Journal of Population Therapeutics & Clinical Pharmacology

**RESEARCH ARTICLE**
**DOI: 10.53555/3zmh9609**

# EXPLORING THE PHYTOCHEMICAL PROPERTIES OF *SYZGIUM CUMINI* PLANT EXTRACT AGAINST DIARRHOEA

**Hajra Sohail[1], Maria Akhtar[2], Sidra Shafique[3], Seema Batool[4], Fatima Hamid[5], Raheela Sarwar[6], Ayesha Tabbasum[7], Muhammad Tayab[8], Dr. Saman Zahra[9], Dr. Farheen Aslam[10*], Usman Minhas[2*]**

[1]University of Central Punjab, Lahore,Pakistan
[2]The University of Lahore, Lahore,Pakistan
[3]Dr. Ikram-ul-Haq Institute of Industrial Biotechnology GC University, Government Collage University Lahore (GCU), Lahore,Pakistan
[4]Department of Development and Environmental Studies, Arctic Studies,Paris-Saclay University,Paris, France
[5]Research and Development Center, University of Health Sciences, Lahore,Pakistan
[6]Center of Biotechnology and Microbiology, University of Peshawar,Peshawar,Pakistan
[7]Department of Biological Sciences, Virtual University of Pakistan,Pakistan
[8]Department of Zoology, University of Education, Lahore, Pakistan
[9]Department of Biology, University of Okara, Okara, Pakistan
[10]Lahore College for Women University,Lahore,Pakistan

***Corresponding Author:** Usman Minhas
*IMBB, The University of Lahore, Corresponding Author ID: usmanminhas060@gmail.com

## Abstract
*Syzgium cumini* (jamun) has long been used to treat *diabetes*, *gallstones*, *diarrhoea* and other diseases. The majority of the positive effects of *Syzgium cumini* leaves are attributed to phytochemicals, present in leaves, including gallic acid, tannins, mallic acid, flavonoids, essential oils, jambolin, ellagic acid, jambosine, antimellin, and betulinic acid. *Syzgium cumini* has medicinal properties, including anticancer, antidiabetic, antifertility, anti-inflammatory, antidiarrheal, antimicrobial, antinociceptive, antioxidant, antiradiation, chemotherapeutic, and gastroprotective. The primary purpose of this article is to provide information about the phytochemical makeup and health benefits of jamun leaves. The phytochemical content and health-promoting properties of *Syzgium cumini* are identified by using the High-Performance Liquid Chromatography (HPLC) technique, suggesting that they could be employed as a component in the creation of pharmaceutical medications.

**Keywords:** Phytochemical Content, Essential Oil, Pharmaceutical Medications, Treatment of Diseases

## 1. Introduction
*Syzgium cumini* is utilized as an alternative to manufactured medications in the Ayurveda and Unani systems of medicine because of its high bioactive content. Since ancient times, this medicine system has used several medicinal plants to heal ailments. It is a prominent organic herbal medication used to treat *diabetes, diarrhoea, oral ulcers,* and loss of appetite. *Syzgium cumini* is found throughout the Asian subcontinent, South America, Eastern Africa, and some mild sections of the United States of

America (Singh *et al*., 2019). These evergreen tropical trees are known for their common names, such as jamun, java plum, black plum, blackberry, and jambolan. They are rich in carbohydrates, minerals, and phytochemicals like anthocyanins, terpenes, and flavonoids. They are also high in fragrance compounds, particularly essential oils. These essential oils are impure, odorous, aquaphobic, extremely concentrated, and volatile at room temperature. *S. cumini* is an Indigenous minor crop in Asia, primarily in Pakistan, India, Sri Lanka, Nepal, and Bangladesh. Minor crops are highly nutritious and have effective medicinal characteristics. Its tree grows to an average height of 30-40 feet and produces plentiful delectable fruit that appears green when fresh and purplish-black when completely ripe, with a sweetish sour flavour. It is a cross-fertilized plant that can produce fruits for 60-70 years (Kumawat *et al*., 2018).

It is also used to prevent specific health issues including cancer, gout, and heart disease (Usman *et al*., 2013). *S. cumini* is a versatile plant due to its therapeutic and medical properties. The seeds are used to prevent diarrhoea and dysentery, while the leaves are used to cure bleeding gums, and the bark is used as an antibacterial agent. All plant extracts contain phytochemical elements such as alkaloids, saponins, steroids, and tannins, but they lack amino acids and proteins, except for seed extract. *Syzgium cumini* leaf extract contains solely flavonoids (Hajra *et al*., 2023).

A plant is a living factory that produces bioactive substances throughout its existence. Some of the plants used by humans to season food produce therapeutic phyto-compounds. Many, such as polyphenols and terpenoids, give plants their unique odours; others, such as tannins and quinones, cause plant colouring. Plant bioactive substances are divided into two categories: antimicrobial phytochemicals and antimicrobial peptides. Phytochemicals are non-nutritive chemical substances produced by plants that have organoleptic qualities and inhibit microbes. Different species create antimicrobial peptides as a natural defence against infections (Hintz *et al*., 2019).

Plant antimicrobial compounds act in a variety of ways, including altering the pathogen's cytoplasmic membrane, interacting with the extracellular protein of microorganisms, varying the ion permeability of the organism's cell wall, coagulating the cytoplasmic content of pathogenic microbes, and inhibiting enzyme production. Bioactive chemicals in plants primarily target the attacking microbes' nucleic acids, proteins, or biomembranes. (Khare, 2012). *S. cumini* demonstrates a variety of pharmacological actions. Many flavonoids and phytochemicals found in plants contribute to their anti-diabetic, anti-oxidative, anti-allergic, and hepatoprotective properties (Ayesha *et al*., 2023). *Syzgium cumini* is one of the most commonly utilized plants for diabetes treatment.

## 2. Material and methods

### 2.1. Phytochemical Screening of *Syzygium Cumini* Ethanolic Extract

Phytochemical screening helps to reveal the essential constituents of plant extract. Phytochemicals are generated from plants through primary and secondary metabolism. Phytochemical screening is of two types i.e. qualitative and quantitative analyses. As qualitative analyses are only concerned with the presence or absence of specific phytochemicals, whereas quantitative analyses give the concentration of particular phytochemicals in plant extract through different analytical techniques (Tyagia *et al*., 2019).

#### 2.1.1. Qualitative Analysis

##### 2.1.1.1. Reagents Preparation

**(a) Ninhydrin Solution**

0.1g of ninhydrin was dissolved in ethanol (5ml).

