

## Elemental Analysis and Biological Activities of *Chrysophyllum roxburghii* G. Don (Sapotaceae) Leaves

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### Abstract

The present study was performed to estimate elementals and to determine bioactivities namely anticariogenic, antioxidant, pancreatic lipase inhibitory and cytotoxic activity of *Chrysophyllum roxburghii* leaves. Elemental analysis revealed that calcium and manganese were present in high concentration among principal and trace elements respectively. Preliminary phytochemical analysis revealed the presence of tannins, alkaloids and terpenoids in the extract. Total phenolic content was found to be 179.05mg Gallic acid equivalents/g of extract. The methanol extract caused a dose dependent inhibition of *Streptomyces mutans* isolates. All the isolates tested were found to be sensitive to extract. In DPPH assay, the extract exhibited marked dose dependent scavenging activity against DPPH free radical with an IC<sub>50</sub> value of 3.54µg/ml. In Ferric reducing assay, the absorbance of the reaction mixture was found to increase with the concentration of extract which is suggestive of reducing power. The activity of chicken pancreatic lipase was affected by the extract and the effect was concentration dependent. Higher inhibition of enzyme (>50%) was observed at extract concentration 50mg/ml. In cytotoxic study, the lethality was found to be directly proportional to extract concentration. Highest mortality (>80%) of shrimps was observed at extract concentration 1000µg/ml. LC<sub>50</sub> of extract was 83.04µg/ml. The bioactivities of the extract could be attributed to the presence of secondary metabolites in the plant material. The plant material could be used as a source of important elements required for the body. In suitable form, the plant could be used in the prevention and treatment of dental caries, oxidative damage, obesity and cancer.

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### INTRODUCTION

*Chrysophyllum roxburghii* G. Don (Syn. *C. lanceolatum* (Bl.) DC.) belongs to the family Sapotaceae. It is commonly called Indian star apple and it is a tree growing up to 15 meters height. It is one of the canopy trees in evergreen forests. It is commonly found in Western Ghats of India and Sri Lanka. The branches are horizontal, leaves are coriaceous, glossy, elliptic, oblong, apex acuminate and nerves numerous. Flowering occurs in April-May and the flowers are greenish white in color and are numerous in axillary fascicles. Seed is a berry (Ramaswamy *et al.*, 2001; Chandrika *et al.*, 2005). The seeds are used in a formulation to treat pneumonia at Sivasagar district of Assam (Acharyya and Sharma, 2004). The ripe fruits are considered edible and tribal people of Anamalais of Western Ghats, India consume

these fruits (Ramachandran, 2007). In Mizoram, the plant is locally called Thei pabuan and the ripen fruits are eaten by people of Mizoram (Kar *et al.*, 2013). The fruit is shown to contain an appreciable quantity of minerals and amino acids (Barthakur and Arnold, 1991). In a previous study, Mallikarjun *et al.* (2011) reported dose dependent antibacterial activity of methanol extract of leaves. Based on literature survey, it has been arrived that the elemental analysis and many of the biological activities of the leaves of *C. roxburghii* are not carried out. Hence, the present study was conducted to determine elemental composition and bioactivities namely anticaries, antioxidant, pancreatic lipase inhibitory and cytotoxic activity of *C. roxburghii* leaves.

## MATERIALS AND METHODS

### Collection and Identification of Plant Material

The plant material was collected in the month of September 2013 at a place called Haniya, Hosanagara taluk of Shivamogga district, Karnataka, India. A voucher specimen (SSC1549) was deposited in the PG department of Studies and Research in Microbiology, Sahyadri Science College (Autonomous), Shivamogga-577203, Karnataka for future reference. The leaves were washed well to remove adhering matter, dried under shade, powdered mechanically and stored in airtight container until use.

