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Research Article

ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL WITH FTIR ANALYSIS OF *AMPELOCISSUS LATIFOLIA* (ROXB.) PLANCH. LEAVES

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ABSTRACT

The antimicrobial activitiy of the methanolic leaf extract of *Ampelocissus latifolia* was evaluated against medicinally important bacteria *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (MTCC 9207), Methicillin-resistant *Staphylococcus aureus* (ATCC 43300), *Propionibacterium acnes* (MTCC 1951) and yeast, *Malaassezia furfur* (MTCC 1374) using the MIC and MBC/MFC analysis. The lowest MIC and MBC value is 200 mg/ml obtained for *Propionibacterium acnes*. The methanolic leaf extracts were subjected to evaluation for antioxidant activity by DPPH free radical scavenging method and Nitric oxide radical scavenging method. Using DPPH method, IC_{50} values for methanolic leaf extract was 738.80 ± 0.3326 µg/ml and for standard ascorbic acid was found to be less than 20 µg/ml respectively. IC_{50} values for methanolic leaf extract and standard ascorbic acid was found to be 48.66 ± 6.36 µg /ml and 45.67 ± 1.49 µg/ml respectively using Nitric oxide radical scavenging method. The functional groups present in the crude powder and methanolic extract of *Ampelocissus latifolia* leaves were identified through Fourier Transform Infrared (FT-IR) spectrometry

Keywords: Antimicrobial, *Ampelocissus latifolia*, MIC, MBC, MFC, Antioxidant activity, Fourier Transform Infrared (FT-IR) spectrometry.

INTRODUCTION

Ampelocissus latifolia belongs to family vitaceae (Grape family). It is a large herbaceous climber, with a tuberous root stock. Stem and branches are hollow, more or less smooth. Leaves circular or broadly heart-shaped with lobes acute and toothed. Leaf stalks are long and inflorescence is a compact cyme, ending in a long bifurcate tendril. Flowers are numerous and deep reddish coloured with 5 oblong petals. Fruits are spherical and black normaly 2 seeded and rarely 3 seeded. Flowering season is from May to August. The roots have been used for the treatment of snake bite and for its astringent effect. The decoction of the root is also used in chronic dysentry. The sandals of Bihar used this plant for muscular pains, sores and fractured bones[1]. The common name of the plant in English language is "Jungli angoor" or the other common name is "Panibel" in India. Juice of tender leaves is used in dental problems and as a detergent for indolent ulcers. The plant bears hypostomatic leaves and stomata are mainly anomocytic with few anisocytic types[2]. The stem ash is applied abdominally for easy delivery in pregnancy[3].

Some studies involving phytochemical screening and physico chemical analysis of acetone, chloroform and alcoholic extracts of *Ampelocissus latifolia* has been performed earlier by some other groups. In acute toxicity studies, these extracts were found to be safe upto a maximum dose of 500 mg/kg. They also exhibited significant anti-inflammatory activity which may be due to its inhibitory effect of histamine kinin and prostaglandins release[1]. Some antimicrobial studies have been done on *Ampelocissus latifolia*, but none of them were performed on the methanolic extract of leaf part of the plant against *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (MTCC 9207), Methicillin-resistant *Staphylococcus aureus* (ATCC 43300), *Propionibacterium acnes* (MTCC 1951) and *Malaassezia furfur* (MTCC 1374). Therefore the present study is focused on Antimicrobial and Antioxidant studies of methanolic extracts of *Ampelocissus latifolia* leaves with its FTIR analysis.

MATERIALS AND METHODS

Collection and authentification of plant

Ampelocissus latifolia was collected from an open field around Mumbai, Maharashtra. The identification of the plant was done at the Blatter Herbarium, St. Xavier's College, Mumbai. The plant has been identified as *Ampelocissus latifolia* (Roxb.) Planch belonging to family Vitaceae. The plant specimen matches with the Blatter Herbarium specimen no. Shah-l of G. L. Shah. Leaves were shade dried and made into coarse powder with mechanical grinder and then passed through sieve, B.S.S Mesh No.60.

