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Research Article

In vitro Antioxidant and Anti-inflammatory activity of Hollyhock *Alcea rosea*

Sabitha B[1], Shoaeb Mohammad Syed[1,2*]

[1]Shri Venkateshwara University, Gajraula, Dist. Amroha - 244236 U.P. India
[2]Dayanand Education Society's Dayanand College of Pharmacy Latur Maharastra, India*

Abstract:
Althea rosea, or Alcea rosea L., is a member of the Malvaceae family. This medicinal herb has long been used to cure a variety of ailments, including chronic bronchitis and asthma. Alcea rosea has both traditional and industrial uses, making it an important medicinal plant for the general people as well as the medical and pharmaceutical communities. The extract from Alcea rosea (ARET) has been found to have antioxidant and anti-inflammatory qualities in this investigation. The Hollyhock Root (Red, White, Pink)-ARET stem extract demonstrated strong in vitro antioxidant activity, as determined by the DPPH assay. The IC50 value was determined to be 141.4214 mg/ml, and the inhibition percentage was 82% at 200 mg/ml of Hollyhock Root (RED)-ARET. The IC50 value was determined to be 141.4214 mg/ml, and the inhibition percentage was 83% at 200 mg/ml of Hollyhock Root (PINK)-ARET. The IC50 value was determined to be 141.4214 mg/ml, and the inhibition percentage was 78% at 200 mg/ml of Hollyhock Root (WHITE)-ARET. Both are contrasted with the typical ascorbic acid concentration of 70.7107 mg/ml. Therefore, even a high level of antioxidant activity is important when compared to vitamin C, which is a common ascorbic acid.

Keywords: Alcea rosea (ARET)**.,** antioxidant, DPPH assay, anti-inflammatory.

***Author of correspondence: Email:** ybccpsh@gmail.com

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Introduction:

Reactive oxygen species (ROS), which include superoxide anions, hydroxyl radicals, hydrogen peroxide, and alkoxyl radicals, are dangerous intermediates that are produced during respiration and injure biological macromolecules.(For example, lipids, proteins, carbs, and DNA.) This ultimately results in a number of degenerative illnesses, including cardiovascular disorders, atherosclerosis, and cancer (Murphy, 2009). It is believed that maintaining a healthy biological system depends heavily on antioxidants and oxidation balance (Gandhi & Abramov, 2012). According to Chiva-Blanch and Visioli (2012), dietary antioxidants can strengthen cellular defenses and shield cellular constituents from oxidative damage. It took widespread agreement to switch to natural antioxidants from synthetic ones due to the potential health hazards and toxicity of the latter (P. Evans & B. Halliwell, 2007). The search for antioxidants from natural sources has garnered a lot of attention as a result of efforts to find new natural resources for active antioxidant compounds (Wong et al., 2006). Throughout Southern Europe and Asia, gardens and parks widely cultivate Alcea rosea (Malvaceae), commonly known as holyhock. Due to its abundance of secondary metabolites, Alcea rosea can be used in a variety of food and medicinal research applications. This plant has been shown in numerous pharmacological investigations to have analgesic, antimicrobial, and anti-inflammatory properties (Mert et al., 2010; Dar et al., 2017). Alcea rosea roots have been used in Iranian traditional medicine to cure a number of ailments, such as angina, bronchitis, diarrhea, constipation, inflammation, and severe coughs (Ahmadi et al., 2012). Studies on the

ethnobotanical and ethnopharmacological properties of many species of Alcea rosea have shown promise in treating a wide range of illnesses. The overall antioxidant activity of Alcea rosea should be measured because of the growing interest in the connection between antioxidants and illnesses. Evaluation of the antioxidant and free radical scavenging properties as well as the total protein content of several Alcea rosea extracts was the aim of this investigation. Monosaccharides, amino acids, phenolic acids (ferulic, syringic, vanillic, p-coumaric, phydroxyphenylacetic, p-hydroxybenzoic, and caffeic), acidic polysaccharides, and pictinic polymers, which include glucorunic, galactorunic, and rhamnose acids (Azab, 2016). This plant has antibacterial, analgesic, and anti-inflammatory properties, according to pharmacological research. The roots of Alcea rosea are used in Iranian traditional medicine to cure a variety of conditions, including angina, bronchitis, diarrhea, constipation, inflammation, and severe coughing.

MATERIAL AND METHODS

Collection of samples:

Alcea rosea (ARET) collected from Ooty, Nilgiris, Tamilnadu for this investigation.

Method of preparation of samples:

10 gram of Alcea rosea (ARET) is heated with 100 ml of water, ethanol and methanol separately for 5 hours under reflux condenser in water bath, cool and filter. The filtrate is evaporated under vacuum to get aqueous and ethanolic extract.

HOLLYHOCK ROOT (RED, WHITE, PINK)-ARET





Alcea rosea

Kingdom : Plantae
Order : Malvales
Family : Malvaceae
Genus : *Alcea*
Species : *Alcearosea*

Parts used : Root
Common Name: Hollyhock .
Composition of Stem: carbohydrate, protein, and elemental compositions

Pharmacological evaluation:
Hollyhock Root (Red, White, Pink)-ARET aqueous extract was investigated for *in-vitro* antioxidant activity by DPPH assay and in-vitro Anti-inflammatory activity by inhibition of albumin denaturation.

In *vitro* Antioxidant activity
Determination of DPPH radical scavenging activity:
Antioxidant activity of the sample Hollyhock Root (Red, White, Pink)-ARET was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (Brand-Williams et al., 1995). About 100µL of sample extract was taken in the microtiter plate. And, 100µL of 0.1% methanolic DPPH was added to the sample and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; wherein colour change from purple to yellow and pale pink were considered as strong and weak positive, respectively. The plates were read using Elisa plate reader at 490nm. Standard ascorbic acid was used as reference. All the analysis were performed in triplicates and the average values were taken.

