Screening and Evaluation of Antimicrobial Activity of Medicinal Plants Collected from Lalitpur District

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ABSTRACT

Objectives: To screen and evaluate antimicrobial activity of crude ethanol extracts of medicinal plants against various bacteria.

Methods: The study was conducted from February - August 2018 in Pinnacle college, Lalitpur with plan to collect Seven different herbs species; *Acorus calamus* (Bojho), *Aloe vera* (Ghiu Kumari), *Artermisia indica* (Titepate), *Azadirachta indica* (Neem), *Mentha arvensis* (Pudhina), *Zanthoxylum armatum* (Timur) and *Zingiber officinale* (Ginger) were subjected to 70% ethanol soxhlet extraction, then extract then heated at 78.37^{0}C to remove ethanol, working solution was prepared in DMSO. Test organisms included mainly enteric isolates i.e. *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Salmonella* Typhi and *Staphylococcus aureus* were selected. The Antibiotic Susceptibility tests of isolates were done by of Modified Kirby Bauer disc diffusion method. The antimicrobial activities of the extracts were determined by Agar well diffusion technique. Minimum Bactericidal Concentration (MBC) was determined by dilution technique.

Results: Among 7 plants that were tested, 6 plants were found to have activity against test bacteria. *Acorus calamus* 3/5(60%) was effective against test bacteria. *Azadirachta indica* and *Menthe arvensis* inhibited 2/5 (40%). *Aloevera* and *Artermisia indica* were effective against *S aureus* only 1/5(20%) and *Zingiber 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 *Acorus calamus*, *Azadirachta indica* and *Mentha arvensis.* The largest ZOI of 15 mm was obtained with *Zanthoxylum armatum* acting upon *S. aureus*. While, the smallest diameter of 8 mm was showed by *Acorus calamus* against *S.* Typhi. The lowest MBC was given 15.63 mg/ml by *Mentha arvensis and Azadirachta indica* against *Pseudomonas aeruginosa* while, *Artermisia indica* against *S. aureus* **.**

Conclusion: Medicinal plants should be possible medication in the future to combat pathogens.

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

INTRODUCTION

Antimicrobial treatments derived from traditional medicine have been utilized for a long time to treat a wide range of illnesses in many different cultures (Maharjan et al. 2012). Traditional medicine's beliefs and practices are still employed in global healthcare as alternatives to modern treatments and as sources of inspiration for contemporary medication research, even if not all of its therapies have been scientifically confirmed to be successful. The increasing development

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of antibiotic resistance and the limitations of traditional antimicrobial drugs have made the search for alternative sources of antimicrobial agents important. Researchers are trying to produce more potent herbal medicines to treat illnesses that are resistant to several drugs because of the short half-lives of newly created antibiotics. Because medicinal plants contain a broad variety of bioactive compounds, they have historically been utilised to treat microbial infections (Sapkota et al. 2020; Khan et al. 2017; Bayoub et al. 2010).

The abundance of active chemicals produced by plants, the majority of which most likely originated as chemical defences against contact or predation, has made them a rich source of medicinal materials. The majority of plants have one or more of the following therapeutic qualities: they are sedatives, laxatives, cardiotonic, diuretics, antibacterial, antifungal, antihelminthic, and anticancer (Bayoub et al. 2010). It was often thought that the active chemical components found in various plant portions were what gave plants their therapeutic qualities (Maharjan et al. 2012). Since the discovery of penicillin and streptomycin, which marked the beginning of the antibiotic era, other antibiotics derived from microbes have been identified. It is surprising how little study has been paid to the antibiotic properties of higher plants. The limited notion that antibiotics can only be derived from microbes is the reason behind this. However, the way that antibiotics are defined now has motivated many researchers to look for these qualities in plants. The active ingredients or secondary plant products have a therapeutic impact on both humans and animals. Alkaloids, glycosides, isoflavonoids, cumarins, terpens, phenolic compounds, saponins, essential oils, mucilage, tannins, bitter principles, etc. comprise the primary group of active ingredients (Khan et al. 2017; Sapkota et al. 2020; Maharjan et al. 2012).

