Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [5]April 2023 : 88-99
©2023 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Standardization and potential antimicrobial activity of Khatami (*Althea Officinalis*)

Aappa Sakharam Patil and Vijay Jagdish Upadhye
Center of Research for Development,Parul Institute of Applied Sciences,
Parul University,Vadodara, Gujarat.

ABSTRACT

Althaea officinalis L., Lavandula angustifolia Mill., and Rosa x damascene Herrm. are used to treat excision or burn lesions in traditional medicine. To study and standardization and anti-microbial activity of Khatami Althea Officinalis after collecting herbs, the extract of Althaea officinalis L was prepared hydroalcoholic procedure. Then organoleptic, physico-chemical analyses were done, along with the standardization method a chemical with HPTLC method, heavy metal analysis, antimicrobial activity, Total antioxidant by DPPH method, Total phenolic, Total Flavonoid, were performed. The HPTLC studies have shown that it is more versatile than ordinary TLC methods, Heavy metal analysis showed a minimum presence of metals, antioxidants, polyphenols, and flavonoids. The antimicrobial activity shows that the hydroalcoholic extracts inhibited good antimicrobial activity against all tested fungi and bacteria It is concluded that the standardization and potential antimicrobial activity of the extracts from A. officinalis against tested bacteria and fungi. Further investigation work is to characterize the bioactive compound and other values for fixing standards for this plant.

Keywords:*antimicrobialactivity, phytochemicalsanlysis, antioxidantactivity, HPTLC*

Received 02 .02.2023 Revised 05.03.2023 Accepted 15.04.2023

INTRODUCTION

For decades, humans have approached medicinal plants as the treatment of disease. It has been a close connection between plants and humans from the time of human civilization development; although not all plant species have been recognized for their medicinal use. Herbal medicines (HM), known as alternative and complementary medicines, have been employed for decades in the treatment of medical ailments through their bioactive ingredients [1]. Over the period, humans have researched and recognized plant species that are effective to treat specific illnesses. It has been a standard practice to use herbal medicine in traditional Ayurveda, Chinese Medicine, Russian herbalism, Unani,and many other medical systems. Furthermore, the botanicals' secondary metabolites'key biological function is what brings about their pharmacological effects[2].

The plants are considered a potential source of biochemicals, although not all are exploited[3,4]. The challenging health issue is to treat infectious diseases because of their prevalence and spread. Hence knowing the plants' resources to treat the disease has been of interest among many scientists and health care units, and these herbal medicinesare more acceptable among people [5, 6,7]. These are the reasons for introducing the anti-microbial effects of different plants and the increasing new wave of international studies [8]. In this study, the known medicinal plant *A. officinalis* have been considered to know its antimicrobial potential.

Scientific name: *Althaea officinalis* L. (Malvaceae)
Synonym(s): Althaea, Marshmallow
Part(s) used: Leaf, root, flowers.
Geographical distribution: The plant is native to Europe, Asia, and the United States of America.

The Malvaceae family plant, generally known as Althaea and Alcea, Marshmallows [9], in Traditional Persian Medicine (TPM), called Khatami, Panirak or Moloukhia[10]; also known as "Althea, Cheeses, Mallards, Moorish Mallow, Mortification Root, Schloss Tea, Sweet Weed, and White Maoow [11]. The plant originated in Europe and has been cultivated for more than a thousand years ago, particularly in wet areas such as Turkey, Iraq, Iran, and the south of Europe [9]. The plant is distinct by its big, sharp apex and indented edge leaves[12]. Both the plant leaves and roots are dried and used for herbal formulation.

Table 1: Althaea officinalis L. classification	
Rank	**Scientific Name and Common Name**
Kingdom	*Plantae* - Plants
Subkingdom	*Tracheobionta* - Vascular plants
Superdivision	*Spermatophyta* - Seed plants
Division	*Magnoliophyta* - Flowering plants
Class	*Magnoliopsida* - Dicotyledons
Subclass	*Dilleniidae*
Order	*Malvales*
Family	*Malvaceae* Juss. - Mallow family
Genus	*Althaea* L. - marshmallow
Species	*Althaea Officinalis* L. - common marshmallow
Source: United States Department of Agriculture Database [13]	

For decades *A.officinalis* is considered a traditional medicine for treating bladder and skin diseases, as well as bronchitis, coughing, inflammation, kidney diseases and laryngopharyngeal mucosal irritation, as well as for treating lipemia, oral cavity and nose inflammations, platelet aggregation, and stomach ulcers [14]. This plant's flower, leaves, and roots are used in traditional medicines for decades over the world [15]. Over the years, it is been used to treat wounds. The water-holding capacity of the plant roots and their high content of polysaccharides can boost the immune system [16]. Pectin in *Althaea officinalis* has a wound-healing and immune-stimulating effect [17]. This plant compound shows phagocytosis and macrophage activation which is reported to be useful in skin disease treatment such as eczema[18]. The presence of polysaccharides in A. officinalis is useful in the treatment of oral and pharyngeal mucosa inflammation and irritation[19].

Therapeutic applications of marshmallows are supported principally by traditional use, phytochemical investigation, and pre-clinical research. Limited human evidence is available to study the efficacy of marshmallow-containing combination products for dermatological conditions. Although clinically unproven, marshmallows may interfere with the absorption of oral medications. No clinical trials assessing marshmallow monotherapy have been conducted for any indication. Therefore, ingestion of marshmallows several hours before or after other medicinal agents may be warranted.

MATERIAL AND METHODS

Collection of Plants:*Althaea Officinalis* (Khatmi) plant collection from Himachal Pradesh India for research study and Extract as Figure:01 and Figure:02.

Extraction a hydroalcoholic procedure

In this study, the hydroalcoholic protocol is used for the test solution extraction, where 2.5g of dry Khatmi Extract sample is added with 50mL of methanol in a beaker, and refluxed for 1 hour. The solution is then cooled and filtered using a 0.45-micron membrane filter.

Organoleptic study

Organoleptic is defined as being perceivable by the senses such as smell, appearance, taste, touch, odor, etc.[20]. There are different ways to test the organoleptic properties of dried samples, such as microscopic or chemical testing, and even by perceiving it directly through the senses. Organoleptic evaluation can be examined through organs of sense and thereby defining some particular characteristics of the material which can be considered as the first step towards the establishment of identity and degree of purity[21]. The organoleptic investigations (condition, color, odor, taste, texture, and nature) were performed.

Physico-chemical analysis

Loss on drying (LOD): In the dried glass stopper shallow weighing bottle, add 1.0g and noted the total weight of the glass stoppered bottle with a sample in it (A). The loaded bottle was placed in the drying chamber (hot air oven) without the stopper, placing the stopper in the same chamber. The sample was dried at 105 + 2°C for about 1 hour. After one hour the lid was placed and the bottle was cooled and then weighed (B). The percentage loss of drying was calculated using the following formula

$$\% \text{ loss of drying} = \frac{(B - A)}{\text{Wt of Taken}} x100$$

pH Value: 1.0 g of sample was transferred into 100mL beaker and 100 water is added and mixed well for about 1.0 minutes. The pH meter electrode was placed into the beaker and 3 readings were taken, the average of which was considered as the pH value of the sample.

