Antibacterial, antioxidant, anticancer effects and GCMS analysis of *Berberis aristata*

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(Received: April 2020 Revised: July 2020 Accepted: August 2020)

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ABSTRACT

Introduction and Aim: *Berberis aristata* is an important medicinal plant, which belongs to Berberidaceae family. It is used to treat skin diseases, jaundice, syphilis, diarrhoea, continual rheumatism and urinary problems in Ayurveda. The aim of the present study was to evaluate the antioxidant and antibacterial activity of aqueous extract of *B. aristata* and GC-MS analysis for active compound identification.

Materials and Methods: Different antioxidant assays were carried out for evaluating the antioxidant activity of aqueous extract of *B. aristata.* The maximum DPPH radical scavenging activity of aqueous extracts, the maximum superoxide radical scavenging activity of *B. aristata* extract, the maximum Fe^{3+} and the maximum phosphomolybdenum of *B. aristata* was analysed along with the anticancer activity of aqueous extract of *B. aristata*. These form MTT assay of the extracts.

Results: The targeted investigations carried at the cytotoxicity interest of bark of *B. aristate* have delivered out a few salient diagnostic features, which allow one to distinguish it from other substitutes and adulterants. The antioxidant activity, antibacterial activity and the usage of GCMS analysis has especially contributed to this differentiation.

Conclusion: Based on these observations, the bark extract of *B. aristata* can be used as a potential drug and thereby is apromising future for further researches.

Keywords: *Berberis aristata*; antibacterial activity; antioxidant activity; anticancer activity; DPPH; GCMS analysis.

INTRODUCTION

Berberis aristata is usually called as Indian barberry or tree turmeric. It belongs to Berberidaceae family. Tree turmeric is native to Himalayas in India. It is cultivated in the Nilgiris in Southern India and in Sri Lanka. It is drastically used in Ayurvedic medicine. *Daruharidra* is the Sanskrit name of *B. aristata* (1, 2). It is an evergreen shrub with a peak height of 3-5metres. It is also woody with yellow to brown outer bark and with darkish yellow inner bark.

The bark is fully blanketed with thorns and leaves in tufts of 5-8 with pinnate venation. The upper surface of leaves are darkish green and lower surface of leaves are mild in colour (3). The vegetation is used traditionally in Indian machine of drugs as an antibacterial, antiperiodic, antidiarrheal and anticancer (4). It is likewise used in the remedy of ophthalmic contamination. Its root, stem and leaves are also extensively used in the treatment of numerous ailments y in Ayurveda. *B. aristata* is used in Ayurveda for treatment of many sicknesses, which include liver infection, bellyache, jaundice and mounds. The aqueous extract of *B. aristata* bark has huge spectrum of activity against 13 test pathogens, starting from 12 to 25mm.

Diterpenes have been the most plentiful phytoconstituent (15.3%) and showed broad spectrum of antimicrobial activity starting from 16.66 to 42.66mm. It is used as a tonic, demulcent, diaphoretic, diuretic and for the treatment of diarrhoea, pores and skin disorder, syphilis, continual rheumatism and urinary issues (5).

Thereby it is clear from a range of pharmacological research that tree turmeric has anti-inflammatory, anti-oxidant, anti-coagulant, anti-diabetic, anti-microbial, anti-ulcer, anti-bacterial and anti-cancer properties. These reviews were more related with leaves and roots of medicinal plants. Thus, this current study is focused to analyse the qualitative, quantitative properties along with antimicrobial and anticancer activities present in bark of *B. aristata.*

MATERIALS AND METHODS

Preparation of extract

Bark of *Berberis aristata* was collected from the economic market, Chennai, Tamil Nadu, India. About 10 g of torn pieces of bark was weighed and soaked in 75mL of distilled water for 72 hours. The supernatant was filtered.

Qualitative phytochemical analysis

The aqueous extract of *B. aristata* was subjected to initial phytochemical screening and the use of precise reagents were done as in standard methods (6).

In vitro antioxidant assays

DPPH radical scavenging activity

The antioxidant activity of aril aqueous extract of *B.aristata* was measured based on the scavenging activity of the strong 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical. 1mL of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (50-300µg/mL) of plant extracts. The sample was incubated in dark for 30 min. Distilled water was used as the reference standard. 1mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm (7).

