Phytochemical Screening and Pharmacological Evaluation of *Colocasia esculenta* stolon

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Abstract
Medicinal plants have played a noteworthy role in various ancient traditional systems ofmedication. Recently, plants provided an inexpensive source of drugs for most of the world's population. The medicinal significance of a plant is due to the existence of chemical constituents like alkaloids, glycosides, resins, volatile oils, gums, tannins etc. *Colocasia esculenta* is a vegetative propagated tropical root having its origin in Southeast Asia. It occupies 9th position among world food crops with its cultivation spread across Africa. Taro tubers are important sources of carbohydrates as an energy source and are used as staple foods in tropical and subtropical countries. It is largely produced for its underground corms and contains 70–80% starch. At first the extraction of the plant stolons is done by drying it and crushed it in fine powder with solvents from low polarity towards high polarity likewise n-hexane, ethyl acetate, methanol and water by using Rotary shaker. Then the phytochemical screening, antioxidant activity, total phenolic content and total flavonoid content and invitro anti-inflammatory activity was performed. This evaluation helps in the investigation show compounds that may help combat many harmful diseases. *C. esculenta* stolon's methanolic extract showed strong antioxidant activity by blocking DPPH. Additionally, it was discovered that the extract includes substantial amounts of total phenols and flavonoids. The findings of these investigations suggest that *C. esculenta* stolon methanolic extract has effective antioxidant and anti-inflammatory properties.

Keywords: *C. esculenta,* alkaloids, glycosides, resins, volatile oils, gums, tannins, anti-inflammatory activity.

1. Introduction

Medicinal plants have played a noteworthy role in various ancient traditional systems ofmedication. Recently, plants provided an inexpensive source of drugs for most of the the world's population. The medicinal significance of a plant is due to the existence of chemical constituents like alkaloids, glycosides, resins, volatile oils, gums, tannins etc [1]. Plant-based substances have recently become of great interest owing to their resourceful applications. These compounds are synthesized by the primary or rather secondary metabolism of living organisms. Phytochemicals may have biological significance, for example, carotenoids or flavonoids, but are not established as essential nutrients. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories [2].
Traditional knowledge of medicine has long been used since ages for curing various human ailments. About 60-80% of the world's population still relies on plant-based medicines. The traditional Indian systems of medicine have a long history of use, yet they lack adequate scientific knowledge. Through phytochemical screening, one could detect the various important compounds which could be used as the base of modern drugs that cure various diseases [3,4]. Plant derived substances have recently become of greatinterest owing to their versatile applications. The medicinal importance of plants is due to the presence of chemical constituents like alkaloids, glycosides, resins, volatile oils, gums, tannins etc. These compounds are synthesized by the primary or rather secondary metabolism of living organisms. Phytochemicals may have biological significance, for example, carotenoids or flavonoids, but are not established as essential nutrients [5]. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories [6].
Colocasia esculenta is a vegetative propagated tropical root having its origin in Southeast Asia. It occupies 9th position among world food crops with its cultivation spread across Africa. Taro tubers are important sources of carbohydrates as an energy source and are used as staple foods in tropical and subtropical countries. It is largely produced for its underground corms and contains 70–80% starch. There are numerous root and tuber crops growing in the world. Taro is one of such crops grown for variouspurposes [7]. It is an erect herbaceous perennial root crop widely cultivated in the tropical and subtropical world belonging to the genus Colocasia in the plant family called Araceae. The crop has been largely produced in Africa even though the time of its spread to the region is unknown nowadays cultivated in Cameroon, Nigeria, Ghana and Burkina Faso where it has gained high importance. It has been suggested that the crop was cultivated to fill seasonal food gaps when other crops were

still in the fields because of its potential in giving reasonable yield under conditions where other crops may be unable to give produce by various crop production constraints [8].
In terms of nutrition, Taro corm is an excellent source of carbohydrate, the majority being starch of which 17-28% is amylose, and the remainder is amylopectin. The size oftaro starch grains is one-tenth that of potato and their digestibility has been estimated to be 98.8%. Because of its ease of assimilation, it is suitable for people with digestive problems [9]. Taro is especially useful to people allergic to cereals and can be consumed bychildren who are sensitive to milk, and as such taro flour is used in infant food formulaand canned baby foods. Taro corm is low in fat and protein; however, the protein content of taro corm is slightly higher than that of yam, cassava or sweet potato. The protein is rich in some essential amino acids, but is low in isoleucine, tryptophan and methionine. Taro leaves contain higher levels of protein and are also excellent sources of carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitaminA, vitamin C and dietary fibre. They also contain greater amounts of vitamin B-complexthan whole milk and are higher in protein and other nutrients, except oil, than tannia (new cocoyam; Xanthosoma sagittifolium). The fresh taro leaf lamina and petiole contain 80 % and 94 % moisture, respectively [10].
C. esculenta is used as a good expectorant, stimulant, appetizer, astringent and juice of taro corm is used to treat alopecia patients. Its leaf juice when applied over scorpion sting poisoning, shows promising results even used to treat food poisoning [11]. Leaf extract also helps in maintaining one's sugar levels. Taro corms provide many nutritious amounts of minerals such as copper, iron, zinc and many more. Taro leaves when cooked have a great effect on the digestive system. It is used as a sorbent material in the treatment of swelled glands and organs. Chemical constituents present in *C. esculenta* like flavonoids help in protection from lung and oral cavity cancers. It has good healing properties and can be applied to the skin to treat skin disorders. Decoction of *C.esculenta* peel happened to be used as a tribal medicine to cure diarrhea [12].
Based on literature obtained from ethnomedicinal documentation *C. esculenta* has been selected to determine phytochemical and pharmacological properties other than nutritional properties. The principal objective of this work was to explore phytochemical characterization and exploration antioxidants and anti-inflammatory activity of *C. esculenta (figure 1)*.

