WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 4, 1380-1391. Research Article ISSN 2277– 7105

EVALUATION OF ESSENTIAL PHYTOCONSTITUENTS OF *AEGLE MARMELOS* USING LEAF EXTRACTS

A. U. Manjula* and Prema Sampath Kumar

Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women, Chennai-600008, Tamil Nadu, India.

Article Received on
06 Feb 2016,
Revised on 27 Feb 2016,
Accepted on 18 Mar 2016
DOI: 10.20959/wjpr20164-5968

***Correspondence for Author**
A. U. Manjula
Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women, Chennai-600008, Tamil Nadu, India.

ABSTRACT

Medicinal plants are incredible source of bioactive constituents. Identification and elucidation of phytoconstituents leads to the discovery of drugs and to attain better approach to various ailments. *Aegle marmelos* (L.) Correa (Rutaceae) is a traditionally used medicinal plant known for siddha and ayurvedic formulations. The objective of this study was to identify and examine the bioactive chemical compounds and green synthesis of silver nanoparticles of dried leaves powder of *A.marmelos* subjected to solvents such as benzene, chloroform, methanol, ethanol and water (Aqueous). The phytochemical screening revealed the presence of essential therapeutic components such as alkaloids, flavonoids, terpenoids, tannin, glycosides, phenolic compounds, etc. The methanol and aqueous leaf extracts showed maximum active phytochemical compounds compared to the other solvents. TLC (Thin layer chromatography) profiling of leaves provides a clear sketch about the presence of potential phytoconstituents in the extract. The Ag^{+} ion reduction for silver nanoparticles synthesis was done using 1 mM silver nitrate ($AgNO_3$) solution. The reduction was recorded at 418 and 422nm using UV-Visible spectrophotometer and SEM imaging confirmed the presence of cluster of spherical nanoparticle with the size of 20nm using ZEISS EVO 40 EP Electron microscope. Thus, the current research may be considered for selection of solvent for an ideal bioactivity studies and pharmacological research and also to obtain novel drugs using *A.marmelos* leaves.

KEYWORDS: *Aegle marmelos*, Phytochemical, TLC, Silver nanoparticles, Characterization.

INTRODUCTION

Since ancient times, medicinal plants have been recorded as the primary source of active ingredients used for treating different diseases. They also serve as food supplements, pharmaceutical formulations, lead compounds for synthetic drugs.[1] Herbal drugs are the source of traditional system of medicine in India viz., Ayurveda, Unani, Siddha, Homeopathy and Naturopathy.[2] Herbal drugs are significantly important because of their effectiveness, consistency, low cost and minimal side effects.[3]

Aegle marmelos (L.) Correa (Rutaceae) commonly known as 'Bael' or 'Bengal quince' (English), 'Vilvam' (Tamil) and has been employed in folk therapy since ancient time for curing human ailments.[4] It is a sacred tree for Hindu Religion, native to India.[5] Leaves, fruits, stem and roots of this plant have been used in ethno medicines for several medicinal properties. The leaves possess astringent, expectorant, anti-catarrhal, anti-asthmatic, antioxidant, anti-ulcerous and ophthalmic properties.[6] The leaves are widely used to treat diarrhea, dyspepsia, dysentery, seminal weakness, diabetes, snake bite, skin and eye diseases. Leaf juice is boiled with coconut oil and massaged on the head for headache.[7] Extraction, characterization and evaluation of the bioactive plant constituents has been a challenging task for the researchers. Silver nanoparticles are nanoparticles made of silver ions widely applied to various fields in day-to-day life such as household and personal care products due to its medicinal properties. Nanoparticles synthesized using plant extracts serves as natural encapsulation film and are toxic free.

The present investigation was initiated to explore the essential bioactive compounds profile in the leaves of *A.marmelos* and thereby to provide the knowledge about utilization of this natural flora as therapeutic agents.

MATERIALS AND METHODS

Fresh leaves of *A.marmelos* were collected, shade dried and ground into fine powder (Fig. 1). The powdered leaves were dissolved in benzene, chloroform, ethanol, methanol and distilled water (aqueous) separately. The mixtures were kept in dark for 24hrs at room temperature, then filtered and kept in water bath at 40°C till all the solvents had completely evaporated. The crude extracts obtained were stored for further use.

