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Determination of antibacterial activity of two medicinally important Indian Taxa

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ABSTRACT

The present paper deals to study of antibacterial activity of two Indian plant taxa viz. Hibiscus sabdariffa and Rumex nepalensis. Study includes antibacterial activity against human pathogenic stains (Salmonella sp. (MTCC); Staphylococcus aureus, Bacillus subtillis. Escherichia coli. Pseudomonas sp. etc).

Key words: *Hibiscus sabdariffa, Rumex nepalensis* and Antibacterial activity.

INTRODUCTION

The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful [3]. Plants serve as the basis of traditional medicine systems for thousands of years in Nigeria, India, China, Indonesia etc. [4].The search for components with antimicrobial activity has gained increasing importance in recent times due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic resistant microorganism [5]. Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacology. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [6]. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more. These natural compounds formed the foundations of modern prescription drugs as we know today. Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as a defense system against disease or more accurately, to protect against disease. Since ancient time, mankind has used plants to cure diseases and relive physical suffering.

Demand on plant based therapeutics has increased many fold because they are natural products having no side effects and easily available at affordable prices. Plants extracts are used for centuries in different traditional system of medicine for the treatment of human aliments, particularly those caused by pathogenic bacteria, fungi as well as virus.

Hibiscus sabdariffa is known for delicacy and also for medicinal properties. Tender young leaves and stems - raw or cooked use in salads, as a pot-herb and as a seasoning in curries, they have an acid, rhubarb-like flavor. Fresh calyx (the outer whorl of the flower) is eaten raw in salads, is cooked and used as a flavoring in cakes etc and is also used in making jellies, soups, sauces, pickles, puddings etc. The calyx is rich in citric acid and pectin and so is useful for making jams, jellies etc. It is also used to add a red colour and to flavor to herb teas.

Whereas leaves of *Rumex nepalensis* and shoots - cooked as a vegetable. The leaves are used in the treatment of colic. The juice of the leaves is applied externally to relieve headaches. A decoction of the plant is used to wash the body in order to alleviate body pain. Medicinally the root is purgative. It is used as a substitute for rhubarb (*Rheum spp*.). A strong decoction of the root is applied to dislocated bones. A paste of the root is applied to swollen gums.

MATERIALS AND METHODS

Plant Material

In the present investigation, two taxa viz, *Hibiscus sabdariffa* L. belonging to the family Malvaceae *and Rumex nepalensis* Spreng. belonging to the family Polygonaceae were selected for the study. Moreover, the medicinal importances of these two species are also documented by several workers.

Screening for antibacterial activity:

Material of the selected plants was dried in shade. The dried material was grounded to a fine powder. This powdered material was stored in paper bags for further use.

Preparation of extract: - Extracts were prepared as that used for antibacterial analysis.

Preparation of Nutrients medium:-Nutrient broth medium was used to culture the bacteria for experiment.

Determination of Antibacterial activity:-

Different bacterial strains were used for the screening are, Gram + ve -*Staphylococcus aureus*, *Bacillus subtillis*, *Bacillus streaothermophilus*, *Rhodococci* sp. Gram – ve , *Proteus valgaris*, *Escherichia coli*, *Pseudomonas* sp, *Salmonella* sp. (MTCC). Pure culture of some bacteria were like *Salmonella sp.* (MTCC), *Escherichia coli* and *Bacillus streaothermophilus* were obtained from the Microbiology department of veterinary college , Seminary Hills, Nagpur, rest of all obtained from biotechnology department of SFS college, Seminary hills, Nagpur and inoculated in 25 ml broth, then incubated at 37o C. for 24 hrs.

d) Well Diffusion Assay: - The modified agar well diffusion method of **[7].** was employed. The petri dishes and nutrient agar medium was sterilized by autoclaving. To this sterilized nutrient medium 10 ml of one day old bacterial culture was added. Culture were inoculated and stirred well, this media was poured in petri dishes and allowed to set. Two well were created using a 5 mm cork borer. In this well 100 ul of the plant extracts/standard was filled.

