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FORMULATION OF HERBAL GEL FOR POTENTIAL ANTIBACTERIAL ACTIVITY AGAINST *PSEUDOMONAS AERUGENOSA*

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Tectona grandis, Antibacterial, Minimum inhibitory Concentration (MIC)

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ABSTRACT: The present work deals with the formulation of containing aqueous extract of *Tectona grandis (Teak)* Leaves and prepared formulation against some Gram-positive and Gram-negative bacteria. The antibacterial activity of various *T. grandis* leaves extracts was measured by determining minimum inhibitory concentration (MIC) by cube dilution method and zone of inhibition by disc diffusion method against *P. aeruginosa*, Aqueous *T. grandis Leaves* extracts exhibited comparatively higher antibacterial potential in comparison with other extracts. All the prepared herbal formulations were subjected to antibacterial study by determining the zone of inhibition by disc diffusion. Herbal formulations containing gel aqueous exhibited better zones of inhibition against *P. aeruginosa* in comparison with others. This type of bacteria (germ) is found commonly in the environment, like in soil and water. From the results of this study, it can be concluded that herbal formulations containing gel aqueous *T. grandis leave* extract are found suitable for use against bacterial infections in the form of topical gels or another suitable dosage form.

INTRODUCTION: The World Health Organization released its first report in 2014 on surveillance of antimicrobial resistance, revealing that this is an increasing global threat and putting our capacity to treat common nosocomial or community-acquired infection at risk [1]. This growing problem was characterized by the infectious diseases caused by multidrug-resistant Gram-negative bacteria that challenge public health policies worldwide to the point of being known as the ESKAPE pathogens. Present periods represent their escape from the effects of the antibacterial agents or the nonexistence of newer and more effective antibiotics [2].

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Pseudomonas aeruginosa is the most important toxigenic pathogen within the genus Pseudomonas because of the quantity and types of invasive infections it produces, as well as the noteworthy morbidity and mortality associated [3]. The resistant mechanisms in *Pseudomonas aeruginosa* are related to the enhancement of the mortality rate of patients infected with this pathogen [6]. Additionally, the rising indiscriminate use of antibacterial in health centers or by people who practice self-medication could lead susceptible patients to get infected by multidrug-resistant microorganisms [7], [8].

The embossing of antibiotic resistance and related toxicity issues limit the use of these drugs and generate a renaissance in phototherapy research [9]. To trace this challenge, there is growing interest in identifying and evaluating antimicrobial compounds in extracts of medicinal plants as a new source of drugs and alternative treatment approach [10]. The present study aimed to evaluate the antibacterial capacity of traditionally used

Tectona grandis plants against *P. aeruginosa* to scientifically validate the inhibitory activity attributed by their popular use and propose new sources of antibacterial agents [12].

Pseudomonas aeruginosa: This type of bacteria (germ) is commonly found in the environment, like in soil and water. Many different sorts of *pseudomonas;* the one that the majority often causes infections in humans is named *Pseudomonas aeruginosa*, which may cause infections within the blood, lungs (pneumonia), or other parts of the body after surgery. These bacteria permanently detect new ways to avoid the effects of the antibiotics used to treat the infections they cause. Antibiotic resistance occurs when the germs did not answer the antibiotics designed to kill them. If they develop resistance to many sorts of antibiotics, these germs can become multidrug-resistant. *P. aeruginosa* resistance mechanisms have been linked to an increase in the death rate of individuals infected with this infection. Furthermore, the increasing indiscriminate need for antimicrobials in health centers or by persons who self-medicate could expose vulnerable individuals to multidrug-resistant bacteria. Carrillo and Nakamura *et al.* [13-15]. Antibiotic susceptibility & associated toxicity issues have reduced the use of such medications, resulting in a resurgence in phytotherapy research. Scientists are becoming engaged in discovering and evaluating antibacterial elements in selected plants as a local alternative to drugs and therapy options to fix this concern Marri *et al.* [16-18].

Plant Profile: *Tectona grandis* is indigenous to East and Southeast Asia, including Bangladesh, India, Indonesian, Malaysian, Myanmar, Thailand, and Sri Lanka, although it has become naturalized & grown in many African and Caribbean nations. Myanmar's rosewood forests contain about half of all naturally occurring teak on the planet.

