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RESEARCH ARTICLE

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# A novel approach on herbal water to reduce water contaminant *Salmonella typhi* - an *in vitro* study

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## ABSTRACT

The aim of the present study is to investigate the antibacterial activity of selected traditional medicinal plants against the clinical isolate of *Salmonella typhi*. The methanol and aqueous extracts of dried leaves of *Aristolochiaindica* Linn., *Melia dubia* Cav., *Andrographis paniculata* Linn. *Enicostemma axillare* Linn and *Tinospora cordifolia* Miers were evaluated for its antibacterial activity against the water communicable *Salmonella typhi*. Among these a significant antibacterial activity was showed by methanol extracts of *Aristolochia indica* and *Melia dubia*. Also their combination showed a maximum zone of inhibition. From the above results we gave an approach for the use of herbal water with these two plants and it could be very effective in action in order to control and prevent the widespread of water contaminant, *Salmonella typhi* among the people.

***Keywords***: *Salmonella typhi, Melia dubia, Aristolochia indica, Herbal water.*

## 1. INTRODUCTION

The most dangerous health hazards and death of many people occurs mainly due to the pathogenic microbial infections worldwide. In the current scenario, microbes have been developed to change their nature and genetic structure to attain a highly resistant capacity for survival against the drugs used in the treatment [1]. To overcome these hazardous problems, plants are described as medicinal origin since ancient period. The traditional plants of Siddha system are highly involved in the treatment of various infectious diseases with suitable curative effects. Scientists are keenly looking forward for the development of best alternative and novel drugs to - invade the drug resistant microbes [2]. Natural plants, algal and marine sources provide an array in drug preparation for treating against the infectious diseases. The investigation on plants by various researchers indicates that plants are one of the major sources for discovery of drugs and development of medicine for prevention of disease and management.

Among various dreadful diseases of microbes, *Salmonella typhi*, a Gram negative pathogen plays a significant role in causing enteric typhoid fever which leads to step-ladder pyrexia, intestinal perforation and hemorrhage resulting in the rose spots of skin and fatal fulminating disease [3]. The

multi drug resistant(MDR) strains of *Salmonella typhi* is highly ubiquitous and causes typhoidal fever mainly community endemic and epidemic fever [2].The major problem of disease is due to its easy spreading nature by water and food, mostly affecting the growing children in the age group of 5-20 years [4]. In United States, about 50,000 cases are reported to have salmonella infection every year, mostly children [5]. The ingestion of contaminated food or water is the only main source of infection causing typhoid fever. Another reason for the cause of typhoid fever is poor sanitation and the failure to maintain proper health and hygiene.

According to our Siddha Pharmacology, the plants *Melia dubia, Andrographis paniculata, Tinospora cordifolia*, *Aristolochia indica* and *Enicostemma axillare* are confined to possess excellent medicinal properties. The leaves of *Melia dubia* possess actions such asantilithic, diuretic and cathartic and used in treating the vaadha disease and spleenomegaly. The whole plant of *Aristolochia indica* has hepatonic action and used highly in cardiac problems, ulcer and vaadha diseases. The leaves of *Andrographis paniculata* possess the actions oftonic, stimulant and alterative and used in curing malaria, dengue, chikun gunya and vaadha type fevers. The whole plant of *Tinospora cordifolia* owns the actions of antiperiodic, demulcent and hepatic stimulant. Its decoction is used in curing fevers and cardiac disorders. The leaves of *Enicostemma axillare* acquires the actions of febrifuge, tonic and alterative and used as curative agent of vaadha disease and nervous problems[6].

Various articles were reported on the barks of *Melia dubia* has anti bacterial activity against Staphylococcus aureus. The leaves of *Melia dubia* was also reported to possess Antiviral [7], Antidiabetic and Antioxidant [8] and Larvicidal property [9]. The Antidiabetic [10], Antimicrobial [11], Anti inflammatory [12] and Anthelmintic action [13] of *Aristolochia indica* has been proved in previous researches. *Andrographis paniculata* has been reported to exhibit antimicrobial activity against some bacteria and fungi [14] except *S.typhi* on our review. *In vivo* antimicrobial and anti-oxidant activity [15] of *Enicostemma axillare* and antiarthritic property against formaldehyde induced arthritis [16] had been proven. *Tinospora cordifolia* acts against *Escherichia coli, Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* [17]. It has been already proven to have anti diabetic activity and involved in treatment of postprandial hyperglycemia [18].