Fig. 1: *A.marmelos* leaves powder

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The benzene, chloroform, ethanol, methanol and aqueous extract of the *A.marmelos* leaves were screened for the presence of essential phytoconstituents using the standard method (Table 1).[8]

Table 1: Procedure for phytochemical screening

S.NO.	REAGENTS USED	OBSERVATION	INFERENCE	REFERENCES
1.	1ml extract+ 1ml Glacial acetic acid+ Few drops of 2% Ferric chloride + 1ml Conc. H_2SO_4 (Keller killiani test)	Brown ring	Cardiac glycosides	[9]
2.	0.5ml extract + 2ml Chloroform+ Conc. H_2SO_4 (Salkowski test)	Reddish Brown ring	Terpenoides	-
3.	0.2ml extract+ 2ml acetic acid+ Conc. H_2SO_4 (Liebermann-Burchard test)	Green color (upper layer)	Steroids	-
		Deep red color (lower layer)	Triterpenoids	
4.	1ml extract+ 2ml 10% NaOH + dilute HCl (Sodium hydroxide test)	Yellow color	Flavonoids	[8]
5.	0.5ml extract+ 10ml distilled water+ few drops 1% Ferric chloride	Blue-black or blue-green	Tannin	[8]
6.	0.5ml extract + 1% HCl - boiled for 5mins	Red precipitate	Phlobatannin	[9]
7.	0.5ml extract+ 5ml distilled water- shaken vigorously for 15mins (Foam test)	Stable foam	Saponin	[10]
8.	0.5ml extract+ Few drops Molisch's reagent+ Conc. H_2SO_4+ Cooled+ 1ml distilled water (Molisch's test)	Red or dull Violet at interphase	Carbohydrate	[11]
9.	1ml extract+ few drops of Dragendorff's reagent (Dragendorff's test)	Orange precipitate	Alkaloids	[10]
10.	1ml extract+ 1ml Conc. H_2SO_4	Red color	Quinone	-
11.	1ml extract+ 1ml distilled water+ 1ml of NaOH solution	Yellow	Glycosides	-

12.	2ml extract+ 5% Ferric Chloride (Ferric Chloride test)	Blue or Black color	Phenols	[12]
13.	1ml extract+1ml 1N NaOH solution (Fluorescence test)	Blue-green fluorescence	Coumarins	-
14.	2ml extract+ 2% Ninhydrin - boiled for 5mins (Ninhydrin test)	Blue color	Protein & Amino acid	-
15.	1ml extract+ 15ml absolute alcohol – constant stirring	Precipitate formation	Gums & Mucilage	-
16.	1 drop extract spotted between filter paper (Spot test)	Oil staining	Fixed oils & Fats	
17.	1ml extract+ 5ml Chloroform+ 1ml Conc. H_2SO_4 (Salkowski test)	Reddish brown color	Phytosterols	-
18.	2ml extract+ 1ml 2N NaOH –heated for 5mins at 100°C	Bluish green color	Anthocyanin	-
		Yellow color	Betacyanin	
19.	0.5ml extract+ 10ml distilled water- shaken for 5mins	Turbidity formation	Resin	-
20.	0.5ml extract+ 5ml Ether- pressed on filter paper & evaporated	Transparence appearance	Fatty acid	[13]

THIN LAYER CHROMATOGRAPHY STUDIES

The presence of bioactive secondary metabolites from the leaves of *A.marmelos* was qualitatively analyzed by thin layer chromatography.[14, 15]

Extract preparation: The aqueous and methanolic extracts of leaves of *A.marmelos* were prepared freshly by grinding in mortar and pestle.

Slurry preparation: The leaves extracts were subjected to thin layer chromatography to separate the active compounds. The glass slides were prepared using slurry of silica gel G in distilled water. The uniform silica gel slurry with thickness of 0.25mm was made and allowed to dry at room temperature and placed in the oven at 100°C for 30 minutes to activate the silica gel. The plate was then cooled to room temperature for 15 minutes.

Using a microcapillary tube, a small drop of methanol and aqueous extract of leaves of *A.marmelos* was placed on the glass slide, 1cm above the bottom. This spot was allowed to dry and the glass slide was then placed in a glass chamber covered with lid and allowed for saturation with the solvent mixture. When the solvent reached 1 cm below the top, the plates were taken out of the chamber and detected with the respective spraying reagents.

