Chemical Fingerprint by HPLC-DAD-ESI-MS, GC-MS Analysis and Anti-Oxidant Activity of Manasamitra Vatakam: A Herbomineral Formulation

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ABSTRACT

Background: Manasamitra Vatakam is a classical ayurvedic herbomineral formulation used for the treatment of neurodegenerative properties and epileptic disorders. The wide range mixture of herbal extracts and minerals were used in the formulation. Aim: The aim of the study implies in performing the chemo-profiling, chromatographic fingerprint analysis by HPLC-DAD-ESI-MS for the selected formulations of Manasamitra Vatakam followed by the identification of bioactive compounds by Gas Chromatography – Mass Spectrometric (GC-MS) analysis, to evaluate the diffusion and dilution methods for the determination of anti-bacterial activity in the methanolic extracts of Manasamitra Vatakam (MMV). Materials and Methods: The antibacterial activity was performed by both diffusion and dilution methods whereas the antioxidant activity was performed by free radical scavenging of 2,2-diphenyl-1-picrylhydrazyl and hydrogen peroxide scavenging assay method. Results: The estimation of bioactive constituents showed positive results by qualitative analysis. Antibacterial activity of MMV was evaluated against two gram positive Staphylococcus aureus and Bacillus cereus, two gram negative Escherichia coli and Klebsiella pneumonia by disk diffusion (0.078-10µg mL⁻¹), broth dilution (0.078-10µg mL⁻¹) and broth micro dilution method (0.39-50µg mL⁻¹) respectively. The bioactive constituents were analysed by GC-MS analysis for the methanolic extract of the formulation. Conclusion: To conclude, the formulation was found abundant with phenolic and flavonoid compounds by HPLC-ESI-MS analysis, the bioactive compounds identified are responsible for the anti-bacterial activity. The broth microdilution method performed by resazurin method was observed as the fast screening, sensitive and accurate method for the quantitative determination of anti-bacterial activity. Key words: Classical formulation, Phytochemicals, MIC, Diffusion and dilution methods, Heavy metals.

INTRODUCTION

Globally, India is the largest producer of medicinal herbs used for enormous ailments in day to day practice. In the traditional medicinal systems, the whole plant is used as the alternative and complementory therapy.1 Medicinal plants have been the mainstream of traditional herbal medicines worldwide since the antiquity to date.2 About 1/4th of the pharmaceutical industries have structural modifications of natural products for the advancement of drug discovery and development. The non-nutrient compounds or the bioactive constituents referred to as “phytochemicals” are responsible against various microbial infections and hence the study of natural products is known as “Phytochemistry”.3 Even, the pharmacological activity of the crude extract is determined by the nature of phyto constituents present in the plant species and these compounds are responsible for the potential activity are known as “Secondary metabolites” like alkaloids, flavonoids, isoprenoids, saponins exerts antioxidant, steroidal activity, modulation of hormones and detoxification of enzymes and anti-cancer properties. Hence, plants are the major source of mankind throughout the history.4,5 With the rise in the clinical toxicity, the use of ayurvedic formulation is enhancing significantly over the decade.6 The standardization of botanicals for the specific markers was confirmed with the fingerprinting analysis. Ayurveda is considered as “Mother of healing”, is the integral part of the traditional medicine claims to aid the various metabolic and chronic disorders by enhances the longevity.7 Manasamitra Vatakam (MMV) is a herbomineral formulation, a Classical traditional medicine from the antique Ayurvedic formulations cited in Sahasrayogam, potentially used for memory impairment, neuroprotective activity and manic illness.8 MMV contains a composite mixture of 55 plant species of varying therapeutic activities like anxiolytic, anti-depressant, Antioxidant and anti-epileptic activities.9 The minerals present in MMV are Pravala pishthi, Tamrachuda Padika, Rajata Bhasma, Swarna Bhasma, Mrigashringa Bhasma, Makshika Bhasma, Mukta Pishthi, Loha Bhasma and Shilajat. 70-80% of the nutrient consumptions were proven to be obtained from the vegetable crops in the form herbal supplements claims to contain the heavy metal and the trace elements in it. The macro and
microelements are used for various biochemical processes in the human body reveals to have a significant effect on combating various ailments. In parallel to which, with the increase in adverse drug events from the synthetic drugs many of the populations finds the herbal drugs are of safe and toxic with lesser amount side effects. But, upon the regular usage of these herbal formulations results in the nephrotoxicity because of the contaminants, heavy metals and adulterants used.

