International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 1237-1244
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

IDENTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM *Withania somnifera* LEAF EXTRACT ITS ANTIMICROBIAL ACTIVITY AND ANTICANCER PROPERTY

Kala. K[2]., Gopinath L. R.[1], Abinaya. K[1]., Bhuvaneswari. R[3]., and Archaya, S[1]

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

[2]Former Regional Joint Director, Collegiate of Education, Tirchy.

[3]Department of Zoology, NKR Govt. Arts College for Women, Namakkal.

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

ABSTRACT

Medicinal plants were identified by traditional communities in ancient times through their constant interaction with them. Modern medicines which were based on non-organic in origin lead to numerous complications interms of side effects and resistant genes. Hence more and more attention is paid to the medicines based on organic origin. Among numerous plants that have been used by traditional communities Withania somnifera one plant which was used for wide range of therapeutic properties. However, in the present study an attempt have been made to identify effective solvent and biologically active compounds for antimicrobial and anticancer property. Among the three-solvent water, methanol and ethanol. Methanol was found to be effective in identifying secondary metabolites and also quantity of secondary metabolites was higher when compared to other solvents. Phenols were found to be higher in the W. somnifera leaves followed by flavanoids and alkaloids indicating their therapeutic potential. GCMS results of methanol crude extract of W. somnifera showed 24 different compounds with alkanes, alkenes, alcohols, esters, etc. which are known for anticancer and antimicrobial activity. Antimicrobial activity among the tested organisms K. pneumonia showed the highest followed by P. mirabilis and P. aeruginosa comparable with the standard antibiotics. Antioxidant capacity of W. somnifera quantified through DPPH and ABTS assay. The DPPH scavenging activity increased up to 86.71 % with 400 µg/ml and ABTS reached up to 92.91 with 400 µg/ml. The methanol crude extract of W. somnifera showed least IC_{50} with HeLa 194.11 µg/ml followed by MCF 210.78 µg/ml HepG2 217.98 µg/ml cell lines.

KEY WORDS

Withania somnifera, DPPH.

INTRODUCTION

Withania somnifera (L.) *Dunal* (Solanaceae) grows in many of the Indian subcontinent and in tropical and subtropical zones of the Mediterranean region and northern Africa to Southwest Asia. *Withania somnifera* (L.) *Dunal*, commonly called as Ashwagandha which is an evergreen woody perennial shrub in its native habitat of the hot and dry topics. It grows about 3 feet tall and 2 feet wide in one season and produces small light green flowers which then form attractive reddish-orange berries concealed inside with transparent paper coverings. It prefers full sun, and average soil with good drainage. In cooler zones, it is grown as an annual shrub. Ashwagandha is grown in farm land as a herbaceous perennial. Its leaves and woody stems are killed off by frost but, as long as the soil is not overly wet, its root remains vital and will sprout new shoots in the spring. If grown as an annual herb, Ashwagandha is best to grow

early in the Spring to provide an extended growing season anytime in April or May, with sizable roots forming by October.

The drug produced from this herb acts as a rejuvenating agent; mainly used in Ayurvedic and Unani preparations. *Withania* is extensively analyzed to have strong aphrodisiac, rejuvenative, sedative and life prolonging geriatric properties. The similarity between the restorative properties and those of ginseng roots has led to ashwagandha roots being called Indian ginseng (Dipankar *et al.,* 2012). The roots are finely used as a nutrient and help to endure health in pregnant women. Fertility of women is enhanced by consuming decoction of the root boiled with milk and ghee.

The root extracts of *Withania* are also used in constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, muscular energy and spermatorrhoea. It has been used due to its extensive antioxidant property to treat rheumatism and neurodegenerative disorders (Raja sankar *et al.,* 2009). The paste prepared out of its leaves is used for curing inflammation of tubercular glands and that of its roots for curing skin diseases, bronchitis, ulcer and dyspepsia and eye diseases. The seeded fruits of Ashwagandha possess diuretic property and an infusion of the bark is given for asthma. Ashwagandha greatly influences adaptogenic effects on the body and thus, it helps to promote weight loss when consumed in amalgamation of other weight loss supporting supplements. An adaptogen is a compound that tends to reduce stress related fluctuations in the diet. This indigenous Ayurvedic herb is supposed to be a withering substance for people who either overeat or under eat due to ecological stress conditions. It may also help boost metabolism and eliminate irregularities in digestion.

