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Assessment of *Annona reticulata* Linn. leaves fractions for invitro antioxidative effect and antimicrobial potential against standard human pathogenic strains

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Abstract Since from long time the plant, *Annona reticulata* Linn. is known for its beneficial effects. Leaves of *A. reticulata* were screened for phytochemicals and in vitro antioxidant, antibacterial and antifungal activity. The shade dried leaves were extracted with methanol and aqueous methanolic extract was partitioned successively with n-butanol, chloroform and acetone solvents. Methanolic extract was subjected to antioxidant screening using DPPH free radical scavenging activity and H_2O_2 scavenging activity. Antibacterial and antifungal activity of extract and fractions were analyzed on eight different clinical bacterial and fungal strains using agar well diffusion method and broth dilution method (MIC and MMC determination). The antioxidant activity showed that the extracts exhibited scavenging effect in concentration-dependent manner. The extract showed potent inhibitory effect against *Bacillus subtilis* and *Escherichia coli* bacterial strains while in case of fungal strains the maximum effect was observed against *Candida blanki*. The maximum zone of inhibition of n-butanol, chloroform and acetone fractions was observed against *B. subtilis*, and *E. coli* respectively while all fractions exhibited potent inhibitory effect against *C. blanki*. MIC and MBC values were determined for active samples, methanol extract and chloroform fraction against *Staphylococcus aureus*, *B. subtilis*, *E. coli* and *Pseudomonas aeruginosa* which revealed lower MIC and MBC values. The fungal strains *Candida albicans*, *Saccharomyces cerevisiae* and *C. blanki* were used to calculate MIC and MFC values for methanol extract and acetone fraction which

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demonstrated lower MIC and MFC values. The results provided evidence that the plant is richly supplied with numerous phytoconstituents that might indeed be potential sources of natural antioxidant, antimicrobial agents and supplementary food.

1. Introduction

Vast number of bacteria and their associated infections are the major challenge in modern medicine. Rapid ability of bacteria to develop resistance to antimicrobial agents produces subsequent failure of most of standard antimicrobial drug treatment thereby increasing chances of chronic infection and risk of mortality.[1] The similarity of eukaryotic fungal cell with human cell is the basis of toxicity of antifungal drugs.[2] Prolong use of standard antifungal drugs such as amphotericin-B and fluconazole leads to several marked adverse effects.[3] Certain broad spectrum antibiotics impede human normal body flora thereby altering normal functions of body.[4] Although free radicals are required for different biological processes but overproduction of radicals under certain conditions such as oxidative stress and abnormal cell physiology leads to different disorders.[5–7] Several oxidants are produced and released by micro-organisms from degradation products of their own metabolism that further leads to numerous harmful effects such as disturbed cell physiology. These destructive effects can be managed by using antioxidants along with antimicrobial agents.[8,9]

Plant contains wide range of secondary metabolites such as carotenoids, phenolics, anthocyanins, and thiols which plays protective role against oxidative damages.[6,10] The health promoting effects of phytochemicals are because of their ability to counter the oxidation process by reacting with free radicals, chelating catalytic metals and scavenging oxygen.[6,11] Also antioxidants of plant origin are devoid of toxic effects whereas synthetic antioxidants[7] such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG), and tert-butylhydroquinone (TBHQ) cause liver toxicity and carcinogenesis.[11] Hence it is essential to explore antioxidant potential of these medicinal plants and their phytochemicals for the effective treatment of variety of diseases.

