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Antibacterial activity and FTIR characterization of herbal plants collected from north-western Himalayas

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ABSTRACT: The present investigation was carried out to evaluate the antimicrobial impact of seven herbal plants extracts i.e., *curry* leaf (*Murraya koenigii*), *bana* leaf (*Vitex negundo*), *bhavadi* leaf (*Ocimum basilicum*), *umre* bark (*Ficus glomerata*), milk thistle seeds (*Silybum marianum*), *mulethi* root (*Glycyrrhiza glabra*), *amla* powder and *amla* juice (*Emblica officinalis*) against six different human pathogenic strains *viz. Shigella flexneri*, *Bacillus cereus*, *Stenotrophomonas maltophilia*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*. Different extracts were prepared by using the method of aqueous infusions, decoctions and juice. *Curry* leaves and milk thistle did not show any antimicrobial activity against any strains by well diffusion method as compared to other extracts used in the study. The varying outcomes observed in the antimicrobial activity of the different extracts could be attributed to the different functional groups like ester, alcohol, phenol, alkane, amino, amide, amines, carboxylic acid, ether present in these extracts, as indicated by the Fourier Transform Infrared Spectroscopy (FTIR) analysis. The presence of different functional groups in extracts may have contributed to the differences in efficacy against pathogenic strains. The presence of antibacterial effects of *bana*, *bhavadi*, *umre*, *mulethi* and *amla* suggests that it could be used against human pathogenic bacteria and to manage other diseases.

KEYWORDS: Antimicrobial; herbal extract; herbal plants; pathogens; FTIR; functional groups.

1. INTRODUCTION

India has identified over 2500 plants with medicinal properties. Herbal plants have been used in traditional medicine for many years. Herbal plants are extremely beneficial to various types of human diseases and have no negative side effects. Plants, particularly traditional, pharmacopeial drugs and various compounds, have a wide range of potential applications. This demonstrates a better understanding of the plant and the compounds derived from it. In the current situation, many plants have scientifically proven their herbal properties and significance [1]. Compounds known as antimicrobial agents have been proven successful in treating infections caused by pathogenic bacteria in both therapeutic and preventative contexts [2]. Infectious diseases account for 41% of all infections globally, making them a major public health concern [3].

Infectious disorders are mostly brought about by bacterial resistance to numerous antibiotics, which naturally arises because of the accumulation of diverse drug residues inside a single strain. Multidrug resistant bacteria can transmit harmful bacteria from person to person and cause treatment failure, both of which have medical and economic repercussions. Drug resistance has risen despite the fact that pharmaceutical corporations are developing new generations of antibiotics [4]. Additionally, administering antibiotics excessively and incorrectly has negative effects that might harm the immune system as well as vital organs including the liver, kidney, pancreas, and spleen [5]. As an alternative to conventional treatment, researchers are now concentrating on creating better-quality drugs with enhanced antimicrobial capabilities [6]. Since the dawn of time, honey has been used as medicine in various cultures that practice "Folk medicine [7]". Today, natural plant remedies like honey and others are employed as medications. Medical grade honey, which was formerly thought to be extinct, is now being employed more frequently as an antibacterial agent to treat infectious diseases [8]. Additionally, honey's antimicrobial, antiviral, anti-inflammatory, anti-tumor, and antioxidant capabilities have been proven [7,9,10,11]. Numerous studies support the systemic use of honey to treat bacteria that are drug-resistant [12] because they have less adverse

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effects than synthetic pharmaceuticals. Many herbal plants are employed as complementary therapy for both human and animal ailments. An understanding of the numerous functional groups crucial to the medicinal activities of phytochemical substances found in herbal plants will be provided by their chemical identification. Using infrared spectroscopy, [13] examined the *in vitro* effectiveness of bioactive extracts from 15 herbal plants against extended spectrum beta-lactamases (ESbetaL) producing multidrug resistant bacteria. Bioactive group screening of compounds in *Calotropis gigantea* dry leaf powder using FTIR analysis [14]. Bioactive plant extracts are promising source for the majority of drugs [15]. *Murraya Koenigii* (*Curry* leaves) were used as a spice for its characteristic flavor and aroma contains high antioxidants like tocopherol, β-carotene and lutein [16]. The whole plant is regarded as tonic and stomachic and has traditional uses [17]. *Ocimum basilicum* L. showed positive effects against viral, fungal, bacterial infections [18]. *Vitex negundo* L. extracts of the leaves have shown bactericidal and antitumor activity [19]. Decoction of their leaves may also improve eyesight [20]. The root of licorice (*Glycyrrhiza glabra* L.) belongs to the Fabaceae family, is widely used as sweetening and flavoring agent in food and tobacco industries but has also been proposed for various clinical applications [21]. *Silybin*, silibinin are major constituent of milk thistle with maximum amount of flavonolignan used particularly for liver disorders, which contains 90 per cent of herb's component in most preparations [22]. *Ficus glomerata* bark is antiseptic, antipyretic and vermicidal, and the decoction of bark is used in the treatment of various skin diseases, ulcers and diabetes [23]. *Emblica officinalis* (popularly known as amla or Indian gooseberry) has high medicinal as well as nutritional value due to its high vitamin C content of about 600 mg/100 g of fresh fruit [24,25]. They are useful in curing diseases like diabetes, cough, asthma, bronchitis, headache, ophthalmic disorders, dyspepsia, colic, flatulence, skin diseases, leprosy, jaundice, scurvy, diarrhea and greyness of hair [26].

In recent years, FTIR spectroscopy has grown in significance in the pharmaceutical industry. The FTIR spectrum has been used as a mandatory method to identify drugs in the pharmacopoeias of many nations. FTIR spectroscopy facilitates the interpretation of various vibrations and spectral bands due to the various functional groups in the sample [27]. The FTIR spectrum is frequently used by scientists to distinguish between species of plants and organisms [28,29]. It is also employed to identify the purity of herbal products in case of any adulteration and even assess the quality of herbal items. Carboxylic acids are potent antibacterial agents and have a significant role in the production of fat in the body. They serve as the main pharmacological ingredients in the management of specific human disorders [30]. Esters and volatile oils combined to produce fruit aroma. Some acids contain alkynes. They have antiviral, antifungal, and anticancer properties. Alkanes are found in the cuticle and epicuticular wax of numerous plant species.

The present study was therefore conducted to investigate the functional groups of herbal plants by FTIR and evaluate the antimicrobial impact of amla juice, aqueous infusions of *Murraya koenigii, Ocimum basilicum, Vitex negundo, Emblica officinalis* and decoctions of *Glycyrrhiza glabra, Silybum marianum, Ficus glomerata* against 6 different pathogenic strains viz. *Shigella flexneri* (MTCC1457), *Bacillus cereus* (MTCC1272), *Stenotrophomonas maltophilia* (MTCC4383), *Listeria monocytogenes* (MTCC839), *Escherichia coli* (MTCC443), *Staphylococcus aureus* (MTCC96).

