Enhanced antimicrobial, antioxidant and anticancer activity of *Rhizophora apiculata*: An experimental report

V. Ramalingam¹ · R. Rajaram¹

Received: 7 June 2017 / Accepted: 17 March 2018 / Published online: 24 March 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

The present study is designed to evaluate the antimicrobial, antioxidant and anticancer activities of *Rhizophora apiculata*. Initially, the phenolic and flavonoid content was quantified in solvent extracts, and gallic acid and rutin were used as a control, respectively. Further, antimicrobial and minimal inhibitory activities of different solvent extracts were assessed against human clinical pathogenic bacteria, and the results showed that butanol and methanol extract has potential antimicrobial activity. FTIR analysis of solvent extracts showed the presence of phenolic compounds at 3409–3430 cm⁻¹ that actively involved in various applications including antioxidant and anticancer activities. The in vitro antioxidant activity of solvent extracts showed excellent antioxidant potential, about 84% of DPPH free-radical scavenging, 76% of hydrogen peroxide, 82% of hydroxyl radical scavenging, and 80% of reducing power. Two-way ANOVA analysis showed that the highly significant effect of antioxidant activity depends on the concentration of extracts. The DNA protection efficiency of extracts against oxidative damage was confirmed by DNA nicking assay using bacterial DNA. The methanol extract effectively inhibited the growth and induces the apoptosis through ROS generation and sensitizes the mitochondrial membrane potential of A549 lung cancer cells. Taken together, the results showed that the solvent extracts of *R. apiculata* could be potential antioxidant and anticancer agents.

Keywords *Rhizophora apiculata* · Flavonoids · Antioxidant activity · DNA nicking assay · Anticancer activity

Introduction

The comprehensive effect of cellular antioxidant resistance becomes weak to produce free radical due to the extreme reproduction of reactive oxygen species (ROS) and loss of antioxidant activity or both (Liochev 2013; Benedetti et al. 2011). The by-products of organic synthesis or extracellular factors are induced ROS generation including hydroxyl radicals, hydrogen peroxide and superoxide radicals. The scavenging efficiency of ROS can serve as a desirable remedy against free radical-mediated diseases including cancer, inflammation. (Rathee et al. 2007). ROS and free transition ions are the cause of complete oxidative stress to DNA, proteins and lipids that promote oxidative stress-related disorder (Droge 2002). Moreover, chemical antioxidants involve

being favourable for toxicity and undesirable reaction that prevent their broad recipe. Consequently, the importance to use of natural antioxidants for prevention of numerous oxidative stress-related syndromes was increased (Ningappa et al. 2008).

In modern technology, natural antioxidants are focused to lead the human from free radicals and decrease the development of many chronic diseases (Meghashri et al. 2010). The edible and non-edible herbs and their secondary products have the diversity of phenolic compounds acquire numerous biological outcomes including antioxidant and anticancer activities. The consumption of plant foods was profitable to the dynamism and supports to avoid the degenerative process, consequently inferior frequency and mortality rate to cancer, cardio and cerebrovascular diseases (Nithiyanantham et al. 2013). Nowadays, the commercial antioxidants, 2- and 3-*tert*-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA) and *tert*-butylhydroquinone (TBHQ), were included in fodder owing to its toxicity, the use is being investigated (Valentao et al. 2002), thus leads to researchers to focus the natural resources.

✉ R. Rajaram
drrajaram69@rediffmail.com

¹ DNA Barcoding and Marine Genomics Lab, Department of Marine Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu 620 024, India

Among the natural resources, mangrove plants have been utilized for many traditional medicines for numerous diseases in India (Loo et al. 2007) that includes *Rhizophora mucronata*, *R. mangle* and *R. apiculata*. The diverse of mangrove plants occurred in Eastern and Southeast Asia and contains many therapeutic applications (Rahim et al. 2008). *R. mucronata* is used in the treatment of angina and hemorrhage while old leaves or roots are used in childbirth (Kusuma et al. 2011). The anti-HIV activity such as HIV replication and HIV-reduced cytopathic effects was reported using polysaccharide from the leaves *R. apiculata* (Premnathan et al. 1999). The tannins are the potential source of reducing power (Gulcin et al. 2003) and scavenging activities (Minussi et al. 2003), while the mangrove tannins are consist of proanthocyanidins (Rahim 2005) and the structural analysis of tannins was characterized by depolymerisation reactions of nucleophiles. Therefore, we have selected *R. apiculata* in this study to determine the efficiency of antimicrobial, antioxidant and anticancer activities.

Lung cancer is the leading cause of cancer-related death among men worldwide and is the second among women, with a 5-year survival rate is only 18%. However, the response rate to chemotherapy was less than 30% and also many patients suffered serious side effects after chemotherapy (Vaikundamoorthy et al. 2016). Thus, to further explore the valuable diagnostic and novel therapeutic targets is an urgent need. Recently, the researchers aiming at developing a novel compound from natural resources due to its less toxicity. The aim of this study is to assess the anticancer activity of *R. apiculata* against A549 human lung cancer cells. Further, the assessment of antioxidant and antimicrobial activity of *R. apiculata* and oxidative DNA damage protecting activity using different solvent extracts was evaluated.