MATERIALS AND METHODS

Withania somnifera was collected from the Namakkal district. The *Withania somnifera* 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 *Withania somnifera* 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 *Withania somnifera* extract against HeLa, MCF7, and HepG2 cell lines.

Results

Phytochemical test of *Withania somnifera* crude extract with different solvents (Table 1). Quantity of secondary metabolites in different crude extract of *Withania somnifera* (Table 2). Phytochemical components identified in the methanol leaf extract of *Withania somnifera* by GC-MS analysis showed 24 active compounds with highest peak area of Benzenetriol (Table 3). The nature of the phytochemical compounds present in methanol extract of *Withania somnifera* majorly belongs to alcohol, carboxylic acid, alkane, ester, aldehyde and alkene. The molecular range of phytochemical components identified in the methanol leaf extract of *Withania somnifera* is in the range of 84 to 256. Phytochemical components identified in the methanol leaf extract of *Withania somnifera* are used as therapeutic agent, flavouring agent, fungicides, preservatives, food additives and antibacterial agents. Antibacterial activity of *Withania somnifera* at different concentrations with different microorganisms showed highest zone of inhibition with *K. pneumonia* at 150µg/ml concentration (Figure 1). (Figure 2).

Average percentage cell inhibition of methanol extract of *Withania somnifera* leaf against HeLa, MCF-7 and HepG2 cells showed highest cell inhibition with HeLa cells (95.32) followed by MCF-7 cells (89.71) and Hep G2 cells (87.44) at 400µg/ml (Figure 3, 4 and 5). Fifty percent inhibition (IC_{50}) concentration of methanol extract of *Withania somnifera* leaf for cancer cells showed lowest IC50 in HeLa cells (194.11) followed by MCF-7 cells (210.78) and Hep G2 cells (217.98) (Figure 6). Antioxidant activity (ABTS, DPPH) of methanol extract of *Withania somnifera* leaf at different concentrations showed highest activity with ABTS assay at 400µg/ml concentration (92.91) (Figure 7 & 8).

Table 1. Phytochemical test of *Withania somnifera* crude extract with different solvents.

S. No.	Phytochemical test		Solvents		
			Water	Methanol	Ethanol
1	Alkaloids	Dragendroff's reagent	+	+	+
		Iodine	-	+	-
		Mayer's	+	+	+
		Wagner's	-	-	+
		Alkaline	-	+	-
2	Flavonoids	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. H_2SO_4	-	-	+
9	Saponins	Molisch's	+	-	+
		Lead acetate	+	+	-
		Foam	-	+	+
		Haemolysis Test	+	+	+
10	Protein	Millons	+	+	+
11		Biuret	+	+	+
12	Carbohydrate	Molisch	+	+	+

Table 2. Quantity of secondary metabolites in different crude extract of *Withania somnifera*

Secondary metaboliotes	Aqueous	Methanol	Ethanol	P value	F value
Alkaloids	119.84 ± 6.31	157.66 ± 4.19	135.23 ± 3.05	0.0002^{VS}	48.826
Flavanoids	128.85 ± 3.49	172.46 ± 4.07	143.99 ± 1.48	$< 0.0001^{ES}$	142.63
Tannin	0.62 ± 0.04	0.83 ± 0.03	0.71 ± 0.03	0.0008^{ES}	29.382
Phenols	184.43 ± 2.02	209.33 ± 3.79	186.87 ± 1.46	$< 0.0001^{ES}$	82.407
Saponins	0.14 ±0.02	0.16 ± 0.01	0.14 ± 0.01	0.2160^{NS}	2.000
P value	$< 0.0001^{ES}$	$< 0.0001^{ES}$	$< 0.0001^{ES}$		
F value	1827.5	3094.0	8348.7		

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

S. No.	RT	Peak area %	Identified Name
1	2.71	0.36	Propane, 1,1diethoxy-2methyl-
2	3.18	11.07	2,3,4,5Tetrahydropyrid azine
3	3.59	0.0761	3-Amino-2-oxazolidinone
4	4.32	2.2794	Furfural
5	4.75	0.0303	1-Butanol, 3-methyl-, acetate
6	5.38	0.5314	2-Cyclopentene-1,4-dione
7	6.17	0.1733	1H-Imidazole, 4,5-dihydro-2-
8	6.41	0.2883	2-Cyclopenten-1-one, 2-hydroxy-

