

## Screening and Evaluation of Antimicrobial Activity of Medicinal Plants

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### ABSTRACT

**Objectives:** To screen and evaluate antimicrobial activity of crude ethanol extracts against various microorganisms.

**Methods:** Ethanoic extracts of 7 different species; *Acorus calamus* (Bojho), *Aloe vera* (Ghiu Kumari), *Artemisia indica* (Titepate), *Azadirachta indica* (Neem), *Mentha arvensis* (Pudhina), *Zanthoxylum armatum* (Timur) and *Zingiber officinale* (Ginger) were subjected to soxhlet extraction. Test organisms included mainly enteric isolates i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Salmonella* Typhi and *Staphylococcus aureus* were selected, pathogens confirmed by Antibiotic Susceptibility test was done by disc diffusion method of Modified Kirby – Bauer method using Amikacin (30 mcg), Ampicillin (10 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (5 mcg), Gentamicin (10 mcg) and Penicillin G (10 mcg). The antimicrobial activities of the extracts were determined by Agar well diffusion technique both individually and in combination. On the other hand, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration was determined by dilution technique.

**Results:** Among 7 plants that were tested, 6 plants were found to have activity against test bacteria. *A. calamus* was effective against 3 out of 5 test bacteria. *A. indica* and *mentha arvensis* inhibited 2 out of 5 bacteria. *A. vera* and *A. indica* were effective against *S. aureus* only and *Z. officinale* had no antibacterial effects over any tested bacteria. *S. aureus* was the most susceptible gram positive bacteria meanwhile *K. oxytoca* stood among Gram negative, emerged as the most resistant species. *S. aureus* showed ZOI with 6 plant extracts excluding ginger. *P. aeruginosa* was inhibited by *A. calamus*, *A. indica* and *M. arvensis*. The largest ZOI of 15 mm was obtained with *Z. armatum* acting upon *S. aureus*. While the smallest diameter of 8 mm was observed with *Acorus calamus* against *S. Typhi*. The smallest MBC value of 13.63 mg/ml was obtained with *M. arvensis* against *P. aeruginosa*. Results showed that *P. aeruginosa* was the most resistant bacteria, only 1 out of 3 antibiotics were sensitive to it. 60% of the test bacteria were sensitive to 2 out of 3 antibiotics tested.

**Conclusion:** To recapitulate, the extract of species can be used to prevent the pathogenic organism.

**Keywords:** Zone of Inhibition, Minimal Bactericidal Concentration, Minimum Inhibitory Concentration, Antibiotic Sensitivity Test

### INTRODUCTION

The use of herbs as flavours for food and drinks, for medicinal purposes eg. In treatment of various ailments

and aesthetic purpose as insecticides, cosmetics, perfumes etc dates back to prehistoric times. Especially in countries like Nepal, there are many rural parts where most of the

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people are out of reach of western pharmaceuticals or prepacked Indian Ayurvedic medicines. The use of traditional medicine is widespread in these rural parts where 75% of patients are being treated by native healers (Manandhar 1980). History doesn't shade any light on initial uses of medicinal and aromatic plants by the primitive men, but it is widely known that plants were eagerly sought during the earliest civilization in all parts of the world.

