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Pharmacological activities of leaf and bark extracts of a medicinal mangrove plant *Avicennia officinalis* L.

Swagat Kumar Das[1], Dibyajyoti Samantaray[1], Archana Mahapatra[1], Nityasundar Pal[2], Rudranarayan Munda[2] and Hrudayanath Thatoi[2*]

Abstract

Background: *Avicennia officinalis* is a medicinally important mangrove plant and used in traditional medicinal practices to treat various ailments like rheumatism, paralysis, asthma, dyspepsia, tumors etc. The objective of the present study was to evaluate the carbohydrate metabolizing enzyme inhibitory, antioxidant, antimicrobial and cytotoxic potentials of ethanol leaf and bark extracts of *A. officinalis*.

Methods: The carbohydrate metabolizing enzyme inhibition potential was studied by α-amylase and α-glucosidase inhibitory activities. The antioxidant activity was investigated by measuring the scavenging potential of extracts against DPPH, ABTS and superoxide radicals. The antimicrobial activity was studied by agar well diffusion method and the cytotoxicity potential by MTT assay.

Results: The study revealed that *A.offiicnalis* bark extract inhibited the activity of α-amylase and α-glucosidase in a dose dependent manner with an IC_{50} value of 0.66 and 0.71 mg/ml respectively. The leaf extract also demonstrated inhibition potential against α-amylase and α-glucosidase with an IC_{50} value of 0.29 and 1.19 mg/ml respectively. The ethanol bark extract also exhibited scavenging potential against DPPH, ABTS and superoxide radicals in a dose dependent manner with IC_{50} values of 112, 114 and 82 μg/ml respectively and ethanol leaf extract with IC_{50} values of 200, 41.9 and 207.6 μg/ml respectively. Both leaf and bark extracts exhibited dose dependent antiproferative activity on TC1 murine cell lines. Both leaf and bark extracts exhibited antimicrobial activity against bacteria (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and fungi (*Candida albicans, C. krusei*). The qualitative phytochemical assay, UV-Vis and FTIR analysis revealed the presence of different phytoconstituents in the leaf and bark extracts of *A.officinalis*.

Conclusion: The results suggest that ethanol leaf and bark extracts of *A.officinalis* were effective in inhibiting α-amylase and α-glucosidase and also have antioxidant, antimicrobial potentials which justify the ethnobotanical use of this plant.

Keywords: *Avicennia officinalis*, Antidiabetic, Antioxidant, Antimicrobial, Cytotoxic

* Correspondence: hn_thatoi@rediffmail.com
[2]Department of Biotechnology, North Orissa University, Baripada, Odisha, India
Full list of author information is available at the end of the article

Background

The plant *Avicennia officinalis* L. belonging to family Acanthaceae is one of the important mangrove species [1]. *A. officinalis* L., is an evergreen mangrove plant usually growing 8 - 18 m tall and 1 m in diameter and widely distributed in Indian subcontinent, Indonesia, Malaysia, Brunei, Myanmar, Philippines, Singapore, Sri Lanka, Thailand, Viet Nam, and southern Papua New Guinea. This mangrove species is widely distributed across the coastal region of Indian coasts and Andaman Nicobar islands [2, 3].

Wide uses of different parts of *A. officinalis* in traditional medicinal practices have been well documented. Seeds of this plant are used as maturative poultices and ulcers and to hasten suppuration of boils and abscesses. Roots are used as aphrodisiac, bark as diuretic, in the management of skin afflictions especially scabies, rheumatism, paralysis, asthma and snake-bites. Fruits are used as plaster for tumors [4, 5]. Plant decoction with sugar and cumin is used in dealing with dyspepsia. A resinous substance exuded from the bark acts as a contraceptive [6]. Different studies have also reported the pharmacological importance of this plant. The leaf, bark roots and fruits of this plant showed antimicrobial activities [7–12]. The leaves of this plant exhibited antiulcer [12], antinociceptive [13], anti-inflammatory [14]; anticancer [15] and antioxidant [16] activities.

In the present study, the ethanol bark and leaf extracts of *A.officinalis* plant were evaluated for their in inhibitory effects against carbohydrate metabolizing enzymes such as α-amylase and α-glucosidase, as well as their antioxidant, antimicrobial and cytotoxic potentials.

Methods

Collection of plant and extraction

Leaves and stem barks of *Avicennia officinalis* were collected from the mangrove forest of Mahanadi delta region of Odisha coast. The plant specimen was identified by Prasanna Kumar Nayak, Herbarium keeper, Integrated Coastal Zone Management Project (ICZMP), Forest Department, Govt. of Odisha. After collection, the plant samples were air dried under shade at room temperature and grounded. The samples were extracted by maceration using 90% ethanol. The obtained crude extracts were evaporated to dryness. Then the extracts were dissolved in DMSO for further studies.

Phytochemical analysis

Qualitative and quantitative phytochemical analysis

Different phytochemicals are determined qualitatively. Total phenol, flavonoid and tannin content of leaf and bark extracts of *A. officinalis* were determined quantitatively using standardized phytochemical assay methods [17].

