Proceedings of National Seminar on
ADVANCED MATERIALS for ENERGY, ENVIRONMENT and MEDICINAL APPLICATIONS (AMEEMA)

11TH & 12TH JULY 2019

Sponsored by

TNSCST

Tamilnadu State Council for Science and Technology

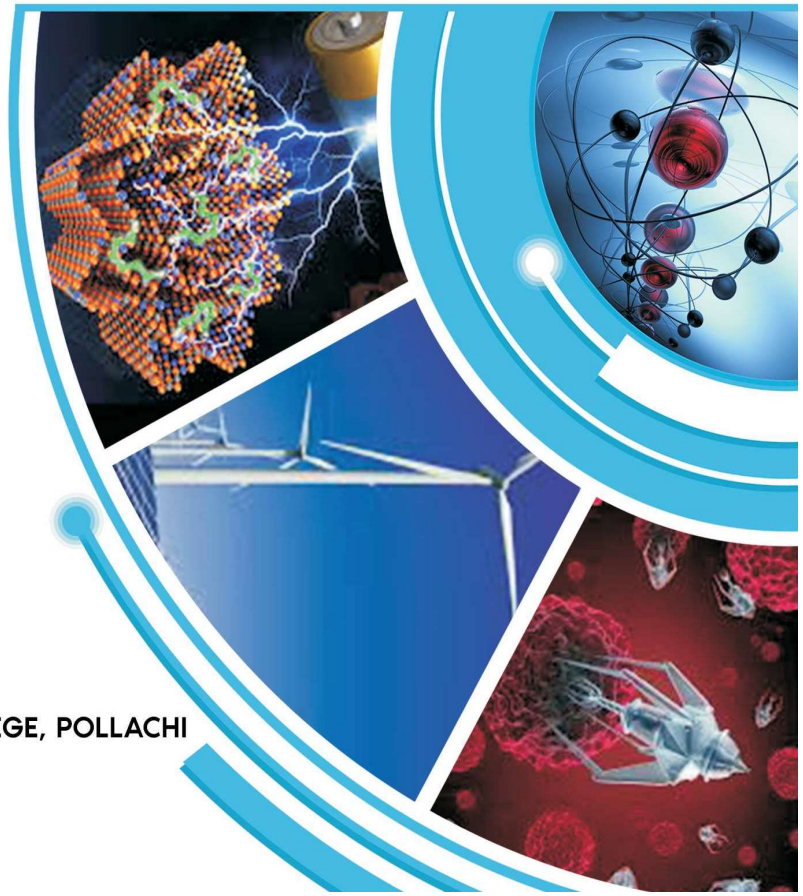
NGM College Pollachi

Chief Editor :

Dr.K.POONKODI

Joint Editors :

Dr.M.SUGANTHI
Mrs.K.VIMALADEVI
Ms.R.MINI
Dr.V.PRABHU
Mrs.M.ANUSUYA



PG DEPARTMENT OF CHEMISTRY
NALLAMUTHU GOUNDER MAHALINGAM COLLEGE, POLLACHI

*****

GC-MS ANALYSIS AND *INVITRO* CYTOTOXICITY POTENTIAL OF ETHANOL EXTRACT OF *AZIMA TETRACANTHA* LAM

K. POONKODI* AND S. SHARMILA DEVI
PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi, Tamilnadu-642001

ABSTRACT

Herbal medicines are very useful for treating various diseases, *Azima tetracanntha* Lam commonly known as Sanghu mull in Tamilnadu belongs to Salvadoraceae family. It is available throughout the year. It is well known for their medicinal uses like, dropsy and skin diseases, reducing inflammation by acting on body mechanism, protects liver from damage. The leaves are also administered with food as a remedy for rheumatism, the leaves juice is used as ear drop. The leaves are found to contain azimine, azcarpine, carpine and isorhamnitine-3-O-rutinoside etc (Friedelin, lupeol, glutinol and β-sitosterol were isolated from the petroleum ether extract of the leaves of *A.tetracantha.* The seeds of this plant have been found to possess novel fatty acids along with other fatty acids. The ethanol extract showed the presence of phenols, carbohydrates, coumarins, steroids, tannins, saponin. The chloroform extract showed the presence of steroids, glycosides, terpenoids, flavonoid and alkaloids. The present study examined the preliminary phytochemicals of ethanol extract of *A. tetracanntha* and was evaluated for *in vitro* cytotoxicity activity against Chung lever cell lines using MTT assay. The ethanol extracts of *A.tetracantha* was incubated with different concentration (25, 50, 75 and 100 µg/ml) affected the viability of Chang liver cell line. Ethanol extract of *A.tetracantha* leaves showed the cytotoxicity effect on the Chang liver cell line in dose dependent pattern and the IC_{50} value was determined as 22µg/ml. The result showed that the ethanol extract of *A.tetracantha* has severe cytotoxicity against Chang liver cancer cell line.