All the nutrient agar plates were incubated at 37 ^{o} C for 24 hrs. After the plates were observed for clear zone of inhibition. The diameter of the zone of inhibition was measured in millimetres. The results were documented with the help of tables, photograph and graphs.

RESULT AND DISCUSSION

The antibacterial activity of leaves, stem and root were carried out from *Hibiscus sabdariffa* and *Rumex nepalensis*. Most of the extract show antibacterial activity against nine bacteria, which are known as human pathogenic bacteria. These are *E. coli (mixed) B. subtilis, Pseudomonas sp., S. aureus P. vulgaris, Salmonella sp., E. coli (positive strain), Rhodococci sp., B. stearothermopelus.* Petroleum ether extracts of leaves, stem and root have not shown any activity at all in *Hibiscus sbadariffa.* On the other hand in *Rumex nepalensis* stem and root extract has no effect but leaf extract shows the effect on *E. coli* (mixed), *S. aureus* and *Salmonella sp.* In case of *Hibiscus sabdariffa* highest zone of inhibition recorded in acetone extract of leaves against *Proteus vulgaris* i.e.18.1 mm, followed by water extract of root against *S. aureus* i.e. 18mm, and then by same extract against *E. coli (mixed).* i.e. 17.1mm. All extracts of leaves, stem and root of *H. Sabdariffa* have not shown any activity against *Pseudomonas sp., Salmonella sp., Rhodococci and Baccilus sp*. at all. In case of *Rumex nepalensis* highest zone of inhibition recorded in water extract of leaves against the *E. coli* (mixed) i.e. 24 mm, followed by water extract of stem against *S. aureus* i.e. 20mm, and then by water extract of root against *Pseudomonas sp.* i.e. 19.1mm. All results obtained are shown in table 1, 2 and Graph 1.

Investigation carried out of plant material as alternative source of antibacterial agent. It has become more common over the past few years, due to the increased rate of development of antibiotic resistance organism. The inhibition of bacterial growth *in-vitro* by the extracts of plants could be due to the presence of some active compounds in the extracts. These active compounds may act alone or in combination to inhibit bacterial growth. It may be due to crude plant extracts containing multiple organic components including flavonoids, tannins, alkaloids, triterpenoids all of which are known to have antibacterial affects. Plant extract contain phenolics compounds like tannins that are very good antimicrobial agent **[8].**. Thus it may be summarized that the class of natural compounds must exhibit the antibacterial activity. The metabolites have been shown to be responsible for various therapeutic activities of medicinal plants **[9].** Flavonoids especially are known to be effective antimicrobial agent a wide array of microorganism; the activity is attributed to their ability to complex with extra cellular and soluble proteins and with bacterial cell wall **[10].** There are several reports on antibacterial activity of different herbal extracts has been published are supported the work done **[11, 12, 13, 14, 15, 16, 17, 18].** In the present investigation all five extracts of leaves, stem and roots of *Rumex nepalensis* and *Hibiscus sabdariffa* were screened for antibacterial activity against nine human pathogenic bacterial strains. Most of the extracts have shown antibacterial activity against these nine bacteria. These are *E. coli (mixed) B. subtilis, Pseudomonas sp., S. aureus P. vulgaris, Salmonella sp., E. coli (positive strain), Rhodococci sp., B. stearothermophilus.*

Bacillus subtilis is a Gram positive rod shaped bacteria. It is an obligate aerobe and an endospore former. They are found in soil and on vegetation. They can contaminate food and may cause food poisoning. *B. streaothermophilus* can withstand temperature of 121 ^{0}C for 12 minutes and is one of the most heat resistant organisms known. *Escherichia coli* are common member of the normal flora of large intestine. It is predominant facultative organism in the gastrointestinal tract and colonizes the tract within hours or few days. It is responsible for

causing diarrhoea which is characterized by rapid onset of watery non bloody fluid. Pathogenic strain of *E. coli* is called as *E coli* positive strain.