UV-Vis spectral analysis

The leaf extracts of *A. officinalis* were scanned in the wavelength range between 240 and 700 nm by using Systronics UV-VIS spectrophotometer 117 and the peaks thus recorded were evaluated for presence of phenolic compounds.

FT-IR study

FT-IR analysis was performed using FT-IR spectrometer (PerkinElmer FT-IR Spectrometer- Spectrum Two) to detect the characteristic peaks and their corresponding functional groups. The scans were made in a wave number range from 4000 and 400 cm^{-1}.

Pharmacological study

In vitro carbohydrate metabolizing enzyme inhibition assay

α-Amylase inhibition assay The in vitro antidiabetic activity of the leaf and bark samples of *A. officinalis* were evaluated employing the α-amylase enzyme inhibition assay [18]. The experiments were performed by taking plant extracts at 0.1, 0.5 and 1 mg/ml concentrations. The porcine α-amylase was dissolved in cold 20 mM phosphate buffer, pH 6.7 containing 6.7 mM of NaCl. The enzyme and extracts at different concentrations were mixed and pre-incubated at 37 °C for 20 min. After incubation 0.5% starch as substrate (dissolved in 20 mM phosphate buffer, pH 6.7) was transferred to the pre-incubated mixture and further incubated for 15 min at 37 °C. After incubation, DNS colour reagent was added to each reaction mixture and reaction mixture was placed in boiling water bath for 10 min. Thereafter, after cooling the absorbance of the mixture was taken at 540 nm. Acarbose was used as positive control. All the experiments were performed in triplicate. The IC_{50} values were determined.

α–Glucosidase inhibition assay The α-glucosidase enzyme inhibition activity was evaluated following the method of Apostolidis, et al. [19] with some modifications. Briefly, α -Glucosidase (1 U/mL) was premixed with 0.1, 0.5 and 1.0 mg of extract in phosphate buffer solution (PBS) at pH 6.8. Then the reaction mixture was incubated for 5 min at 37 °C. After the incubation, 1 mmol/L of paranitrophenyl alpha D glucopyranoside in 50 mmol/L of phosphate buffer was added to initiate the reaction. The reaction was terminated by the addition of 1 mol/L sodium carbonate. α -Glucosidase activity of the mixture was determined by measuring the quantity of nitrophenol released from pNPG. The absorbance of the mixture was measured at 405 nm. Acarbose was used as positive control. The results were expressed in concentration of extract required to inhibit 50% of α -glucosidase (IC_{50}).

In vitro antioxidant assay

DPPH radical scavenging assay The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging properties of ethanol bark and leaf extracts of *A. officinalis* were evaluated employing the method of Povichit et al. [20]. Briefly, the leaf and bark samples of different concentrations (50 μg/ml to150 μg/ml) were mixed with 0.1 mM DPPH solution and incubated for 30 min at room temperature under dark condition. The scavenging potential was estimated by taking absorbance at 517 nm. BHT was used as positive control under the same condition. The percentage of DPPH scavenging was calculated as:

$$\%\text{of DPPH scavenging} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

Where, Abs $_{\text{Control}}$ is the absorbance of the control reaction, and Abs $_{\text{Sample}}$ is the absorbance of the test sample.

ABTS radical scavenging assay The ABTS scavenging activity was measured following the method of Hsu et al. [21], with modifications. $ABTS^{+}$ solution was prepared by mixing 7 mM of ABTS and 2.45 mM of $K_2S_2O_8$ in water, which was incubated for 12 h in dark at room temperature. Before use, the ABTS solution was diluted with ethanol to get an absorbance of 0.7 ± 0.02 at 734 nm by using a UV-visible spectrophotometer (Systronics). Briefly, 2 mL of the ABTS solutionwas added to test samples at different concentrations (50, 100, 150 μg/ml). The samples were mixed thoroughly, the reaction mixtures were incubated at room temperature for 10 min, and the absorbance was recorded immediately at 734 nm. The % of ABTS scavenging activity was calculated as:

$$\%\text{of ABTS scavenging activity} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

Where, Abs $_{\text{Control}}$ is the absorbance of the control reaction, and Abs $_{\text{Sample}}$ is the absorbance of the test sample.

Superoxide scavenging assay The super oxide scavenging properties of ethanol bark and leaf extracts of *A. officinalis* were evaluated employing the method of Kono [22]. Briefly, the reaction mixture contains EDTA (0. 1 mM), NBT (24 mM), sodium bicarbonate (50 mM, pH 10.2). The reaction was initiated by the addition of 400 μl hydroxylamine hydrochloride, followed by various concentrations (50, 100 and 150 μg) of the samples and were incubated at 25 °C for 15 min. The % of scavenging of superoxide radical was evaluated by recording the rate of NBT reduction at an absorbance of 560 nm. The percentage inhibition of NBT reduction was calculated as below:

$$\%\text{ of Superoxide scavenging activity} = \frac{\text{Change in absorbance per min}_{\text{Control}} - \text{Change in absorbance per min}_{\text{Sample}}}{\text{Change in absorbance per min}_{\text{Control}}} \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the sample.

