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Metabolic profiling and biological activities of *Artabotrys hexapetalus* (L.f.) Bandari leaves

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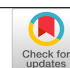
ABSTRACT



Artabotrys hexapetalus (L.f.) Bandari an endemic shrub in India producing fragrant flowers belongs to Annonaceae family and is mainly used traditionally for the treatment of cholera and scrofula. The present study reveals antimicrobial, antioxidant and anticancer activities of *Artabotrys hexapetalus*.To screening and evaluate the major secondary metabolites presents, antioxidant, anticancer potential by *in vitro* assay in *A.hexapetalus* leaves. All successive extracts of *Artabotrys hexapetalus* with ethyl ethanoate, chloroform, methanol, ethanol were obtained by Soxhlet method and subjected to phytochemical screening and *in vitro* antioxidant activity. Antibacterial activity and anticancer activity were determined by Disc diffusion and MTT methods, respectively. Compared to other solvents extracts, the methanol extract of *A.hexapetalus* contains the higher phenolic content (48.45 μg/mg), flavonoid content (49.15 μg/mg) and potential antioxidant capacity (IC_{50}:10.15 $\pm$ 0.85 μg/mg). Due to the high potential of methanol extract of *A hexapetalus* was subjected to the separation of active molecules. Further, trisaccharide, n-Hexadecanoic acid and Z-8-Methyl-9-tetradecenoic acid was isolated and charecterised by UV-vis, FT-IR, NMR spectral studies. Trisaccharide and partially separated fractions from *A.hexapetalus* contain higher antioxidant, anticancer (MCF-7) and antimicrobial activity. Here we conclude that, the methanol extract of *A.hexapetalus* leaves and their fractions possess highly activities against various free radicals, human pathogens and cancer cell line (MCF-7). This is the primary lead to make a new biological agent from *A.hexapetalus* leaves.

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INTRODUCTION

Plants are the major source for of pharmaceutical medicines and drugs. Medicinal flora is the major natural source to cure a variety of human diseases due to the presence of their active phytomolecules, which have greater therapeutic value with lesser side effects (Nisa *et al.*, 2011). In plants, multiple active metabolites have been studied and reported by scientific experts.

Secondary metabolites are compounds that can be involved in response to plant physiological

stress (Hättenschwiler and Vitousek, 2000). Secondary metabolite includes compounds like alkaloids, tannins, flavonoid, saponins and terpenoids. These are very efficient against free radicals and also against various diseases like diabetes mellitus, cancer, neurological disorders (Reddy *et al.*, 2003). Antioxidants are well-known metabolites which will act against free radical and protect oxidative injury (Ajith and Janardhanan, 2002). Free radicals are produced by an oxidation reaction, which leads to chain reactions that may damage cells. Natural antioxidants are provided by plants, that protects from biological oxidants. Plants are an important source of accepted antioxidants, such as carotenoids, flavonoids, tannins, vitamin C and E (Park *et al.*, 2010) and have potent free radical scavengers (Mathew and Abraham, 2006). Synthetic antioxidants have been proved to cause many side effects (Ito *et al.*, 1983). Thus, the present study attempted to find out the efficient natural antioxidants with lesser side effects.

A conventional remedial system using herbal material leads to produce novel antibiotics and drugs (Deba *et al.*, 2008). A range of herbs and herbal extracts have potential phytochemicals which have healing property as well as anti-tumor potential (Hammer *et al.*, 1999). *A. hexapetalus* (L.f.) is habituated in India, Sri Lanka, Java and South China. In India, *A. hexapetalus* is cultivated in gardens for its fragrant flowers. This plant's leaves are used for the treatment of cholera and abdominal and kidney pain. The leaves contain an alkaloid, artabotrine and gallic acid and are reported to have Cardiac stimulant, uterine stimulant and muscle relaxant.

MATERIALS AND METHODS

The fresh leaves of *A. hexapetalus* have been collected from Udumalpet and Tiruppur in Tamil Nadu for this study. The collected leaves were washed, chopped and desiccated for 15– 20 days. The dried leaves were crushed using an electric grinder. 150g of dry leaf powder was extracted with a gradient solvent system on increasing polar order Soxhlet extraction method, and further, the extract was concentrated to 15 g by vacuum drier.

Phytochemical screening

The phytochemical selection of the plant extract was conceded by following the methods of (Trease and Evans, 1989; Harborne, 1970).

Estimation for the amount of phenolic substance

The total phenolic content of plant extracts (*A. hexapetalus*) was determined by gallic acid equivalence (GAE) (Singleton *et al.*, 1999) Plant extracts were diluted in the concentration of mg /ml 25μl of the sample was taken test tubes and add 0.5ml of Folin's ciocalteau reagent. After one to two minutes, 1.5ml of 20% Na_2Co_3 was added and makeup with distilled water. The test tubes were incubated at 25ºC for 1 hour. The absorbance is measured at 760 nm. The endpoint of the reaction mixture is the formation of blue colour.

