International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 927-934
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

IDENTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM *Adhatoda vasica* LEAF EXTRACT ITS ANTIMICROBIAL ACTIVITY AND ANTICANCER PROPERTY

Gopinath L. R., Ramya Bharathi, V., Archaya, S and Premalatha, K.

Department of Biotechnology, Vivekanandha College of Arts and Sciences for Women (A), Namakkal, Tamilnadu, India.

***Corresponding Author Email: lrgopinathnamakkal@gmail.com**

ABSTRACT

Adhatoda vasica is one such plant which is used widely for a variety of purposes which forms part of many other traditional herbal medicines. Biologically active compounds present in the plants are responsible for the such curative activity. These compounds could be identified by dissolving them in appropriate solvents. In the present study water, methanol and ethanol were used as solvents. Most of the secondary compounds were found in all the solvents but methanol gave positive result more than the other. Quantification of important solvents showed that high tannin content 65.61 µg/ml followed by saponins 19.09 µg/ml and alkaloids 12.87 µg/ml with methanol crude extract of A. vasica. GCMS analysis of methanol crude extract of A. vasica showed 21 compounds of alcohols, steroids, ester, etc. mostly having antimicrobial and antioxidant property. Antimicrobial property with well diffusion method showed effective antimicrobial activity for B. subtilis 12.17mm followed by V. cholera 11.83mm and K. pneumonia 11.50mm. Antioxidant activity was performed with DPPH assay and ABTS assay with DPPH assay 81.81% antioxidant activity was recorded at 160µg/ml and 94.84% antioxidant activity with 160µg/ml using methanol crude extract of A. vasica. Anticancer property was estimated through cytotoxicity study with cell lines showed least IC_{50} with HeLa 88.24 µg/ml followed by MCF 92.80 µg/ml HepG2 111.08 µg/ml cell lines using methanol crude extract of A. vasica.

KEY WORDS

Adhatoda vasica, cytotoxicity study

INTRODUCTION

Adhatoda vasica is a member of family *Acanthaceae* (synonyms of Justicia adhatoda, *Adhatoda zeylanica)* (Malik and Ghafoor, 1988). The plant *i*s about 1- 6 m tall, evergreen, perennial shrub. It is spread in the open/meagre tree shade habitat conditions particularly in tropical and sub-tropical areas with 1450 m height. It has been used in Ayurvedic and Unani medicines and used locally for the last 2000 years in India (Atal, 1980). It is distributed in Indonesia, Malaya, Southeast Asia, India and Pakistan (Malik and Ghafoor, 1988). Several ethno medicinal uses of different parts of *J. adhatoda* from Pakistan, India, Nepal, Sri Lanka and Thailand have been reviewed by Claeson *et al*., (2000).

Its high medicinal value and local use for fuel have fragmented populations of *J. adhatoda*. *Vasica* has played significant criteria not only in the traditional Indian system of medicine popularly called "the Ayurveda", but also confers potential investment in modern pharmaceutical and cosmetic industries. It is a predominant herb of the ayurvedic system used in the treatment of coughs, bronchitis, asthma and symptoms of common cold.

A vast variety of pharmacological uses of *Adhatoda* is believed to be the result of its rich concentration of

alkaloids. The leaves of *Adhatoda vasica* are rich in vitamin C and carotene and also yield certain amount of essential oil. Chemical compounds found in leaves and roots of this plant also include fats, resins, sugar, gum, amino acids, proteins etc. (Dymock, 1972). The leaves contain a very small amount of an essential oil and a crystalline acid according to previous reports (Tofazzal Hossain and Obydul Hoq, 2016).

MATERIALS AND METHODS

Adhatoda vasica was collected from the Namakkal district. The *Adhatoda vasica* leaves were collected, washed, dried, grinded and extract was filtered using Soxhlet apparatus. The extract was qualitatively analysed. Quantitative analysis of 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 *Adhatoda vasica* 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 *Adhatoda vasica extract against*e HeLa, MCF7, and HepG2 cell lines.