1. TLC study of alkaloids: The alkaloids were separated using the solvent mixture chloroform and methanol (15:1). The color and R_f value of the separated alkaloids were

recorded both under Ultra Violet (254nm) and visible light after spraying with Dragendroff's reagent.[16]

2. TLC study of flavonoids

The flavonoids were separated using chloroform and methanol (19:1) solvent mixture. The color and R_f value of these spots were recorded under ultraviolet light (254nm) and visible light after spraying with Aluminium chloride solution (1 gm aluminum chloride in 100 ml 95% ethanol).[16]

3. TLC study of saponins

The saponins were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The color and R_f values of these spots were recorded under ultraviolet light (254nm) and visible light after spraying with formaldehyde-phosphoric acid reagent (0.03g formaldehyde in 100ml of 85% phosphoric acid).[16]

4. TLC study of sterols

The sterols were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The color and R_f values of these spots were recorded under ultraviolet light (254nm) and visible light after spraying with vanillin- H_2SO_4 spray reagent (10% vanillin in ethanol: conc. H_2SO_4 in 2:1 ratio).[16]

5. TLC of tannins

The tannins were separated using chloroform, methanol and water (65:35:10) as mobile phase. The color and R_f values of these spots were recorded under ultraviolet light (254nm) and visible light by spraying plate with a freshly prepared solution of Fast Blue B (0.5g Fast Blue B in 90ml acetone and 10ml water).[17]

After detection, the plates were examined to locate the compounds. The plates were first viewed under normal light and the separation zone is marked. Then, the plate is examined under UV light (254nm) and the fluorescence is marked.

The R_f values of the spots were calculated by the formula,

$$R_f = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

SILVER NANOPARTICLE SYNTHESIS

Synthesis of silver nanoparticles (SNP): The fresh leaves of *A.marmelos* (20g) was washed thoroughly and finely cut into pieces and added to 100 mL of sterilized double distilled water. The mixture was boiled for 15 min, left undisturbed overnight and then decanted using Whatman filter paper no 1. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution and incubated at room temperature. As a result, a brown-yellow solution was developed indicating the formation of silver nanoparticles and it was further confirmed by UV-Vis spectrum analysis and SEM imaging.[18]

Characterization of the synthesized silver nanoparticles using UV-Vis spectroscopy

Synthesis of silver nanoparticles solution with leaves extract may be visualized by Ultraviolet-Visible spectroscopy. The bio-reduction of the Ag+ ions was monitored and UV-Vis spectra absorption peak were recorded on a Perkin Elmer UV Spectrophotometer in 400-600 nm range operated at a resolution of 1 nm.[19]

SEM imaging: The prepared SNPs were characterized using high resolution. The samples were prepared by a coating a drop of suspended silver solution on to a copper grid and allowing the solvent (water) to evaporate. The samples were left to dry at room temperature. SEM observations were carried out on a ZEISS EVO 40 EP Electron microscope and the image was photographed and recorded.[20]

RESULTS AND DISCUSSION

Based on the literature, *A.marmelos* was selected for its enviable pharmacological properties and the active medicinal constituents in the plant.

Qualitative phytochemical analysis: Qualitative phytochemical analysis was performed to detect the active components in the plant which play an important role in drug discovery and chemical biology. The phytochemical analysis of leaves of *A.marmelos* was done with Benzene, Chloroform, Ethanol, Methanol and Aqueous extracts (Table 2). The following results were observed.

The phytochemical screening has revealed the presence of essential secondary metabolites in the leaves which are considered as active medicinal chemical constituents. The most of the active phytochemical constituents were found to be higher in the methanol and aqueous extracts compared to the other extracts. It was also reported in literature, that different

organic extracts of the leaves of *A.marmelos* possess alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids.[21] Each phytocompound has its own medicinal and economical benefits such as Alkaloids as anesthetics; Glycosides aid in digestion; Flavonoids and other phenolic compounds as antioxidants, antimicrobial; Terpenoids for acute inflammation; Saponin for soap and anticancer activity; Tannin for dyeing; Steroids for asthma, chronic inflammation, etc.,

