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EVALUATION OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ACTIVITY OF *GARCINIA LANCIFOLIA* ROXB.

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ABSTRACT

Keywords:

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Flavonoids have been reported to have many pharmacological activities, antimicrobial, antioxidant, cytotoxic, chemoprevention activities along with strong antiproliferative effects. The fruits of *Garcinia lancaefolia* Roxb., family Cluseaceae, a plant endemic to Assam has been used in locally to treat many diseases for which it was selected for investigating its antimicrobial properties. No scientific validation has been made so far on its phytochemical content and the antibacterial activity for which the methanolic and water extract of plant parts were investigated by Agar Well Diffusion method. Furthermore, our phytochemical studies indicated that methanolic extract of *Garcinia lancaefolia* flowers and fruits contains flavonoids, tannins, reducing sugar, steroid terpene and cardiac glycosides. The total phenolic and flavonoids content studies were quantified for all the plant parts. Antibacterial activities of the crude extract were screened against wound infection causing 5 human pathogens. The most susceptible bacteria were *S. aureus*, followed by beta hemolytic *Streptococcus pyogenes*, while the most resistant bacteria were *E. coli*, followed by *Bacillus subtilis*. From the screening experiment, the crude fruit extracts showed the best antibacterial activity; hence this plant can be further subjected to isolation of the therapeutic antimicrobials and pharmacological evaluation. The largest zone of inhibition (ZOI) was obtained with the fruit juice against *Staphylococcus aureus* (19mm) followed by beta hemolytic *Streptococcus pyogenes* (16.2mm) and *Bacillus subtilis* (15mm).

INTRODUCTION: Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions ¹.

According to Biswas and Mukherjee ², 70% of the wound healing Ayurvedic drugs are of plant origin, 20% of mineral origin, and the remaining 10% consisting of animal products and these drugs are stated to be effective in different conditions. Many traditional remedies are based on systematic observations and methodologies and have been time-tested but for

many of them, scientific evidence is lacking and there are only few prospective randomized controlled trials that have proved the clinical efficacy of these traditional wound healing agents ³.

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Plants in the genus *Garcinia* belonging to the family Clusiaceae are

known to be a rich source of bioactive compounds such as xanthenes, biflavonoids, benzophenones, benzoquinones, and triterpenes having antibacterial, antifungal, antioxidant and cytotoxic effects⁴. Preparations containing flavonoids as active constituents have been used for centuries in Indian Ayurveda for the treatment of human diseases and in anti-infective research. With this concept the antimicrobial screening results of *Garcinia lancaefolia* (Family-Clusiaceae) was selected for our study.

One of the lesser known species of *Garcinia*, *Garcinia lancaefolia*, also known as "*Rupahi Thekera*" in Assam is facing extinction even in its native place. Its fruit is eaten raw and also cooked as meals. It is tangy when raw and becomes sweet on maturity. It is a glabrous shrub growing up to 12ft. height generally grown under dense shade of other trees. It is found in the evergreen forest of North-east India. It is facing rarity in nature and is often cultivated at homestead. Young leaves and shoots which are slightly acidic in taste, are eaten cooked by Karbis & Mishings. The leaves of *Garcinia lancifolia* are used as stomachic, diuretic and the fruit is used for dysentery and diarrhoea. Leaves are also cooked as vegetables⁵.

However, these therapeutic potentials of the plant have not been scientifically evaluated. Therefore, the present study was undertaken to investigate its phytochemical components and antimicrobial properties against some common and wound infection causing organisms.

MATERIALS AND METHODS:

Plant material: The plant materials such as fresh leaves, flowers and fruits were collected in April –May 2011 from cultivated area in Sonitpur district, Assam, India and then shade dried at room temperature. The voucher specimen was deposited and identified at Department of Botany, Gauhati University, Assam and Botanical Survey of India (BSI), Shillong, Meghalaya, India. The collected plant material was air-dried in darkness at ambient temperature (20°C). The dried plant material was cut up and stored in tightly sealed dark containers until needed. The leaves were washed with 70% alcohol and rinsed with sterilized distilled water. Then, the leaves were air dried and homogenized to powder and stored in airtight bottles.

Preparation of the extract: The air-dried plant material (10 g) was coarsely crushed in small pieces of 2-6 mm using the cylindrical crusher and extracted with organic solvents (water, methanol, acetone, ethyl acetate, petroleum ether) in a ratio of 1:10 (w/v) and kept on a rotary shaker for 24 h at 20°C. The extract was filtered through a paper filter (Whatman No. 1) and evaporated under reduced pressure by the rotary evaporator. The obtained extracts were stored in dark glass bottles and used as stock samples for further processing.