The beneficiality and medicinal properties of plants have been known and used by human being in some form or other (Jain and Saklani, 1991). According to the Hindu mythology, earliest mention of the medicinal use of plants has been found in "Rig Veda" which was written between 4000 and 1600 BC. In the "Atharva Veda" we find the more varied use of drugs. It is in the "Ayurveda" (600 B.C.) which is considered as "Upaveda" where definite proportions of drugs and their uses have been given in detail. "Charak Samhita" is another earliest treatise on "Ayurveda" (600 B.C.) which lists a total of 341 plants and plants products for use in health management. "Susruta Samhita" also dealt with plants related to medicine. Subsequent authors of later treatises have extended the use of Ayurvedic single plant drugs to six hundred species of plants (Bhattacharjee 1998). Plants have been a rich source of medicine because they produce a lot of active molecules, most of which probably evolved as chemical defences against predation of interaction. Most of the plant possess one or more of the medicinal properties, viz. antibacterial, antifungal, antihelminthic, anticancer, sedative, laxative, cardiogenic, diuretic and others (Parajuli et al 1998). It was believed that medicinal properties of plants were due to active chemical constituents present in different parts of the plants. (Mitscher et al 1980). Since the inception of antibiotic era with penicillin and streptomycin, a large number of antibiotics have been discovered from microorganisms. Surprisingly, the antibiotic principles of higher plants have gained relatively very little attention. This is because of the narrow concept that antibiotics can be extracted from microorganisms only. However, modern definition of antibiotics has inspired many workers to explore plants for these properties. Plants are composed of cell materials such as chlorophyll, starch, sugars, proteins, lipids, vitamins etc.

The medicinal effect on human and animals is due to the active components or secondary plant products. The main group of active components are alkaloids, glycosides, saponins, essential oils, mucilage, tannins, bitters principles, etc (Kruger 1992).

Generally, these active components are extractable with different kinds of solvents. The active compounds which inhibit and/or kill microorganisms are called antimicrobials. Different kinds of medicinal plants may contain such to different extent. These antimicrobial substances in medicinal plants are generally alcohol extractable (Sasidharan 1997). The crude herbal extracts, as such or in the purified form play significant role in the treatment of diseases to human beings. They are used in the treatment of large number of bacterial and fungal infections such as infected wounds, diarrhoea, dysentery, vomiting, fever, cough, itching of skin, etc., specially in Ayurvedic system. In Nepal too, herbal products have been used for remedy for several diseases since indefinite time. (Shrestha 2006).

Screening of medicinal plants for antimicrobial activities has so far yielded any drug of high therapeutic value like the antibiotics from microbial sources. However, some of the plant products like quinine, berberine and conessine have established their clinical usefulness through long, selective or specific usage for the treatment of malaria, gastroenteritis and amoebiasis respectively (Fernandes et al 2007). This represents research work is intended to verify whether the traditional use of medicinal plants in the rural areas possess antimicrobial activity or not. Nevertheless, demonstrating activity in a bioassay is necessarily a first step in the drug development process (Paul and Balick 2012). So this work is a first step towards investigating antimicrobial activity of medicinal plants as claimed by users.

## **MATERIALS AND METHODS**

### **Collection of samples**

Seven different medicinal plants which either includes roots /rhizomes /aerial parts (stems /leaves), seeds and fruits were collected from different of lalitpur area.

**Identification and documentation of sample plant**

Voucher herbarium specimens, usually 3-4 in number were made simultaneously with sample collection on the spot. Medicinal plants were identified according to the description given on different books viz. Flora of Kathmandu Valley by HMG/N (2002), flora of British India (1992), Medicinal plants of Nepal by HMG/N (2008 and 2010) and other pertinent taxonomic literature. The collected plant parts were washed with clean water and left to remove moisture. The plants were then pressed inside the paper sheet in between blotting paper. The paper sheets were changed in regular intervals too. General description of the plant morphology was also noted on the spot for better identification.

**Processing of the samples**

**Washing and chopping:** Barks and roots were washed to remove soil and other extraneous matter. Collected samples were then cut into fragments into 3-5 cm pieces and split longitudinally into several sections.

**Drying of the sample:** The samples were dried in shade in room temperature. Turning up and down at least twice a day is necessary to fasten drying.

**Packaging and storage of the samples:** The completely dried plants were packed in water proof bags. In case of incomplete drying, cotton bags were chosen.