Table 2: Phytochemical analysis of *A.marmelos* leaves - Results

Phytochemical Tests	Benzene Extract	Chloro-Form Extract	Ethanol Extract	Methanol Extract	Aqueous Extract
Alkaloids	--	++	++	++	++
Anthocyanin & Betacyanin	A(--) B(--)	A(++) B(--)	A(--) B(--)	A(--) B(--)	A(++) B(--)
Carbohydrate	++	--	++	++	++
Cardiac Glycosides	--	--	++	++	++
Coumarin	++	--	++	++	++
Fatty Acid	--	--	--	++	--
Fixed Oil	--	--	--	++	--
Flavonoids	--	++	++	++	++
Glycosides	--	++	++	++	++
Gum & Mucilage	--	--	++	++	++
Phenols	++	++	++	++	++
Phlobatanin	--	++	--	--	--
Phytosterol	--	++	--	++	++
Protein & Aminoacid	--	--	++	++	++
Quinone	--	--	--	++	--
Resin	++	++	--	++	++
Saponin	++	++	++	++	++
Steroids & Triterpenoids	S(++) T(--)	S(++) T(--)	S(--) T(++)	S(++) T(++)	S(++) T(--)
Tannin	++	--	++	++	++
Terpenoids	--	--	++	++	++

++ - *PRESENT* -- - *ABSENT*

A - *ANTHOCYANIN* B - *BETACYANIN*

S - *STEROIDS* T- *TRITERPENOIDS*

TLC Screening: The TLC was studied for aqueous and methanol extracts of leaves of *A.marmelos* using different solvent phase for different secondary metabolites. Different colored bands were observed under UV (254nm) and visible light and the R_f values were calculated.

The TLC study of alkaloids revealed the presence of 2 spots in aqueous leaf extract with R_f value 0.218 and 0.327, while methanol leaf extract showed 2 spots with R_f value of 0.200 and 0.236 (Table 3). The TLC study of flavonoids revealed presence of minimum of 1 spot in aqueous leaf extract with R_f value of 0.909, while methanol leaf extract showed 2 spots with R_f value of 0.636 and 0.854 (Table 4). The TLC study of saponin shows presence of 1 spot in aqueous leaf extract with R_f value of 0.836, while methanol leaf extract showed maximum of 3 spots with R_f value of 0.836, 0.872 and 0.909 (Table 5). The TLC study of sterols reveals presence of 1 spot in aqueous leaf extract with R_f value of 0.636, while methanol leaf extract also showed only1 spot with R_f value of 0.654 (Table 6). The TLC study of tannin have shown the presence of only 1 spot in aqueous leaf extract with R_f value of 0.636, while methanol leaf extract also showed only 1 spot with R_f value of 0.654 (Table 7). Profiles obtained for different samples using different solvent system showed difference in the number of bands. When methanolic extract of *A.marmelos* was used, separation and intensity of bands were more.[22] The variations in the Rf values helps in understanding the polarity of the phytoconstituents and selection of suitable solvent system.

Table 3: TLC study of Alkaloid – *A.marmelos* leaves

S.NO.	SOLVENT	TEST SAMPLE	Rf VALUES
1.	Chloroform : Methanol (15:1)	Aqueous extract	0.218 0.327
2.		Methanolic extract	0.200 0.236

Table 4: TLC study of Flavonoid – *A.marmelos* leaves

S.NO.	SOLVENT	TEST SAMPLE	Rf VALUES
1.	Chloroform : Methanol (19:1)	Aqueous extract	0.909
2.		Methanolic extract	0.636 0.854

Table 5: TLC study of Saponin – *A.marmelos* leaves

S.NO.	SOLVENT	TEST SAMPLE	Rf VALUES
1.	Chloroform : Glacial acetic acid: Methanol : Water (64:34:12:8)	Aqueous extract	0.836
2.		Methanolic extract	0.836 0.872 0.909

Table6: TLC study of Sterols – *A.marmelos* leaves

S.NO.	SOLVENT	TEST SAMPLE	Rf VALUES
1.	Chloroform : Glacial acetic acid: Methanol : Water (64:34:12:8)	Aqueous extract	0.636
2.		Methanolic extract	0.654