These active ingredients may often be extracted using a variety of solvents. Antimicrobials are active substances that either suppress or kill microbes. To varying degrees, several types of therapeutic plants might contain such. Most of these antimicrobial compounds found in medicinal plants may be extracted using alcohol (Khan et al.2017). In their natural or refined form, raw herbal extracts are useful in the treatment of many human diseases. Particularly in the Ayurvedic system, they are used to treat a wide range of bacterial and fungal illnesses, including infected wounds, diarrhoea, dysentery, vomiting, fever, coughing, and skin itching (Purkayastha & Dahiya 2012). Herbal items have long been utilised as a cure for a variety of illnesses in Nepal as well (Shrestha 2020). Screening of medicinal plants for antimicrobial activities has so far yielded any drug of high therapeutic value like the antibiotics from microbial sources. Some plant compounds, such as quinine, berberine, and conessine, have been used for a long time to treat malaria, gastroenteritis, and amoebiasis, respectively, demonstrating their therapeutic effectiveness (Fernandes et al. 2007; Maharjan 2012). This research aims to confirm whether or not the traditional uses of medicinal herbs in rural regions have antibacterial activity. However, proving activity in a bioassay is unavoidably the first step towards developing a new medication (Paul and Balick 2012). Therefore, this study is an initial step towards examining the antibacterial activity of medicinal herbs as reported by their users.

MATERIALS AND METHOD

Collection of samples: Seven different medicinal plants which either includes roots /rhizomes /aerial parts (stems /leaves), seeds and fruits were collected from different regions of Lalitpur and process in microbiological lab of Pinnacle College in Febuary – August 2018 .

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 (HMG 2015). 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 (Mistry et al. 2014; Maharjan et al. 2012; Sapkota et al. 2020).

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 (Rajendhran et al. 2008; Tewari et al. 2012; Maharjan et al. 2012; Sapkota et al. 2020).

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 at 78.37^{0}C. 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 (maharjan et al. 2012). Percentage yield was calculated using following formula

$$Percentage\ yield\ (\%) \frac{Weight\ of\ extract \times 100\%}{Weight\ of\ dried\ plant\ part}$$

Preparation of stock/ working solution: One gram of the dried extract was dissolved in 1 ml of 10% dimethyl sulphoxide (DMSO) and made a homogeneous solution of 1gram/ ml or 1000 mg/ml stock/ working solution (Purkayastha & Dahiya 2012; Maharjan et al. 2012).

Collection of standard cultures: Five different types of bacteria were selected; *Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa, Salmonella* Typhi, and *Staphylococcus aureus*. These test bacteria were also confirmed by their Gram staining, culture on selective media characteristics, biochemical characteristics (Cheesbrough 2013).

Qualitative screening and determination of antimicrobial activity: The crude extract of medicinal plant was screened for its antimicrobial activity. The fresh test bacterial culture comparable with turbidity standard 0.5 was swabbed on MHA agar surface and left to dry. 50 µl 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^{0}C overnight. Then they were viewed for zone of inhibition, measured using a scale and the mean was recorded (Bayoub et al. 2010, Mahrjan et al. 2019, Purkayastha & Dahiya, 2012).

Determination of Minimum Bactericidal Concentration (MBC): The crude extracts which showed antimicrobial activity were subjected for MIC and MBC by broth dilution technique where the stocks of 1000 mg/ml (tube no 1) of the extracts were resuspended again in 10% DMSO and final concentration of tube no 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 becomes 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.92 µg/ml. Each dilution was seeded with bacterial suspension (1×10^8 cfu/ml) and incubated for 24 h at 37° 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 ≥ 99.9%. Tubes without visible turbidity were streaked on agar plate and observed for 99.9% killing (Maharjan et al. 2019; Bayoub et al. 2010).

