*Section: Research Paper*

**Original Article**

# Synthesis, Optimization and Characterization Of *Abelmoschus Esculentus* Mucilage As A Potential New Age Nano- Therapeutic Intervention

**Shivani Agarwal*[1,2], Prashant Upadhyay [3],**

1*.*Faculty of Pharmacy, IFTM University, Moradabad , Uttar Pradesh, India.*

*2. Moradabad Educational Trust Group of Institutions Faculty of Pharmacy, Moradabad , Uttar Pradesh , India.*

*3. School of Pharmaceutical Science , IFTM University, Moradabad ,Uttar Pradesh, India.*

E-mail- shivaniagarwal mbd2013@gmail.com , Tel no-9456689876

## ABSTRACT

**Objective**: To establish the quality control parameters for the isolated mucilage, the primary goal was to concentrate on qualitative and quantitative analysis of isolated okra mucilage using High-performance Liquid chromatography and Thin Layer Chromatography.

**Methods:** The components present in the methanolic and ethanolic fruit extract of okra were identified using high performance liquid chromatography (HPLC) at 254 nm and 356 nm. Ascorbic acid, an amino acid, and the overall amount of polyphenols were quantified**.**

**Results:** According to the findings, 11.5 and 10.5%, respectively, of the okra fruit mucilage were found to be yielded by methanolic and ethanolic fruit extracts. When detected at wavelengths of 254 nm and 356 nm, respectively, HPLC analysis of methanolic and ethanolic okra fruit extract revealed the existence of 8 components with Rf values in the range of 0.14 to 0.62 and 0.14 to 0.54. The okra fruit methanolic extract was found to have 11.45%w/w of the total amino acids. It was discovered that the ascorbic acid content in methanolic and ethanolic okra fruit extracts was 0.24% w/w and 0.18% w/w, respectively. The methanolic and ethanolic okra fruit extracts were calculated to have total phenolic contents (tannic acid equivalents, mg/g) of 4.6% w/w and 5.3% w/w, respectively.

**Conclusion**: Due to the presence of ascorbic acid and total phenolics, the data revealed two benefits: first, it might potentially serve as a novel functional ingredient with health-promoting applications, and second, it could provide guidelines for quality control requirements for the isolated okra fruit.

**Keywords**: Mucilage, Okra. HPLC, thin layer chromatography , fluorescence study.

## 1. Introduction

Natural polymers are frequently derived from the plant and animal kingdoms. High molecular weight, water solubility, and the presence of monosaccharide units joined by glycosides characterize the majority of naturally occurring polymers (1)

Gummy exudates of natural polymers such protein, enzyme, muscle, fibre, and polysaccharides have been used to make a variety of medicinal medications (1,2). Among the well-known natural polymers are gelatin, aloe mucilage, guar gum, karaya gum, bhara gum, sodium alginate, locust bean gum, okra gum, and linseed mucilage.

These natural polymers can be used to make a variety of pharmacological dosage forms, including buccal films, microspheres, nanoparticles, matrix controlled systems, and viscous liquid formulations (3). Natural polysaccharides are specifically used in pharmaceutical preparation to aid in the production, protection, enhancement of stability, bioavailability, and patient acceptance of drug delivery systems (4).Gums have many uses in medicine, including adhering to troche masses and pills, emulsifying resin oils, and suspending insoluble solid components in mixtures(5).

Abelmoschus esculentus, the biological name for okra, is an upright annual plant (Family: Malvaceae). D-galactose, L-rhamnose, and L-galacturonic acid make up the polysaccharide. When okra is squeezed and extracted in water, a gelatinous mucilage solution is what is left behind (6).

Okra gum has been used with a variety of pharmaceutical excipients, including binder, control release, film coating, bio-adhesive, and suspending agent (7)[10]. Okra gum has been investigated as a controlled release agent in customised release matrices containing medication in comparison to sodium carboxy methyl cellulose (NaCMC) and hydroxyl-propyl-methyl cellulose (HPMC) (8)[11]. With time-independent kinetics controlling the rate of release, okra gum matrices provide a protracted, regulated drug release of more than 6

hours (9). The results showed that Okra gum matrices were useful in producing sustained-release tablets with a potential half-life of up to 6 hours.

Additionally, unripe fruit has traditionally been used to cure urinary diseases, prevent scurvy, moisturise skin, and reduce discomfort. Blood volume expander and plasma replacement have both been accomplished with okra mucilage. Okra mucilage, which exhibits hypoglycemic propert(9)ies, is a polysaccharide made up of galacturonic acid, rhamnose, and glucose. Fresh fruits have been shown to have anti-cancer, antibacterial, and anti-ulcer properties when used medicinally to treat a variety of illnesses (10)

Microspheres and nanoparticles are created using these polymer-based materials that are orientated. On the creation of these carriers, which have been employed in the creation of microspheres, numerous investigations have been documented to date.

The treatment of waste water, immobilisation of enzymes, and the preparation of alginate, polystyrene, polyacrylamide, polyvinyl alcohol, nitrocellulose, etc. are among the most well-known uses of microspheres. Recently, dosage forms with precise release rate control and site-specific drug administration have had a significant impact on the development of innovative drug delivery systems. In innovative drug delivery methods, microspheres have played a key role(11) [18].

The majority of multi-particulate drug delivery methods are oral dosage forms made up of numerous small discrete units that each have certain desirable properties. These subunits are packed into a sachet, enclosed in a capsule, and compacted into a tablet to produce the appropriate total dose. Due to benefits including enhanced bioavailability, decreased risk of systemic toxicity, decreased risk of local irritation, and predictable gastric emptying, multi-particulate dose forms have been developed instead of single unit systems (10).

## 2. MATERIALS AND METHODS

All of the chemicals and solvents used were of the analytical grade and were obtained from laboratories. Distilled water was used throughout the entire experiment. Preliminary phytochemical/physicochemical studies were conducted using a variety of pharmacognostic techniques, including examination of ash values, moisture content, viscosity, swelling index (SI), pH value, etc. For the purpose of studying powder characterization, characteristics including bulk density, tapped density, Hausner's ratio, and angle of repose were established. Qualitative testing for mucilage and organic components were also conducted. High-

performance thin-layer chromatography (HPTLC) was used in an analytical investigation to set the proper standards(12).

### 2.1 Extraction of okra polysaccharide

Okra (*Abelmoschus esculentus*) was obtained from the local market of Moradabad, Uttar Pradesh India .The harvested okra was meticulously cleaned, dried for 24 hours in the shade, and then dried for a further 30–40 hours at 30–40°C to get a constant weight. The grinder was used to minimize the size. Fruit powder was put into an airtight container after passing through sieve number.(13)

Mucilage extraction involves two steps:

**STEP1: Extraction of mucilage:** The powdered fruit in 500ml of distilled water. heated for about 4 hours at 60°C while stirring continually. Filtered concentrated solution was cooled at 4°C to 6°C after passing through muslin cloth.

**STEP2: Isolation of mucilage:** Mucilage has been separated from extracted gum using acetone. This enables muslin cloth filtration. acetone-washed, then the mucilage was filtered through muslin fabric. Pressed mucilage underwent a fourth hour of drying after a constant weight in a hot air oven at 35–45°C. The hard mucilage cake was mashed and sieved using sieve #22 before being placed in a desiccators to be utilized later.(14)

### 2.2 Percentage yield of isolated mucilage

Based on the quantity of fresh okra and the quantity of dry mucilage obtained in accordance with solvents, the percentage yield for extracted mucilage was calculated and expressed as mucilage percentage (%). the weights of the dried mucilage obtained and the weight of the fresh material are used to calculate the percentage yield ratio. By using the formula, the yield of mucilage was determined by weighing the fresh material and dried, isolated mucilage .

