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IDENTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM *Alternanthera sessilis* LEAF EXTRACT ITS ANTIMICROBIAL ACTIVITY AND ANTICANCER PROPERTY

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ABSTRACT

Human beings consume maximum of grains followed by fruits and leaves, among this leaf occupy a unique status generally known for its vitamins minerals and therapeutic compounds. Among these therapeutic compounds recently gaining importance Alternanthera sessilis is one such leafy vegetable used on regular basis for general health maintenance. However, identification of compounds and their therapeutic activity needs their isolation which occur through dissolving in solvents. In the present study three solvents water, methanol and ethanol were used to identify the secondary metabolites though all the compounds were identified from all the three solvents methanol lead to positive result in most of the tests for secondary metabolites. Quantification of major secondary metabolites showed high saponins followed by alkaloids and tannins. GCMS analysis of methanol crude extract of A. sessillis showed 16 compounds were most of them were alkane, alcohols and esters which were known for antimicrobial and anticancer property. Methanol crude extract of A. sessillis was with K. pneumonia and B. subtilis 13.33mm followed by P. mirabilis 12.83mm and S. pneumonia 12.67mm. The DPPH scavenging activity reached up to 73.31% with 800 µg/ml and ABTS reached up to 84.29 with 800 µg/ml when methanol crude extract of A. sessillis was used. The methanol crude extract of A. sessilis showed least IC_{50} with HeLa 458.64 µg/ml followed by MCF 497.76 µg/ml HepG2 513.21 µg/ml cell lines.

KEY WORDS

Alternanthera sessilis, therapeutic compounds.

INTRODUCTION

Alternanthera sessislis (L.) R. Br. (Amaranthaceae) is popularly called as dwarf copperleaf or sessile joyweed and in Bangladesh as Chanchi shak. It is an aquatic plant and can be commonly observed in marshy areas and wetlands of Bangladesh (Singh *et al.,* 2009). *Alternanthera sessilis* Linn. (*Amaranthaceae*) is a perennial herb bearing short petiole, simple leaves and white flowers found throughout India but native to bangaldesh. This herb has been reported to have antioxidant, antimicrobial and wound healing activity and used as a galactogogue, cholagogue and febrifuge and in indigestion purposes (Sivakumar *et al.,* 2016).

The decoction of leaves are used to treat eye diseases, wound, cuts and an antidote to snake bite and for curing skin diseases. Ancient medical practitioners of Bangladesh believe that the plant possess medicinal properties and hence used as a leafy vegetable in dietary supplements. In Noakhali district of Bangladesh, the plant is used to treat gonorrhea, low sperm count,

and leucorrhea (Ahamad *et al.,* 2014). Various communities of Uttara Kannada district of Karnataka, Tamilnadu cook and consume this joyweed as spinach which indirectly possess therapeutic importance for the treatment of ulcers. The plant is well grown in Kaptipada Forest Range in Orissa in which the inhabitants such as local tribals (Santals, Gonds, Kolha) used this plant for treatment of fevers, ophthalmia and pruritis.

Phytochemical screening of *A. sessilis* leaf extract shows the presence of alkaloids, tannins, carbohydrates, proteins, ascorbic acid and synthesized AgNPs from leaves of *Alternanthera sessilis* shows elevated presence of tannins, carbohydrates, proteins and ascorbic acid and possess higher efficiency than the extract obtained from its leaves (Niraimathi *et al.,* 2013). Previous phytochemical studies have reported the isolation of flavonols, triterpenoids, steroids and tannins; β-sitosterol, stigmasterol, campesterol, and lupeol being very few of its important constituents (Lalitha *et al.,* 2011).

