Quantification of bioactive compounds in selected medicinal plants of Dakshina Kannada district

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Abstract

Introduction: Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. The present study was conducted to quantify the bioactive compounds present in leaf methanol extract of selected medicinal plants.

Methods: Primary and secondary metabolites like carbohydrate, protein, fat, L-ascorbic acid, phenolic, alkaloids, flavonoid, tannin and saponin content was determined by following standard procedures.

Results: C. papaya showed highest protein (884.2±0.1 µg/ml), fat (8.616±0.104 %), ascorbic acid (162.96±0.05 mg/g), phenolic (255.82±0.01 mg/g) and tannin (32.46±0.408 mg/g) content. K. pinnata showed high amount of carbohydrate (45.13±0.11 mg/100ml) and saponin (99.67±0.323 %) content. A. ficoidea showed high alkaloid (12.11±0.01 mg/g) content and E. perfoliatum showed highest flavonoid (222.46±0.202 mg/g) content.

Conclusion: The bioactive compounds such as phenolics, flavonoids and tannins which will contribute to the antioxidant and antimicrobial activity of these plants. They also play important role in the growth and development, including protection from predators and environmental stress.

Keywords: Bioactive compounds, medicinal plants, primary metabolites, secondary metabolites.
Polyphenolic flavonoids occurring as glycosides were found to have strong antioxidant properties, to be effective in scavenging free radicals and reactive oxygen species with their phenolics hydroxyl groups [13] and to decrease the risk of diseases such as cardiovascular and degenerative diseases by counteracting macromolecular oxidation [14, 15]. Gallic acid (3, 4, 5-trihydroxybenzoic acid) is an endogenous plant phenolic compound that is found in many phytotherapeutics [16]. Many gallic acid rich plants exhibit protective effects against liver injury [17-19] and they have been found to have various pharmacological properties, including neuroprotective, antioxidant [20], anti-inflammatory [21, 22], antiobesity [23-25] and anticancer [26] properties. Several studies have reported that gallic acid and its derivatives can selectively induce cancer cell death by apoptosis without harming healthy cells [27-29]. A major class of phenolic compounds is hydroxycinnamic acids, which are found in almost every plant and the major representative of hydroxycinnamic acids is caffeic acid [30, 31]. Caffeic acid possesses both antioxidant and prooxidant properties. The prooxidant action of polyphenols may be an important mechanism in their anticancer and apoptosis-inducing properties [32, 33].

So the objective of my study to quantify the bioactive compounds present in leaf methanol extract of selected medicinal plants.

Materials and Methods

*Alternanthera ficoidea* L., *Carica papaya* L., *Eupatorium perfoliatum* L., *Kalanchoe pinnata* L., *Mussaenda belilla* L., and *Talinum fruticosum* L., were collected from in and around the area of Mangalore and Belthangady (Dakshina Kannada, District). The plants were identified and authenticated by the botanist of Department of Applied Botany, Mangalore University. These leaf samples were separated from each plant and were washed with running tap water, surface sterilized in distilled water, blot it dried and powdered. 150 grams of the samples were weighed and extraction was carried out using soxhlet apparatus. The extracts obtained from the solvents were concentrated using rotary vacuum evaporator and then dried. The yielded crude extracts were stored in a deep freezer at -20 ºC for the further study. Since most of bioactive compounds were qualitatively determined in methanol extract. Methanol leaf extract was used for quantification of bioactive compounds in selected plant materials.

Quantification of primary metabolites

**Total carbohydrate**

The total carbohydrate content was estimated by the anthrone method [34]. 100 mg of the dried powder sample was hydrolyzed with 5.0 ml of 2.5 N HCl in a test tube and kept it in a boiling water bath for three hours. The cooled to room temperature and neutralized with solid sodium carbonate until the effervescence ceases out. The sample was made up to 100 ml, centrifuged and supernatant was collected. 0.2-1.0 ml of glucose served as the working standards. To 0.5 and 1.0 ml test sample was made up to 1.0 ml with distilled water. To this solution, 0.2 ml of test sample was taken and standard (Bovin Serum Albumin) was pipetted out into a series of test tubes. 0.2 ml of test sample was taken and made up to 1ml with distilled water. 1 ml of distilled water serves as the blank. 5 ml of Folin-ciocalteau reagent was added, mixed thoroughly and incubated at room temperature in the dark for 30 min. Blue colour was read at 660 nm. The amount of protein content was calculated using standard graph and expressed in mg/g.