**Percentage yield = Weight of dried mucilage obtained/weight of drug used × 100**

### 2.3 Preparation of Plant mucilage Extracts:

#### 2.3.1. Methanolic Extract of *Abelmoschus esculentus* mucilage (ME).

A Soxhlet extractor (1000 mL) was used to extract the powdered material in 80% v/v methanol for 72 hours at room temperature while stirring continuously after being sonicated for 1 hour. After filtering the extract, the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator to create a crude solid extract, which was then freeze-dried to completely remove the solvent. The yield obtained was 12% weighted(15).

**2.3.2. Aqueous Extract of *Abelmoschus esculentus* mucilage (AE).**

A 72-hour extraction process using distilled water and sufficient quantity of mucilage powder was carried out at room temperature. To keep the mixture free of microbiological contamination, chloroform was added. As soon as the extraction process was finished, it was filtered, the solvent was evaporated in a rotary evaporator, and the solid mass was then freeze-dried. The yield obtained was 10 % weighted in table-2.(16)

## 3. Solubility behavior

Visual observation of the solubility of polysaccharide in various solvents was made in accordance with (Indian pharmacopoeia, 1996). The medication is soluble in distilled water, phosphate buffer pH 6.8, ethanol, and was found to be insoluble in acetone, chloroform, and ether, among other solvents, when it was dissolved in each of the appropriate solvents (1 ml each)in table-3.(17)

**4. Phytochemical analysis of the isolated mucilage:**

**4.1 Characterization of extracted natural plant polysaccharide [11]**

The isolated polymer was evaluated for their chemical and physical characteristics such as its identification and purity test, percentage yield, organoleptic evaluation, solubility, pH, swelling index, loss on drying, micromeritics property etc in table-4.(18)

**4.2 Preliminary Phytochemical Screening**

**1. Detection of mucilage:** Powdered drug show used for the identification of mucilage

**1**.**Ruthenium red:** A few drops of ruthenium red were added to the powdered medication, which was then placed on a watch glass to produce a red tint**.**

**2**.**Polymer Test:** The Powdered drug were swells in water or aqueous KOH.

**2. Detection of carbohydrates :** Each extract was individually diluted in 5 ml of distilled water and then filtered. The filtrates were examined to determine whether carbs were present.

**1**.**Molisch's Test** (General test): Two drops of an alcoholic -naphthol solution were applied to filtrates in a test tube. The violet ring that forms at the junction is a sign of the presence of carbohydrates.

**2**.**Fehling's Test:** Filtrates were heated with Fehling's A & B solution after being hydrolyzed with dilute HCl, neutralised with alkali, and hydrolyzed. The presence of reducing sugars is indicated by the precipitate turning crimson.

**3.Benedict's test:** Benedict's reagent was applied to the filtrates and they were gently heated. Precipitate that is orange or red suggests the presence of reducing sugars.

**3. Detection of alkaloids**: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

**1.Dragendroff's Test:** Dragendroff's reagent was used to treat the filtrates (solution of Potassium Bismuth Iodide). Alkaloids are present when red precipitate is formed.

**2.Hager's Test:** Hager's reagent was used to process the filtrates (saturated picric acid solution). The appearance of a yellow-colored precipitate is evidence of the presence of alkaloids.

**3.Wagner's Test:** Wagner's reagent was used to process the filtrates (Iodine in Potassium Iodide). Alkaloids are present when a precipitate that is brown or reddish forms.

**4. Detection of glycosides** After hydrolyzing the extracts with diluted HCl, the glycosides were tested

**1.Borntrager's Test:** After being treated with a solution of ferric chloride, the extracts were placed in boiling water for roughly five minutes. After cooling, the mixture was extracted using equal parts benzene. After being separated, the benzene layer underwent ammonia solution treatment. Anthranol glycosides cause the ammoniacal layer to become rose-pink, indicating their presence.

**2.Keller-Killiani test:** Add 1 ml of pyridine and 1 ml of sodium nitroprusside to 2 ml of extract. A pink to crimson colour is visible.

**3.Baljet's test:**. With sodium picrate, a thick portion has a yellow to orange tint.

**4**.**Legal's Test:** Sodium nitroprusside in pyridine and sodium hydroxide were used to treat the extracts. cardiac glycosides are present when a pink to blood red hue forms**.**

**5. Detection of Saponins:**

**Froth Test:** Distilled water was used to dilute the extracts to 20ml, which was then agitated in a graduated cylinder for 15 minutes. The presence of saponins is indicated by the formation of a 1 cm layer of foam.

**6.Detection of Proteins:**

**Biuret test** (general test): Add 4% NaOH and a few drops of 1% CuSO4 solution to 3ml T.S. It seems to be pink or violet.

**Warming test:** Heat is applied after mixing 5 mg of powder with 10 mg of distilled water to create coagulated protein, which indicates the presence of protein.

**7. Detection of Tannins (Phenolic compounds):**

**Ferric Chloride Test:**. Three to four drops of a ferric chloride solution were added to the extracts. Blueish black colour formation suggests the presence of phenols.

**8. Detection of Amino acids:**

**Ninhydrin Test:** 0.25% w/v Ninhydrin reagent was added to the extract and heated for a short period of time. A blue colour is produced when an amino acid is present.

**9. Detection of starch:**

**Iodine test:** Combine a few drops of diluted iodine solution with 3ml of test solution. Blue colour first appears; it vanishes during boiling and then returns upon chilling.

**4.3 Phytochemical screening of okra mucilage**

Preliminary phytochemical tests for the detection of carbohydrates, phenols, flavonoids, alkaloids, terpenoids, steroids, tannins, saponins, and cardiac glycosides were conducted using standard phytochemical methods as described elsewhere in table-5 (19)**.**

**4.3.1 FTIR Spectra of Abelmoschus esculentus polysaccharide:**

The chemical structure of the components of okra fibers was analyzed using FTIR. The main absorbance peaks of interest in this study have been identified. The FTIR spectrum of the okra shows absorption bands of chemical groups characteristic of lingo cellulosic fiber compounds: cellulose, hemicellulose and lignin. Such components are mainly composed of alkenes and aromatic groups and various oxygen containing functional groups (ester, ketone and alcohol).

The distinctive functional groups in the selected extracts were located using an FTIR (Fourier transform infrared) spectrophotometer. To make thin, translucent sample discs, potassium bromide (KBr) was fully mixed with the AE and ME (5 mg), respectively, and then pressed at a pressure of 6 bars within 2 minutes. The Perkin Elmer 2000 spectrophotometer equipment with a scan range of 400 to 4000 cm1 was used to generate the FT-IR spectra, which was then analysed using Bruker OPUS software in figure-1 (20)

**4.3.2 Fluorescence analysis:**

One of the pharmacognostic techniques that can help identify genuine samples and adulterants is fluorescence analysis. (20) The drug powder underwent a fluorescence examination after being subjected to several solvent treatments. The study was carried out in both short- and long-wavelength daytime and UV radiation. The behaviour of okra mucilage after treatment with various reagents such as diluted ammonia, sulphuric acid, nitric acid,

acetic acid, picric acid, and water was studied using visible and ultraviolet light. After being exposed to several chemical reagents, the mucilage took on its usual coloring in table-8.