### Determination of Elemental Composition

For elemental analysis, a known amount of powdered leaf material (1.0gm) was digested using mixture of concentrated nitric acid and perchloric acid (10ml) in a beaker. The powdered material was left in acid mixture for 24 hours and was digested on the hot plate until complete digestion. After digestion, 10ml of 10% nitric acid was added to the beaker and the beaker was left for two hours for residue to settle down. The supernatant liquid was filtered through Whatman No. 1. The filtrate was subjected to estimation of elements namely calcium, magnesium, potassium, phosphorus, zinc, iron, copper and manganese using Atomic absorption spectrophotometer (Sarangi *et al.*, 2002).

### Preparation of Extract

The powdered leaf material (25g) was extracted in Soxhlet apparatus using methanol (HiMedia, Mumbai) as solvent. After extraction, the content was filtered through Whatman filter paper no. 1 and concentrated at 40°C under reduced pressure. The weight of the extract was taken. The condensed methanol extract was stored at 4°C until use (Kekuda *et al.*, 2012a).

### Phytochemical Analysis of Leaf Extract

The extract obtained after solvent evaporation was subjected to standard tests for detection of phytoconstituents namely alkaloids (Dragendorff's reagent and Mayer's reagent), tannins (ferric chloride test), saponins (frothing test and hemolysis test), glycosides (Salkowski test and Keller-Kiliani test), sterols (Burchard test), flavonoids (Shinoda test) and terpenoids (Salkowski test) (Mallikarjuna *et al.*, 2007; George *et al.*, 2010).

### Total Phenolic Content of Leaf Extract

The content of total phenolics in leaf extract was estimated by Folin-Ciocalteu reagent (FCR) method with some modifications (Rekha *et al.*, 2012). Here, a dilute concentration of leaf extract (0.5 ml) was mixed with 0.5 ml diluted Folin-Ciocalteu reagent (1:1) and 2 ml of sodium carbonate (7%), allowed to stand for 30 minutes and the absorbance was measured colorimetrically at 765nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/ml). The concentration of total phenolic compounds was determined as µg Gallic acid equivalents (GAE) from the graph.

### Anticaries Activity of Leaf Extract

The anticaries efficacy of leaf extract was determined by Agar-well-diffusion method (Kekuda *et al.*, 2012a) against 24 oral isolates of *Streptococcus mutans* (S-1 to S-24) recovered from teeth samples of dental caries patients. The test bacteria were inoculated into sterile Brain heart infusion broth (HiMedia, Mumbai) tubes and

incubated at 37°C for 24 hours. The broth cultures were swabbed uniformly on solidified sterile Brain heart infusion agar (HiMedia, Mumbai) plates using sterile cotton swab. Then, wells of 6mm diameter were punched in the inoculated plates with the help of sterile cork borer and the leaf extract (10 and 20mg/ml of 10% dimethyl sulfoxide [DMSO; HiMedia, Mumbai]), Standard antibiotic (Chloramphenicol, 1mg/ml) and Control (10% DMSO) were added into respectively labeled wells. The inoculated plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition formed around the well was measured with a ruler. The experiment was carried in triplicates to get average reading.

### Antioxidant Activity of Leaf Extract

#### DPPH Free Radical Scavenging Activity

The radical scavenging ability of leaf extract and ascorbic acid (reference standard) was tested on the basis of the radical scavenging effect on the DPPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical (Rekha *et al.*, 2012). Here, 2ml of different concentrations of leaf extract and ascorbic acid (1 to 200µg/ml) were mixed with 2ml of DPPH solution (0.002% in methanol) in clean and labeled tubes. The tubes were incubated at room temperature in dark for 30 minutes and the absorbance was measured at 517nm using UV-Visible Spectrophotometer (Elco, SL-159). The absorbance of the DPPH control (2ml DPPH+2ml methanol) was also noted. The scavenging activity of the extract was calculated using the formula:

$$\text{Scavenging activity (\%)} = (A - B) / A \times 100,$$

where A is absorbance of DPPH control and B is absorbance of DPPH and extract/standard combination. The IC<sub>50</sub> value for the extract was calculated by Origin 6.0 software. IC<sub>50</sub> denotes the concentration of extract required to scavenge 50 % of DPPH free radicals.