Ethyl acetate fraction of *Alternanthera sessilis* has been reported to reduce fasting blood glucose level, triglyceride level, when administered to obese type 2 diabetic rats induced by high fat diet and streptozotocin (Boah *et al.,* 2011). Previous study reports validate the folk medicinal use of *Alternanthera sessilis* in Bangladesh for treatment of severe pain, and further suggests that the aerial parts of the plant can be a potential mean for lowering blood glucose levels (Ahamad *et al.,* 2014). Since the plant is widespread in Bangladesh, it proves a better favourable assistance in being a source of a low-cost and effectual medicine for people with diabetes and persons suffering from chronic pain in diseases like rheumatoid arthritis and osteoporosis. Notably, the plant is highly consumed and demanded by the people of Bangladesh as a vegetable in a healthy dietary supplement.

MATERIALS AND METHODS

Alternanthera sessilis plant was collected from the Namakkal district. The *Alternanthera sessilis* leaves were collected, washed, dried, grinded and extract was filtered using Soxhlet apparatus. The extract was qualitatively and quantitative analysed for Secondary Metabolites was done by using the standard procedure prescribed by Harborne (1973), Kumaran, *et al*., (2006), Obadani (2001), and Mc. Donald, *et al*., (2001). The extracted samples were analyzed using gas-chromatography mass spectrometer (GC-MS). The antibacterial activity of *Alternanthera sessilis* extract using Muller Hinton agar against different microorganisms. The concentration of the unreacted DPPH radical after its reaction with the examined antioxidants was estimated by the slightly modified Brand-Williams method (Brand Williams *et al*. 1995). The estimation of antioxidant properties of examined compounds by ABTS method. ABTS cation radical was prepared according to Nenandis *et al*. (2004). The cell culture and MTT assay were done for *Alternanthera sessilis* extract against HeLa, MCF7, and HepG2 cell lines.

RESULTS

Phytochemical test of *Alternanthera sessilis* crude extract with different solvents (Table 1). Quantity of secondary metabolites in different crude extract of *Alternanthera sessilis* (Table 2). Phytocomponents identified in the methanol leaf extract of *Alternanthera sessilis* GC-MS analysis showed the presence of 16 active secondary metabolites with highest peak is of Hexamethyl-Cyclotrisiloxane (Table 3). The nature of 16 active compounds present in methanol extract of *Alternanthera sessilis* includes in the group of alcohol, alkane, carboxylic acid, ester, diene and aldehydes. Phytochemical compounds present in methanol extract of *Alternanthera sessilis* and its molecular weight ranged between 142 to 530

Antibacterial activity of *Alternanthera sessilis* at different concentrations with different microorganisms showed highest zone of inhibition with *B. subtilis* (13.33) and *K. pneumonia* (13.33) at 150µg/ml concentration (Figure 1).

Antifungal activity of *Alternanthera sessilis* methanol crude extract at different concentrations showed highest zone of inhibition with *C. albicans* (10.33) followed by *F. graminearum* (9.83) and *A. fumigates* (9.67) at 150µg/ml concentration (Figure 2).

Average percentage cell inhibition of methanol extract of *Alternanthera sessilis* leaf against HeLa, MCF-7 and HepG2 cells showed highest cell inhibition with HeLa cells (82.84) followed by MCF-7 cells (82.54) at 800µg/ml concentration (Figure 3, 4 & 5).

Fifty percent inhibition (IC50) concentration of methanol extract of *Alternanthera sessilis* leaf for cancer cells showed lowest IC50 with HeLa cells (458.64) followed by MCF-7 cells (497.76) and Hep G2 cells (513.21) (Figure 6).

Antioxidant activity (ABTS, DPPH) of methanol extract of *Alternanthera sessilis* leaf at different concentrations showed highest antioxidant activity in ABTS at 800µg/ml concentration (84.29) (Figure 7 & 8).

Table 1. Phytochemical test of *Alternanthera sessilis* crude extract with different solvents.

