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Phytochemical screening, anti-oxidant and anti-microbial activity of leaf, stem and flower of Rangoon creeper: a comparative study

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Abstract
Rangoon creeper, *Quisqualis indica* Linn. is an important shrub belonging from family Combretaceae. This plant is traditionally used for cell aging or other oxidative stress-related diseases. This study was done to quantify the polyphenols, flavonoids and to investigate the anti-oxidant and anti-microbial activity of aqueous and ethyl acetate crude and dry sample extracts of leaf, stem and flowers. The phytochemical quantitative assay was done by the Aluminium chloride, Folin-Ciocaltue Method and the anti-oxidant activity was measured by ABTS and H_2O_2 radical scavenging assay. To investigate the anti-microbial activity, disk diffusion method was used. The highest amount of total polyphenolic content was found in flower of crude (102.24±0.48 mg GAE/g of tissue) and dry (83.75±0.47 mg GAE/g of tissue) samples in ethyl acetate extracts. The highest amount of total flavonoids content was found in crude leaf (60.67±0.31 mg QE/g of tissue) and dry flower (54.27±0.20 mg QE/g of tissue) of ethyl acetate extract. The aqueous extracts of leaf and flower showed the higher anti-oxidant activity than the ethyl acetate extracts. The crude and dry samples of leaf and flower extracts showed maximum zone of inhibition for both the solvents rather than stem against *Staphylococcus aureus* as well as *Escherichia coli*. The results revealed that the leaves and flowers of *Quisqualis indica* contained more bioactive substances than stem, which may be responsible for high anti-oxidant properties. So, Rangoon creeper can be used to prevent various oxidative stress-related diseases.

Keywords: Rangoon creeper, polyphenols, flavonoids, anti-oxidants, anti-microbial

Introduction
Quisqualis indica Linn. Commonly known as Rangoon creeper or Chinese Honeysuckle, basically found in Asia. In West Bengal or Bangladesh it is known as Malati or Madhumalati. It belongs to the family Combretaceae. In this plant, leaves are opposite or elliptical. Flowers are nearly 8 cm long and 4 cm wide. The flower cluster of this plant is mainly observed in three colours as white, pink and red. The colour of flower is change due to decreasing of temperature and time [1]. The flower is initially white in colour. Then it turns to pink and after that it turns attractive red in colour. In this plant, all the essential phyto-constituents are present that are required for normal and good human health.

Phytochemicals are naturally occurring plant derived compounds that are present in the various parts of medicinal plants and that have defense mechanisms and protect from various diseases. Chlorophyll, proteins and common sugars are included in primary metabolites and plant secondary metabolites are terpenoid, alkaloids, flavonoids and phenolic compounds [2]. In all plants there are numerous bioactive compounds are present and they have various structures and functions [3]. Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens [4]. Flavonoids are phenolic compounds which are widely distributed in the human diet as well [5]. The bioavailability, metabolism, and biological activity of flavonoids depend on the configuration, total number of hydroxyl groups, and substitution of functional groups about their nuclear structures [6]. Phytochemicals are known to be having powerful chain breaking, lipid oxidation and anti-oxidant activities as well as polyphenols are very important plant constituents because of their radical scavenging ability due to presence of their hydroxyl groups[7], [8]. Anti-oxidant activity and phytochemical properties may reduce the risks of liver and cardiovascular disease, cancers, and degenerative diseases [9]. Anti-oxidant compounds are necessary to improve the quality of a healthy lifestyle [10].

The main focus of this study is to estimate the total polyphenols, flavonoids content, and to determine the anti-oxidant and anti-microbial activity of crude and dry extracts of leaf, flower and stem of the plant using two different solvents i.e. aqueous and ethyl acetate.

Fig 1: Leaves, stem and flowers of *Quisqualis indica*

Material and Methods

Plant Material

The leaves, stems and flowers of *Quisqualis indica* Linn.were collected from Salt Lake, Kolkata and identified by Dr. Madhusudan Mondal, Former Additional Director of Botanical Survey of India, and West Bengal. The samples were cleaned using tap water to remove the soil and then rinsed with double distilled water. The plant samples were dried for 15-20 days at room temperature. These samples were then ground to a fine powder and were stored in polythene bottles at 4°C till the time of experiments.

