Evaluation of in vitro antioxidant and anticancer activity of *Allophylus cobbe* leaf extracts on DU-145 and PC-3 human prostate cancer cell lines

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Abstract *Allophylus cobbe* (L.) Raeusch. belonging to the family Sapindaceae, is a commonly distributed small shrub in Western Ghats of India previously reported for its traditional medicinal properties. It is used for the treatment of various ailments. The present study is aimed at investigating preliminary phytochemicals, inducing the determination of the total phenolic contents, antioxidant assays and anticancer activity of *A. cobbe* leaf extracts on (DU-145) and (PC-3) cell lines. Preliminary phytochemical screening showed a broad spectrum of secondary metabolites. Highest amount of phenolic content was present in aqueous extract (91.96 ± 0.61 mg/g GAE) and it also proved to have the most potent antioxidant activity at a concentration of 100 mg/ml ($64.71 \pm 0.15\%$). IC₅₀ value was (431.10 ± 15.05 µg/mL) for DU-145 and (362.08 ± 24.17 µg/mL) for PC-3 cell lines while the standard drug paclitaxel showed an

IC₅₀ value of 0.3 µM/mL. Morphological changes was observed in cancerous cells undergoing apoptosis in human prostate cancer cell lines (DU-145 and PC-3) while the extract showed no cytotoxicity towards normal cells (MEF-L929). It can be concluded that the tested extracts holds significant antioxidant and anticancer activities. However further investigation on lead compounds of *A. cobbe* will enable its therapeutic use.

Keywords *Allophylus cobbe* · Phytochemicals · Antioxidant · Anticancer · Prostate cancer

Introduction

The incidence of prostate cancer differs greatly in various parts of the world and between different ethnic groups. It is an age related disease in men older than 50 years of age (Hariharan and Padmanabha 2016). Studies have shown that prostate cancer is an increasing public health problem and leading cause of morbidity and mortality in men around the world next to cardiovascular disease (Jain et al. 2014). The mainstay for treatment of prostate cancer are surgical, radiation therapy and chemotherapy. However, radical prostatectomy is the most effective therapy for the treatment of prostate cancer in patients with a life expectancy of over 10 years (Bianco et al. 2005). Recently, with increased patient awareness and

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attempts to increase the quality of life, the aim of treatment has been not only to eradicate cancer but also to minimize the morbidity rate and to improve quality of life as much as possible (Wiygul et al. 2005). Thus, alternative medicines are brought into practice to minimize the side effects caused by the synthetic drugs used in the treatment. Herbal medicines are being practice at large scale for primary health care in developing countries (Rackley et al. 2006). According to World Health Organization (WHO) about 80% of world population relies on such systems of medicine (Ankad et al. 2014). About 2000 drugs of plant origin are derived from the evidences of different traditional and folktale practices as reported by Indian Materia Medica (Muralidharan and Narasimhan 2012). Thus the focus towards the use of traditional medicines is increasing rapidly in developing countries.

Allophylus cobbe (L.) Raeusch belong to the family of Sapindaceae, is a small shrub and grows widely in the Western Ghats of India. It is traditionally used in the treatment of bone fractures and other ailments while the juice of the leaves is used to relieve rashes. Leaves powdered with lime are applied to relieve gastrointestinal disorders. Roots are used to treat diarrhea (Punekar and Lakshminarasimhan 2011). The leaves are widely used as antiseptic for treatment of lesions and cuts due to its antibacterial property as reported by the local folklore healers in the Uttara Kannada District of Karnataka (Bhat et al. 2012). Plant derived drugs are safer than synthetic medications, thus resulting in their wider utility. Biological activities in plants are attributed to polyphenols and hence have been studied comprehensively. Radical scavenging activity is one of the significant properties of phenols studied in plants (Rastogi and Mehrotra 2004; Rastogi et al. 1995). The presence of antioxidants at higher levels in plants, are responsible for these functionalities which have been reported by several studies (Kumar et al. 2010; Sen et al. 2010). The antioxidant activity is based on capacity to donate hydrogen atom to free radicals. Due to this nature, these compounds have potency in inhibiting cancer cells, atherosclerosis and diabetes (Pardhi et al. 2010). Based on the traditional claims on the plant and lack of scientific evidences to show its potential pharmacological properties and paucity of reliable literature on cytotoxic investigation of this plant on human prostate cancer cell lines this study was designed. The work herein deals with preliminary

screening of phytochemicals, determination of total phenolic contents of *A. cobbe* leaves extracts with their antioxidant activity and anticancer activity on DU-145 and PC-3 human prostate cancer cell lines.

