Comparative phytochemical analysis of aerial parts of A. procumbeans, F. dichotoma, S. sponteneum, S. nigra and T. angustifolia

Hemali Padalia, Sumitra Chanda

Abstract
Traditional medicinal plants can be good source of herbal drug. This herbal or traditional medicine have a use of various phytochemical or the bioactive compounds. Phytochemicals also known as secondary metabolites, natural plant constituents present in all plant parts which are responsible for medicinal properties of plants. The aim of the present work is comparative phytochemical analysis of aerial part of five different plants viz. A. procumbean, F. dichotoma, S. sponteneum, S. nigra L, T. angustifolia. Qualitative phytochemical analysis was done by various phytoconstituent like flavonoid, tannins, phlobatannis, steroid, saponins, cardiac glycosides, triterpenes and alkaloid. Determination of total alkaloids content was carried out. The result shows the presence of cardiac glycosides and steroids in all the five plants maximum being in A. procumbeans. Maximum alkaloids content was found in T. angustifolia aerial part. Phytochemical screening of plant extracts shows the presence of different level of the cardiac glycosides, steroids, saponins, tannins, alkaloids and triterpenes in the all five plants. The present study suggested that use of these plants in herbal medicine is recommended and makes for recommending new therapeutic agents needs.

Keywords: Medicinal plants, Phytochemicals, Cardiac glycosides, Alkaloid content

1. Introduction
Nature has been an important source of medicine and has helped mankind in the maintenance of health since ancient time. The plant kingdom is a wealth house of potential drugs and at the present year there has been an increasing consciousness about the magnitude of medicinal plants[1]. All plant parts like leaves, stem, flowers, roots, bark, seeds, oil, rhizome etc. have been used as herbal medicine individually or in combinations with each other. According to the World Health Organization (WHO), almost 80% of the world’s population depends on conventional medicines for treatment of many disease, because of green medicine easily available, safe, fewer side effects and better compatibility with the human body[2]. Phytochemicals present in the plants are non-nutritive plant bioactive chemical constituents that produce specific physiological action on the human body[3]. In the plant cell phytochemicals are synthesized by specific biochemical pathways. Phytochemicals can be classified into two groups viz. primary and secondary. Primary metabolites such as proteins, sugars, lipids, amino acids, chlorophyll etc. are required for growth and development of plants while secondary metabolites such as phenolic compounds, alkaloids, flavanoids, terpenoids, tannins, saponins, cardiac glycosides, essential oils, etc. which are important in plant defense against herbivory and adaption to environmental stress[4, 5, 6]. Secondary metabolites are structurally and chemically diverse group of compounds. They have a wide range of application in field of medicine, agriculture, veterinary and numerous other areas. Flavonoids are ubiquitous plant secondary metabolites. They are comprising major subgroups like flavonols, catechins, tannins, anthocyanins, flavones, and flavonones[7]. These compounds are present in plant tissue as red, blue, and purple anthocyanin pigments which help the plant in reproduction by recruiting pollinators and seed dispersers[8]. Flavonoids exhibit a wide range of pharmacological effects including anti-oxidant[9], anticancer, cardiovascular, and anti-inflammatory activity[10], anti-allergic effects[11], etc. Alkaloids are a highly diverse group of low molecular-weight, nitrogen-containing organic compounds derived mostly from amino acids or from the transamination process. Plants produce approximately 12,000 different alkaloids, which can be classify into groups according
to their carbon skeletal structures. Alkaloids exhibit a wide range of pharmacological effects including anti-oxidant and antibacterial activity. Tannins are the high molecular polymeric phenolics produced by secondary plant metabolism. Tannins have a range of ecological functions, such as important constituents in nutrient cycling, provide defense against herbivore and pathogen and plant growth regulating activities. Tannins exhibit various pharmacological effects including anti-oxidant, antibacterial, and anticancer activity etc. Glycosides are characterized by a sugar portion attached by specific bond to non-sugar portions; it may be phenol, alcohol or sulfur compounds. Cardiac glycosides have been reported to have anti-arrhythmic activity and anti-proliferative activity. Plant saponins are a group of naturally occurring secondary metabolites in which glycosyl residues are attached to a triterpenoid (triterpene or steroidal) aglycon. In plants, saponins are mostly found in angiosperms and they have a large number of biologically and pharmacologically active compounds. Saponins have been reported to have wide range of biological effects including antioxidant, anti-inflammatory and anti-cancer activities.

In the present work, five plants viz. A. procumbean, F. dichotoma, S. spontaneum, S. nigra L, T. aungustifolia were selected to evaluate and compare their phytochemical constituents. Such analysis will give a clue to their probable biological activity and thus they can be further studied. Some of the reported biological activities of these five plants are given in Table 1.

