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Evaluation of phytochemicals and *in vitro* antimicrobial activity of aqueous and ethanolic extract from seeds of *Ricinus communis* Linn

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Abstract

The current study has been carried out to evaluate phytochemical components from aqueous and ethanolic extract of seeds of *Ricinus communis* and also deals with its *in vitro* antimicrobial activity. The aqueous extract showed presence of steroids, tannin, saponin, alkaloids, phytosterol and cardiac glycosides while ethanolic extract showed presence of diterpenes, steroids, flavonoids, tannin, coumarin, phenols, alkaloids and saponin. The *in vitro* antimicrobial activity of aqueous and ethanolic extract of seeds of *Ricinus communis* were tested against *Bacillus subtilis*, *Salmonella typhimurium*, *Pseudomonas auruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Aspergillus niger* and *Penicillium chrysogenum* by agar well diffusion method. The aqueous extract of the sample showed significant range of inhibitory effect against *Salmonella typhimurium* and *Pseudomonas auruginosa*, however the ethanolic extract is more effective and showed inhibitory effect against all the bacteria. The GCMS data revealed that novel bioactive components are present in both the extract from seeds of *Ricinus communis*. The aqueous and ethanolic extract doesn't showed antifungal activity against *Aspergillus niger* and *Penicillium chrysogenum*. Thus, these results confirms the presence of active antibacterial compounds in seeds of *Ricinus communis*, might be used as a source of antibiotics.

Keywords: *Ricinus communis* Linn, Phytochemical screening, *in vitro* Antimicrobial activity

1. Introduction

The medicinal plants are an enormous source of valuable antimicrobial components, useful as an alternative to synthetic ones; used to develop various drugs. *Ricinus communis* L. is a small soft woody tree, occurs in tropics and warm temperature regions of the world,^[1] comes under the fourth largest family of the angiosperms Euphorbiaceae. Ricin from *Ricinus communis* is a well known poisonous component which elicits violent purgative action in human being^[2]. The seed of *Ricinus communis* contains ricin, a toxic water soluble protein which also present in all parts of the plant in lower concentrations.

The studies on phytochemical analysis and antimicrobial activity of *Ricinus communis* has been carried out by various workers^{[3];[4];[5];[6]}, since present study deals with estimation of wide range phytochemicals and antimicrobial activity against unstudied microorganisms.

Materials & Methods:

Collection of Plant material:

Fresh and healthy seeds of *Ricinus communis* Linn were collected from the farmer's fields at Gajargaon of Kolhapur district, Maharashtra, India during November 2014. Seeds were washed with distilled water and dried in sunlight for three days. Then the dried seeds were grinded with ordinary grinder.

Identification

Ricinus communis Linn and its seeds were authenticated by Mr. R. S. Sawant, Associate Professor, Department of Botany, Dr. Ghali College, Gadhinglaj, Kolhapur district, Maharashtra, India.

Preparation of Test extract

The aqueous and ethanolic extract of *Ricinus communis* seeds were prepared by addition of 0.5 gm and 1.0 gm of powder into 10 ml of respective solvents to obtain the concentration 5 % and 10 % and kept at room temperature (25°C) for overnight. Samples were further used after centrifugation.

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Phytochemical analysis

Phytochemical analysis of *Ricinus communis* seeds were carried out for the aqueous and ethanolic extracts to evaluate the presence of secondary metabolites like steroids, saponins, phytosterols, phenolic compound, tannins, flavonoids, etc. "Table 1" by using various standard methods [7];[8];[9] with slight modifications.

Test for Steroids

1 ml test extract was added in 10 ml of chloroform and equal quantity of conc. H₂SO₄ acid was added in test tube from side. The upper layer turns red in colour while H₂SO₄ layer shows yellow colour with green fluorescence, which indicate the presence of steroids.

Test for Diterpenes: Copper acetate test

Test extract was dissolved in distilled water and treated with 8-10 drops of copper acetate solution; the formation of emerald green colour indicates presence of the diterpenes.

Test for Tannin:-

Lead acetate test: 2ml extract was added to 1% lead acetate and observed for yellowish precipitate which indicates the presence of tannin.

FeCl₃ test: 4ml extract was treated with 4 ml of FeCl₃. The formation of green colour indicates that presence of condensed tannin.

Test for Cardial Glycosides: Keller-Killani Test

2 ml glacial acetic acid containing a drop of FeCl₃ used to treat the extract. Formation of brown colour ring indicates the presence of cardial glycosides.

