



# Antibacterial activity of selected medicinal plants and GC-MS analysis of *Withania somnifera*

Ponmozhi V, Pavithra S, Naga Lakshmi M, Jaganathan J, Karthik L, Shree Devi M.S\*.

Department of Gunapadam, Sivaraj Siddha Medical College, Salem, Tamilnadu, India. \*For correspondence: shreemd@gmail.com

Article Info: Received 04 Sep 2014; Revised: 24 Nov 2015; Accepted 28 Nov 2015.

## ABSTRACT

The present study was designed to screen the anti-bacterial activity of some traditional medicinal plants against *Escherichia coli* by *in vitro* methods. The leaves of *Lagenaria vulgaris*, *Withania somnifera*, *Acalypha indica*, *Psidium guajava*, combination of *Phyllanthus amarus* and *Terminalia bellerica*, combination of *Terminalia bellerica* and *Terminalia chebula* were screened for anti-bacterial activity by agar well diffusion method on Muller Hinton agar. Among these, significant anti-bacterial activity was showed by *Withania somnifera* (24±1mm). Further we screened the phytochemicals present in *Withania somnifera* extract using GC-MS analysis.

**Keywords:** *Withania somnifera*, *Escherichia coli*, Muller Hinton Agar, anti-bacterial activity

## 1. INTRODUCTION

Medicinal plants represent a rich source of anti microbial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts are used for extract as well as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant parts. Hundreds of plants species have been tested for anti-microbial properties [1].

Amukkara belongs to the division Angiosperm, class Dicotyledons, order Polymoniales [2]. It is also known as *Physalis somnifera* Dunal, *Withania kansuensis* Kaung, *Withania microphysalis* Suess.

The species name *somnifera* means “sleep-inducing” in Latin. It belongs to the family Solanaceae (also known as ‘nightshade’ family). The berries of Amukkara can be used as a substitute for rennet in cheese making [3]. In other languages Amukkara was named as Winter cherry, Indian ginseng (English), Aswagandha (Sanskrit), Asgandh (Urdu). In Siddha aspect, *Withania somnifera*'s properties are Suvai (taste) – Inippu (Sweet), Kaippu (Bitter), Gunam (Character) – Light, oily, Veeriyam (Potency) – Hot, Vibavam (after digestion) – Sweet [4]. Aswagandha is used in many cases, for virtually every condition. It is also especially good for restoring vitality and full sexual potency. The root is very potent and it unquestionably builds sexual vitality-*Chris Kilham* [5]. As it has many medicinal properties, it is not only used as a single drug, but also combination with other

drugs in Unani and Ayurveda system of medicine for centuries. Initially it has been described by Dioscorides in his book "Kitab-ul-Hashaish" [6].

Two varieties of Asgand in classical Unani literature 1. *Asgand nagori*, 2. *Asgand dakani*. Amukkara (*Withania somnifera*) has been given for the various illness like polyarthritis, rheumatoid arthritis, lumbago, painful swellings, spermatorrhoea, asthma, leucoderma, general and sexual debility, anxiety neurosis, ulcer, leucorrhoea, marasmus [6]. Withaferin A show signs of moderately potent anti-arthritis and anti-inflammatory activities. The shrub's extract is active against Vaccinia virus and *Entamoeba histolytica*. Amukkara exhibit the defensive exploit also against *Aspergillus* sp. infections. Anti-biotic activity of Withaferin A is due to the existence of unsaturated lactone-ring. Withaferin A, Withanolide D & E display significant Anti tumour activity *in vitro* against cells derived from human epidermoid carcinoma of nasopharynx and *in vivo* against Ehrlich ascites carcinoma, sarcoma 180, sarcoma Black. They also act as a mitotic poison stunning the division of cultured human larynx carcinoma cells at metaphase. Anti stressor effect of Amukkara scrutinized in rats using cold water swimming stress test. The drug treated animal exhibit improved stress tolerance. Total alkaloidal fraction of root extract illustrated prolonged hypotensive bradycardiac and respiratory stimulant activity in dogs. The leaves of *Withania somnifera* contain 12 withanolides, 5 unidentified alkaloids, amino acids, chlorogenic acid, glycoside, glucose, tannin, flavanoids. And the most important steroidal lactone is withaferin A. It is a thermostable and inactivated at pH 7.2. It is insoluble in water. It shows anti-arthritis, anti-inflammatory activities. The anti-biotic activity of the leaves contain withaferin-A inhibited the cultivation of different gram positive bacteria, acid-fast bacilli, aerobic bacilli, pathogenic fungi.