Chemo-profiling and marker analysis plays a critical role in the herbal drug for ensuring the therapeutic efficacy, pharmacokinetic profile. The profiling and fingerprinting of phytoconstituents explores the plant analysis better and is the best alternative for the classical analytical methodology. Nevertheless, it helps to differentiate the species are of authentic or adulterated by its origin and allows for the proper discrimination between the species or the herbal medicines. LC-MS fingerprinting analysis can be laid as a platform for the additional testing to identify the fingerprinting analysis as it emphasizes on the systematic characterization of herbal compositions. No methodology has stated for the qualitative or quantitative estimation of the MMV for the quality control of the herbs, hence from the quality aspects of the classical formulation the fingerprinting analysis was performed to be the emerging need for its identification. The HPLC-DAD-ESI-MS analysis was performed for the fingerprinting analysis and further confirmation of the constituents were determined qualitatively. GC-MS is the versatile analytical technique which is unsuited to rapid high sensitivity analysis of specific compounds, represents the mass of specific particle (Da) to number (z) of electrostatic changes (e). The inborn error of metabolism in new-born are detectable, isotopic labelling of metabolites helps in metabolic activity. In traditional medicine, GC-MS is the feasible technique for the analysis of the liposoluble compounds like volatile or essential oils.

The Minimum inhibitory concentration (MIC) is the minimum (lowest) concentration of a test compound that will inhibit the growth of a bacterial strain. It can be done in several methods like micro dilution or macro dilution test, extended break point sensitivity test and e-strip test methods. Conventionally, this is determined using a series of doubling dilutions of the antibiotic in liquid culture medium, to produce a range of concentrations in test tubes (macro dilution) or in a microtiter tray (micro dilution). After inoculation of the test strain into each antibiotic concentration, bacterial growth is determined by visible turbidity after 18–24h of incubation. The MIC is the lowest concentration of compound with no visible bacterial growth.

In the present study, four different marketed formulations of MMV were chosen, and analyzed for the estimation of bioactive constituents, heavy metal analysis for the determination of elemental and trace analysis, HPLC-DAD-ESI-MS fingerprinting analysis. Based on the results, comparative profiling was studied for all the four formulations of MMV, of all the formulations the formulation found to be comparatively better was subjected to GC-MS analysis to study the possible volatile compounds present and also the evaluation of antibacterial activity was studied by both the diffusion and dilution techniques in the herb mineral formulation.

**MATERIALS AND METHODS**

**Materials**

All the chemicals of analytical grade were procured and used for the analysis. HPLC grade Methanol 99.9% (Merck Specialties’ Pvt. Ltd., Mumbai, India), Milli-Q water (Milli-Q10 TS, Millipore water Purification system, Merck) was used. Formulations A, B, C and D of MMV were prescribed by Ayurvedic Physician and procured from Ayurvedic pharmacy. Quercetin, glucose, gallic acid, ursolic acid, diosgenin were purchased form M/s Natural remedies, Bangalore. Cefotaxime susceptibility test discs (SD040-1VL) were procured from HIMEDIA lab Pvt. Ltd.

**Sample preparation**

A pooled mix of tablets of twenty was finely weighed and powdered. 1 g of powdered mix was transferred into a 10 mL standard flask. Sample extraction was exerted by adding 5 mL of methanol followed by sonication for about 30min and made to the mark with the diluent. The sample matrix prepared was then subjected to prior filtration with Whatmann filter paper and followed by 0.2 µm membrane filter. The filtered solution was used to perform the HPLC-DAD-ESI-MS and GC-MS analysis.