Materials and methods

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), potassium ferricyanide [$K_3Fe(CN)_6$], L-ascorbic acid, trichloroacetic acid (TCA) and deoxyribose were obtained from Sigma Chemicals St. Louis, MO, USA. Hydrogen peroxide, ferrous chloride ($FeCl_2$), ferric chloride ($FeCl_3$), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], ethylene diamine tetra acetic acid (EDTA), methanol, butanol, hexane, chloroform and ethanol were purchased from Fisher scientific and Mueller–Hinton agar and Muller–Hinton broth purchased from HiMedia, Mumbai, India. Absorbance spectrometry analysis was done using Shimadzu model 1800 UV spectrophotometer (Japan) and Fourier transform infrared spectrophotometer (FT-IR) analysis was carried out in JASCO 460 plus FT-IR.

Sample collection and preparation of solvent extract

The fresh mangrove plant *R. apiculata* was collected from Muttukuda, Southeast coast of India (Lat 9.93715 and Long. 79.14915) and identified (Gurudeeban et al. 2015). The collected mangrove was washed thoroughly with artificial seawater and brought to the laboratory. The mangrove was cut into small pieces and dried at room temperature. The dried leaves were powdered using mortar and pestle and 2 g of powder was used for extraction with different solvents such as ethanol, methanol, hexane, butanol and chloroform (each 20 ml) using Soxhlet apparatus. The supernatant was filtered through no. 1 Whatman filter paper (40 μ m) to attain the solvent extracts and stored at 4 °C until further analysis.

Phytochemical analysis and characterization of solvent extracts

The solvent extracts were evaluated for phytochemical composition such as phenols and flavonoids using gallic acid and rutin as standard (Roopashree et al. 2008). The presence of phenols and flavonoids were expressed in microgram of gallic acid and rutin that equivalent of 1 g/ml of solvent extracts was achieved from the following linear relationships: $f(\text{concentration of gallic acid/rutin}) = \text{yield of phenols/flavonoids}$. The silica gel TLC plate was prepared with solvent chloroform–methanol–water (65:25:4, v/v/v) and extracts were spotted on the bottom of the plate and run in the solvent. The single spot was visualized by ultraviolet light and scraped for FTIR analysis. The scraped compound was dried and blended with KBr to obtain a pellet. FTIR spectra were collected at a resolution of 4 cm^{-1} in transmission mode range between 4000 and 400 cm^{-1} . Methanol extracts of *R. apiculata* were further characterized using GC–MS and 1H NMR analysis.

Antimicrobial activity

Agar-well diffusion assay

Human clinical pathogenic bacteria such as *E. coli*, *Vibrio cholerae*, *Salmonella* sp., *Corynebacterium* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella* sp., *Klebsiella* sp., *Enterobacter* sp. and *Mycobacterium* sp. were collected from Government Hospital, Trichy, Tamil Nadu, India and used for antimicrobial activity via agar well-diffusion assay and minimum inhibitory concentration. All bacterial pure cultures were sub-cultured in nutrient broth and incubated on the shaker for 24 h at 30 °C. The liquid bacterial culture was swabbed on the Mueller–Hinton agar plates and

6 mm hole was bored at the centre of plates. The holes were packed with 50 μ l (10 μ g/ml) of different solvent extracts of mangroves and kept for 1 h to settle extracts. The plates were incubated another 24 h and evaluated for antimicrobial activity (Farjana et al. 2014).

Minimum inhibitory concentration (MIC)

The concentration of solvent extracts used for the MIC as same as in agar-well diffusion assay. MIC was resolved in accordance with the standard microdilution method (Andrews 2001). The test organisms were diluted into a 96-well microtiter plate with different solvent extracts (10 μ g/ml) were dissolved and incubated for 24 h at 37 °C. The MIC of solvent extracts was evaluated by measuring the culture growth in UV Spectrophotometer at 600 nm.

Anti-oxidant activity

DPPH free radical scavenging activity

The DPPH radical scavenging activity of different solvent extracts of *R. apiculata* was assessed according to the previous method (Apostolou et al. 2013). Briefly, 1 ml of freshly prepared methanol solution of DPPH (1 mM) was mixed with different solvent extracts at various concentrations (10–100 μ g/ml). The reaction mixture was shaken and incubated at 37 °C for 30 min in dark place, absorbance was recorded at 517 nm. In every experiment, methanol was considered as blank while ascorbic acid as control was also run simultaneously. The antioxidant BHT was used as a positive control in all assays. The inhibition of DPPH radical scavenging capacity of tested extracts was calculated according to the following equation:

$$\% \text{ scavenging} = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

H₂O₂ scavenging activity

Hydrogen peroxide scavenging activity was measured by incubating the reaction mixture containing various concentrations (10–100 μ g/ml) of solvent extracts (1 ml), 2.5 ml of phosphate buffer (pH 7.4; 100 mM) and 400 μ l of H₂O₂ (5 mM) for 20 min (da Silva et al. 2011). After incubation, the reaction mixture was read at 610 nm. For each and every experiment, the mixture without sample was considered as blank and ascorbic acid was used as a control.