Keywords: *Azima tetracanntha,* cytotoxicity, Chung liver cell line, MTT assay and GC-MS

INTRODUCTION

The usage of plant based products has been used from ancient times to cure many diseases, now a days various types of cancer are prevails due to environmental factors. There are many commercial anticancer drugs are available with high cost and produce side effects. So the need of cost effective and side effect free drugs are needed. The *Azima tetracantha* Lam commonly known as Sanghu mull in Tamilnadu belongs to Salvadoraceae family. It is distributed in Peninsular India, West Bengal and Orissa. Also found in Saudi Arab, Somalia, East and Central Africa, South Africa and Sri Lanka. Due to its powerful diuretic activity, it is given in rheumatism, dropsy, dyspepsia and chronic diarrhea. It is also given as a stimulant tonic after confinement. The leaf juice given in internally relieves cough phthisis and applied as ear drops against earache. The crushed leaves are placed on painful teeth.

The leaves are found to contain azimine, azcarpine, carpine and isorhamnitine-3-O-rutinoside etc (Rall *et al*., 1967; Williams and Nagarajan, 1988; Bennett *et al.,* 2004) Friedelin, lupeol, glutinol and β-sitosterol were isolated from the petroleum ether extract of the leaves of *A.tetracantha* (Rao and Prasada Rao PRS, 1978). The seeds of this plant have been found to possess novel fatty acids along with other fatty acids (Daulatabad *et al.,* 1991). Presence of glucosinolates and neoascorbinogen has also been reported (Bennett *et al.,* 2004). The ethanol extract showed the presence of phenols, carbohydrates, coumarins, steroids, tannins, saponins. The chloroform extract showed the presence of steroids, glycosides, terpenoids, flavonoid and alkaloids.

The *A.tetracantha* Species were reported to possess antipyretic activity (Nargis *et al*., 2011; Ekbote *et al.,* 2009), *In vitro* antioxidant activity (Muthuswamy *et al.,* 2012; Thendral *et al.,* 2010; Rani *et al.,* 2013; Ekbote *et al.,* 2010; Vinoth *et al.,* 2015; Josephinol *et al.,* 2017), antibacterial activity (Gowthami *et al.,* 2012), *In vitro* anticancer activity (Nandhini *et al.,* 2016), Anti-Proliferative and cytotoxic effect (Gayathri *et al.,* 2015), Anti-Diabetic activity (Amaranth *et al.,* 2013), *In vitro* hepatoprotective activity (Prakash *et al.,* 2015; Ekbote *et al.,* 2010), Antimicrobial activity (Hema *et al.,* 2012; Jaganthan *et al.,* 2015; Vinoth *et al.,* 2014). There are only a few reports are available for its anticancer activity of *A. tetracantha.* The aim of the present investigation was to evaluate the GC-MS analysis and *in vitro* Cytotoxicity potential of ethanol extract of *Azima tetracantha* Lam.

MATERIALS AND METHODS

Plant material

A. Tetracantha of plant family Salvadoraceae collected from an area near Udumalpet, Tamil Nadu, South India. The plant material was identified and authenticated by Dr.Satishkumar Department of Botany, NGM College, Pollachi, Coimbatore, Tamil Nadu.

Extraction Process

The leaves of *A.tetracantha* were first washed well and dust was removed from the plant. The plants were washed several times with water to remove the traces of impurities from the plant. Then the plants were dried at room temperature and powdered.

Extraction with ethanol

The 250 g of powder leaves of *A.tetracantha* was extracted with ethanol by using Steam distillation. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract was concentrated to yield black residues.

