Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8196
JPP 2014; 3(4): 77-82
Received: 07-10-2014
Accepted: 13-10-2014

Sivaperumal Gopalan
Department of Chemistry, Government College of Engineering, Salem-11. Tamil Nadu, India.

Kannan Kulanthai
Department of Chemistry, Government College of Engineering, Salem-11, Tamil Nadu, India.

Gnanavel Sadhasivam
Department of Chemistry, Government College of Engineering, Salem-11, Tamil Nadu, India.

Ramachandran Arumugam
Department of Chemistry, Government College of Engineering, Salem-11

Sridevi Arumugam
Department of Chemistry, A.V.S. College of Arts and Science, Salem-636106.

Correspondence:
Kannan Kulanthai
Department of Chemistry
Government College of Engineering Salem-636011,
Tamilnadu, India
Email:
kannan_k2002@yahoo.co.in
Contact No: 94431-92718
Tel: 0427-2346157, 0427-2346102

Evaluation of antibacterial activity, phytochemical screening and characterization of *Mimusops elengi* seeds

Sivaperumal Gopalan, Kannan Kulanthai, Gnanavel Sadhasivam, Ramachandran Arumugam, Sridevi Arumugam

Abstract
Objective: The objective of the present work is the extraction, phytochemical analysis characterization and biological studies of *M. elengi* seeds by using a solvents extraction method.
Methods: The present work focused on the extraction of seeds *M. elengi* using different solvents like n-Hexane, Ethyl acetate and Methanol. In this work the methods of drying, grinding, extraction, screening, characterization and biological studies are done. The seeds of *M. elengi* were prepared and screened for their antibacterial activity of four different bacterial strains, including the gram-positive strains such as *S. aureus, B. subtilis* and *Escherichia coli*, *Pseudomonas aeruginosa* gram-negative strains. The extracts were evaluated at 50 µl/ml by using Petri Disc Diffusion method.
Results: All the extracts exhibited moderate anti-bacterial activity in a dose-dependent manner. Among the various extracts of *M. elengi* seeds, hexane extract exhibited high inhibitory zone followed by ethyl acetate and methanol. The *In vitro* study supports its traditional approach as a preventive remedy for the treatment of microbial diseases. The extracts revealed the presence of alkaloids, flavonoids, tannin, saponin, amino acid, carbohydrates, terpenoids, and phytosterol .The resulting extracts sample was analysed by FT-IR spectrum.

Keywords: *Mimusops elengi* seeds, bacterial strains, Petri Disk Diffusion plate method.

1. Introduction
Natural products are known to play an important role in the pharmaceutical studies. Plants have been an important source of medicine for thousands of years. The everyday World Health organisation estimate those up to 80% of peoples are mainly used as the traditional medicines [1]. *M. elengi* plant considers as a sacred plant among Hindus are has obtained an important place in religious texts as well as in Sanskrit literature. It is fragrant flowers are celebrated in the puranas and even placed amongst the flowers of the Hindu paradise [2]. The small and large evergreen trees found in the Andaman Islands and frequently cultivated in gardens. It is also grown as an avenue of the tree throughout the greater part of the India [3]. It has made an important contribution to the field of science from ancient period and also to modern research fields it's used as the large number of medicinal properties [4, 5, 6]. The traditional medicines are based on hundreds of year's belief, observations and analysis, which is helping in the developed modern medicine. Today there is widespread interest in ayurvedic drugs. This interest is based upon the belief that herbal medicines are safe, inexpensive and less adverse effect. The World Health organisation estimates two-third of the world population still depends upon traditional medicines for the treatment of various types of diseases. The important traditional medicinal plant is *M. elengi* belongs to Ayurvedic medicine. It has been used as the indigenous system of medicine for the treatment and cured different types of diseases. All parts of the plant have used as medicinal properties. The barks are used as the acrid and sweet biliousness and diseases of the gum and teeth. There is also used as liver complaints, diseases of the nose and headache. The smoke of the flower is used for treatment of asthma. The leaves were found to have antioxidant, cytotoxic, analgesic, wound healing and antipyretic activities. Plants are the basis of the life on earth and are central to peoples live hoods [7].

Herbal medicines are comparatively safer than synthetic drugs. Plant based traditional knowledge has become an organized tool in the search for new sources of drugs and nutraceuticals [8, 9]. The ethno botanical survey can bring out many different clues for the development of drugs to treat human diseases. Herbal medicines are used as to be great importance in the many developing countries [10]. Considering the current rate of deforestation with the concurrent loss of biodiversity, there is a need for most accurate documentation of the knowledge and experience of the traditional herbalists [11].

