ABSTRACT

Plant and plant products are being used as a source of medicine since long. Plant extracts increasingly used as phytotherapeutics and are still a large source of natural antioxidant. In the present study to investigate the Phytochemical screening and in vitro antioxidant activity of Furcraea foetida leaves. The results of the present study indicated that the preliminary phytochemical analysis of the leaves of Furcraea foetida revealed presence of Flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids, phlobatannins. Steroids, alkaloids and anthroquinones. The inorganic elemental analysis of Furcraea foetida leaves revealed the presence Calcium, Magnesium, Sodium, Potassium, Sulphate, Phosphate, Chloride and Nitrate. Vitamin A, C and D were found to be in Furcraea foetida leaf. In vitro antioxidant activity of Furcraea foetida leaves confirmed through DPPH, Superoxide radical scavenging activity, total antioxidant assay, Iron chelating activity and reducing power assay. Thus, it can be concluded that Furcraea foetida leaves can be used as an accessible source of natural antioxidants with consequent health benefits.

KEYWORDS: Furcraea foetida leaves, Phytochemicals, Antioxidant, Vitamins, inorganic elements
INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics. About 150 phytochemicals have been studied in detail.

These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Keeping in view, the present study to investigate the Phytochemical screening and in vitro antioxidant activity of Furcraea foetida leaves.

MATERIALS AND METHODS

Plant materials
The fully mature Furcraea foetida leaves were collected in January 2015 from New Bus stand, Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation of alcoholic extract
The leaves of Furcraea foetida plant were first washed well and dust was removed from the rhizome. Leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 50% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.
Phytochemical screening
Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by.[6, 7, 8 and 9] Qualitative analysis of Vitamins.[10]

Qualitative analysis of Inorganic elements
Ash of drug material (500mg) was prepared and treated with HNO₃ and HCl (3:1 v/v) for 1 hour. After the filtration, the filtrate was used to perform the following tests.[11]

In vitro antioxidant activity
DPPH radical-scavenging activity
DPPH radical-scavenging activity was determined by the method of.[12] Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

\[
\text{Radical scavenging activity (\%) = } 100 \left( \frac{A_C - A_S}{A_C} \right) \times 100
\]

Where \( A_C \) = control is the absorbance and \( A_S \) = sample is the absorbance of reaction mixture (in the presence of sample).

Determination of Total Antioxidant Capacity
The antioxidant activity of the extracts was evaluated by the phosphomolybdenenum method according to the procedure of.[13] The assay is based on the reduction of Mo(VI)–Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 ml extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid. The scavenging activity was calculated according to the following equation: % Inhibition
% of Inhibition = \frac{(A_0-A_1)}{A_0} \times 100

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

**Superoxide anion scavenging activity assay**

The superoxide anion radicals scavenging activity was measured by the method of [14]. In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 μM) solution, 0.75 ml of NADH (936 μM) solution and 0.3 ml of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 μM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation:

\%
\text{Inhibition} = \frac{(A_0-A_1)}{A_0} \times 100

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

**Reducing power assay**

The Fe^{3+} reducing power of the extract was determined by the method of [15]. The extract (0.75 ml) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate (K_3Fe(CN)_6) (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 800g for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride (FeCl_3) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

**Statistical analysis**

Tests were carried out in triplicate for 3 separate experiments. The scavenging activity of sample was expressed as 50% effective concentration (EC_{50}), which represented the concentration of sample having 50% of radical scavenging effect. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Ms- Windows based graphpad Instat (version 3) software. Results were expressed as graphically / mean± standard deviation.
RESULTS AND DISCUSSION
Medicinal plants are assumes greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These ‘secondary compounds’ instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures.[14]

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the Furcraea foetida leaves investigated and summarized in Table-1. The phytochemical screening Furcraea foetida leaves showed that the presence of flavonoids, phenolics, steroids, tannin, saponins, anthroquinones and terpenoids. Alkaloids glycosides and phlobatannins were absent.