### 4.4 Phytochemical screening of okra mucilage extracts

The physicochemical analysis of Okra Mucilage are summarized in table 6. The PH of 1 % w/v solution of methanolic and ethanolic Okra extract in distilled water at 25 °C was found to be 6.60 and 6.80 respectively. Thus the obtained pH of extracts of okra mucilage indicates that this mucilage will be less irritating to GIT and suitable for various type of formulation including suspending agent in suspension. The loss on drying of the Okra methanolic and ethanolic extract was found to be 7.86 and 8.10% respectively. The results indicates that mucilage need to be stored in air-tight containers as it is hygroscopic Ash values were calculated for both methanolic and ethanolic extract to characterize mucilage; for methanolic extract total ash, acid insoluble ash and water-soluble ash were found 9.69% 1.21% and 4.38% respectively. For ethanolic extract total ash, acid insoluble ash and water-soluble ash were found to be 10.0%, 1.0% and 4.60% respectively. The isolated mucilage was also studied for swelling index. The swelling index was found to be 78.62%in water, 66.45%in 0.1N HCL and 48.65%in Phosphate buffer for methanolic extract. For ethanolic extract the swelling index was found to be 77.85%in water, 65.42 %in 0.1N HCL and 47.56 %in Phosphate buffer(21).

**Solubility**

Solubility of the mucilage powder was determined in different solvents such as water, chloroform, ethanol, acetone, petroleum ether and benzenein table-2 (22)

**Solubility profile of okra mucilage extracts :**

Numerous studies have examined the bioactive potential of okra mucilage and its rheological properties, which have been published in recent bibliographic surveys .Okra's significance as a cheap functional meal still has to be emphasised, nevertheless. Functional foods are a significant area for innovation since they aim to not only sate hunger and give people with the nutrients they need, but also to prevent diseases linked to poor nutrition and improve customers' physical and mental health. Vegetables are the richest sources of chemicals with healthy benefits due to their abundance in polyphenols, a powerful source of antioxidants**. (17)**

**2.6.1. Thin Layer Chromatography (TLC) Analysis. AE and ME**

The results described in table -7.

### 2.6.4. HPLC Procedure for the identification of components in Okra methanolic and ethanolic extract

100 mg each of the methanolic extract and ethanolic extract of Okra powder was dissolved in 10 ml of the suitable solvent and centrifuged at6500 rpm for 5 min. These supernatant was used as test solution for HPLC analysis to identify the components in Okra mucilage. The analysis was carried out by application of the 5 mg/ml methanolic extract of Okra mucilage and 10 mg/ml ethanolic extract of Okra mucilageon HPLC Precoated silica gel 60 (10 cm×10 cm) F254 plate [Merck, Darmstadt, Germany, 1.05554.0007] at different volumes 5,10,20 and 40 µl. were applied with proper supply of nitrogen gas to provide an inert environment to the sampling process. The plate was developed using the mobile phase Ethyl acetate: Acetic acid: formic acid: water: :100:11:11:25 in CAMAG twin trough glass Chamber (20×10 cm)up to the distance of 85.8 mm at room temperature. The plates were air-dried and viewed using CAMAGTLC Scanner at wavelength 254 nm and 356 nm. 8 numbers of tracks were studied and the distance between the tracks maintained as 11.4 mm with band length of 6.0 mm.**(25)** On a Shimadzu HPLC with a 250 mm (Kinetex) column and gradient injection of the mobile phase of 1% of acetic acid (solvent A): acetonitrile (solvent B) at a flow rate of 0.7 ml/min, isoquercitrin quantitative analysis was carried out in accordance with a modified method as described by Seal.

### 2.6.5. Development of Polyherbal Composition for antioxidant evaluation:

The polyherbal formulation combines MUE, ME, and AE. While equal volumes of the aforementioned extracts were used for phytochemical screening, the proportion of each extract used in the composition was chosen based on its unique in-vitro efficacy, or percentage free radical scavenging activity. MUE > AE > ME was determined to be the order of the methanol extracts' antioxidant activity. The extracts were collected in the following ratios: 5:3:2 in table -9.**(26)**

#### 2.6.5.1 Synergistic free radical scavenging activity of Polyherbal extract

Table 5.6 shows the results of the DPPH method testing of the free radical scavenging activity of PZME, DSME, AMME, and PHME. With increasing doses, it was discovered that all test samples showed an increase in percentage inhibition. With a maximum% inhibition of 82.79% at 8 mg/ml in comparison to standard ascorbic acid (92.92%), the comparative antioxidant activity across the test samples clearly demonstrated the combination synergistic

impact of PHME. This was in contrast to the individual extracts (PZME, DSME, and AMME). The test samples' antioxidant activity was determined to be in the following order: PHME>PZME>AMME>DSME in table-10 **(20).**

**Screening of extracts by *in vitro* antimicrobial assays:**

**Determinatio**(27)**n of zone of inhibition of individual and polyherbal extract by agar well diffusion technique:**

The antimicrobial spectrum showed that all the test samples were found to be effective against both gram positive and gram-negativestrains (Fig. 5.8) in a concentration dependent manner. The zone of inhibition of the test samples when compared at each concentration level forindividual strains, it was found that AMME exhibited antimicrobialefficacy with least zone of inhibition (ZI) against *S. aureus, S. bacillus, E.coli and P. aeruginosa* at 200 mg/ml. The sample PHME was found to be significantly more effective (Maximum ZI among all testsamples) against S. aureus, S. bacillus, E. coli and *P. aureginosa* atmaximum concentration level (200 mg/ml) which clearly showed the combinational effect of methanolic extracts (Fig. 5.3to 5.6). The results were showed antimicrobial efficacy in order viz. PHME > PZME >DSME > AMME. Brivaracetam shows its maximum antimicrobial efficacy as standard drug as compared to PHME under consideration (Fig. 5.7).The results showed the synergistic effect of PHME in comparison to individual methanolic fractions against both gram positive and negative strains. The antimicrobial activity of the selectedplant extracts and its polyherbal composition were consistent withthe ethnopharmacological relevance**.(28)**

**Determination of MIC of individual and polyherbal plant extract by Broth dilution method**

MICs are used to evaluate the antimicrobial efficacy of test drugs by broth dilution method. This method is used to measure the effect of decreasing concentrations of test drug in terms of inhibition of microbial growth. These evaluations can be used to determine appropriate concentrations required to produce the effect. The MICs of the test drug are quite less thanthe concentration found in the finished dosage form. The different methanolic test extracts showed variable MICs against both gram negative and gram-positive strains. The PHME was found to exhibit atleast MIC against *S. bacillus. E. coli* and *P. aureginosa* as comparedto PZME, DSME and AMME. PHME showed the same MIC value asPZME against *S. aureus*

(Fig.5.9)., The average MICs of all them ethanolic extract and combinations were found to be in order viz.PHME < PZME < DSME < AMME (Fig. 5.10) which clearly showed an inverse relation with the zone of inhibition. It can be concluded thatPHME showed combinatorial effect with least MIC value. The phenolic and flavonoids contents of the selected herbs probably killed the microorganisms either through inhibition of cell wall synthesis or disruption in permeability of bacterial cell membrane. These increased the probability of loss of membrane function and all key cellular constituents, resulting in mutation, cellular damage and finally cell death**(29)**

**Result And Discussion:**

1. **Percentage yield of okra mucilage**

In the present research analysis, after isolation of the mucilage from the fresh fruit of okra by means of methanol and ethanol, the percentage yield of purified extracts was found to be 11.5 and 10.5 %w/w respectively and was mentioned in table 1.

