DISINFECTANT PRODUCTION USING *SYZYGIUM CUMINI* SEED EXTRACT BY ANALYZING AND CHARACTERIZING ITS PROPERTIES

Jessie Gifta S[1], Dr. J. Helan Chandra[2]

[1] *Jessie Gifta S, Student, Department of Biotechnology, Jeppiaar Engineering College, Chennai, TamilNadu, India*

[2]*Dr.J. Helan Chandra, Professor, Department of Biotechnology, Jeppiaar Engineering College, Chennai, Tamilnadu, India*

ABSTRACT

The Syzygium cumini seeds have many properties in different biological field. The present study was done to produce disinfectant from the Syzygium cumini seed extract against pathogenic organisms and to analyse the Phytochemical, GC-MS, preservative property, cytotoxic activity of the extract. The methanolic extract of S. cumini was used to prepare the Disinfectant along with Lemon grass oil. Phytochemical tests were done to characterize the compounds responsible for activity. Alkaloids, Jambosine, Glycoside, Saponins, Terpenoids, Resins and Quinones were found to be present in the extract. The extract was analysed by GC-MS and 9 compounds were found to be present in the Syzygium cumini seed extract. Cytotoxic activity of the S. cumini seed extract was tested in Human Erythrocytes (RBC). Active components present in S. cumini seed extract have got a good preservative property by increasing the Shelf life of the food products, so its preservative property was studied in Chicken, Meat and Fish. Disinfectant produced from S. cumini seed extract has got antimicrobial activity against many pathological organisms and was tested in Bacillus cereus, Bacillus subtilis (gram positive) and Pseudomonas aerogenosa, Brucella (gram negative). The Syzygium cumini seeds can used be in all biological fields and it is a cost effective method to produce disinfectant against pathogenic organisms thus it can be used for cleaning purposes in laboratories and hospitals.

Keywords: *Syzygium cumini, Phytochemicals, GC-MS, Cytotoxic activity, Shelf life, Disinfectant.*

---

1. INTRODUCTION:

The jambolan (*Syzygium cumini*) is a traditional plant which is native to India, Burma, Ceylon and the Andaman Islands. It is also found in the islands of Zanzibar, Pemba and Mombasa, and the adjacent coast of Kenya. Jambolan is an evergreen tropical tree, height 50 to 100 ft., with smooth and glossy leaves that has a terpentine smell. Jamun has purplishblack oval edible berries which come from the fragrant white flowers in branched clusters at stem tip [1]. There are several drugs of plant origin containing substantial amounts of alkaloids, the treatment of many diseases, which are described in ancient literature. *Syzygium cumini* belonging to the family *Myrtaceae*, proved to possess many medicinal properties like hypoglycemic, antibacterial, anti-HIV activity, anti-diarrhea and anti-inflammatory activity and it is found throughout India. It has been used in Ayurveda and Unani system of medicine for variety of therapeutic properties. The jambolan seeds are astringent and diuretic used to control urinary discharge and also used as a remedy for diabetes [2]. Jambolana plants are rich in a wide variety of secondary metabolites, such as tannins, alkaloids, terpenoids, flavonoids which have been found in vitro to have antimicrobial property and

may serve as an alternative, effective, cheap and safe antimicrobial for the treatment of microbial infections. Multiple drug resistant has been improved to replace the existing antimicrobial drugs for treating the infectious organisms which has become the global health problem. Therefore, it is necessary to find the new antimicrobial plant and incorporating it as folk medicine and to isolate its active principle and using it as drug [3]. GC-MS is an analytical method that has an ideal method of combining the gas chromatography and mass spectroscopy to identify different volatile and semi-volatile bioactive compounds in a test sample. The identification of compounds in this study is based on the peak area of the compound (which represents the percentage of that compound), its molecular formula and molecular weight. Disinfectants are antimicrobial agents produced from natural sources which are applied to remove the microorganisms. Disinfectants work by destroying the cell wall of the microbes or interfering with their metabolism. They are used in laboratories, hospitals, sewage sludge. The screening on antibacterial potential of jambolana seed extract against multidrug-resistant Grampositive and Gram-negative human bacterial pathogens seems to be important. Cytotoxic potential was evaluated on human erythrocytes by haemolytic assay method and acute oral toxicity study was done in mice. Cytotoxic activity is the ability of the sample to break the cell wall and release the components present in the cell. It is also used to kill the pathogens by breaking their cell wall [3]. The demand of consumers for natural and organic products as well as the deleteriousness of artificial antioxidants has grabbed the attention of researchers towards the edible herbs and plants as natural resources of harmless and effectual antioxidants and preservatives for use in food industry [4].