Water soluble extractive for Extract and powder: In a 250mL conical flask 5.0 g of sample was transferred and 100 mL water and was placed on a shaker for 6 hours and then allowed to stand for 18 hours, and then filtered and evaporated 25mL of the filtrate to dryness in a weighed evaporating dish. Further dried at 105oC to a constant weight and weigh. Then the percentage of water-soluble extractives was calculated using the following formula.

$$\text{Wt. taken (A): a g in 100 mL, 25 mL, taken i.e., } \frac{25xa}{100}$$

$$\%\ \text{Of water-soluble extractive} = \frac{\text{Wt.of residue}}{Wt.of\ sample\ (A)} x100$$

Alcohol soluble extractive: In a 250mL conical flask 5.0 g of sample was transferred and 100 mL alcohol of specified strength was placed on a shaker for 6 hours and then allowed to stand for 18 hours, and then filtered and evaporated 25mL of the filtrate to dryness in a weighed evaporating dish. Further dried at 105°C to a constant weight and weigh. Then the percentage of alcohol-soluble extractives was calculated using the following formula.

$$\text{Wt. taken (A): a g in 100 mL, 25 mL, taken i.e., } \frac{25xa}{100}$$

$$\%\ \text{Of alcohol-soluble extractive} = \frac{\text{Wt.of residue}}{Wt.of\ sample\ (A)} x100$$

Total ash: To a pre-weighted crucible 1.0g of air-dried sample was added and then again weighed. The sample was then incinerated gently at 450 °C, until free from carbon. And then weighed. Then the percentage of total ash was calculated using the following formula.

$$\%\ \text{Of Total Ash} = \frac{\text{Wt.of Ash}}{Wt.of\ sample} x100$$

Acidic Insoluble Ash: The Ash obtained from the total ash was taken to determine the acid-insoluble ash content. 10% of diluted HCl was added and boiled in a water bath for 10 min, and filtered through the ashless filter paper. The soluble matter was washed with hot water. The insoluble matter of ash was transferred to the crucible (one used for total ash) and dried on the hot plate. And then in France, it was ignited for an hour at 800°C. The crucible is then cooled in a desiccator and weighed. Then the percentage of Acidic Insoluble Ash was calculated using the following formula.

$$\%\ \text{Of Acidic Insoluble Ash} = \frac{\text{Wt.of acidic insoluble ash}}{Wt.of\ sample} x100$$

HPTLC method

Sample preparation: The extract was filtered using Whatman filter paper No.1. Extract residue was re-dissolved in 1ml of chromatographic grade alcohol, which was used for sample application on pre-coated MERCK-TLC/ HPTLC silica gel $60F_{254}$ aluminum sheets.

Developing solvent system: Several solvent systems were obtained in the solvent of Chloroform: Methanol: Glacial acetic acid (9:1:0.2 v/v)

Sample application: Application of bands of 10ul extract was performed using the spray technique by using Anisaldehyde-sulphuric acid reagent, which was prepared by mixing 0.5mL Anisaldehyde, 10 mL Glacial acetic acid, 85 mL Methanol, and 5 mL Sulphuric acid (98%). The sample was duplicated on pre-coated silica gel $60F_{254}$ aluminum sheets, considering the start point 10 mm on the Y axis and 80 mm as the end point from the plate, with the assistance of CAMAG Linomat 5 applicator connected to the CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of chromatogram: After the application of spots, the chromatogram was developed in CAMAG TLC twin through the chamber for 30 min.

Drying condition: The drying was done through TLC Plate Heater preheated at 100+ 5°C for 3 minutes.

Detection of spots: The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at 254nm, 366nm, and 540 nm after spraying with anisaldehyde sulphuric acid The Rf values and fingerprint data were recorded by WIN CATS software.

Heavy metal analysis by ICPMS Method

The 10ppm working Standard solution was prepared, by adding 1mL of standard stock solution (1000 ppm) in a 100mL volumetric flask and made up to the volume with Type-1 water. From this working standard solution 2ppb, 5 ppb, 10ppb standard solutions were made by adding 100 µL, 500 µL, and 1 mL of10 ppm working standard solution in respective 100 mL volumetric flask and made up to the volume with Type-1 water, respectively.

The sample solution was prepared by adding 0.4g sample in PFA(Perfluoroalkoxy) Teflon vessels and adding 5mL trace metal grade Nitric acid (HNO_3). The vessels were tightly closed and kept on the turner by setting the following parameters.

Power		**Ramp (Min)**	**°C (Control)**	**Hold Time (min)**
MAX	**%**			
800 W	100	30	180	10

After the digestion procedure, the vessels were kept for 10 min fumigation. After which the solution was transferred to a 50mL vessel and made up the volume with distilled water and filtered using 0.22 u filter paper. Using this solution, the blank solution was prepared simultaneously.

Detection: The calibration curve was plotted on ICPMS, and the metals were detected in samples using the calibration curve.

Antimicrobial Activity

Culture Preparation: The freshly prepared slant of *S. aureus, P. aeruginosa, E. coli, B. subtilis, C. albicans & A. brasiliensis* was used, and washed the slant by using 10 mL of sterile Normal saline solution.

Media Preparation-Muller Hinton Agar (MHA) was used for determining the activity of *S. aureus* (ATCC6538), *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC8739) &*B. subtilis* (ATCC 6633). Sabroud Dextrose Agar (SDA) was used for determining the activity of *C. albicans* (ATCC 10231). Potato Dextrose Agar (PDA) was used for determining the activity of *A. brasiliensis (ATCC 16404)*. Media was prepared as per the Manufacturer's Instructions. The media was then autoclaved at 121˚C temp. & 15 lbs pressure for 20 minutes.

Sample Preparation: Test samples were weighed 5 gm and transferred into different 150 ml labeled flasks. 2 ml of Dimethyl sulfoxide, 9 ml of methanol & 9 ml of water were added in it & sonicated for 10 mins. After sonication, samples were refluxed in the water bath at 90˚C for 1 hr. Samples were filtered with Whatman filter paper & concentrated up to 5 ml. These samples were used for anti-microbial activity.

Testing Procedure: For Antimicrobial activity-10µl of bacterial culture was pipetted into MHA flasks, and 10µL of yeast culture was pipetted into the SDA flask. 10µL of fungal culture was pipetted into the PDA flask. Mixed it slowly. labeled the plates & then poured 25 ml of media into a sterile measuring cylinder. Upon solidification, wells were made using a sterile borer into agar plates containing inoculums. Then, 100 µl of each sample was added to respective wells including blank. Upon the diffusion of samples, the MHA plates were incubated in a Bacteriological incubator at 35°C for 24 hours & the SDA & PDA plates were incubated in a Biological Oxygen Demand incubator at 25°C for 48 hours. The extract was tested against two Gram-positive and two Gram-negative bacteria, as well as two fungi. Antimicrobial activity was detected by measuring the zone of inhibition (including the diameter of the well) that appeared after the incubation period.