Radical scavenging activity was calculated by the following equation:

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \frac{Absorbance\ of\ Control - Absorbacne\ of\ Test\ Sample}{Absorbance\ of\ Control}\ x\ 100$$

***In-vitro* Anti-inflammatory activity - Inhibition of albumin denaturation:**
The reaction mixture was prepared separately by mixing 0.5mL of aqueous extract of Hollyhock Root (Red, White, Pink)-ARETand its compounds A, B, and C (1mg/mL) with 0.45 mL aqueous solution of bovine albumin fraction (5%). The pH (6.3) of the solution was adjusted using a small amount of 0.1N HCl at 37 ˚C for 20 min, then heat to 57 ˚C for 30 min. Cool the solution and transfer it to a 96 well plate and measure the absorbance at 660nm. Diclofenac sodium was used as the standard at the concentration of 1000µg/mL and the control contains 0.05mLof distilled water. The percentage of inhibition of albumin denaturation was calculated by the following formula,

$$Percentage\ of\ Inhibition\ (\%) = \frac{Absorbance\ of\ Control - Absorbacne\ of\ Test\ Sample}{Absorbance\ of\ Control}\ x\ 100$$

Where, Control – Reaction mixture except drug, Test Sample – Reaction mixture containing the sample

Principle of In vitro Egg Albumin Denaturation Method
The principal objective behind the egg albumin denaturation assay is to determine whether agents or compounds can stop or hinder egg albumin from becoming denatured under particular circumstances. Denaturation is the term used to describe how a protein changes in structure and loses its biological activity. Egg albumin is employed as a model protein in the experiment, and denaturation is brought about by exposing it to extremes of heat, pH, or other denaturing agents. Egg albumin's original conformation is disrupted during denaturation, changing its physical characteristics, and causing it to lose its functional activity. The egg albumin denaturation assay measures a drug or compound's capacity to prevent or lessen egg albumin denaturation to evaluate its anti-inflammatory effects. The egg albumin denaturation assay is based on the idea that substances with anti-inflammatory qualities may be able to stabilize protein structures and prevent denaturation, which is frequently linked to inflammation and tissue damage. As a result, agents or chemicals that significantly decrease the denaturation of egg albumin in this assay may have potential anti-inflammatory property. One of the causes of inflammation is assumed to be protein denaturation. NSAIDs prevent protein denaturation and inhibit the COX enzyme at the same time. The different concentrations of the test sample can be incubated with egg albumin solution in controlled experimental conditions and let the reactions happen and then the determination of absorbance to calculate the percentage inhibition.

Phytochemical Studies:
Qualitative Analysis
Detection of Carbohydrates and glycosides
Small amount of extract was treated with few drops of dilute hydrochloric acid and filtered. The filtrate was collected and subjected for following tests.
A) Molisch's test
1 ml of filtrate was mixed with 2 drops of molisch's reagent and 1 ml of concentrated sulphuric acid was added along the sides of the test tube.(Brown to violet ring indicates the presence of carbohydrates) (Surekha, 1991, Kokate, C.K., et al., 2006).
B) Legal's test
Filtrate was hydrolysed with hydrochloric acid on water bath. 1 ml of pyridine and few drops of sodium nitro prusside were added and then made alkaline with sodium hydroxide solution. (Pink to red colour indicates the presence of glycosides)
C) Borntrager's test
Filtrate was hydrolysed with hydrochloric acid on water bath and then treated with chloroform. Chloroform layer was separated and dil. Ammonia solution was added to it. (If ammonia layer acquire pink or red or violet colour, indicates the presence of glycosides).

Detection of Alkaloids:

Small quantity of extract was treated with few drops of dil. HCl and filtered. The filtrate was collected and subjected for following tests.

A) Dragendroff's test

Methanol extract of the plant (2 ml) and dilute hydrochloric acid (0.2 ml) were taken in a test tube. After adding 1 ml of Dragendroff's reagent. If reddish brown precipitate with filtrate, alkaloids are present

B) Mayer's test

2 ml of concentrated HCl was added to 2 ml of the respective plant extract samples followed by an addition of few drops of Mayer's reagent. If cream precipitate with filtrate, alkaloids are present.

C) Wagner's test

Two drops of Wagner reagent was added to 2 ml of extract and mixed well. If reddish brown precipitate with filtrate, alkaloids are present.

D) Hager's test

The extract was treated with few drops of Hager's reagent. A yellow precipitate was formed, indicating the presence of alkaloids. (Surekha, 1991).

Detection of Steroids

Small quantity of extract was treated with 5 ml of chloroform and subjected to the following tests.

A) Salkowsky test

To 1 ml of chloroform solution, 2ml of Conc. Sulphuric acid was added. If chloroform layer appears red color, and acid layer shows greenish yellow fluorescence indicates the presence of steroids.

B) Liebermann-Burchard test

To 2 ml of root extract add 1-2 ml of acetic anhydride was added and2 drops of Conc. Sulphuric acid was added along the sides of the test tube. First red, then blue and finally green color appearance indicates the presence of steroids.

Detection of Proteins and Amino acids:

Small quantity of extract was treated with in few ml of water and subjected to the following tests

A) Biuret Test:

Filtrate was treated with 4% NaOH and few drops of CuSO4 solution. Violet or pink color indicates the presence of proteins.

B) Millon's Test:

Filtrate was treated with Millon's reagent. White precipitate turns to brick red indicate the presence of proteins.

C) Ninhydrin Test:

Filtrate was treated with 3 drops of 5% Ninhydrin solution in boiling water bath for10 min. Purplish or bluish color appearance indicates the presence of Aminoacids (Khandelwal, K.R., 2004).

Detection of Flavonoids

Shinoda Test:

Alcoholic extract treated with 5 ml of 95 % ethanol, few drops of Conc. HCl and 0.5 g Magnesium turnings. Pink color indicates the presence of flavonoids. (Khandelwal, K. R., 2004). To small quantity of extract, lead acetate solution was added. Formation of yellow colored precipitate indicates the presence of flavonoids. Extract was treated with sodium hydroxide; yellow coloration indicates the presence of flavonoids. (Kumar et al., 2008).

Detection of Tannins

To 5 ml of extract, 1ml of 10 % aq. Potassium dichromate solution was added. Red precipitate indicates the presence of tannins. To 5 ml of extract, 1 ml of 5 % ferric chloride solution was added, deep blue-black color, tannins. Small quantity of extract was treated with Dil. HNO3, reddish to yellow color (tannins). Extract treated with Dil. Iodine solutions, transient red color (tannins). Extract treated with Dil. KMnO4, decoloration (tannins) (Khandelwal, K.R., 2004).

Detection of Saponins

Foam Test:

Dried root extract of plant was shaked with 20 ml distilled water vigorously. Persistent foam indicates the presence of Saponins.

Detection of Terpenoids

A) Knoller's Test

The extract was diluted with 2 ml of 0.1 % stannic chloride in pure thionylchloride. Pink to red to green to purple color indicates the presence of terpenoids.

Detection of Phenol:

A) Test by Ferric chloride:

Three drops of newly produced, 1% ferric chloride and potassium ferro cyanide were added to the aqueous extract solution to spike it. A bluish- green tinge was thought to be appealing. The extract of methanol was diluted with water. A few crystals of ferric sulphate were added to the mixture as well. The presence of phenolic compound is attested to by their deep violet hue.

