UGC MINOR RESEARCH PROJECT FINAL REPORT

Project Title: Standardization and Evaluation of Antimicrobial and Antioxidant Potential of Selected Medicinal Plants.

Principal Investigator: Dr.(Mrs.)Vanita Kulkarni.

Co-Investigator: Dr. Bhanu Raman.

File No: 47-184/12 (WRO)

Rationale

Herbal medicine is the oldest known form of medicine in the world. It has been used since the earliest times to treat illness and restore good health. Today, herbs are still used globally for their medicinal properties and remain the most extensively used remedies worldwide for the treatment and prevention of various diseases.

In the present work 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.

Many antioxidant compounds, occurring naturally in plant sources have been identified as free radical or active oxygen scavengers. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to cellular

damage. The methanolic leaf extracts were subjected to evaluation for antioxidant activity by DPPH free radical scavenging method and Nitric oxide radical scavenging method.

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.

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.

Chemicals for Antimicrobial Studies

Methanol A.R. grade (Merck, India), 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 for Antimicrobial & Antioxidant Studies

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 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 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 at106 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

Apparatus for Antioxidant Studies

Jasco V-630 spectrophotometer was used for the measurement of absorbances of solution mixtures for antioxidant studies.

Chemicals for Antioxidant Studies

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),

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. Radical scavenging activity was expressed as inhibition percentage of free radical by the sample and was calculated using following formula:

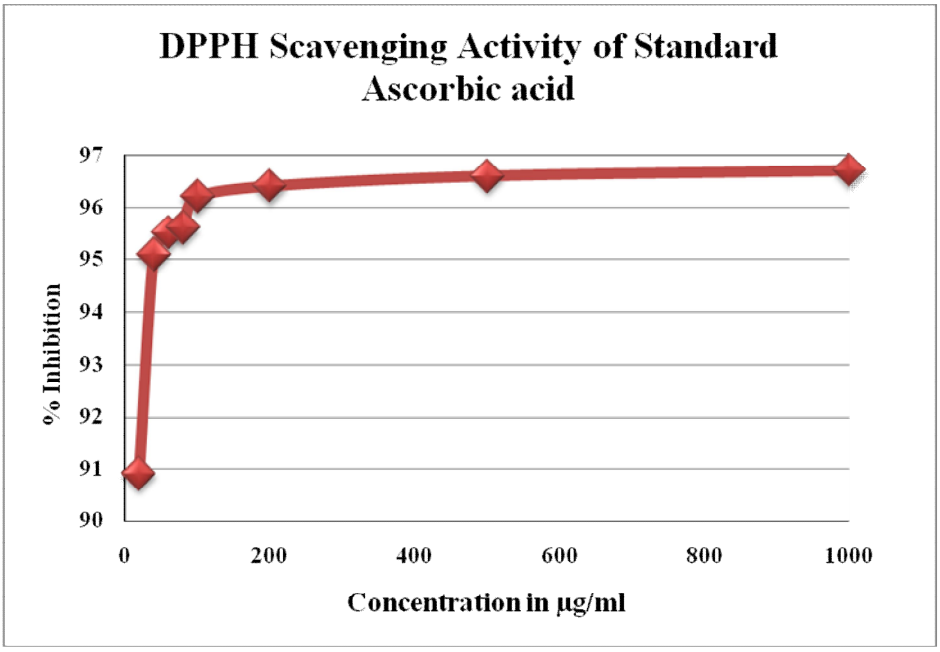
$$\% \text{ DPPH Scavenged} = \frac{\text{AControl} - \text{ATest}}{\text{AControl}} \times 100$$

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

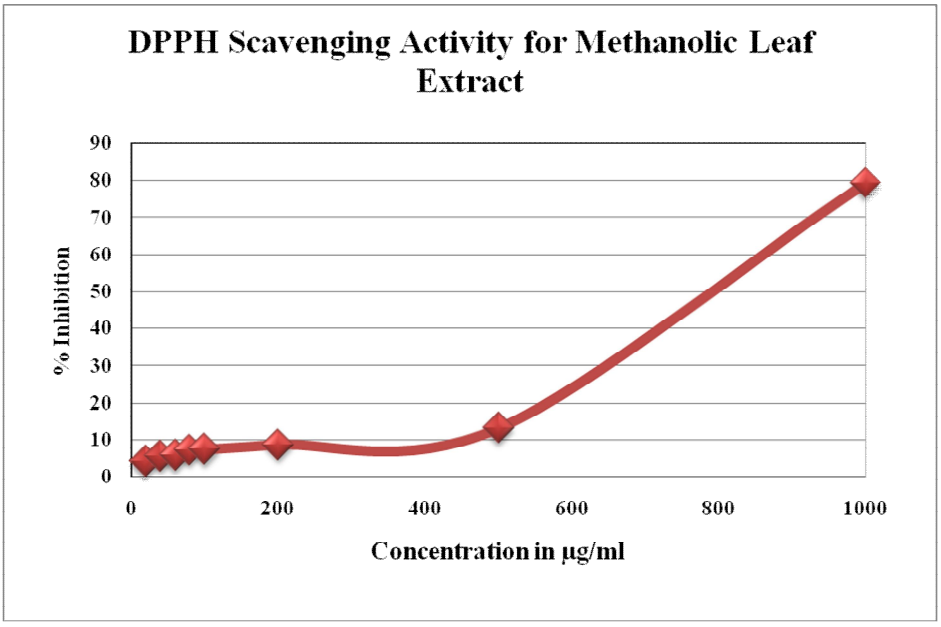
Table 2: Data of DPPH scavenging activity of *Ampelocissus latifolia* methanolic leaf extract.