#### Ferric Reducing Activity

The reducing ability of leaf extract was tested by ferric reducing assay. Briefly, different concentrations (1 to 200µg/ml) of extract and ascorbic acid (reference standard) in 1ml of methanol were mixed in separate tubes with 2.5ml of phosphate buffer (200mM, pH 6.6) and 2.5ml of potassium ferricyanide (1%). The tubes were placed in water bath for 20 minutes at 50°C, cooled rapidly and added with 2.5ml of trichloroacetic acid (10%) and 0.5ml of ferric chloride (0.1%). The amount of iron (II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussian blue at 700nm after 10minutes. The increase in absorbance of the reaction mixtures indicates increased reducing power (Rekha *et al.*, 2012).

#### Pancreatic Lipase Activity of Leaf Extract

The inhibitory effect of leaf extract and Orlistat (reference standard) against lipase was tested against lipase extracted from the pancreas of chicken. Lipase inhibitory activity of different concentrations of leaf extract and Orlistat was tested by mixing 100µl of each concentration of leaf extract, 8ml of oil emulsion and 1ml of chicken pancreatic lipase followed by an incubation of 60 minutes. The reaction was stopped by adding 1.5 ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against NaOH (0.02M) using phenolphthalein as an indicator (Kumar *et al.*, 2011). The inhibition of lipase activity (%) was calculated using the formula:

$$\text{Lipase inhibition (\%)} = (A - B) / A \times 100,$$

where A is lipase activity, B is activity of lipase when incubated with leaf extract.

### Cytotoxic Activity of Methanol Extract

Brine Shrimp Lethality bioassay was performed in order to determine cytotoxic nature of the leaf extract. The eggs of brine shrimp *Artemia salina* (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water which was prepared with 10 g of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500 ml of distilled water. After 36-48 hours, the phototropic shrimps were collected. 25 shrimps were transferred into each vial containing 5ml of artificial sea water with different concentrations of leaf extract (10, 100 and 1000 µg/ml). The vials were incubated at 25°C and surviving shrimps were counted microscopically after 24 hours. Potassium dichromate was used as positive control (Kekuda *et al.*, 2012a). LC<sub>50</sub> value was calculated and the value >1000 µg/ml was considered non-toxic.

### Statistical Analysis

The experiments were performed in triplicates. The results are represented as Mean±Standard deviation. The IC<sub>50</sub> values were calculated by Origin 6.0 software.

## RESULTS

An appreciable quantity of elements was detected in the leaf material. Among the major elements, Calcium was detected in high quantity followed by potassium, magnesium and phosphorus. In case of minor elements, manganese was detected in high concentration followed by iron, copper and zinc (Table 1).

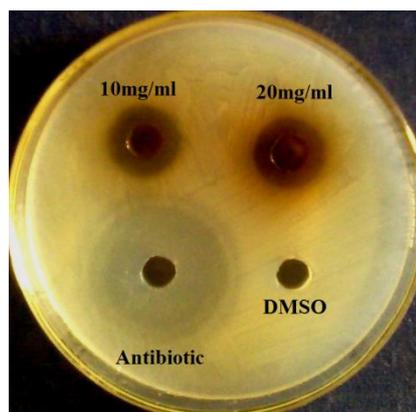
**Table 1:** Elemental composition of leaf material.

| Element    | Quantity (ppm) |
|------------|----------------|
| Calcium    | 10125.10±10.33 |
| Potassium  | 7000.05±11.10  |
| Magnesium  | 4125.65±08.45  |
| Phosphorus | 900.45±10.00   |
| Manganese  | 640.47±06.56   |
| Iron       | 330.20±05.12   |
| Copper     | 17.25±02.15    |
| Zinc       | 16.49±01.14    |

The extract yield was 4.16%. Preliminary phytochemical screening of leaf extract revealed the presence of phytoconstituents namely of tannins, alkaloids and terpenoids. The total phenolic content of leaf extract was found to be 179.05±0.5mg GAE/gram of extract.

