-Project Report –

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Title: "ANTIBACTERIAL ACTIVITY OF FOUR MEDICINAL PLANTS OF JODHPUR REGION"

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INTRODUCTION

Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. In many developing countries traditional medicine is one of the primary health care systems. India is well known for Ayurveda, which is one of important traditional medicine practiced. Antibacterial active principles isolated from higher plants is appears to be one of the important alternative approaches to contain antibiotic resistance and the management of disease. It is believed that plant based drugs cause less or no side effect when compared with synthetic antibiotics. With the rising prevalence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Since antiquity, plants have been used to treat common infectious diseases. The healing potential of many plants have been utilized by Indian traditional medicines like Siddha, Ayurvedha and Unani. Being nontoxic and easily affordable, there has been a resurgence in the consumption and demand for medicinal plants[1].

Achyranthes aspera (Family: Amaranthaceae) commonly known as *latjira* is known as a medicinal plant in different systems of folk medicine in India. *A. aspera* is a perennial herb growing upto three meters. Juice of *A. aspera* root is used in the treatment of diabetes, juice from leaf is used to treat earache[2]. It is also used in the treatment of gonorrhea. The whole part of the plant is claimed to posses medicinal properties in the traditional medicinal system. Extracts from this plant is given to cure diabetics in mammals. Phytochemical or biological works were scanty in *A. aspera* [3]. There are scanty reports on its antibacterial activities of this plant. In order to demonstrate the antibacterial efficacy, test were conducted against human pathogenic bacteria including those responsible for causing inflammation.

Tinospora or Giloy or Amrita (Family: Menispermaceae) is a well known medicinal herb contains anti-inflammatory and antipyretic properties. This herb has been used in Ayurveda in India since centuries as a medicine in building up the immune system and the body's confrontation against definite infecting organisms. In a scientific study conducted using human WBC (white blood corpuscles), this herb helps in increasing the killing ability of macrophages, the resistant cells those are accountable for fighting foreign materials as well as microorganisms

Tinospora or Giloy is used as an immune-modulator in immune-suppression of certain ailments like as obstructive jaundice, hepatic fibrosis, peritonitis and sepsis. Guduchi or Giloy improve the functioning of protective cells, macrophages and will improve body's resistance to infections.

Cleome viscosa Linn. (Capparidaceae) is also known as Tickweed, or Spider plant. It occurs in woodland and grassland, and is a weed of fallow land, fields, roadsides and wasteland, often occurring on sandy soils, but sometimes on calcareous and rocky soils. In Asia and Africa the leaves and seeds used as a rubefacient and vesicant and to treat infections, fever, rheumatism and headache. The whole herb is used in treatment of inflammation of the middle ear and applied on wounds and ulcers. A decoction is used as an expectorant and digestive stimulant and the vapour from a steaming decoction of the whole plant is inhaled to treat headache [4]. The seeds and its oil have antihelminthic properties but they are ineffective in treating roundworm infections [5]. The roots are a remedy for scurvy and rheumatism [6]. An aqueous seed extract displayed significant analgesic activity in mice and local anaesthetic activity in guinea pigs [7,8]. In tests with rats the anti-diarrhoeal [9] and antipyretic [10] activities of the extracts have been confirmed.

Withania somnifera belongs to the family Solanaceae, commonly known aswagandha. It is a shrub reaching about 150 cm in height. This plant grows widely in all drier parts of sub tropical India. It has general animating and generative qualities and is used among others for the treatment of nervous exhaustion, memory related conditions and insomnia, it helps countered, chronic fatigue, weakness, dehydration, bone weakness. It involves in the activation of macrophages, Phagocytosis and increased activity of the lysosomal enzymes.

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antibacterial and antifungal activity of *Achyranthus aspera, Tinospora cordifolia, Cleome viscosa and Withania somnifera*.

MATERIALS AND METHOD

Collection of Plant Material:

Fresh Root, Stem, Leaves, and flower of four plants viz., *Achyranthes aspera, Tinospora cordifolia, Cleome viscosa and Withania somnifera*, free from disease were collected from Jodhpur region.

Solvent Extraction:

The plant parts were washed thoroughly 2-3 times with running water and dried in shade for four days and then powdered with the help of Warring blender. 25 g of shade-dried powder was filled in the thimble and extracted successively with methanol solvent in Soxhlet extractor for 48h. The solvent extracts was concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Preparation of Inoculum:

The gram positive (*Streptococcus mutans* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) were obtain from IMTECH and grown in nutrient broth overnight in a rotary shaker at 37°C.