M. elengi is a large glabrous evergreen tree 12-15 m height, with a compact leafy head and short, bark smooth, scaly and [12]. Taxonomy and nomenclature (common names) is as follows

Kingdom	:	Plantae,
Order	:	Ericales,
Family	:	Sapotaceae,
Genus	:	Mimusops,
Species	:	*M. elengi*.
Binomial name	:	*Mimusops elengi*

The plant names in different languages are given below

S. No	Language	Plant Name
1.	Tamil	Magilam
2.	English	Bullet wood
3.	Hindi	Bakul
4.	Sanskrit	Bakula
5.	Malaysian	Elengi

The fragment flowers bloom from January form March .It starts bearing fruits from January to May .The fruit is a berry, yellow in colour and ovoid in shape. [15, 16].

2. Materials and Methods

2.1 Plant collection

The seeds of *M. elengi* were collected from TNHB (Opp. Periyar University) in Salem district, Tamil Nadu, India during the month of November. The fresh plant material was air dried as shown in Fig.1 A and then homogenized to fine the powder as shown Fig.1 B. The powder was sieved by using further use.

Fig 1: A) *M. elengi* seeds

Fig 1: B) *M. elengi* seeds powders

2.2 Extraction of Plant Materials

The extraction of plant material seeds was carried out was extracted by using a Soxhlet apparatus at 50-60 ^{0}C. In the extraction procedure, the total amount of 80 gms powdered seeds was used. The extraction was carried out by using solvents of increasing polarity starting from the extract of n-Hexane, ethyl acetate and methanol respectively. The extraction was carried out with 800 ml of each solvent for the period of 6-8 hours. At the end of the extraction, the respective solvents were concentrated by using the rotary evaporator at 40-50 ^{0}C under reduced pressure. The following extracts of *M. elengi* were obtained and named as

HEME – Hexane extract of seeds of *Mimusops elengi,*

EAME- Ethyl acetate extract of seeds of *Mimusops elengi*,

MEME - Methanol extract of Seeds of *Mimusops elengi*

The percentage yields of *M. elengi* seed in n-Hexane (40%) methanol (15.4%), ethyl acetate (9.3%).The prepared extracts were tested for anti-bacterial activity.

2.3 Phytochemical Screening

The Phytochemical screening of the plant extracts was carried out using standard chemical procedure [24].

2.4 FT-IR: (Fourier Transform Infra Red) Spectrum

FT-IR-7600 is a single-beam FT-IR spectrometer (Lambda Scientific).The FT- IR spectra were recorded using the KBr disc for the successive solvent extracts.

2.5 Anti-Bacterial Studies

The extracts were screened *In vitro* for their antibacterial activities against both gram positive and gram negative bacterial strains by using the Disc Diffusion method using cultivated in a suitable agar medium under optimal incubation conditions to obtain a fresh overnight grown culture Bauer *et al* 1966 [25]. The bacterial strains used for the determination of antibacterial activities are Escherichia coli, Staphylococcus aureus, and Bacillus subtilis *Pseudomonas aeruginosa*.

DAY 1
The solutions of the extracts were prepared at 5ml in dimethyl sulfoxide (DMSO).

DAY 2
Harvest a number of distinct colonies from the fresh grown plate culture to suspend in a tube containing broth until turbidity (visually) corresponding to the 1.0 McFarland standard is reached. Using a sterile cotton swab dipped into the adjusted culture medium and squeezed. Then made a lawn culture on Muller – Hinton Agar media. Allow to dry the plates for max. 15 minutes. Longer drying times allow pre-incubation of the cells, which that should be avoided. Plates should be incubated as soon as possible after the application of the discs using sterile forceps and the discs (Antibiotic or tested compound loaded) are applied onto the agar surface. Discs must not be relocated once they have made contact with the agar surface. Incubate the plates under optimal incubation conditions.

DAY 3
The diameter of the inhibition zones is measured to the nearest mm from the point of abrupt inhibition of growth (using a calipers or mm ruler).

3. Results and Discussion
3.1 Phytochemical screening
The preliminary qualitative phytochemical screening of the crude extracts of the seeds was done to assess the presence of bioactive components. The presence of alkaloids, flavonoids, tannin, saponin, amino acid, carbohydrates, terpenoids, and phytosterol were determined and included in table 1.