Fig 2: Crude sample of *Quisqualis indica*

Chemicals and Reagents

All the chemicals and reagents used in the experiments were of analytical grade. Folin-ciocalteu, aluminum chloride and ascorbic acid were obtained from Merck Life Science Private Limited, Mumbai. Sodium nitrite and sodium carbonate were purchased from RFCL Limited, New Delhi. Gallic acid, sodium hydroxide and hydrogen peroxide were supplied by SD Fine-Chem Limited, Mumbai. Quercetin was obtained from Sisco Research Laboratories Pvt. Ltd., Maharashtra and ABTS from Tokyo Chemical Industry Co. Ltd., Japan.

Preparation of extracts

Preparation of crude extracts

Crude extract of leaves, stems and flowers were obtained by grinding finely cut 1 g of fresh leaves, stems and flowers of *Quisqualis indica* Linn. with 25mL of solvents. All the extracts were heated and centrifuged at 10,000 rpm for 10 min. The supernatant was used for further studied.

Preparation of dry extracts

1 g of dry powder of leaves, stem and flower of the plant were dissolved in 25mL of solvents separately and kept on the rocker for 24 hrs. for extraction. Then the extracts were centrifuged at 10,000rpm for 10min. The supernatant was used for further studied.

Determination of polyphenols content [11]

The polyphenol contents of leave, stem and flower were determined by using the Folin-Ciocalteu method with slight modifications. The standard curve was prepared by using Gallic acid as standard reagent. The results were expressed as mg Gallic acid equivalents/g of dry or crude tissue.

Determination of flavonoid content [12]

The flavonoid content of leave, stem and flower were determined by the aluminum chloride colorimetric assay with slight modifications. The standard curve was prepared by using Quercetin as a standard reagent. The results were expressed as mg Quercetin equivalents/g of dry or crude tissue.

Study of anti-oxidant activity

Hydrogen peroxide scavenging assay [13]

The solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). In case of control, the absorbance of hydrogen peroxide at 230 nm without sample extracts was taken. The percentage inhibition activity was calculated from.

% Inhibition= $[(A_0-A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of extract or standard. A calibration curve was plotted with % inhibition activity versus concentration of standard Gallic acid.

ABTS free radical scavenging assay [14]

Plant extracts were allowed to react with the ABTS solution and the absorbance was taken at 734 nm after 30mins using a spectrophotometer. Percentage inhibition of absorbance was calculated using the formula,

% Inhibition = $[(A_0-A_1)/A_0] \times 100$,

Where, A_0 is absorbance of ABTS radical + methanol; A_1 is absorbance of ABTS radical + sample extract/standard. A standard curve was plotted with % inhibition versus concentration of ascorbic acid used as standard.

Study of anti-microbial activity

Test Organism [15]

Two test organisms were used to investigate the anti-microbial activity of extracts of leaf, stem and flower of *Quisqualis indica*, 1) *Staphylococcus aureus* (J) 2) *Escherichia coli* (H), collected from Department of Microbiology, Calcutta University, West Bengal, and India. *Escherichia coli* are a gram negative bacteria and *Staphylococcus aureus* is gram positive bacteria.

Preparation of inoculums [16]

One loopful of both strains was taken from subculture and inoculated in two separate test tubes containing 10 ml sterile nutrient broth. Two test tubes were incubated at 37°C for 18 hrs.

Preparation of disc [17]

Anti-microbial activity of *Quisqualis indica* was performed by Disc Diffusion Method. The discs were produced from Whatman filter paper, punched into 5mm in size and sterilized.

Anti-microbial activity using disc diffusion method [18]

Disc diffusion assay was carried out to determine the anti-microbial activity of *Quisqualis indica* against test microorganisms using standard protocol with slight modifications. For aqueous extract, double distilled water and for ethyl acetate extract, ethyl acetate was used as control. Petri plates were incubated at 37^0C for 16-18 hrs. After that the zone of inhibition was measured in triplicate.

Statistical Analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. All the statistical analyses were done by using MS Excel Software.

Results and Discussions

Estimation of total polyphenol content

The total polyphenol content of crude and dry sample of leaf, flower and stem of aqueous (Aq.) and ethyl acetate (E.A.) solvent were showed in Figure 3.

