



PHYTOCHEMICAL AND ANTI-MICROBIAL SCREENING OF THE LEAVES EXTRACT OF *PROSOPIS GLANDULOSA* TORR

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ABSTRACT

Prosopis Grandulosa Torr belonging to the family Fabaceae (alt. Leguminosae), a plant of immense medicinal values was taken for the present study. The dry powder of the leaves of the plant was successfully extracted with Ethanol and Aqueous. All the extracts were subjected to Preliminary Phytochemical and anti-microbial screening. It showed the presence of Sterols, Carbohydrates, Glycosides, Gums, mucilage and Flavonoids and the results obtained indicate that the ethanolic extract was found to have significant anti-microbial activity in microorganism.

Keywords: *Prosopis Grandulosa*, Phytochemical, Anti-microbial, Leaves extract.

INTRODUCTION

Antimicrobial drugs are drugs designed to kill, or prevent the growth of microorganisms (bacteria, fungi, and viruses). Bacteria, fungi, and viruses are responsible for almost all of the common infectious diseases found in North America from athlete's foot, to AIDS, to ulcers (as of 2001). Interestingly enough, many disorders formerly thought to be caused by other factors, like stress, are now known to be caused by bacteria. For example, it has been shown that many ulcers are caused by the bacteria *Helicobacter pylori*, and not by stress, as many originally thought. Thus, antimicrobials represent an important part of medicine today¹⁻².

*Prosopis Grandulosa Torr*³ (Family: Fabaceae) used as Honey mesquite, though the major endemic woody plant in West Texas⁴ Still, this and other mesquite have been favored firewoods for years, and this use is expanding. Not differentiating the American desert species⁵ says, "Mesquite was the most widespread and important resource of the diverse native peoples in southwestern North America. It was utilized for food, fuel, shelter, weapons, tools, fiber, dye, cosmetics, medicine, and a multitude of other practical as well as aesthetic purposes: every part of the plant was used. Since no information available on an anti-microbial property of the plant *Prosopis Grandulosa Torr* the present study was undertaken to investigate the anti-microbial effect of the leaves extracts of the *Prosopis Grandulosa Torr*.

MATERIALS AND METHODS

Preparation of extracts

a) Ethanolic extract of leaves of *Prosopis glandulosa Torr*.

The powdered leaves of *Prosopis glandulosa* is extracted with 5-6 litres of Ethanol 99% by hot soxhlet extraction

using round bottomed flask. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. A brown colour residue was obtained the extract was then stored in a desiccator. The colour and consistency of leaves extracts of *Prosopis glandulosa Torr* is shown in the Table 1.

b) Aqueous extract of leaves of *Prosopis glandulosa Torr*.

The marc left after Ethanolic extract, was dried and subsequently extracted with 4-5 litres of distilled water by continuous hot percolation process using round bottomed flask for 72hrs. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. A light brown colour residue was obtained and the extract was then stored in a desiccator. From the weight of the each extractive residue, the extractive values were calculated in percentage. All the above extracts were used for identification of constituents by phytochemical tests and for the pharmacological studies. The colour and consistency of leaves extracts of *Prosopis glandulosa Torr* is shown in the Table 1 and the yields of various extracts were shown in the Table 2.

Anti-Microbial activity⁶⁻⁸

Materials required

Ethanolic and aqueous extracts of *Prosopis glandulosa torr*, Norfloxacin, Trypticase Soy Broth, 5 ml (or any other suitable broth), Mueller Hinton Agar, (MHA) pH 7.3±0.1, Sterile Saline or Broth and Petri dish.

Preparation of plates

Sterile Mueller Hinton Agar, (ph 7.3±0.1) is poured into plates (Petri dishes) kept on a leveled surface. The depth of the medium should be approximately 4mm. After the medium has solidified, dry the plates for 30 minutes in an incubator (35-37°C) to remove excess moisture from the



surface. While pouring into the plates, 5% defibrinated sterile sheep blood should be aseptically added to MHA for testing Streptococci and other fastidious organisms.

Preparation of Inoculum

1. Use only pure culture for sensitivity testing. Perform Gram staining before preparing an inoculum.
2. Select 4 to 5 similar colonies and transfer them into a tube containing 5 ml of Trypticase Soy Broth (or a similar broth) with the help of a wire loop.
3. Incubate the broth culture at 35-37°C for 2 to 5 hours to obtain moderate turbidity.
4. Dilute the broth culture of actively growing organisms with sterile broth or saline to obtain a turbidity equivalent to that of Barium Sulphate Standard (equivalent to half the density of McFarland Standard No. 1) which is prepared by adding 0.5 ml of 1.175% BaCl₂ 2H₂O solution to 99.5 ml of 0.36 NH₂SO₄. Overnight cultures of aerobes non-fastidious organisms generally have too much growth to be used undiluted. When time does not permit for the development of a sufficiently turbid broth culture, colonies can be suspended directly into a small volume of a saline or broth which is then further diluted up to the turbidity equivalent to half that of McFarland Standard No. 1. In order to prevent further growth the diluted and standardised inoculum should not be allowed to stand longer than 15 to 20 minutes before the plates are inoculated.

