



# PREPARATION AND PHYTOCHEMICAL INVESTIGATION OF PANCHVALKAL ARISTA IN CONTRAST TO PANCHVALKAL DECOCTION

<sup>1</sup>Vaidehikumari Patel, <sup>2</sup>Dr. Rajesh K. Patel

<sup>1</sup>Research Scholar, <sup>2</sup>Professor

<sup>1</sup>Hemchandracharya North Gujarat University Patan 382465, India

<sup>2</sup>Department of Biosciences, Veer Narmad South Gujarat University, Surat 395007, India

**Abstract:** The *Asava-Arista* are traditional Ayurvedic pharmaceutical dosage forms that are easy to use due to their improved palatability, enhanced therapeutic action, and long shelf life. The fermentation process extracted the active principles from raw plant material through self-generated alcohol. Panchvalkal is a well-known Ayurvedic anti-infective polyherbal formulation. The present study used Panchvalkal plant mixers to prepare an Ayurvedic dosage form Arista. To prepare the Panchvalkal Arista, Panchvalkal decoction was subjected to a fermentation process by a fermenting agent, *Saccharomyces cerevisiae*. Non-fermented Panchvalkal decoction and fermented Panchvalkal Arista were then investigated for their phytoconstituents by High-Resolution Liquid Chromatography-Mass Spectrometry coupled with Quadrupole Time-Of-Flight (HRLCMS-QTOF) analysis. This study's result clarified the changes that occurred after the fermentation process and revealed the formation of new phytochemicals in the Panchvalkal Arista formulation.

**Keywords:** Ayurveda, Panchvalkal, Arista, Fermentation, HRLCMS-QTOF

## 1. INTRODUCTION

Ayurveda describes the process of making alcoholic liquid formulations prepared as per the principles of the classical fermentation process that conserves the alcohol soluble extractives of herbal ingredients in self-generated alcohol[1]. It includes the *Asava* and *Arista*, which are traditional Ayurvedic dosage forms and are frequently prescribed to treat several diseases[2]. The self-generated alcohol in these preparations potentiates the products pharmaceutically and therapeutically[3].

Panchvalkal is a mixture of five plant barks with properties like cleaning and healing infected wounds. Individually and in combination, it has astringent properties and is useful in treating wounds and managing inflammation[4,5]. Panchvalkal formulations also possess the quorum-modulatory potential and moderate prebiotic properties[6]. Panchvalkal includes the stem barks of five plants-*Ficus benghalensis* Linn., *Ficus glomerata* Linn., *Ficus religiosa* Linn., *Ficus virens* W. T. Aiton, and *Thespesia populnea* Soland Ex Correa[1,7,8].

A novel approach was applied to prepare Arista from Panchvalkal barks, and phytochemical investigations were carried out to determine the changes in the fermented Panchvalkal decoctions. The phytochemical investigation of the Ayurvedic formulation using phytochemical parameters and thin layer chromatography of raw materials and formulations is insufficient for proper standardization[9]. Nowadays, innovative and advanced methods are available for standardizing herbal drugs, like the combination of chromatographic and spectrophotometric methods[10]. Therefore, modern analytical methods such as High-Resolution Liquid Chromatography-Mass Spectrometry coupled with Quadrupole Time-Of-Flight (HRLCMS-QTOF) analysis was applied to investigate both non-fermented Panchvalkal decoction as well as fermented formulation Panchvalkal Arista.

## 2. MATERIAL AND METHOD

### 2.1 Collection of Panchvalkal Plant Barks

The stem barks of Panchvalkal plants named *Ficus benghalensis*, *Ficus virens*, *Ficus religiosa*, *Ficus glomerata*, and *Thespesia populnea* were collected from local regions of Ahmedabad, Gujarat, India. The botanical identification and qualitative evaluation were carried out using different parameters from data mentioned in the Ayurvedic pharmacopeia of India. The side branches were collected, and bark from each stem was removed and cut into small pieces. All barks pieces were allowed to shade dry and stored in an air-tight container for further use.

## 2.2 Preparation of Panchvalkal Arista

To prepare Panchvalkal decoction, all the barks were weighted equally (10gm each). The decoction was prepared by boiling this bark (1 part) in a specified volume of water (16 parts) for a definite time (until 1/4th part of the water remains). The decoction was allowed to cool at room temperature and then filtered using a clean muslin cloth[11]. Baker's yeast *Saccharomyces cerevisiae* was used as a fermenting agent[3]. Baker's yeast *Saccharomyces cerevisiae* MTCC 170 was obtained from the microbiology laboratory of SKKPGSC, Nanikadi, Gujarat, India.

The madhur dravya- jaggery (39.0625 gm) was added into 100 ml Panchvalkal decoction (kwath) and mixed well to dissolve jaggery[12]. 10ml of fermenting agent *Saccharomyces cerevisiae* was added aseptically into the previously prepared mixture of jaggery and decoction. Then mixer was transferred aseptically to sterile air-tight glass bottles and placed in an incubator at 28°C temperature for 30 days to complete the fermentation[13,3]. After the completion of incubation time, the fermented extract was taken out and filtered using a clean muslin cloth.