Figure 1: *Colocasia esculenta* tree and stolon

2. Materials & Methods

2.1. Materials

Gallic acid and quercetin are purchased from Sigma-Aldrich (Seelze, Germany). Methanol, ethyl acetate, water and n-hexane were purchased from Merck (India). Sulphuric acid, Hydrochloric acid, glacial acetic acid, Fehling's solution A & B, draggendroff's reagent, ammonia, aluminium chloride, magnesium ribbon, iodine, potassium iodide, potassium acetate, L-ascorbic acid, trichloroacetic acid, ferric chloride, potassium ferricyanide, Folin-ciocalteu reagent, sodium carbonate, sodium chloride, disodium hydrogen phosphate, sodium di- hydrogen phosphate and methanol were purchased from Sisco Research Laboratories (SRL) Pvt. Ltd., Thomas Baker Pvt. Ltd., and Merck (India). All other chemicals were used in analytical grade. α-amylase, DNSA (3,5- Dinitro salicylic acid), starch, and 1,1-diphenyl-2-picryl-hydrazil (DPPH) were purchased from Hi Media, India.

2.2. Plant materials

Stolon's of *Colocasia esculenta* were collected in November 2022 from the nearby local market of shiv mandir, Siliguri. The plant was authenticated by Botanical Survey of India, Shibpur, Howrah (West Bengal). The plant was authenticated (Specimen no. NBU/KP-001) by the Botanical Survey of India, Shibpur, Howrah (West Bengal). The voucher specimen has been preserved in our laboratory for future reference.

2.3. Extraction

Stolon' s of *C. esculenta* were dried under shade to protect from direct sunlight and crushed in a mechanical grinder to make fine powder. The powder was then successively extracted with solvents from low polarity towards high polarity likewise n-hexane, ethyl acetate, methanol and water by using Rotary shaker (Figure 2). Then theresulting extracts were filtered and dried using water bath. Finally, we get n- hexane (HECE), ethyl acetate (EAECE), methanol (MECE) and aqueous (AECE) extract of *C. esculenta* [13].

Figure 2. Image of Rotary Shaker

2.4. Phytochemical screening:

Phytochemical screening is a means of determining qualitatively or quantitatively the amount of substance of therapeutic and nutritional benefit in plants. Plants that are rich in certain phytoconstituents have always been of pharmacological importance. In this study, the condensed extracts likewise HECE, EAECE, MECE & AECE were used for preliminary screening of phytochemical such as alkaloid, glycosides, carbohydrates, flavonoids, terpenes, saponins, phenols, tannins, quinones, and steroids. The standard protocol has been used for this test as per Nayak et al. [11,14].

2.4.1. Test for Alkaloids

Mayer's Test: The test sample was mixed with a few drops of diluted hydrochloric acid, and then filtered. Mayer's reagent was used to treat the filtrate. The appearance of a yellowish precipitate indicates the presence of alkaloids [15].

Dragendroff's Test: First, a few drops of diluted hydrochloric acid were added to the test sample before it was filtered. Filtrate was mixed with Dragendroff's reagent. Precipitate with an orange- brown colour suggests the presence of alkaloids [16].

Wagner's Test: A test sample was treated with a few drops of dilute hydrochloric acid and filtered. Wagner's reagent was added to the filtrate. A reddish-brown colour precipitate was observed which confirms the presence of alkaloids [17].

Hager's Test: Firstly, a small quantity of test sample with a few drops of dilute hydrochloric acid and filtered. The filtrate was treated with Hager's reagent. The yellow precipitate wasformed which indicates the presence of alkaloids [18].

2.4.2. Test for flavonoids

Shinoda's Test:

A small quantity of test sample was dissolved in methanol. One piece of magnesium ribbon followed by concentrated hydrochloric acid was added dropwise to the test sample and heated. The appearance of magenta colour demonstrated the presence of flavonoids [11].

