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A STUDY OF ANTIMICROBIAL ACTIVITY OF SIDDHA HERBAL DRUG NARUVILI VER CHOORANAM (*CORDIA DICHOTOMA* ROOT)

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ABSTRACT

The current study is to evaluate the antimicrobial activity oh Siddha herbal drug Naruvili ver Chooranam (*Cordia dichotoma*) commonly called as Indian cherry belongs to the family Boraginaceae. Presently there is a great interest on herbal medicine and integrating their use into modern medicine because of their low cost, drug resistance, limits of medicine, medicinal value, availability and cultural exchange. Several plant extracts have both phytochemical and antimicrobial properties, typically resulted from combining secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids. Nowadays drug resistance has become an important issue to modern medicine and the effectiveness of antibiotic therapy. The plant is used as anthelminthic, astringent, diuretic, and beneficial in diseases of lungs, spleen and urinary tract, powdered kernels mixed with oil applied to ringworms, dyspepsia, and fever. The dried plant material was extracted into aqueous, ethanol, and chloroform fraction. The strains of used in testing anti-microbial activity of CD were obtained from microbial type culture collection (MTCC). The bacteria used in the study are gram negative bacteria *(Escherichia coli, Klebsiella pneumonia, pseudomonas aeruginosa, shigella flexneri, salmonella typhi*); gram positive bacteria *(Staphylococcus aureus, Bacillus subtillis, Strepococcus pyogens*) and one fungal species

Candida albicans were used for assessing the antimicrobial activity with standard drug tetracycline (10 mg/ml).

KEYWORDS: Naruvili ver chooranam, Antimicrobial activity, disc diffusion method, broth dilution, *Cordia dichotoma*.

INTRODUCTION

Globally antibiotic resistance has been seen in every country according to World Health Organization (WHO) and drug resistance bacteria are estimated to cause 700000 deaths every year. If no action is taken they are expected to kill 10 million people annually by 2050. The risk of deaths from a resistance pathogens is two to three times greater said Dr.Carmen pessoa de silva coordinator of antimicrobial resistance at WHO.

This increasing prevalence of antibiotic-resistant microorganisms has made a need to replace alternative sources of antibiotic products. The bacteria are divided into three categories based on the urgency with which new antibiotics needed against them. They're classified as three priorities; critical, high, and medium. In critical priority *Actinobacter baumannii, Pseudomonas aeruginosa, Enterobacteriaceae,* then in high priority *Enterococcus faecium, Staphylococcus aureus, Helobacter pylori, Campylobacter Spp, Neisseria gonorrhea.*[2]

Herbal medicine is the World most ancient form of medicine as it is evident from the fact that every ancient civilization used plants for healing and in many culture. The fact is that herbal knowledge was said to have been handed down from generation to generation. Several plant extracts have both phytochemical and antimicrobial properties, typically resulted from combining secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids. Nowadays drug resistance has become an important issue to modern medicine and the effectiveness of antibiotic therapy.[3]

There is a great interest on herbal medicine and integrating their use into modern medicine because of their low cost, drug resistance, limits of medicine, medicinal value, availability and cultural exchange. Antimicrobial properties of various plant parts like root, stem, leaves, seeds, flowers, and fruits have well documented for some of the medicinal plants for the past two decades.[3]

Figure: 1 Flowers of Cordia dichotoma.

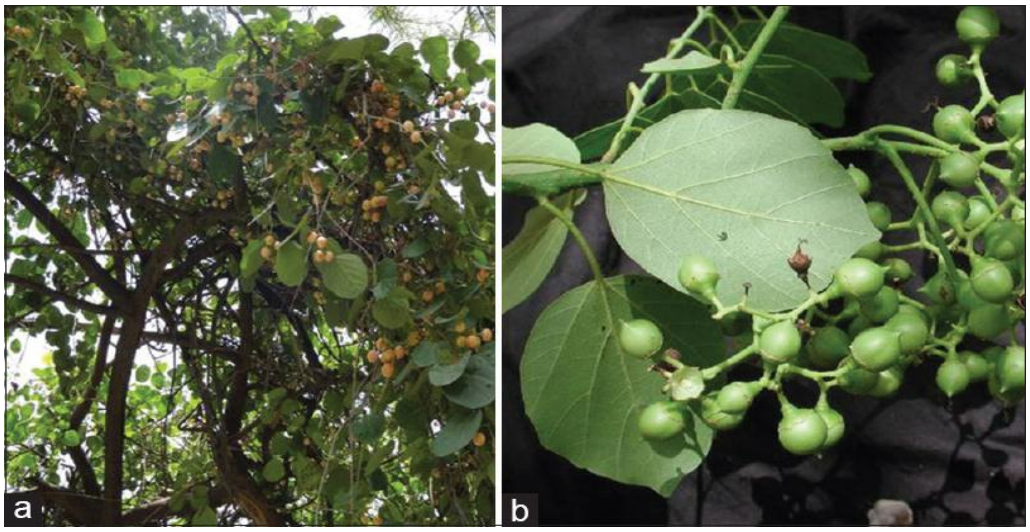


Figure: 2 Plant of Cordia dichotoma.