## 2.3 Phytochemical investigation by HRLCMS-QTOF Analysis

Both non-fermented Panchvalkal decoction (PD) and Panchvalkal Arista (SC-Y-PA) were analyzed to find the changes that occurred after the permutation process. For HRLCMS-QTOF analysis, both samples were sent to SAIF, IIT Bombay, India. Both the samples were analyzed using an Agilent (6550 iFunnel QTOFs) system consisting of a hip sampler (G4226A), a binary pump (G4220B), a column component (G1316C), and Q-TOF. Chromatographic separation was performed on a 1290 infinity UHPLC system fitted with a Hypersil GOLD column C18 (100 X 2.1 mm-3Micron).

Nitrogen was used as drying and collision gas. The parameter such as gas flow was set at 13 l/min with a 250 °C temperature, the sheath gas flow rate was 11 l/min at 300 °C, and the nebulizer gas was set at 35 psi gas flow pressure. The capillary tension was set at 3500 V, nozzle voltage was 1000 V, fragmentor voltage was 175 V, and skimmer voltage was set at 65 V. The mobile phase contained 0.1% formic acid in water (A) and 90% acetonitrile, 10% water, and 0.1% formic acid (B). The flow rate was adjusted to 0.300 ml/min with a 5.00 µl injection volume. The elution program is given in Table 1. Positive and negative ion chromatogram was recorded, and Q-TOF data acquisition and analysis of mass spectrometry were carried out using Agilent MassHunter software.

Table 1: Chromatography timetable

|   | Time   | A      | B     | Flow         | Pressure    |
|---|--------|--------|-------|--------------|-------------|
| 1 | 1 min  | 95.00% | 5.00% | 0.300 ml/min | 1200.00 bar |
| 2 | 20 min | 0.00%  | 100%  | 0.300 ml/min | 1200.00 bar |
| 3 | 25 min | 0.00%  | 100%  | 0.300 ml/min | 1200.00 bar |
| 4 | 26 min | 95.00% | 5.00% | 0.300 ml/min | 1200.00 bar |
| 5 | 30 min | 95.00% | 5.00% | 0.300 ml/min | 1200.00 bar |

## 3. RESULT AND DISCUSSION

### 3.1 Phytochemical Investigation by HRLCMS-QTOF analysis

Phytochemical screening for both non-fermented Panchvalkal decoction (PD) and fermented Panchvalkal Arista (SC-Y-PA) was carried out, which showed the presence of different phytochemicals before fermentation and at the end of fermentation. Analysis of these results indicated that certain compounds were retained during the entire course of fermentation while many disappeared. Likewise, the new compound formation was also traced (Table 2). Furthermore, the identified compounds from both the samples were divided into three categories, i.e., retained compounds disappeared compounds, and newly formed compounds. Based on HRLCMS-QTOF analysis, eight compounds were retained during the entire course of fermentation of Panchvalkal Arista. Further, sixty-one compounds were known to diaper, and fifty-eight compounds were newly formed as a result of fermentation.

Table 2: Phytochemicals that are retained, disappeared, and newly formed due to fermentation of Panchvalkal decoction (PD) to Panchvalkal Arista (SC-Y-PA).

| Retained compounds                                                       | Disappeared compounds                        | Newly formed compounds                    |
|--------------------------------------------------------------------------|----------------------------------------------|-------------------------------------------|
| Plumieride                                                               | Dihydrocaffeic acid 3-O-glucuronide          | 8-Hydroxy-3-chlorodibenzofuran            |
| Gentisic acid                                                            | Tranexamic acid                              | O-Demethylfonsecin                        |
| Quinic acid                                                              | Oxacyclotetradecan-2-one                     | Isoamyl nitrite                           |
| b-D-Glucuronopyranosyl-(1->3)-a-D-galacturonopyranosyl-(1->2)-L-rhamnose | Retronecine                                  | 2,4-Di-tert-butylphenol                   |
| Lusitanicoside                                                           | L-isoleucyl-L-proline                        | alpha-Santalal                            |
| Corchorifatty acid F                                                     | Pirbuterol                                   | Eremopetasidione                          |
| N-acetyl-LTE4                                                            | Buformin                                     | 1-[(5-Methyl-2-furanyl)methyl]pyrrolidine |
| Sphinganine                                                              | D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose | Dambonitol                                |