Plants of the *Annona* genus (Family: Annonaceae) are the notable source of potential therapeutic agents. The plant *Annona reticulata* Linn., commonly known as Ramphal, Bullock's heart, and Custard apple and it is native to India.[12] It grows naturally in tropical and subtropical region. In the rural area plant parts like leaves, bark, seed and root are used as folk medicine to combat against different disease conditions. Various extracts of different plant parts have shown antihyperglycemic,[13] cytotoxic and recombinant caspase inhibitory activity,[14] antinociceptive,[15] analgesic and CNS depressant,[16] analgesic and anti inflammatory,[17] tumor inhibitor,[18] and antiproliferative[19] effects. Dopamine, salsolinol, coclaurine, sesquiterpenes and acetogenin are the bioactive metabolites commonly present in leaves.[20] Other species of plant reported to have antibacterial potential but *A. reticulata* is still not confirmed for antimicrobial potential. Therefore, the objective of present study was to investigate the antioxidant and antimicrobial activity of *A. reticulata* leaves using different antioxidant assay and pathogenic strains of micro-organism respectively.

2. Materials and methods

2.1. Chemicals

Chemicals like dimethyl sulphoxide (DMSO), hydrogen peroxide (H_2O_2), Sodium hydroxide and potassium dihydrogen phosphate were purchased from Rankem (India). 1,1-diphenyl-2-picrylhydrazyl (DPPH), nutrient broth (NB), bacteriological agar, yeast potato dextrose broth (YPD), potato dextrose broth (PD) and antibiotic disk (amoxicillin, ciprofloxacin, amphotericin B and nystatin) were obtained from Himedia (Mumbai, India). Ascorbic acid was purchased from Oxford laboratory, India. All the reagents were of analytical grade purity and obtained from Rankem (India).

2.2. Plant material

The leaves of *A. reticulata* were collected from rural areas of Beed district, in the state of Maharashtra, India in the month of January 2013. The leaves were identified and authenticated by a Botanist, Dr. B.D. Gachande, Associate Professor of Botany department, N. E. S. Science College, Nanded, India. The voucher specimens were deposited at herbarium of School of Pharmacy, SRTM University, Nanded, Maharashtra, India.

2.3. Extraction and fractionation

The leaves of *A. reticulata* were cleaned thoroughly with distilled water and shade dried in clean and dust-free environment. Dried leaves were finely powdered (80 mesh) by using dry grinder. The powder (200 g) was Soxhlet-extracted with 1 L methanol for 8 h at 64 °C. Extract was filtered using Whatman filter paper (No. 1) and concentrated by rotary evaporation (Superfit, India). Dried methanol extract (20 g) was redissolved in 150 ml of distilled water and sequentially partitioned (3×) in separatory funnel with an equal volume of pet ether, n-butanol, chloroform and acetone solvents. Phytochemical solubility and polarity were considered for solvent selection.[21] All the fractions were concentrated under reduced pressure in a rotary evaporator. Yield of pet ether, n-butanol, chloroform and acetone fractions was 6%, 12.5%, 16% and 12% respectively.

2.4. Qualitative phytochemical investigation

Freshly prepared extract of leaves was subjected to standard phytochemical analysis to ensure the presence of alkaloids, flavonoids, steroids, terpenoids, reducing sugars, tannins, saponins, glycosides, aleurone grains, and proteins.[22,23]

2.5. Antioxidant activity

2.5.1. Determination of free radical scavenging activity (DPPH)

The radical scavenging activity of *A. reticulata* leaves extract was estimated using stable free radical of 1,1-diphenyl-2-picrylhydrazyl assay (DPPH).[24] About 1 ml of methanolic extract at different concentrations (20, 40, 60, 80 and 100 μg/ml) was added with 1 ml solution of 0.1 mM DPPH-methanol solution and allowed to incubate at room temperature for 30 min. The absorbance of the resulting mixture was measured at 517 nm against methanol as blank by using UV–Visible spectrophotometer (Shimadzu Kyoto 1800). Known antioxidant such as ascorbic acid was used as positive control.[25] The percentage of radical scavenging activity was calculated using the formula:

$$\%\text{inhibition} = [(A_C - A_T)/A_C] \times 100$$

where A_C and A_T are the absorbance of blank and extract respectively.

Decrease in the absorbance of the DPPH mixture indicates an increase in radical scavenging activity of DPPH. Antioxidant activity of the extract was expressed as IC_{50}, the extracts concentration (in μg/mL) that inhibits the formation of DPPH radicals by 50%. IC_{50} was calculated by plotting the graph of inhibition percentage versus the extract concentration.[26] Assay was carried out in triplicate and the mean values with ±SEM are presented.