S. No.	Phytochemical test		Solvents		
			Water	Methanol	Ethanol
1	Alkaloids	Dragendroff's reagent	+	+	+
		Iodine	-	+	+
		Mayer's	+	+	+
		Wagner's	+	+	+
2	Flavonoids	Alkaline	-	+	-
		Pews	-	+	+
		Shinoda	+	+	+
3	Lignin	Lignin	-	+	+
		Labat Test	+	+	-
4	Tannins	Ferric chloride	-	+	+
		Gelatin	+	-	-
5	Phenols	Ferric chloride	-	+	-
		Ellagic	-	+	+
		Phenol	+	-	-
6	Terpenoids	Libermann's Burchard Test	-	+	+
7	Sterols	Libermann's Sterol Test	-	-	+
		Libermann's Burchard Test	-	+	+
8	Glycosides	Legals	+	-	-
		Keller Killani Test	+	+	+
		Glycosides Test	+	+	+
		Conc. H2SO4	+	-	+
		Molisch's	+	-	+
9	Saponins	Lead acetate	+	+	-
		Foam	-	+	+
		Haemolysis Test	+	+	+
10	Protein	Millons	+	+	+
11		Biuret	+	-	+
12	Carbohydrate	Molichs	+	+	+

Table 2. Quantity of secondary metabolites in different crude extract of *Alternanthera sessilis*

Secondary metabolites	Aqueous	Methanol	Ethanol	P value	F value
Alkaloids	8.54 ± 0.26	10.53 ± 0.27	8.96 ± 0.42	0.0007^{ES}	31.247
Flavanoids	1.83 ± 0.06	2.09 ± 0.05	1.93 ± 0.06	0.0040^{VS}	15.959
Tannin	4.76 ± 0.09	6.41 ± 0.17	5.34 ± 0.09	$< 0.0001^{ES}$	139.82
Phenols	0.35 ± 0.03	0.56 ± 0.03	0.45 ± 0.03	0.0004^{ES}	36.778
Saponins	11.58 ± 0.19	12.51 ± 0.22	11.10 ± 0.05	0.0002^{ES}	53.162
P value	$< 0.0001^{ES}$	$< 0.0001^{ES}$	$< 0.0001^{ES}$		
F value	2795.8	2614.0	1597.7		

Table 3. Phytocomponents identified in the methanol leaf extract of *Alternanthera sessilis* GC-MS analysis

S. No.	RT	Peak area %	Identified Name
1	2.02	0.07	2-Methyl-2-decanol
2	2.13	1.62	1-Hexen-4-ol, 1-chloro-3,5-dimethyl-
3	2.29	0.38	Phtytol
4	2.50	0.72	5-Hydroxy-4-methyl-6-hepten-3-one
5	2.75	2.25	2,3,4-Trimethylpentanoic acid
6	3.21	8.99	Methyl 2-*O*-methyl-*α*-*D*-xylofuranoside
7	3.49	0.76	Methyl 2,4-dimethylhexanoate
8	4.89	14.37	Hexamethyl-Cyclotrisiloxane
9	5.11	1.27	1,1-Diethoxydecane
10	10.10	1.33	1-Fluorododecane
11	26.56	0.19	Cyclohexane-1,3-dione,2-allylaminomethylene-5, 5-dimethyl-
12	42.94	2.47	1,10-Hexadecanediol
13	43.01	13.91	10,13-Dimethyl-Tetradecanoic acid, methyl ester
14	43.78	1.64	*Z,Z*-6,28-Heptatriactontadien-2-one
15	44.95	5.96	(*Z,Z*)-9,12-Octadecadien-1-ol
16	45.14	5.06	1-Pentatriacontanol

Figure 1. Comparative antibacterial activity of Alternanthera sessilis at different concentration