RESULTS

Phytochemical test of *A. vasica* crude extract from different solvents (Table 1). Quantity of secondary metabolites in different crude extract of *A. vasica* showed in Table 2. Phytochemical components identified in the methanol leaf extract of *A. vasica* by GC-MS analysis showed the presence of 21 active compounds and the higher percentage of phytol (19.44) was observed (Table 3). The antibacterial activity was showed in Figure 1. Average percentage cell inhibition of methanol extract of *A. vasica* leaf against HeLa, MCF-7 and HepG2 cells showed highest cell inhibition against HeLa cells (91.18) followed by MCF-7 cells (88.21) and Hep G2 cells (75.34) at 160µg/ml concentration with corresponding p value and f value about < 0.0001^{ES}, 136.49. Among the three different cell line highest cell inhibition was observed with HeLa cells in all the concentrations with respective p value and f value of < 0.0001^{ES}, 2109.3 (Figure 2-4). Antioxidant activity (ABTS, DPPH) of methanol extract of *A. vasica* leaf at different concentrations showed highest antioxidant activity with ABTS at 160µg/ml concentration (94.84) with respective p value and f value about < 0.0001^{ES}, 3814.5 (Table 6 & Figure 7).

Table 1. Phytochemical test of *A. Vasica* 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	+	-	-

S. No.	Phytochemical test		Solvents		
			Water	Methanol	Ethanol
		Keller Killani Test	+	+	+
		Glycosides Test	+	+	+
		Conc. H2SO4	+	-	+
		Molisch's	+	-	+
		Lead acetate	+	+	+
9	Saponins	Foam	+	+	+
		Haemolysis Test	+	+	+
10	Protein	Millons	+	+	+
11		Biuret	-	-	+
12	Carbohydrate	Molichs	+	+	+

Table 2. Quantity of secondary metabolites in different crude extract of *A. vasica*

Secondary metabolites	Aqueous	Methanol	Ethanol	P value	F value
Alkaloids	10.13 ± 0.46	12.87 ± 0.32	11.34 ± 0.09	0.0002^{ES}	52.682
Flavanoids	6.21 ± 0.46	8.41 ± 0.15	7.41 ± 0.19	0.0003^{ES}	40.415
Tannin	60.20 ± 2.04	65.61 ± 1.55	64.02 ± 0.74	0.0129^{S}	9.784
Phenols	2.31 ± 0.17	3.48 ± 0.31	2.46 ± 0.11	0.0010^{VS}	26.606
Saponins	16.89 ± 0.63	19.09 ± 0.47	16.94 ± 0.60	0.0050^{VS}	14.520
P value	$< 0.0001^{ES}$	$< 0.0001^{ES}$	$< 0.0001^{ES}$		
F value	1663.4	3324.0	9676.8		

Table 3. Phytochemical components identified in the methanol leaf extract of *A. vasica* by GC-MS analysis.

S. No.	RT	Peak area %	Identified Name
1	6.21	0.38	Benzenemethanol, a-[1(methylamino)ethyl]-
2	7.28	1.23	Cyclopentaneethanamine, N,à-dimethyl-
3	8.10	0.18	Phosphoric acid, diethyl pentyl ester
4	8.56	3.28	Pseudoephedrine, (+)-
5	11.02	6.35	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
6	12.68	0.56	n-Hexadecanoic acid
7	14.16	6.35	1-Eicosanol
8	14.63	19.44	Phytol
9	15.35	0.26	9,12,15-Octadecatrienal
10	16.59	1.28	8,11,14-Eicosatrienoic acid, (Z, Z, Z)-
11	18.91	2.36	Ethanethioic acid, S-[2(dimethylamino)ethyl] ester
12	23.65	9.76	Squalene
13	25.18	8.97	7-Octadecyne, 2-methyl
14	28.52	2.37	Vitamin E
15	29.99	5.28	Campesterol
16	30.48	19.17	Stigmasterol
17	30.68	15.34	rans-Z-à-Bisabolene epoxide
18	31.35	6.35	5à-Androstan-16-one, cyclic ethylene mercaptole
19	31.65	13.63	β-Sitosterol
20	32.35	2.36	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-
21	33.42	10.82	Lupeol

Figure 1. Comparative antibacterial activity of A. vasica at different concentration