Table 1: Phytochemical screening of Furcraea foetida leaves

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemical analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Amino acid</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Anthroquinone</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Polyphenol</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Glycoside</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) Presence  (-) Absence

Vitamins
Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the
molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams.\cite{16}

Vitamin A, also known as retinol, is needed for skin and body tissue repairs. Children need vitamin A to build bones and teeth. Vitamin A is part of the body’s defense system against infections. Vitamin A deficiency is a problem in countries where people eat very few dairy products, fruits, or vegetables. One of the first signs of a vitamin A deficiency is difficulty seeing at night because the retina of the eye needs the vitamin to function well. However, taking in extra vitamin A will not help healthy people see better. Skin creams and moisturizers with vitamin A might smooth skin, but it does not rewind the clock. The skin does not react in that way to vitamin A because it lacks the genetic information and does not know how to use the vitamin. Vitamin A is in fish, meat, and dairy foods, especially concentrated in the liver of fish and animals. Many vegetables also supply vitamin A, such as carrots, pumpkins, and squash; as well as the yellow fruits such as cantaloupes and peaches. Dark green vegetables, tomatoes, and sweet potatoes are also a good source. Most of these fruits and vegetables do not actually contain vitamin A, but rather contain beta carotene which the body converts to vitamin A. Carotene is the pigment that makes egg yolks yellow and carrots bright orange. Most adults carry enough vitamin A in their livers to supply them for months. Large doses of vitamin A can cause liver damage, and this is why most multi-vitamin supplements have some of the vitamin A come from beta carotene rather than retinol. Many studies have made claims that beta carotene in fruits and vegetables helps reduce the risk of some cancers.\cite{17}

Vitamin C, or ascorbic acid, is one vitamin humans cannot make; they have to get it from food. Vitamin C helps hold the cells together, heal wounds, and build bones and teeth. The best sources for vitamin C are citrus fruits, strawberries, melons, and leafy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers, and avoiding high temperatures and long cooking times.\cite{16} The vitamins of the \textit{Furcraea foetida} leaves investigated and summarized in Table-2.
Table 2 Qualitative analysis of vitamins in *Furcraea foetida* leaf

<table>
<thead>
<tr>
<th>S.no</th>
<th>Vitamins</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vitamin A</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Vitamin C</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Vitamin D</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin E</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) Presence  (-) Absence

MINERALS

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water.[18] Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body. Plants are the rich source of all the elements essential for human beings. There is a relationship between the element content of the plant and its nutritional status. Some elements are essential for growth, for structure formation, reproduction or as components of biologically active molecules while others have some other beneficial effects.[19] The inorganic elements of the *Furcraea foetida* leaves investigated and summarized in Table 3

Qualitative or quantitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food. The quality of many foods depends on the concentration and type of minerals what they contains, also play a very significant role against a variety of degenerative diseases and processes, they may also prevent and reduce injury from environmental pollutants and enhance the ability to work and learn, some minerals are essential to a healthy diet (e.g. Calcium, Phosphorus, Potassium and Sodium) where as some can be toxic (e.g. Lead, Mercury, Cadmium and Aluminium). It is clear that mineral nutrition is important to maintain good health and because of that determination of As, Ca, Fe, Mg, Na, K, Zn, Ni, Co etc. have been added to Ayurvedic Pharmacopoeia of India.[20]

Table 3 Qualitative analysis of inorganic element in *Furcraea foetida* leaf

<table>
<thead>
<tr>
<th>Inorganic elements</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>+</td>
</tr>
<tr>
<td>Magnesium</td>
<td>+</td>
</tr>
<tr>
<td>Sodium</td>
<td>+</td>
</tr>
<tr>
<td>Potassium</td>
<td>+</td>
</tr>
<tr>
<td>Iron</td>
<td>--</td>
</tr>
<tr>
<td>Sulphate</td>
<td>+</td>
</tr>
</tbody>
</table>
In vitro antioxidant activity

A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. Numerous studies were carried out on some of these plants, e.g. rosemary, sage and oregano, which resulted in a development of natural antioxidant formulations for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those which are less widely used in culinary and medicine is still rather scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals. In the present study, methanol extract was prepared from powdered leaf part of plant material. The antioxidant activity was examined using different antioxidant assays such as DPPH radical scavenging, superoxide anion radical scavenging, total antioxidant assay, reducing power and metal ion chelating activities.