**Table: 1 Percentage yield of okra mucilage**

| Plant name | Percentage yield (%) |
|---|---|
| *Abelmoschus esculentus* | Methanolic Extract 11.5 |
| | |
| *Abelmoschus esculentus* | Ethanolic Extract 10.5 |

**Table: 2 Organoleptic Properties of okra mucilage**

| S.NO. | Parameters | Observation |
|---|---|---|
| **1** | Description | Brownish |
| **2** | Odor | Characteristic |
| **3** | Shape | Irregular |
| **4** | Taste | Mucilaginous |

**Table 3: Solubility profile of okra mucilage**

| SR.No. | Solvent | Extract | Solubility |
|---|---|---|---|
| 1. | Warm water | Ethanolic<br>Methanolic | Soluble |
| 2. | Cold water | Ethanolic | Swell to form gel |

| | | | |
|---|---|---|---|
| | | Methanolic | |
| 3. | Ethanol | Ethanolic<br>Methanolic | Insoluble |
| 4. | Acetone | Ethanolic<br>Methanolic | Insoluble |
| 5. | Benzene | Ethanolic<br>Methanolic | Insoluble |
| 6. | Methanol | Ethanolic<br>Methanolic | Insoluble |

**2.Phytochemical analysis of the isolated mucilage:**

**Table :4 Chemical Test for Characterization *of Abelmoschus esculentus* polysaccharide**

| **S.No** | **Chemical test** | **Colour** | **Inference** |
|---|---|---|---|
| **1** | **Test for Mucilage** | | |
| | **Ruthenium red** | Light Pink | +ive |
| | **Polymer test** | Gelatinous mass | +ive |
| **2** | **Carbohydrate test** | | |
| | **Molisch's test** | Reddish violet ring | +ive |
| | **Fehling's test** | Brick red | +ive |
| | **Benedict's test** | Green colour | +ive |
| **3** | **Alkaloid test** | | |
| | **Dragendroff's test** | Orange brown colour | -ive |
| | **Hager's test** | Orange yellow precipitate | -ive |
| | **Wagner's test** | Orange yellow precipitate | -ive |

| | | | |
|---|---|---|---|
| **4** | **Test for Glycosides** | | |
| | **Borntrager's test** | Reddish pink | -ve |
| | **Killer-killiani test** | Reddish brown | -ve |
| | **Baljet test** | Orange | -ve |
| | **Legal test** | Orange brown | -ve |
| **5** | **Test for Saponins** | | |
| | **Froth formation test** | Froth formed | -ve |
| **6** | **Test for Proteins** | | |
| | **Biuret test** | Violet or pink colour | +ve |
| | **Warming test** | Protein coagulated | +ve |
| **7** | **Test for Tannins(phenolic compounds)** | | |
| | **$FeCl_3$ test** | Formation of green and blue colour | +ve |
| **8** | **Test for Aminoacids** | | |
| | **Ninhydrin test** | Purple colour | +ve |
| **9** | **Test for Starch** | | |
| | **N/50 Iodine solution** | Blue colour | +ve |

**Phytochemical Screening of okra mucilage extracts :**

Preliminary phytochemical studies of AE and ME revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, proteins, terpenoids, tannins, and sterols. Saponins and cardiac glycosides were found to be absent in mucilaginous extract.

**Table-5 Chemical Constituents of ethanolic and methanolic extract**

| Chemical Components | Ethanolic Extract | Methanolic Extract |
|---|---|---|
| **Tannins** | ++ | +++ |
| **Steroids** | ++ | +++ |
| **Flavonoids** | +++ | +++ |
| **Saponins** | - | - |
| **Alkonoids** | - | - |
| **Anthraquinons** | ++ | ++ |
| **Phenols** | +++ | +++ |
| **Resins** | ++ | ++ |
| **Fixed oil** | - | - |
| **Cardiac Glycosides** | - | - |
| **Uronic acid** | ++ | +++ |
| **Mucilage** | +++ | +++ |
| **carbohydrates** | +++ | +++ |
| **Proteins and aminoacids** | +++ | +++ |
| **Sugar** | ++ | ++ |

**Sign: ---indicates absent: + indicates minimum: ++ indicates Moderate: +++ indicates Maximum.**

**Table-6 Experimental studies of extracts**

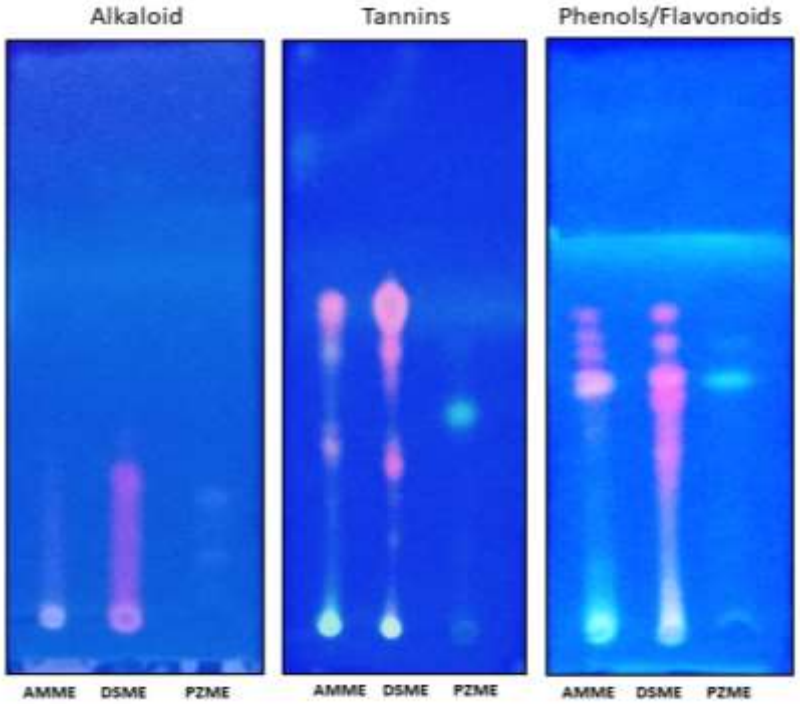
| S.R No | Experimental Studies | Extracts | Observation % |
|---|---|---|---|
| 1. | Total Ash Value | Methanolic<br>Ethanolic | 8.1<br>8.7 |
| 2. | Acid Insoluble Ash Value | Methanolic<br>Ethanolic | 1.32<br>1.1 |
| 3. | Water Soluble Ash Value | Methanolic<br>Ethanolic | 3.95<br>4.18 |
| 4. | Swelling Index | Methanolic | 83.16 in distilled water |

| | | | |
|---|---|---|---|
| | | Ethanolic | 54.16 in HCL<br>32.6 in phosphate buffer<br>84.15 in distilled water<br>50.13 in HCL<br>31.65inphosphate buffer |
| 5. | PH OF 1% w/v solution | Methanolic<br>Ethanolic | 6.6<br>6.8 |
| 6. | Loss on drying | Methanolic<br>Ethanolic | 7.45<br>7.96 |
| | | | |

**1. Thin Layer Chromatography**

There were several bands seen after extract component partitioning with a solvent solution, indicating that phyto chemicals were divided based on polarity. The chromatogram in Figure 1 was utilised to identify phenols, terpenoids, and carbohydrates after the spraying of specific detecting agents. The Rf values of samples MUE, ME, and AE demonstrated the presence of phenols/flavonoids, terpenoids, and carbohydrates. After contrasting the values and criteria, a decision was made (Table 4). Four different sites in the sample ME indicated the presence of tannins, phenolic compounds, and flavonoids (5 spots). Quercetin and its derivatives (2-quercetin) were discovered to be phenolic and flavonoid compounds. Similar to this, were identified as tannins based on their Rf values.