**(b) Ferric Chloride Solution**

0.2g of ferric chloride was dissolved in distilled water (10ml).

**(c) Sodium Hydroxide Solution**

NaOH (0.2g) was dissolved in 10 ml of distilled water.

**(d) Wagner's Reagent**
0.2g of iodine and 0.6g of potassium iodide was dissolved in 10 ml of distilled water.

**(e) Mayer's Reagent**
0.135g mercuric chloride was dissolved in 2 ml distilled water. The 0.5g of potassium iodide was mixed in the same volume of distilled water (2 ml). These solutions were joined in a specific ratio and the final volume was obtained through distilled water.

**2.1.1.2. Alkaloids**
**(a) Wagner's Test**
1ml of the extract was added to 2ml HCL and heated in a water bath. Then a few drops of Wagner's reagent were added to this solution. The configuration of precipitates was taken as an indication of the existence of alkaloids.

**(b) Mayer's Test**
1ml of the extract was added to 2ml HCL and heated in a water bath. Then a few drops of Mayer's reagent were added to this solution. The formation of precipitates was taken as confirmation of the occurrence of alkaloids.

**2.1.1.3. Terpenoids**
5ml of the extract was added to chloroform (2ml). Then concentrated sulfuric acid (3ml) was mixed carefully. The appearance of a reddish-brown colour indicated the existence of terpenoids.

**2.1.1.4. Protein**
**(a) Ninhydrin Test**
1ml of the extract was boiled with a solution of Ninhydrin (1ml). The Violet colour indicated the presence of proteins and amino acids.

**2.1.1.5. Tannins**
1ml of the extract was added to the Ferric chloride (1ml) solution. The blackish-green colour showed the existence of tannins.

**2.1.1.6. Flavonoids**
2ml of the extract was mixed with a few drops of 20% sodium hydroxide solution. The deep yellow colour was then converted into a colourless solution by adding a few drops of diluted HCL. That indicated the presence of flavonoids.

**2.1.1.7. Steroids**
1 ml of extract was added to 2 ml of chloroform. Then concentrated sulfuric acid was poured along the sides of the test tubes. Red-coloured rings were produced that indicated the existence of steroids.

**2.1.1.8. Carbohydrates**
**(a) Iodine Test**
Extract (1ml) was added to the iodine solution (1ml). A dark blue or purple colour indicated the occurrence of carbohydrates.

**2.1.1.9. Carotenoids**
1g of extract was mixed with 10 ml of chloroform with vigorous shaking. The mixture was filtered, and 85% sulfuric acid was added to it. The appearance of a blue colour at the interface indicated the presence the carotenoids.

### 2.1.2. Quantitative Analysis

#### 2.1.2.1. Estimation of Total Phenolic Compounds

Phenols called phenolics are a class of chemical compounds comparing a hydroxyl group directly bonded to a hydrocarbon group with an aromatic ring (Keerthiga *et al*., 2022).

**Reagents**

Gallic acid 0.129g/100ml, 100ul leaves extract, 1ml Folin-Ciocalteu, 20% $Na_2CO_3$.

**Procedure**

For the determination of total phenolics in *Syzygium cumini* leaves Folin-Ciocalteu was used. Gallic acid was used as standard. Different dilutions of Gallic acid were used i.e. 12.9µg/ml-258µg/ml. In five different test tubes the 1ml of FC and 1ml of $Na_2CO_7$ and, left for 10 min. Volume was made up to 25ml. Absorbance was taken at 760nm. The standard graph was plotted against conc. (µg/ml) at the x-axis and absorbance at the y-axis. The absorbance of the sample was also taken by adding 100µl of leaves extract into 1000µl of FC reagent and 1000µl of $Na_2CO_3$. The total phenolic content of *S. cumini* leaves was calculated by the calibration of the standard curve (Keerthiga *et al*., 2022).

#### 2.1.2.2. Estimation of Total Flavonoid Compounds

Flavonoids are vital and different phenolic compounds. Generally, flavonoids had 15 C-atoms and compared two phenol rings associated by a chain of three carbons to shape tricyclic compounds (Toobah *et al*., 2024).

**Reagents**

Quercetin 0.0018g/25ml, 100µl leave extract, methanol, 10% Aluminum chloride, 1M Potassium acetate.

**Procedure**

Different dilutions of quercetin were prepared from a stock solution ranging from 14.4µg-144µg. Added 1.3ml methanol, 0.25ml of 10% $AlCl_3$, and the same amount of potassium acetate in all the samples. Made the volume up to 25ml in a volumetric flask and incubated the solution for 10 min at room temperature. Absorbance was taken at 415nm on a spectrophotometer against a reagent blank. The standard graph of quercetin was plotted between conc. (µg/ml) on the x-axis and absorbance on the y-axis. The same procedure was repeated for *S. cumini leaves* extract to get absorbance and TFC was calculated by standard graph (Sonawane *et al*., 2013).

### 2.1.3. Estimation of Antioxidant Activity in *Syzygium Cumini* Leaves Extract

Antioxidant activity was engaged with the barrier instrument of the living being against the pathological related to the assault of free radicals. This process blocked the process of oxidation. Used for the adjustment purpose of foodstuff, makeup, pharmaceutical and polymeric products (Iyer *et al*., 2014). *S. cumini* is a great source of essential antioxidants. Antioxidants such as carotenoids, flavonoids, benzoic acid, ascorbic acid, cinnamic acid and tocopherols are the secondary metabolites obtained from the different parts of the plant. From all of them, beta-carotene and ascorbic acid are the most useable antioxidants. (Rao *et al*., 2015).

**Reagents**

Ethanol (pure form), DPPH Assay: weighed the 0.04g of DPPH on added in flash containing 100ml of ethanol placed on a shaking incubator for 30 min. Covered with foil paper. It was stored at 4°C in the dark.

**Procedure**

DPPH radical scavenging activity was estimated according to the Brand-Williams method (Brand Williams *et al*., 1995). The stock solution of BHT (butylated hydroxytoluene) was prepared by mixing

its 0.01g in 100ml methanol. From the stock solution, different dilutions were prepared in conc. range from 16.28µg/ml-50.76µg/ml. all these dilutions were taken as initial dilutions. The wavelength was set at 515nm. By absorbance, %age antioxidant activity was calculated and plotted graph of BHT against concentration was on the x-axis and %age antioxidant activity on the y-axis. The BHT antioxidant is used as a positive control. Each sample was diluted with DPPH. 3ml of DPPH was added in different volumes of Syzigium cumini leaves extract in the range from 10µl-50µl.

