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Screening of Anti Microbial Activity of Manjistha

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Abstract

Quest for Antimicrobial agents from plant sources is a prime area of research in the last decade. Manjistha *(Rubia Cardifolia Linn)* is Popular medicinal plant in Ayurveda which has multiple indications includes Krimighna,Vishaghnaetc Krimighna property is often taken has Antimicrobial property hence present study was conducted to screen the Antimicrobial activity of Manjista Root on selected Organisms viz Staphylococcus Aureus E-Coli, Candid albicans, and Aspergillosisflavus. The study showed significant results in higher zone of inhibition in Alcoholic extract and lower in aqueous extract, and antifungal activity of aqueous extract at 500mcg Showed moderate activity for candid albicans & Aspergillosisflavus.

Key words: Rubiacordifolia; Antimicrobialactivity.

Introduction

Manjisthsa is one among the varnya Mahakashaya[1]Vishaghna dravya[2] and jwarahara Mahakashaya[3] according to caraka where as in sushruta samhita Manjishta is one among priyangwadi [4] pittasamshamana gana[5] Nighantukaras Like *Shaligrama* and *Nighantusangrahakar* are explained. The *Manjistha* is used in *Raktaja*a*Krimi*. And in Ayurvedic classics explained that Manjistha is used in Kusta and other skin diseases [6,7,8].

It is used in various formulations like Mahamanjisthadikada, Manjisthadikwatha, Laghumanjisthadikvatha, Manjisthadilepa, Manjisthadi tail and sinduradi tail etc. [9,10].

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Taxonomic description it is perennial climbers; stem acutely 4-angled with minute, recurved prickles on the angles; leaves in whorls of 3-4, ovate or ovate-oblong to lanceolate, glabrous. Flowers greenish-yellow in cymes; fruit glabrous, 1-seeded, bluish black. Flowering and fruiting – July to December, and part used root, root contains purpurin, Manjistin, garancin, purpuroxanthin, resin, glucose, sucrose, triterpenes, lucidin, anthroquinones fatty acids, gum[11].

The in vitro antibacterial studies of the aqueous and alcohol extracts of the root were carried out by disc agar diffusion method. The extracts were found to be effective against Gram negative (Escherichia coli) pathogens when compared to Gram Positive (Staphylococcus aureuss) pathogen. The phytoconstituents, present in the extracts may be responsible for the antimicrobial activity. The mechanism is yet to be identified.

Materials and Methods

Collection of plant material and extraction

The Roots of *Rubiacordifolia* were collected from Belgaum local market it was sent for Authentication to Botanical survey of India pune. Then the following studies were performed systematically. The roots were collected, dried and coarsely powdered. The powder was subjected to extraction using soxhlet apparatus with ethanol 95% and water separately. The two extracts were concentrated into paste consistency. From each extract test compound 5mg and 10mg was dissolved in 2ml of Dimethyl sulphoxide (DMSO) and stored in airtight containers.

Micro organisms

The following strains of bacteria were used E-Coli (gram –ve)

And Staphylococcus (Gram +ve)

Antibacterial activity

The above mentioned bacterial isolates were grown in nutrient agar at 37° C and reactivated them for further use in nutrient broth. The different extracts of Rubiacordifolia and standard allopathic drugs were tested for antimicrobial activity against the test organism using the agar diffusion Method of (Van. C.J. Kurata H. et.at. (1994) Mueller Hinton agar Media was prepared and the plates were swabbed with 24 hrs cultures of respective bacteria grown in nutrient broth overnight. Sterile discs of 8mm diameter were impregnated with 0. 25ml and 0.5ml of each extract and DMSO separately. DMSO was used negative control and discs of standard drugs as positive control. The plates were then incubated at 37° C for 24hrs. After the incubation period was over, the plates were observed for zone of inhibition and were measured using transparent scale or slide calipers each reading was taken[12,13,14].

Results and Discussion

The inhibitory effect of the alcoholic and aqueous extracts of roots of *Rubiacordifolia,* An Invitro a study were carried out for antibacterial and antifungal effect and is compared with the standard allopathic antibacterial drugs. Like ampicilline, tetracycline, norfloxin gentamycin and the

Table 1: Organoleptic characters of extract (Macroscopic characters extractive)

Sl.No.	Extract.	Aqueous	Alcohol
1	Colour	Reddish brown	Reddish brown
2	Consistency	Semi solid	Semi solid
3	Odour	Pungent odous	Pungent
4	Taste	Bitter, acrid	Bitter acrid

Table 2: Zone of Inhibition in mm, concentration of the drug 0.25/ml

Sl. No.	Organism	I Aqueous ext.	II Alcohol ext.	III DMSO	Ampi-cillin	Tetra-cycline	Norflo-xacin	Genta-mycin
1	E-coli (Gram – ve Bacilli)	4 mm	5 mm	0 mm	18 mm	25 mm	22 mm	20 mm
2	Staphylococcus (Gram +vecocci)	5 mm	4 mm	0 mm	20 mm	21 mm	19 mm	20 mm

Table 3: Zone of Inhibition on (In Millimeters)

Concentration of the drug 0.5 / ml

Sl. No	Organism	I Aqueous ext.	II Alcohol ext.	III DMSO	Ampi-cillin	Tetra-cycline	Norflo-xacin	Genta-mycin
1	E-coli (Gram – ve Bacilli)	18 mm	24 mm	0 mm	17 mm	20 mm	24 mm	19 mm
2	Staphylococcus (Gram +vecocci)	20 mm	22 mm	0 mm	19 mm	22 mm	20 mm	21 mm

