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ANTIBACTERIAL ACTIVITY OF *SIMAROUBA GLAUCA* LEAF EXTRACTS AGAINST FOOD BORNE SPOILAGE AND PATHOGENIC MICROORGANISMS

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ABSTRACT

Crude ethanol and methanol extracts from dried and fresh leaves of *Simarouba glauca* were tested for their inhibitory activity against two food borne pathogenic microorganisms (*Staphylococcus aeureus* and *Escherichia coli*) and two food spoilage microorganism (*Bacillus subtilis* and *Pseudomonas aeurogenosa*). Screening for antimicrobial activity using well diffusion assay showed the inhibition against entire tested microorganisms. On the other hand the maximum zone of inhibition was recorded of fresh leaves methanol extract (FLM) about 11 mm against *Escherichia coli* and the lowest zone of inhibition was recorded of fresh leaves methanol extract (FLM) about 2 mm against *Bacillus subtilis*. Minimum inhibitory concentrations (MIC's) of extracts were determined using agar dilution method on the same test microorganisms. Fresh leaves methanol (FLM) extract gave MIC value ranging from 160 to 10,240 parts per million (ppm). Result showed that the *Bacillus subtilis* was the most sensitive microorganism.

INTRODUCTION: In developing countries, Infectious diseases remain the main cause of high mortality rates recorded by WHO (1996) [1]. Food infection and intoxification also considered as the most common causes of food borne diseases worldwide. Food borne pathogens causing these diseases find their way in foods through cross contamination, improper handling and temperature abuse. *Staphylococcus aeureus* and *Escherichia coli* are among the common food borne microorganisms that cause infection. Food spoilage microorganisms, on the other hand, cause products to lose their quality which renders them unacceptable to consumers. Short shelf life of food products because of spoilage is one of the major problems of food industry. Examples of food spoilage microorganisms are *Bacillus subtilis* and *Pseudomonas aeurogenosa* [2]. The treatment of diseases is mainly based on use of antibiotics. In recent years, a number of antibiotics have lost their effectiveness due to development of resistant strains [3] mostly through the expression of resistant genes [4, 5]. In addition to these problems, antibiotics are sometimes associated with adverse effect including hypersensitivity and allergic reactions [6].

Preservation of pathogenic and spoilage microorganisms in foods is usually achieved by using chemical preservatives. These chemical preservative acts as antimicrobial compounds which inhibit the growth of undesirable microorganisms. Some of chemical preservative have chemical toxicity and thus food manufacturer demanded to find alternative source of antimicrobial compounds [7, 8]. Medicinal plants are also known to be used as food preservative

due to its antimicrobial activity [9]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases but only few of them have studied chemically and biologically in order to identify their active constituents [10]. In 1998 WHO estimated that 80 % of the people living in developing countries almost exclusively use traditional medicine. Most traditional medicine relies heavily on medicinal plants [11].

Considering the vast potentiality of medicinal plants as antimicrobial agents, a systematic investigation was undertaken to screen the antibacterial activity of *Simarouba glauca* against food borne pathogens namely *Staphylococcus aeureus*, *Escherichia coli* and food spoilage microorganisms namely *Bacillus subtilis* and *Pseudomonas aeurogenosa*.

Simarouba glauca (*Laxmitaru*, Family- *Simaroubaceae*) is a flowering tree and show antimicrobial and insecticidal activity [12] (Joshi and Joshi, 2007). It also has been used as febrifuge, antidysentric, antiherpetic, antihelminthic [13] and antiprotozoal [14] activities.

MATERIALS AND METHODS:

Collection of plant material: Fresh leaves of *Simarouba glauca* were collected from Puria Park, K. K. Wagh College of Agriculture, Saraswati Nagar, Nashik. (M.S). The leaves were washed thoroughly 2 to 3 times with water and with autoclaved distilled water and chopped in to small pieces. The cut leaves were divided in to two lots: Fresh leaves of *Simarouba glauca* (FL) and dried leaves of *Simarouba glauca* (DL).