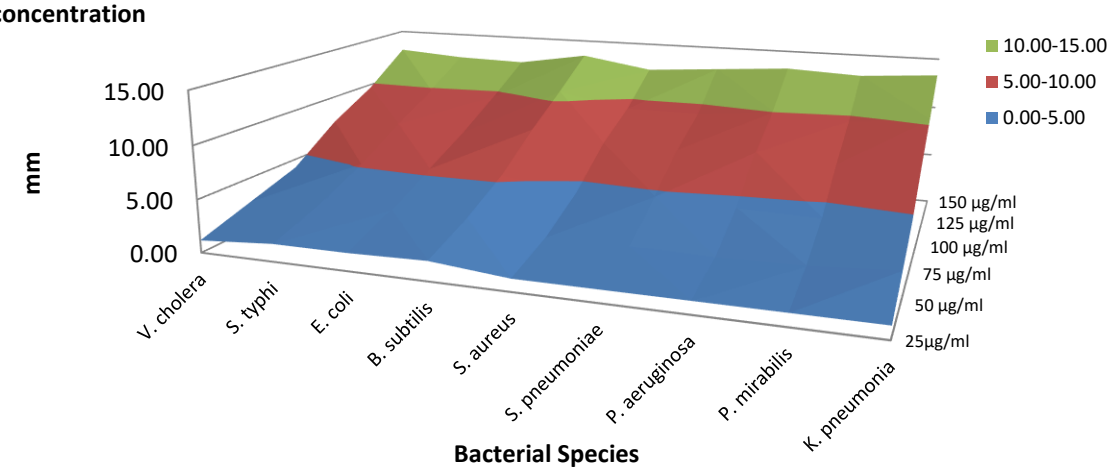


Figure 2. Comparative graph for antifungal activity of Alternanthera sessilis methanol crude extract at different concentrations

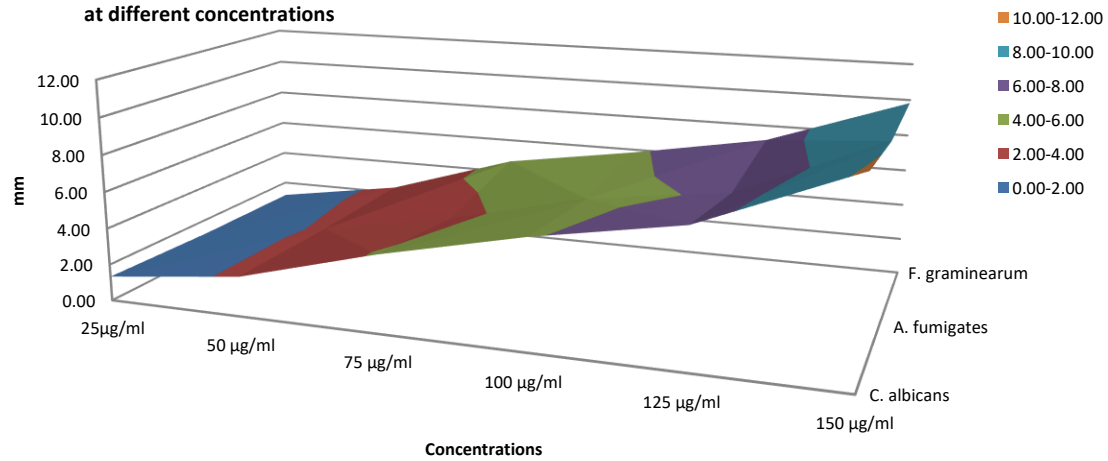


Figure 3. Average percentage cell inhibition of methanol extract of Alternanthera sessilis leaf against HeLa Cells