In this study the dried leaves of *Melia dubia* (Meliaceae), *Aristolochia indica* (Aristolochiaceae), *Andrographis paniculata* (Acanthaceae), *Enicostema axillare* (Gentianaceae), *Tinospora cordifolia* (Menispermaceae) were processed and tested for its antibacterial activity against *S.typhi* by Agar well diffusion method based on Antibiotic Susceptibility test.

## 2. MATERIALS AND METHODS

### *2.1. Materials Required*

Muller Hinton Agar (MHA) and Agar-Agar was purchased from Hi Media Pvt. Ltd., Mumbai. Also Glassware, Plant extracts, Solvents (water and methanol), Clinical samples of *Salmonella typhi*, Incubator were required.

### *2.2. Collection of plants*

*Aristolochia indica* and *Enicostemma axillare* are collected from the foothills of Oorachikottai malai, Bhavani at a latitude (11º45'N) and longitude (77º68'E), Erode District, Tamilnadu, India. *Andrographis paniculata, Melia dubia* and *Tinospora cordifolia* were collected at the latitude (10º73'N) and longitude (77º76'E) in an area belongs to Namakkal District, Tamilnadu, India, during October 2014. The collected plants are brought to the Laboratory and they are identified properly in the herbal garden of Sivaraj Siddha Medical College, Salem, Tamilnadu, India and they were allowed to dry under sun shade at room temperature.

### *2.3. Processing of the plants*

The leaves of*Melia dubia, Andrographis paniculata, Tinospora cordifolia* and the whole plant of *Aristolochia indica* and *Enicostemma axillare* were dried and processed into fine powers by using mechanical grinders based on the preparation of choorana and purified. These powders are extracted with the help of solvents.

### *2.4. Preparation of crude extract with solvents*

The one gram of the powder to be extracted was weighed and then it was transferred into a sterile screw cap tube containing solvent (both water and methanol) of 10ml for every plant respectively. They were mixed and loaded in the orbital shaker for a day at a speed of 120 rpm. The filtrates are filtered by the use of Whatman's filter paper No. 1.

### *2.5. Bacterial Specimen*

The clinical isolates of *Salmonella typhi* grown in a nutrient agar were maintained at4º C in refrigerator and they were sub cultured properly for further use.

An inoculum of bacterium is mixed in 1 ml of distilled water for the process of swabing.

### *2.6. Agar Well Diffusion Method*

The culture plates with the specified media (Muller Hinton Agar) were made into well for Agar well diffusion method. The bacterial sample is swabbed onto the culture plate and the wells are pipetted with the plant extracts and they were labeled. Then they were kept in an incubator at 37ºC for 24 hours. After incubation, the zone of inhibition obtained was measured.

### *2.7. GC-MS Analysis*

The methanol extract of *Melia dubia* and *Aristolochia indica* was analyzed by gas chromatography-mass spectrometry (GC/MS)PerkinElmer Clarus 500 GCMS Turbomass ver5.2.0 Capillary Column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane) Column length: 30m, Column id: 250µm. The temperature of the column was programmed from 50ºC at 6ºC/min to 200ºC (5min) at 7ºC/min to 280ºC (5min). Injector temperature was set as 280ºC. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. (Split ratio 1:10) The identification of the chemical constituents was based on matching their recorded mass spectra with those obtained from the NIST 2005 library spectrum provided by the software on a GC / MS system.

### *2.8. Statistical Analysis*

All the tests were conducted in triplicates and the values were recorded. Using Microsoft Excel 2007 (Roselle, IL, USA) the data are expressed as mean ± standard deviation.