Result:

Hollyhock Root (RED)-ARET has shown antioxidant properties. Hollyhock Root (Red)-ARET extract has shown in vitro antioxidant activity by DPPH assay in the current investigation. At 200 mg/ml Hollyhock Root (RED)-ARET, the inhibition percentage is **82%** and the IC_{50} value was found to be 141.4214 mg/ml compared to the standard ascorbic acid IC_{50} value of 70.7107 mg/ml. As a result, even a high concentration of antioxidant activity is **significant** compared to the standard ascorbic acid vitamin C.

The inhibition of the albumin denaturation method was used to measure the anti-inflammatory activity in vitro. In comparison to standard diclofenac sodium, neither the crude extract of Hollyhock Root (PINK) - ARET nor its separated constituents exhibit any appreciable anti-inflammatory efficacy. When compared to standard diclofenac sodium, the aqueous extracts of Herbal face toner exhibit moderate anti-inflammatory efficacy. At 300 mg/ml, Hollyhock Root (PINK)-ARET had a **42%** IC_{50} value of 317.0714 mg/ml compared to the standard diclofenac sodium IC_{50} value of 106.6798 mg/ml. As a result Hollyhock Root (PINK)-ARET is **moderate** anti inflammatory activity.

Hollyhock Root (WHITE)-ARET has shown antioxidant properties. Hollyhock Root (WHITE)-ARET extract has shown in vitro antioxidant activity by DPPH assay in the current investigation. At 200 mg/ml Hollyhock Root (WHITE)-ARET, the inhibition percentage is **78%** and the IC_{50} value was found to be 141.4214 mg/ml compared to the standard ascorbic acid IC_{50} value of 70.7107 mg/ml. As a result, even a high concentration of antioxidant activity is **significant** compared to the standard ascorbic acid vitamin C.

The inhibition of the albumin denaturation method was used to measure the anti-inflammatory activity in vitro. In comparison to standard diclofenac sodium, neither the crude extract of Hollyhock Root (WHITE) - ARET nor its separated constituents exhibit any appreciable anti-inflammatory efficacy. When compared to standard diclofenac sodium, the aqueous extracts of Herbal face toner exhibit moderate anti-inflammatory efficacy. At 200 mg/ml, Hollyhock Root (WHITE)-ARET had a **34%** IC_{50} value of 141.4214 mg/ml compared to the standard diclofenac sodium IC_{50} value of 106.6798 mg/ml. As a result Hollyhock Root (WHITE)-ARET is **moderate** anti inflammatory activity.

Discussion:

A previous investigation demonstrated that the egg albumin denaturation assay evaluates a drug or compound's capacity to inhibit or diminish egg albumin denaturation, serving as an indicator of its anti-inflammatory effects (Dharmadeva S et al., 2016). According to these findings, Alcea rosea extracts have the ability to scavenge free radicals, which makes them a promising antioxidant agent that can be used to treat a variety of pathological disorders linked to oxidative stress (Nazir S. et al., 2021). In ethnopharmacology, alcea rosea is used to treat a variety of ailments. The antioxidant qualities of medicinal plants typically contribute to their therapeutic effect. Antioxidants combat free radicals, which shields us from a range of illnesses.

Table: 1 HOLLYHOCK ROOT (RED)-ARET Invitro antioxidants activity by DPPH assay

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)
1	100 mg	0.38	0.08	78%	**77%**
2		0.38	0.12	68%	
3		0.38	0.05	86 %	
1	200 mg	0.38	0.06	84%	**81%**
2		0.38	0.07	81%	
3		0.38	0.08	78 %	
1	300 mg	0.38	0.09	76%	**75%**
2		0.38	0.07	81%	
3		0.38	0.12	68%	
1	Vit C	0.38	0.03	92%	**93%**
2		0.38	0.02	94%	
3		0.38	0.02	94%	

Table 2: HOLLYHOCK ROOT (RED)-ARET ANTIOXIDANT IC_{50} VALUE (mg/ml) Compared to STANDARD VIT C IC_{50} VALUE (mg/ml)

S.NO	Concentration (mg)	Average	IC_{50} value(mg/ml)
1	100	77%	141.4214 **mg/ml**
2	200	81%	
3	300	75%	
Standard Vitamin C			
S.NO	**Concentration (mg)**	**Average**	**IC_{50} value (mg/ml)**
1	50	91%	70.7107 **mg/ml**
2	100	88.6%	
3	150	92%	

Table 3: HOLLYHOCK ROOT (RED)-ARET Invitro anti-inflammatory activity

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)
1	100 mg	0.31	0.18	41%	**40%**
2		0.31	0.18	41%	
3		0.31	0.19	38 %	
1	200 mg	0.31	0.23	25%	**33%**
2		0.31	0.19	38%	
3		0.31	0.19	38 %	
1	300 mg	0.31	0.21	32%	**23%**
2		0.31	0.24	22%	
3		0.31	0.26	16%	
1	Diclofenac sodium	0.31	0.10	67%	**66%**
2		0.31	0.11	64%	
3		0.31	0.10	67%	

Table 4: HOLLYHOCK ROOT (RED)-ARET ANTIINFLAMMATORY IC_{50} VALUE (mg/ml) Compared to STANDARD DICLOFENAC SODIUM IC_{50} VALUE (mg/ml)

S.NO	Concentration (mg)	Average	IC_{50} value
1	100	40%	215.7172 **mg/ml**
2	200	33%	
3	300	23%	
Standard Diclofenac sodium			
S.NO	**Concentration (mg)**	**Average**	**IC_{50} value**
1	50	84%	106.6798 **mg/ml**
2	100	82%	
3	150	78%	

Table 5: HOLLYHOCK ROOT (WHITE)-ARET Invitro antioxidants activity by DPPH assay

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)
1	100 mg	0.39	0.14	64%	**70%**
2		0.39	0.12	69%	
3		0.39	0.08	79 %	
1	200 mg	0.39	0.07	82%	**78%**
2		0.39	0.08	79%	
3		0.39	0.10	74 %	
1	300 mg	0.39	0.13	66%	**71%**
2		0.39	0.11	71%	
3		0.39	0.09	76%	
1	Vit C	0.38	0.03	92%	**93%**
2		0.38	0.02	94%	
3		0.38	0.02	94%	

Table 6: HOLLYHOCK ROOT (WHITE)-ARETANTIOXIDANT IC_{50} VALUE (mg/ml) Compared to STANDARD VIT C IC_{50} VALUE (mg/ml)

