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Comparative phytochemical screening, antioxidants, cytotoxic, and in-vitro anti-inflammatory activity of non-polar and polar solvent extracts of Ipomoea quamoclit

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Abstract
The Ipomoea quamoclit is known as konjolata, commonly called the cypress vine Convolvulaceae family. I. quamoclit leaves found several medicinal uses such as antioxidant, anticancer, anti-inflammatory, etc. It is also traditionally used as an antidote to snake bites.
Aerial parts of the plant are selected to evaluate the comparative phytochemical screening, antioxidants such as DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, total phenolic content, total flavonoids content, total antioxidant capacity, cytotoxicity, and in-vitro anti-inflammatory activity of the nonpolar and polar solvent extracts. The aerial part of the plant was successively extracted with dichloromethane (DCM), ethyl acetate, methanol, and 95% ethanol.
Phytochemical screening of the extracts of I. quamoclit reveals the presence of components such as alkaloids, flavonoids, saponins, tannins, steroids, carbohydrates, etc. The results of the antioxidant study exhibit good antioxidant properties in different methods such as DPPH free radical scavenging, total phenolic content, total flavonoids content, and the total antioxidant capacity of the extracts of I. quamoclit. In which nonpolar solvent DCM extract showed significant antioxidant activity compared to the other extracts. Cytotoxicity study of the extracts of I. quamoclit indicates that the ethyl acetate and methanolic extract have good cytotoxic activity compared to other extracts LC_{50} 6.092 and 1.688 µg/mL, All the extracts of the polar and nonpolar solvent of I. quamoclit inhibit thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity. The extracts (25-200µg/ml) showed significant inhibition of denaturation of egg albumin and bovine albumin in a concentration-dependent manner, highest inhibition found at 200µg/mL at 98.07%.
The finding of the study suggested that nonpolar solvents dichloromethane has good antioxidant property, and the polar solvents methanol extract has good cytotoxic and anti-inflammatory activity.
Key Words: *Ipomoea quamoclit, phytochemicals, antioxidants, cytotoxicity, anti-inflammatory.*

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I. Introduction

Plants and man are inseparable. Because plants not only provide food, shelter, and medicine but also life-sustaining oxygen gas. Since disease and decay have always co-existed with life, the early man had to think about disease and its treatment at the dawn of human intellect. Thus, the human race started using plants as a means of treatment of diseases and injuries from the early days of civilization on earth and its long journey from ancient times to the modern age the human race successfully used plants and plant products as effective therapeutic tools for fitting against disease and various other health hazards [1].

Plant products are used from the time of immemorial as flavoring agents, cosmetics, pharmaceuticals, and biological probes. However, their importance as a drug, popularly known as folklore medicine, has been recognized from ancient times. Medicinal plants have been serving as the major sources of medicine for the maintenance of the health and wellbeing of human beings from the very beginning of their existence on earth. As therapeutic uses of plants continued with the progress of civilization and development of human knowledge, scientists endeavored to isolate different chemical constituents from plants, put them to biological and pharmacological tests thus have been able to identify and isolate therapeutically active compounds, which have been used to prepare modern medicines [2]. The plants that possess therapeutic properties or exert beneficial

pharmacological effects on the animal body are generally called medicinal plants. A large number of important modern drugs and most the traditional medicines are derived from medicinal plants. Some secondary metabolites of plants and animal origins like alkaloids, glycosides, tannins, and volatile oils possess medicinal properties [3]. However, since a single plant contains widely diverse phytochemicals (such as alkaloids, terpenoids, glycosides, flavonoids, etc.), thus the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of plants have medicinal potential by rigorous scientific research to define efficacy and safety [4].

Bangladesh possesses a rich flora of medicinal plants spread over an area of about 55000 square miles and endowed by nature with a very favorable climate, which possesses one of the richest flora of all other areas of similar size on the surface of the globe. A great variety of plants grow in its forests, jungles, western lands, and along the roadsides. Out of the estimated 5000 species of higher plants growing in this country more than a thousand are regarded as having medicinal properties. These plants grow here both wild and under cultivation. Many of the food, vegetable, beverage, spice, and ornamental plants are grown in this country contain medicinal useful chemical substances and constitute important items of therapeutic agents of various medicinal preparations, particularly of Unani, Ayurvedic medicines. Out of this estimated 1000, wild & cultivated medicinal plants available in this country only about 450 to 550 have so far been enumerated with documentation of information on their chemical constituents and medical property of uses [5].

I. quamoclit belonging to the Convolvulaceae family is an annual, herbaceous plant, commonly known as mayil manikkam, akasamulla, kunjalata, tarulata, kamalata, getphul in India and distributed throughout the tropical areas of the world. *I. quamoclit* is used as folk medicine around the world for various illnesses. This paper reviews the important biological activities of *I. quamoclit* reported over the last few decades. These include antioxidant activity, antimicrobial activity, anticancer activity, antidiabetic activity as well as insecticidal activity. These studies reveal that *Ipomoea quamoclit* has various biological activities; hence, it is encouraging to evaluate the comparative study of non-polar and polar solvent extracts and identify which is better. It is also traditionally used as an antidote to snake bites [6-10]. That's why we are interested to review the biological study of extracts of *I. quamoclit* available in Bangladesh.