Superoxide radical scavenging activity

Superoxide radical scavenging activity was done with the reaction combination, which contained distinct concentrations of aril aqueous extract of *B. aristata*, 50 mM of phosphate buffer (pH 7.8), 1.5mM of riboflavin, 12 mM of EDTA and 50 mM of NBT. These were added in the sequence. The reaction was initiated by illuminating the reaction mixture for 15 min. Immediately after illumination, the absorbance was measured at 590 nm and the IC_{50} was calculated. Ascorbic acid was used as positive control (8).

Phosphomolybdenum reduction assay

The antioxidant capacity of the aril aqueous extract of *B. aristata*, the plant extract with concentrations ranging from 20 to 120 µg/mL was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water tub at 90ºC for 90 min. The absorbance was detected using ultraviolet spectrophotometer at 695nm.The standard reference used was distilled water (9).

Ferric (Fe^{3+}) reducing power assay

The reducing power of aril aqueous extract of *B. aristata* was studied.1mL of plant extract of different concentrations (20 - 120 µg/mL) was mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1 % (w/v) potassium ferricyanide [$K_3Fe(CN)_6$]. The mixtures were then incubated at 50°C for 20 min. 1mL of 10%(w/v) trichloroacetic acid was added to each mixture. Then to the 1 mL mixture of 0.1%(w/v) $FeCl_3$was added and the absorbance was measured at 700 nm using Spectrophotometer. Distilled water was again used as the standard reference (10).

Antibacterial activity

Microbial strains

The microorganisms of Gram advantageous strains such as *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus* were considered in addition to Gram negative strains such as *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*. These were used for the evaluation of antibacterial activity (11).

Nutrient broth agar medium

Nutrient broth agar medium was changed into organized, in step with the usual strategies (peptone-5 g, yeast-3 g, NaCl-5 g, distilled water- a thousand mL, agar-20 g) and changed into suspended form in 200 mL of distilled water in a 500 mL conical flask, stirred, boiled to dissolve and then autoclaved at 15lbs and at 121°C for 15 mins. The warm medium is poured in sterile petri plates, which were saved inside the aseptic laminar chamber. The medium is allowed to solidify for 15 min.

Agar well diffusion method

Antibacterial activity of aril aqueous extract of *B. aristata* was carried out by using agar well diffusion method. The solidified nutrient agar in the petri plates was inoculated by dispensing the inoculum by using sterilized cotton swabs, which was previously immersed in the inoculum-containing test tube and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The tuber root extract were poured into the wells made on agar plates containing various concentrations. All the plates with extract loaded wells were incubated at 37ºC for 24 h and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. Tetracycline (25 µg) was used as positive control (12).

Preparation of HeLa cell suspension

A sub-cultured HeLa cell lines in DMEM (Dulbecco's Modified Eagle's Medium) were separately trypsinized. 10% FSC and 25ml DMEM was added to disaggregated cells. The cells were suspended in the medium by the pipette and the cells were homogenized (13).

Cytotoxicity assay

The test was carried out using (3-(4, 5-dimethyl thiazol-2yl)- 2, 5-diphenyltetrazolium bromide (MTT). MTT will be divided by two enzymes namely mitochondrial succinate dehydrogenase and reductase enzymes of viable cells, resulting in a purple coloured product called formazan. After 48 h incubation the wells were added with MTT and left for 3hours in room temperature. All wells were removed using pipette and 100µl SDS in DMSO were added to dissolve the formazan crystals. Absorbance was read in Lark LIPR-9608 micro plate reader at 540nm (14).

Gas chromatography-Mass Spectrometry (GC-MS)

A Shimdzu GC-2010 Plus gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ

I.D., 0.25µ film thickness (15). The sample was introduced using split injection with a ratio of 10:1. The oven temperature program was programmed to start at 35°C, hold for 2minutes, then ramp at 20°C per minute to 450°C and hold for 5 minutes. Then the carrier gas of Helium was set to the flow rate of 2ml/min (16).

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical analysis of aqueous extract of *B. aristata* showed the presence of terpenoids, flavonoids, glycosides and saponins (Table 1).

