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Screening of Antibacterial Activity of *Cissus Quadrangularis* and *Boerhavia diffusa* against Clinical Pathogens

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Abstract: ***Medicinal plants available locally in nature are now in eradication. Many plants used in traditional medicine represent rich sources of natural bioactive substances with health promoting effects and no side effects. The use of modern medicines is developing. Even though it is necessary, the medicinal plants and it uses should be protected. Researches should be conducted on medicinal plants to develop medicines. The chief significance of the below study was to test antibacterial activity of methanol and ethanol extracts of Cissus quadrangularis and Boerhavia diffusa against clinical pathogens. The leaves of Cissus quadrangularis and Boerhavia diffusa were collected from Kattumannarkoil village, Cuddalore district, Tamilnadu. . The methanol and ethanol extracts of dried leaves were prepared. The methanol and ethanol extracts were tested for antibacterial activity against Escherchiacoli, Staphylococcus aureus, Klebsiella and Pseudomonas in different concentrations. The ethanolic extract of Cissus quadrangularis showed manimum zone of inhibition (21±0.3mm) against Klebsiella in the concentration of 150 µl. The minimum antibacterial activity exhibited by Boerhavia diffusa against tested clinical pathogens. In this present study Cissus quadrangularis showed maximum antibacterial activity. The extract of this plant leaves can be used to replace chemical antibiotics.***

Keywords: *Cissus quadrangularis*, *Boerhavia diffusa*, Clinical pathogens, Ethanol and Methanol extract

1. Introduction

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. The antimicrobial compounds produced by plant a reactive against plant and human pathogenic micro organisms. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens (Amenu2014).

The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs. These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as 'forgotten plants'. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.

Medicinal plants have been used as a source of medicine since the early times of Civilization. India is a sub - continent which contains a huge and enormous medicinal plants and well popular for preparation of medicine by using plants. *(*Nair *et al., 2004*). On recent years, the use of plant based products, organic products have been enormously increasing in developing as well as developed countries. The most powerful and promising elements of plants are their secondary metabolites, on which humans depend upon. Significantly, natural products and their derivatives contribute to more than half of the Food and Drug Administration (FDA) approved drugs. The disciplines of ethnobotany and ethnopharmacology define "medicinal plant" as those species used in traditional medicine that contain beneficial elements in healing diseases in humans and/or animals. The objective of ethnopharmacology is to develop a drug to treat patients, and ultimately to validate traditional use of medicinal plants.

Herbal plants consist of high source of secondary metabolites such as antiviral, antifungal and antibacterial agents. Screening of bioactive molecules from plants leads to the development of new therapeutic drugs that not only shown efficient activity against various disease causing pathogens, also gives a ray of hope to treat deleterious disease including cancer and Alzheimer's disease (Sheeja & Kuttan, 2007). Further investigation of the antimicrobial activities of the medicinal plants will expose the plants as potential source of therapeutic agents. .

According to World Health Organisation (WHO) more than 80% of the World's population relies on traditional medicine for their primary health care need. (Ammara *et al.*, 2009). The use of Medicinal plants is well known in rural area sof many developing countries. Medicines obtained from plants

are relatively safer than synthetic drugs. (Idu *et al.*, 2007). According to National Health Experts 2000 different plants are used for medicinal preparation for both internal and external use in India. Rigveda mentions 67 plants having therapeutivc effects, Yajurveda lists 81 plants and Atharva 290 plants. (Nabachandra *and* Manjula *1992).* The World Health Organization recently complied an inventory of more than 20, 000 species of medicinal plants. Indian medicinal plants and their products are used to control diverse disease such as Bronchitis, Pneumonia, Ulcers and Diarrhoea. (Galal *et al., 1991).* The other side the number of Multi - drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. (Graybill1998).