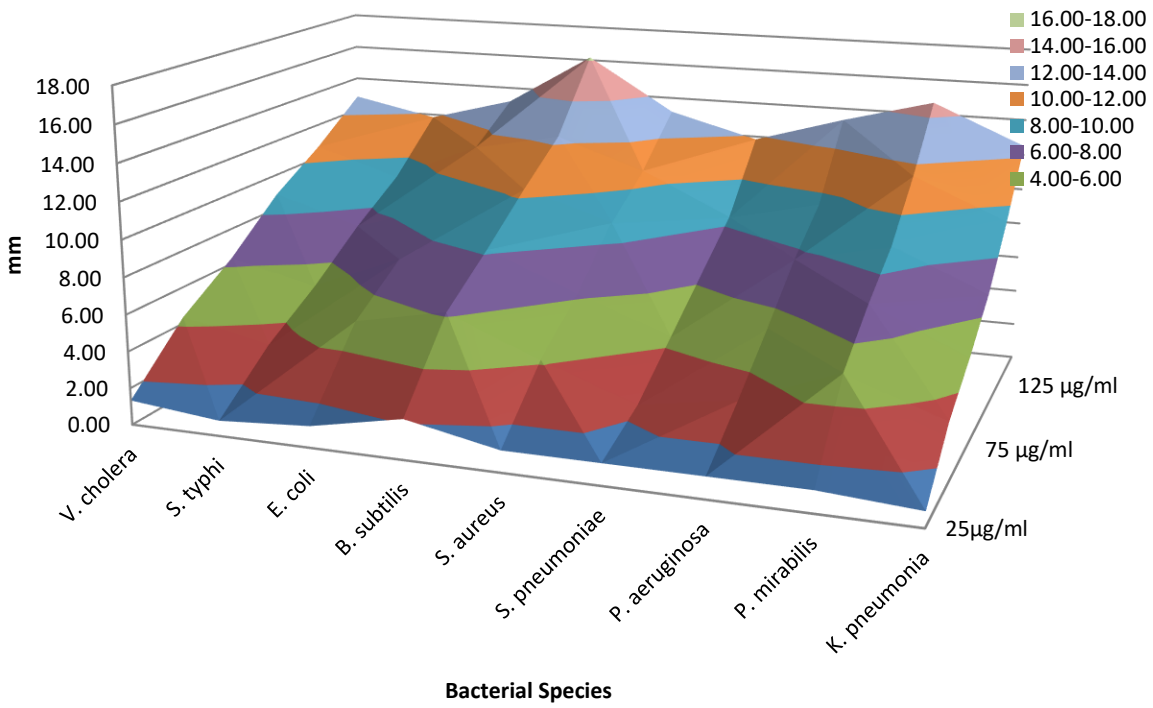


Figure 2. Average percentage cell inhibition of methanol extract of A. vasica leaf against HeLa Cells

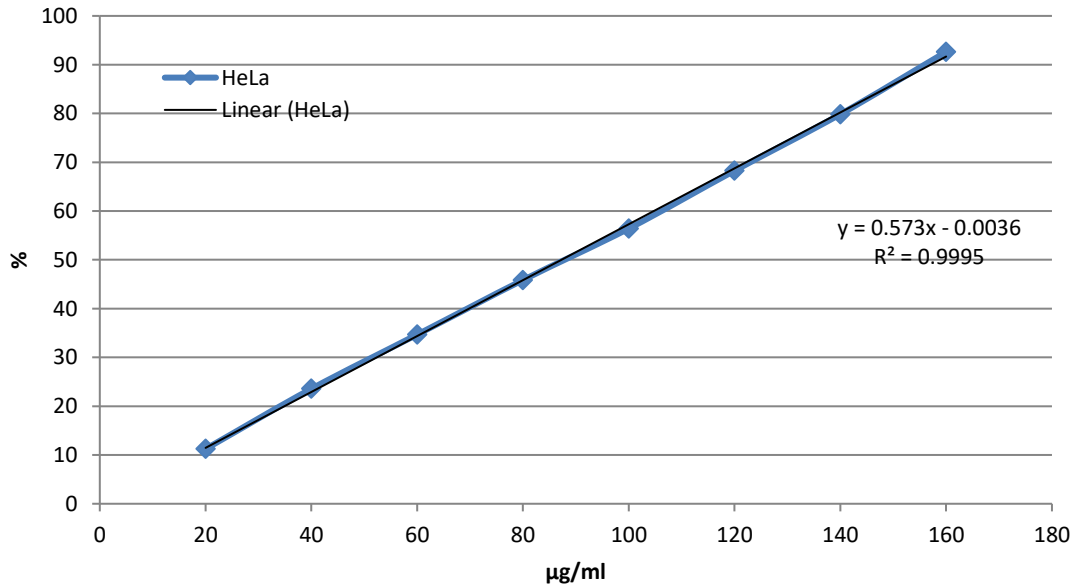


Figure 3. Average percentage cell inhibition of methanol extract of A. vasica leaf against MCF7 cells