**Estimation of bioactive constituents**

Bioactive compounds often termed as secondary metabolites are essential for eliciting the pharmacological effects. Besides the primary metabolites, secondary metabolites are the compounds regarded as "biological side tracks" functioning of the plant. Irrespective of the pharmacological activity they themselves help in shielding the plant as a whole i.e., flavonoids protect against free radicals during photosynthesis, terpenoids attract pollinators and inhibit the competing plants, and alkaloids rid the phytoalexins and herbivore animals. However, exceptionally few plants are highly poisonous due to the increased concentration of secondary metabolites. The determination of the bioactive contents i.e., alkaloids, flavonoids, glucoside, phenolic, saponins, steroid saponins, sterols and terpenoids were estimated in all the four formulations of MMV.

**In vitro antioxidant activity**

The Antioxidant activity was performed by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity. 0.1mM of DPPH was prepared using methanol as vehicle from which 1mL was added to the formulation extract diluted to various concentrations. The aliquots were incubated in the dark at room temperature for 30min and the absorbance was recorded at 517nm, where ascorbic acid was kept as positive control. The samples were performed in triplicated and expressed as % incubation percentage. The IC50 value was calculated using Graph pad prism.

**Elemental analysis**

The glassware and Teflon tube used for digestion were soaked in dilute nitric acid and rinsed with Milli-Q-water. All the standard solutions were made by diluting 1000 mg/L of stock solutions (Merck). For the estimation of Hg, As and Se 5%v/v HCl was used for both standards and sample preparations, Whereas Milli-Q-water was used as diluent for Zn, Cr, Ni, Cu, Fe, Na, Pb and Cd standards and sample analysis.

Elemental analysis was carried out by using atomic absorption spectroscopy (AAS) system (AA 7000 AAS, Lab India Instruments Pvt Ltd).

The sample was subjected to digestion in microwave digestor as per EPA Method 3052. All the standard solutions were made by diluting 1000 mg/L of stock solutions (Merck). For the estimation of Hg, As and Se 5%v/v HCl was used for both standards and sample preparations, Whereas Milli-Q-water was used as diluent for Zn, Cr, Ni, Cu, Fe, Na, Pb and Cd standards and sample analysis.

The sample obtained was taken up for analysis and also expressed as % incubation percentage. The IC50 value was calculated using Graph pad prism.
2% sodium borohydride (NaB\textsubscript{H}\textsubscript{4}) in 0.5 % (w/v) sodium hydroxide (NaOH) was used as reducing agent. Milli-Q-water and 5% v/v HCl was used as diluents for FAM and HAG-AAS method respectively.

Instrument calibration was performed with a blank solution made to autozero followed by standardization with different working standards of different calibration sets. Standard samples were analysed prior to the samples to plot the calibration curve. The analysis was carried out in triplicate and the absorbance was measured.

**INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS**

**HPLC-DAD-ESI-MS analysis**

The HPLC system comprises of a Shimadzu binary solvent delivery module (LC10ADVP), SPD M20A PDA detector and manual Rhodyne injector with the loop capacity of 0.02ml. loop volume and column oven CT0-20A on the whole controlled by the communication module CBM-20A. The chromatographic separation was achieved using Phenomenex C\textsubscript{18} column (25cm x 4.6mm; 5µ) and the solvent system of water-formic acid (A; 100:0.1% v/v) and methanol (B) at 45:55 v/v with an isocratic flow of 0.8mL/min. The detection was set at 254nm.24-26

The separation was made on an (G1) LC-MS 2020 system equipped with single quadrupole mass spectrometer with electrospray ionization ESI (+) source with the positive ionization mode. The temperature was set at 280°C and 320°C for curved desolvation line (CDL) and heat block. The interface parameters as; nitrogen gas was used to assist nebulization with a flow rate of 1.5 mL/min. The drying gas flow was maintained at 15L/min.

