ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 7(A), pp. 27744-27750, July, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

IN-VITRO ANTIBACTERIAL ACTIVITY SCREENING OF SELECTED MEDICINAL PLANTS AGAINST DIFFERENT ENTERIC PATHOGEN ISOLATED FROM SOIL AND WATER

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0907.2322

ARTICLE INFO

Article History:

Received 4th April, 2018
Received in revised form 18th May, 2018
Accepted 16th June, 2018
Published online 28th July, 2018

Key Words:

Antibacterial activity, plant extract, soil and water borne bacteria

ABSTRACT

Contaminated food, water, & soil are the most common habitats of pathogens, causing infections**.** The development of new antimicrobial agents against resistant pathogens is of increasing interest as many bacteria are resistant to chemical antibiotics. The use of plants in treatment of infectious diseases is common in traditional medicine. Therefore, crude extracts of eleven plants commonly used in traditional medicine, and honey were evaluated for their antibacterial property by disc and well diffusion assays. In this study, different bacterial strains were isolated from soil and water. Although biochemical characterization of the different bacterial isolates has not been done, their identification was carried out done by gram staining. The antibacterial activity of extracts of traditionally used medicinal plants namely *Curcumin longa* (Turmeric), *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Allium cepa* (Onion), *Zingiber officinale* (Ginger), *Datura stramonium* (Dhatura), *Syzygium aromaticum* (Clove), *Cinnamomum zeylanicum* (Cinnamon), *Piper nigrum* (Black pepper), *Mentha spicata* (Mint), *Allium sativum* (Garlic); and honey were tested against the different enteric bacterial strains isolated from the soil and water. The tested plant extracts and honey exhibited variable degrees of antibacterial activity. Compared with standard antibiotic amoxicillin, the extracts showed low to moderate activity against the bacteria. Our study suggests that cinnamon, garlic, neem , ginger, tulsi, black pepper, clove , turmeric and honey could be the alternatives to the conventional antibiotics to cure the bacterial infections without the associated side effects of these antibiotics.

INTRODUCTION

Bacteria, as common microorganisms found in air, food, water and soil; remain a major problem in developing countries where disease outbreaks frequently occur in congested areas. Pathogenic bacteria are the most common cause of illness. Billions of people around world lack access to clean water and an access to basic sanitation. Each year millions of people get sick due to the contaminated food, air and water. Antibiotics are used to treat common bacterial diseases such as respiratory, ear, gastrointestinal, urinary tract, and skin infections but lately bacteria develop resistance to most of the drugs.

Conventional antibiotics usually provide effective therapy for bacterial infections [1]. However, these bacteria have become resistant to one or more antibiotics and the population of Multidrug Resistant (MDR) bacteria is increasing [2]. Mechanisms which microorganisms have developed to resist antibiotics include inactivation of antibiotics by enzymes, alteration of drug target sites, blockage of drugs from entering into the cell membrane, chromosomal and plasmid mediated resistance. The drugs also have serious side effects and the cost of medication is high [3].

Due to the resistance emerging in organisms against antimicrobial drugs, it is an immediate need to develop alternate new antimicrobial drugs, more active against the pathogens, to cater to containment of the problem. Apart from resistance, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reactions [4, 5]. Medicinal plants are a source of great economic value all over the world. Since ancient times, herbs and oils are known for their varying degree of antimicrobial and antibacterial activity [6-8]. Due to immense diversity, India is a vast repository of medicinal plants that are used in traditional medicinal treatments, as reported in

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literature and commonly used in ayurvedic medicines. In the recent past years, few reports have already been documented about prevalence of herbal microbial treatments which are thought to do permanent treatments of ailments caused by bacteria as also that herbal products are not toxic [9-12] . Drugs which are extracted from plants are very effective, easily available and less expensive, and they rarely have side effects associated with them.

Crude extracts of plants may be used for initial screening for the potential antibacterial compounds from plants. Over the last few decades, there have been many investigations on natural remedies like crude extracts of medicinal plants as sources of new microbicidal agents. Therefore, in the present study, the main objective was to evaluate the antibacterial activities of the extracts of some traditionally used medicinal plants against enteric bacterial isolates from soil and water. These plants and honey are used by people in the Indian traditional healthcare system for treatment of common ailments including gastrointestinal disorders.