The result of anticaries activity of leaf extract against oral isolates of *S. mutans* is shown in Table 2 and Figure 1. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and reported as positive and the absence of zone as negative. It was found that the leaf extract caused inhibition of *S. mutans* isolates in a dose dependent manner i.e., the diameter of inhibition zone increased with the increase of extract concentration. The zone of inhibition ranged from 1.2 to 2.3cm and 0.8 to 1.9cm at extract concentration of 20mg/ml and 10mg/ml respectively. Inhibition caused by standard antibiotic was

higher than that of leaf extract. DMSO did not cause any inhibition of bacterial isolates.



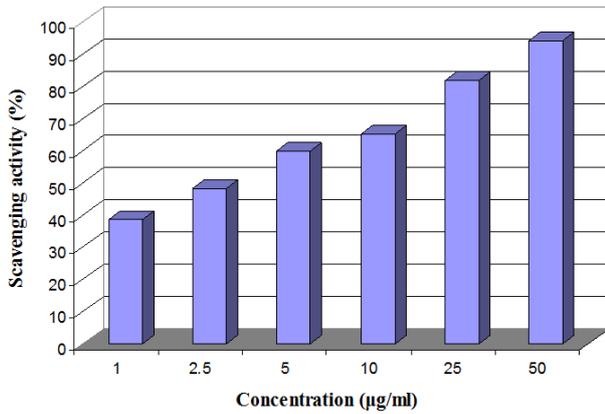
**Figure 1:** Inhibitory effect of leaf extract against isolate S-11.

**Table 2:** Anticaries activity of leaf extract against *S. mutans* isolates.

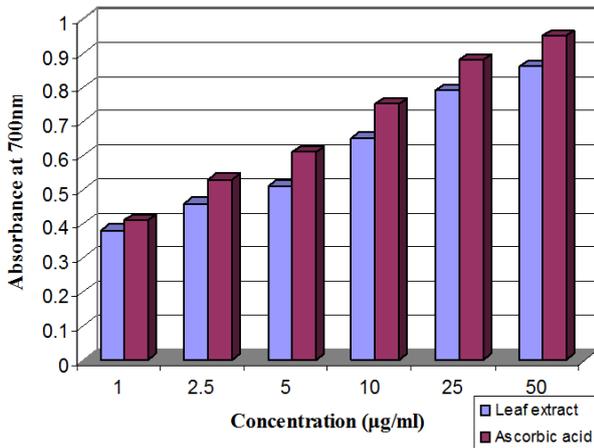
| Isolates | Zone of inhibition in cm |         |          |        |
|----------|--------------------------|---------|----------|--------|
|          | Methanol extract         |         | Standard | DMSO   |
|          | 20mg/ml                  | 10mg/ml |          |        |
| S-1      | 1.7±0.1                  | 1.5±0.0 | 2.4±0.2  | 00±0.0 |
| S-2      | 1.6±0.0                  | 1.4±0.1 | 2.6±0.2  | 00±0.0 |
| S-3      | 1.4±0.0                  | 1.2±0.0 | 2.3±0.1  | 00±0.0 |
| S-4      | 1.5±0.1                  | 1.3±0.0 | 2.2±0.0  | 00±0.0 |
| S-5      | 1.8±0.2                  | 1.6±0.0 | 2.6±0.0  | 00±0.0 |
| S-6      | 1.5±0.0                  | 1.1±0.0 | 2.8±0.2  | 00±0.0 |
| S-7      | 1.9±0.1                  | 1.6±0.1 | 2.4±0.1  | 00±0.0 |
| S-8      | 1.8±0.0                  | 1.5±0.1 | 2.1±0.0  | 00±0.0 |
| S-9      | 1.5±0.0                  | 1.2±0.0 | 2.3±0.1  | 00±0.0 |
| S-10     | 1.9±0.2                  | 1.6±0.1 | 2.9±0.2  | 00±0.0 |
| S-11     | 1.2±0.0                  | 1.1±0.0 | 2.6±0.0  | 00±0.0 |
| S-12     | 1.2±0.0                  | 0.8±0.0 | 2.3±0.0  | 00±0.0 |
| S-13     | 1.6±0.1                  | 1.4±0.0 | 2.4±0.0  | 00±0.0 |
| S-14     | 1.6±0.1                  | 1.3±0.0 | 2.4±0.1  | 00±0.0 |
| S-15     | 1.8±0.1                  | 1.5±0.0 | 2.7±0.2  | 00±0.0 |
| S-16     | 2.1±0.2                  | 1.8±0.1 | 2.3±0.0  | 00±0.0 |
| S-17     | 1.8±0.1                  | 1.6±0.0 | 2.1±0.0  | 00±0.0 |
| S-18     | 2.0±0.2                  | 1.7±0.1 | 2.6±0.2  | 00±0.0 |
| S-19     | 1.5±0.0                  | 1.2±0.0 | 2.7±0.2  | 00±0.0 |
| S-20     | 2.3±0.2                  | 1.9±0.2 | 2.3±0.0  | 00±0.0 |
| S-21     | 1.6±0.0                  | 1.2±0.0 | 2.1±0.0  | 00±0.0 |
| S-22     | 1.4±0.0                  | 1.2±0.0 | 2.6±0.2  | 00±0.0 |
| S-23     | 1.8±0.1                  | 1.5±0.1 | 2.5±0.2  | 00±0.0 |
| S-24     | 1.9±0.2                  | 1.6±0.0 | 2.9±0.2  | 00±0.0 |