2. RESULTS AND DISCUSSION

2.1 Comparative results of zone of inhibition against microbial pathogens by selected herbal plants in present study

Well diffusion method was used to assess the antibacterial activity of medicinal herbs (*Curry* leaf, *Bana* leaf, *Bhavadi* leaf, *Umre* bark, Milk thistle seeds, *Mulethi* root, *Amla* powder and *Amla* juice) against bacterial pathogens *S. flexneri* (MTCC 1457), *B. cereus* (MTCC 1272), *S. maltophilia* (MTCC 4383), *L. monocytogenes* (MTCC 839), *E. coli* (MTCC 443), and *S. aureus* (MTCC 96). The antimicrobial activity of the seven plant extracts against the test pathogenic microorganisms is shown in Table 1 and Figure 1. Aqueous infusions of *curry* leaves, *bhavadi* leaves, *bana* leaves, and *amla* powder were potentially effective with variable efficiency against the tested bacterial strains, whereas aqueous decoctions of *umre* bark, milk thistle seeds, *mulethi* root, and fresh *amla* juice were potentially effective with variable efficiency.

Zone of inhibition of herbal plants to six different pathogenic strains were compared as stated in Table 1 and Figure 1. *Amla* juice exhibited highest antimicrobial resistance against six pathogenic strains. The zone of inhibition of *amla* juice was recorded as 6.53±0.031 mm for *E. coli* (MTCC 443), 6±0.028 mm for *L. monocytogenes* (MTCC 839), 5.33±0.105 mm for *S. flexneri* (MTCC 1457), 4±0.109 mm for *B. cereus* (MTCC 1272), 4±0.027 mm for *S. aureus* (MTCC 96) and 3±0.036 mm for *S. maltophilia* (MTCC 4383). The zone of inhibition was highest for *E. coli* (MTCC 443) and *L. monocytogenes* (MTCC 839) strains.

Mulethi root inhibits all strains with a zone of inhibition of 6±0.031 mm in *E. coli* (MTCC 443), 5.66±0.036 mm in *S. maltophilia* (MTCC 4383), 5.33±0.028 mm in *L. monocytogenes* (MTCC 839), 5±0.109 mm in *B. cereus* (MTCC 1272), 3.52±0.027 mm in *S. aureus* (MTCC 96) and 2±0.105 mm in *S. flexneri* (MTCC 1457). These observations clearly shows that *mulethi* root has significant antibacterial activity and may be an excellent natural alternative preservative for controlling food poisoning.

Medicinal plant i.e., *Umre* bark also has high zone of inhibition in all strains, *viz.*, 6.33±0.105 in *S. flexneri* (MTCC 1457), 6.11±0.031 mm in *E. coli* (MTCC 443), 2.33±0.028 mm in *L. monocytogenes* (MTCC 839), 2.32±0.027 mm in *S. aureus* (MTCC 96), 1.25±0.109 mm in *B. cereus* (MTCC 1272), 1.15±0.036 mm in *S. maltophilia* (MTCC 4383). The zone of inhibition is highest in *S. flexneri* (MTCC 1457) and *E. coli* (MTCC 443). *Umre* bark was also resistant to all the pathogenic strains thus possessing good antimicrobial activity. The decoction of bark can be utilised for the development of herbal nutraceuticals that can provide a natural and plant- based approach to addressing microbial challenges, potentially supporting immune health.

On other hand, if we see aqueous extract of *amla* powder zone of inhibition is 5±0.105 mm in *S. flexneri* (MTCC 1457), 3.65±0.027 mm in *S. aureus* (MTCC 96), 3.33±0.109 mm in *B. cereus* (MTCC 1272), 2±0.036 mm in *S. maltophilia* (MTCC 4383) whereas no zone of inhibition is therein *L. monocytogenes* (MTCC 839) and *E. coli* (MTCC 443) strains.

The inhibition zone of *bhavadi* herbal plant is 2±0.027 mm in *S. aureus* (MTCC 96), 1.86±0.105 mm in *S. flexneri* (MTCC 1457) and 1±0.036 mm in *S. maltophilia* (MTCC4383) strains and no zone of inhibition against other remaining strains. The *bhavadi* herbal plant is less susceptible to antimicrobial activity**.** On the other hand, *bana* showed no zone of inhibition against five pathogenic strains except for *B. cereus* (MTCC 1272) as 1.00±0.109 zone of inhibition (mm).

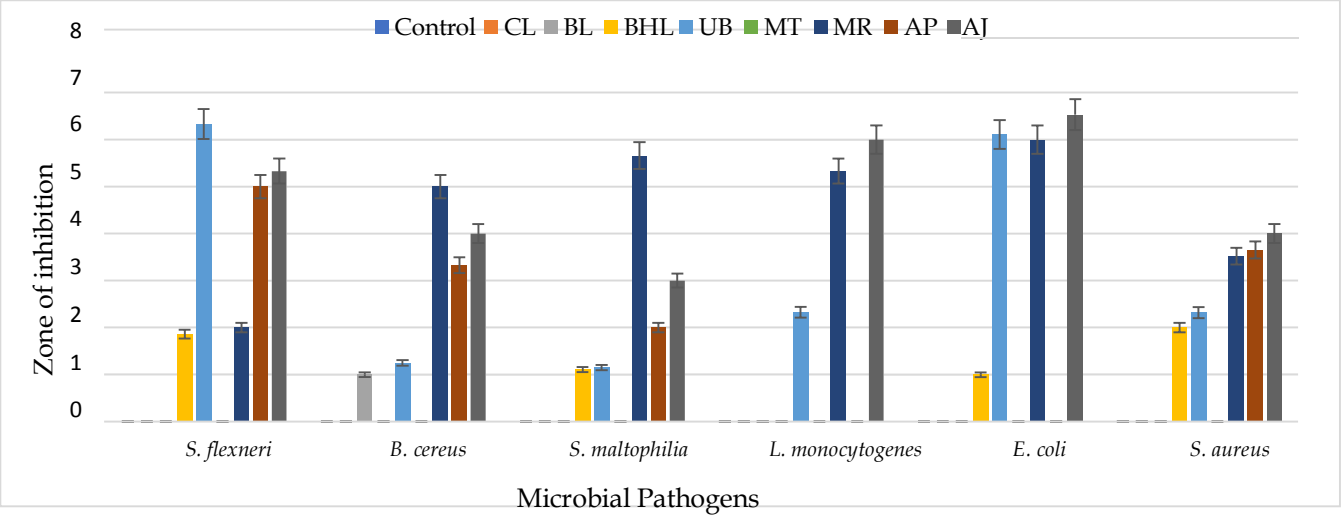


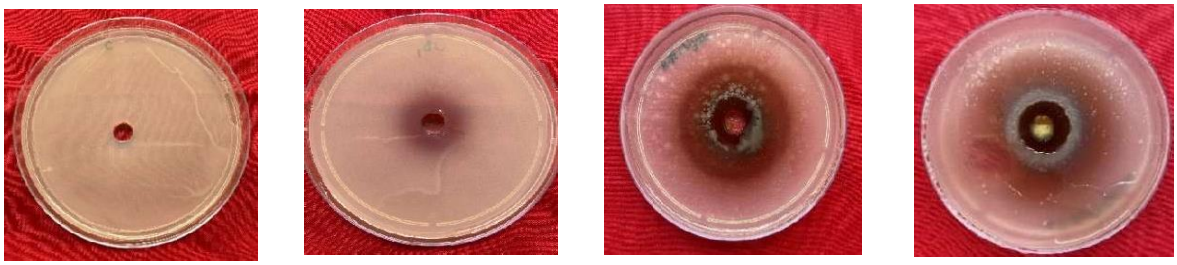
Figure 1. Comparative results of zone of inhibition (mm) against microbial pathogens by selected herbal plants
CL: *Curry* leaves (*Murraya koenigii)*, BHL: *Bhavadi* leaves (*Ocimum basilicum)*, BL: *Bana* leaves (*Vitex negundo)*, UB: *Umre* bark (*Ficus glomerata)*, MT: Milk thistle (*Silybum marianum)*, MR: *Mulethi* root (*Glycyrrhiza glabra)*, AP: *Amla* powder (*Emblica officinalis)*, AJ: *Amla* juice (*Emblica officinalis)*

