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Pharmacognostic, phytochemical and antioxidant studies on roots of *Cochlospermum religiosum* linn

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Abstract
Cochlospermum religiosum (Linn.) Alston. (Bixaceae) Traditionally, this plant is used for the treatments of jaundice, coughs, tuberculosis, asthma, inflammation, fever gonorrhea, and diarrhea. The current paper reports on morphology and histology studies of the roots of Cochlospermum religiosum Linn. Some unique and distinct characters have been identified in the root category. Physiochemical parameters and preliminary phytochemical studies of leaf powder were also performed. Also measure the amount of phenolic content, flavonoid content, flavonone determination and evaluate the *in vitro* antioxidant activity of alcohol and aqueous extract from the leaves of the Cochlospermum religiosum Linn. The total phenolic content was calculated calorimetrically using a folin ciocalteu reagent, and the total flavonoid content was determined by aluminum chloride method, while a modified 2, 4-dinitrophenylhydrazine method was used to determine flavonone. Total phenolic content of hydroalcoholic extract, a fraction of chloroform, a fraction of butanol and an insoluble portion of butanol hydroalcoholic extract of the root (active extract) of C. religiosum was found to be 43.50, 18.66, 26.50 and 13.86 mg / g respectively, the total flavo content for the same extraction was found to be 14.2, 11.2, 18.2 and 3.8 mg / g respectively. The total flavonone content for the same extract was found to be 6.0, 0.6, 5.4 and 1.0 respectively. Antioxidant activity was measured based on DPPH solid extracts test, IC50 hydroalcoholic extract, chloroform fraction, butanol fraction and soluble butanol were found to be 117.9 µg / ml 143.45 µg / ml 124.6 20m ml.

Keywords: pharmacognostical, phytochemical, antioxidant activity, *Cochlospermum religiosum* Linn

Introduction
Antioxidant means “anti-oxidation” and lipid protection function in each oxidation by radicals. The human body is a powerful antioxidant defense system. A key feature of the antioxidant is its ability to absorb free radicals. The most effective free radicals and types of oxygen are present in biological systems from a variety of sources. These free radicals can make nucleic acids, proteins, lipids or DNA and trigger degenerative disease. Antioxidant compounds such as phenolic acid, polyphenols, and flavonoids release free radicals such as peroxide, hydroperoxide, or lipid peroxyl, thereby blocking the oxidative pathway leading to degenerative diseases. Several clinical studies suggest that antioxidants in fruits, vegetables, tea, and red wine are the most important indicators of the effectiveness of these diets in reducing the incidence of chronic diseases, including heart disease and other cancers [1-2].

Cochlospermum religiosum (Linn.) Alston. (Bixaceae) also known as the silk yellow cotton tree because of its bright yellow flowers and soft cotton-like flowers around the seeds. It is a leafy tree found in India, Burma, and Thailand. Traditionally, this plant is used to treat coughs, asthma, jaundice, tuberculosis, inflammation, gonorrhea, and diarrhea [3 - 9]. In addition, dried leaves and flowers are used as a stimulant, laxative, antipyretic, and sedative [10]. Attachment prepared from the base of the stem to the nail to treat fractures [11]. Phytochemical experiments have shown that most of the phytoconstituents present in a plant include alkaloids, glycosides, flavonoids, phenolic, saponins, steroids, coumarins and leucoanthocyanins[12-13]. Isorhamnetin-3-glucosides (methylated quercetin) and myricetinare are phytoconstituents isolated from Cochlospermum religiosum [14 - 15]. Many of its properties have been scientifically proven to be antibacterial, antifungal, insecticidal, antioxidant and hepatoprotective [16 - 17].

Materials and methods:
Plant collection and verification
The roots of the plants were collected in the Nagzira forest. A sample of the plant was dried and their herbarium sheet prepared. It was scientifically proven by Drs. Dongarwar sir, Department of Crops, R.T.M. Nagpur University of Nagpur. The template voucher number is 9240. The roots of the Cochlospermum religiosum were collected and thoroughly washed with water. Cleaned roots were included in macroscopic and microscopic studies. The roots are dried followed by a fine powder with the help of a mechanical grinder and stored in an airtight container for further experimental studies.