Preliminary phytochemical screening

Ethanol extract of *A.tetracantha* was subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in it.

GC-MS analysis

GC-MS analysis of the phytoconstituents of *A.tetracantha* was carried out using thermo GC – trace ultra-version: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non – polar column (30m x 0.25mm), film thickness 0.25µm. The carrier gas was Helium, used at a constant flow rate of 1.0 ml/min. The oven temperature was set from 70 to 260˚Cat 6˚C/min. One ml of the sample was injected with split less mode. Mass spectra were recorded over 50-500 amu range with electron impact ionization energy 70 eV. The MS transfer line temperature was set at 280˚C. The total running time of GC-MS was 37.49 min.

MTT assay

3-[4,5-dimetylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hour of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline.

The culture medium from the Chang liver monolayer was replaced with fresh medium. Test sample in duplicate were added on the cells. After incubation at 37±1˚C for 18 hrs, MTT was added in all the wells and incubated for 4 hrs. After incubation, DMSO was added in the wells and read at 570 nm using photometer. The % cell inhibition was determined using the following formula.

% cell inhibition=100-Abs (sample) /Abs (control) X 100

RESULT AND DISCUSSION

Preliminary Phytochemical Screening

Ethanol extract of *A.tetracantha* was subjected to preliminary phytochemical screening for the presence of various phyto compounds like, terpenoids, alkaloids, flavonoids, steroids etc. The results showed that Glycosides, Terpenoids and Steroids give positive to the screening. This is shown in table (1).

Table 1: Preliminary Phytochemical Screening of ethanolic extract of *A.tetracantha*

S.No	COMPOUNDS	ETHANOL EXTRACT
1	Glycosides	+
2	Alkaloids	_
3	Terpenoids	+
4	Flavonoids	_
5	Steroids	+
6	Saponins	_
7	Phenols	_
8	Proteins	_

GC-MS Analysis

The ethanol extract of *A.tetracantha* was subjected to GC-MS analysis. The analysis revealed that the presence of 22 compounds from ethanol leaf etract of *A.tetracantha* (table 2). The major components are Hexadecanoic acid, ethyl ester (11.55%), 1-Ethyl-2-phenyl-3-phenylimino-3H-indole (6.55%), Homotropan-7à-ol (6.38%), Flavanone, 3-hydroxy-2',4'5,7-tetramethoxy-,acetone, trans- (5.26%), Cannabidiol (4.67%), 8-chloro-5-quinoline carboxylic acid (3.96%), Methyl 4-bromo-5,7-dimethoxyindole-2-carboxylate (3.80), along with 15 other constituents. The structures of the components are shown in table (2) and GC-MS chromatogram was shown in fig (1).

Table 2: Chemical composition of ethanol extract of *A.tetracantha*

S.No	Compound name	RT	Area %
1	Homotropan-7à-ol	14.66	6.38
2	Cyclohexane,1,4-dimethyl-2-octadecyl-(CAS)	21.38	3.44
3	Hexadecanoic acid, ethyl ester	23.01	11.55

4	3-(1-Indenylidene)-2,2-dimethylpropanal oxime	23.50	1.49
5	3-(methylthio)-quinoline	25.70	2.89
6	2-(2-Methoxyphenyl)-1-[1-(2-cyclohexylethyl)indol-3-yl]ethanone	26.05	1.86
7	Benz[a]anthracene, 3-methyl-(CAS)	26.35	3.27
8	1-Ethyl-2-phenyl-3-phenylimino-3H-indole	26.80	6.55
9	4-Hydroxy-5-isopropyl-5Hfuran-2-one	27.25	2.76
10	Sorbitol hexaacetate	28.23	2.19
11	27-Hydroxy-3,11-dioxoolean-12-en-28-oic acid	28.86	2.87
12	Succinic acid, 3,5-dimethylcyclohexyl tetradecyl ester	30.47	2.35
13	Cannabidiol	31.14	4.67
14	1,2-O-Isopropylidene-3-C-vinyl-à,D-allo-furanose	31.77	2.64
15	Propyl-5-oxo-6,7,8,9-tetrahydro-5H-pyridazino[4,3-c]azepine-3-carboxylate	32.22	2.24
16	1-(2-Naphthyl)-2-[(tert-butoxycarbonyl)methylene]pyrrolidine	32.93	1.85
17	Flavanone, 3-hydroxy-2',4',5,7-tetramethoxy-, acetate,trans-	34.60	5.26
18	2,2-Dimethyl-7-methylthio-4-chromanone	35.76	2.22
19	4-Phenyl-6-methoxybenzothiopyran	36.27	1.71
20	8-chloro-5-quinolinecarboxylic acid	37.68	3.96
21	9-Octadecenamide.(Z)-	39.21	2.99
22	Methyl 4-bromo-5,7-dimethoxyindole-2-carboxylate	39.57	3.80

