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Antibacterial and Phytochemical Analyses of Bioactive Compounds of Maytenus emarginata (Will.)

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Abstract : Maytenus emarginata is a dry deciduous tree in common from the cost, in scrub jungle, up to 1000m Sri Lanka, Southeast Asia, Malaysia to north Queensland. The following results have been observed from the leaf extract of M. emarginata. The Acetone extract of M. emarginata (Leaf) exhibited high inhibitory activity against twelve bacterial strains. High level of inhibitions was found against Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella paratyphi, Vibrio cholerae, Streptococcus pneumoniae, Proteus vulgaris, Escherichia coli, Enterobacter aerogenes, Bacillus cereus and Serratia marcescens while others had shown very moderate inhibitions against the test strains. Preliminary Phytochemical analysis on the methanol and acetone leaf extracts of M. emarginata revealed the presence of alkaloids, flavonoids, saponins and sterols etc., In the GC-MS analysis, the acetone leaf extract of M. emarginata showed 24 phytochemical compounds, Namely Decane, Cyclopentene, Phthalic acid, isobutyl, octadecyl ester etc., Therefore the plant species of M. emarginata is highly potential medicinal plants with several essential phytocontituents which are used mainly as flavour ingredient, antimicrobial agent.

Key Words: M. emarginata, Antibacterial assay and GC-MS

INTRODUCTION

Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning. The screening of plant products for antimicrobial activity has shown that the higher plants represent potential source of novel antibiotic prototypes (Afolayan, 2003).

Maytenus is a genus of flowering plants in the staff vine family, Celastraceae. Members of the genus are distributed throughout Central and South America, Southeast Asia, Micronesia and Australasia, the Indian Ocean and Africa. They grow in a very wide variety of climates, from tropical to subpolar.

Botanical Name:

Maytenus emarginata (Willd.) Local Name: Kankera

Tamil: Kattangi, Nandunarani, Valulu-Va

English: Thorny staff tree

MATERIALS AND METHODS

Specimen Collection and Authentication

M. emerginata (RHT67543) was collected from Kolli hills in the month of 17.010. 2021. The plant materials were identified and authenticated by John Britto, the former Director and Head, Rapinat Herbarium, Centre for Molecular Systematic, St Joseph's College (Autonomous), Tiruchirapalli, Tamilnadu, India. The voucher specimens were deposited at Rapinat Herbarium.

Qualitative Phytochemical Analysis

Detection of Alkaloids

Solvent free extract 50 mg was stirred with few ml of dil. HCl and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

a) Mayer's Test

To a few ml of filtrate, one or two drops of Mayer's reagent were added by the side of the test tube. A white creamy precipitate indicated the test as positive.

b) Wagner's Test

To a few ml of filtrate, few drops of Wagner's reagent was added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

c) Hager's Test
To a few ml of filtrate 1 or 2 ml of Hager's reagent (Saturated aqueous Solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

d) Dragendroff's Test
To a few ml of Filtrate 1 or 2 ml of Dragendroff's reagent was added. A prominent yellow precipitate indicated the test as positive.

Detection of Carbohydrates and Glycosides
The extract (100mg) was dissolved in 5 ml water and filtered. The filtrate was subjected to the following tests:

a) Molish's Test
To 2 ml of filtrate two drops of alcoholic solution of α- naphthol was added, the mixture was shaken well and 1 ml of con H_2SO_4 was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

b) Fehling's Test
1 ml of filtrate was boiled on water bath. To this, 1 ml of Fehling solutions A and B were added. A red precipitate indicated the presence of sugar. Fehling's solution A: $CuSO_4$(34.66 g) was dissolved in distilled water and made up to 500 ml using distilled water. Fehling's solution B: (Potassium sodium tartarated (173 g) and NaOH (50g) was dissolved in water and made up to 500 ml.

c) Barfoed's Test
To 1 ml of filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 min. Red Precipitate indicated the presence of sugar Barfoed"s Reagent. Copper acetate 30.5 g was dissolved in 1.8 ml of glacial acetic acid.

d) Benedict's Test

To 0.5 ml of filtrate, 1 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 mins. A characteristic-colored precipitate indicated the presence of sugar.