Cissus quadrangularis (family Vitaceae) is an indigenous medicinal plant of India and distributed throughout the tropical region in the world. C. quadrangularis belongs to the family Vitaceae, which is a perennial plant commonly known as Veldt Grape or Devils backbone (Kumbhojkar *et al*., 1991) It is known to be an ancient medicinal plant, with optimal healing in white tissue area of the body (tendon, ligament, etc.). (Justin Raj *et al*., 2011). Phytochemical analysis of Cissus quadrangularis indicates the presence of carotene, phytosterol, terpenoids, β - sitosterol, δ - amyrin, δamyrone and calcium (Garima Mishra *et al*., 2011) C. quadrangularis stem and leaves are used for the treatment of Hemorrhoid, Menstrual disorder, Scurvy and as Anti - oxidant, Anti - flatulence, Anti - bacterial, and Antifungal. *Cissus quadrangularis* is used for diabetes (Syed *et al*., 2021) obesity, high cholesterol, digestive tonic, bone fractures, allergies, cancer (Siddiqui et al., 2021), stomach upset, painful menstrual periods, analgesic, malaria, wound healing, peptic ulcer disease, weak bones, weak bones (osteoporosis) and as bodybuilding supplements as an alternative to anabolic anthelmintic, anti - dyspeptic and treatment for scurvy, asthma (Murthy *et al*., 2003);) (Sundaran *et al*., 2020), preserving the cellular integrity during DMBA induced oral carcinogenesis (Velvizhi *et al*., 2017) and antibacterial (Kashikar *et al*., 2006).

Boerhavia diffusa is a species of flowering plant in the four O' clock family, which is commonly known as Punarnava (meaning that which rejuvenates or renews the body in Ayurveda). It is a herbal medicine for pain relief and other uses. (Wagh *et al*., 2010). *Boerhavia diffusa* has Anti-oxidant properties. It has Anti-cancer and Anti-inflammatory Properties (Sri *et al*., 2017). Punarnava rejuvenates the kidney cells and help to balance the body fluids by eliminating the waste from the body. It is a best herb for chronic kidney disease. It has anti bacterial and anti spasmodic properties which this herb effective in Urinary tract infections (Vineeth *et al*., 2014). It helps to induce edama which decreases the risk of congestive heart failure. It stimulates bile secretion which helps to keep liver healthy and improves the function of liver. It is a best medicine to fight Obesity. It is an effective medicine for Joints and Connective tissues. It helps to treat Arthritis.

2. Materials and Methods

Cleaning of Glassware: All the glassware were soaked in cleaning solution (100g Potassiumdichromate) was added to 100ml of distilled water followed by addition of 500ml of concentrated Sulphuric acid for about 12 hours and washed in tap water. They were thoroughly rinsed in tap water and dried. Theyweresterilizedat180degreeCelsius for3 hours in Hot air oven.

Sterilization: All the media are sterilized in an autoclave at 15 lbs pressure for 20 minutes the glassware were sterilized at180 degree Celsius 3minutes, in Hot oven air.

Collection, Identification, and Processing of Plant material

The leaves of *Boerhavia diffusa* and *Cissus quadrangularis* were collected from Kattumannarkoil village, Cuddalore district, Tamilnadu. The leaves were washed with 70% alcohol and then rinsed with distilled water and sun - dried for 9days. The dried leaves were grinded and made into a fine powder using mortor and pestles. The powder was kept in a sterile airtight container to avoid contamination.

Preparation of methanolic extracts: 5g of dried powder was dissolved in 50ml of methanol in conical flask separately, plugged with cotton and kept at room temperature for 3 days filtered through Whatmann filter paper and stored tightly in a screw cap tube to avoid contamination.

Preparation of Ethanolic extracts: 5g of dried leaf powder was dissolved in 50ml of alcohol (95% ethanol) in conical flask separately, plugged with cotton and kept at room temperature for 3days filtered through Whitmann filter paper and stored tightly in a screw cap tube to avoid contamination.

Collection of test bacteria: The test bacteria were collected from the Department of Microbiology, Annamalai University. The bacteria were inoculated in broth and maintained at 4 degree Celsius in Refrigerator.

Determination of Antimicrobial activity of *Cissus quadrangularis* and *Boerhavia diffusa*: Antibacterial activity of methanol and ethanol extracts of *C issusquadrangularis* and *Boerhavia diffusa* were evaluated by Agar Well Diffusion Method. Sterilized Mueller Hinton Agar was poured into Petri dishes to the level of obtaining a standard well and allowed to set. Nutrient broth was prepared and inoculated with the test bacteria by using Swab method and uniform distribution was ensured. Sterile cork borer of 10mm diameter was used to punch holes in the agar. Each hole in the Petri dish was filled with extracts and a positive control was filled with Gentamicin (20µl) and a negative control was filled with 10% DMSO. The inoculated plates were incubated for 24 hours at 37 degree Celsius. The active extracts had zones of inhibition which were measured to indicate the degree of sensitivity.