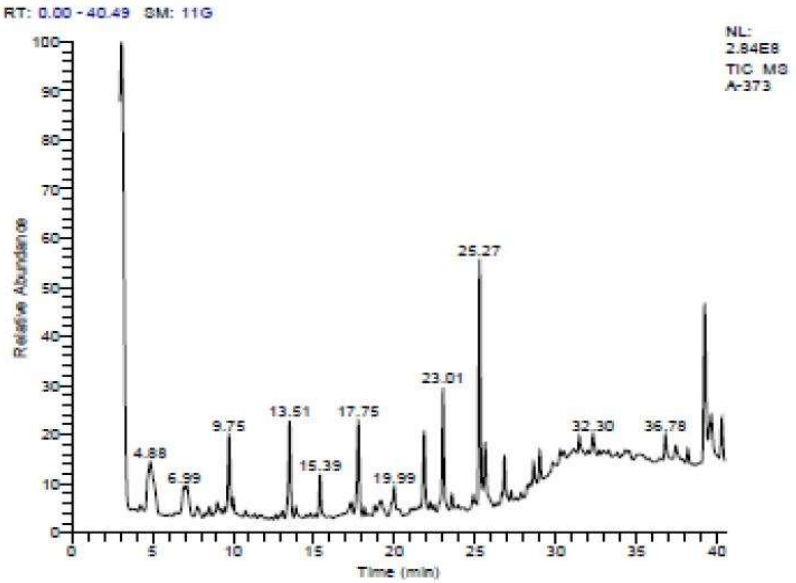


Figure 1: Chromatogram obtained from the GC-MS with the extract of *A.tetracantha*

***In Vitro* Anti-cancer activity**

In MTT assay, a simple and reliable technique, which measures cell viability for screening *In-vitro* cytotoxicity of the ethanol extract and the results shown in table (3). The viability of cancer cells after incubation with different concentrations of *A.tetracantha* leaves are depicted in Chang liver. The incubation with different concentration of ethanol extract of the sample (25, 50, 75 and 100 µg/ml) affected the viability of Chang liver cell line. The Ethanol extract of *A.tetracantha* leaves showed the cytotoxic effect on the Chang liver cell line in dose dependent pattern and the IC_{50} value was determined as 22 µg/ml given in table (4). The result showed that the ethanol extract of *A.tetracantha* has severe cytotoxicity against Chang liver cancer cell line.

Table 3: % of cell inhibition of *In-Vitro* anticancer activity of *A.tetracantha* ethanol extract