Table 1: Preliminary phytochemical screening of different extracts of the seeds of *M. elengi.*

S. No	Test	HEME	EAME	MEME
1.	**Test for alkaloids**			
	a) Dragendorff's test	+	+	+
	b) Wagner test	-	+	+
	c) Hagers test	+	-	+
2.	**Test for Flavonoids**			
	a) Lead test	-	-	-
	b) NaOH	-	+	+
3	**Test for Phenols**			
	a) $Fecl_3$	-	-	-
4.	**Test for Tannins**			
	a) $Fecl_3$	-	-	-
	b) $K_2Cr_2O_7$	-	-	+
	c) Lead acetate	+	+	-
	d)			
5.	**Test for saponin**			
	a) Form test	+	+	-
6.	**Test for Amino Acid**			
	a) Xantho proteic test	-	+	-
	b) Biuret Test	-	-	-
7.	Test for Coumarin	-	-	-
8.	Test for Starch	-	-	-
9.	Test for Quinone	-	-	-
10.	**Test for Carbohydrates**			
	a) Fehling test	-	-	+
	b) Benedict test	-	-	-
	c) Molishs test	-	-	-
11.	**Test for cardiac glycosides**			
	Test for Killer –Killani test	-	-	-
12.	**Test for terpenoids**			
	a) Salkovaki test	+	-	+
	b) Libermans test	-	-	+
13.	**Test for phytosterol**			
	a) Salkovaki test	-	-	+
	b) Libermans test	+	-	-

+ Present, - Absent

The table .1 indicated that the hexane extract the presence of *M. elengi* seeds extract contains alkaloids, tannins, saponins, terpenoids, phytosterol. The ethyl acetate extract indicates that the presence of alkaloids, flavonoids, tannins, saponins, amino acid. The methanol extract indicates that the presence of alkaloids, flavonoids, tannins, terpenoids, carbohydrates and phytosterol. It is clearly evident from the table that the other phytoconstituents like phenols, coumarins and glycosides were absent in all the three solvent extracts. These results suggest the presence of primary bioactive metabolites that acts as the precursors for the synthesis of secondary metabolites. These turns help in the development of new bio

products for the future.

4. Phytochemical analysis by spectrophotometer

4.1 FT-IR – Spectrum

The spectroscopy can also be usefully contributed to structural elucidation when new compounds are identified in the plants. FT-IR spectra were taken for hexane, ethyl acetate and methanol extract of *M. elengi* seeds. The FT-IR spectrum profile is illustrated in the Figures. 2, 3, 4. The FT-IR spectrum confirmed the presence of alcohol, phenols, alkanes, aldehyde, ketones, ethers, amines and aromatic compounds present in the extracts.

The spectrum was recorded in the wavelength region between 400 cm^{-}; to 4000 cm^{-};. The spectrum shows peaks at 3422 cm-1, 3404 cm-1, and 3427 cm-1 (strong O-H bonding) which indicates the presence of –O-H stretching of carboxyl group and N-H stretching of secondary amides. These peaks indicate the presence of bonded hydroxyl groups. Further, the peaks observed at 2926 cm-1, 2922 cm-1 represents the C- H stretching bonds of alkenes. The peak observed at 1740 cm-1, 1642 cm-1, 1348 cm^{-}1, 1381 cm-1, 1588 cm-1 and 1268 cm-1 represent the C=C aromatic conjugates. The sharp peak at 1046 cm^{-}1 is assigned to C-N stretching vibrations. The peak observed at 712 cm-1, 666 cm-1 and 589 cm-1 represent the presence different functional groups like secondary Alcohol (O-H stretches, H-bonded)