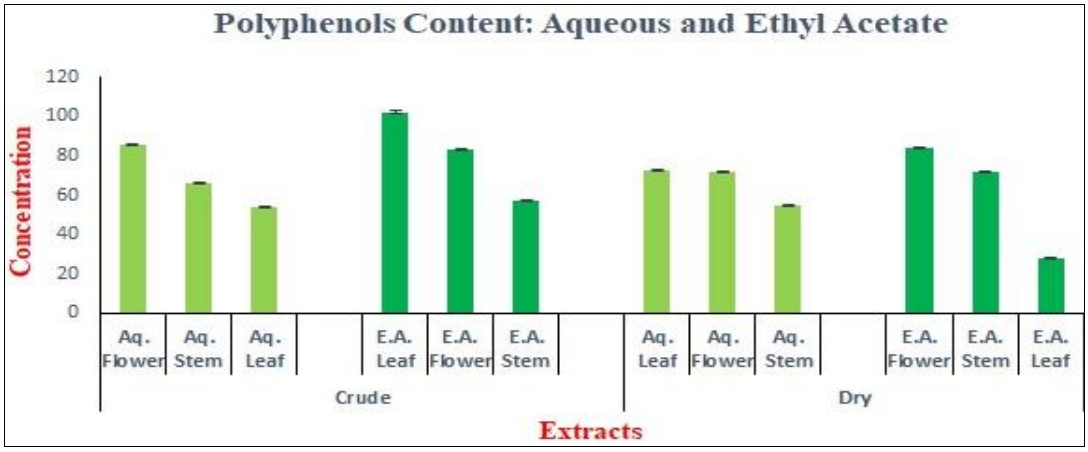


Fig 3: Total polyphenol content (mg GAE/g of crude or dry tissue)

It was clearly observed that the total polyphenol content in ethyl acetate solvent was significantly higher than that of aqueous solvent for both crude and dry samples. Among all the crude samples, flower in ethyl acetate solvent showed highest amount of polyphenol (102.24±0.48 mg GAE/g of tissue) while the lowest content was observed in leaf of aqueous solvent (54.02±0.47 mg GAE/g of tissue). In case of dry samples, flower in ethyl acetate solvent showed highest amount of polyphenol (83.75±0.47 mg GAE/g of tissue) and leaf in ethyl acetate solvent exhibited lowest amount (28.23±0.040 mg GAE/g of tissue).

Estimation of total flavonoids content

The total flavonoids content of crude and dry sample of leaf, flower and stem of aqueous (Aq.) and ethyl acetate (E.A.) solvent were shown in Figure 4. The flavonoids content for crude samples is found to vary between 21.25±0.30 (stem of aqueous solvent) to 60.67±0.31 mg QE/g of tissue (leaf of ethyl acetate solvent). Among all the dry samples, the lowest value was observed for stem in ethyl acetate solvent (5.26±0.28mg QE/g of tissue) and highest for flower in ethyl acetate solvent (54.27±0.20 mg QE/g of tissue).

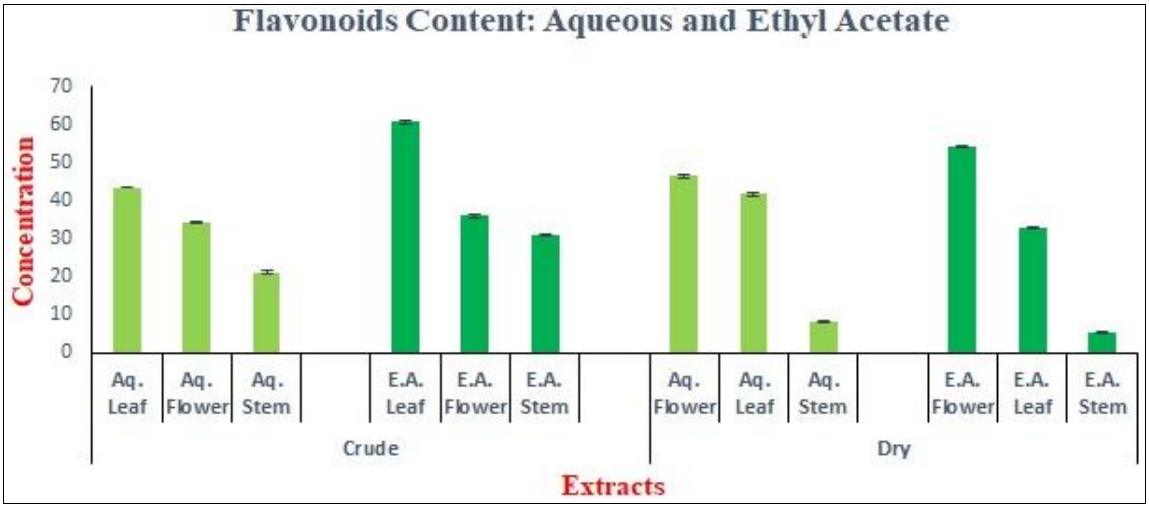


Fig 4: Total flavonoid content (mg QE/g of crude or dry tissue)

