Biochemical Profiling and Total Flavonoids Contents of Leaves Crude Extract of Endemic Medicinal Plant
*Corydyline terminalis* L. Kunth

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ABSTRACT

Now a day the importance of medicinal plants has been increasing both for pharmaceutical industry and traditional users. Most of the countries either it is developing and under developing mostly rely on traditional medicines. This herbal or traditional medicine involves the use of different type organic extracts or the bioactive pure chemical constituents. This type of biochemical investigation provides the health application at minimum cost. This survey such as ethnomedicine keenly represents one of the best avenues in searching new economic plants for medicine. Keeping this view in mind, the present study is carried out in *Corydyline terminalis* L. Kunth leaves collected from rainforest area at Sabah state, Malaysia. This plant has several beneficial properties. The powder leaves of *Corydyline terminalis* L. Kunth was extracted with organic solvent such as hexane, ethyl acetate, chloroform, butanol and methanol. The total phenolic contents of the extracts was determined by Willet method with modification. The results for total flavonoids content of the extracts as caffeic acid equivalents were found to be highest in hexane extract (68.02%) followed by ethyl acetate (61.50%), methanol (39.27%), butanol (19.08%) and chloroform (15.75%). Based on these results it can be suggested that the biochemical properties of the leaves for curing various ailments.

Key words: Biochemical screening, Flavonoids content, Soxhlet extraction, Organic extracts, *Corydyline terminalis* L. Kunth.

INTRODUCTION

Ancient man is well known for their knowledge of utilizing vast variety of drugs for millennia. The crude form of many traditionally used herbs and plants are known to be very useful based on current knowledge. There are at least 121 chemical substances of known structure are still extracted from plants that are useful as drugs around the globe. The World Health Organization (WHO) estimates that more than 4 billion people, 80% of the total world population, presently medication herbal medicine for some aspect of primary health care. In the third world countries, herbal medicine is a major component or ingredient in all indigenous peoples’ traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. WHO had noted that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. The major pharmaceutical companies in the world are presently conducting extensive research on plant materials which are gathered from the rain forests and other places for their potential medicinal value.

Today, the U.S. Pharmacopoeia, with its reliance on herbal compounds, has been all but forgotten. The *Physician's Desk Reference*, an extensive listing of chemically manufactured drugs is where most of the modern physicians rely on. It is important to note that each entry in this enormous volume, in addition to specifying the chemical compound and actions of a particular drug, also includes an extensive list of contraindications and possible side effects. Rather than using a whole plant as different types of organic extracts, pharmacologists identify, isolate, extract, and synthesize individual components, thus capturing the active properties.
This can create problems, however, in addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavonoids, and other substances that are important in supporting a particular herb’s medicinal properties. These elements also provide an important natural safeguard isolated or synthesized active compounds can become toxic in relatively small doses; it usually takes a much greater amount of a whole herb, with all of its components, to reach a toxic level. Herbs as traditional medicine can have powerful effects. They should not be taken lightly. Now a day the suggestions to practitioner for herbal treatments in this book are not intended to substitute for consultation with a qualified health care, but rather to support and assist you in understanding and working with your physician’s advice.

Chemical substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems. Some medicinal herb used in ‘Traditional Chinese Medicine’ for more than two thousand years to treat asthma and other respiratory problems. Ephedrine, the active ingredient in ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems. It helps the patient to breathe more easily.

There are so many groups or families of phytochemicals and they help or accelerate to the human body in several ways. Chemical constituents inside the plants may be protecting human body from a host of diseases. Chemical constituents are non-nutritive plant bioactive chemicals that have protective or disease preventive properties. Plant produces itself these bioactive chemicals to protect itself but recent research demonstrates that many chemical constituents can protect humans against diseases. There are so many groups of bioactive chemicals in fruits, vegetables and herbs and each works differently.

So many possible ways to fight or kill these diseases is to improve our body’s antioxidant defenses. Comparatively high consumption of vegetables and fruits has been associated with a lowered incidence of such degenerative and incurable diseases. Fruits constituents also help to improve health in other ways. Due to active chemical constituent of fruit juice, can also be taken to alleviate sore throat and seasickness. The functional bioactivity of a plant organic extract, in general, depends upon the presence of compounds such as polyphenols, carotenoids, terpenoids and chlorophyll. Plants also can contribute in this area primarily due to the antioxidant activity of phenolic and flavonoids compounds.