2. MATERIALS AND METHODS:

2.1 Sample Collection:

The *Syzygium cumini* fruits were purchased from local market, Madipakkam, Chennai. The fruits were kept in sterile place and the seeds were collected by removing the pulp. The seeds were dried in shade and stored.

2.2 Extraction:

Methanolic extracts of *Syzygium cumini* seed powder were prepared by immersing 100 g seed powder the conical flask stoppered with rubber cork containing 600 ml of Methanol with occasional shaking at room temperature for 24 hours. The mixture were kept for three days for Methanol extract and filtered. The process was repeated twice using remaining residues. All the dried extracts were stored at 4◦C in air-tight jars until further use [3].

2.3 Phytochemical Screening:

2.3.1 Test for Alkaloids

1ml of the *Syzygium cumini* seed extract was taken and 2ml of Wagner's reagent was added to it. The appearance of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test of Glycosides

1ml of the *Syzygium cumini* seed extract was taken and 2ml of Benedict's reagent was added to it. The appearance of orange red precipitate indicates the presence of reducing sugars (glycosides)

2.3.3 Test for Tannins

A small amount of seed extract was heated in water bath. Few drops of Ferric chloride were added. The appearance of dark green colour indicates the presence of Tannins.

2.3.4 Test for Saponin

0.2 gram of extract was shaken with 5 ml of distilled water. It was then heated to boil. The appearance of frothing bubbles indicates the presence of Saponins.

2.3.5 Test for Flavonoids

0.2 gram of extract was shaken with 5 ml of distilled water. Few drops of 10% lead acetate solution were added. The appearance of yellow or dirty white precipitate indicates the presence of flavonoids.

2.3.6 Test for Proteins

The test solution was treated with 10% sodium hydroxide solution. Two drops of 0.1% of copper sulphate was added. Formation of pink or violet colour indicates presence of proteins.

2.3.7 Test for free aminoacids

The solution was boiled with 0.2% solution of ninhydrin. Appearance of purple colour indicates presence of free amino acids.

2.3.8 Test for steroids

Red color produced in lower chloroform layer when 2ml extract was dissolved in 2ml chloroform and 2ml of sulphuric acid was added to it. This indicates the presence of steroids.

2.3.9 Test for diterpenes

The extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald red indicates the presence of diterpenes.

2.3.10 Test for quinones

Small amount of extract was treated with conc. hydrochloric acid. Formation of yellow colour precipitate indicates the presence of quinines.

2.3.11 Test for antroquinone

The powdered plant material is mixed with organic solvent and filtered. Sodium hydroxide was added to it. Pink or violet color indicates the presence of antroquinone.

2.3.12 Test for triterpenoids

5 ml extract was mixed with equal volume of chloroform along with few drops of sulphuric acid. The mixture was shaken and kept aside. Appearance of yellow colour in the lower layer indicates its presence.

2.3.13 Test for resins

1ml of extract was treated with few drops of Acetic anhydride. 1ml of conc. Hydrochloric acid was added. The colour change from red to orange indicates the presence of resins.

2.3.14 Test for Xanthoproteins

1ml of extract was treated with few drops of nitric acid and ammonia. Formation of reddish orange precipitate indicates the presence of xanthoproteins.

2.4 Preservative Property:

The Preservative property of *Syzygium cumini* seed extract was analyzed in Chicken, Meat, and Fish. The clean and sterile chicken, fish and meat was dipped in seed extract and kept in refrigerator. The sample was checked once in 10 days. The bacterial colonies developing on the sample was noted [4].

2.5 Disinfectant Production:

The Disinfectant was produced by adding 0.2 W% of lemon grass oil, 85 W% of *Syzygium cumini* seed extract, 0.2 W% of lauryl dimethyl amine oxide, 14.6 W% of distilled water. The mixture was kept in shaker for 1 hr.