In milk thistle extract, there is no zone of inhibition against all the strains tested in this study. Milk thistle decoction extract does not possess antimicrobial potential. Similarly, aqueous extract of *curry* leaves was found to have no antibacterial activity against different pathogenic strains. As it is clear from Figure 1, *Curry* leaves and milk thistle extracts do not show any antimicrobial activity. Figure 2 shows the antimicrobial activity of seven different herbal plants against *S. flexneri* (MTCC 1457) strain. The values of zone of inhibition (mm) are given in Table 1. The zone of inhibition was highest in *umre* bark followed by *amla* juice against this strain.

Among the seven different herbal plants tested for their antimicrobial activity against *B. cereus* (MTCC 1272) strain, it was found that *mulethi* root displayed the highest zone of inhibition as depicted in Figure 3. Microbial activity of seven different herbal plants against *S. maltophilia* (MTCC 4383) strain showed in Figure 4. The values of zone of inhibition (mm) are given in Table 1. The zone of inhibition was highest in *mulethi* root followed by *amla* juice and *amla* powder against this strain.

Table 1. Comparative results of zone of inhibition against microbial pathogens by selected herbal plant

Samples	Zone of inhibition (mm)					
	S. flexneri (MTCC 1457)	*B. cereus* (MTCC 1272)	*S. maltophilia* (MTCC 4383)	*L. monocytogenes* (MTCC 839)	*E. coli* (MTCC 443)	*S. aureus* (MTCC 96)
Curry leaves	0.00	0.00	0.00	0.00	0.00	0.00
Bana leaves	0.00	1.00±0.109	0.00	0.00	0.00	0.00
Bhavadi leaves	1.86±0.105	0.00	1.00±0.036	0.00	1.00±0.031	2.00±0.027
Umre bark	6.33±0.105	1.25±0.109	1.15±0.036	2.33±0.028	6.11±0.031	2.32±0.027
Milk thistle	0.00	0.00	0.00	0.00	0.00	0.00
Mulethi root	2.00±0.105	5.00±0.109	5.66±0.036	5.33±0.028	6.00±0.031	3.52±0.027
Amla powder	5.00±0.105	3.33±0.109	2.00±0.036	0.00	0.00	3.65±0.027
Amla juice	5.33±0.105	4.00±0.109	3.00±0.036	6.00±0.028	6.53±0.031	4.00±0.027



Control *Umre* bark *Amla* powder *Amla* juice

Figure 2. Antimicrobial Activity of different herbs against *Shigella flexneri*

As illustrated in Figure 5, the microbial activity of seven different herbal plants against *L. monocytogenes* (MTCC 839) strain. The values of zone of inhibition (mm) are given in Table 1. The zone of inhibition is highest in *amla* juice followed by *mulethi* root and *umre* bark against this strain and for all remaining strains, it shows no zone of inhibition. The values of zone of inhibition (mm) of microbial activity of seven different herbal plants against *E. coli* (MTCC 443) strain are given in Table 1. The zone of inhibition was highest in *amla* juice than in different extracts of *umre* bark, *mulethi* root and very less in *bhavadi* leaf.

The zone of inhibition (mm) of microbial activity of seven different herbal plants against *S. aureus* strain are given in Table 1. The zone of inhibition is highest in *amla* juice followed by aqueous extract of *amla* powder, decoction of *mulethi* root and *umre* bark.

Different researchers examined the effectiveness of plant extracts as antimicrobial agents. The enzymes and proteins of microbial cell membranes are thought to interact with antimicrobial terpenoid, alkaloid, and phenolic compounds present in plant extracts, disrupting them to release protons that lead to cell death or may prevent the enzymes required for amino acid biosynthesis [31,32]. Others have connected the inhibitory properties of these plant extracts to their hydrophobicity, causing structural disruption and changing the permeability of proteins [33,34].

The disc diffusion method was employed to investigate the antibacterial activity of 18 plant species used in folk medicine as mentioned in current study [35]. Well diffusion method was used to check the antibacterial activity against different bacterial pathogens using *Ficus racemose* ethanolic and ethyl acetate extract [36]. In current study, aqueous plant extract was used to test the effectiveness of medicinal herbs on bacterial pathogens. Aqueous and ethanolic medicinal plant extracts were used to test the antibacterial activity of the plants [37]. Zone of inhibition of herbal plants to six different pathogenic strains was compared. *Mulethi* root inhibits all strains with a maximum zone of inhibition of 6 mm in *E. coli*. In respect to this study, Panthi and Choudhary [38] studied the antibacterial activity of *G. glabra* and found positive results against *Acinetobacter bohemicus*, *Kocuria kristinae, Micrococcus luteus, Staphylococcus auricularis, and Bacillus megaterium* with MICs between 0.31 and 1.25 mg/ml.

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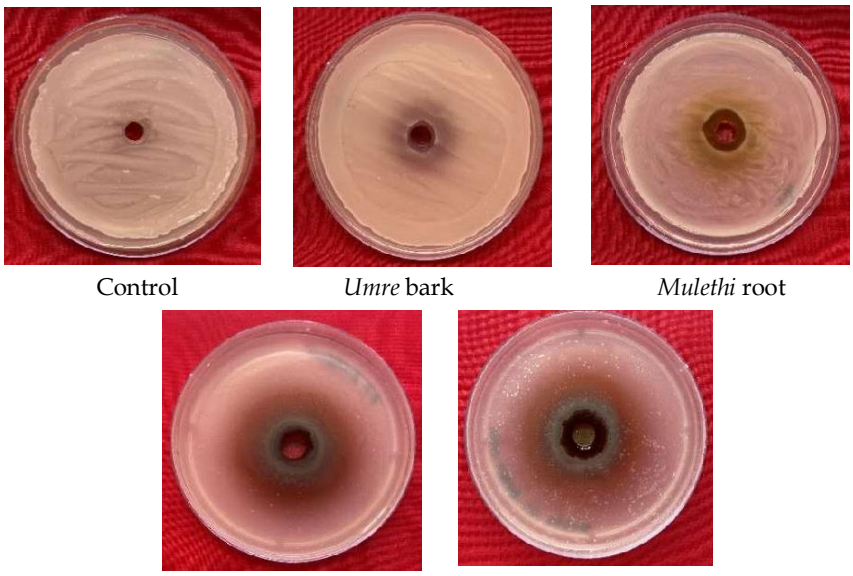