Inoculation

1. Dip a sterile cotton swab into the approximately diluted culture inoculum and rotate it while pressing against the upper inside wall of the tube, above the fluid level to remove excess inoculum.
2. Streak the agar surface of the plate in three directions, turning the plate by 60° between each streaking.
3. Replace the lid of the petridish and keep it at room temperature for 5 to 10 minutes, but no longer than 15 minutes to dry the inoculum. Confluent growth is desirable for accurate results.

Application of Sensitivity-disc

Remove one Sensitivity disc from its container with the help of a flamed forceps and carefully place it on the surface of the medium. Finally, press it lightly with the forceps to make complete contact with the surface of the medium. Allow the plate to stand at room temperature for 30 minutes (prediffusion time) before proceeding for the next step.

Incubation

Incubate the plates at 35-37°C for 16 to 18 hours.

Reading of Zones

Measure the diameter of the zone of inhibition at the end of the incubation period. If plates are showing proper growth, they may be read after 6 to 8 hours in clinically urgent situations. Measure only those zones that are showing complete inhibition and record the zone diameter to the nearest millimeter. If only isolated colonies grow instead of confluent growth, the inoculum is too light and the test should be repeated.

Quality Control

Quality parameters are important to be ensured in the use of combidiscs to ensure that correct and reproducible results are obtained. For quality control of disc diffusion, NCCLS has recommended the following ATCC reference strains namely *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Ps. Aeruginosa* ATCC 27853, *H. influenzae* ATCC 49247, *S. pneumoniae* ATCC 49619, & *N. gonorrhoeae* ATCC 49226. When these strains produce the results that fail within the specified limits (as per NCCLS guidelines), the susceptibility test results are considered to be valid.

Interpretation

Although there is some correlation between the size of the zone of inhibition and the susceptibility of the organism to the antibiotic, the former is a function of many variables e. g. density of the inoculum, depth of the medium, diffusibility of antibiotic etc. The size of the inhibition zone at which the organism is considered Resistant, Intermediates or Sensitive is given in the Zone size interpretative chart as a part of this literature.

RESULTS AND DISCUSSION

Based on the ethno pharmacology literature the leaves of *Prosopis glandulosa Torr* was collected in and around Salem district of Tamilnadu of plant and the collected plant was identified and authenticated by a (B.S.I). The leaves were shade dried at room temperature for 10 days and coarsely powdered.

The phytoconstituents were extracted by using two solvents like alcohol and aqueous by continuous hot percolation process. The colour and consistency of two extracts of the leaves of *Prosopis glandulosa Torr* is shown in Table 1. The extractive values were given in Table 2.

The phytoconstituents were identified by chemical tests, which showed the presence of various phytoconstituents (presented in Table 3) mainly in the following extracts, Alcohol extract - Carbohydrate, Glycosides and Flavonoids, Aqueous extract - Sterols, Flavonoids, Gums and mucilage's.

Table 1: The colour and consistency of leaves extracts of *Prosopis glandulosa Torr*.

Name of extract	Colour	Consistency
Ethanol	Brown	Powder
Aqueous	Light brown	Powder



Table 2: Extractive Values of the leaves of *Prosopis glandulosa* Torr.

Part used	Ethanol	Aqueous
Leaves	5.1%	4.8%

Table 3: Qualitative chemical test leaves extracts of *Prosopis glandulosa* Torr.

Constituents	Tests	Ethanol	Aqueous
Alkaloids	Mayer's reagent	-	-
	Dragondraff's reagent	-	-
	Hager's reagent	-	-
	Wagner's reagent	-	-
Sterols	Libermann's test	-	+
	Salkowski's test	-	+
Carbohydrate and glycosides	Molish reagent	+	+
	Fehlings reagent	-	-
	Barfoed's reagent	-	-
	Borntrager's reagent	-	-
	5% KOH	-	-
Fixed oils and fats	Spot test	-	-
	Saponification	-	-
Phenolic compounds	Extract + FeCl ₃	-	-
Test for Tannins	Gelatin test	-	-
	FeCl ₃ test	-	-
Protein and amino acids	Biuret test	-	-
	Ninhydrin test	-	-
	Xanthoprotein test	-	-
	Millon's reagent	-	-
Triterpenoids And Saponins	Tin + Thionyl chloride	-	-
	Foam test	-	-
	Haemolysis test	-	-
Gums and mucilages	Precipitation with 95% alcohol	-	+
	Molish's test	-	+
	Ruthenium test	-	+
Flavone and flavonoids	Aqueous NaOH	+	+
	Con.H ₂ SO ₄	+	+

