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Antibacterial Activity of *Coccinia grandis* Leaf Extract on Selective Bacterial Strains

[1]Hannah Elizabeth.S, [2]Channamma. G. M, [3]Joel Durairaj.G, [4]Reshma Karkera, [5]D.Gnanasekaran

[2]Dept.of Pharmaceutical Chemistry, RMES'S College of Pharmacy, Kalburagi, India
[3]Dept. of Hospital Administration, Father Muller College of Allied Health Sciences, Mangaluru, India
[4]Dept. of Prosthodontics A. J. Institute of Dental Sciences, Mangaluru, India
[5]Dept. of Pharmacology, NeelsarojInstituteof Pharmacy, Bangaluru, India
[1]Department of Zoology
[1]Auxillium College Gandhinagar, Vellore, India

Abstract **:***Coccinia grandis* belongs to the family Cucurbitaceae and it is popular in Mexico and central America and now presently available in and around southern part of India. It possesses many uses anti-diabetic, obesity, kidney stones, hemorrhoids, acne, eye problems antimicrobial. The phytochemical prospection of dried leaves extracts showed the presence of different classes of secondary metabolites, as saponins, cardenolides, flavonoids and polyphenols that have demonstrated antimicrobial action. Fresh and dried leaves presented significantly antimicrobial activity against all bacterial strains tested, specially *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Escherichia coli*. Ethanol and hexane fractions were effective against *Escherichia coli* and *Bacillus cereus* respectively.

Index Terms-*Coccinia grandis,* Hemorrhoids, Polyphenols, Antimicrobial action.

1. INTRODUCTION

Man has been using vegetal material for medicinal purposes from time immemorial. It may be difficult to ascertain how and where this aspect of man- plant relationship has started, but it is undesirable that on the basis of empirical experience through generations, human societies developed a certain system of herbal medicines.

The herbal wealth of Indian and knowledge of their medicinal properties have a long tradition, as referred to in Rig Veda and other ancient literature. The topography of India which is in the tropical belt with its varied climatic zones makes it a vast storehouse of medicinal plants.

Indigenous people all over the country have an instinctive knowledge of the therapeutic uses of the plants of their environment. The tribal bodies of India and Africa are a treasure house of knowledge of traditional herbal medicines.

Bacteria are a large group of unicellular microorganisms. Typically a few micrometers in length, bacteria exhibit a wide range in shapes, ranging from spheres to rods and spirals. Bacteria is ubiquitous in every habitat on earth, growing in soil, acidic hot springs, radioactive wastes[1] water, and deep in the earth's crust, as well as in organic matter and the alive bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and million bacterial cells in a millilitre of fresh water, in all, there are approximately five nonillion ($5x10^{30}$) bacteria on earth, forming much of the world's biomass. However, most bacteria have not been characterized, and only about half of the phyla of bacteria have species that can be grown in the laboratory.

PATHOGENIC BACTERIA

Approximately ten times more number of bacterial cells is found in skin and gut of human beings than in the other parts of the human body. Typically bacteria live in balance. The majority of the bacteria in the body are rendered harmless by the protective effect of the immune system, however, a few species of bacteria are pathogenic and causing infectious diseases, like cholera, syphilis, anthrax, leprosy, plague etc.

GRAM POSTIVE PATHOGENIC BACTERIA

Staphylococcus aureus

Staphylococcus aureus cells can survive for months on any type of surface[2]. Hands are the main vector for transmitt in *S.aureus*

Streptococcus pyogenes

Streptococci are gram positive cocci arranged in chains or pairs. They are the part of normal flora of humans and animals. Some of them are human pathogens. The most important of them is *Streptcoccus pyogenes* causing pyogenic infections, with a characteristic tendency to spread; it is also responsible for the non suppurative lesions acute rheumatic fever and glomerulonephritis which occur as sequelae to infection[3]. *Streptococcus pyogenes* causes a variety of suppurative infections of wounds or burns, the two typical streptococcal infections of the skin are erysipelas and impetigo.

Bacillus cereus

Bacillus cereus is ubiquitous in nature. Some strains are associated with food poisoning from the ingestion of improperly stored foods such as rice or milk[4].

Bacillus cereus in a contaminant in various foods and it has been implicated in food poisoning outbreaks, with generally benign manifestations as well as in opportunistic non gastrointestinal infections in a hospital environment[5].

GRAM NEGATIVE PATHOGENIC BACTERIA

Escherichia coli

Escherichia coli is gram negative, facultative anaerobic and non-sporulating. Routes of transmission include: Unhygienic food preparation, farm contamination due to manure fertilization[6], irrigation of crops with contaminated grey water or raw sewage. Uropathogenic *E.coli* (UPEC) is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy.

Klebsiella pneumonia

Klebsiella pneumonia is the most predominant member of this genus in Klebsiella related infections as well as more generally in gram negative sepsis[7]. *Klebsiella pneumonia* is a common cause of nosocomial infections. *Klebsiella pneumonia* is a serious disease with high case fatality.