3. Results

In this study, Ethanol and Methanol extracts of two different medicinal plants [*Cissus. Quadrangularis* and *Boerhavia diffusa*] were tested against *Escherichia coli, Klebsiella , Pseudomonas*, and *Staphylococcus aureus by agar well* diffusion method. The extracts of both plants showed

antibacterial activity against all tested bacteria.

The ethanolic extract of *Cissus quadrangularis* exhibited the maximum antibacterial activity against the clinical pathogens. The methanol extract of *Cissus quadrangularis* showed less activity against the tested clinical pathogens. Both Methanol and ethanol extracts of *Cissus quadrangularis* and *Boerhavia diffusa* showed antibacterial activity and it represented in the Figure 1 and 2. The maximum zone of inhibition (21±0.3) was attained from the ethanolic extract of *Cissus quadrangularis* against *Klebsiella* in the concentration of 150 µl followed by *Staphylococcus aureus* with zone of inhibition zone (19±0.3). The methanolic extract showed maximum zone (17±0.72) against *Pseudomonas* and *Klebsiella* followed by *Staphylococcus aureus* with the inhibition zone (15±0.4) (Table 1)

Table 1: Antibacterial activity of *Cissus quadrangularis* against clinical pathogens

S. No	Name of the Microorganisms	Concentration of the Extract and Zone of inhibition (mm)						Positivecontrol (Gentamicin)
		Methanol			Ethanol			
		50µl	100µl	150µl	50µl	100µl	150µl	
1.	*Escherichiacoli*	12±0.3	14±0.3	13±0.3	15±0.47	16±0.816	17±0.62	21±0.2
2.	*Pseudomonas*	13±0.34	13±0.92	17±0.72	11±0.57	15±0.47	16±0.96	31±0.314
3.	*Klebsiella*	11±0.2	16±0.3	17±0.6	12±0.2	19±0.5	21±0.3	35±0.4
4.	*Staphylococcus aureus*	12±0.4	13±0.2	15±0.4	12±0.3	13±0.4	19±0.3	23±0.3

Negative control-DMSO

Figure 1: Antibacterial activity of Ethanol extracts of *Cissus quadrangularis*

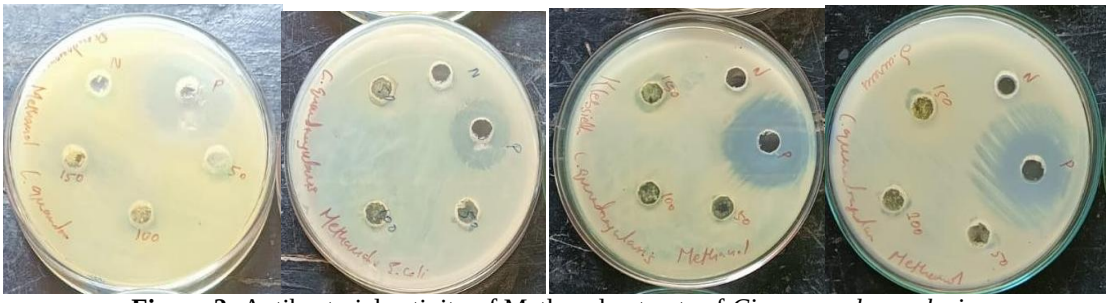

Figure 2: Antibacterial activity of Methanol extracts of *Cissus quadrangularis*

The ethanolic extract of *Boerhavia diffusa* exhibited the maximum antibacterial activity against the tested clinical pathogens. The methanol extract of *Boerhavia diffusa* showed less activity against the clinical pathogens. Both Methanol and ethanol extract of *Cissus quadrangularis* and *Boerhavia diffusa* showed antibacterial activity and it represented in Figure 3 and 4. The maximum zone of inhibition (19±0.3) was attained from the ethanolic extract of *Boerhavia diffusa* against *Staphylococcus aureus* in the concentration of 150 µl followed by *Escherichiacoli* with zone of inhibition zone (16±0.2). The methanolic extract showed maximum zone (18±0.2) against *Staphylococcus aureus* followed by *Klebsiella* with the inhibition zone (16±0.3) in Table 2