2.4.3. Test for Tannins

The test sample was dissolved in a small quantity of distilled water and then filtered. The filtrate was taken in a test tube and a 10% aqueous potassium dichromate solution was added to it. The formation of a yellow colour precipitate indicates the presence of tannins.

A small amount of the test sample was dissolved in a minimum quantity of distilled water and filtered. The filtrate was treated with a 10% lead acetate solution. Thedevelopment of yellowish-brown precipitate demonstrated the presence of tannins.

At first, the test sample was dissolved in a minimum quantity of distilled water and filtered. Then the filtrate was treated with 1ml of 5% ferric chloride solution. The occurrence of greenish black colour in the solution indicates the presence of tannins [12].

Test for reducing sugar

Benedict's Test: In a test tube small amount of test sample was dissolved in a little amount of distilled water and filtered. The filtrate was taken and an equal amount of Benedict's reagent was added. The mixture was heated for a few minutes. The formation of a brick-red precipitate indicates the presence of reducing sugars [13].
Fehling's Test: A small quantity of test sample was treated in a minimum amount of distilled water and filtered. To the filtrate, an equal volume of Fehling's A & B solution was added and heated for a few minutes. The development of the brick red colour demonstrated the presence of reducing sugars [14].

2.4.4. Test for triterpenoids and steroids

Liberman-Burchard Test: First, a test sample of 10 mg was placed in a test tube with 1 ml of chloroform and 1 mlof acetic anhydride. 2 ml of strong sulfuric acid were then added to the mixture. The existence of triterpenoids and steroids was established by the formation of a reddish- violet ring at the intersection of two layers [15].
Noller Test: A test tube containing 2 ml of pure thionyl chloride and 0.01 percent anhydrous stannic chloride was used. Five milligrams of the test sample were added to the solution, turning it purple. Triterpenoids are present when the purple colour changes further to deep crimson [16].
Salkowski Test: One millilitre of concentrated sulfuric acid and ten milligrams of the test sample were dissolved in one millilitre of chloroform. The presence of steroids is shown by the chloroform layer's reddish-blue and the acid layer's green fluorescence [17].

2.4.5. Test for Saponins

Take 2ml of extract and add 8 ml of distilled water to it. The solution in the test tube shaken vigorously for approx. 5 minutes and allow the solution in the test tube to stand for about 30 minutes. The presence of froth indicates the presence of saponins [11].

2.4.6. Test for cardiac glycosides

Keller- killani Test: Take 2 ml of filtrate in the test tube in which 3 ml of glacial acetic acid was added to it. Add 2 drops of 5 % ferric chloride to it which is followed by the addition of concentrated sulphuric acid through the sidewall of the test tube. A blue coloration was observed in the acetic acid layer which shows the presence of cardiac glycoside [18].

2.5. Determination of Total phenolic content:

The Folin-Ciocalteu assay was performed as per the method indicated by Sheikh et al. [14] to determine the total phenolic concentrations of HECE, EAECE, MECE and AECE with a minor change. At first stock solution of standard and extract is prepared in a concentration of (1 mg/1ml) in distilled water from which 0.4 ml sample/standard was withdrawn into the test tube and then, 0.4 ml of Folin-ciocalteu reagent and 4 ml of distilled water was added in samples/standard, which was incubated for 5 minutes in the dark place. After 5 minutes, 4 ml of 7 % Sodium carbonate solution was added in each tube and volume makeup was done up to 10 ml of distilled water, which was further incubated in a dark place for 90 minutes at room temperature. At 730 nm, measurement of absorbance was taken, where distilled water was retained as a blank and gallic acid as standard. A calibration curve was made with gallic acid concentrations in the range of (50, 100, 150, 200 and 250µg/ml). Total phenolic content was expressed in mg Gallic acid equivalents (GAE) per gram of dry weight [19].

2.6. Determination of Total flavonoid content:

Aluminium chloride colorimetric method was used according to Parajuli et al. [12] for Total flavonoids determination with some modifications. In brief, 1 ml of extracts or standard solution of Quercetin (500 µg/ml) was added to 10 ml ofvolumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5 % Sodium nitrite was added. After 5 minutes, 0.3 ml of 10 % aluminium chloride was added and allowed to stand for 6 minutes. Then after, 2 ml of 1 M Sodium hydroxide was added and the final volume was made up to 10 ml with distilled water. The mixture was shaken and the absorbance was measured at 510 nm using UV spectrophotometer. Total flavonoid values of HECE, EAECE, MECE and expressed as mg quercetin equivalent per gram dry extract weight[20,21].