Experimental Section

Sample collection for enteric bacteria: Samples of polluted water and soil were collected aseptically in sterile bottles, brought to the laboratory in the Department, and stored at 4°C till further use.

Isolation of bacterial strains and preparation of test bacteria: Serial dilution method is one of the old and usable methods for the isolation of bacterial colony [13] and therefore was employed in the present study**.** Briefly, 1g of each sample was taken into tubes containing 10 ml saline and mixed (master test tube). 1ml from the master test tube was taken and mixed with 9 ml of distilled water (1/10 dilution) and again further dilution was serially done up to 1/100 dilution. Nutrient agar media was prepared by pouring plate method. After solidification of agar, 0.5 ml of sample solution from desired tube was poured over agar plate and spread over the plate by the help of spreader. Plates were incubated at 37ºC for 24 hours or till the colonies appeared. To obtain pure cultures, subcultures were done. Gram staining was done to confirm the bacterial populations in each sub cultures.

Collection of plant materials: Based on extensive literature survey, and on the basis of easy availability, eleven Indian medicinal plants most widely reported for their medicinal properties viz., *Allium sativum* (garlic) , *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Allium cepa* (Onion), *Zingiber officinale* (Ginger), *Datura stramonium* (Dhatura), *Syzygium aromaticum* (Clove), *Cinnamomum zeylanicum* (Cinnamon), *Piper nigrum* (Black pepper), *Mentha spicata* (Mint); and honey were selected for *in vitro* antimicrobial screening. The plant samples were collected by the authors from the local area (Lucknow) from their natural habitats or purchased from the local market as required and identified in the Department of Applied Plant Science of the University. The voucher specimens are maintained in the department. Natural honey was purchased from local markets of Lucknow.

Preparation of plant/part crude extracts

All extracts were prepared using standard protocol with few minor modifications [14]. Samples of plants/parts were weighed (10 g), and washed thoroughly with tap water. Samples were surface sterilized with absolute ethanol for 5 min. After washing thoroughly with autoclaved distilled water three times to remove extra ethanol, the samples were air dried in a laminar air flow chamber. The sterilized samples were homogenized aseptically using a sterile mortar and pestle in 10 ml either autoclaved distilled water or with absolute alcohol. The mixture was centrifuged at 5,000 rpm for 10 min and supernatant was filtered through Whatman filter paper No. 4. This extract was considered as 100% concentration which was further diluted to 30% & 50 % with appropriate volumes of sterile distilled water. The extract was preserved aseptically at 4°C until further use.

Natural honey was used as 100% as procured from local market and further dilutions of 30% and 50 % were made using distilled water.

Preparation of test inoculums

Bacteria were sub- cultured overnight at 37^0C in agar plates. The bacterial growth was harvested using 5 ml of sterile saline water; its absorbance was adjusted at 580 nm using spectrophotometer and diluted to attain viable cell count of 10^6 CFU/ml.

Preparation of sensitivity disc

Discs of 5 mm diameter were punched out using Whatman No. 1 filter paper with the aid of a paper punch and placed in glass bottles. The glass bottles containing discs were then sterilized by autoclaving at 121^0C for 15 min after which they were allowed to cool. Stock solutions of the ethanolic and aqueous crude extracts were prepared by grinding at a concentration of 10 g/ 10 ml (stock solution 100% concentration). From this stock solution different concentrations (30 and 50%) of each the plants extract were prepared. This was followed by introducing sterile discs into each concentration. The discs were allowed to soak in solution and kept for further analysis.

Antibiotic sensitivity test

Different concentrations (100, 50 & 30%) of natural crude plant extracts and honey were tested for their antibacterial activity.10 µg amoxicillin served as standard drug (positive control), while broth containing 20 µl of methanol served as negative control. Observations were recorded by measuring the areas (in mm) of the inhibition zones after 24 h incubation at 37°C [15].

Antibacterial activity assessment

Well diffusion method: The antimicrobial activity of the extracts was determined by the method in agar well diffusion [16]. Briefly the method is described as follows. Each plate was inoculated by swabbing with bacterial suspension (10^5–10^6 colony forming unit "CFU"/ml), which was swabbed evenly on the surface of solid agar media by the help of cotton swab. After 20 min, wells of 5 mm diameter were made in solid agar medium with the help of gel puncher and filled with 50 µl of various test samples of plant extracts. The positive control wells were filled with amoxicilin (Standard drug). All the plates were incubated at 37°C for 24 h. After the test duration of 24 h, the diameter of the zone of inhibition was measured in millimetres [17]. The measurements were made with a ruler held

on the under surface of the plate without opening the lid and inhibition zone ≥ 9.0 mm showed that the bacteria was susceptible to the antibacterial extract [18,19] .