II. Materials And Methods

2.1. Collection and identification of plants

The aerial part of *Ipomoea quamoclit* (konjolata) *was* collected from Manikgonj, Bangladesh. The plants were identified by the experts of the Department of Botany Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. The plants are distributed around Bangladesh, especially in Bandarban, Chattogram, and Khagrachari districts.

2.2. Preparation of Extract

The aerial part of the plants was taken and selected to be studied. They were first dried in the shade for several weeks, crushed by hands, and dried again. Then the crushed parts of the plants were reduced into a coarse powder with the help of a mechanical grinder. The powders of the plant were stored in an airtight container and kept in a cool, dark, and dry place until analysis commenced. The powered materials of the medicinal plant's aerial part namely *I. quamoclit* (400g) were taken in clean, flat-bottomed glass containers and then soaked with dichloromethane (1000mL). The containers with their contents were sealed and kept for 3 days accompanying occasional shaking and stirring. The whole suspensions were then filtered by a piece of clean, white cotton cloth and then filtered by a filter paper. The filtrate was collected in a clean conical flask. The wet powder was dried at room temperature and soaked with ethyl acetate (1000mL) and maintained the above-mentioned process. Then the powder was extracted with methanol (1000mL) in the same fashion. Finally, the powder was extracted with the ethanol of 95% (1000mL). After filtration, the filtrated extracts were dried on the rotary evaporator followed by dried in a desiccator for several days and gives four solid extracts such as deep green solid (1.05g) DCM extract *I. quamoclit* denoted as IQDCM, a Light brown solid (0.59g) ethyl acetate extract *I. quamoclit* represents as IQEA, a brownish-black solid (2.46g) methanol extract of *I. quamoclit* marked as IQM, and a brown solid (1.18g) 95% ethanol extract *I. quamoclit* designated as IQE.

2.3. Phytochemical screening

Preliminary phytochemical screening of the extracts of *I. quamoclit* was carried out. The freshly prepared crude extracts were qualitatively tested for the identification of chemical constituents, such as alkaloids, flavonoids, glycosides, saponins, carbohydrates, terpenoids, and tannins. The tests were carried out by the method described by Harborne and Sazada et al and in each test, 10% (w/v) solution of the extract was taken unless otherwise mentioned in the individual test [11,12].

2.4. Tests for determination of antioxidant activity

2.4.1. DPPH Free Radical Scavenging Assay

DPPH is a reactive free radical that acts as an electron acceptor (oxidant/ oxidizing agent) and causes the oxidation of other substances. On the other hand, antioxidants act as electron donors (reductant/ reducing agent). Antioxidants neutralize DPPH by being oxidized themselves. DPPH is found as a dark-colored crystalline powder composed of stable free-radical molecules and forms deep violet color in solution. The scavenging of DPPH free radical (neutralization) is indicated by the deep violet color being turned into pale yellow or colorless [13].

Calculation:

$$\% \text{ Inhibition} = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ Control}\right) \times 100$$

2.4.2. Determination of Total Phenolic Content

The total phenol content in extracts was determined using the Folin-Ciocalteu reagent based on colorimetric reaction. In brief, 1 mL of plant extract (200 µg/mL) or standard of different concentrations was taken in test tubes. Then, 5 mL of FCR and 7.5 % Na_2CO_3 were added to it and mixed well. Moreover, test tubes containing standard or extract solution were incubated for 30 minutes and 1 hr respectively at room temperature. Absorbance was taken at 765 nm by UV-VIS spectrophotometer [14]. Gallic acid was used as a standard to produce a calibration curve. The phenol content in extracts was expressed as mg of gallic acid equivalents (GAE) /g of extract.

2.4.3. Determination of Total Flavonoid Content

Total flavonoid content in plant extracts was determined using the colorimetric method involving aluminum chloride [15]. In this method, 1 mL of plant extract (200µg/mL) or standard of different concentrations was taken in the test tube. 3 mL of methanol, 200µl of 10% aluminum chloride solution, and 200 µl of 1M potassium acetate solution were added to the previously mentioned test tube. Finally, 5.6 ml of distilled water was mixed with the reaction mixture. The reaction mixture was then incubated for 30 minutes at room temperature and the absorbance of the solution was measured at 415 nm using a spectrophotometer against of blank. Quercetin was used as a reference standard and flavonoid contents of extracts were calculated as mg of QE/ g of extract.

