RESEARCH PAPER

Phytochemical Screening and Anti TB Activity of stems of Portulaca Grandiflora Hook

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ABSTRACT
This article focuses on the use of herbal medicine, rooted in pharmacognosy, and the therapeutic application of medicinal plants, commonly known as herbalism, phytomedicine, or phytotherapy. It delves into the potential of *Portulaca grandiflora* Hook in combating *tuberculosis* (TB), a disease with ancient origins. The focus is on the anti-tubercular properties of *Portulaca grandiflora* Hook. The study involves the two-extraction method. Using the two extracts (ethanol and aqueous extract) isolating the various phytochemical compounds such as flavonoids, alkaloids, tannins, carbohydrates, saponins, and steroids from this plant. These compounds are identified using Thin Layer Chromatography (TLC). Furthermore, the isolated compounds undergo thorough characterization through techniques including UV spectroscopy and infrared spectroscopy (IR) shedding light on their potential effectiveness against TB. The quercetin was found in this study. Docking study was carried using 2FK8 PDB ID.

Keywords: *Portulaca grandiflora, physicochemical analysis, Anti tuberculosis activity, Flavonoid compound, docking study of anti TB.*
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1. INTRODUCTION

The scientific and pharmaceutical communities have shown a great deal of interest in medicinal plants. The medical potential of natural substances has been extensively proven in several papers, hence supporting claims about their biological effectiveness. For conventional and alternatives medical treatments, nutraceuticals, dietary supplements, folk cures, pharmaceuticals intermediates, and chemical components of synthesized medications, these plants provide an abundant bioresource.[1]

The World Health Organization (WHO) has defined traditional medicine as treating patients using practices that date back hundreds of years, or even beyond, before modern medicine appeared and gained widespread recognition. This definition includes herbal drugs. [2] The age-old illness tuberculosis (TB) continues to be a major worldwide health concern. [3] The illness is multidrug-resistant (MDR) in around half a million patients. The World Health Organization (WHO) plans to eradicate tuberculosis (TB) by the year 2050; however, the primary obstacles to this aim are multidrug-resistant (MDR) and extensively drug-resistant (XDR) types of TB. [4] The development of extensively drug-resistant TB patients that do not react well to first-line antitubercular medications (rifampicin, isoniazid, pyrazinamide, and ethambutol) and multidrug-resistant *Mtb* strains makes treating TB difficult. These medications have adverse effects as well. As a result, a lot of TB patients have turned to complementary and alternative medicine, with the most popular form of treatment for TB being herbal therapies. [5] Rose purslane, or *Portulaca grandiflora* Hook., is a wild plant that is occasionally considered a weed. *P. grandiflora* belongs to the species *Portulaca grandiflora* Hook, family Portulacaceae, genus Portulaca L., class Magnoliopsida,

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and order Caryophyllales. [6] The "moss rose," or *Portulaca grandiflora* Hook, is a popular plant. Succulent ornamental plant *Portulaca grandiflora* is an attractive plant with a creeping habit. [7] *Portulaca grandiflora*. Hook., often known as "ten o'clock," is one of the most widely grown annual flower plants in the tropical world owing to its ease of cultivation, prolific blooming, and flowers in a variety of colors such as red, yellow, pink, purple, white, orange, and/or mixed. [8]

2. MATERIAL AND METHODS:

2.1. Plant Collection: -

P. grandiflora was collected in the October 2023 saw the collection of the plant kind from the nearby sangli in Maharashtra. Identification of the plant was done by The Department of Botany at Kasturbai Walchand College in Sangli.

2.1.1. Drying and size reduction of plant material:
After being gathered, the plant stems were cleansed and cleared of any dirt or debris. To dry, we let the stem air. To ensure a consistent drying process, the stem was often twisted and arranged in thin layers. For an extended period, dried plant material stores well. When they had dried completely, the stem was crushed into a coarse powder using a mixer grinder.

2.1.2. Materials: Fresh stems of the plant purslane (*Portulaca grandiflora*) were used. The extraction procedure involved the use of ethanol, acetone, and chloroform.