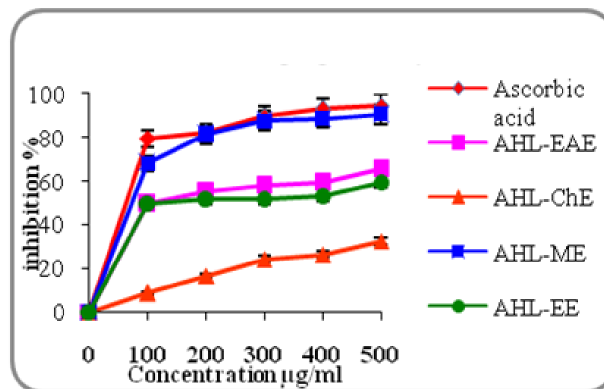

Figure 1: DPPH radical scavenging activity of different solvent extract of *A.hexapetalus* leaves

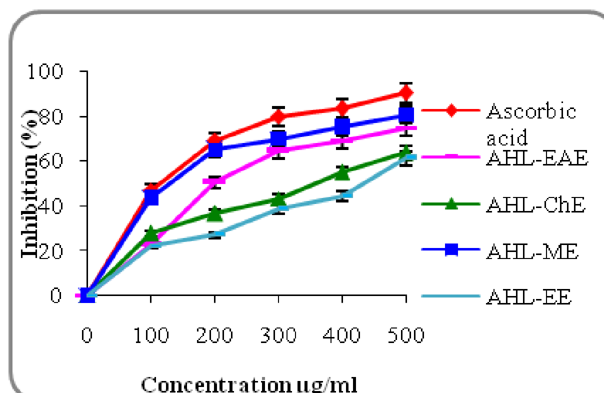

Figure 2: H_2O_2 radical scavenging activity of differentsolvent extract of *A.hexapetalus* leaves

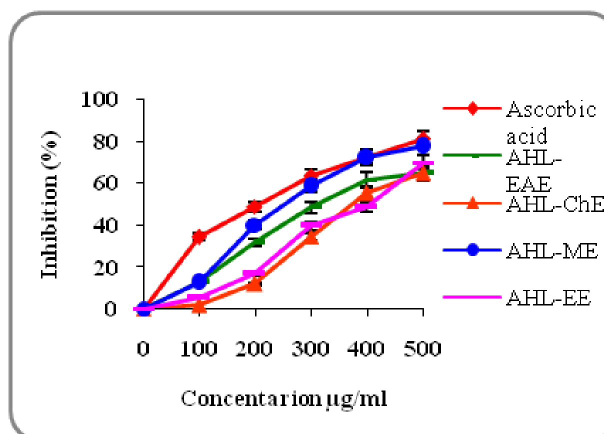

Figure 3: Fe^{2+} chelating activity of different solvent extract of *A.hexapetalus* leaves

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Table 1: Phyto-chemical screening of *Artabotrys hexapetalus* leaves

Extract Major Phytochemical	Artabotrys hexapetalus leaves extract using				
	Petroleum ether	Ethyl acetate	Chloro-form	Methanol	Ethanol
Alkaloids	-	-	-	+	-
Flavonoids	-	-	-	+	-
Tannins and Phenolic compounds	-	+	+	+	+
Proteins	+	+	-	+	-
Steroids and sterols	-	+	+	+	-
Carbohydrates	-	-	+	+	+
Terpenoids	+	+	-	-	-
Cardio glycosides	-	-	-	+	+

+ denotes presence of major phytochemicals; - denotes absence of phytochemicals

Table 2: Antibacterial activity of *A.hexapetalus* against different human bacterial pathogens

Concentration (mg/ml)	Extracts EA(μg/ml)			CH(μg/ml)			ME (μg/ml)			E (μg/ml)			Std Tet (μg/ml)
	25	50	75	25	50	75	25	50	75	25	50	75	25
S.pyogens	+	+	++	-	+	+	+	++	+++	+	++	++	+++
S.aureus	+	+	++	-	+	+	+	++	+++	+	++	++	+++
E.coli	+	+	++	-	+	+	+	++	+++	+	++	++	+++
K.planticola	+	+	++	-	+	+	+	++	+++	+	++	++	+++

+, Zone of inhibition (0-5mm) indiameter ; ++ Zone of inhibition(6-10mm)in diameter; +++,Zone of inhibition(11-15mm)indiameter; -, No inhibition

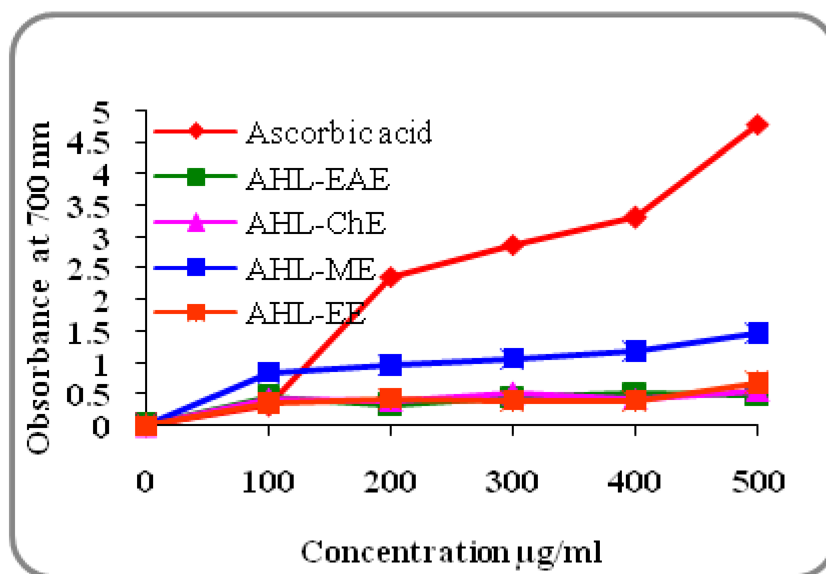

Figure 4: Ferrous reducing power of different solvent extract of *A.hexapetalus* leaves