9	7.19	0.1791	2,4-Dihydroxy-2,5-dimethyl-3(2H)-
10	8.53	1.7692	Glycerin
11	9.16	0.0690	4-Ethyl-4-methyl-5-methylene-[1,3]dioxolan-2-one
12	9.94	0.1406	2,5-Dimethyl-4-hydroxy-3(2H)- furanone
13	10.14	0.4052	5-Ethylcyclopent-1- enecarboxaldehyde
14	10.76	0.5048	Levoglucosenone
15	11.10	0.1419	1,3,2-Dioxaborolane, 4,4-dimethyl-5-oxo-, 2-ethyl
16	11.59	0.0595	Piperidin-4-ol, 2,5-dimethyl-
17	11.83	2.0060	4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl-
18	12.43	0.1277	Hexanoic acid
19	13.20	0.1801	1-Propanol, 2,2-dimethyl-, benzoate
20	19.21	35.1557	1,2,3-Benzenetriol
21	20.87	2.8053	D-Allose
22	23.26	0.8604	1,6-Anhydro-à-d-galactofuranose
23	28.06	11.3818	n-Hexadecanoic acid
24	32.51	23.0288	E-9-Tetradecenoic acid

Figure 1. Comparative antibacterial activity of Withania somnifera at different concentration

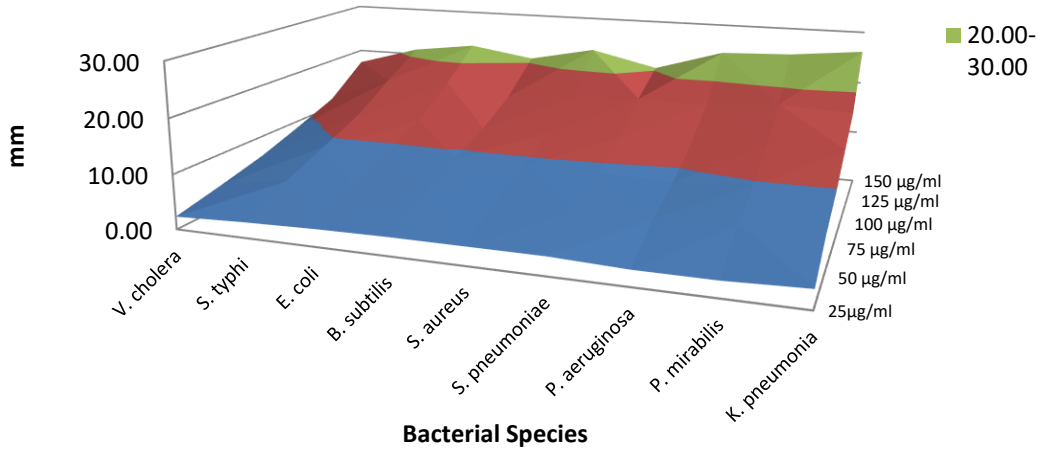
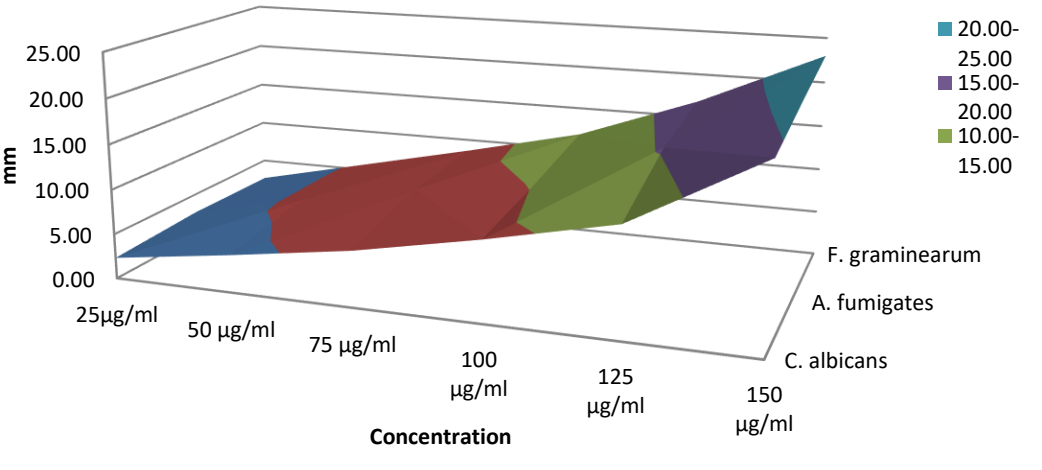