S.NO	Concentration (mg)	Average	IC_{50} value(mg/ml)
1	100	70%	141.4214 **mg/ml**
2	200	78%	
3	300	71%	
Standard Vitamin C			
S.NO	**Concentration (mg)**	**Average**	**IC_{50} value (mg/ml)**
1	50	91%	70.7107 **mg/ml**
2	100	88.6%	
3	150	92%	

Table 7: HOLLYHOCK ROOT (WHITE)-ARET Invitro anti-inflammatory activity

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)
1	100 mg	0.33	0.26	21%	**25%**
2		0.33	0.23	30%	
3		0.33	0.25	24 %	
1	200 mg	0.33	0.25	24%	**34%**
2		0.33	0.20	39%	
3		0.33	0.20	39 %	
1	300 mg	0.33	0.26	21%	**24%**
2		0.33	0.26	21%	
3		0.33	0.23	30%	
1	Diclofenac sodium	0.31	0.10	67%	**66%**
2		0.31	0.11	64%	
3		0.31	0.10	67%	

Table 8: HOLLYHOCK ROOT (WHITE)-ARET ANTIINFLAMMATORY IC_{50} VALUE (mg/ml) Compared to STANDARD DICLOFENAC SODIUM IC_{50} VALUE (mg/ml)

S.NO	Concentration (mg)	Average	IC_{50} value
1	100	25%	141.4214 **mg/ml**
2	200	34%	
3	300	24%	
Standard Diclofenac sodium			
S.NO	**Concentration (mg)**	**Average**	**IC_{50} value**
1	50	84%	106.6798 **mg/ml**
2	100	82%	
3	150	78%	

Table 9: HOLLYHOCK ROOT (PINK)-ARET Invitro antioxidants activity by DPPH assay

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)
1	100 mg	0.34	0.05	85%	**78%**
2		0.34	0.08	76%	
3		0.34	0.09	73 %	
1	200 mg	0.34	0.05	85%	**83%**
2		0.34	0.05	85%	
3		0.34	0.07	79 %	
1	300 mg	0.34	0.07	79%	**82%**
2		0.34	0.06	82%	
3		0.34	0.05	85%	
1	Vit C	0.38	0.03	92%	**93%**
2		0.38	0.02	94%	
3		0.38	0.02	94%	

Table 10: HOLLYHOCK ROOT (PINK)-ARET ANTIOXIDANT IC_{50} VALUE (mg/ml) Compared to STANDARD VIT C IC_{50} VALUE (mg/ml)

S.NO	Concentration (mg)	Average	IC_{50} value(mg/ml)
1	100	78%	141.4214 **mg/ml**
2	200	83%	
3	300	82%	
Standard Vitamin C			
S.NO	**Concentration (mg)**	**Average**	**IC_{50} value (mg/ml)**
1	50	91%	70.7107 **mg/ml**
2	100	88.6%	
3	150	92%	

Table 11: HOLLYHOCK ROOT (PINK)-ARET Invitro anti-inflammatory activity

S.NO	Concentration(mg)	COD	SOD	%inhibition	Average(%)
1	100 mg	0.35	0.25	28%	**33%**
2		0.35	0.22	37%	
3		0.35	0.23	34 %	
1	200 mg	0.35	0.23	34%	**33%**
2		0.35	0.25	28%	
3		0.35	0.22	37 %	
1	300 mg	0.35	0.20	42%	**42%**
2		0.35	0.22	37%	
3		0.35	0.18	48%	
1	Diclofenac sodium	0.31	0.10	67%	**66%**
2		0.31	0.11	64%	
3		0.31	0.10	67%	

Table 12: HOLLYHOCK ROOT (PINK)-ARET ANTIINFLAMMATORY IC_{50} VALUE (mg/ml) Compared to STANDARD DICLOFENAC SODIUM IC_{50} VALUE (mg/ml)

S.NO	Concentration (mg)	Average	IC_{50} value
1	100	33%	317.0714 **mg/ml**
2	200	33%	
3	300	42%	
Standard Diclofenac sodium			
S.NO	**Concentration (mg)**	**Average**	**IC_{50} value**
1	50	84%	106.6798 **mg/ml**
2	100	82%	
3	150	78%	

Table 13: HOLLYHOCK ROOT (RED)-ARET Phytochemical test
[- absence ; + presence]

S.No	PhytochemicalTest	Ethyl acetate extract
1.	Alkaloids	-
2.	Flavonoids	+
3.	Tannins	+
4.	Phenols	_
5	Carbohydrates	+
6.	Proteins	-
7.	Steroids	+
8.	Saponins	_

Table 14: HOLLYHOCK ROOT (PINK)-ARET Phytochemical test
[- absence ; + presence]

S.No	PhytochemicalTest	Ethyl acetate extract
1.	Alkaloids	-
2.	Flavonoids	+
3.	Tannins	+
4.	Phenols	_
5	Carbohydrates	+
6.	Proteins	-
7.	Steroids	+
8.	Saponins	_

Table 15: HOLLYHOCK ROOT (WHITE)-ARET Phytochemical test
[- absence ; + presence]

S.No	PhytochemicalTest	Ethyl acetate extract
1.	Alkaloids	-
2.	Flavonoids	+
3.	Tannins	+
4.	Phenols	_
5	Carbohydrates	+
6.	Proteins	-
7.	Steroids	+
8.	Saponins	_

Table 16: HOLLYHOCK ROOT (ARET) RF VALUE

ARET	Red (A)	Pink (B)	White (C)	Quercitin (Q)	Rutin (R)
Distance travelled by solvent	**9.6**	**9.5**	**9.3**	**9.5**	**9.6**
Distance travelled by solute	**2.7**	**2.1**	**2.3**	**1.9**	**2.1**
Rf Value	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**

Fig 1: HOLLYHOCK ROOT –ARET Standard



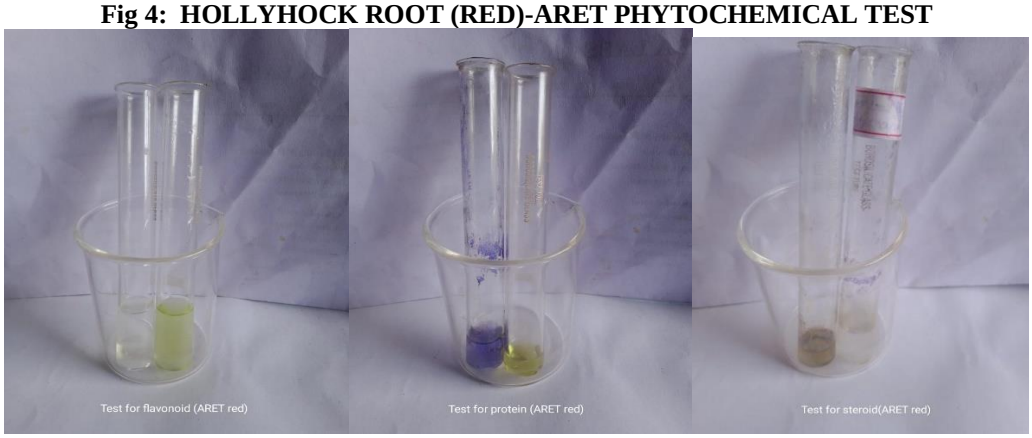
Fig 2: HOLLYHOCK ROOT (RED)-ARET ANTIOXIDANT ACTIVITY