2.4.4. Total antioxidant capacity

An antioxidant is defined as "any substance that, when presented at low concentration compared to those of an oxidizable substrate (proteins, lipids, carbohydrates, and DNA), significantly delays or prevents oxidation of that substrate" [16, 17]. The main function of antioxidants is to protect the body against the destructive effects of free radical damage [18]. The total antioxidant activity of the extract can be evaluated by the phosphomolybdenum method described by Prieto *et al.* 1999 [19]. The assay is based on the reduction of Mo (VI)-Mo (V) by the extracts and subsequent formation of green PO_4/Mo (V) complex at acidic p^H.

2.5. Cytotoxicity study

2.6. Brine Shrimp Lethality Bioassay

Cytotoxicity of extract of *I. quamoclit* was determined by Brine Shrimp lethality bioassay described by Meyer *et al.* 1982 [20]. Brine Shrimp lethality bioassay is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin. By this method, natural product extracts, fractions as well as pure compounds can be tested for their bioactivity. The method utilizes in vivo lethality in a simple zoological organism as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. Brine toxicity is closely correlated with 9KB (human nasopharyngeal carcinoma) cytotoxicity ($p=0.036$ and kappa= 0.56). ED_{50} values for cytotoxicity are generally about one-tenth of the LC_{50} values found in the Brine Shrimp test. Thus, it is possible to detect and then monitor the fractionation of cytotoxic, as well as 3PS (P_{388}) active extracts using the Brine Shrimp lethality bioassay. The Brine Shrimp assay has the advantages of being rapid (24 hours), inexpensive, and simple. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample (2-20 mg or less). Furthermore, it does not require animal serum as is needed for cytotoxicity [21].

2.7. Anti-inflammatory activity

When cells in the body are damaged by microbes, physical agents, or chemical agents, the injury is in the form of stress. Whether loss of function occurs depends on the size and extent of the injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to

heat, radiation, and bacterial or viral invasion [22]. 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 acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site [23].

2.6.1 Inhibition of Protein Denaturation

To evaluate the anti-inflammatory effects of the extracts, the protocol described by Padmanabhan and Jangle [24 and Elias and Rao [25] was used with small modifications. A volume of 1 ml of extracts (aqueous and ethanolic) or of diclofenac sodium at different concentrations (25,50, 100, 200μg/ml) was homogenized with 1 ml of the aqueous solution of bovine serum albumin/ egg albumin (5%) and incubated at 27°C for 15 minutes. The mixture of distilled water and albumin constituted the control tube. Denaturation of the proteins was caused by placing the mixture in a water bath for 10 minutes at 70°C, the mixture was cooling inside the ambient room temperature, and the activity of each mixture was measured at 660 nm.

Calculation of percent of inhibition:

$$\% \text{ Inhibition} = \frac{Absorbance\ of\ Control - Absorbance\ of\ Test}{Absorbance\ of\ Control} \times 100$$

III. Results And Discussions

3.1 The percentage yield of plant extracts by Maceration process

Powders of *I. quamoclit* were extracted successively with DCM, ethyl acetate, methanol, and ethanol by maceration process yielded IQDCM 0.26%, IQEA 0.14%, IQM 0.62%, and IQE 0.29%

3.2 Phytochemical Screening

Preliminary phytochemical screening of the extracts reveals the presence of various bioactive components such as phenols, flavonoids, alkaloids, terpenoids, tannins, saponins, glycosides, steroids, and carbohydrates. The results of the phytochemical screening are shown in Table 1.

Table 1: Qualitative analysis of phytochemical constituents of the extracts

Phytochemical Investigated	IQDCM	IQEA	IQM	IQE
Tannins	-	-	+	+
Flavonoids	+	-	-	-
Saponins	-	+	+	+
Carbohydrate	+	-	-	+
Steroids	-	-	-	+
Alkaloids	+	-	+	-
Terpenoids	-	+	+	-

Note: Symbol (+) indicates presence and symbol (–) indicates the absence

Phytochemical analysis showed the presence of various phytochemicals which are responsible for the therapeutic activity of medicinal plants [26]. These constituents are responsible for the antioxidant activity of these plants.

3.3. In-Vitro Antioxidant Assays

3.3.1. DPPH Free Radical Scavenging Assay

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC_{50}values of fractions dichloromethane (IQDCM), Ethyl acetate (IQEA), methanol (IQM), and 95% ethanolic (IQE) extracts of *I. quamoclit* are presented in Table 2 and figure 1.