Figure 2. Comparative graph for antifungal activity of Withania somnifera methanol crude extract at different concentrations



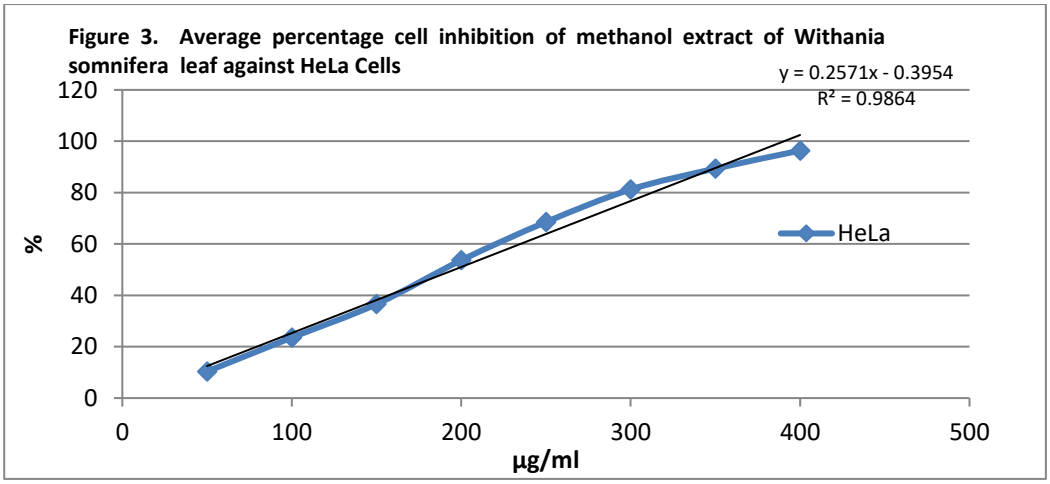


Figure 3. Average percentage cell inhibition of methanol extract of Withania somnifera leaf against HeLa Cells

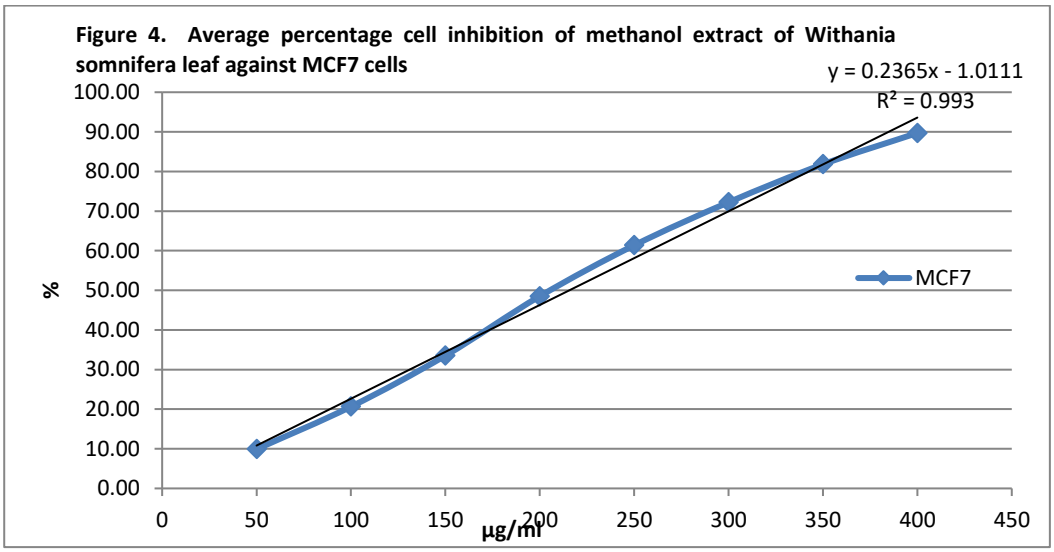


Figure 4. Average percentage cell inhibition of methanol extract of Withania somnifera leaf against MCF7 cells