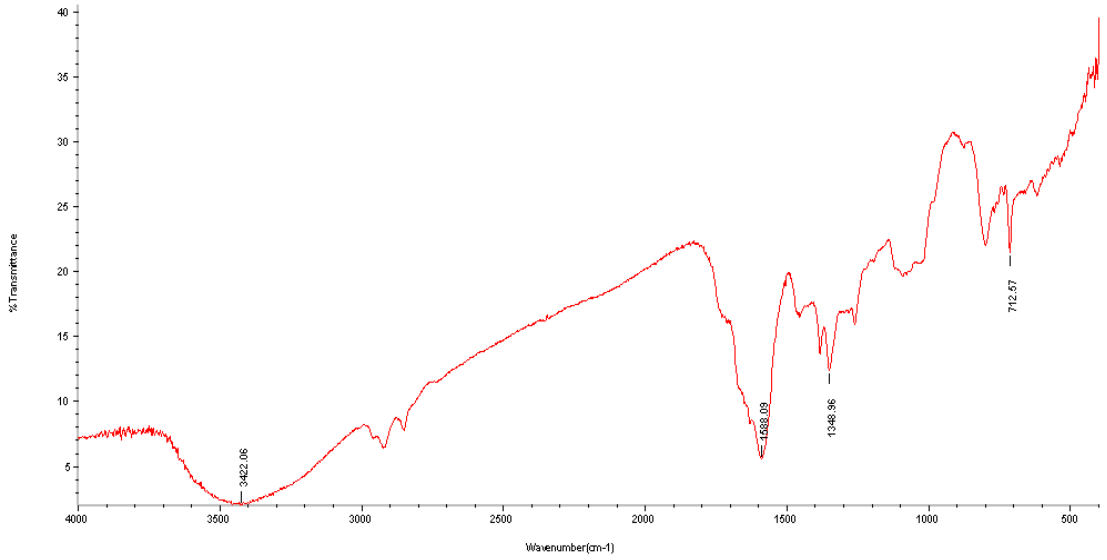


Fig 2: FT-IR Spectrum of Hexane Extraction of *M. elengi* seeds

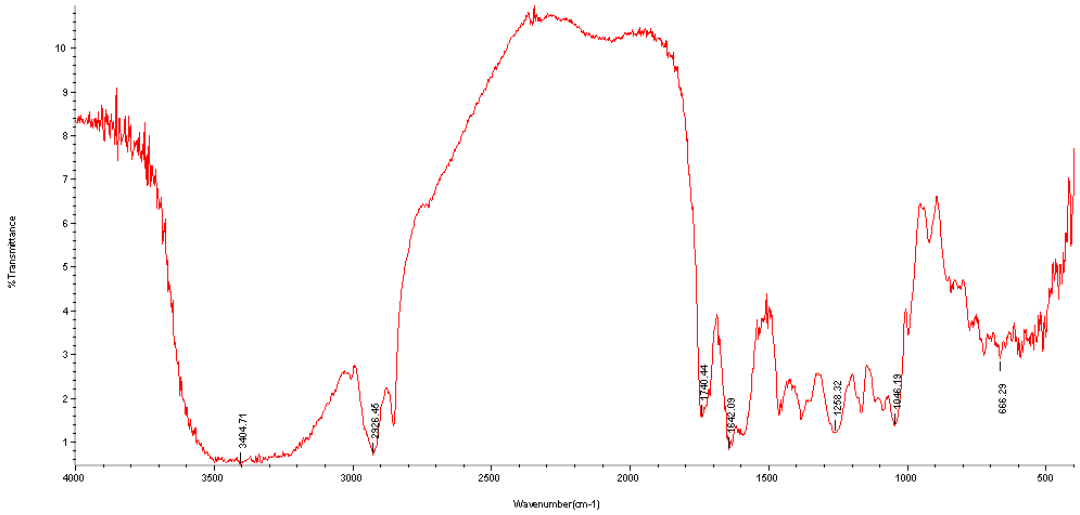


Fig 3: FT-IR Spectrum of Ethyl Acetate Extraction of *M. elengi* seeds

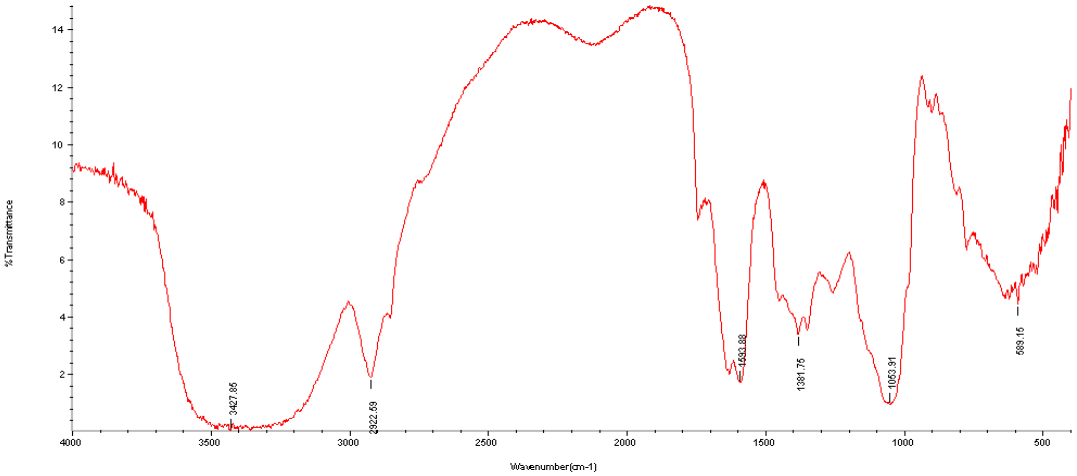
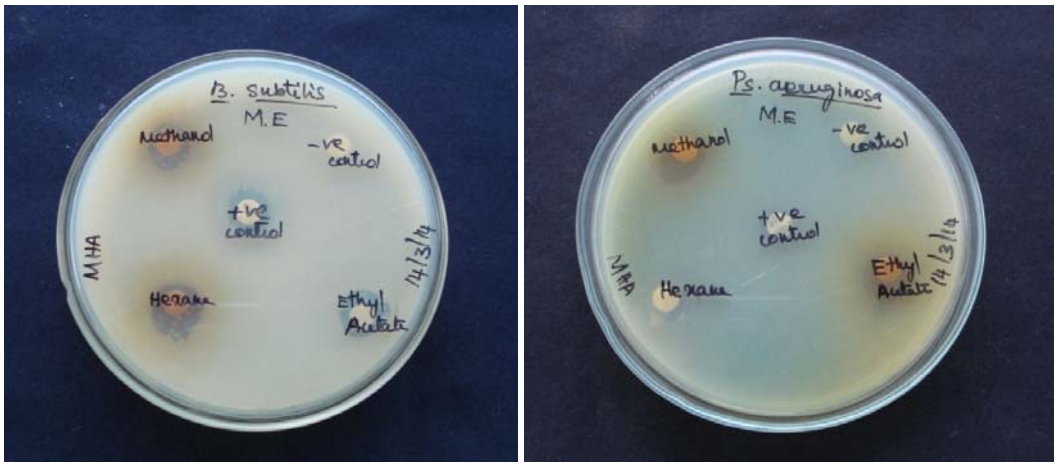


Fig 4: FT-IR Spectrum of methanol Extraction of *M. elengi* seeds

4.2 Antibacterial activity

Antibacterial activity of *M. elengi* seeds extracts was carried out for human pathogens, such as Streptococcus aureus , Bacillus subtilis are gram +ve and *Pseudomonas Aeruginosa*, Escherichia coli are gram -ve bacteria [Fig-5]. The results for anti-bacterial activity of *M. elengi* extracts showed clear zone of inhibition as indicated in Table -2 against Streptococcus aureus, *Bacillus subtilis*, *Pseudomonas Aeruginosa*, Escherichia coli. The concentration of 50 µl/ ml was used for +ve as well as –ve control. The crude *M. elengi* seeds extracts may show anti-bacterial activity against *B. Subtilis* for higher antibacterial activity



A) *B. Subtilis* **B)** *P. aeruginosa*

Fig 5: Zone of inhibition of *M. elengi* seeds extracts using different solvents against Bacterial Pathogens.

Table 2: Antibacterial activity extracts of *M. elengi* seeds. (Zone of inhibition in mm)