**Grinding the samples:** Then the dried samples were subjected to grinding.

**Soxhlet extraction with 70% ethanol**

Known weight of a dried plant powder was loaded in a clean and dried thimble of a soxhlet extractor. It was then fit in a 250 ml round bottom flask. 150 ml of 70% ethanol was slowly poured from the upper mouth. Then it was fit with a condenser. The flask was heated with heating mantle. The solvent vapor reached the cylinders through the side tube and condensed on passing into the condenser. The condensed solvent dropped on the powder of medicinal plant and dissolved soluble compounds. The solution filtered and passed out back into the flask through the siphon tube. In this way, a continuous supply of solvent vapor was maintained top the cylinder and dissolved soluble compounds flows back to the flask. This process was allowed to run for 8 to 10 hours or till the colored solvent appeared in siphon.

**Removal of the solvent**

After the completion of the extraction process, the round bottom flask containing extract was poured in evaporating dish made up of porcelain. The shell of the dish was flat, so large liquid surface promoted the evaporation as it was constantly heated. Solvent was completely removed and collected in the bottom. The extract was weighed and noted. Then it was transferred in a bottle, labelled and kept in a refrigerator.

**Preparation of stock/ working solution**

One gram of the extract was transferred into 20 ml screw cap test tube. 9 ml of distilled water was added in it and made a homogeneous solution of 1 gram/ 10 ml or 100 mg/ml stock/ working solution.

**Collection of standard cultures**

Five different types of bacteria were selected; their cultures were obtained in a nutrient agar plate after incubation. Gram staining was done and they were grown in different media like: Mendo Agar, Mac conkey agar, Cetrimide agar, XLD, MSA, respectively for *Escherichia coli*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Salmonella Typhi*, and *Staphylococcus aureus*. They were also incubated in the biochemical test media and tested for their biochemical characteristics.

**Qualitative screening and determination of antimicrobial activity**

The crude extract of medicinal plant was screened for its antimicrobial activity. Sterile Muller Hinton agar plates were prepared. Before using the plates, they were dried under laminar airflow or incubation at 37°C for 30 minutes. The fresh bacterial culture comparable with turbidity standard 0.5 McFarland was prepared. Sterile cotton swab was dipped in to prepared inoculums, rotated and pressed against the upper walls of the tubes above the liquid culture. The plate was then rotated through an angle of 60° after each swabbing. Finally, the swab was passed round the edge of agar surface and left to dry. Then, 6m diameter's wells were made in media plates. 50 microliter of the working solution was transferred into the well. The solvent itself was also tested for its antimicrobial activity. The plates were left for half an hour. After diffusion, the inoculated plates were incubated at 37°C overnight. Then they were viewed for zone of inhibition, measured using a scale and the mean was recorded.

### Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The crude extracts which showed antimicrobial activity were subjected to two fourth serial dilution method of fine gold and barren to determine MBC. For each bacterium, a set of 11 dry screw cap test tubes were taken and labelled. Tube number 1 was as positive control and 11 as negative control. The total of 2 ml of plant extract was poured in all the tubes. This was carried up to the tenth tube. So two fourth dilution of the plant extract was prepared with equal volume but decreasing concentration. The first tube contains 2 ml of broth and extract, similarly second tube was homogenized and 2 ml; of this content was transferred to the third tube. no plant extract was added to the tube labelled as the positive control. Now with the help of a micro pipette, 50 ml of culture inoculation of test bacteria was prepared. All tubes were incubated at 37°C for 24 hours and observed for turbidity. The test results were interpreted on the basis of fact that the growth occurs in positive control and any other tube in which the concentration of the extract is not sufficient to inhibit the growth and the lowest concentration of agents that inhibits growth of organism, detected by lack of visible turbidity by inhibition of 99% is designed as the MIC. The MBC is identified by determining the lowest concentration of extract solution that reduces the viability of the initial bacterial inoculum by a predetermined reduction such as  $\geq 99.9\%$ . Tubes without visible turbidity were streaked on agar plate and observed for 99.9% killing.