Figure 2 shows the radical scavenging activity of different concentrations of leaf extract and ascorbic acid. Both extract and ascorbic acid exhibited marked scavenging activity and the activity was dose dependent. The scavenging activity of ascorbic acid (IC<sub>50</sub> 2.29µg/ml) was greater than that of leaf extract (IC<sub>50</sub> 3.54µg/ml).

The reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> was investigated in the presence of extract to examine the reducing power of extract and the result is shown in Figure 3. The absorbance of the reaction mixture was found to increase with the increase in concentration of extract and reference standard which is suggestive of reducing power.

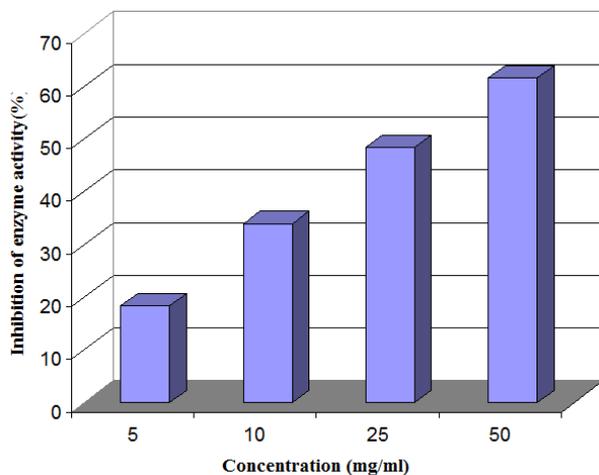


**Figure 2:** DPPH free radical scavenging activity of leaf extract.

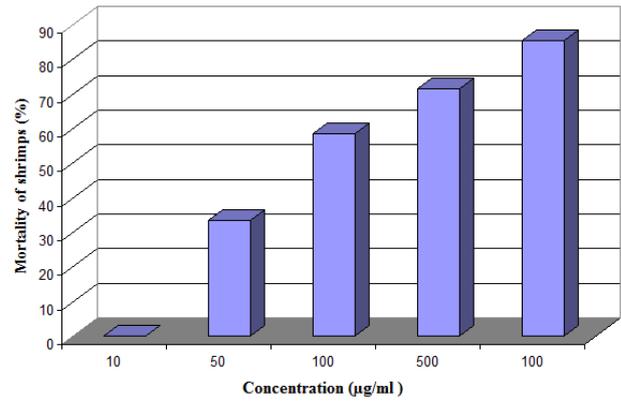


**Figure 3:** Ferric reducing activity of leaf extract.

Inhibitory activity on chicken pancreatic lipase of different concentrations of leaf extract was determined using olive oil as the substrate. It was observed that the activity of lipase was affected when incubated with the methanol extract. The inhibitory activity was found to be dose dependent i.e., higher inhibition of enzyme was observed on increasing the concentration of extract. An inhibition of greater than 50% was observed at extract concentration 50mg/ml (Figure 4). Orlistat showed high inhibitory activity (IC<sub>50</sub> 6.03µg/ml) against lipase when compared to leaf extract.



**Figure 4:** Pancreatic lipase inhibitory activity of methanol extract.



**Figure 5:** Cytotoxic activity of leaf extract.

The cytotoxic activity of different concentrations of methanol extract in terms of mortality of brine shrimps is presented in Figure 5. The degree of lethality was found to be directly proportional to the concentration of the extract. Highest mortality (>80%) was observed at extract concentration 1000µg/ml where as no mortality was observed at 10µg/ml. LC<sub>50</sub> of extract was found to be 83.04µg/ml and thus the extract is toxic. Potassium dichromate (LC<sub>50</sub> 32.04µg/ml) showed marked cytotoxic effect when compared to leaf extract.

**DISCUSSION**

All individuals required carbohydrates, proteins, fats, vitamins and minerals in their nutrition. Nutrients such as carbohydrates, proteins and fats are consumed in larger quantity and are called macronutrients. However, vitamins and minerals are consumed in lesser quantity and are referred to as micronutrients. The mineral elements are inorganic substances present all body tissues and fluids in the body and their presence is essential to life. Elements are broadly classified into principal elements or major elements and trace elements or minor elements based on the quantity being consumed. Minerals such as calcium, potassium, magnesium and phosphorus forms the principal elements while iron, copper, manganese and zinc forms the trace elements. Although minerals yield no energy, they are important for calcification of bone, blood coagulation, neuromuscular activity, acid base equilibrium, enzyme activity, osmotic regulation etc. (Soetan *et al.