Anti-bacterial Activity:

The disc diffusion method was used. Different concentration of the extracts (100 µg/ml) was prepared by reconstituting with methanol. The test micro organisms were seeded into respective medium by spread plate method and 10 µl (10 cells/ml) bacteria was seeded in nutrient medium. After solidification the filter paper discs (5 mm in diameter) was impregnated with the extracts and placed on test organism-seeded plates. Streptomycin sulphate (10 µg/ml) was used as positive control and methanol 1solvent (100 µg/ml) was used as negative control The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.

Table 1. Antibacterial activity of some medicinal plant methanol extracts (100 µg/ml) and antibiotic (10 µg/ml) against bacterial species tested by disc diffusion assay:

Plant	Plant Part	Zone of Inhibition (mm)			
		S. mutans	*S. aureus*	*E. coli*	*P. aeruginosa*
Achyranthes aspera	Root	4	-	-	3
	Stem	6	4	-	11
	Leaves	6	3	3	11
	Flower	7	5	4	**12**
Tinospora cordifolia	Root	5	2	7	5
	Stem	5	3	-	4
	Leaves	7	-	6	5
	Flower	8	7	5	9
Cleome viscose	Root	8	6	3	4
	Stem	6	6	7	3
	Leaves	4	9	8	6
	Flower	**14**	9	8	9
Withania somnifera	Root	3	-	-	2
	Stem	6	8	-	7
	Leaves	8	6	-	8
	Flower	10	9	6	10

Results and Discussion

The antibacterial activity of all the four plants against *S. mutans, S. aureus, E. coli and P. aeruginosa* are shown in table 1. Results obtained in the present study relieved that the tested four medicinal plants extracts posses potential antibacterial activity against all the tested bacteria. The results are as follows:

***Achyranthes aspera*:** All the extracts exhibited antibacterial activity against *S. mutans* and *P. aeruginosa*. The highest antimicrobial activity was shown by flower extract against *P. aeruginosa* followed by leaves and stem extracts. The extracts presented least antibacterial activity against *E. coli*. The present investigation confirmed the previous work on antimicrobial activity of *A. aspera* against pathogenic organisms reported by Handa [11] and Ellof [12]. Geetha *et al* (2010) also studied the effect of ethanolic extract on *S. aureus* and *E. coli*[13] .

***Tinospora cordifolia*:** The root extract showed maximum antibacterial action against all tested bacteria with a maximum inhibition zone against *P. aeruginosa*. All the extracts exhibited moderate activity against *S. mutans*. Rose *et al,* reported *in vitro* antibacterial activity of

methanolic extracts of roots against *E. coli, S. aureus* and *P. Aeruginosa* [14]. Mahesh and Satish (2008) reported the antibacterial activity of methanolic extract of *Tinospora cordifolia* against *E. coli* and *S. Aureus*[15].

***Cleome viscosa*:** All the tested plant parts reflected good antimicrobial activity against all experimental micro organisms. The maximum antibacterial action was exhibited by flower extract against *S. mutans* with a maximum inhibition zone of 14 mm followed by leaves and stem extract against *S. aureus*. Saradha and Subba rao (2010) also reported antibacterial activity of *Cleome viscosa* against *E. coli* and *P. Aeruginosa* [16]. Antimicrobial effect of seeds of *C. viscosa* against *S. aureus, E. coli* and *P. aeruginosa* was also reported by wake *et al* [17].

***Withania somnifera*:** Again flower extract greatly inhibited growth of *S. mutans* and *P. aeruginosa*. The least antimicrobial activity was recorded against *E. coli* followed by *S. aureus*. Santhi and Swaminathan (2011) evaluated antibacterial activity and phytochemical analysis of leaves of *Withania somnifera* [18]. Jain and Varshney (2011) indicate that the methanolic and aqueous root extracts of *W. somnifera* might be exploited as natural drug for the treatment of several infectious diseases caused by these organisms [19].

Conclusion:

All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these plants were more effective than traditional antibiotics to combat the pathogenic microorganisms studied. The flower extracts of all the plants proved to be good antibacterial agents. The results of present investigation clearly indicate that the antibacterial and antifungal activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.

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Achyranthus aspera

Tinospora cordifolia

Cleome viscose

Withania somnifera