Microbial Limit Test

Sample preparation of 1 gm/mL or 10 gm/mL samples was taken in 10 mL/100 mL sterile Soyabean casein digest broth. Samples were mixed using a cyclomixture.

For the microbial limit,the test Pour –plate method was used.

Total microbial plate count: From the prepared sample, 1 mL of the sample was pipetted into two different sterile Petriplates and Labelled properly. Autoclaved Soyabean casein digest agar medium was cooled for about 40°C and approximately 25 mL of media was poured into each label plate and rotated slowly for proper mixing. And allowed the plates to solidify then incubated at 30° to 35°C for 3-5 days. The colonies from two plates were counted which have not more than 250 colonies and the mean was taken. The colony-forming unit was counted by the following formula.

$$\text{Colony forming unit (cfu): } \frac{\text{No. of colonies x dilution}}{\text{Weight of sample}}$$

Total yeast & mold count: From the prepared sample, 1 mL of the sample was pipetted into two different sterile Petriplates. And labeled. Autoclaved medium of Sabouraud dextrose agar was cooled for about 40°C and approximately 25 mL of media was poured into each label plate and rotated the plates slowly for proper mixing. And allowed the plates to solidify and then incubate at 20° to 25°C for 5 days. The colonies from two plates were counted which have not more than 20 to 25 colonies and the mean was taken. The colony-forming unit was counted by the above-mentioned formula.

Test for Specified Organisms:

Escherichia coli: 0.1 mL sample was pipetted and transferred the same to 10mL of MacConkey Broth and incubated at 42° to 44°C for 24 to 48h. The Subculture on a plate of MacConkey Agar was made and incubated at 30° to 35°C for 18 to 72h. The growth of pink, non-mucoidal colonies indicated the possible presence of *E.coli* which was confirmed by identification tests.

Identification Test: Suspected colony was streaked on Eosin Methylene Blue agar (EMB) and incubated on the plate at 30° to 35°C for 18 to 24h. The growth with greenish metallic confirmed the presence of *E.coli.*

Indole Test: 0.1 mL sample from MacConkey broth was pipetted into 10 mL peptone broth and mixed well. The tube was incubated at 30° to 35°C for 18 to 24h. The next day Kovach's reagent was added to peptone broth. The pink ring observation confirmed the presence of *E.coli.*

Salmonella: 0.1 mL sample was pipetted and transferred the same to 10 mL of *Rappaport Vassiliadis Salmonella* enrichment Broth and incubated at 30° to 35°C for 24 to 48h. The subculture on a plate of Wilson and Blair's BBS agar was made and incubated at 30° to 35°C for 24 to 48h. Green colonies with black centers developed and in 48h the colonies become uniformly black. Colonies surrounded by a dark zone and metallic observed indicated the possibility of the presence of *Salmonella*. And if subcultured on plates of XLDA and incubated at 30 to 35°C for 24 to 48h, developed red colonies with or without black centers indicate the possibility of *Salmonella*, which was confirmed by identification tests.

Identification Test: The suspected colony was streak on Triple sugar iron agar (TSI) slant and Incubated at 30 to 35°C for 24 to 48h,the formation of acid & gas with or without blacking butt and the absence of acidity on the surface growth observation confirmed the presence of Salmonella.

Pseudomonas aeruginosa: From the prepared sample, a loopfull sample was taken and streaked on a plate of Cetrimide agar and incubated at 30° to 35°C for 18 to 72h. A greenish-colored colony indicated the possible presence of Pseudomonas aeruginosa, which was confirmed by identification tests.

Identification Test: Suspected colony was streaked on Pseudomonas agar with fluorescent& Pseudomonas agar with pyocyanin media-containing plates and incubated the plates at 30° to 35°C for 18 to 24h. The greenish colonies observation on Pseudomonas agar with fluorescent& colorless to yellow colony observation on Pseudomonas agar with pyocyanin confirmed the presence of pseudomonas. Oxidase test: Suspected colonies were picked & put on the Oxidase disc, the white-to-blue transformation, confirmed the presence of *pseudomonas*.

***Staphylococcus aureus*:** From the prepared sample, a loopful sample was taken and streaked on a plate of Mannitol salt agar and incubated at 30 to 35°C for 18 to 72h. Yellow or white colonies with yellow zones indicated the possibility of *S.aureus*., which was confirmed by identification tests.

Identification test: Catalase test: Suspected colonywere picked on the sterile slide and 1-2 drop of Hydrogen peroxide was added. The bubble formation confirmed the presence of *S.aureus.*

Positive Control: A positive Control was used with a known amount of the organism to check the viability of the organism and also to check the property of the medium used for its detection. There should be characteristic growth on all the different types of medium.

Negative Control: To test the sterility of the medium and the diluent, (without organisms) is known as the negative control. There should not be any growth of microorganisms in this control.

Total antioxidant by DPPH method:

1 g of sample was taken and extracted in 20 mL of 50 % methanol in a conical flask. Next day filtered with Whatman filter paper No. 1 and volume made up to 50 mL with 50 % methanol. This is a Stock solution. From this stock solution, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL, and 1.2 mL was taken in different volumetric flask and volume made up to 100 mL with 50 % methanol to get working solutions with a concentration of 80 µg, 120 µg, 160 µg, 200 µg, 240 µg respectively. The working solutions thus obtained were used for the antioxidant study.

Quantification of total polyphenols by UV Method:

Standard Preparation: The standard solution was prepared by adding 100mg standard Gallic acid and 75 mL of 50& methanol solution and sonicated for 15 min (until the standard is completely dissolved). The solution volume was made up to 100 mL by adding 50% methanol solution. From which 5mL solution was transferred and made up to 50 mL by adding DM water.

Sample Preparation: The sample was prepared using 500mg of extract/ powder in an iodine flask to which 75 mL 50% methanol solution was added and reflexed in a water bath for30min, cooled, and filtered with Whatman filter paper in a volumetric flask. The solution is made up to the volume of 100 mL with 50% methanol solution, from which 5mL of the solution was taken and made up to 50mL using DM water.

Assay: The Assay was performed by pipetting 2 mL of standard and sample solution in a 2-separate volumetric flask (50 mL). 20 mL of water, 2.5 mL of Folin-Ciocalteu reagent, and 5 mL of 30% Na_2CO_3 were added and allowed to stand for 30mins. The volume of the solutions was made up to 50mL using DM water, Shaked well, and allowed to stand for 20min. The absorbance of the standard and sample was taken using a UV spectrophotometer at 750nm using DM water as blank. Then the percentage of Total Polyphenols was calculated using the following formula.

$$\%\ \text{of Total Polyphenol} = \frac{T}{S} x \frac{Cs}{Ct} x \frac{P}{(100 - LOD)} x100$$

Where: T = absorbance of test solution; S = absorbance of standard solution; Cs = concentration of standard solution; Ct = concentration of test solution; P = Purity of standard; LOD = loss on drying of extract.