Sectioning
The embedded paraffin model was separated with the help of a rotary microtome. The diameter of the section was 10 - 12 mm. passed the phases into a water glass containing water, filtered and the phases were contaminated with Phloroglucinol and conc. hydrochloric acid; then added to glycerin and observed under 10X and 45X low power. The horizontal sections were studied. Digital photographs are taken using a Motic Digital telescope installed DCM(USB

2.0) resolution 350k pixels camera imaging accessory and using Motic analysis software) [18- 19].

Photomicrographs
Small tissue profiles are added with micrographs where needed. The magnification images were taken with a Motic Digital microphone mounted on DCM (USB 2.0) 350k pixel camera recording device and using Motic analysis software).

Physicochemical Research
Physicochemical investigation of the root of Cochlorspermum religiosum Linn. were classified as extraterrestrial organic matter, whole ash, soluble ash, soluble alcohol and extractive soluble water were decimated [18 - 20].

Preparation of crude plant extract:
Approximately 400 g of plant material was dried in the shade under normal natural conditions and reduced in size (and passed through a sieve to remove fines that could clog the soxhlet coupling tube during extraction). The powdered substance was charged in a soxhlet machine and the reduction was done using petroluim ether. The extract is made with a hydroalcoholic solvent (alcohol: water, 6: 4). Hydro alcoholic extract was also diluted with chloroform solvent. Residues obtained after separation were further separated by butanol. The extract and its components were then concentrated using a Rotary vacuum evaporator and the residues were collected in glass bottles [21 - 22].

Preliminary phytochemical investigation
The plant can be considered as a biosynthetic laboratory for a wide range of compounds such as alkaloids, glycoside, thermal oils, tannins, saponins, flavonoids etc. These compounds are called secondary metabolites and are responsible for therapeutic effects. To investigate the presence or absence of primary and secondary metabolites, all extracts were subjected to a chemical experimental battery [18 - 22].

Determination of total Phenolic, total flavonoid and flavanone content.
Total Phenolic Content
Procedure: Total phenolic content was measured using the folin ciocalteu colorimetric method. Briefly, 0.1 ml of experimental sample was mixed with 1 ml of phenol reagent for diluted folin ciocalteu (1:10 with distill water; 0.2 N) (Sigma – Aldrich Chemie, Steinheim, Germany). After 3 minutes, 1 ml of saturated solution of sodium carbonate (75g / l) was added to the mixture and prepared in 10 ml of distilled water. The reaction was kept in the dark for 90 minutes and the absorption was read at 725 nm against the corresponding experiments and normal spaces prepared in the same way except for folin ciocalteu's phenol reagent. Gallic acid has been used as a reference for building a normal curve (20-200 mg / ml). The results were expressed as mg of gallic acid equivalents (GAE) / g extract [23 - 24].

Total flavonoid content
The total Flavonoid content is determined by the aluminum chloride method. Briefly, in 1 ml of experimental solution (1mg / ml), 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate (AlCl3.¬¬6H2O), 0.1 ml of 1 M **sodium acetate (CH3COONA) and 2.3 ml of distill water** were added.After incubating at room temperature for 40 minutes, the absorption of the reaction mixture was measured at 435 nm against the corresponding solvent, prepared in the same manner without the addition of AlCl3. Rutin was used as a reference level and the results were presented as mg of rutin equivalents (RE) / g extract [25].

Determination of flavonone
A modified version of the 2, 4-dinitrophenylhydrazine was used for the extraction of flavonones (Nagy and Grancai, 1996). Hesperidin has been used as a reference level. Twenty milligrams of hesperidin was dissolved in methanol and then diluted to 500, 1000, 1500, 2000 µg / ml. One milliliter of each diluted solution react separately with 2ml of 1% 2, 4-DNPH reagent and 2ml of methanol at 500c for 50 minutes. After cooling at room temperature, the reaction mixture was mixed with 5ml of 1% KOH to 70% methanol and placed in a preheated oven for 2 minutes. Then 1ml of the mixture was mixed with 5 ml of methanol and centrifuged at 1,000 x g for 10 minutes to remove the rain. The supernatant was collected and prepared in 25 ml. Supernatant absorption was measured at 495 nm. Hesperidin was used as a reference level and the results were presented as mg of hesperidin equivalents (HE) / g extract [26].

Antioxidant activity
Testing of the antioxidant activity of hydroalcoholic extract and its components of Cochlospermum religiosum was performed with an *in vitro* assay.