**Calculation of % Antioxidant Activity**

$$\% \text{ Antioxidant activity} = \frac{\text{Abs (blank)-Abs (Sample)}}{\text{Abs (Blank)}} \times 100$$

**2.2. Characterization and Quantification of Phytochemicals by HPLC**

HPLC (High-Performance Liquid Chromatography) is a widely used and functional analytical technique to isolate natural products and separate the specific components from the mixture. It is also used for the identification, quantification and purification of individual constituents of the mixture. In HPLC, reversed phase chromatography does 65% constituent separations as it is the most frequently used separation technique due to its wide operational range. It is the most simplest and versatile method to operate the compounds with distinct polarity and molecular mass e.g. secondary metabolites identification and characterization. The buffers used as a mobile phase are strong acids with low pH in reverse-phase chromatography. For the components, an identification detector must be selected. The UV detector stands out among various detectors due to its high specificity and sensitivity. It effectively detects many natural essential compounds, such as plant phenolic compounds, which are found to absorb UV light at wavelengths between 190 to 380 nm. When plant material needs to be separated using HPLC and is in dried powdered form, it is necessary to use organic solvents like methanol, ethanol, or chloroform for preparing extracts. Before loading the extract into the HPLC system, it is filtered through different types of available filters to ensure optimal performance of the analytical columns. (Boligon *et al*., 2014).

**2.3. Antimicrobial Activity of Extracted Phytochemicals**

**2.3.1. Preparation of Culture Media and Inoculation**

The nutrient medium and petri plates were sterilized at 120°C for 20 minutes. Media pouring was carried out in laminar airflow. Approximately 25 ml of media was poured into sterilized petri plates and allowed to solidify. The bacterial strains were swabbed on the medium with the help of cotton swabs after the agar plates were properly solidified. Sterile nutrient agar plates were prepared and inoculated through the spread plate method under aseptic conditions.

**2.3.2. Preparation of Extracted Phytochemicals**

HPLC-separated phenolic phytochemicals were dried into a concentrated extract by evaporating the solvent phase through the freeze-drying method with the help of a rotary evaporator at 60-70°C under reduced pressure until a constant extract weight was attained. After that make dilutions of extracted phytochemicals by adding organic solvent (ethanol) for testing its antimicrobial activity (Kiran *et al*., 2023).

**2.3.3. Antibacterial Activity**

The antibacterial activity of *S.cumini* leaves extracted phytochemicals was tested by using the agar well diffusion method as described by (Maria *et al*., 2023). In each well 100µl extracted phytochemicals were added and organic solvent (ethanol) was used as a control. The antibacterial activity was performed against six bacterial strains e.g. *E.coli. S.aureus, Pseudomonas, Salmonella typhi, Klebsiella* and *Bacillus subtilis.* The antibacterial activity of polyphenolic phytochemicals against specific bacterial strains was expressed in terms of the diameter of the zone of inhibition (in mm).

### 2.4. Identification of Phytochemicals by GC-MS

Gas chromatography-mass spectroscopy (GC-MS) is an analytical technique used to determine and identify the essential components present in a plant extract. GC-MS plays a beneficial role in the analysis of phytochemicals collected from High-Performance Liquid Chromatography (HPLC). GC separates the mixtures into individual components by temperature-controlled capillary columns whereas MS recognises the variety of components based on their mass spectra (JF Mahdi *et al*., 2019). The collected phytochemicals for GC-MS analyses were first treated with anhydrous sodium sulphate (pretreated at 400°C for 4 hrs) for the separation of phytochemicals from the solvent e.g. acetonitrile, distilled water or ethanol. The concentrated percentage of identified constituents will be calculated by standard method using calibration curves that will be generated by running GC analysis of selected authentic compounds

## 3. Results

### 3.1. Qualitative Analysis of Phytochemicals

Qualitative phytochemical analysis was investigated by biochemical testing with the leaf extract of *S. cumini* to detect the presence or absence of numerous primary and secondary metabolites with the help of standard protocols.

#### 3.1.1. Alkaloids

The identification of alkaloids in *S. cumini leaves* extract was taken by Wagner's and Mayer's test. The configuration of precipitates shows the presence of alkaloids in the extract as shown in figure (3) a.

#### 3.1.2. Terpenoids

The existence of terpenoids is shown by the colour change in leaf extract. The green colour of *S. cumini* leaves extract converted into reddish-brown as shown in Figure (3) b.

#### 3.1.3. Tannins

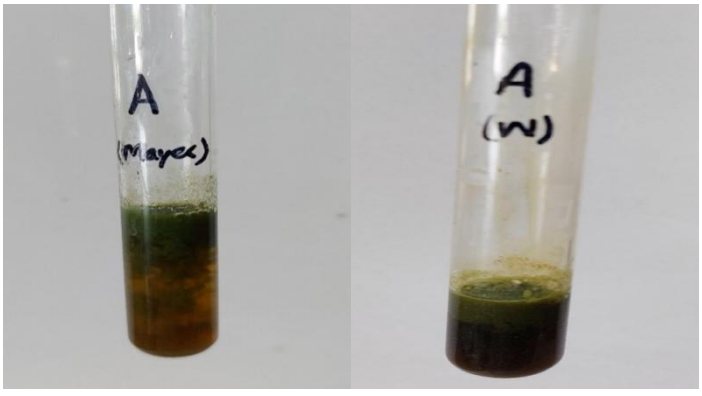
Tannins are identified when the colour of *S. cumini* leaves extract is converted into blackish-green in colour as shown in the figure. (3) c

#### 3.1.4 Proteins

The presence of proteins in the *S. cumini* leaves extract is undertaken by the conversion of the green colour of the extract into the violet colour as shown in Figure (3) d.

#### 3.1.5. Steroids

The existence of steroids in *S. cumini* leaves extract is identified by the production of the red ring on the top of extract as shown in figure (3) e.