Thin layer chromatography (TLC) was performed utilising methods that are common in the industry (Harborne, 1998). All of the selected samples of MUE, ME, and AE (2 mg/ml) were dissolved in methanol. Solvent systems made by varying the concentration of the mobile phases were used. The plates were immediately observed using a UV-TLC viewer after drying (Figure 3). The reference values from studies by Sharma OP et al., 1998, Medi-ari M et al., 2004, and Svendsen AB, 1983 were then compared to the estimated Rf values for the various sites.




| **Carbohydrates (Cyclohexane: Chloroform: Diethyl amine 5:4:1)** | | | |
|---|---|---|---|
| **Sample extract** | **No. of spots** | ***$R_f$* value** | **Inference** |
| MUE | 1 | 0.511 | Protropin derivatives |
| ME | 2 | 0.13 | Antioxidant activity |
| | | 0.48 | |
| AE | 2 | 0.37 | |
| | | 0.23 | |
| **Terpenoids (Chloroform: Ethyl acetate: Acetic acid 50:50:10)** | | | |
| MUE | 3 | 0.48 | Rutin derivatives |
| | | 0.57 | Cinnamic acid |
| | | 0.71 | Cinnamic acid derivatives |
| ME | 4 | 0.76 | Cinnamic acid derivatives |
| | | 0.53 | Ferulic acid |
| | | 0.25 | Paragallol |
| | | 0.68 | Cinnamic acid derivatives |
| AE | 1 | 0.60 | Cinnamic acid derivatives |
| **Phenol/Flavonoid (Toluene: Ethyl acetate: Formic acid 36:12:5)** | | | |
| MUE | 3 | 0.56 | Quercetin dervivatives |
| | | 0.77 | 2- quercetin derivatives |
| | | 0.88 | ()-epigallocatechin2329 |
| ME | 5 | 0.46 | quercetin |
| | | 0.56 | 2-1, quercetin derivatives |
| | | 0.67 | ()-epigallocatechin |

| | | | |
|---|---|---|---|
| | | 0.70 | ()-epigallocatechin |
| | | 0.88 | Flavones |
| AE | 2 | 0.67 | rutin |
| | | 0.70 | epigallocatechin2330 |

**Table:7 MUE- Mucilaginous Extract , ME- Methanolic Extract , AE- Alcoholic Extract**

**Preparation of standard curve of Rutin**

The plant extractives (both methanolic and ethanolic extract) contains various constituents. The flavonoids are most active against microbial viability and are effective in tissue remodeling. Hence flavonoid content was estimated by taking rutin flavanoid as standard using Aluminum chloride colorimetric method. Standard curve was prepared separately by dissolving rutin in methanol followed by serial dilution in the concentration range from 1 – 50 µg/ml. (Fig 1). Rutin act as efficient radical inhibitors and have a neuroprotective effect against ischemia and reperfusion-induced cerebral injury .

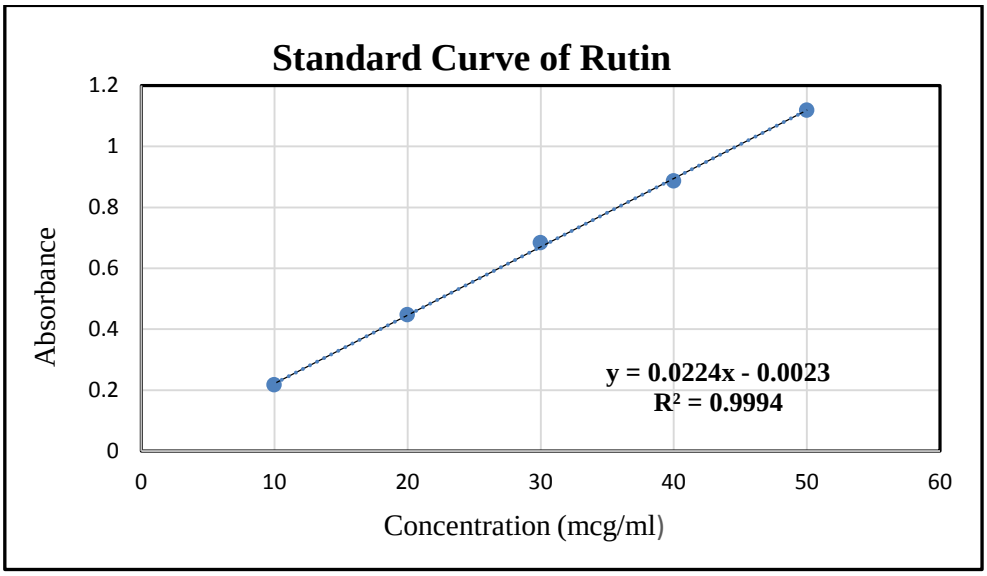



Figure.1: Standard curve of Rutin

**5.3.2.1 Rutin content of methanolic extract**

The Rutin content for MUE, ME was found to be 10.30 ± 1.84% and 13.26 ± 1.65 % w/w respectively

**5.3.2.2Rutin content of ethanolic extract extract**

The Rutin content for MUE, AE was found to be 10.30 and 8.26 ± 1.65 % w/w respectively

## 2. FTIR of MUE, ME. AE

FTIRspectroscopic studies revealed the presence of various functionalgroups such as alkyl, ketone, aldehyde, carboxyliccids, esters, and amide in aqueous and methanolic extracts of *Abelmoschus esculentus*, respectively (Table 1 and Figures2(a) and 2(b)).

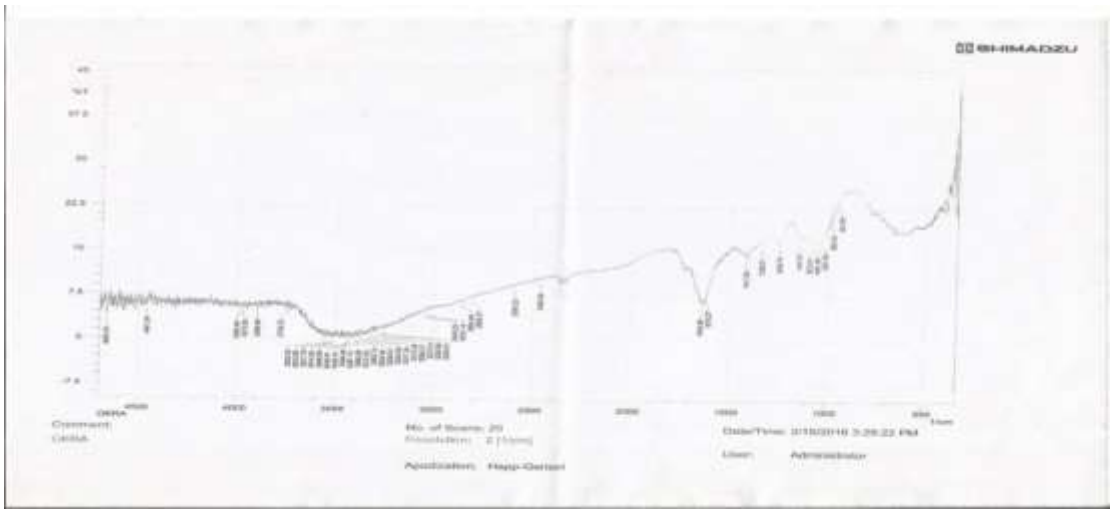


**Figure 2: FTIR of MUE**

## 3. Fluorescence analysis

**Table 8: Fluorescence analysis exhibited by okra mucilage Powdered drug**

| | **Visible/day light** | **UV 254 nm (short)** | **UV365 nm (long)** |
|---|---|---|---|
| Powdered drug as such | Light brown | brown | Yellowish brown |
| Powder+dil NH3 | Yellowish brown | Light brown | brown |
| Powder+conc HNO3 | Brown | Blackish Brown | Dark brown |
| Powder+10% NaOH | Yellowish brown | DarkYellowish | Bluish brown |
| Powder+1M H2SO4 | Brown | Dark brown | Blackish brown |
| Powder+10% FeCl3 | Brown | Dark brown | Blackish brown |
| Powder+10% Iodine | Yellowish brown | Dark brown | Blackish brown |

