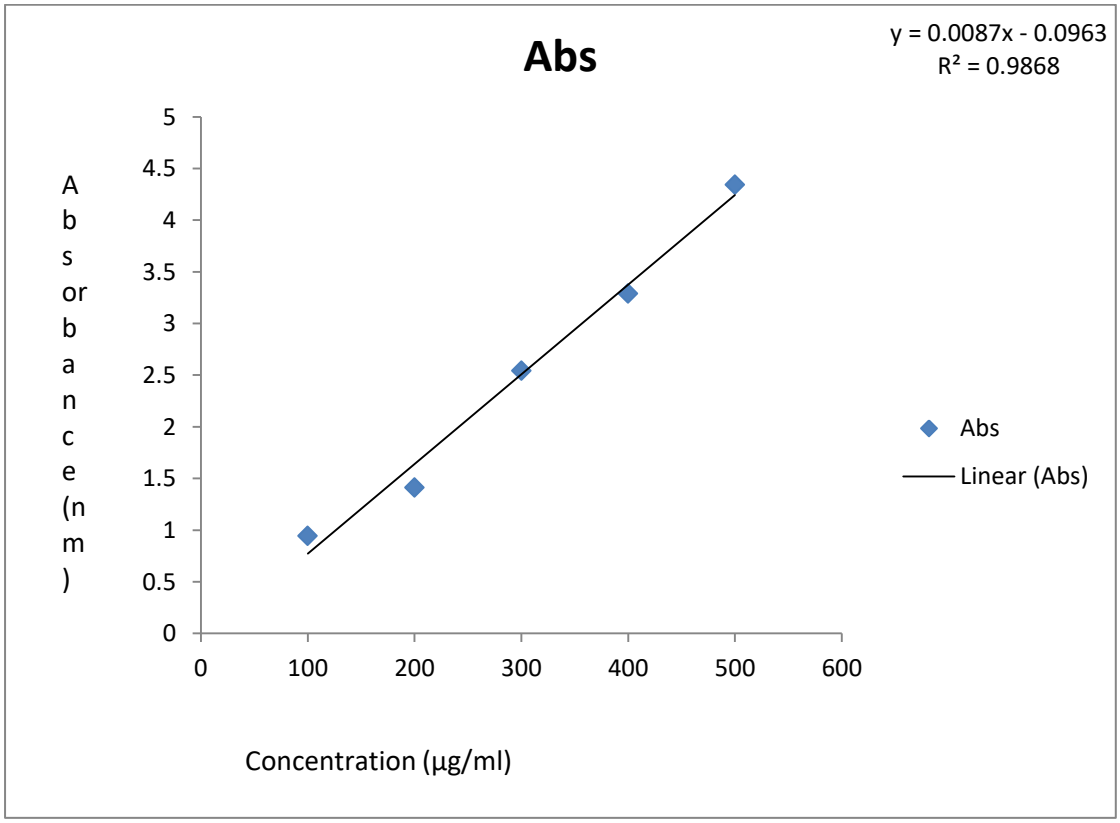
Y= mx + c

0.331= 0.127 x + 0.0843

X= 3.27 µg QE/mg plant extract

Quantitative Analysis of Phenolic Compound

Fig 2: Standard curve of Gallic acid for Quantification of Phenolic compound



Calculation

Y= absorbance of extract

X= Concentration of extract

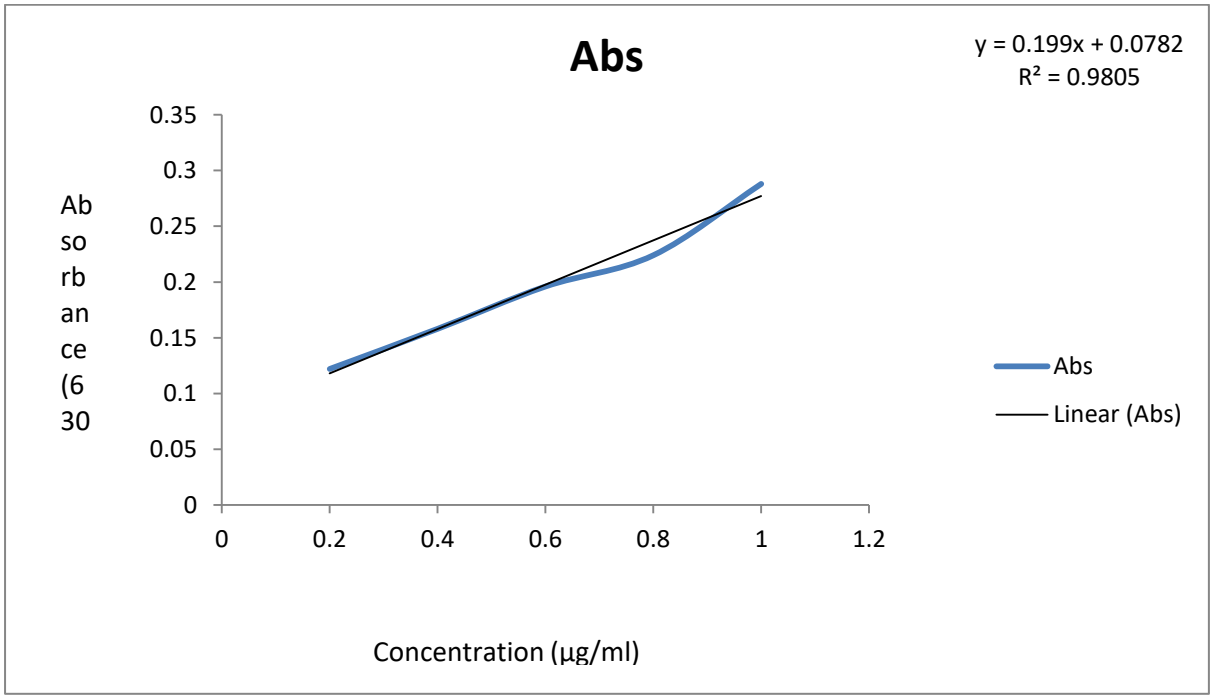
Y= mx + c

2.359 = 0.0077x + 0.1049

X = 319.98 mg/g

Quantification of Carbohydrates

Fig 3: Standard curve of Glucose for Quantification of Carbohydrates



Calculation

Y= absorbance of extract

X= Concentration of extract

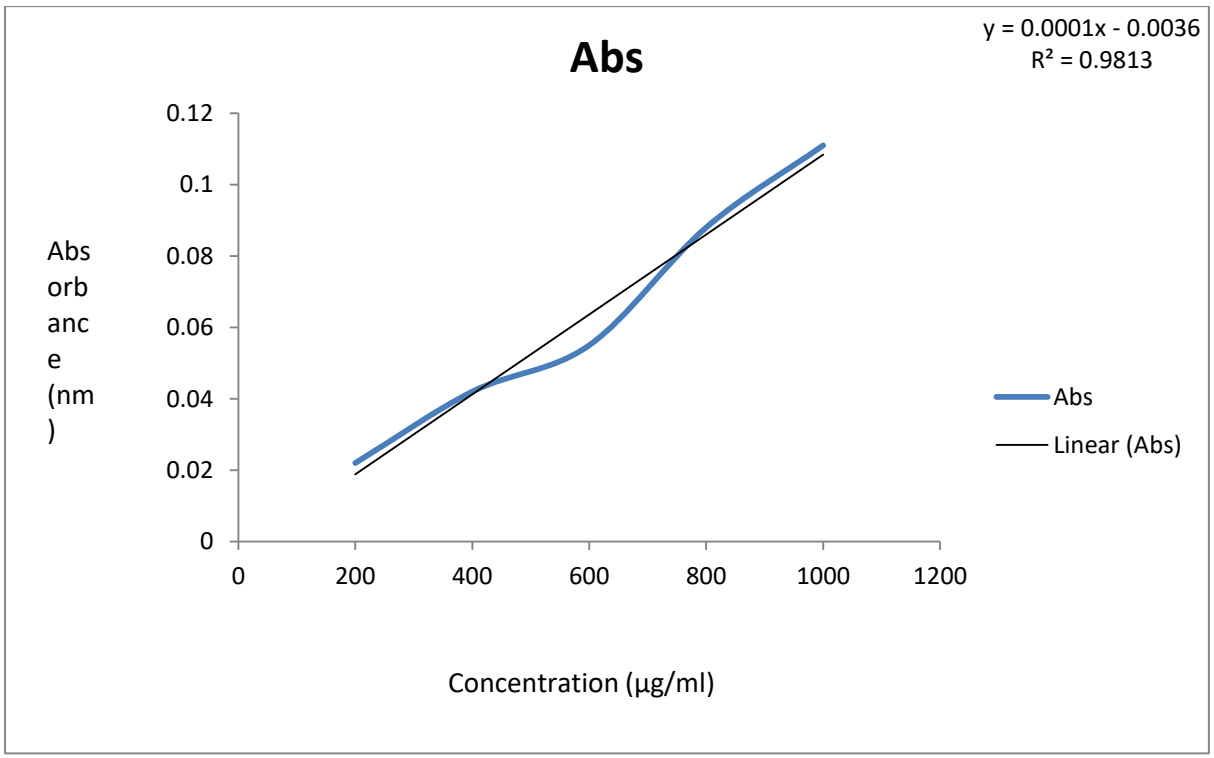
Y= mx + c **µ**

0.403=0.199x+0.0782

X=1.632%

Quantification of Protein

Fig 4: Standard curve of BSA for Quantification of Proteins



Calculation

Y= absorbance of extract

X= Concentration of extract

Y= mx + c

0.126=0.0001x-0.0036

X=1296 or 12.96%

Discussion

Arisaema, has been taken into consideration due to its high pharmacological and commercial significance. This plant is known to be enriched with phytochemicals, that constitute