Fig 3: HOLLYHOCK ROOT (RED)-ARET ANTI-INFLAMMATORY ACTIVITY



Fig 4: HOLLYHOCK ROOT (RED)-ARET PHYTOCHEMICAL TEST



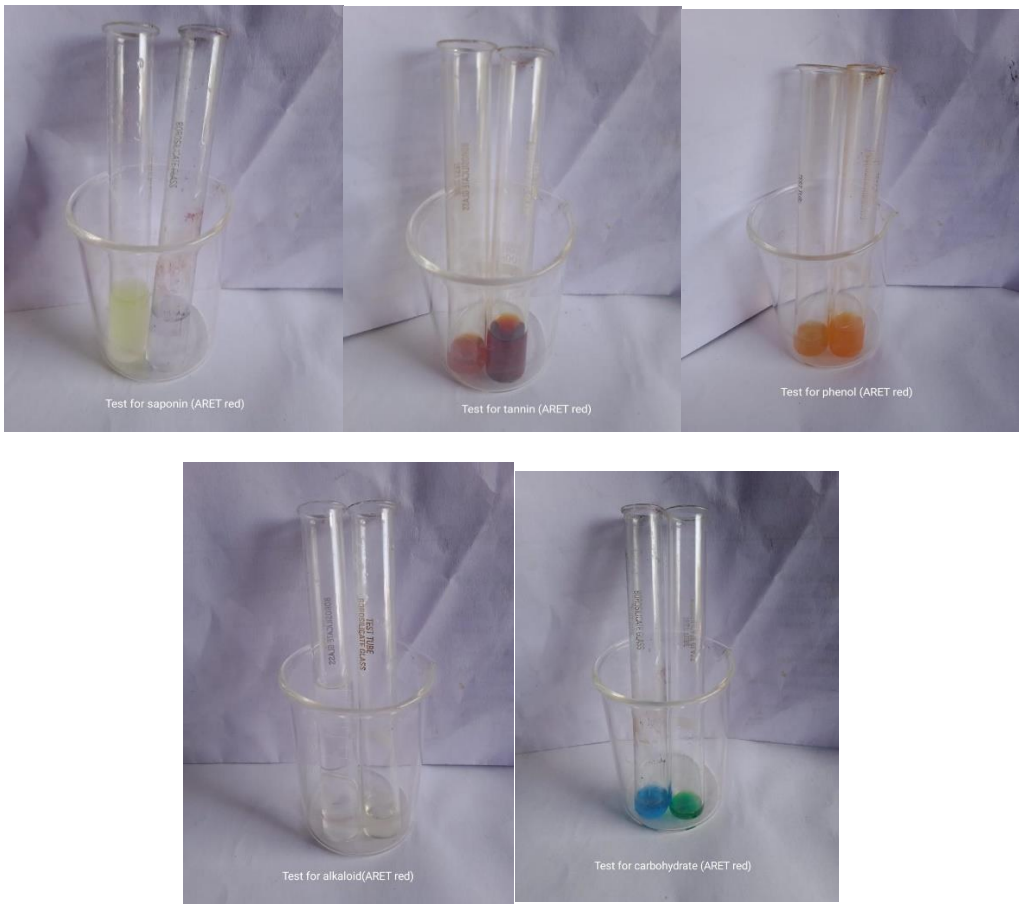


Fig 5: HOLLYHOCK ROOT (PINK)-ARET ANTIOXIDANT ACTIVITY

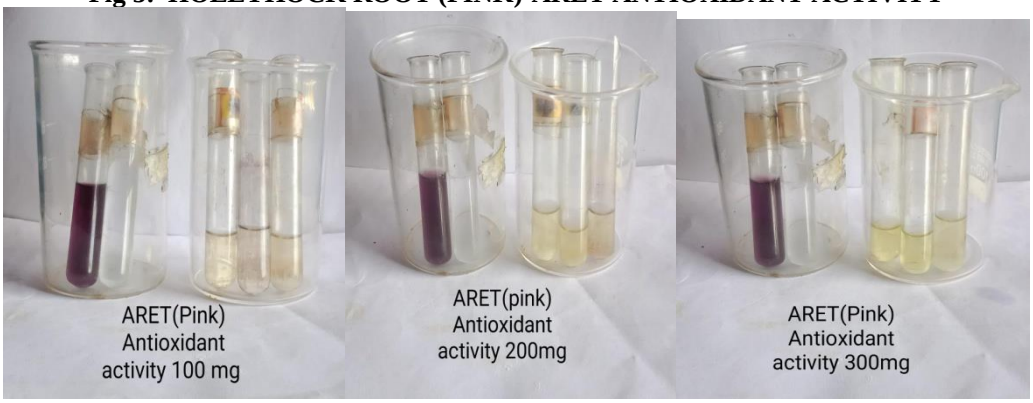


