

Comparative Study on Phytochemical Screening of Juniperous Erecta on Leaf and Bark with Different Solvents

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Abstract

The aim of study was investigating the phytochemical screening of water and ethyl acetate extract from fresh leaves and bark of JUNIPEROUS ERECTA. The phytochemical screening for extracted from fresh leaves and bark of JUNIPEROUS ERECTA was tested and shown positive result for phenol, saponins and lactone components. However, the extract of fresh leaves and bark not show the positive result for alkaloids, tannins, protein, carbohydrate and anthocyanins components. The extract were characterized for Ultraviolet-visible spectroscopy and found that wavelength of our compounds are present. The extract were characterized for Anti-microbial and found that no zone of inhibition.

Keywords- *JUNIPEROUS ERECTA, saponins and lactone, carbohydrate and anthocyanins*

INTRODUCTION

Phytochemical components are biologically active, naturally occurring chemical constituents found in plants, which provide health benefit for the humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg 1999). They contributes to

the colour, aroma and flavor and also protect the plants from diseases. In general, the plant chemicals that protect plant cells from environmental hazards are pollution, stress, UV exposure and pathogenic attack is called as phytochemicals (Mathai, 2000). Recent, studies they have roles in the protection of

human health, when their dietary intake is significant. (Meagher and Thomson 1999). Medicinal plant is the richest bio-resource of the drugs for traditional systems of the medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

MATERIALS AND METHODS

PREPARATION OF LEAF EXTRACT

WITH DIFFERENT SOLVENTS:

PREPARATION OF AQUEOUS EXTRACT :

About 5g of finely divided leaf of a Juniperous Erecta was weighted and transferred into 250ml conical flask containing 100 ml distilled water was added and it was corked and heated in water bath for 30 minutes and kept aside for again 30 minutes and filtered.

PREPARATION OF ETHYL ACETATE EXTRACT :

About 5g of finely divided leaf of a Juniperous Erecta was weighted and transferred into 250ml conical flask containing 100 ml ethyl acetate was added and it was corked and heated in water bath for 30 minutes and kept aside for again 30 minutes and filtered.

PREPARATION OF BARK EXTRACT WITH DIFFERENT SOLVENTS:

PREPARATION OF AQUEOUS EXTRACT :

About 5g of finely divided bark of a Juniperous Erecta was weighted and transferred into 250ml conical flask containing 100 ml distilled water was added and it was corked and heated in water bath for 30 minutes and kept aside for again 30 minutes and filtered.

PREPARATION OF ETHYL ACETATE EXTRACT:

About 5g of finely divided bark of a Juniperous Erecta was weighted and transferred into 250ml conical flask containing 100 ml ethyl acetate was added and it was corked and heated in water bath for 30 minutes and kept aside for again 30 minutes and filtered.

MATERIAL FOR SCREENING STUDY

The chemicals used for phytochemical screening are Sodium hydroxide, Glacial Acetic acid, Concentrated Sulphuric acid, Acetic anhydride, Concentrated Nitric acid, Wagner's reagent, Lead acetate, Ferric chloride, Molisch reagent, Sodium nitroprusside.

PHYTOCHEMICAL SCREENING:

The methods used for the screening of phytochemical are given below as follows,

1. Test for alkaloids: Wagner's test

To 1ml of the extract, a few drops of wagners reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

2. Test for flavanoids: Lead acetate Test

To 1ml of the extract, a few drops of 10% lead acetate solution were added, appearance of yellow colour indicates the presence of flavanoids.

3. Test for phenols: Lead acetate Test

To 1ml of the extract, few ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of phenolic compounds.

4. Test for Tannins: Ferric chloride test

To 2ml of the extract and 2ml of ferric chloride were mixed. Formation of blue colour indicates the presence of tannins.

5. Test for steroids: LibermannBurchard Test

To 1ml of the extract 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated

sulphuric acid were added. The solution becomes blue, indicates the presence of steroids.

6. Test for saponins:

To 1ml of the extract was taken in a test tube and 20 ml of water is added. The mixture was shaken vigorously and kept for 3 minutes. Formation of forth shows the presence of saponins.

7. Test for proteins: Biuret test

To the plant extract, a Drop of copper sulphate and sodium hydroxides are added. Appearance of violet colour indicates the presence of proteins.

8. Test for carbohydrates: Molisch test

To the extract with Molisch reagent, add concentrated sulphuric acid along the sides to form layers a reddish violet ring at the interference shows the presence of carbohydrates.