Dried leaves powder were mixed with extracting solvent like Water (W), Methanol (M) and Dichloromethane (DCM). Then the mixture was filtered and sterilized by using Sintered glass filter (Grade 5, pore size 1-2 μ , Borosil). The filtrate was freeze dried. These extract were used for various phytochemical screening.

About 20 g of the fruit of *G. lancifolia* were sliced, homogenized, and squeezed in two-layered muslin cloth, to extract the complete juice. The juice (GFJ) was centrifuged at 3000 rpm for 5 min and used for determination of total phenolic content and antimicrobial activity.

The pulp (residue) was homogenized with methanol and chloroform (50:50 v/v). The extraction was repeated until it became colorless. The extract (GFP) was filtered and a final volume was made up to 10 mL with methanol.

Seeds were washed with water, surface sterilized with 10% sodium hypochlorite solution, then rinsed with sterile distilled water and air dried using a laminar air flow. The seeds were grounded into a fine powder. For the seeds exactly 1000 g of the powdered seeds were soaked in mixture of methanol and sterile distilled water in ratio 3:2 for 72 hours and later filtered to obtain the methanolic extract. The mixture was first concentrated *in vacuo* using rotary evaporator to remove the methanol. Filtered extracts were dried using a rotary evaporator at 45°C. The aqueous residue was later lyophilized to get the crude extract which was white in colour. The yield collected was 250 g. Then the extract was stored at 4°C for further use.

Preliminary Phytochemical Screening: The preliminary phytochemical screening of *G. lancaefolia* Roxb was carried out for the detection of various phytoconstituents. All the plant extracts were qualitatively tested for the presence of chemical constituents as Phenols, Tannins, Alkaloids, Flavonoids and reducing sugar. Phytochemical tests of *G. lancifolia* extracts were carried out using standard procedures to identify the constituents as described by Sofowara ⁶, Trease and Evans ⁷ and Harborne ⁸.

Chemicals: Penicillin (10ug/ml), Ciprofloxacin (10ug/ml), Ofloxacin (10ug/ml), Gentamycin (10ug/ml), Molten Mueller Hinton (MH) Agar and Nutrient agar medium were obtained from Hi-media laboratories, Mumbai. All other chemicals were of analytical grade and obtained locally.

Microorganisms: Pure cultures of the test organisms obtained from Down Town Hospitals Pvt.Ltd. in Guwahati, Assam, India from wound infections were used for the purpose. Antibiotic susceptibility was determined from the size of the inhibition zone, according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997). The microorganisms used were *Staphylococcus aureus*, *Streptococcus pyogenes* (β -hemolytic) and *Pseudomonas aeruginosa*, *E. coli* and *Bacillus subtilis* identified to the species level.

Screening for Antibacterial Activity: *In vitro* antibacterial activity was determined by agar-well diffusion method described by Mukherjee *et al* ⁹. All the pathogenic microorganisms mentioned above were incubated at 35±0.1°C for 24 h by inoculation into Nutrient Broth. The plating was carried out by transferring bacterial suspension (10⁵ CFU/ ml) to sterile Petri plate and mixed with Nutrient agar medium (Hi-Media Laboratories Limited, Mumbai, India) and Molten Mueller Hinton (MH) Agar medium

and allowed to solidify. About 75 μ l of the sample (5 mg /ml) was placed in the wells and plates were then transferred to an incubator at 37°C for 24 hours. The negative control was included without adding the cultures to know the sterile conditions. The antibacterial activity was recorded by measuring the width of the clear inhibition zone (ZOI) around the wells and compared with the standard antibiotics.

RESULTS AND DISCUSSION:

Phytochemical Analysis Results: Phytochemical screening of the crude ethanolic extracts of *Garcinia lancaefolia* Roxb revealed the presence of cardiac glycosides, terpenoids, saponins, tannin, flavonoids, steroids and reducing sugars but saponin and phlobatanin showed negative results. The methanolic extract was found to contain more flavonoids. The preliminary phytochemical screening of methanolic extract reveals the presence of alkaloids, flavonoids, tannins, and triterpenes (**table1**). The tannins were observed in the leaf, stem and fruit extracts. The saponins were observed only in water extracts of leaf, stem and fruit extracts. The flavonoids were observed in methanol and water extracts. In case of cardiac glycosides, water, methanol and ethanol extracts were found to be tested positive along with dichloromethane.

The *G. lancifolia* leaf extracts showed the presence of phenolic compounds. Highest concentration was observed in methanol extract followed by dichloromethane and water extracts. The stem extracts also showed the presence of phenolics, being highest in content in methanol extract; whereas the water extract showed very less amount. The *G. lancaefolia* fruit was also rich in phenolic compounds. The juice component was high in free phenolics contents compared to pulp component.