Apparatus

Jasco V-630 spectrophotometer was used for the measurement of absorbances of solution mixtures for antioxidant studies. Bruker Alpha-T FT-IR equipped with universal sampling module, equipped with a room temperature DTGS detector. The scan range was taken from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The powder and dried extracts were mixed with KBr salt using mortar and pestle and compressed into a thin pellet for FT-IR analysis.

Chemicals

Methanol A.R. grade (Merck, India), Ascobic acid (S.D Fine Chemicals, Mumbai), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Sigma Aldrich, Germany), Sodium nitroprusside (Fisher scientific, Mumbai), Sulphanilamide (S.D Fine Chemicals, Mumbai), N(1-Naphthyl) ethylene diamine dihydrochloride (S.D Fine Chemicals, Mumbai), DMSO (Himedia, India), Sabouraud's agar (Himedia, India), Tween 80 (Himedia, India), Tryptone soy broth (TSB) (Himedia, India), HiAnero Gas Pack (Himedia India), Equitron Anaerobic jar.

Preparation of plant extract

The leaf powder of *Ampelocissus latifolia* (20 gms) was extracted with 250 ml each of methanol by soxhlet extraction for 8 hrs. The extracts obtained were later kept for evaporation to remove the excess solvent. These extracts were then stored in plastic bottle in refrigerator for further antimicrobial, antioxidant and FTIR studies.

Bacterial strains

Four bacterial strains, namely *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (MTCC 9207), Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), *Propionibacterium acnes* (MTCC 1951) and yeast *Malaassezia furfur* (MTCC 1374) were used for antimicrobial testing. The microbial isolates were procured from National Chemical Laboratories (NCL), Pune, Maharashtra, India and Microbial Type Culture Collection (MTCC) Chandigarh, India. The microorganisms were maintained at 4°C temperature.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal and Fungicidal Concentration (MBC / MFC)

Determination of the minimum inhibitory concentration (MIC) was carried out only on leaves using the Broth dilution method[4-6] with slight modification. The extracts were reconstituted in 10% v/v aqueous dimethyl sulfoxide (DMSO) at the required concentration of 1600 mg/ml. A serial two fold dilution of reconstituted extract was

prepared to obtain 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.391, 0.195 mg/ml concentration. Then 100 µl of an 18 hrs old culture of each of the bacteria earlier adjusted at10^6 Colony Forming Unit per milliliter (CFU/ml) was added to all tubes and thoroughly vortexed. The tubes were incubated at 37°C for 48 hrs and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC. The MBC / MFC value was determined by spot innoculating of bacterial suspension from the MIC tubes that did not show any growth and subcultured onto tryptone soy broth (TSB) agar plates and incubated at 37°C for 48 hrs for *S. epidermidis*, *M. luteus*, MRSA. For *M. furfur* Sabouraud's media with 0.2% corn oil and 0.5% tween 80 was used and the incubation period was 37°C for 72 hrs. For *P.acne* media used was tryptone soy broth and the incubation period was 7 days at 37°C under anaerobic conditions. After incubation, the concentration at which no visible growth was seen on the agar plate was recorded as the MBC / MFC (Table 1).

Table 1: Minimum Inhibitory (MIC) and Minimum Bactericidal / Fungicidal Concentration (MBC / MFC) of *Ampelocissus latifolia* methanolic leaf extract.

Microorganism	MIC (mg/mL)	MBC & MFC(mg/mL)
Staphylococcus epidermidis (ATCC 12228)	400 mg/ml	400 mg/ml
Micrococcus luteus (MTCC 9207)	800 mg/ml	800 mg/ml
Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	400 mg/ml	400 mg/ml
Propionibacterium acnes (MTCC 1951)	200 mg/ml	200 mg/ml
Malaassezia furfur (MTCC 1374)	200 mg/ml	800 mg/ml

DPPH Scavenging Activity

The free radical scavenging activity of the methanolic leaf extract of *Ampelocissus latifolia* was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (2, 2-diphenyl-2-picryl hydrazyl). 0.1 mM of DPPH in methanol was prepared.