9. Test for Terpenoids: Salkaowski Test

A few drops of concentrated sulphuric acid was added to the plant extract, shake well and allow to stand for 2 minutes, lower layer turned yellow, indicates the presence of terpenoids

10. Test for quinones:

To 1ml of the extract, 1ml of concentrated sulphuric acid was added, formation of red colour indicates the presence of quinones.

11. Test for Anthocyanins:

To the substance, 10% of sodium hydroxide was added, appearance of blue colour indicates the presence of Anthocyanins.

12. Test for coumarins:

To 1ml of extract, 1ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

13. Test for lactones:

To 2ml of extract, few drops of sodium nitroprusside and glacial acetic acid were added. Formation of deep red colour indicates the presence of Lactones.

UV SPECTROSCOPY STUDIES

UV spectroscopy is a type of absorption spectroscopy in which light of the ultra-violet region is absorbed by the molecule which results in the excitation of the electrons from the ground state to a higher energy state. UV-visible wavelength spectrum, most of the molecules absorb

light. The UV frequency is between 100 and 400 nm, and the visible spectrum is between 400 and 700 nm.

UV spectroscopy of the plant extract Leaf of (JEA & JEEA) and Bark of (JEA & JEEA) was discussed for it is characteristic peak for different phytochemicals present in the extract.

ANTIMICROBIAL STUDIES

Antimicrobial activity can be defined as a collective term for all active principles (agents) that inhibit the growth of bacteria, prevent the formation of microbial colonies, and may destroy microorganisms.

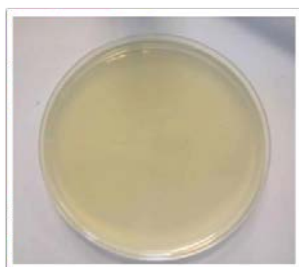
Antimicrobial activity of different natural substances such as medicinal plant extract have been investigated against food borne bacteria.

ANTI-BACTERIAL ACTIVITY

The effect of various plant extracts on the several bacterial strains were assayed by Agar well diffusion method and further confirmed by Disc diffusion method. The minimum concentrations of the plant extracts to inhibit the microorganisms were also determined by micro dilution method using plant fraction serially diluted in sterile nutrient broth.

PREPARATION OF NUTRIENT AGAR MEDIUM

The medium was prepared by dissolving 28g of the commercially available Nutrient Agar Medium in 1000ml of distilled water. The dissolved medium was autoclaved at 15lbs pressure at 121°C for 20 minutes. The autoclaved medium was mixed well and passed onto 100mm petriplates while still molten. Tetracycline were used as standard antibacterial drug.



AGAR WELL DIFFUSION METHOD PRINCIPAL

The anti-microbial present in the plant extract are allowed to diffuse out into the medium and interact in a plant freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeter.

STAPHYLOCCUS AUREUS:

Staphylococci are Gram-positive bacteria, with diameters of 0.5- 1.5 µm and characterized by individual cocci, which

divide in more than one plan to form grape-like clusters. There are 33 species and eight sub-species in the genus. Staphylococcus, many which preferentially colonize the human body. The staphylococci are non-motile, sporeforming facultative anaerobes that grow by aerobic respiration or by fermentation. Most species have a relative complex nutritional requirement, however, in general they require an organic source of nitrogen, supplied by 5 to 12 essential amino acids, e.g. arginine, valine and B vitamins, including thiamine and nicotinamide.

BACILLUS SUBTILIS:

Bacillus Subtilis of a genus of rod-shaped, gram-positive, aerobic or anaerobic bacteria widely found in soil and water. One that could be killed upon exposure to heat and one that was resistant to heat. He called the heat-resistant forms “spores” (endospores) and discovered that these dormant forms could be converted to a vegetative or actively growing state.

PSEUDOMONAS AERUGINOSA:

Pseudomonas aeruginosa is a Gram-negative, aerobic rod shaped bacterium, which belongs to the bacterial family pseudomonadaceae, a member of proteobacteria – aeruginosa is one of the

subtypes among a group of 12 members. The pathogen also can form biofilm and is responsible for 10-20% of infection in hospitals. *Pseudomonas aeruginosa* is one of the most clinically and epidemiologically important bacteria. It is the main cause of nosocomial infection among non-fermenting, Gram-negative bacilli and the major cause of opportunistic infection in immune compromised patients.

KLEBSIELLA PNEUMONIAE

Klebsiella pneumoniae is a gram-negative, encapsulated, non-motile bacterium found in the environment and has been associated with pneumonia in patient populations with alcohol use disorder or diabetes mellitus. The bacterium typically colonizes human mucosal surfaces of the oropharynx and gastrointestinal (GI) tract. Once the bacterium enters the body, it can display

high degrees of virulence and antibiotic resistance.