Detection of Glycosides
50 mg of extract was hydrolyzed with concentrated HCl for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following tests:

a) Borntrage's Test
To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicated the presence of glycosides.

b) Legal's Test

50 mg of the extract was dissolved in pyridine. Sodium nitro preside solution was added and made alkaline using 10% NaOH. Presence of glycosides was indicated by pink color.

Detection of Saponins
To 1ml of extract, add 2ml of distilled water and shaken vigorously and allowed to stand for 10 min. There is the development of foam on the surface of the mixture. Then shake for 10 minutes, it indicates the presence of saponins.

Detection of Proteins and Amino Acids
The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through Whatman no: 1 filter paper and filtrate were subjected to tests for proteins and amino acids.

a) Millon's Test
To 2 ml filtrate, few drops of millon's reagent were added. A white precipitate indicated the presence of proteins.

b) Biuret Test
An aliquot of 2 ml of filtrate was heated with 1 drop of 2 % $CuSO_4$ solution. To this 1 ml of ethanol (95%) was added, followed by excess of KOH Pellets. Pink color in the ethanolic layers indicated the presence of proteins.

c) Ninhydrin Test
2 drops of Ninhydrin solution (10 mg of Ninhydrin in 200 ml of acetone) were added to 2 ml of aqueous filtrate. A characteristic purple color indicated the presence of amino acids.

Detection of Phytosterols

a) Libermann – Burchard's Test
The extract (50 mg) was dissolved in 2 ml acetic anhydride. To these one or two drops of concentrated H_2SO_4 were added slowly along the sides of the test tube. An array of color change showed the presence of phytosterols.

Detection of Phenolic Compounds and Tannins
a) Ferric Chloride Test
The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drop of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

b) Lead Acetate Test
The extract (50 mg) was dissolved in distilled water and to this 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

c) Gelatin Test
The extract (50 mg) was dissolved in 50 ml of distilled water 2 ml of 1% solution of gelation containing 10% sodium chloride was added to it. White precipitate indicated the presence of phenolic compounds.

d) Alkaline Reagent Test
An aqueous solution of the extract was heated with 10% NH4OH solution. Yellow fluorescence indicated the presence of flavonoids.

e) Magnesium and Hydrochloric Test
The extract (50 mg) was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated HCl were added (dropioire). If any pink to crimson developed, presence of flavanol glycoside was inferred.

Detection of Volatile Oil
In volatile oil estimation apparatus, 50 g of powdered material (crude drug) was taken and subjected to hydro distillation. The distillate was collected in graduated tube of the assembly wherein the aqueous portion automatically separated out from the volatile oil.

Test for Steroids
10 ml of all extract of the test plant was evaporated to a dry mass and the mass dissolved in 0.5 ml of chloroform. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green color or a mixture of these two shades shows the presence of steroidal compounds.

Test for starch

To mix 3 ml test solution was added a few drops of dilute iodine solution. Blue color indicated the presence starch. Color disappears on boiling and reappears on cooling.

Test for flavonoids

a) Shinoda test

To 2ml test solution, a few fragments of magnesium ribbon were added and to it con. sulphuric acid was added drop wise. Pink scarlet or crimson red appeared.

b) Zinc chloride reduction test

To 2ml test solution, a mixture of zinc dust and con. HCl were added. A red color was obtained after few minutes.

c)Alkaline reagent test

To 2ml test solution, sodium hydroxide solution was added to give a yellow or red color.

Gas Chromatography - Mass Spectrometry

Gas Chromatography - Mass Spectrometry (Finnigan Matt GCO Mass Spectrometer) is one of the so called hyphenated analytical techniques. As the name implies, it is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. Gas chromatography separates the components of a mixture and mass spectrometry characterizes each of the components individually. By combining the two techniques one can evaluate a solution (both qualitatively and quantitatively) containing a number of chemicals. They are used extensively in the medical, pharmacological and law enforcement fields.

One microliter of the filtrate was injected into the Gas Chromatography column. The sample gets evaporated and carried away by carrier gas helium. It gets segregated into individual components. The sample fraction coming out of the column was led into mass detector and the mass spectrum of each component was recorded. The mass spectrum of unknown component was compared with the known spectrum of NIST library and the components were identified.

Specifications

Column: Elite – 1 (100% Dimethyl poly siloxane), 30m×0.25mm ID ×1µm df.

Equipment: GC Clarus 500 Perkin Elmer.