Materials and methods

Materials

Collection of plant material

A. cobbe plant was collected from the Uttar Kannada District in the Western Ghats of North Karnataka in the month of May 2015. The plant was identified and authenticated by Dr. Kotresha, and a voucher specimen was submitted to the Department of Botany, Karnataka Science College, Dharwad, Karnataka. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder. The powder was stored in airtight bottles at -4°C until use.

Chemicals and solvents

The chemicals and solvents used during the experiments were of AR grade. Standard gallic acid and ascorbic acid, was procured from Sigma Aldrich (Mumbai, India). Folin-Ciocalteu reagent, Ferric reducing antioxidant power (FRAP), Molybdate reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and other chemicals used during the experiments were procured from Hi media Laboratories, Pvt. Ltd. (Mumbai, India) and Merck Specialities Pvt. Ltd. (Mumbai, India).

Cell lines

The cell lines of human prostate cancer tissues samples DU-145 (moderately metastatic) and PC-3 (highly metastatic) and MEF-L929 (normal mouse embryo fibroblast cell line) were procured from the National Centre for Cell Science (Pune, India). Cells were routinely grown in culture flasks (Techno Plastic Products, Trasadingen, Switzerland) and subcultured in Dulbecco Modified Eagle Medium (DMEM) from Sigma-Aldrich (St. Louis, MO, USA) supplemented with 10% fetal bovine serum, (FBS, from Sigma-

Aldrich) 1% penicillin–streptomycin and 1% non-essential amino acids from (Abbott Healthcare Pvt Ltd, Mumbai, India) and incubated in a CO₂ incubator in a 5% CO₂ and 95% humidity atmosphere at 37 °C.

Methods

Preparation of extract

About 100 g dried leaves were coarsely powdered and subjected to extraction by Soxhlet extractor. The extraction was done with different solvents in their increasing order of polarity such as chloroform, ethyl acetate, methanol, ethanol and aqueous. Each time fresh plant material was taken and later extracted with other solvents. All extracts obtained were concentrated under reduced pressure at 40 °C using a rotary vacuum evaporator and evaporated to dryness.

Preliminary phytochemical analysis

The crude powder of *A. cobbe* was qualitatively tested for the presence of various secondary metabolites namely alkaloids, flavonoids, glycosides, phenols, lignins, saponins, sterols, tannins, anthraquinone and reducing sugar following the standard procedure of Deepti et al. (2012).

Total phenolic content (TPC)

The total phenolic content of *A. cobbe* was quantified using a modified Folin–Ciocalteu method as previously described (Wolfe et al. 2003). Extracts prepared (0.01% w/v in respective solvents) were quantified spectrophotometrically at 760 nm. Gallic acid was used as reference and the results were compared to the standard curves and total phenolic content was expressed as mg/g Gallic acid equivalent (GAE) per gram dry powder for the samples.

Determination of antioxidant activity by using in vitro methods

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability assay The DPPH antioxidant activity of *A. cobbe* (methanol, ethanol and aqueous extracts) was determined using the assay described by Brand-

Williams et al. (1995). 0.1% leaf extract was prepared in ethanol at different dilutions (basically it is mentioned as ‘concentration’ only). DPPH solution (0.004%) was prepared in ethanol and 1 mL of this solution was mixed with the same volume of extract and standard solution separately. The mixture was incubated for 30 min in dark and ascorbic acid was used as a standard reference. The absorbance at 517 nm was measured using ethanol as blank. The scavenging activity against DPPH was calculated using the equation:

$$\text{DPPH scavenging activity (\%)} = \frac{A_c - A_t}{A_c} \times 100$$

where A_c is the absorbance of the control reaction (1 mL of ethanol with 1 mL of DPPH solution), and A_t is the absorbance of the test sample. The results were analyzed in triplicate. The IC₅₀ value is the concentration of sample required to inhibit 50% of the DPPH free radical.