RESULT AND DISCUSSION

The Leaf and Bark of *Juniperus Erecta* are taken separately in a conical flask. The water and ethyl acetate is used as a solvent and the extract was prepared. The phytochemical screening of the aqueous extract and ethyl acetate extract were conducted and following results were observed.

On comparing the water extract of leaf and bark phenol, saponins, lactones are present. Alkaloids, tannins, proteins, carbohydrates, anthocyanins are absent in both leaf and bark extract.

On comparing the ethyl acetate extract of leaf and bark phenol, saponins, lactones are present.

Table :-1 PHYTOCHEMICAL SCREENING OF WATER EXTRACT

S.NO	PHYTO CHEMICALS	JUNIPEROUS ERECTA OF WATER EXTRACT	
		LEAF	BARK
1	Alkaloids	-	-
2	Flavonoids	+	-
3	Phenol	+	+
4	Tannins	-	-
5	Saponins	+	+

6	Protein	-	-
7	Carbohydrates	-	-
8	Terpenoids	+	-
9	Quinones	-	+
10	Anthocyanins	-	-
11	Coumains	+	-
12	lactones	+	+

Table-2 Phytochemical Screening of Ethyl Acetate Extract

S. NO	PHYTO CHEMICALS	JUNIPEROUS ERECTA OF ETHYL ACETATE EXTRACT	
1.	Alkaloids	-	-
2.	Flavonoids	-	-
3.	Phenol	+	+
4.	Tannins	-	-
5.	Saponins	+	+
6.	Protein	-	-
7.	Carbohydrates	-	-
8.	Terpenoids	+	-
9.	Quinones	-	+
10.	Anthocyanins	-	-
11.	Coumains	+	+
12.	lactones	+	+

UV SPECTROSCOPY ANALYSIS

UV Spectrum of Juniperous Erecta of Leaf and Bark extract shows the presence of possible wavelength of the compounds like Cytidine, Adenosine, Pyrimidine, carbonyl, Aldehyde.

On comparing the water extract of Leaf 271nm indicates the presence of Cytidine and the Bark 267nm indicates the presence of Adenosine, 343nm indicates the presence of Pyrimidine.

TABLE-3 UV SPECTRUM OF JUNIPEROUS ERECTA ETHYL ACETATE EXTRACT

S.NO	JUNIPEROUS ERECTA OF WATER		CLASS OF COMPOUNDS
	LEAF	BARK	
1.	271	-	Cytidine
2.		267	Adenosine
3.		343	Pyrimidine