Pseudomonas sp. is the epitome of an opportunistic pathogen to human. It is gram negative aerobic bacteria. It causes urinary tract infection, respiratory system infection, dermatitis soft tissue infection, gastrointestinal infection and a variety of systemic infection. *Staphylococcus aureus* is gram positive bacteria that occur in microscopic cluster resembling groups. It is a facultative anaerobe that grows by aerobic respiration or by fermentation which yields lactic acid. These are pathogenic to human beings. They cause a wild range of superlative infection as well as food poisoning and toxic shock syndrome. *Salmonella sp.*, it includes a large number of pathogens of human beings as well as mammals. These are gram negative bacilli. These are pathogenic when acquired by oral route. Broadly they may cause enteric fever, septicemia and enteritis. The enteric fever and septicemia are caused by thousand of *Salmonella*. Thus the plant extracts can be used as an important antibiotic to cure above mentioned disorders caused by the different strains of bacteria.

Antibacterial activity of plant extract

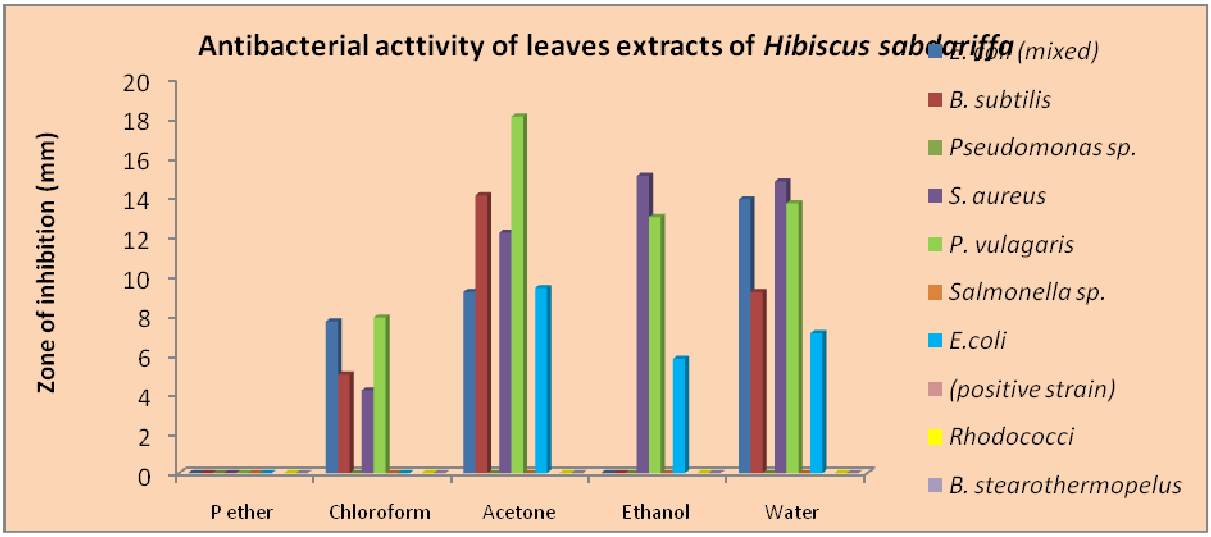
Table 1: Screening of *Hibiscus sabdariffa* for antibacterial activity