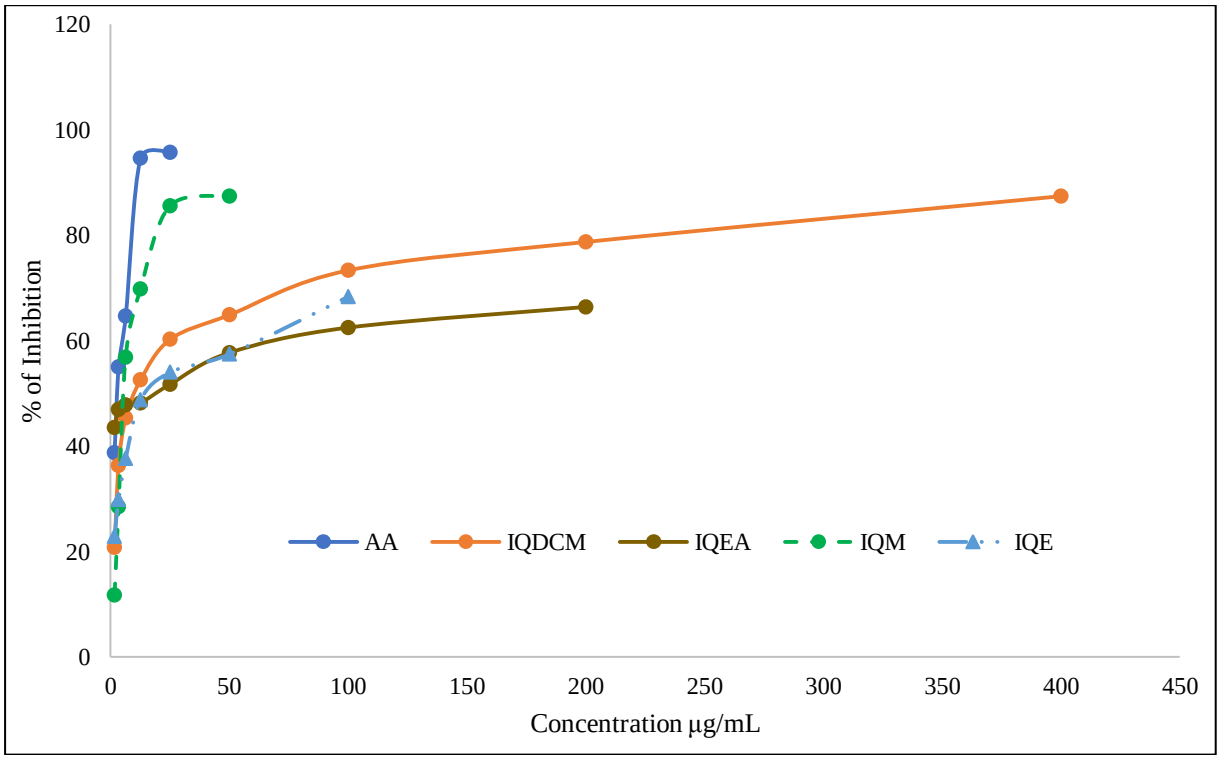

Figure 1: Comparative % Scavenging of standard and different extracts of *Ipomoea quamoclit*

Table 2: IC_{50} values of the extracts of *Ipomoea quamoclit*

Sample/Standard	IC_{50} (µg/ml)
Ascorbic acid	1.50
IQDCM	26.67
IQEA	86.28
IQM	45.52
IQE	318.74

DPPH radical scavenging is a widely used method to evaluate the free radical scavenging activity of compounds or the antioxidant capacity of plant extracts. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. The greater the decolorizing action, the higher the antioxidant activity, and is reflected by a lower IC_{50} value. Substances that can perform this reaction can be considered antioxidants and, therefore, radical scavengers [27]. In the present study, the DPPH free radical scavenging assay of the fraction indicates that the DCM part has more power of free radical scavenging compared to the others parts. But comparatively lower than ascorbic acid. IC_{50} values are ascorbic acid, IQDCM, IQEA, IQM and IQE are 1.50, 26.67, 86.28, 45.52, 318.74 µg/ml. The results of the DDPH free radical scavenging assay exhibit IQDCM have good scavenging power than other extract and IQE have lass power compare to extract.

3.3.2. Total Phenol Content Determination (TPC)

Total phenolic contents of the ethyl acetate and methanolic extract of the leaves of *I. quamoclit were* determined by using the Folin-Ciocalteu reagent and were expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the ethyl acetate and methanolic extracts are calculated using the standard curve of gallic acid ($y = 0.0114x+0.3101$; $R^2 = 0.9932$). The result of phenolic content is given in table 3 and figure 2.