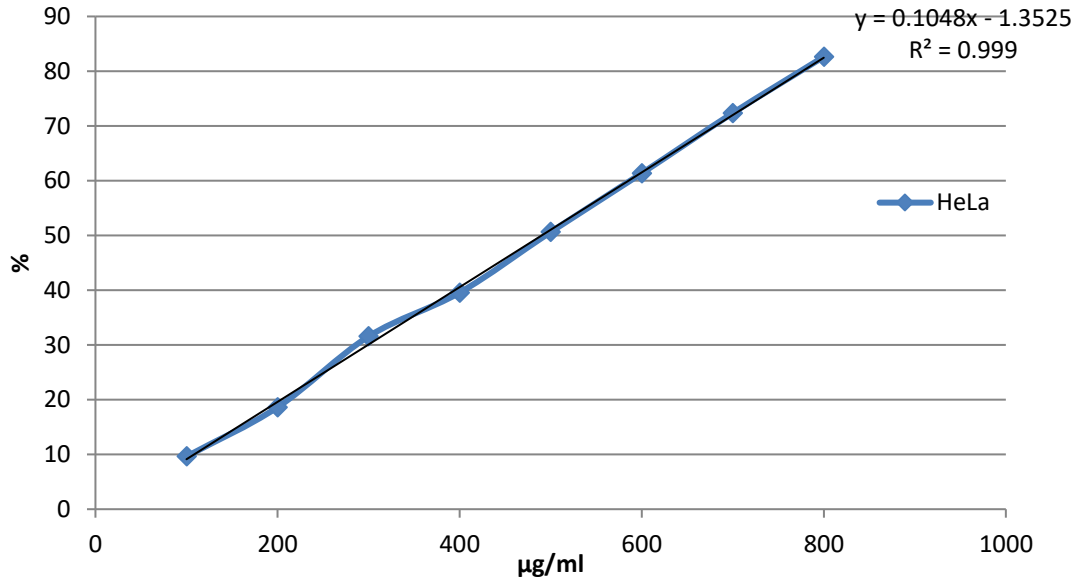


Figure 4. Average percentage cell inhibition of methanol extract of Alternanthera sessilis leaf against MCF7 cells

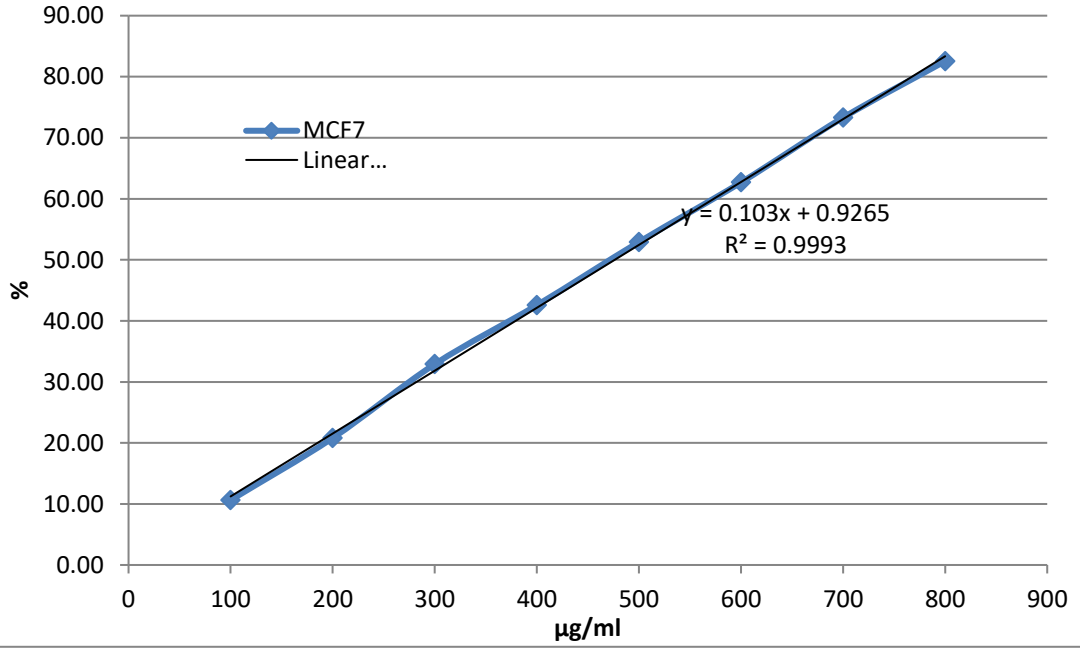


Figure 5. Average percentage cell inhibition of methanol extract of Alternanthera sessilis leaf against HepG2 cells