Estimation of anti-oxidant activity by hydrogen peroxide radical scavenging assay

Phenolics can scavenge or stabilize the free radicals either by reduction process or by forming complex with oxidizing compounds. In this study, we have evaluated the hydroxyl radical scavenger activity of aqueous (Aq.) and ethyl acetate (E.A.) extracts of both crude and dry samples of leaf, flower and stem of *Quisqualis indica*, mentioned in Figure 5.

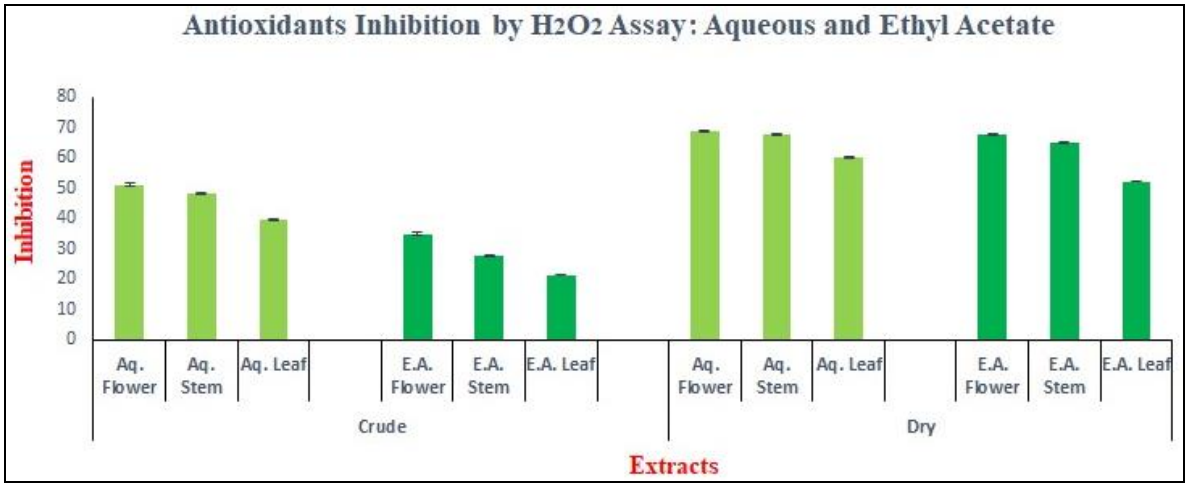


Fig 5: Free radical scavenging (%) by Hydrogen Peroxide

In case of crude samples, the percentage inhibition by hydrogen peroxide scavenging assay ranged from 21.36±0.17% in ethyl acetate leaf sample to 51.09±0.43% in aqueous flower sample. The free radical scavenging of dry samples was found to range between 52.27±0.12% (leaf in ethyl acetate solvent) to 68.8±0.42% (flower in aqueous solvent).

Simultaneously, we also calculated the total anti-oxidant content of crude and dry sample of leaf, stem and flower of *Quisqualis indica* for both the solvents aqueous (Aq.) and ethyl acetate (E.A.) by inhibiting hydrogen peroxide radicals, given in Figure 6. Both crude and dry flower sample of aqueous extract exhibited the highest anti-oxidant content of 6.57±0.06 and 9.05±0.12mg GAE/g of tissue respectively, and the lowest amount was observed in crude and dry leaf sample of ethyl acetate extract, 2.4±0.08 and 6.74±0.03 mg GAE/g of tissue respectively.