2.7. Determination of antioxidant activity

2.7.1. Determination of DPPH radical scavenging activity

The free radical scavenging activity of HECE, EAECE, MECE and AECE was measured by DPPH (1, 1-diphenyl-2-picryl-hydrazil) employing the method described by Anastasia Wheni Indrianingsih et al. [8]. 0.3 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of various concentrations (50, 100, 150, 200, 250 and 300 µg/ml) of extracts. After 30 minutes, absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. All the tests were performed in triplicate. Ascorbic acid (AA) was used as a reference compound.
The ability of the samples to scavenge the DPPH radical was calculated by using the following equation:
DPPH scavenging activity (%) = $(Abs(c) - Abs(s) \times 100) / Abs(c)$
Where Abs(c) is the absorbance of the control and Abs(s) is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extracts was expressed as IC_{50}. The IC_{50} values were defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50 % [22].

2.7.2. Ferric Reducing Antioxidant Power (FRAP)

The reducing power assay of HECE, EAECE, MECE and AECE was calculated as per the method described by Kumar et al. [9] with few changes init. Stock solution of 1 mg/ml, extract and standard (Ascorbic acid) was prepared in methanol. The stock was diluted using methanol at concentrations of 100-300 µg/ml, and 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide at 1 % were then added. After

giving the mentioned solution of good shake,then incubated at 50 °C for 15 min in a water bath. After incubation, the solution was allowed to cool before being mixed well with 2.5 ml of 10% w/v trichloroacetic acid, which was then added to the appropriate solution. 2.5 ml of the solution was taken outof the mixture and diluted with 2.5 ml of distilled water, respectively. Finally, 0.5 ml of ferric chloride (0.1%) was added and combined. The absorbance was measured at 700 nm. The blank solution contains everything except standard/extract [23].

2.8. Determination of in vitro anti-diabetic activity:

α-amylase inhibitory assay-

The amylase activity of HECE, EAECE, MECE and AECE was performed by Chukwuma et al. [7] with minor changes in it. Solution of acarbose (1 mg/ml) was prepared in sodium phosphate buffer (0.02 M and pH 6.9,which was maintained by 0.006 M NaCl solution). The dilution of stock was done in theconcentration of 100-1000 µg/ml, from which 500 µl was withdrawn from each dilution tube mixed with 500 µl of α-amylase solution (13U/ml) and thus, the mixture of tubes was kept for incubation at 37ºC for 30 minutes. After this, 500 µl of 1% starch solution was added to each tube and incubated at 37ºC for 10 minutes. 1 ml DNSA solution was added to each tube and thus, the tubes were set down for boiling at water bath for 10min to stop the reaction. After the tubes get cooled, 10ml distilled water was added tothe tube, and absorbance were measured at 540 nm using phosphate buffer as blank and 100% enzyme activity solution (except sample/ standard, everything was added) as control. The percentage of inhibition for α-amylase activity was calculated by the belowequation:

% inhibition = Abs (c) − Abs (s) × 100)/ Abs (c)

Where Abs(c) is the absorbance of the control and Abs(s) is the absorbance of standard/sample. IC_{50} represents the concentration (µg/ml) of the extracts and acarbose required to inhibit 50% of α-amylase [24].

2.9. Determination of in vitro anti-inflammatory activity:

This study aims at evaluating the in vitro anti-inflammatory potential of HECE, EAECE, MECE and AECE. In vitro anti-inflammatory potentials were evaluated usinga standard experimental protocol such as Inhibition of albumin denaturation assay at different concentrations and diclofenac used as the standard drug [10,13].

To prepare the reaction mixture, at first stock solution was prepared in the buffer. Thensix dilutions were done at different concentrations. Then withdrawn 100 µl from each dilution. After the withdrawn 0.45 ml (0.5%) of Bovine Serum Albumin was added. The solutions were mixed well. The samples were incubated at 37 ºC for 30 min and then heated to 57 º C for 3min, after cooling the samples 2.5 ml of phosphate buffer solution was added. The absorbance was measured at 255 nm using UV-Vis spectrophotometer. Diclofenac sodium was used as reference drug and treated similarlyfor determination of absorbance and viscosity [25].

% inhibition = Abs (c) − Abs (s) × 100)/ Abs (c)

Where Abs(c) is the absorbance of the control and Abs(s) is the absorbance of standard/sample. The percentage inhibition of protein denaturation was calculated using the following equation, and the results were reported as IC_{50} values [26,27].

3. Results And Discussion

3.1. Phytochemical Screening

As secondary metabolites, plants generate phytochemicals on their own. Phytochemicals are essential for the regulation of plant cell functions in addition to providing plants their particular colour, flavor, and fragrance. In addition, an expanding corpus of studies has shown that Phytochemicals have a few positive medical effects and few negative ones. Therefore, the assessment and characterization of phytochemicals is a vital step in the pharmacological discovery of drugs derived from plants. According to both contemporary scientific study and conventional medical practices, medicinal plants are important species that can be utilized to treat ailments and enhance human health.

According to the findings of the phytochemical investigation (Table 5.1.), numerous chemical groups exist, including carbohydrates, flavonoids, terpenoids, tannins, etc. extract from *C. esculenta* stolon.