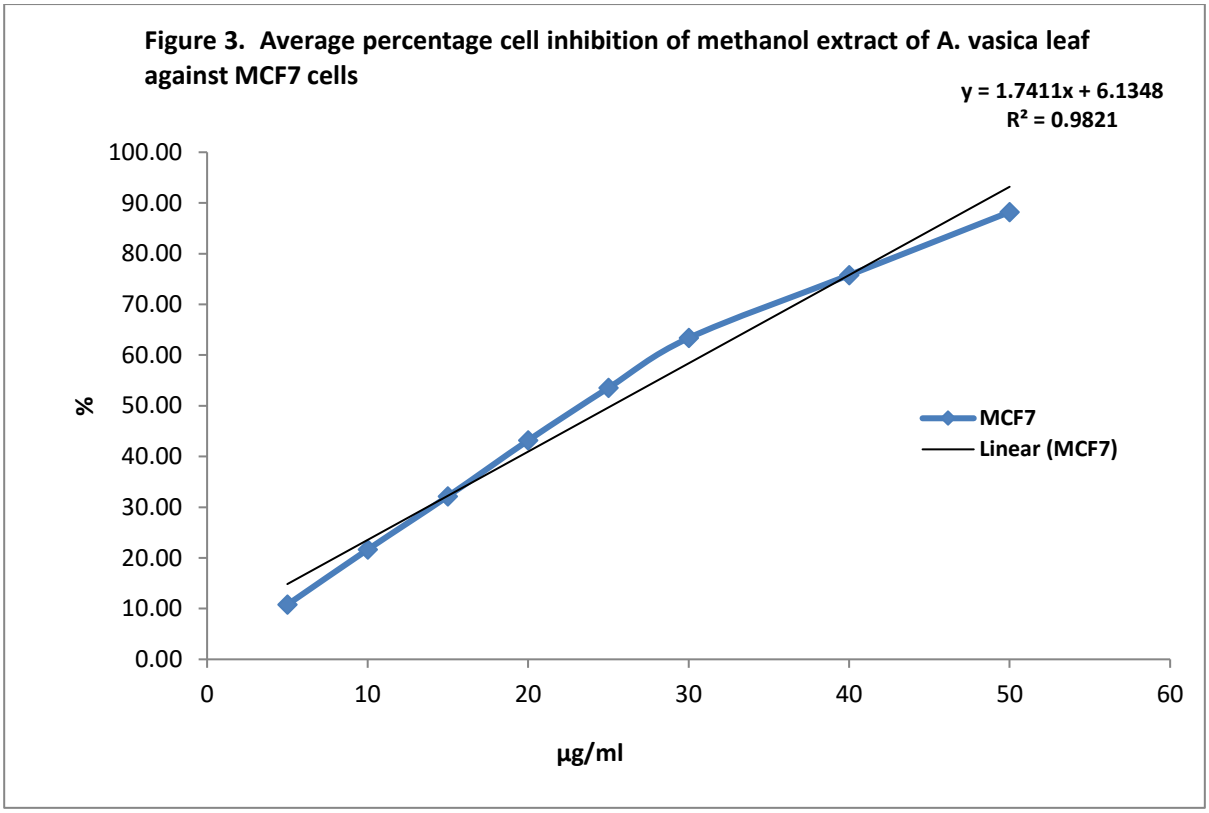


Figure 4. Average percentage cell inhibition of methanol extract of A. vasica leaf against HepG2 cells