2.6 Cytotoxic Activity:

Cytotoxicity study on human erythrocytes, blood was collected from six healthy volunteers, mixed with isotonic buffer (0.9% NaCl) solution and centrifuged at 600 rpm for 10 min and the supernatant was discarded. This was repeated for three times. The solid pellets were then taken and 1% erythrocyte suspension was prepared in phosphate buffered saline (10 mM PBS, pH 7.4). The seed extract was incubated with an equal volume of 1% erythrocyte suspension at 37 ◦C for 1 h in a shaking water bath. Non-haemolytic and 100% haemolytic controls were the buffer alone. Cell lysis was monitored by measuring the release of haemoglobin spectrophotometrically at 540 nm. Percentage haemolysis was calculated by using the following formula [3]

% Haemolysis = (Absorbance of Sample – Absorbance of Blank) / 100

2.7 Anti-microbial Activity:

Well Diffusion Method:

The petriplates were sterilized and nutrient agar was prepared (2.8 grams 100 ml distilled water) and sterilized in autoclave. The Gram negative bacteria *Aero pseudomonas, Brucella* and Gram positive bacteria *Bacillus subtilis, Bacillus cereus* were plated in different petriplate containing agar medium. In the plates the wells were punched and the disinfectant containing methanolic seed extract of Syzygium cumini, lemon grass oil and water along with control was placed in the wells. The plates were incubated overnight at 37^{o}C and the results were observed [3].

3. RESULT AND DISCUSSION:

3.1 Phytochemical Test:

Table 1: Phytochemical Screening of *Syzygium cumini* Seed Extract

S.NO	TEST	RESULTS
1.	Alkaloids	+
2.	Glycosides	+
3.	Tanins	-
4.	Saponins	+
5.	Flavonoids	+
6.	Proteins	-
7.	Free amino acids	-
8.	Anthraquinones	+
9.	Diterpenoids	+
10.	Triterpenoids	+
11.	Steroids	-

12.	Quinones	+
13.	Resins	+
14.	Xanthoproteins	-

1.2 GC-MS:

The GC-MS analysis was given for outsourcing in IIT Madras and it was performed using a JEOL GC MATE II (Quadrapole double focusing mass analyser). The operating conditions were 1ml/min flow rate at a temperature of about 220o C – 2500 C. About 9 compounds were identified in the study. For *Syzygium cumini* seed extract, the compounds were identified at the different retention time of 26.32, 20.22, 21.8, 14.62, 18.03, 21.47, 22.67, 23.73, 25.15 minutes. The compounds were identified with the help of NIST library and the compounds are listed below along with its structure.

FIGURE 1: Chromatogram obtained for GC-MS analysis of *Syzygium cumini* seed extract