DPPH Measurement Function: (1, 1-Diphenyl -2-Picrylhydrazyl (DPPH) radical scavenging method (DPPH)).
The free radical activity of hydroalcoholic extract and its components of Cochlospermum religiosum and L-ascorbic acid (Vitamin C) is measured by the ability to supply hydrogen or to dispose of radical-scavenging using stable stable DPPH.

A. Preparation of acetate buffer (PH 5.5)
6.8g of sodium acetate is dissolved in 100 ml of distilled water. Add 3ml of glacial acetic acid and keep at PH 5.5.Make the volume to 600ml with distilled water.

B. Preparation of buffered methanol
Take 150ml methanol and volume make up to 250ml with acetate buffer.

C. Preparation of 500mM (stock) DPPH
Dissolved 9.85mg DPPH in 100ml buffered methanol.

D. Preparation of 50mM (working solution) DPPH
Transferred 20ml stock solution in 100ml volumetric flask and make upto 100ml with buffered methanol.

Procedure: Approximately 50mM of DPPH solution was prepared with methanol in the buffer and 4.9 ml of this solution was added to 0.1 ml of solution extracted at different concentrations (10-100 µg / ml).
Incubate for 30 minutes at 370c.30 minutes later; absorption is rated at 517 nm. The low absorption of the reaction mixture indicates high free radical emissions activity. The ability to hunt the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = \frac{(A_{cont} - A_{test})}{A_{cont}} \text{ X } 100$$

$A_{cont:}$ means absorbance of the control reaction
A_{test}: means absorbance in the existence of the extracts.
The antioxidant activity of the extracts was stated as IC_{50}. the IC_{50} value represents the minimal concentration of a drug that is required for 50% inhibition *in vitro*. The outcomes of anti-oxidant action of extracts of *Cochlospermum religiosum* by DPPH free radical scavenging method [27, 28, 29, 30].

Result and Discussion

Pharmacognostic studies

In Pharmacognostic study of roots of *Cochlospermum religiosum (Linn)* macroscopy, microscopy, powder characteristic, physical parameters, and extractive values were studied.

Deciduous trees, to 10 metre high, bark 20-25 mm dense, external surface dark grey, fibrous; branchlets thick.

Fig 1: Plant *Cochlospermum, religiosum* Linn.

Fig 2: Herbarium sheet of *Cochlospermum religiosum* Linn.

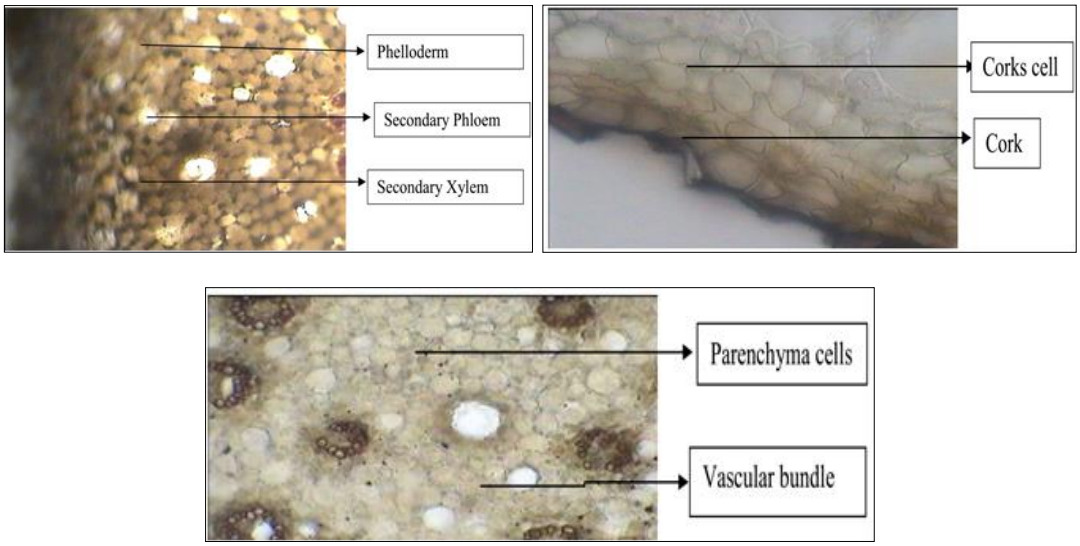
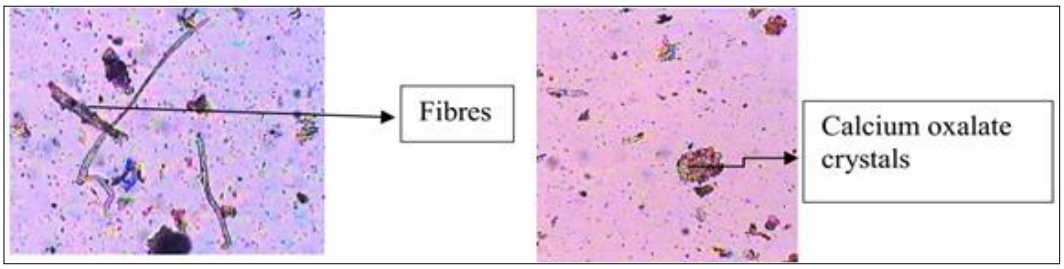