Ferric ion reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was used to measure the total antioxidant power of the extracts. Antioxidant activity assays of *A. cobbe* (methanol, ethanol and aqueous extracts) were performed by the method described by Benzie and Strain (1996) with slight modifications. Different dilutions of 0.1% leaf extract was mixed with 2.5 mL of 0.2 mM phosphate buffer (pH 7.4) and 2.5 mL of potassium ferricyanide (1% W/V). The resulting mixture was incubated at 50 °C for 20 min followed by the addition of 2.5 mL of trichloroacetic acid (10% W/V). 2.5 mL of distilled water was added and later 0.5 mL of ferrous chloride (0.1% W/V). Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard.

Phosphomolybdenum (PM) assay

In this study, total antioxidant activity was determined with the PM assay described by Prieto et al. (1999). 0.1% of *A. cobbe* (methanol, ethanol and aqueous extracts) leaf extracts was added to each test tube individually containing 3 mL of distilled water and 1 mL of molybdate reagent solution. These tubes were kept incubated at 95 °C for 90 min. After incubation

the absorbance was measured at 695 nm. Ascorbic acid was used as reference standard.

Cell line assay

Culturing of cell lines The cells were subcultured in Dulbecco Modified Eagle Medium supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, 1% non-essential amino acids in tissue culture flasks and incubated in a CO₂ incubator in a 5% CO₂ and 95% humidity atmosphere. After trypsinization the cells were counted and the cell viability was tested by trypan blue using haemocytometer. A known number of cells (2×10^3 cell/well in 100 μ L of medium) was seeded into 96-well plates, respectively, for carrying out the MTT assay.

Treatment groups 5 mg/mL of *A. cobbe* leaf methanol, ethanol and aqueous extract were prepared in 0.1% di-methyl sulfoxide (DMSO) for treating human prostate cancer cell lines (DU-145 and PC-3) and the normal cell line (MEF-L929). The reactant mixtures were diluted with media and cells were treated with different concentrations of the extract (3.125–200 μ g/mL) and incubated for 72 h, respectively. The following treatment groups are set up of the study. Negative control: cells alone. Positive control: cells + paclitaxel (Standard drug). Test groups: cells + methanol extract of *A. cobbe* leaves; cells + ethanol extract of *A. cobbe* leaves; and cells + aqueous extract of *A. cobbe* leaves. A similar treatment pattern was used for the normal mouse embryo fibroblast cells (MEF-L929).

MTT cell viability assays The MTT cell viability assay was performed using the method described by (Igarashi and Miyazawa 2001). After 72 h the media of the treated cells (100 μ L) were removed and the cell cultures were incubated with 25 μ L of MTT at 37 °C for 4 h. After incubation, the formazan produced was solubilized by addition of 100 μ L DMSO. The suspension was placed on a microvibrator for 5 min and the absorbance was recorded at 540 nm by an ELISA reader and the results were analyzed in triplicate and percentage of inhibition was calculated.

Statistical analysis All results were expressed as mean $\pm$ SD for three replications. Statistical analysis of data was performed by analysis of variance (ANOVA) and level of statistical significance between groups using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). At least three independent analyses were carried out per sample.

Results

Total yield of crude extract

The total yield of crude extracts from *A. cobbe* leaves by using the solvents, viz. chloroform, ethyl acetate, methanol, ethanol and aqueous were 5.2 g, 6 g, 37.2 g, 19.68 g and 47.2 g w/w, respectively, with reference to the air dried plant material.

Phytochemical analysis

Leaf extracts of *Allophylus cobbe* showed the presence of a wide variance of rich secondary metabolites. Alkaloids and flavonoids were present in chloroform, methanol, ethanol and aqueous extract, glycosides, sterols and tannins were highly present in all five solvents, the highest quantity of phenols was detected in methanol, ethanol and aqueous extracts, lignins in chloroform, ethyl acetate and methanol, saponins in ethyl acetate, sterols were present in all extracts, tannins were detected in ethyl acetate, methanol, ethanol and aqueous extracts. Anthraquinone, phlobatannins and reducing sugar were absent in all extracts and volatile oil was present in chloroform and ethanol extract (Table 1).