Different concentrations of sample and standard used were 20, 40, 60, 80, 100, 200, 500, 1000µg/ml. After 30 minutes the absorbance was measured at 517 nm. Ascorbic acid was used as the standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity[7-9]. Radical scavenging activity was expressed as inhibition percentage of free radical by the sample and was calculated using following formula:

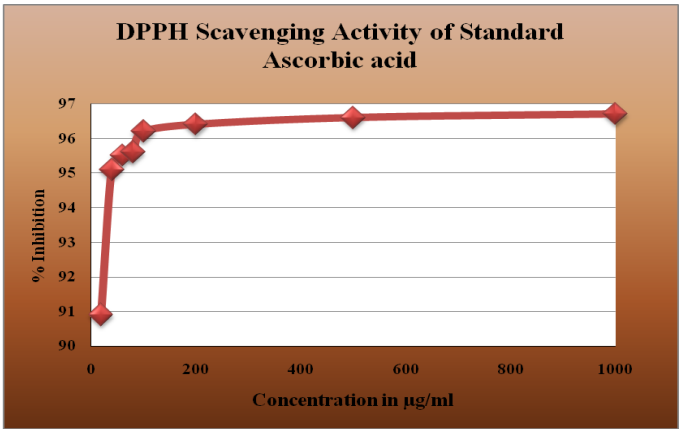
$$\% \text{ DPPH Scavenged} = \frac{A_{Control} - A_{Test}}{A_{Control}} \times 100$$

Where $A_{Control}$ was the absorbance of control (Methanol and DPPH). A graph is plotted using % Inhibition v/s Concentrations (Table 2 & Fig1 & 2).

Table 2: Data of DPPH scavenging activity of *Ampelocissus latifolia* methanolic leaf extract.

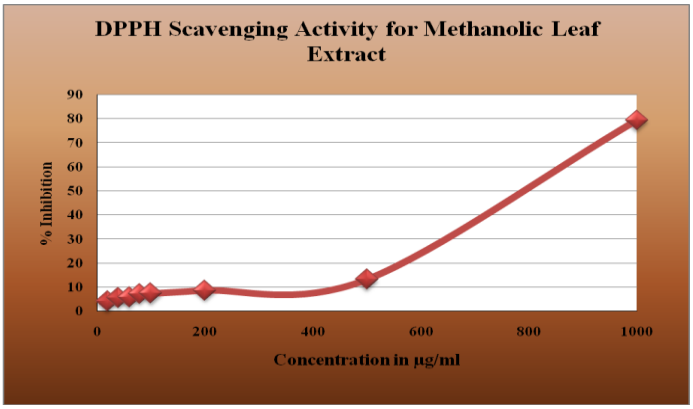
Concentration Used (µg/ml)	Standard Ascorbic acid		Leaf Extract Sample	
	Ascorbic acid Absorbance Mean ± SD	Ascorbic acid % Inhibition Mean ± SD	Sample Absorbance Mean ± SD	Sample % Inhibition Mean ± SD
20	0.0196 ± 0.0003	90.9 ± 0.1	0.2057 ± 0.0002	4.2 ± 0.1
40	0.0105 ± 0.0002	95.1 ± 0.1	0.2024 ± 0.0004	5.7 ± 0.2
60	0.0096 ± 0.0003	95.5 ± 0.1	0.2023 ± 0.0003	5.8 ± 0.1
80	0.0094 ± 0.0001	95.6 ± 0.1	0.1990 ± 0.0002	7.3 ± 0.1
100	0.0081 ± 0.0002	96.2 ± 0.1	0.1986 ± 0.0003	7.5 ± 0.2
200	0.0077 ± 0.0003	96.4 ± 0.1	0.1961 ± 0.0003	8.7 ± 0.2
500	0.0071 ± 0.0002	96.6 ± 0.1	0.1861 ± 0.0002	13.3 ± 0.1
1000	0.0070 ± 0.0001	96.7 ± 0.1	0.0443 ± 0.0003	79.3 ± 0.2