*, 2010; Vinayaka *et al.*, 2013). In the present study, an appreciable quantity of major and minor elements was detected in the leaf of *C. roxburghii*. Among the principal elements, calcium was present in high concentration followed by potassium, magnesium and phosphorus. Among trace elements, manganese was detected in high concentration followed by iron, copper and zinc. In a previous study, Barthakur and Arnold (1991) evaluated chemical composition of fruit of *C. roxburghii*. Of the 14 macro and micronutrients studied, potassium was the most highly concentrated. The fruit also contained considerably higher concentrations of calcium, iron, manganese, phosphorus and others.

Among the oral diseases, dental caries is one of the most prevalent infectious diseases affecting people of all age groups all over the world. It is chronic and is caused by an interaction between microflora of oral cavity, diet, dentition and oral environment. It affects 60-90% of young population. A large number of microorganisms viz., mutans streptococci, lactobacilli and *Actinomyces* are

known to be involved in causing dental caries. Among cariogenic flora, *Streptococcus mutans* is considered as the principal aetiological agent, however, additional acidogenic microorganisms such as lactobacilli may also be involved. The cariogenicity of *S. mutans* is ascribed to its ability to metabolize dietary carbohydrates, to produce acid and tolerate acid, to adhere to and to form biofilm on tooth surfaces (Ferrazzano *et al.*, 2009; Almeida *et al.*, 2012; Brighenti *et al.*, 2012; Narotzki *et al.*, 2012). Antimicrobial mouth-rinses and antibiotics are most commonly used to prevent and treat dental caries. However, these chemicals have shown to have some drawbacks such as development of resistance among cariogenic flora, high cost and possible side effects (Guimaraes *et al.*, 2006; Dzidic *et al.*, 2008). Hence, several investigators have screened the efficacy of natural products, in particular plants, against microorganisms implicated in causing dental caries. In our study, the leaf extract showed a dose dependent inhibitory activity against oral isolates of *S. mutans*. It has been experimentally shown that plants and their products exhibit marked inhibitory activity against cariogenic flora (Fani and Kohanteb, 2007; Jose and Beegum, 2007; Ferrazzano *et al.*, 2009; Almeida *et al.*, 2012; Vivek *et al.*, 2013).