Solvent Extraction: Thoroughly washed dried leaves and fresh leaves of *S. glauca* were powdered with the help of blender, 5 gm dried leaf powder was mixed in 100 ml of each methanol (DLM) and ethanol (DLE) respectively and 5 gm fresh leaf powder was mixed in 100 ml each methanol (FLM) and ethanol (FLE) respectively. The extraction was successfully done by Soxhlet extractor for 48 hrs. The solvent extracts were concentrated and reduced by rotary vacuum evaporator and preserved in air tight bottles at 5^{o}C until further use.

Growth and Maintenance of Test Microorganisms: Bacterial culture of *Bacillus subtilis, Escherichia coli, Pseudomonas aeurogenosa* and *Staphylococcus aeureus* were obtain from National Chemical Laboratory (NCL), Pune. The bacterial culture were pre-cultured in nutrient broth overnight in a rotary shaker at 37^{o}C and centrifuged at 10,000 RPM for 5 min. Further pellet was suspended in sterile double distilled water and cell density was standardized spectrophotometrically (A_{610}nm) to obtain a final concentration of 10^5 cfu / ml.

Determination of Antimicrobial Assay: Antimicrobial activity of various extracts of *S. glauca* was evaluated by the well diffusion method on nutrient agar medium [15]. The sterile nutrient agar medium (20 ml) in petri dishes was uniformly smeared using sterile cotton swab with tested pure culture of *E. coli, B. Subtilis, P. aeroginosa* and *S. aureus*. The wells of 5 mm diameter were made using sterile cork borer in each petri plates and various extracts of *S. glauca* were added, a blank well loaded without test compound was regarded as test control. For each treatment triplicates were maintained. The plates were incubated at 37^{o}C for 24 hrs and zone of inhibition was measured by comparing control and standard antibiotics.

Determination of Minimum Inhibitory Concentration (MIC): The method used to determine the MIC was the agar dilution method of the European Society of Clinical Microbiology and Infectious Diseases (2000). Freeze dried extracts was dissolved in sterile distilled water in two fold dilutions from 100 to 204800 PPM. 1 ml of each extract dilutions were individually added to 19 ml Muller Huntingson Agar (MHA) and poured in petriplates giving final concentration from 5 to 10240 PPM. The final concentration was calculated from following equation.

$$C_f = \frac{C_i}{20}$$

Where, C_f is final concentration of extract in agar and C_i is initial concentration of extracts in the sterile solution. Test microorganism was spotted on the surface of the solidified extract-agar mixture. Four spots were placed in each plate at an amount of 10 ml (Ci 10^4 cfu for bacteria) per spot. The plates were inoculated by starting from the lowest concentration up to the highest concentration. Controls (agar without extract) were also inoculated at the start and at the

end of the dilutions. After inoculation, plates were allowed to dry for 30 min. Plates were incubated at 37 ± 1°C for 18 hrs. The lowest concentration which showed no visible growth of the test microorganism was considered as the Minimum Inhibitory Concentration (MIC) for the extract.

RESULTS AND DISCUSSION:

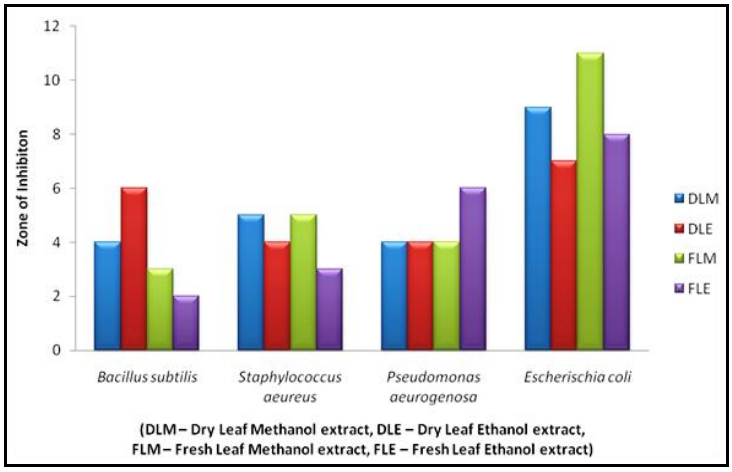
Determination of Antibacterial Activity: Medicinal plants are important in Indian traditional medicine and most frequently used in *Ayurveda*. The ethanol and methanol extracts of dried and fresh leaves were recorded earlier by soxhlet apparatus. Among the extracts used dry leaves extracts (DLE) of *Simarouba glauca* was found to be averagely effective against *Bacillus subtilis, Staphylococcus aeureus, Pseudomonas aeurogenosa, Escherischia coli.*