Absorbance of Control: 0.2148 ± 0.0001



IC_{50} value of Standard Ascorbic acid: less than 20 µg/ml.

Fig1. DPPH scavenging activity of Standard Ascorbic acid.



IC_{50} Value of methanolic leaf extract of *Ampelocissus latifolia*: 738.80 ± 0.3326 µg/ml.

Fig2. DPPH scavenging activity of *Ampelocissus latifolia* methanolic leaf extract.

Nitric oxide Scavenging Activity

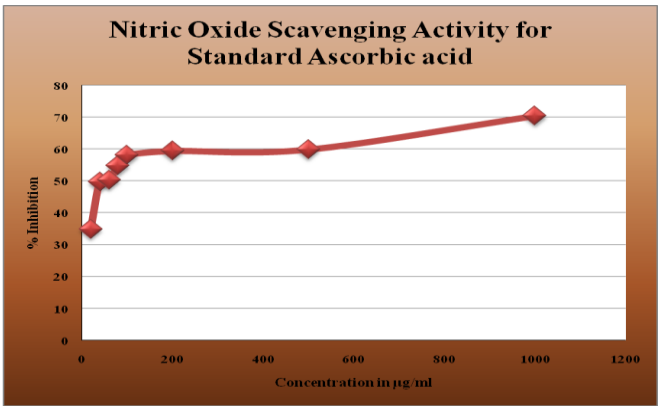
The chemical source of Nitric oxide was sodium nitroprusside (10mM) in phosphate buffer (pH 7.4), which spontaneously generates nitric oxide. Nitric oxide interacts with oxygen to produce stable products, leading to the production of nitrites. About 0.5 ml sodium nitroprusside (10mM) in phosphate buffer was mixed with 2 ml of different concentrations 20, 40, 60, 80, 100, 200, 500, 1000µg/ml of the sample and standard and incubated for 150 min at room temperature. After 150 min the samples from the above were reacted with 1.2 ml Greiss reagent (1gm sulphanilamide in 5 ml ortho phosphoric acid and 104 mg N-(1-napthyl) ethylene diamine dihydrochloride in 100 ml distilled water. The absorbance of the chromophore formed during the diazotization of nitrite with

sulphanilamide and subsequent coupling with N-(1-napthyl) ethylenediamine was read at 546 nm. The reaction mixture without the extracts of plants but with methanol and sodium nitroprusside served as control. Ascorbic acid was used as positive control[10,11]. The results of anti-oxidant activity of methanolic extracts using Nitric oxide radical scavenging method were shown below (Table 3 and Fig 3 & 4).

Table 3: Data of Nitric oxide scavenging activity of *Ampelocissus latifolia* methanolic leaf extract.