Tools: - The Soxhlet apparatus, glassware, heating mantle, analytical balance, IR spectrophotometer (ALPHA. CVB), and UV-spectrophotometer (JASCO V-730) were the tools used in this investigation.

2.2. Method

2.2.1. Extract preparation: [9]
There are two extraction method.

1) Soxhlet extraction
2) Maceration

1) Method of soxhlation: The extraction procedure made use of organic solvents such as ethanol, acetone, and chloroform. Continuous Soxhlet extraction was used along with 150 millilitres of chloroform to extract 30 grams of dry *portulaca grandiflora* hook stem powder. After the extraction procedure, the solvent had lost all of its colour. The extractor's powder was removed, dried, and used for acetone and ethanol extractions after the chloroform extract was filtered. Etoile, chloroform, and ethanol extracts were all labelled and stored in separate bottles.

2) Maceration method: 500 ml of a 1:19 chloroform: water IP was made in a single beaker. After adding and blending around 25 grams of dry powder, foil paper was placed on top. Mixture maximum of seven days, this mixture was swirled three times daily. Following the filtering process, the filtrate was gathered, and the marc was compressed. It was kept cold in the refrigerator.

This formed the process of extracting water. Then, carry out steps B and C of the aqueous extraction process once more.

2.2.2. Phytochemical screening: [10,11]

Test for flavonoids:

a) **Alkaline Reagent Test:** Plant extract is treated with 10% ammonium hydroxide solution and test solution is treated with 2 milliliters of 2% NaOH. If there was any yellow fluorescence, the response was affirmative.

b) **Lead Acetate Test:** a little amount of 10% lead acetate solution was added to the test solution. The yellow precipitate was seen.

c) **Test for Ferric Chloride:** Add extract or test solution together with a few drops of Fecl3. If a green precipitate was formed, the reaction was positive.

Test for alkaloid:

a) **Dragendorff's Test:** After adding one drop of the reagent solution to the filtrate, alkaloids are detected by the development of a brown-colored precipitate or turbidity.

b) **Mayer's Test:** the presence of alkaloids is indicated by the production of a white precipitate, or turbidity, when a single drop of the Mayer's reagent solutions is dripped into the filtrate.

c) **Wagner's Test:** Wagner's reagent is applied to a drops of test solution. When Brown precipitate was seen, the alkaloid was present.

Test for steroids:

a) **Salkowski test:** Add 2 ml of extract, 2 ml of chloroform, and 2 ml of concentrated H2SO4.Give it a good shake. Steroids are present when the acid layer fluoresces a greenish yellow color and the chloroform layer becomes red.

b) Perform the Liebmann Burchard test by combining 2 ml of extract with chloroform. Add 2 drops of conc. H2SO4 from the test tube's side and 1-2 milliliters of acetic anhydride. The outcome displays the colors red, blue, and green in order of appearance.

Test for carbohydrates:

a) **Barfoed's Test:** mix a few drops of Barfoed's reagent into 2 milliliters of test solution. For two minutes, the mixture was heated. There was a crimson precipitation.

b) **Molisch Test:** Test solutions treated with a little amount of conc. H2SO4 and alcoholic alpha naphthol. One saw a violet ring.

Test for saponin:

a) Foam test: Shake the 2ml extract or dried powder briskly with water. Long-lasting foam that is stable was seen.

Test for glycosides:

a) Baljet's test: The test 1 ml solution turns yellow to orange when treated with sodium picrate.

Test for tannins:

a) Lead acetate test: after adding 1% lead acetate solution to 2 milliliters of extract, the presence of tannin components is indicated by the production of white precipitate.

b) Iodine test: A few drops of di. iodine solution was added to the 2 ml test solution. The existence of tannin is indicated by the creation of a fleeting red color.

2.2.3.- Isolation and Identification of Phytoconstituents:

1. Thin layer chromatography: [12]
TLC was used to evaluate the ethanol extract. The glass plate measured 7.5 by 2.5 cm. A 0.2 mm thick layer of the silica gel G slurry was applied to it. At 110 0C, the plates were left to air dry for an hour before being activated. The best component resolution was found with methanol: chloroform, despite the evaluation of many solvent solutions. The dots were visible under a UV lamp once the development plates had been taken out and dried.