Table 3: GC-MS profile of partially purified fraction from *A.hexapetalus*

S.No.	Peak Name; chemical formula; Molecular weight	Retention time	Peak area	%Peak area
1.	2-Cyclopentene-1,4-dione; C5H4O2; 96	4.32	312153	0.31
2.	1-(2-Tetrahydrofurylmethyl) piperidine; C10H19NO; 169	5.16	1991538	2.03
3.	2-Methyl-1-methyl pyrrolidine; C7H15N; 113	7.91	436588	0.44
4.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; C6H8O4; 144	10.24	1242542	1.27
5.	Propanoic acid, 2-methyl-, pentyl ester; C9H18O2; 158	11.48	7684587	7.87
6.	Galacto-heptulose; C7H14O7; 210	13.19	3979247	4.07
7.	Phenol, 2,6-dimethoxy-; C8H10O3; 154	14.81	565458	0.57
8.	3-Hexadecene, (Z)-; C16H32; 224	15.05	442589	0.45
9.	4,5-Dimethyl-2-isopropyloxazole; C8H13NO; 139	15.27	1083447	1.10
10.	Phenol, 2,4-bis(1,1-dimethylethyl)-; C14H22O; 206	18.96	2527961	2.58
11.	E-14-Hexadecenal; C16H30O; 238	20.06	1129061	1.15
12.	Bicyclo[3.1.0]hexan-2-one, 1,5-bis(1,1-dimethylethyl)-3,3-dimethyl-; C16H28O; 236	20.23	1160225	1.18
13.	Octahydro-2(1H)-quinolinone; C9H15NO; 153	24.14	3096080	3.17
14.	α-d-Mannofuranoside, methyl; C7H14O6; 194	26.34	1221874	1.25
15.	n-Hexadecanoic acid; C16H32O2; 256	27.39	58118912	59.52
16.	Z-8-Methyl-9-tetradecenoic acid; C15H28O2; 240	29.96	12647529	12.95

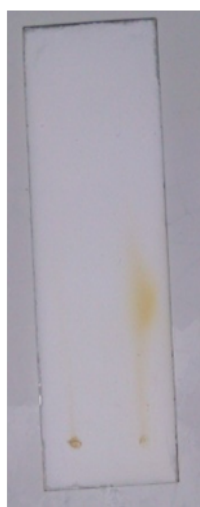

Figure 5: TLC profile of Compound 1from *A. hexapetalus*

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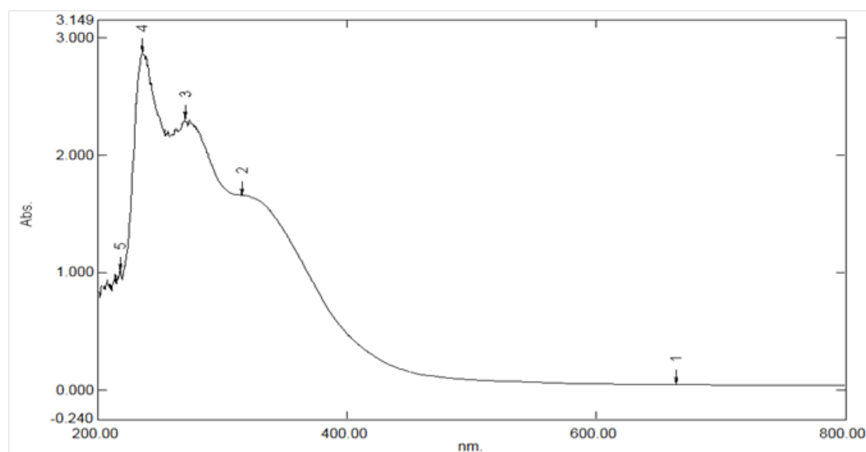

