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Estimation of total carbohydrate content of *Pinus roxburghii* from Almora district by Anthrone method

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Abstract
For living things to carry out their regular bodily activities, they need to consume carbohydrates. A state of hypo hydration, ketosis, and catabolic muscle metabolism are brought on by consuming fewer carbohydrates. The goal of the current study was to calculate and contrast the total amount of carbohydrates in various sections of the *Pinus roxburghii* plant that was gathered from Almora. The Anthrone method was used to estimate the total amount of carbohydrates. The findings indicated that the leaves had the highest overall glucose content of any plant element.

Keywords: Carbohydrates, plant extracts, *Pinus roxburghii*, anthrone method

Introduction

Carbohydrates are found in all living things, including microbes, plants, and animals. Their biological duties are irreplaceable, no question about that. There are many medications on the market right now that contain carbohydrates. The creation of medications containing carbohydrates has slowed down in recent years. Only nine of the more than 200 medications that were approved between 2015 and 2020 are small-molecule carbohydrates [1]. The metabolism of reserve carbohydrates occurs during respiration and development. These carbohydrates can be found in many plant sections. It helps plants develop and recuperate from stress, herbivory, and other disturbances [2]. A substantial amount of the human diet is made up of wild plants, which provide critical biochemical components including proteins, lipids, and carbohydrates that are necessary for maintaining metabolic functions and general health. In addition to offering vital biochemical substances and energy, these wild plants are also additional sources of vitamins and minerals, which are critical for preserving the body's physiological balance. Wild plants frequently have nutritional qualities that are on par with or even better than those of their domesticated equivalents [3]. Recently, a lot of research has been done on the use of wild plants as food and medicine, looking into how well they might work to cure conditions including diabetes, cancer, wounds, jaundice, and more [4, 6]. *Pinus roxburghii* is one such gymnospermic plant species that is grown abundantly in the Himalayan region and is categorize in least concern species [7, 8]. Using the anthrone method, which measures the total amount of carbohydrates in the sample, the current study was carried out to determine the total amount of carbohydrates.

Materials and Methods Sample collection

Parts of *Pinus roxburghii* were collected from Almora region. After removing dust with tap water, the gathered plant parts were rinsed with double-distilled water, allowed to dry in the shade, and then ground into a powder using a mixer grinder. The powder was stored in sealed polybags at 4°C.

Extract preparation

For three hours, a 100 mg sample powder was hydrolyzed in a boiling tube with five milliliters of 2.5 N hydrochloric acid in a boiling water bath. After letting the mixture cool to room temperature, solid sodium carbonate was added to neutralize it until the effervescence stopped. Following a 100 mL dilution with distilled water, the contents of the boiling tube were centrifuged for 10 minutes at room temperature at 3000 rpm. After that, the supernatant was gathered and used for estimate [9].

Quantitative estimation

Using the Anthrone technique, the total carbohydrate content was estimated [9]. The first step was to dispense different quantities of 0.2, 0.4, 0.6, 0.8, and 1 mL aliquots from a working standard solution into different test tubes. After that, 0.5 mL of the sample's extract was put into a different test tube, and distilled water was added to each test tube until the total volume was 1 ml. One milliliter of pure water was used as the blank. After that, each test tube—including the blank—was filled with 4 mL of Anthrone reagent. A vortex was used to mix the contents of each test tube, and the tubes were then boiled for eight minutes in a water bath. The After quickly cooling the test tubes, a spectrophotometer was used to detect the absorbance of the resultant green to dark green at 630 nm. Three duplicates of each experiment were carried out. Plotting the glucose concentration on the x-axis and the associated absorbance on the y-axis allowed for the creation of a standard graph. Ultimately, grams of glucose equivalents per 100 grams of plant material were used to calculate the sample's total carbohydrate content.

Statistical analysis

All the tests were performed in triplicates and the data was entered in MS Excel 19 for descriptive statistics such as mean, and standard deviation, and results were compared by one-way ANOVA followed by Tukey's test and considered significant at $p < 0.05$.

Result and discussion

The data presented in (Table 1 and Figure 1) reveals the quantitative estimation of the total carbohydrate content of *Pinus roxburghii* needles (leaves), bark and female cone. The maximum total carbohydrate content of *Pinus roxburghii* was noted in the leaf extract and the minimum total carbohydrate content was observed in the bark extract. The total carbohydrate content of *Pinus roxburghii* bark extract showed (0.85 ± 0.0008 mg GLU /g DW) which is lower than the previous report of Bianchi *et al.* [10]. They found that the carbohydrate contents in the root was 12.3 g/kg dry bark. Needles showed the maximum content of carbohydrates (1.82 ± 0.052 mg GLU/g DW). The content of total carbohydrates in the female cone was obtained 0.93 ± 0.058. In previous studies by Nepi *et al*., the total carbohydrate content of the ovular sections of few species of *Pinaceae* family showed 103.23±54.7 (mg/ml) and 49.74±15.6 (mg/ml) respectively [11]. One more study by S. Cheikh-Rouhou *et al.* found that the total carbohydrate content in the seeds of *Pinus halepensis* and Pinus pinea seeds were 25.7±0.2 g/100g and 13.9 g/100g respectively which is also more than our study [12]. The maximum total carbohydrate content for Needles, Bark, and Female cone was in the following order:

Needles> Female Cone> Bark

The Needles are the main photosynthetic part of the plants hence showed the maximum carbohydrate content as studied by Sharma *et al.* and Chaudhary *et al.* [13, 14]. Therefore, the presence of the maximum amount of carbohydrates in the needles of *Pinus roxburghii* is justified.

Table 1: Comparison of the total carbohydrate content [Mean ± SD(n=3)] in *Pinus roxburghii* of different parts.

Parts of *Pinus roxburghii*	Total carbohydrate content (mg GLU/g DW)
Needles	1.82 ± 0.052
Bark	0.85 ± 0.008
Cone	0.93 ± 0.058

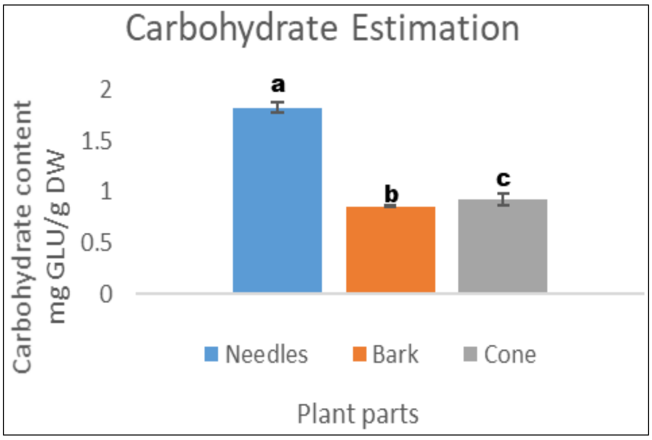

Fig 1: Variations in carbohydrate content within different parts of *Pinus roxburghii* extract. Data denoted by distinct letters indicate significant differences ($p < 0.05$) between them. Experiments were conducted in triplicates (Mean ± SD).

Conclusion

Pinus roxburghii displayed a low amount of total carbohydrate content. The reason could be the species inhabiting at higher altitude which can be a major reason. *Pinus roxburghii* can be used as a commercial source of carbohydrates only if the proportion of its carbohydrate content is known. Due to its abundance in the Himalayan region, it is an appropriate raw resource.

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