2.5.2. Determination of hydrogen peroxide (H_2O_2) scavenging activity

Scavenging of H_2O_2 by extract was used to determine antioxidant ability of extract according to method of Gulcin.[27] Solution of H_2O_2 (0.2 M) was prepared in phosphate buffer (pH 7.4). 1 ml extract of different concentrations (20, 40, 60, 80 and 100 μg/ml) was added to 0.6 ml solution of 40 mM hydrogen peroxide solution. Absorbance of mixture was measured at 230 nm using UV–Visible spectrophotometer (Shimadzu Kyoto1800) against a blank solution containing phosphate buffer solution without H_2O_2. Known antioxidant such as ascorbic acid was used as positive control.[28] IC_{50} value was determined by linear regression analysis. The percentage of H_2O_2 scavenging was calculated by following formula:

$$\%\text{inhibition}(H_2O_2) = [1 - (\text{Absorbance of extract}/\text{Absorbance of control})] \times 100$$

2.6. Antimicrobial activity

2.6.1. Microbial strains and growth conditions

The methanolic extract and fractions were selectively tested against eight different strains of microorganisms. Strains were obtained from School of Life science, Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded, Maharashtra, India. Gram positive bacteria strains used include *Staphylococcus aureus* (MTC 96), *Staphylococcus epidermidis* (MTCC 1228), *Proteus vulgaris* (ATCC 33420) and *Bacillus subtilis* (B 28). *Escherichia coli* (MTCC 170), *Pseudomonas aeruginosa* (CC 488), *Klebsiella pneumoniae* (ATCC 15380), *Salmonella typhi* (ATCCB 23564) were Gram negative strains used for determination of antibacterial activity. Fungal strain *Aspergillus niger* (MTCC A), *Aspergillus flavus* (MTTC 873), *Aspergillus fumigatus* (MTCC 2551), *Vestilago myditis* (MCIM 983), *Microsporum canis* (MTCC 2520), *Candida albicans* (MTCC 3018), *Saccharomyces cerevisiae* (MCIM 170), *Candida blanki* (MTCC 1442) were employed for determination of antifungal activity. The bacterial strains were cultured overnight at 37 °C in nutrient agar while fungal strains were cultured overnight at 30 °C using potato dextrose agar.

2.6.2. Determination of inhibitory effect

Antimicrobial activity methanolic extract (ME) and n-butanol (NB), chloroform (CH) and acetone (AC) fractions were determined using agar well diffusion method. Molten and cooled agar (20 ml/dish) were poured into sterilized petri dishes. The plates were left overnight at room temperature and observed for contamination. Wells of 10 mm diameter were prepared with the help of sterilized stainless steel cork borer. 100 μl culture of each strain was used to prepare lawn on agar plates by using spreader. ME and fractions (100 μg/ml) were prepared in DMSO and 100 μl of each was used for activity. One well in each plate was loaded with ME and three wells were loaded with NB, CH, AC fraction respectively. Amoxicillin and ciprofloxacin were used as standard drug for antibacterial activity whereas griseofulvin was used for antifungal activity. The bacterial plates were incubated at 37 °C for 24 h and fungal plates were incubated at 27 °C for 48 h. The antimicrobial activity was assessed by measuring diameter of zone of inhibition at cross angles after incubation and compared with zone of inhibition of the standard antimicrobial drug.

2.6.3. Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined by broth dilution method.[29] It is the lowest concentration of the sample at which the tested microorganisms did not demonstrate any visible growth after incubation.[25] 0.5 ml of sample (90, 80, 70, 60, 50, 40, 30, 20, 10 μg/ml) was added to 2 ml of nutrient broth and a loopful of the test organisms was introduced into the tubes. Same procedure was repeated using the standard antibiotic drug. A tube containing nutrient broth was seeded with the test organisms that which serve as control. Tubes were incubated at 37 °C temperature for 24 h for bacteria and at 28 °C temperature for 48 h for fungi. After the incubation period, the tubes turbidity was observed and it was considered as the indication of growth.