## 3. RESULTS AND DISCUSSION

Pathogenic microbes are the major problem of health disorders in humans and animals and their contagious nature make it difficult to control their widespread. Microbial infections are the major cause of many uncontrolled death all over the world in the Pre Antibiotic era [5, 14]. Therefore, newer antimicrobial compounds with low/no side effects are desirable for pharmaceutical applications. The phytochemicals present in the plants are the key compounds in several plants play the role of medicinal properties which is highly recommended for the development of new pharmaceutical molecule.

In this survey, the antibacterial activities of the five different plant extracts from different parts are reported against the *Salmonella typhi* and were depicted in Table 1. The most significant result is obtained by the methanolic extracts of *Melia dubia* and *Aristolochia indica* when compared to the results revealed by other plant extracts (Figure 1). In our view of search no other studies had gone on *Melia dubia* and *Aristolochia indica* over the bacteria, *Salmonella typhi*. But *Aristolochia indica* and *Melia dubia* had already proven by various authors to possess the anti microbial activity other than *Salmonella typhi* [11]. This study reveals the Anti salmonella activity of both *Aristolochia indic*a and *Melia dubia*.

**Table 1.** Antibacterial activity of some medicinal plants against *S.typhi*

| Plant Name | Mortality ± SD (Aqueous extract ) | Mortality ± SD (Methanol extract ) |
|---|---|---|
| *Melia dubia* | 13. 00 ± 1.0 | 13.50 ± 0.5 |
| *Aristolochia indica* | 03. 00 ± 1.0 | 15.38 ± 0.3 |
| *Andrographis paniculata* | 11.17 ± 0.7 | 11.17 ± 0.7 |
| *Tinospora cordifolia* | 11.17 ± 1. 0 | 13. 00 ± 1. 0 |
| *Enicostemma axillare* | 1. 017 ± 0.7 | 8.167 ± 1.2 |
| *Melia dubia and* | 13. 00 ± 1. 0 | 13. 00 ± 1. 0 |
| *Aristolochia indica* | | |

All the data values are expressed in mean ± standard deviation (n=3)

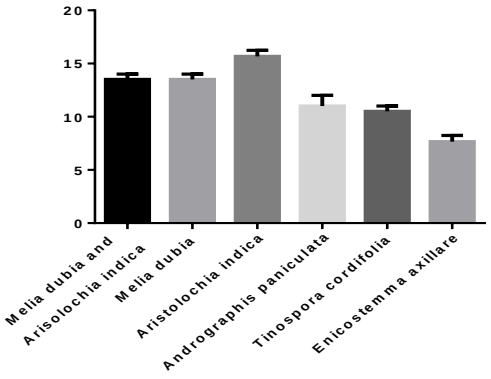



**Figure 1.** Antibacterial activity of methanol extracts of some medicinal plants against *S.typhi*

**Table 2.** List of compounds present in ethanolic extract of *Melia dubia* leaves

| S.No. | Peak Name | Retention Time(min) | Peak Area | % Peak area |
|---|---|---|---|---|
| 1. | Name: Hexanal, 3-methyl-<br>Formula: $C_7H_{14}O$<br>MW: 114 | 3.62 | 372208 | 0.0436 |
| 2. | Name: Octane<br>Formula: $C_8H_{18}$<br>MW: 114 | 3.81 | 375272 | 0.0440 |
| 3. | Name: 2-Pentanone, 4-hydroxy-4-methyl-<br>Formula: $C_6H_{12}O_2$<br>MW: 116 | 4.34 | 50037648 | 5.8610 |
| 4. | Name: 1-(2-Hydroxymethylpyrrolidin-1-yl)ethanone<br>Formula: $C_7H_{13}NO_2$<br>MW: 143 | 5.41 | 817625 | 0.0958 |
| 5. | Name: Butanoic acid, 4-hydroxy-<br>Formula: $C_4H_8O_3$<br>MW: 104 | 6.02 | 1493231 | 0.1749 |
| 6. | Name: 2-Nonanone<br>Formula: $C_9H_{18}O$<br>MW: 142 | 14.42 | 221859 | 0.0260 |
| 7. | Name: Thymol<br>Formula: $C_{10}H_{14}O$<br>MW: 150 | 15.23 | 7707298 | 0.9028 |
| 8. | Name: 6-Azacytosine<br>Formula: $C_3H_4N_4O$<br>MW: 112 | 15.83 | 602510 | 0.0706 |
| 9. | Name: Geranic acid<br>Formula: $C_{10}H_{16}O_2$<br>MW: 168 | 16.48 | 2405235 | 0.2817 |
| 10. | Name: 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 17.48 | 263044 | 0.0308 |
| 11. | Name: ç-Elemene<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 17.58 | 399244 | 0.0468 |
| 12. | Name: 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 17.89 | 840836 | 0.0985 |
| 13. | Name: Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 18.05 | 453984 | 0.0532 |
| 14. | Name: Di-epi-à-cedrene<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 18.24 | 3551778 | 0.4160 |
| 15. | Name: 1H-Benzocycloheptene, | 18.55 | 5081222 | 0.5952 |