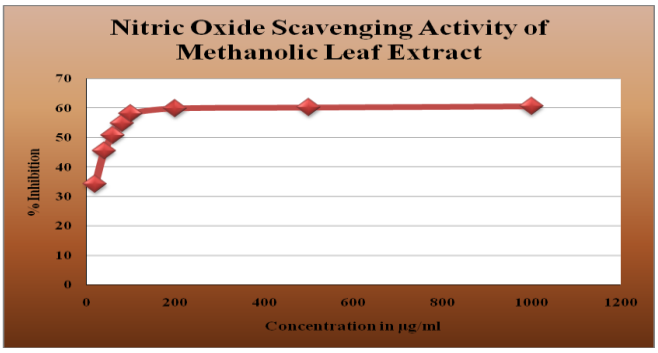
Concentration Used (µg/ml)	Standard Ascorbic acid		Leaf Extract Sample	
	Ascorbic acid Absorbance Mean ± SD	Ascorbic acid % Inhibition Mean ± SD	Sample Absorbance Mean ± SD	Sample % Inhibition Mean ± SD
20	0.1117 ± 0.0002	34.89 ± 0.12	0.1127 ± 0.0002	34.26 ± 0.09
40	0.0862 ± 0.0001	49.74 ± 0.06	0.0934 ± 0.0003	45.51 ± 0.19
60	0.0852 ± 0.0001	50.32 ± 0.06	0.0842 ± 0.0003	50.90 ± 0.16
80	0.0775 ± 0.0001	54.81 ± 0.06	0.0774 ± 0.0002	54.88 ± 0.12
100	0.0718 ± 0.0001	58.11 ± 0.07	0.0716 ± 0.0003	58.25 ± 0.18
200	0.0695 ± 0.0002	59.46 ± 0.09	0.0687 ± 0.0002	59.96 ± 0.09
500	0.0687 ± 0.0002	59.92 ± 0.09	0.0681 ± 0.0002	60.27 ± 0.09
1000	0.0507 ± 0.0002	70.42 ± 0.09	0.0675 ± 0.0002	60.66 ± 0.09

Absorbance of Control: 0.1715 ± 0.0002



IC_{50} Value of Standard Ascorbic acid: 45.67 ± 1.49 µg/ml

Fig 3:Nitric oxide scavenging activity of Standard Ascorbic acid



IC_{50} Value of methanolic leaf extract of *Ampelocissus latifolia*: 48.66 ± 6.36 µg/ml.

Fig 4:Nitric oxide scavenging activity of *Ampelocissus latifolia* methanolic leaf extract.

The capability to scavenge the NO radical was calculated using the following equation:

$$\% \text{ NO Scavanged} = \frac{A_{Control} - A_{Test}}{A_{Control}} \times 100$$

Where $A_{control}$ is the absorbance of the control (Methanol and Sodium nitroprusside). A_{test} is the absorbance in the presence of the extracts. The antioxidant activity of the extracts were expressed as IC_{50}.

RESULTS

The minimum inhibitory concentrations of the methanolic leaf extract of *Ampelocissus latifolia* on the selected microorganisms are shown in Table 1. The MIC values were tested from 800 to 0.195 mg/mL. For *S. epidermidis,* MIC and MBC values both were found to be 400 mg/ml whereas for *M.Luteus* both MIC and MBC values were at 800 mg/ml. Again MRSA also showed MIC and MBC values both at 400 mg/ml. The lowest MIC and MBC value was for *P.acne* at 200 mg/ml. *M. furfur* showed MIC at 200 and MFC 800 mg/ml respectively.

Using the DPPH scavenging activity, the IC_{50} value of methanolic leaf extract of *Ampelocissus latifolia* was found to be 738.80 ± 0.3326 µg/ml and for standard ascorbic acid it was less than 20 µg/ml respectively. By using Nitric oxide scavenging activity of methanolic leaf extract of *Ampelocissus latifolia*, the IC_{50} value was found to be 48.66 ± 6.36 µg/ml and for standard ascorbic acid was 45.67 ± 1.49 µg/ml respectively.

The FT-IR analysis of leaf powder of *Ampelocissus latifolia* showed the presence of alkanes, alkenes, alkynes, alcohols, carboxylic acids, ethers, esters, nitro compounds, phenols, polysulfides and Aliphatic iodo compounds. The FT-IR of methanolic leaf soxhlet extract of *Ampelocissus latifolia* revealed the presence of alkanes, alkenes, alkynes, amines, amides, carboxylic acids, ethers, transitional metal carbonyl and Aliphatic fluoro compounds. The FT-IR of stem powder of *Ampelocissus latifolia* revealed the presence of alkanes, alkenes, alcohols, amines, amides, carboxylic acids, ethers, esters, aldehydes, ketones, phenols and nitro compounds [Table 4-6, Fig 5-7].