Cytotoxicity assay

Maintenance of Cancer cell line

The TC1 murine cancer cell line obtained from National Centre for Cell Science (NCCS), Pune, India and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fatal bovine serum (FBS) and 1% antibiotic solution. The culture conditions were 37 °C, 5% CO_2. The cells were harvested after attainment of 75-80% confluence using trypsin (0.025%) and EDTA (0.52 mM) in phosphate buffered saline (PBS).

Assessment of cell morphology

The TC1 murine cells were grown in 96-well plates with 10^4 cells per well. DNA fragmentation or chromatin condensation of the cells were detected by 4′,6′-diamidino-2-phenylindole (DAPI) staining followed by observation of cells under fluorescence microscope. The apoptotic cells and necrotic cells were differentiated from live cells by staining the cells with acridine orange/ethidium bromide (AO/EtBr).

Determination of cytotoxicity by MTT assay

The effect of bark and leaf extracts of *A. officinalis* on the viability of TC1 murine cell line was investigated by MTT assay. About 10^4 cells per well in triplicate were seeded in a 96-well culture plate followed by treatment with *A. officinalis* extracts at different concentrations for 72 h. Then to each well, 20 μl of MTT (5 mg/ml) solution were added and incubated at 37 °C for 4 h. The viability of the cells was observed by taking the absorbance at 595 nm.

Antimicrobial study

The bacterial strains of *Bacillus subtilis* (MTCC736), *Escherichia coli* (MTCC443), *Pseudomonas aeruginosa* (MTCC424), *Staphylococcus aureus* (MTCC737) and fungal strains viz. *Candida albicans* (MTCC227*), Candida krusei* (MTCC9215) were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh and the cultures were maintained as per the MTCC instructions. The antimicrobial activity of ethanol bark and leaf extracts of *A. officinalis* were assessed by agar well diffusion method against the different bacterial and fungal strains as mentioned above. Overnight broth culture of the bacterial isolates and fungal isolates were seeded over the

Table 1 Preliminary phytochemical screening of *A. officinalis* extracts

Sample	Steroids	Saponins	Phenols	Terpenoids	Flavonoids	Tannins
EB	+	+	+	+	+	+
EL	+	–	+	–	+	+

(+) = present (–) = absent

nutrient agar and potato dextrose agar plates respectively using sterile cotton swab to make lawn culture. Wells of 5 mm diameter were punched over the agar plates using sterile gel puncher and 5 μl (20 mg/ml dissolved in DMSO) of each extract was poured into each well. The plates were incubated for 24 h at 37 °C in case of bacteria and 48 h at 28 °C hr in case of fungal strains. The clear zones formed around each well were measured and average diameter of the inhibition zone was expressed in mm. Tetracycline (10 μg/disc) was used as positive control. DMSO served as negative control. Each experiment was carried out in triplicates. The minimum inhibitory concentrations of the ethanol leaf and bark extracts as well as the positive control Tetracycline were determined using a microdilution method [23].

Statistical analysis

All experiments were conducted in triplicate and the data obtained were expressed as mean ± standard error mean (SEM). The concentration giving 50% inhibition (IC_{50}) was calculated by non-linear regression analysis.

Table 2 Total phenol, flavonoids and tannins contents of *A. officinalis* extracts

Sample	Polyphenolic content (mg/g of GAE)	Flavonoids content (mg/g of QAE)	Condensed tannins content (mg/g of GAE)
EB	48.22 ± 0.51	0.051 ± 0.002	36 ± 1.11
EL	29.84 ± 1.2	0.012 ± 0.002	9.61 ± 0.15

Results and discussion

Phytochemical analysis

Mangrove plants contain biologically active phytochemicals such as steroids, terpenoids, flavonoids, tannins, saponins, and phenols that are responsible for their bioactivities including their blood glucose lowering effects, antioxidant, anticancer and antimicrobial properties [24]. In the present study, the preliminary phytochemical analysis were undertaken for the ethanol leaf (EL) and ethanol bark (EB) extracts of *A. officinalis* to ascertain different phytochemicals responsible for its different bioactivities. The qualitative phytochemical screening in *A. officinalis* revealed the presence steroids, flavonoids, tannins and phenolics both in the ethanol bark and leaf extracts. However, presence of saponins and terpenoids were only reported in ethanol bark extracts (Table 1). The quantitative analysis of EB and EL of *A.officinalis* for total phenol content, total flavonoid content and total tannin content was carried out and reported in Table 2. Total phenol content (TPC) in the ethanol bark and leaf extracts of *A.officinalis* were evaluated by

