

Invitro Phytochemical Investigation, Chracterization & Antimicrobial Study Of Latex Of Euphorbia tirucalli Linn.

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Abstract: The present work deals with phytochemical investigation of latex of Euphorbia tirucalli linn. The aqueous extract by maceration and the latex of plant extracted with ethanol and chloroform by successive solvent treatment. Characterization of latex is performed by thin layer chromatography. Antimicrobial activity of all extract where screened by paper disk method against *Staphylococcus aureus*, *Bacillus subtilus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Candida albicans*.

Keywords: Antimicrobial activity, Latex of Euphorbia tirucalli linn.

Introduction:

Euphorbia tirucalli is commonly known as dugdhika & is grown in India. It belongs to family Euphorbiaceae¹. The plant is native of America but has become acclimatized & grows freshly in all parts of India. Milky juice and bark were used traditionally. The plant is useful in affection of spleen & acts as purgative in colic & bowel complaints, it is used in whooping cough, asthma, in java the bark is used in applying to fractures.^{1,2,3} Many pharmacological activities of *E. tirucalli* have been documented by many workers as molluscicidal activity⁴, The inhibition of the ascetic tumor in mice has also been reported⁵.

Materials and Method:

Collection and preparation of extracts

Fresh plant of Euphorbia tirucalli was collected from Hatkanangle area & authenticated by Dr. G. G. Potdar, Dept. of Botany, Y.C. College of Science, Karad, Maharashtra.

Extraction of Plant Material:

The plant was washed and was shaded dried to obtain coarse powder. This powder was subjected to different extraction procedures. The aqueous extract was prepared by maceration process⁶, in which the latex was macerated with 10% chloroform water for seven days with occasional shaking. The menstrum obtained after the filtration was evaporated to get thick past. The latex of plant material extracted⁷ with chloroform and methanol. Finally the drug macerated with chloroform water. The thick past of extract was obtained. The solubility of thick paste extract was checked in different solvents.

Preparation and suspension of test bacteria:

18 to 24 hours old culture of broth gram positive and gram negative growing bacteria were used for Preparation of suspension of test bacteria in sterile normal saline. The gram positive bacteria used were *Staphylococcus aureus*, *Bacillus subtilis*, the gram negative bacteria were used *Pseudomonas aeruginosa*, *Proteus vulgaris*, and the fungi *Candida albicans* and the turbidity of the suspension was adjusted to the turbidity of solution of the McFarland standard⁹.

Detection of Antimicrobial activity:

During this work, tryptone Soya Moll (himedia) was used for testing Antimicrobial activity⁸. The Antimicrobial activity was evaluated by "Paper disk plate method" in which sterile paper of 4 mm diameter punched by punching machine. Plates were previously inoculated with the suspension of the test microorganism. Small paper disk impregnated with 10 mg /10ul of each extract were placed upon the surface of an inoculated plate. Along with extract, paper disk impregnated with 10 mg /10ul of ethanol was also placed on the surface of inoculated plate as a negative control. A standard disk containing 25 ug of cotrimazole was also placed on the agar surface as positive control⁹. The plates were then incubated at 37°C. After 24 hrs incubation results were recorded by measuring the zone of inhibition.

Phytochemical investigation by TLC

Phytochemical investigation of latex of plant. TLC is a rapid and economical procedure for the determination of the main active principles of medicinal plants e.g., alkaloids, cardiac glycosides, saponin & most of the steroidal containing compound. TLC is also used for fractionation of the extract obtained by extraction procedure by using different solvent compositions. The latex of plant was analyzed on silica gel layers with the aid of three solvent systems and six spray reagents, each one applied for the identification of active principles according to their polarity. Spots were visualized under short and long wavelength ultraviolet lights and, the plates were sprayed with a specific spray reagent. The extent of the surface of the spot is a measure for the quantity of the material present. The volume of the spots applied on the chromatographic plates was 5µl, corresponding to approximately 300µg for each dry extract¹⁰. Chromatography was performed in the following solvent systems: Nonpolar solvent: toluene-acetone (8:2); semi-polar solvent: toluene-chloroform-acetone (4:2.5:3.5); polar solvent: n-butanol-glacial acetic acid-water (5:1:4). The chromatograms were observed first without chemical treatment, under UV 254 nm and UV 365 nm light, and then using the spray reagents¹¹.

Table no 1: Phytochemical investigation of latex of plant:

Sr no.	Extract	Alkaloids	Tannins	Carbohydrate	Sterols	Glycosides
1	Aqueous extract	-	+	++	-	-
2	Chloroform extract	+	+	+	++	-
3	Ethanollic extract	+	+	++	++	-

Table 2: Antimicrobial Activity of latex of Euphorbia tirucalli linn extract.

Sr no.	Name of microorganism	Aqueous extract	Chloroform extract	Ethanollic extract
1	<i>Pseudomonas aeruginosa</i>	+	-	+
2	<i>Proteus vulgaris</i>	+	-	+
3	<i>S aureus</i>	++	-	+
4	<i>Bacillus subtilis</i>	--	-	++
5	<i>Candida albicans</i>	+	++	+

Table 3: Estimation of Phytochemical constituents by TLC

Sr. No	Phytochemical Constituents	Spray reagents	Light used Observation	Presence in latex of <i>E.tirucalli</i>
1.	Alkaloid	Dragendorff reagent	Visible light Stable Orange color	++
2.	Saponin	Liebermann- Burchard	Visible light Green, redbrown, violet or blackish zone.	+
3.	Ployphenols and Tannins	3% FeCl ₃	Visible light Dark zones	+
4.	Cardiac glycosides	Liebermann-Burchard	Visible light	+
5.	Triterpenes	Liebermann-Burchard	Visible light Red-brown zones	++

Result and discussion:

From the above experiment it was observe that glycosides was not present in latex while alkaloids, carbohydrate & sterol ware present in more concentration where as tannins in low concentration. The phytoconstituents are characterized by using thin layer chromatography. It can be concluded that the aqueous extract showed antimicrobial activity against all test microorganism except *Bacillus subtilus*. The successive ethanolic extract shows antimicrobial activity against all test microorganisms. Chloroform extract shows activity against *Candida albicans*. Hence from this observation it was revealed that aqueous and successive ethanolic extract showed significant anti microbial activity against most of the microorganism. Ethanolic extract shows grater zone of inhibition against as compare to all extracts of *Euphorbia tiruculli* linn.

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