| Retained compounds | Disappeared compounds                                                                                              | Newly formed compounds                                         |
|--------------------|--------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|
|                    | 1,2,3,4-Tetrahydro-alpha,7-dihydroxy-beta-(hydroxymethyl)-9-methoxy-3,4-dioxocyclopenta[c][1]benzopyran-6-propanal | CI Basic red 9                                                 |
|                    | (S)-N-[3-(3,4-Methylenedioxyphenyl)-2-(mercaptomethyl)-1-oxopropyl]glycine                                         | Amantadine                                                     |
|                    | Hydroxyatrazine                                                                                                    | N-Methacrylylglycine methyl ester                              |
|                    | Carindacillin                                                                                                      | Harpagoside                                                    |
|                    | Saccharopine                                                                                                       | Verproside                                                     |
|                    | Procyanidin B7                                                                                                     | 3-(2-Furanylmethylene)pyrrolidine                              |
|                    | Leonuriside A                                                                                                      | Herierin IV                                                    |
|                    | (1S,2S,4R,8S)-p-Menthane-1,2,8,9-tetrol 2-glucoside                                                                | 1-(Methylsulfanyl)-1-oxopropan-2-yl acetate                    |
|                    | Vanillactic acid                                                                                                   | Dehydrojuvabione                                               |
|                    | Decarbamoylsaxitoxin                                                                                               | Ethyl 3,4,5-trimethoxybenzoate                                 |
|                    | Diphenylcarbazide                                                                                                  | Mirtazapine                                                    |
|                    | Lycoperdic acid                                                                                                    | Stilbamidine                                                   |
|                    | Glucosylgalactosyl hydroxylysine                                                                                   | 7-Hydroxy-2-methyl-4-oxo-4H-1-benzopyran-5-acetic acid         |
|                    | 2,4,6-Triethyl-1,3,5-trioxane                                                                                      | Metyrapone                                                     |
|                    | Flavonol 3-O-beta-D-glucoside                                                                                      | trans,trans-1,4-Diphenyl-1,3-butadiene                         |
|                    | Menthyl ethylene glycol carbonate                                                                                  | 3-Phenoxypropionic acid                                        |
|                    | Cinnamodial                                                                                                        | Methoxyeugenol                                                 |
|                    | cis-p-Coumaric acid                                                                                                | 3,4,5-Trimethoxyphenyl acetate                                 |
|                    | Geranyl 2-ethylbutyrate                                                                                            | Flocoumafen                                                    |
|                    | Ethyl vanillin isobutyrate                                                                                         | 6-Cinnamoyl-1-galloylglucose                                   |
|                    | Dihydrodeoxystreptomycin                                                                                           | Procerin                                                       |
|                    | Manumycin A                                                                                                        | 4-Methylburimamide                                             |
|                    | 9,10,13-trihydroxy-11-octadecenoic acid                                                                            | Hesperetin 7-O-glucuronide                                     |
|                    | 16-Hydroxy hexadecanoic acid                                                                                       | Podolide                                                       |
|                    | 3-Methylbutyl 2-methylpropanoate                                                                                   | PGE2                                                           |
|                    | 4,4-Difluoropregn-5-ene-3,20-dione                                                                                 | 2-(Methylthiomethyl)-3-phenyl-2-propenal                       |
|                    | C16 Sphinganine                                                                                                    | Citric acid                                                    |
|                    | Palmitic amide                                                                                                     | (Z)-5-[(5-Methyl-2-thienyl)methylene]-2(5H)-furanone           |
|                    | 2alpha,3alpha-(Difluoromethylene)-5alpha-androstan-17beta-ol acetate                                               | Sulfuric acid                                                  |
|                    | Nigakilactone B                                                                                                    | D-Lombricine                                                   |
|                    | Guazatine                                                                                                          | Halosulfuron-methyl                                            |
|                    | Methyl 2-furoate                                                                                                   | Resorcinol                                                     |
|                    | Lucidenic acid M                                                                                                   | 2,6-dihydroxybenzoic acid                                      |
|                    | Avocadene                                                                                                          | 2-(2-Thienylmethylene)-1,6-dioxaspiro[4.4]non-3-ene            |
|                    | N-Hexadecanoylpyrrolidine                                                                                          | (R)-(Homo)2-citrate                                            |
|                    | L-Malic acid                                                                                                       | Chorismic acid                                                 |
|                    | 6-(Allylthio)purine                                                                                                | 2-Succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate |
|                    | Bismuth subsalicylate                                                                                              | m-Hydroxybenzoic acid                                          |
|                    | Trichotomine                                                                                                       | (±)-Glycerol 1,2-diacetate                                     |
|                    | IPSP                                                                                                               | Dihydroferulic acid 4-O-glucuronide                            |
|                    | Mitoxantrone                                                                                                       | Methyl helianthoate F glucoside                                |
|                    | Mecarbinzid                                                                                                        | Kaempferol 3-rhamnoside 7-xyloside                             |
|                    | Pteridine                                                                                                          | Hypericin                                                      |
|                    | Robinetinidol-(4alpha->8)-catechin-(6->4alpha)-robinetinidol                                                       | Trovafloxacin                                                  |

| Retained compounds | Disappeared compounds                                            | Newly formed compounds                  |
|--------------------|------------------------------------------------------------------|-----------------------------------------|
|                    | 6-(2-Carboxyethyl)-7-hydroxy-2,2-dimethyl-4-chromanone glucoside | 3-(4-Hydroxyphenyl)propionic acid       |
|                    | Sudachiin A                                                      | Torachryson 8-beta-gentiobioside        |
|                    | Fluopicolide                                                     | 1-O-Caffeoylquinic acid                 |
|                    | Lauryl hydrogen sulfate                                          | 9,10-Dihydroxy-12,13-epoxyoctadecanoate |
|                    | Hexazinone                                                       | Cis-5-Caffeoylquinic acid               |
|                    | Calpeptin                                                        | Chlorogenic acid                        |
|                    | 2-Dodecylbenzenesulfonic acid                                    |                                         |
|                    | C16 Sphinganine                                                  |                                         |
|                    | 5Z-Caffeoylquinic acid                                           |                                         |