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. The results of anti-oxidant activity of methanolic extracts using Nitric oxide radical scavenging method were shown below The capability to scavenge the NO radical was calculated using the following equation:

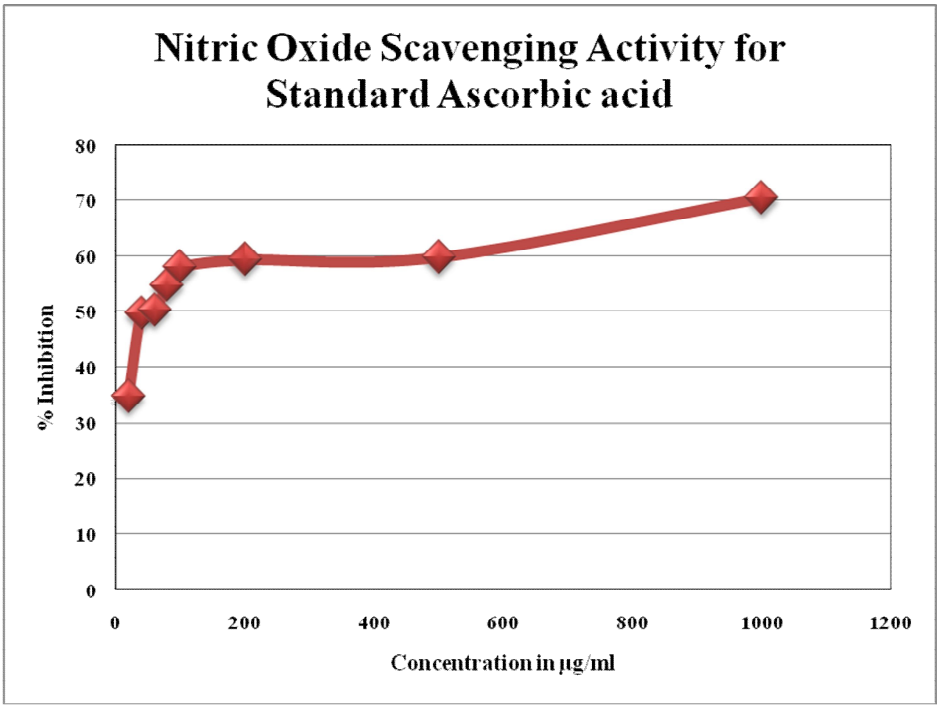
$$\% \text{ NO Scavanged} = \frac{\text{AControl} - \text{ATest}}{\text{AControl}} \times 100$$

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}. (Table 3 & Fig 3 & 4).

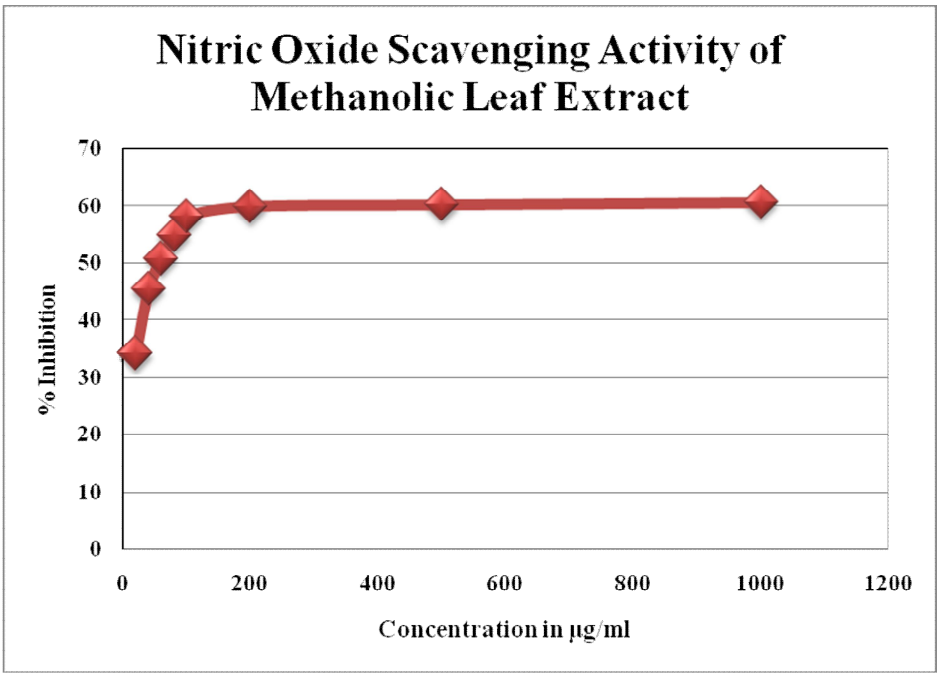
Table 3: Data of Nitric oxide scavenging activity of *Ampelocissus latifolia* methanolic leaf extract.

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.

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.

The methanolic leaf extracts were subjected to evaluation for antioxidant activity by DPPH free radical scavenging method and Nitric oxide radical scavenging method shown in Table 2, 3 & Figure 1-4. 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.

CONCLUSIONS

The methanolic leaf extract of *Ampelocissus latifolia* was evaluated for antimicrobial activity against *Staphylococcus epidermidis, Micrococcus luteus,* Methicillin-resistant *Staphylococcus aureus, Propionibacterium acnes* and *Malaassezia furfur* before. Antioxidant studies was done to demonstrate that the methanolic *Ampelocissus latifolia* leaf extract can effectively scavenge various reactive oxygen species or free radicals under in vitro conditions.