(a.1) (a.2)

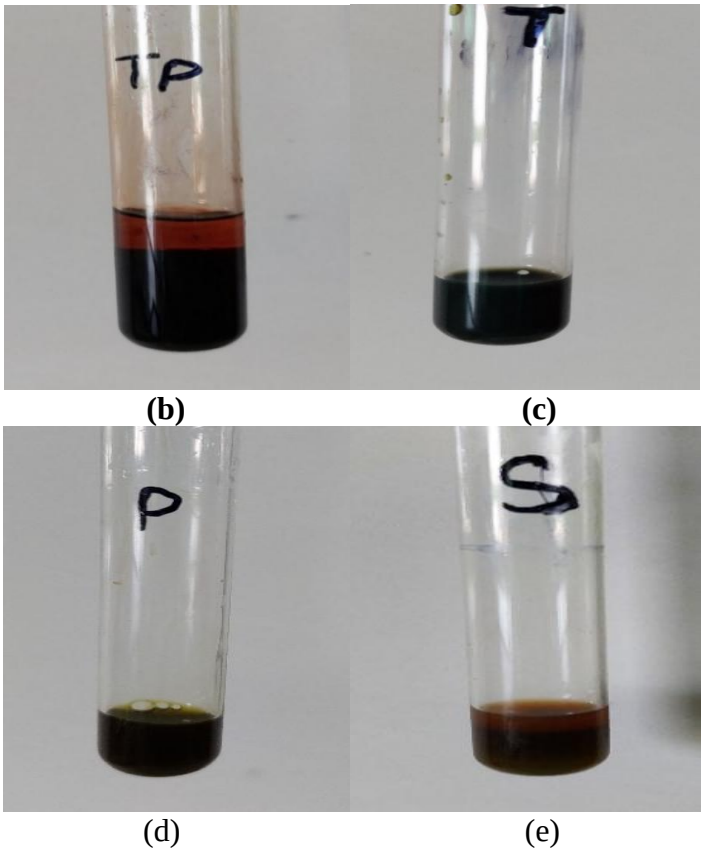



**Figure 3.1:** Qualitative Analysis of Phytochemicals in *S.cumini* Leaves Extracts
(a) Alkaloids (Mayer's Test) (b) Terpenoids (c) Tannins (d) Proteins and (e) Steroids

### 3.2. Quantitative Analysis of Phytochemicals

Quantitative phytochemical screening was done by the spectrophotometer method of ethanolic extract of *S. cumini* to measure the total phenols and total flavonoids with the help of standard protocols.

#### 3.2.1. Estimation of Total Phenolic Compounds

Various dilutions of gallic acid with known concentrations (12.9µg, 32.25µg, 64.5µg, 129µg and 258µg) showed different ranges of absorbance and a straight line curve was obtained as shown in Fig. 3.2.

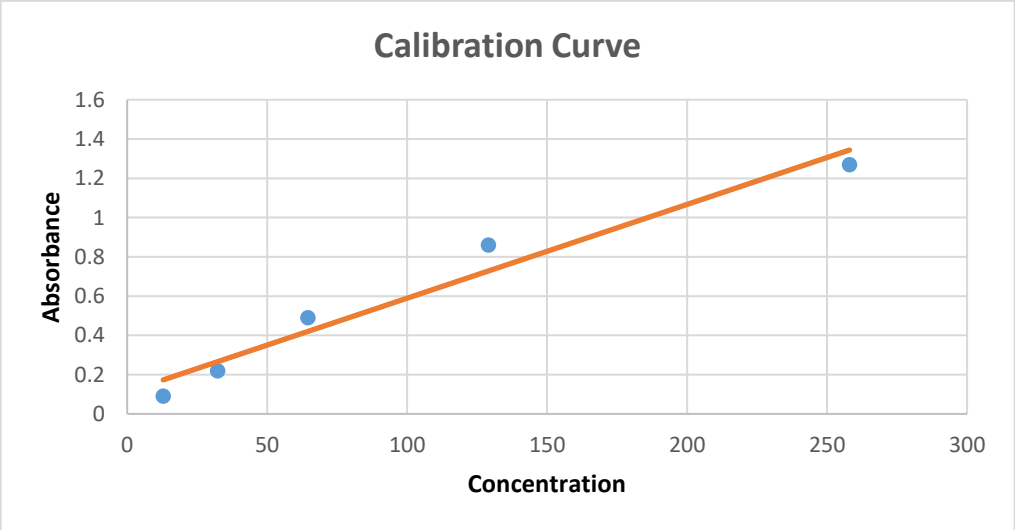



**Figure 3.2:** Standard Curve Graph of Gallic Acid

* From this standard curve, total phenolic content was interpreted and equivalent of gallic acid was mg per gram weight of powder. Total phenolic compounds were found to be 130mg in *S. cumini* leaves.

### 3.2.2. Estimation of Total Flavonoid Compounds

Various dilutions of quercetin with known concentrations (14.4µg, 36µg, 72µg and 144µg) plotted on the x-axis and different ranges of absorbance on the y-axis and a straight line curve was obtained as shown in Fig. 3.3.

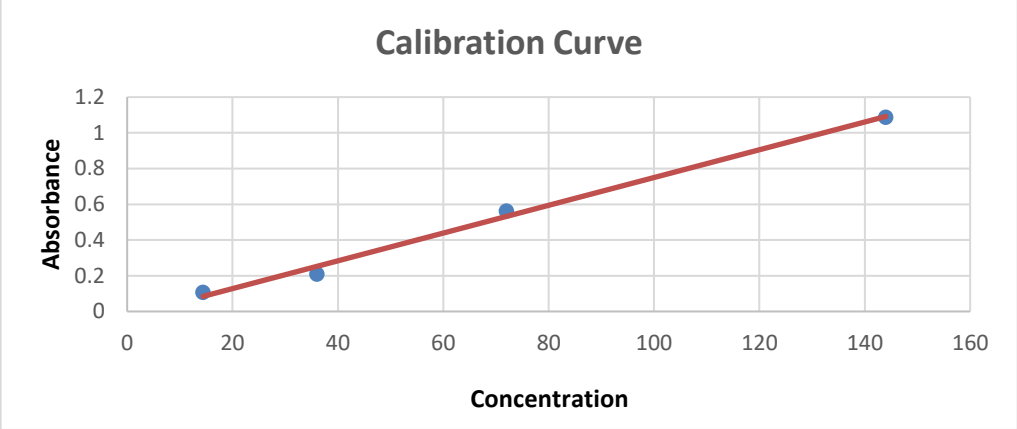



**Figure 3.3:** Standard Curve Graph of Quercetin

* From this standard curve, total flavonoid content was calculated. Results were expressed in mg quercetin equivalent per gram of dry weight. *S. cumini* leaves showed 30.6 mg of total flavonoid compounds.