It is well known that reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, singlet oxygen, peroxy radical, hydrogen peroxide are produced during oxidative stress. These radicals are known to be involved in several pathological conditions such as aging, cancer, neurodegenerative disease, coronary heart diseases etc. Antioxidant defenses in organisms against ROS may be of endogenous (enzymatic and nonenzymatic) or dietary origin (vitamins, carotenoids, flavonoids, etc.). An antioxidant is defined as any substance when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Synthetic antioxidants such as BHA, BHT, PG and others are known to possess some possible toxic or carcinogenic effects. Hence, search for antioxidants from natural origin, in particular from plants, has been greatly increased (Jayaprakasha *et al.*, 2001; Chang *et al.*, 2007; Kim *et al.*, 2006; Stoilova *et al.*, 2007; Mohamed *et al.*, 2013). Polyphenolic compounds including flavonoids are found in various parts of both edible and inedible plants. They are reported to exhibit multiple biological effects, including antioxidant activity. Phytochemicals such as flavonoids, tannins, anthocyanins and other phenolic constituents present in food of plant origin are potential antioxidants. Phenolic compounds present in dietary supplements can protect the body against oxidative damage (Yuan *et al.*, 2005; Wojdylo *et al.*, 2007; Kim *et al.*, 2006; Stoilova *et al.*, 2007).

DPPH radical scavenging assay is one of the important antioxidant assays which is simple, easy to perform and can be done in relatively shorter period of time. DPPH, the relatively stable, organic, nitrogen centered free radical having absorption maxima at 515-520nm in alcoholic solution. It is widely used in the determination of antioxidant activity, in terms of radical scavenging activity, of single compounds as well as of different plant extracts (Hinneburg *et al.*, 2006; Katalinic *et al.*, 2006; Wojdylo *et al.*, 2007; Kim *et al.*, 2006; Rekha *et al.*, 2012; Junaid *et al.*, 2013; Kekuda *et al.*, 2013). The scavenging effect of antioxidants is due to their hydrogen

donating ability. The antioxidants reduce the purple colored DPPH radical to a yellow colored compound diphenylpicrylhydrazine, and the extent of reaction will depend on the hydrogen donating ability of the antioxidants (Bondent *et al.*, 1997). In the present study, leaf extract exhibited a marked scavenging potential. However, scavenging potential of leaf extract was lesser when compared to ascorbic acid. Although the scavenging abilities of leaf extract was lesser than that of ascorbic acid, it was evident that the extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants (Chung *et al.*, 2006).

We investigated the  $Fe^{+3}/Fe^{+2}$  transformations in the presence of different concentrations of leaf extract in order to measure the reducing power. Here, the presence of reductants (antioxidants) in the extract would reduce  $Fe^{+3}$  to  $Fe^{+2}$  by donating an electron. The amount of  $Fe^{+2}$  complexes formed after incubation can be determined by measuring the formation of Perle's Prussian blue at 700nm. Increasing absorbance at 700nm indicates an increase in reductive ability. The reducing capacity of an extract or a compound usually serves as a significant indicator of its potential antioxidant activity (Chung *et al.*, 2006; Hsu *et al.*, 2006). Ferric reducing assay is routinely performed to investigate reducing potential of various kinds of samples (Jayaprakasha *et al.*, 2001; Hinneburg *et al.*, 2006; Kim *et al.*, 2007; Rekha *et al.*, 2012; Junaid *et al.*, 2013). In the present study, it was found that the reducing power of leaf extract increased with the increase of its concentration. It is evident that the leaf extract possess reductive potential and could serve as electron donors, terminating the radical chain reactions (Chung *et al.*, 2006).