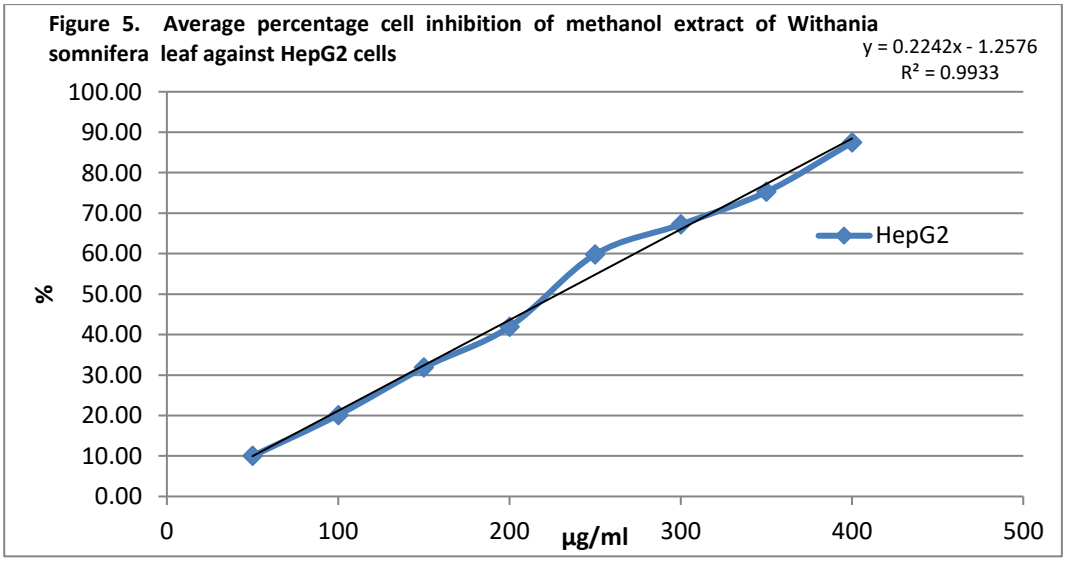


Figure 5. Average percentage cell inhibition of methanol extract of Withania somnifera leaf against HepG2 cells

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

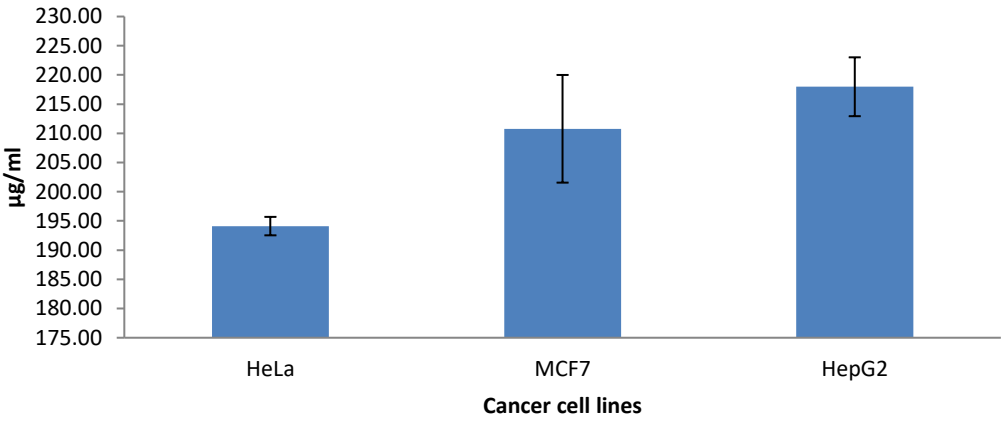


Figure 7. Antioxidant activity (ABTS) of methanol extract of Withania somnifera leaf