### 3.3. Antioxidant Activity (DPPH Assay)

DPPH is a stable free radical that exists in purple colour and transforms into a yellow colour (non-radical form) when abstracting one of its electrons. So it is widely used to measure the electron-donating capacity of any antioxidant under the assay conditions. The standard graph was plotted against the percent antioxidant activity on the y-axis and concentration on the x-axis. This graph is used for the estimation of the antioxidant activities of *S. cumini* leaves extract. The %age antioxidant activity of *S.cumini* leaves extract was found to be 28.84%, 42.30%, 56.41%, 71.79% and 84.61% at different ranges of concentration from 10-50 µg/ml (Table 3.1).

**Table 3.1:** % Radical Scavenging Activity and IC50 Values of *S.cumini* Leaves Extract

| **Calculation of % Radical Scavenging and IC50 from DPPH Assay** | | | |
|---|---|---|---|
| **Absorbance Measurement Data** | | | |
| **Concentration µg/ml** | **Sample** | **% RSA** | **IC50** |
| 10 | 0.555 | 28.84 | -3.17733816 |
| 20 | 0.45 | 42.3 | 3.913351769 |
| 30 | 0.34 | 56.41 | 11.00404169 |
| 40 | 0.22 | 71.79 | 18.09473162 |
| 50 | 0.12 | 84.61 | 25.18542154 |
| **Control** | 0.778 | | |

### 3.3. HPLC Extracted Phytochemicals:

High Performance Liquid Chromatography (HPLC) is a functional analytical technique used to separate natural components and specific products from the mixture. Ethanolic extract of *S. cumini* leaves extract showed multiple peaks of different heights and peak areas in the HPLC chromatogram.

(Figure 3.4). The chromatogram showed 7 phenolic compounds with different heights, peak areas, concentration% and time (Table 3.2).