#### Antibiotic susceptibility test

All the bacterial isolates which were employed in this study were subjected to in-vitro antibiotic susceptibility test by disc diffusion method of modified Kirby Bauer method as described by CLSI (2018) guideline. The antibiotic disc was amikacin, ampicillin, chloramphenicol, ciprofloxacin, gentamycin and penicillin G.

## RESULTS

Six different Shade dried parts medicinal plants viz. roots, rhizomes, leaves, stems and seed etc were subjected to continuous extraction with 70% alcohol for 8-10 hours by using soxhlet extractor to obtain crude 70% ethanol extracts of the respective plants. After the extraction was complete, the solvent was totally removed by evaporating

above 78.37°C for about 8 minutes using evaporating dish. Then percentage yields of the crude extracts were calculated. Aloe vera gave the highest yield of 38.33%, followed by *Acorus calamus* (33.41%), *Azadirachta indica* (32.15%), *Mentha arvensis* (30.56%), *Artemisia indica* (30.38%), *Zingiber officinale* (28.29%) and *Zanthoxylum armatum* (13.32%).

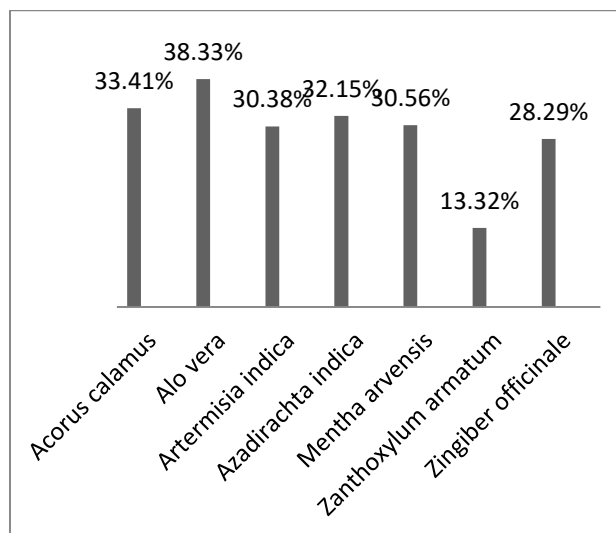


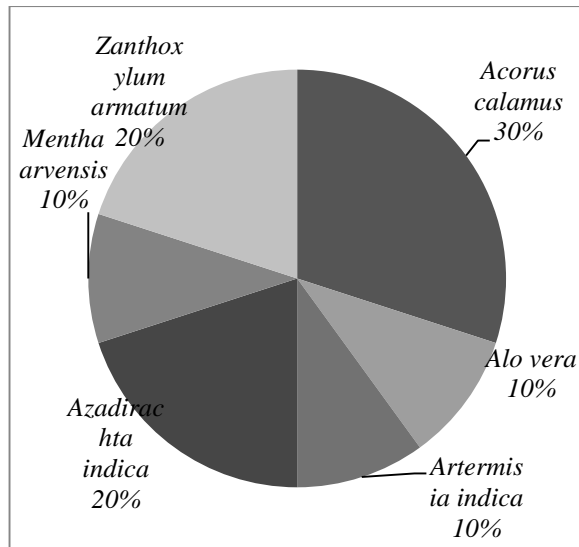
Figure 1: Percentage Yields of Crude Ethanol Extracts

#### Evaluation of Antimicrobial Activity

Antimicrobial activity was evaluated by two ways viz. measuring zone of inhibition and quantitative determination of plant extract for minimum bactericidal concentration (MBC). The diameter of zone of inhibition (ZOI) produced by plant extract on particular microorganism was measured for the estimation of potency of plant extract. Similarly, the minimum amount of the extract usually expressed in terms of microgram per milliliter of the bacterial broth solution required to inhibit the bacterial growth as well as to kill the bacteria altogether clearly expresses the better effectiveness of the extract against the corresponding bacteria.