Table 5.1. Preliminary phytochemical tests of *C. esculenta* stolon extract

Phytochemicals	Name of the test	Result			
		n- hexane extract	Ethyl acetate extract	Methanolic extract	Aqueousextract
Reducing sugar	Fehling's test	-	-	-	-
	Benedict's test	-	-	+	-
Flavonoids	Shinoda test	-	-	-	-
	Lead acetate test	-	-	-	-
Terpenoids	Salkowski test	-	-	+	-

Glycosides	Keller-killani test	-	-	+	-
	Legal test	-	-	-	-
Saponins	Foam test	-	-	+	-
Resins	Turbidity test	-	+	+	-
Tannins	Ferric chloride test	-	-	-	-
Alkaloids	Wagner's test	-	+	-	-
	Hager's test	+	+	-	-

(+) present, (-) absent

3.2. Determination of Total Phenolic Content

By using the Folin-Ciocalteu method, which creates the reagent from the combinationof two substances, phosphor tungstic acid and phosphomolybdic acid, which oxidize to blue colour, the total phenolic content of several extracts of the Colocasia esculenta stolon was determined. The reduction of acid into tungsten and molybdenum causes the blue colour when phenyl group is present. Here, the standard was gallic acid. Hence, a calibration curve of the standard was established which is shown in Figure **3**. The phenolic compound content was determined as gallic acid equivalents using the following linear equation based on the calibration curve: $A = 0.0109x + 0.1843$, $R^2 = 0.9924$. A is the absorbance, C is gallic acid equivalent (µg). The total phenolic content of the extract was expressed as mg of gallic acid equivalent per gram of dry weight of the extract. The level of total polyphenolic compounds of HECE, EAECE, MECE and AECE were 40 mg, 296 mg, 368 mg and 336 mg gallic acid equivalent per gram of the extract.

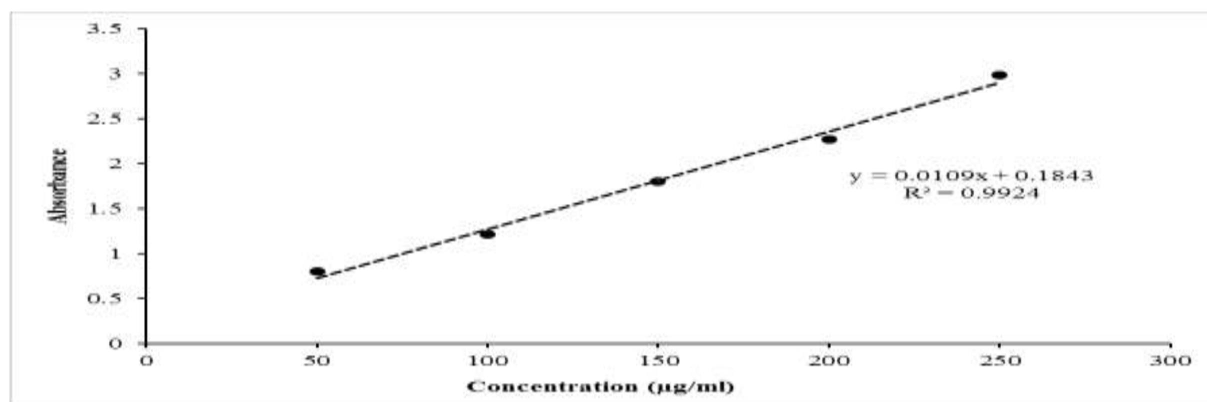

Figure 3. Standard curve of Gallic acid

3.3. Determination of Total Flavonoid Content

The total flavonoid content of different extracts of *Colocasia esculenta* was assessed by utilizing the aluminum chloride method, where this reagent forms a complex with flavones and flavanol which can be easily detected at 415nm in a UV-vis spectrophotometer. Here, quercetin was used as a standard whose calibration curve was established**. Figure 4** Linear equation based on the calibration curve is A= 0.006C - 0.2472, R2 = 0.9869. A is the absorbance, and C is the quercetin equivalents (µg/ml). The total flavonoid content of HECE, EAECE, MECE and AECE were found to be 224.64, 290.64, 191.32 and 190 mg/g of equivalent per gram of dry weight.