many secondary metabolites such terpenoids, flavanoids, tannins, steroids, reducing sugars and cardiac glycosides, which can help in cure of many diseases such as cancer, obesity, ulcers, cardiovascular diseases and many more. This study concentrates phytochemical Profiling and Quantification in rare *Arisaema_propinquum*. Methods used in extraction is: Maceration process was used to find and study the bioactive compounds, in which Phytochemicals including flavonoid and phenolic compound and also the neutraceutical traces including carbohydrates and proteins were found. After that, methanolic extract of the *Arisaema propinquum* was evaluated for the quantitative research of the mentioned compounds which resulted in values. As reported by **P.Mir et.al.** in *Arisaema_propinquum*,various phytochemicals are present such as alkaloids, carbohydrates, proteins, amino acids, phenols, tannins, flavonoids and terpenoids wherein my plant phytochemical screening of the extract reveals the presence of flavonoids, phenolic compound, protein and carbohydrates. The quantification values are 3.27 µg QE/mg plant extract, 319.98 mg/g, 12.96% and 1.63% respectively.

CHAPTER 6: Conclusion

In this research, qualitative test were performed for *Arisaema propinquum*, in which phytochemicals including flavonoid and phenolic compound and also the neutraceutical traces including carbohydrates and proteins were found. After that, methanolic extract of the *Arisaema propinquum* was evaluated for the quantitative research of the mentioned compounds which resulted in values. The plant extract contained carbohydrates 1.63% and proteins 12.96%s which were quantified by standardized protocols. In the present study the plant extract contained flavonoid 3.27 µg /mg and phenolic compounds 319.98 mg/g. However, till today, this plant is unexplored for the scientific evidences that authenticate the biosynthesis and accumulation of different secondary metabolites and also further exploration for bioactive compounds having pharmacological properties.

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