Hydroxyl radical scavenging activity (HRSA)

Hydroxyl radical scavenging activity of extracts was evaluated as described previously (Rathee et al. 2007) with slight modifications. 100 μ l of different concentrations of extracts (10–100 μ g/ml) was dissolved in buffer (0.6 ml of PO₄ buffer

(pH 7.4), 10 mM deoxyribose and 170 mM EDTA) and the reaction was commenced by adding 100 μ l of ascorbic acid (2 mM) and 100 μ l of 10 mM H₂O₂. After incubation at 80–90 °C for 20 min, the reaction was stopped by the addition of 1 ml of TCA (1%) and heat at boiling water bath for 5 min. The amount of pink-colored chromogen was measured at 532 nm against reaction mixture without sample as blank and ascorbic acid as a control.

Reducing power

The various concentrations (10–100 μ g/ml) of solvent extracts (1 ml) were dissolved into 0.2 M phosphate buffer (pH 6.6) with 1% of potassium ferricyanide (2.5 ml) and vigorously mixed. After incubation at 50 °C for 20 min, the reaction was terminated by adding 1 ml of 10% TCA. The reaction mixture was centrifuged at 3000 rpm for 15 min and ferric chloride was dissolved into supernatant. The solution was recorded at 700 nm against ascorbic acid as control and reducing power was calculated (Loo et al. 2007).

DNA damage protecting activity

The potential of solvent extracts was assessed for DNA damage protection assay according to previous method (Kaur et al. 2008). DNA was isolated from *Staphylococcus aureus* (KF554244) by previously described method (Ramalingam et al. 2014). The isolated DNA was destructed by Fenton's reagent (FR) (30 mM H₂O₂, 50 mM ascorbic acid and 80 mM FeCl₃). 3 μ l of DNA was mixed with 10 μ l of FR and incubated for 45 min at room temperature. Consequently, different solvent extracts (100 μ g/ml) and BHT was also incubated with the reaction mixture. DNA was visualized by running 1.2% of the agarose gel stained with ethidium bromide.

Anticancer activity

Cancer cell lines

The lung cancer cell line A549 and normal cell line HBL100 were purchased from National Centre for Cell Sciences (NCCS), Pune, India. Cancer and normal cells were grown in Dulbecco's modified eagles medium (DMEM) supplemented with L-glutamine with 10% fetal bovine serum and cells were maintained with 1% penicillin/streptomycin in a 5% CO₂ humidified atmosphere at 37 °C.

MTT assay

The cytotoxic activity of methanol extracts of *R. apiculata* was evaluated using MTT assay (Ramalingam et al. 2016). Lung cancer cells (1×10^{-4} cells/ml) were taken into 96-well

culture plate and incubated for 48 h at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. After incubation, the medium was replaced with different concentrations of methanol extracts and further incubated for 24 h. After 24 h, MTT solution (5 mg/ml) was added and incubated for 4 h and 200 µl of DMSO was added to solubilise the formazan crystals. The absorbance was measured at 570 nm using microplate reader (Bio-rad, USA). The IC₅₀ concentration of methanol extracts was calculated using SPSS software (version 16.0).

Morphological changes

A549 cells were seeded on a coverslip in six-well plate and allowed to attach overnight. The next day, the medium was replaced with fresh medium containing left alone/treated with methanol extracts (IC₅₀ concentration) and allowed to incubate for 24 h. For ROS measurement, the coverslip was stained with 10 mM DCFH-DA and incubated at 37 °C for 45 min. For AO-EB staining, the coverslip was stained with 50 mg/ml of AO/EB and for mitochondrial membrane potential ($\Delta\psi$ m) the washed A549 cells were incubated with 50 ml of Rhodamine-123 dye (10 mg/ml) for 30 min (Ramalingam et al. 2017). Finally, the stained coverslip was washed with 1× PBS to remove excess dye, then coverslip was fixed on slide and the images were captured using 40× objectives under the fluorescence microscope (Carl Zeiss, Jena, Germany).

Statistical analysis

All experiments were executed in triplicates and results were presented at the mean. Statistical analysis was performed with multiple variance analysis (two-way ANOVA) using Tukey's test by comparing all the extracts with BHT for the dose–response relationship.

Results

Solvent extraction was more commonly used for the separation of antioxidant and antimicrobial potential, depending upon the nature of the solvent. In this present study, the five different polar and non-polar solvents of *R. apiculata* were used to prepare extracts and assessed for antioxidant and antimicrobial potential. The phytochemical screening of all solvent extracts was revealed that the rich amount of phenols and flavonoids (Fig. 1) was presented in all the extracts of *R. apiculata*.