Test for Flavonoid:-

Alkaline reagent test: Test extract was treated with 10 % NaOH solution; formation of the intense yellow colour indicates presence of flavonoid.

NH₂OH test: 10 % NH₂OH was used to treat the 3 ml extract, development of yellow fluorescence indicated the positive test.

Mg turning test: Test extract was treated with Mg followed by few drops of conc. HCl, finally 5 ml of 95 % ethanol was added. Formation of the crimson red colour indicates presence of flavonoid.

Zinc dust test: 2 ml test extract were treated with Zn dust and few drops of conc. HCl, the development of red colour taken as an evidence for flavonoid.

Test for Anthocyanin

2 ml of aqueous test extract was added to 2 ml of 2 N HCl and NH₃, the appearance of pink red colour turns to blue violet which indicates positive test for anthocyanin.

Test for Phytosterol: Salkowskis test

Test extract was treated with chloroform and filtered. Then the filtrate was treated with few drops of conc. H₂SO₄, well shaken, allowed for stand, the appearance of golden red colour indicates positive test.

Test for Saponin: Foam test

5 ml test extract was mixed with 20 ml of distilled water and agitated in graduated cylinder for 15 minutes. The formation of foam indicates presence of saponin.

Test for Phenol: Ferric Chloride test

Test extract was treated with 4-5 drops of Alcoholic FeCl₃ solution. Formation of the bluish black colour indicates positive test for presence of Phenol.

Test for Emodins

2 ml of NH₄OH and 3 ml of benzene was added to the extract. The appearance of red colour indicates emodins.

Test for Coumarin

3 ml of 10% NaOH was added to 2 ml of aqueous test extract, the formation of yellow colour indicates the positive test.

Test for Leucoanthocyanin

In 5 ml of aqueous extract, 5 ml of isoamyl alcohol was added. The upper layer appear red in colour which indicates the presence of Leucoanthocyanin.

Test for Phlobatannins

Aqueous extract of sample is boiled with 1% aqueous HCl, the deposition of red ppt indicates the presence of Phlobatannins.

Test for Alkaloids: Wagner's reagent test

Filtrate (1 ml HCl was added into a test tube containing 3 ml of conc. test extract. The mixture was heated gently for 20 minutes and filtered after it gets cooled) was treated with Wagner's reagent, formation of reddish precipitate shows presence of alkaloids.

Test.organisms

The following standard test microorganisms were used for this study: *Bacillus subtilis* NCIM 2635, *Salmonella typhimurium* NCIM 2501, *Pseudomonas auruginosa* NCIM 5032, *Staphylococcus aureus* NCIM 2654, *Proteus vulgaris* NCIM 2813, *Aspergillus niger* (Isolated) and *Penicillium chrysogenum* (Isolated).

Antimicrobial activity

In vitro testing of extracts for antimicrobial activity of the aqueous and ethanolic extract of seeds of *Ricinus communis* against various microorganisms "Table 2" was determined by using agar well diffusion method, using Nutrient agar medium for antibacterial activity while Potato dextrose agar (PDA) for antifungal activity.

Results and Discussion

Phytochemical analysis of aqueous and ethanolic extracts of seeds of *Ricinus communis* was performed and the results were presented in Table 1. The results of chemical test with aqueous extract contains phytochemicals like steroids, tannin, alkaloids, phytosterol, cardial glycosides and saponin while ethanolic extract shown presence of steroids, tannin, saponin, coumarin, alkaloids, flavonoids, diterpenes and phenols. According to Harborne and Willians [10], flavonoids shows anti-inflammatory, antioxidant, antimicrobial, vascular activities along with other medicinal properties. Several reports on the antimicrobial activity of flavonoids are available in the literature [11];[12];[13]. As per Harborne [14] tannin may be toxic to bacteria, yeast and filamentous fungi. It also shown potential

antiviral^[15] as well as antibacterial activity^{[16];[17]}. The ethanolic extract found rich in steroids and tannin in comparison with aqueous extract.