Fig 6: HOLLYHOCK ROOT (RED)-ARET ANTI-INFLAMMATORY ACTIVITY



Fig 7: HOLLYHOCK ROOT (PINK)-ARET PHYTOCHEMICAL TEST

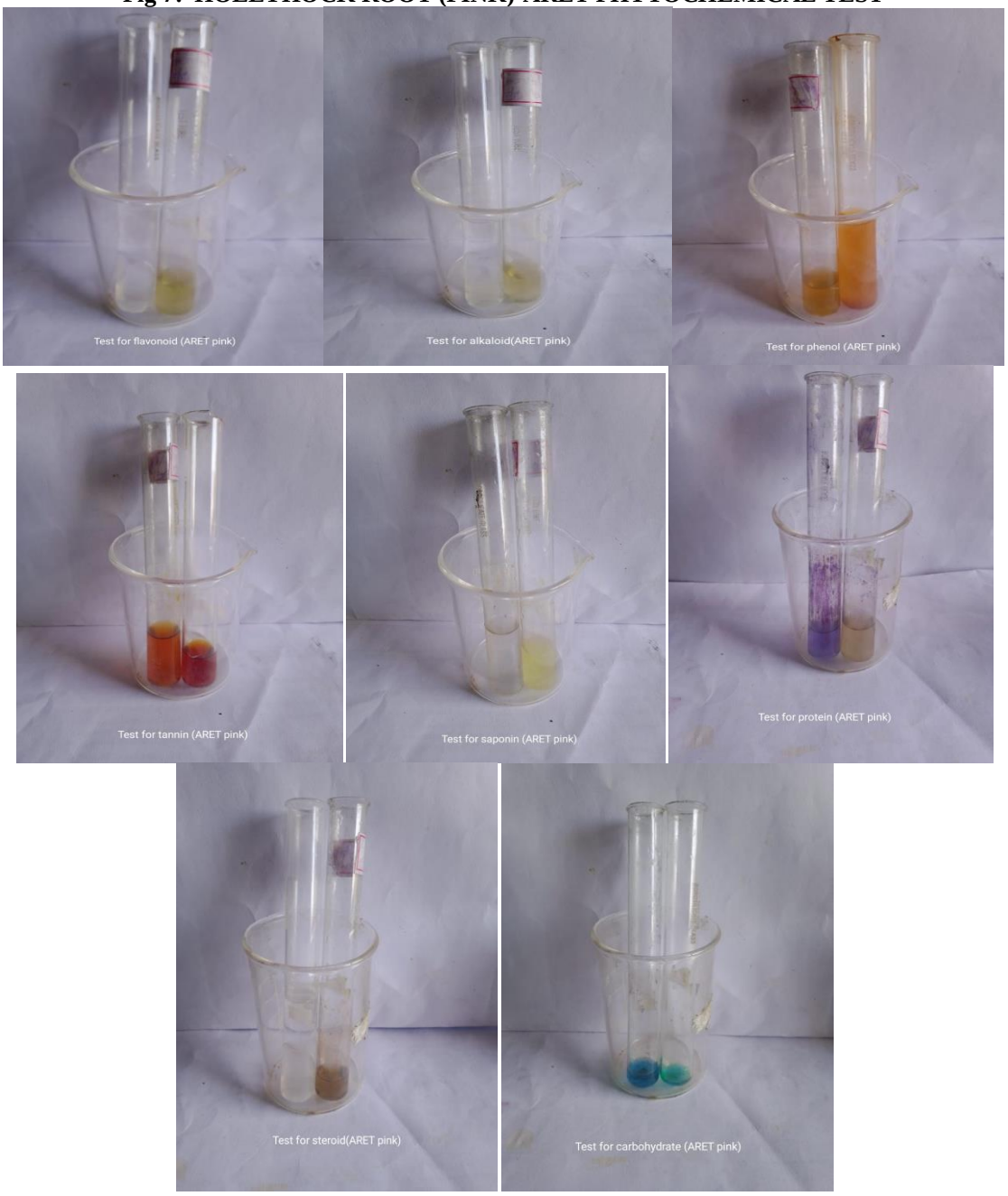


Fig 8: HOLLYHOCK ROOT (WHITE)-ARET ANTIOXIDANT ACTIVITY

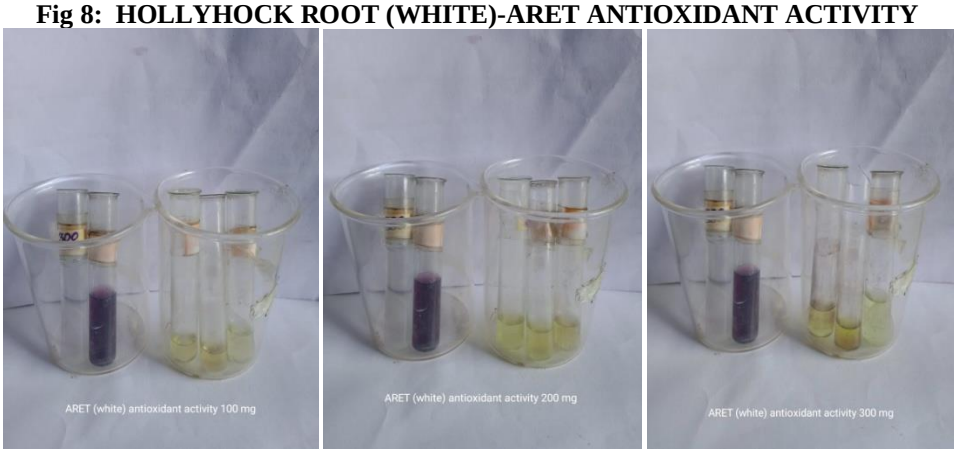
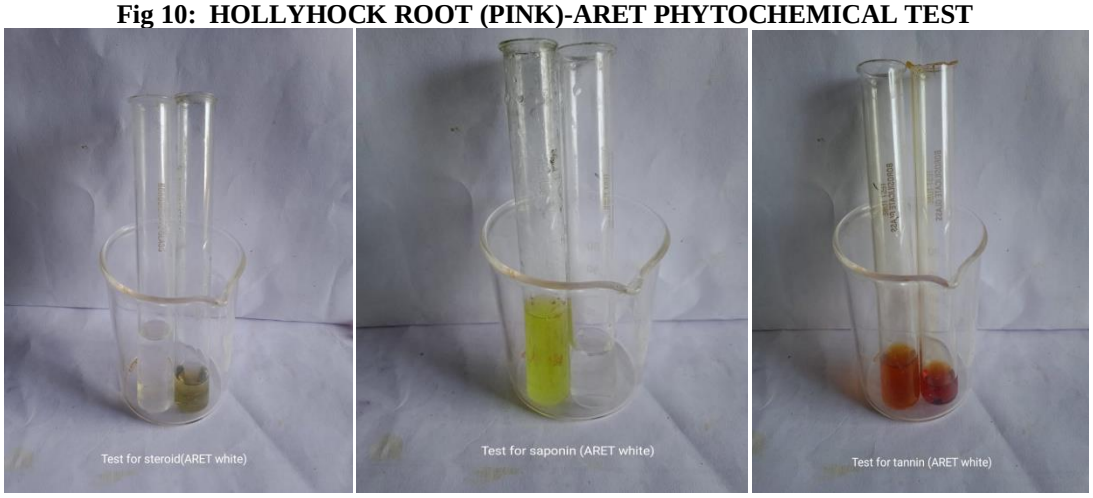