Fig 3: T.S. of *Cochlospermum religiosum*

Description of Microscopy of root of *Cochlospermum religiosum*: The detailed transverse section shows Phelloderm, Secondary Phloem, and Secondary Xylem, Corks, Corks cell, Parenchyma cells and Vascular bundle.

Powder characteristic



Fibre stained with Phloroglucinol + conc. HCL. (1:1) Calcium oxalate crystals stained with dil. Sulphuric acid

Fig 4: Powder charecterstics of *Cochlospermum religiosum*

Physicochemical investigation

Table 1: Results of Ash and Extractive value

(%w/w) Extractive values	Water soluble Extractive value	16.24%
	Alcohol soluble Extractive value	19.26%
(%w/w) Ash values	Total ash Value	7.33%
	Acid insoluble ash value	1.25%

Table 2: Phytochemical screening of crude extract and its fractions

Plant Constituent	Identification Test	HACR	CFCR	BFCR	BIFCR
Sterols	Libermann Burchard test	_	_	_	_
	Salkowaski test	_	_	_	_
Alkaloids	Dragendorff's test	+	+	+	_
	Mayer's test	+	+	+	_
	Hager's test	+	+	+	_
	Wagner's test	+	+	+	_
Tannins	Potassium dichromate test	+	+	+	_
	Lead acetate test	+	+	+	_
	Ferric chloride Test	+	+	+	_
Flavonoids	Shinoda test	+	+	+	+
Carbohydrates	Molish's test for carbohydrates	+	+	+	_
	Biuret test	+	+	+	_
Proteins	Fehling's test	_	_	_	_
	Xantho-protein test	_	_	_	_
Saponins	Foam test	_	_	_	_

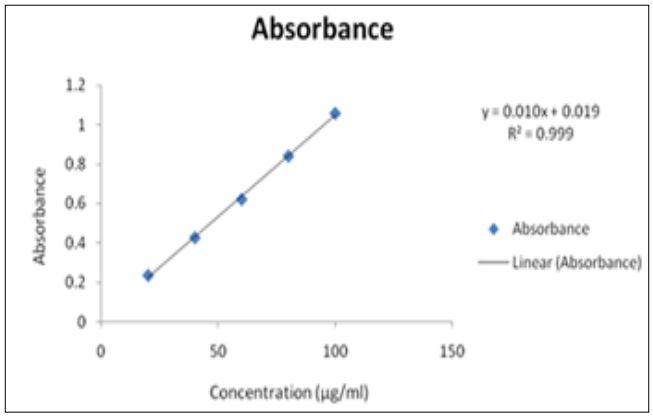
1] HACR=Hydroalcoholic extract
2] CFCR=Chloroform soluble fraction
3] BFCR=Butanol soluble fraction
4] BIFCR=Butanol insoluble fraction
+:- Found to be present, −:- Found to be absent

The outcomes of preliminary phytochemical screening of various crude extract shown the presence of carbohydrates, alkaloids, tannins in hydroalcoholic extract, as well as butanol soluble and chloroform soluble fraction. Furthermore flavonoids were shown to be existing in hydroalcoholic extract and all fractions.