Figure: 3 Ripened fruits Cordia dichotoma.

The plant Naruvili (*Cordia dichotoma*) commonly known as Indian cherry, Clammy cherry and Sebesten plum and it belongs to the family Boraginaceae.[4] It is distributed commonly throughout India ascending up to 1000m. It is a crooked tree about 14m high, sometimes a shrub. Leaves alternate, variable in form and size, ovate or obolong, repand or sublobate. Flowers borne in many-flowered lax corymbs, polygamous, hermaphrodite. Calyx glaborous or minutely pubescent, much accrescent, tubular campanulate, corolla tube hairy within. Fruits berries, ovoid, sub-acute, yellow or pinkish, glossy, usually one seeded. The plant is used as anthelminthic, astringent, diuretic, and beneficial in diseases of lungs, spleen and urinary tract, powdered kernels mixed with oil applied to ringworms, dyspepsia, and fever.[5]

In this plant root contains α-L- rhamnopyranoside; holocellulose, liginin, tannin in bark; α–β and γ-eudesmol, guaiol in wood; protein and tannin in leaves; taxifolin, 3,5-dirhaminoside, galactose, mannose, and N-acetylglutamine in mucilage; α-arabinofuranosyl residues in fruits; D-arabinose, L-fructose, D-glucose, galacturonic and β-glucoronic acids in seeds.[6]

MATERIALS AND METHODS

Plant Material

The plant *Cordia dichotoma* (CD) in this study was collected in and around Coimbatore district on Jan 2017. It was the identified and authenticated by the botanist and Gunapadam (Pharmacology) experts of Govt. Siddha Medical College Arumbakkam Chennai. After the identification the whole plant material was thoroughly washed in running tap water in order to remove dust and debris. Then it was allowed to complete drying at room temperature for one week. Then the dried root was powdered well by stone mortar sieved by thin white cotton cloth and stored in an airtight sealed container.

Aqueous Extract

It is prepared by dissolving 20gm of fine powder of the plant root separately in 100ml distilled water. The contents were kept on a shaker for 48hr. The extract was filtered and dried in air-oven at 40^0C. The extract was stored under refrigeration at 4^0C for further studies.

Ethanol extract

Dried plant material was grounded in a percolator with 95% ethanol. About 10ml 0f ethanol per gram of the plant sample was used. Then the extract was dried under a reduced pressure at 40^0C. The dried extract was stored in sterile bottle which was used for further studies.

Chloroform extract

Chloroform extract of *Cordia dichotoma* (CD) was conducted as per standard guide line. The plant material (100 g) was extracted with chloroform using a soxhelt extractor for continuously 10hr until solvent changed in to pure and colorless. The solvent was removed using a rotary vacuum evaporator at 40^0C to give a concentrated extract, which was then frozen and freeze-dried until use.

Test Microorganisms

The strains used in testing anti-microbial activity of CD were obtained from microbial type culture collection (MTCC). The bacteria used in the study are gram negative bacteria *(Escherichia coli, Klebsiella pneumonia, pseudomonas aeruginosa, shigella flexneri, salmonella typhi*); gram positive bacteria *(Staphylococcus aureus, Bacillus subtillis, Strepococcus pyogens*) and one fungal species *Candida albicans* were used for assessing the antimicrobial activity with standard drug tetracycline (10 mg/ml).

Antimicrobial screening

Antimicrobial susceptibility test was done by disc diffusion method by Kiry-bauer of the isolated organisms and minimum inhibitory concentration (MIC) values were obtained by using micro-broth dilution assay method as per the guideline of National Committee for Clinical Laboratory Standards.

In the conical flask containing 100ml of nutrient broth were inoculated by the strains of different microorganisms. Then these conical flasks were incubated at 37^0C for 24hrs and referred as seeded broth. Muller Hinton Agar (Himedia, India, Mumbai) plates were prepared for the bacterial strains and the test microorganisms were inoculated by the spread plate. 20µl of the CD root extract were impregnated in 6mm of filter paper discs and placed on the upper layer of the prepared agar plates. The these agar plates were incubated for about 24hrs at 37^0C. Sabouraud dextrose agar media was prepared for fungal strain and incubated for 24hrs at 28^0C. For about 4^0C the stock cultures were maintained. For antibacterial screening Ciprofloxacin (10µg/disc) was used as the standard drug. Then for antifungal screening Ketoconazole (10µg/disc) was used as the standard drug. After incubation, antibacterial and antifungal activity of the test drug aqueous, ethanol extracts of CD root were assessed by measuring the inhibition zone formed around the discs. Suppose if there is an inconsistent result among two MIC values, a particular test was repeated in triplicate under same

incubation conditions. The MIC proves the lowest concentration of the CD root extract has inhibited the growth of microorganisms compared with the standard drug.