S.No	Conc (µg)	% cell inhibition
1	25	58
2	50	72
3	75	74
4	100	83

Table 4: IC_{50} values for Ethanol extract of *A.tetracantha*

Name of the Extract	IC_{50} µg/ml	Name of the cell line
Ethanol	23	Chang liver

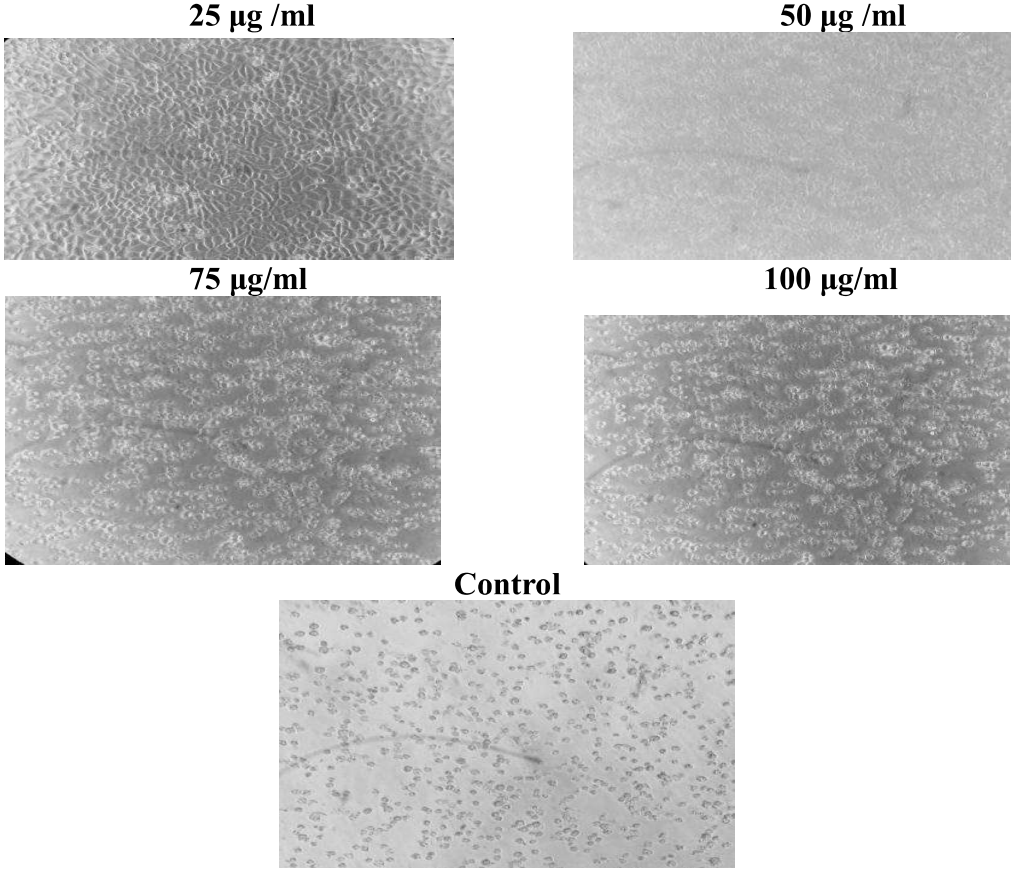


Figure 2 : Anti-cancer activity – Ethanol extract of *A.tetracantha* on Chang liver cell line

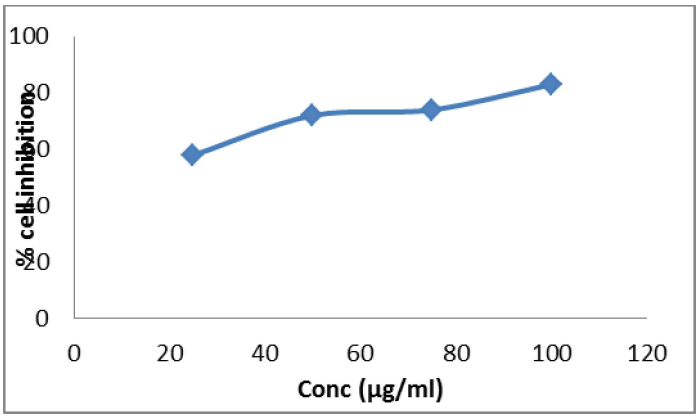


Figure 3: % of cell inhibition at various concentrations of *A.tetracantha* extract

CONCLUSION

The phytoconstiuents from ethanol extract of *A.tetracantha* was analyzed by GC-MS method. Hexadecanoic acid, ethyl ester, 1-Ethyl-2-phenyl-3-phenylimino-3H-indole and Homotropan-7à-ol, Flavanone, are the major components of *A.tetracantha.* Preliminary phytochemical analysis showed the presence of Glycosides, Terpenoids, and Steroids. The anticancer activity of ethanol extract of *A.tetracantha* was tested against Chang liver cell line. Ethanol extract of *A.tetracantha* showed concentration dependent activity and the IC_{50} value is 22 µg/ml. The results indicated that the ethanol extract of *A. tetracantha* showed severe *in vitro* anticancer activity.