Table 3: Quantitative estimation of total polyphenol in hydro alcoholic extracts and its fractions

Sr. No.	Concentration (µg/ml)	Absorbance	Gallic acid equivalent mg/g
	20	0.236	-
	40	0.428	-
	60	0.623	-
	80	0.843	-
	100	1.06	-
1.	HACR	0.454	43.50
2.	CFCR	0.205	18.66
3.	BFCR	0.284	26.50
4.	BIFCR	0.157	13.86

The total polyphenols concentration was expressed as mg/g of dry extract. The amout of total polyphenol in the extract was determined as mg of Gallic acid equivalent using an equation obtained from the standard Gallic acid graph.



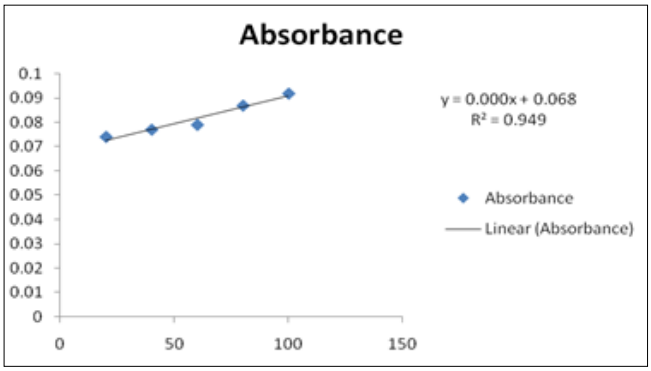
Graph 1: Calibration curve for Gallic acid

Calculation

Using the above equation the total phenolic content in the hydroalcoholic extract, chloroform fraction, butanol fraction and butanol insoluble fraction of hydroalcoholic extract of roots (active extract) of *C. religiosum* were found to be 43.50, 18.66, 26.50 and 13.86 mg/g respectively.

Table 4: Quantitative estimation of total flavonoids in hydroalcoholic extracts and its fractions. (Standard: Rutin)

Sr. No.	Concentration (µg/ml)	Absorbance at 435nm	Rutin equivalent mg/g
	20	0.074	-
	40	0.077	-
	60	0.079	-
	80	0.087	-
	100	0.092	-
	HACR	0.21	14.2
	CFCR	0.18	11.2
	BFCR	0.25	18.2
	BIFCR	0.106	3.8



Graph 2: Calibration curve of Rutin

Calculation

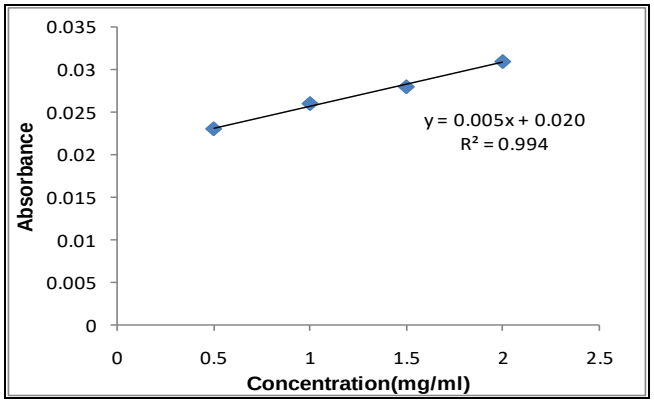
Accordind to given above above equation the total flavonoid content in the hydroalcoholic extract, chloroform fraction, butanol fraction and butanol insoluble fraction of hydroalcoholic extract of roots (active extract) of *C.religiosum* were found to be 14.2, 11.2, 18.2 and 3.8 mg/g respectively.

Result of Flavonone

Table 5: Quantitative estimation of total flavonones in hydroalcoholic extracts and its fractions

Sr. No.	Concentration (µg/ml)	Absorbance at 495nm	Hesperidin equivalent mg/g
	500	0.023	
	1000	0.026	
	1500	0.028	
	2000	0.031	
	HACR	0.05	6.0
	CFCR	0.023	0.6
	BFCR	0.047	5.4
	BIFCR	0.025	1.0

The amount or concentration of flavonone was expressed as mg/g of dry extract. The amount of total flavonone in the extract was determined as mg of hesperidin equivalent using an equation obtained from the standard hesperidin graph.