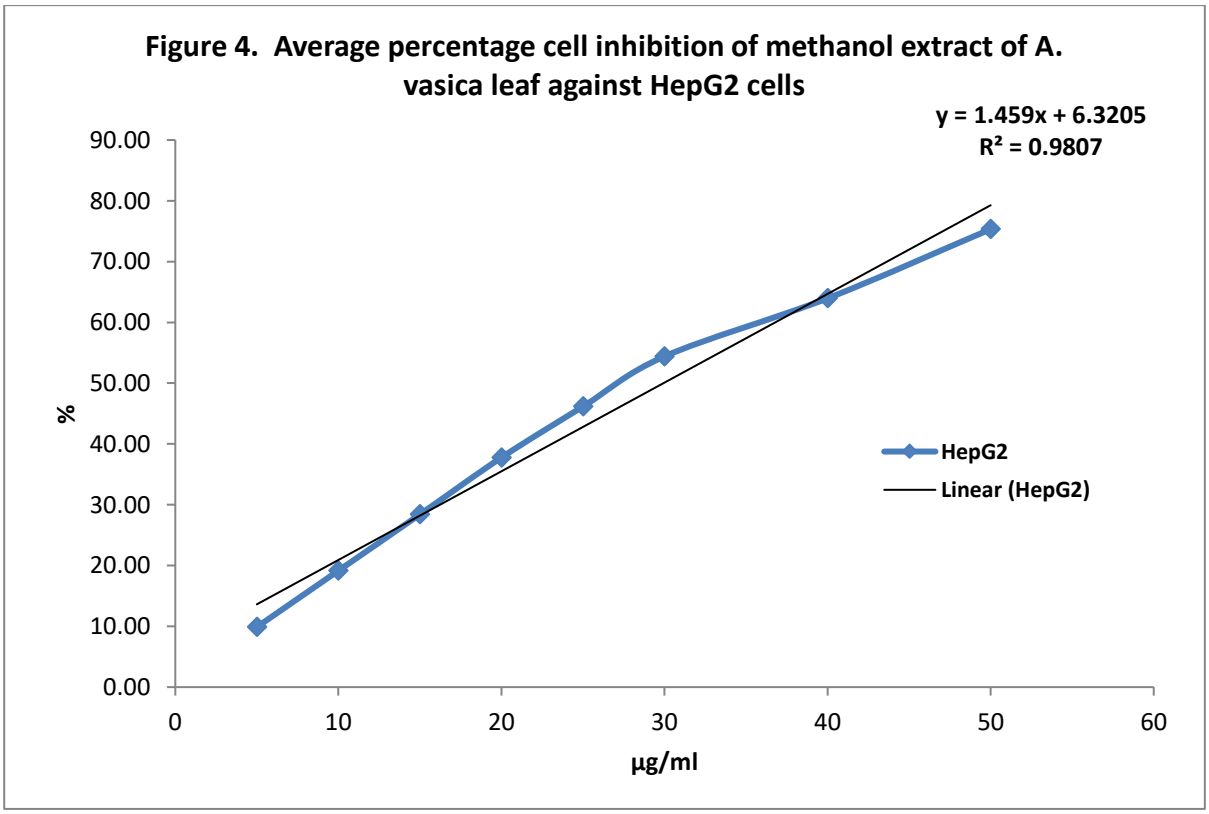


Figure 5. Fifty percent inhibition (IC50) concentration of methanol extract of A. vasica leaf for cancer cells