Table 7: TLC study of Tannin – *A.marmelos* leaves

S.NO.	SOLVENT	TEST SAMPLE	Rf VALUES
1.	Chloroform : Methanol : Water (65:35:10)	Aqueous extract	0.636
2.		Methanolic extract	0.654

Silver nanoparticle synthesis: Reduction of Ag^{+} ion into silver nanoparticles during exposure of *A.marmelos* leaves extracts to silver nitrate solution and followed by a color change of dark yellowish-brown in aqueous solution (Fig. 2). The color change is the characteristic phenomenon for visual identification of the reduction property of the extract. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded with the absorption peaks at 418 and 422nm for leaves extract (Fig. 3). The absorbance usually depends on the nano size of the particle. The so obtained nanoparticles of *A.marmelos* from SEM image were found to be highly dense and stable with an average size of 20nm in leaves extract (Fig. 4). The nanoparticles are aggregated together during drying of sample. Therefore, the morphology resembles colloidal nature.[23]

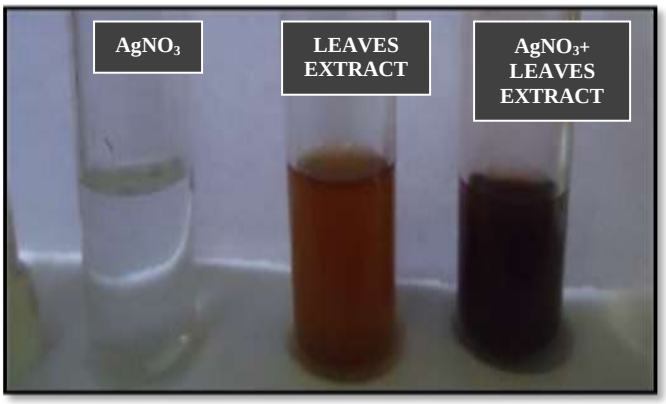

Fig. 2: Synthesis of SNP

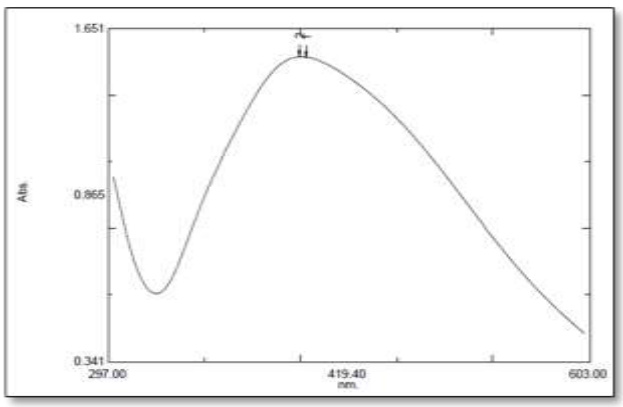

Fig. 3: UV-Vis spectrum of synthesized SNP

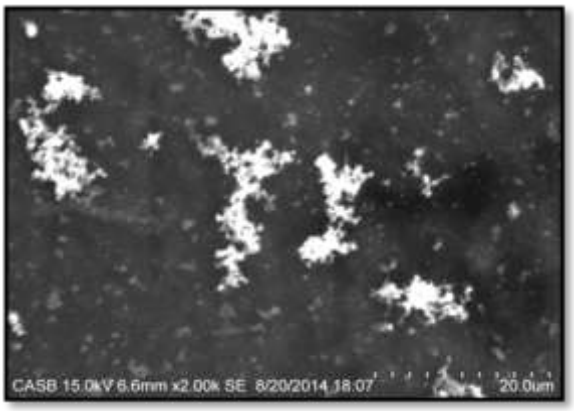

Fig. 4: SEM image of synthesized extract

CONCLUSION

The phytoconstituents in the leaves of *A.marmelos* provides pathway for pharmacological and bioactivity investigations in finding solution for prevailing diseases and discovering new drugs. Synthesis of silver nanoparticle is the latest approach in the medical application and promising tool for diagnostic and therapeutic applications. *A.marmelos* act as a substrate for silver nanoparticle synthesis. The investigations provide valuable knowledge of the plant on Ethnomedicinal benefits. It is suggested that *A.marmelos* could be a potential natural drug for various chronic diseases and pharmaceutical applications.