**HPLC of okra extracts**

| Time (min) | Pump A: 1% acetic acid | Pump B: acetonitrile |
|---|---|---|

| | | |
|---|---|---|
| 0 | 90 | 10 |
| 28 | 60 | 40 |
| 39 | 40 | 60 |
| 50 | 10 | 90 |
| 55 | 90 | 10 |

## 4. Development of Polyherbal Composition for antioxidant evaluation:

**Table 9 : Synergistic antioxidant effect of polyherbal methanol extract (PHME)**

| Sample | % inhibition at different concentration | | | |
|---|---|---|---|---|
| | 2mg/ml | 4mg/ml | 6mg/ml | 8mg/ml |
| MUE | 63.84±1.263<br>b**** | 67.59±0.304<br>b*** | 87.26±1.909<br>b**** | 92.92±0.790<br>b**** |
| ME | 20.52±0.397<br>a****<br>b**** | 56.77±0.447<br>a****<br>b* | 57.82±0.599<br>a****<br>b**** | 74.13±0.520<br>a****<br>b**** |
| AE | 17.09±1.064<br>a****<br>b**** | 44.44±0.150<br>a****<br>b**** | 47.77±1.611<br>a****<br>b**** | 75.35±1.267<br>a****<br>b**** |
| ME+AE | 13.77±0.728<br>a****<br>b**** | 45.88±0.193<br>a****<br>b**** | 61.93±0.220<br>a****<br>b**** | 78.64±0.939<br>a****<br>b* |
| ME+AE+MUE | 40.43±0.872<br>a**** | 61.10±0.728<br>a*** | 71.78±1.599<br>a**** | 82.79±1.718<br>a**** |

MUE: Mucilaginous extract, ME:Methanolic extract , AE: *Alcoholic extract* All values are represented as mean ± SEM, n = 3 for each group, Data were analyzed by two-way ANOVA, for each bacterial strain, followed by Dunnett's multiple comparisons test Multiple Comparisons Test, ****p< 0.0001 ***p< 0.001, **p< 0.01, *p< 0.1. **(a)** Signifies significant difference as compared to ascorbic acid (standard drug) and **(b)** denotes significant difference as compared to test drug PHME

**Table -10 Antimicrobial efficacy of methanolic extract against bacterial strains**

| Strains | Test drug | Zone of inhibition (in mm), mean ±SEM | | | |
|---|---|---|---|---|---|
| | | 50 mg/ml | 100 mg/ml | 150 mg/ml | 200 mg/ml |
| | PZME | 18.50±0.545<br>**** | 20.50±0.514<br>**** | 24.10±0.514<br>**** | 25.00±0.824<br>*** |

| | | | | | |
|---|---|---|---|---|---|
| *S. aureus* | DSME | 18.60±0.545 **** | 21.20±0.465 *** | 22.00±0.544 **** | 23.30±0.874 **** |
| | AMME | 11.75±1.480 **** | 13.00±1.154 **** | 18.50±0.589 **** | 19.32±0.200 **** |
| | PHME | 24.50±0.542 | 26.00±0.576 | 29.25±0.521 | 30.00±0.968 |
| | | | | | |
| *S. bacillus* | PZME | 18.30±0.566 **** | 21.75±0.520 **** | 23.75±0.556 **** | 24.00±0.521 **** |
| | DSME | 16.20±0.525 **** | 18.40±0.573 **** | 20.50±0.485 **** | 21.75±0.580 **** |
| | AMME | 13.50±0.520 **** | 15.25±0.560 **** | 17.75±0.150 **** | 18.75±0.100 **** |
| | PHME | 25.25±0.358 | 27.50±0.345 | 28.50±0.500 | 29.75±0.658 |
| | | | | | |
| *E. coli* | PZME | 16.10±0.480 **** | 18.20±0.532 **** | 19.00±0.547 **** | 20.55±0.510 **** |
| | DSME | 22.50±0.559 | 25.25±0.850 | 26.50±0.480 | 27.50±0.400 |
| | AMME | 16.45±0.564 **** | 17.10±0.570 **** | 19.50±0.250 **** | 20.50±0.560 **** |
| | PHME | 21.50±0.125 | 25.70±0.587 | 27.75±0.582 | 28.00±0.814 |
| | | | | | |
| *P. aureginosa* | PZME | 16.30±0.558 **** | 19.15±0.400 **** | 22.50±0.529 **** | 23.50±0.454 **** |
| | DSME | 16.40±0.514 **** | 18.70±0.565 **** | 20.50±0.540 **** | 21.00±0.100 **** |
| | AMME | 11.50±0.458 **** | 12.75±0.590 **** | 15.50±0.555 **** | 16.80±0.450 **** |
| | PHME | 23.25±0.598 | 25.75±0.367 | 28.75±0.522 | 29.00±0.586 |

All values are represented as mean ± SEM, n = 3 for each group, Data were analysed by two-way ANOVA, for each bacterial strain, followed by Dunnett's multiple comparisons test Multiple Comparisons Test, ****p< 0.0001, Asterisk (*) denotessignificant difference as compared to test drug PHME.

**Table -11: Antimicrobial activity of standard drug against different microbial strains**

| **Brivaracetam (10µg/ml)** | |
|---|---|
| *S. aureus* | 31.00±0.816 |
| *S. bacillus* | 31.50±0.530 |
| *E. coli* | 32.50±0.577 |
| *P. aureginosa* | 33.75±0.500 |

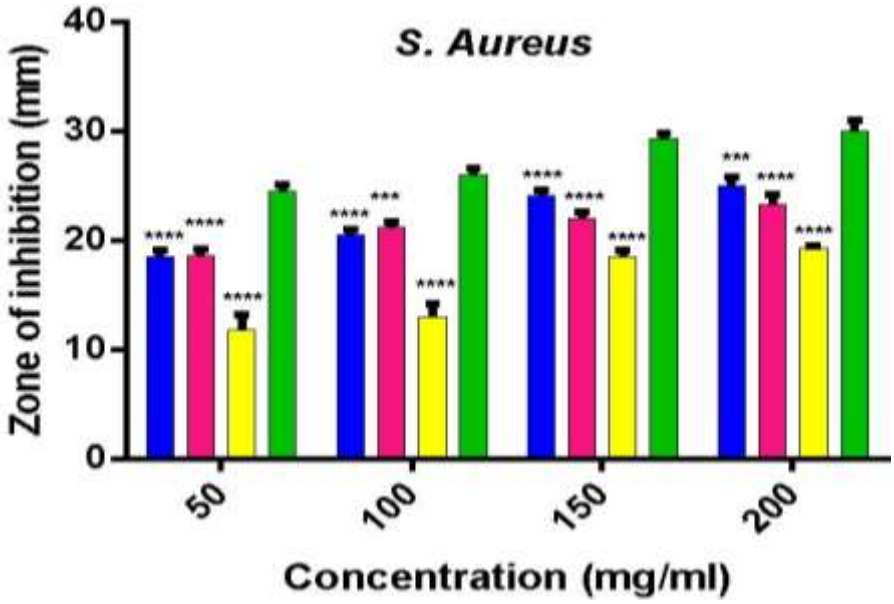



Figure 3: Antimicrobial spectrum of different plants extract and polyherbal against *S.Aureus*