Figure 6: UV-visible spectra of Compound 1 from *A.hexapetalus*

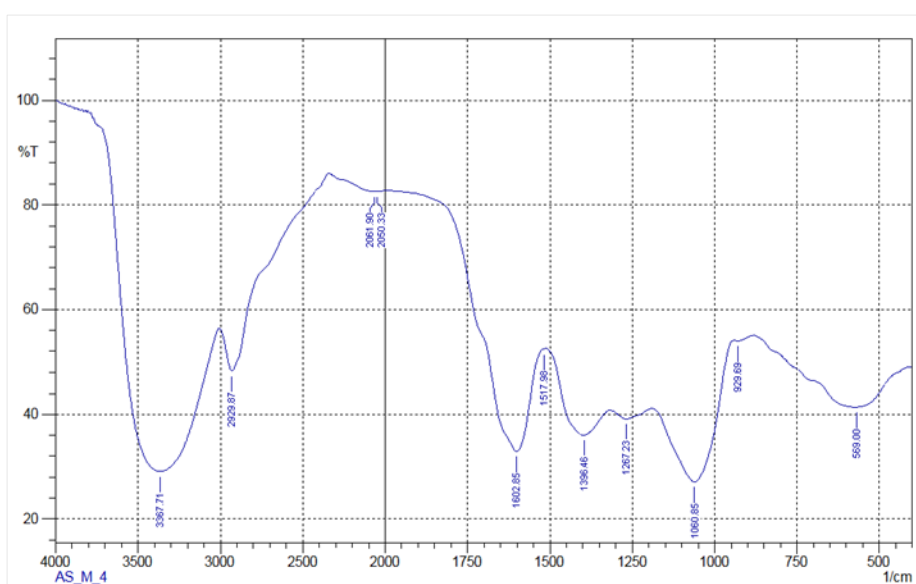

Figure 7: FT-IR spectrum of acompound – 1

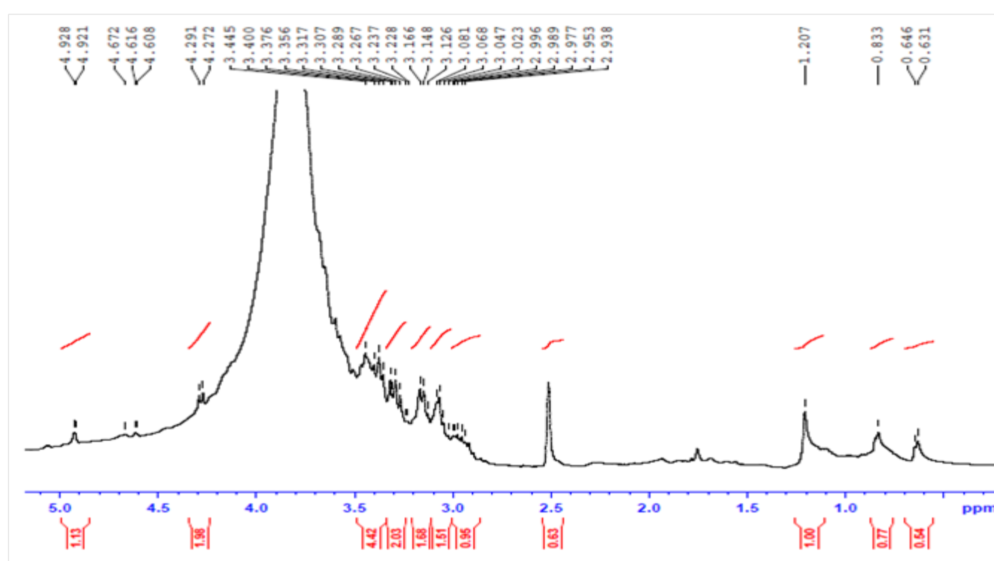

Figure 8: 1H spectrum of Compound 1 from of *A.hexapetalus*

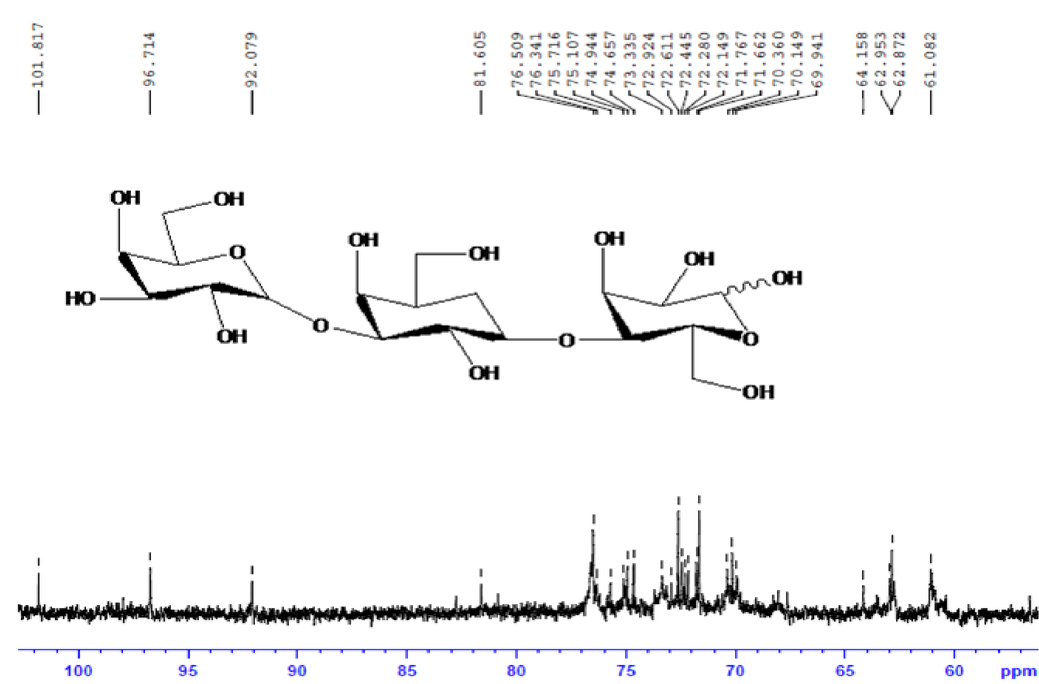

Figure 9: ^{13}C NMR spectrum of Compound 1 from of *A.hexapetalus* and its chemical structure