The data Table 2 revealed that aqueous extract of seeds of *Ricinus communis* was effective against only *Salmonella typhimurium* and *Pseudomonas aeruginosa*. More specifically 50 µl of 10 % aqueous extract (Fig 3) found effective against *Salmonella typhimurium* while, 100 µl of 5 % concentration inhibit the growth of *Pseudomonas aeruginosa* along with *Salmonella typhimurium* (Fig 2). 50 µl of 5 % concentration was found effective against *Proteus vulgaris* (Fig 1). The antimicrobial assay implies that only 5 % of ethanolic extract can inhibit the growth of *Proteus vulgaris* (Fig 1 and 2). 10 %

of 100 µl aqueous extract from *Ricinus communis* seeds was found effective against *Salmonella typhimurium* while the ethanolic extract shown significant zone of inhibition against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhimurium* (Fig 4). Although *Ricinus communis* have the water soluble protein Ricin, which act as poison, the ethanolic extract were found most effective. The isolated fungal cultures were shown resistance against all the extracts. The GCMS data shows presence of novel compounds in both the samples. The results obtained may support the use of *Ricinus communis* seeds in traditional medicine for the treatment of various diseases.

Table 1: Phytochemical analysis of aqueous and ethanolic extract from *Ricinus communis* seeds

Sr. No.	Phytochemical	Results	
		Aqueous extract	Ethanolic extract
1	Steroids	+	++
2	Diterpenes: Copper acetate test	-	+++
3	Tannin:	Lead acetate test	+
		FeCl ₃	++
4	Cardial Glycosides: Keller-Killani test	+	-
5	Flavonoid:	Alkaline Reagent Test	-
		NH ₄ OH	+
		Mg turning test	-
		Zn dust test	+
6	Anthocyanin	-	-
7	Phytosterol: Salkowski's test	+	-
8	Saponin: Foam test	+++	+
9	Phenols: FeCl ₃ test	-	+
10	Emodins	-	-
11	Coumarin	-	+
12	Leucoanthocyanin	-	-
13	Phlobatannins	-	-
14	Alkaloids (Wagner's reagent)	+	+

Key: (+) Positive test, (-) Negative test, '+' low; '++' moderate; '+++' high

Table 2: Antimicrobial activity of aqueous and ethanolic extract of *Ricinus communis* seeds

Organism used	Zone of inhibition (mm)	Zone of inhibition (mm)							
		Aqueous extract				Ethanolic extract			
		5%		10%		5%		10%	
		50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl
<i>Bacillus subtilis</i> NCIM 2635	-	-	-	-	11.6±1.5	15.6±1.5	12.3±1.5	15.0±1.0	
<i>Salmonella typhimurium</i> NCIM 2501	10.6±0.5	12.0±1.0	15.0±1.0	15.0±1.0	-	13.0±1.0	-	12.0±1.0	
<i>Pseudomonas aeruginosa</i> NCIM 5032	-	12.3±1.1	-	-	13.3±0.5	16.0±1.0	10.6±0.5	15.6±0.5	
<i>Staphylococcus aureus</i> NCIM 2654	-	-	-	-	-	12.6±0.5	10.3±0.5	16.3±0.5	
<i>Proteus vulgaris</i> NCIM 2813	-	-	-	-	14.0±1.0	14.0±1.0	-	-	
<i>Aspergillus niger</i> (Isolated)	-	-	-	-	-	-	-	-	
<i>Penicillium chrysogenum</i> (Isolated)	-	-	-	-	-	-	-	-	

Note: Each value is the mean of three readings ± SD.