Total phenol content

Total phenolic content determined of *A. cobbe* methanol, ethanol and aqueous extracts are presented in Table 2. The amount of total phenolic content were calculated using the r^2 values from the equation ($y = mx + a$), however, y–Absorbance of unknown, m–Value from the graph, x–Concentration, a–Value from the graph and the results were expressed as mg/g GAE respectively.

Table 1 Preliminary phytochemical screening of *A. cobbe* leaves extracts

Constituent	Test	Chloroform	Ethyl acetate	Methanol	Ethanol	Aqueous
Alkaloids	Iodine	—	—	—	—	—
	Wagner's	+	—	+	+	+
	Dragendroff's	+	—	++	+	+
Flavonoids	Pew's	—	—	—	—	—
	Shinoda	—	—	++	+	++
	NaOH	+	++	+	++	++
Glycosides	Keller-Killani	++	++	++	++	++
	Glycosides	++	++	++	++	++
	Conc. H ₂ SO ₄	++	++	++	++	—
	Molisch's	++	++	++	++	++
Phenols	Ellagic acid	—	—	++	++	++
	Phenol	—	—	++	++	++
Lignin's	Labat	+	++	++	—	—
Saponins	Foam test	—	+	—	—	—
Sterols	Salkowski's	++	++	++	++	++
Tannins	Gelatine	+	—	++	+	++
	Lead acetate	++	++	++	++	++
Anthraquinone	Bomtrager's	—	—	—	—	—
Phlobatannins		—	—	—	—	—
Reducing sugar		—	—	—	—	—
Volatile oil		+	—	—	+	—

Notes: The method/tests were carried out following the standard procedure as published by Deepti et al. (2012)

—, Absent; +, moderately present; ++, high presence

Table 2 Total phenolic content (TPC) of *A. cobbe* leaf methanol, ethanol and aqueous extracts

Leaf extracts	TPC	Units equivalent	r ² values
Methanol extract	89.16 ± 0.88	mg/g GAE	R ² = 0.9819
Ethanol extract	76.37 ± 0.02	mg/g GAE	R ² = 0.9759
Aqueous extract	91.96 ± 0.61	mg/g GAE	R ² = 0.9832
Gallic acid (reference)	96.56 ± 0.73	mg/g GAE	R ² = 0.9941

Antioxidant activities

DPPH assay

Antioxidant activity of *A. cobbe* leaves methanol, ethanol and aqueous extracts was determined using DPPH radical scavenging assay. The results for 0.01% leaf extracts concentration are presented in Table 3. The antioxidant capacity of the extract was compared with ascorbic acid as standard antioxidant.

FRAP and PM assay

In the present study, the presence of antioxidants in the sample would result in the reduction of ferri cyanide Fe³⁺ to ferro cyanide Fe²⁺ by donating an electron.

Methanol, ethanol and aqueous extracts were subjected to FRAP assay along with standard ascorbic acid. Similarly the extracts were subjected to PM assay using ascorbic acid as reference. The results are presented in triplicates with mean ± SD (Fig. 1a, b).

Anticancer assay

Anticancer activity of A. cobbe leaf methanol, ethanol and aqueous extracts on DU-145 and PC-3 human prostate cancer cells lines

The effect of methanol, ethanol and aqueous extracts of *A. cobbe* leaves on cell viability were evaluated individually by MTT assay and the IC₅₀ values were calculated from dose-dependent response studies

Table 3 Determination of percentage inhibition of DPPH radical scavenging activity of *A. cobbe* leaf extracts

Concentration	100 mg/mL	200 mg/mL	300 mg/mL	400 mg/mL	500 mg/mL
Percentage of inhibition					
Methanol extract	45.67 ± 0.15	56.49 ± 0.91	71.73 ± 0.13	80.47 ± 0.17	84.80 ± 0.38
Ethanol extract	52.68 ± 0.27	64.39 ± 0.28	76.09 ± 0.82	82.74 ± 0.13	85.37 ± 0.40
Aqueous extract	64.71 ± 0.15	70.96 ± 0.82	74.15 ± 0.36	85.69 ± 0.26	89.28 ± 0.76
Ascorbic acid	68.54 ± 0.19	75.11 ± 0.58	87.73 ± 0.38	89.64 ± 0.44	93.59 ± 0.57

The values presented are mean ± SD, n = 3. Results were analyzed using One-way ANOVA