Inhibitory activity of solvent extracts was checked against ten different pathogenic microorganisms using well diffusion method (Fig. 2a) and MIC (Fig. 2b) expressed potential activity. Commencing all solvent extracts, butanol has high antimicrobial effect against *Corynebacterium* sp. (29 mm), *Staphylococcus aureus* (28 mm) and *Vibrio cholera* (25 mm) while hexane extracts *Corynebacterium* sp. (27 mm) and *Mycobacterium* sp. (21 mm) although other extracts such as

Fig. 1 Presence of phenols and flavonoids of *R. apiculata* was analysed using gallic acid (a) and rutin (b) as standard. The yield of phenol (c) and flavonoids (d) was quantified using linear relationship

Fig. 2 Antimicrobial activity of solvent extracts using agar-well diffusion method (a) and toxicity on pathogenic organisms using minimal inhibitory concentration assay (b)

methanol, chloroform and ethanol have moderate activity against all pathogens. Ethanol has no activity against *Enterobacter sp.* and *Staphylococcus aureus* due to its low

polarity. Among the solvent extracts, methanol extracts have potential inhibitory activity against all the tested pathogenic microorganisms.

FTIR analysis was evaluated to determine the functional compound in solvent extracts that may influence the antimicrobial activity. The solvent extracts have the peak around $3409\text{--}3430\text{ cm}^{-1}$ correspond to the hydroxyl functional group of alcohol and phenol compounds (Fig. 3) and the peak at approximately $1113\text{--}1155\text{ cm}^{-1}$ owing to the characteristic of aromatic C–O bond stretching. The result of FTIR analysis revealed that the presence of phenol compound in all extracts has been proved. The GC–MS analysis (Fig. 4a) of methanol extracts showed the presence of high amount of hexadecanoic acid, oleic acid, pentadecanoic acid and tridecanoic acid, the probability suggested that these represented the presence of flavonoids compounds. The NMR spectrum of methanolic extracts showed the characteristic of flavonoids that reveals the presence of aromatic protons in the range between δ 7.102 and 8.48 (Fig. 4b).

All the solvent extracts were assessed for DPPH scavenging activity that had substantial activity, especially butanol extract has 84% of activity that almost equal to DPPH scavenging activity of commercial antioxidant BHT (89%) at the concentration of $100\text{ }\mu\text{g/ml}$. Further, the methanol and hexane also have moderate scavenging activity; it has 64 and 63% of activity, respectively (Fig. 5a). The increasing concentration of extract was increased $> 80\%$ of scavenging

activity due to the presence of phenols and flavonoids in extracts. The results revealed that butanol extract was a strong free radical scavenger compared to other extracts.

Hydrogen peroxide was the important reactive oxidative species that play a main role in oxidative stress. All solvent extracts of *R. apiculata* were adequate of hydrogen peroxide scavenging activity, depending upon the dose. The butanol extract was high scavenging potential followed by methanol, ethanol, hexane and chloroform with 76, 72, 43, 40 and 27%, respectively. The H_2O_2 scavenging activity of butanol and methanol was nearly equivalent to commercial antioxidant BHT at the concentration of $100\text{ }\mu\text{g/ml}$ (Fig. 5b).

Among the oxidative radicals, hydroxyl radical was one of the most compassionate and induces severe damage to nearby biomolecules. In this study, methanol extracts were scavenged hydroxyl radicals up to 82% while the other extracts such as butanol, chloroform, hexane and ethanol have 75, 70, 61 and 60% of scavenging activity at $100\text{ }\mu\text{g/ml}$ concentration, respectively (Fig. 5c). This result expressed that the solvent extracts almost equal with BHT (86%). The ferric-reducing power of *R. apiculata* solvent extracts also increased with an increase of concentration ($10\text{--}100\text{ }\mu\text{g/ml}$). The antioxidant potential of methanol extract was found to be an impressive reducing power compared to other extracts although lesser than BHT at $100\text{ }\mu\text{g/ml}$ (Fig. 5d).

Two-way analysis of variance of antioxidants assays revealed statistically significant effects owing to the concentration of extracts as well as solvents and interactions of the concentration of extraction solvents (Table 1). But the comparison study of chloroform (> 0.081), ethanol (> 0.17) and hexane extracts showed the insignificant effects over the activity compared with BHT ($p > 0.05$). A significant interaction of concentration and solvents indicates that the antioxidant activity of butanol and methanol extracts does not vary to different concentration (< 0.001).

The DNA damage was induced using FR (lane 2) and the extracts were used as protecting agent (lanes 3–7) in mitochondrial DNA of *S. aureus* (lane 1). Interestingly, the methanol and butanol (lanes 5 and 6) extracts with high antioxidant activity protect the DNA damage from FR. In conclusion, the methanol and butanol extracts scavenged the effects of hydroxyl radicals produced by FR. It is also confirmed by the quantification of DNA and the results showed that methanol and butanol extract treated DNA has high amount of DNA and rest of the extracts were obtained with fewer amounts when compared with control DNA (Fig. 6).