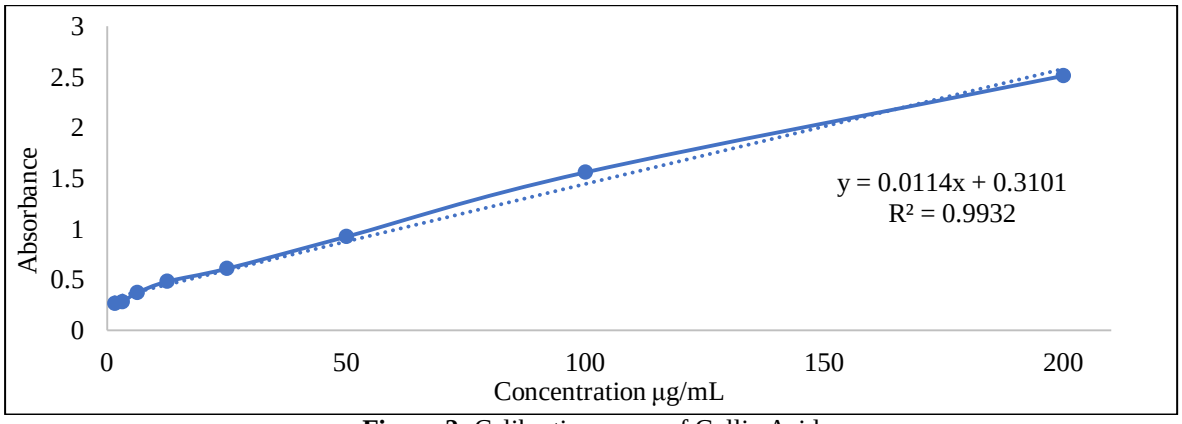

Figure 2: Calibration curve of Gallic Acid

Table 3: Total phenolic contents present in extracts of *Ipomoea quamoclit*

Extracts	Weight of dry extract per g/mL	Absorbance	GAE concentration (C) (µg/mL)	GAE concentration (C) (mg/mL)	TPC as GAE, $A=\frac{c-v}{m}$ (mg/g)	Mean value ± SEM
IQDCM	0.0002	0.622	27.36	0.027	136.80	128.68±11.47
	0.0002	0.585	24.11	0.024	120.57	
IQEA	0.0002	0.428	10.34	0.010	51.71	49.96±2.48
	0.0002	0.420	9.64	0.010	48.20	
IQM	0.0002	0.570	22.80	0.023	113.99	113.55±0.62
	0.0002	0.568	22.62	0.023	113.11	
IQE	0.0002	0.319	0.78	0.006	28.33	29.17±1.18
	0.0002	0.327	1.48	0.006	30.00	

The phenolic compounds are potential non-enzymatic antioxidants acting as reactive oxygen species (ROS) scavenging compounds. Polyphenols process structural chemistry for free radical scavenging activity, and they are more effective in antioxidant activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors which can stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction). Phenolic antioxidants interfere with the oxidation of lipids and other molecules by the rapid donation of a hydrogen atom to radicals. Phenoxy radicals intermediate are relatively stable, so they don't initiate further radical reactions. [28] The results of the present study exhibited high phenolic content (128.68±11.47 mg/g) in the IQDCM extract and (113.55±0.62 mg/g) in the methanolic extract of leaves of *I. quamoclit (IQM)* equivalent to standard GAE others two have less phenolic contents such as IQEA 49.96±2.48 mg/g, and IQE is 29.17±1.18 mg/g. The high phenolic content indicates the higher antioxidant activity of the extracts.

3.3.3 Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was used to determine the total flavonoid contents of the ethyl acetate and methanolic extracts of *Ipomoea quamoclit* leaves. The total flavonoid contents were calculated using the standard curve of quercetin (0.0069x -0.0065; $R^2 = 0.995$) and were expressed as quercetin equivalents (QE) per gram of the plant extract. The results of flavonoid content are given below in table 4 and figure 3.