Table 1: Qualitative analysis of aril aqueous extract of *B. Aristata*

S. No	Phytochemicals	Tests	Results
1	Alkaloids	Mayer's test	+
2.	Terpenoids	Lieberman Burchardt test	+
3.	Flavonoids	NaOH solution	+
4.	Tannins	Ferric chloride	-
5.	Glycosides	Sodium nitroprusside solution + Con. H_2SO_4	+
6.	Saponins	Foam test	+

Total phenols and flavonoids

Oxidative stress is considered to be the main source for the initiation and development of many diseases. The diseases like autoimmune diseases, cataract, cancer, Parkinson's disease, heart diseases, neurodegenerative diseases, etc. Phenolic compounds are physiologically active against herbivores or pathogens, used as insecticides, fungicides or pharmaceuticals. These evidences suggests that oxidative damage plays an important role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants lower the risk of disease.

Flavonoids help in preventing injuries caused by free radicles by scavenging method. It stabilises the reactive oxygen site by reacting with radicle. Antioxidant also protects living organisms from reactive oxygen species (ROS).

An imbalance between antioxidants and free radicals result in oxidative stress, which may lead to cellular damage. Today most of the antioxidants are synthetically manufactured. The phenol and flavonoid compounds quantified in the aril aqueous extract of *B. aristata* seemed to be responsible for the antioxidant activity. The total phenol content was 11.04±2.20 µg/mg of GAE and the total flavonoid content was 6.08±0.50 µg/mg of QE in the extract. These results provide a comprehensive profile of the antioxidant activity of aril of *B. aristata* with respect to their phenols and flavonoids content. Quantitative analysis of aqueous extract of *B. aristata* shows the presence of phenols and flavonoids (Table 2).

Table 2: Quantitative estimations of aril aqueous extract of *B. aristata*

S. No	Phytochemicals	Amount (µg/mg)
1.	Phenols	11.04±2.20
2.	Flavonoids	6.08±0.50

DPPH radical scavenging assay

Scavenging of DPPH free radical is one of the popular antioxidant assays. DPPH radical scavenging assay is a decolorization assay that will measure the capacity of antioxidants to directly scavenge DPPH radicals by monitoring its absorbance using spectrophotometer at wavelength of 517 nm. The ability of aril aqueous extract of *B. aristata* to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH radical scavenging activity was 99.29±0.95% at 300 µg/mL concentration (Table 3). Aril aqueous extract of *B. aristata* demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. The IC_{50} was found to be 491.40 µg/mL concentration and was compared with standard (Ascorbic acid, IC_{50} = 11.98 µg/mL concentration).

Table 3: DPPH radical scavenging activity of aril aqueous extract of *B. aristata*

S. No.	Concentration(µg/mL)	Aril aqueous extract % of inhibition
1	50	94.30±0.82
2	100	97.80±0.76
3	150	99.00±0.26
4	200	99.05±0.87

5	250	99.24±0.85
6	300	99.29±0.95

Superoxide ($O_2^{\cdot-}$) radical scavenging activity

The anionic forms of superoxide are relatively harmful and its effects are increased when it produces other forms of free radicles. The Flavonoids helps in scavenging the superoxide anions. In this system, the Superoxide anions that is derived from (DO) dissolved oxygen by the riboflavin-light-NBT system will reduce NBT. n this method, superoxide anion reduces the yellow dye (NBT2+) to blue formazan, which is measured at 590 nm in UV-Vis spectrophotometer. Antioxidants inhibits the blue coloured NBT formation. The resuts were interpreted by measuring the consumption of superoxide in the reaction mixture.The maximum superoxide radical scavenging activityof Berberis aristata was 99.94±0.38% at 120 µg/mL concentration (Table 4) and the IC50 was 192.38 µg/mL concentration.The results were compared with standard ascorbic acid.

Table 4: Superoxide ($O_2^{\cdot-}$) radical of Aril aqueous extract of *B. aristata*

S. No.	Concentration (µg/mL)	Aril aqueous extract % of inhibition
		Superoxide ($O_2^{\cdot-}$) radical
1	20	99.77±0.98
2	40	99.81±0.26
3	60	99.87±0.37
4	80	99.91±1.06
5	100	99.93±1.03
6	120	99.94±0.38

Phosphomolybdenum reduction activity

By phophomolybdenum reduction method which is based on the reduction of (Mo (VI) to Mo(V) by green phosphate/Mo (V) complex at acidic pH) , with 695 nm as maximum absorption the total antioxidant activity of Aril fluid concentrate was estimated of B. aristata.