RESULTS AND DISCUSSION

The antimicrobial activity has been evaluated with the aqueous, ethanolic and chloroform extracts of Cordia dichotoma (CD) against different pathogens include gram negative bacteria (*Escherichiacoli, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhi*); gram positive bacteria (*Staphylococcus aureus, Bacillus subtillis, Strepococcus pyogens*) and one fungal species *Candida albicans*. Through diffusion disc method to inhibit the growth of microorganisms by the antimicrobial experiment conducted with the plant extracts, using the ciprofloxacin and ketoconazole as positive control which was represented in the table.

The aqueous extract of CD exhibited no activity against *Candida albicans* (MIC-12.5gm) and the inhibition zone diameter of 0mm.Ketoconazole was very effective in inhibiting candida albicans used for this study (10µg) and inhibition zone diameter of 22mm. Thus the aqueous extract of CD has efficient antibacterial effect against gram negative bacteria but not against gram positive and fungi.

The antibacterial activity was most significant in ethanol extract as compared to chloroform extract for all tested microorganisms. But the ethanolic and chloroform extracts of CD showed significant inhibitory effect against all the microorganisms studied. It was also described that the anti-microbial activity of ethanolic extract of CD was more specific on both gram positive and gram negative bacteria compared with aqueous and chloroform extract as represented in Table 1. Ethanolic extract of CD with MIC values ranging between 0.78 to 12.5. In combination with anti-fungal activity, extract exhibited marked inhibitory effect than ethanol extract of CD and the inhibition zone diameter of 23mm for chloroform extract and inhibition zone diameter of 20mm for ethanol extract.

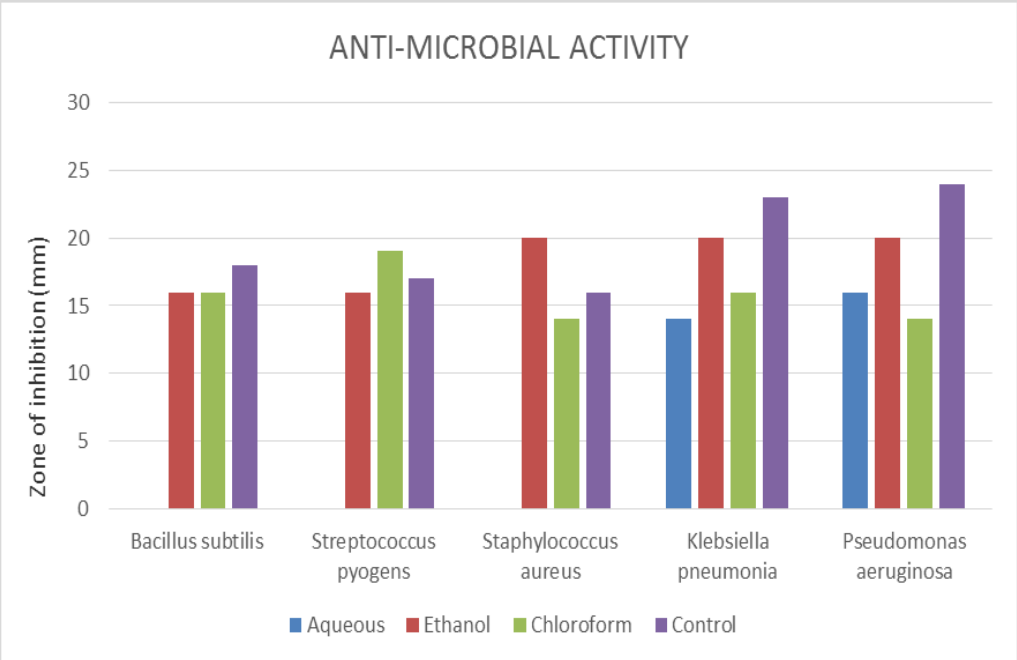
Due to the biologically active principles present in the plant CD are best extracted with ethanolic and chloroform extracts showed potent anti-microbial activity. Alkaloids, flavonoids tannins, terpenoids, carbohydrate compounds and cyanogenetic compounds found in this plant may be responsible for antimicrobial activity. The potency of the antimicrobial herbs is due to the presence of tannins. In addition, the strong inhibitory effects of CD against microorganisms are due to the presence of lignin. Finally the results obtained in this present

study exhibits potent anti-bacterial activity against the gram negative bacterial strains and anti-fungal activity against *Candida albicans* of CD root.