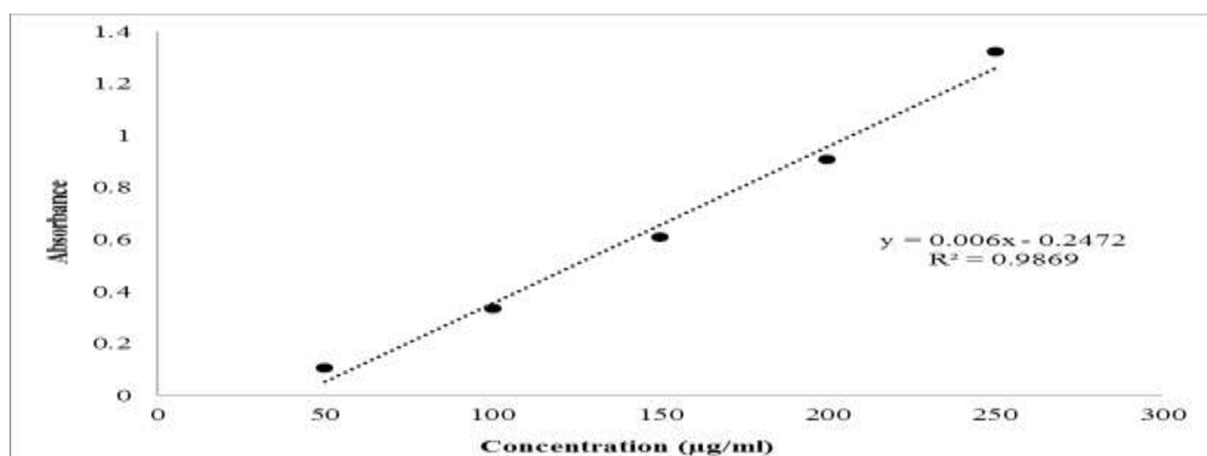

Figure 4. Standard curve of Quercetin

3.4. In-vitro antioxidant assay

Compound's antioxidant activity is linked to a variety of processes and reactions. To undertake a systematic prediction of the antioxidant activity of natural substances,more than one method is recommended. Antioxidant abilities were assessed utilizing DPPH free radical scavenging activity and ferric reducing antioxidant power (FRAP), in this study HECE, EAECE, MECE, AECE have significant antioxidant properties.

3.4.1. DPPH radical scavenging activity

This assay helps in the detection of the reduction of DPPH, where DPPH is reduced to DPPH-H due to the presence of an antioxidant compound present in the extract, and because of this reduction colour changes from purple to yellowish, which can be easily detected in absorbance at 517 nm. It is a very commonly used method for the evaluationof antioxidant activity spectrophotometrically. **Figure 5** represents the graph between the percentage of inhibition and concentrations, which shows a constant increment in scavenging property of all extracts and standard i.e. ascorbic acid. The IC_{50} values were found to be 7.29µg/ml, 10.27 µg/ml, 97.50 µg/ml, 24.26 µg/ml and 194.89 µg/ml in ascorbic acid, methanolic, n- hexane, ethyl acetate, aqueous extract respectively.

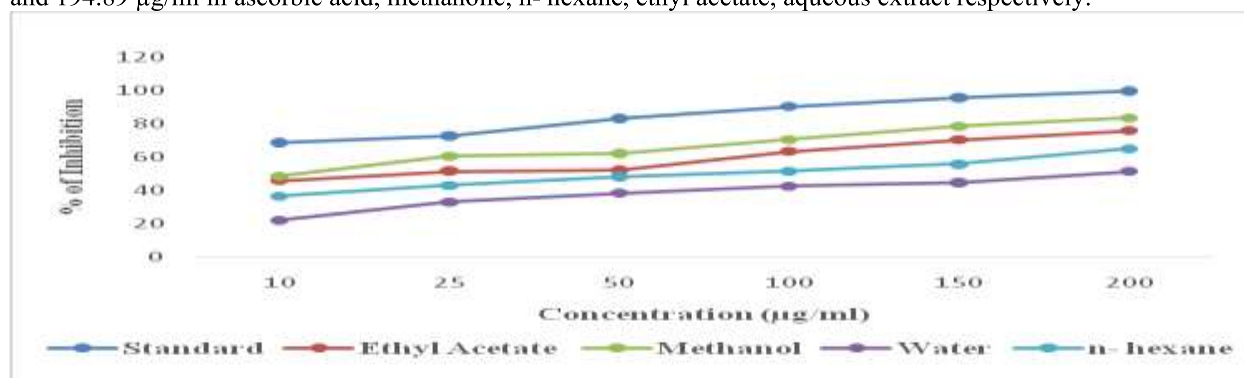

Figure 5: DPPH radical scavenging activity of HECE, EAECE, MECE, AECE andascorbic acid.

3.4.2. Ferric reducing antioxidant power (FRAP)

Reducing power assay, which reflects the capacity of an antioxidant to donate anelectron, is another commonly used in vitro model to evaluate the antioxidant potential of the plant extracts. In this assay, the reducing capacity of the extracts was determined by their ability to reduce the Fe3+ ferricyanide complex to the ferrous form. The reaction could be monitored by the spectrophotometer due to the appearance of the blue colour of ferrous ions. The reducing activity of all the extracts was evident by the formation of the colour of ferrous ions. In this experiment, the extract changes colour from pale yellow to greenish blue or dark blue depending on the concentration. The colour change is due to the presence of an antioxidant group present in the plant which reduces ferricyanide (Fe2+) to ferrocyanide (Fe3+) and when the ferric chloride was added, the ferric-ferrous complex was formed which will be measured at 700nm. The reducing power assay of plant extract/standard increases with concentrations, **Figure 6** represents the reducing power assay of both standard and extracts, from this figure itis concluded that the reducing power assay of plant extracts is greater than standard (Ascorbic acid). So, it is responsible for antioxidant activity.