The maximum phosphomolybdenum reduction was 99.83±0.19% at 120 µg/mL focus (Table 5) and the RC50 was 88.71 µg/mL concentrations. Phosphomolybdenum assay is a quantitative method used to measure the reduction reaction rate in antioxidant, oxidant and molybdenum ligand. During prolonged incubation at higher temperature it requires thermally generating auto-oxidation.

Table 5: Phosphomolybdenum reduction activity of aril aqueous extract of *B. aristata*

S. No.	Concentration (µg/mL)	Aril aqueous extract % of reduction
		Phosphomolybdenum reduction at 695nm
1	20	98.83±0.35
2	40	99.63±0.44
3	60	99.78±0.19
4	80	99.78±0.32
5	100	99.81±0.13
6	120	99.83±0.19

Ferric (Fe^{3+}) reducing power activity

The reducing power assay was carried out by the reduction of Fe^{3+} to Fe^{2+} by the aril aqueous concentrate of *B. aristata* and the subsequent formation of ferro-ferric complex. With the increase in concentration of extract, the reduction ability also decreases. The maximum Fe^{3+} reduction was 99.81±1.02% at 120 µg/mL concentrations (Table 6) and the RC50 was 270.44 µg/mL concentrations. It was compared with the standard ascorbic acid (RC50 = 7.72 µg/mL concentration). We observed in this assay, higher absorbance of the reaction mixture indicated higher reduction potential. The reducing capacity of aqueous extract poses as a significant indicator of its potential antioxidant activity. The reducing capacity of the extract was performed using Fe^{3+} to Fe^{2+} reduction assay as the yellow colour changes to green or blue colour depending on the concentration of antioxidants. The antioxidants such as phenolic acids sand flavonoids were present, considerable amount in aril aqueous extract of *B. aristata* and showed the reducing capacity in a concentration dependant manner.

Table 6: Ferric (Fe^{3+}) reduction activity of aril aqueous extract of *B. aristata*

S. No.	Concentration (µg/mL)	Aril aqueous extract % of reduction
		Fe^{3+} reducing power @ 700nm
1	20	99.75±0.72
2	40	99.77±0.26

3	60	99.77±1.13
4	80	99.78±0.53
5	100	99.79±0.37
6	120	99.81±1.02

Antibacterial activity

The Aril fluid concentrate of *B. aristata* were explored for *in-vitro* antibacterial action against microorganism including Gram-positive microscopic organisms (*Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus*) and Gram-negative microbes (*Escherichia coli, Shigella flexneri*). The diameter of the clear zone in culture plates was measured to detect the antibacterial sensitivity of the extract. Their potency were assessed quantitatively. The maximum zone for *Staphylococcus aureus* was 27 mm at 500 µg/mL fixation. The antibacterial activity may be due to the presence of secondary metabolites such as phenolic compound, terpenoids, tannin and alkaloids that adversely affect the growth of microbes. The antibacterial activity of aqueous extract of *B. aristata* along with zone of inhibition is clearly given (Table 7; Fig. 1).

Table 7: Antibacterial activity of aril aqueous with extract of *B. aristata*

S. No	Organisms	Zone of inhibition mm			Standard (Tetracycline)
		250 µg	375 µg	500 µg	
1	*Bacillus subtilis*	-	-	10	18
2	*Micrococcus luteus*	-	-	12	18
3	*Staphylococcus aureus*	23	24	27	27
4	*Escherichia coli*	22	23	24	34
5	*Shigella flexneri*	16	20	22	36
6	*Pseudomonas aeruginosa*	18	20	23	34

