Research Paper
Impact Factor: 6.089
Peer Reviewed Monthly Journal
www.ijmdrr.com

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

"ANTIMICROBIAL AND PROTEIN (SDS PAGE) INVESTIGATION IN THE MEDICINAL PLANT *MORINGA OLEIFERE* COLLECTED FROM TRIVANDRUM DISTRICT"

Parvathy Balakrishnan* Dr. Sreeja mole KL* Dr.Mary Reena Jacob**
**S N College ,Cherthala.*

Abstract
The study indicates that the medicinal plants Moringa oleifera gives a basis of application in traditional medicinal and the bioactivity of phytochemicals constituents was more valuable. The presence of bioactive compounds is an indication of the presence of compounds which can be inhibitory against clinical isolates. The two concentrations of the extract were found to be effective against the selected strains (Streptococcus and E. coli). Because the growth of these organisms were inhibited the extracts by forming an inhibitory zone of different diameters. The highest inhibition was observed with E. coli which was found to be 1.7cm at 100mg/ml concentration. Similarly, a satisfactory inhibitory zone (1.4cm) was also observed with Streptococcus at 100mg/ml concentration. These results revealed the significant antibacterial activity of the extract against studied bacteria and the extract could be a promising reservoir for antibacterial agents with potential application in treating bacterial infection. The results of the SDS PAGE analysis show that there are 3 major bands present in the total protein extract of this sample which are of sizes 130 kDa, 40 kDa and 15 kDa. So, further investigation is necessary to confirm the presence of these compounds can be confirmed through the MALDI-TOF protein sequencing of the protein bands obtained.

Keywords: Screening, Antimicrobial, Medicinal, Extract.

- Collection of the plants from five different locations of Trivandrum district
- Establishment of the various steps involved in the SDS PAGE method.
- Analysis of the amount of protein concentrations in this plant.
- Analysis of Antimicrobial Study

Antibacterial screening using agar well diffusion method

- 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplate, after solidification, 100 µl (10^5 c.f.u./mL) of test organism were swabbed on the respective plates.
- Wells of 6 mm diameter were punched into the agar medium and filled with 100 µL of plant extract (of 100 mg/mL and 50 mg/mL concentration), antibiotic solution (positive control) and solvent blank (methanol) (negative control).
- The plates were incubated for 24 hours at 37C.
- After incubation the diameter of inhibitory zones formed around each disc were measured in mm and recorded.

Sodium dodecyl sulphate polyacylamide gel electrophoresis (SDS Page)

Preparation of stock solutions

1X running buffer (TGB Tris Glycine Buffer) was prepared by dissolving 3 g Tris base (25 mM), 14.4g ofGlycine (190 mM) and 1g SDS (0.1 %) in 900 ml distilled water. The pH of the solution was adjusted to 8.3 and makeup to 1L using distilled water. Acrylamide/bisacrylamide solution (30%) was prepared by adding 14.6g of acrylamide powder and 0.4g of bisacrylamide powder in 50 ml distilled water and stored at 4^{o}C until use. Also, 50 ml each of 1.5 M Tris pH 8.8 and 1 M Tris pH 6.8 were prepared for SDS gel preparation.

Stacking and Resolving gel composition for 15% SDS-PAGE gel

• **Resolving Gel (1mm plate) 15%**	
Water	1.76 ml
30% Acrylamide/Bisacrylamide solution	2.14 ml
1.5M Tris pH	1.4 ml
10% SDS	54 µl
10% APS (freshly prepared)	54 µl
TEMED	5.4 µl

• **Stacking gel (1mm plate) 8%**	
Water	1.82 ml
30% Acrylamide/Bisacrylamide solution	454 µl
1M Tris pH	334 µl
10% SDS	27 µl
10% APS (Freshly prepared)	27 µl
TEMED	2.7 µl

Sample loading and electrophoresis

Gel plates were placed in the electrophoresis apparatus carefully to prevent bubbles formation at the bottom of the gel plates. Running buffer was added to the lower tray and then the upper tray was filled with the same buffer. About 10 µl each of the samples (supernatant) and SDS PAGE loading dye was taken in a fresh vial, mixed thoroughly for 30-60 seconds and boiled for about 10 minutes in order to denature and neutralise the charge in the proteins in the sample. After the denaturation, the samples were chilled on ice and then loaded at the bottom of each well using a micropipette. Close the lid of the

apparatus tightly and connect the power supply at 100 V until the dye front reached the bottom of the gel plate

