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Phytochemical profiling, Antioxidant and Antibacterial potentials evaluation of the methanolic extract of Sarcochlamys *pulcherrima* (Roxb.) Gaud, a medicinal plant.

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ABSTRACT

***Sarcochlamys pulcherrima* (Roxb.) Gaud is commonly known as Dogal tree. The tender leaves are traditionally used a special dish in *Baikhow* religious festival by Rabha tribe of Assam. The plant leaves are also used as natural medicine in local health care system by tribe's people. The present study intended to evaluate the antibacterial potentiality of the plant against bacterial strains, including Gram-positive bacteria e.g., *Staphylococcus aureus* and Gram-negative bacteria e.g., *Escherichia coli.* Moreover, antioxidant potential was evaluated on the experimental material by DPPH. In-vitro qualitative study on this plant revealed that it has a natural rich source of potent antioxidant properties. It has anti-microbial activity against human pathogen namely *S. aureus* and *E. coli.* The tested sample showed positive zones of inhibition against *S. aureus* but no zone of inhibition against *E. coli.* The positive controls kanamycin and gentamycin showed zones of inhibition against both the organism. The present investigation results revealed that *S. pulcherrima* (Roxb.) Gaud has antibacterial potentiality facilitating inhibition of bacterial growth, reproduction, or survival. The qualitative phytochemical analysis of the plant extract shows that it contents alkaloids, reducing sugar, steroids, phenolic, flavonoids, and acidic compounds which attributes rich source of antioxidant. IC_{50} values of the tested samples tender leaves of *S. pulcherrima* shows high concentration of antioxidant activity. IC_{50} values of the sample shows high concentration of antioxidant activity (241.04 ± 0.11 µg/ml). Traditionally, tender leaves are consumed as a vegetables and natural medicinal purposes to treat various diseases by Rabha tribes. Therefore, this plant may be antibacterial potential to medicine, food preservation, cosmetics, water treatments and textile industry..**

1. INTRODUCTION:

Rabha tribe is distinctly prime habitants of Rabha Hasong Autonomous Council area of Assam. They normally collected green wild leafy vegetables for edible, as well used traditionally in natural medicinal purposes. Green leafy vegetables have a very high protective food value. Leafy vegetables contain natural antioxidant, phytochemicals, phytonutrients such as vitamins, proteins, dietary fibre, essential fatty acid and minerals but no

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cholesterol, very little fats, sugar and sodium, and calories[1]. Leafy vegetables are good source of phytochemicals[2]. Over the years green leafy vegetables are used for edible as well as medicinal purposes[3]. The tribe indiscriminately collects leafy vegetables from natural habitat and sells them in market to earn their livelihood. *Sarcochlamys pulcherrima* (Roxb.) Gaud plant species is called fok xaak by Rabha tribe peoples including several of its sub-caste such as Rongdani, Mai-Turi, Kosha Rabha, Totala Rabha, Pati Rabha, Dahari Rabha, Bitla Rabha and Modahi Rabha. The tender leaves of the targeted plant are popularly ingested as a vegetable and used as medicinal purposes[4]. The tribe prepare a special dish for their religious festival by using the delicate leaves of the targeted plant. At the onset of Bohag Bihu, the utmost predominant agro-based festival of state of Assam, conventionally the Assamese people gather hundred and one leafy vegetables to prepare a unique local recipe, which include *S. pulcherrima* also. Traditionally local dwellers have an opinion that the prepared recipe bears natural medicinal values that promote good health[5]. Thus, this indicates that the tender leaves of the ethno-medicinal plants have high potency of antioxidant and antimicrobial properties[6].

S. pulcherrima (Roxb) Gaud is an evergreen shrub commonly of the tropical rain forest. However, these are also seen to be dwelling in dank secondary forests on the tidalplains of countries like Bhutan, Myanmar, Indonesia and Thailand [4]. In several hilly and plains region of the Northeastern region of India the targeted plant is seen to be growing in the wilds It is reported that several ethnic tribes residing in different states of Northeast India namely Assam, Meghalaya, and Nagaland, and even in its neighbouring country Bangladesh traditionally use this plant leaves as edible vegetable and medicines.[5]. Different communities has named *S. pulcherrima* by different vernacular names such as Mechaki (Assamese)[5, 7], Fok xaak (Rabha), Ombe (Missing)[8], Ad umbra (Bodo), Michiaki (Garo)[7]. Traditionally the plant is used as ethnomedicine in eye problems, itching and intestinal disorder.