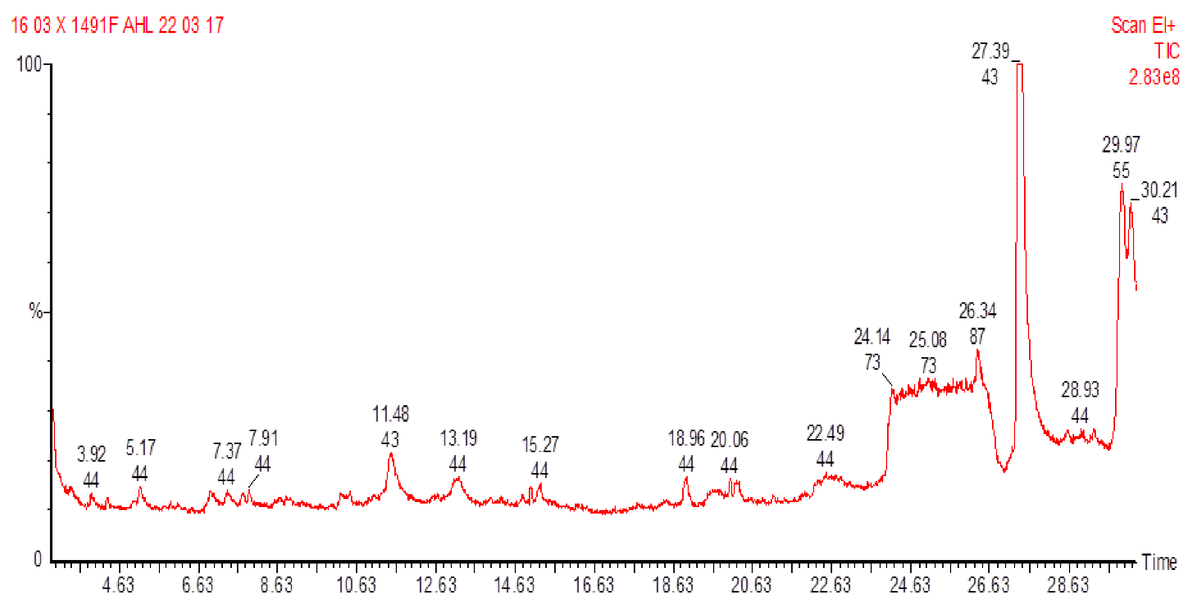

Figure 10: GC-MS Chromatogram of partiallypurified fraction from *A.hexapetalus*

Estimation for the amount of flavonoid substance

Total flavonoid content assay was followed by (Marinova *et al.*, 2005). Th standard used for this test is quercetin. 1mL of extracts was taken in test tubes and mixed with 0.5 mL of $AlCl_2$ ethanol solution. At room temperature, the tubes are incubated for 1 hour and measure the absorbance at 510 nm. The yellow colour indicates the presence of flavonoid.

Estimation for Antioxidant activity DPPH radical scavenging activity

The ability of A. hexapetalus L. f. extracts to scavenge DPPH radical was assessed using (Mondal *et al.*, 2006) method with modification. Briefly, an aliquot of the extract 100-500 μg /ml was mixed with 3 ml DPPH. The resultant absorbance was recorded at 517 nm after 30 min. Incubation at 37^{0}C.

The percentage of scavenging activity was derived using the Equation (1),

$$Percentage\ of\ inhibition\ (\%) = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100$$

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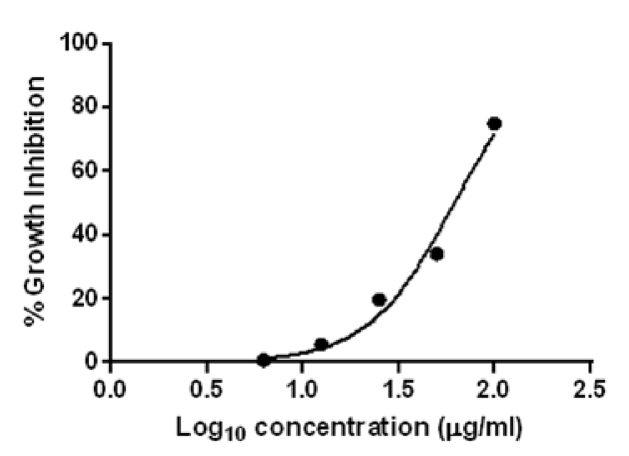

Figure 11: Cytotoxicityassay of partial purified fraction of *A.hexapetalus* leaves

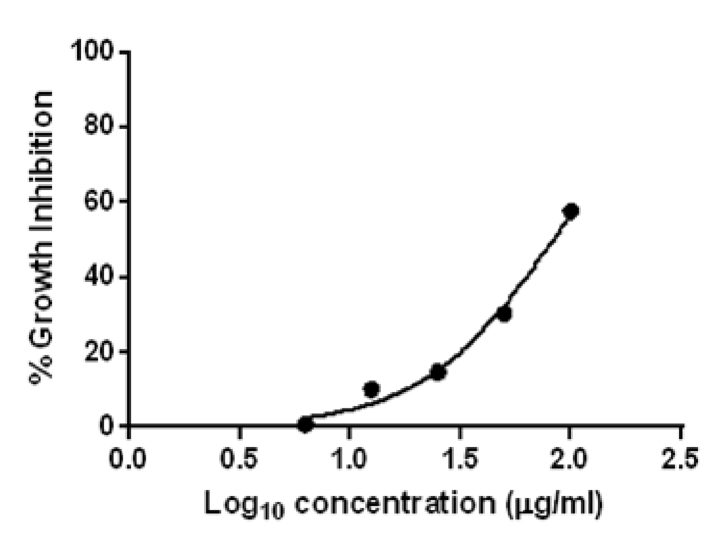

Figure 12: Cytotoxicityassay of compound -1 isolated from *A.hexapetalus*

Where $A_{control}$ - absorbance of reaction mixture without sample

A_{sample} - absorbance reaction mixture with a sample.