TABLE 1: TEST FOR VARIOUS PHYTOCHEMICAL CONSTITUENTS IDENTIFIED IN THE PLANT EXTRACTS

Parts used	tannin	Saponin	Reducing sugar	Steroid	Terpene
Leaf	++ (W)	+W	+ (WMC)	+ (WMC)	++ D, M
Stem	+ (D W,M)	+W	++ (WMC)	+ (WM)	+M
Fruit pulp	++(W)	-	+++ (WMC)	++ (WMC)	+M
Fruit juice	+++ (W,D,M,E)	+W	++++ (WMC)	+++ (WMC)	+M

*Extract made in Water (W) , Methanol(M) , Chloroform (C) , Dichloromethane (D) and Ethanol(E). +++++ High rate +++ medium ++ low -- absent

Plant phenolics present in the fruit and vegetables have received considerable attention because of their potential antioxidant activity¹⁰. Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants¹¹. These compounds, one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants. These compounds play an important role in growth and reproduction, providing protection against pathogens and predators¹², besides contributing towards the colour and sensory characteristics of fruits and vegetables¹³.

Various amount of cardiac glycosides were found in different extracts of the plant which suggest their antioxidant property (table 2).

TABLE 2: RESULTS OF PRESENCE OF CARDIAC GLYCOSIDES IN VARIOUS FORMS OF EXTRACTS

Type of Extracts	Leaf	Stem	Fruit
Water	+++	+++	+++
80% ethanol	+++	++	+++
70% methanol	+++	++	+++
Chloroform	+	+	++
Acetone	-	-	-
Dichloro methane	++	+	+++
Petroleum ether	-	-	-

TABLE 3: ANTIBACTERIAL ACTIVITY OF *G. LANCAEFOLIA* AGAINST SELECTED PATHOGENS

Antibiotic/ZOI (mm)	Zone of inhibition(ZOI) (mm)						
	LLD	LLM	LLW	LSM	LSW	LFJ	LFP
<i>Pseudomonas aeruginosa</i> Gentamycin/9mm	-	12	12	-	-	15	13
<i>Escherichia coli</i> Ciprofloxacin/11mm	-	-	-	-	-	12.8	8
<i>Bacillus subtilis</i> Ciprofloxacin/16mm	-	-	-	-	-	15	11.3
<i>Staphylococcus aureus</i> Ofloxacin/5mm	7.3	12.4	-	10	-	19	14
<i>Beta hemolytic Streptococcus pyogens</i> Penicillin/18mm	11.2	10.2	11	14	-	16.2	11.2

In this study, the results of the investigation show that the plant extracts from *Garcinia lancaefolia* have good antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and β -hemolytic *Streptococcus pyogens* may be due to the presence of various phytochemical constituents. But in case of *Bacillus subtilis* and *Beta hemolytic Streptococcus pyogenes*, the standard antibiotic was seen to be more effective than the extracts.

CONCLUSION: The *G. lancifolia* fruit is used against dysentery and diarrhoea by many ethnic people of Assam. The antibacterial activity of fruit juice and pulp extracts proved the efficacy of the fruit against dysentery and diarrhoea.

Their presence in the extracts is an indication of the plants' potent antioxidant and membrane-stabilizing properties.

Antibacterial activity of *G. lancifolia*: Among all the crude extracts, the fruit extracts were highly effective against Gram-positive bacteria compared to Gram-negative bacteria. The high antibacterial activity may be attributed to the presence of phenolics, flavonoids, terpenoids.

In case of *Pseudomonas aeruginosa*, the fruit extract showed a 15mm as compared to Gentamycin which exhibited a zone of inhibition of 9mm; hence this plant extract was summarized to be effective against gram negative *Pseudomonas aeruginosa*. In the case of *Escherichia coli*, the control drug ciprofloxacin showed less activity (about 11.0mm) when compared with the plant fruit juice extract having a ZOI of 12.8 mm. But the other extracts did not inhibit the test organism in any form.

The fruit juice extracts were found to have inhibitory activity against all the test pathogens(table 3) among which the highest ZOI was seen for *Staphylococcus aureus* (19mm).

The fruit juice was very effective with high antibacterial activity against both Gram negative and Gram-positive organisms. The high antibacterial activity may be attributed to the presence of phenolics, flavonoids, terpenoids.

This research work states that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids, and steroids in the ethanolic extract of *Garcinia lancaefolia* were responsible for its antimicrobial activity. Moreover, this study reveals that such unexplored plants show much promise in the development of phytomedicines having antimicrobial properties.

In this endeavor, traditional herbal medicines and the drugs derived from these plants may have the possibility of use in medicine because of their antibacterial activity and benefit the modern world of medicine to fight against many multidrug resistance organisms.

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