Table 4: Active Index of Anti Batcerial study

Sl. No.	**Organism**	**Drug compound conit 0.25ml**	**Standard drugs**			
			Ampi-cillin (18) mm	**Tetra-cycline (25)** mm	**Norflo-xacin (22)** mm	**Genta-mycin (20)** mm
1	E-Coli Grame – ve Bacilli	Aqueous (4) ext.	0.22	0.16	0.181	0.2
		Alcohol (5) ext.	0.27	0.2	0.22	0.25
2	Staphylococcus Grame +vecocci	Aqueous (4) ext.	A (20) 0.25	T (2) 0.19	N . (19) 0.27	G. (20) 0.25
		Alcohol (5) ext.	0.2	0.19	0.27	0.2
Sl. No	**Organism**	**Drug compound conit. 0.5ml**	**Standard drugs**			
			A (17)	**T (20)**	**N (24)**	**G (19)**
1	E-Coli Grame –ve Bacilli	Aqueous (18) ext.	-1.05	0.9	0.75	0.94
		Alcohol (24) ext.	-1.41	-1.2	1.0	-1.26
2	Staphylococcus Grame +vecocci	Aqueous (20) ext.	A (19) -1.05	T (22) 0.90	N (20) 1	G (21) 0.95
		Alcohol (22) ext.	-1.15	1	-1.1	-1.04

Active Index = Zone of inhibition of the test compound

Zone of inhibition of the standard compounds

standard antifungal like grisofelvinOne gram +ve bacteria Staphylococcus and one gram – ve bacteria E-Coli were selected the two extracts. 1) Alcoholic and 2) Aqueous extracts of Manjishta were dissolved in nutral solvent DMSO and two concentration of 0.25ml and 0.5ml of each were prepared and studied for Antibacterial property by disc agar diffusion Method using Mueller Hinton agar.

The study showed higher zone of inhibition in 0.5ml conc. of Alcoholic extract and lower in 0.25ml conc. of aqueous extracts. *E-Coli(Gram –ve)* the study showed higher zone of inhibition in 0.5ml conc. of Alcoholic extracts 24mm, aqueous extracts 18mm, and DMSO 0mm, and standard drugs showed zone of inhibition Ampicillin 17mm, Tetracycline 20mm, Norflxacin 24mm, Gentamycin 19mm.

Table 5: Anti Fungal Screening

Zone of Inhibition in mm

Sl. No.	Compound	Candid Albicans			AspergillosisFlavus		
		500 mg	250 mg	125 mg	500 mg	250 mg	125 mg
1	I Aquenousext	12 mm	R	R	12 mm	12 mm	R
2	II Alcohol ext	18 mm	14 mm	10 mm	14 mm	R	R
3	III D.M.S.	0	0	0	0	0	0

Staphylococcus (Gram +ve)

In the study sowed zone of inhibition in 0.5ml conc. of Alcoholic extract 22mm, aqueous extract 20mm, and DMSO 0mm, and Standard drugs showed zone of inhibition Ampicillin 19mm, Tetracycline 22mm, Norflxacin 20mm, Gentamycin 21mm, Antifungal activity of the 2 extracts of concentrations 125mcg 250mcg and 500mcg were tested on Candid albicans and Aspergillosisflavus. One alcoholic extract at 500mcg conc.was better against both the fungus, by showing 18mm,and 14mm of zone of inhibition respectively. 250mcg and 125mcg conc. of alcoholic extraction were resistant against aspirgillosisflavus.

In aqueous extract 500mcg conc. showed good activity by showing 12mm of zone of inhibition for both fungus. In 250 mcg conc. it was again 12mm for Aspergillosisflavus. Where as for Candida albicans is resistant. But in 125mcg conc. it was resistant to both microorganisms.The aim of the study is not the prevalence of a disease, but it is aimed at evaluation of antimicrobial activity of manjishta roots and hence statistical analysis of data is not considered.

Discussion

Invitro studies were carried out for anti bacterial and antifungal effect and is compared with the standard allopathic, antibacterial drugs like ampicillin, tetracycline, norfloxin gentamycin and the standard antifungal like grisofelvin.

One gram +ve bacteria staphylococcus and one grame – vebacteria.E.coli were selected the two extracts. A) Alcoholic & b) Aqueous extracts of Manjishsta were dissolved in neutral solvent DMSO & 2 concentration of 0.25 ml and 0.5 ml of each were prepared and studied for antibacterial property by disc agar diffusion method using Mueller Hinton agar.

The study showed higher zone of inhibition in 0.5 ml conc of Alcoholic extract and lower in 0.25 ml conc of aqueous extract. Antifungal activity of the 2 extracts of concentrations 125 mcg 250 mcg and 500 mcg were tested on candid albicans and aspergillosisflavus. One alcoholic (Alco) extract at 500 mcg conc was better against both the fungus, by showing 18 mm and 14 mm of zone of inhibition respectively. 250 mcg and 125 mcg conc of alcoholic extraction were resistant against aspirgillosisflavus.In aqueous extract 500 mcg conc showed good activity by showing 12 mm of zone of inhibition for both fungus. In 250 mcg conc it was again 12 mm for aspirgillosisflavus, where as for candida albicans is resistant. But in 125 mcg conc it was resistant to both micro organisms.

The aim of the study is not the prevalence of a disease, but it is aimed at evaluation of antimicrobial activity of Manjishsta roots and hence statistical analysis of data is not considered.

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