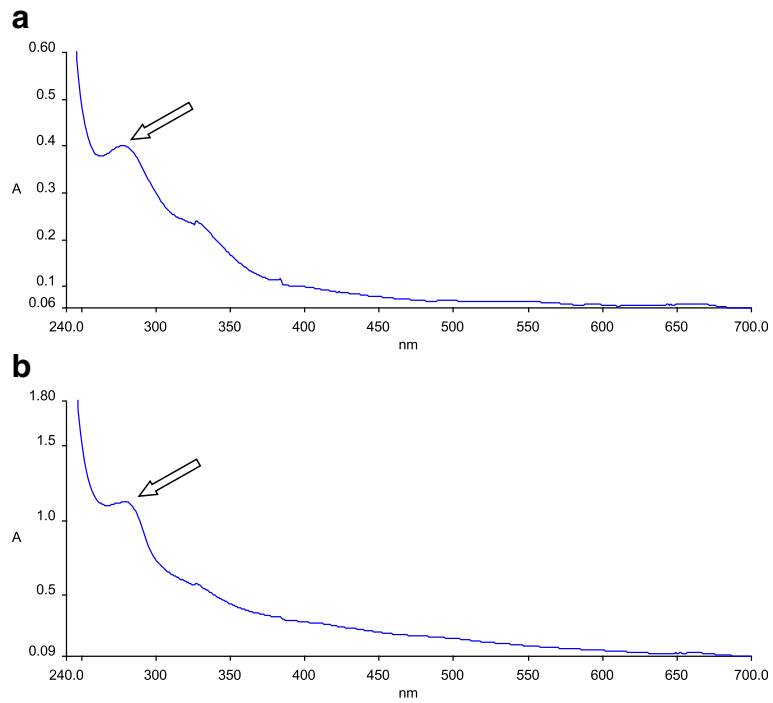

Fig. 1 UV-Vis spectra of *A. officinalis* ethanol bark (1a) and leaf (1b) extracts

plotting a standard curve using different concentrations of GAE (Gallic acid equivalent) with their respective absorbance at 700 nm. The total amount of phenolic contents present in each extract was evaluated by using the calibration curve which was expressed as mg of GAE/g. The EB extract exhibited higher amount of phenol content (48.22 mg/g GAE) as compared to EL extract (29.84 mg/g GAE). The EB also showed higher flavonoid content (0.051 mg/g QE) as compared to EL extract (0.012 mg/g QE). The tannin content in the ethanol bark of *A.officinalis* was recorded as 36 mg/g GAE which was much higher than the ethanol leaf extract having 9.61 mg/g GAE.

UV-visible and FT-IR analysis provide reliable and sensitive method for detection of bio molecular composition of plant species. Therefore in the present study UV-visible and FT-IR techniques are employed to evaluate the UV visible and IR finger print in ethanol solvent extracts of EL and EB of *A. officinalis*. The UV-VIS spectroscopy offers a simple, technique to identify the main phytochemicals. The qualitative UV-Visible spectrum profile of *A. officinalis*, ethanol extract was selected from 200 to 700 nm due to sharpness of peaks and proper baseline. The profile showed the peak at 270 nm for ethanol bark (Fig. 1a) and ethanol leaf (Fig. 1b) extracts confirming the presence of phenolic derivatives in the extract of *A. officinalis*. The FTIR analysis is one of the most commonly used analytical technique used to identify the functional groups of any plant extracts. The functional groups are identified depending on their corresponding peak values in the infra red (IR) spectral region. The FTIR spectrum of ethanol leaf and bark extracts of *A. officinalis* were given in Fig. 2a and b. The ethanol leaf extract exhibited characteristic bands at 721, 1053, 1239, 1338, 1401, 1630, 1728, 2944 and 3516 cm^{-1} for -C-O-H, C-O-C, -CH_3, C=C, -C=O, -C-H, N-H groups. Similarly, the EB extract showed bands at 539, 655, 884, 1040, 1233, 1323, 1377, 1512, 1697, 2867, 2941 and 3455 cm^{-1} (Table 3). Earlier studies have also shown that different solvent extracts from leaf, bark and fruit extracts of *A. officinalis* were rich in alkaloids, glycosides, terpenoids,