S. No	Type of Extractions	Concentration (ml)	Diameter of zone of inhibition (mm)			
			G+ve		G-ve	
			S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
1.	HEME	50 µl	NA	6 mm	5.5 mm	NA
2.	EAME	50 µl	NA	6.5 mm	5 mm	NA
3.	MEME	50 µl	NA	5.5 mm	6.5 mm	NA

NA-No Activity

The gram +ve pathogens using *M. elengi* seeds hexane extract was shown to be as potent as Zone of inhibition is 6.0 mm ethyl acetate shown to be a zone of inhibition 6.5 mm followed by methanol shown the a zone of inhibition 5.5 mm. The gram –ve pathogens *M. elengi* seeds hexane, ethyl acetate and methanol extracts was show zone of inhibition is same values (5.5 mm, 5.0 mm and 6.5 mm). The order of the antibacterial activities are Ethyl acetate > Methanol > Hexane extract. The results clearly show that alkaloids. Phytosterols, Flavonoids, tannins and terpenes, which were

abundantly found in Methanol, Ethyl acetate and Hexane extracts were responsible for the anti-bacterial activity of *M. elengi* seeds. The antibacterial studies were given in table 2 and shown in figure-5 reveal the significant antibacterial potential of all the three extracts of *M. elengi* seeds. The antibacterial activity of the extracts might be attributed to the presence of the foresaid secondary metabolites in the extracts.