Figure 3. Antimicrobial activity of different herbs against *Bacillus cereus*

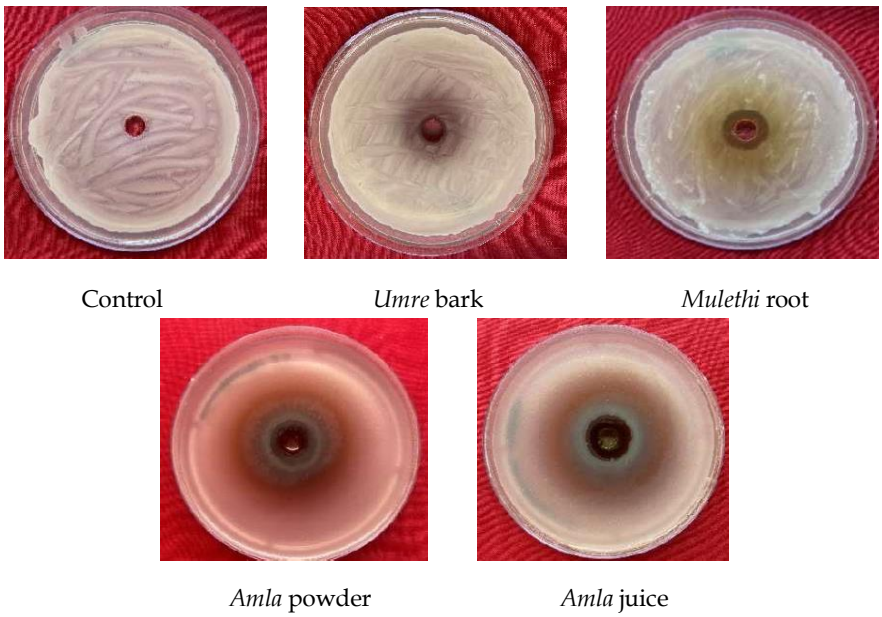

Figure 4. Antimicrobial Activity of different herbs against *Stenotrophomonas maltophilia*

Figure 5. Antimicrobial Activity of different herbs against *Listeria monocytogenes*

Umre bark also had high zone of inhibition in all strains, and maximum of 6.33 mm in *S. flexneri* (MTCC 1457). *Ficus racemose* ethanoic and ethyl acetate extract showed more favorable antibacterial activity against *E. coli*, *S. aureus*, *Salmonella typhi, Klebsiella pneumoniae* at concentration of 1.32 mg/ml, 0.98 mg/ml, 1.76 mg/ml, 1.52 mg/ml, respectively and fungistatic against *Aspergillus niger* and *Candida albicans* at

concentration of 1.39 mg/ml and 2.19mg/ml, respectively as compared to extract of water, hexane and Chloroform in well diffusion method [39].

Amla juice was highly susceptible to antimicrobial activity against all strains. The maximum zone of inhibition of 6.53 mm was against *E. coli*. On other hand, aqueous extract of *amla* powder showed antibacterial activity against all strains tested and maximum zone of inhibition i.e., 5 mm against *S. flexneri*. Similarly, showed that the extract of *E. officinalis* exhibited antibacterial activity against the seven bacterial species- *Pseudomonas aeruginosa*, *Salmonella paratyphi A*, *Proteus vulgaris*, *E. coli*, and *S. aureus* isolated from bore water, which were in comparison to current study [25].

Bhavadi herbal plant showed antibacterial activity against three strains namely *S. aureus*, *S. flexneri* and *S. maltophilia*. Also, the nanoemulsion essential oil at a concentration of 5 per cent to 25 per cent of *O. basilicum* and found moderate adequate to strong inhibition zone in *E. coli* and a moderate to strong inhibition in *S. aureus* [18].

In current study, *bana* showed inhibitory effects only against *B. cereus* and no zone of inhibition against five bacterial strains. Similarly, the study conducted on plant extract of *V. negundo* against both gram positive (*S. aureus*) and gram negative (*E. coli*, *K. pneumoniae*) bacteria [19]. The control used was 98 per cent ethanol. It exhibited a small degree of inhibition, possibly as a result of the evaporation of control drug due to its high concentration and small amount (50 µl).

On the other hand, *curry* leaves and milk thistle showed no inhibition against all the six pathogenic strains tested in this study. In contrast to these results, [40] studied the effect of methanolic extract of *M. koenigii* leaves against different species of Gram –ve, Gram +ve bacteria and fungus *Candida albicans.* The results showed moderate antibacterial activity against *S. aureus* and good antibacterial activity against *E.coli* and *P. aeruginosa*, antifungal activity against *C. albicans* and no zone of inhibition against *Klebsiella*. Antibacterial activities of the ethanolic seed extract of milk thistle exhibited a strong zone of inhibition against *S. aureus* and *Salmonella enterica* as compared to the control using modified Kirby-Bauer disc diffusion technique [41].

These observations clearly shows that *mulethi* root, *umre* bark, *bhavadi* leaves, and *amla* has significant antibacterial activity and may be an excellent natural alternative preservative for controlling food poisoning and other pathogenic bacteria. Similar results of different herbal plants were supported by Zeedan et al. [36] who used herbal plants extracts of clove and black cumin using a water/ethanol solvent to check the antibacterial activity and found inhibitory effects against the studied microorganism *E. coli* (57.14%), *K. pneumonia* (42.28%), and *S. aureus* (28.57%) at concentrations of 50 per cent, 20 per cent and 10 per cent. Ethanolic extracts of *Pimenta dioica* leaves have antimicrobial and chemo preventive activity against *S. entiritica*, *L monocytogenes*, *S. flexneri*, *Shigella sonnei* and *Aspergillus* sp. Essential oil of *P. dioica* also controlled the growth of food spoiling and pathogenic bacteria *P. aeuginosa*, *S. aureus* and *E. coli* [42]. The extracts of *Combretum album* showed a linear antibacterial efficacy against all the tested pathogenic bacteria i.e., *Bacillus licheniformis*, *P. aeruginosa*, *B subtilis*, *P fluorescens*, *Bacillus mycoides*, *E. coli*, *Pseudomonas putida* and supported that there is no correlation between the susceptibility shown by *C. album* extracts [43]. *Amla* juice followed by *mulethi* root and *umr*e bark showed maximum inhibition against all the strains tested and useful for the treatment of infectious diseases.

2.2 FTIR Analysis

FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 2 and the FTIR spectrum profile was illustrated in the Figure 6. FTIR spectrum confirmed the presence of alcohol, phenol, alkanes, alkyl halide, amino acids, carboxylic acid, aromatic, amines in the leaves and seeds of the herbal plants taken.