Important Unani formulations contains Asgand are Habbe Asgand, Kushta Gaodanti, Majoon Salab, Majoon Zanjabeel [6]. Other therapeutic uses in Indian medicine system, Amukkara is regarded as a very best sex stimulant and also cures rheumatism, gout, skin disease, nervous disorder (Parkinson's and Alzheimer's disorders, tardative dyskinesia, cerebral ischemia), insanity, hypertension, dyspepsia, lumbar pains, abortion and relieves hand and limb tremors. Amukkara is an herbal medicine that possesses memory-enhancing properties. bronchitis, fibromyalgia, menstrual problem, hiccups, chronic liver disease, hemiplegia[7].

Important Siddha formulations contains Amukkara are Amukkara chooranam (*Kunmam, Vettai, Vikkal, Pandu*), Amukkara kudineer (*Vatha noigal, Nervous debility*), Maha elathi kuligai (*Janni, Jwaram, Swasa noi*), Naraththai lehyam (*Pitha kunmam, Vanthi, Agni mantham, Arochakam, Neerkattu*), Idi valladhi (*Vatham, Kushtam, Megam, Soothaga soolai, Vellai, Granthi*), Nandhi mezhugu (*Kanta malai, Kabala-kushtam, Elumbusilaippun, Moolam, Pilavai*), Rasagandhi mezhugu (*Sori, Pavuthiram, Puzhu vettu, Putru, Megam, Vatha rogam*), Gandaka rasayanam (*Meganeer, Vellai, Saruma noi, Ven kushtam*), Parangi chakkai rasayanam (*Marbu noi, Parangi pun, Kandamalai, Lingaputru*), Visha jwara kudineer (*For all Visha jwaras*), Vilva ver kudineer (*Cough, Elaippu ayasam, Dryness of skin*), Amukkara lehyam (*Vatha disorders, General tonic for strength and vitality*) [8].

*Withania somnifera* Dunal is the most important medicine widely known as Asgand. It is also called as "Medhya Rasayan" or "Mind Rejuvenate" used enhances the overall brain function [9]. In Ayurvedha, it is known as Ashwagandha which is Sanskrit, it is combination of "Ashva" means "horse", and "Gandha" means "smell". Its medicinal properties are Anti-stress, adaptogenic, aphrodisiac, sedative, diuretic, anti-spasmodic, anti-inflammatory, vermifuge, febrifuge, alterative, deobstruent, tonic, soporific. It is a nervine tonic. It enhances immunity and endurance. It is a natural nutrient for insomnia. It is a good hypnotic in alcoholism. It is bitter in taste and hot in potency so it alleviates vata and kapha. It stimulates thyroid activity. It enhances anti-peroxidation of liver. Amukkara is a revitalizing plant that preserves proper nourishment of muscle and bone tissues. It amplifies body's resistance towards adverse influence. It is also cure amnesia. It sustains proper functions of adrenal gland and reproductive system. Being a powerful aphrodisiac used for sexual vitality and as an adaptogen. It is blood tonifier that recovers circulation and nutrients absorption of cells. In tissues peroxidation is a toxic function hence it is protective to liver [9].

*Escherichia coli* belongs to the family Enterobacteriaceae, a Gram negative bacteria. It is one of the normal floras in the intestine of human being. It may cause systemic inflammatory response syndrome (SIRS). As *E. coli* generally exhibit multiple drug resistance, Anti-biotic sensitivity testing is important in treatment aspect. The genus is named after Eschrich who was the first to describe the colon bacillus under the name *Bacterium coli commune*. Currently, five different types of diarrheagenic *E. coli* of (1) Enteropathogenic *E. coli*

(EPEC) (2) Enterotoxigenic *E.coli* (ETEC) (3) Enteroinvasive *E.coli* (EIEC) (4) Enterohemorrhagic *E.coli* (EHEC) (5) Enteroaggregative *E.coli* (EAEC) [9].

We took a step to give remedy for diarrhea, urinary tract infection, pyogenic lesions caused by *Escherichia coli* via leaves of *Withania somnifera* (Tamil name: Amukkara). Most of the researches focused on the root of Amukkara, we focused on the leaves of Amukkara to cure the above mentioned diseases.

## 2. MATERIALS AND METHODS

### 2.1. Collections of plant material

Fresh leaves of different plants viz *.Withania somnifera*, *Lagenaria siceraria*, *Acalypha indica*, *Tinospora cordifolia*, *Psidium guajava*, *Phyllanthus amarus*, *Terminalia bellerica*, *Terminalia chebula*. Disease freed leaves collected from Pudhu road (Latitude 11°40'25" N to 11°40'30" N, Longitude 77°47'20" E to 77°47'32" E) (Figure1) in Salem. The leaves were washed thoroughly 6-7 times in running water and once with sterile distilled water and then it perfectly dried.