**Secondary metabolites**

**L-Ascorbic acid content**

Ascorbic acid content in the samples was estimated by the method of Roe and Keuther [37]. 1g of powdered sample extract was homogenized in 10 ml of 4% TCA and centrifuged at 2000 rpm for 10 min. The supernatant was treated with a pinch of activated charcoal, shaken well and kept it for 10 min. Centrifugation was repeated twice to remove the charcoal residues. The volume of the clear supernatants obtained was noted. 0.5 ml and 1 ml of the supernatant was taken for the assay and the volume was made up to 2.0 ml with 4% TCA. Series of working standard ascorbic acid solution (0.2 to 1.0 ml) was made up to 2.0 ml with 4% TCA and to this 0.5 ml of Dinitrophenylhydrazine (DNPH) reagent and two drops of thiourea solution after 3 hrs. The osazones formed after incubation at 37 ºC for 3 hrs, were dissolved in 2.5 ml of 85% H2SO4 in ice cold, with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea solutions were added after the addition of 85% H2SO4. After incubation for 30 min. at room temperature, the solutions were read at 540 nm and the levels of ascorbic acid in the samples were determined using the standard graph and expressed as mg of ascorbate /g extract.

**Total protein**

Protein content in sample was determined by using Lowry’s method [38]. 500 mg of powdered sample was mixed with 10 ml of the phosphate buffer and centrifuged. Supernatant was used for protein estimation. 0.2 ml to 1ml of the working standard (Bovin Serum Albumin) was pipetted out into a series of test tubes. 0.2 ml of test sample was taken and made up to 1ml with distilled water. 1 ml of distilled water serves as the blank. 5 ml of Folin-ciocalteau reagent was added, mixed thoroughly and incubated at room temperature in the dark for 30 min. Blue colour was read at 660 nm. The amount of protein content was calculated using standard graph and expressed in mg/g.
was extracted thrice with 20 ml of CHCl₃, pooled the organic layers and evaporated to dryness. The basic fraction basified the acidic layer with Conc. NaOH to pH-12 and extracted with CHCl₃ (20 ml) thrice, pooled the CHCl₃ layers, dry over absorbent cotton and evaporated to dryness, weighed the fraction that contains ajmalicine and serpentine expressed as mg/100g of extract.

**Total phenolic content**

0.5 ml of the sample extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% AlCl₃ followed by 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of flavonoid content was expressed as µg of quercetin/mg of extract [40].

**Tannin content**

The tannin content was estimated by the method of Price [41] with slight modifications. 20 μl of the sample aliquoted into a test tube containing 980 μl of distilled water. After 4 hrs with continuous stirring at about 55 ºC. The plant sample extract was dispersed in 200 ml of 20% ethanol. The combined extracts were washed twice with 10 ml of 5% aqueous sodium chloride. 60 ml of n-butanol was added to aqueous layer and washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven into a constant weight. The saponin content was calculated in percentage.

**Statistical analysis**

The experimental results were expressed as mean ± Standard Error Means (SEM) of triplicates. Analysis of data was carried out by applying alkaline copper solution was added to all the test tubes, mixed well and allowed to stand for 10 min. Then 0.5 ml of independent’t’ test and one-way analysis of variance (ANOVA) using SPSS. P-value lesser than 0.05 (p<0.05) was considered as statistically significant.

**Result and Discussion**

Results of the quantitative analysis of primary and secondary metabolites of selected medicinal plants were represented in the table 1.