| | | | | |
|---|---|---|---|---|
| | 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-, (R)-<br>Formula: $C_{15}H_{24}$<br>MW: 204 | | | |
| 16. | Name: Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-<br>Formula: $C_{15}H_{22}$<br>MW: 202 | 18.67 | 35301644 | 4.1349 |
| 17. | Name: 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 18.92 | 86370976 | 10.1167 |
| 18. | Name: à-Farnesene<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 19.01 | 17699712 | 2.0732 |
| 19. | Name: Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 19.18 | 30062872 | 3.5213 |
| 20. | Name: Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 19.56 | 65128456 | 7.6286 |
| 21. | Name: 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-<br>Formula: $C_{15}H_{26}O$<br>MW: 222 | 20.33 | 7013739 | 0.8215 |
| 22. | Name: 1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-<br>Formula: $C_{15}H_{26}$<br>MW: 206 | 20.48 | 212992 | 0.0249 |
| 23. | Name: Dodecanoic acid<br>Formula: $C_{12}H_{24}O_2$<br>MW: 200 | 20.85 | 29367056 | 3.4398 |
| 24. | Name: à-Bisabolol<br>Formula: $C_{15}H_{26}O$<br>MW: 222 | 21.07 | 898917 | 0.1053 |
| 25. | Name: 1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-<br>Formula: $C_{15}H_{24}$<br>MW: 204 | 21.52 | 4318344 | 0.5058 |
| 26. | Name: Cubenol<br>Formula: $C_{15}H_{26}O$<br>MW: 222 | 21.91 | 7024571 | 0.8228 |
| 27. | Name: 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-à,à,4a,8-tetramethyl-, [2R-(2à,4aá,8á)]-<br>Formula: $C_{15}H_{26}O$<br>MW: 222 | 22.59 | 7871117 | 0.9220 |
| 28. | Name: ç Dodecalactone<br>Formula: $C_{12}H_{22}O_2$ | 22.88 | 12538542 | 1.4687 |

| | | | | |
|---|---|---|---|---|
| | MW: 198 | | | |
| 29. | Name: Oxiranemethanol, 3-methyl-3-(4-methyl-3-pentenyl)-<br>Formula: $C_{10}H_{18}O_2$<br>MW: 170 | 24.01 | 923070 | 0.1081 |
| 30. | Name: Tetradecanoic acid<br>Formula: $C_{14}H_{28}O_2$<br>MW: 228 | 24.51 | 22669942 | 2.6554 |
| 31. | CYCLOPROPANEMETHANOL, .ALPHA.,2-DIMETHYL-2-(4-METHYL-3-PENTENYL)-, [1.ALPHA.(R*),2.ALPHA.]- | 25.44 | 2397035 | 0.2808 |
| 32. | Name: (E)-3(10)-Caren-4-ol<br>Formula: $C_{10}H_{16}O$<br>MW: 152 | 27.06 | 2301126 | 0.2695 |
| 33. | Name: Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1à,3à,5à)-<br>Formula: $C_{10}H_{16}O$<br>MW: 152 | 27.77 | 2893188 | 0.3389 |
| 34. | Name: Hexadecanoic acid, ethyl ester<br>Formula: $C_{18}H_{36}O_2$<br>MW: 284 | 28.02 | 18138544 | 2.1246 |
| 35. | Name: n-Hexadecanoic acid<br>Formula: $C_{16}H_{32}O_2$<br>MW: 256 | 28.36 | 327794528 | 38.3949 |
| 36. | Name: 9,12-Octadecadienoic acid, methyl ester<br>Formula: $C_{19}H_{34}O_2$<br>MW: 294 | 30.36 | 6357145 | 0.7446 |
| 37. | Name: 9-Octadecenoic acid (Z)-, methyl ester<br>Formula: $C_{19}H_{36}O_2$<br>MW: 296 | 30.50 | 10999760 | 1.2884 |
| 38. | Name: 9,12-Octadecadienoic acid, ethyl ester<br>Formula: $C_{20}H_{36}O_2$<br>MW: 308 | 32.18 | 30248502 | 3.5430 |
| 39. | Name: (E)-9-Octadecenoic acid ethyl ester<br>Formula: $C_{20}H_{38}O_2$<br>MW: 310 | 32.33 | 50590664 | 5.9257 |