By the researcher several studies have done and revealed that the antioxidant capacity may be from compounds inside the plants samples such as flavonoids, isoflavones, flavones, anthocyanins, catechins and other phenolics. Various curable and incurable diseases has been linked to the oxidative stress but the food industry has been concerned with issues such as rancidity and oxidative spoilage of foodstuffs. The oxidation for enzyme as well as auto oxidation of amino acid or lipids during storage and processing is the major reaction responsible for the deterioration in food quality affecting the colour, flavour, texture and nutritive value of the foods. They are often added antioxidants to the foods to prevent the radical chain reactions of oxidation by inhibiting the initiation and propagation step leading to the termination of the reaction and a delay in the oxidation process.

Corydline terminalis L. Kunth, Asparagaceae (monocotyledonous flowering plant) is one of the popular traditional plant used extensively as remedy in Southeast Asia for the treatment of wide range of diseases. Cordylines are known to the world by many names and are crowned as “King of tropical foliage”. Corydline terminalis L. Kunth is a shrub plant, with woody stem that can reach a height of 6 metres. This plant which is native to tropical Asia, Australia and New Zealand does not have many branches and its roots can swell to form starch extract, usually occurs in root plants. The name Cordyline comes from the Greek word koridyle, meaning “club,” a reference to the enlarged underground stems or rhizomes. The leaves are simple and in an arrangement of twisted leaves, long, oval or in shape of spear as long as 45 cm and between 7.5-15 cm in width. The stalk consists of long and flat leaves. Consisting of leaves of various colour; red, pink, purplish strips and also with borders of whitish leaves it is reaching high popularity among gardeners, landscapers and collectors alike. The flowers grow in a bunch which is in medium size up to 70 cm long, yellow or purple in colour. Meanwhile the fruits form in ball shapes, reddish in colour with diameter of 2 cm.

In Malaysia, especially Sabah, Kadazandusun’s ethnic use Corydline terminalis L. Kunth as medicinal use for curing cough, bloody cough, dysentery, high fever, difficulties in urine, bloody urine, kidney diseases, Tibiae, headache and inflammation in the digestive tract. Furthermore, it is also can also be used to heal scurf and joint pain. People also used Corydline terminalis L. Kunth as popular ornamental plant, with numerous cultivars available, many of them selected for green or reddish or purple foliage despite its variety of medicinal uses. This species is usually planted by the stem or leafy shoots. Some of the villagers plant it by a local method name ‘ut’. It is easily planted where the stem part easily grows into a new plant. It is convenient to be planted in pots and on the soil individually, in rows or in groups. This is the first time a research work is being carried out on this tropical traditional medicinal plant. During
our study on the biologically active constituents of this plant, we examined the biochemical screening of the leaves of *Corydyline terminalis* L. Kunth widely used in Sabah community, Malaysia. Hence, the aim of this present study has been made to investigate the biochemical screening and total flavonoids contents of the leaves crude extracts of *Corydyline terminalis* L. Kunth.

**MATERIALS AND METHODS**

**General**
All the solvent used in this experiment were of analytical grade or GC grade. UV-Visible spectra measurements were done using Spectro (Thermo Fisher Scientific, model 4001/4) spectrophotometer Ultrospeck in methanol (\(\lambda_{\text{max}}\) in nm). The water was purified from water distillation plants in our laboratory. All solvents were analytical reagent grade.

**Plant Material**
The fresh green leaves of *Corydyline terminalis* L. Kunth were collected from the rainforest hilly area of southern part of Sabah, Malaysia. The leaves of this plant were harvested during the month of May, 2011. The leaves were collected at afternoon on May 21, 2011 and packed in polyethylene bags and bring to the laboratory and stored at 4°C until required.