Additionally, literatures were studied to determine the antimicrobial efficacy of identified compounds. Both retained and newly formed compounds were screened for their recorded antimicrobial potential. From retained compounds, Plumieride, Gentisic acid, Quinic acid, Corchorifatty acid F, and Sphinganine exhibited antimicrobial effects against diverse pathogenic microorganisms. And some newly formed compounds like 2,4-Di-tert-butylphenol, alpha-Santalal, Amantadine, Mirtazapine, Stilbamidine, Methoxyeugenol, Citric acid, Sulfuric acid, 2,6-dihydroxybenzoic acid, Hypericin, Trovafloxacin, 3-(4-Hydroxyphenyl) propionic acid and Chlorogenic acid were also recorded for its potent antimicrobial efficacy. Complete details regarding the antimicrobial potential of some identified compounds are given in Table 3.

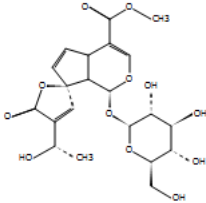
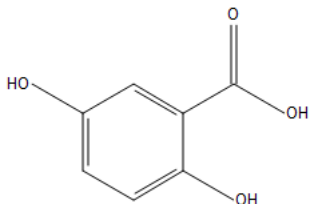
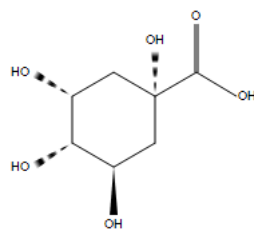
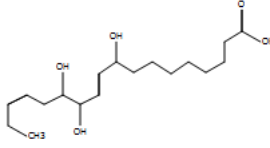
Table 3: Antimicrobial activity of some identified compounds based on literature review

| Name of compound               | Antimicrobial activity                                                                                                                                                                                                                                                                                                                                                      | Reference    |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| <b>Retained compounds</b>      |                                                                                                                                                                                                                                                                                                                                                                             |              |
| <b>Plumieride</b>              | Antibacterial against <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Escherichia coli</i><br>Antifungal against <i>Candida albicans</i> and plant pathogenic fungi                                                                                                                                                                                            | [14][15][16] |
| <b>Gentisic acid</b>           | Antifungal activity against <i>Candida albicans</i> ATCC 10231 and <i>Schizosaccharomyces octosporus</i>                                                                                                                                                                                                                                                                    | [17]         |
| <b>Quinic acid</b>             | Antibacterial activity against <i>E. coli</i> ATCC 35218, <i>P. aeruginosa</i> ATCC 10145, <i>P. mirabilis</i> ATCC 7002, <i>K. pneumoniae</i> RSKK 574, <i>A. baumannii</i> RSKK 02026, <i>S. aureus</i> ATCC 25923, <i>E. faecalis</i> ATCC 29212, <i>B. subtilis</i> ATCC 6633<br>Antifungal against <i>C. albicans</i> ATCC 10231 and <i>C. parapsilosis</i> ATCC 22019 | [18]         |
| <b>Corchorifatty acid F</b>    | Antifungal activity                                                                                                                                                                                                                                                                                                                                                         | [19]         |
| <b>Sphinganine</b>             | Antibacterial against <i>Staphylococcus aureus</i>                                                                                                                                                                                                                                                                                                                          | [20]         |
| <b>Newly formed compounds</b>  |                                                                                                                                                                                                                                                                                                                                                                             |              |
| <b>2,4-Di-tert-butylphenol</b> | Antibacterial activity against <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i>                                                                                                                                                                                                                                                                               | [21]         |
|                                | Antifungal activity against <i>Aspergillus niger</i> , <i>Fusarium oxysporum</i> and <i>Penicillium chrysogenum</i>                                                                                                                                                                                                                                                         | [22]         |
| <b>alpha-Santalal</b>          | Antibacterial against <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i>                                                                                                                                                                                                                               | [23]         |
| <b>Amantadine</b>              | Anti-Influenza A Virus                                                                                                                                                                                                                                                                                                                                                      | [24]         |
| <b>Mirtazapine</b>             | Antibacterial against <i>Proteus vulgaris</i> , <i>Acinetobacter baumannii</i> and <i>Escherichia coli</i>                                                                                                                                                                                                                                                                  | [25]         |
|                                |                                                                                                                                                                                                                                                                                                                                                                             | [26]         |
| <b>Stilbamidine</b>            | Antibacterial activity against <i>Staphylococcus aureus</i>                                                                                                                                                                                                                                                                                                                 | [27]         |