2.6.4. Minimum microbicidal concentration (MMC)

Minimum microbicidal concentration (MMC) includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC) of sample which was determined according to the MIC values.[25] The tubes showing no turbidity were streaked on nutrient agar medium and incubated at 37 °C for 24 h for calculating MBC. Similarly to calculate MFC, tubes showing no turbidity were streaked on yeast potato agar medium and incubated at 28 °C for 48 h. The nutrient agar and potato dextrose agar streaked with the test organisms were taken as control. The lowest concentration at which had no visible growth was taken as the minimum microbicidal concentration.

2.7. Statistical analysis

All the analyses were performed in triplicate. The results were expressed as mean ± SEM. Statistical analysis was carried out by one way ANOVA followed by *post hoc* Tukey test using GraphPad InStat version 3 USA. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Preliminary phytochemical screening and extraction yield

Preliminary phytochemical investigation of ME revealed the presence of different phytoconstituents which are listed in Table 1.

3.1.1. DPPH radical scavenging activity

Radical scavenging ability of ME has been determined by DPPH radical scavenging assay, a standard and rapid technique for the identification of antioxidant compounds. The DPPH is stable free radicals which get reduced in presence of antioxidant compound that intern decreases absorbance ability of DPPH at 517 nm. A lower absorbance at 517 nm indicates a higher radical-scavenging activity of ME. The scavenging activity of ME and standard, ascorbic acid on DPPH was expressed as IC_{50} value. Fig. 1 represents concentration dependent change in absorbance. The IC_{50} value of ME and ascorbic acid was 52.08 μg/ml and 21.80 μg/ml respectively.

3.1.2. Hydrogen peroxide (H_2O_2) scavenging activity

The ability of ME to scavenge hydrogen peroxide is shown in Fig. 2 and compared with that of ascorbic acid as reference standard. ME showed dose dependent H_2O_2 scavenging activity as that of ascorbic acid. The hydrogen peroxide radical scavenging activity of extract was (27.38 ± 0.21)%, (35.71 ± 0.57)%, (46.42 ± 0.01)%, (54.76 ± 0.03)% and (63.09 ± 0.05)% at the concentration of 20, 40, 60, 80 and 100 μg/ml respectively. The IC_{50} values for ME and ascorbic acid were 70.06 μg/ml and 18.85 μg/ml respectively.

Table 1 Phytochemical screening of *Annona reticulata* leaves extract.

Sr. No.	Phytoconstituents	Result
1	Alkaloid	+
2	Amino acids	+
3	Carbohydrates	–
4	Fats and fixed oils	–
5	Flavonoid	+
6	Glycosides	+
7	Phenolic compound	+
8	Proteins	+
9	Starch and waxes	–
10	Steroids and triterpenoid	+
11	Naphthoquinones	–
12	Aleurone grains and inulin	+
13	Acidic compound	–

+, positive; –, negative.

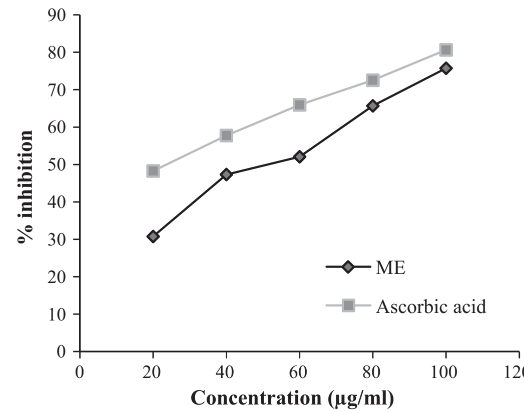

Figure 1 DPPH radical scavenging activity of ME of *Annona reticulata* leaves and ascorbic acid.