**Plant material grinding**
The samples were washed thoroughly with fresh running water, dried under shade room temperature (25 ± 1 °C) for 3 days. The plant initially identified by morphological features and data base present in the library, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Sabah, Malaysia and a voucher specimen has been deposited at the Borneo Herbarium, Sabah, Malaysia with voucher number 031. Approximately 50 g of leaves were ground using a grinder (Blender 80115) for 20 s. The unfermented *Corydyline terminalis* L. Kunth leaves were kept in the oven at 40 °C and put in a desiccator for at least 24 h prior to analysis. The small pieces of the samples were homogenised in a grinder for 3 min to 40-mesh size. The air-dried leaves and stems of *Corydyline terminalis* L. Kunth were pulverized into powdered form.

**Extraction**
Coarse powders (500 g) were separately Soxhlet extracted with methanol solvent (1 L, 72 h) at the temperature between 45-65 °C. The viscous semi solid masses were dried at 40 °C in rotary evaporator frozen and lyophilized (Buchi Labortec AG, model 1, R-215). The methanol extract was (30 g) suspended in water and then defatted and finally extracted successively with hexane, chloroform, ethyl acetate and butanol to give hexane (5.03 g), ethyl acetate (4.67 g), chloroform (3.39 g), and butanol (2.58 g) and residual methanol fractions (7 g), respectively. The extract was filtered through Whatman No. 41 filter paper to obtained particle free crude extract. The residue was re extracted twice follow the same procedure and filtered. The combined extracts were concentrated and dried under vacuum.

**Determination of total flavonoids**
The total flavonoids content of the crude extracts of *Corydyline terminalis* L. Kunth were determined by using colorimetric method as described by Willet,[15-17] with some modifications. Methanol extracts (0.5 mL), 10% aluminium chloride (0.1 mL), 1M potassium acetate (0.1 mL) and distilled water (4.3 mL) were mixed. At room temperature the mixture samples was incubation for 30 min. The absorbance of the crude extracts was measured at 415 nm using Spectro (Thermo Fisher Scientific, model 4001/4) spectrophotometer. Quercetin was used to make the calibration curve. The calculation of total flavonoids content in the extracts was carried out in triplicate and the results were averaged.

**Preliminary phytochemicals screening**
One gram of hexane, ethyl acetate, chloroform, butanol and aqueous methanol crude plant extracts of the powder leaves of *Corydyline terminalis* L. Kunth were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The obtained crude extracts were subjected to preliminary biochemical screening following the methodology of Hatano[18] and Birt.[19]

**SCREENING PROCEDURE**

**Test for alkaloids**
The stock crude extract (5 ml) was added hydrochloric acid (2 ml). One milliliter of Dragendroff’s reagent was added to this acidic medium. An orange or red precipitation immediately produced that indicates the presence of alkaloids.

**Test for amino acids**
The crude stock extract solution (1 ml) was added few drops of Ninhydrin reagent. The purple colour appearance shows the presence of amino acids.

**Test for anthraquinones**
The crude stock extract solution (5 ml) was hydrolysed by diluted concentrated sulphuric acid extracted with benzene. Finally dilute ammonia solution was added to it. The rose pink coloration obtained. It is suggested that the positive response for anthraquinones.

**Test for flavonoids**
The crude stock extract solution (1 ml) and a few drops of dilute sodium hydroxide were added. An intense yellow colour was appearance in the plant crude extract, which
become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids.

**Test for glycosides**
The crude extract was hydrolysed by hydrochloric acid for few hours on a water bath. Pyridine (1 ml) was added to the hydrolysate and a few drops of sodium nitroprusside solutions were added to it and then it was made alkaline with sodium hydroxide solution. The pink to red colour obtained shows the presence of glycosides.

**Test for phytosterol**
The plant crude extract solution was refluxed with solution of alcoholic potassium hydroxide till to complete saponification takes place. The whole mixture was diluted with water and then extracted with ether. The ether layer was evaporated by water bath and the residue was tested for the presence of phytosterol. The residue was dissolved with few drops of diluted acetic acid then 3 ml of acetic anhydride was added followed by few drops of Conc. H$_2$SO$_4$. The bluish green colour was appearance showed the presence of phytosterol.

**Test for saponins**
The crude extract stock solution (1 ml) was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm foam layer showed the presence of saponins.