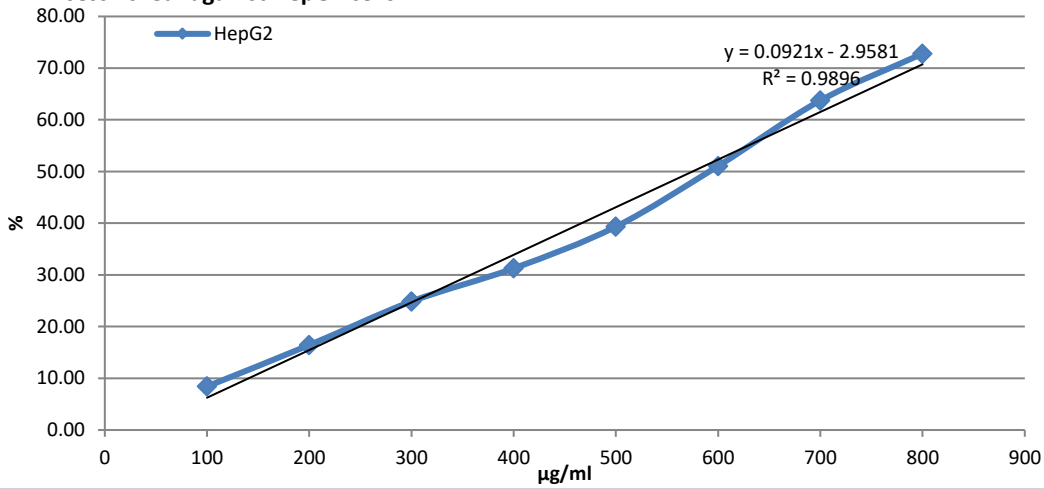


Figure 6. Fifty percent inhibition (IC50) concentration of methanol extract of Alternanthera sessilis leaf for cancer cells

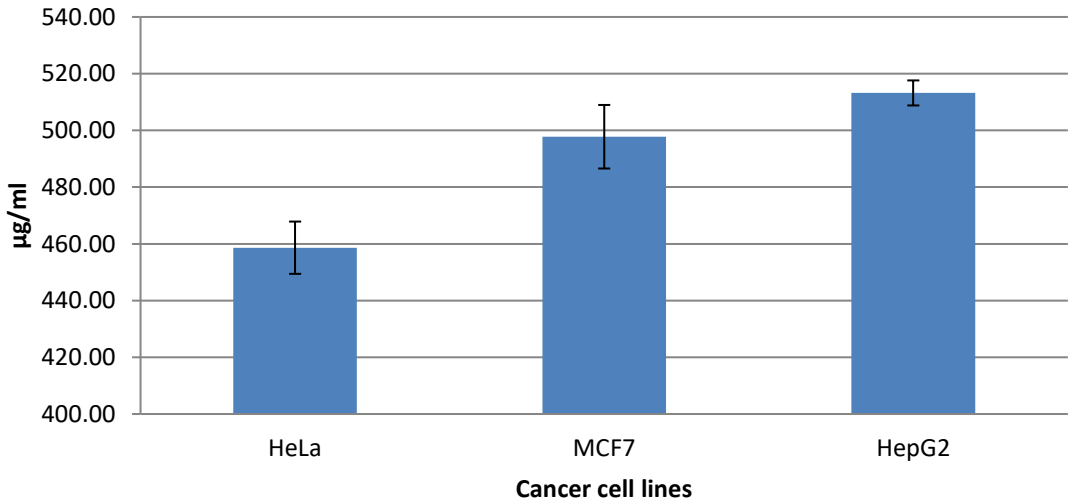


Figure 7. Antioxidant activity (ABTS) of methanol extract of Alternanthera sessilis leaf