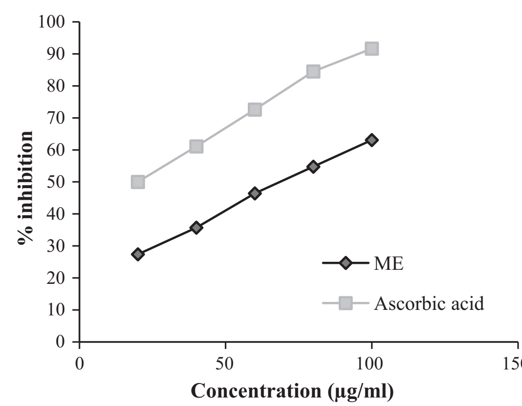

Figure 2 Hydrogen peroxide scavenging activity of ME of *Annona reticulata* leaves and ascorbic acid.

3.2. Antimicrobial study

3.2.1. Antibacterial activity

The antimicrobial activity of different concentrations of ME, NB, CL and AC of *A. reticulata* leaves was determined against eight bacterial and fungal strains and recorded as zone of inhibition. The results are presented in Table 2. The inhibition zone of ME was obtained against all the strains of bacteria were in the range of 11–17 mm. The ME exhibited potent inhibitory activity against *B. subtilis* (16.33 ± 0.33) and *E. coli* (14.66 ± 0.33). The NB fraction had maximum zone of inhibition against *B. subtilis* (14.33 ± 0.33) whereas minimum zone of inhibition for *K. pneumoniae* (10.66 ± 0.33). No zone of inhibition observed for *S. epidermidis*, *E. coli* and *P. aeruginosa*. The CL fraction showed inhibitory effect against all bacterial strains with highest inhibition zone for *B. subtilis* (17.33 ± 0.33) and *E. coli* (16.66 ± 0.33). The highest inhibitory activity of AC fraction was observed against *E. coli* where as the weakest inhibitory activity was determined against *S. epidermidis*. AC fraction was not effective against *P. vulgaris* and *P. aeruginosa*. Antibacterial activity of ME and

Table 2 Antibacterial activity of ME and NB, CL, AC fractions from *Annona reticulata* leaves.

Bacterial strain	Diameter of inhibition zone (mm)					
	ME	NB	CL	AC	Amoxicillin	Ciprofloxacin
S. aureus	13.66 ± 0.33	12.66 ± 0.33	12.66 ± 0.33	11.00 ± 0.00	15.33 ± 0.33	16.66 ± 0.66
S. epidermidis	–	–	14.33 ± 0.33	10.66 ± 0.33	17.33 ± 0.33	15.66 ± 0.66
P. vulgaris	11.33 ± 0.33	13.33 ± 0.33	14.33 ± 0.33	–	16.33 ± 0.33	16.66 ± 0.66
B. subtilis	16.33 ± 0.33	14.33 ± 0.33	17.33 ± 0.33	10.66 ± 0.33	15.33 ± 0.33	15.00 ± 1.00
E. coli	14.66 ± 0.33	–	16.66 ± 0.33	14.33 ± 0.33	16.33 ± 0.33	17.33 ± 0.33
P. aeruginosa	11.00 ± 0.00	–	10.66 ± 0.33	–	15.33 ± 0.33	16.00 ± 0.57
K. pneumoniae	11.00 ± 0.57	10.66 ± 0.33	11.33 ± 0.33	13.00 ± 0.57	17.33 ± 0.33	17.00 ± 0.57
S. typhi	13.33 ± 0.33	13.00 ± 0.57	11.66 ± 0.33	12.00 ± 0.57	16.33 ± 0.33	15.33 ± 0.33

Table 3 MIC and MBC of ME and CL fraction of *Annona reticulata* leaves.

Bacterial strain	MIC (μg/ml)		MBC (μg/ml)	
	ME	CL	ME	CL
S. aureus	40	50	40	60
B. subtilis	10	10	20	10
E. coli	30	20	30	30
P. aeruginosa	60	70	70	80

Table 5 MIC and MFC of ME and AC fraction of *Annona reticulata* leaves.