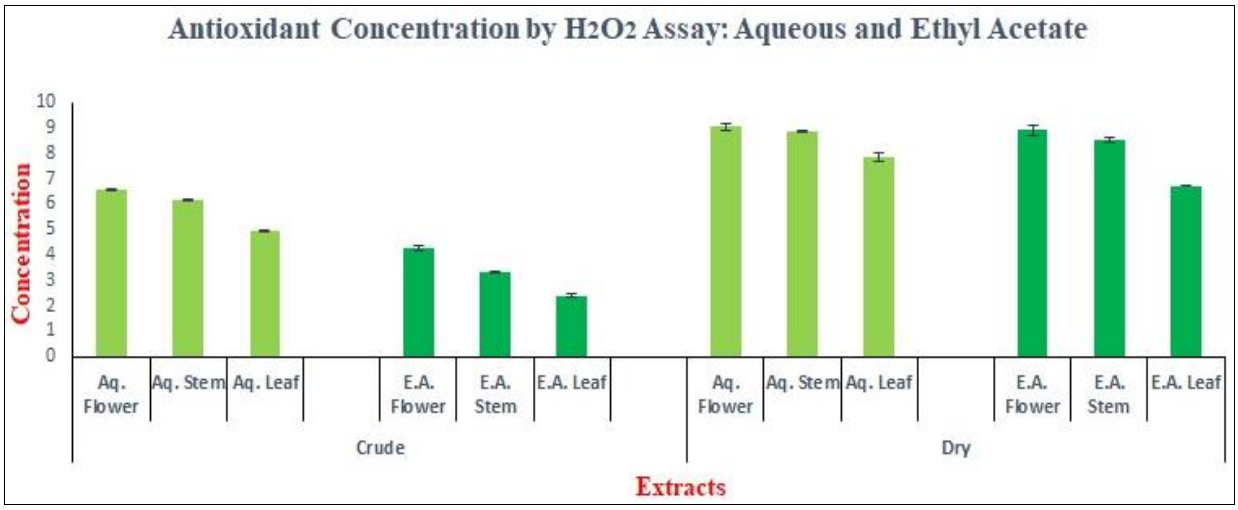


Fig 6: Total anti-oxidant concentration (mg GAE/g of crude or dry tissue) by H_2O_2 scavenging assay.

Estimation of anti-oxidant activity by abts free radical scavenging assay

The results of percentage inhibition of leaves, stem and flower of *Quisqualis indica* in aqueous (Aq.) and ethyl acetate (E.A.) extracts by ABTS free radical scavenging assay were shown in Figure 7. In case of crude samples, the percentage inhibition ranged from 24.78±0.1% in ethyl acetate stem sample to98.71±0.14% in aqueous flower sample. The free radical scavenging of dry samples was found to range between 36.38±0.07% (stem in ethyl acetate solvent) to 97.99±0.35% (leaf in aqueous solvent).

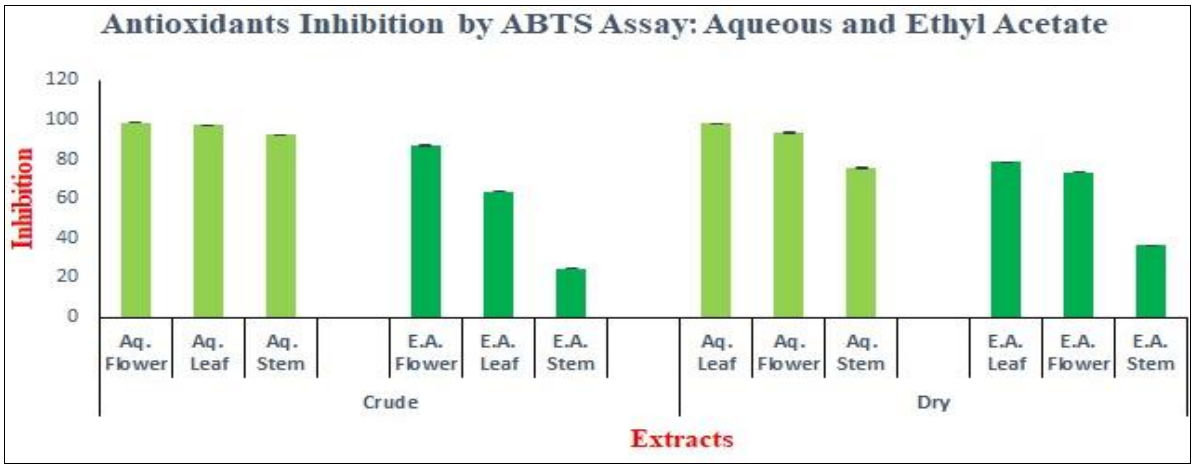


Fig 7: Free radical scavenging (%) by ABTS Assay

Based on the results of free radical scavenging, total anti-oxidant content of crude and dry sample of leaf, stem and flower of *Quisqualis indica* for the solvents, aqueous (Aq.) and ethyl acetate (E.A.) extracts by ABTS assay were calculated and showed in Figure 8.