2. NEED OF THE STUDY

Traditional medicines are used by about 60 percent of the world's population. These are used for primary health care not just in rural areas in developing countries, but also in developed countries where modern medicines are predominantly used[8]. In the Indian systems of medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research. In the western world also, the use of herbal medicines is steadily growing with approximately 40 percent of the population reporting the use of the herbs to treat medical illness within the past year[9]. Because of the concern about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various disease has been on the rise in the last few decades.

In this study, the above mentioned five selected gram positive and gram negative human pathogenic strains were selected for *in-vitro* antibacterial studies by using plant extract *Coccinia grandis*.

Coccinia grandis Linn (Family: Cucurbitaceae) is a climber here cultivated throughout India. It is commonly known as kovai in Tamil. In Folklore Medicine, the fruit is used to treat leprosy, fever, asthma, infective hepatitis, jaundice and sore throats. It is also used as expectorant and astringent[10]. *Coccinia grandis* is a climbing perennial here distributed almost all over the world. The leaves of the plant posses anti diabetic, anti-inflammatory, antipyretic, analgesic and antimicrobial properties[11].

3. RESEARCH METHODOLOGY

Plant Collection And Authentication

The leaves of *Coccinia grandis* (Family; Cucurbitaceae) were collected during the month of January, from in and around Karnataka, India. The plant material was cleaned with distilled water and shade dried at room temperature. The plant material was authenticated by Prof. Nagaraj H department of Botony, Bharathi college, Bharathinagar. The shade dried plant materials were powdered by using electric blender.

Preparation of Plant Extracts

The powdered plant material was extracted separately to exhaustion in a soxhlet apparatus using acetone, ethanol, methanol, aqueous and hexane solvent (Merk chemicals, India) systems. All the extracts were filtered through a cotton plug followed by what man filter paper (No.1) and then concentrated by using a rotary evaporator at low temperature (40-50^{0}C) and reduced pressure to get yield from acetone, ethanol, methanol, aqueous and hexane fractions separately. The extracts were preserved in airtight containers and kept at 4^{0}C until further use. Another set of aqueous, acetone, ethanol, methanol and hexane solvents plant crude extracts were taken for *in-vitro* antibacterial studies.

Bacterial Strains

The following gram positive and gram negative bacterial species were used for *in-vitro* antibacterial studies. *E.coli* (MTCC 443), *Bacillus cereus* (MTCC 430), *Klebsiella pneumonia* (MTCC139), *Staphylococcus aureus* (MTCC-2940), *Streptococcus pyogenes* (MTCC 442). All the stock cultures were obtained from the institute of microbial Technology (IMTECH, Chandigarh, India) (MTCC-Microbial Type Culture Collection).

Maintenance of Test Bacterial Strains

All the microorganisms used in the study were maintained in nutrient agar (Himedia, Mumbai) slants and kept in refrigerator sub-cultures were made after every fifteen days.

Antibacterial activity (Agar well diffusion method)

The extracts obtained from plant material were studied for antimicrobial activity. A loopful of standard strains such as *E.coli*, *Bacillus cereus. Klebsiella pneumonia*, *Staphylococcus aures* and *Streptococcus pyogenes* were inoculated in 30 ml of nutrient broth in a conical flask and incubated for 24 hrs to activate the stain. In agar well diffusion method[12], the media and the test bacteria cultures were poured into petridishes. The test strain 0.25 ml was inoculated into the media. Care was taken to ensure proper homogenization.

The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5mm). The extract compound (50µl) was introduced into the well and the plates were incubated at 37^{0}C for 24 to 48hrs. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of the zone of inhibition. Ciprofloxacin (Himedia, Mumbai, India) is a reference drug usedas a control for test organisms.

4. RESULTS & DISCUSSION

Agar well diffusion method

In the present study the antibacterial activity of *Coccinia grandis* leaf extracts using solvents such as acetone, ethanol, methanol, aqueous and hexane was evaluated against five bacterial spp. (Table-1) Ethanol leaf extract of *Coccinia grandis* showed high antibacterial activity against *S.aureas B.Cereus,. E.coil* (Table-1), *K.pneumoniae* and *S.pyogens*. (Table-1) the hexane leaf extract showed high antibacterial activity against *B.cereus, E.coli, K.pneumoniae* and *S.pyogens* (Table:1) whereas, acetone, methanol and aqueous extracts showed intermediate activity against *S.aureus, B.cereus, E.coli, K.pneumoniae*, and *S.pyogens*. The inhibitory activity of plant extracts reported in (Table-1) was compared with standard reference antibiotic Ciprofloxacin (30µg /0.1ml).