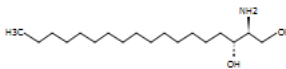
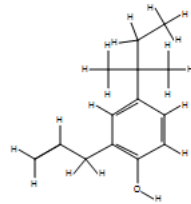
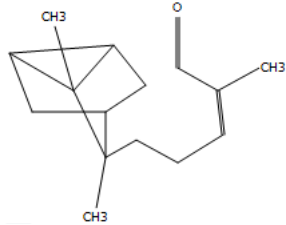
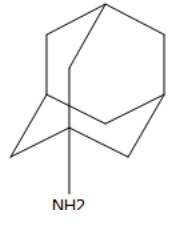
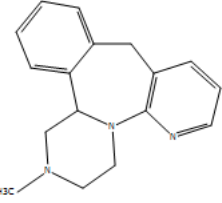
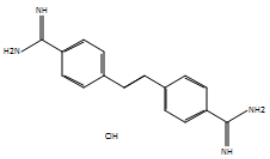
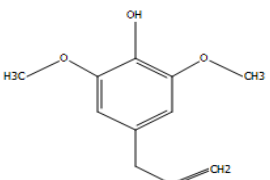
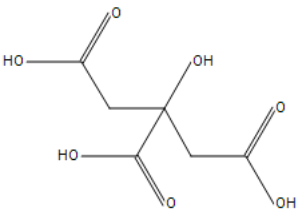
| Name of compound                          | Antimicrobial activity                                                                                                                                                                                                                                                                                                  | Reference            |
|-------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| <b>Methoxyeugenol</b>                     | Antibacterial activity against <i>Bacillus subtilis</i> ATCC 8272 <i>Staphylococcus aureus</i> 25923, <i>Pseudomonas aeruginosa</i> 27853 and <i>Escherichia coli</i> 25922                                                                                                                                             | [28]                 |
| <b>Citric acid</b>                        | Antibacterial Against <i>Salmonella typhimurium</i>                                                                                                                                                                                                                                                                     | [29]                 |
| <b>Sulfuric acid</b>                      | Antibacterial against <i>Salmonella Montevideo</i> , <i>Salmonella Typhimurium</i> , <i>Salmonella Heidelberg</i> , <i>Salmonella Enteritidis</i> , and <i>Salmonella Newport</i>                                                                                                                                       | [30]                 |
| <b>2,6-dihydroxybenzoic acid</b>          | Antibacterial against <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>B. subtilis</i> , <i>S. enteritidis</i><br>Antifungal against <i>C. albicans</i>                                                                                                                                                    | [31]                 |
| <b>Hypericin</b>                          | Antibacterial against <i>Staphylococcus aureus</i>                                                                                                                                                                                                                                                                      | [32]                 |
| <b>Trovafloxacin</b>                      | Antibacterial against <i>Staphylococcus aureus</i>                                                                                                                                                                                                                                                                      | [33]                 |
| <b>3-(4-Hydroxyphenyl) propionic acid</b> | Antibacterial against <i>Salmonella enterica</i> subsp. enterica serovar Typhimurium and <i>S. enterica</i> subsp. enterica serovar Infantis                                                                                                                                                                            | [34]                 |
| <b>Chlorogenic acid</b>                   | Antibacterial against <i>S. pneumoniae</i> ATCC 49619, <i>B. subtilis</i> 9372, <i>S. aureus</i> 6538, <i>Shigella dysenteriae</i> 51302, <i>E. coli</i> ATCC 25922 and <i>Salmonella Typhimurium</i> 50013<br>Antifungal activity against <i>Candida albicans</i> ATCC 10231 and <i>Schizosaccharomyces octosporus</i> | [35]<br>[17]<br>[18] |

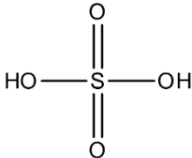
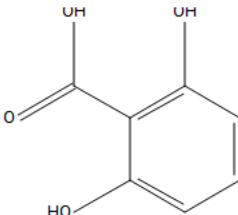
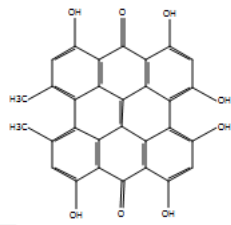
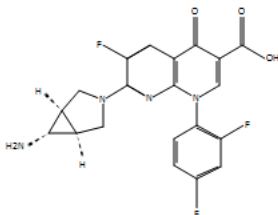
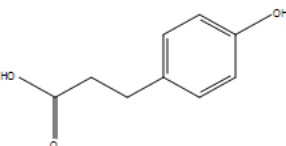
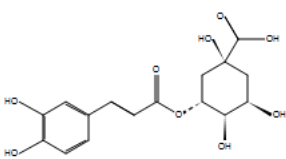
Above mentioned compound's HRLCMS-QTOF analysis data showing their molecular formula, retention time, m/z, mass, and chemical structures are tabulated in Table 4.