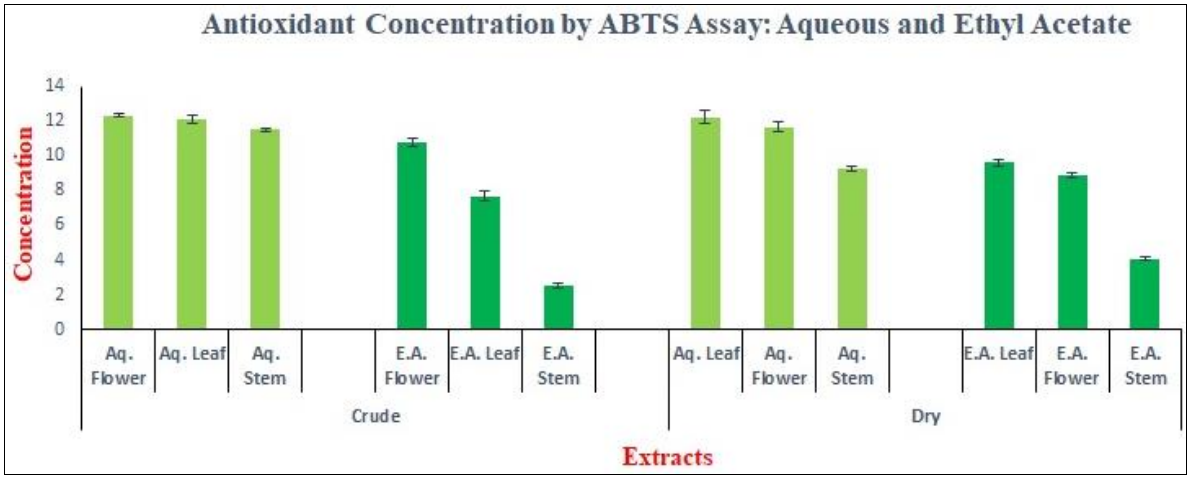


Fig 8: Total Anti-oxidant concentration (mg AAE/g crude or dry tissue) by ABTS Assay

The anti-oxidant content for crude samples by ABTS assay was found to vary between 2.53±0.14 (stem of ethyl acetate solvent) to 12.33±0.08 mg AAE/g of tissue (flower of aqueous solvent). Among all the dry samples, the lowest value was observed for stem in ethyl acetate solvent (4.07±0.11 mg AAE/g of tissue) and highest for leaf in aqueous solvent (12.24±0.41 mg AAE/g of tissue).

Determination of anti-microbial activity

Anti-microbial activity of crude samples

The results of the study indicated that the aqueous and ethyl acetate extracts prepared from the crude sample of leaves, stem and flower of *Quisqualis indica* showed inhibitory activity against these two bacteria, shown in Table 1.

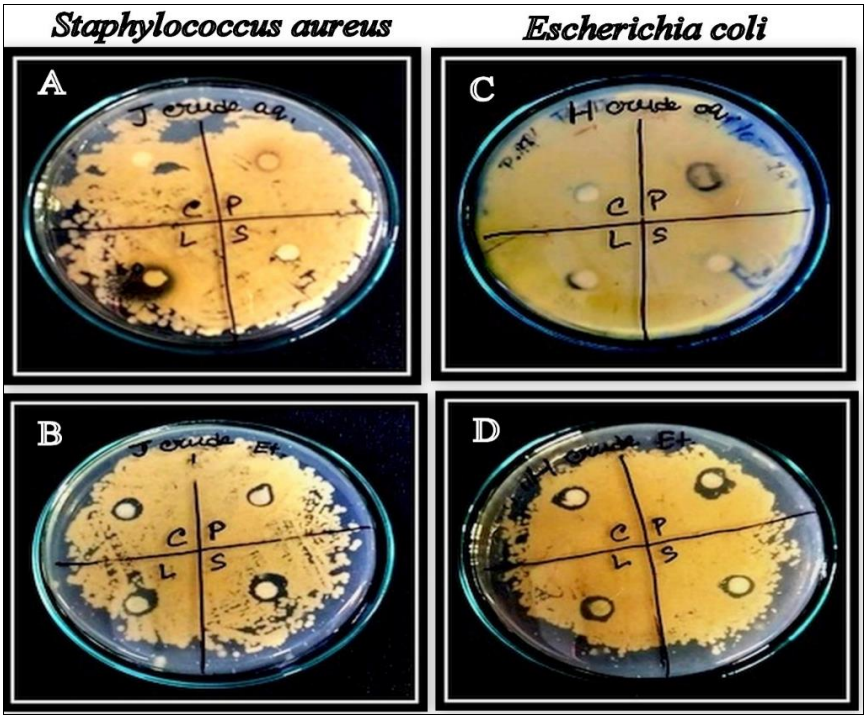
Table 1: Zone of inhibition produced by crude samples