The mean diameter of zone of inhibition and minimum bactericidal concentration of 70% ethanol extract of different medicinal plants which showed significant zone of inhibition ( $> 8\text{mm}$ ) during qualitative screening process (as indicated by + sign in table) are shown in the Tables 1.

The antimicrobial activity of Medicinal Plant Extracts *Acorus calamus* was shown by 30%, followed by *Azadirachta indica* and *Zanthoxylum armatum* (20%), *Mentha arvensis*, *Aloe vera* and *Artemisia indica* was shown 10% against the test isolates



**Figure 2:** Antimicrobial activity of Medicinal Plant Extracts among the test isolates

The MBC values obtained for *E. coli* was above 50 µg/ml with *Zanthoxylum armatum* (Timur). *Pseudomonas aeruginosa* showed wide range of MBC ranged from 13.63 µg/ml with *Zanthoxylum armatum* (Timur) to 63.33 µg/ml with *Azadirachta indica*.



**Photograph 1:** ZOI produced by different plant extracts against *P. aeruginosa* in agar well diffusion method. Top left: *Acorus calamus* Top right: *Azadirachta indica* and Bottom: *Mentha arvensis*.

With *Acorus calamus*, it gave MBC of 25 µg/ml. Among all, the widest range of MBC was shown by *Staphylococcus aureus* (15.63 µg/ml to above 50 µg/ml). *Acorus calamus* gave MBC of 31.25 µg/ml, *Aloe vera* and *Zanthoxylum armatum* gave MBC above 50 µg/ml, *Artemisia indica* gave

MBC of 15.63 µg/ml and *Azadirachta indica* gave MBC of 25 µg/ml against *Staphylococcus aureus*. Lowest value of MBC was given by *Mentha arvensis* against *Pseudomonas aeruginosa* (13.63 µg/ml).

## DISCUSSION

Nepal is a developing country where more than 70% of the population rely directly or indirectly on agriculture and other natural resources for their food. Income and existence including medicinal plants for the treatment of various disease. In Nepal, >40% of the total population is below poverty line. This further accentuates their dependence of herbal medicines. Nepal is botanically very rich with nearly 7000 species of vascular plants and 4500 species of non-vascular plants reported till date. The distribution is approximately 49.2%, 53.96%, 35.7%, 18.09% and 7.4% respectively in tropical, subtropical, temperate, sub alpine and in alpine zones (Malla and Shakya 2006/2007).

Herbal medicine has formed the core of disease treatment in Nepal since ancient time. From this point of view, it seems high time that an extensive study is necessary to be carried out to ascertain the therapeutic value of different medicinal plants. Moreover, there is need of proper documentation, observation and conservation of the useful species.

The aim was to explore the efficacy of various medicinal plants against different microorganisms and to verify various claims regarding the same. In line with professed objective of this study only those plants were selected which were believed to be used for different common diseases like diarrhea and dysentery fever boils wound and ulcer intermittent fever Bronchitis and lungs disease etc. About 7 plants were selected. Herbarium specimen were prepared on the spot and identification was done. Samples were labelled properly and left for drying. The plants need to be chopped down before drying and that the dried sample wood ground to find powder consistency. Rabe and var standen (1997) found that the majority of antibacterial activity was present in alcohol rather than aqueous extracts. Alcohol has been found to be good cheap and all-purpose solvent for preliminary extraction. Ethanol extracts of *Adhatoda vasica* possess significant antimicrobial activity than aqueous extract of the same plant. It was found that for the same plant different solvents gave different percent yields.