Fungal strain	MIC (μg/ml)		MFC (μg/ml)	
	ME	AC	ME	AC
C. albicans	30	–	30	–
S. cerevisiae	40	50	50	60
C. blanki	20	10	20	10

NB, CH, AC fractions was compared with amoxicillin and ciprofloxacin as a standard.

3.2.2. Minimum microbicidal concentration (MMC) and minimum bactericidal (MBC)

The ME and CL fraction were selected for MIC and MBC study as they found to be effective against all bacterial strains. Potent antibacterial effect was observed against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. MIC and MBC values of ME and CL fraction were listed in Table 3. The MIC values of ME against tested bacterial strains were in between 10 μg/ml to 60 μg/ml while MBC was in between 20 μg/ml to 70 μg/ml. MIC and MBC values of CL fraction were in the range of 10 μg/ml to 70 μg/ml and from 10 μg/ml to 60 μg/ml respectively.

3.2.3. Antifungal activity

Antifungal effect of ME and NB, CL, AC fractions was tested against selected strains of fungi. According to results mentioned in Table 4, all the samples exhibited potent antifungal effect against *C. Albicans*, *S. cerevisiae* and *C. blanki*. ME showed highest inhibition zone against *C. Blanki* (14.66 ± 0.33) and lowest against *S. cerevisiae* (12.66 ± 0.88). All the samples exhibited potent inhibitory effect against *C. blanki*. The AC showed significant inhibition zone against *C. blanki* (17.00 ± 0.57). No inhibition zone was observed against *A. niger*, *A. fumigates*, *A. flavus*, *V. myditis* and *M. canis*.

3.2.4. Minimum microbicidal concentration (MMC) and minimum fungicidal concentration (MfC)

The MIC and MFC for ME, AC fractions were investigated against *C. albicans*, *S. cerevisiae* and *C. blanki* and listed in Table 5. The MIC and MFC values for ME were 30 μg/ml, 40 μg/ml, 20 μg/ml and 30 μg/ml, 50 μg/ml, 20 μg/ml for *C. albicans*, *S. cerevisiae* and *C. blanki* respectively. The MIC value of AC fraction was 50 μg/ml for *S. cerevisiae* and 10 μg/ml for *C. blanki*, while MFC was 60 μg/ml for *S. cerevisiae* and 10 μg/ml for *C. blanki*.

4. Discussion

In search of effective antimicrobial agent, current research is focusing over plants as a potential source of medicine as

Table 4 Antifungal activity of ME and NB, CL and AC fractions from *Annona reticulata* leaves.

Fungal strain	Diameter of inhibition zone (mm)				
	ME	NB	CL	AC	Griseofulvin
C. albicans	13.33 ± 0.33	12 ± 0.57	11.33 ± 0.33	–	17.33 ± 0.33
S. cerevisiae	12.66 ± 0.88	11.66 ± 0.88	11.66 ± 0.66	11.33 ± 0.33	16.00 ± 0.57
C. blanki	14.66 ± 0.33	14.33 ± 0.66	14.66 ± 0.33	17.00 ± 0.57	15.33 ± 0.33
A. niger	–	–	–	–	–
A. fumigates	–	–	–	–	–
A. flavus	–	–	–	–	–
V. myditis	–	–	–	–	–
M. canis	–	–	–	–	–