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 discs were amikacin (30 mcg), ampicillin (10 mcg), chloramphenicol (30 mcg), ciprofloxacin (5 mcg), gentamicin (10 mcg) and penicillin G (10 mcg).

RESULTS

Seven 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. Then percentage yields of the crude extracts were calculated. Aloe vera gace the highest yield of 38.33%, followed by *Acorus calamus* (33.41%), *Azadirachta indica* (32.15%), *Mentha arvensis* (30.56%), *Artermisia indica* (30.38%), *Zingiber officinale* (28.29%) and *Zanthoxylum armatum* (13.32%).

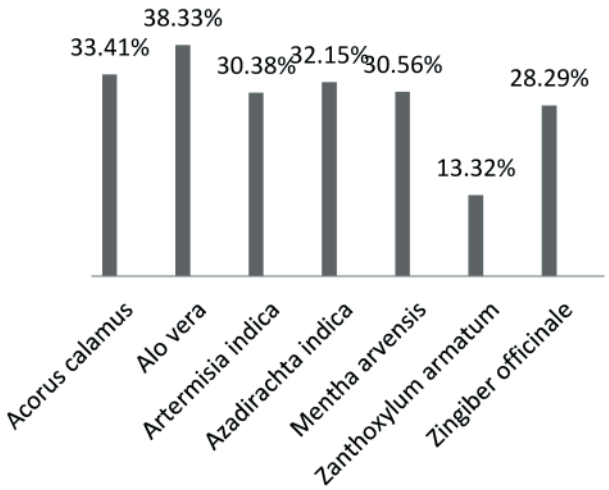

Figure 1: Percentage Yields of Crude Ethanol Extracts

Evaluation of Antimicrobial Activity: 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 (>8mm) during qualitative screening process (as indicated by + sign in table) are shown in the figure 2.

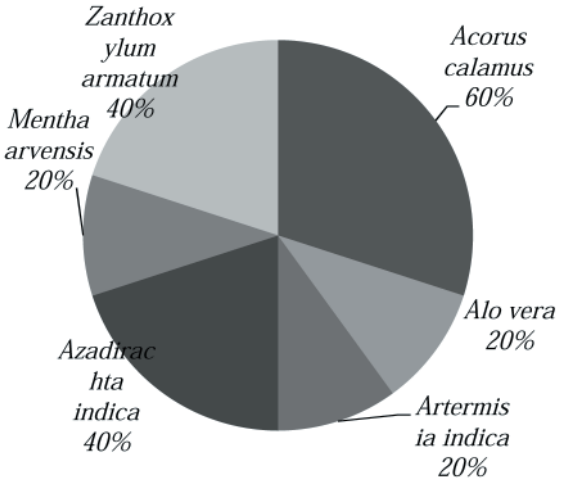

Figure 2 : Antimicrobial activity of Medicinal Plant Extracts among the test isolates

Acorus calamus exhibited 60% antibacterial activity against the five test isolates, followed by *Azadirachta indica* and *Zanthoxylum armatum* (40%), *Mentha arvensis, Aloe vera,* and *Artermisia indica* (20%). *Zanthoxylum armatum* produced MBC values of 62.5 mg/ml against *S. aureus* and *E. coli*. Similarly, MBC 15.63 mg/ml against *P. aeruginosa* and 31.25 mg/ml against *S. aureus* were demonstrated by *Azadirachta indica*.