Calculation:
Retention factor (Rf value) was determined using the formulabelow:

$$\text{Rf value} = \frac{\text{The length of the solute front (in cm)}}{\text{length of solvent front travelled (cm)}} \times 100$$

2. Column chromatography: [13]

Selection of Stationary Phase and Column
Column chromatography was used to an ethanol extract using a silica gel glass column. The stationary phase was 150–200 gram of silica gel (60–120 mesh). For one hour, it was turned on in a hot air oven at 110°C. A mobile phase consisting of 9:1 v/v chloroform: methanol was used to prepare an activated silica gel slurry, which was then added to a dried column. To keep the prepared column from drying out, a little amount of solvent was placed on top of it. The *portulaca grandiflora* hook ethanolic extract was added to the column without disturbing it after it had set.

2.2.4.- Spectroscopy analysis for total flavonoid content:

1. Ultraviolet spectroscopy:
Analysis of the isolated compound using UV light was done. A 10 g/ml concentration sample was produced by mixing an isolated compound with ethanol. At wavelengths between 200 and 800 nm, the extract was scanned. An JASCO V-730 UV spectrophotometer was used for the UV analysis at A.B.C.P., Sangli, where the distinctive peaks were found.

2. IR spectroscopy:
The obtained compound from the column chromatography was dried using desiccation and concentration, according to infrared spectroscopy. Following the dry chemical's trituration with KBr, a pellet was created using a KBr press. An infrared beam and an infrared spectrum were sandwiched by this pellet. Appasaheb Birnale College of Pharmacy in Sangli conducted the analysis utilizing FTIR technology from the JASCO firm.

2.2.5- Pharmacological screening [14]

1. Anti-TB activity (Alamar Blue Assay)

Procedure:
At an optical density (OD) of 0.6 (about 5 x 107 colony-forming units [CFU]/ml) at 600 nm, *Mycobacterium* avium was grown to the mid-log phase. The 96-well microplates were filled with 100 microliters (5 × 10 4 CFUs) of bacterial suspension each well. Each well was then filled with 10 µm of the chemical. For the negative control, DMSO was utilized, and for the positive control, rifampicin (RIF). Following six days of incubation at 37°C, the plates were sealed. Then, each well received an addition of 10% (v/v) Alamar Blue. The hue shift indicated each compound's anti-*Mtb* action. If the hue changed from blue to pink, the chemical was thought to be active against *Mtb*.The absorbance of the plate was measuredat 600 nm by using micro plate reader.

2.2.6.- Docking study:
The technique known as "molecular docking" allows one to precisely expect how two molecules will align with one another to create stable complexes. Auto dock software's commercial docking study was used to conduct this investigation. At the Appasaheb Birnale College of Pharmacy in Sangli, docking research was carried out.

Drug molecule: Quercetin

Target Selection and Preparation: PDB ID: 2FK8, *m tuberculosis.* **[15]**

3. RESULTS AND DISCUSSION:

3.1. Phytochemical Analysis
Various phytochemical components present are listed in Table 1 below, where (+, -) denotes the existence or absence of respective phytochemical ingredients. The flavonoid, alkaloid, steroid, carbohydrate, saponin and glycoside are present in ethanol extract and flavonoid, alkaloid, carbohydrate, saponin and glycoside are present and steroids are absence in aqueous extract.

Table 1. List of the chemical constituents present in stems of *portulaca grandiflora*

Sr.no	Chemical constituents	Ethanol	Aqueous
1.	Flavonoids	+ ve	+ ve
2.	Alkaloids	+ ve	+ ve
3.	Steroids	+ ve	- ve

4.	**Carbohydrates**	+ ve	+ ve
5.	**Saponin**	+ ve	+ ve
6.	**Glycosides**	+ ve	+ ve

3.2. Qualitative analysis

3.2.1. Thin layer chromatography

TLC analysis *portulaca grandiflora* hook. ethanol extract. solvent system chloroform: methanol (9:1) was used and 1 spot was visible and the Standard quercetin's Rf value of 0.88 and test quercetin's Rf value of 0.84 roughly match one another