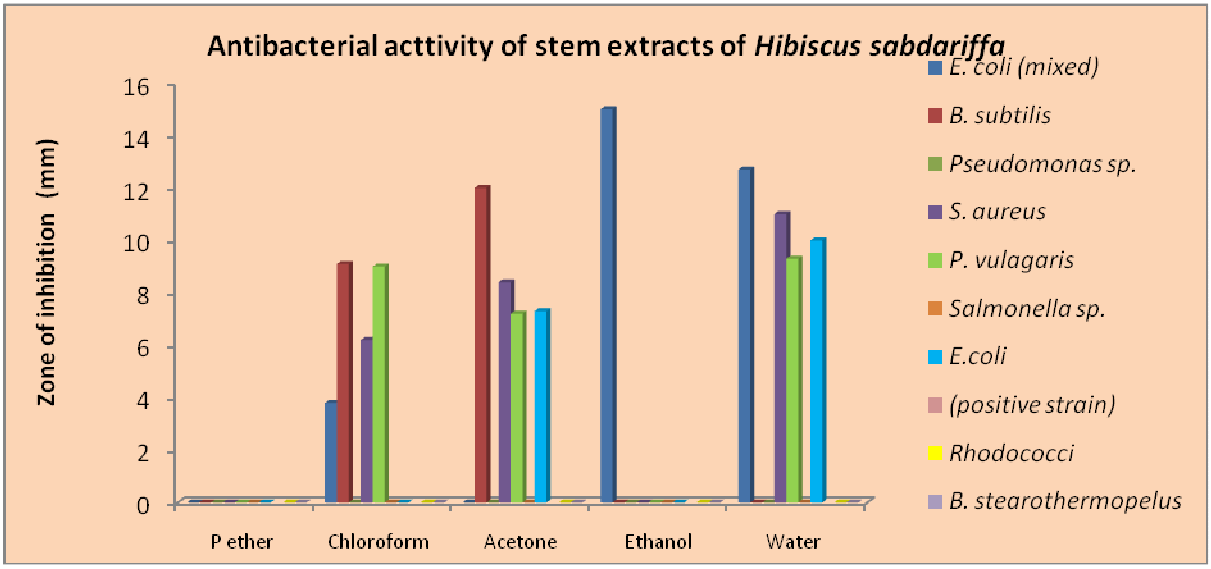
Bacterial strain	Zone of inhibition in mm along without well diameter (5mm)														
	Leaves extracts					Stem extracts					Root extracts				
	1a	**2a**	**3a**	**4a**	**5a**	**1a**	**2a**	**3a**	**4a**	**5a**	**1a**	**2a**	**3a**	**4a**	**5a**
E. coli (mixed)	0	7.7	9.2	0	13.9	0	3.8	0	15	12.7	0	3.9	11	0	17.1
B. subtilis	0	5	14.1	0	9.2	0	9.1	12	0	0	0	14.2	0	6.3	13.1
Pseudomonas sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. aureus	0	4.2	12.2	15.1	14.8	0	6.2	8.4	0	11	0	11.4	7	2.1	18
P. vulgaris	0	7.9	18.1	13	13.7	0	9	7.2	0	9.3	0	0	0	0	0
Salmonella sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E.coli (positive strain)	0	0	9.4	5.8	7.1	0	0	7.3	0	10	0	9.7	10	4	12
Rhodococci	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. stearothermopelus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1a- petroleum ether , 2a- chloroform, 3a- acetone, 4a – ethanol and 5a – water extract															