Crude Aqueous Extract		**Crude Ethyl Acetate Extract**	
Organisms Name	**Zone Diameter (mm)**	**Organisms Name**	**Zone Diameter (mm)**
Staphylococcus aureus		*Staphylococcus aureus*	
Control	Nil	Control	8.66
Flower	Nil	Flower	1.66
Stem	Nil	Stem	5.66
Leaf	10.66	Leaf	3.33
Organisms Name	**Zone Diameter (mm)**	**Organisms Name**	**Zone Diameter (mm)**
Escherichia coli		*Escherichia coli*	
Control	Nil	Control	8.33
Flower	3.66	Flower	4.66
Stem	Nil	Stem	3.66
Leaf	1.66	Leaf	2

In case of crude aqueous extracts, only leaf of *Quisqualis indica* showed highest activity against *Staphylococcus aureus*, producing 10.66 mm zone of inhibition, shown in Figure 9. For ethyl acetate extracts, stem showed the largest zone of inhibition of 5.66 mm and flower showed the lowest zone of inhibition of 1.66mm against *Staphylococcus aureus*, shown in Figure 10.

The crude aqueous extracts of flower of *Quisqualis indica*

showed the maximum zone of inhibition of 3.66 mm & leaf showed 1.66 mm of minimum zone of inhibition against *Escherichia coli*, shown in Figure 11. In case of ethyl acetate extracts, crude flower showed the highest activity by producing 4.66mm zone of inhibition and leaf produced the lowest zone of inhibition of 2mm against *Escherichia coli*, shown in Figure 12.



A =>**Fig 9:** Anti-microbial Activity of Crude Aqueous Extracts against *Staphylococcus aureus.*
B =>**Fig 10:** Anti-microbial Activity of Crude Ethyl Acetate Extracts against *Staphylococcus aureus.*
C =>**Fig 11:** Anti-microbial Activity of Crude Aqueous Extracts against *Escherichia coli*.
D =>**Fig 12:** Anti-microbial Activity of Crude Ethyl Acetate Extracts against *Escherichia coli*.

Anti-microbial activity of dry samples

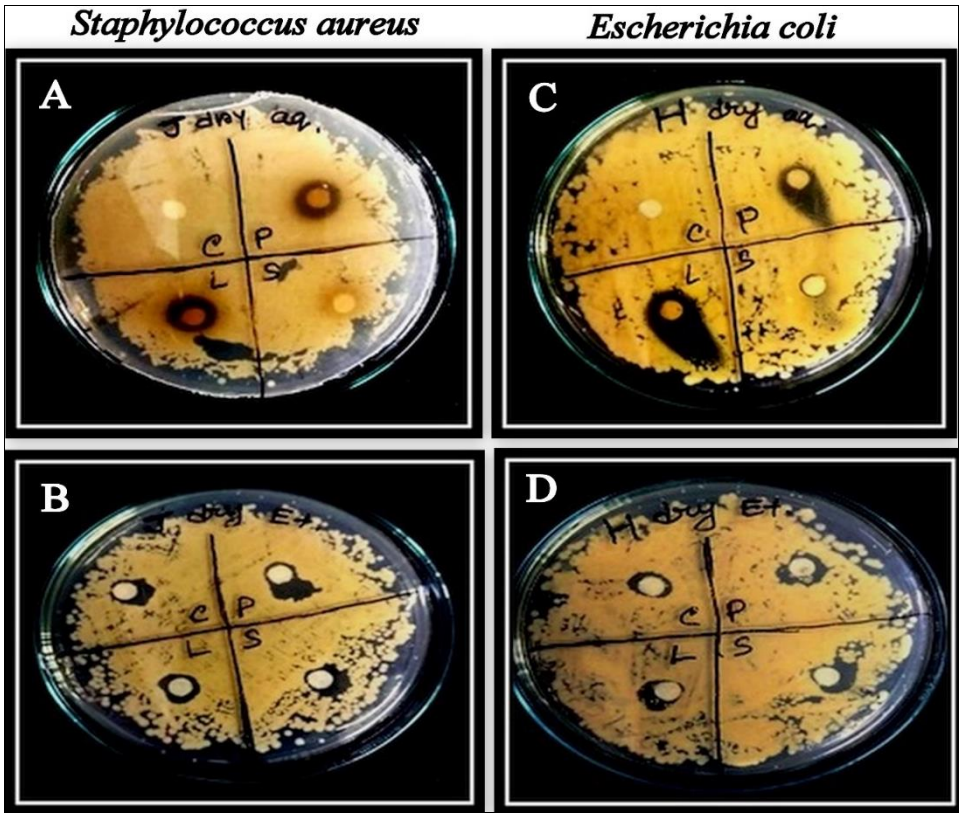
The inhibitory activity against *Staphylococcus aureus* and *Escherichia coli* by the aqueous and ethyl acetate extracts prepared from the dry sample of leaves, stem and flower of *Quisqualis indica* was shown in Table 2