The more intense band occurring at 3273.50 cm^{-1}, 2916.66 cm^{-1}, 2349.5 cm^{-1}, 1733.2 cm^{-1}, 1615.8 cm^{-1}, 1146.2 cm^{-1} and 1017.6 cm^{-1} corresponding to O-H/N-H/C-H/C=O stretching, bending, vibrations respectively indicate the presence of alcohol, alkane, amines, amides, amino acids substituted compounds in leaves of *curry* leaves as shown in Figure 6a.

The intense bands occurring at 3274.5 cm^{-1}, 2916.6 cm^{-1}, 2849.5 cm^{-1}, 1604.6 cm^{-1}, 1243.1 cm^{-1}, 1146.2 cm^{-1}, 1012 cm^{-1} and 766.0 cm^{-1} corresponding to O-H / C-O stretch (str)/ N-H/ O-H str/ C-H/ C=O stretching, bending, vibrations respectively indicate the presence of alcohol, phenol, amines, amides, carboxylic group, ester, ether, amino acids group in *bana* leaves as shown in Figure 6b.

In *bhavadi* leaves as shown in Figure 6c intense bands occurring at 3272.6 cm^{-1}, 2918.5 cm^{-1}, 2849.5 cm^{-1}, 2309.1 cm^{-1}, 1742.40 cm^{-1}, 1615.8 cm^{-1}, 1385.18 cm^{-1}, and 1015.81 cm^{-1} corresponding to COOH/ O-H/C=C-

O-C/NH/ O-H str/ C=O/phenol/ C-O str/ stretching, bending, vibrations respectively indicate the presence of amines, amides, carboxylic groups, ester, amino acids, phenol, ether groups.

The intense bands occurring at 3272.6 cm^{-1}, 2920.4 cm^{-1}, 17332.29 cm^{-1}, 1602.8 cm^{-1}, 1558.0 cm^{-1}, 1243.1 cm^{-1}, 777.18 cm^{-1} corresponding to N-H/ C-H/ C=O/ phenol C-O str/ C=N/ C-H aromatic str/ stretching, bending, vibrations respectively as shown in Figure 6d indicate the presence of amines, amides, ester, ether, phenol C-O bond, O-H aromatic mono substituted compounds in the *umre* barks.

Milk thistle seeds powder showed intense bands occurring at 3274.50 cm^{-1}, 2920.04 cm^{-1}, 2851.4 cm^{-1}, 2092.9 cm^{-1}, 1707.08 cm^{-1}, 1656.52 cm^{-1}, 1541.62 cm^{-1}, 1457.43 cm^{-1} and 1032.5 cm^{-1} corresponding to COOH-O –H / N-H / O-H / C-H / N-H /C=O/Phenol O-H / C=N / stretching, bending, vibrations respectively indicate the presence of alcohol, amines, alkane, carboxylic acid, ester, C-H bond for alkanes, amino acids etc. as shown in Figure 6e.

The more intense band occurring at 3902.5 cm^{-1}, 3270.7 cm^{-1}, 2922.2 cm^{-1}, 1770.5 cm^{-1}, 1634.4 cm^{-1}, 1149.9 cm^{-1}, 1015.7 cm^{-1} and 833.1 cm^{-1} corresponding to O-H/N-H/C-H/C=O respectively indicate the presence of alcohol, alkane, amines, amides, amino acids and meta substituted compounds in *mulethi* root as shown in Figure 6f.

Amla powder depicted intense band occurring at 3272.6 cm^{-1}, 2916.6 cm^{-1}, 2115.3 cm^{-1}, 1716.4 cm^{-1}, 1675.8 cm^{-1}, 1112.6 cm^{-1}, and 689.6 cm^{-1} corresponding to O-H/N-H/C-H/C=O stretching, bending, vibrations respectively indicate the presence of alcohol, alkane, amines, amino acids and meta substituted compounds as shown in Figure 6g.

Though the compounds behind this antibacterial activity have not been identified, preliminary phytochemical analysis of the aqueous extract revealed the presence of tannins, alkaloids, and flavonoid compounds. FTIR analysis also confirmed the presence of alcoholic, amine, carboxylic acids, and aromatic groups, which supports these findings. It helps to identify the chemical constituents, elucidate the chemical structure.

Table 2. FTIR peak values and functional groups of herbal plant extracts

S. No.	Herbal Plants	O-H	Phenol C-O	N-H Str alkane	COOH O-H str	C-H For alkane	Ether C-O Str	Ether C=C-O-C str	Ester C=O	C-H bending	Amine C=N str	Amine N-H bond	Amino acid N-H str	C=O str	Aromatic Meta di substituted
1	*Curry* leaves	3273.50	ND	2349.5	ND	2916.66	1146.2	ND	ND	ND	1017.6	ND	1615.8	1733.2	ND
2	*Bana* leaves	3274.50	1243.10	ND	2849.5	2916.6	1146.2	ND	ND	ND	1012	ND	1604.6	ND	766.0
3	*Bhavadi* leaves	3272.60	1385.18	2309.1	2918.5	28.49.5	ND	1015.81	1742.40	ND	ND	ND	1615.8	ND	ND
4	*Umre* bark	3272.60	1243.10	ND	ND	2920.4	ND	ND	1732.29	1558.0	ND	ND	1602.8	ND	777.18
5	Milk thistle	3274.50	1457.43	2092.9	2920.04	2851.4	ND	ND	1707.08	ND	1032.5	1656.52	ND	1541.62	ND
6	*Mulethi* root	3270.70	ND	2922.2	3902.5	ND	1149.9	ND	1770.50	ND	1015.7	1634.4	ND	ND	833.1
7	*Amla* powder	3272.60	ND	2115.3	ND	2916.6	1112.6	ND	1716.40	ND	ND	ND	1675.8	ND	689.6

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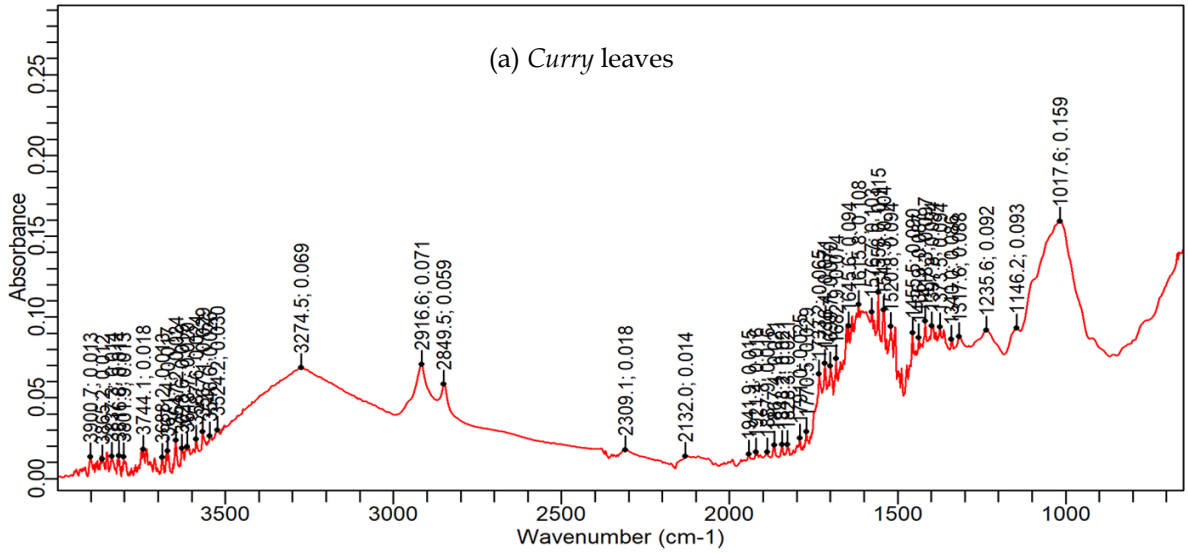