**Table 3:** Evaluation of antimicrobial activity diameter of zone of inhibition (ZOI) and minimal bactericidal concentration (MBC) given by *different extract* against test bacteria

Test Organisms	<i>Acorus calamus</i>		<i>Aloe vera</i>		<i>Artemisia indica</i>		<i>Azadirachta indica</i>		<i>Mentha arvensis</i>		<i>Zanthoxylum armatum</i>		<i>Zingiber officinale</i>	
	ZOI	MBC	ZOI	MBC	ZOI	MBC	ZOI	MBC	ZOI	MBC	ZOI	MBC	ZOI	MBC
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	8	>50	-	-
<i>K. oxytoca</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. aeruginosa</i>	12	25	-	-	-	-	9	>50	10	13.63	-	-	-	-
<i>S. Typhi</i>	8	>50	-	-	-	-	-	-	-	-	-	-	-	-

ZOI expressed in mm and MBC in µg/ml.

Aloe Vera give the highest yield of 38.33% , followed by *Acorus calamus* 33.4 1%, *Azadirachta indica* 32.15%, *Mentha arvensis* 30.56%, *Artemisia indica* 30.38%, *Zingiber officinale* 28.29% and *Zanthoxylum armatum* 13.32%. Indeed, these remarkable difference in total sales may be due to various factors such as part of plant material, consistency of powder, time of extraction, extent of dryness are of prime importance. Plant material with large and thick leaves gives larger yield. Where older and right plants give less in comparison with younger plant materials. In this research, thin slices of Aloe Vera leaves were used raw without drying so it gave the highest yield. *Zanthoxylum armatum* gave the lowest yield, its seed which was highly sun dried was used for extraction. Also if extracting solvent is not completely removed, there are chances of obtaining high value of yield.

Total of five bacterial species were chosen to include those which have more prevalent pathogenicity. The bacteria chosen cause common diseases like respiratory tract disease diarrhea, enteric fever, nosocomial infection, septicemia, wound infection, urinary tract infection, gallbladder infection, appendicitis, peritonitis, bacteremia, meningitis, pyogenic infections, food poisoning etc. Test bacteria included gram positive *Staphylococcus aureus* and Gram Negative *Klebsiella oxytoca*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella Typhi*. These bacteria included active motile pathogens, and commensals. This bacterial species was isolated from pus, blood stool and

water samples at microbiology laboratory, Pinnacle college. Collected organisms were tested for purity. M Endo Agar was used for *E coli* mannitol salt agar was used for *S. aureus*, also Cetrimide Agar was used for *P. aeruginosa*, XLD agar was used for *S. Typhi* and Mac conkey Agar was used for *K. oxytoca*.

In this study microbiological activity of 70% ethanol extract 7 different medicinal plants were tested against by bacterial species using Agar well diffusion method in MHA and nutrient Agar. After overnight incubation at 37°C, the plants were examined for clear zone of inhibition more than 8 mm which is considered as positive and lesser is considered as negative. During this study, it was found that *Acorus calamus* (bojho) showed zone of inhibition against *S. aureus*, *P. aeruginosa* and *S. Typhi* the plant was found in effective against *E. coli* and *K. oxytoca*. However, zone of inhibition for *S. Typhi* was 8 mm in diameter. So it was considered as intermediate in the study according to Mahesh Baidhya *et al* (2012) ethanolic extracts of *Acorus calamus* demonstrated antimicrobial activity against *S. aureus* and *P. aeruginosa* but not against *S. Typhi*. This study contradicted his results with *S. Typhi* only. Meanwhile aloe vera produced zone of inhibition against *Staphylococcus aureus* only. Zone of inhibition was 12 mm in diameter. Kaveri (2013) show that aloe vera had the maximum inhibitory activity against *E. coli* and moderate inhibitory activity against *Klebsiella* species. This result does not support the result of our study. *Artemisia Indica* (tite pate) was also found to have antimicrobial activity against *Staphylococcus aureus* only