Classification of Teak Plant:

Kingdom:	Plantae
Clade:	*Tracheophytes*
Clade:	*Angiosperms*
Clade:	*Eudicots*
Clade:	*Asterids*
Order:	*Lamiales*
Family:	*Lamiaceae*
Genus:	*Tectona*
Species:	*T. grandis*

Teak has two genetic origin centers, according to molecular analysis: one in India and another in Myanmar and Laos *Shuid et al.* [19]. *Teak* is a huge fruit shrub with gray to whitish branches that grow up to 40 meters (131 feet) tall and is noted for its top-quality wood.

It has ovate-elliptic to ovate leaves that are 15–45 cm (5.9–17.7 in) long by 8–23 cm (3.1–9.1 in) broad and are supported on sturdy 2–4 cm (0.8–1.6 in) long petioles. The leaf edges are complete. From June to October, fragrant white flowers are produced on panicles of 25–40 cm (10–16 in) long and 30 cm (12 in) wide.

The corolla tube is 2.5–3 mm long and has obtuse lobes that are 2 mm broad. *Tectona grandis* Fruits are oblong and 1.2–1.8 meters long, ripening from November to October. The petals mature before the label that pollen is discharged in a few hrs. of the flowering stage, making blooms weakly protandrous.

The blooms are mostly text analysis (pollinated by insects), although they can also be anemophilous (wind-pollinated). In its natural range in Thailand, members of the bee genus Ceratina were found to be the most important pollinators [20].

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: Leaves of *T. grandis* were collected in the month of January, 2021, from the Goel Group of Institution, Lucknow. The collected plant with complete herbarium was authenticated at Goel Institute of Pharmacy and Sciences, Lucknow, Uttar Pradesh (India). (Vide Letter No. GIPS/PhCog. /006/2021/001). Also, a sample specimen was deposited there.

Preparation of Extract: 1kg of the air-dried leaves of *T. grandis* was reduced to a fine powder and extracted using a maceration process with petroleum ether, Acetone, 80% Methanol, and Hexane. Each extract was concentrated using a Rota evaporator. The extraction was dried in a hot air oven below 30°C and preserved by Dimethyl sulfoxide (DMSO).

Microorganism: The microorganisms were obtained from the microbiological laboratory MRD life sciences, Lucknow (India).

Antibacterial Screening:

Determination of Minimum Inhibitory Concentration (MIC): The cube dilution method determined the minimum Inhibitory Concentration of extract. Taken 10 Test tubes for each extract per microorganism were thoroughly washed and sterilized. 0.5 ml of test solutions (7.8 µg/ml to 1000 µg/ml) was taken in 8 test tubes. Freshly prepared and sterilized 0.5 ml nutrient broth & 0.5 ml 6 % DMSO was added to each of 8 test tubes. Test tubes were thoroughly shaken. To each test tube, 50 µg of 24 hours sub-culture of bacteria was added and shaken properly.

The 9th test tube contained 0.5 ml of nutrient broth & 0.5 ml 6 % DMSO, and 50 µg of subculture of bacteria to act as positive control and last test tube contained 0.5 ml of nutrient broth & 1 ml of 6 % DMSO to act as a negative control. All the test tubes were incubated in an incubator at 37 °C for 24 hours and observed for turbidity comparing with both the controls.

Determination of Zone of Inhibition: The zone of inhibition was determined for each extract at a concentration of 1000 µg/ml and compared to that of standard tetracycline 5 µg/disc by disc diffusion method. Agar plates were made by pouring nutrient agar suspension (sterilized) in a cleaned Petri dish to get 4 mm thickness (approx.). Plates were kept under laminar airflow. Stock solution of each extract 100 mg/ml were prepared by dissolving the extract in 6 % DMSO, a paper disc of 6 mm diameter was cut and sterilized in a hot air oven. 10 µl of every extract was taken in a sterile pipette and soaked in paper disc. The disc was dried. The disc was carefully kept on the agar media and slightly pressed for proper fixing. The standard tetracycline disc was taken, placed on the media, and fixed properly. Plates were incubated at 37°C for 24 hours. Plates were observed for zone of inhibition.

Formulation and Preparation of Herbal Gels: Accurately weighed Sodium Carboxymethyl Cellulose was soaked in distilled water and was allowed to swell. The accurate quantity of aqueous extract was weighed and dispersed in distilled water. The drug expansion was added to the soaked polymer with stirring until a gel was formed. The formulated gel was filled in a collapsible tube and sealed by crimping the ends.

Evaluation of Herbal Gels:

Color: The compositions' color was tested with white and black backgrounds.

Odor: The smell of the gels was tested by dissolving a little amount of gel in water and sniffing it.