ACKNOWLEDGEMENT

The authors wish to thank the Staffs of Department of Plant Biology and Plant Biotechnology and The Principal, Ethiraj College for Women, for their constant support and encouragement.

REFERENCES

1. Ncube NS, Afolayan AJ and Okoh A. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotechnol, 2008; 7 (12): 1897–1906.
2. Ashok DB Vaidya and Thomas PA Devasagayam. Current Status of Herbal Drugs in India: An Overview, J Clin Biochem Nutr , 2007; 41(1): 1–11.
3. Valiathan MS. Healing Plants. Curr Sci, 1998; 75(11): 1122–1126.
4. Ayurvedic Pharmacopoeia of India (1988). The Ayurvedic Pharmacopoeia of India. Vol-IV, Part- I, 1st Edition, Govt. of India, Ministry of health and family welfare, Dept. of AYUSH, New Delhi.
5. Bailey LH: How Plants Get Their Names. Dover Publications Inc., New York, New York; 1963.
6. Warrier PK, Nambiar VPK and Ramankutty C, Indian medicinal plants: A compendium of 500 Species. Orient Blackswan, Hyderabad, India. 1993; I: 62–66.
7. Kingston C, Nisha BS, Kiruba S, Jeeva S. Ethnomedicinal plants used by indigenous community in traditional healthcare system. Ethnobot Leaflets, 2007; 11: 32–37.
8. Trease GE and Evans WC. Pharmacognosy, 15th ed., London: Saunders Publishers, 2002, 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.
9. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol, 2005; 4: 685–688.

10. Sofowora A, Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd. 1993; 134–156.
11. RafiqKhan Mohammed, Dhanya Radhakrishnan, Mufeedha Mohamed, Mohamed Shamseer, Sheethal Johnson. Phytochemical screening of Aegle marmelos (L.) Correa fruit pulp: a potential source of ethnomedicine. World J Pharm Res, 2013; 2(6): 2919-2927.
12. Mace ME. Histochemical localization of phenols in healthy and diseased banana roots. Physiol Plant, 1963; 16(4): 915–925.
13. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello A, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Trop J Pharm Res, 2008; 7(3): 1019.
14. Harborne JB. Phytochemical methods. 2nd ed., Springer, Chapman and Hall, London and New York, 1984.
15. Stahl E. Thin Layer Chromatography, 2nd edition, Springer-Verlag, NY, 1969.
16. Dheeba B, Sampathkumar P, Sathiya Priya RR and Kannan M. Phytochemical studies and evaluation of antioxidant potential of various extracts of Aegle marmelos Bark. Pharmacologyonline, 2010; 3: 831–839.
17. Gangwal A. Extraction, Estimation and Thin Layer Chromatography of Tannins: A Review. International Journal of Pharmaceutical and Chemical Sciences, 2013; 2(3): 1585–1588.
18. Alagumuthu G and Kirubha R. Biogenic Synthesis of Silver Nanoparticles using Aegle marmelos fruit extract and their Antibacterial potential. Asian Journal of Research in Chemistry, 2013; 6(9): 839–844.
19. Ajithadas Aruna, Ramraj Nandhini, Venkatachalam Karthikeyan and Pandi Bose. Synthesis and Characterization of Silver Nanoparticles of Insulin Plant (costus pictus D. Don) Leaves. Asian Journal of Biomedical and Pharmaceutical Sciences, 2014; 4(34): 1–6.
20. Dinesh S, Karthikeyan S and Arumugam P. Biosynthesis of silver nanoparticles from Glycyrrhiza glabra root extract. Archives of Applied Science Research, 2012; 4(1):178–187.
21. Venkatesan D, Karrunakarn CM, Kumar SS and Swamy PTP. Identification of phytochemical constituents of Aegle marmelos responsible for antimicrobial

activity against selected pathogenic organisms. Ethnobotanical Leaflets, 2009; 13, 1362–1372.

22. Kumar Ashwani and Bhardwaj Ashish. Comparitive qualitative and quantitative characterization among North Indian Tribulus terrestris. International Research Journal of Pharmacy, 2012; 3(6): 212–218.
23. Sadowski Z, Maliszewska IH, Grochowalska B, Polowczyk I , Kozlecki T. Synthesis of silver nanoparticles using microorganism. Materials science Poland, 2008; 26(2): 420–423.