Ferrous reducing power

The ferrous reducing power was measured by (Oyaizu, 1986) method. The reducing sugars were compared with the standard L-ascorbic acid. 2.5 mL of phosphate buffer (0.2 M, pH 6.6), 2.5 mL of potassium ferricyanide was mixed with the plant extracts. After a few minutes, 2.5 mL of 10% TCA was added and incubated at 50°C for 20 mins. After incubation, the mixtures were centrifuged at 3000 rpm for 10 mins and transfer the supernatant to another tube. Then the supernatant was mixed with 0.5mL ferric chloride and 2.5 mL of distilled water. Absorbance is measured at 700 nm—the increasing absorbance of the reaction mixture, indicating high antioxidant power.

Hydrogen peroxide radical scavenging activity

This radical scavenging activity was followed by (Ruch *et al.*, 1989) method. Add 0.6 mL of hydrogen peroxide (40mM) and 0.5 mL of phosphate buffer (pH 7.4) to the extracts, the extract aliquot like 100, 200, 250, 300, 400, 500 μg/ml with DMSO; after adding the extract the reaction mixture incubate at room temperature for 10 mins. After incubation, absorbance is measured at 230 nm against the blank solution with phosphate buffer. The percentage inhibition calculated by Equation (1).

Fe^{2+} chelating activity assay

The chelating activity of *A. hexapetalus* leaves extracts were evaluated by measuring the Fe^{2+} chelating activity according to the method of (Dinis *et al.*, 1994). Aliquot of 100 -500 μg /ml of extract, 1.6 ml of distilled water and 0.05 ml of $FeCl_2$ (2 mM) were added and after 30 s, 0.1 ml ferrozine (5 mM) added. The reaction mixture was incubated for 10 min at 30^0C and the absorbance of the Fe^{2+} fer-

rozine complex was measured at 562 nm. A lower absorbance indicates a higher chelating power. The chelating activity of the extracts on Fe^{2+} was compared with that of EDTA (0.01 mM) and Citric acid (0.025 M). The percentage of chelating activity calculated using Equation (1).

Antibacterial activity

Test organism

Streptococcuspyogenes and *Staphylococcus aureus, Escherichiacoli, Klebsiella planticola* are used for antibacterial activity test. The strains were collected from the branch of Microbiology, KAHE, Coimbatore-21, Tamil Nadu.

The antibacterial activity of the extract was carried out at 4ºC using the pre-prepared inoculums on slopes of nutrient agar by (Takahashi *et al.*, 2004). The Muller–Hinton agar was used as a nutrient medium for the growth of microbes. The extracts were dissolved in DMSO during the amount of mg/mL and aliquot 25, 50, 75 μl of extracts. The well was punched by well puncher and different concentrations of extracts were added in that well. The effects of the plant extract were compared with tetracycline as standard drugs at 1mg/mL concentration.

Isolation and characterization

The composed plant resources (leaves) were washed methodically for 2-3 weeks at 35-40°C and strike stream chop and pulverized in a stimulating chopper. 21 gram of methanol extract of *A. hexapetalus* was used to isolate the Phyto molecule by using column chromatography. Parallel fractioning was assorted collectively for additional study, and next it was subjected to make clear the composition. The homogenized solo spot fractions analyzed the arrangement by using spectral studies. UV-Visible spectroscopy is a single cheap and cost-effective method. The presence of chromophore may be identified with the UV-Vis spectroscopy, and FT-IR spectral data were used to arrive at the functional group present in the fractions (IR Affinity-1, Shimadzu, Japan). ^{1}H NMR was to find out the protons and ^{13}C NMR the carbon atoms in the unknown sample matrix. Based on the signals found within ^{1}H NMR and ^{13}C NMR, structure for compound could be predicted (Yadav *et al.*, 2016).

GC-MS Analysis

Fractions from *A. hexapetalus* which contains 2-5 spots on TLC have taken for GC-MS analysis to find phytochemical composition by using Gas chromatography-Mass spectrometry. Analyses were carried out using Perkin Elmer-Clarus 500 with capillary column directly coupled to the mass spectrometer system column (Elite-5ms) (30mx 0.25mmID fused of 5% phenyl 95% dimethylpolysiloxane). Oven temperature 70°C @ 6° C mins to 170°C (2 min) @ 6° C/min to 290°C (5min); injector temperature 290°C, carrier gas He, flow rate 1ml/min; split ratio 1:10; mass spectral were taken ionization energy at 70ev, 200°C, scanning. The scanning range was 40-450m/z. All peak values of fractions were compared with those compounds stored in the database of the NIST 2005 library and identified the volatile compounds (Yadav *et al.*, 2015).

Antioxidant and antibacterial activity of isolated fractions from *A. hexapetalus*

Antioxidant potentials of fractions were screened as per, (Mondal *et al.*, 2006) method. The radical scavenging assay DPPH was performed to screen the potentials of the plant fractions.