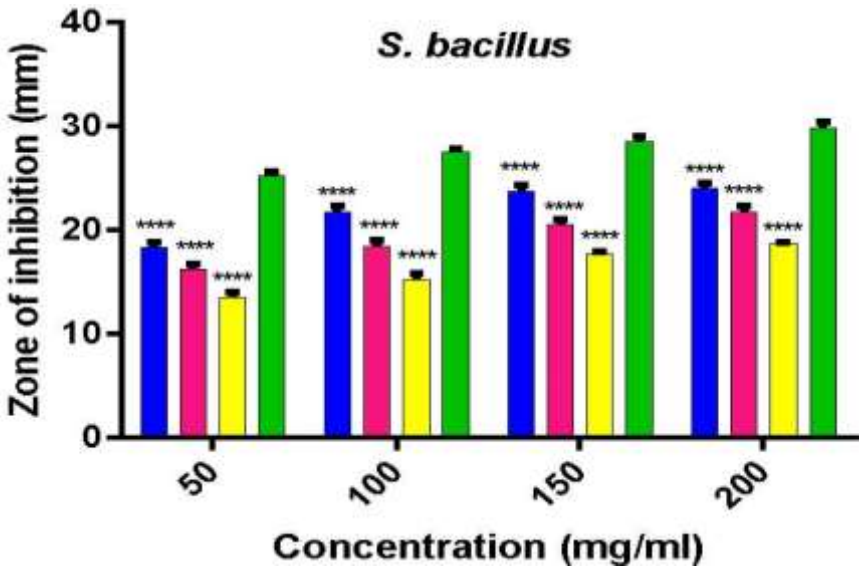



Figure 4: Antimicrobial spectrum of different plants extract and polyherbal against *S. bacillus*

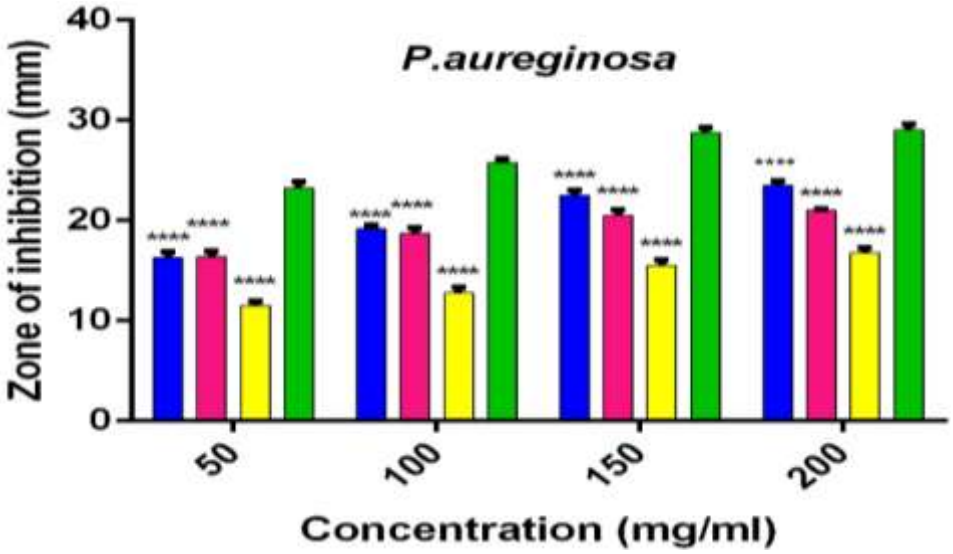



Figure 5: Antimicrobial spectrum of different plants extract and polyherbal against *P. aureginosa*

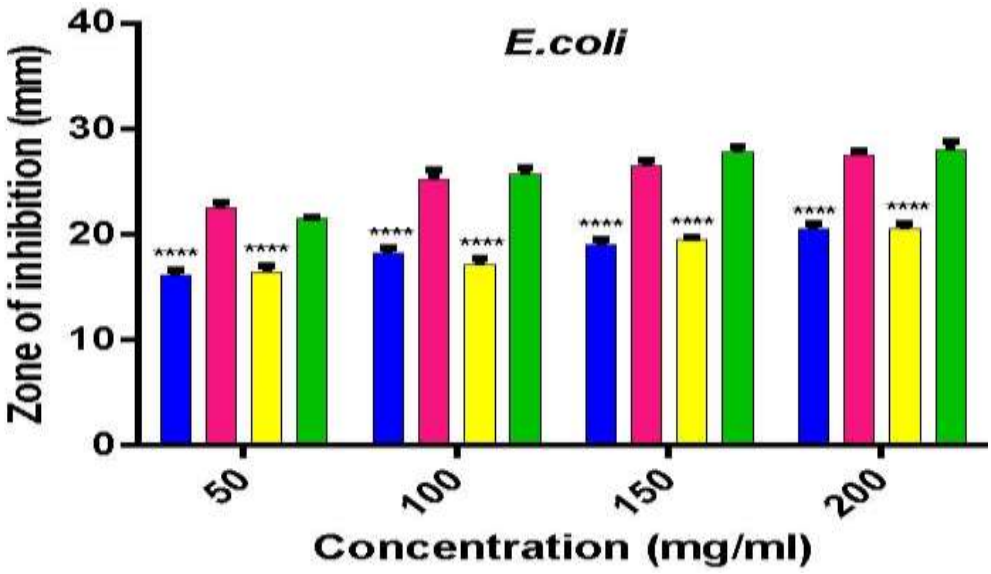



Figure 6: Antimicrobial spectrum of different plants extract and polyherbal against E.coli.

**Antimicrobial efficacy of methanolic extract against bacterial strains at different concentration (Fig.: 5.3, 5.4, 5.5 and 5.6)**

All values are represented as mean ± SEM, in triplicate ($n$ = 3). Data were analysed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test. Data represented as significant difference as compared to PHME group and ****P < 0.0001