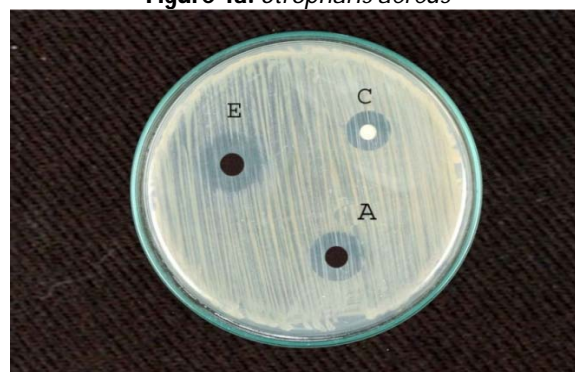
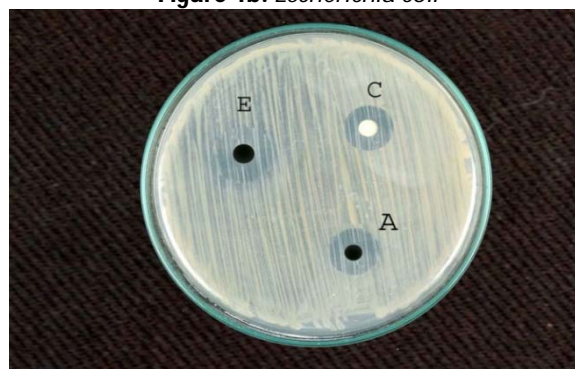
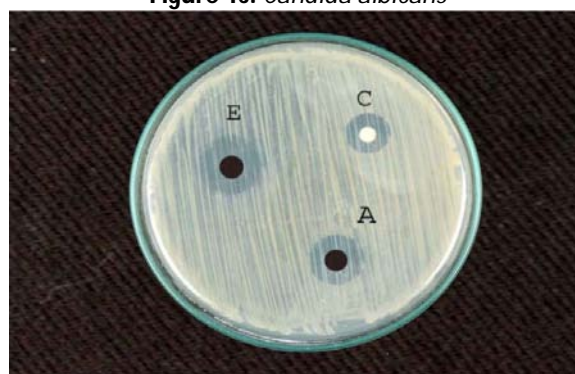
+ = Presence; - = Absence

Anti-microbial activity

The effect of ethanolic and aqueous extract of *Prosopis glandulosa* Torr on microbial organism Table 4 and Fig 1a-c. The results obtained indicate that the ethanolic extract was found to have significant anti-microbial activity in microorganism. The ethanolic extract of *Prosopis glandulosa* shows a significant zone of inhibition of the micro-organism is about 26mm, 22mm, 10mm respectively in *Escherichia coli* as compared to the control group Norfloxacin. (24mm)

Table 4: Zone of Inhibition of *Prosopis glandulosa* Torr on microbial organism in millimeters.

	<i>Escherichia coli</i>	<i>Stropharis aereus</i>	<i>Candida albicans</i>
Control (Norfloxacin)	24mm	16mm	12mm
Alcohol extract	22mm	26mm	12mm
Aqueous extract	10mm	8mm	8mm

Figure 1a: *Stropharis aereus***Figure 1b:** *Escherichia coli***Figure 1c:** *Candida albicans*

The ethanol and aqueous extracts zone of inhibition on gram+ve organism, *Stropharis aereus* is about 14mm, 26mm, 12mm respectively as compared to that of control *Norfloxacin* (16mm). The same ethanol and aqueous extract of *Prosopis glandulosa* has a zone of inhibition on fungi organism (*Candida albicans*) shows 14mm, 12mm, 8mm respectively when compared with the control drug *Norfloxacin* (12mm). Hence we confirm that our drug is having excellent anti-microbial activity when compared with standard drug.

CONCLUSION

The phytochemical constituents were identified by chemical tests and these showed the presence of various constituents like sterols, carbohydrates, glycosides, gums, mucilage and Flavonoids. Hence, the alcohol extract which has less polarity than the aqueous extract, also shows the presence of majority of phytoconstituents, and has been selected for antimicrobial studies. From the

above studies it was concluded that the leaves of *Prosopis glandulosa* Torr. Possess good anti-microbial activity. However, the future studies are proposed to find out the active principle responsible for the activity.

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