The effect of methanol extracts on the proliferation of lung cancer and normal cells was evaluated using MTT assay. The percentage of viable cells was decreased with increasing the concentration of methanol extracts (Fig. 7). The IC_{50} of the methanol extracts was found to be at $30\text{ }\mu\text{g/ml}$ against A549 cells. According to the American National Cancer Institute (NCI) guidelines, the crude extracts were

Fig. 3 FTIR spectrum of different solvent extracts. **a** Butanol, **b** chloroform, **c** ethanol, **d** hexane and **e** methanol

Fig. 4 GCMS analysis of methanol extracts **a** showed the presence of hexadecanoic acid, oleic acid, pentadecanoic acid and tridecanoic acid and ^1H NMR spectrum **(b)**. The list of compounds present in the methanol extracts was included as inset table **a**

accepted for cytotoxicity when the IC_{50} values were less than $30 \mu\text{g/ml}$. The toxicity on HBL100 normal cells was showed minimal toxicity at $100 \mu\text{g/ml}$ of methanol extracts. The cause of extracts on morphological changes in A549 cells was observed in control (Fig. 7b) and treated cancer cells (Fig. 7c). The extracts initiated the cell breakdown, interruption of cell growth, cell bunching and loss of membrane stability in A549 cells.

Apoptotic morphological changes affected by methanolic extracts of *R. apiculata* were studied using AO/EB staining method. The treated cells were characterized to live (light green fluorescence), early apoptotic (bright fluorescence) and late apoptotic (red fluorescence) cells (Fig. 8a, b). DCFH-DA was used to measure the ROS formation and the results indicated that the control samples showed no green

fluorescence, and high green fluorescence intensity was obtained in treated cells due to the formation of ROS and induce the apoptosis (Fig. 8c, d). The MMP was estimated using Rhodamine 123 staining and the results showed that the $30 \mu\text{g/ml}$ concentration of methanol extracts decreased the MMP of A549 cells that was confirmed by low fluorescence intensity in treated cells than the control cells (Fig. 8e, f).

Discussion

Since the last decade natural antioxidants especially phenols and flavonoids have been exploited as the potential remedial agent against the number of diseases including cancer,

Fig. 5 Antioxidant activity of *R. apiculata* solvent extracts at different concentration. **a** DPPH activity, **b** hydrogen peroxide scavenging activity, **c** hydroxyl radical scavenging activity and **d** reducing power

ability. The values are mean of triplicate. BHT was used as positive control in all assays

diabetes, inflammatory diseases and aging. In the present study, the natural antioxidant was extracted from different extracts of *R. apiculata* for protecting the DNA damage and antimicrobial activity. Butanol and methanol extracts of mangrove leaf have good antioxidant activity against all tested assays and also effectively protect the DNA damage from Felton's reagent. Initially, the phytochemical screening of solvent extracts showed good phenol and flavonoids content in all the extracts that leads the antimicrobial and antioxidant activities. The amount of phenolic compounds was increased with the concentration of *R. apiculata* extracts (Loo et al. 2007). Phenols play a significant role in radical scavenging activity owing to their hydroxyl groups (Asha et al. 2012) present in the extract. Further, the previous

reports states that the antioxidant property of tannin (phenol) depends upon the existence of C-ring (Gulcin et al. 2003) and the number of -OH group present in the extracts (Aleksic and Knezevic 2014). The GC-MS characterization was also showed that the methanolic extract has flavonoid derivatives and it was further confirmed by ^1H NMR. Li et al. (2007) reported that the methanol extract has flavonoid derivatives that were confirmed by the presence of aromatic protons, oxymethine protons and methylene protons. The NMR characterization of *R. apiculata* confirmed the presence of poly flavonoid due to the inter flavonoid linkage with high degree polymerization (Oo et al. 2009).

Further, the antimicrobial activity of solvent extracts was tested against human clinical pathogens and the results

Table 1 Shows the two-way analysis of variance performed for all the four antioxidant assays

Antioxidant assays	Concentration of extracts	Solvents	Concentration of extracts × solvents	Residual
DPPH				
df	5	6	30	84
F value	40,815	23,982	321.2	
p value	<0.001	<0.001	<0.001	
H₂O₂ scavenging				
df	5	6	30	84
F value	8772	13,010	443.9	
p value	<0.001	<0.001	<0.001	
Hydroxyl radical scavenging				
df	5	6	30	77
F value	4.255	203.7	1.314	
p value	>0.081	<0.001	>0.17	
Reducing power				
df	5	6	30	84
F value	74.98	386.4	4.831	
p value	<0.001	<0.001	<0.001