Agar disk-diffusion method: Agar disk-diffusion test is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing [20]. In this well known procedure, agar plates were inoculated with inoculums of the bacterial suspension (10^5–10^6 colony forming unit CFU/ml). Then, the filter paper discs (about 5 mm in diameter) soaked in different concentration of each plant extract for 2-3 hrs was placed on the agar surface. The petri dishes were incubated under suitable conditions during which the test agent would diffuse into the agar and inhibit germination and growth of the test microorganisms. After 24 hrs of inoculation the diameters of inhibition growth zones were measured and interpreted.

RESULT & DISCUSSION

Isolation and confirmation of bacteria

Morphologically different colonies were appeared on LB agar medium and characterized by gram staining and it showed that colonies were of gram negative and gram positive bacteria from different samples of water and soil.

The antibiotic sensitivity test of the environmental bacterial strains was tested by standard antibiotic Amoxicillin (10 µg).

Antibacterial effect of plant/part extract

The antimicrobial activity of selected eleven medicinal plant species and honey was evaluated *in vitro* against the enteric bacterial strains isolated from soil and water and the results are shown in Tables 1 and 2. In general, most plant extracts of the different plant parts exhibited broad spectrum of antibacterial activity. All extracts exhibit antibacterial activity in dose-dependent manner against the test bacteria.

Most of the aqueous plant extracts exhibited antibacterial activities against all the tested organisms in varying degrees in both disc diffusion and well diffusion assays except Dhatura (*Datura stramonium*). As shown in Tables 1 & 2, honey was found to have an inhibitory effect against bacteria using the disc diffusion and agar well experiments. The aqueous extracts of cinnamon and clove exhibited the lowest antibacterial activity. The neem and garlic aqueous extracts, as well as honey, showed the best antibacterial activity in the disc diffusion method. The test bacteria were found to be sensitive to the ethanolic extracts of cinnamon, neem, and ginger (Tables 1, 2). The Zones of inhibition by the different plant/part extracts with well diffusion method were more pronounced as compared to the disc diffusion method

Antibiotic resistance has been an emerging problem worldwide in the last two decades. This has led to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant/products [21, 22].

Table 1 Antibacterial activity of plant/part extracts (aqueous and ethanolic) against bacterial isolates by disc diffusion method

Plant names & parts used/ standard antibiotics	Zone of inhibition (mm) by disc diffusion method by ethanolic plant extract			Zone of inhibition (mm) by disc diffusion method by aqueous plant extract		
	30%	50%	100%	30%	50%	100%
Natural honey	7.4±1.2	10.2±1	16.5±2	-	8.3±1.3	15.2±2.2
Allium sativum (garlic) bulbs	9.2±1.4	13.1±1.2	18.2±2	-	7.5±1.2	12.4±1
Cinnamomum zeylanicum (cinnamon) bark	7±1	10.5±2	16.2±1	-	7.3±1	10.3±2.1
Piper nigrum (black pepper) seeds	-	8.5±1.2	12.1±1	-	-	9.2±2
Mentha spicata (mint) leaves	-	7.8±1.1	12.5±1.1	-	-	10.1±1
Syzygium aromaticum (clove) buds	-	8.3±1	11.5±1	-	-	9.5±1
Curcuma longa (turmeric) rhizome	7.1±1	10.4±2	13.5±1	-	7.5±1	10.5 ±1
Azadirachta indica (neem) leaves	7±1	13.6±1	18.7±2	-	11.4±2	15±2
Ocimum sanctum (tulsi) leaves	-	9.1±1	12.4±1	-	-	9.6±1
Allium cepa (onion) bulbs	-	8.2±1	10.6±1	-	11.4±1	14±1
Zingiber officinale (ginger) rhizome	-	9.6±2	13.3±2	-	8±1	10.4±1
Datura stramonium (dhatura) leaves	-	-	7.4±1.6	-	-	-
Amoxicillin (10 µg) (reference standard drug)	20±2					