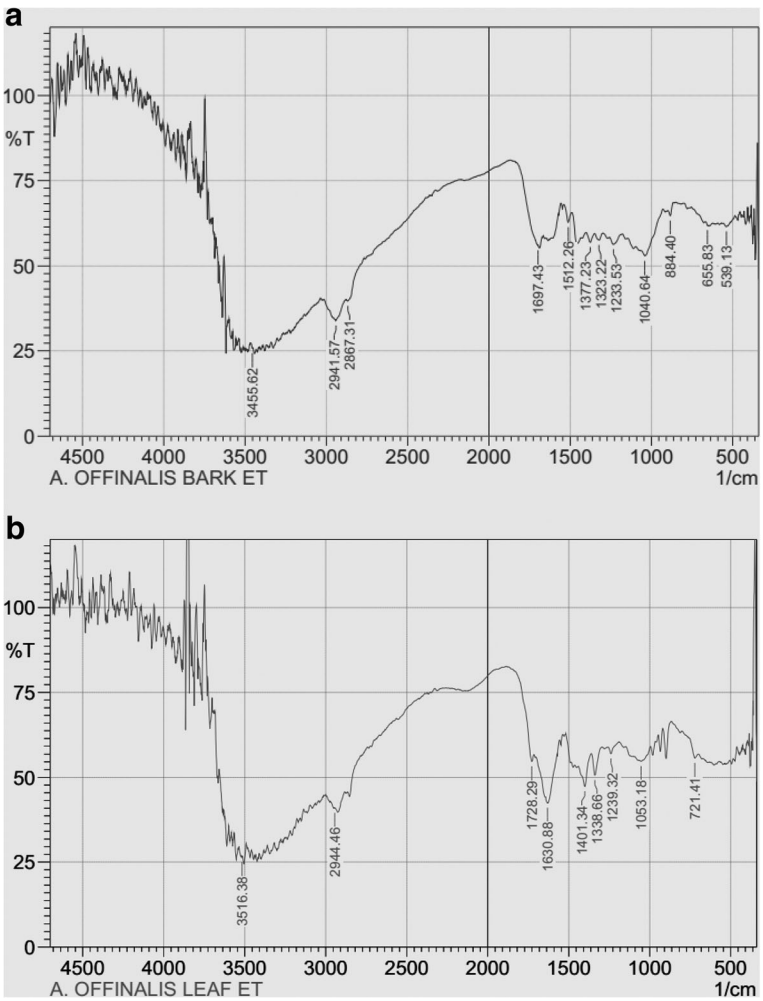

Fig. 2 FT-IR spectra of *A. officinalis* ethanol bark (2a) and leaf (2b) extracts

Table 3 FTIR spectral peak values and functional groups obtained for ethanol leaf and bark extracts of *A. officinalis*

Wave number (cm^{-1})	Assigned functional group
539	torsion and ring torsion of phenyl
600-900	-CH out of plane bending vibrations
1053	C-OH
1233, 1239, 1323, 1338, 1377	C-O stretching vibrations
1401	–OH bending vibrations
1630	=C–H stretching vibrations
1697, 1728	–C=O stretching vibrations
2867, 2941, 2944	Asymmetric $–CH_2–$, symmetric $–CH_3$ and $–CH_2–$ stretching vibrations
3455, 3516	Stretching vibration of bonded and non-bonded –O–H groups

flavonoids, steroids, tannins, saponins, phenols etc. [3, 25]. Taking these results into account, the antioxidant, antidiabetic, cytotoxic and antimicrobial potential of the ethanol leaf and bark extracts of *A. officinalis* attributed to the presence of different phytoconstituents of leaf and bark extracts of *A.officinalis*.

Carbohydrate metabolizing enzyme inhibition study

Amongst various therapeutic approaches for treating diabetes, decrease in the postprandial hyperglycemia is the major one. This condition can be achieved by retarding the absorption of glucose through the inhibition of carbohydrate hydrolysing enzymes (α-amylase and α-glucosidase) in the digestive tract. Presences of inhibitors of these enzymes delays and prolong carbohydrate digestion time. The effect causes a reduction in the rate of glucose absorption and consequently blunts the postprandial plasma glucose rise [26]. The antidiabetic potential of ethanol bark and leaf extracts of *A. officinalis* were evaluated by α-amylase and α-glucosidase inhibition assays. In the present study the carbohydrate metabolizing enzyme inhibition potentials were evaluated at 0.1, 0.5 and 1.0 mg concentrations since below 0.1 mg concentration the inhibition capacities were non-significant (data not shown). The study revealed that both ethanol bark and leaf extracts of *A. officinalis* were able to inhibit both α-amylase and α-glucosidase enzymes in a dose dependent manner except for α-glucosidase inhibition activity of EL. An increase in the α-amylase inhibition was observed with increase in concentration of extracts. The EL showed better potential to inhibit α-amylase enzyme with an IC_{50} value of 0.29 mg/ml as compared to EB with IC_{50} value of 0.66 mg/ml. The standard drug Acarbose exhibited better α-amylase inhibition potential with IC_{50} value of 0.15 mg/ml. However, the EB showed better potential than EL extracts in inhibiting α-glucosidase enzymes. The IC_{50} values for EB and EL were observed as 0.71 and 1.19 mg/ml respectively in reference to standard drug Acarbose having IC_{50} value of 0.11 mg/ml towards their capacities to inhibit α-glucosidase enzymes (Tables 4 and 5). The carbohydrate metabolism enzyme inhibition potentials of plants are associated with presence phytochemicals like phenol and their polyphenolic derivatives like flavonoids, tannins [27]. Hence, the presence of phenolics, flavonoids, and tannins in *A. officinalis* leaf and bark extracts would have been contributed toward α-amylase and α-glucosidase inhibition.

Antioxidant activity

Oxidative injury now appears the fundamental mechanism underlying a number of human disorders such as inflammation, viral infections, autoimmune pathologies, diabetes, cancer *etc* [28]. So it is quite possible that antioxidants of natural origin can play a pivotal role in management of oxidative stress mediated complications. The antioxidant potential of plant extracts can be evaluated by various in vitro and in vivo antioxidant methods. In the present study, the antioxidant activities of the EB and EL extracts of *A.officinalis* were evaluated at 50, 100 and 150 μg/ml concentrations by employing DPPH, ABTS and superoxide scavenging assays Table 5.