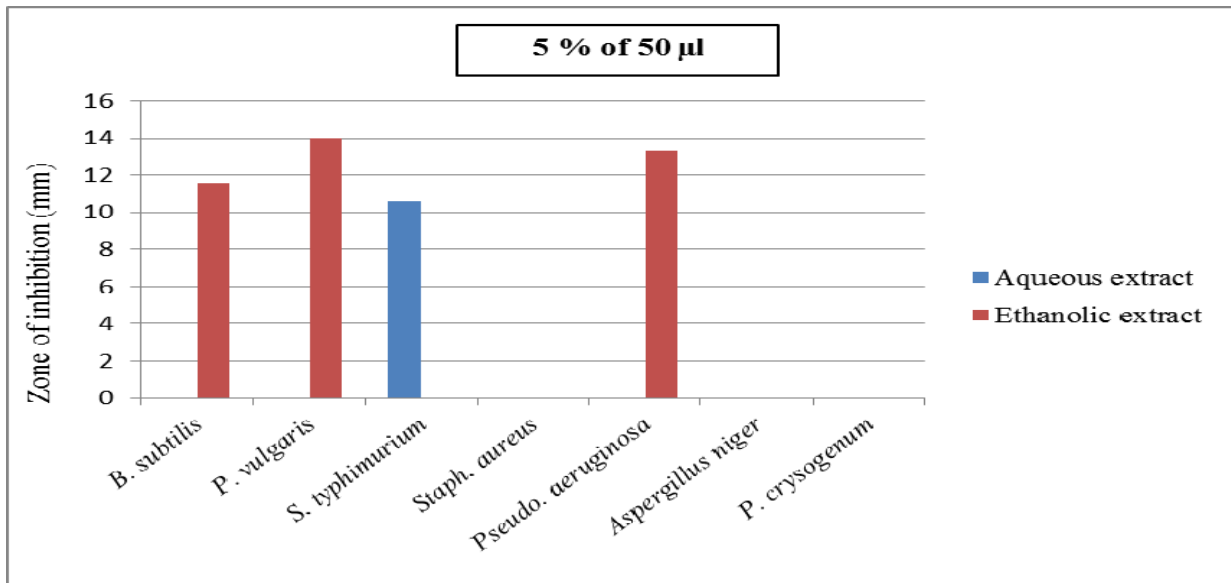


Fig 1: Effect of 5% (50 µl) *Ricinus communis* seed extracts against test organisms

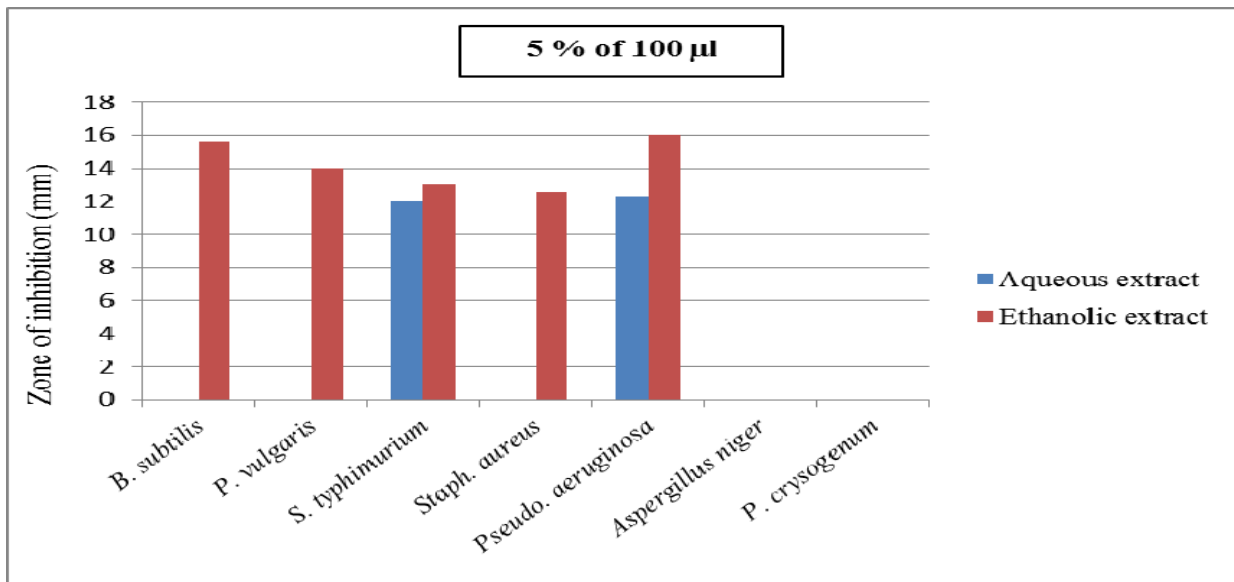


Fig 2: Effect of 5% (100 µl) *Ricinus communis* seed extracts against test organisms

