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The Antibacterial Activity of Various Solvent Extracts of Leaves of *Raphunus sativus*

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ABSTRACT

Raphinus sativus extract was prepared by Soxhlet extraction method using various solvents such as ethyl acetate (polar solvent) water and ethanol (nonpolar). The chemical test was performed to identify the presence of flavonoid content. Among them ethyl acetate extract shows a high flavonoid content.So it was used for further studies. By using the in vitro agar well diffusion method, antibacterial activity was measured in all extracts. 2 strains of bacteria were chosen for testing, including gram-positive (Staphylococcus aureus, ATCC25923) and gram-negative (E. coli, ATCC25922) bacteria. The diameter of the zone of inhibition were measured and compared to the amoxicillin reference standard.Compared to gram-positive bacteria, the extract exhibits higher potential activity against gram-negative bacteria. Concentration directly relates to how much action is taken.

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INTRODUCTION

The recognition of the importance of medical plants as potential sources of leaf compounds in the drug discovery process has led to an increase in the use of medicinal plants, which are the foundation of traditional medicine in recent decades. Bioactive compounds from medicinal plants are requires as the basis for more pharmacological research, as well as microbial resistance, which is a global issue. In this research, we proved leaves of Raphinus sativus could be therapeutic importance in the treatment of bacterial infections [1].

Raphinus sativus var. longipinnatusis belonging to the family Brassicaceae (Cruciferae) is a mild-flavoured winter radish usually characterized by a long, white, and nap form root. They are also known as Daikon (Japanese for 'big root') or Mooli in HINDHI [2].

Leaves are arranged in a rosette. Radishes are fast-growing leaves, and annual, cool-season crops. It acts as an appetizer and has laxative effects on the intestine. It is also used to treat liver disease and poor digestion. It also have antioxidant, anti-tumorigenic, anti-diabetic, and anti-proliferative properties [3].

Leaf was collected at Madurai local market then it was authenticated by Dr. Stephen at American College. Then phytocompounds were extracted by soxhlet apparatus. An antibacterial study was conducted [4].

MATERIALS AND METHODS

Preparation of Raphinus sativus extract

The leaves of Raphinus sativus were prepared from local market at Madurai. Then it was soaked in each solvent (polar,non-polar and neutral) for 10 hours. Then it was placed in a Soxhlet apparatus, percolation was performed continuously for 24 hrs...

Then the extract was dried by distillation followed by rotator flash evaporator. The extract residue was stored in cold storage equipment [5–7].

Evaluation of extract

Diluted HCl was added to the extract, shaken, and filtered. Then each of solvent extracts were undergoes various chemical tests and it shows the ethyl acetate extract has a higher concentration of flavonoids which is responsible for anti-bacterial activity. So, only ethyl acetate extract was used for further activities Chemical test data were shown in Table 1 [8, 9].

Tests for alkaloids

Wagner's test: To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicates the presence of Alkaloids.

Tests for Carbohydrates

Molish's test

2 ml of aqueous extract was treated with 2 drops of alcoholic a-naphthol solution in a test tube and then 1 ml of concentrated sulfuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

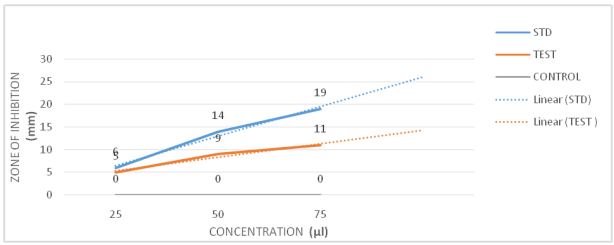

Figure 1: Antibacterial Activity of ethyl acetate extract of leaves from *Raphinus sativus* against *Staphylococcus aureus*

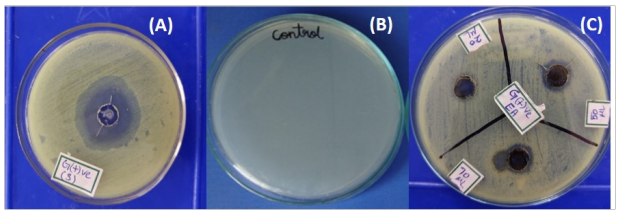

Figure 2: Antibacterial Activity of ethyl acetate extract of leaves from *Raphinus sativus* against *Staphylococcus aureus* (A) STD (B) CONTROL (C) TEST

Tests for flavonoids

Lead acetate test

The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

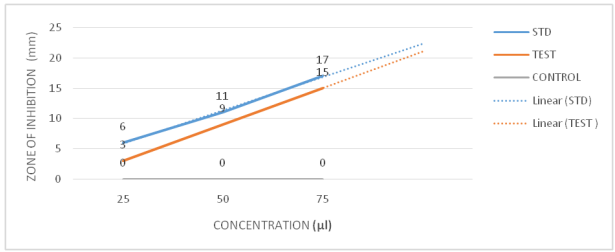

Figure 3: Antibacterial Activity of ethyl acetate extract of leaves from *Raphinus sativus*

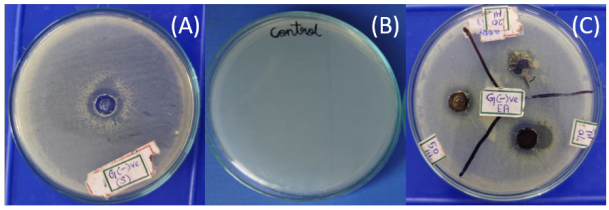

Figure 4: Antibacterial Activity of ethyl acetate extract of leaves from *Raphinus sativus* against *E. coli* (A) STD (B) CONTROL (C) TEST

Sinoda's test

A few magnesium turnings and 5 drops of concentrated hydrochloride acid was added drop wise to 1 ml of the extract solution. A pink, scarlet, crimson red occasionally green colour appeared after few minutes. Confirm the presence of flavonoids.