Plant Description

- **Alysicarpus procumbean (Roxb.)**
  - Family: Fabaceae
  - Habit: Perennial herb
  - Distribution: Throughout India
  - Vernacular name: Nano Saneravo
  - Parts used: Aerial part
  - Action/uses: Fever, malaria, indigestion and pain

- **Fimbristylis dichotoma L.**
  - Family: Cyperaceae
  - Habit: Grasses
  - Distribution: Throughout India
  - Vernacular name: Chio, Moth
  - Parts used: Aerial part
  - Action/uses: Cattle may graze on F. dichotoma but it has low nutritional value. It is considered a poor manure crop and has been used to make inferior mats in Philippines

- **Schaarum spontaneum L.**
  - Family: Poaceae
  - Habit: Herb
  - Distribution: Throughout India
  - Vernacular name: Dabh, Kans
  - Parts used: Aerial part
  - Action/uses: whole plant is used to treat diseases of vatam and pittam, vomiting, mental diseases, abdominal disorders, dyspnoea, anaemia, and obesity. Roots are useful in treatment of dyspepsia, burning sensation, piles, sexual weakness, gynecological troubles, respiratory troubles, etc

- **Suaeda nigra L.**
  - Family: Amaranthaceae (Suaedoideae)
  - Habit: Shrub
  - Distribution: Throughout India
  - Vernacular name: Seepweed and Mojave sea-blime
  - Parts used: Aerial part
  - Action/uses: Leaves - cooked A salty flavour. They are used as a condiment to add a salty flavour when cooking other foods. Plants for a Future cannot take any responsibility for any adverse effects from the use of plants. Always seek advice from a professional before using a plant medicinally.
Typha angustifolia L.

- **Family:-** Typhaceae
- **Habit:-** Grass
- **Distribution :-** Throughout India
- **Vernacular name :-** Ghabajariyu
- **Parts used :-** Aerial part
- **Action/uses :-** Dried pollen is used in the treatment of kidney stones, internal haemorrhage of almost any kind, painful menstruation, abnormal uterine bleeding, post-partum pains, abscesses and cancer of the lymphatic system. Externally, it is used in the treatment of tapeworms, diarrhoea and injuries.

### Table 1: List of studied plants, part used, solvent extracts and their biological activity.+

<table>
<thead>
<tr>
<th>No</th>
<th>Plant</th>
<th>Parts</th>
<th>Extracts</th>
<th>Activity</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Harpagothyum procumbens</td>
<td>Whole</td>
<td>ET</td>
<td>Analgesic</td>
<td>[29]</td>
</tr>
<tr>
<td>2</td>
<td>Justica procumbens</td>
<td>Leaf</td>
<td>ME</td>
<td>Antidiarrheal</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Fimbristyli slitoralis Gaud</td>
<td>Whole</td>
<td>ET, AQ</td>
<td>Antioxidant</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>Fimbristyli smilaecea</td>
<td>Whole</td>
<td>AQ</td>
<td>Allelopathic</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>Fimbristyli saphylla L.</td>
<td>Whole</td>
<td>ME</td>
<td>Antimicrobial, Cytotoxic,</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Antidiarrheal</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Saccharum spontaneum Linn.</td>
<td>Whole</td>
<td>ME</td>
<td>Anti-diarrheal, CNS Depressant</td>
<td>34</td>
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<td>7</td>
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<td>35</td>
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<tr>
<td>8</td>
<td>Saccharum spontaneum Linn.</td>
<td>Root</td>
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<td>Antilithiatic</td>
<td>36</td>
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<tr>
<td>9</td>
<td>Saccharum spontaneum Linn.</td>
<td>Flower</td>
<td>CH</td>
<td>Antimicrobial, Cytotoxic,</td>
<td>37</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Antioxidant</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Saccharum spontaneum Linn.</td>
<td>Root</td>
<td>ET</td>
<td>Total Phenol and Flavonoid</td>
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</tr>
<tr>
<td>11</td>
<td>Saccharum spontaneum Linn.</td>
<td>Root</td>
<td>PE, ET, CH, AQ</td>
<td>Antioxidant</td>
<td>39</td>
</tr>
<tr>
<td>12</td>
<td>Saccharum spontaneum Linn.</td>
<td>Root</td>
<td>PE, EA, CH, ET, AQ</td>
<td>Antioxidant</td>
<td>40</td>
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<td>13</td>
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<td>ET</td>
<td>Urolithiatic</td>
<td>41</td>
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<td>14</td>
<td>Saccharum spontaneum Linn.</td>
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<td>Antioxidant</td>
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</tr>
<tr>
<td>15</td>
<td>Suaeda fruticosa</td>
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<td>AQ</td>
<td>Diabetic</td>
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<tr>
<td>16</td>
<td>Suaeda asparagoidesMiq.</td>
<td>Whole aerial</td>
<td>ET</td>
<td>Antioxidant</td>
<td>44</td>
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<td>17</td>
<td>Suaeda baccata</td>
<td>Aerial</td>
<td>ET</td>
<td>Antimicrobial</td>
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<td>18</td>
<td>Suaeda vermiculata</td>
<td>Aerial</td>
<td>B4</td>
<td>Antimicrobial</td>
<td>46</td>
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<tr>
<td>19</td>
<td>Typha angustata</td>
<td>Leaves</td>
<td>PE, ME, AQ</td>
<td>Anti-inflammatory</td>
<td>47</td>
</tr>
<tr>
<td>20</td>
<td>Typha angustifolia L.</td>
<td>Leaves</td>
<td>ME, CH, AQ</td>
<td>Anti-thrombolytic, Cytotoxicity</td>
<td>48</td>
</tr>
<tr>
<td>21</td>
<td>Typha angustifolia L.</td>
<td>Rhizome</td>
<td>ME, AQ</td>
<td>Anti-inflammatory</td>
<td>49</td>
</tr>
<tr>
<td>22</td>
<td>Typha angustifolia L.</td>
<td>Pollen grains</td>
<td>ME, AQ</td>
<td>Anti-inflammatory</td>
<td>50</td>
</tr>
</tbody>
</table>