Assay of total Flavonoids by gravimetry method:

1g of weighed sample was taken and 60mL of methanol was added, shaken for 1 hour on a shaker, and allowed to stand for 18 hours. The next day it is then filtered and the residue is used and given for

washing with 50mL methanol and repeated. All the methanolic filtrate was combined and concentrated to 10 mL. then this 10 mL solution was added dropwise with continuous string to the beaker containing 100mL diethyl ether. And kept stand for 10 min. and filtered with Whatman filter paper and collected the filtrate was in a pre-weighed porcelain dish and the solution was evaporated in a water bath to dryness and further dry at 100°C in an oven to a constant weight. Then the percentage of Total Flavonoid was calculated using the following formula.

$$\% \text{ of Total Flavanoids} = \frac{\text{Weight of residue}}{\text{Wt.of sample taken}} \text{x100}$$

RESULTS

Organoleptic analysis

The organoleptic analysis reported Brown colored powder with characteristic odor and taste.

Physicochemical analysis

The physicochemical parameters of the Shati extract are represented in Table 2. The pH of the solution was found to be 5.24. The percent moisture content (Loss of drying) was 2.23%. Low moisture content could prevent the sample from microbial infection during storage. The ash values like total ash and acid insoluble ash values were found to be 4.75% and 0.93% respectively. And solubility like water soluble and alcohol soluble was found to be 92.83% and 87.18% respectively.

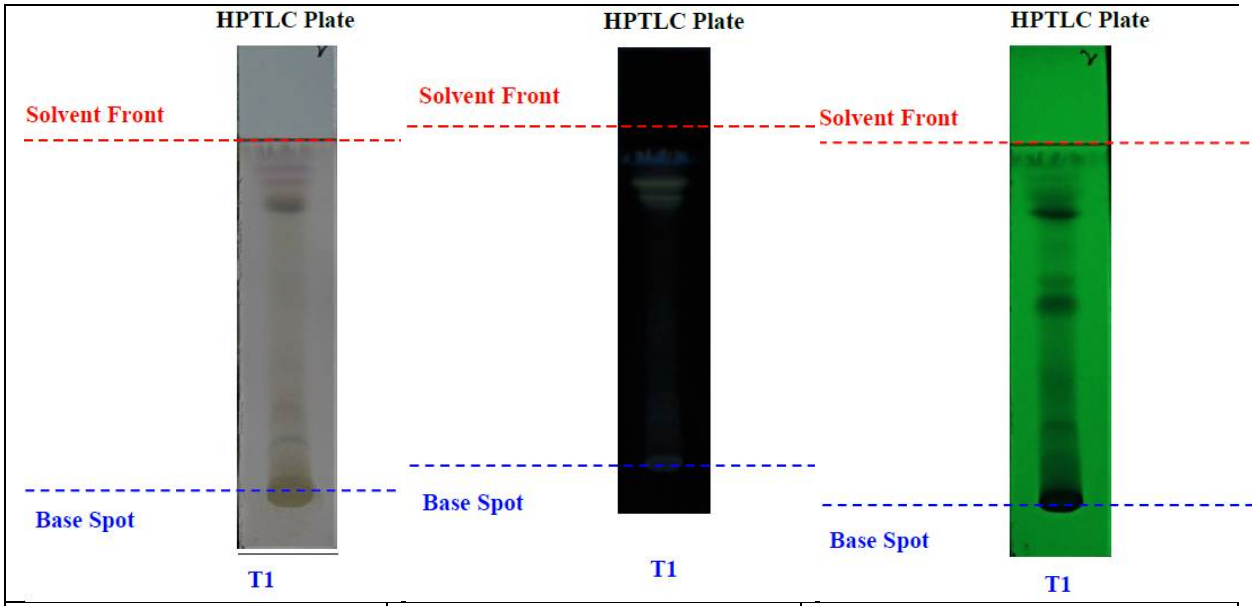
Table 2: Results of Physico-Chemical Analysis

Parameters	Result
Loss on Drying	2.23 %
pH (1%)	5.24
Water Soluble Extractive	92.83 %
Alcohol Soluble Extractive	87.18 %
Total Ash	4.75 %
Acid Insoluble Ash	0.93 %

HPTLC

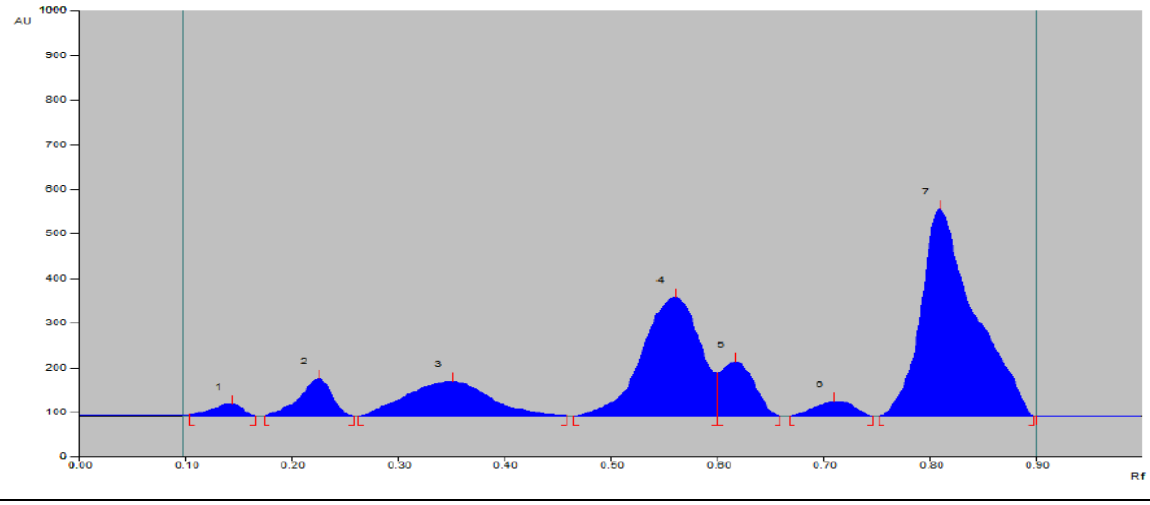
In this study, the HPTLC fingerprinting of the alcoholic extract revealed seven spots for HPTLC Chromatogram at 254 nm at the following Rf values 0.14, 0.23, 0.35, 0.56, 0.62, 0.71, 0.81. HPTLC Chromatogram at 366nm revealed ten spots at the following Rf values 0.14, 0.23, 0.28, 0.35, 0.43, 0.48, 0.56, 0.71, 0.81, 0.87. And at 540 nm revealed five spots at the following Rf values 0.14, 0.28, 0.35, 0.81, 0.87, and the purity of the sample was confirmed by comparing the absorption spectra at the start, middle, and end positions of the band.