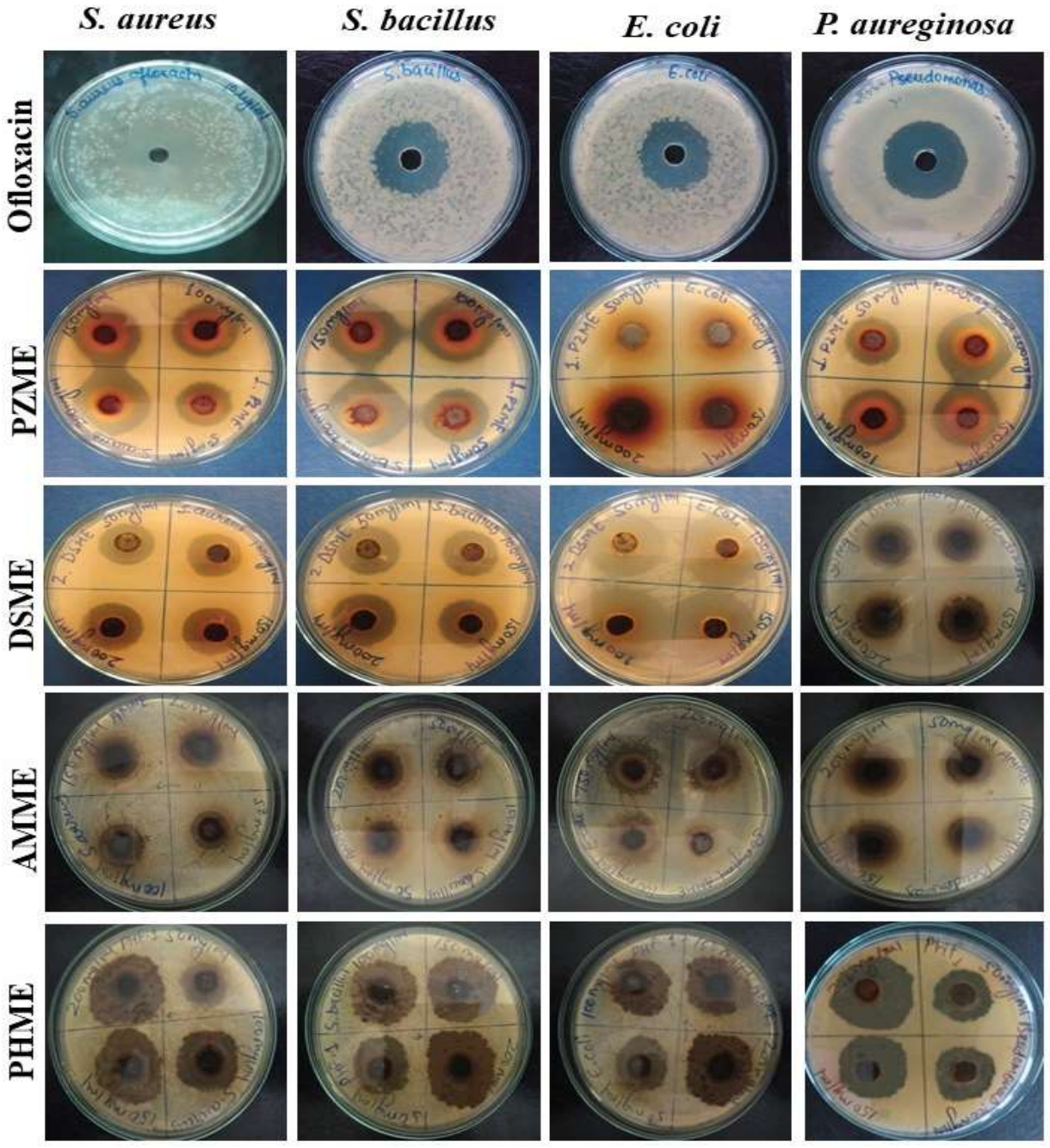



Figure -7 :*In-vitro* culture plates (Agar cup plate method) of standard drug (brivaracetam) and Methanolic extracts (PZME, DSME, AMME and PHME) showing zone of inhibition (ZI)

against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli andPseudomonas aeruginosa*at 50, 100, 150 and 200mg/ml concentrations.

**Determination of MIC of individual and polyherbal plant extract by Broth dilution method**

.**Table-12: Minimum Inhibitory Concentration of different methnolic extracts**

| **Extracts** | ***S. aureus*** | ***S. bacillus*** | ***E. coli*** | ***Pseudomonas*** | **Average MIC** |
|---|---|---|---|---|---|
| PZME | 3120µg/ml | 3120µg/ml | 1560µg/ml | 6250µg/ml | 3510µg/ml |
| DSME | 1560µg/ml | 6250µg/ml | 780µg/ml | 6250µg/ml | 3690µg/ml |
| AMME | 780µg/ml | 3120µg/ml | 1250 µg/ml | 6250µg/ml | 5650µg/ml |
| PHME | 3120µg/ml | 1560 µg/ml | 780µg/ml | 3120µg/ml | 2130µg/ml |
| Brivaracetam | 0.62µg/ml | 0.31µg/ml | 0.15µg/ml | 1.25µg/ml | 0.58 µg/ml |

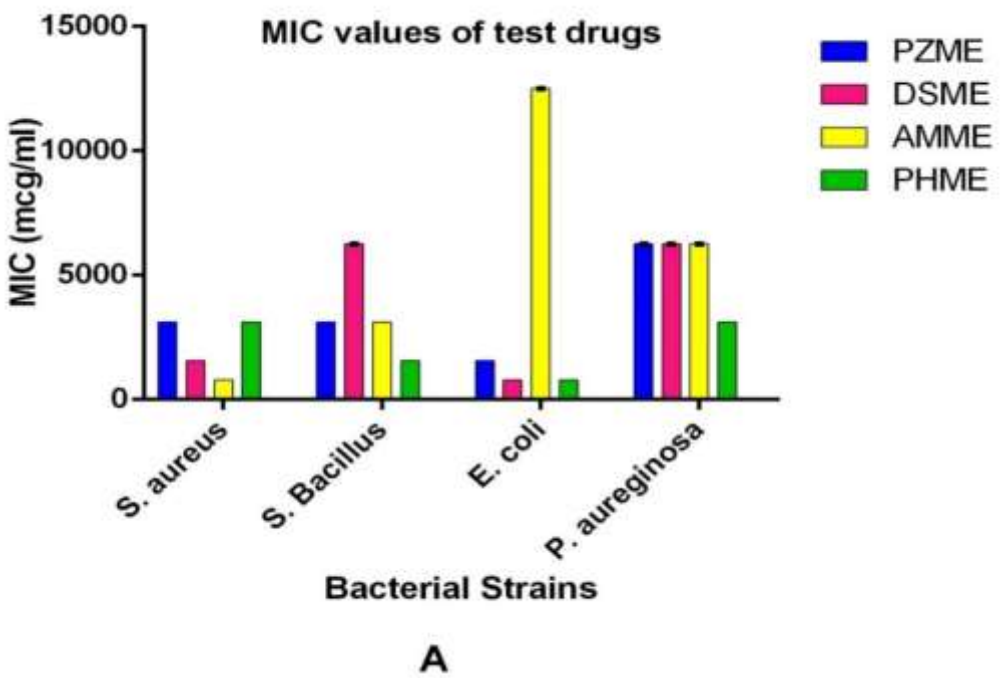



Figure.8: Represented comparative minimum inhibitory concentration (MIC) values of test drugs extracts against different bacterial strains

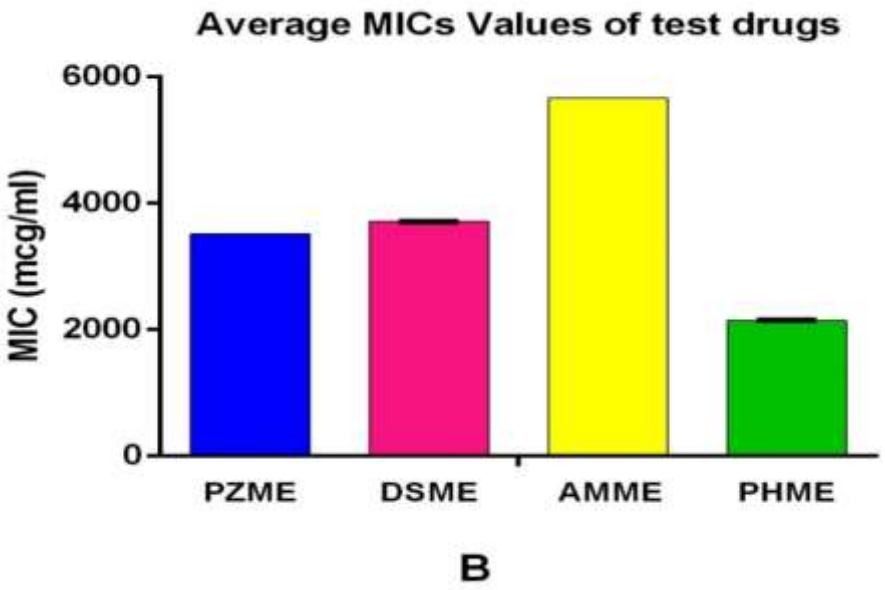



Figure 9 **:** Represented average MIC values of test drugs

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