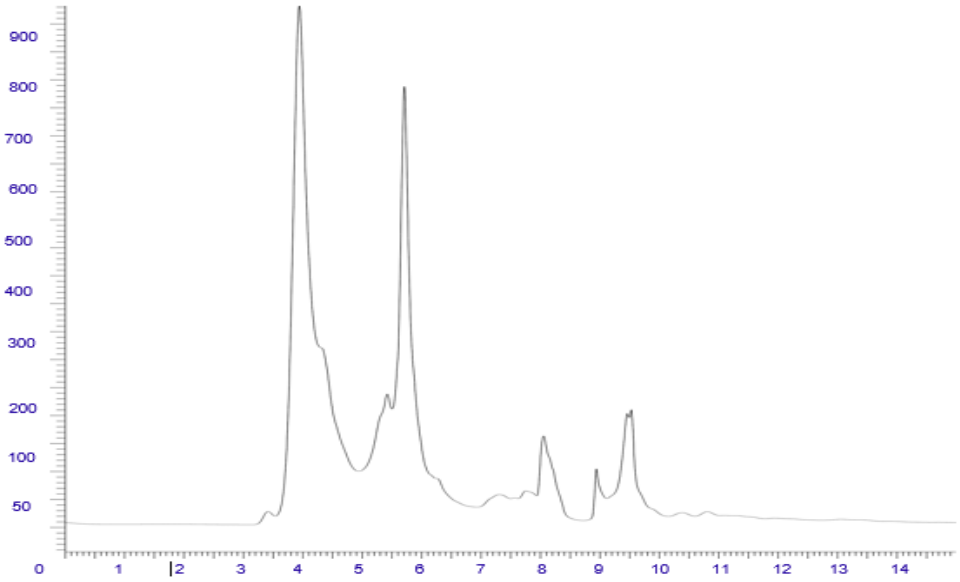


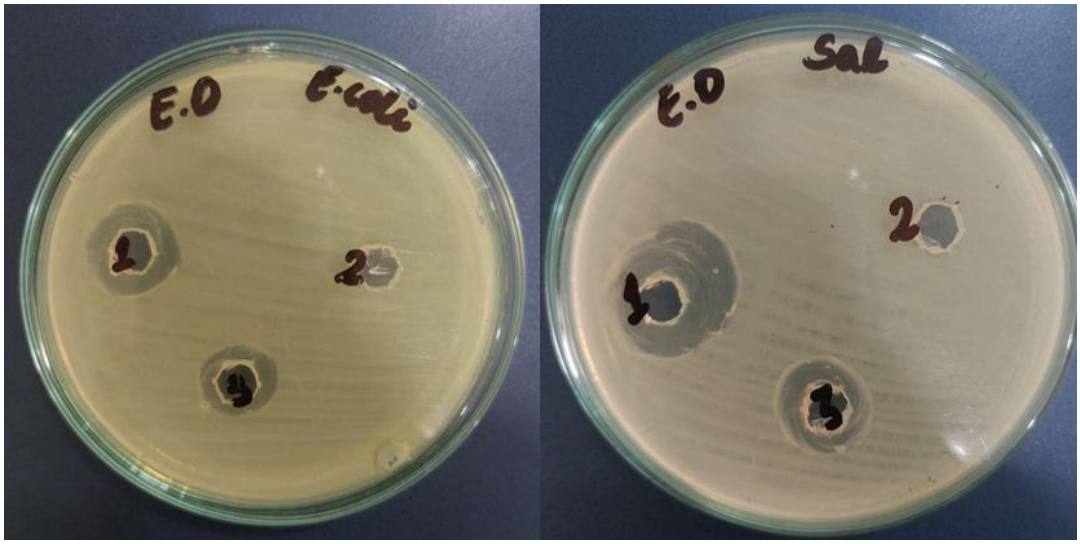

**Figure 3.4:** HPLC Chromatogram of *S.cumini* Leaves Extract

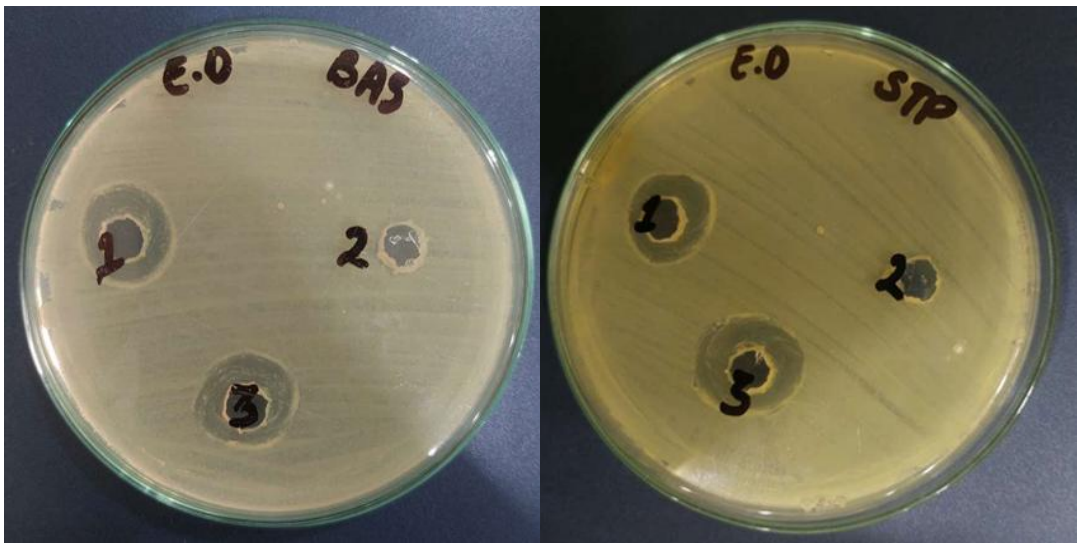
**Table 3.2:** Chromatographic Characteristics of HPLC Detected Peaks of *S.cumini* Leave Extract

| **Peak #** | **Time (min)** | **Area (µV-s)** | **Height (µV)** | **Area (%)** | **Area/Height (S)** |
|---|---|---|---|---|---|
| **1** | 3.939 | 23123454.54 | 906921.14 | 50.70 | 25.4967 |
| **2** | 5.420 | 3453102.95 | 172928.87 | 7.57 | 19.9683 |
| **3** | 5.708 | 9423417.37 | 715192.35 | 20.66 | 13.1761 |
| **4** | 8.051 | 2245539.52 | 142622.90 | 4.92 | 15.7446 |
| **5** | 8.940 | 776832.14 | 92123.31 | 1.7 | 8.4325 |
| **6** | 9.526 | 1874931.24 | 197698.05 | 4.11 | 9.4838 |

### 3.3.1. Antibacterial Activity:

The extracted leaves phytochemical constituents from HPLC, only fraction 1 and 2 showed antibacterial activity against bacterial strains e.g., E.coli and Pseudomonas with zone of inhibition (ZOI) of 11mm to 12mm in (Fig 3.5). Whereas no antibacterial activity was showed against other bacterial strains i.e., *B.subtilis* and *S.aureus.*






**(c)** **(d)**

**Figure 3.5:** Antibacterial Activity of *S.cumini* Extracted Essential Oil against Bacterial Strains (a)*E.coli* (b) *Salmonela sp.* (c) *B.subtilis* and (d) *S.aures*

### 3.3.2. GC-MS Analysis:

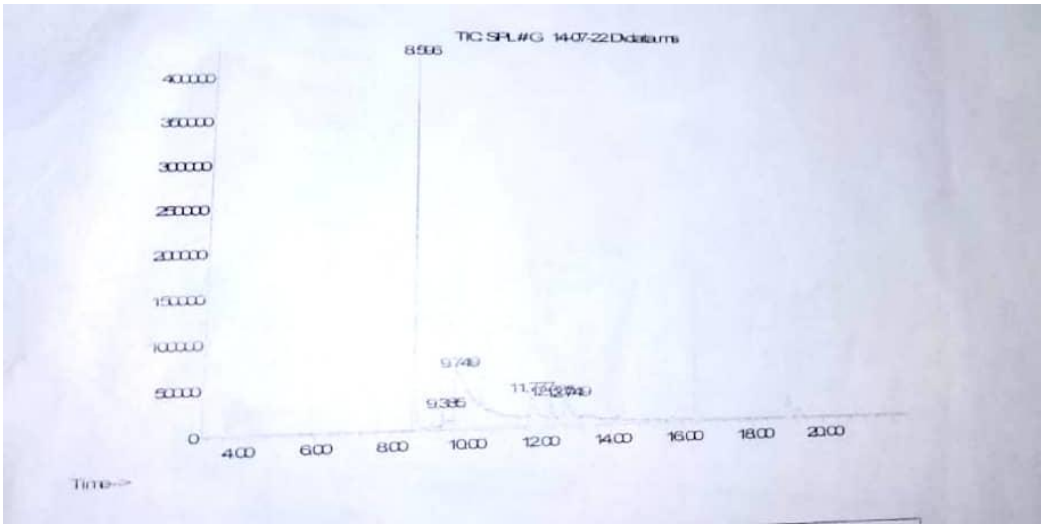
The fractions that showed effective zone of inhibition (ZOI) against bacteria were identified by GC/MS through which the chemical composition of particular peak were analyzed. Fraction 1 was recognized as Galic acid (GA) according to GC/MS chromatogram and fraction 2 was declared as Caffeic acid (CA) in Fig 3.6. But these fractions also contained other minor phytochemical constituents along with them that have similar structural and chemical compositions in Table 3.3 and 3.4.

**Table 3.3:** GC-MS Analysis of HPLC Extracted Fraction 1

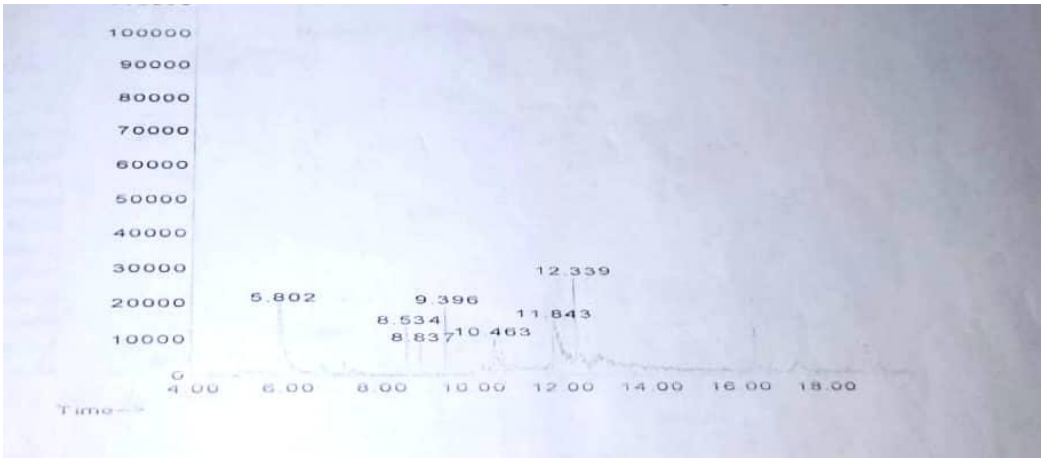
| Sr. # | Retention Time | Compound Found | Area % |
|---|---|---|---|
| **1** | 8.596 | Bisphenol C | 20.43 |
| **2** | 9.385 | Phenol, 2,5-bis(1,1-dimethylethyl) | 1.87 |
| **3** | 9.749 | 1,2,3-Benzenetriol | 56.6 |
| **4** | 11.777 | n-Hexadecanoic acid | 9.2 |
| **5** | 12.335 | Phytol | 4.3 |
| **6** | 12.749 | 3,4,5 tri-hydroxybenzoic acid | 7.5 |