Table 4: HRLCMS-QTOF analysis data for the compound having antimicrobial potential

| Name of compound     | Molecular Formula                               | Retention time (Rt) (min) | Mass-to-Charge (m/z) | Mass     | Structure                                                                             |
|----------------------|-------------------------------------------------|---------------------------|----------------------|----------|---------------------------------------------------------------------------------------|
| Plumieride           | C <sub>21</sub> H <sub>26</sub> O <sub>12</sub> | 2.678                     | 515.14               | 470.1413 |  |
| Gentisic acid        | C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>    | 2.771                     | 153.018              | 154.0249 |  |
| Quinic acid          | C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>   | 4.108                     | 191.055              | 192.0621 |  |
| Corchorifatty acid F | C <sub>18</sub> H <sub>32</sub> O <sub>5</sub>  | 8.676                     | 327.217              | 328.2246 |  |



| Name of compound        | Molecular Formula                                | Retention time (Rt) (min) | Mass-to-Charge (m/z) | Mass     | Structure                                                                             |
|-------------------------|--------------------------------------------------|---------------------------|----------------------|----------|---------------------------------------------------------------------------------------|
| Sphinganine             | C <sub>18</sub> H <sub>39</sub> N O <sub>2</sub> | 10.965                    | 302.3083             | 301.301  |    |
| 2,4-Di-tert-butylphenol | C <sub>14</sub> H <sub>22</sub> O                | 1.239                     | 229.1574             | 206.1681 |    |
| alpha-Santalal          | C <sub>15</sub> H <sub>22</sub> O                | 1.246                     | 241.1571             | 218.1678 |    |
| Amantadine              | C <sub>10</sub> H <sub>17</sub> N                | 2.096                     | 174.1258             | 151.1365 |   |
| Mirtazapine             | C <sub>17</sub> H <sub>19</sub> N <sub>3</sub>   | 3.673                     | 288.1469             | 265.1577 |  |
| Stilbamidine            | C <sub>16</sub> H <sub>16</sub> N <sub>4</sub>   | 3.764                     | 265.1464             | 264.1391 |  |
| Methoxyeugenol          | C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>   | 4.629                     | 195.1039             | 194.0965 |  |
| Citric acid             | C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>     | 1.29                      | 191.018              | 192.0247 |  |

| Name of compound                   | Molecular Formula                                                               | Retention time (Rt) (min) | Mass-to-Charge (m/z) | Mass     | Structure                                                                             |
|------------------------------------|---------------------------------------------------------------------------------|---------------------------|----------------------|----------|---------------------------------------------------------------------------------------|
| Sulfuric acid                      | H <sub>2</sub> O <sub>4</sub> S                                                 | 1.659                     | 96.9586              | 97.9659  |    |
| 2,6-dihydroxybenzoic acid          | C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>                                    | 3.058                     | 153.018              | 154.0249 |    |
| Hypericin                          | C <sub>30</sub> H <sub>16</sub> O <sub>8</sub>                                  | 5.323                     | 297.036              | 504.074  |    |
| Trovafloxacin                      | C <sub>20</sub> H <sub>15</sub> F <sub>3</sub><br>N <sub>4</sub> O <sub>3</sub> | 5.325                     | 461.108              | 416.11   |   |
| 3-(4-Hydroxyphenyl) propionic acid | C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>                                   | 5.529                     | 165.054              | 166.0614 |  |
| Chlorogenic acid                   | C <sub>10</sub> H <sub>10</sub> O <sub>6</sub>                                  | 3.546                     | 285.06               | 226.0464 |  |

#### 4. CONCLUSION

Arista preparation is a biomedical fermentation mediated by microorganisms. This fermented product has been well standardized since the Samhita period. Most importantly, it is considered a unique dosage form in Ayurveda as it has several advantages like it possesses better keeping quality due to self-generation of alcohol by fermentation. In this dosage form, multiple phytochemicals with therapeutic values are transformed into liquid form to provide a safe, potent, and better administered liquid form. The study was conducted to determine the changes that occurred due to the fermenting agents on natural plant material. Fermenting agent *Saccharomyces cerevisiae* plays an essential role in the fermentation of Panchvalkal Arista. Furthermore, the HR-LCMS chromatographic data indicates the changes produced in non-fermented Panchvalkal decoction and Panchvalkal Arista. The comparative data indicated the presence of newly formed compounds in Panchvalkal Arista after the fermentation process.

#### 5. ACKNOWLEDGEMENT

A special thanks to SAIF IIT Bombay for analyzing the research sample in their facilities and providing the accurate results in limited time.