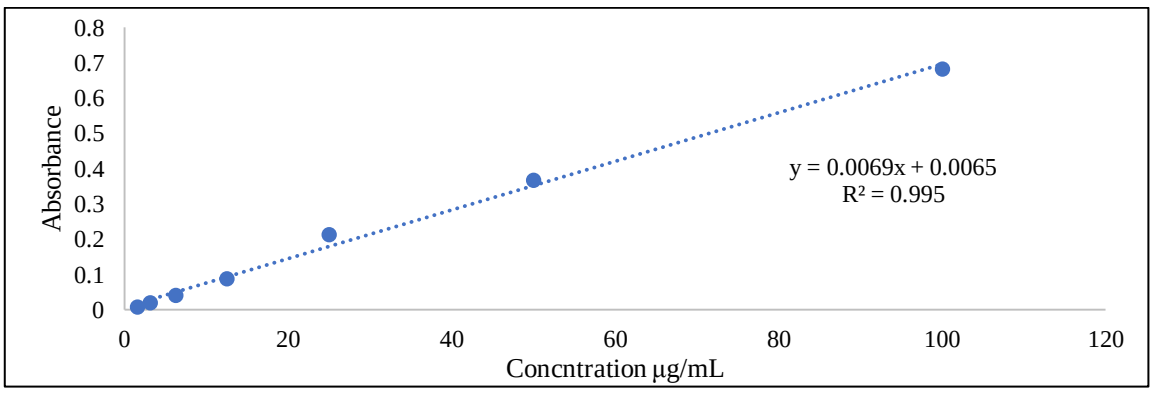

Figure 3: Calibration curve of quercetin

Table 4: Total flavonoid contents present in extracts of *Ipomoea quamoclit*

Extracts	Weight of dry extract per g/mL	Absorbance	QE concentration (C) (µg/mL)	QE concentration (C) (mg/mL)	TFC as QE, $A=\frac{c-v}{m}$ (mg/g)	Mean value ± SEM
IQDCM	0.0002	0.713	102.391	0.1024	511.96	509.06±4.10
	0.0002	0.705	101.232	0.1012	506.16	
IQEA	0.0002	0.064	8.333	0.0083	41.67	38.41±4.62
	0.0002	0.055	7.029	0.0070	35.14	
IQM	0.0002	0.052	6.594	0.0066	32.97	29.71±4.61
	0.0002	0.043	5.290	0.0053	26.45	
IQE	0.0002	0.033	3.841	0.0038	19.20	17.75±2.05
	0.0002	0.029	3.261	0.0033	16.30	

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure. They have miscellaneous favorable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease, atherosclerosis, etc. Oxidative stress may lead to cellular damage which is related to various health ailments such as diabetes, cancer, CVD, neurodegenerative disorders, and aging. Oxidative stress can also damage many biological molecules and proteins and DNA molecules are significant targets of cellular injury. Antioxidants interfere with radical-producing systems and increase the function of endogenous antioxidants, protecting the cells from damage by these free radicals and in vitro antioxidant capacity of most common in flavonoids as well as *in vitro* antioxidant activity and effects on endogenous antioxidants. Flavonoids are very effective in preventing lipid peroxidation and lipid peroxidation is responsible for various diseases such as atherosclerosis, diabetes, hepatotoxicity, and inflammation, along with aging [29]. Flavonoids are known for their antioxidant properties, and the flavonoid content of the investigated plant is of interest when evaluating its antioxidant properties. A positive correlation between the content of flavonoids and the antioxidant capacity in plant extracts has been found. Principally, the procedure involves the formation of a complex between flavonoid and $AlCl_3$ that produces a yellow-colored solution. The absorbance is then measured Spectro photometrically to determine the presence of flavonoid compounds. The result of the present study of total flavonoid content exhibit, in which the IQDCM extract showed higher flavonoid content 509.06±4.10 mg/g compare to other extracts such as ethyl acetate extract 38.41±4.62 mg/g, methanol extract 29.71±4.61 mg/g, and 95% ethanol extract 17.75±2.05mg/g. The results indicate that the leaves of *I. quamoclit* have higher antioxidant properties due to the presence of higher total flavonoids content.

3.3.4. Determination of total antioxidant capacity

The antioxidant effect is mainly due to phenolic compounds, such as phenolic acid, and phenolic diterpenes. The antioxidant activity of plant extracts is not limited to phenolics. Activity may also come from the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids, and vitamins, among others. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. (Javanmardi et al., 2003). [30] The phosphomolybdenum method is based on the reduction of M0 (V1) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with maximum absorption at 695 nm. The total antioxidant capacity of plant extracts is expressed as the number of grams equivalent to ascorbic acid. The results of the total antioxidant study showed in table 5 and figure 4.

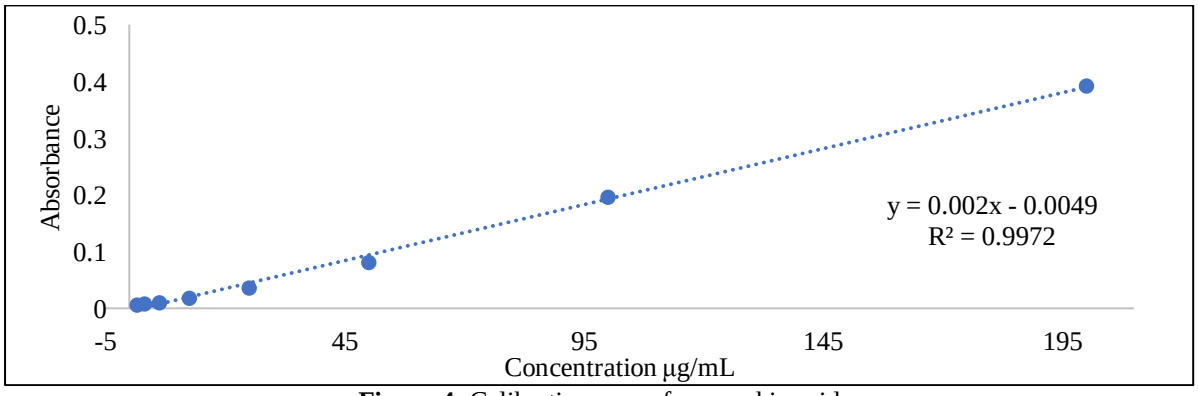

Figure 4: Calibration curve for ascorbic acid

Table 5: Total antioxidant capacity of the extracts of *Ipomoea quamoclit.*

Extracts	Weight of extract per g/ml	Absorbance	AAE concentration (C) (µg/ml)	AAE concentration (C) (mg/ml)	TAC as AAE, $A=\frac{c-v}{m}$ (mg/ml)	Mean ± SEM
IQDCM	0.0002	0.361	178.05	0.178	890.25	886.5±5.3
	0.0002	0.358	176.55	0.177	882.75	
IQEA	0.0002	0.114	54.55	0.055	272.75	265.25±10.6
	0.0002	0.108	51.55	0.052	257.75	
IQM	0.0002	0.086	40.55	0.041	202.75	194.0±12.4
	0.0002	0.079	37.05	0.037	185.25	
IQE	0.0002	0.012	3.55	0.004	17.75	21.5±5.3
	0.0002	0.015	5.05	0.005	25.25	