DPPH antioxidant activity test is a direct and dependable method for determining the radical scavenging action. An odd electron from the DPPH radical is responsible for the absorbance at 517 nm and also for a noticeable deep purple colour. When DPPH accepts an

Table 4 Antidiabetic activities of ethanol leaf and bark extracts of *A. officinalis*

Sample	Concentration (mg/ml)	α-Amylase inhibition		α-Glucosidase inhibition	
		% of inhibition	IC_{50}(mg/ml)	% of inhibition	IC_{50}(mg/ml)
EB	0.1	31.0 ± 2.71	0.66	25.24 ± 1.09	0.71
	0.5	48.14 ± 3		41.34 ± 1.09	
	1.0	63.57 ± 7		64.02 ± 2.07	
EL	0.1	68.2 ± 0.01	0.29	31.8 ± 0.01	1.19
	0.5	75 ± 0.01		36.6	
	1.0	81.7 ± 0.01		39.9 ± 0.01	
Acarbose			0.15		0.11

Table 5 Radical scavenging potentials of ethanol leaf and bark extracts of *A. officinalis*

Sample	Concentration (μg/ml)	DPPH scavenging		ABTS scavenging		Superoxide scavenging	
		% of scavenging	IC_{50} (μg/ml)	% of scavenging	IC_{50} (μg/ml)	% of scavenging	IC_{50} (μg/ml)
EB	50	26.94 ± 2.22	112	35.89 ± 2.13	114	34.55 ± 0.47	82
	100	49.81 ± 1.25		49.33 ± 1.15		64.02 ± 1.49	
	150	61.75 ± 2.08		56.96 ± 1.63		85.6 ± 0.95	
EL	50	25.1 ± 0.42	200	88.91 ± 0.34	41.9	8.88 ± 0.0.74	207.6
	100	29.36 ± 2.05		92.49 ± 0.34		15.16 ± 1.04	
	150	33.69 ± 2.52		93.94 ± 0.68		40.47 ± 0.96	
Standard			47.5		76.5		83

electron from an antioxidant compound, the DPPH becomes colourless, which can be quantitatively measured from the changes in absorbance. The present study showed that both EB and EL extracts of *A. officinalis* could scaveng DPPH radical. The EB extract scavenging potential varied between 26.94 to 61.75% with IC_{50} value of 112 μg/ml. The EL potential for scavenging DPPH radical varied between 25.1 to 33.69% at the different concentrations in the present study with IC_{50} value of 200 μg/ml. BHT under the similar condition could scavenge DPPH radical with IC_{50} value of 47.5 μg/ml. The results were in line with the previous study by [29], and showed 34.9% scavenging activity at highest concentration (150 μg/ml) of the extract.

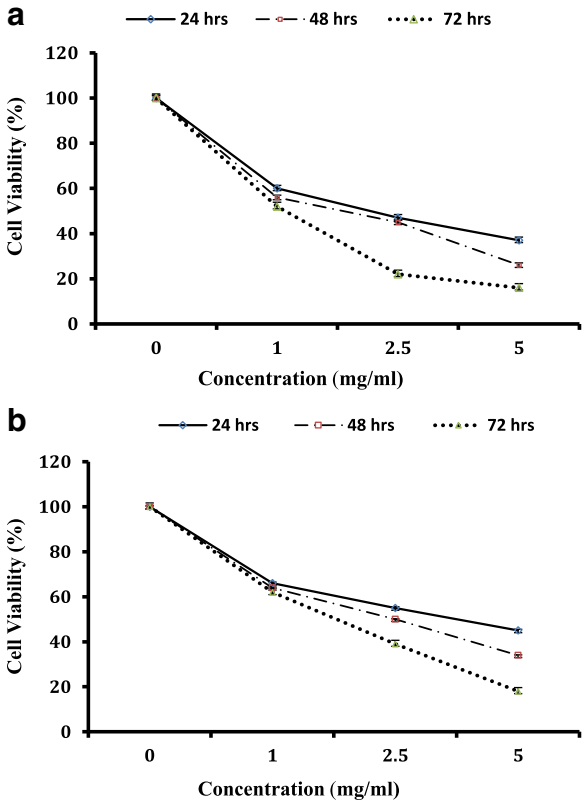

Fig. 3 Viability were measured by MTT assay using different concentrations (0,1,2.5,5 mg/ml) of *A.officinalis* bark (3**a**) and leaf (3**b**) for 24, 48 and 72 h . All the experiments were done in triplicate and data are represented as mean ± SEM from triplicate independent experiments

The ABTS assay is based on the inhibition of the absorbance of radical cation, $ABTS^+$ by antioxidants. The principle behind the assay involves the reaction between ABTS and potassium per sulphate to produce the ABTS radical cation ($ABTS^+$) which is a blue green chromogen. In the presence of antioxidants the coloured radical is converted back to colourless ABTS [30]. The present study revealed that both EB and EL extracts of *A. officinalis* could scavenge ABTS radical. However, EL exhibited better ABTS radical scavenging activity as compare to EL with IC_{50} value of 41.9 μg/ml which was even better than the ABTS scavenging potential of BHT with IC_{50} value of 76. 5 μg/ml. The EB extract demonstrated ABTS radical scavenging activity with IC_{50} value of 114 μg/ml.