1.1. Taxonomic position:

Botanical Name: *Sarcochlamys pulcherrima*, Family: Urticaceae (NBC, 2011), Local name: fok xaak (Rabha), Mechaki (Assamese). Parts used: tender leaves and seed.

1.2. Botanical description of the plant:

S. pulcherrima (Roxb.) Gaud is a medium tree; it is called Dougal tree in English. The marked plant has fresh branchlets which remains enveloped with soft hair, narrowly lanceolate leaves are alternate positioned and toothed. The leaves are also caudate acuminate in structure. The plant has inflorescence spike, and flowers are dioecious. The one seeded remains enclosed in fleshy perianth[9]. Blossoming and blooming of flowers and fruits happens in winter seasons and is Height up to 16.4 feet. Small white flowers appear in axillary spikes. *S. pulcherrima* (Roxb.) Gaud is an unexplored medicinal plant, as well as very scanty of literature (Figure 1).

The main objectives of this research paper war exploring antibacterial potential evaluating against bacterial strains. The bacterial strains targeted to understand the antimicrobial activity includes a gram-positive bacterium namely *Staphylococcus aureus* and a gram-negative bacterium namely *Escherichia coli.* Moreover, antioxidant potential was also aimed to evaluate on the experimental material.

(a) (b)

Figure 1: (a) Fresh twigs and (b) Herbariums sheet of Tender leaves of *S. pulcherrima* (Roxb)

2. MATERIALS AND METHODS:

2.1. Study area: *Sarcochlamys pulcherrima* was acquired from villages of Rabha Hasong, an Autonomous Council Area of Assam, India which is geographically located at 25° 50' to 26º10'' N and 90° 00E to' 15'' E. The area is sharing its boundary with East Kamrup district, west Dhubri district, North Barpeta, Bongaigoan and Dhubri district of Assam. The major tribes are Rabha, Bodo, Garo and other communities[10]. The topology of Rabha Hasong is characterized by small hills, valleys and plains. Main water bodies are Dudhnoi river, Krishnai river, Jinijiram and Jinari of Brahmaputra. The area with a sub-tropical climate experience maximum temperature 36°C during July-August which falls to a minimum temperature of 6ºC during January. The average rainfall is of 2169mm. The soil quality is acidic were sandy and clayey loam constitute the major portion.

2.2. Authentication of the target sample: Several fresh, healthy, young parts namely leaves (S1), roots (S2), twig (S3) and fruit (S4) of the plant species were collected from natural habitat of Assam. The plant materials were identified with consultation of standard literature such as “Flora of Assam” written by Kanji Lal, U.N[11]. The Plant was authenticated by Botany Department of Guwahati

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University, Assam and also by Department of Botany, Dudhnoi College, Dudhnoi, Assam. The information of traditional uses of the plant were collected from respondents of this area, through standard questionaries.

2.3. Preparation of plant Extracts: The experiment was performed in the Physiology and Biochemistry Laboratory of the Department of Zoology, Gauhati University, Assam. Freshly collected and washed with plain water, the plant parts (S1-4) of selected species namely *S. pulcherrima* (Roxb) were then diced into small chunks. The chunks were then desiccated under shades for two weeks and then powered in an electric blender. The powdered materials were kept in airtight containers to avoid the humidity and then stored at room temperature until use in laboratory. Methanol was used as solvent because of low boiling point and polar characteristics. 200gram of each dry powered sample was methanol extracted in a Soxhlet extractor for 24 hours. The sample methanol extract was filtered using What man no. 41 filter paper followed by methanol evaporation from the sample extract by employing a rotary evaporator in vacuum at 60ºC. Complete methanol evaporation was ensured by exposing the extract to a temperature of 40-50ºC for 8 hours in an oven. Subsequently, the residues were stored at 4 ºC in a sealed condition for further use.