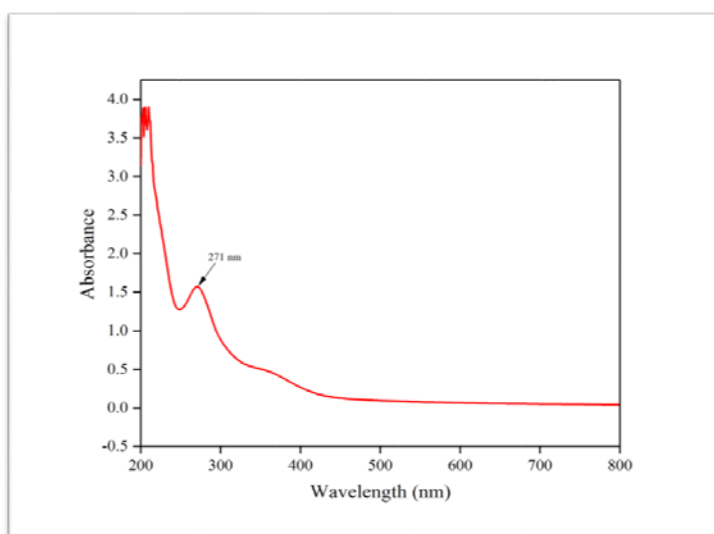


Figure 5.1 UV Spectrum of Juniperous Erecta of leaf extract

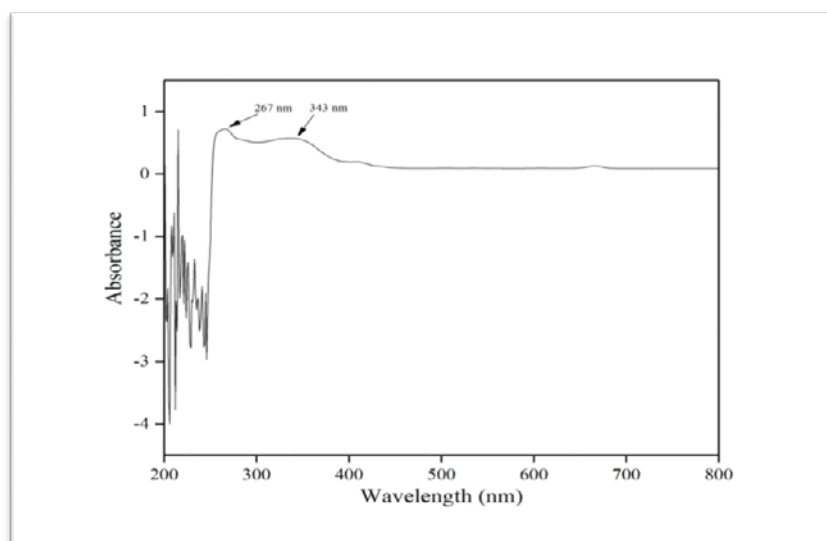


Figure 5.2 UV Spectrum of Juniperous Erecta of Bark extract

TABLE -4 UV SPECTRUM OF JUNIPEROUS ERECTA AQUOUS EXTRACT

S.NO	JUNIPEROUS ERECTA OF WATER		CLASS OF COMPOUNDS
	LEAF	BARK	
1	-		
2		279	Carbonyl, Aldehyde

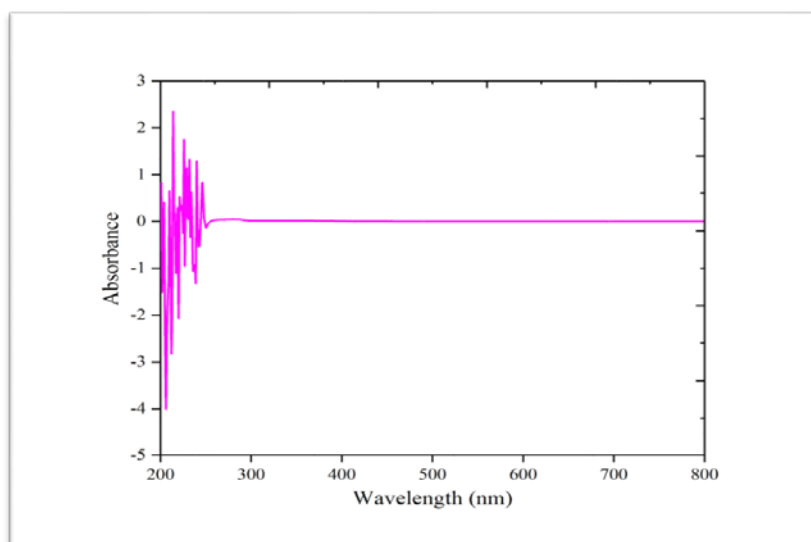


Figure 5.3 UV Spectrum of Juniperous Erecta of Leaf extract

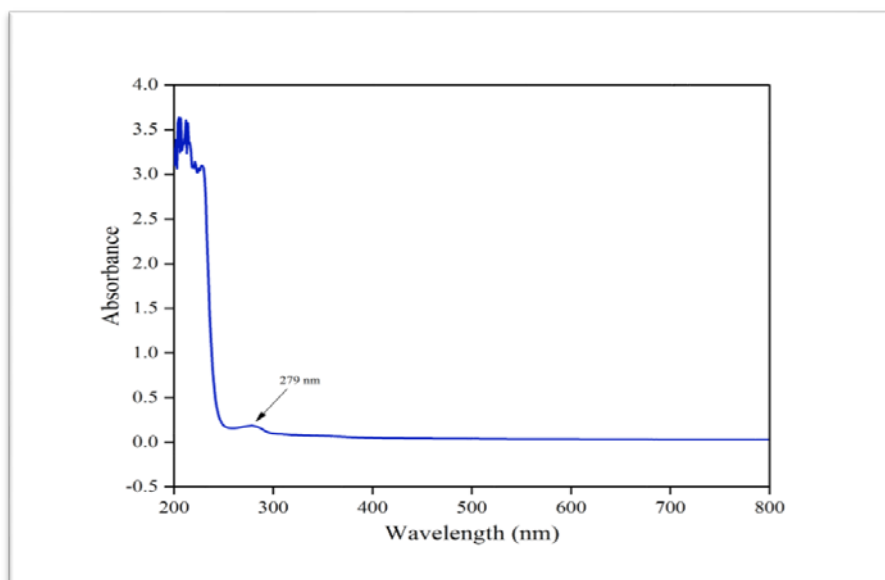


Figure 5.4 UV Spectrum of Juniperous Erecta of Bark extract

ANTIMICROBIAL ACTIVITIES:

ANTIBACTERIAL ACTIVITIES:

Antimicrobial activity of the given extract of Leaf and Bark of JUNIPEROUS ERECTA was done by using well deficient method by using tetracycline as standard and the samples were diluted. By using nutrient agar as medium, well deficient method was carried and their inhibition was studied against both gram- positive bacteria-*Bacillus Subtilis* and *Staphylococcus aureus*, gram-negative bacteria-*Klebsiella pneumonia* and

Pseudomonas aeruginosa on comparing these two gram-positive and gram-negative bacteria there is zone of inhibition not present in the plant extract are shown in the figure 5.9. Hence the compound JuniperousErecta can be studied further for the application of antibacterial activity since it is very effective when compare with the standard against gram-negative bacteria.