Fig. 1 a FRAP assay for extracts of *A. cobbe*. b PM assay for extracts of *A. cobbe*. Data are presented as mean SEM (n = 3)

assessed 72 h post-treatment. *A. cobbe* leaf extracts inhibited the growth of both (DU-145 and PC-3) human prostate cancer cells in a dose dependent manner. The cytotoxicity for normal mice embryo fibroblast (MEF-L929) cell line was also evaluated and there was no inhibition observed on normal cell lines. The cell viability of the *A. cobbe* leaf extracts was compared to standard chemotherapeutic drug paclitaxel which is commonly used in the treatment of prostate cancer (Fig. 2a–c). The IC₅₀ values were calculated in a dose-dependent response and are presented in Table 4. The combination of the extracts along with the standard drug also induced a higher cytotoxicity in cancer cells. These results revealed morphological changes and shrinkage of cells leading to cell apoptosis induced by the extracts in the human prostate cancer cell lines as depicted in Fig. 3a–c.

Discussion

The practice of medicinal plants as herbal remedies in the treatment of various ailments, has been

recorded way back since the ancient history. These plants remarkably serve as strong anti-oxidants and free radical scavenging agents, which ultimately results in prevention of autoimmune diseases and cancer (Ghate et al. 2014). The plant collected from Uttar Kannada district of Western Ghats was identified according to their taxonomical features as *A. cobbe* and screened for the presences of phytochemical constituents with five solvent extracts. Phytochemical investigation of *A. cobbe* leaf led to the presences of rich secondary metabolites in the selected leaf extracts. These secondary metabolites are found to have various biological and therapeutic properties (Eldahshan and Abdel-Daim 2015). The phytoconstituents present were quantified by different biochemical tests. In the present study, the phytochemical analysis of *A. cobbe* leaf extract revealed a wide-range of secondary metabolites in chloroform, ethyl acetate, methanol, ethanol and aqueous extracts. Phytoconstituents were evaluated by different quantitative biochemical tests (Table 1). The studied phytoconstituents and the presences of phenolic compounds in the leaves of *A. cobbe*

Fig. 2 a, b Percent viability inhibition of *A. cobbe* methanol, ethanol, aqueous leaf extracts and standard drug on prostate cancer cell lines (DU-145 and PC-3). c Percent viability of

normal cells (MEF-L929) treated with *A. cobbe* methanol, ethanol and aqueous leaf extracts. Data are presented as mean SEM (n = 3)

Table 4 IC₅₀ values of cell proliferation inhibition of *A. cobbe* leaf extracts (µg/mL)

Cells	Methanol	Ethanol	Aqueous	Paclitaxel (µM/mL)
DU-145	623.24 ± 11.05	652.25 ± 21.02	431.10 ± 15.05	0.3
PC-3	523.25 ± 13.08	554.15 ± 22.10	362.08 ± 24.17	
MEF-L929	89,842.51 ± 12	98,745.14 ± 08	73,625 ± 11	

The values presented are mean ± SD, n = 3. Results were analyzed using One-way ANOVA

extracts are found to have pharmaceutically importance. The current study indicates that these phenolic compounds are promising molecules with useful cytotoxic, analgesic, and antioxidant activity profiles

(Luximon-Ramma et al. 2002). In the present study the total phenolic contents of *A. cobbe* extract exhibited good quantity of phenolic compounds in methanol, ethanol and aqueous extract but to

Fig. 3 a–b Morphological changes of DU-145 and PC-3 human prostate cancer cell lines and MEF-L929 normal cell line at 72 h of treatment. c Morphological changes of DU-145 and PC-3 human prostate cancer cells treated with the standard

drug Paclitaxel for 72 h. CS cellular shrinkage, BL membrane blebbing (magnification for DU-145 and PC-3 was $\times 40$ and MEF-L929 was $\times 20$)

different extent. Aqueous extract exhibited the highest amount of phenolic content (91.96 ± 0.61 mg/g GAE) as compared to methanol (89.16 ± 0.88

mg/g GAE) and ethanol (76.37 ± 0.02 mg/g GAE) extracts. However, the phenolic content of *A. cobbe* extracts was at least an order of magnitude

Fig. 3 continued**Standard Drug (Paclitaxel)**

lower than the phenolic content of standard Gallic acid (96.56 ± 0.73 mg/g GAE) (Table 2).