Diameters of zone of inhibition is measured in mm. Data is expressed as mean of 4 independent test and ± standard deviations (SD), - no inhibition

Table 2 Antibacterial activity of plant extracts (aqueous and ethanolic) against bacterial isolates by well diffusion assay

Plant names/ standard antibiotics	Zone of inhibition (mm) by well diffusion method by ethanolic plant extract			Zone of inhibition (mm) by well diffusion method by aqueous plant extract		
	30%	50%	100%	30%	50%	100%
Natural honey	7.2±1	12.5±2	18.3±2	-	10.6 ± 2	18.2± 2
Allium sativum (garlic) bulbs	8 ±1	14±1.5	20 ±1	-	10.2±1.3	15.2± 1
Cinnamomum zeylanicum (cinnamon) bark	8.2±1	9.4 ± 1	18.1±1.1	-	7±1.4	11.1±2
Piper nigrum (black pepper) seeds	7±1	9.3±1	14.3±2	-	7.4±1	8 .5±1.2
Mentha spicata (mint) leaves	-	8.2±1	13.1±1	-	7±1	11.2±1.3
Syzygium aromaticum (clove) buds	-	9.1±2	11.4±2	-	-	9.3±1.2
Curcuma longa (turmeric) rhizome	8±1	11.4±1.2	15.3±1	-	8.1±1	11.4±1
Azadirachta indica (neem) leaves	9 ± 1	15.3 ±1	20.1±2	7±1	13.3 ±2	17.7±2
Ocimum sanctum (tulsi) leaves	7±1	10.1±1.2	13.5±1	-	7±1	9.4±1
Allium cepa (onion) bulbs	-	9.2±1	11.2±1	8±2	12.5±2	16±1
Zingiber officinale (ginger) rhizome	7±1	10±2	15±2	7±1	9±1	11±1
Datura stramonium (dhatura) leaves	-	8.6±1.8	10.4±1.2	-	-	7.6±1.5
Amoxicillin (10 µg) (reference standard drug)		20±2				

Diameter of inhibition zone was measured in mm. Data is expressed as mean of 4 independent tests and ± standard deviation (SD), - no inhibition

The antimicrobial property of these plants/parts may differ depending on whether they are fresh or dried extracted. In order to use the plant extract to control food borne bacteria or from contaminated water supply, it is essential that antibacterial effects of crude plant extracts are investigated against soil and water bacterial isolates.

Keeping the above in mind, the present study was designed to evaluate the antibacterial activities of the selected plant/ parts against bacteria isolated from soil and water. In this study the *in vitro* antibacterial activity of the aqueous and ethanolic extracts of the selected 11 different plant/parts were tested against bacterial isolates from soil and water. The different plants selected for *in vitro* antimicrobial screening were *Allium sativum* (garlic) , *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Allium cepa* (Onion), *Zingiber officinale* (Ginger), *Datura stramonium* (Dhatura), *Syzygium aromaticum* (Clove), *Cinnamomum zeylanicum* (Cinnamon), *Piper nigrum* (Black pepper), *Mentha spicata* (Mint); and honey were selected. Here, we used different plant parts which included leaves (Tulsi, Mint), flower (Clove), bulbs (Garlic, Onion), fruits (Black pepper), bark (Cinnamon), rhizomes (Ginger, Turmeric) and nectar (Honey). The present study has used established methods such as the agar well and disc diffusion techniques to establish the antibacterial activity of plant extracts.

The results showed that the extracts of the medicinal plants tested were effective antibacterial agents and the bacteria were sensitive to the tested plant extracts in varying degrees. The variation in antimicrobial potentiality of examined plants could be attributed to their disparate contents of biocidal agents. This is in accordance with previous studies which found that bacterial strains had varying degrees of sensitivity against phyto-compounds present in plant extract [20, 23].

The antibacterial effects of honey are well studied and it is said that it exerts its effect due to presence of an enzyme which produces hydrogen peroxide, and also due to the high sugar content which dehydrates bacteria sufficiently to kill them [24]. In this study also, we observed sensitivity of bacterial isolates (both gram positive and negative) from soil and water to the honey in both agar well and disc diffusion assays (18 ±2; 16.2± 2 mm respectively).