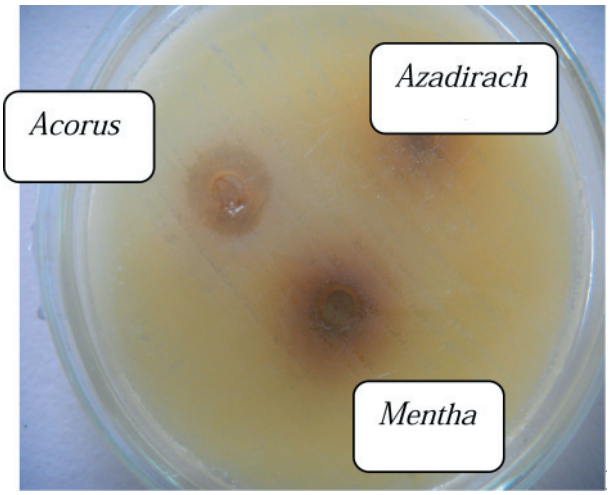

Figure 3: ZOI produced by different plant extracts against *S*. Typhi in agar well diffusion method.

MBC values for *Acorus calamus* were 31.25 mg/ml for *Pseudomonas aeruginosa* and *S. aureus* and 62.5 mg/ml for *Salmonella* Typhi. Similarly, *Mentha arvensis* administered MBC (15.63 mg/ml) against *Pseudomonas aeruginosa*. For *S. aureus*, *Aloevera* provided MBC greater than 62.5 mg/ml. Furthermore, against *S. aureus*, *Artermisia indica* produced MBC of 15.63 mg/ml. Whereas, MBC was obtained against every test isolate with no inhibitory effect.

Table 1: Evaluation of Antimicrobial Activity Diameter of Zone of Inhibition (ZOI) and Minimal Bactericidal Concentration (MBC) given by *different extract* against Test Bacteria.

Test Organisms	*Acorus calamus*		*Aloevera*		*Artermisia indica*		*Azadirachta indica*		*Mentha arvensis*		*Zanthoxylum armatum*		*Zingiber officinale*	
	ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)
Escherichia coli	–	–	–	–	–	–	–	–	–	–	8	62.5	–	–
Klebsiella oxytoca	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pseudomonas aeruginosa	12	31.25	–	–	–	–	9	15.63	10	13.63	–	–	–	–
Salmonella Typhi	8	62.5	–	–	–	–	–	–	–	–	–	–	–	–
Staphylococcus aureus	12	31.25	10	62.5	9	15.63	10	31.25	–	–	15	62.5	–	–

DISCUSSION

Since ancient times, herbal therapy has been the mainstay of disease treatment in Nepal (Shrestha et al. 2020). From the perspective presented in the discussion above, it appears that a thorough investigation is required to determine the therapeutic efficacy of various medicinal plants. Furthermore, accurate recording, observation, and protection of the valuable species are required.

Therefore, this study was conducted to investigate and validate claims about the effectiveness of different medicinal herbs against different bacteria. As stated in the study's stated goal, only those plants were chosen that were thought to be effective in treating a variety of common illnesses, including dysentery, diarrhoea, fever, boils, wounds, ulcers, intermittent fever, pneumonia, and lung conditions. They chose around seven plants. Identifiability was completed and herbarium specimens were prepared immediately. Following accurate labeling, the samples were set aside to dry. To get a powder consistency, the dried sample must be processed after the plants have been cut down. Alcohol had more antibacterial action than aqueous extracts, according to research by Rabe and Van (2007).

It has been discovered that alcohol is an excellent, affordable, all-purpose solvent for first extraction. *Adhatoda vasica* ethanol extracts have significantly more antibacterial activity than the plant's aqueous extract. It was discovered that various solvents produced varying percent yields for the same plant. A similar study by Bayoub et al. (2010) shown that thirteen distinct ethanol plant extracts had varying degrees of antibacterial activity against *L. monocytogenes*. In this study, 70% ethanolic crude extract gave antimicrobial activity against gram positive and gram negatives test bacteria.

Aloe vera yields 38.33%, which is more than any other plant. *Acorus calamus* comes in at 33.41%, *Azadirachta indica* at 32.15%, *Mentha arvensis* at 30.56%, *Artemisia indica* at 30.38%, *Zingiber officinale* at 28.29%, and *Zanthoxylum armantum* at 13.32%. It is possible that a variety of factors, including the amount of plant material, the powder's consistency, the extraction period, and the degree of dryness, contributed to the notable variation in overall yields. A higher yield is produced by plant material with big, thick leaves. In this study, thin slices of *Aloe Vera* leaves were employed raw without drying, giving the best output. However, compared to younger plant materials, older plants generate a lower yield. *Zanthoxylum armatum* give 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.