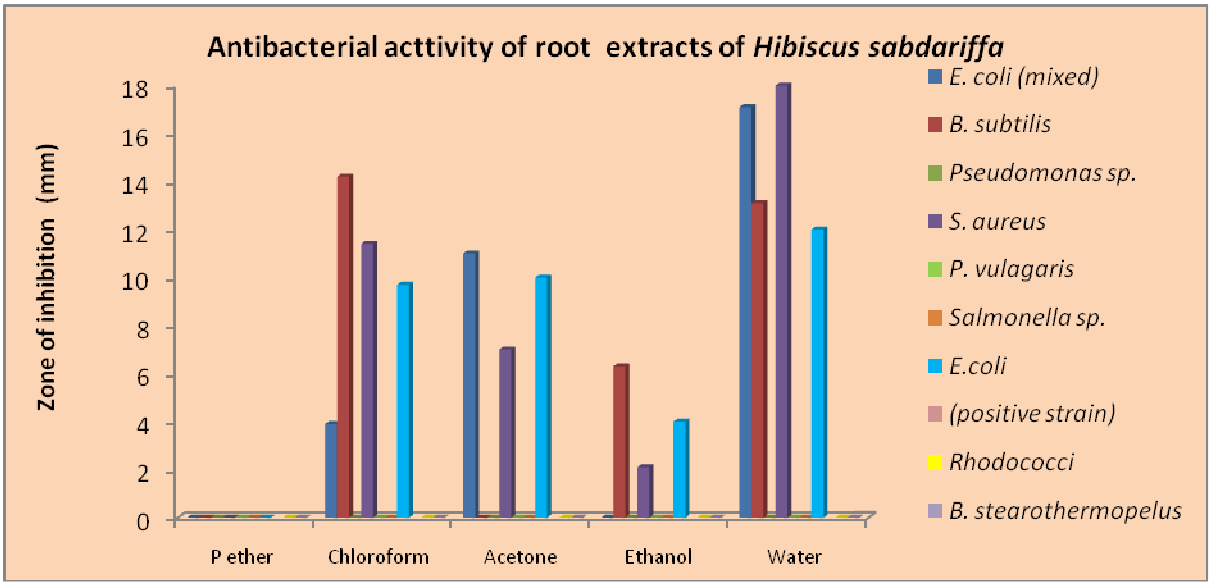
Table 2: Screening of *Rumex nepalensis* for antibacterial activity

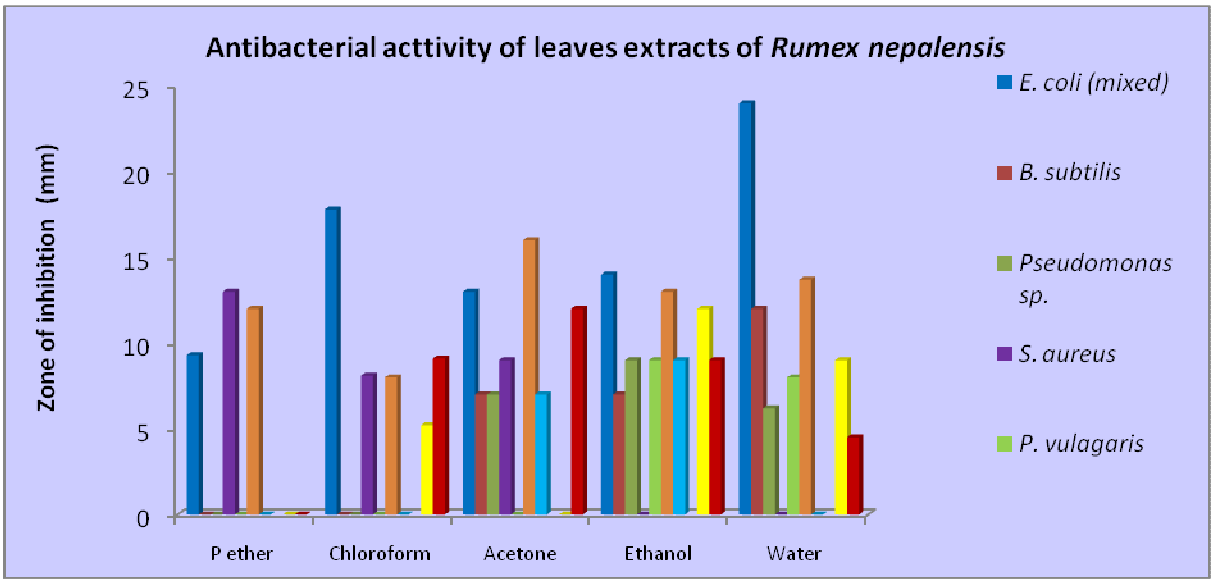
Bacterial strain	Zone of inhibition in mm along without well diameter (5mm)														
	Leaves extracts					Stem extracts					Root extracts				
	1a	**2a**	**3a**	**4a**	**5a**	**1a**	**2a**	**3a**	**4a**	**5a**	**1a**	**2a**	**3a**	**4a**	**5a**
E. coli (mixed)	9.3	17.8	13	14	24	0	11	12	16	0	0	8.3	9	19	17.4
B. subtilis	0	0	7	7	12	0	12	7	0	0	0	0	9	9	0
Pseudomonas sp.	0	0	7	9	6.2	0	0	8	8	13	0	8.2	9	10	19.1
S. aureus	13	8.1	9	0	0	0	11.7	8	0	20	0	0	7	0	0
P. vulagaris	0	0	0	9	8	0	0	0	11	0	0	0	0	9	18.3
Salmonella sp.	12	8	16	13	13.7	0	7	11	9	18	0	9	14	14	0
E.coli (positive strain)	0	0	7	9	0	0	0	0	13	9.2	0	5.2	0	11	7.2
Rhodococci	0	5.2	0	12	9	0	3.2	8	13	17	0	0	12	15	0
B. stearothermopelus	0	9.1	12	9	4.5	0	7.7	0	9	16	0	9.9	12	11	6.1
1a- petroleum ether , 2a- chloroform, 3a- acetone, 4a – ethanol and 5a – water extract															