DPPH Assay

DPPH radical scavenging activity of extract of *Furcraea foetida* and standard as ascorbic acid are presented in Fig 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants. Recently, the use of the DPPH’ reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH’ free radical by a scavenger (A-H) causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH’ is thought to be due to their hydrogen donating ability. The half inhibition concentration (IC$_{50}$) of plant extract and ascorbic acid were 52.74 μg ml$^{-1}$ and 34.91 μg ml$^{-1}$ respectively. The plant extract exhibited a significant dose dependent inhibition of DPPH activity.

| Phosphate | + |
| Chloride  | + |
| Carbonate | --|
| Nitrate   | + |

(+) Presence  (-) Absence
potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

![DPPH Radical Scavenging Activity](image)

**Fig 1 DPPH radical scavenging activity of *Furcraea foetida* leaves**

**Total antioxidant activity**

The yield of the ethanol extract of the plant extract and its total antioxidant capacity are given in Fig. 2. Total antioxidant capacity of *Furcraea foetida* is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract.[13] Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract. The half inhibition concentration (IC₅₀) of plant extract and ascorbic acid were 57.64 μg ml⁻¹ and 42.41 μg ml⁻¹ respectively.
Fig 2 Total antioxidant activity of *Furcraea foetida* leaves

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system.[24] The superoxide anion radical scavenging activities of the extract from *Furcraea foetida* assayed by the PMS-NADH system were shown in Fig 3. The superoxide scavenging activity of *Furcraea foetida* was increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of *Furcraea foetida* was 52.82 μg ml\(^{-1}\) and ascorbic acid were 31.62 μg ml\(^{-1}\) respectively. These results suggested that *Furcraea foetida* had notably superior superoxide radical scavenging effects.

Fig 3 Superoxide radical scavenging activity of *Furcraea foetida* leaves
Reducing power activity

For the measurements of the reducing ability, the Fe$^{3+}$–Fe$^{2+}$ transformation was investigated in the presence of *Furcraea foetida*. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.\[25,26\] Fig. 4 depicts the reductive effect of *Furcraea foetida*. Similar to the antioxidant activity, the reducing power of *Furcraea foetida* increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Furcraea foetida* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

![Reducing power activity](image)

**Fig 4 Reducing power activity o of *Furcraea foetida* leaves**

**CONCLUSION**

The present study concluded that the preliminary phytochemical analysis of the leaves of *Furcraea foetida* revealed presence of Flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids, phlobatannins. Steroids, alkaloids and anthroquinones. The inorganic elemental analysis of *Furcraea foetida* leaves revealed the presence Calcium, Magnesium, Sodium, Potassium, Sulphate, Phosphate, Chloride and Nitrate. Vitamin A, C and D were found to be in *Furcraea foetida* leaf. *In vitro* antioxidant activity of *Furcraea foetida* leaves confirmed through DPPH, Superoxide radical scavenging activity, total antioxidant assay, Iron chelating activity and reducing power assay.
This work has gathered experimental evidence on the *Furcraea foetida* leaves as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect cells/organism from oxidative damage and thus could be an effective against oxidative stress. In addition, the *Furcraea foetida* leaves found to contain a secondary metabolites which plays a major role in controlling antioxidants. Thus, it can be concluded that *Furcraea foetida* leaves can be used as an accessible source of natural antioxidants with consequent health benefits.

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