### 2.2. Powder preparation

Thoroughly washed leaves of above mentioned plants were dried in shade for six days and they were powdered on the basis of soxhlet method and stored in separate air tight container.

### 2.3. Growth and maintenance of test microorganism

Bacterial culture of *Escherichia coli* (*E.coli*) was obtained from a hospital in Salem. It was maintained on nutrient agar at 4° C in refrigerator.

### 2.4. Media preparation and cultivation of bacteria

Muller Hinton Agar is purchased in Hi-Media Pvt. Ltd, Mumbai, India. About 38.0 gm of MHA was suspended in 1000 ml of distilled water and heated to dissolve the media completely. The media with required glassware were sterilized by autoclave at 15 lbs. pressure (121° C) for 15 minutes. In semi hot condition (40° C) the media was poured aseptically in sterile Petri plate (20ml) and allowed to solidify at RT (room temperature). After solidification of

media, 3-4 wells were cut per plate on the basis of Agar Well Diffusion Method (Kirby Bayer's method). One loop of bacterial sample was diluted in 1ml distilled water taken in a sterile test tube and using sterile cotton swab it was swabbed on the surface of the Petri plate. The extracts prepared were poured in these wells and incubated at 37°C for 18 to 24 hours. On the next day clear zone of inhibition around the wells were obtained and the values were recorded.

### 2.5. GC-MS analysis

The GC-MS analysis of *Withania somnifera* leaf extract with in ethanol was performed using Clarus 500 Perkin Elmer gas chromatography equipped with Capillary Column Elite-5MS (5% Phenyl 95% dimethylpolysiloxane) (Column length: 30m, Column id: 250µm) and mass detector turbomass(version 5.2.0) which was operated in Electron Ionization(70ev). Helium was the carrier gas at a flow rate of 1 ml/min, the injector was operated at 280° C and the oven temperature was programmed as follows; 50°C@6°C/min to 200°C (5min)@7°C/min to 280°C (5min). The mass range was 40-450amu. The transfer line and source temperature were 200°C, 160°C. The identification of components was based on comparison of their mass spectra with that NIST 2005 Library. The quantity of injected sample was 1.4 micro liters.

### 2.6. Statistical analysis

For each plant, the antibacterial assay was performed for three times and the inhibitory zones obtained were recorded. With the help of Microsoft Excel 2007 (Roselle, IL, USA), the data are stated as mean ± standard deviation (n=3).

## 3. RESULTS AND DISCUSSION

Plants are the principle source of prospectively useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* anti-bacterial assay. Many reports are available on the anti-viral, anti-bacterial, anti-fungal, anthelmintic, anti-molluscal and anti-inflammatory properties of plants. Some of these observations have facilitated in identifying the active

**Table 1.** Compounds identified using GC-MS analysis

S.No.	Peak Name	Retention Time(min)	Peak Area	% Peak area
1.	<u>Name:</u> 3-Amino-2-oxazolidinone <u>Formula:</u> C <sub>3</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub> <u>MW:</u> 102	3.59	209439	0.0761
2.	<u>Name:</u> Furfural <u>Formula:</u> C <sub>5</sub> H <sub>4</sub> O <sub>2</sub> <u>MW:</u> 96	4.32	6274845	2.2794
3.	<u>Name:</u> 1-Butanol, 3-methyl-, acetate <u>Formula:</u> C <sub>7</sub> H <sub>14</sub> O <sub>2</sub> <u>MW:</u> 130	4.75	83525	0.0303
4.	<u>Name:</u> 2-Cyclopentene-1,4-dione <u>Formula:</u> C <sub>5</sub> H <sub>4</sub> O <sub>2</sub> <u>MW:</u> 96	5.38	1462939	0.5314
5.	<u>Name:</u> 1H-Imidazole, 4,5-dihydro-2-methyl- <u>Formula:</u> C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> <u>MW:</u> 84	6.17	477177	0.1733
6.	<u>Name:</u> 2-Cyclopenten-1-one, 2-hydroxy- <u>Formula:</u> C <sub>5</sub> H <sub>6</sub> O <sub>2</sub> <u>MW:</u> 98	6.41	793604	0.2883
7.	<u>Name:</u> 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one <u>Formula:</u> C <sub>6</sub> H <sub>8</sub> O <sub>4</sub> <u>MW:</u> 144	7.19	493007	0.1791
8.	<u>Name:</u> Glycerin <u>Formula:</u> C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> <u>MW:</u> 92	8.53	4870390	1.7692
9.	<u>Name:</u> 4-Ethyl-4-methyl-5-methylene-[1,3]dioxolan-2-one <u>Formula:</u> C <sub>7</sub> H <sub>10</sub> O <sub>3</sub> <u>MW:</u> 142	9.16	189924	0.0690
10.	<u>Name:</u> 2,5-Dimethyl-4-hydroxy-3(2H)-furanone <u>Formula:</u> C <sub>6</sub> H <sub>8</sub> O <sub>3</sub> <u>MW:</u> 128	9.94	387150	0.1406
11.	<u>Name:</u> 5-Ethylcyclopent-1-enecarboxaldehyde <u>Formula:</u> C <sub>8</sub> H <sub>12</sub> O <u>MW:</u> 124	10.14	1115327	0.4052
12.	<u>Name:</u> Levoglucosenone <u>Formula:</u> C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> <u>MW:</u> 126	10.76	1389678	0.5048
13.	<u>Name:</u> 1,3,2-Dioxaborolane, 4,4-dimethyl-5-oxo-, 2-ethyl <u>Formula:</u> C <sub>6</sub> H <sub>11</sub> BO <sub>3</sub> <u>MW:</u> 142	11.10	390656	0.1419
14.	<u>Name:</u> Piperidin-4-ol, 2,5-dimethyl- <u>Formula:</u> C <sub>7</sub> H <sub>15</sub> NO <u>MW:</u> 129	11.59	163829	0.0595