The total antioxidant activity study of the extract showed the antioxidant capacity of DCM extract is 886.5±5.3, ethyl acetate extract is 265.25±10.6, methanol extract 194.0±12.4, and 95% ethanol 21.5±5.3 mg/g. The results indicate a higher total antioxidant capacity of the extract but DCM extracts revealed a highly significant total antioxidant capacity.

3.4. Cytotoxicity study of Ipomoea quamoclit

3.4.1. Results of Brine Shrimp Lethality Bioassay for Cytotoxic Activity

The ethyl acetate and methanolic extract of *I. quamoclit were* found to be lethal to brine shrimp nauplii. The results are present in Tables 6-9 and figure 5.

Table 6: Data for Brine Shrimp lethality bioassay for cytotoxic activity of IQDCM

Conc.	log conc.	No. of nauplii taken (N_0)	No. of nauplii alive (N_1)	No. of nauplii dead	%Mortality, $\%M=\frac{N0-N1}{N0}\times 100$	Log LC_{50}	LC_{50} (µg/ml)
25	1.397	10	6	4	40	1.75	55.625
50	1.698	10	5	5	50		
100	2	10	5	5	50		
200	2.301	10	3	7	70		
400	2.602	10	1	9	90		
800	2.903	10	1	9	90		

Table 7: Data for Brine Shrimp lethality bioassay for cytotoxic activity of IQEA

Conc.	log conc.	No. of nauplii taken (N_0)	No. of nauplii alive (N_1)	No. of nauplii dead	%Mortality, $\%M=\frac{N0-N1}{N0}\times 100$	Log LC_{50}	LC_{50} (µg/ml)
25	1.397	10	4	6	60	0.785	6.092
50	1.698	10	2	8	80		
100	2	10	2	8	80		
200	2.301	10	1	9	90		
400	2.602	10	0	10	100		
800	2.903	10	0	10	100		

Table 8: Data for Brine Shrimp lethality bioassay for cytotoxic activity of IQM

Conc.	log conc.	No. of nauplii taken (N_0)	No. of nauplii alive (N_1)	No. of nauplii dead	%Mortality, $\%M=\frac{N0-N1}{N0}\times 100$	Log LC_{50}	LC_{50} (µg/ml)
25	1.397	10	3	7	70	0.227	1.688
50	1.698	10	2	9	80		
100	2	10	1	10	90		
200	2.301	10	1	10	90		
400	2.602	10	0	10	100		
800	2.903	10	0	10	100		

Table 9: Data for Brine Shrimp lethality bioassay for cytotoxic activity of IQE

Conc.	log conc.	No. of nauplii taken (N_0)	No. of nauplii alive (N_1)	No. of nauplii dead	%Mortality, $\%M = \frac{N0 - N1}{N0} \times 100$	Log LC_{50}	LC_{50} (µg/ml)
25	1.397	10	8	2	20	1.098	80.97
50	1.698	10	6	4	40		
100	2	10	5	5	50		
200	2.301	10	2	8	80		
400	2.602	10	1	9	90		
800	2.903	10	0	10	100		