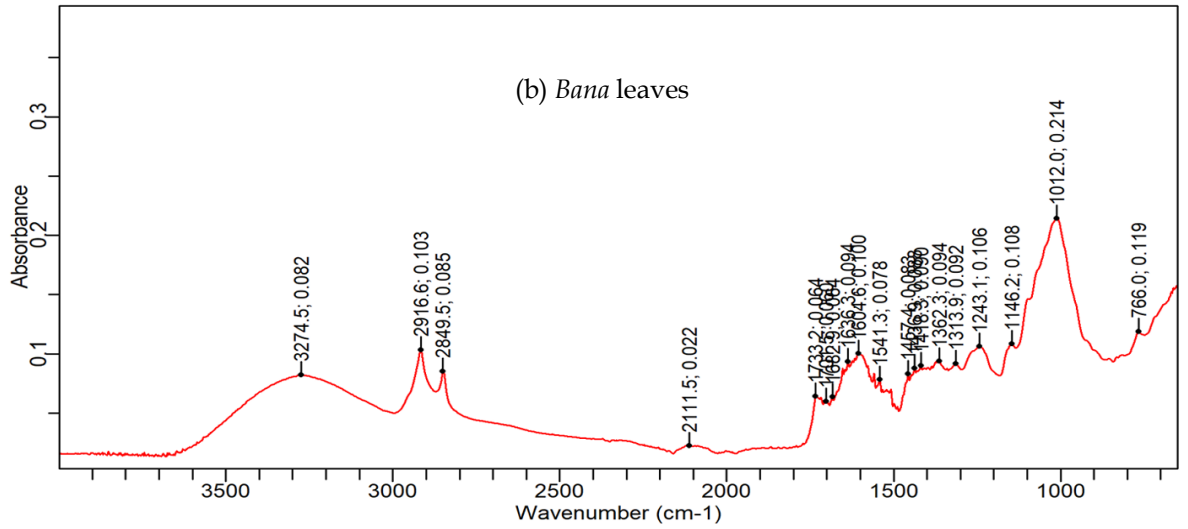

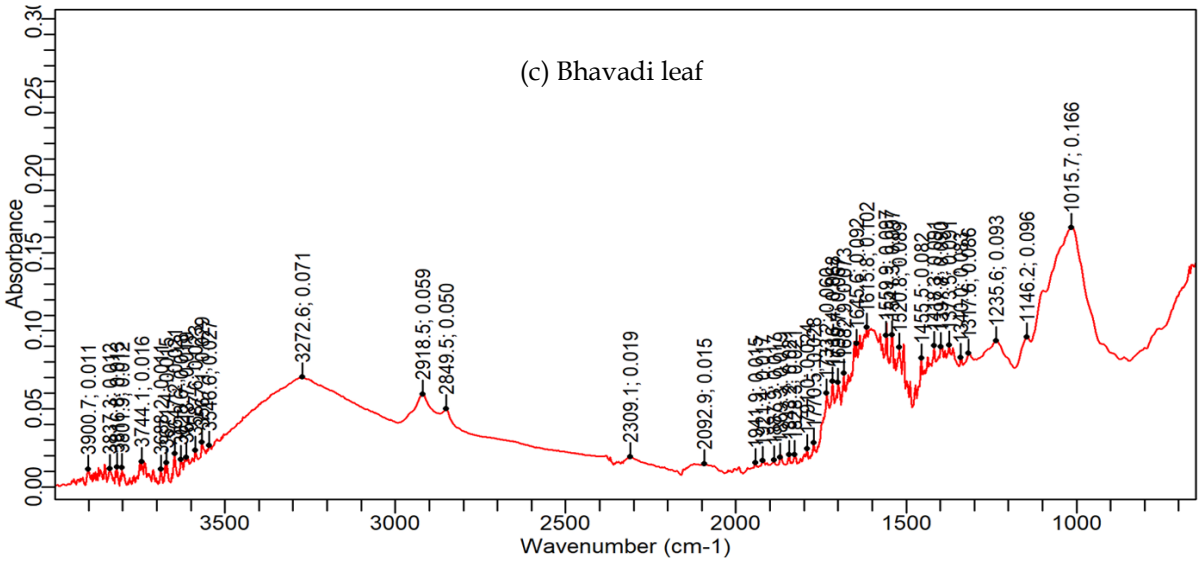

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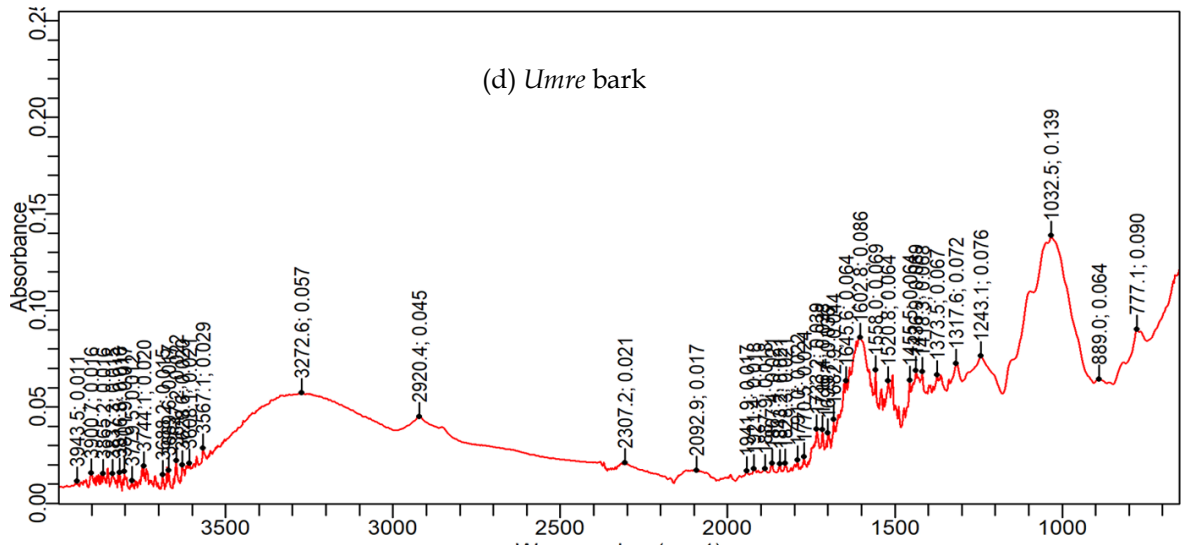