Fig 9: HOLLYHOCK ROOT (WHITE)-ARET ANTI-INFLAMMATORY ACTIVITY



Fig 10: HOLLYHOCK ROOT (PINK)-ARET PHYTOCHEMICAL TEST



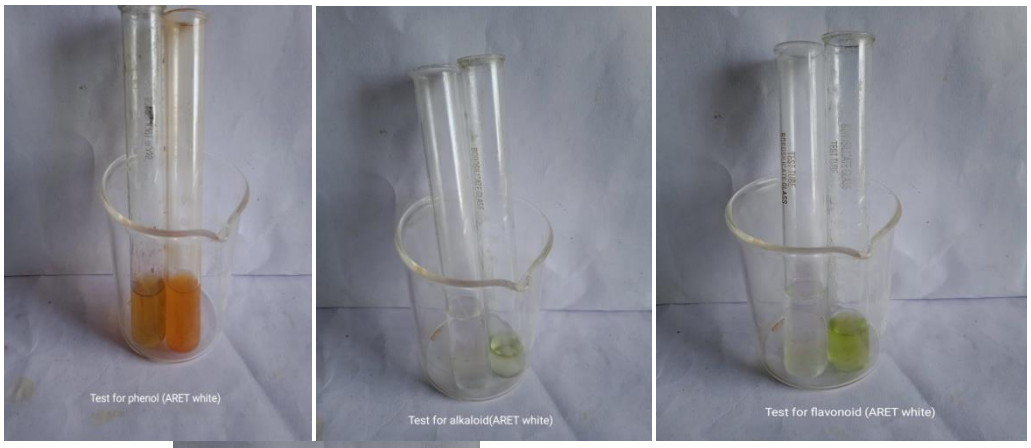




Fig 11: ARET TLC

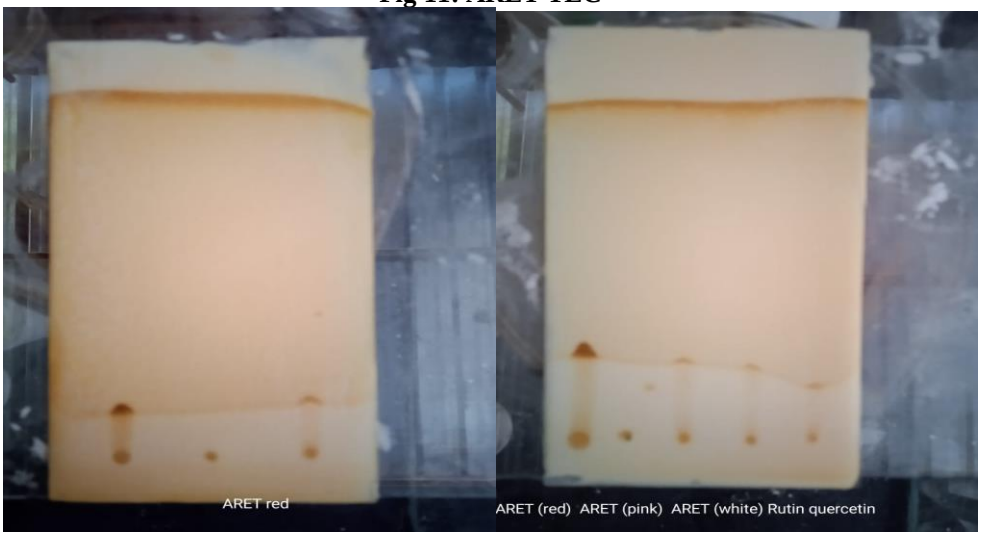


Fig 12: HOLLYHOCK ROOT (RED)-ARET ANTIOXIDANT ACTIVITY

IC_{50} VALUE

Chart

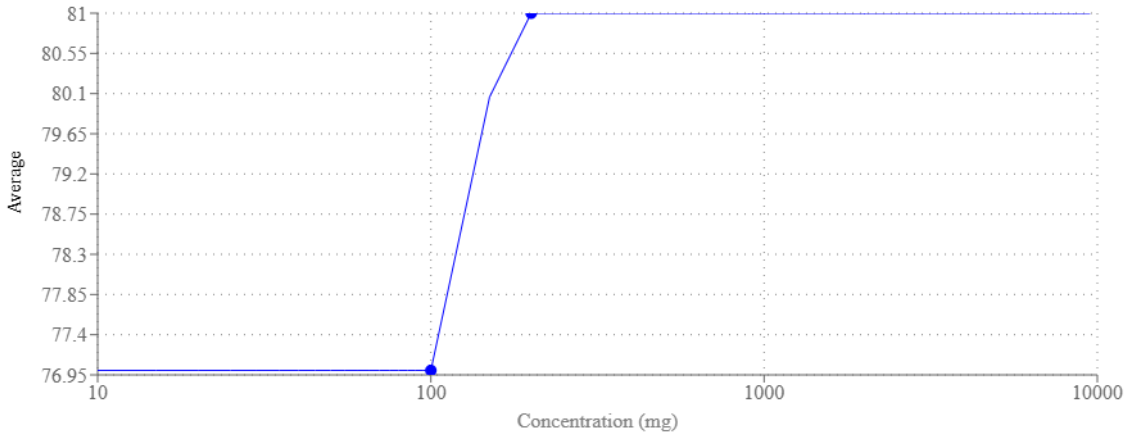


Fig 13: HOLLYHOCK ROOT (RED)-ARET ANTI-INFLAMMATORY ACTIVITY

IC_{50} VALUE

Chart

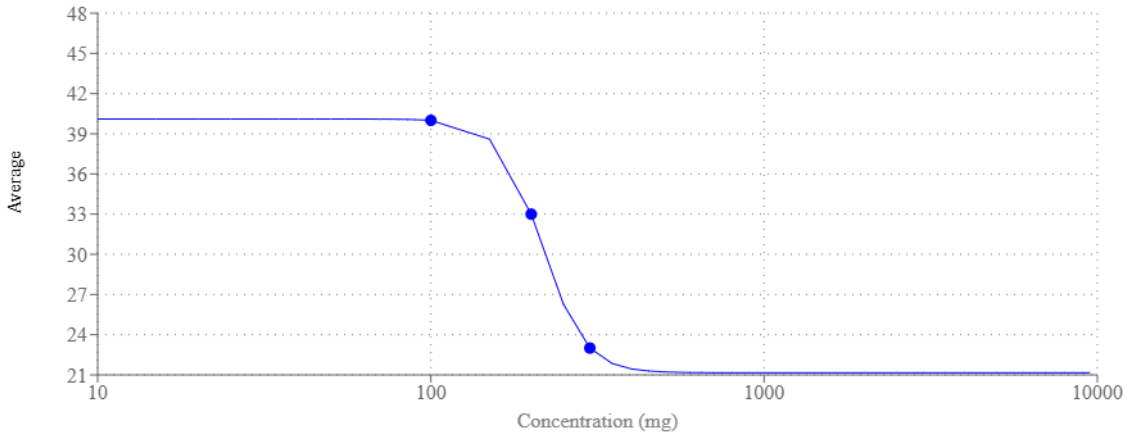


Fig 14: HOLLYHOCK ROOT (WHITE)-ARET ANTIOXIDANT ACTIVITY