2.4. Qualitative phytochemical analysis: The presence of several bioactive secondary compounds was estimated in leaf extract (S1) of the plant by using standard methods[12-14].

2.5. Antimicrobial activity: The antimicrobial activity of the leaf extract (S1) of the targeted plant was analysed by using disk diffusion with 4 quadrant method against *Escherichia coli* and *Staphylococcus aureus*[15, 16]. The provided sample weighing 200mg/ml was assessed for the antimicrobial activity. The extracts were prepared by Soxhlet extraction method and weighed using DMSO as the solvent. A stock extract was taken as concentrated sample. 100 µl of concentrate extract was mixed with 900 µl of DMSO in each tube and final volume of all tube was made up to 1000 µl. Thereafter, a serial dilution of each of the extract was made up to 10-1, 10-2, 10-3 (dilution factor 10). Nutrient agar (medium) was prepared by dissolving required amount in distilled water and sterilized in an autoclave for 15 minutes at 121°C at 15 psi and then directly taken inside Laminar Air Flow to maintain the sterile environment. In an aseptic condition pre sterilized media was poured into petri-plates and later inoculated with standardized inoculum of *E. coli* and *S. aureus*. For the study, Kanamycin and gentamycin was taken as positive controls at the centre (30 mcg each) against *E. coli* & *S. aureus* respectively. A total of 4 extracts (one concentrate and three diluted samples: 10-1, 10-2, 10-3 dilution) for the sample (S1) was taken for disc diffusion with 4 quadrant method [15, 16]. The plates were incubated at 37°C and observed after 24 hours to 48 hours for examining the zone of inhibition formation around the wells.

2.6. Determination of antioxidant activity using DPPH (2,2-Diphenyl-1- Picrylhydrazyl): DPPH is a stable free radical that reacts with antioxidants, resulting in a decrease in absorbance. Methanolic extracts of all the four samples (S1-4) were subjected to determination of antioxidant activity. Along with the samples the standard ascorbic acid was also measured in terms of hydrogen donating ability by using DPPH. It is most commonly employed method to evaluate antioxidant activity of plant extract since it is easy and rapid to use[17]. On interaction with antioxidants the purple-coloured stable DPPH free radical reduces to yellow-coloured diphenyl picryl hydrazine.

The DPPH solution was prepared by mixing 4 mg of DPPH in 100 ml of methanol (40 µg/ml). The DPPH solution was further diluted by adding methanol (working solution) at an absorbance of 0.98±0.02 at 517 nm using the spectrophotometer. Similarly, the standard solution was prepared by mixing 18 mg of ascorbic acid in 2 ml of methanol (9 mg/ml).

The ascorbic acid standard curve was adopted by following the methods of published reports with modifications [18-20]. From the stock solution (1 mg/ml), different working solutions were prepared as: 10, 150, 350 and 500 µg/ml concentrations by using methanol for dilution. It was followed by adding DPPH (as given in the following table 1) and then incubation in dark condition for 5 minutes. The coloured solution was then measured for absorbance using UV-VIS spectrophotometer at 517 nm.

Each of the samples were prepared for determination of antioxidant activity by mixing 1 gram of each powdered sample (S1-4) in liquid nitrogen. It was then dissolved in 20 ml of methanol and kept at RT for 72 hours following the maceration technique to complete the extraction process[21]. After 72 hours, the samples were filtered and desiccated in hot air oven. Then weighed 3 mg of the dried sample and dissolved in 3 ml of solvent (methanol) making the concentration to 1mg/ml. From the stock solution (1 mg/ml), different working solutions will be prepared as: 10, 150, 350 and 500 µg/ml concentrations by using methanol for dilution.1.5 ml of each diluted sample was

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taken in different test tubes followed by addition of 1.5 ml DPPH (as given in the following table 2). The final volume of all the test tubes was made up to 3 ml with methanol. After that all the test tubes were incubated for 5 minutes in dark condition at room temperature. Finally, absorbance for all the tubes was measured at 517 nm.

The percentage inhibition was calculated using equation:

% DPPH scavenging effect or % inhibition = 100 x (A *control* – A *sample*)/A *control*)

IC_{50} values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis.