Superoxide radical is known to be very harmful to cellular components and acts as a precursor of the more reactive oxygen species. It contributes to tissue damage and various diseases [31]. In superoxide radical scavenging assay, superoxide derived from hydroxylamine hydrochloride reduces NBT to a chromogenic complex formazan. Colour reduction indicated the consumption of superoxide anion by antioxidants. The present study showed that both EB and EL extracts of *A. officinalis* have moderate to better superoxide anion scavenging potential. The EL extracts showed better superoxide scavenging potential with IC_{50} value of 82 μg/ml. In the similar

Table 6 Cytotoxic activities of ethanol leaf and extracts of *A. officinalis*

Extracts	IC_{50} values (mg/ml)		
	24 h	48 h	72 h
EB	3.0 ± 0.04	2.9 ± 0.01	2.4 ± 0.13
EL	3.91 ± 0.33	3.27 ± 0.16	2.8 ± 0.10

condition the standard antioxidant compound ascorbic acid could scavenge superoxide anion with IC_{50} value of 83 μg/ml. Phenolic compounds account for the most antioxidant activities of plant extracts [32]. Our results observed high content of polyphenolic compound and flavonoids in EB and EL extracts of *A.officinalis* which might have contributed for the acclaimed antioxidant activities of *A.officinalis*.

Cytotoxicity activity

The MTT is a non-radioactive, colorimetric quantification assay that assesses the cell proliferation and viability and cytotoxicity. The assay is based in the enzymatic cleavage of the tetrazolium salt to insoluble purple formazan by cellular mitochondrial dehydrogenases present in viable cells [33]. In order to investigate the cytotoxic effects of *A.officinalis* ethanol leaf and bark extracts, murine TC1 cell lines were adopted in the present study. The MTT assay is a commonly used for measurement of cell proliferation and viability against different factors such as cytokines, mitogens and nutrients. This is also useful for analysis of different cytotoxic and cytostatic compounds, such as anticancer drugs, pharmaceutical compounds and different phytochemicals as well. The ethanol leaf and bark extracts of *A. officinalis* at different doses (1, 2.5 and 5 mg/ml) were incubated with TC1 cells and the cell viability was determined at 24, 48 and 72 h intervals. Results of this assay indicated that the ethanol leaf and bark extracts of *A.officinalis* exhibited cytotoxicity in a concentration dependent manner (Fig. 3a and b). Both EB and EL extracts of *A.officinalis* showed significant effect on TC1 cell in concentration range between 5 mg/ml to 1 mg/ml as compared with control. The highest cytotoxicity of EB and EL extracts against TC1 cell were found in 5 mg/ml concentration with 82.45 and 80.49% of cell growth inhibition. The EB and EL extracts showed prominent cytotoxic activities at all the three concentrations i.e. 1, 2.5 and 5 mg/ml after 24 h of incubation with IC_{50} values of 3 and 3.91 mg/ml. At 48 h, the EB and EL extracts exhibited cytotoxic activity on TC1 cells with IC_{50} values of 2.9 and 3.27 mg/ml. After incubation with TC1 cell line for 72 h, both EB and EL exhibited prominent cytotoxic activities with IC_{50} value of 2.4 and 2.8 mg/ml (Table 6). The morphological changes in TC1 murine cells were observed after 48 h with EB and EL extracts of *A.officinalis*. TC1 murine cells upon staining with DAPI and AO/EtBr dye revealed apoptotic changes like condensed and fragmented nuclei in both EB and EL treated TC1 cells after 48 h treatment (Figure 4). The microscopic study indicated death of TC1 cells induced by both EB and EL probably due to apoptosis. These results can be attributed to the presence of bioactive phytoconstituents present in the ethanol bark and leaf extracts of *A.officinalis*. Previous studies have also highlighted the anticancer properties of *A. officinalis* [15, 34, 35]. The present study clearly demonstrated the cytotoxicity properties of *A. officinalis* which was in line with the previous study carried out by Reddy and Ratna (2016)