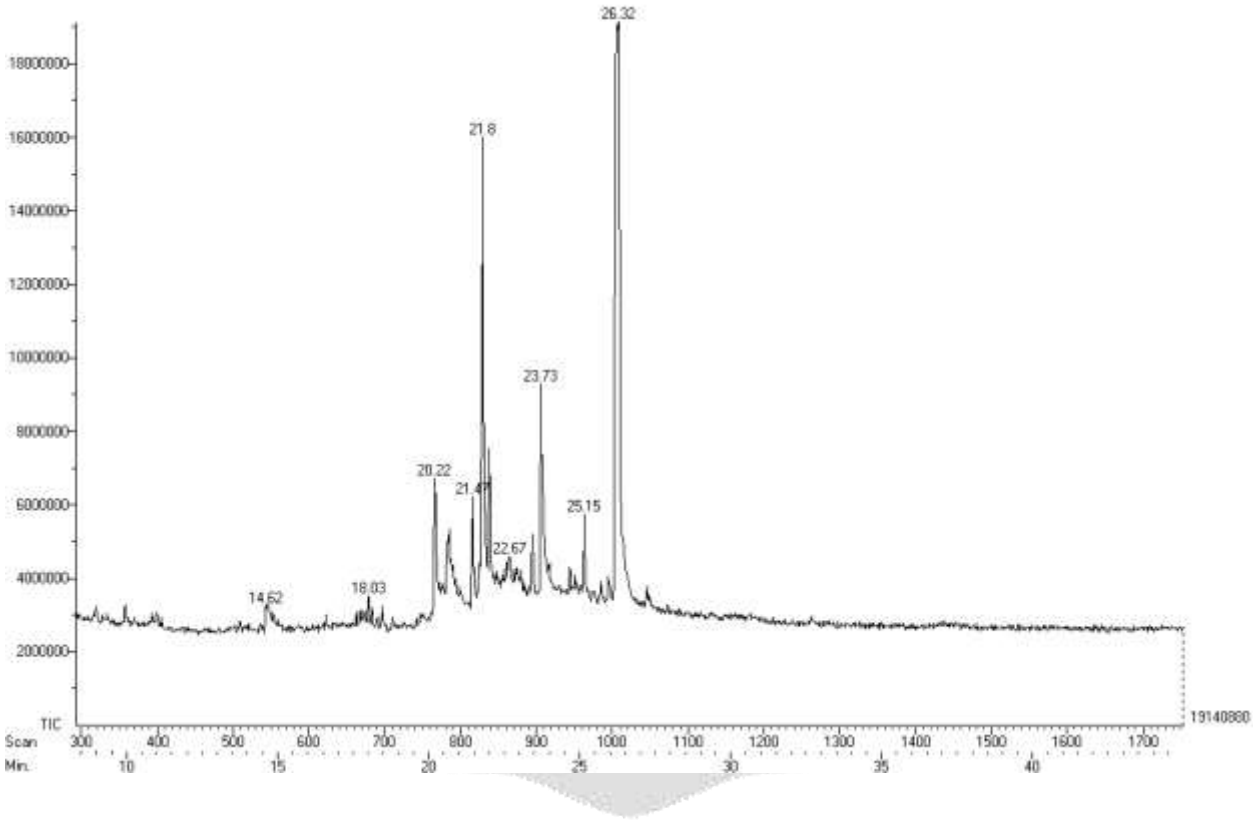
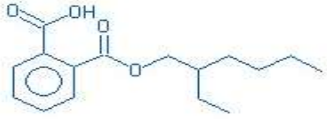
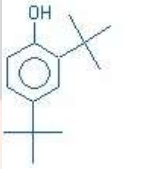
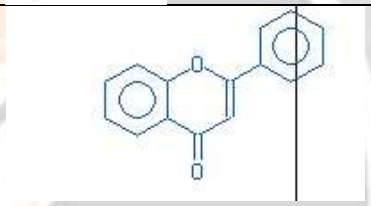
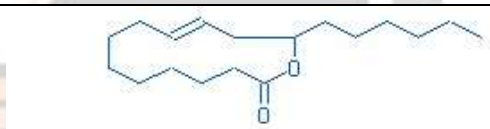
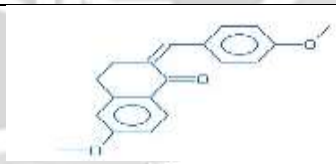
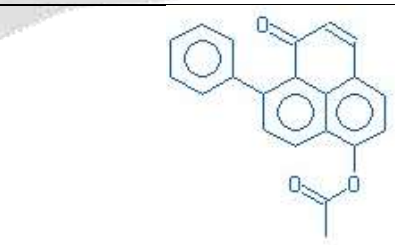


Table 2: GC-MS analysis of *Syzygium cumini* seed extract

S.NO	Compound name	Retention time	Molecular structure
1.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	26.32	
2.	Tridecanoic acid, methyl ester	20.22	
3.	Phytol	21.08	
4.	Phenol,2,4-bis(1,1-dimethylethyl)	14.62	
5.	Flavone	18.03	
6.	13-hexyloxacyclotridec-10-en-2-one	21.47	
7.	Oleic acid	22.67	
8.	2H-Naphtalen-1-one,3,4-dihydro-6-methoxy-2-(4-methoxybenzylideno)	23.73	
9.	Benzonaphth-1-one,6-acetate-9-phenyl	25.15	

3.3 Preservative Properties:

The Preservative property of *Syzygium cumini* seed extract was tested in Chicken, Meat and Fish. The sample treated with the seed extract was stored in refrigerator. It was checked for once in 10 days. The *Syzygium cumini* seed extract showed a good preservative property by inhibiting the microbial development on chicken, meat and fish. The active component Tridecanoic acid, methyl ester present in the *Syzygium cumini* seed extract was responsible for the preservative property in *S.cumini* seed.