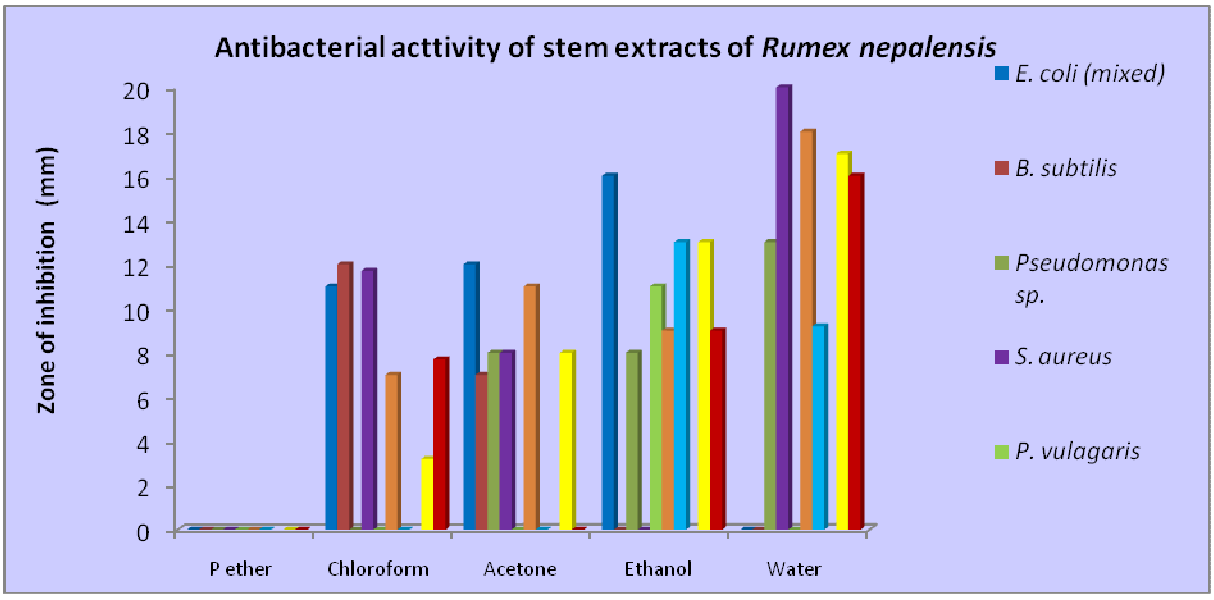
Graph 1: Showing antibacterial activity of leaf, stem and root extracts of *Hibiscus sabdariffa* and *Rumex nepalensis*