Fig. 6 Shows protecting the mitochondrial DNA damage of *S. aureus* using DNA nicking assay. Lane 1—negative control (water + DNA), lane 2 control (DNA + FR), lane 3 DNA + FR + chloroform extract, lane 4 DNA + FR + hexane extract, lane 5 DNA + FR + methanol extract, lane 6 DNA + FR + butanol extract and lane 7 DNA + FR + ethanol extract

showed that butanol and methanol extracts have excellent antimicrobial activity against all tested pathogens. *R. apiculata* contains moderate antimicrobial activity against many bacteria and *Saccharomyces cerevisiae*, the severe

morphological changes observed against *Candida albicans* (Lim et al. 2006). Methanol extracts showed high antimicrobial activity than the ethanol extracts and also the cold methanol proved to be a good solvent to extract the antimicrobial compounds from extracts (Mukesh et al. 2012a). The MIC of *R. apiculata* showed the inhibitory effect at 1 mg/ml of concentration against *S. aureus* and *C. albicans* (Mukesh et al. 2012b). The antimicrobial tannin extracted from *R. apiculata* can be used as additive and synthetic preservatives in food (Suraya et al. 2011). The presence of phenolic compounds in the *R. apiculata* extracts has been confirmed through analysis of extract in FT-IR. The phenolic compounds are the sources of antimicrobial and antioxidant activities, and play a vital role in DNA nicking assay. The major peak observed in analysis corresponds to the OH group of phenolic compounds and aromatic C–O bond stretching that direct the total activity of the extracts.

Butanol extract of mangrove leaf has excellent DPPH scavenging activity, although methanol and hexane extracts have moderate scavenging activities. Rahim et al. (2008) reported that the increase the dose of bark extracts of *R. apiculata* increases the DPPH scavenging activity. The number of the hydrogen atom in hydroxyl group of phenyl ring plays a critical role in the scavenging activity and stabilize the free radicals (Nimse and Pal 2015). DPPH was a free radical exhibit the deep purple colour and take the hydrogen from antioxidant becomes diamagnetic molecule and exposes the faded purple colour (Verma et al. 2009). Loo et al. (2008) stated that the pyroligneous acid catechol extracted from *R. apiculata* showed excellent DPPH activity than the reference compounds such as ascorbic acid, alpha-tocopherol and butylated hydroxyanisole.

The dose-dependent hydrogen peroxide scavenging activity of *R. apiculata* solvent showed excellent scavenging at 100 µg/ml. The acetone extracts of condensed *R. apiculata* have strong H₂O₂ activity (56%) at 50 µg/ml compared to BHT and α-tocopherol (Suraya et al. 2011). Hydrogen peroxide can destruct the cells over oxidation of lipids, protein and DNA, and consequently cell death through apoptosis (Sheline and Wei 2006). Hydroxyl radical present in the hydrogen peroxide was reacted with biomolecules and cause the tissue damage and cell death (Khan et al. 2012). The scavenging activity of hydrogen peroxide by *R. apiculata* could reduce the tissue damage as well as oxidative-related disorders.

High reactive and short lifespan hydroxyl radical species are the source of damage to neighbour essential biomolecules (Sakanaka et al. 2005). In the present study, the methanol and butanol extracts of mangrove leaf have the excellent activity of scavenging the hydroxyl radical that could inhibit the cell damage. Nithiyanantham et al. (2013) reported that the competence of extracts to destroy the hydroxyl radicals was directly involved for pertinent

Fig. 7 Cytotoxic activity of methanol extract **a** on lung cancer cells (A549) and normal cells (HBL100) and morphology changes in A549 lung cancer cells in control (**b**) and methanol extract treated (**c**)

with preventing the multiplication of lipid peroxidation. Butanol fraction of *R. apiculata* showed 85% of hydroxyl radical scavenging activity at 70 μg/ml of concentration (Gao and Xiao 2012) could reduce the cell damage.

Reducing the power of potassium ferricyanide was one of the excellent antioxidant mechanisms to evaluate the capability of leaf extracts (Jayaprakasha et al. 2001). In the present study, the ferric reducing ability of *R. apiculata* depends on the concentration of extracts, the increase concentration also increases the reducing the power of ferric chloride (Rahim et al. 2008). Donation of electron-hydrogen atom affects the reducing power of compounds correlated with oxidative stress and related disorders (Ak and Gulcin 2008). The evaluation of DNA nicking assay also proved and correlated with antioxidant activity of extracts. The results showed that the addition of extracts with mitochondrial DNA containing FR reduces the formation of DNA damage. Taken together, these results showed that

the methanol extract of *R. apiculata* could be a potential antioxidant and also to be a DNA damage protective agent.

The methanol extract of *R. apiculata* showed excellent cytotoxic activity against A549 lung cancer cells and the affect the morphology of the cells. Hong et al. (2011) reported that the tannin extracted from *R. apiculata* showed significant in vitro cytotoxic activity against HepG2 cancer cells at 12.06 μg/ml and in vivo cytotoxicity against brine shrimp at 4.67 mg/ml as acute cytotoxicity and 2.10 mg/ml as chronic toxicity. The IC₅₀ concentration of methanolic extracts of *R. apiculata* was actively induced the apoptosis, ROS generation and reduce the MMP in A549 cancer cells. Vinod Prabhu and Guruvayoorappan (2012a) reported that the methanolic extracts of *R. apiculata* reduce the tumour development in the melanoma-injected mice and also showed anti-inflammatory activity against carrageenan- and formalin-induced paw edema (Vinod Prabhu and Guruvayoorappan 2012b). The present

Fig. 8 Methanolic extracts induces the apoptosis in A549 cells and confirmed by AO/EB staining (a, b), DCFH-DA staining (c, d) and rhodamine staining (e, f)

study reported that the extract of *R. apiculata* was rich in biomolecules such as phenols and flavonoids that leads the antimicrobial, antioxidant and anticancer activities.