Consistency: The gel's texture was tested by rubbing it to the epidermis.

Greasiness: By rubbing the solution to the skin, the greasiness of the compositions was noticed.

Homogeneity: After the gel was set in the container, all formulated gels were visually inspected for homogeneity. They were examined for the presence of any aggregates and their manifestation.

pH Measurements: The pH of the formulations was measured using a digital pH meter, and the glass electrode was completely dipped into the gel system to cover the electrode. The pH of gels was measured within 5 minutes.

Determination of Viscosity: Formulated gels viscosity was determined using a Brookfield viscometer using the spindle No. 4 at 1.5 rpm.

Determination of Extrudability: Extrudability is a useful empirical test to measure the force required to extrude the material from a bottle or tube since the passing of gels has gained considerable importance in delivering the desired quantity of gel from collapsible tubes. With this, the extrudability measurement becomes an important criterion for gels. As not strictly a test of product characteristics due to the inclusion of force necessary to deform the whitener, the method applied is for determining applied shear in the zone of the rheo-gram corresponding to the shear rate exceeding.

The yield value was exhibiting. Formulated gels were filled in standard capped collapsible tubes and sealed by crimping the ends. The weight of the tube was recorded. The sealed tube was placed between two glass slides and was clamped. A 500-gm gel weight was placed over the glass slide, and the cap was removed. The gel extruded amount was collected and weighted [21].

Determination of Spreadability: 2 glass slides 6 cm long were used, containing gel. The second slide was fixed on a wooden plate, and the upper one was tied to a hook with a balance at the other end in which a weight was kept to pull that slide. Five gm gel was uniformly placed on the lower slide, and the upper slide was placed on it. The one-kilogram weight was kept on the slides for five minutes to expel the entrapped air. The excess discharged gel was carefully scrapped off. A weight of 80 gm. was kept on the balance. The time in seconds required to separate the slides completely was noted. Less time indicates more slip and better spreadability. These experiments were repeated thrice, and mean value was taken.

Spread ability is calculated using the formula

$$S = M \times L / t$$

Where, spreadability of gel (S); weight tied to the upper slide (M); length of a glass slide (L); time taken (t).

RESULTS AND DISCUSSION: The nature of gels is semisolid, which has gained a greater deal of importance in the medical field for their topical effect in the treatment of pain, strain, inflammation, infections, and other diseases. In the current work, topical herbal gels containing aqueous extract of *T. grandis* leave were developed using sodium carboxymethyl cellulose. This formulated herbal gel potency was investigated against *Pseudomonas aeruginosa*. MIC of *T. grandis* leave extracts was determined by cube dilution method. It was observed that all the leave extracts showed MIC ranging from 1.44 to 1000 µg/ml.

The aqueous extract showed lowest MIC value against *P. aeruginosa*. Against *P. aeruginosa*, it was 125 µg/ml. From the above observation, it was clearly concluded that the aqueous extract of leaves showed the lowest MIC value [22-25].

TABLE 1: MIC OF *T. GRANDIS* LEAVE EXTRACTS BY DILUTION METHOD:

Bacteria	*T. grandis* leave extract (MIC/µg/ml) 80% Methanol
P. aeruginosa	1.44

In order to evaluate the antibacterial activity of extracts having MIC up to 1000 µg/ml, the disc diffusion method was adopted, and zone of inhibition was recorded. The antibacterial sensitivity of extracts and that of standard (Tetracycline) were compared. Aqueous extract of *T. grandis* leaves at 1000 µg/disc showed a higher zone of inhibition against *P. aeruginosa* (22 mm). Other extracts showed less zone of inhibitions. Thus, antibacterial screening of *T. grandis* leaves extracts by disc diffusion method finally showed comparatively higher antibacterial potential in case of aqueous extract of leave, and hence, it was taken for further formulation study.

Zone of Inhibition of *T. Grandis* Leave Extracts and Tetracycline (Standard) by Disc Diffusion Method: Prepared herbal gels of aqueous *T. grandis* leave extract prepared using 4.5% sodium carboxymethyl cellulose were tested for pH, viscosity, extrudability and spreadability. pH of these gels was within the range between, 6.25 – 7.30. Viscosities of these herbal gels were measured within 43000 – 52000 cps.