Carrier Gas: Helium 1 ml/min.

Detector: Mass detector– Turbo mass gold– Perkin Elmer, Software –Turbomass 5-1.

Sample injected: 1µl (one Micro litre) was injected with a Hamilton syringe to the GC-MS manually.

Split: 10:1.
Oven Temperature Programme
110˚ – 2 min hold
Up to 280˚ at the rate of 5˚ / min – 9min hold
Injector temp: 250˚C.
Total GC time: 45min.

MS Programme

Library used: NIST ver.2.1.

Inlet line temperature: 200˚C

Source temperature: 200˚C

Electron energy: 70ev

Mass scan: (m/z) 45-450

MS time: 46min

Identification of Components

Interpretation of mass spectrum (GC-MS) was conducted using database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared with the spectrum of known components stored in the NIST library. The retention time, molecular weight, molecular formula, and composition percentage of the sample material was recorded.

Identification of Compound Nature and Activity

Identification of Compound Nature and Activity was conducted using database of Duke's Phytochemical and Ethno-botanical Database.

Anti-Microbial Studies

Disc diffusion assay

Disc diffusion method is used for the rapid determination of the drug or a particular substance on a specific bacterium. This method consists of impregnating small circular disc of standard filter paper with given amount of a chosen concentration of substance. The discs are placed on plates of culture medium that has been seeded with a test bacterial inoculum. After incubation the diameter of the clear zone of inhibition surrounding the deposit of substance is taken as a measure of the inhibitory power of the particular substance against the particular test organism.

Disc preparation

The filter paper discs of uniform size are impregnated with the compound (plant extract) usually consisting of absorbent paper. It is most convenient to use Whatman No.1 filter paper for preparing the discs. Dried discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These dried discs were used for the test.

Procedure

The vacuum dried extract was reconstituted with DMSO to obtain a stock solution of, 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. Nutrient agar (Hi Media Laboratories Pvt. Ltd. Mumbai) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. Discs of 6 mm were punched from Whatman No.1 filter paper. 10µl of each stock concentration (i.e., 1000µg, 500µg, 250µg and 125µg) of the extract were respectively introduced in the discs using sterile automatic pipettes. The discs were allowed to dry at room temperature for 2 hrs. and were placed at equidistance in each of the plates using a sterile forceps. The plates were incubated to 37°C for 24 hrs for bacteria and at 25˚C for 48 hours for fungi. The control antibiotic was Streptomycin (10µg) for bacteria and Nystatin (15 µg) for fungi (Hi Media Laboratories Pvt. Ltd. Mumbai) was used. Diameters of the inhibition zones were measured. The antibacterial/antifungal activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract at the four concentrations.

Test Bacteria

The microbial strains used are Escherichia coli (MTCC # 119), Vibrio cholerae (ATCC #14104), Enterobacter aerogenes (MTCC # 2990), Klebsiella pneumonia (MTCC # 3040), Serratia marcescens (MTCC # 2645), Salmonella paratyphi (MTCC # 734), Pseudomonas aeruginosa (MTCC # 2474), Staphylococcus aureus (MTCC # 3163), Proteus mirabilis (MTCC # 1429), Proteus vulgaris (MTCC # 1771), Bacillus subtilis (MTCC#441), Bacillus cereus (ATCC # 4342), Streptococous pneumoniae (ATCC # 7066).

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

In the plant M. emerginata extract of acetone, ethanol, methanol shows the presence of alkaloids. The acetone, ethanol, ethyl acetate and methanol extract of the plant shows the presence of Flavonoids. Phenol and Tannin also present in the plant extract of acetone, ethanol, ethyl acetate and methanol. Carbohydrate is present in all the five-plant extract revealed the result from the molish test. Acetone, ethanol, ethyl acetate, methanol shows the presence of Glycosides. Little amount of sterol compound also presents in the plant extract. Terpenoids also present in the plant extract of acetone, ethanol, ethyl acetate, methanol and aqueous. On doing this preliminary phytochemical analysis in M. emerginata plant, the result shows the presence of such secondary metabolites Alkaloids, Flavonoids Tannin/Phenol, Steroid, Carbohydrate, Glycoside, Terpenoids and Proteins. the entire details shown by Table

