JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

# ANTIFUNGAL ACTIVITY AND PHYTOCHEMICAL STUDY OF ETHANOLIC EXTRACT OF ALTHAEA OFFICINALIS SEEDS.

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**Abstract***:* The objective of the study was to estimate the phyto-constituents of Althaea Officinalis seeds and its Microplate Quantative Analysis (MIC). Althaea Officinalis is used to treat a variety of ailments like dry cough, constipation, peptic ulcers, mouth ulcers, pneumonia, etc. Althaea Officinalis seeds also give good anti-inflammatory, anti-microbial, anti-bacterial, antioxidant and anti-fungal properties. Phytoconstituents present in Althaea Officinalis are Lauric acid, altheahexacosanyl lactone, lanstanol and althaecoumaryl glucoside. Potato Dextrose Broth and active cultures of fungus Candida Albicans were used. The seeds were found to be secure, non-toxic, and safe to use for preparation of other formulations.

**Key Words** – Microplate Quantitative Analysis(MIC), Microscopy, Phytochemical Investigation.

## I. INTRODUCTION

The genus Althaea belongs to the family Malvaceae, the most major species of the genus is Althaea officinalis L., Althaea officinalis (Khatmi) generally understood as Marshmallow is actually common remedial plant utilized in Unani medication. It's a perennial herb; it's indigenous to western Asia, Europe and United States of America. Root, flowers and seeds are particularly utilized in Unani medication. Carpals are big and pubescent and are understood as Tukhm- eKhatmi. The seeds are small to moderate size, about 6 mm, brownish black in shade and reniform in shape. The darkish black seed is better. Seeds are singular in each carpet. Each carpel has one seed. Seeds are fat and dark brown with a mucilaginous insipid flavor. Collection of seeds is produced, when they turn ripened, before stumbling on the ground. Shade desiccated seeds are kept in cold and arid places. Seeds are relatively tough retaining hairy exterior face. External periphery of seed is around 0.5-0.6 cm. The load of one seed is around 11.1 mg. (100 seeds = 1.11 gm.) The seeds are hairy at periphery. The temperament of seed is moderate towards cool and moisture.

## II. MATERIALS AND METHODS

### *2.1 Collection of plant materials*

The seeds of plant Althaea Officinalis were purchased from K.R Implex stores, Punjab in the month of July 2022 and authenticated at Infinite Biotech, Sangli.

### *2.2 Chemicals*

Ethanol, Methanol, Distilled Water, Dragendorffs' reagent, Ferric Chloride solution, Phloroglucinol solution, glacial acid, PDB was purchased from Himedia. Analytical grades chemicals were used.

### *2.3 Equipment's*

Digital balance, digital water bath, magnetic stirrer, muffle furnace, dessicator, hot-air oven, UV Spectrophotometer.

### *2.4 Method-*

#### 2.4.1 Extraction of Althaea Officinalis:

The seeds of Althaea Officinalis were collected and bruised into fine particles. About 500gm of crushed Althaea Officinalis powder were extracted using ethanol as a solvent by hot extraction method using Soxhlet apparatus. When the solvent becomes colorless, the process is discontinued. Then, the extract was evaporated to dryness and the powder obtained was stored for further use.

**2.4.2 Evaluation Parameters:**

**2.4.2.1 Microscopy-**

1) Clear the powder with a clearing reagent. Chlorinated Soda.
2) Stain the cleared powder with staining reagents Phloroglucinol.
3) Make mount free from bubbles to determine-
   a) Type of cells.
   b) Fibers
   c) Calcium Oxalate Crystals, etc.

**2.4.2.2 Solubility-**

Solubility of the extract powder was determined in various solvents like ethanol, methanol, chloroform, distilled water, phosphate buffer pH 6.8 The drug was dissolved in solvents respectively, and solubility of drug was determined.