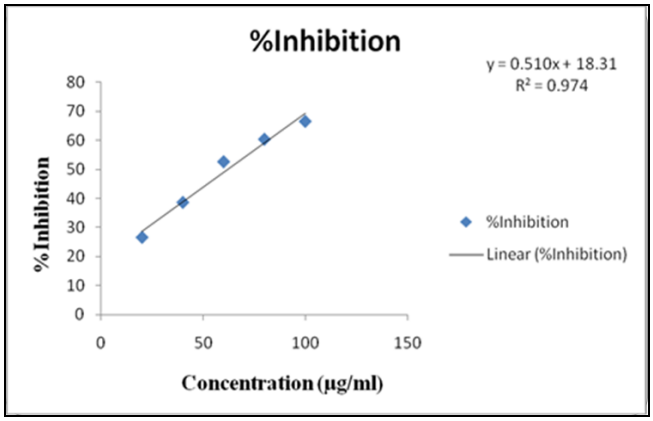
Graph 3: Calibration curve for Hesperidin

Calculation

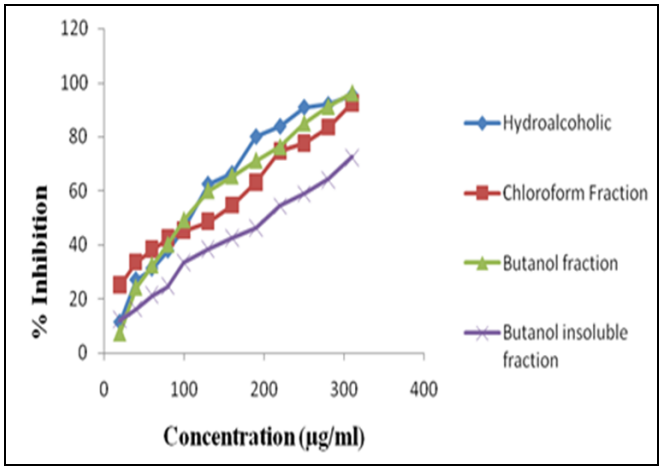
With the help of above equation the amount of total flavonone in the hydroalcoholic residue, chloroform fraction, butanol fraction and butanol insoluble of hydroalcoholic extract of roots (active extract) of *C. religiosum* were found to be 6.0, 0.6, 5.4 and 1.0 respectively of the extract.

Antioxidant activity

DPPH scavenging method used for the antioxidant activity of the extracts and was expressed as IC_{50} value. The results of anti-oxidant activity of extracts of *Cochlospermum religiosum* using DPPH free radical scavenging method were shown below.



Graph 4: Calibration curve of Ascorbic acid



Graph 5: Antioxidant activity by DPPH method of hydroalcoholic extract and its fractions

Table 6: DPPH radical scavenging activity IC_{50} (µg) of hydroalcoholic extract and its fraction

Sr. No.	Extract / Fractions	IC_{50} (µg)
1.	Hydroalcoholic extract	117.93
2.	Chloroform fraction	143.45
3.	Butanol fraction	124.66
4.	Butanol insoluble fraction	201.67
5.	Ascorbic acid	62.13

Graph 4 and 5 revealed a significant ($p < 0.01$) reduction in the concentration of DPPH radical due to the scavenging capacity of soluble solids of hydroalcoholic extract and its different fractions of *C.religiosum* and the standard ascorbic acid as a standard compound, revealed the maximum activity at all concentrations. The free radical scavenging capacity of hydroalcoholic extract and its fractions was evaluated by its capacity to scavenge the constant DDPH radical. This test provided information on the activity of the test compound with a constant free radical since its odd electron DPPH gives strong absorption bands at 517nm in visible spectroscopy (deep violet color).

The concentration needed for 50% inhibition of hydroalcoholic extract, chloroform fraction, butanol and butanol insoluble fraction was found to be 117.9 µg / ml 143.45 µg / ml 124.66 µg / ml and 201.67 µg / ml respectively.

Conclusion

The DPPH scavenging ability of root of *Cochlospermum religiosum* in hydroalcoholic extract, chloroform fraction, butanol and butanol insoluble fraction was completed. The result shown as that the hydroalcoholic extract showed superior activity than the other extract. But standard ascorbic acid showed significantly higher activity than that of all extracts; the IC_{50} value of ascorbic acid was 62.13µg/ml. The IC_{50} value of Chloroform fraction was 143.45 µg/ml when compared to their corresponding Butanol fraction and Butanol insoluble fraction with Ic50 values 124.66 µg/ml and 201.67 µg/ml respectively.

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Conflicts of Interest

Conflict of interest declared none.

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