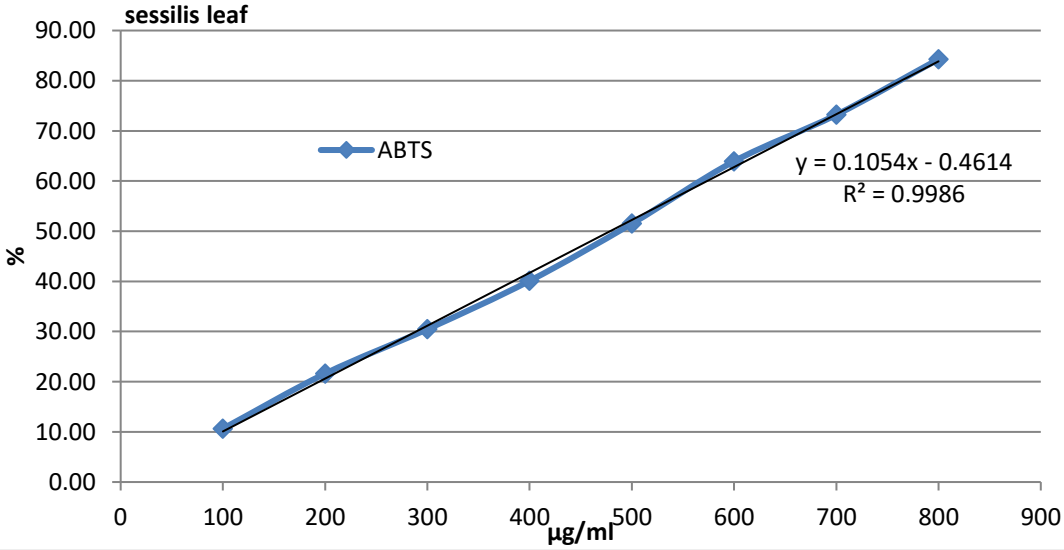
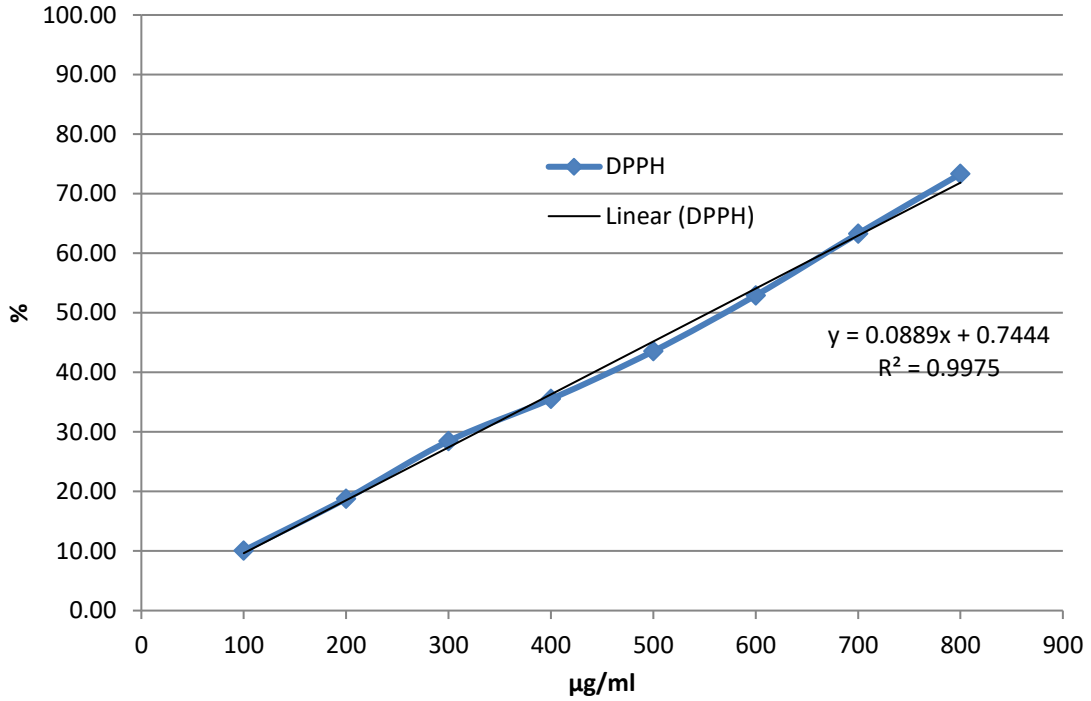


Figure 8. Antioxidant activity (DPPH) of methanol extract of Alternanthera sessilis leaf