with 9mm ZOI. *Azadirachta Indica*(Neem) producer zone of inhibition against to best bacteria *P. aeruginosa* and *S. aureus* with ZOI of 9mm and 10mm respectively. Results of Challa (2013) suggest that Aqueous extracts of *Azadirachta Indica* leaf and bark exhibit high antimicrobial activity against *P. aeruginosa* and *S. aureus* and this result also supports the result obtained in our study. *Mentha arvensis* (pudina) was found to have antimicrobial activity against *P. aeruginosa* only with ZOI of 10 mm in diameter. *Zanthoxylum armatum* (Timur) give the lowest yield of 13.3 2% among all the medicinal plants used in study full stop however it produced a ZOI with equal and *S. aureus* of 8mm and 15 mm in diameter. Essential oil of *Z. armatum* also exhibited moderate antimicrobial activity. Results of Bhandari (2012) shows that Gram Positive Bacteria like *S. aureus* are more sensitive to *Zanthoxylum armatum* Gram Negative bacteria. This finding is consistent with the result obtained from our study.

*Zingiber officinale* (ginger) did not show zone of inhibition with any of the test bacteria. So it was found to be the medicinal plant having least antimicrobial property among 7. The finding of Shahidul (2014) showed a potential antimicrobial activity of ginger which contradicts our result. Antimicrobial substance supplied in the agar well diffuses readily in concentric circles and inhibits or kills the microorganisms that are susceptible. This effect manifested by ozone of clearing is observed up to a point where the decreasing concentrations of the diffusing antimicrobial substance is still sufficient to inhibit or kill the organism. Beyond the point the concentration is insufficient and hence growth starts. Thus by measuring the zone of inhibition of the antimicrobial for various organisms we can estimate the degree of susceptibility or sensitivity.

In this experimental study, *E. coli* was inhibited by *Zanthoxylum armatum* to small extent. All other plant extracts failed to produce zone of inhibition with *E. coli*. *Zanthoxylum armatum* produced ZOI for 8 mm against *E. coli*. *K. oxytoca* was found to be the most resistant test bacteria as ZOI was not observed with any of the plant extracts used in this study.

Three plant extracts showed positive result against opportunistic pathogen *Pseudomonas aeruginosa* are maximum of 12mm was recorded with *Acorus calamus*. *Mentha arvensis* stands next to *Acorus calamus* (9mm) and

*Azadirachtha indica* (9mm). this showed that the antimicrobial activity of the *Acorus calamus* is greatest against *P. aeruginosa* than any other plant extract used in the experimental study. Similarly experiment with *S. typhi* pointed out towards its significant resistance. Only one plant extract could destroy the defense mechanism of bacilli to some extent. *Acorus calamus* developed zone of inhibition of 8mm diameter against *S. Typhi*, so it was considered as intermediate in the study. Only Gram positive cocci used in the study, *S. aureus* gave ZOI with all the plant extract except, *Mentha arvensis* and *Zingiber officinale*. Highest value of ZOI of 15 mm was observed with *Zanthoxylum armatum* followed by *Acorus calamus* (12mm), *Aloe vera* (10 mm), *Azadirachtha indica* (10 mm) and *Artramecia indica* (9mm).

Analysis of different plant extract showed that *Zingiber officinale* (ginger) had no activity against any test organism. *Acoruscalamus* (bojho) showed antimicrobial activity against three out of five test organism. Neem and timur showed antimicrobial activity against two out of five test organism. Lastly, *Aloe vera* and *Artramecia indica* showed antimicrobial activity against only one organism. The largest ZOI was obtained with *Zanthoxylum armatum* (timur) against *S. aureus*. The smallest ZOI was obtained twice with *Acorus calamus* (bojho) against *S. typhi* and *Zanthoxylum armatum* (timur) against *E. coli*. The diameter of zone of inhibition produced depends on different factors broadly classified as intrinsic and extrinsic factors. Extrinsic parameters like pH of the medium, period and temperature of incubation, volume of the well, concentration of the plant extract and size of the inoculum can be fixed and standardized during the experiment. Hence, no error results due to extrinsic parameters occurred during the experiment. However, intrinsic factors such as nature of medicinal plants, including its component, solubility and diffusing properties are pre-determined.