phytochemicals are generally safe with no or few side effects.[30] Very few plant species have been investigated for their medicinal properties and still plant species is the richest source for novel bioactive molecules.[31] *A. reticulata* is one of the most popular nutritional and medicinal plant which is rich in variety of secondary metabolites including polyphenols.[20] Suresh et al.[19] revealed anticancer effect of root extract against human melanoma cell which might be due to presence of acetogenins and alkaloids. The antibacterial and antifungal effect of methanol extract and fractions against different pathogenic bacterial and fungal strains had supported traditional claims of plant as a medicine. The presence of phenolic and polyphenolic compounds in the methanolic extract may be attributed to potent inhibitory effect of methanol extract against seven bacterial strains. It is already reported that polyphenolic compounds are biologically active and possesses antimicrobial property[32] suggesting that antibacterial and antifungal activity of methanolic extract and fractions may be due to the presence of some phenolic compounds. Methanolic extract and fractions were not effective against certain strains of bacteria and fungi which may be attributed to presence of lesser amount of antimicrobial compounds or lesser susceptibility of these strains to phytochemicals present in extract and fractions. Several mechanisms has been explained for antimicrobial effect of polyphenols that mainly includes ability of polyphenols to neutralize bacterial toxins, biofilm inhibition, reduction of host ligands adhesion, enzyme inhibition, interaction with eukaryotic DNA and membrane disruption.[33] Methanol extract and chloroform fraction also exhibited broad antibacterial activity against tested bacterial strains. Several previous studies revealed that antimicrobial activity of plant extract is due to presence of alkaloids[34], saponins[35,36] and flavonoids.[37] The MIC and MMC values were relevant to results obtained in antibacterial and antifungal screening, showing that methanol extract exhibited potent antimicrobial activity. Similar potent antibacterial and antifungal activity were demonstrated by chloroform fraction and acetone fraction respectively. The erratic antimicrobial activity of extract and fractions was due to presence of other inactive and inert plant metabolites that do not posses antimicrobial activity.[38]

The compounds having antioxidant property may own additional antimicrobial activity.[25] Antioxidants exert several functions in biological system which mainly involve protection from oxidative injury and in cell signaling pathways. The protective effects of antioxidants are because of its ability to scavenge reactive oxygen species such as superoxide radical, hydroxyl radical, peroxide radical and nitric acid radical that are produce during abnormal metabolic processes.[39] Due to association of different complex mechanisms with antioxidant property[40,41] it is difficult to predict antioxidant efficacy of phytochemicals. The antioxidant activity of extract and fractions of *A. reticulata* was determined by two spectrophotometric methods, DPPH and hydrogen peroxide scavenging method. Reduction in DPPH absorption might be due to phytochemicals which have scavenging ability either by transfer of hydrogen or of an electron.[10] Ascorbic acid restrains potent ability to scavenge DPPH so it is used as a standard antioxidant. The methanolic extract represented dose dependent free radical scavenging corresponding with the results of Ebrahimabadi et al.[42,43] showing that plant metabolites like flavonoids, tannins and other phenolic posses antioxidant activity.

Hydrogen peroxide has ability to oxidize thiol (–SH) group thereby causes inactivation of enzymes that contains thiol group. It rapidly crosses cell membrane and reacts with Fe^{2+}, Cu^{2+} which intern generates hydroxyl radicals. Hydroxyl radicals are the most reactive and cyto-toxic among the oxygen radicals.[6] Extract exhibited pronounced dose dependent hydrogen peroxide scavenging activity as that of ascorbic acid which may be attributed to the presence of several electron donating compounds such as alkaloids, flavonoids, phenolics and leads to neutralization of H_2O_2 to water.[11]

5. Conclusion

On the basis of data obtained by the study, we concluded that tested extract and fraction samples had substantial antimicrobial activity and leaves of *A. reticulata* could be source of bioactive antimicrobial components. Methanolic extract obtained from the leaves possessed strong antimicrobial and antioxidant activity. The samples demonstrated potent antimicrobial activity against pathogenic bacterial and fungal strains. The antioxidant activity might be attributed to the presence of several natural antioxidants. Finally, the results obtained in the present study demonstrated that *A. reticulata* possesses good antioxidant and antimicrobial activity, suggesting that it could be useful in the treatment of free radicals and associated diseases in the form of antimicrobial agent or supplementary food. Further studies will be required to explore precise mechanisms and component underlying these beneficial biological effects.

Conflict of interest statement

The authors declare no conflict of interests.

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