**GC-MS analysis**

For the identification of the chemical compounds, the methanolic extract was subjected to analysis on Agilent GC-MS system (GC 7890B equipped with Agilent 5977A MSD mass detector) using HP\textsubscript{5} Phenyl Methyl Silox (60°C-325°C) 30mx250mmx0.2µm capillary as stationary phase. The oven temperature programming employed was with the initial temperature of 80°C for 1min, raised for 70°C linearly to 220°C and held for 5min, followed with the linear raise of 10°C/min to 290°C and held for 10min. The injector port was maintained at 290°C, helium gas was used as carrier gas with a flow rate of 1.2mL min\textsuperscript{-1}. Samples were given through split less mode of 1µL injection volume i.e., in the ratio of 1:10. The ionization voltage of 70ev and ion source temperature was kept at 280°C. The injection was performed by split mode with a split ratio of 10:1. Solvent delay time was set for 3min for all samples generated by different methods. MSWS V 8.0 workstation was used to process data. Interpretation on mass spectrum of GC-MS was generated by different methods. MSWS V 8.0 workstation was used to process data. Interpretation on mass spectrum of GC-MS was generated by different methods. The ionization of samples was performed through single quadrupole mass spectrometer with electrospray ionization ESI (+) source with the positive ionization mode. The temperature was set at 280°C and 320°C for curved desolvation line (CDL) and heat block. The interface parameters as; nitrogen gas was used to assist nebulization with a flow rate of 1.5 mL/min. The drying gas flow was maintained at 15L/min.

**Broth microdilution assay**

The resazurin based dilution method is the most appropriate method for the determination of MIC values for the anti-bacterial activity, as the possibility was enhanced for two-fold dilution of the anti-bacterial agent with the smaller volumes using 96-well microtitration plate. Staphylococcus aureus and Bacillus cereus (Gram-positive), Escherichia coli and Klebsiella pneumonia (Gram-negative) were grown on Nutrient agar by streak plate technique. After 48h, the individual colonies were picked and inoculated in the nutrient broth. Both the cultures were monitored for growth using a UV-1800 spectrophotometer (Shimadzu, Japan) after 24h at a wavelength of 600 nm to obtain a final OD 1.0. These liquid cultures were used for further inoculation on 96-well microtitration plate. After well mixing without any agitation the 96-well microtitration plate was cautiously incubated under 35 ± 2° C for 20h (Table 3). Eventually, the resazurin solution was prepared with the concentration of 6 mg mL\textsuperscript{-1} with sterile water as vehicle and the mixture was vortexed to ensure for the homogenous solution.

**RESULTS AND DISCUSSION**

**Phytochemical analysis**

Nonetheless, in all the four formulation A, B, C and D the composition of the plants is similar to as cited in Sahasrayogam, but the manufacturing and processing varies results in change in the presence of active constituents thereby results in the variation of the active principles present in the formulation. The methanolic extracts of the formulation A, B, C and D were subjected to bioactive estimations i.e. total alkaloid, flavonoid, glycoside, saponin, steroidal saponin and terpenoid content and the results were given in Figure 1 and depicted in the Table 1. From the phytochemical analysis, the determination of the major bioactive content was identified for all the formulations. Indeed, the formulation B has shown better availability of the bioactive contents followed by formulation D, A and C. The long-term utilization of the herbal medicines results in the deposition of the trace elements and the heavy metals in the human body resulting in the potential risk and it also depends on the daily dietary intake of the herbal medicines. Hence, the heavy metal and trace elemental analysis was performed and was present to be in varied amounts in all the formulations but Was found to be present in permissible limits and the results were listed in Table 2. From, the HPLC chromatograms of all the four formulations were submitted to LC-MS analysis.
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Quantitative parameters | Formulation A | Formulation B | Formulation C | Formulation D
--- | --- | --- | --- | ---
Alkaloid | 41.7 ± 0.0009 | 50.3 ± 0.0024 | 35.7 ± 0.0007 | 40.1 ± 0.0003
Flavonoid | 39.60 ± 0.003 | 47.40 ± 0.003 | 31.80 ± 0.003 | 45.2 ± 0.003
Glycoside | 10.7 ± 0.0052 | 23.1 ± 0.0140 | 6.6 ± 0.0048 | 12.9 ± 0.0083
Phenolic | 88.90 ± 0.0014 | 86.10 ± 0.0031 | 67.90 ± 0.0010 | 81.1 ± 0.0017
Saponin | 106.9 ± 0.0032 | 149.8 ± 0.0038 | 94.2 ± 0.0026 | 136.2 ± 0.0056
Sterol Saponin | 54.9 ± 0.2117 | 86.65 ± 0.0510 | 35.5 ± 0.0328 | 78.4 ± 0.0746
Terpenoid | 23.3 ± 0.0091 | 44.6 ± 0.0129 | 17.7 ± 0.0212 | 32.6 ± 0.0146

Table 1: Estimation of phytochemical constituents in Manasamitra Vatakam of various formulations.