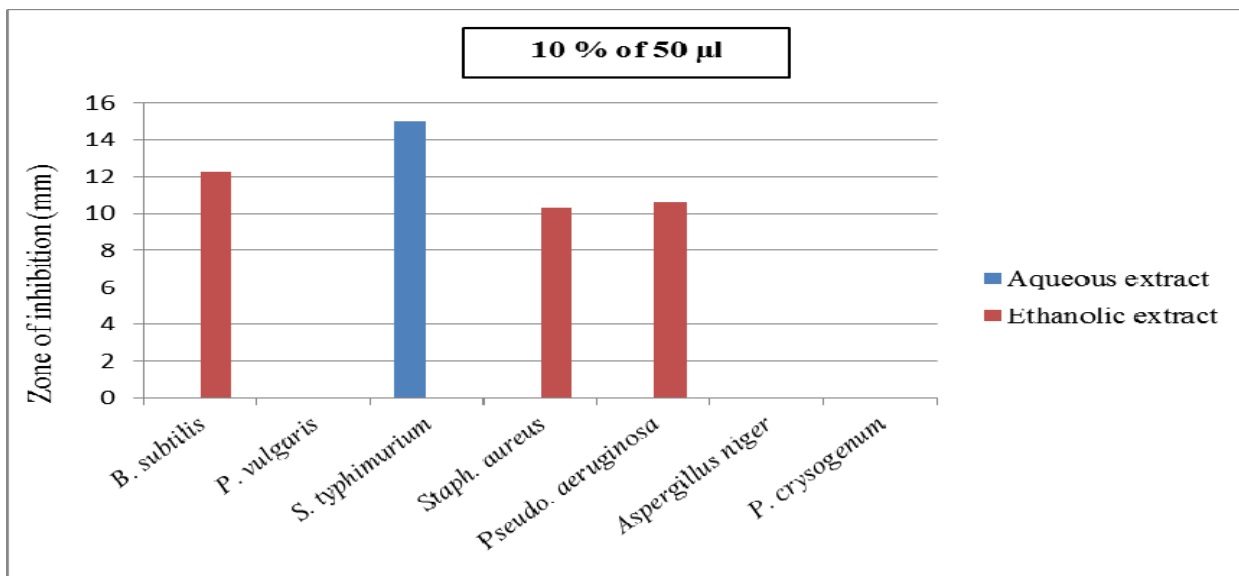


Fig 3: Effect of 10% (50 µl) *Ricinus communis* seed extracts against test organisms

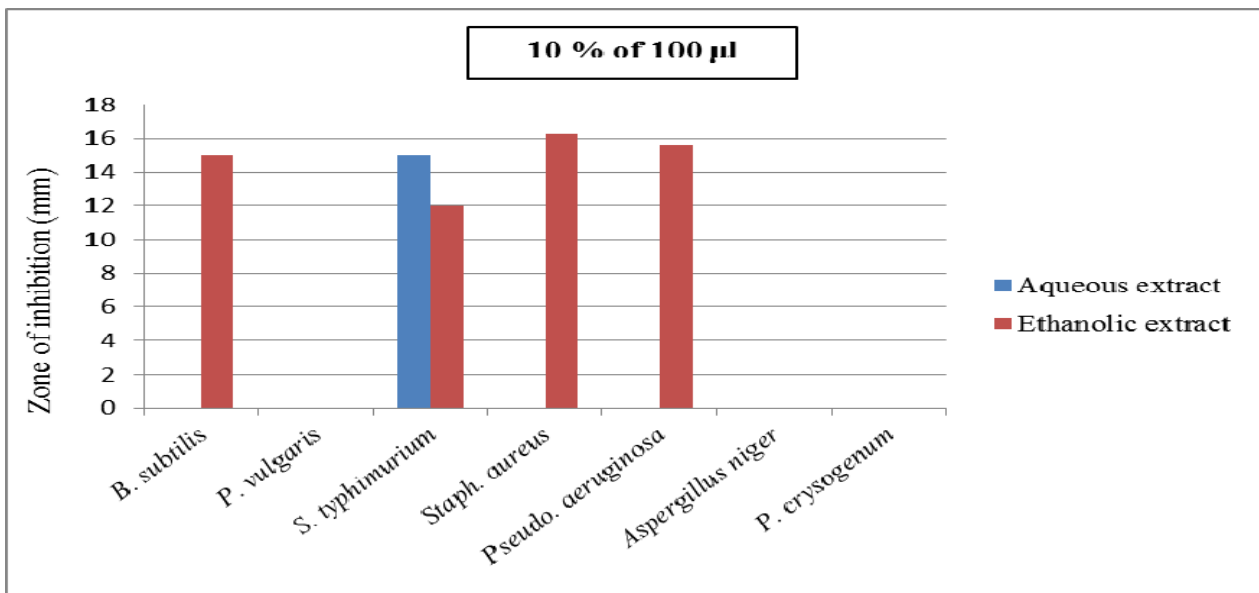


Fig 4: Effect of 10% (100 µl) *Ricinus communis* seed extracts against test organisms

Conclusion

Present study concludes that ethanolic extract of seeds of *Ricinus communis* were rich source of various phytochemicals. Both the extracts were shown antibacterial properties but comparatively the ethanolic extract of *Ricinus communis* seeds was rich in phytochemicals by the means of quantity and shown higher inhibition zone. Qualitatively ethanol extracts yields more phytochemicals than aqueous extract. The seed extract of *Ricinus communis* from both the solvent system may be used as a source of antibiotics, more specifically ethanol extract is efficient.

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