Ethanolic extract of Garlic at 100 % concentration showed 20±1 mm and 18± 2 mm zones of inhibition in well and disc diffusion assays respectively. The antibacterial action of garlic is attributed mainly due to its active component allicin [25, 26].

The sensitivity of various bacterial and clinical isolates to pure preparations of allicin has been reported and is in consonance with the results of the present study. Allicin is the most abundant thiosulfinate found in garlic and is generated when the enzyme alliinase reacts with its substrate alliin [27].

In this study, we found that the bacteria exhibited susceptibility towards cinnamon extracts. The aqueous extract of cinnamon in the disc & well diffusion experiment had an inhibitory effect (10.3±2.3; 11.2±1.2 mm) against bacteria but to lesser extent as observed with the ethanolic extract. The ethanolic extract of cinnamon in agar well diffusion assay (18±1 mm) showed greater zone of inhibition in comparison to disc diffusion assay (16±1mm). The limited inhibitory activity of cinnamon extract can be explained by the low water solubility of the essential oil and its components (the active components of the extract) [28]. Cinnamon has also been used as a health-promoting agent for the treatment of diseases such as inflammation, gastrointestinal disorders, urinary infections, diabetes and neurological disorders. Another potential medical use of cinnamon would be with regards to its antimicrobial properties, especially antibacterial activity [29, 30]. This has also been demonstrated by the results of this study.

The antibacterial activity of clove extract as determined by disc and agar well diffusion methods, showed that it caused inhibition of the growth of the bacteria. In the disc diffusion experiment, ethanolic extract of clove had an inhibitory effect against bacteria whereas aqueous extract had mild sensitivity towards the bacterial isolates. The results of the present study were similar to those previously reported [7, 31] in which it was reported that clove oil was active against both gram positive and negative bacteria and its minimum inhibitory concentration was 300mg /ml.

Curcumin is the most important fraction which is responsible for the biological activities of turmeric. It is soluble in ethanol and acetone, but insoluble in water. Curcumin, a potent antioxidant is believed to be the most bioactive and soothing portion of the herb turmeric and posses the properties like antioxidant, anti-inflammatory, anti-platelet, cholesterol lowering, antibacterial and anti-fungal effect [32]. Different fractions of *C. longa* rhizome in organic solvents viz., petroleum ether, methanol etc. were found to be more effective antimicrobial agents than the crude extract [33]. Turmeric has reported inhibitory action against bacterial growth when extracted in alcohol, and similar results were obtained in the present study also, where the ethanolic extract of turmeric had more sensitivity than the aqueous extract both in disc diffusion (13.5±1 & 10.5±1 mm) and agar well diffusion (15±1 &11.4±1 mm) assays .

Neem is an important native medicinal plants of India, it has a wide spectrum of biological activity, and is the most useful traditional medicinal plant in India. Each part of neem tree has some medicinal property. Neem leaves, bark extracts and neem oil are commonly employed for therapeutic uses. Neem oil suppresses several species of pathogenic bacteria [34]. The results of the present study confirmed the antibacterial properties of 100 % Ethanolic and aqueous extracts of neem leaves which showed sensitivity against bacterial growth both by the method of disc (18.7±2 &15±2 mm) and well diffusion (20.1±2 & 17.7±2) assays. Lower concentrations of neem leaves extract showed mild sensitivity in both ethanolic and aqueous extracts (Table 1 &2).

The ethanolic extract of black pepper exhibited sensitivity towards bacterial growth both in disc diffusion and agar well diffusion assays (12.1±1 & 14.3±2 mm respectively). Aqueous extract of black pepper was found to be relatively resistant (7-9 mm) towards bacteria in both the diffusion assays. The ethanol extract of black pepper showed antibacterial activity, due to the presence of phytochemical compounds like tannins and alkaloids, but the bacterial isolates were found to be insensitive to its aqueous extract. The black pepper is traditionally used to

treat palsy, gout and lumbago, as a tonic to the liver, and shows stomachic, emmenagogue, abortifacient, aphrodisiac and digestive properties [35].