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>A. ficoidea</th>
<th>C. papaya</th>
<th>E. perfoliatum</th>
<th>K. pinnata</th>
<th>M. belilla</th>
<th>T. fruticosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (mg/100ml)</td>
<td>29.1±0.1</td>
<td>45.1±0.1</td>
<td>35.66±0.11</td>
<td>45.13±0.11</td>
<td>24.46±0.41</td>
<td>18.8±0.17</td>
</tr>
<tr>
<td>Protein (µg/ml)</td>
<td>692.1±0.1</td>
<td>884.2±0.26</td>
<td>548.03±0.15</td>
<td>292.3±0.3</td>
<td>356.1±0.15</td>
<td>224.4±0.41</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>6.51±0.010</td>
<td>8.616±0.104</td>
<td>2.93±0.060</td>
<td>5.33±0.293</td>
<td>4.66±0.030</td>
<td>3.53±0.026</td>
</tr>
<tr>
<td>L-Ascorbic acid (mg/g)</td>
<td>37.06±0.11</td>
<td>162.96±0.05</td>
<td>48.1±1</td>
<td>87.8±0.72</td>
<td>11.2±0.2</td>
<td>69.1±0.26</td>
</tr>
<tr>
<td>Total Alkaloid (mg/g)</td>
<td>12.11±0.01</td>
<td>10.31±0.02</td>
<td>11.76±0.033</td>
<td>10.38±0.012</td>
<td>10.42±0.002</td>
<td>9.27±0.030</td>
</tr>
<tr>
<td>Total Phenolic (mg/g)</td>
<td>189.44±0.41</td>
<td>255.82±0.01</td>
<td>173.17±0.124</td>
<td>157.38±0.364</td>
<td>112.98±0.522</td>
<td>226.20±0.105</td>
</tr>
<tr>
<td>Total Flavonoid (mg/g)</td>
<td>70.56±0.011</td>
<td>76.88±0.07</td>
<td>222.46±0.202</td>
<td>42.49±0.052</td>
<td>52.49±0.005</td>
<td>91.42±0.085</td>
</tr>
<tr>
<td>Total Tannin (mg/g)</td>
<td>32.23±0.195</td>
<td>21.13±0.157</td>
<td>31.10±0.105</td>
<td>28.47±0.066</td>
<td>32.46±0.408</td>
<td>31.14±0.026</td>
</tr>
<tr>
<td>Total Saponin (%)</td>
<td>92.46±0.007</td>
<td>55.51±0.040</td>
<td>52.62±0.248</td>
<td>99.67±0.323</td>
<td>9.54±0.037</td>
<td>99.39±0.081</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=3, p<0.05 considered as significant.

Data are expressed as mean±SEM. Independent ‘t’ test was used to compare the carbohydrate, fat and proteins in the selected parts of medicinal plants. p=0.001, α=0.05, p<0.05 there was a significant difference between the tests. Since ‘p’ value was equal to 0.001 was < than our chosen significant level α=0.05. We concluded that the mean of the different tests i.e., anthrone method for carbohydrate, fat content, Lowry’s content method for protein content was significantly different. One-way ANOVA statistics were used to compare the samples between all secondary metabolites. p=0.001, α=0.05, p< 0.05 was considered as significant.

Organic compounds produced in the plant kingdom have metabolic functions essential for plant growth and development produced in every plant [43]. Carbohydrate is one such group of carbon compounds, which are essential to life. Almost all organisms use carbohydrates as building blocks of cells and as a matter of fact, exploit their rich supply of potential energy to maintain life [44]. Proteins are essential to maintaining the structure and function of all life and vital for growth and development. The presence of higher protein level in the plants parts points towards their possible increase in food value or that a protein based bioactive compound could also be isolated in future [45]. Plant sugars can be used as artificial sweetener and they can even help in diabetes by supporting the body in its rebuilding [46]. Vitamins are essential to maintain normal metabolic processes and homeostasis within the body. The amount of a specific vitamin required by an individual varies

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considerably and it is influenced by such factors as body size, growth rate, physical activity, and pregnancy [47]. Ascorbic acid (vitamin C) has been gaining attention as a potential treatment for human malignancies. Various experimental studies have shown the ability of pharmacological doses of vitamin C alone or in combinations with clinically used drugs to exert beneficial effects in various models of human cancers [48]. The alarming increase in the rate of infection by antibiotic-resistant microorganisms has urged scientists to search for compounds which have potential antimicrobial activity [49]. The ability to synthesize compounds by secondary metabolism possessing antimicrobial potential makes plants an invaluable source of pharmaceutical and therapeutic products [50]. More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders [51-54]. Due to the presence of various compounds that are essential for good health, it can also be used to improve the health status of society [55].

Conclusions
Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional uses [56], then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional ‘herbal’ drug. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, column chromatography, flash chromatography, sephadex chromatography and HPLC, should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity [57].

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