**Table 3.** List of compounds present in the ethanolic extract of *Aristolochia indica* leaves

| S.No. | Peak Name | Retention Time(min) | Peak Area | % Peak area |
|---|---|---|---|---|
| 1. | Name: Glycerin<br>Formula: $C_3H_8O_3$<br>MW: 92 | 8.70 | 693202 | 0.3436 |
| 2. | Name: Sorbic Acid<br>Formula: $C_6H_8O_2$<br>MW: 112 | 9.50 | 475382 | 0.2356 |

| | | | | |
|---|---|---|---|---|
| 3. | Name: Nonanal<br>Formula: $C_9H_{18}O$<br>MW: 142 | 9.95 | 203218 | 0.1007 |
| 4. | Name: Phenol, 2-methoxy-<br>Formula: $C_7H_8O_2$<br>MW: 124 | 10.08 | 1824351 | 0.9043 |
| 5. | Name: 2-[2-(4-Methyl-furazan-3-yloxy)-ethyl]-2H-tetrazol-5-ylamine<br>Formula: $C_6H_9N_7O_2$<br>MW: 211 | 10.56 | 1798108 | 0.8913 |
| 6. | Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-<br>Formula: $C_6H_8O_4$<br>MW: 144 | 11.85 | 5803331 | 2.8765 |
| 7. | Name: Octanoic Acid<br>Formula: $C_8H_{16}O_2$<br>MW: 144 | 12.39 | 1211118 | 0.6003 |
| 8. | Name: 2-Decenal, (E)-<br>Formula: $C_{10}H_{18}O$<br>MW: 154 | 13.82 | 174571 | 0.0865 |
| 9. | Name: Benzaldehyde, 4-methyl-<br>Formula: $C_8H_8O$<br>MW: 120 | 14.28 | 19174904 | 9.5043 |
| 10. | Name: 2-Methoxy-4-vinylphenol<br>Formula: $C_9H_{10}O_2$<br>MW: 150 | 15.51 | 3464966 | 1.7175 |
| 11. | Name: Phenol, 2,6-dimethoxy-<br>Formula: $C_8H_{10}O_3$<br>MW: 154 | 16.29 | 4892199 | 2.4249 |
| 12. | Name: Benzaldehyde, 3-isopropoxy-4-methoxy-<br>Formula: $C_{11}H_{14}O_3$<br>MW: 194 | 17.87 | 822253 | 0.4076 |
| 13. | Name: cis-à-Copaene-8-ol<br>Formula: $C_{15}H_{24}O$<br>MW: 220 | 19.35 | 423388 | 0.2099 |
| 14. | Name: 1,3-Dioxolan-2-one, 3-methyl-3-(4,8-dimethylnona-3,7-dienyl)-4-methylene-<br>Formula: $C_{16}H_{24}O_3$<br>MW: 264 | 19.49 | 778706 | 0.3860 |
| 15. | Name: Sucrose<br>Formula: $C_{12}H_{22}O_{11}$<br>MW: 342 | 19.67 | 6450432 | 3.1973 |
| 16. | Name: 4-(2-Acetyl-5,5-dimethylcyclopent-2-enylidene)butan-2-one<br>Formula: $C_{13}H_{18}O_2$<br>MW: 206 | 20.85 | 535931 | 0.2656 |
| 17. | Name: (-)-Spathulenol<br>Formula: $C_{15}H_{24}O$<br>MW: 220 | 21.03 | 1275933 | 0.6324 |