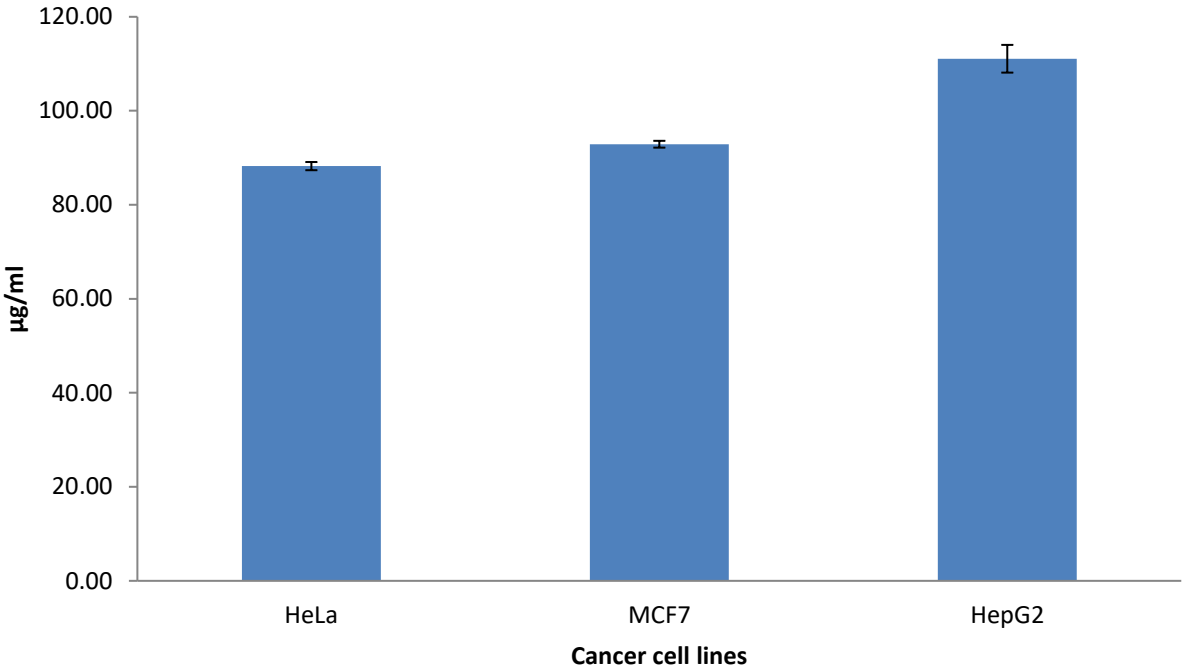


Figure 6. Antioxidant activity (ABTS) of methanol extract of A. vasica leaf

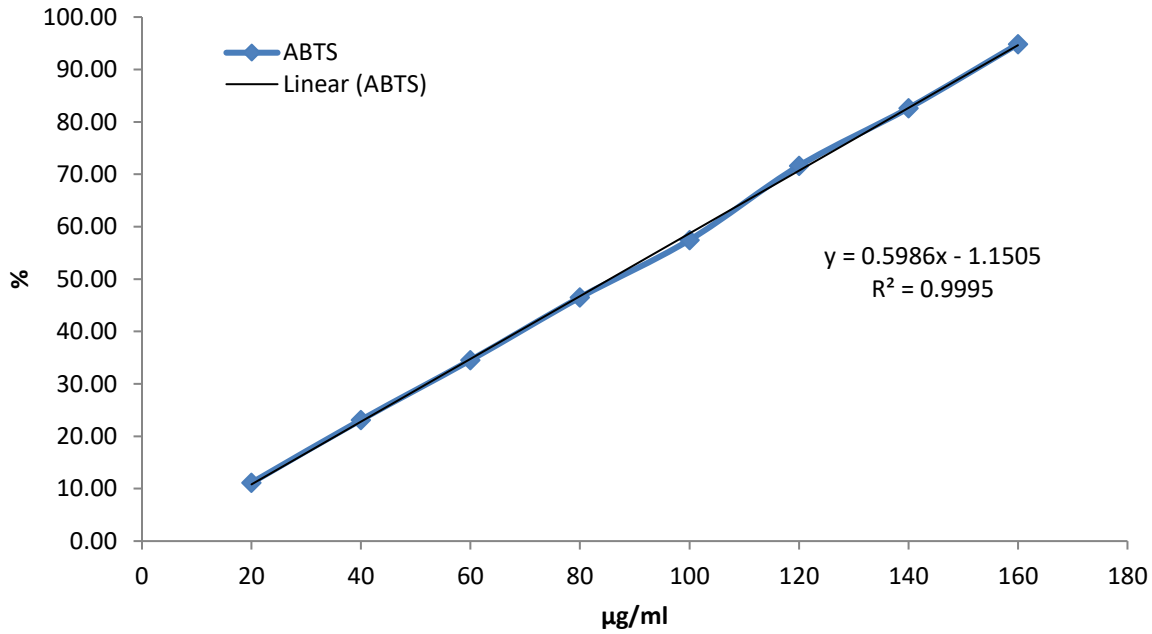
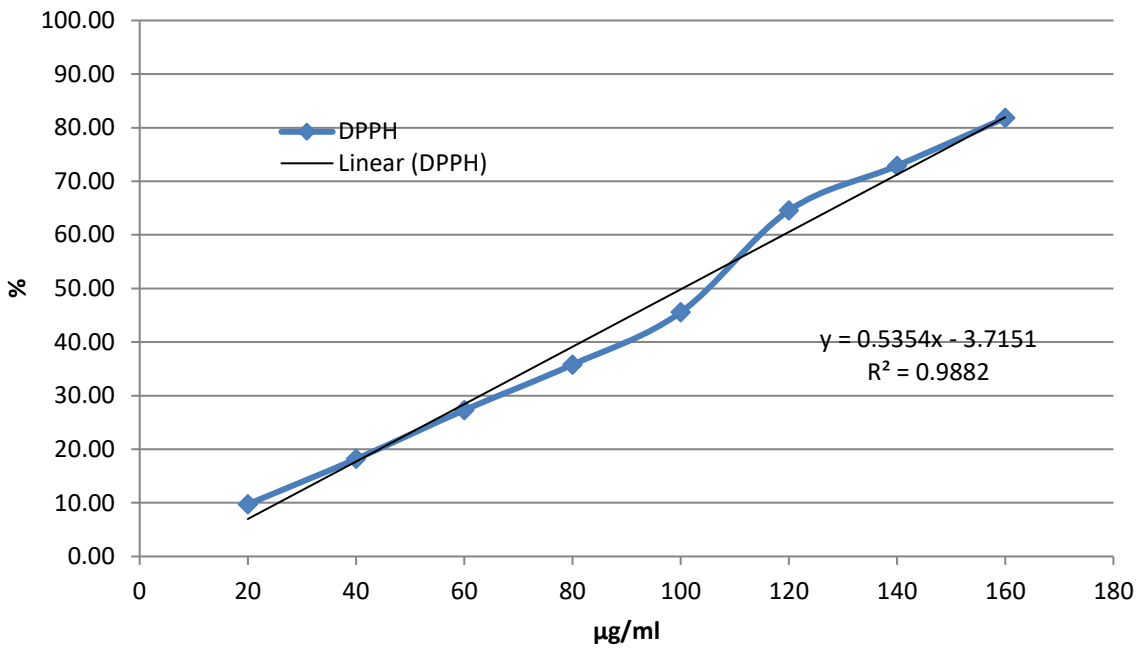