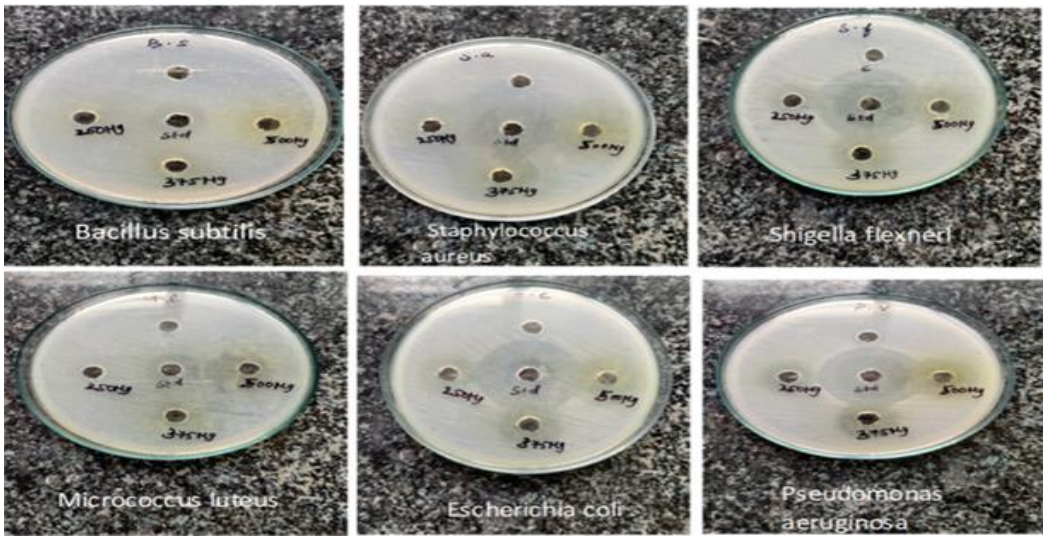


Fig. 1: Antibacterial activity of aril aqueous extract of *B. aristata*

Anticancer activity

The *invitro* cytotoxicity activity results of the sample Berberine extract against HeLa cells were inhibited, however the increasing of sample concentrations showed huge increment of cytotoxicity which is clearly observed in the results (Table 8). It was evident that the samples tested at high as 200 µg/ml showed cytotoxicity activity as high as 32.81% against HeLa cell lines. It was proven that the cytotoxicity effect of the test sample showed cell disintegration after 48 h of treatment against the selected tested cell lines at higher concentrations (Fig. 2). In this cell lines studies, cytotoxicity effect was observed in tested sample concentrations in 48 hours treatment, it also revealed that all tested concentration of test samples shows good cytotoxicity over the tested cell line (Fig.2). The IC50 of the tested sample Berberine extract against HeLa cells was calculated as 118.97 µg/ml.

Table 8: *In vitro* cytotoxicity effect of sample Berberine extract against HeLa cell lines

Sample Conc. (µg/ml)	% Cell Viability
0	100.00
1.625	96.28
3.125	93.51
6.25	84.95
12.5	76.45