Table 2: Zone of inhibition produced by dry samples

Dry aqueous Extract		**Dry Ethyl Acetate Extract**	
Organisms Name	**Zone Diameter (mm)**	**Organisms Name**	**Zone Diameter (mm)**
Staphylococcus aureus		*Staphylococcus aureus*	
Control	Nil	Control	13.66
Flower	11.66	Flower	3.66
Stem	Nil	Stem	3.33
Leaf	11	Leaf	4.66
Organisms Name	**Zone Diameter (mm)**	**Organisms Name**	**Zone Diameter (mm)**
Escherichia coli		*Escherichia coli*	
Control	Nil	Control	9
Flower	7.66	Flower	3.66
Stem	Nil	Stem	2.66
Leaf	10.33	Leaf	3.33

In case of dry aqueous extract, both flower and leaf of *Quisqualis indica* showed significant zone of inhibition of 11.66 mm & 11 mm against *Staphylococcus aureus*, shown in Figure 13. Among ethyl acetate extracts, leaf showed maximum 4.66 mm zone of inhibition and stem showed minimum 3.33 mm zone of inhibition against *Staphylococcus aureus*, shown in Figure 14.

Similarly, In dry aqueous extract, both leaf and flower of *Quisqualis indica* showed significant anti-microbial activity by producing 10.33mm and 7.66mm zone of inhibition against *Escherichia coli*, respectively, shown in Figure 15.In case of dry ethyl acetate extracts, flower showed 3.66mm maximum zone of inhibition and stem showed the lowest 2mm zone of inhibition against *Escherichia coli*, shown in Figure 16.



A =>**Fig 13:** Anti-microbial Activity of Dry Aqueous Extracts against *Staphylococcus aureus.*
B =>**Fig 14:** Anti-microbial Activity of Dry Ethyl Acetate Extracts against *Staphylococcus aureus.*
C =>**Fig 15:** Anti-microbial Activity of Dry Aqueous Extracts against *Escherichia coli.*
D =>**Fig 16:** Anti-microbial Activity of Dry Ethyl Acetate Extracts against *Escherichia coli.*

Conclusion

The crude and dry sample of leaf and flower of *Quisqualis indica* for both the solvents exhibited strong anti-oxidant activity by inhibiting hydrogen peroxide radicals and ABTS free radicals when compared with standards like Gallic acid and Ascorbic acid, respectively. In addition to this, leaf and flower contain a significant amount of polyphenols and flavonoids than stem, which play a major role in controlling oxidation reaction. These samples exhibited their anti-bacterial activity against *Staphylococcus aureus* as well as *Escherichia coli* for both the solvents. Thus, this easily available medicinal plant can be used as a source of potential anti-oxidant. The highest amount of total polyphenol and flavonoids content were found in leaf and flower of crude and dry sample of ethyl acetate extracts, respectively. The bioactive compounds may vary due to environmental factors or other biogeochemical stresses [19, 20, 21]. The aqueous extracts of leaf and flower showed the higher anti-oxidant activity than the ethyl acetate extracts. The leaf showed maximum zone against *Staphylococcus aureus*, and flower showed maximum zone against *Escherichia coli*. The results revealed that the leaves and flowers of *Quisqualis indica* contained more bioactive substances than stem, which may be responsible for high anti-oxidant as well as anti-microbial properties. So, *Quisqualis indica* can be used to prevent various oxidative stresses related diseases. Though further research work is recommended in this context.

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Contributions

Marked authors contributed equally.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this research article.

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