Table 4: FT-IR Peak Values of *Ampelocissus latifolia* Leaf Powder

Wave number cm^{-1}	Bond	Functional Group Assignment	Group Frequency, cm^{-1}
3327.12	O-H	Hydrogen bonded alcohols, phenols	3200-3600
2919.68	C-H	Alkanes	2850-2970
2142.86	C≡C	Alkynes	2100-2260
1567.19	NO_2	Nitro compounds	1500-1570
1375.55	C-H	Alkanes	1340-1470
1079.06	C-O	Alcohols, ethers, carboxlic acids, esters	1050-1300
874.84	C-H	Alkenes	675-995
564.83	C-I stretch	Aliphatic Iodo compounds	500-600
472.81	S-S stretch	Polysulfides	470-500

Table 5: FT-IR Peak Values of *Ampelocissus latifolia* Methanol Soxhlet Leaf Extract

Wave number cm^{-1}	Bond	Functional Group Assignment	Group Frequency, cm^{-1}
3398.12	N-H	Amines, Amides	3300-3500
2945.69	C-H	Alkanes	2850-2970
2833.32	C-H stretch	Methoxy, Methyl ether O-CH_3	2815-2850
2523.08	O-H	Hydrogen bonded carboxylic acids	2500-2700
2224.34	C≡C	Alkynes	2100-2260
2044.32	Carbonyl stretch	Transition metal carbonyl compounds	1800-2100
1654.57	C=C	Alkenes	1610-1680
1449.61	C-H	Alkanes	1340-1470
1417.03	C-H	Alkanes	1340-1470
1114.63	C-F Stretch	Aliphatic Fluoro compounds	1000-1150
1032.64	C-F Stretch	Aliphatic Fluoro compounds	1000-1150

Table 6: FT-IR Peak Values of *Ampelocissus latifolia* Stem Powder

Wave number cm^{-1}	Bond	Functional Group Assignment	Group Frequency, cm^{-1}
3429.29	O-H	Hydrogen bonded alcohols, phenols	3200-3600
3054.16	C-H	Alkenes	3010-3095
2918.05	C-H	Alkanes	2850-2970
1727.98	C=O	Aldehydes, Ketones, Carboxylic acids, Esters	1690-1760
1605.39	N-H bend	Secondary amine	1550-1650
1449.02	C-H	Alkanes	1340-1470
1318.92	NO_2	Nitro Compounds	1300-1370
1241.98	C-N	Amines, Amides	1180-1360
1064.27	C-O	Alcohols, ethers, carboxlic acids, esters	1050-1300
783.98	C-H	Alkenes	675-995