| | | | | |
|---|---|---|---|---|
| 18. | Name: Ledol<br>Formula: $C_{15}H_{26}O$<br>MW: 222 | 21.52 | 8884979 | 4.4040 |
| 19. | Name: 4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one<br>Formula: $C_{14}H_{22}O$<br>MW: 206 | 21.73 | 272171 | 0.1349 |
| 20. | Name: 3-Buten-2-one, 4-(6,6-dimethyl-1-cyclohexen-1-yl)-<br>Formula: $C_{12}H_{18}O$<br>MW: 178 | 22.09 | 3792152 | 1.8796 |
| 21. | Name: Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, à,à,6,8-tetramethyl-, stereoisomer<br>Formula: $C_{15}H_{24}O$<br>MW: 220 | 22.39 | 225514 | 0.1118 |
| 22. | Name: 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol<br>Formula: $C_{15}H_{24}O$<br>MW: 220 | 22.47 | 1343568 | 0.6660 |
| 23. | Name: 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1à,4aá,8aà)]-<br>Formula: $C_{15}H_{26}O$<br>MW: 222 | 22.65 | 637965 | 0.3162 |
| 24. | Name: 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one<br>Formula: $C_{15}H_{22}O$<br>MW: 218 | 23.15 | 17427678 | 8.6383 |
| 25. | Name: Humulane-1,6-dien-3-ol<br>Formula: $C_{15}H_{26}O$<br>MW: 222 | 23.56 | 6276971 | 3.1113 |
| 26. | Name: 7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene<br>Formula: $C_{15}H_{24}O$<br>MW: 220 | 23.96 | 980912 | 0.4862 |
| 27. | Name: 7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane<br>Formula: $C_{15}H_{26}O_2$<br>MW: 238 | 24.16 | 2233856 | 1.1072 |
| 28. | Name: Tetradecanoic acid<br>Formula: $C_{14}H_{28}O_2$<br>MW: 228 | 24.47 | 3733627 | 1.8506 |
| 29. | Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol<br>Formula: $C_{20}H_{40}O$<br>MW: 296 | 25.19 | 10141586 | 5.0268 |
| 30. | Name: 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-à,à,4a,8-tetramethyl-, [2R-(2à,4aá,8á)]-<br>Formula: $C_{15}H_{26}O$ | 25.84 | 4712397 | 2.3358 |

| | | | | |
|---|---|---|---|---|
| | MW: 222 | | | |
| 31. | Name: ç-Gurjunenepoxide-(2)<br>Formula: $C_{15}H_{24}O$ | 26.48 | 3293316 | 1.6324 |
| | MW: 220 | | | |
| 32. | Name: n-Hexadecanoic acid<br>Formula: $C_{16}H_{32}O_2$ | 28.08 | 77840000 | 38.5826 |
| | MW: 256 | | | |
| 33. | Name: 5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol<br>Formula: $C_{15}H_{26}O_2$ | 30.62 | 3675326 | 1.8217 |
| | MW: 238 | | | |
| 34. | Name: Phytol<br>Formula: $C_{20}H_{40}O$ | 30.90 | 6274721 | 3.1102 |
| | MW: 296 | | | |

The phytochemical constitutions of *Melia dubia* has been reported to have steroids, phytosterols, triterpenoids, saponin, flavanoids, tannins, protein and aminoacid, carbohydrates, glycosides, fats and fixed oils and essential oils [8,19]. GC MS chromatogram of leaf extract of *Melia dubia* (Figure 2) showed 39 different compounds which contributed to the medicinal activity of the plant, with n-Hexadecanoic acid showing maximum peak area 38.39% at a retention time 28.36 minutes. Other compounds with prominent peak are 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl followed by Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene.

Likewise Phytochemical analysis of leaf extracts of *Aristolochia indica* has been reported to have steroids, glycosides, alkaloids, triterpenoids, volatile oils, anthracine glycosides[12] and the GC MS analysis of the leaf extract of *Aristolochia indica* (Figure 3) showed the presence of n-Hexadecanoic acid with 38.58% peak at a retention time of 28.8 minutes which is followed by 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one.