25	69.33
50	61.40
100	47.54
200	32.81

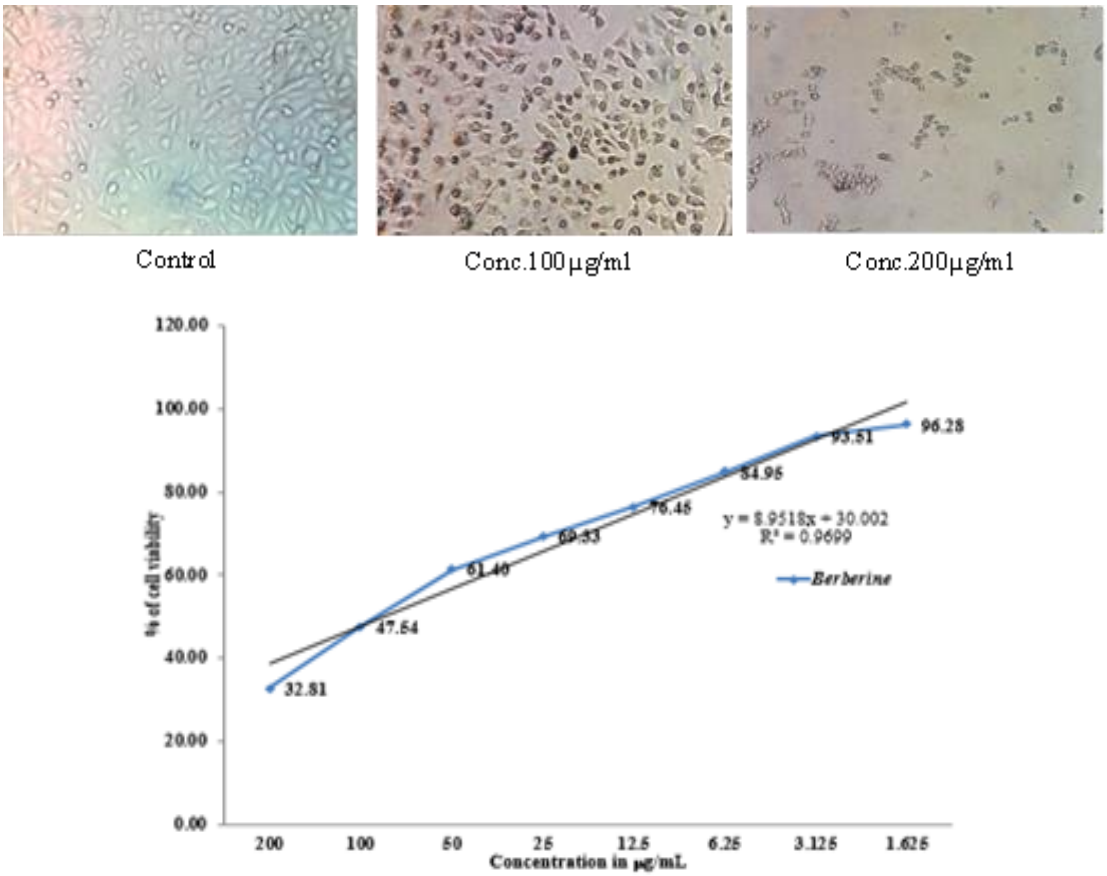


Fig. 2: Cytotoxicity activity of Berberine extract against the HeLa cell lines

GC-MS analysis

GC-MS analysis of aril aqueous extract of *B. aristata* was shown in (Table 9). An antioxidant compound, flavone (5,7-dihydroxy-3-phenylchromen-4-one) and phytol were eluted and recorded. The detailed gas chromatography-mass spectrometry chromatogram of aqueous extract of *B. aristata* along with name of the component is given (Fig. 3).

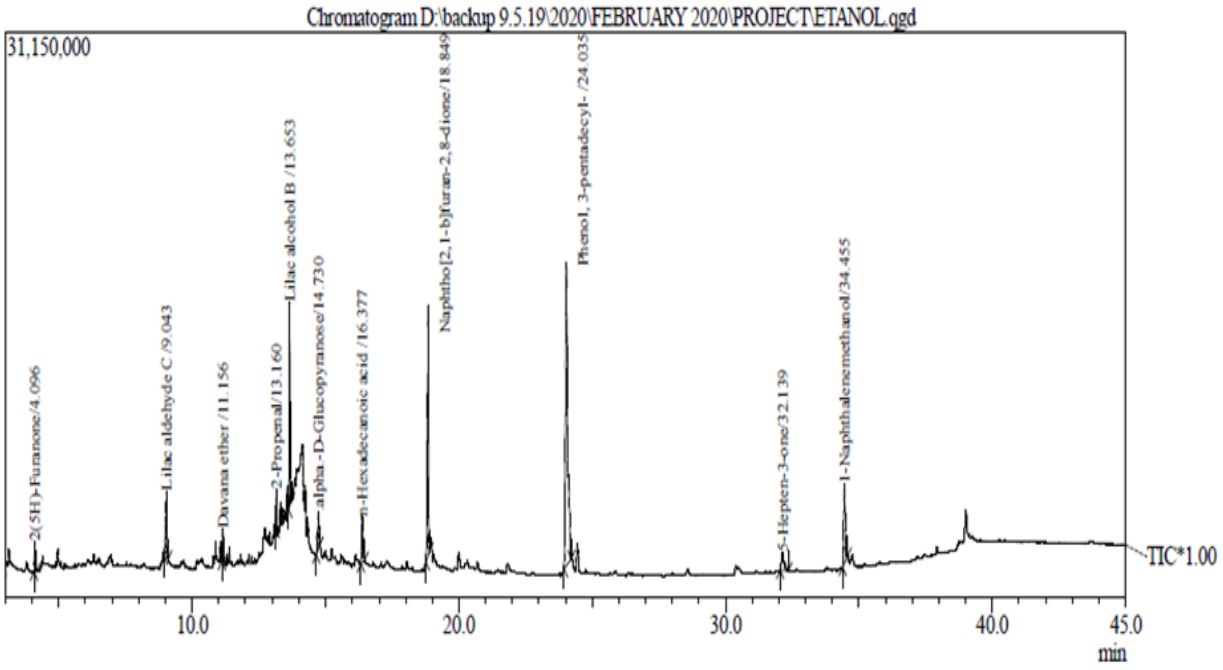
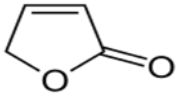
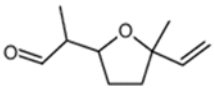
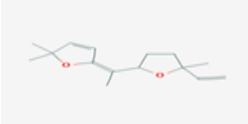
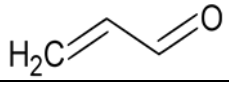
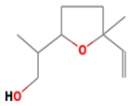
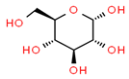
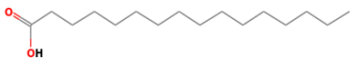
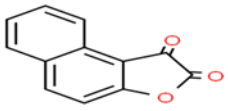