The corresponding HPTLC chromatograms are presented in Fig. 1 and 2, for the respective densitometer

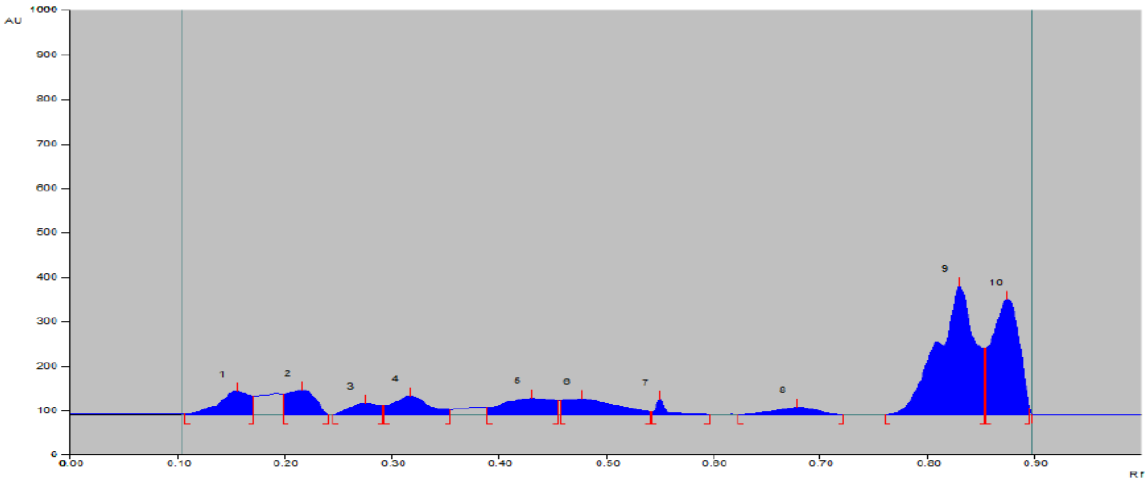


@ 254	@366	@540

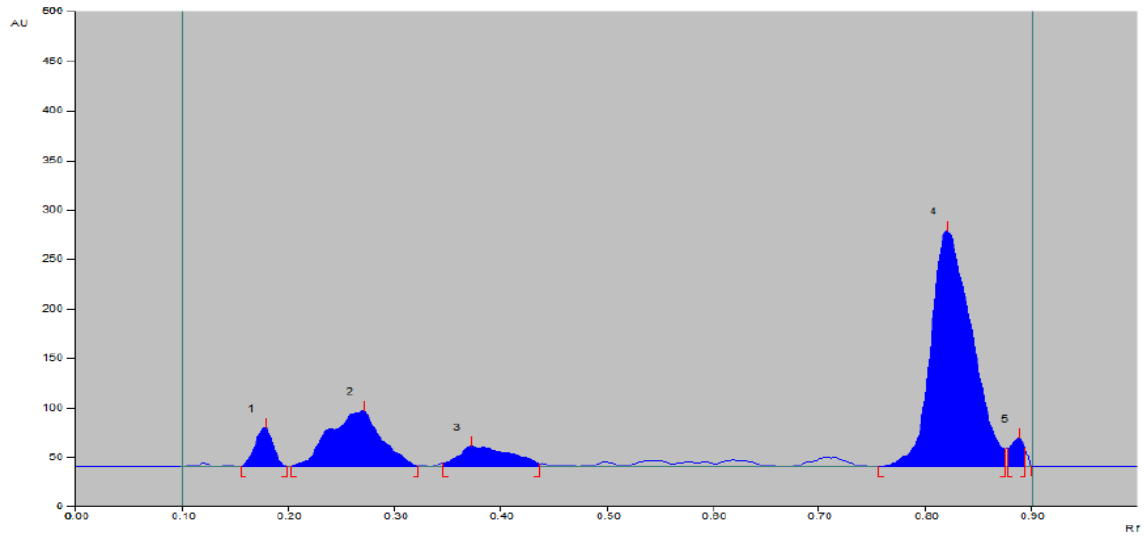
Figure 3: HPTLC chromatogram of the sampleat 254nm, 366nm, and 540nm in alcoholic extract (anisaldehyde sulphuric acid sprayed data).



@254nm



@366 nm



@540

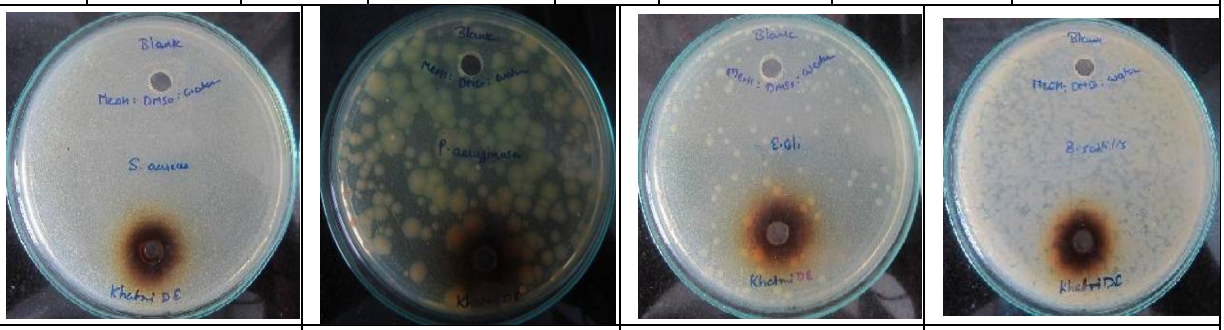
Figure 3: HPTLC chromatogram of alcoholic extract at 254nm, 366nm, and 540nm showing different peaks (bands) of phytoconstituents.

Heavy Metal Analysis:
The Heavy Metal Analysis is tabulated in TableNo.3 below:

Table 3: Results of Heavy metal Analysis		
Parameters	**Result**	**Limit as per API**
Lead	0.075 ppm	NMT 10 ppm
Cadmium	0.001 ppm	NMT 0.3 ppm
Mercury	0.002 ppm	NMT 1 ppm
Arsenic	0.026 ppm	NMT 3 ppm