Solvents : Petroleum ether (PE), Ethyl acetate (EA), Ethanol (ET), Methanol (ME), Chloroform (CH), Butanol (BU), Aqueous (AQ), distilled water and water.

### Material and methods

#### Plant Collection
The aerial parts of five plants (A. procumbens, F. dichotoma, S. spontaneum, S. nigra, and T. angustifolia) were collected in August, 2014 from Jamnagar and Surendranagar districts of Gujarat, India. They were thoroughly washed with tap water and air dried under shade. The dried aerial parts were homogenized to fine powder and stored in air tight bottles.

#### Qualitative Phytochemical Analysis
The crude powder of aerial parts of different plants was subjected to qualitative phytochemical analysis. [51]

#### Flavonoids
Alkaline reagent test was performed for checking the presence of flavonoids. The crude powder of aerial parts was treated with a few drops of diluted sodium hydroxide (NaOH) on of intense yellow colour which turned colourless on addition of a few indicated the presence of flavonoids.

#### Tannins
The crude powder of aerial parts was treated with alcoholic ferric chloride (FeCl₃) reagent blue colour indicated presence of tannins.

#### Phlobatanins
The crude powder of aerial parts was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence of the presence of phlobatanins.

#### Saponins
The presence of saponins was determined by Frothing test. The crude powder of aerial parts was vigorously shaken with distilled water and was allowed to stand for 10 minutes and classified for saponin content as follows: no froth indicates absence of saponins stable froth of more than 1.5 cm indicated the presence of saponins.

#### Steroids
Liebemann-Burchard reaction was preformed for checking the presence of steroids. A chloroformic solution of the crude powder of flower was treated with acetic anhydride and a few drops of concentrated H₂SO₄ were added down the sides of the test tube. A blue green ring indicated the presence of steroids.

#### Cardiac glycosides
Keller-kiliian test was performed for checking the presence of
cardiac glycosides. The crude powder of aerial parts was treated with 1.0 ml mixture of 5% FeCl$_3$ and glacial acetic acid (1. 199 v v$^{-1}$). To this solution, a few drops of concentrated H$_2$SO$_4$ were added. Appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides.

**Triterpenes**
Chloroform extract of the crude powder of aerial parts was treated with concentrated sulphuric acid (H$_2$SO$_4$). Appearance of reddish brown ring indicated the presence of triterpenes.

**Alkaloids**
The crude powder of aerial parts was dissolved in 2 N HCl. The mixture was filtered and filtrate was divided into 2 portions. One portion was treated with few drops of Mayer’s reagent and the other portion was treated with equal amount of Wagner’s reagent. The creamish precipitate and brown precipitate indicate the presence of respective alkaloids. A (+) score was recorded if the reagent produced only a slight opaqueness; A (+++) score was recorded if a definite turbidity but no flocculation was observed and A (++++) score was recorded if heavy precipitate of flocculation was observed.

**Determination of total alkaloid content**
Two gram of dried powder was taken in 150 ml flask and 80 ml 20% glacial acetic acid in methanol was added to it and was allowed to stand for 4 h at room temperature. This was filtered and the extract was concentrated in a water bath to one quarter of the original volume. Concentrated ammonium hydroxide (25%) was added drop wise to the extract until the precipitation was completed. Then it was taken in a separating funnel and an equal volume of chloroform was added. It was washed with distilled water three times to make it pH neutral. Sodium sulphate (Na$_2$SO$_4$) was added to remove moisture. It was filtered and dried. Crude alkaloid content was collected and weighed. Crude alkaloid content is expressed in mg/g of dried leaf powder (Harborne, 1973) [51].