Table-1: Antibacterial activity of *Coccinia grandis* by Agar well diffusion method

Extracts / Reference drug	**Zone of Inhibition (in mm)**				
	S.aureus	*S.pyogenes*	*B.cereus*	*E.coli*	*K.pneumoniae*
AE	-	5.2± 0.23	7.23± 0.15	7.45 ±0.10	6.90± 0.36
ET	15.2± 0.30	9.9 ± 0.10	12.34±0.32	16± 0.15	15.13±0.32
MT	12.98 ± 0.26	-	4.98 ± 0.32	6.63 ± 0.41	4.12 ± 0.32
AQ	-	7.98±0.25	-	2.45±0.34	5.12±0.49
HE	4.23±0.47	13.2 ± 0.26	15.86±0.32	11.87±0.43	15.23±0.55
Ciprofloxcin (30µg / 0.1 ml).	23.13 ± 0.32	19.16± 0.15	26.10 ±0.10	25.03± 0.25	22.13 ± 0.15

AE-Acetone, ET-Ethanol, MT-Methanol, AQ-Aqueous, HE-Heaxane,(-) no zone of inhibition, Reference drug –Ciprofloxcin (30µg / 0.1ml).

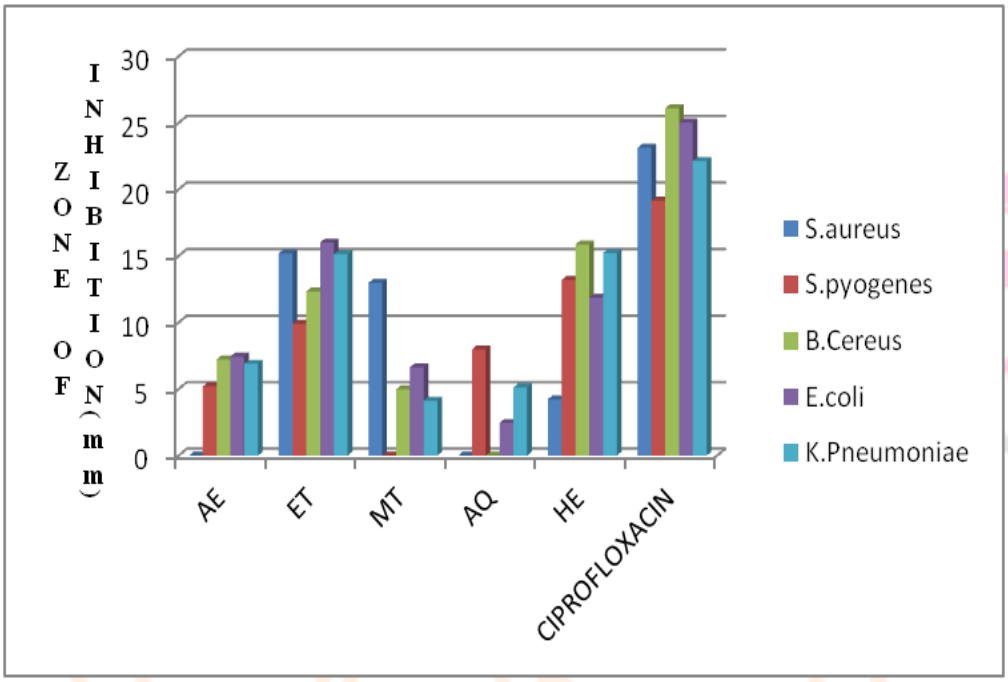

Figure-1: Antibacterial activity of *Coccinia grandis* by Agar well diffusion method

Due to importance of the above mentioned conditions. The *Coccinia grandis* leaves was used for testing their antibacterial activity and showed high activity against those organisms such as *S.aureus, B.cereus, K.pneumoniae* and *E.coli* (Zone of Inhibitions in mm -12.98, 15.86, 15.23 and 16 respectively) in different extracts used with reference to the standard drug (Ciprofloxacin) used as shown in Table-1. These indicate that the herbal preparations could be used for preventing and treat the disease caused by those selected organisms.

This is to be expected because the outer membrane of gram-negative bacteria is known to be present barrier for the penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes, which are able of breaking down foreign molecules introduced from outside[13]. It was interesting that the extracts of above selected plants showed their response on both gram positive and gram-negative bacteria.

Phytochemical screening of *Coccinia grandis* reported the presence of saponins, cardenolides, flavonoids and polyphenols. Flavonoids is also an important active chemical compounds against microorganisms. They have been found *in-vitro* and to be effective for antimicrobial activity against a wide array of microorganisms[14]. Main chemical constituents of the plants are glycosides picroside, I II and III, Picrorhizin, Kutkoside, Kurrin, Kuthinol, Kutkiol, Kutkisterol, Kutkoside, Androsin, Apocynin, Drosin and Cucurbitacin. These compounds are effective against anti-inflammatory, hepatoprotective

properties against toxins, antioxidant activity, and lower the high cholesterol and antibacterial. Selected medicinal plant has been very effective in pathogenic bacterial infections were also recorded.

CONCLUSION

The plants have potent antibacterial effect as confirmed by selective antibacterial tests. A Potent antibacterial drug could probably be formulated from the plant extract of *Coccinia grandis* to combat the effects of bacterial infections.

CONFLICTS OF INTEREST

There is no conflicts of interest by all authors.

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