In conclusion, the present work indicates the methanol and butanol extracts of *R. apiculata*, showing excellent antimicrobial and antioxidant activities based on various

in vitro assays. The presence of phenols and flavonoids in the solvent extracts of *R. apiculata* could play a vital role in the in vitro antimicrobial and antioxidant activities. Moreover, the methanol and butanol extracts protect DNA from ROS-induced damage, recommending their use against free radical-induced pathological conditions. The methanol extracts showed anticancer activity against human adenocarcinoma lung cancer cells and less toxicity against normal cells. Consequently, the present findings strongly suggest that the extract carries beneficial use for human health and could be used for food additives.

Acknowledgements We acknowledge the Science and Engineering Research Board, Department of Science and Technology, Ministry of Science and Technology, Government of India for funds in carrying out this work (Ref. no. SR/FT/LS-050/2009 Dated July 23, 2010). We would like to thank Professor S. Dhanuskodi, Head, Department of Physics, Bharathidasan University, Tiruchirappalli–24, India for providing facility to FTIR analysis of our sample.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest to disclose.

References

- Ak T, Gulcin I (2008) Antioxidant and radical scavenging properties of curcumin. *Chem Biol Interact* 174:27–37
- Aleksic V, Knezevic P (2014) Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. *Microbiol Res* 169(4):240–254
- Andrews JM (2001) Determination of minimum inhibitory concentrations. *J Antimicrob Chemother* 48(1):5–16
- Apostolou A, Stagos D, Galitsiou E, Spyrou A, Haroutounian S, Portet N, Trizoglou I, Wallace Hayes A, Tsatsakis AM, Kouretas D (2013) Assessment of polyphenolic content, antioxidant activity, protection against ROS-induced DNA damage and anticancer activity of *Vitis vinifera* stem extracts. *Food Chem Toxicol* 61:60–68
- Asha KK, Suseela M, Lakshmanan PT (2012) Flavonoids and phenolic compounds in two mangrove species and their antioxidant property. *Indian J Mar Sci* 41(3):259–264
- Benedetti S, Catalani S, Palma F, Canestrari F (2011) The antioxidant protection of CELLFOOD® against oxidative damage in vitro. *Food Chem Toxicol* 49:2292–2298
- da Silva LCN, Silva-Junior CA, Souza RM, Macedo AJ, Silva MV, Correia MTS (2011) Comparative analysis of the antioxidant and DNA protection capacities of *Anadenanthera colubrina*, *Libidibia ferrea* and *Ptyrocarpa moniliformis* fruits. *Food Chem Toxicol* 49:2222–2228
- Droge W (2002) Free radicals in the physiological control of cell function. *Physiol Rev* 282:47–95
- Farjana A, Zerín N, Kabir MS (2014) Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. *Asian Pac J Trop Dis* 4(2):S920–S923
- Gao M, Xiao H (2012) Activity-guided isolation of antioxidant compounds from *Rhizophora apiculata*. *Molecules* 17:10675–10682
- Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI (2003) Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chem* 83:371–382
- Gurudeeban S, Satyavani K, Ramanathan T (2015) Identification of medicinal mangrove *Rhizophora apiculata* Blume: morphological, chemical and DNA barcoding methods. *Int J Sci Eng Res* 6(2):1283–1290
- Hong LS, Ibrahim D, Kassim J (2011) Assessment of in vivo and in vitro cytotoxic activity of hydrolysable tannin extracted from *Rhizophora apiculata* barks. *World J Microbiol Biotechnol* 27:2737–2740
- Jayaprakasha GK, Singh RP, Sakariah KK (2001) Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chem* 73:285–290
- Kaur R, Arora S, Singh B (2008) Antioxidant activity of the phenol rich fractions of leaves of *Chukrasia tabularis* A. Juss. *Biore-sour Technol* 99:7692–7698
- Khan RA, Khan MR, Sahreen S, Ahmed M (2012) Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. *Chem Cent J* 6:1–7
- Kusuma S, Anilkumar P, Kamala B (2011) Potent antimicrobial activity of *Rhizophora mucronata*. *J Ecobiotechnol* 3(11):40–41
- Li DL, Li XM, Peng ZY, Wang BG (2007) Flavanol derivatives from *Rhizophora stylosa* and their DPPH radical scavenging activity. *Molecules* 12:1163–1169
- Lim SH, Darah I, Jain K (2006) Antimicrobial activities of tannins extracted from *Rhizophora apiculata* barks. *J Trop For Sci* 18(1):59–65
- Liochev SI (2013) Reactive oxygen species and the free radical theory of aging. *Free Radic Biol Med* 60:1–4
- Loo AY, Jain K, Darah I (2007) Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, *Rhizophora apiculata*. *Food Chem* 104:300–307
- Loo AY, Jain K, Darah I (2008) Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. *Food Chem* 107:1151–1160
- Meghashri S, Vijay Kumar H, Gopal S (2010) Antioxidant properties of a novel flavonoid from leaves of *Leucas aspera*. *Food Chem* 122:105–110
- Minussi RC, Rossi M, Bologna L, Cordi L, Rotilio D, Pastore GM, Duran N (2003) Phenolic compounds and total antioxidant potential of commercial wines. *Food Chem* 82:409–416
- Mukesh P, Jadhav RN, Jadhav BL (2012a) Evaluation of antimicrobial principles of *Rhizophora* species along Mumbai coast. *J Adv Sci Res* 3(3):30–33
- Mukesh P, Pramod S, Vijay S, Vaishali J, Jadhav BL (2012b) Comparative performance of activity of antimicrobial principles of mangroves *Rhizophora* species along Mumbai coast. *Indo Glob Res J Pharm Sci* 2(4):42–429
- Nimse SB, Pal D (2015) Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv* 5:27986–28006
- Ningappa MB, Dinesha R, Srinivas L (2008) Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. *Food Chem* 106:720–728
- Nithiyanantham S, Siddhuraju P, Francis G (2013) A promising approach to enhance the total phenolic content and antioxidant activity of raw and processed *Jatropha curcas* L. kernel meal extracts. *Ind Crops Prod* 43:261–269
- Oo CW, Kassima MJ, Pizzi A (2009) Characterization and performance of *Rhizophora apiculata* mangrove polyflavonoid tannins in the adsorption of copper (II) and lead (II). *Ind Crops Prod* 30:152–161
- Premanathan M, Arakaki R, Izumi H, Kathiresan K, Nakano M, Yamamoto N, Nakashima H (1999) Antiviral properties of a mangrove plant, *Rhizophora apiculata* Blume, against human immunodeficiency virus. *Antivir Res* 44:113–122