In this study, we observed the antibacterial activity of both aqueous and ethanolic extracts of fresh mint leaves. The effect was more pronounced in the ethanolic extract (disc & well assays; 12.5±1.1 & 13.3±1mm) when compared to the aqueous extract (disc & well assays; 10.1±1 & 11.2±1.3mm) by both the diffusion assays. Mint has been used as appetite stimulant, a treatment for gastrointestinal infection and to lower blood sugar in diabetics. Its use for the treatment of certain types of cancer and viral infections has also been reported. Herbalists consider it as an astringent, antiseptic, antipuritic, antipasmodic, antimicrobial, stimulant and emmenagogue [36]. Leaf extracts of mint showed broad spectrum antimicrobial activity since various water, ethanol and methanol extracts of the leaves have exhibited antibacterial activity.

Ethanolic extract of ginger has antibacterial activity against both gram negative and positive bacteria and our results (tables 1 and 2) were in accordance with other studies [6, 7, 37]. Fresh ginger has been used for cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, loss of appetite, and in rheumatism all over world.

Both ethanolic and aqueous extracts of onion have antibacterial effect but it is more prominent in ethanolic extract as shown by this study and as also reported by earlier workers [8, 38]. The antibacterial activity of onion juice can be attributed to the presence of flavanoids and polyphenols which have been reported to show broad spectrum antibacterial activity [39]. Several authors have reported pharmaceutical activity of extracts of *Allium cepa* (onion) including anti-tumor, anti-diabetic, antioxidant, antimicrobial, anti-allergic activity. *In vitro* studies have shown onion to also possess antibacterial, antiparasitic, and antifungal activity [39, 40].

In this study, the aqueous extract of tulsi at lower concentration (30%) was insensitive to the bacterial growth but higher concentrations demonstrated bactericidal effect. Ethanolic extract showed mild activity at lower concentration, but was found to be sensitive at higher concentration. *Ocimum sanctum* L., known as 'tulsi' in Hindi and 'holy basil' in English, is traditionally used as a medicinal plant in day-to-day practice in Indian homes for treatment of various ailments. The essential oil extracted from the tulsi leaves contains eugenol, a phenolic compound which may be attributed to its antimicrobial, antidiabetic, and anticancer properties [41, 42].

In our study, Dhatura was relatively sensitive against bacterial inhibition as 100% concentration had mild effect. Other studies have reported its effect as antibacterial [43].

Our results indicate that the extracts of the test medicinal plants were sensitive against the bacterial growth but this was not found to be significant when compared with the standard drug. The effects of climatic and soil conditions, method of growth, mode of transportation, harvesting time, use of pesticides and inorganic fertilizers on the plant samples were not considered in this study. Methods of preparation of plant extracts differed in the various studies and this may give rise to the differences in antibacterial activity reported by other studies. In this study, we isolated bacteria from soil and water and identified them by simple gram staining, no further characterization of bacteria was done. The present study focussed mainly on the assessment of broad spectrum antibacterial activity of plant /parts extracts which were being regularly used by the society at large. Herbalists prefer using crude extracts rather than extracting single components from them. This gave the direct effect of plant/part extracts on the bacteria. The majority of herbs are used in their dried form in various studies, however, plants must be used in their fresh form to be useful as medicines, since they lose their healing properties when dried [44]. Whole plant extracts have many components and these components work together to produce therapeutic effects and also to lessen the chances of side effects from any one component.

However, when the published articles on the anti- microbial effect of these natural products are viewed, the comparison between results is often rendered difficult, because of the use of different non- standardized approaches, inoculums preparation techniques, inoculums size, growth medium, incubation conditions and end- point determinations.

CONCLUSION

The findings of the present study showed that neem, garlic, ginger, cinnamon, honey, turmeric, tulsi, clove, black pepper had sensitivity against the test bacteria. Also, the ethanolic extracts of most of tested plants exhibited more sensitivity against bacterial growth in comparison to their aqueous extracts. However, the aqueous extracts of neem, garlic, and honey showed sensitivity against the test bacteria. The results of this study serve to give scientific evidence for the uses of these plants in traditional medicine and that they possess antibacterial activity, thus giving leads to their further exploration for treatment of enteric bacteria and gastro-intestinal disorders.

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How to cite this article:

Leena Rastogi *et al*.2018, In-Vitro Antibacterial Activity Screening of Selected Medicinal Plants Against Different Enteric Pathogen Isolated From Soil And Water. *Int J Recent Sci Res.* 9(7), pp. 27744-27750.
DOI: http://dx.doi.org/10.24327/ijrsr.2018.0907.2322

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