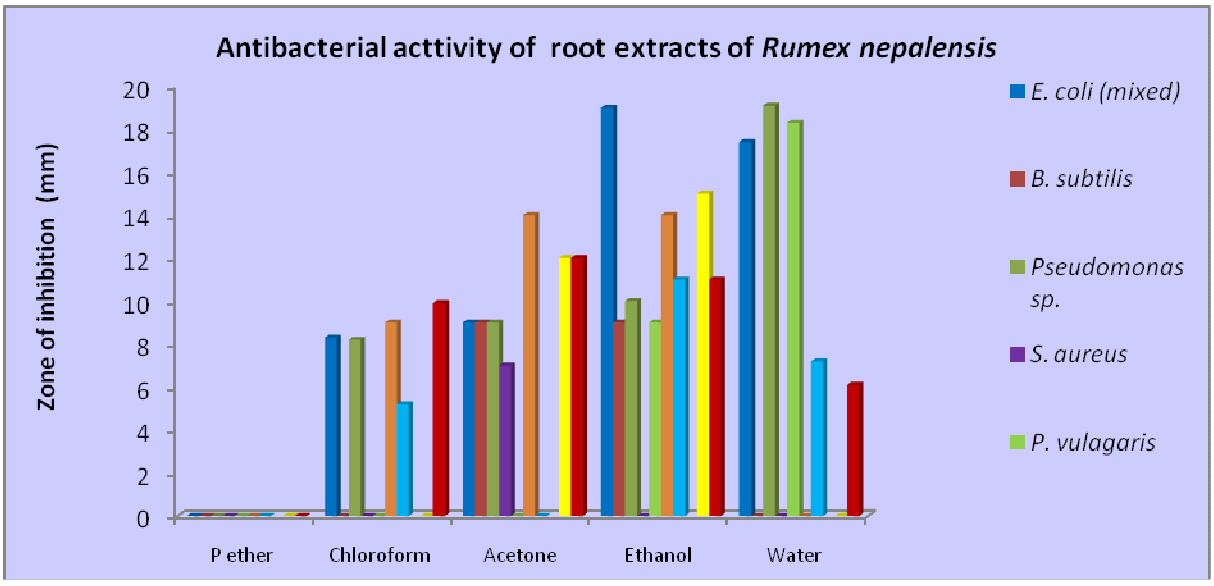







Antibacterial acttivity of leaves extracts of *Rumex nepalensis*
Zone of inhibition (mm)
25
20
15
10
5
0
P ether
Chloroform
Acetone
Ethanol
Water
E. coli (mixed)
B. subtilis
Pseudomonas sp.
S. aureus
P. vulagaris


Antibacterial acttivity of stem extracts of *Rumex nepalensis*
Zone of inhibition (mm)
20
18
16
14
12
10
8
6
4
2
0
P ether
Chloroform
Acetone
Ethanol
Water
E. coli (mixed)
B. subtilis
Pseudomonas sp.
S. aureus
P. vulagaris


Antibacterial acttivity of root extracts of *Rumex nepalensis*
Zone of inhibition (mm)
20
18
16
14
12
10
8
6
4
2
0
P ether
Chloroform
Acetone
Ethanol
Water
E. coli (mixed)
B. subtilis
Pseudomonas sp.
S. aureus
P. vulagaris

CONCLUSION

Antibacterial activity of the extract of stem, leaves, and root were carried out for *Hibiscus sabdariffa* and *Rumex nepalensis*. Most of the extract showed activity against nine human pathogenic strains. Antibacterial activities conclude that these plants stop bacterial growth. The results are encouraging but scientific scrutiny is absolutely necessary before being put in practice as well as the most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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