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 37^0C, the plants were examined for clear zone of inhibition more than 8 mm which is considered as positive and lesser is considered as negative. In this study, *Acorus calamus* (bojho) showed zone of inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* Typhi. The extreact plant was found ineffective against *E. coli* and *Klebsiella* oxytoca. However, zone of inhibition for *Salmonella* Typhi was 8 mm in diameter. So it was considered as intermediate. According to study conducted by Baidhya et al. (2012) ethanolic extracts of *Acorus calamus* demonstrated antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* but not against *Salmonella* Typhi. This study contradicted his results with *Salmonella* Typhi only. Meanwhile *Aloevera* 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* spp. This result does not support the result of our study.

Artemisia indica (tite pate) was also found to have antimicrobial activity against S*taphylococcus aureus* only with 9mm zone of inhibition. *Azadirachta indica* (Neem) producer zone of inhibition against to best bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* with zone of inhibition of 9mm and 10mm respectively. Results of Challa (2013) suggest that Aqueous extracts of *Azadirachta Indica* leaf and bark exhibit high antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and this result also supports the result obtained in this study. *Mentha arvensis* (pudina) was found to have antimicrobial activity against *Pseudomonas aeruginosa* only with zone of inhibition of 10 mm in diameter.

Two methods were used to assess antimicrobial activity: quantifying the zone of inhibition and determining the minimum bactericidal concentration (MBC) of plant extract. To assess the plant extract's efficacy, the width of the zone of inhibition (ZOI) it created on a specific microbe was evaluated. The higher effectiveness of the extract against the corresponding bacteria is also expressed clearly by the minimum amount of the extract, which is typically expressed in terms of micrograms per millilitre of the bacterial broth solution, needed to both inhibit and kill bacterial growth. Essential oil of *Z. armatum* also exhibited moderate antimicrobial activity. Results of Bhandari (2012) shows that Gram Positive Bacteria like *Staphylococcus 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. Study conducted by (Purkayastha & Dahiya 2012) also suggested that Neem, tulsi, and aloe vera ethanol extracts revealed flavonoids and tannins as the main active ingredients against methicillin-resistant *Staphylococcus aureus*.

The antimicrobial agent used in the agar well quickly diffuses in circles, inhibiting or killing the sensitive bacteria. Up to a point where the dispersing antimicrobial substance's reducing concentrations are still adequate to inhibit or kill the organism, this effect—represented by ozone of clearing—is observed. After that, the concentration becomes insufficient, at which point growth begins. Therefore, we may determine the level of susceptibility or sensitivity by evaluating the antimicrobial's zone of inhibition for different species. 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*. *Klebsiella 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.

A Study conducted by Sapkota et al. (2020) examined nine distinct medicinal plants and assessed for their antibacterial efficacy against ten different bacterial species by Among these, it was found that *Euphorbia hirta*, *Azadirachta indica, and Artemisia vulgaris* were efficient against gram-positive bacteria, which included Methicillin-resistant *Staphylococcus aureus* MRSA, *Bacillus subtilis*, and *Staphylococcus aureus*. The remaining six medicinal plants, however, were found to be ineffective against all microorganisms (*Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aerogenosa, Salmonella typhii, Shigella dysentriae*, MRSA.