Visualization of proteins in the gels

To visualize the protein in the gel, the gel was placed in 40% distilled water, 10% acetic acid, and 50% methanol solution containing 0.25% Coomassie Brilliant Blue R-250 (staining solution) and incubated for 1 hr at room temperature with continuous shaking. The gel was then transferred to a mixture of 40% distilled water, 10% acetic acid, and 50% methanol (detaining solution) and incubated room temperature with continuous shaking until the excess dye has been removed. As a result, the proteins in the gel can be seen as deep blue bands (Figure no. 2).

Data analysis

Total number of bands obtained from the SDS PAGE gel were calculated for protein profiling

Table 1: Antibacterial activity of different concentrations (100mg/ml and 50mg/ml) of methanolic extracts of Muringa by agar well-diffusion method.

Sl.no	Organism	Concentration of the extract	Resistant	Sensitive	Zone diameter (in cm)
1.	*Escherichia coli*	100mg/ml		+	1.7
		50mg/ml		+	1.2
2.	*Streptococcus* sp.	100mg/ml		+	1.4
		50mg/ml		+	1.0

Table 3: Antimicrobial activity of standard antibiotic (Amoxycillin)

Organism	Zone diameter of amoxicillin
E. coli	2.1
Streptococcus sp.	2.6

Fig: Antibacterial activity of methanolic extract of Murringa and standard antibiotic amoxicillin.

In the present study, the total protein profile of different varieties of moringa plant from Trivandrum district using SDS PAGE is under consideration. (Figure no. 1).

Total protein extraction

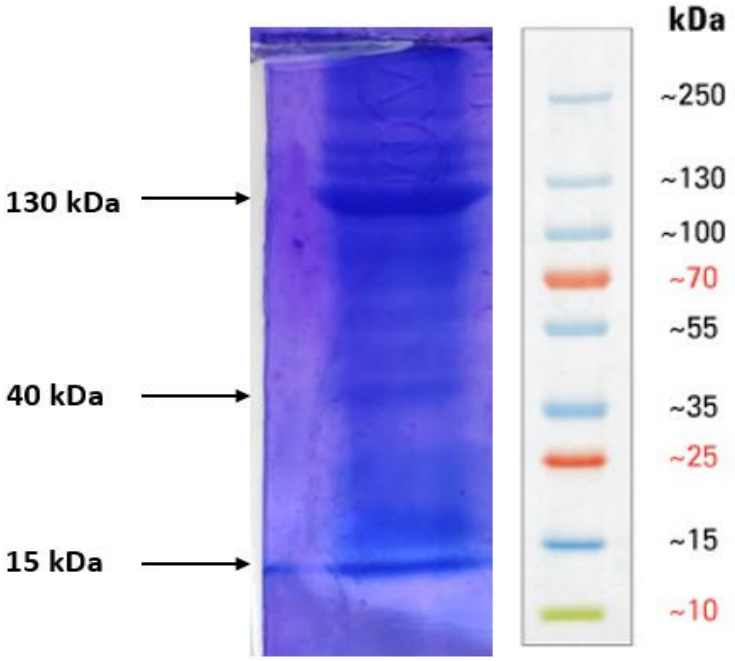

Figure no. 2: Electrophorogram of 15% polyacrylamide gel banding pattern showing diversity of total proteins of muringa plant.

References

1. lexander J MacLeod, Nirma M Pieris. Analysis of the volatile essential oils of the *Murrayakoenigii*and *Pandanus latifolius*. Phytochemistry21(7), 1653-1657, 1982.
2. A. Ludwiczuk, M I Georgiev, Pharmacognosy, 2017.
3. Anon(1962). The Wealth of India (Raw materials), volume 6. Publications and Information Directorate (CSIR) , New ,Delhi.
4. Araruna MK, Santos KK, da Costa JG, et al.(2013). Phenolic composition and in vitro activity of the Brazilian fruit tree *Caryocarcoriaceum*Wittm. Eur J Integral Med 5:178-83.