3. RESULTS AND DISCUSSION:

India is observed as the seventeen 'megabiodiverse countries' internationally and Assam constituted in the Eastern Himalayan Biodiversity of India is one of the twenty-five 'megabiodivese' regions of the world.

Indigenous medical plants of the state Assam is mostly known to the local people and tribes of the state but remains unknown to the scientific research world. The dynamic and potent phytochemicals enhanced with pharmacological properties still remains unexplored. Therefore several enlivening live saving potent formulation of drug can be revealed from such unexplored indigenous plants of Assam which are popularly used with conviction by tribal community and villagers dwelling in the state. Such discoveries can me marked as boon to the mankind worldwide[22-24].

3.1. Qualitative Phytochemical Analysis:

The qualitative evaluated phytochemical constituents of *S. pulcherrima* (Roxb) in S1 by qualitatively test are displayed in Table 1. A broader range of bioactive phyto-compounds such as alkaloids, flavonoids, steroids, glycosides, and reducing sugar was revealed to be present in the plant leaf (S1) extracts.

Table 1: Qualitative phytochemical analysis of leaves extracts of *S. pulcherrima* (Roxb)

Sl. No.	Phytochemical constituents	Test/Reagent	Results
1	Alkaloid	Wagner's test (crude extract+2ml 1% HCL+ 2-3 drops of Wagner's reagent)	+
2	Reducing sugar	Fehling test	+
3	Steroids	Acetic acid+ 2ml Chloroform+conc. H_2SO_4+ crude extract	+
4	Proteins	Millions test (crude extract+ 2ml millions reagent)	+
5	Saponins	Foam test (crude extract+ 5ml distilled water)	-
6	Glycosides	Liebermann's test (crude extract+2ml chlroform+2ml acetic acid)	+
7	Carbohydrate	Benedict's test (crude extract+ 2ml benedict reagent)	+
8	Flavonoids	Alkaline reagent test/ Shinoda test	+

*Note: '+' = presence; '-' = absent

In favour of the current research phytochemical profiling, a study by Paul et al., 2010 also revealed presence of phenolics along with saponins, flavonoids and other acidic compounds[25, 4].

3.2. Antibacterial analysis:

Antimicrobial activity analysis of provided test (S1) sample against *E. coli* and *S. aureus* using disc diffusion method with 4 quadrants against *E. coli* & *S. aureus* showed positive zones of inhibition against *S. aureus* (Table 2) but no zone of inhibition against *E. coli*. The positive controls kanamycin and gentamycin showed zones of inhibition against both the organism (Table 2).

Table 2: Antibacterial analysis of young tender leaves of S. pulcherrima against *Escherichia coli* and *Staphylococcus aureus*

Zone of Inhibition *for E. coli* (in mm)					**Antibiotic (K)**
Sample ID	**Concentrated**	**10^{-1}**	**10^{-2}**	**10^{-3}**	
S1	NIL	NIL	NIL	NIL	32mm
Zone of Inhibition for *S. aurues* (in mm)					**Antibiotic (Gen)**
S1	12mm	9mm	9mm	9mm	21mm

The study suggests that phytochemical components of *S. pulcherrima* are thought to be responsible for the plant's antimicrobial, as well as antioxidant properties, which have been observed in traditional medicine and communities of Assam in India. It is reported that *S. pulcherrima* plant has rich source of antioxidant activity[25]. Qualitative surveillance is a very important and common approach to evaluate presence of bioactive compounds in plants extract and it is reported that *S. pulcherrima* contains antioxidant and antimicrobial properties[26]. Previous study reported that the biological activities of the detected phytochemicals components include activities like anti-microbial, anti- fungal, anti-inflammatory, anti-diabetic, hepatoprotective and anti-cancer[27]. *S. pulcherrima* contains significant exploratory and sedatives properties[28]. Another study reveals anti-biofilm potential of the triterpenoids compound extracted from *S. pulcherrima* (Roxb.)[29]. Detection of several secondary metabolites in the methanolic leaf extract of *S. pulcherrima* in the present study can

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draw conclusion of presence of antioxidant and antimicrobial activities in the extract. Further quantitative estimation of the bioactive compounds and in dept studies on *S. pulcherrima* can open on to uncovering of new drug(s) and even lead to new therapeutics approach for diverse ailments.