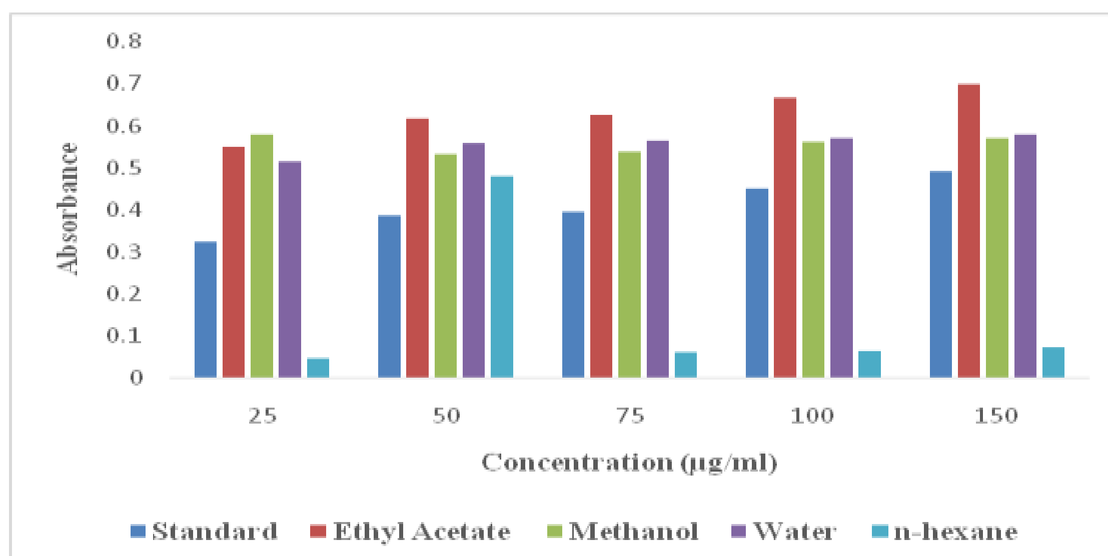

Figure 6: Reducing power assay of HECE, EAECE, MECE, AECE and ascorbic acidat different concentrations.

3.5. *In vitro anti-diabetic activity*:

3.5.1. α-amylase inhibition assay

The α-amylase breaks down the starch and is responsible for the production of glucose, hence when this enzyme activity is controlled it helps to overcome postprandial hyperglycemia. In this study, acarbose was used as a standard. The $_{IC50}$ value was reported in *Colocasia esculenta* methanolic extract (355.12 µg/ml), ethyl acetate

extract (370.86 µg/ml), n-hexane extract (362.14 µg/ml), and aqueous extract (155.81 µg/ml) and standard (186.56 µg/ml) (**Figure 7**). Low IC50 contains aqueous extract and shows more antidiabetic activity than others extract. From the bar diagram (Figure.7) we can say that the order of antidiabetic activity of a *Colocasia esculenta* is aqueous extract > methanolic extract > n- hexane extract> ethyl acetate extract.