The antimicrobial activity of Naruvili Ver (Cordia dichotoma root) against various bacterial strains and yeast by disc diffusion and micro broth dilution methods.

Bacterial strains	SOLVENT EXTRACTS						Positive control	
	Aqueous		Ethanol		Chloroform			
	Disc (mm)	MIC (mg/ml)	Disc (mm)	MIC (mg/ml)	Disc (mm)	MIC (mg/ml)	C	K
Bacillus subtilis	0	25	16	12.5	16	12.5	18	NT
Streptococcus pyogens	0	50	16	6.5	19	6.25	17	NT
Staphylococcus aureus	0	50	20	3.125	14	12.5	16	NT
Klebsiella pneumonia	17	3.125	20	1.56	16	6.25	23	NT
Pseudomonas aeruginosa	19	3.125	20	1.56	14	12.5	24	NT
Escherichia coli	16	6.25	20	3.125	17	6.25	22	NT
Salmonella typhi	14	12.5	19	3.125	15	12.5	21	NT
Shigella flexneri	16	6.25	20	0.78	10	12.5	19	NT
Proteus vulgaris	15	6.25	21	0.78	19	3.125	21	NT
YEAST *Candida albicans*	17	12.5	20	3.125	20	3.125	NT	23

ANTIMICROBIAL ACTIVITY



The inhibitory diameter was measured by Himedia zone of inhibition scale.

C: Ciprofloxacin 10 µg. K: Ketoconazole 10 µg. NT: Not Tested.

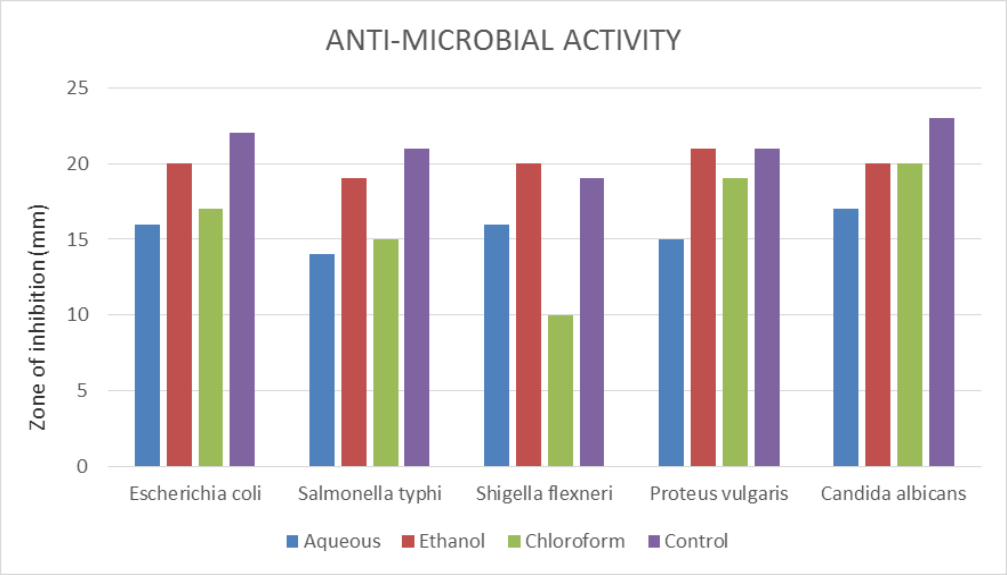


Figure 2: Average Zones of Inhibition of Naruvili Ver (*Cordia dichotoma*). Control: Ciprofloxacin 10 µg / Ketoconazole 10 µg.

CONCLUSION

The aqueous, ethanol and chloroform extracts of *Cordia dichotoma* exhibited significant antimicrobial activity against gram negative bacteria *(Escherichia coli, Klebsiella pneumonia, pseudomonas aeruginosa, shigella flexneri, salmonella typhi*). Ethanol and Chloroform extracts showed moderate activity against gram positive bacteria (*Staphylococcus aureus, Bacillus subtillis, Strepococcus pyogens*). The antibacterial activity was more significant in ethanol extract as compared to chloroform extract for all the tested microorganisms. Then the chloroform extract of *Cordia dichotoma* exhibited marked inhibitory effect against *Candida albicans*. The aqueous and ethanol extracts presented no activity against the yeast *Candida albicans*. The results of the study showed the effective source of plant root extracts of *Cordia dichotoma* for its antimicrobial activity against the common pathogens.

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