One of the most important choices of treatment of obesity is the development of inhibitors of nutrient digestion and absorption. Inhibition of pancreatic lipase is the most widely studied mechanism to determine the efficacy of natural products as antiobesity agents. Pancreatic lipase is the main lipid-digesting enzyme that removes fatty acids from  $\alpha$  and  $\alpha'$  position of dietary triglycerides yielding the lipolytic product  $\beta$ -monoglyceride and long-chain saturated and polyunsaturated fatty acids. Orlistat, one of the two clinically approved drugs for treatment of obesity, act by inhibiting pancreatic lipase. Even though it is one of the best-selling drugs worldwide, it has side effects such as oily stools, oily spotting, and flatulence (Shi and Burn, 2004; Birari and Bhutani, 2007; Yun, 2010; Kumar *et al.*, 2011 Phcog J). This prompted research for the identification of inhibitors that lack the side effects. Phytochemicals of plants present an exciting opportunity for discovering newer anti-obesity agents. Several plants, for example *Taraxacum officinale*, *Nomame herba*, *Vitis vinifera* etc, have to shown to be promising sources of anti-obesity agents (Zhang *et al.*, 2008; Moreno *et al.*, 2003; Yamamoto *et al.*, 2000). In the present study, the leaf extract was found to inhibit the activity of chicken pancreatic lipase in a dose dependent manner. Inhibition of chicken pancreatic lipase has been studied by researchers in order to screen anti-obesity activity of a variety of samples. In a study, ethyl acetate extract of *Terminalia bellerica* seeds has shown concentration dependent inhibition of chicken pancreatic lipase (Kumar *et al.*, 2010). The methanol extract of a macrolichen *Everniastrum cirrhatum* was found to inhibit activity of chicken pancreatic lipase in a concentration dependent manner (Kumar *et al.*, 2011 phcog j). In

another study, Kekuda *et al.* (2011) observed dose dependent inhibition of chicken pancreatic lipase by extract of *Streptomyces* species from Western Ghats of Karnataka.

Brine shrimp lethality bioassay is a rapid method that utilizes only 24 hours and is inexpensive. This assay is based on the ability of the extract to exhibit lethality in laboratory cultured brine shrimp. The assay utilizes a large number of organisms for validation and a relatively small amount of sample. It does not need animal serum as needed for other methods of cytotoxicity testing. This test is considered to be very useful in determining biological activities such as cytotoxic, phototoxic, pesticidal, trypanocidal, enzyme inhibition, and ion regulation activities. It can also be extrapolated for cell-line toxicity and antitumor activity (Luis *et al.*, 2002; Hossain *et al.*, 2009; Raghavendra *et al.*, 2010; Kekuda *et al.*, 2010). In the present study, we evaluated cytotoxic nature of leaf extract by brine shrimp lethality assay. The extract demonstrated dose dependent cytotoxic effect with LC<sub>50</sub> of 83.04µg/ml. Studies have shown that plant extracts exhibit lethal effect on brine shrimp. Raghavendra *et al.* (2010) showed cytotoxic effect of methanol extract of *Putranjiva roxburghii* seeds with LC<sub>50</sub> of 427.74µg/ml. The leaf extract of *Abrus pulchellus* was found to cause dose dependent lethal effect with LC<sub>50</sub> of 281.70µg/ml (Kekuda *et al.*, 2010). Leaf extract of *Croton gibsonianus* was found to exhibit cytotoxicity with LC<sub>50</sub> of 109.48µg/ml (Kekuda *et al.*, 2012b).

## CONCLUSION

The study reveals the presence of an appreciable quantity of various minerals in the leaf material of *C. roxburghii*. The plant can be a potential source of minerals that are required for normal physiology of the individual. The leaf extract also displayed anticaries, pancreatic lipase inhibitory, antioxidant and cytotoxic properties. The observed bioactivities could be ascribed to the presence of secondary metabolites. Further studies are under progress to separate active principles from the leaf extract and to determine their bioactivities.

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