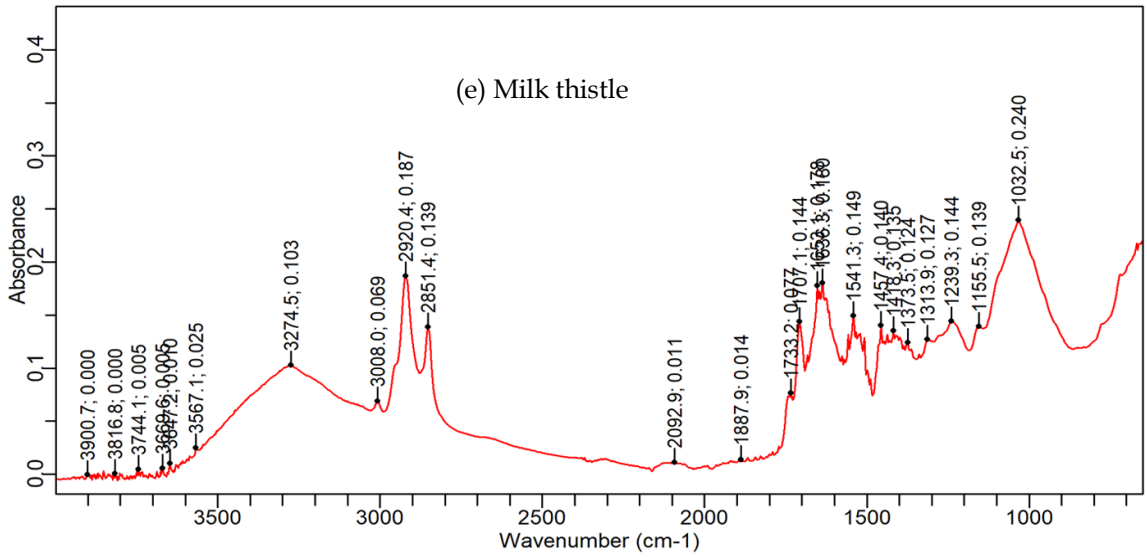

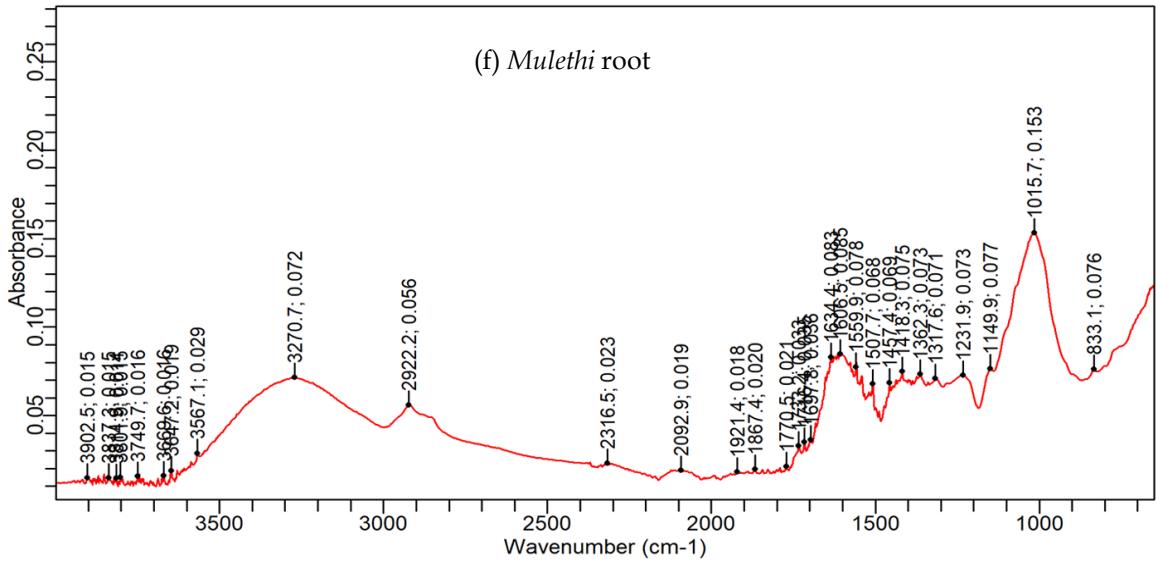

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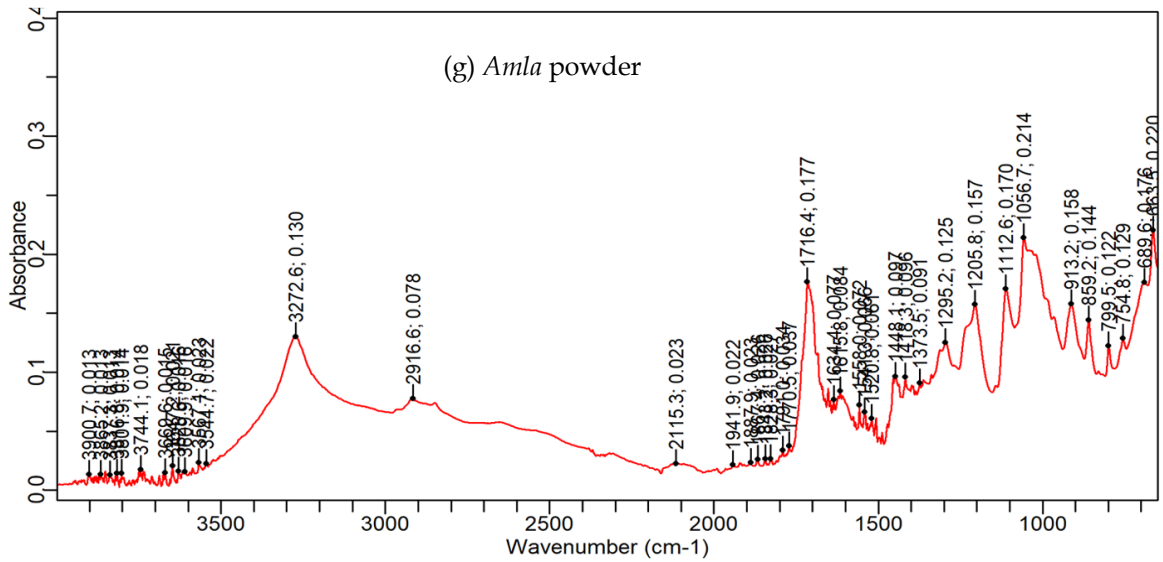

Figure 6. FTIR Spectrum extraction values of (a) *Curry* leaves (b) *Bana* leaf (c) *Bhavadi* leaf (d) *Umre* bark (e) Milk thistle (f) *Mulethi* root (g) *Amla* powder

The presence of common functional g (g) *Amla* powder nines, amides and amino acids are mostly present in all the herbal plants used in the s different functional groups like alcohol, alkane, amines, amides, amino acids in *curry* leaves; alcohol, phenol, amines, amides, carboxylic group, ester, ether, amino acids group in *bana* leaves; presence of amines, amides, carboxylic groups, ester, amino acids, phenol, ether groups in the *bhavadi* leaves; presence of amines, amides, ester, ether, phenol C-O bond, O-H aromatic mono substituted compounds in the *umre* bark; presence of alcohol, amines, alkane, carboxylic acid, ester, C-H bond for alkanes, amino acids etc. in the milk thistle seeds powder; presence of alcohol, alkane, amines, amides, amino acids and meta substituted compounds in *mulethi* root; presence of alcohol, alkane, amines, amino acids and meta substituted compounds in *amla* powder viz. tannins, alkaloids, and flavonoids, which can be extracted from these medicinal plants and used in herbal drug preparations beneficial for the treatment of many bacteria-borne diseases, are believed to be responsible for the plant's antibacterial potency. The specific plant compounds that can act as antibacterial agents may have therapeutic significance as a result of this finding, which is obviously possible.