FIG. 1: ZONE OF INHIBITION OF *T. GRANDIS* LEAVE EXTRACTS AND TETRACYCLINE (STANDARD) BY DISC DIFFUSION METHOD

It was observed that gel containing 15 % aqueous *T. grandis* leave extract showed good extrudability in comparison with other formulated gels. The gel spreadability was almost similar. These results may be suggested that the gel containing 15% aqueous extract exhibited satisfactory extrudability and spreadability. The dismemberment of gels from the collapsible tube is important during the application,

whereas spreadability plays an important role in helping of uniform application of gels. Good gel quality takes less time to spread and should have high spreadability.

TABLE 2: pH, VISCOSITY, EXTRUDABILITY AND SPREADABILITY OF HERBAL GELS CONTAINING AQUEOUS *T. GRANDIS* LEAVE EXTRACT

Formulation no.	pH	Viscosity	Extrudability*	Spread ability
F5	7.30	45000	++	27.00
F6	7.15	48000	++	27.10
F8	6.60	52000	+++	26.00
F9	6.38	51000	++	25.00
F10	6.25	43000	+++	25.50

Antibacterial study of all prepared gels determining zones of inhibition by disc diffusion method. Zone of inhibitions against *P. aeruginosa* was compared with the standard gels containing tetracycline (1%). Tetracycline gel (1%) exhibited zones of inhibition in the range of 29 – 39 mm for *P. aeruginosa*. It was observed that herbal gel containing 15 % aqueous *T. grandis* leave extract exhibited better zones of inhibition against *P. aeruginosa* in comparison with that of 5 % and 10 %.Zones of inhibitions of herbal gel containing 15 % aqueous *T. grandis*leave extract against *P. aeruginosa* is shown in **Fig. 2.** The antibacterial potential of these herbal gels was found to be increased with the increasing percentage content of the aqueous *T. grandis* leave extract present in herbal gels.

Zone of Inhibition of Herbal Gels Containing Aqueous *T. grandis* Leave Extract by Disc Diffusion Method:

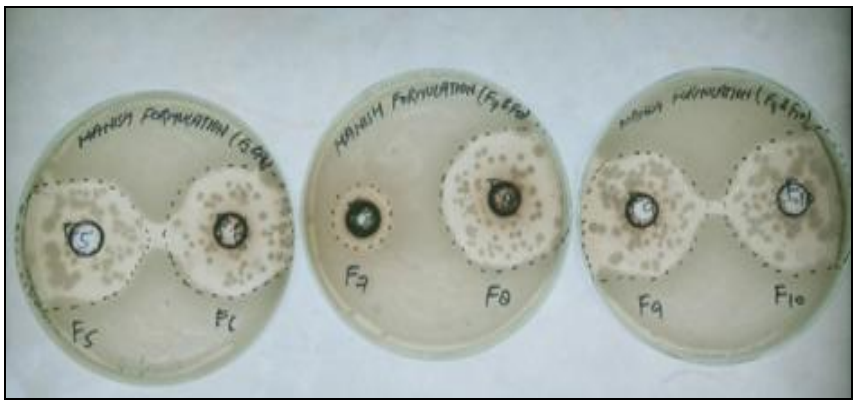

FIG. 2: ZONES OF INHIBITIONS OF HERBAL GEL CONTAINING AQUEOU S*T. GRANDIS*LEAVE EXTRACT AGAINST *P. AERUGINOSA*

CONCLUSION: Extracts from *T. grandis* leave were tested for antibacterial activity against *P. aeruginosa*. MIC (by cube dilution method) and zone of inhibition (by disc diffusion method) of aqueous *T. grandis* leave extracts exhibited comparatively higher antibacterial potential than other extracts. On the basis of it, topical herbal gels containing aqueous extract of *T. grandis* leave were formulated using 4.5 % sodium carboxymethyl cellulose as a gel base and evaluated for their antibacterial potential against *P. aeruginosa*. pH and viscosities of these gels were within the range 6.25 – 7.30and 43000 – 52000 cps, respectively. Gel containing 15 % aqueous extract exhibited satisfactory extrudability and spreadability. Antibacterial study of all prepared gels determining inhibition zones by disc diffusion method. Herbal gels containing 15 % aqueous *T. grandis* leave extract exhibited better zones of inhibition against *P. aeruginosa* in comparison with that of 5 % and 10 %. From the results of this study, it can be concluded that herbal gels containing 15 % aqueous *T. grandis* leave extract was found suitable for topical use against bacterial infections. It may be suggested that before its commercialization, the gel formulations should be subjected to detailed clinical studies using animals and human subjects. Also, extensive accelerated stability studies should be examined.

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CONFLICTS OF INTEREST: Nil

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