15.	<u>Name:</u> 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- <u>Formula:</u> C <sub>6</sub> H <sub>8</sub> O <sub>4</sub> <u>MW:</u> 144	11.83	5522083	2.0060
16.	<u>Name:</u> Hexanoic acid <u>Formula:</u> C <sub>6</sub> H <sub>12</sub> O <sub>2</sub> <u>MW:</u> 116	12.43	351457	0.1277
17.	<u>Name:</u> 1-Propanol, 2,2-dimethyl-, benzoate <u>Formula:</u> C <sub>12</sub> H <sub>16</sub> O <sub>2</sub> <u>MW:</u> 192	13.20	495725	0.1801
18.	<u>Name:</u> 2-Furancarboxaldehyde, 5-(hydroxymethyl)- <u>Formula:</u> C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> <u>MW:</u> 126	14.26	45527032	16.5384
19.	<u>Name:</u> 2-(4'-Methoxyphenyl)-2-(2'-methoxyphenyl)propane <u>Formula:</u> C <sub>17</sub> H <sub>20</sub> O <sub>2</sub> <u>MW:</u> 256	17.21	3489257	1.2675
20.	<u>Name:</u> 1,2,3-Benzenetriol <u>Formula:</u> C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> <u>MW:</u> 126	19.21	96777080	35.1557
21.	<u>Name:</u> D-Allose <u>Formula:</u> C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> <u>MW:</u> 180	20.87	7722487	2.8053
22.	<u>Name:</u> 1,6-Anhydro- $\alpha$ -D-galactofuranose <u>Formula:</u> C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> <u>MW:</u> 162	23.26	2368539	0.8604
23.	<u>Name:</u> n-Hexadecanoic acid <u>Formula:</u> C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> <u>MW:</u> 256	28.06	31331798	11.3818
24.	<u>Name:</u> E-9-Tetradecenoic acid <u>Formula:</u> C <sub>14</sub> H <sub>26</sub> O <sub>2</sub> <u>MW:</u> 226	32.51	63394000	23.0288

principle responsible for such activities and in developing drugs for the remedy of diseases in human beings. However, not many reports are available on the exploitation of anti-bacterial activity of plants for developing commercial formulations. [1]. According to our view of search, most of the researches are done in root of *Withania somnifera*. This made us interested on leaves of Amukkara. Some of the researches have done with leaves of *Withania somnifera* and reported to have Anti-cancer activity and Anti-bacterial activity against clinically threatened bacteria. On the continuation of those researches we have decided to reveal the secret of the activity of Amukkara leaves. In our another point of view, root based medicines causes death to the whole

plant and also it takes more time to collect the root but in case of leaves, the collection is easy and it conserve the plant and environment. The above mentioned plants were screened for their antibacterial activity against *E.coli* and the obtained results were depicted in Table 1 and Figure 2. The zone of inhibition obtained were *Withania somnifera* (Amukkara- Solanaceae)-24 mm, *Euphorbia hirta* (Ammaan pacharisi- Euphorbiaceae)-21 mm *Tinospora cordifolia* (Seenthil- Menispermaceae)-18 mm, *Psidium guajava* (Koyya- Myrtaceae)-19mm, *Phyllanthus amarus* (Keezhanelli- Euphorbiaceae)-20mm, *Terminalia bellerica* with *Terminalia chebula* (Thandrikai with Kadukai- Combretaceae)-23mm. Among these plants such as *Withania somnifera* shows the maximum inhibitory zone (Figure 1).