Values are in mean ± SD, n=3, phenolic equivalent to gallic acid, flavonoid equivalent to quercetin, glycoside equivalent to glucose, saponin, sterol saponin equivalent to diosgenin, terpenoid equivalent to ursolic acid.

Concentration range (mg kg⁻¹) of Heavy metal and trace elements present in MMV of various formulations.

| Elements | Formulation A | Formulation B | Formulation C | Formulation D | Limits |
|---|---|---|---|---|---|
| Arsenic (As) | ND | ND | ND | ND | NMT 3 ppm |
| Cadmium (Cd) | 0.027 | 0.032 | 0.040 | 0.039 | NMT 0.3 ppm |
| Mercury (Hg) | ND | ND | ND | ND | NMT 0.5 ppm |
| Lead (Pb) | 1.231 | 1.744 | 1.979 | 2.681 | NMT 10 ppm |
| Calcium (Ca) | 2.385 | 1.184 | 2.210 | 1.557 | NMT 15 ppm |
| Copper (Cu) | 0.275 | 0.074 | 0.865 | 0.115 | NMT 40 ppm |
| Iron (Fe) | 12.25 | 9.48 | 21.177 | 12.074 | NMT 55 ppm |
| Potassium (K) | 0.955 | 0.497 | 1.566 | 0.686 | NMT 20 ppm |
| Selenium (Se) | ND | ND | ND | ND | NMT 2 ppm |
| Sodium (Na) | 2.872 | 1.151 | 2.863 | 1.308 | NMT 10 ppm |

Table 2: Concentration range (mg kg⁻¹) of Heavy metal and trace elements present in MMV of various formulations.

HPLC-DAD-ESI-MS fingerprinting analysis

LC-MS fingerprinting analysis and sown in Figure 3; the process of selection includes the identification of common peaks, normalization of their retention times and determination of the mass value for all the common peaks present in the formulations. Out of all the peaks, 11 peaks are termed as common peaks as they are present in all the four formulations. ESI-MS was performed to further confirm the fingerprints of the peaks identified. Analysis was performed with both the positive and negative ionization modes. On comparison with the m/z values all the peaks were unambiguously identified and were detected as Berberine, jatrorrhizine, curcumin, vitexin, rutin, quercetin, catechin, piperine, palmatine, naringenin, and ellagic acid respectively and the compounds were listed in the Table 3. All the formulations have shown similar fingerprinting pattern and molecular mass. But, formulation B has showed higher peak abundance and the variations caused may be difference in the preparation, manufacturing, drying process and the storage conditions. Upon the comparative note, the order of phytochemical, heavy metal analysis and HPLC-DAD-ESI-MS for the formulation Manasamitra Vatakam analysis was found to be Formulation C < Formulation A < Formulation D < Formulation B.

GC - MS analysis

For the better understanding of the secondary metabolites present, formulation B was subjected to GC MS analysis and the GC MS chromatogram was depicted in Figure 4 and tabulated in Table 4. The results appertain to GC MS analysis for the methanolic extract of formulation B, Manasamitra Vatakam lead to the identification of numerous compounds. The compounds interpreted from the mass spectra are Cyclopropyl carbinol, Camphor (levo), Isobornyl alcohol, Safranal, Decanoic acid, Dodecanico acid, Ascaridole epoxide, Cuminic acid, Undecanoic acid, 10-methyl-, methyl ester, Benzoic acid, Lauric acid, 4-Octadecanial, Diethyl Phthalate, α-Asarone, 2-Naphthaleneacetol, Ar-tumerone, β-bisabolol, β-Asarone, Methyl tetradecanoate, Isocalamendiol, 3,3,8,8-tetramethyl-, β-Eudesmol, n-Pentadecanoic acid, 13-Heptadecyn-1-ol, 5(1H)-Azulenone, Phthalic acid, Phytol, α-Copaen-11-ol, Naphthalene, Benzenepropanoic acid, Eudesma-5,11(13)-dien-8,12-olide, naphthalene, n-Hexadecanoic acid, 18-2-Hydroxy-10-pentyl-11-oxa-1,5-dithia-spiro(5.13)nonadec-15-yn-12-one, Hexadecanoic acid, Heptadecanoic acid, 9,12-Hexadecadienoic...