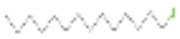
Table 4.1: Phytochemical Analysis in Maytenus emarginata plant (Leaf)

Name of Components	Test	Acetone	Ethanol	Ethyl Acetate	Petroleum Ether	Methanol	Aqueous
Alkaloids	Wager's	++	++	++	-	++	-
	Hager's	++	++	-	-	++	-
	Mayer's	++	++	-	-	+++	-
Protein	Biuret	-	-	-	+	+	-
	Con.H_2SO_4	-	+	+	-	+	+
	Xanthoproteins	+	+	+	-	-	-
Flavonoids	Pew's	+++	++	+++	-	++	-
	Shinoda	++	+++	+++	-	+++	+
	Alkaline	+++	+++	++	-	++	++
	Con.H_2SO_4	++	+++	++	-	++	++
Phenols/	$FeCl_3$	+++	+++	+	+	+++	++

Tannins							
	$K_2Cr_2O_7$	++	++	+	-	+++	+
	Lead Acetate	++	++	++	-	+++	+
	Braymer's	+++	+++	+++	+	+++	++
Saponins	Foam	-	+	-	-	-	-
	$HaHCO_3$	-	-	-	-	-	-
Glycosides	Keller-Kiliani	++	++	++	+	++	++
	Glycosides	++	++	+	-	++	+
	Libermann's	-	-	-	-	-	-
Carbohydrates	Molish	+++	++	+++	++	+++	+++
	Iodine	+	+	-	+	-	-
	Benedict's	+	+	+	+	+	++
	Fehling's	++	+	+	-	++	++
Sterols	Salkowski's	+	+	+	-	+	++
Emodins		-	-	-	-	-	-
Quinones		+	+	+	-	+	++
Anthocyanins		-	-	-	-	-	-
Terpenoids		++	++	++	-	+++	++