The presence of similar types of functional groups like alcohols, ethers, esters, carboxylic acids, anhydrides, deoxyribose, alkenes, organic halogen compounds, carbohydrates, amines, amides, amino acids, polymeric hydroxyl derivatives, primary amine, methylene group have been reported in powdered samples of different plants [29,44-47].

Absence of spectral peaks or bands in between the region 2220- 2260 cm^{-1} indicates that there are no cyanide groups in all the extracts of the herbal plants studied. This shows that samples taken for the study does not contain any toxic substances [48].

Carboxylic acid present in the herbal plant serves as main pharmaceutical product in curing ulcers, jaundice, headache, stomatitis, hemicranias, fever, pain in liver, wound in cattle, treatment of edema and rheumatic joint pains [30]. Amines, amides and amino acids are the main groups of protein synthesis and herbs serves as herb oil and hair tonic. Sulphur derivative compounds were used as disinfectants and dermal cream. Polysaccharides, carbohydrates, chlorates and nitrates play the role of the disinfectants [49].

3. CONCLUSION

The present investigation reveals a real solution for antimicrobial resistance by using natural herbal extracts. The five plant extracts (*Bana*, *bhavadi* leaf, *umre* bark, *mulethi* root, and *amla*) out of seven mentioned above have a powerful antimicrobial activity that inhibit or at least stop the growth of six pathogenic bacterial strains; so those extract may be used as food additives and food preservatives to control such microbial growth and conserve human and animal health. The plant extracts which proved to be potentially effective as *E. officinalis* (*amla*), *G. glabra* (*mulethi*) and *F. glomerata* (*umre* bark) can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding health hazards of chemically antimicrobial agent applications. The results of the FTIR analysis showed the presence of alcohol, alkane, amines, amides, amino acids and meta substituted compounds in selected herbal plants. FTIR spectrum reflecting objectively the panorama of chemical constituents in a complex system is the most

credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine.

The results of this study suggest how the pharmaceutical industry might exploit these novel herbal plants for ethnomedicine. Further research may require to identify the new bioactive compounds present in these herbal plants responsible for antibacterial activity.

4. MATERIALS AND METHODS

4.1 Sample Collection

The herbs/medicinal plants and wild *amla* utilized in the current study were procured from the local vicinities of Palampur, Himachal Pradesh, India (Table 3). The herbs were thoroughly rinsed. They were dried in hot air oven at 60°C and grinded into powders. Powders were stored in air tight containers for further analysis.

Table 3. Plants used in present research work

Common name of Plants	Botanical name	Plant part used
Curry	*Murraya koenigii*	Leaf
Bhavadi	*Ocimum basilicum*	Leaf
Bana	*Vitex negundo*	Leaf
Mulethi	*Glycyrrhiza glabra*	Root
Milk thistle	*Silybum marianum*	Seeds
Umre	*Ficus glomerata*	Bark
Amla	*Emblica officinalis*	Fruit

4.2 Preparation of Aqueous Infusions

Aqueous infusions (cold extract) were prepared using leaves of *Curry* (*M. koenigii*), *bhavadi* (*O. basilicum*) and *bana* (*V. negundo*) using different ratios of herbs and water using modified method of Olurinola [50]. The herbs were weighed separately for preparing different proportions of extract. Cleaned them thoroughly cut into shreds and placed in a container having filtered water. The container was then covered and placed at 4°C for 3 h. The soaked herbs were then grinded in pestle and mortar. The extract was strained using muslin cloth and cold extract was ready. All three aqueous infusions were prepared using this method.

4.3 Preparation of Aqueous Decoctions

Aqueous decoctions of roots, bark and seeds were prepared using *mulethi* root (*G. glabra*), milk thistle seeds (*S. marianum*) and *umre* bark (*F. glomerata*) using different ratios of herbs and water using modified method of Olurinola [50]. The herbs were weighed separately for preparing different proportion of decoction. Cleaned them thoroughly and coarsely grounded them using pestle and mortar. The grounded powder was then put in a pan having water and simmered for 15 min. The decoction was then filtered using muslin cloth. All three aqueous decoctions were prepared using this method.

4.4 Preparation of *Amla* Juice

Chaikiya variety of *amla* (*E. officinalis*) was procured from CSK Himachal Pradesh Agricultural University, Palampur (HP). The fruit was thoroughly rinsed in tap water and then in distilled water, separately. The whole fruit was pureed well using a sterilized juicer, and then filtered through a muslin cloth to obtain a clear aqueous fraction of fruit, free from pulp.

4.5 Bacterial Culture

Bacteria *viz.*, *S. flexneri* (MTCC1457), *B. cereus* MTCC1272), *S. maltophilia* (MTCC4383), *L. monocytogenes* (MTCC839), *E. coli* (MTCC443), *S. aureus* (MTCC96) were procured from the Microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The pure bacterial cultures were maintained on nutrient agar medium. Each bacterial culture was further maintained by sub-culturing regularly, on the same medium and stored at 4°C, until used in experiment.

4.6 Well Diffusion Technique for Antimicrobial Activity

The antimicrobial activity of extract against pathogenic bacteria was evaluated by using agar well diffusion method [51]. The isolates were inoculated into 10 ml of sterile nutrient broth, and incubated at 35±2°C overnight. The turbidity of culture was checked. The cultures were swabbed on the surface of sterile

nutrient agar plates using a sterile cotton swab and allowed to dry for 3- 5 min. Agar wells were prepared with the help of sterilized borer with 8mm diameter and of each infusion and decoction of selected herbs was introduced into the well. Sterile distilled water was used as control. The inoculated plates were incubated at 35±2°C for 24 h and zone of inhibition was measured to the nearest millimeters (mm).

4.7 FTIR Assay

For FTIR analysis, dried powder of each herbal plant was used. The FTIR spectra of samples was developed using Agilent Cary 630 FTIR instrument. The respective samples were placed directly onto the attenuated total reflectance (ATR) crystal before cleaning the crystal with methanol. All the samples were evaluated (number of scans was 10 per sample) at room temperature, and the spectra were collected in the 4000-400 cm^{-1} wave number range. The spectral bands obtained for the various samples were interpreted according to the instructions provided by Kaur et al. and Stuart [31,32].

4.8 Statistical Analysis

All the analytical parameters were recorded in triplicates and the data was analyzed using SPSS software. The zone of inhibition was expressed as mean ± SD.

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