**2.4.2.3 Phytochemical Test-**

Preparatory qualitative phytochemical analysis was carried out to identify the active constituents present in the alcoholic extracts of Althaea Officinalis. Different procedures were adopted to test the presence of various constituents in the Althaea Officinalis extract.

Test for Alkaloids-

a) Dragendorff' Test- To 2-3ml of filtrate, add few drops of Dragendorff' reagent. Orange brown precipitate is formed.
b) Mayer's test-2-3ml filtrate with few drops Mayer's reagent. It gives white precipitate.

Test for Flavonoids-

Sulphuric Acid test-On addition of sulphuric acid flavonoids dissolve in it and gives a deep yellow solution.

Test for Steroids-

Salkowski reaction-To 2ml of extract, add 2ml chloroform and 2 ml conc. $H_2SO_4$.Shake well. The chloroform layer appears red and acid layer shows yellowish fluorescence.

Test for Glycosides-

Keller Killani test-Solution of 0.5ml glacial acid and 2 drops of ferric chloride was mixed with 2ml of extract. Later,1ml of $H_2SO_4$ was added. Appearance of deep blue color at junction of two liquids.

Test for Anthraquinone Glycosides-

Borntrager's test-To 3ml of extract, add dilute $H_2SO_4$.Boil and filter. To cold filtrate add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

**2.4.2.4 Physicochemical Evaluation-**

**Determination of Foreign Matter**

Weigh 100-500g of the extract powder to be examined and spread it out in a thin layer. The foreign matter should be detected inspection with the unaided eye. Separate and weigh it and calculate the percentage present.

**Determination of Total Ash**

2-3g weighed of extract powder in a tarred platinum or silica dish at a temperature not exceeding 450°C was incinerated until free from carbon, then cooled and weighed. Percentage of total ash was calculated in reference to air dried drug.

**Determination of Acid-Insoluble Ash**

The ash obtained from total ash was kept to boil for 5 minutes with 25ml of dilute hydrochloric acid. The insoluble matter was collected in a silica crucible. Further, it was washed with hot water and burned to constant weight. The percentage of acid-insoluble ash with reference to air-dried drug, was calculated.

**Determination of Alcohol-Soluble Extractive**

5g of air-dried extract powder was mixed with 100ml of ethyl alcohol in a closed flask for twenty-four hours, for six hours it was shaken frequently and allowed to stand for eighteen hours. The 25 ml of solution was kept for filtration, then the filtrate was evaporated in a flat-bottomed dish, dried at 105C to constant weight. Percentage of alcohol-soluble extractive was calculated in reference to air-dried drug.

**2.4.2.5 Comparison of powder concentration with marker concentration by using UV Spectrophotometer-**

10mg of powder (Althaea Officinalis) and active constituent (Lauric acid) was dissolved in 100 ml of Ethanol. Further to prepare solution of 10µg/ml,1ml from the stock solution was diluted with 9 ml of Ethanol and absorbance was noted at wavelength ranging from 200-210nm.The wavelength at which maximum absorbance is shown is the Lambda Max (λmax) of powder and active constituent.

**2.4.2.6 Determination of MIC by Microplate based Quantitative Analysis-**

Potato Dextrose Broth (PDB) and active cultures of Fungus Candida albicans NCIM-3471 was used in this experiment. Plates were prepared under aseptic conditions. Sterile 96-well plate was labeled as per the designed experiment. Each drug was tested under duplicates (two rows) of each drug was made. For each drug the first well was loaded with 300µl of drug. All other wells were loaded with 100µl of sterile PDB. After serially diluting the drugs 100µl of fungal suspension was loaded from 2nd to 11th well. The plates were sealed and kept for incubation at 37°C for 18 hours. After incubation of plates MIC was observed through visible turbidity and analyzed using micro-plate reader. The absorbance was taken at 600nm.