Tests for protein and amino acids

Ninhydrin test

3 ml of the test solution was heated with 3 drops of 5% ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino Acids.

Tests for tannin and phenolic compounds

Ferric chloride test

Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet colour indicates Presence of phenolic compounds.

Test for reducing sugar

Fehling's test

To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

Preparation of culture media

2.8g of nutrient agar was dissolved in 100ml of dissolved water then it was autoclaved at 15lbs pressure and 121c temperature for 15 min. The

Table 1: Chemical test

Test for alkaloids	Test for carbohydrates	Test for flavonoids		Amino acid and protein test	Tannin and phenol test	Test for reducing sugar
Wagner's test	Molisch's test	Sinoda's test	Lead acetate test	Ninhydrin test	Ferric chloride test	Fehling's test
+	-	+	+	+	+	+
+	+	+	+	+	+	-
-	-	-	-	-	-	-

+ Compound presents, - Compounds absents

Table 2: Antibacterial Activity of ethyl acetate extract of leaves from *Raphinus sativus* against *Staphylococcus aureus*

Method	Microorganism	Compound Code	Concentration (μl)	Zone of Inhibition (mm)
CUP PLATE METHOD	Staphylococcus aureus [Gram-positive]	STANDARD (A)	50	15
		NEGATIVE CONTROL(B)	50	00
			25	05
		TEST SAMPLE (C)	50	09
			75	11

Table 3: Antibacterial Activity of ethyl acetate extract of leaves from *Raphinus sativus* against *E. coli*

Method	Microorganism	Compound Code	Concentration (μl)	Zone of Inhibition (mm)
CUP PLATE METHOD	E. coli [Gram-negative]	STANDARD (A)	50	11
		NEGATIVE CONTROL(B)	50	00
			25	03
		TEST SAMPLE (C)	50	09
			75	14

media was transferred to Petri dish in an aseptic room. Petridish was incubated overnight at room temperature. For the inoculation, a total of 2, one in each strains of gram positive (Staphylococcus aureus, ATCC25923) and gram negative (E. coli, ATCC25922) bacteria were chosen [10].

Evaluation of Antibacterial Activity

By using the Cup plate method (also known as the agar well method), the extracts' in-vitro antibacterial activity was assessed. On Petri dish plates, 0.1ml of each of the test inoculums was equally disseminated using a sterile glass spreader. In the inoculated media, wells were drilled using sterile 6 mm cork borer. 25, 50, 75 μl of the extracts (re-dissolved in the appropriate solvents) and 1:1 negative controls (solvent: water) were added to the wells. For 24 hours, the inoculation plates were incubated at 37^0C. A clear zone surrounding the well on the plates served as a visual cue that bacterial growth had been

inhibited. Anti Bacterial activity was quantified in terms of the average diameter of the zone of inhibition, and the size of the zone of inhibition was assessed [11, 12].

RESULTS AND DISCUSSION

The collected leaves were authenticated and phytocompounds were extracted by Soxhlet apparatus using various solvents such as ethyl acetate, water and ethanol. The chemical test were performed for above extracts, among them ethyl acetate extracts shows good results. It was shown in Table 1. That is have high flavonoid content. So it was used for the following studies.

By using the cup plate method, the isolated compounds were tested for their antibacterial activity against Staphylococcus aureus [Gram-positive]. The results are shown in Table 2. Amoxicillin, the standard compound showed a zone of inhibition of 18 mm at 25 microliter concentration whereas as compounds at various concentration showed less antibacterial activity. Among all the concentrations tested, 25(μl) showed less antibacterial activity. Concentration 75(μl) shows high degree of action among the series against Staphylococcus aureus. The results was shown in Figure 2 and it is shown as a graphical representation in Figure 1.

In gram-negative bacteria, the same procedure was performed. Standard shows a zone of inhibition at radius of 11mm. whereas test compound at 50 microlitre shows 9 mm zone of inhibition which is less than that of STD. But as concentration increases test compound shows more activity than that of STD at the same concentration. The results were shown in Table 3 and it was also shown in graphical representation in Figure 3. The picture were shown in Figure 4.

CONCLUSION

Then leaves were extracted using water, ethanol and ethyl acetate as solvent. Chemical test was performed for all, among them ethyl acetate shows more flavonoids concentration. So ethyl acetate extract was used for further studies. Antibacterial studies were performed using agar plate method using E.coli and Staphylococcus aureus as test organisms. For gram-positive bacteria were shows less activity than standard. But for gram-negative bacteria, at higher concentration it shows similar potential to that of standard.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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