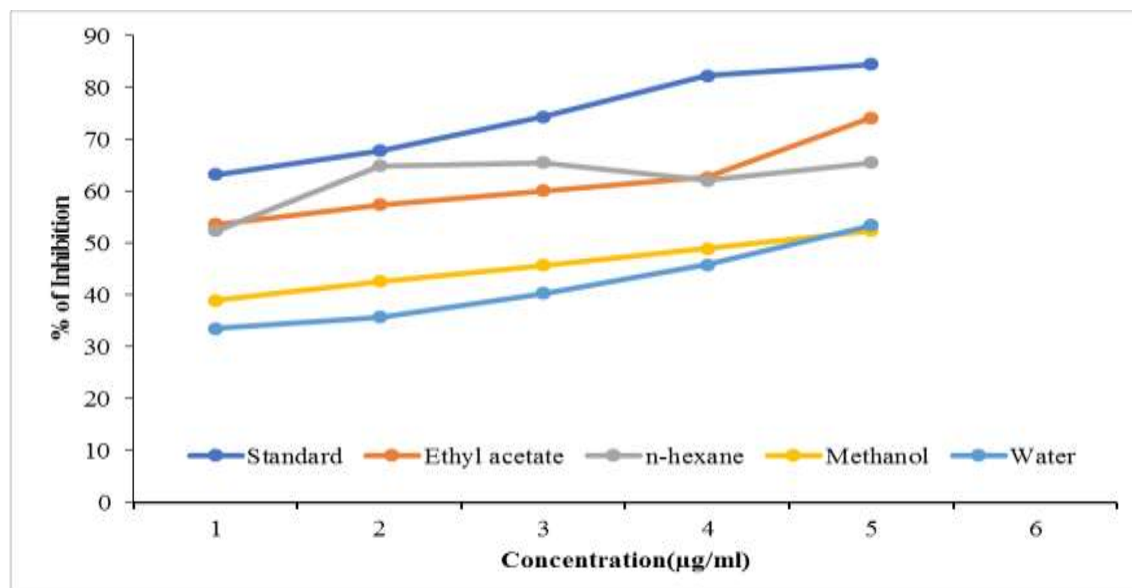

Figure 7: Anti-diabetic activity of different *Colocasia esculenta* crude extract andstandard.

3.6. In vitro anti-inflammatory activity:

3.6.1. Albumin denaturation assay

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acidor base, a concentrated inorganic salt, an organic solvent or heat. Most biologicalproteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. In this study, the in vitro anti-inflammatory activity of HECE, EAECE, MECE, AECE were measured for inhibitory activity against protein denaturation. **Figure 8** table presents the inhibitory effect of different *C. esculenta* extracts on albumin denaturation of all extracts and reference standard i.e. Diclofenac Sodium. The IC50 values were found to be 234.25 µg/ml, 598.21 µg/ml, 346.05 µg/ml, 334.09 µg/ml and 382.01 µg/ml for HECE, EAECE, MECE, AECE and Diclofenac Sodium respectively.

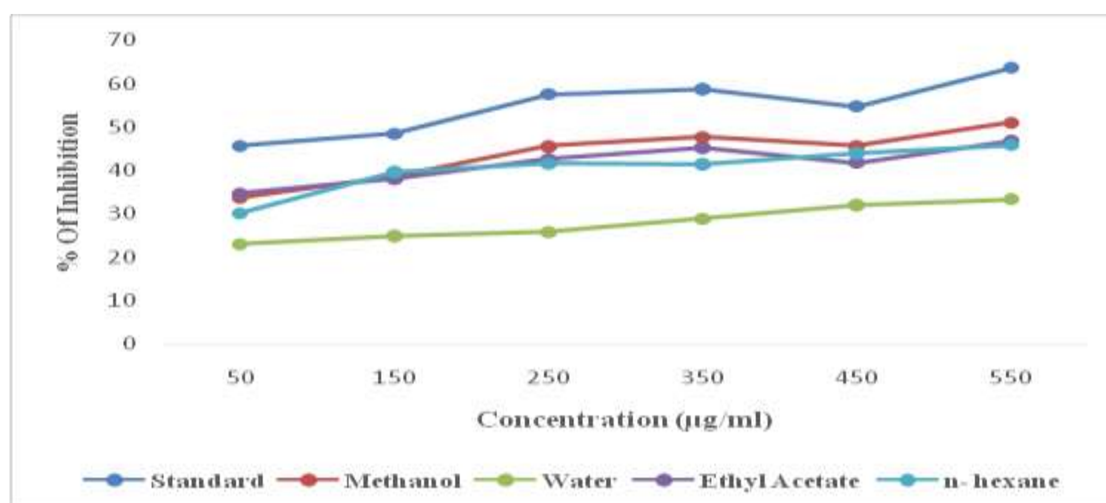

Figure 8: Albumin assay of HECE, EAECE, MECE and AECE and standard

4. Conclusion

Phytochemical investigation confirmed the presence of triterpenoid, alkaloid, carbohydrate, tannin, and flavonoid, glycoside compounds in *C. esculenta* stolon. It contains a significant number of phenolic and flavonoid groups of chemicals, accordingto this experiment. The presence of these compounds is responsible for the entrapment of free radical and act as a natural antioxidant as well as antidiabetic activity of the stolon of *C. esculenta*. Phenolic acids and flavonoids have been discovered to beamong the other active substances that are responsible for antioxidant activities. This evaluation helps in the investigation show compounds that may help combat many harmful diseases. *C. esculenta* stolon's methanolic extract showed strong antioxidant activity by blocking DPPH. Additionally, it was discovered that the extract includes substantial amounts of total phenols and flavonoids. The

findings of these investigations suggest that *C. esculenta* stolon methanolic extract has effective antioxidant and anti-inflammatoryproperties.

Availability of data and materials:
All data obtained or evaluated during this study are included in this manuscript, and are available from the corresponding author upon reasonable request.

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Krishna Pal, Soumik Patra accomplished the conceptualization. Literature review and analysis were conducted by Sharmila Mondal, Preeta Bose. Santanu Manna, Soumik Patra, Priya Roy, Titin Debnath wrote the manuscript.Yogendra Singh reviewed and edited the manuscript. Supervision of this research study was carried out by Krishna Pal. The final draft of the manuscript has been read and approved by all writers.

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