## REFERENCES

- [1] A. Chaudhary, M. Dalvi, N. Singh, and A. Wele, "A progressive review of Sandhana kalpana (Biomedical fermentation): An advanced innovative dosage form of Ayurveda," *AYU (An Int. Q. J. Res. Ayurveda)*, vol. 32, no. 3, p. 408, 2011, doi: 10.4103/0974-8520.93925.
- [2] N. Bhatt, M. Deshpande, and A. Valvi, "A CRITICAL REVIEW OF STANDARDIZATION OF AYURVEDIC ASAVA-ARISHTA PART I - REVIEW AND STATUS," *World J. Pharm. Res.*, vol. 5, no. 5, pp. 5041–5048, 2016, doi: 10.20959/wjpr20165-6204.
- [3] C. Das, G. Ghosh, and D. Das, "Ayurvedic liquid dosage form Asava and Arista: An overview," *Indian J. Pharm. Educ. Res.*, vol. 51, no. 2, pp. 169–176, 2017, doi: 10.5530/ijper.51.2.22.
- [4] P. N. Narayan, "A Case Study of Panchvalkal Kwath Dhawan in Dushta Vrana W.S.R. to Varicose Ulcer," *World J. Pharm. Res.*, vol. 7, no. 10, pp. 1040–1045, 2018, doi: 10.20959/wjpr201810-12389.
- [5] A. M. Kulkarni and D. Amarprakash, "CLINICAL STUDY TO EVALUATE EFFICACY OF ' PANCHVALKAL OINTMENT ' IN CLINICAL STUDY TO EVALUATE EFFICACY OF ' PANCHVALKAL OINTMENT ' IN THE M ANAGEMENT OF INFECTED WOUND Abstract :," *Int. J. Anal. Exp. modal Anal.*, vol. 13, pp. 250–258, 2021.
- [6] P. Patel, C. Joshi, H. Palep, and V. Kothari, "Anti-infective potential of a quorum modulatory polyherbal extract (panchvalkal) against certain pathogenic bacteria," *J. Ayurveda Integr. Med.*, vol. 11, no. 3, pp. 336–343, 2018, doi: 10.1016/j.jaim.2017.10.012.
- [7] P. Singh *et al.*, "Panchvalkal (polyherbal formulation) mitigates STZ induced type 2 DM by modulating the expression of hexokinase (HX), lactate dehydrogenase (LDH), triphosphate isomerase (TPI)," *Phytomedicine Plus*, vol. 2, no. 1, p. 100193, 2022, doi: 10.1016/j.phyplu.2021.100193.
- [8] Rajeev Ranjan *et al.*, "Antimicrobial Activity (In Vitro) of Panchvalkal Mentioned In Himvan Agada: An Ayurvedic Formulation," *Int. J. Ayurvedic Herb. Med.*, vol. 4, pp. 2806–2814, 2017, doi: 10.18535/ijahm/v7i4.24.
- [9] P. S. Jadhav and S. A. Gosavi, "A Review on Analytical Method for Standardization of Ayurvedic Asava and Aristas," *Int. J. All Res. Educ. Sci. Methods*, vol. 8, no. 8, pp. 246–248, 2020.
- [10] P. N. Dongare, S. J. Dighade, T. B. Kadu, and A. L. Pohane, "Review on Herbal Drug Formulation and Standardization," *Int. J. Sci. Acad. Res.*, vol. 03, no. 02, pp. 3513–3518, 2022, [Online]. Available: <http://www.scienceijsar.com>.
- [11] A. Thakur, R. Thakur, and D. S. Baghel, "Impact of fermenting agents on marketed patent proprietary formulation Ashwagandha Kalpa," *Asian J. Pharm. Clin. Res.*, vol. 10, no. Special Issue September, pp. 63–66, 2017, doi: 10.22159/ajpcr.2017.v10s4.21338.
- [12] A. Sharangdhara, *Sharangdhara Samhita, Madhyam Khanda 10/44-46, Eglish tanslation by Prof.K.R. Srikantha Murthy, Reprint edition, Chaukhamba orientalia, Varanasi, 2012.* 2012.
- [13] S. Sekar and S. Mariappan, "Traditionally fermented biomedicines, arishtas and asavas from Ayurveda," *Indian J. Tradit. Knowl.*, vol. 7, no. 4, pp. 548–556, 2008.
- [14] T. T. Sint, A. A. Tun, and M. M. Htay, "Antibacterial Screening on Phytoconstituents of Bark from Plumeria acutifolia Poir . ( Tayoke Saga )," in *The Third Myanmar National Conference on Earth Sciences (MNCES, 2019)*, 2019, pp. 1455–1467.
- [15] M. Mone, M. A. U. Saiced, K. M. G. Dastogeer, M. A. Ali, and M. B. Meah, "Plumieride from Allamanda cathartica as an inhibitory compound to plant pathogenic fungi," *Arch. Phytopathol. Plant Prot.*, vol. 47, no. 11, pp. 1311–1326, 2014, doi: 10.1080/03235408.2013.840103.
- [16] M. Afifi, O. Salama, A. Gohar, and A. Marzouk, "Iridoids With Antimicrobial Activity From Plumeria Alba L.," *Bull. Pharm. Sci. Assiut*, vol. 29, no. 1, pp. 215–223, 2006, doi: 10.21608/bfsa.2006.65194.
- [17] J. Vandal, M. M. Abou-Zaid, G. Ferroni, and L. G. Leduc, "Antimicrobial activity of natural products from the flora of Northern Ontario, Canada," *Pharm. Biol.*, vol. 53, no. 6, pp. 800–806, 2015, doi: 10.3109/13880209.2014.942867.
- [18] B. Özçelik, M. Kartal, and I. Orhan, "Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids," *Pharm. Biol.*, vol. 49, no. 4, pp. 396–402, 2011, doi: 10.3109/13880209.2010.519390.
- [19] J. D. Fudyma *et al.*, "Untargeted metabolomic profiling of Sphagnum fallax reveals novel antimicrobial metabolites," *Plant Direct*, vol. 3, no. 11, pp. 1–17, 2019, doi: 10.1002/pld3.179.
- [20] B. Melnik, "Disturbances of antimicrobial lipids in atopic dermatitis," *JDDG - J. Ger. Soc. Dermatology*, vol. 4, no. 2, pp. 114–123, 2006, doi: 10.1111/j.1610-0387.2006.05902.x.
- [21] K. K. Varsha, L. Devendra, G. Shilpa, S. Priya, A. Pandey, and K. M. Nampoothiri, "2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp.," *Int. J. Food Microbiol.*, vol. 211, pp. 44–50, 2015, doi: 10.1016/j.ijfoodmicro.2015.06.025.
- [22] N. Aissaoui *et al.*, "Antibacterial Potential of 2,4-Di-tert-Butylphenol and Calixarene-Based Prodrugs from Thermophilic Bacillus licheniformis Isolated in Algerian Hot Spring," *Geomicrobiol. J.*, vol. 36, no. 1, pp. 53–62, 2019, doi: 10.1080/01490451.2018.1503377.
- [23] L. Jirovetz *et al.*, "Comparative study on the antimicrobial activities of different sandalwood essential oils of various origin," *Flavour Fragr. J.*, vol. 21, no. 3, pp. 465–468, 2006, doi: 10.1002/ffj.1625.
- [24] D. B. Burlington, G. Meiklejohn, and S. R. Mostow, "Anti-influenza a activity of combinations of amantadine and ribavirin in ferret tracheal ciliated epithelium," *J. Antimicrob. Chemother.*, vol. 11, no. 1, pp. 7–14, 1983, doi: 10.1093/jac/11.1.7.
- [25] S. Kalaycı, S. Demirci, and F. Sahin, "Antimicrobial Properties of Various Psychotropic Drugs Against Broad Range Microorganisms," *Curr. Psychopharmacol.*, vol. 3, no. 3, pp. 195–202, 2015, doi: 10.2174/2211556004666150520230121.
- [26] G. Rukavishnikov, L. Leonova, E. Kasyanov, V. Leonov, and G. Mazo, "P.0603 The antimicrobial effects of mirtazapine and fluvoxamine: results of the pilot experiment," *Eur. Neuropsychopharmacol.*, vol. 53, no. May 2022, pp. S442–S443, 2021, doi: 10.1016/j.euroneuro.2021.10.568.
- [27] A. A. Richtarik, K. Lindemulder, and B. De Boer, "The antibacterial activity of selected pharmacological agents.," *Can. J. Microbiol.*, vol. 11, no. 4, pp. 637–640, 1965, doi: 10.1139/m65-085.
- [28] D. L. Custódio *et al.*, "Antimicrobial activity of essential oils from Pimenta pseudocaryophyllus and Tynanthus micranthus," *Brazilian Arch. Biol. Technol.*, vol. 53, no. 6, pp. 1363–1369, 2010, doi: 10.1590/S1516-89132010000600013.