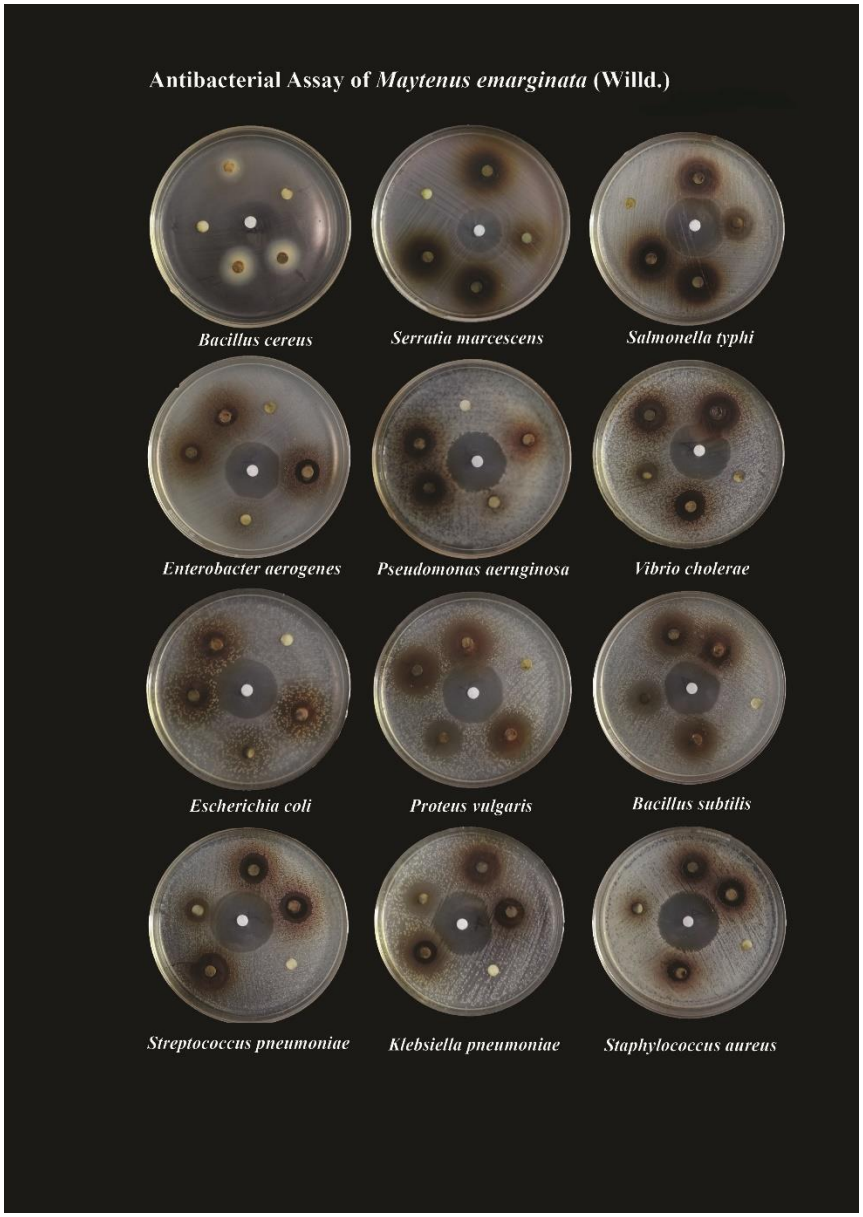
Gas Chromatography - Mass Spectrometry

The GC-MS studies on the leaf of M. emerginata shows the highest peak area (%) of 35.06 was obtained by 2-Nonen-1-ol and the lowest peak area (%) of 4.72 by Oleic acid, 3-hydroxypropyl ester was obtained. The other compounds were obtained between the peak areas of 4.72 to 35.06. The compounds such as 2,4,5- Tetradecane, 1-fluoro- / Carbonic acid, 2,2,2-richloroethy l undec-10-enyl ester/ Benzaldehyde, 2-nitro-, diaminomet Hylidenhydrazone were recorded. The detailed tabulation of GC-MS analysis has been given in table.

S.No	Name	Mole. Form	Mole. Weight	Ratio	Area	Molecular structure	Uses
1	Oleic acid, 3-hydroxypropyl ester	$C_{21}H_{40}O_3$	340.5405	3.516	4.72		Free fatty acid is obtained from the glyceride by hydrolysis, steam distillation & separation by crystallization or solvent extraction.
2	Tetradecane, 1-fluoro-	$C_{14}H_{29}F$	216.3785	3.985	33.55		Laboratory chemicals, Manufacture of substances
3	2-Nonen-1-ol	$C_9H_{18}O$	142.2386	20.648	35.06		In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. ... Recommendation for (E)-2-nonen-1-ol usage levels up to the level
4	Carbonic acid, 2,2,2-trichloroethy l undec-10-enyl ester	$C_{14}H_{23}Cl_3O_3$	345.690	21.494	15.47		medicine for the treatment of diabetes
5	Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone	$C_8H_9N_5O_2$	207.189	23.108	11.19		It is an additive in medicinal products, and is used as a flavoring agent

Antibacterial screening of the Selected plant species of M. emerginata

The acetone extract of M. emerginata (leaf) exhibited high inhibitory activity against twelve bacterial strains. Strong inhibitions were found against Streptococcus pneumonia, Escherichia coli, Klebsiella pneumonia and Proteus vulgaris. And the other hand Salmonella paratyphi, Vibrio cholera, Enterobacter aerogenes, Bacillus subtilis have shown either moderate or poor inhibitions. The ethanol extract exhibited the inhibitory activity against towel bacterial strains. Strong inhibitions were found against Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Vibrio cholerae, Enterobacter aerogenes, Bacillus subtilis, and Proteus mirabilis. And others have shown either moderate or poor inhibitions. The ethyl acetate extract exhibited high inhibitory activity against towel bacterial strains. Strong inhibitions were found against Streptococcus pneumonia, Pseudomonas aeruginosa, Bacillus cereus, Vibrio cholerae, Enterobacter aerogenes and Proteus mirabilis. And while the others had shown either moderate or poor inhibitions against the test strains. The methanol extract of M. emerginata (leaf) exhibited high inhibitory activity against towel bacterial strains. Strong inhibitions were found against Streptococcus pneumonia, Pseudomonas aeruginosa, Bacillus cereus Klebsiella pneumoniae, Staphylococcus aureus, Salmonella paratyphi, Vibrio cholerae, Enterobacter aerogenes, Bacillus subtilis, and Proteus mirabilis, and while others had shown very moderate inhibitions. The aqueous extract of M. emerginata (leaf) exhibited high inhibitory activity against towel bacterial strains. Strong inhibitions were found against Klebsiella pneumonia and the remaining showed either moderate or poor inhibition. The following plate, chart and table gives more precise information regarding antibacterial activity.