## III. RESULT AND DISCUSSION

### *3.1 Microscopy-*

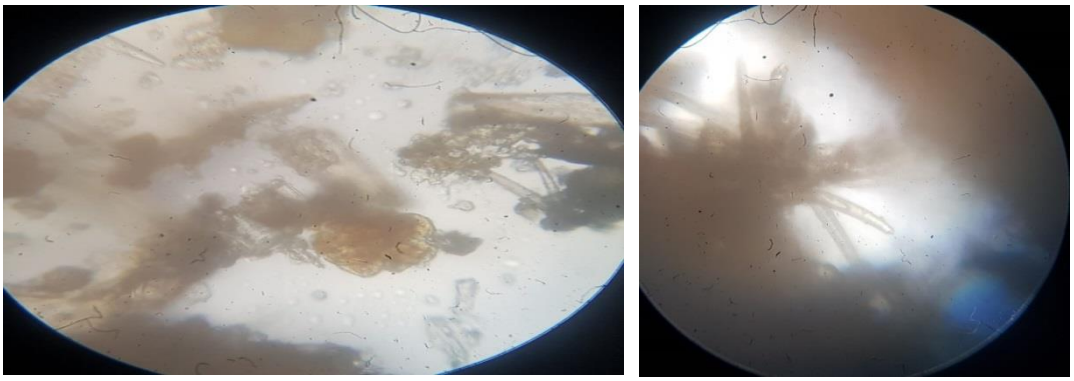

**Figure no 1- Microscopy of seed powder**

Microscopy shows the presence of fibres,calcium oxalate crystals,aleurone grains.

### *3.2 Solubility-*

It was observed that the extract powder showed good solubility in distilled water as compared to ethanol, methanol and chloroform.

**Table no1-Solubility of extract powder in different solvents**

| Solvents | Solubility |
|---|---|
| Distilled Water | Soluble |
| Ethanol | Sparingly Soluble |
| Methanol | Sparingly Soluble |
| Chloroform | Slightly Soluble |

### *3.3 Phyto-chemical Test-*

**Table no 2-Phyto-Chemical Test of extracted powder**

| Test | Results |
|---|---|
| **Test for Alkaloids-**<br>a) Dragendorff' Test-<br>b) Mayer's test- | + + |
| **Test for Flavonoids-**<br>a) Sulphuric Acid test- | + + |
| **Test for Steroids-**<br>a) Salkowski reaction- | + + |
| **Test for Glycosides-**<br>a) Keller Killani test- | + + |
| **Test for Anthraquinone Glycosides-**<br>a) Borntrager's test- | + + |

+ + Positive

The phytochemical test conducted confirms the presence of alkaloids, flavonoids, glycosides, steroids in seed extract.

→

### *3.4 Physicochemical Evaluation-*

**Table no 3-Physicochemical Evaluation of extract powder**

| Evaluation Parameters | Standard Range | Results |
|---|---|---|
| Foreign Matter | Not more than 2% | 1% |
| Total Ash | Not more than 8% | 4% |
| Acid-Insoluble Ash | Not more than 1.5% | 0.5% |
| Alcohol-Soluble Extractive | Not less than 10% | 14% |

A test was performed to determine foreign matter in seed powder and was found to be 1%, which is in the acceptable range. Total ash, Acid-insoluble ash, Alcohol-soluble extractive was calculated and found to be 4%,0.5% and14%which is in the acceptable level.