The less zone of inhibition was recorded of FLM about 2mm and 3mm against *B. subtilis* and *S. aeureus* respectively. The maximum zone of inhibition was recorded of fresh leaves methanol extract (FLM) about 11 mm against *E. coli*. The tested bacterial strain showed different pattern of inhibition (**Table 1**). Thus leaves extracts of *S. glauca* showed maximum zone of inhibition against *E. coli* in comparison with other bacterial species. (**Graph 1**) The broad spectrum antimicrobial activity of *S. glauca* was also reported by Banger *et al*. 2009 [16].

TABLE 1: ANTIMICROBIAL ACTIVITY OF *S. GLAUCA* EXTRACTS (100,000 PPM) USING WELL DIFFUSION ASSAY

Medicinal Plants	Leaves Extract	Zone of Inhibition (mm)			
		Bacterial Species			
		Bacillus subtilis	*Staphylococcus aeureus*	*Pseudomonas aeurogenosa*	*Escherischia coli*
Simarouba glauca	DLM	4 ± 1.50	5 ± 1.00	4 ± 0.57	9 ± 2.01
	DLE	6 ± 1.00	4 ± 1.50	4 ± 2.01	7 ± 0.57
	FLM	3 ± 1.00	5 ± 0.50	4 ± 0.52	11 ± 0.55
	FLE	2 ± 1.00	3 ± 0.57	6 ± 0.50	8 ± 0.50
Streptomycin Sulphate	5 µg/ml	8 ± 0.33	7 ± 0.33	7 ± 0.33	9 ± 0.33

(DLM – Dry Leaf Methanol extract, DLE – Dry Leaf Ethanol extract, FLM – Fresh Leaf Methanol extract, FLE – Fresh Leaf Ethanol extract)



GRAPH 1: COMPARISON OF *S. GLAUCA* PLANT EXTRACT

Determination of Minimum Inhibitory Concentration (MIC): Fresh leaves methanol extract (FLM) inhibited the growth of the test microorganisms with minimum concentration ranging from 160-10240 ppm as opposed to the DLM, DLE and FLE showed the inhibition of microbial growth at the highest concentration used (**Table 2**). Among the test microorganisms *B. subtilis* was the most sensitive to FLM which required 160 ppm to inhibit its growth followed by *S. aeureus* inhibited by 640 ppm concentration and *P. aureoginos* inhibited by 2560 ppm concentration (Table 2).

TABLE 2: MIC (PPM) OF PLANT EXTRACTS AGAINST TEST MICROORGANISMS

Test Microorganism	MIC (ppm)			
	DLM	DLE	FLM	FLM
Bacillus subtilis	2,560	>10, 240	>2,560	160
Staphylococcus aeureus	2,560	>2,560	2,560	640
Pseudomonas aeurogenosa	2,560	2, 560	>2,560	2,560
Escherischia coli	2,560	2,560	2,560	>2,560

(DLM – Dry Leaf Methanol extract, DLE – Dry Leaf Ethanol extract, FLM – Fresh Leaf Methanol extract, FLE – Fresh Leaf Ethanol extract)

The results are in accordance with the finding in well diffusion assay. Jenie *et al.* (2001) [17] reported the same activity with extracts of *Piper betle* Linn. against *S. aureus* and *E. coli.* This result cannot be compared with the values obtained by the mentioned authors since different plant material, extraction process and MIC determination methods were used.

CONCLUSION: This study showed that crude ethanol and methanol extracts from medicinal plants could inhibit certain food borne spoilage and pathogenic microorganisms. Extract from fresh and dried leaves of *S. glauca* inhibited all test microorganisms with minimum inhibitory microorganism ranging from 160 to 10240 ppm. Results revealed that the extract can be used as source of natural antimicrobial compounds which can be applied to foods to prevent growth of undesirable microorganisms. In addition, with the advent of novel food preservation techniques, extract from fresh and dried leaves of *S. glauca* could prove useful in antimicrobial food packaging. Incorporation of the extract in food packaging materials and determination of its effect on the shelf-life of food products are some topics for further research.

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