**Table 3.4:** GC-MS Analysis of HPLC Extracted Fraction 2

| Sr. # | Retention Time | Compound Found | Area % |
|---|---|---|---|
| 1 | 5.802 | Cyclotrisiloxane, hexamethyl | 24.3 |
| 2 | 8.534 | Caryophyllene | 3.5 |
| 3 | 8.837 | Biscyclo (2,2,1)heptane, 7,7-dimethyl-2-methylene | 1.94 |
| 4 | 9.396 | Diethyl Phthalate | 9.4 |
| 5 | 10.463 | 3,4-Dihydroxybenzeneacrylicacid | 12 |
| 6 | 11.843 | Pentanoic acid | 31.9 |
| 7 | 12.339 | Napthalenone | 16.7 |




**(a)**




**(b)**

**Figure 3.6:** GC-MS Analysis of HPLC Extracted Fractions (Resistant To Pathogenic Bacterial Strains)
(a) Fraction 1 and (b) Fraction 2

## 4. Discussion

In impoverished countries, diarrhoea is a leading cause of morbidity and mortality. Diarrhoea is characterised as an unusual rise in stool fluidity, frequency, and volume when compared to a person's typical discharge pattern. Every year, around 2.5 million people die from diarrhoeal infections, of whom 50-70% are children under the age of five (Ogino *et al*., 2024). *S. cumin* extracts have remarkable ability against gram-positive and gram-negative bacteria that cause diarrhoeal illness. This investigation found that several *S.cumini* extracts, including ethanolic, methanolic, and aqueous, have significant antibacterial properties against diarrhoeal isolates. (Ahmad et al., 2019).

Phenolic compounds are extremely essential because of their antioxidant properties. Plants contain a wide range of phenolic content, particularly in their seeds and leaves. *S. cumini leaves* extract contains phenolic components such as tannic acid, gallic acid, ellagic acid, caffeic acid, catechin, quercetin, ferulic acid, and myricetin. According to studies, phenolic components have several biological actions, including antioxidant, anti-inflammatory, anti-aging, and antibacterial properties. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable free radical that is extensively used to estimate antioxidant activity (Surana *et al*., 2021). Antioxidants are secondary metabolites that can protect our bodies from oxidative stress induced by free radicals. Their actions are determined by the position of the hydroxyl group and the structure of the molecule. Another study compares the antioxidant activity of *S. cumini*-derived essential oils to standard antioxidants (BHA).The IC50 values for essential oil and BHA were 1.2mg/ml and 0.011mg/ml, respectively (Stankovic *et al*., 2010). As a result of the presence of

phenolic components, essential oil has a higher reduction potential than BHA. The current study evaluates the antioxidant effect of an ethanolic extract of *S. cumini* leaves in comparison to the antioxidant (BHT) as a standard. The leaves extract scavenged 84.61% of DPPH at 50 µg/ml, but the BHT standard only demonstrated 50.76% activity at the same dose.

*S. cumini* extract has antibacterial properties against both gram-positive and gram-negative bacteria, including *E.coli, Pseudomonas, B. subtilis,* and *Neisseria gonorrhoea*. It demonstrates the greatest inhibition zone. Essential oil derived from *S. cumini* leaves has been shown to have a strong inhibitory effect against pathogenic microorganisms. (Elansary *et al*., 2012). The current study found that *S. cumini* extract had outstanding antibacterial properties against four pathogenic bacteria (*E. coli, B. subtilis, Salmonella,* and *S. aureus*) that cause diarrhoea. Phenolic chemicals are extremely essential because of their antioxidant properties. Plants contain a wide range of phenolic content, particularly in their seeds and leaves. *S. cumini leaves* extract contains phenolic components such as tannic acid, gallic acid, ellagic acid, caffeic acid, catechin, quercetin, ferulic acid, and myricetin. According to studies, phenolic components have several biological actions, including antioxidant, anti-inflammatory, anti-ageing, and antibacterial properties (Jaiswal *et al*., 2011).

Phenolics' antimicrobial activity was directly tied to the inhibition of cellular enzymes. This was determined by the substance's (phenolic compound) penetration rate into the cell and resulted in alterations in membrane permeability. Increased membrane permeability is the most important factor in the mode of action of antimicrobial activity. Phenolic chemicals (phenolics and flavonoids) damage cell membrane integrity and lead to cell death (Moreno *et al*., 2006). The similarities and differences in phenolic acid and antibacterial properties are due to changes in cell surface structures between Gram-positive and Gram-negative bacteria. Gram-positive bacteria such as *B. subtilis* and *S. aureus* are more vulnerable to the method of action of phenolic acids than Gram-negative bacteria such as E.coli and Pseudomonas sp. (Faria *et al*., 2011).

## CONCLUSION

This study found that S.cumini leaf extract and essential oil, along with multi-resistant antibiotics, were effective against diarrhoea-causing bacteria. The study demonstrated the antibacterial activities of S.cumini leaf extract and essential oil. Different peaks of the extract's phytochemicals were obtained using HPLC and validated using GC-MS, revealing the existence of certain beneficial compounds with antibacterial properties against E.coli and Pseudomonas sp. particularly. In future investigations, these peaks could be separated further and their individual effects investigated. Overall, the study found *S.cumini* leaves and oil to be helpful against diarrhoeal bacteria.