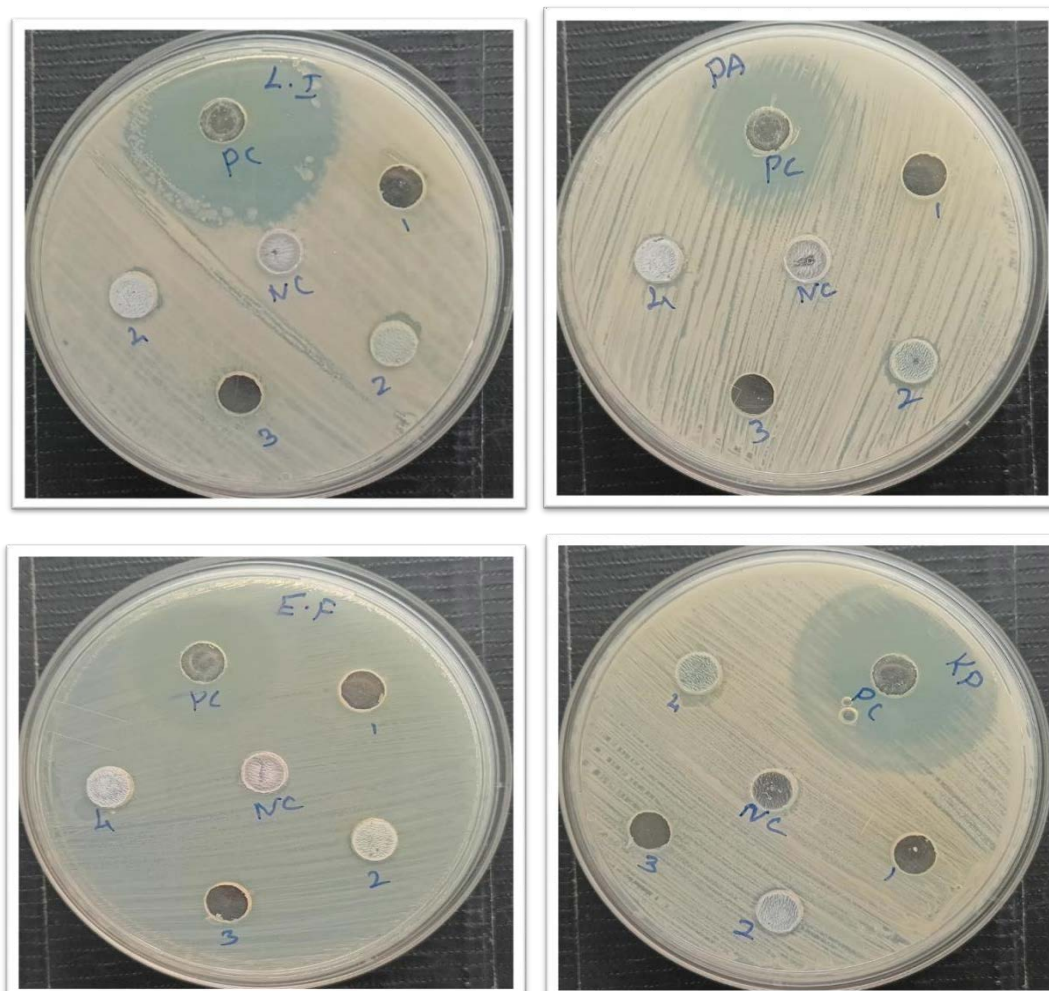


Figure 5.5 Antibacterial Activity of gram-positive *Bacillus subtilis* *Staphylococcus aureus* and gram- negative *Klebsiellapneumoniae* *Pseudomonas aeruginosa*

CONCLUSION

- The leaf and bark of JUNIPEROUS ERECTA are phytochemically

screened for aqueous and ethyl acetate and presence of various phytoconstitutes were observed.

- The extract were characterized for Ultraviolet–visible spectroscopy and found that wavelength of our compounds are present.
- The extract were characterized for Anti-microbial and found that no zone of inhibition.

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