In vitro Anti-cancer Activity of isolated fractions

MCF 7, the human breast cancer cell line was obtained from National Centre for Cell Science (NCCS) Pune, India. The cell line was grown in Eagles Minimum Essential Medium (EMEM) that containing 10% fetal bovine serum (FBS). Then all cells were maintained at 37^{0} C, 5% CO_2, 95% air and 100% relative humidity. Maintained cultures were passage weekly, and the culture medium was changed twice in a week.

The monolayer cells were detached with trypsin-ethylene diamine tetraacetic acid (EDTA) to make single-cell suspensions. And viable cells were counted by tryphan blue stain by using a haemocytometer. The cell suspension was diluted with medium containing 5% FBS to give a final density of 1x10^{5} cells/ml. After 24 h, the cells were treated with test samples. They were initially dispersed in dimethylsulfoxide (DMSO). Aliquots of 100 μl of these different sample dilutions (0.5 to 2.5 μg/ml) were added to the appropriate wells that are already containing 100 μl of the medium. Following drug addition, the plates were incubated for 48 h at 37^{0} C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as blank and triplicate was maintained for all concentrations.

MTT assay

MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide) is a yellow, water-soluble tetrazolium salt. Living cell mitochondrial enzyme,succinate-dehydrogenase that cleaves the tetrazolium ring and converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to

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the number of viable cells. After 48h of incubation, 15μl of MTT (5mg/ml) in phosphate-buffered saline (PBS) was added to each well. And incubated that at 37^{0}C for 4h. The medium with MTT was then flicked off and the formed Formosan crystals. That should be solubilised in 100μl of DMSO, then measured the absorbance at 570 nm using a microplate reader (Mosmann, 1983; Vistica *et al.*, 1991). The % cell inhibition was determined using the following formula,

$$\% \ Cell \ Inhibition = 100 - \left[\frac{Abs \ (sample)}{Abs \ (control)}\right] \times 100$$

Graph of nonlinear regression was plotted between % Cell inhibition and Log concentration and also IC_{50} was determined using software called Graph Pad Prism.

RESULTS AND DISCUSSION

Phytochemical screening

The extracts of *A. hexapetalus* leaves (Ap L) contains the major metabolites such as alkaloids, steroids, flavonoids, phenols, tannins, protein, carbohydrates and Terpenoids and showed in the Table 1.

Estimation for the amount of phenolic substance

The total phenolic contents in *A. hexapetalus* leaves extract were obtained using the Gallic acid linear curve (y=0.771x-0.665 (R^2= 0.996). The ethyl acetate, chloroform, methanol and ethanol extract of *A.hexapetalus* leaves contain higher phenolic content as 9.15, 7.26, 48.45 and 11.65 μg/mg respectively. The full amount of phenolic content was more in methanol leaf extract compared to the other solvent extract (Yadav *et al.*, 2012).

Estimation for the amount of Flavonoid substance

The full amount of flavonoid substance was obtained via the deterioration calibration curve y=0.014x-0.015 (R^2= 0.988) with quercetin equivalent. The ethyl acetate, chloroform, methanol and ethanol extract of *A.hexapetalus* leaves contain 36.1, 22.45, 49.15 and 48.35 μg/mg of total flavonoids respectively. Comparatively, the methanol extract of A.hexapetalus leaves contains high flavonoid contents (Yadav *et al.*, 2012).

Antioxidant activity

DPPH radical scavenging activity

A.hexapetalus leaf extract has the capability to scavenge the DPPH gratis radicals as equivalent to the standard L - ascorbic acid. The leaf extract of *A.hexapetalus* shows a good antioxidant capacity in the photometric evaluation of free radical scavenging ability (Figure 1). Due to the scavenging ability of the extract, there is more reduction of absorbance in the DPPH radical scavenging activity. The IC_{50} value for leaf extract was compared with the standard L- ascorbic acid 10.15 $\pm$ 0.85 and 07.55 $\pm$ 0.45 μg/mg, respectively. The lesser IC_{50} value indicates the privileged free radical scavenging activity (Yadav *et al.*, 2012).

Hydrogen Peroxide Radical Scavenging Activity

The scavenging capability of *A.hexapetalus* leaves is shown in the Figure 2. IC_{50} values for standard L-ascorbic acid and *A.hexapetalus* leaf extract is 42.35 $\pm$ 0.75 μg/ml and 47.68 $\pm$ 1.95 μg/mg. The presence of H_2O_2 in the cell civilization possibly will show the way to the oxidative DNA injury. So, removing H_2O_2 is extremely necessary for antioxidant resistance in cells (Yadav *et al.*, 2016).

Fe^{2+} chelating activity assay

The capability to chelate changeover metals can be measured as an essential antioxidant manner of action. IC_{50} values for standard L-ascorbic acid and *A.hexapetalus* leaf extract is 97.50 $\pm$ 2.10 and 172.45 $\pm$ 2.15 μg/mg, respectively (Figure 3).

Ferrous Reducing Power Assay

The ferrous reducing power of extracts was screened with a decrease of Fe^{3+} to Fe^{2+} after adding the extract. The elevated absorbance value indicates the occurrence of elevated antioxidant ability of the extract. The golden colour of the reaction combination changes to a variety of sunglasses of blue with green depending upon the antioxidant activity of the extract (Figure 4).