## REFERENCES

1. Agarwala P, PK Gaurb, N Tyagia, D Purib, N Kumarc, and SS Kumard. (2019). An overview of phytochemical, therapeutic, pharmacological and traditional importance of Syzygium cumini. *Asian J Pharmacogn*. ***3*(1): 5-17.**
2. AH AJ, JF MAHDI, M FAROOQUI, and S YH. (2019). Gas Chromatography-mass spectroscopic analysis of black plum seed (Syzygium cumini) extract in hexane. *GAS*. ***12*(2).**
3. Andleeb HA (2023).Comparative Study of Antibacterial, Antifungal, and Antioxidant activity of bitter apple *Citrullus colocynthis* plant parts in Methanol and n-Hexane. Tobacco Regulatory science (TRS) **1264-1279**.
4. Boligon AA., and ML Athayde. (2014). Importance of HPLC in analysis of plants extracts. *Austin Chromatogr*. ***1*(3): 2**.
5. Eshwarappa RSB, RS Iyer, SR Subbaramaiah, SA Richard, and BL Dhananjaya (2014). Antioxidant activity of Syzygium cumini leaf gall extracts. *BioImpacts:* ***BI*: *4*(2), 101**.
6. Ilyas SI Ishfaq , DK Fatima, A Tariq, K Shahzadi, M Rashid, H Sohail, F Aslam, U Minhas, and T Arshid. (2024). Synergistic Effect of Zingiber Officinale (Ginger) and Curcuma Longa L. (Curcumin Analogs) for Anti-Inflammatory, Anti-Nociceptive Activity AND Analgesic Potentials. *Journal of Population Therapeutics and Clinical Pharmacology*. ***31*(3): 1363-1369**.

7. Margaret E, AM Shailaja, and VV Rao. (2015). Evaluation of antioxidant activity in different parts of Syzygium cumini (Linn.). *International Journal of Current Microbiology and Applied Sciences*, ***4*(9): 372-379**.
8. Minahil DM, H Sohail, H Tariq, M Jahangir, S Rehmat, M Akhtar, W Kiran, R Waseem, U Minhas, F Saleem. Preparation of Citrus Paradisi Vinegar from Varieties and Its Antioxidant And Nutritional Analysis. Journal of Survey in Fisheries Sciences. **10(1) 3305-3312**.
9. Minhas U. (2023). Identification of Antibacterial and Antioxidant Activity of Syzigium Cumini Leaves Extracted Essential Oil Against Diarrhoea. Tobacco Regulatory Science (TRS). **1839-1847**.
10. Mutha RE. AU Tatiya, and SJ Surana. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: An overview. *Future journal of pharmaceutical sciences*. ***7*: 1-13**.
11. Sohail H. (2023). In Vitro Antibacterial Approach of Syzigium Cumini Leaves Extract against 13. Diarrhea Causing Bacteria by Using Different Biochemical Test. Tobacco Regulatory Science (TRS). **1526-1541**.
12. Sonawane SK, SS Arya, and S Gaikwad (2013). Use of Jambhul powder in the development of bioactive components enriched milk kulfi. *The Journal of Microbiology, Biotechnology and Food Sciences*, ***2*(6): 2440**.
13. Stankovic MS. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts. *Kragujevac J Sci*. ***33*(2011): 63-72**.
14. Waseem R, A Asif, K Najam, U Minhas, R Asif, MM Arif, A Seerat, H Sohail, and M Akhtar (2023). Exploring Medicinal Plants Extracts For Antibacterial and Antiproliferative Properties: A Focus On Cannabis Sativa, Ficus Carica and Dodonea Viscosa. *Journal of Population Therapeutics and Clinical Pharmacology*. ***30*(17): 1497-1507**.
15. Zhou W, Z Zhao, Z Yu, Z Hou, R Keerthiga, AND A Fu. (2022). Mitochondrial Transplantation therapy inhibits the proliferation of malignant hepatocellular carcinoma and its mechanism. *Mitochondrion*. ***65*: 11-22.**
16. Ihara E, N Manabe, H Ohkubo, N Ogasawara, H Ogino, K Kakimoto and K Higuchi. (2024). Evidence-based clinical guidelines for chronic diarrhea 2023. *Digestion*. ***105*(6): 480-497**.
17. Ahmed R, M Tariq, M Hussain, A Andleeb, MS Masoud, I Ali, and A Hasan. (2019). Phenolic contents-based assessment of therapeutic potential of Syzygium cumini leaves extract. *PloS one*. ***14*(8): e0221318**.
18. Faria AF, MC Marques, and AZ Mercadante (2011). Identification of bioactive compounds from jambolão (Syzygium cumini) and antioxidant capacity evaluation in different pH conditions. *Food chemistry*. ***126*(4): 1571-1578**.
19. Elansary HO, MZ Salem, NA Ashmawy, and MM Yacout, M. M. (2012). Chemical composition, antibacterial and antioxidant activities of leaves essential oils from Syzygium cumini L., Cupressus sempervirens L. and Lantana camara L. from Egypt. *Journal of Agricultural science*. ***4*(10): 144**.
20. Atale N, A Jaiswal, A Chhabra, U Malhotra, S Kohli, S Mohanty, and V Rani. (2011). Phytochemical and antioxidant screening of Syzygium cumini seed extracts: a comparative study. *J Pharm Res*. ***4*(12): 4530-4532**.
21. Gupta K, SP Singh, AK Manhar, D Saikia, ND Namsa, BK Konwar, and M Mandal, M. (2019). Inhibition of Staphylococcus aureus and Pseudomonas aeruginosa biofilm and virulence by active fraction of Syzygium cumini (L.) Skeels leaf extract: in-vitro and in silico studies. *Indian journal of microbiology*. ***59*: 13-21**.
22. Kumawat M, J Damor, J Kachchhwaha, AK Garg, and C Singh, C. (2018). Pharmacological properties and therapeutic potential of Syzygium cumini (Jamun): A review. *World Journal of Pharmaceutical Sciences*. ***7*: 312-322**.
23. Irfan M, O Polonskyi, A Hinz, C Mollea, F Bosco, T Strunskus, and M Ferraris. (2019). Antibacterial, highly hydrophobic and semitransparent Ag/plasma polymer nanocomposite coating on cotton fabric obtained by plasma based co-deposition. *Cellulose*. ***26*: 8877-8894**.

24. Khare D. (2012). *Pharmacognostical and Therapeutical Evaluation of Swietenia Mahagoni Jacq. Seeds and Jambu [Syzygium Cumini (Linn) Skeels.] Seeds in Madhumeha WSR to Diabetes Mellitus-a Comparative Study* (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).