Radical scavenging activities are very crucial due to the toxic effects of free radical ions present in biological systems through reactive oxygen species (ROS) which eventually affects many human diseases including cancer (Diplock 1997). Free radical scavenging activity is one of the mechanisms for anti-oxidation (Sini and Devi 2004). Antioxidant activity of the extracts were studied by screening its ability to decolorize the stable DPPH radical. In the present study antioxidant activity of *A. cobbe* leaves extracts was determined using DPPH free radical scavenging assay, FRAP method and PM assay. The results for 100 mg/mL leaf extracts concentration are presented in Table 3. Aqueous leaf extract proved more potent with $64.71 \pm 0.15\%$ radical scavenging activity for a minimum concentration of 100 mg/ml as compared to methanol (45.67 ± 0.15) and ethanol extracts (52.68 ± 0.27). However, the radical scavenging effect of aqueous extract was slightly lower in degree as compared to the standard ascorbic acid (68.54 ± 0.19). As in the DPPH radical scavenging activity assay, aqueous leaf extract also proved to be more potent in FRAP and PM assay as compared to that of methanol and ethanol extract. The presence of antioxidant, in the different extract have been shown to impart antioxidant activity by breaking the free radical chain. This is because antioxidants are the compounds capable of donating electron or hydrogen

molecule for reduction. (Oliveira et al. 2008). A higher concentration determined was directly proportional to higher antioxidant potential of the leaf extract (Fig. 1a, b).

The evaluation of anticancer activity of *A. cobbe* leaf methanol, ethanol and aqueous extracts on human prostate cancer cell lines (DU-145 and PC-3) and a normal cell line (MEF-L929) was investigated for growth and morphology of the cells, respectively. MTT assay an in vitro model system was used to determine the percentage inhibition that reveals changes in basic cellular functions and morphology. As a result significant growth inhibition was observed in prostate cancer cell lines (DU-145 and PC-3), while there was no effect on the growth of normal cells (MEF-L929). The cells treated with varying concentration (3.125, 6.25, 12.5, 25, 50, 100, 200 μ g/mL) of extracts showed inhibition after 72 h at and the percent inhibition was calculated (in triplicates). Aqueous and methanol extracts were most effective in inhibiting the growth of cells at a concentration of 200 μ g/mL. The ethanol extract showed less effect throughout the range of tested concentrations and was not statistically significant on human prostate cancer cell lines (DU-145 and PC-3) for a single time point of 72 h. The results were compared with those obtained with the standard paclitaxel drug used in the treatment of prostate cancer which led to significant viability inhibition of the cancer cell lines than the extracts (Fig. 2a–c). However, aqueous extract was most

effective with IC_{50} value $431.10 \pm 15.05 \mu\text{g/mL}$ for DU-145 and $(362.08 \pm 24.17 \mu\text{g/mL})$ for PC-3 cells. Variation in IC_{50} value was observed among methanol and ethanol extracts (Table 4) while the standard drug paclitaxel showed IC_{50} value of $0.3 \mu\text{M/mL}$. The results revealed changes at morphological level where the cancerous cells underwent shrinkage and membrane blebbing leading to apoptosis in human prostate cancer cell lines (DU-145 and PC-3) while the extract showed no cytotoxicity towards normal cell lines (Fig. 3a–c).

Conclusion

Considering the selectivity of *A. cobbe* plant in the treatment of cancer cells, it needs to be validated on several other cell lines. However, using in vitro model we observed that aqueous leaf extracts of *A. cobbe* had significant anticancer activity on human prostate cancer cell lines DU-145 and PC-3 using in vitro model. At this point, the exact mechanism behind the anticancer activity of *A. cobbe* could not be evaluated. Thus, the present work is a comprehensive study of phenolic content along with antioxidant and anticancer activities of *A. cobbe*. The antioxidant activity is positively correlated to the phenolic contents in the plant. Aqueous extract of *A. cobbe* leaves possess radical scavenging activity with significant anticancer activity. Our study supports suitability of this plant as an herbal medicine. However, further studies on isolation, identification of bio-active molecules and pharmacological studies in animal models will give evidence to validate the traditional medicinal claims of *A. cobbe*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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