### *3.5 Comparison of drug concentration with marker concentration by using UV Spectrophotometer-*

**Lambda Max ($\lambda_{max}$) of Drug Althaea:**

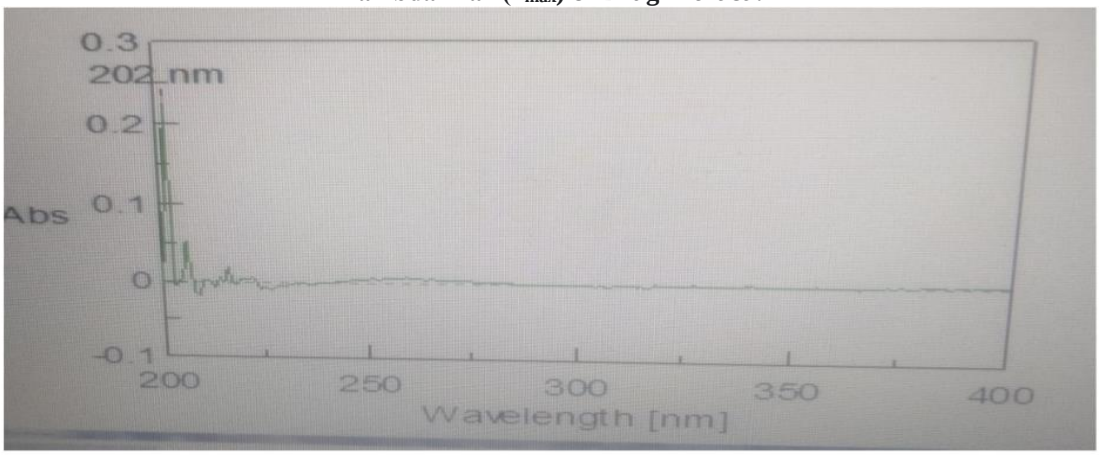


**Figure no 2- Lambda Max ($\lambda_{max}$) of Extracted Powder**

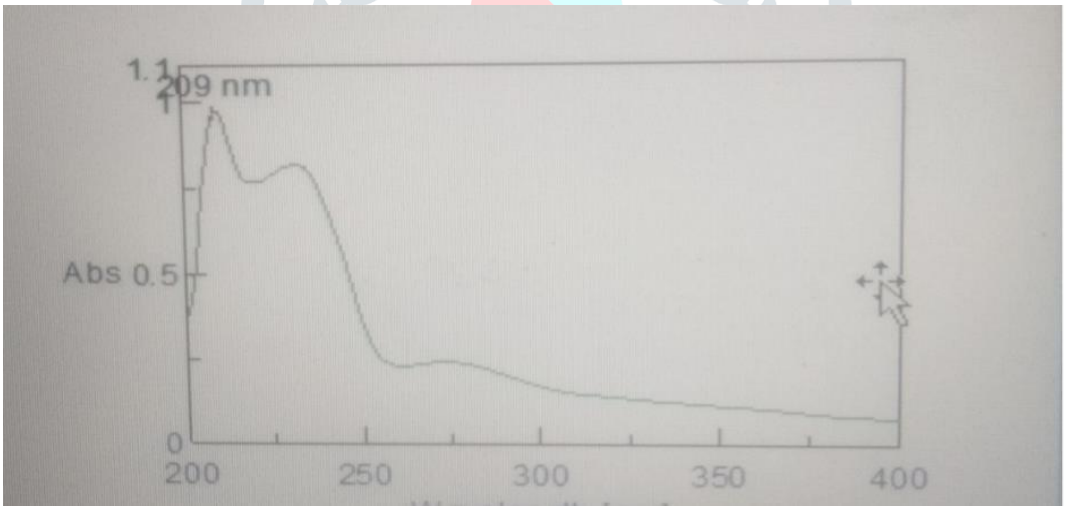


**Figure no 3- Lambda Max (λmax) of Active Constituent Lauric Acid:**

To confirm the presence of Lauric acid, UV Spectrophotometric comparative analysis was performed with the powder. The powder and marker samples show $\lambda_{max}$ of 202 and 209nm, respectively.