5. Conclusion

The chemical constituents found in the seed extracts through phytochemical screening are such as alkaloids, flavonoids, tannin, saponin, amino acid, carbohydrates, terpenoids, and phytosterol. FT-IR spectroscopy confirmed the presence of alkane, alkene, aldehyde ether ester and aromatic phytoconstituents. The crude extracts of *M. elengi* showed moderate antibacterial activity. The hexane fraction comparatively shows more antibacterial activity. *M. elengi* is a valuable plant source for traditional drug preparations. This study may be a lead for further ethno pharmacological investigation to identify new antibacterial compounds with therapeutic benefits. It is hoped that this study would lead to the establishment of extracts new and more potent anti-microbial drugs of natural origin.

6. Acknowledgement

The authors are very much thankful to Dr. R.S.D. Wahida Banu, Principal, Government College of Engineering, Salem-636 011, Tamil Nadu for providing the facilities to carry out this research work and for her constant encouragement.

7. References

1. Kirtikar KR, Basu BD. Indian medicinal plants with illustrations, Oriental Enterprises, India, 2001.
2. Shah PJ, Gandhi SM. Study of *M. elengi* bark in experimental gastric ulcers. J Ethno Pharmacology 2003; 305-311.
3. Manjeshwar SB, Ramakrishnan JP. Chemistry and medicinal properties of the *M. elengi*, A review. Food Res Int 2011; 1823-1829.
4. Bharat G, Parabia MH. Pharmacognostic evaluation of bark and seeds of *M. elengi*. Inter Journal Pharmaceutical Sci 2010; 110-113.
5. Shanmugam S, Annadurai M. Ethno medicinal plants used to cure diarrhoea and dysentery in Pachalur hills, Tamil Nadu. Journal of Appl Pharmaceutical Sci 2011; 94-97.
6. Khare CP. Encyclopaedia of Indian medicinal plants, Spr. Publication, 2004, 314-315.
7. Sakshi S, Vineet G, Analgesic and antipyretic activity of *M. elengi*, Pharmacology, 2011, 3:1-6.
8. Wagner H, Bladt S. Plant drug analysis, Berlin, Germany, Springer-Verlag, 1984.
9. Sharma PP, Mujundar AM. Indian journal Traditi Knowledge 2003; 2:292-296.
10. Ghosh A. Indian Jour. Traditi, Knowledge 2003; 2:393-396.
11. Grierson DS, Afolayan. Journal Ethno pharmacology 67:327-332.1999
12. Kirtikar KR. Indian medic. Plants with illustrations 2001.
13. Almeida MR, Vol 3A, Orient Press, Mumbai, 2001, 168.
14. Bharat G, Parabia MH. Int J Pharm Pharmaceut Sci 2010; 2, 110.
15. Dagne K, Jonsson A. J. of the Science of Food and Agriculture 1997; 73:274.
16. Almeida MR. Vol 3A Orient Press, Mumbai, 168, 2001.
17. Sushruta Samhita of Sushruta, chapter 39, verse no. 5 Varanasi, 423, 2005.
18. Singh KL, Srivastava P. *M. elengi* Linn. Potential medicinal plant Archives of Biomedical Sciences, 2014, 18-29.
19. Kumar B, Suresh RB, Karmakar, Asian Pacific Journal of Tropical Biomedicine vol. 2. 2012.
20. War DS, Raza MA. *In vitro* antibacterial activity of extracts of *M. elengi*, African journal. Jour microbiological Research 2009; 458-462.
21. Gopalkrishnan B, Shimpi SN. Seeds of *M. elengi* Pharmacognosy and Phytochemitry. Int J Pharmacog Phytochem Res 2010; 3(1):13-17.
22. Venkateswara Rao, Sharlene C. Secondary metabolites from the flowers of *M. elengi* Linn. G. Scholars Research Library, Der Pharmacia Letter 2012; 4(6):1817-1820.
23. Shahwar D, Raza MA. Antioxidant potential of phenolic extracts of *M. elengi*. Asian Pacific Journal of Tropical Biomedicine 2012; 2(7):547.
24. Harborne JB. Phytochemical methods, A guide to marital techniques of plant analysis. Edn 3, champan and hall London, 1998.
25. Bauer AW, Kirby W. Antibiotics susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45:493-496.