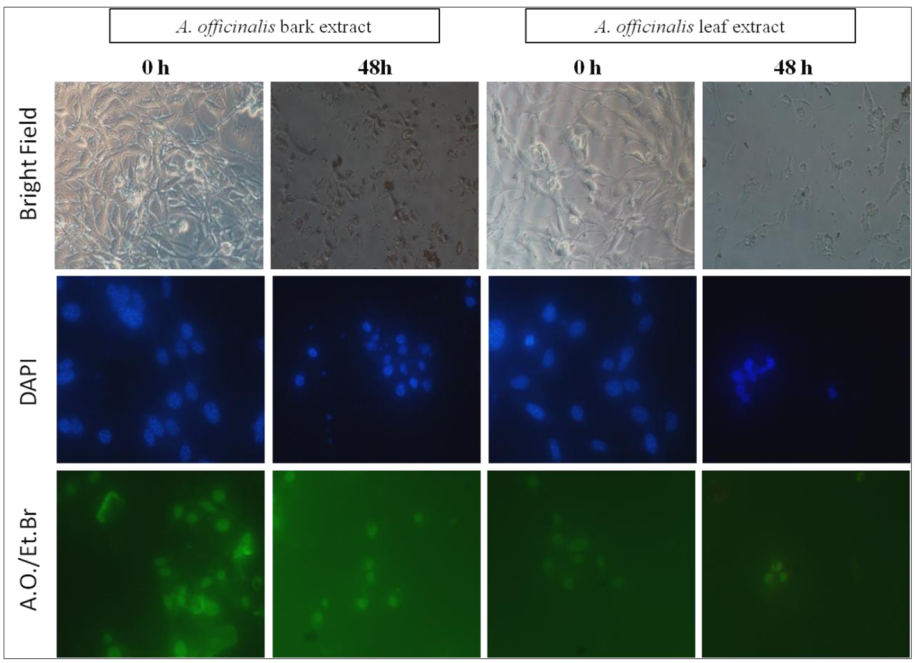

Fig. 4 Growth inhibitory effect of ethanol bark and leaf extracts of *A. officinalis* on TC1 cell line (A) Morphological and nuclear changes in TC1 cells after addition of 3 mg/ml for 48 h. Upper row: Morphological changes seen under light microscope. Middle and lower row: Nuclear changes seen under fluorescence microscope after DAPI and A.O./Et.Br staining respectively

Table 7 Antimicrobial activities of ethanol leaf and extracts of *A. officinalis*

Microorganisms	EB		EL		Tetracycline	
	Zone of inhibition (in mm)	MIC (μg/ml)	Zone of inhibition (in mm)	MIC (μg/ml)	Zone of inhibition (in mm)	MIC (μg/ml)
B. subtilis	16.5 ± 0.5	200	20.5 ± 0.5	200	28.5 ± 0.5	25
E.coli	16.5 ± 0.5	200	15.2 ± 0.7	200	25.5 ± 0.5	50
P. aeruginosa	25.6 ± 0.7	100	22.8 ± 0.3	100	30.5 ± 0.5	25
S. aureus	14.5 ± 0.5	200	–	–	25.5 ± 0.5	50
C.albicans	25.5 ± 0.5	100	23.8 ± 0.7	200	30.5 ± 0.5	25
C. krushi	25.5 ± 0.5	100	25 ± 0.5	100	31.8 ± 0.7	25

[35]. As per the US National Cancer Institute plant screening program, a crude extract considered effective for in vitro cytotoxic activity if the IC_{50} is less than 20 μg/ml following incubation between 48 and 72 h in carcinoma cells [36]. However, the present study demonstrated that both EB and EL extracts exhibited cytotoxic activities against TC1 cell line after 72 h of incubation with IC_{50} value > 1 mg/ml 12.4 and 2.8 mg/ml for 72 h; hence, may not be considered promising. The result in the present study is not in line with earlier studies which may be due to the difference in the extracts or cell line.

Antimicrobial activity

The antimicrobial activities of EB and EL extracts of *A. officinalis* are shown in Table 7. Antimicrobial study in the present study revealed that both ethanol leaves and barks of *A.officinalis* were able to inhibit the growth of bacterial and fungal strains to different extent. The EB and EL extracts exhibited antimicrobial activity on both bacterial and fungal strains studied and inhibition zone diameters were 14.5-25.5 mm. The EB and EL extracts exhibited better antibacterial effect against *P. auruginosa* (MIC value as 100 μg/ml) as compared to *B. subtilis*, *E. coli* and *S.aureus* strains (MIC value as 200 μg/ml). Further, EL exhibited no antibacterial effect against *S.aureus* (Table 7). The MIC values for fungal microbe *C. albicans* and *C.krushi* were 100 μg/ml each for EB extracts and 200 and 100 μg/ml respectively for EL extracts. It has been reported that crude plant extracts of plants having MIC values < 100 μg/mL are considered potentially useful for antimicrobial study; therefore, the present study demonstrated the use of the leaf and bark extracts of *A.officinalis* for development of lead compounds for antimicrobial therapeutics. Results of the present findings also corroborates with previous published reports [8, 9, 12]. The antimicrobial activity may be attributed to the presence of different polyphenolic compounds in the *A.officinalis* leaf and bark extracts. The results of the antimicrobial study indicated that that this plant has a scientific basis in traditional use and can be further investigated as antibacterial source.

Conclusion

The present study highlights the possible use of bark and leaf extracts of *A. officinalis* as source of antioxidant, antimicrobial agents. The study showed that both leaf and bark extracts could inhibit carbohydrate metabolizing enzymes (α-Amylase and α-Glucosidase). The study also demonstrated that both leaf and bark extracts contain considerable quantity of phenols, flavonoids and tannins that were found to be major contributor for their antioxidant, antmicrobial and carbohydrate metabolizing enzyme inhibitory activities. However, the present study demonstrated that the cytotoxicity potential of the extracts were not promising. In conclusion, these preliminary in vitro results are encouraging for further biological and phytochemical studies aimed at isolating and identifying the active principles, which could provide scientific evidence for the use of bark and leaf extracts of *A. officinalis* for therapeutic purpose.

Abbreviations

A.O.: Acridine Orange; ABTS: 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid; *DMEM*: Dulbecco's Modified Eagle Medium; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EtBr: /Ethidium Bromide; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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Authors' contributions

HNT has edited the manuscript. SKD, DS and AM have conducted all the experiments. RM and NP conducted the cytotoxicity study. All authors read and approved the final manuscript.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author details
[1]Department of Biotechnology, College of Engineering and Technology, Bhubaneswar, Odisha 751029, India. [2]Department of Biotechnology, North Orissa University, Baripada, Odisha, India.

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