- [29] A. El Baaboua *et al.*, “Evaluation of Antimicrobial Activity of Four Organic Acids Used in Chicks Feed to Control *Salmonella typhimurium*: Suggestion of Amendment in the Search Standard,” *Int. J. Microbiol.*, vol. 2018, 2018, doi: 10.1155/2018/7352593.
- [30] B. R. Scott *et al.*, “Antimicrobial efficacy of a sulfuric acid and sodium sulfate blend, peroxyacetic acid, and cetylpyridinium chloride against *Salmonella* on inoculated chicken wings,” *J. Food Prot.*, vol. 78, no. 11, pp. 1967–1972, 2015, doi: 10.4315/0362-028X.JFP-15-170.
- [31] M. Kalinowska *et al.*, “Plant-derived and dietary hydroxybenzoic acids—a comprehensive study of structural, anti-/pro-oxidant, lipophilic, antimicrobial, and cytotoxic activity in mda-mb-231 and mcf-7 cell lines,” *Nutrients*, vol. 13, no. 9, 2021, doi: 10.3390/nu13093107.
- [32] G. Wang *et al.*, “Hypericin enhances  $\beta$ -lactam antibiotics activity by inhibiting sarA expression in methicillin-resistant *Staphylococcus aureus*,” *Acta Pharm. Sin. B*, vol. 9, no. 6, pp. 1174–1182, 2019, doi: 10.1016/j.apsb.2019.05.002.
- [33] J. R. Aeschlimann, E. Hershberger, and M. J. Rybak, “Activities of trovafloxacin and ampicillin-sulbactam alone or in combination versus three strains of vancomycin-intermediate *Staphylococcus aureus* in an in vitro pharmacodynamic infection model,” *Antimicrob. Agents Chemother.*, vol. 44, no. 5, pp. 1153–1158, 2000, doi: 10.1128/AAC.44.5.1153-1158.2000.
- [34] H. L. Alakomi *et al.*, “Weakening of *Salmonella* with selected microbial metabolites of berry-derived phenolic compounds and organic acids,” *J. Agric. Food Chem.*, vol. 55, no. 10, pp. 3905–3912, 2007, doi: 10.1021/jf070190y.
- [35] Z. Lou, H. Wang, S. Zhu, C. Ma, and Z. Wang, “Antibacterial activity and mechanism of action of chlorogenic acid,” *J. Food Sci.*, vol. 76, no. 6, 2011, doi: 10.1111/j.1750-3841.2011.02213.x.