Figure 1: Comparative Profiling of Bioactive constituents in MMV of various formulations.

Figure 2: Antioxidant activity with DPPH assay.
Figure 3: The characteristic compounds identified through mass spectrum by HPLC-DAD-ESI-MS analysis were berberine (1), jatroprhizine (2), curcumine (3), vitexin (4) rutin (5), quercetin (6), catechin (7), piperine (8), palmatine (9), naringenin (10) and ellagic acid (11).
Table 3: Compounds identified in MMV by HPLC-DAD-ESI-MS.

| Peak No | Retention time | Area | Molecular Formula | m/z value | Compound name |
|---------|----------------|------|-------------------|-----------|---------------|
| 01      | 1.007          | 3.201| C_{20}H_{18}NO_{4} | 336.05    | Berberine     |
| 02      | 1.392          | 3.492| C_{20}H_{20}NO_{4} | 338.92    | Jatrorrhizine |
| 03      | 1.507          | 3.477| C_{21}H_{20}O_{6}  | 367.05    | Curcumin      |
| 04      | 1.807          | 3.283| C_{15}H_{10}O_{6}  | 286.21    | Vitexin       |
| 05      | 1.907          | 3.972| C_{27}H_{30}O_{16} | 301.26    | Rutin         |
| 06      | 2.107          | 5.120| C_{15}H_{12}O_{5}  | 321.26    | Naringenin    |

Table 4: Compounds identified in formulation B of MMV by GC-MS.

| Peak No | Retention time | Area | Molecular Formula | m/z value | Compound name |
|---------|----------------|------|-------------------|-----------|---------------|
| 1       | 7.621          | 4.624| C_{10}H_{18}O      | 154.21    | Isobornyl alcohol |
| 2       | 19.024         | 2.773| C_{12}H_{16}O      | 208.10    | Asarone        |
| 3       | 24.291         | 2.413| C_{19}H_{28}O      | 320.19    | Phthalic acid  |
| 4       | 25.406         | 1.288| C_{17}H_{34}O      | 270.25    | Palmitic acid  |
| 5       | 25.918         | 1.726| C_{15}H_{22}O      | 218.16    | Naphthalenone  |
| 6       | 26.267         | 18.828| C_{16}H_{32}O      | 256.24    | n-Hexadecanoic acid |
| 7       | 29.395         | 6.613| C_{18}H_{32}O      | 280.24    | 9,12-Octadecadienoic acid (Z,Z)- |
| 8       | 29.506         | 9.983| C_{18}H_{34}O      | 282.25    | 9,12-Octadecadienoic acid (Z,Z)- |
| 9       | 29.893         | 3.386| C_{18}H_{36}O      | 289.13    | Octadecanoic acid |
| 10      | 32.693         | 1.215| C_{18}H_{36}O      | 414.38    | γ-Sitosterol    |
| 11      | 33.458         | 4.181| C_{29}H_{50}O      | 284.27    | Dihydrolycorine |

Antioxidant activity

The free radical scavenging activity was determined by DPPH assay activity. The activities were analysed statistically by graph pad prism and the IC50 value was calculated. Of all the four formulations, formulation B showed better Antioxidant property on a comparative note. The formulation was evident to have rich in flavonoids and phenolic compounds which have direct inhibitory activity on the free radical mechanism and hence mitigates the Antioxidant nature thereby ceasing the oxidation. The effect free radical scavenging activity was depicted in Figure 2.