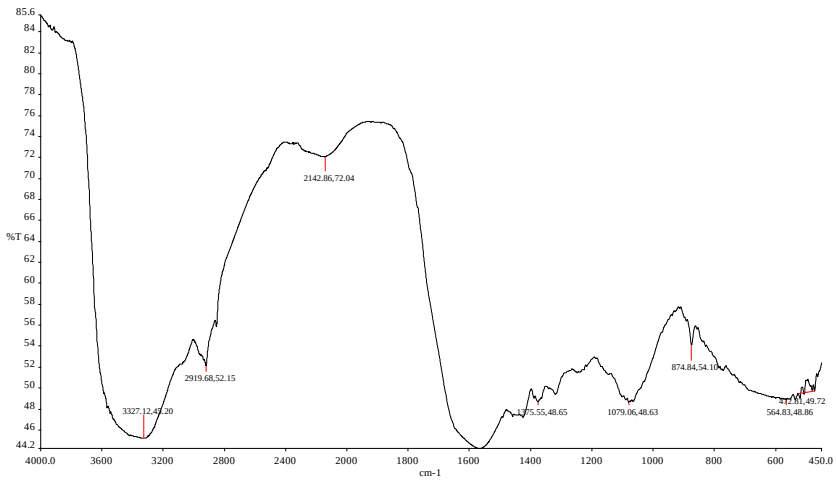


Fig 5:FT-IR Spectrum of *Ampelocissus latifolia* Leaf Powder

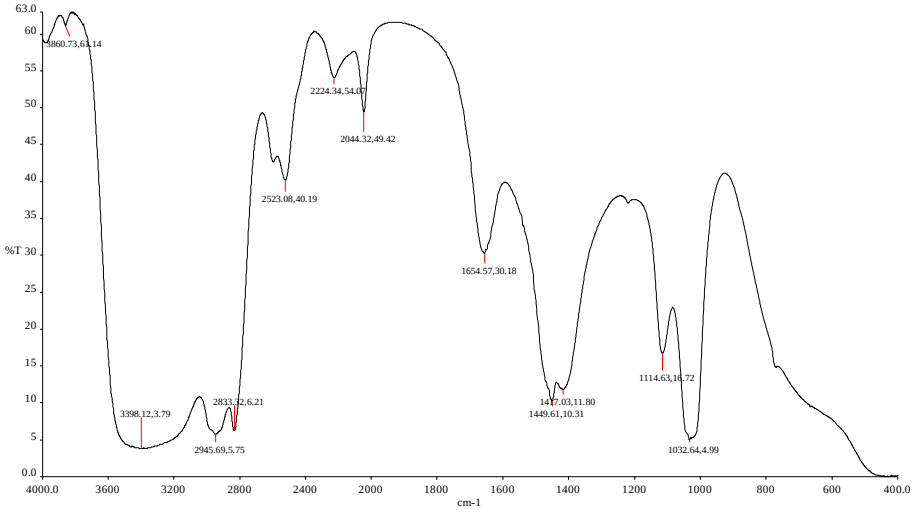


Fig 6:FT-IR Spectrum of *Ampelocissus latifolia* Leaf Methanol Soxhlet Extract

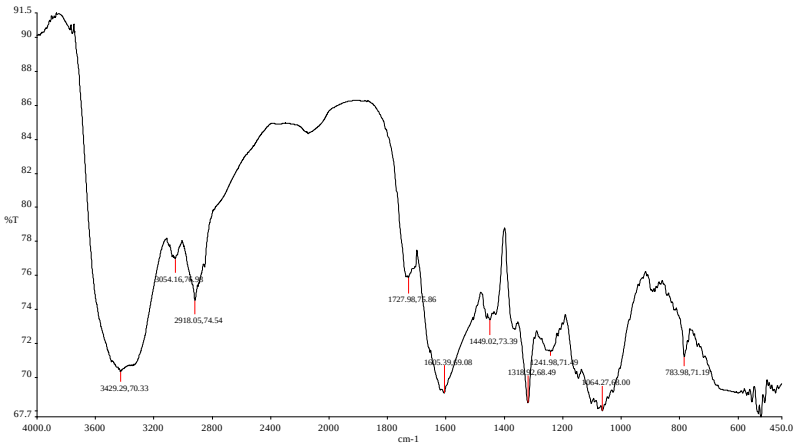


Fig 7. FT-IR Spectrum of *Ampelocissus latifolia* Stem Powder

DISCUSSION

The reported phytochemical studies revealed the presence of alkaloids, reducing sugars, gums, tannins and anthraquinones, etc. in the methanolic extract of the leaf [2] whereas in continuous hot soxhlet extraction using solvents like acetone, chloroform and alcohol, the presence of alkaloids, carbohydrate, phytosterols, fixed oils and fats, saponins, tannins, aminoacids, protein gun and mucilage were revealed[1]. Earlier antimicrobial studies reported that the MBC values can either be the same or higher than the corresponding MIC values[12]. In the antimicrobial study proposed in the present paper for *S. epidermidis,* the MIC was same as the MBC value at 400 mg/ml. Higher values were seen for *M. luteus,* at 800 mg/ml for both MIC and MBC. The lowest values were obtained for *P.acnes* at 200 mg/ml for both MIC and MBC under anaerobic conditions. For Methicillin-resistant *Staphylococcus aureus*, MIC and MBC was at 400 mg/ml. The yeast *M. furfur,* showed MIC at 200 mg/ml and MFC at 4 times MIC value i.e. at 800 mg/ml. The MBC / MFC values which are obtained after plating various dilutions of the extracts are more reliable than the MIC values, obtained using turbidity as a measure of growth. Lower MIC and MBC values indicate higher efficacy[13]. Thus, the low MIC and MBC values exhibited by the leaf extract against *Propionibacterium acnes* is of potential importance in the health care delivery system, since it could be used as an alternative to antibiotics in the treatment of infectious diseases caused by these microbes.

The values of DPPH scavenging activity of methanolic extract are given in Table 2. From the analysis we can interpret that the effect of extract DPPH radicals increased with concentrations. The measurement of the scavenging activity of DPPH radical allows one to determine exclusively the intrinsic ability of the antioxidant compound to donate hydrogen atoms or electrons to this reactive species in a homogenous system. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for the visible deep color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Higher reduction of DPPH is related to the high scavenging activity performed by particular sample. The values of Nitric oxide scavenging activity of methanolic extract are given in Table 3. Nitric oxide is a potent pleiotropic mediator of different physiological process and plays a vital role in smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical, important as an effectors molecule in different biological systems including neuronal messenger, vasodilatation and antimicrobial and anti-tumor activities. But over production of the radical is responsible for pathogenesis of different inflammatory diseases[14].