Table 2: Antibacterial activity of *Boerhavia diffusa* against clinical pathogens

S. no	Name of the Microorganism	Concentration of the extract and zone of inhibition (mm)						Positive control (Gentamicin)
		Methanol			Ethanol			
		50µl	100µl	150µl	50µl	100µl	150µl	
1.	*Escherichiacoli*	13±0.3	12±0.2	15±0.2	11±0.3	13±0.3	16±0.2	23±0.2
2.	*Pseudomonas*	11±0.2	15±0.3	14±0.16	12±0.2	14±0.2	13±0.3	31±0.3
3.	*Klebsiella*	11±0.5	13±0.2	16±0.3	12±0.2	16±0.83	14±0.2	35±0.2
4.	*Staphylococcus aureus*	13±0.2	16±0.3	18±0.2	14±0.3	15±0.2	19±0.3	23±0.17

Negative control - DMSO

Figure 3: Antibacterial activity of Ethanol extracts of *Boerhavia diffusa*

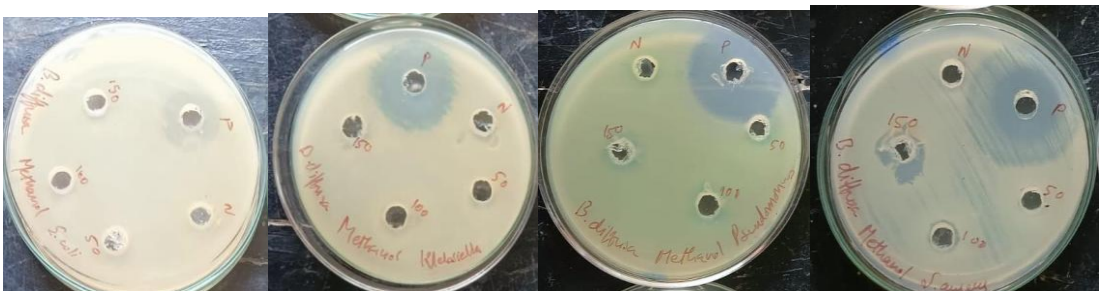

Figure 4: Antibacterial activity of Methanol extracts of *Boerhavia diffusa*

4. Discussion

Methanol and ethanol extract of *Cissus quadrangularis* and *Boerhavia diffusa* showed antibacterial property. The ethanolic extract of *Cissus quadrangularis* showed maximum zone of inhibition (21±0.3) against *Klebsiella* in the concentration of 150µl. The methanolic extract of *Cissus quadrangularis* showed maximum zone of inhibition (17±0.72) against *Pseudomonas* and *Klebsiella* 150 µl. The ethanolic extract of *Boerhavia diffusa* maximum of zone of inhibition (19±0.3) was against *Staphylococcus aureus* in the concentration of 150µl. The methanolic extract of *Boerhavia diffusa* showed maximum zone (18±0.2) against *Staphylococcus* in the concentration of 150µl. The ethanolic extract of *Cissus quadrangularis* exhibited the maximum antibacterial activity against the clinical pathogen than *Boerhavia diffusa*. In the present study ethanol extract of *Cissus quadrangularis* showed maximum antibacterial activity against clinical pathogen. This result can be corelated with Ruskin *et al.*, 2014 the antibacterial activity of *Cissus quadrangularis* against some pathogenic bacteria. They used the extracts of *Cissus quadrangularis* such as ethanol, methanol and ethyl acetate showed varying levels of antibacterial species. The result showed that the ethanol, methanol and acetate extract of *Cissus quadrangularis* exhibited maximum activity against *Klebsiella pnuemonia*

5. Conclusion

In conclusion, we concluded that the *Cissus quadrangularis* was extracted using two solvents. The suitable extraction solvents was ethanol. The ethanolic extract of *Cissus quadrangularis* can be used for potential source of natural antimicrobial compound against clinical pathogenic bacteria when compared with both methanol and ethanol extract of *Boerhavia diffusa*. This research is useful and beneficial for new antibiotic drug development and can be combined with antibiotics to increase the efficiency of the disease treatment. This preliminary study can be further extended in determining the active components of the *Cissus quadrangularis* so that effective medicinal preparations can be made.

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