**Results and Discussion**

**Phytochemical Screening**
The phytochemical screening of five medicinal plants tested was summarized in the table-2. The results showed presence of active secondary metabolites in the five plants studied. Analysis of the dried powder of plants revealed the presence of phytochemicals such as tannins, flavonoids, saponins, glycosides, steroids, and alkaloids. In *A. procumbeans*, *F. dichotoma* and *S. nigra* aerial part, cardiac glycosides were found in maximum amount. Essiet and Udo, (2015) [51] reported presences of maximum amount of cardiac glycosides in stems, leaves and flowers of *Allamanda cathartica*. [52]

Maximum amount of steroid were present in aerial part of *A. procumbeans* and *S. spontaneum*. In *F. dichotoma*, *S. nigra* and *T. angustifolia* aerial parts, steroids were absent. Shah and Hossain, (2014) reported presence of steroids in leaves solvent extracts of *Merremia borneensis* [3], [53].

In *S. spontaneum* and *T. angustifolia* aerial part, triterpenes and flavonoids were found in maximum amount. In *A. procumbeans*, *F. dichotoma*, and *S. nigra* aerial parts, triterpenes were absent. Dey et al., (2014) and Padalia et al., (2014) reported presence of triterpenes and flavonoids in leaves of *Clerodendrum viscosum* and flowers of *Tagetes erecta* [5], [54].

Saponins and alkaloids by Wagner’s reagent were present in the *A. procumbeans* and *F. dichotoma* aerial part. Tannins were present in *A. procumbeans* and *T. angustifolia* aerial part, while absent in the other three plants. Iqbal et al., (2015) reported presence of saponins, alkaloids and tannins in leaf extracts of *G. velutina* [55]. Phlobatannins and alkaloids by Mayer’s reagent were completely absent in all the five plants. Total alkaloid content of crude powder of all the five plants is given in Table.3. The total alkaloid content in all the five plants varied between 0.41 mg/g to 0.83 mg/g. Maximum alkaloid was in *T. angustifolia*. Alkaloids and their derivatives have an important biological function and are used in analgesic, antispasmodic and bactericidal activities. Alkaloids are mainly found in large amount in flowering plants and they have an important physiological effect on mankind [56]. From the results of preliminary phytochemical analysis, it can be concluded that different aerial part of plants possessed different level of phytoconstituents in different amounts. Moteriya et al., (2015) reported phytochemical analysis in dried powder of seven different flowers, various phytochemical are present in varied level and maximum amount of flavonoid was present [57]. Phytochemicals are the heart of phytomedicine. The healing power of phytomedicines directly correlates with the presence of various phytochemicals. Various phytochemical constituents present in the plants may be used as a basic medicinal agent which show divers pharmacological activity [58].

**Conclusions**
Finally, it can be concluded that, the preliminary phytochemical analysis revealed mainly the presence of cardiac glycosides and steroids in all the five plants, maximum being in *A. procumbeans*. This plant can be essentially used as a source of antibacterial, antioxidant, anticancer agent and it may possess other pharmacological activities which can be explored.

<table>
<thead>
<tr>
<th>NO.</th>
<th>Test</th>
<th><em>A. procumbeans</em></th>
<th><em>F. dichotoma</em></th>
<th><em>S. spontaneum</em></th>
<th><em>S. nigra</em></th>
<th><em>T. angustifolia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>4</td>
<td>Saponins</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>++++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Cardiac glycosides</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Triterpenes</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>Alkaloids</td>
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<td></td>
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<tr>
<td></td>
<td>(1)Mayer’s reagent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(2)Wagner’s reagent</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>
Table 3: Total alkaloid content of different plants

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant name</th>
<th>Total alkaloid content (mg/g)</th>
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<tr>
<td>1</td>
<td>Alysicearpusprocumbenii</td>
<td>0.46</td>
</tr>
<tr>
<td>2</td>
<td>Fimbristylis dichotoma</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>Saccharum spontaneum</td>
<td>0.41</td>
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<tr>
<td>4</td>
<td>Suedanigrana</td>
<td>0.54</td>
</tr>
<tr>
<td>5</td>
<td>Typha angustifolia</td>
<td>0.83</td>
</tr>
</tbody>
</table>

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References

34. Vhuyian MMI, Biva IJ, Saha MR, Islam MS. Anti-diarrhoeal and CNS depressant activity of methanolic