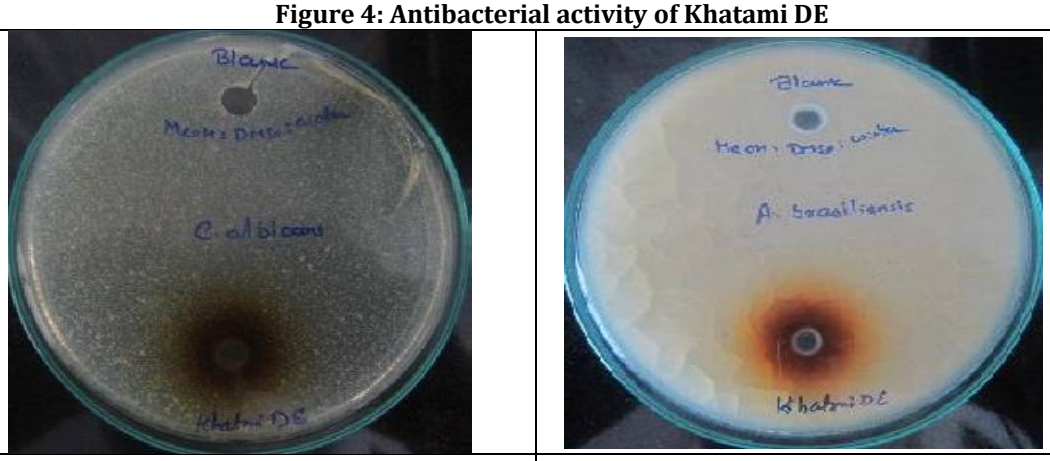
Antimicrobial activity
The extracts from A. officinalis were tested against two Gram-positive and two Gram-negative bacteria, as well as two fungi. The results, presented in Table no. 4show that the hydroalcoholic extracts inhibited good biological activity against all tested fungi and bacteria. The most sensitive microorganism against extracts was *Staphylococcus aureus* with inhibition zones of 16mm. Other microorganisms such as *Pseudomonas aeruginosa* with inhibition zones for 12mm, *Escherichia coli* 10mm, *Bacillus subtilis* 11mm, *Candida albicans* 10mm, *Aspergillus brasiliensis*10mm,respectively were found to be moderately sensitive to the extracts with inhibition zones. It is conceivable that the antimicrobial property of the hexane extracts from *A. officinalis* ascribed to its high content of fatty acid compounds.

Table 4: Zone of inhibition of bacteria and fungi							
Sr. No.	**Name of Sample**	**Zone of Inhibition(mm)**					
		S.aureus	*P.aeruginosa*	*E.coli*	*B.Subtilis*	*C.albicans*	*A. brasiliensis*
1	Blank	NZI	NZI	NZI	NZI	NZI	NZI
2	Khatmi	16	12	10	11	10	10



S.aureus | *P.aeruginosa* | *E.coli* | *B.Subtilis*

Figure 4: Antibacterial activity of Khatami DE



C.albicans | *A. brasiliensis*

Figure 5: Antifungal activity of Khatami DE

Total antioxidant by DPPH scavenging effect (%):
The DPPH showed the following results at different concentrations. Of which the graph was plotted (figure 5).

Table 5: Results of DPPH Scavenging effect at different concentrations	
Concentration (µg)	**DPPH Scavenging effect (%)**
80	32.46
120	43.29
160	52.38
200	58.44
240	67.96

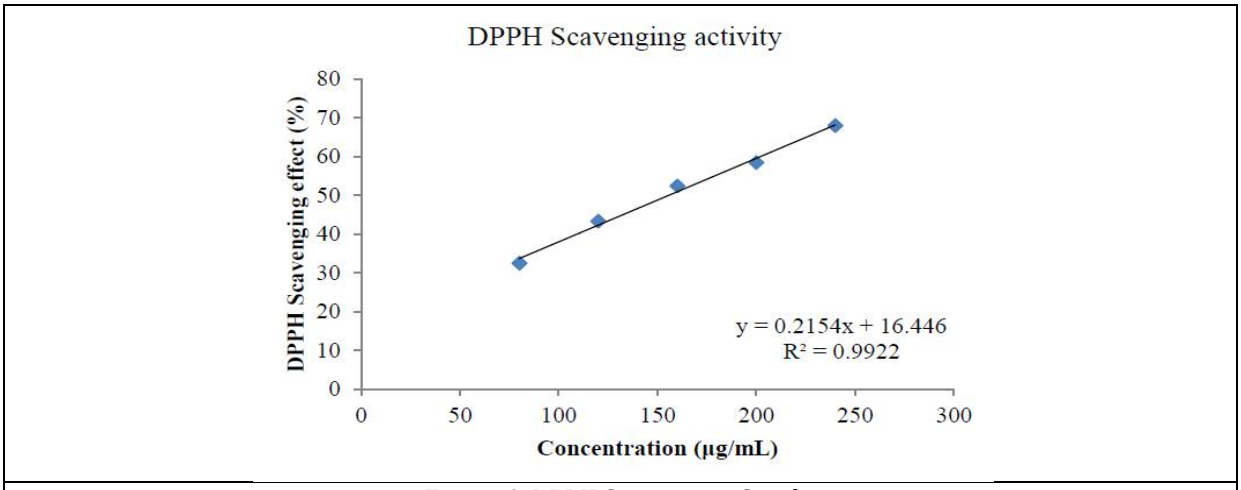


Figure 6: DPPH Scavenging Graphs

Total polyphenol and Total flavonoid calculated in Percentage (%) as below table No.06

Sr.No.	Test Parameter	The result calculated in Percentage (%)	Limit as per API
1	Total Flavonoid	1.48	NA
2	Total Polyphenols	1.98	NA

Microbial limit test

Test Parameter	Results	Limit as per API
Total Microbial Plate Count	Nil cfu/g	10^5 cfu/g
Total Yeast and Mold Count	Nil cfu/g	10^3 cfu/g
Staphylococcus aureus	Absent	Absent/g
Salmonella Sp.	Absent	Absent/g
Pseudomonas Aeruginosa	Absent	Absent/g
Escherichia coli	Absent	Absent/g

As above test Positive control: Growth Observed in Qualitative and Quantitative analysis during Interpretation of Results.
Negative Control: No Growth was Observed in Qualitative and Quantitative analysis during the Interpretation of Results.

DISCUSSION

The food moisture level affects the texture, and the more over the endosperm structure, the lower the moisture content rate. This specifies that freeze-dried samples could be kept over a period without becoming moldy [22]. The moisture content was less in our study which agreed with the study of Ullah *et al.,*[23]. The total ash content signifies the effectiveness and purity of various drugs. Ash contents were low in our study reports, which agreed with the study of Ullah *et al.,*[23].

Physicochemical parameters of the Khatami extract showed high solubility in water and high ash content and a pH to be neutral. Studies have suggested that ash value determination is the key step to analyzing the quality and purity of the sample[24]. Biologically active compounds are chemicals that have the potential to cure various diseases [25].

Phytochemistry is a foundational principle for authenticating numerous therapeutic industries, recognizing crude drugs, and a positive anthropological action [26]. For this, the phytochemical screening of the plant extract was performed to know the possible presence of different phytoconstituents. The HPTLC was performed on the plant extract and reported the presence of different phytoconstituents in

different concentrations. The present study showed the presence of flavonoids and polyphenols, which is supported by the study of [27], who reported similar phytochemicals, including phenol, flavonoids, terpenoids, etc. The study on the flower extract of *A. officinalis* has shown the presence of phytochemicals such as Saponins, phytosterols, steroidal glycosides, proteins, reducing sugar, terpenes, and flavonoids, but the absence of phenols, which contradict our study results [23]. Other studies suggest that A. officinalis has a variety of substances that have been extracted, including starch, pectins, saccharose, mucilage, flavonoids, caffeic acid, p-coumaric acid, isoquercitrin, coumarins, phytosterols, tannins, and several amino acids [28].