Spectral differences are the objective reflection of componential differences. By using the macroscope fingerprint characters of FT-IR spectrum, we can judge the origin of different extracts accurately and effectively, trace the constituents in the extracts, identify the medicinal materials and even evaluate the qualities of medicinal materials. So, FT-IR spectrum reflecting objectively the panorama of chemical constituents in complex system is a most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine[15]. The FT-IR analysis revealed the similarity and variation between the leaf and stem parts of *Ampelocissus latifolia* based on the functional group presence and infrared spectrum. It showed characteristic infrared absorbances [Table 4-6]. The listed infrared functional group absorptions characteristic were cited from the literature[16-18]. The crude powder of leaf and stem of *Ampelocissus latifolia* exhibited similar functional groups with respect to the methanolic leaf extract like alkanes, alkenes, carboxylic acids and ethers. In addition to the above mentioned functional groups the leaf powder showed alkynes, alcohols, esters, nitro compounds, phenols, polysulfides and aliphatic iodo compounds which were not present in methanolic leaf soxhlet extract. The stem powder showed alcohols, esters, aldehydes, ketones, phenols and nitro compounds which were not present in methanolic leaf extract. Transition metal carbonyl compounds and aliphatic fluoro compounds were only present in methanolic soxhlet leaf extract.

CONCLUSIONS

The methanolic leaf extract of *Ampelocissus latifolia* has never been evaluated for antimicrobial activity against *Staphylococcus epidermidis*, *Micrococcus luteus,* Methicillin-resistant *Staphylococcus aureus*, *Propionibacterium acnes* and *Malaassezia furfur* before. Also, it is for the first time a detailed study has been undertaken to demonstrate that the methanolic *Ampelocissus latifolia* leaf extract can effectively scavenge various reactive oxygen species or free radicals under in vitro conditions. FT-IR method reveals clearly the differences of categories of chemical constituents in *Ampelocissus latifolia* powder and methanolic extract of leaf. This study will add a new data regarding the uses of ethnomedicine to the state as well as national level inventory of the medicinal plant resources of our country.

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