**Test for steroids**
The crude plant extracts solution (1 ml) was dissolved in chloroform (10 ml) and added equal volume of concentrated sulphuric acid by sides of the test tube. The upper layer turns into red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

**Test for tannins**
The crude extract solution (3 ml) and a few drops of 1% lead acetate were added. A yellow precipitate was formed, indicates the presence of tannins.

**Test for triterpenoids**
The dry crude plant extract (5 mg) was dissolved in chloroform (2 ml) and then acetic anhydride (1 ml) was added following the addition of 1 ml of Conc. H$_2$SO$_4$. Formation of reddish violet colour indicates the presence of triterpenoids.

**Statistical analyses**
Experimental results were mean ± S.D. of three parallel measurements and analyzed by SPSS 10 (SPSS Inc. Chicago, IL). Differences between means were determined using Tukey multiple comparisons and least significant difference (LSD). By using the Pearson correlation coefficient the correlation is obtained in bivariate correlations. $P$ values < 0.05 were regarded significant.

### RESULTS AND DISCUSSION

The percentage yields of extraction of the leaves powder of Corydalyne terminalis L. Kunth were hexane (3.19 g), ethyl acetate (16.31 g), chloroform (1.59 g), and butanol (9.50 g) and residual methanol fractions (6.34 g), respectively. The total flavonoids contents of the different organic crude plant extracts were determined by Willet method are reported as quercetin equivalents (Table 1). Among the extracts, hexane extract was containing highest (68.02%) amount of flavonoids content compounds followed by ethyl acetate (61.50%), methanol (39.27%), butanol (19.08%) and chloroform (15.75%) (Table 1). In our previous several studies, it has been reported that the yield of extractable compounds was highest in methanol extract from the leaves, peel and seeds of pomegranate in comparison with the solvents such as chloroform, butanol, ethyl acetate and hexane. Furthermore, the extraction of flavonoids contents from the leaves, peel and seeds is commonly achieved with methanol or aqueous ethanol.

The result obtained in the present investigation (Table 2), the ethyl acetate, chloroform, butanol, aqueous ethanol and methanol extracts of the leaves powder of Corydalyne terminalis L. Kunth showed the presence of alkaloids, amino acids, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids. Further, all the organic extracts of the leaves showed the absence of anthraquinones. In the hexane extract, all the group alkaloids, amino acids, flavonoids, glycosides, saponins and triterpenoids was absence except triterpenoids and steroids.

All the herbs and herbal extracts contain different chemical constituents with biological activity that can be show of valuable therapeutic and medicinal index. Most of the protective effect of medicinal plants, fruits and vegetables has been attributed by active biochemicals, which are the non-nutrient plant compounds. Different type’s active biochemicals have been found to possess a wide range of activities, which may help in protection against uncureable

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total flavonoids (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane extract</td>
<td>68.02 ± 2.15</td>
</tr>
<tr>
<td>Ethyl extract</td>
<td>61.50 ± 1.51</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>15.75 ± 0.06</td>
</tr>
<tr>
<td>Butanol extract</td>
<td>19.08 ± 1.92</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>39.27 ± 1.72</td>
</tr>
</tbody>
</table>

The values are means ± SD of three replicates.
Table 2: The analysis of biochemical in the hexane, ethyl acetate, chloroform, butanol and methanol crude extract of Corydyline terminalis

<table>
<thead>
<tr>
<th>Biochemicals</th>
<th>Hexane</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Butanol</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterol</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = presence; - = absence

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CONCLUSION

In this present study, we have found that biologically active biochemical and phytochemicals were present in the ethyl acetate, chloroform, butanol and methanol extracts of Corydyline terminalis L. Kunth. The high flavonoids contents of Corydyline terminalis L. Kunth leaves crude extracts may be due to the presence of bioactive phytochemicals. Further studies are in progress in our laboratory to isolate the bioactive components from the leaves of Corydyline terminalis L. Kunth.
Hossain, et al.: Biochemical Profiling and Total Flavonoids Contents of Leaves Crude Extract of Endemic Medicinal Plant Corydyline terminalis L. Kunth