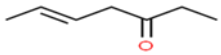
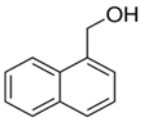
Fig. 3: GCMS chromatogram of aril aqueous with extract of *B. aristata*

Peak Report TIC

Peak#	R.Time	Name	Area	Area%	Height	Height%
1	4.096	2(5H)-Furanone	3132121	1.40	1748631	2.77
2	9.043	Lilac aldehyde C	13548791	6.07	3840747	6.08
3	11.156	Davana ether	3966655	1.78	1874263	2.97
4	13.160	2-Propenal	3441058	1.54	2433455	3.85
5	13.653	Lilac alcohol B	19721825	8.84	11603339	18.36
6	14.730	.alpha.-D-Glucopyranose	6977838	3.13	2090833	3.31
7	16.377	n-Hexadecanoic acid	8096180	3.63	2615482	4.14
8	18.849	Naphtho[2,1-b]furan-2,8-dione	43938065	19.69	14207469	22.48
9	24.035	Phenol, 3-pentadecyl-	97149240	43.54	17205888	27.23
10	32.139	5-Hepten-3-one	6151361	2.76	1034615	1.64
11	34.455	1-Naphthalenemethanol	17012920	7.62	4540012	7.18
			223136054	100.00	63194734	100.00

Table 9: GCMS analysis of aril aqueous extract of *B. aristata*

S. No	Compound name	RT	Compound structure	Mol. weight g/mol	Mol. formula
1.	2(5H)-Furanone	4.096		84.07336	$C_4H_4O_2$
2.	Lilac aldehyde C	9.043		168.23	$C_{10}H_{16}O_2$
3.	Davana ether	11.156		234.33	$C_{15}H_{22}O_2$
4.	2-Propenal	13.160		71.08	C_3H_4O
5.	Lilac alcohol B	13.653		170.25	$C_{10}H_{18}O_2$
6.	Alpha-D-Glucopyranose	14.730		700.7	$C_6H_{12}O_6$
7.	n-Hexadecanoic acid	16.377		256.43	$C_{16}H_{32}O_2$
8.	Naphtho[2,1-b]furan-2,8-dione	18.849		198.17	$C_{12}H_6O_3$
9.	Phenol,3-pentadecyl-	24.035		304.5	$C_{21}H_{36}O$

10.	5-Hepten-3-one	32.139		126.2	$C_8H_{14}O$
11.	1-Naphthalenemethanol	34.455		158.2	$C_{11}H_{10}O$

CONCLUSION

The study clearly indicates that the extract possesses antioxidant, antibacterial, and anticancer properties. At the specific space and time, abiotic factors induce more ability to suppress growths of cancer cell contamination and anticancer activities in comparison to underground biotic factors. These findings justify the traditional uses of this bark for the treatment of cancer.

ACKNOWLEDGEMENT

I would like to express my special thank of gratitude to faculty members in Department of Biotechnology, Dr. M. G. R. Educational and Research Institute. I would also thank my friends and family members for their invaluable contribution and help in finalizing the article.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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