Antibacterial Activity

Antibacterial activity of *A. hexapetalus* leaves was performed using agar well diffusion method resulting in a zone of inhibition against bacterial organisms. The preliminary screening of different solvent extracts of *A. hexapetalus* leaves contains higher antioxidant capacity, antibacterial potentials and anti-cancer potential with the following order of solvent extract.

Table 2 shows, the methanol extract of *A.hexapetalus* has higher inhibitory activity against *S.pyogens*, *S.aureus*, *E.coli* and *K.planticola* compared to ethyl acetate, chloroform, ethanol extracts. The antioxidant activity, antibacterial activity and anticancer activity of methanol extract of *A.hexapetalus* leaves were demonstrated. This shows the methanol extract of *A.hexapetalus* leaves contains more activity compared to other solvent extracts. The same has been taken for further active phytochemical isolation and characterization (Sreedharan *et al.*, 2008).

Column Chromatography Separation

The methanol extract of *A.hexapetalus* has sequentially fractionated and separated through column chromatography. Totally 63 fractions were collected and monitored their TLC profile to find a single active molecule. Fractions 60-63 (100% methanol) were pooled to be homogenous by TLC showing two spots in TLC with the solvent system of 20:80 $CHCl_3$: MeOH and the R_f value was 0.49 and 0.88. The same fractions were taken for preparative TLC (Figure 5) for further structural characterization. This compound was marked as compound-1 and the concentration yield as 117±1.5mg. The UV-visible spectrum of compound-1 shows the highest absorbance peak at 315nm and 269nm (Figure 6). The IR range of compound 1 exhibited a large group at 3367cm^{-1} showing the presence of –OH groups, band at 2929cm^{-1} for C-H group and at 1060 cm^{-1} for C-O group (Figure 7).

The ^{1}H-NMR spectrum exhibited signal at δ 4.92, 4.67 and 4.60 showing the presence of 3 anomeric carbon atoms. The bunch of signal from δ 3.0 to δ 4.0 were attributed to the methane protons under oxygen functions (-OH group). It suggests that the compounds may be a trisaccharide molecule (Figure 8). In the ^{13}C NMR of Compound1, the signal is shown at δ 101.8, 96.7 and 92.0 due to 3 anomeric carbon atoms confirming the presence of a trisaccharide. The signal at δ 61.08, 62.87 and 62.95 are due to three –CH_2 OH group (Figure 9). The rest of the signal between 69.94 to 81.60 is due to carbon atoms under the hydroxyl group. Based on the spectral data, compound 1 is a trisaccharide molecule (Figure 9).

GC-MS study of partially purified fractions

GC-MS analysis of partially purified fractions from *A. hexapetalus* leaves is carried out to find out the metabolic profiles, as shown in Table 3 ; (Figure 10). Sixteen phytoconstituents were identified, including n-Hexadecanoic acid (59.52) and Z-8-Methyl-9-tetradecenoic acid (12.95) being the major compounds (Yadav *et al.*, 2016).

Biological activity of isolated fractions

The isolated compound and partial purified fractions were tested for their antioxidant, antibacterial and anticancer potential revealing they were highly active against radicals, human pathogens and cancer cell lines (MCF-7). DPPH is a constant free radical to accept an electron of hydrogen radical and to turn into a stable molecule. And it produces hydrazine by converting the unpaired electrons to the corresponding electron due to the hydrogen donating capability of the extract. The decreased ability of DPPH radicals by the extract was observed at 517 nm. The isolated tri-saccharide molecule contains moderate antioxidant activity compared to other fractions and L-ascorbic acid and showed the IC_{50} value of the trisaccharide, partial purified fractions and L-ascorbic acid as 34.60±0.85, 26.21±1.51 and 12.30±0.33 μg/mg respectively.

Antibacterial potentials were monitored for isolated fractions and partial purified fractions against *S.pyogens, S.aureus, E.coli* and *K.planticola* (Narayanan and Nagarajan, 2011) and found that partially isolated fractions from *A. hexapetalus* leaves contained more antibacterial activity compared to the isolated trisaccharide and tetracycline. The outcome of cytotoxicity assay, when conceded in triplicates, exposed that partial purified fractions, and isolated trisaccharide from *A.hexapetalus* leaves caused an augment in proportion loss of cells during a dose-dependent way (Takahashi *et al.*, 2004). The IC_{50} of partial purified fractions and trisaccharide was found to be 61.84 and 89.77 g/mg in cytotoxicity activity. This shows partially purified fraction from *A.hexapetalus* leaves contains high cytotoxicity against MCF-7 cell line (Figures 11 and 12).

CONCLUSIONS

The methanol extract of *Artabotrys hezapetalus* leaves possesses high free radical scavenging activity, antibacterial activity and anti-cancer activity. From the methanol extract, trisaccharide was isolated, and it is found to contain moderate biological properties. The remaining partially purified fractions contain high antioxidant capacity with various *in vitro* models, antibacterial activity as well as anticancer activity owing near the occurrence of n-Hexadecanoic acid and Z-8-Methyl-9-tetradecenoic acid. Here we concluded that the methanol extract of *A.hexapetalus* leaves and their fractions are highly potent against various free radicals, human pathogens and MCF-7 cell line. This is the primary lead to make a new biological agent from *A.hexapetalus* leaves.

Statistical analysis

The arithmetical study is performed via Student's't'-test, one-way analysis-of-variance (ANOVA) Followed by Dennett's test for being an assessment of groups through control.

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