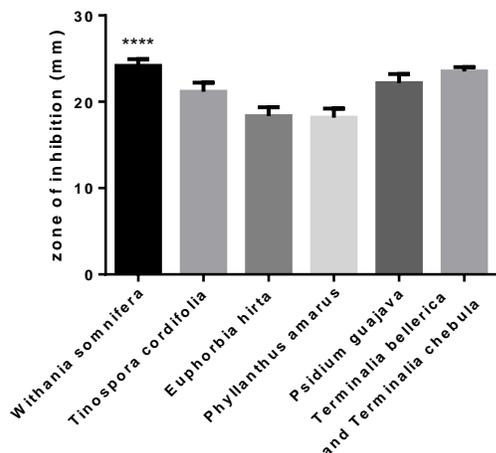


Figure 1. Zone of inhibition observed for different plants

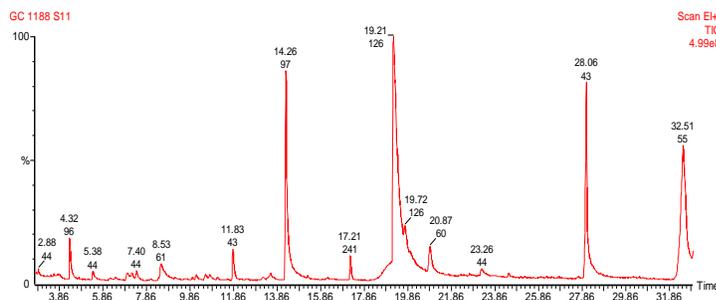


Figure 2. Chromatogram of GC-MS analysis

Amukkara has a high potency to inhibit the *E.coli* (24mm). According to GC-MS analysis, the major compounds present in the leaf extract of *Withania somnifera* are 1,2,3 Benzenetriol (35.1557%), E-9,Tetradecenoic acid (23.0288). In our research aspect, it may be responsible to cure diarrhea, septicemia and urinary tract infection.

#### 4. CONCLUSION

*Withania somnifera* could provide evidence to be a first-rate natural source of an intoxicating and relatively safe anti-bacterial agent. Also the results of our evaluation gave a proof on the antibacterial activity of *Withania somnifera* against *E.coli*. From these results we can conclude that *Withania somnifera* has a high potential to inhibit *E.coli* and it may be use in the treatment of UTI, diarrhea, septicemia after several clinical based researches.

#### Acknowledgement

The authors wish to thank Department of Carism, Sastra University, Tanjore for providing GC-MS facility.

#### Conflict of Interest

The authors declare that they have no conflicts of interest.

#### References

1. Mahesh.B and Satish.S Anti-microbial activity of some important medicinal plant against plants and human pathogens. *World Journal of Agricultural Science* 4(S): 839-843, 2008.
2. Somasundaram.S, Medicinal Botony, Volume 2.
3. *Withania somnifera* Wikipedia, the free encyclopedia.Aavailable from: <http://www.herbalbloom.com>
4. Murugesu muthaliyar.K.S. Siddha Materia Medica (Medicinal Plants Division) Volume 1
5. Qamar Uddin, Samiulla.L,Singh.V.K, Jamil.S.S. Phytochemical and Pharmacological Profile of *Withania somnifera* Dunal: A Review, *Journal of Pharmaceutical Science* 02 (01); 2012: 170-175.
6. Uthamarayan.C.S., Pharmacopoeia of Hospital of Indian Medicine.2<sup>nd</sup> Edition.
7. Usharani Pingali, Raveendranadh Pilli, Nishat Fathima, Effect of Standardized aqueous extract of *Withania somnifera* on tests of Cognitive and psychomotor performance in healthy Human participants.Available from:<http://www.ncbi.nlm.nih.gov> > NCBI > Literature > PubMed Central (PMC)
8. Ananthanarayanan and Panikar(2013), Text Book of Microbiology(9<sup>th</sup> edition),Universities Press,India.
9. Singh. G, Sharma.P.K, Dudhe.R, Singh.S, Biological activities of *Withania somnifera*,*Annals of Biological Research* 2010,1(3);56-63.