Antibacterial activity

The bacterial strains Staphylococcus aureus (9 mm) and Bacillus cereus (11 mm) and Escherichia coli (7 mm) and Klebsiella pneumonia (9 mm) showed zone of inhibition i.e., the MIC values were found to be 5.0 µg mL⁻¹ for gram positive and 5.0 and 2.5 µg mL⁻¹ for gram negative bacteria respectively and were shown in Figure 5. For the determination of the antibacterial activity of the plant extracts it is evident that the disk
diffusion method could not be persistently reliable method and hence it was performed by agar dilution method where the anti-bacterial activity was shown at the lower concentration on a comparative note. The evaluation of the present study includes disk diffusion, broth dilution assay and broth microdilution assay and was depicted in Figure 6 and 7. Where the broth microdilution assay was performed using resarzurin to enhance the detection of the bacterial growth. The extent of bacterial activity was determined based on the intensity of colour i.e., the blue coloured fluorescence indicated the effect of anti-bacterial nature of the sample whereas the pink indicates mild to moderate activity. The MIC values were found to be 0.390 and 0.781 µg mL⁻¹ for the gram positive stains i.e., Staphylococcus aureus and Bacillus cereus and the 6.250 and 3.125 µg mL⁻¹ gram negative bacteria i.e., Escherichia coli and Klebsiella pneumonia respectively and showed better quantitative determination of the bacterial activity than disk diffusion method and broth dilution assay were shown in Table 5.

Figure 5: Disk Diffusion method for Gram Positive Staphylococcus aureus (A), Bacillus cereus (B) and Gram Negative Escherichia coli (C), Klebsiella pneumonia (D) at 2.5, 5 and 10 µg mL⁻¹ concentration.

Figure 6: Broth Dilution Assay method for Gram Positive Staphylococcus aureus (A), Bacillus cereus (B) and Gram-Negative Escherichia coli (C), Klebsiella pneumonia (D) at 10 mg mL⁻¹ to 0.15625 mg mL⁻¹ concentration.

Figure 7: Broth Microdilution Assay method for Gram Positive Staphylococcus aureus, Bacillus cereus (A) and Gram Negative Escherichia coli, Klebsiella pneumonia (B) at 10 mg mL⁻¹ to 0.07812 mg mL⁻¹ concentration (each in triplicate).
CONCLUSION

To the best of our knowledge, the study provides the first HPLC-DAD-ESI-MS and GC-MS analysis with antibacterial activity with the major primary and secondary metabolites. The phytochemical analysis proves the formulation B contains major bioactive constituents. The elemental analysis inferred the presence of heavy and the trace elements were found to be in lesser quantity in formulation B on comparison with the others. The HPLC-DAD-ESI-MS analysis revealed the major amounts of alkaloids, flavonoids and the phenolic moiety in all the formulations. In relevance to the phytochemicals identified in HPLC-MS analysis the Antioxidant activity exerted was anticipated due to the presence of phenol and flavonoids. Based on this data, formulation B was selected for GC-MS analysis revealed the major bioactive compounds like asarone, phthalic acid, hexadecanoic acid and octadecanoic acid which are majorly mitigating the anti-bacterial activity. The presence of phytochemicals like α and β asarone, γ-sitosterol, tocopherol may be responsible for the neuroprotective mechanisms. With this inference it was proved that the herbomineral formulation Manasamitra Vatakam showed anti-bacterial activity against both the gram positive and the gram negative stains.

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GRAPHICAL ABSTRACT

Dr. K. Ilango received his Ph.D in Faculty of Pharmacy from Sri Ramachandra Institute Higher education & Research, Chennai and his Master’s degree from Birla Institute of Technology and Science (BITS), Pilani, Rajasthan. Dr. K. Ilango has been an active Academic Professional in several reputed Institutions. His academic journey in SRM began in 1994. Since then, Dr. K. Ilango’s involvement in academics had been remarkable, starting from his service as Head the Department of Pharmaceutical Chemistry, SRM College of Pharmacy, to leading dual roles as Vice-Principal cum Prof & Head of the Department of Pharmaceutical Chemistry and as Dean for Interdisciplinary Institute of Indian System of Medicine (IIISM) SRM University, his contribution and sharing of knowledge has benefited several aspiring students.

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