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 *Azadirchtha indica* (9mm). This showed that the antimicrobial activity of the *Acorus calamus* is greatest against *Pseudomonas aeruginosa* than any other plant extract used in the experimental study. Similarly experiment with *salmonella* Typhi pointed out towards its significant resistance. Only one plant extract could destroy the defense mechanism of bacilli top some extent. *Acorus calamus* developed zone of inhibition of 8mm diameter against *Salmonella* Typhi, so it was considered as intermediate in the study. Only Gram positive cocci used in the study, all the plant extract except, *Mentha arvensis* and *Zingiber officinale* gave ZOI against *staphylococcus aureus*. 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 offficinale* (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 *Staphylococcus aureus.* The smallest ZOI was obtained twice with *Acorus calamus* (bojho) against *Salmonella typhi* and *Zanthozylum armatum* m (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, and concentration of the plant extract and size of the inoculums 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 two fold serial dilution method MBC is the lowest concentration of antimicrobial substance required to produce a sterile culture (Cheeseberg 2013).

The bacteria was 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.

Zanthoxylum armatum (Timur) demonstrated MBC values of 62.5 mg/ml against *S. aureus* and *E. coli*. Comparably, *Azadirachta indica* demonstrated MBC of 15.63 mg/ml and 31.25 mg/ml, against *Pseudomonas aeruginosa* and *S. aureus* respectively. MBC values for *Acorus calamus* against Salmonella Typhi were 62.5 mg/

ml, while MBC values for *Pseudomonas and S. aureus* were 31.25 mg/ml. Likewise; *Mentha arvensis* administered MBC against *Pseudomonas aeruginosa* (15.63 mg/ml). *Aloe vera* gave MBC above 62.5 mg/ml against *S. aureus.* And, *Artermisia indica* gave MBC of 15.63 mg/ml against *S. aureus*. Wheareas, no inhibitory effect and MBC was obtained against all test isolates. Mistry et al. (2014) has also shown the leaf extract of Neem is very effective against *S. aureus* with MIC value of 125 µg. Similarly, Sapkota et al. (2020) also reported minimum bactericidal concentration (MBC) of *Euphorbia hirta* against *Bacillus subtilis* and *Staphylococcus aureus* was found to be 12.5mg/ml while MBC of *Artemisia vulgaris* against *Bacillus subtilis* and MRSA was also found to be 12.5 mg/ml while of *Staphylococcus aureus* was 25mg/ml. Likewise, 25 mg/ml was *Azadirachta indica's* MBC against *Staphylococcus aureus*.

All the test 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. *E. coli* was found to be sensitive to all three antibiotics. Diameter of zone of inhibition was maximum for gentamicin i.e. 26 mm and minimum for chloramphenicol i.e. 19mm. Gram Negative *Klebsiella oxytoca* was found to be sensitive to penicillin G and intermediate to Ampicillin. But it was found to be resistant to chloramphenicol. Gram Negative *Pseudomonas aeruginosa* was found to be sensitive to only Amikacin. It was resistant to ampicillin and penicillin G. Gram Negative *Salmonella* 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*taphylococcus 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.

CONCLUSIONS

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 using soxhlet extractor with 70% ethanol to assay their antimicrobial property. 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%) *Artermisia indica* (30.38%) *Zingiber officinale* (28.29%) *and Zanthoxylum armatum* (13.32%). Similarly, The antimicrobial activity of Medicinal Plant Extracts *Acorus calamus* was shown by 60%, followed by *Azadirachta indica* and *Zanthoxylum armatum* (40%), *Mentha arvensis , Aloe vera* and *Artermisia indica* was shown 20% against the 5 tested isolates

The crude extracts were then tested for antimicrobial activity against 5 microorganisms that include Gram positive *Staphylococcus aureus* and gram negative *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Salmonella* Typhi. *Zingiber officinale* gave ZOI against *staphylococcus aureus*. 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).

Similarly, the lowest MBC was given 15.63 mg/ml by *Mentha arvensis and Azadirachta indica* against *Pseudomonas aeruginosa* while, *Artermisia indica* against *S. aureus.* Whereas, the highest the MBC values was obtained 62.5mg/ml for *Zanthoxylum armatum* (Timur) against *E. coli and S. aureus, Aloe vera* against *S. aureus. Acorus calamus* against *Salmonella* Typhi.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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