DISCUSSION

In the present study *A. sessilis* is the plant used as leafy vegetables for a variety of ailments. Identification of bioactive compounds were done by dissolving them in appropriate solvents three different polar solvents were used in the present study (water, methanol and ethanol). Among the three-methanol found to be effective in dissolving most of the compounds like alkaloids, flavanoids, tannins, saponins, phenols, glycosides, terpenoids, etc. Methanol being mid polar solvent was able to extract most of the compound. The quantity of secondary metabolites saponins were found to be highest 12.51 mg/100ml followed by alkaloids 10.53 mg/100ml and tannins 6.41 mg/100ml with methanol crude extract of *A. sessilis*. Saponins improves immune system, supports absorption of good cholesterol, acts as anti-cancer agent etc. alkaloids posses effective in antimicrobial and anticancer properties. Tannins were identified for its anti-inflammatory, analgesic, astringency, etc.

GCMS analysis of methanol extract of *A. sessillis* in the present study revealed 16 compounds many of the compounds were alkane, alcohols and ester having antimicrobial and anticancer property apart from aromatic compounds. GCMS analysis supports the present study with their nature of the compound for both antimicrobial and anticancer property (Eswaran *et al*., 2012).

Killing of microbial organisms takes place through the compounds that rupture the cell wall, leaking the cellular material, protein precipitation, disrupting the enzyme systems. Highest zone of inhibition of microbes by the methanol crude extract of *A. sessillis* was with *K. pneumonia* and *B. subtilis* 13.33mm followed by *P. mirabilis* 12.83mm and *S. pneumonia* 12.67mm where as common antibiotic compounds showed 21.00mm with Ciproflaxin and tetracycline and gentamycin for *K. pneumonia* and 22.00mm for *B. subtilis* with Ciproflaxin. Metabolism in living organism produce super oxide anion, hydrogen peroxide, hydroxyl radical which were known as reactive oxygen species (ROS). Most of these ROS were essential for normal function of cell but accumulation of the same leads to damage DNA. Protein, lipids, etc. resulting in hyper tension, cancer, diabetes etc. (Gafrikova *et al.,* 2014; Santharam *et al.,* 2015). These oxidative stresses were reduced by producing antioxidant synthesized by living organism within their cell and also obtaining from ingested foods like vegetables, nuts, fruits, oils, etc. that reacts with free radicals and neutralize (Raju, 2005). Within the cell, cells undergo two different pathways in neutralizing the free radical's vitamin E and b-carotene and coenzyme

are water soluble compounds that neutralize the free radicals produced. Antioxidant scavengers were produced by the cell within the cell also act as potent antioxidant. Compounds like vitamin E ascorbic acid and phenolics in the ingested food also reduce oxidative damage (Santharam *et al*., 2015).

Antioxidant capacity of the *A. sessillis* was estimated through DPPH and ABTS assay were purple colour free radicals were reduced by antioxidant to colourless (Murthy *et al*., 2003). The DPPH scavenging activity reached up to 73.31% with 800 µg/ml and ABTS reached up to 84.29 with 800 µg/ml when methanol crude extract of *A. sessillis* was used.

Cellular toxicity of biochemical compounds occur in two ways they arrest the cell cycle and cause apoptosis. The methanol crude extract of *A. sessilis* showed least IC_{50} with HeLa 458.64 µg/ml followed by MCF 497.76 µg/ml HepG2 513.21 µg/ml cell lines.

CONCLUSION

Plant species are rich in variety medicinal properties which are used in day to day food. More than 2000 plant species were found to be used as effective therapeutic properties (Rukayh *et al*., 2006). Many of the plant species used as food were found to have antioxidant property which cures periodical ailments. The important biological activity of compounds of plant origin were antimicrobial, antioxidant, antibiotic, anticarcinogenic, antiallergic, etc. *Alternanthera sessillis* is one such medicinal plant that used as leafy vegetable in a day to day food process variety of pharmacological properties belong to the family Amaranthaceae and grow in varied agro ecological conditions. Most of the parts of the plants are used for therapeutic purposes which vary in their quantity.

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