### *3.6 Determination of MIC by Microplate based Quantitative Analysis-*

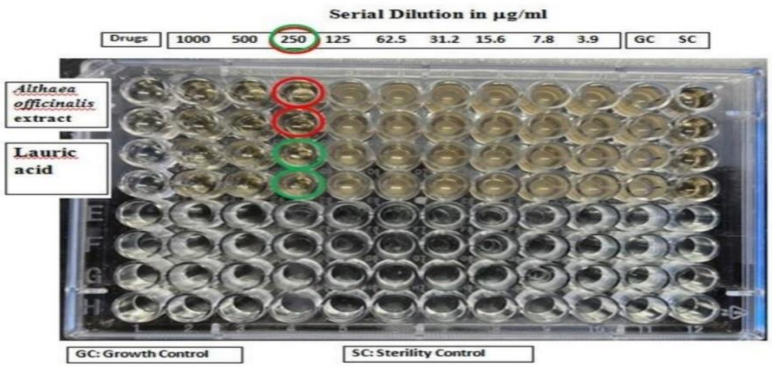


***Figure no 4- MIC by Microplate based Quantitative Analysis***

**Table no 4- Target MIC(µg/ml) for Candida albicans NCIM-3471 in potato dextrose broth (PDB).**

| Compound/Drugs/Antifungal agent | MIC (µg/ml) |
|---|---|
| Sample 1 – Althaea Officinalis | 250 |
| Sample 2 – Lauric acid | 250 |

## IV. CONCLUSION-

The Phytochemical constituents and anti-fungal were analyzed for the seed powder extract of Althaea Officinalis. The presence of alkaloids, flavonoids, steroids, glycosides were confirmed in phytochemical profiling. Alkaloids are already known to have antimicrobial activity. MIC and in-vitro antimicrobial assay supports the antifungal activity against Candida Albicans. Hence, the Althaea seed extract could be an alternative for herbal antifungal products.

## V. REFERENCES-

1) Shah SA, Akhtar N, Akram M, shah PA, Saeed T, Asif HM. Pharmacological activity of althaea Officinalis L. Journal of medicinal plants research 2011 Oct 30,5(24):5662-6.

2) Fahamiya N, Shiffa M, Aslam M, Muzn F. Unani perspective of Khatmi(Althaea Officinalis).Journal of Pharmacognosy and Phytochemistry.2016 Nov 1;5(6):357-60.

3) Joy P, Thomas J, Samuel M, Baby SP(1998)Medicinal Plants, Kerala agriculture University: Aromatic and medicinal plant research station pp.1-211.

4) S.Sandhya, Joyeeta Talukdar and Debabrat Bhaishya(2016)Chemical and biological properties of Lauric Acid: A Review. Int J of adv research vol 4,Issue 7,1123-1128.

5)Mazhar Husain,M.Zaigham,Hamiduddin,AbdulWadud,Mohd.Akbar Ali;A review on Pharmacological and Phytochemical , Profile of Khatmi (Althaea Officinalis):An important mucilaginous plant and its utilization in Unani Medicine,Int.Journal Res Ayurveda Pharm,12(3) 2021;(ISSN:2229-3566).

6) Ali Esmail Al-Snafi; The Pharmaceutical Importance of Althaea officinalis and Althaea rosea: A Review; International Journal of Pharm Tech Research;Vol.5, No.3, pp 1378-1385;July-Sept 2013.

7) Parisa Sadighara,Soraya Gharibi, Amir Moghadam Jafari, Golamreza Jahed Khaniki,and Samira Salari ; The antioxidant and Flavonoids contents of *Althaea officinalis* L. flowers based on their color; Avicenna.J.Phytomed,2(3): 113–117;2012.

8) Alieh Kianitalaei, Zohre Feyzabadi, Shokuhsadat Hamedi, Marzieh Qaraaty. Althaea Officinalis in Traditional Medicine and modern phytotherapy J Adv Pharm Edu Res 2019;9(S2):154-161.

9) Funda Ozbek, Mehmet Erkan Uzunhisarcikli; Mericarp and Seed Morphology of the genus Althaea L. (Malvaceae) in Turkey; GU J Sci 33(3): 622-628 (2020).

10) Hage-Sleiman R, Mroueh M, Daher CF. Pharmacological evaluation of aqueous extract of Althaea officinalis flower grown in Lebanon. Pharm Biol. 2011;49(3):327-33.