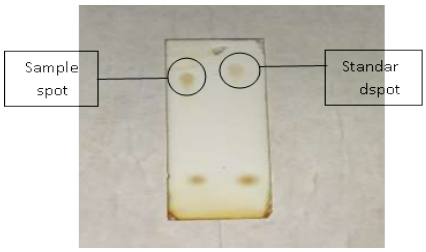


Figure No.1- TLC of standard and sample spot

3.2.2. Ultraviolet Spectroscopy:

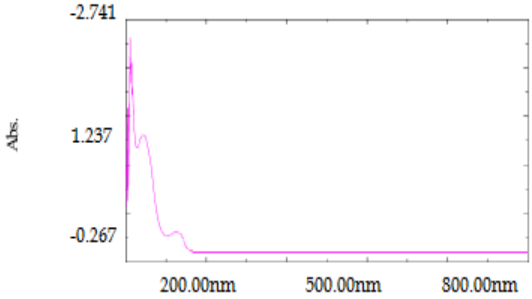


Figure No: 2.- UV of isolated component (quercetin)

Table no. 2. UV spectroscopy wavelength

Sr. No.	Wavelength	Abs.
1	201.00	-0.004
2	250.00	0.231
3	226.00	1.373

Given that the isolated part of the *portulaca grandiflora* hook's ethanolic extract had estimated λ max values of 250 nm, 226 nm, and 201 nm all of which were close to the standard quercetin's λ max of 258 nm. Fig. 5 and Table 3 prove the presence of quercetin in the sample.

3.2.3. Infrared Spectroscopy

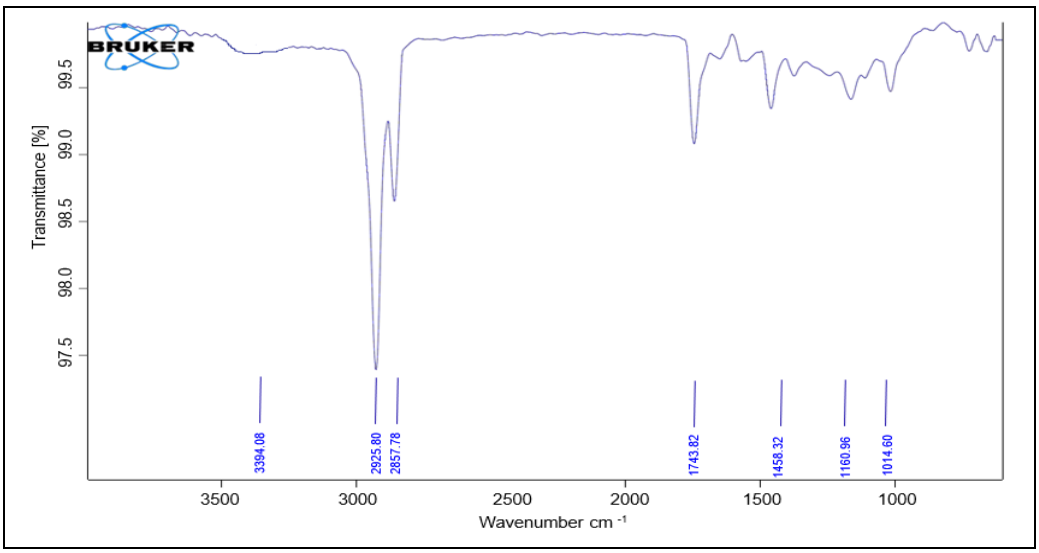


Figure. No. 3 IR spectroscopy of isolated compound (quercetin)

Table No.3. IR ranges and functional group

Sr.no.	Range	Functional group
1	3394.08	**OH**
2	2925.80	**CH**
3	2857.78	**CH**
3	1742.82	**C=O**
4	1458.32	**C=C**
5	1160.96	**C-O**
6	1014.60	**C-O**

3.2.4. Anti-tuberculosis:

These results demonstrated that both extracts have very similar potential for anti- tuberculosis activity. Comparing both the extracts indicates that aqueous samples show moderate activity compared to ethanolic samples Which is 54.33% Ethanol and 30.11% Aqueous samples.