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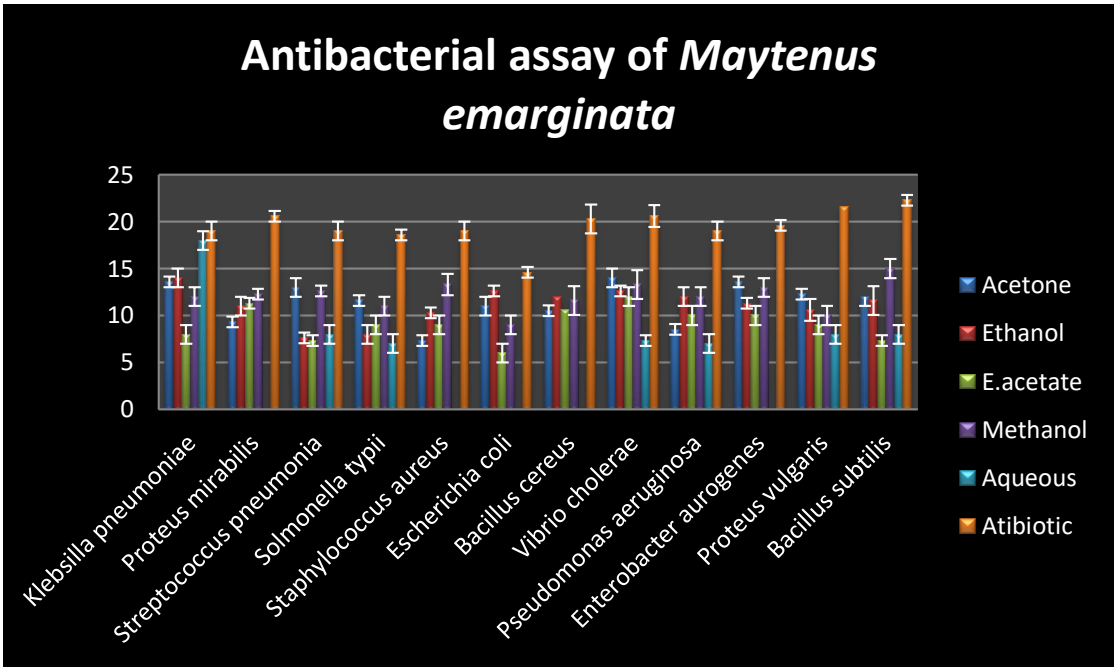

Antimicrobial Activity in Maytenus emarginata (Willd.) plant (Leaf)							
S.NO	Name of the Bacteria	Zone Inhibition (mm)					
		Acetone	Ethanol	Ethyl Acetate	Methanol	Aqueous	Streptomycin
1	Klebsilla pneumoniae	13.6±0.57	14±1	8±1	12±1	18±1	19±1
2	Proteus mirabilis	9.3±0.57	11±1	11.3±0.57	12.3±0.57	-	20.6±0.57
3	Streptococcus pneumonia	13±1	7.6±0.57	7.3±0.57	12.6±0.57	8±1	19±1
4	Salmonella typii	11.6±0.57	8±1	9±1	11±1	7±1	18.6±0.57
5	Staphylococcus aureus	7.3±0.57	10.3±0.57	9±1	13.3±1.15	-	19±1
6	Escherichia coli	11±1	12.6±0.57	6±1	9±1	-	14.6±0.57
7	Bacillus cereus	10.5±0.57	12±1	10.6±0.57	11.6±1.52	-	20.3±1.52
8	Vibrio cholerae	14±1	12.6±0.57	12±1	13.3±1.52	7.3±0.57	20.6±1.15
9	Pseudomonas aeruginosa	8.5±0.57	12±1	10±1	12±1	7±1	19±1
10	Enterobacter aurogenes	13.6±0.57	11.3±0.57	10±1	13±1	-	19.6±0.57
11	Proteus vulgaris	12.3±0.57	10.6±1.15	9±1	10±1`	8±1	21.6±1.52
12	Bacillus subtilis	12±1	11.6±1.52	7.3±0.57	15±1	8±1	22.3±0.57

Conclusion:

The Preliminary Phytochemical Screening and GCMS in M. emarginata shows the best result in the secondary metabolites such as alkaloids flavonoids and other components. From the present studies and the research findings of others, it can be ascertained that the selected species were highly medicinal plants since they contained very essential phytoconstituents which are responsible for curing many ailments. The antimicrobial studies in M. emarginata shows the good anti-bacterial activity against twelve bacteria. However, the species of M. emarginata have been extensively studied and the plant extract of acetone ethanol ethyl acetate and methanol showed a wide range of activity against some bacteria such as Klebsilla, Streptococcus and other few batteries. This research could also lead to the development of the pharmacogenetic drugs in the near future.

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Reference

1. Agnihotri S, Vaidya A.D.B .1996. A novel approach to study antibacterial properties of volatile components of selected Indian medicinal plants. Indian Journal of Experimental Biology, 34: 712-715.

2. Anjaneyulu ASN, Raju DVSN, Srinivasa Rao S. 1998. Chemical evaluation of Pergularia daemia, Indian journal of Chemistry, 37B: 318-320.

3. Afolayan AJ, Extracts from the shoots of Arctotis artotoides inhibit the growth of bacteria and fungi. Pharm. Biol.2003; 41: 22-25

4. Abramowisz M. 1990. The choice of antimicrobial drugs. Medical Leter on Drugs and Therapeutics, 32: 41–48.

5. Akgul, C. and Saglikoglu, G. 2005. Antibacterial activity of crude methanolic extract and its fractions of aerial parts of Anthemis tinctoria. Indian Journal of Biochemistry and Biophysics.42: 395-397.

6. Basha KS, Sudarsanam G, HariBabuRao D, NiazParveen. Evaluation of antibacterial activity of some medicinal plants used by Sugali tribe of Yerramalaisforest of Andhra Pradesh, India. J. Res in Plant Sci. 2011, 1:027-031.

7. Dorman H. J. D, Deans S. G. 2000. Antimicrobial agents from plants, antimicrobial activity of plant volatile oils, Journal of applied microbiology, 88: 308-316.

8. Gahalot D. and Bohara A. 2000. Toxic effect of various plant parts on the casual organism of typhoid fever, Current science, 7: 780-781.

9. Gupta A.K. 2003.Quality standards of Indian medicinal plants, Indian Council of Medical Research, 1: 221-225.

10. Kritikar KR, Basu BD, Indian Medicinal Plants, Sri Satguru Publication, New Delhi, 3rd revised enlarged edition, 1, 2002, 120

11. Larrondo J.V, Agut M, Calvo T.M. 1995. Antimicrobial activity of essences from labiates, Microbios, 82(332):171-172.

12. Lalitharani S, Kalpanadevi V and Mohan V.R. 2013.Pharmacognostic studies on the spine of Zanthoxylum rhetsa (Roxb.) Dc. Bioscience Discovery, 4(1): 05-11.

13. Meena A. K, Rao M. M, Meena R. P, Panda P and Renu. 2011. Pharmacological and Phytochemical Evidences for the Plants of Wedelia. Asian Journal of Pharmaceutical and Clinical Research, 1(1): 07-12.

14. Savrikarand S. S.,Ravishankar B., Introduction to 'Rasashaastra'-The Iatrochemistry of Ayurveda. Afr J Tradit Complement Altern med, 8 (S):66-82, (2011).

15. Shrestha, P.R., Sharma, S., Giri, R.K. and Karki, H. 2009. Antibacterial activity of medicinal plant extracts. Plant resources.31: 51-55.

16. Sheela D and Uthayakumari F. 2013. GC-MS Analysis of bioactive constituents from coastal sand dune taxon Sesuviumportula castrum (L.). Bioscience Discovery, 4(1): 47-53.

17. Vijaya Packirisamy, Vijayalakshmi, Krishna Moorthy. 2014. Antibacterial and Phytochemical Evaluation of Pergularia daemia from Nagapattinam Region. International Journal of Scientific and Research Publications, 4 (11).

18. Wani M, Sarvar F.A, Agrawal J, Deshpande J, Mathew S, Khetmalas M. 2012. Qualitative phytochemical analysis and antimicrobial activity studies of Gymnema sylvestre. R. Br. Acta Biologica Indica, 1(1): 121-124.