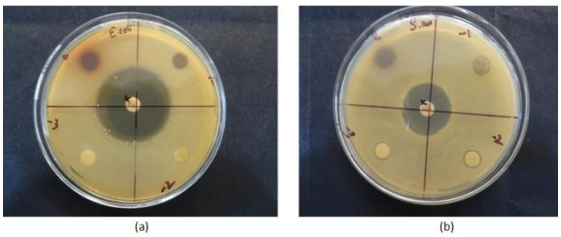

Figure 2: Zone of inhibition of young tender leaves of *S. pulcherrima* against *E. coli* and *S. aureus*

3.4 Antioxidant activity:

In the current study among the four samples (S1-4), plant leaf (S1) extract reveals highest antioxidant activity with IC_{50} 241.04±0.11 µg/ml. followed by fruit (S2) extract with slightest lower antioxidant activity with IC_{50} of 247.47±0.11 µg/ml. The IC_{50} measure for all the plant part extracts are mentioned in Table 3 with graphical representation of the antioxidant activity determination in Figure 2.

Table 3: IC_{50} values of the tested samples (Data is expressed in IC_{50} value ± Standard deviation)

SL No.	Sample ID	IC_{50} (µg/ml)
1	S1	241.04 ± 0.11
2	S2	617.29 ± 0.07
3	S3	467.21 ± 0.08
4	S4	247.47 ± 0.11

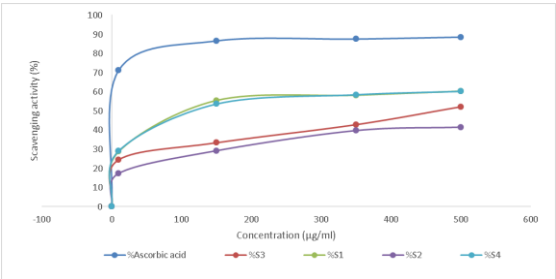

Figure 2: Determination of antioxidant activity by DPPH for all the targeted samples (S1-4) and ascorbic acid (Data is expressed in IC_{50} value ± Standard deviation).

The studied tree, *S. pulcherrima*, is a popular medicinal plant in many tribal communities of Assam, including the Rabha community. Although no proper documentation of the concerned plant is available, a significant event took place of declaring it as an endangered species. Chakma tribe inhabiting in distant hilly districts in Bangladesh use paste of the plant leaves to medicate fever blisters and boils [30]. The Rabha community traditionally practices the use of leaves of *S. pulcherrima* for various health remedies like eye itching, intestinal disorder, diarrhoea, dysentery etc. Seeds of the plant is also been used by tribes of Assam to treat tongue ulcer[8]. Different communities of Bangladesh also use the entire plant including fruits against cold, boil, sore and lactation[31, 32]. The plant has many unexplored antibacterial and antioxidant potential which has been researched in the present study through In Vitro experiments. The result of the present study suggests that further research on the Dogal plant *S. pulcherrima*, can potentially provide drug developments in the pharmaceutical industry.

A study investigated the antioxidant activity in the methanolic extracts of five plants which includes the targeted plant of the present study as well and concluded leaves extract of *S. pulcherrima* displays [25]. Thus, the study holds up utilizing this antioxidant supremacy in providing defend against several health issues arising from free radicals. Similarly, a study announced potential inhibition activity against *Candida albicans* by leaf methanolic extract of *S. pulcherrima*[9].

4. CONCLUSION:

Rabha Hasong Autonomous Council area of Assam is treasure house of leafy vegetables, herbs, shrubs and tree. *S. pulcherrima* plant traditionally used as edible tender leaves and natural medicine to treat various ailments by Rabha community. From the above study, it can be concluded that the methanolic Soxhlet's extraction of *S. pulcherrima* contains a lot of significant antioxidant and antibacterial activity. However further insight study is necessary which may lead the way to drug discovery and natural therapeutic approach.

CONFLICT OF INTEREST:

All the authors declare that there is no conflict of interest.

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AUTHOR'S CONTRIBUTION:

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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