Due to variable diffusibility the antibacterial with very high potency may not demonstrate ZOI commensurate to its efficiency. Therefore, minimum bactericidal concentration (MBC) values have been computed here by to fold serial dilution method MBC is the lowest concentration of antimicrobial substance required to produce a sterile culture (Cheeseberg 2013).

The bacteria were inoculated in a series of tubes with

decreasing concentration of antimicrobial substance. After proper incubation the results were compared with positive and negative growth control tubes. The tubes which showed no growth hair subculture onto nutrient Agar devoid of antimicrobial substance. The minimum concentration which failed to show growth in nutrient Agar plate was taken as MBC value.

The MBC values obtained for *E. coli* was above 50 µg/ml with *Z. armatum* (timur). *P. aeruginosa* showed wide range of MBC range from 13.6 3 µg/ml with Timur to 63.33 microgram/ml with *Azadirachta indica*. With *Acorus calamus* it gave MBC of 25 µg/ml. Among all the widest range of MBC were shown by *S. aureus* 15.6 3 microgram per ml to 50 micrograms per ml. *Acorus calamus* gave MBC of 31.25 µg/ml, aloe Vera and *Z. armatum* gave MBC above 50 µg/ml, *Artemisia indica* give MBC of 15.63 to µg/ml and *Azadirachta indica* give MBC of 25 µg/ml against *S. aureus*. Lowest value of MBC was given by *Mentha arvensis* against pseudomonas aeruginosa. All the bacteria employed in the study were also subjected to antibiotic sensitivity test on MHA played by disc diffusion method. Antibiotic disc was chosen according to their clinical uses against the bacteria (Cheeseberg 2013) and their sensitivity patterns. After incubation at 37°C for 18-24 hours, ZOI of each disc was measured and bacteria was classified as sensitive, resistant or intermediate by referral to the standard chart of antibiotic susceptibility test. *E. coli* was found to be sensitive to all three antibiotic. Diameter of zone of inhibition was maximum for gentamicin i.e. 26 mm and minimum for chloramphenicol i.e. 19mm. Gram Negative *K. oxytoca* was found to be sensitive to penicillin G and intermediate to Ampicillin. but it was found to be resistant to chloramphenicol. Gram Negative *P. aeruginosa* was found to be sensitive to only Amikacin. It was resistant to ampicillin and penicillin G. Gram Negative *S. Typhi* was found to be sensitive to ciprofloxacin and gentamicin but resistant to ampicillin. Diameter of zone of inhibition was maximum for ciprofloxacin i.e. 30 mm. Gram Positive *S. aureus* was found to be sensitive to ciprofloxacin and amikacin but resistance to penicillin G. The diameter of ZOI was maximum for ciprofloxacin i.e. 30 mm.

## CONCLUSION

Altogether seven different medicinal plants were selected on the basis of their use for common diseases and indigenous

ethobotanical knowledge. Different parts of these plants were taken for extraction with 70% ethanol to assay their antimicrobial property, if present using soxhlet extractor. Leaves required longer time for complete extraction in comparison with the extraction from seeds. *Aloe vera* had the highest percent yield of 38.33% followed by *Acorus calamus* (33.41%), *Azadirachta indica* (32.15%) *Mentha arvensis* (30.56%) *Artemisia indica* (30.38%) *Zingiber officinale* (28.29%) and *Z. armatum* (13.32%).

The crude extracts were then tested for antimicrobial activity against 5 microorganisms that include Gram positive *S. aureus* and gram negative *E. coli*, *P. aeruginosa*, *K. oxytoca* and *S. Typhi*. Both the zone of inhibition (ZOI) and minimum bactericidal concentration (MBC) values were determined by agar well diffusion method and two-fold serial dilution method respectively. Only those extracts which produced ZOI were assayed for MBC.

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## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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