3.4 Cytotoxic Activity:

The Cytotoxic activity was tested on human erythrocytes (RBC). The results showed that the *Syzygium cumini* seed extract has active component Phenol present in it, the phenolic compound enhances the cytotoxic activity of the *Syzygium cumini* seed extract. From this test *Syzygium cumini* seed extract has high haemolytic activity.

3.5 Antimicrobial Activity:

Antimicrobial activity of Disinfectant made of *Syzygium cumini* seed extract is shown in the table. It showed sufficient inhibitory actions against the test microbes. The disinfectant *Syzygium cumini* seed extract showed activity against the gram positive and gram negative organisms. The negative control used here was Erythromycin and positive control was ditilled water. The maximum inhibitory zone was obtained for *Pseudomonas aerogenosa* (gram negative).

TABLE 3: Zone of Inhibition for Disinfectant *Syzygium cumini* seed extract

Microorganism	Diameter of Zone of Inhibition obtained for Disinfectant			
	Disinfectant	Erythromycin	Labolene	Dis. Water
Pseudomonas aerogenosa	25	17	5	-
Brucella	23	18	-	-
Bacillus subtilis	24	15	-	-
Bacillus cereus	19	16	8	-

4.CONCLUSION:

The *Syzygium cumini* fruit was purchased from local market and the seeds were removed powdered. The extract was prepared using Methanol and stored in air tight container for further use. The phytochemicals present in the *Syzygium cumini* seed extract was screened and confirmed the presence of alkaloids, glycosides, flavonoids, di and tri terpenoids, quinones, resins. The active components present in the seed extract was analyzed using GC-MS. The Chromatogram of GC-MS analysis showed the presence of 9 components. The preservative property of *Syzygium cumini* seed extract was tested in Chicken, Meat and Fish and it showed the good preservative property of *Syzygium cumini* seed extract by the active component *Tridecanoic acid, methyl ester* present in it. Disinfectant was produced by keeping the *Syzygium cumini* seed extract as principle ingredient and lemon grass oil for fragrance. The disinfectant produced was tested for its activity. The *Syzygium cumini* seed extract was studied for cytotoxic activity. The seed extract was treated with human erythrocytes (RBC) and the study reveals the haemolysis activity of *Syzygium cumini* seed extract by the active component *Phenol* present in it. The *Syzygium cumini* seed extract can also be used for disrupting the cell wall of organism and releasing the intracellular proteins present inside the cell. The disinfectant was tested in 4 organisms like *Pseudomonas aerogenosa, Brucella* (gram negative) and *Bacillus subtilis, Bacillus cereus* (gram positive). The *Syzygium cumini* seed extract showed maximum zone of inhibition against the *Pseudomonas aerogenosa* (gram negative). Thus *Syzygium cumini* seed extract can be used in all the field of biology. They are cost effective and easily available raw material for all the needs in biological field.

5. ACKNOWLEDGEMENT:

We would like to thank the Department of biotechnology, Jeppiaar Engineering College, for supporting and encouraging us in completing this work.

6. REFERENCE:

[1]. Alok Ranjan1, Akanksha Jaiswal and R. Balaji Raja "Enhancement of *Syzygium cumini* (Indian jamun) active constituents by ultra-violet (UV) irradiation method" Scientific Research and Essays Vol. 6(12), pp. 2457-2464, 18 June, 2010.

[2]. K. S. Chitnis, S. B. Palekar , D. R. Koppar and D. Y. Mestry , "Evaluation Of *Syzygium Cumini* Linn. Seed Formulations Available In The Market Using Spectrophotometric And Chromatographic Techniques" Chitnis *et al.*, IJPSR, 2012; Vol. 3(2): 556-560 ISSN: 0975-8232

[3]. Anwesa Baga, Subir Kumar Bhattacharyyaa, Nishith Kumar Palb, Rabi ranjan Chattopadhyay, "In vitro antibacterial potential of Eugenia jambolana seed extracts against multidrug-resistant human bacterial pathogens" Elsevier, Microbiological research 2012.

[4]. Sameer Shaikh 1*, Hashim Bin Yaacob 2, Zubaidah Haji Abdul Rahim, "Prospective Role in Treatment of Major Illnesses and Potential Benefits as a Safe Insecticide and Natural Food Preservative of Mint (*Mentha spp.*): A Review" Asian journal of Biomedical and Pharmacutical sciences, 2014.