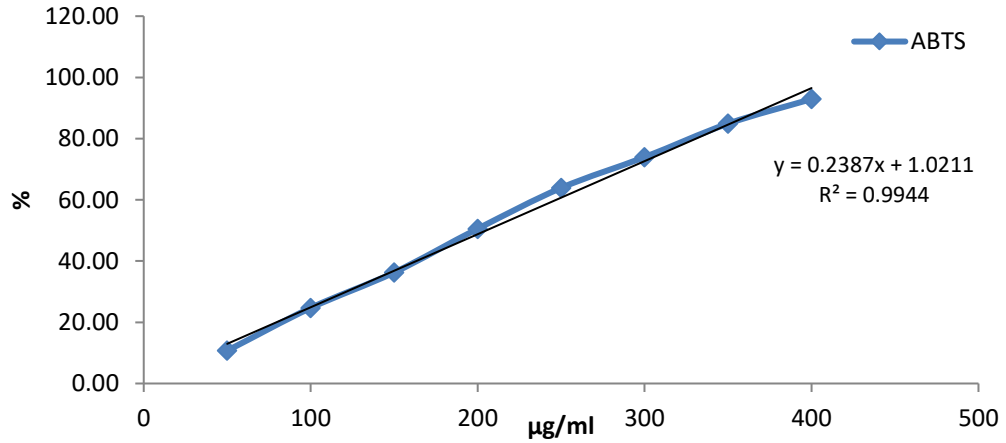
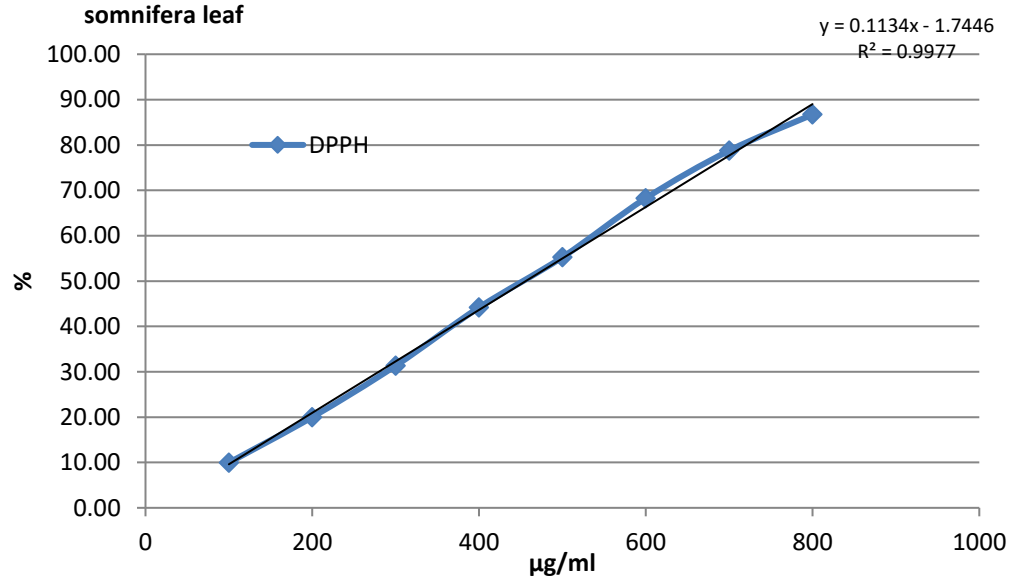


Figure 8. Antioxidant activity (DPPH) of methanol extract of Withania somnifera leaf



DISCUSSION

Biochemical compounds could be derived through dissociating with solvents. Solvents posses' different polarity which dissolves compounds depending upon their nature. In the present study three solvents (water, methanol and ethanol) with different polarity were used to identify the compound presence. All the three solvents positive for most of the compounds however as expected methanol being mid polar solvent was able to show higher positive result compound (Deka *et al*., 1994; Demling and Osandrolone, 2000; Mallika and Devi, 2005; Shah, 2011; Kumar *et al*., 2017). Quantity of secondary metabolites was also higher compared to other solvents however higher quantity of phenols was recorded in all the solvent 209.33 mg/100mg followed by 172.46 mg/100mg and alkaloids 157.66 mg/100mg. GCMS results of the methanol crude extract *W. somnifera* showed 24 compounds which are mostly alkane, alkene, alcohols, fatty acids known for antimicrobial and antioxidant property.

Antimicrobial activity of biochemical compounds exhibits by several ways by rupturing the cell wall, disrupting the enzyme production, leaking the cellular content, precipitation of protein. *W. somnifera* methanol crude extract showed high zone of inhibition 26.17mm for *K. pneumonia* followed by *P. mirabilis* and *P. aeruginosa* amongst most of the pathogenic microbes were above 20mm with 150µg/ml. Whereas common antibiotic compounds showed 21.00mm with Ciproflaxin and tetracycline and gentamycin for *K. pneumonia*.