Figure 7. Antioxidant activity (DPPH) of methanol extract of A. vasica leaf



DISCUSSION

The *vasica* flowers also acts as antiseptic which improves blood circulation and hectic heat of blood (Kirtikar and Basu, 1975). Previous research reports identified and stated that elevated levels of *Adhatoda vasica* alkaloids that are primarily, vasicine and vasicinone have been acknowledged to be a major bioactive component and with the combination of both produces *in vitro* and *in vivo* bronchiodilatory activity to that of theophylline (Prithvi simha *et al.,* 2016).

The leaves, roots and young plants of *Adhatoda vasica* contain the quinazoline alkaloids such as 7-hydroxy vasicine, vasicinolone, 3-deoxyvasicine, vasicol, vasicoline, vasicolinone, adhatodine and anisotine as main compounds (Joshi *et al.*, 1994). Among various constituents of *A. vasica*, vasicine (a pyrroloquinazoline alkaloid) finds an important place due to its significant therapeutic properties (Sweta Bhambhani *et al.,* 2011). The antibacterial activity of *A. vasica* showed high inhibition with *S. typhi.*

The cytotoxic potential of *Adhatoda vasica* was evaluated by monitoring MTT assay. Methanolic and ethanolic extracts of the plant were analyzed against Estrogen Receptor positive breast cancer, MCF-7 cell line. The cell viability was determined after 48 h of treatment with indicated concentrations of each extract. The anti-proliferative effect on MCF-7 cells to increase concentrations of extracts showed better results. Reduction capacity is finely correlated with the antioxidant property and may serve as a significant reflection on the quenching effect. In the present study highest antioxidant activity with ABTS at 160µg/ml concentration

Compounds with reduction capacity indicate that they are tending to donate electrons and can reduce the oxidized intermediates of lipid peroxidation processes. The MTT assay of isolated ethyl acetate fractions of *Adhatoda vasica* flowers shows that all concentrations are having anticancer activity. *Adhatoda vasica* is used against ferric nitrilotriacetate (Fe-NTA)-induced renal oxidative stress, hyper proliferative response and two-stage renal carcinogenesis. *Adhatoda vasica* also shows the antioxidant and anti-clastogenic efficacy against cadmium chloride ($CdCl_2$) - induced renal oxidative stress and genotoxicity in Swiss albino mice that support its anti-mutagenic efficacy (Jahangir *et al.,* 2006).

Acetylation of vasicine isolated from *Adhatoda vasica* leaves forms vasicine acetate and showed moderate antibacterial activity compared to vasicine. The radical scavenging activity of the leaf extract was observed maximum at 1000 µg/mL (66.15%). The cytotoxic studies against A549 lung adenocarcinoma

cancer cell line revealed that vasicine acetate had IC_{50} value of 2000 μg/mL. Aqueous methanolic extracts of *Adhatoda vasica* with minimum and maximum drug doses have shown its potentiality as a radio-protector against the therapeutically induced mutations which can prove to be a great contributor in cancer management in future. Such indigenous herbal drug will definitely be a potential adjuvant to cancer treatments to patient. In the present study methanol extract of *Adhatoda vasica* comparative to other cell lines HeLa cells showed high significant with 120µg/ml concentration.