NO Conflict of interests

REFERENCES

1. Amaranth V, Banagar B, Shivakumar B, Jayaveera KN. Anti-Diabetic Activity of Ethanol and Chloroform Extract of *Azima tetracantha* leaves in High Fructose Induced Diabetic Rats. *International Journal of drug Formulation and Research*, **2013**, 4(3).
2. Bennett RN, Mellon FA, Rosa EA, Perkins L and Kroon PA, Profiling Glucosinolates, Flavonoids, Alkaloids, and other secondary metabolites in tissues of *Azima tetracantha* Lam (*Salvadoraceae). Journal of agricultural and food chemistry*, **2004**, 52 (19), 5856-5862.
3. Daulatabad C D, Desai V A, Hosamani K M and Jawkhande A M. Novel fatty acids in *Azima tetracantha* seed oil. *Journal of the American Oil Chemists Society*. **1991**, 68, 978-979.
4. Gayathri G, Nair B.R, Babu V. Anti-Proliferative and cytotoxic effect of *Azima tetracantha* Lam. On cervical cancer cell line [HeLa] and human peripheral Lymphocyte [HPL]. *Journal of Pharmacognosy and Phytochemistry*, **2015**, 4(4): 161-164.
5. Gowthami M, Tamil selvi S, Senthil kumar G, Panneerselvam A. Phytochemical analysis and antibacterial properties of leaf extract of *Azima tetracantha* (Lam). Asian *Journal of Plant Science and Research*, **2012**, 2 (2):110-114.
6. Hema TA, Shiny M, Parvathy J. Anti -microbial activity of leaves of *Azima tetracantha* against clinical pathogens. *International Journal of Pharmacy and Pharmaceutical Science*. **2012**, 4(4):317-319.

7. Muthuswamy P, Elakkiya S, Manju priya K, Deepa K, Ramachandran S, Shanmugapandiyan P. Preliminary phytochemical and *in vitro* antioxidant perspectives of the leaf extract of *Azima tetracantha* Lam [Family: Salvdoraceae]. *International Journal of Pharmacy and Biological Science*, **2012**, 3 (1): 50-58.
8. Nanthini S, Raddha R and Muthusam P. *In-vitro* anticancer activity of leaves and stem of *Azima tetracantha* Lam. *World Journal of Pharmacy and Pharmaceutical Sciences*, **2016**, 5(3).
9. Nargis begam T, Muhammed Ilyas M H, Vijaya Anand A. Antipyretic activity of *Azima tetracantha* in experimental animals. *International Journal of Current Biomedical and Pharmaceutical Research*, **2011**, 1 (2):41-44.
10. Pawlowska A M, Olesek W, Braca A. Quali-quantitative analysis of flavonoids of *Morusnigra L* and *Morusalba L* (*Moraceae*) fruits. *Journal of Agricultural and Food Chemistry*, **2008**, 56:3377-3380.
11. Phan T T, Wang L, See P, Grayer R J, Chan S Y and Lee S T. Phenolic compounds of Chromolaenaodorata Protect Cultured Skin Cells from Oxidative Damage: Implication for cutaneous Wound Healing *Biol. Pharm. Bull*, **2001**, 24(12):1373-1379.
12. Prajapati N D, Purohit S S, Sharma A K, Kumar T. Hand book of medicinal plants. Jodhpur: Agarbios, **2003**.
13. Prakash E, Jeyadoss T and Velavan. Invitro hepatoprotective activity of ATC leaf extract and Silver nanoparticle in hepatocytes. *Der Pharma Chemica*, **2015**, 7(10):381-390.
14. Rall GJH, Smallberger TM, De Waal HL and Arndt RR. Dimeric Piperidine alkaloids from *Azima tetracantha. Tetrahedron Letts*, **1967**, 3465-3469.
15. Thendral Hepsibha B. validation of ameliorative efficacy of *Azima tetracantha* leaf extracts on asthma through mast cell stabilization assay. Journal of Chemical and Pharmaceutical Research, **2015**, 7(10):907-914.
16. Vinoth B, Manivasagaperumal R. Antimicrobial activity of different extract of *Azima tetracantha* root. International Journal of Pharma and Bio sciences, **2015**, 6(2): 613-620.

*****