Respiration of cell produce number of free radicals like super oxide, hydrogen peroxide, hydroxyl radical which were generally known as reactive oxygen species (ROS). These ROS were essential for normal respiration of the cell but when it goes beyond certain threshold level it also damages DNA, lipids, proteins, etc causing diabetics, cancer, hyper tension, etc. Such oxidative stress was reduced by the cell by producing antioxidant within the cell and also obtain antioxidant from food like vegetables, nuts fruits, oils etc which reacts with free radicals and neutralize them (Raju, 2001). Within the cell, two different pathways are available in neutralizing the water-soluble free radical's vitamin E and b-carotene and coenzyme that neutralize the free radicals produced on the cell wall. Antioxidant scavengers were produced by the cell within the cell also act as potent antioxidant. In take of compounds like vitamin E ascorbic acid and phenolics also reduce oxidative damage (Santharam *et al*., 2015).

Antioxidant capacity of *W. somnifera* quantified through DPPH and ABTS assay were free radicals with purple colour were reduced by antioxidant to colourless (Murthy *et al*., 2003). The DPPH scavenging activity increased up to 86.71 % with 400 µg/ml and ABTS reached up to 92.91 with 400 µg/ml.

Cellular toxicity of biochemical compounds occur in two ways they cause apoptosis and arrest the cell cycle. The methanol crude extract of *W. somnifera* showed least IC_{50} with HeLa 194.11 µg/ml followed by MCF 210.78 µg/ml HepG2 217.98 µg/ml cell lines.

CONCLUSION

Human beings coevolved along with plant species and consumed them as food through trial and error process and evolved empirical knowledge on variety of plant species for their health problems. These plant remedies proved their efficacy through long time-tested medications. Modern medicines are evolved in a short time proved to be effective but with number of complications at times with dangerous systems. However, repeated empirical experiments with natural plant remedies also evolved into phytomedicines which are given as dietary supplements as capsules, powder, extracts, tablets, both as fresh and dried conditions which works in multiple pathway by not only healing but also improves the health. It is also quite often reported that environmental factors influence the production of secondary metabolites which are also used by traditional healers whiled collecting the plant material. Hence in the recent past such information are used in modern technique to increase the production of therapeutic compounds like tissue culture.

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. Rajasankar S, Manivasagam T, Surendran S (2009) Ashwagandha leaf extract: a potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson‟s disease. Neurosci Lett, 454: 11–15.
6. Dipanakar Chaudhuri., Ghate, N.B., Sarkar, R., Mandal, N. Phytochemical analysis and evaluation of antioxidant and free radical scavenging activity of Withania somnifera root. Asian J. Pharm. Clin. Res. 2012; 5, 193–199.
7. 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.
8. Deka, D. K., Lahon, L. C., Saikia, J., Mukit, A. 1994. Effect of *Cissus quadrangularis* in accelerating healing process of experimentally fractured Radius-Ulna of dog: A preliminary study. Indian Journal of Pharmacology; 26: 44-48.
9. Demling, R. H. 2000. Oxandrolone, an anabolic steroid enhances the healing of a cutaneou wound in the rat. Wound Repair and Regeration. 8: 97-102.
10. Kumar, M., Talreja, T., Jain, D., Dhuria, R. K., Goswami, A. and Sharma, T. 2017. Comparative evaluation of *in vitro* antibacterial activity of several extracts of *Achyranthes aspera, Azolla pinnata, Cissus quadrangularis* and *Tinospora cordifolia,* International Journal of Chemical Studies; 5(1): 154-157.
11. Mallika, J., Shyamala Devi, C. S. 2005. *In vitro* and *In vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*. African Journal of Biomedical Research; 8: 95-99.
12. 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.
13. Raju S.M and Nageshwara Rao J.S. 2005. Jaypee's review of medical biochemistry. New Delhi: Jaypee Brothers Publishers. Chapter 7, Vitamins; p. 65-87.
14. Santharam, E., Ganesh, P. and Soranam, R. 2015. Evaluation of in vitro free radical scavenging potential of various extracts of whole plant of Calycopteris floribunda (Lam). J Chem Pharm Res.7(1):860-864.
15. Shah U. 2011. *Cissus quadrangularis* l.: phytochemicals, traditional uses and pharmacological activities - a review, *Int J Pharm Pharm Sci, Vol 3, Suppl 4, 41-44*

Received:09.08.18, Accepted: 10.09.18, Published:01.10.2018

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