- Rahim AA (2005) Physico-chemical characterization of mangrove tannins as corrosion inhibitors. Ph.D. Thesis, Universiti Sains Malaysia
- Rahim AA, Rocca E, Steinmetz J, Kassim MJ, Ibrahim MS, Osman H (2008) Antioxidant activities of mangrove *Rhizophora apiculata* bark extracts. *Food Chem* 107:200–207
- Ramalingam V, Rajaram R, Premkumar C, Santhanam C, Dhinesh P, Vinothkumar S, Kaleshkumar K (2014) Biosynthesis of silver nanoparticles from deep sea bacterium *Pseudomonas aeruginosa* JQ989348 for antimicrobial, antibiofilm and cytotoxic activity. *J Basic Microbiol* 54(9):928–936
- Ramalingam V, Varunkumar K, Ravikumar V, Rajaram R (2016) Development of glycolipid biosurfactant for inducing apoptosis in HeLa cells. *RSC Adv* 6:64087–64096
- Ramalingam V, Revathidevi S, Shanmuganayagam T, Muthulakshmi L, Rajaram R (2017) Gold nanoparticle induces mitochondria-mediated apoptosis and cell cycle arrest in nonsmall cell lung cancer cells. *Gold Bull* 50(2):177–189
- Rathee JS, Hassarajani SA, Chattopadhyay S (2007) Antioxidant activity of *Nyctanthes arbor-tristis* leaf extract. *Food Chem* 103:1350–1357
- Roopashree TS, Dang R, Rani RHS, Narendra C (2008) Antibacterial activity of antipsoriatic herb: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *Int J Appl Res Nat Prod* 1(3):20–28
- Sakanaka S, Tachibana Y, Okada Y (2005) Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chem* 89:569–575
- Sheline CT, Wei L (2006) Free radical-mediated neurotoxicity may be caused by inhibition of mitochondrial dehydrogenases in vitro and in vivo. *Neuroscience* 140:235–246
- Suraya S, Darah I, Jain K, Lim SH (2011) Antimicrobial and antioxidant activities of condensed tannin from *Rhizophora apiculata* barks. *J Chem Pharm Res* 3(4):436–444
- Vaikundamoorthy R, Sundaramoorthy R, Krishnamoorthy V, Vilwanathan R, Rajendran R (2016) Marine steroid derived from *Acropora formosa* enhances mitochondrial-mediated apoptosis in non-small cell lung cancer cells. *Tumour Biol* 37(8):10517–10531
- Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos ML (2002) Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical hydroxyl radical, and hypochlorous acid. *J Agric Food Chem* 50:4989–4993
- Verma AR, Vijayakumar M, Mathela CS, Rao CV (2009) In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food Chem Toxicol* 47:2196–2201
- Vinod Prabhu V, Guruvayoorappan C (2012a) Anti-inflammatory and anti-tumor activity of the marine mangrove *Rhizophora apiculata*. *J Immunotoxicol* 9(4):341–352
- Vinod Prabhu V, Guruvayoorappan C (2012b) Evaluation of immunostimulant activity and chemoprotective effect of mangrove *Rhizophora apiculata* against cyclophosphamide induced toxicity in BALB/c mice. *Immunopharmacol Immunotoxicol* 34(4):608–615