The study on the rats, where the topical application of an A. officinalis extract on a rat excision wound model, resulted in the wound healing percentage being higher in the extract-treated wounds in comparison to the controls. Even other studies have stated that A. officinalis hydroethanolic extract consists of phytochemicals that can act as antibiotics to kill gram-positive bacteria and can faster the wound healing process through other mechanisms [29]. The phytochemical analysis concluded that the plant poses potential therapeutic phytochemicals that make the plant worthy of local use.

The DPPH Study suggests a significant antioxidant property of the plant extract which is also proved in the study by Sadighara *et al.,*[30].

Our study reports suggest that *A.officinalis* extracts exhibit goodanti-microbial activity against different bacteria and fungus that causes skin, lung, and blood infections, exhibiting good anti-inflammatory properties.A similar study on gram-positive and gram-negative bacteria viz. *E. coli, P. aeruginosa, K. pneumonia, B. subtilis, Enterococcus faecalis, S. aureus,* and *Staphylococcus epidermidis*, the hexane extracts of root and flower showed positive results, exhibiting anti-bacterial activity; even the study proved anti-fungalactivity of the extract on three fungi viz. *Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae*[31]. In one more study on leaves, flower and root extracts of A.officinalis showed an anti-bacterial effect against gram-positive bacteria, S. aureus, and gram-negative bacteria, *E. coli, P. aeruginosa*, with the highest antibacterial effect on *S. aureus*, whereas the gram-positive bacteriashowed high sensitivity as compared to gram-negative bacteria [32]. The root methanolic extract is studied to inhibit the activity and reduce pathogens in the oral cavity. The Antimicrobial effects of the plant extract against *Proteus vulgaris, Pseudomonas aeruginosa,* and *Staphylococcus aureus* have been proven. Also, the hexane extracts' antimicrobial activity has been proven against gram-positive and gram-negative bacteria [33,34,35]. The plant seeds' antifungal activity was proved to be effective on *Microsporumcanis, Microsporum gypseum,* and *trichophyton mentagrophytes* [36], as well as Aspergillus (fumigatus, niger, and flavus) species [33, 37]. The topical application of an *A. officinalis* extract on a rat excision wound model was studied, and the wound healing percentage was much higher in the extract-treated wounds compared to the control. Moreover, the A. officinalis hydroethanolic extract includes phytochemicals that can serve as antibiotics to kill gram-positive bacteria and can quicken the healing of wounds through other mechanisms[29].

Active compounds are mucilage polysaccharides arabinogalactans, galacturonorhamnans, glucans, and arabinans[38]. The extract of the marshmallow root stimulates phagocytosis and the release of oxygen radicals and leukotrienes from human neutrophils[39]. The release of cytokines: interleukin-6 and tumor necrosis factor (TNF), from monocytes, by the extracts, demonstrated their potential anti-inflammatory activity [40,41]. In an in-vivo study, an ointment consisting of an aqueous marshmallow root extract (20%) released the irritation caused by UV radiation or tetrahydrofurfuryl alcohol. The ointment containing dexamethasone 0.05% (anti-inflammatory synthetic drug), showed weaker anti-inflammatory effect, but higher in an ointment containing both active ingredients[40]. The extract is studied and reported to treat local irritations, inhibit mucociliary activity, function as an anti-complementary and anti-inflammatory agent, and stimulate the immune system[11].

Chewing of A.officinalis dried roots is considered good for teething children, and even used as a toothbrush, it is studied to have mechanical effects on the gums [36].

Regarding wound healing, the main constitutes responsible for pain reduction and inflammation suppression are known to be polyphenols, flavonoids, anthocyanins, hydroxybutyric acid, and terpenoids[20,21], while, pectins in this plant have an immune-stimulating effect and can accelerate the process of wound healing. The aqueous extract of this plant stimulates phagocytosis, the production of oxygen radicals, and the release of leukotriene from neutrophils. Besides, by stimulating monocytes, it secretes cytokines, IL-6, and tumor necrosis factors from these cells[39].

CONCLUSION

It is concluded that the standardization and potential antimicrobial activity of the extracts from A. officinalis against tested bacteria and fungi. further investigation work to characterize the bioactive compound and other values for fixing standards to this plant.