CONCLUSION

Herbal remedy has been identified across the globe receiving importance due to their broad spectrum of healing capacity with not permitting microbes to evolve resistant strains. Folklore tradition evolved and identified more than 6000 plants *Adhatoda vasica* is one such plant used across folklore medicines was primarily known for bronchodilator property belongs the family *Acanthaceae*. *A. vasica* is the shrub distributed variety of ecological conditions used for bronchitis, caugh, asthma, tuberculosis, lumber pain, joint pain, sprains, eczema, malaria, swellings, rheumatism, veneral diseases, diarrhea, hyperglycemia, convulsant and cytotoxic. Almost all parts of the plant like seed, fruit, root, stem, leaves, flowers, bark, etc. are used for medicinal purposes. Bioactive compounds vary in different parts of the plant according to their physiological conditions. However, *Adhatoda vasica* has traditional communities along with their medicinal uses.

ACKNOWLEDGEMENT

We thank our Chairman and Secretary Dr. M. Karunanithi, Vivekanandha Educational Institutions, Namakkal for providing infrastructure and lab facilities.

REFERENCES

1. Harborne, J. B. 1973. Phytochemicals Methods Chapman and Hall Ltd., London. 49: 188.
2. Kumaran, A. and Joel Karunakaran, R. 2007. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT – Food Science and Technology., 40(2):344-52.
3. Obadoni, B. O. and Ochuko, P. O. Global J. Pure Appl. Sci., 2001. 8: 203-208.
4. Makkar, H. P. S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Res. 49: 241-256.
5. Tofazzal Hossain and Obydul Hoq. 2016. Review Therapeutic use of Adhatoda vasica. Asian J. Med. Biol. Res. 2 (2), 156-163.
6. Nenandis, N., Wang, L.F., Tsimidou, M., Zhang, H.Y. 2004. Estimation of scavenging activity of phenolic compounds using the ABTS·+ assay. J Agric Food Chem 52:4669–4674.
7. Atal, C. K., Jain, M. P., Koul, S. K., Dhar, K. L. 1980. Novel nor-harmal alkaloid from Adhatoda vasica., Phytochemistry:19(8),1880-1882.
8. Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci Technol. 28:25–30.
9. Claeson, U. P., Malmfors, T., Wikman, G., Bruhm, J. G. 2000. Adhatoda vasica: a critical review of ethnopharmacological and toxicological data. J Ethno Pharmac 72: 1–20.
10. Dymock, I. W., Cassar M. B. J., Pyke M. D. D. A., Oakley, M. D. W.G., Roger, M. D. and Williams, M. D. 1972. Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of haemochromatosis.,American Journal of medicine. 52 (2): 141-282.
11. Jahangir Muhammad. 2009. "Health-affecting compounds in Brassicaceae." Comprehensive Reviews in Food Science and Food Safety, 8 (2): 31-43.
12. Joshi, B. S., Bai, Y., Puar, M. S., et al., 1994. 1H and 13C NMR assignments of some pyrroloqunazoline alkaloids of Adhatoda vasica. J Nat prod 57:953 – 962.
13. Kirtikar, Basu, B. D. 1935. Indian Medicinal Plants. International book distributors, Dehradun, 3rd edition, part II: 1548.
14. Malik, K. A. and Ghafoor, A. 1988. Acanthaceae. University of Karachi.
15. Prithvi Simha, Melvin Mathew and Mahesh Ganesapillai. 2016. Empirical modeling of drying kinetics and microwave assisted extraction of bioactive compounds from Adathoda vasica and Cymbopogon citratus. Alexandria Engineering Journal.
16. Sweta Bhambhani, Vijai Singh Karwasara, Vinod Kumar Dixit and Smita Banerjee. 2012. Enhanced production of vasicine in Adhatoda vasica (L.) Nees. Cell culture by elicitation. Physiol Plant, 34:1571–1578.

Received:04.08.18, Accepted: 07.09.18, Published:01.10.2018

***Corresponding Author:**
Gopinath L. R
Email: lrgopinathnamakkal@gmail.com