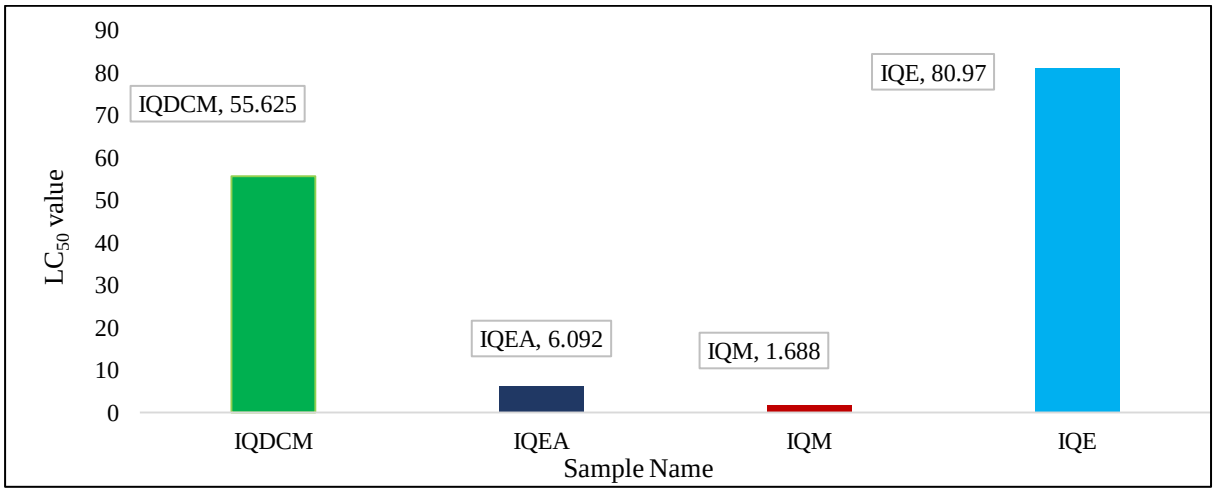

Figure 5: LC_{50} of extracts of the *Ipomoea quamoclit in* Brine Shrimp lethality bioassay

A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT). It appears that BSLT is predictive of cytotoxicity and pesticide activity, and is widely used in the bioassay for bioactive compounds (Zhao *et al.*, 1992) [31]. The results of the cytotoxicity study of the extracts of *I. quamoclit* leaves indicate that the ethyl acetate and methanolic extract have significant cytotoxic activity compared to other extracts LC_{50} 6.092 and 1.688 µg/mL, which indicates that will be auspicious candidates for the anti-cancer study of *I. quamoclit* for development of strategies uses and underlying mechanism, isolation of responsible agents for cancer treatment.

3.5. In vitro anti-inflammatory activity by protein denaturation method

The results of anti-inflammatory activity are given below in Table 10,11, as a percent of inhibition of denaturation of protein.

Table 10: Percent inhibition of denaturation of the egg albumin by standard and extracts of *I. quamoclit*

Concentration (µg/ml)	% Inhibition of denaturation				
	STD	IQDCM	IQEA	IQM	IQE
25	78.48	44.28	78.26	40.20	80.18
50	89.35	75.65	88.22	81.99	86.64
100	93.54	92.07	94.90	91.62	91.17
200	94.00	92.30	95.36	98.07	94.22

Table 11: Percent inhibition of denaturation of the bovine albumin by standard and extracts of *I. quamoclit*

Concentration (µg/ml)	% Inhibition of denaturation				
	STD	IQDCM	IQEA	IQM	IQE
25	78.25	53.77	71.28	56.21	79.47
50	79.66	60.92	77.87	79.66	81.83
100	79.76	80.79	84.65	87.57	82.67
200	80.70	82.49	85.59	88.23	86.91

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation, and acts by removing injurious stimuli and initiating the healing process. Inflammation is, therefore, a defense mechanism that is vital to health. Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to the restoration of tissue homeostasis and the resolution of acute inflammation. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases. At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular, and inflammatory cell responses to infection or injury. Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release. Various pathogenic factors, such as infection, tissue injury, or cardiac infarction, can induce inflammation by causing tissue damage. The etiologies of inflammation can be infectious or non-infectious. In response to tissue injury, the body initiates a chemical signaling cascade that stimulates responses aimed at healing affected tissues. These signals activate leukocyte chemotaxis from the general circulation to sites of damage. These activated leukocytes produce cytokines that induce inflammatory responses [32]. Denaturation of proteins is a well-documented cause of inflammation. Several anti-inflammatory drugs have shown dose-dependent ability to inhibit thermally-induced protein denaturation [33]. The commonly used drugs for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects, especially gastric irritation leading to the formation of gastric ulcers [34]. Therefore, the search for natural antioxidants with anti-inflammatory activity has greatly increased in recent years. The ability of the extracts of *Ipomoea quamoclit* to inhibit the thermal denaturation of protein is possibly a contributing factor to its anti-inflammatory activity. The inhibitory effect on protein denaturation is shown in Tables 10 & 11. The extracts (25-200µg/ml) showed significant inhibition of denaturation of egg albumin and bovine albumin in a concentration-dependent manner.

IV. CONCLUSION

In the present experimental study, it is suggested that DCM extracts of the leaves *I. quamoclit* have good antioxidant activity. The significant antioxidant activity of extract may be due to the presence of the higher amount of phenolic content and flavonoids content in IQDCM, also confirmed by the presence of higher total antioxidant capacity. The results of cytotoxicity indicate that ethyl acetate and methanol extract of *I. quamoclit* exhibited good cytotoxicity activity compared to the other extract. But methanol extract showed highly significant activity compared to all the extracts. These indicate that will be a good candidate for anticancer agents. A protein denaturation study found that ethyl acetate and methanol extract showed good anti-inflammatory activity compared to others but the response is dependent on the dose of the drug. The finding of the study suggested that nonpolar solvents dichloromethane has good antioxidant property, and the polar solvents methanol extract has good cytotoxic and anti-inflammatory activity.

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