REFERENCES

1. Enioutina, E. Y., Salis, E. R., Job, K. M., Gubarev, M. I., Krepkova, L. V, & Sherwin, C. M. T. (2017). Herbal Medicines: challenges in the modern world. Part 5. status and current directions of complementary and alternative herbal medicine worldwide. Expert Review of Clinical Pharmacology, 10(3):327–338.
2. Quave, C. L. (2018). Wound healing with botanicals: a review and future perspectives. Current Dermatology Reports, 7:287–295.
3. Digrak, M., Alma, M. H., & Ilçim, A. (2001). Antibacterial and antifungal activities of Turkish medicinal plants. Pharmaceutical Biology, 39(5):346–350.
4. Skaltsa, H. D., Demetzos, C., Lazari, D., & Sokovic, M. (2003). Essential oil analysis and antimicrobial activity of eight Stachys species from Greece. Phytochemistry, 64(3):743–752.
5. Burton, A., Smith, M., & Falkenberg, T. (2015). Building WHO's global strategy for traditional medicine. European Journal of Integrative Medicine, 7(1):13–15.
6. Mosadegh, M., & Naghibi, F. (2002). Iran Traditional Medicine: Past & Present.
7. Organization, W. H. (1978). The promotion and development of traditional medicine: report of a WHO meeting [held in Geneva from 28 November to 2 December 1977]. World Health Organization.
8. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4):564–582.
9. Mozaffarian, V. (2013). Identification of medicinal and aromatic plants of Iran. éditeur non identifié.
10. Aghili Khorasani, M. (2008). Makhzan al-Advie Iran university of Medical Science: Research Institute for Islamic and Complementary Medicine. Tehran.
11. Jean, B. (2009). Pharmacognosie, phytochimie, plantes médicinales (4e éd.). Lavoisier.
12. Strzelecka, H., & Kowalski, J. (2000). Encyclopedia of herbalism and phytotherapy. Warsaw: PWN, 42.
13. Classification for Kingdom Plantae Down to Species *Althaea officinalis* L. (n.d.). United States Department of Agriculture Database.
14. Albahri, G., Badran, A., Hijazi, A., Daou, A., Baydoun, E., Nasser, M., & Merah, O. (2023). The Therapeutic Wound Healing Bioactivities of Various Medicinal Plants. Life, 13(2):317.
15. Banaee, M., Soleimany, V., & Haghi, B. N. (2017). Therapeutic effects of marshmallow (*Althaea officinalis* L.) extract on plasma biochemical parameters of common carp infected with *Aeromonas hydrophila*. Veterinary Research Forum, 8(2):145.
16. Rheder, D. T., Guilger, M., Bilesky-José, N., Germano-Costa, T., Pasquoto-Stigliani, T., Gallep, T. B. B., Grillo, R., Carvalho, C. dos S., Fraceto, L. F., & Lima, R. (2018). Synthesis of biogenic silver nanoparticles using Althaea officinalis as reducing agent: evaluation of toxicity and ecotoxicity. Scientific Reports, 8(1):12397.
17. Blumenthal, M., Goldberg, A., & Brinckmann, J. (2000). Herbal medicine. Expanded commission E monographs. Integrative Medicine Communications.
18. Visser, J., & Voragen, A. G. J. (1996). Pectins and pectinases. Elsevier.
19. Franz, G. (1989). Polysaccharides in pharmacy: current applications and future concepts. Planta Medica, 55(06):493–497.
20. Arya, V., & Thakur, R. (2012). Organoleptic and microscopic analysis of *Gentiana regeliana*. Journal of Pharmacognosy and Phytochemistry, 1(2):32–35.
21. Shah, B. N. (2009). Textbook of pharmacognosy and phytochemistry. Elsevier India.
22. Dastagir, G., Hussain, F., & Khattak, K. F. (2014). Nutritional evaluation of plants of family zygophyllaceae and euphorbiaceae. Pak. J. Bot,46(5):1703–1707.
23. Ullah, Saqib & Dastagir, Ghulam & Daud, Fajr & Naz, Mehik & Noor, Laila & Khan, Aamad & Khan, Ismail & Pakhtunkhwa, Khyber. (2023). Assessment Of Medicinal Flowers With Special Emphasis on Elemental and Nutritional Analysis. 19:1136-1151.
24. Mulla, S. K., & Paramjyothi, S. (2010). Preliminary pharmacognostical and phytochemical evaluation of Portulaca quadrifida Linn. International Journal of PharmTech Research, 2(3):1699–1702.
25. Sreenu, P., Samatha, T., Gandhi, N., & Azmeera, S. N. (2014). Phytochemical analysis of root, stem and leaf extracts in Rubia cordifolia L. an important medicinal plant. World Journal of Pharmacy and Pharmaceutical Sciences,3(10):826–838.
26. Mukherjee, P. K., Bahadur, S., Chaudhary, S. K., Kar, A., & Mukherjee, K. (2015). Quality related safety issue-evidence-based validation of herbal medicine farm to pharma. In Evidence-based validation of herbal medicine (pp. 1–28). Elsevier.
27. Farhat, C., Younes, H., Alyamani, O. A., Mrad, M., Hourani, N., Khalifeh, H., El-Makhour, Y., Dbaibo, G., & Hage-Sleiman, R. (2022). Chemical characterization and in vitro biological evaluation of aqueous extract of Althaea officinalis L. flower grown in Lebanon. Journal of Herbal Medicine, 34, 100575.
28. Bonaterra, G. A., Bronischewski, K., Hunold, P., Schwarzbach, H., Heinrich, E.-U., Fink, C., Aziz-Kalbhenn, H., Müller, J., & Kinscherf, R. (2020). Anti-inflammatory and Anti-oxidative Effects of Phytohustil® and Root Extract of *Althaea officinalis* L. on Macrophages in vitro. Frontiers in Pharmacology, 11:290.
29. Nicolaus, C., Junghanns, S., Hartmann, A., Murillo, R., Ganzera, M., & Merfort, I. (2017). In vitro studies to evaluate the wound healing properties of Calendula officinalis extracts. Journal of Ethnopharmacology, 196:94–103.
30. Sadighara, P., Gharibi, S., Jafari, A. M., Khaniki, G. J., & Salari, S. (2012). The antioxidant and Flavonoids contents of *Althaea officinalis* L. flowers based on their color. Avicenna Journal of Phytomedicine, 2(3):113.
31. Mahdi, V., Ali, S., & Farshid, S. (2011). Chemical composition and antimicrobial activity of the flower and root hexane extracts of *Althaea officinalis* in Northwest Iran. Journal of Medicinal Plants Research, 5(32):6972–6976.

32. Walter, C., Shinwari, Z. K., Afzal, I., & Malik, R. N. (2011). Antibacterial activity in herbal products used in Pakistan. Pak. J. Bot, 43:155–162.
33. Al-Snafi, A. E. (2013). The pharmaceutical importance of *Althaea officinalis* and *Althaea rosea*: A review. Int.J. Pharm. Tech. Res, 5(3):1385–1387.
34. Al-Snafi, A. E. (2015). Therapeutic properties of medicinal plants: a review of their antibacterial activity (part 1). International Journal of Pharmacology and Toxicology, 6(3):137–158.
35. Choopani, R., Sadr, S., Kaveh, S., Kaveh, N., & Dehghan, S. (2015). Pharmacological treatment of catarrh in Iranian traditional medicine. Journal of Traditional and Complementary Medicine, 5(2):71–74.
36. Kumar, S. S., Sudhakar, S., Kapil, S., & Snigdha, T. (2016). Ethno-pharmacological review on Althaea officinalis. World J. Pharmacy and Pharm. Sci., *5*(7):425–432.
37. Rashidi, A., Mousavi, B., Rahmani, M. R., Rezaee, M. A., Hosaini, W., Motaharinia, Y., Davari, B., & Zamini, G. (2011). Evaluation of antifungal effect of *Lavandula officinalis*, *Salvia officinalis* L., Sumac, *Glycyrrhiza glabra*, and *Althaea officinalis* extracts on *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus flavus* species. Journal of Medicinal Plants Research, 6(2):309–313.
38. Abbaspour, M., Hoseini, H., Sobhani, Z., Emami, S. A., Bazzaz, B. S. F., & Javadi, B. (2023). Development and Evaluation of Vaginal Suppository Containing *Althaea officinalis* L. Polysaccharide Extract. Brazilian Journal of Pharmaceutical Sciences, 58.
39. Scheffer, J., Wagner, H., & Proksch, A. (1991). Radix althaeae und Flores chamomillae Extrakte auf Entzündungsreaktionen humaner neutrophiler Granulozyten, Monozyten und Rattenmastzellen. Third Phytotherapy Congress Lübeck-Travemünde.
40. ESCOP, & Phytotherapy, E. S. C. on. (2003). *ESCOP* Monographs: the scientific foundation for herbal medicinal products. George Thieme Verlag.
41. Organization, W. H. (1999). WHO monographs on selected medicinal plants (Vol. 2). World Health Organization.

CITATION OF THIS ARTICLE

A. S. Patil and V. J. Upadhye: Standardization and potential antimicrobial activity of Khatami (Althea Officinalis) .Bull. Env.Pharmacol. Life Sci. Vol 12[5] April 2023: 88-99.