IC_{50} VALUE
Chart

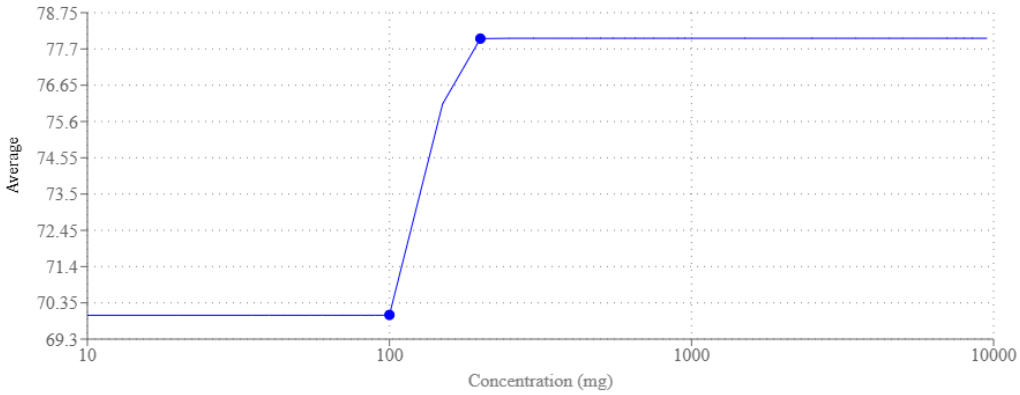


Fig 15: HOLLYHOCK ROOT (WHITE)-ARET ANTI-INFLAMMATORY ACTIVITY
IC_{50} VALUE
Chart

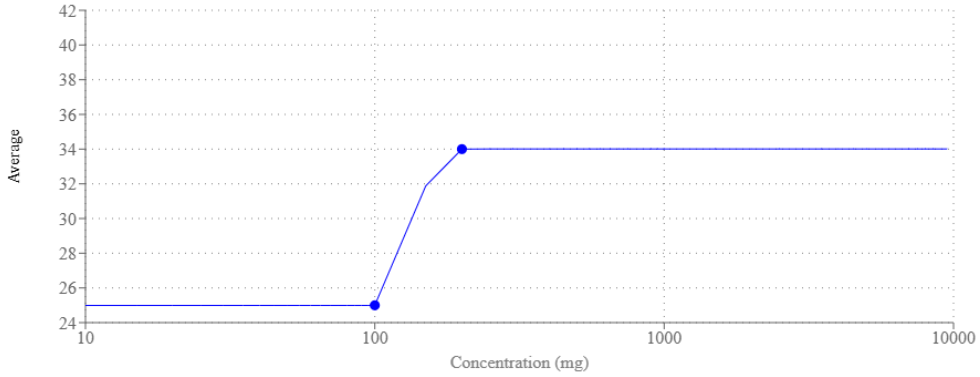


Fig 16: HOLLYHOCK ROOT (PINK)-ARET ANTIOXIDANT ACTIVITY
IC_{50} VALUE
Chart

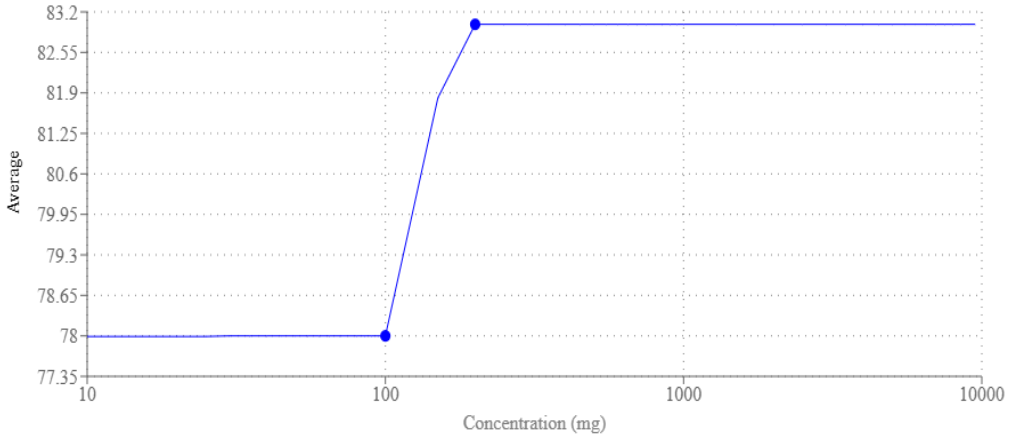
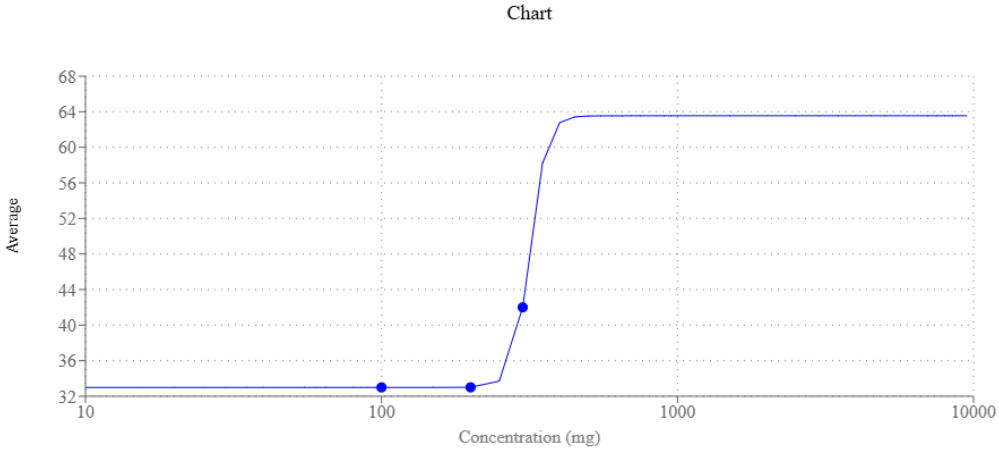


Fig 17: HOLLYHOCK ROOT (PINK)-ARET ANTI-INFLAMMATORY ACTIVITY IC_{50} VALUE



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