Table No. 4- Microplate Alamar Blue Assay

Sample code	Concentration (µg/ml)	Absorbance at 600nm				% Inhibition
		Test 1	Test 2	Test 3	Mean	
Control	**-**	1.603	1.603	1.603	**1.603**	-
Std. Rifampicin	**1000**	0.422	0.422	0.422	**0.422**	**73.67%**
Ethanol	**1000**	0.732	0.732	0.732	**0.732**	**54.33%**
Aqueous	**1000**	0.977	0.976	0.977	**0.976**	**39.11%**

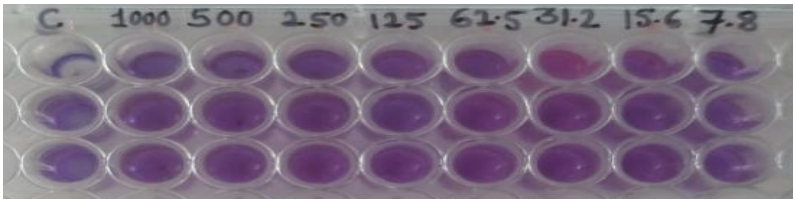


Figure No: 4. Standard Drug photograph for Anti-TB activity

Figure No. 5. Ethanol sample photograph

Figure No. 6. Aqueous sample photograph

The minimal inhibitory concentration of the aqueous and ethanolic extracts of the *portulaca grandiflora* hook stem extract was determined to be in the ethanol sample at 54.33% and in the aqueous sample at 39.11%, respectively, based on the findings of the Microplare alamar blue test, which measures anti-tubercular activity. Compared to aqueous extract, ethanolic extract had the greatest minimal inhibitory concentration. And shown potential anti-tubercular action as compared to the standard group.

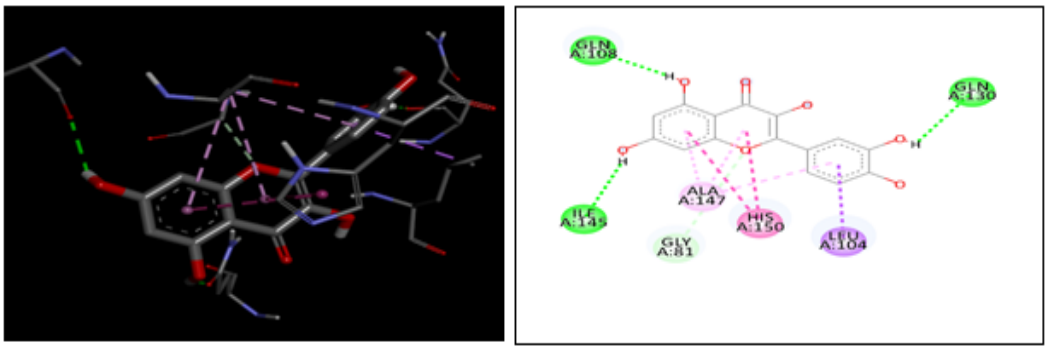
3.2.5. Docking

Table no. 5. Docking Interaction for 2FK8

Sr. no	Amino acid	Interaction
1.	GLN130A	Hydrogen Bond
2.	ILE145A	
3.	GLN108A	
4.	GLY81A	
5.	LEU104A	Hydrophobic Bond
6.	HIS150A	
7.	ALA147A	

Table no. 6. Docking score for 2FK8

Receptor	Drug/ Ligand	Affinity
2FK8	Quercetin	-8.9 (kcal/mol)



Figre.No.7.- 3D and 2D images of docking for anti TB activity

4. CONCLUSION

As a result, in this study, anti-tuberculosis activity analysis of two different extracts obtained from stems of portulaca grandiflora hook. And component analysis was carried out using the IR spectroscopy and NMR spectroscopy method of ethanol extract. The presence of flavonoid compounds is responsible for the activity. Compared to aqueous extract, ethanolic extract had the greatest minimal inhibitory concentration. And shown potential anti-tubercular action as compared to the standard group.

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