In previous research, n-Hexadecanoic acid has been reported as an inhibitor of phospholipase A(2), hence, an anti-inflammatory compound [20]. Both the plants has high amount of n-Hexadecanoic acid and it might be responsible for the antibacterial activity. There were many other compounds present in the extracts and they were given in table 2 and table 3 for *Melia dubia* and *Aristolochia indica* respectively

**Figure 2.** GC-MS analysis of leaf extract of *Melia dubia*

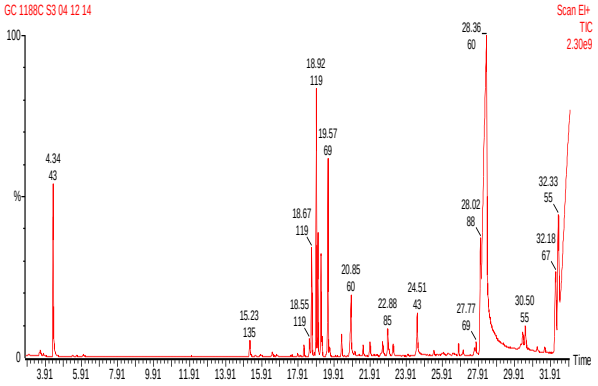
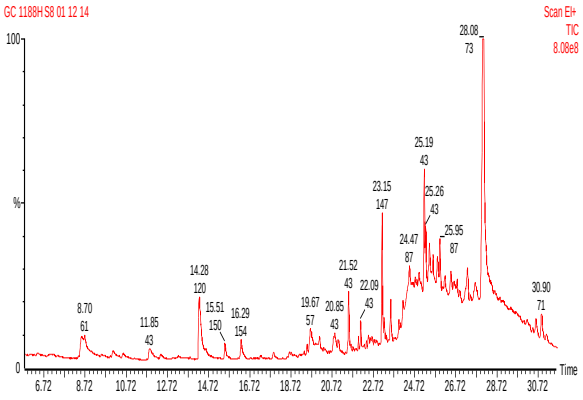



**Figure 3.** GC-MS analysis of leaf extract of *Aristolochia indica*



### *3.1. Proposal of herbal water*

In ancient days, people were supposed to use water from hills and spring which is full of herbal nutrients. Unfortunately now-a-days people are using water from other sources such as lakes, ponds which are contaminated with disposal of human wastes. We are very well known about the tragedy of Typhoid Mary (Mary Mallon) who acts as a source of infection and spreaded typhoid bacilli more than 200 people. This type of epidemic disease can be reduced by the use of Herbal water. We came to know about the practice instructed by Government of Kerala for the welfare of the rural people. People of Kerala were instructed to drink herbal water with some traditional plants [21, 22]. It inspired us, for the development of herbal water. Based on the considerable activity shown by *Aristolochia indica* and *Melia dubia*, we conducted an experiment. The plant extracts were mixed with contaminated water of *S.typhi* and the comparison study was performed. We observed only a few colonies in the culture plates treated with herbal water when compared to untreated culture of *S.typhi*.

### *3.2. Ideal use of Herbal water*

*In vitro* scavenging activity of herbal water with *Melia dubia* and *Aristolochia indica* is now verified and it is considered to be a natural biological purification technology (Green Technology) [23]. Moreover, these herbs are widely available in rural area. Hence surely it will effective and it decreases the virulence of contaminants present in the ponds and lakes of rural areas.

## 4. CONCLUSION

This study reveals the antibacterial activity of *Aristolochiaindica*, *Melia dubia*, *Andrographis paniculata*, *Enicostemma axillare* and *Tinospora cordifolia*. Also our comparison study with *Aristolochiaindica* and *Melia dubia* explored the better scavenging activity against the pathogen in the insanitary water and so we conclude that the usage water with these herbs will be highly useful in controlling the spreading nature of water contaminant, *Salmonella typhi.*

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## Conflict of Interest

The authors declare that they have no conflicts of interest.

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