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DETERMINATION OF PIGMENTS, ANTIOXIDANT POTENTIAL AND VITAMIN CONTENTS OF BENGUET PINE (*Pinus kesiya*) POLLEN

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ABSTRACT

The study determined the total carotenoid, β-carotene, chlorophyll a and chlorophyll b and total phenolic contents of *P. kesiya* pine pollen. The study also evaluated the antioxidant property of *P. kesiya* pollen using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and determined its Vitamin A and Vitamin C contents. Results showed that Benguet (*P. kesiya*) pine pollen contained 2.90 µg/g total Carotenoid, 0.06 µg/g β-carotene, 409.41 µg/g Chlorophyll A and 704.68 µg/g Chlorophyll B,0.103 µg/g Vitamin A and 0.500 µg/g Vitamin C and displayed an antioxidant component with 271.10 mg/L that can scavenge 50% of radicals. This study is of great potential remedy to reduce antibiotic resistance in fish and provide a plant-based anti-oxidant.

Keywords: Pigments, Antioxidant, Vitamin, Benguet pine, *Pinus kesiya*

INTRODUCTION

The Benguet Pine (*Pinus. kesiya)* also known as Benguet Pine is a conifer species which is considered as one of the most economically and culturally important species in Benguet province [1]. *Pinuskesiya* of the family Pinaceae are common in northern Philippines, particularly in highland regions with an altitude of approximately 1,500 meters that provides optimum conditions for its growth [2]. It is commonly found in elevated areas of Baguio City, the mountain province, Zambales and Mindoro, Philippines [3].*P. kesiya* is fast growing and has the capacity to adapt to various growing conditions (high plasticity), long living and produces high quality and long-fibered pulp [4].

Medicinal plants are of potential as immunostimulants fortifying the immune response of cultured species to effectively prevent and control occurrence of diseases caused by different bacterial and viral pathogens. The role of medicinal plants in disease prevention or control has been attributed to the antioxidant properties of their constituents, which are usually associated with a wide range of amphipathic molecules that are broadly referred to as polyphenolic compounds [5]. Pine pollen contains an incredibly wide spectrum and high concentration of unique and rare nutrients that are essential for the human body. Pine pollen reportedly promotes rapid growth, rejuvenation, and healing due to its high vitamins, minerals, and enzymatic contents that nourish the body at a fundamental level [6].

Preliminary studies on Phytoandrogen components of *Pinus kesiya* was conducted to screen phytochemicals and identify the phytoconstituents present in the different parts of *P. kesiya*. It was reported that tannins flavonoids, steroids and alkaloids are present in branch, needles, pollen, twigs and roots and that glycosides are positively detected in the pollen and twigs but none in the other parts while alkaloids and sterols are observed in bark [3].

MATERIALS AND METHODS

Collection of Pine Pollen

The Department of Environment and Natural Resources - Cordillera Administrative Region, Ecosystem Research and Development Services DENR-ERDS) facilitated the scouting and collection of pine pollen in Baguio City. Two boxes of around 518 pieces of catkins of Benguet pine were collected, segregated, air-dried and transported to the laboratory for another week of air drying. The granules from each pollen cone from the catkins were removed manually, sieved and the starchy powder pine pollens were placed in a tightly sealed container.

Analyses of Pigments

The method of Tao et al. (2010) was adapted in the analysis of pigment with slight modifications. Five hundred (500) mg of the air dried pine pollen was placed in a 50 mL centrifuge tube and extracted three times with 10 mL ethanol using vortex mixer for 1 minute. The mixture was allowed to stand until the particles of pine pollen settled at the bottom of the tube.

Supernatants were collected and measured. The final volume of the extract was adjusted to 75 mL by adding 95% ethanol. The absorbance value of the extract at 450 nm was determined by the use of spectrophotometer (A200).

The total carotenoid yield (microgram/g dried weight) was calculated using the formula:

$$\text{Total carotenoid yield (µ/g dried weight)} = \frac{V(A-0.0051)}{0.175W}$$

Where: A = absorbance value
V = final volume of the extract (mL)
0.175 = extinction coefficient of carotenoids
W = weight of dried powder.

β-carotene content was calculated based from the Vitamin A content using the formula as follows:

β--caraotene (µg/g): y= µg/g RE (Vitamin A) x 0.6 IU x 10 µg/g

Where: y = the value of Vitamin A in RE

The concentration of chlorophylls A and B were determined [8]. Methanolic solutions of plant extracts for chlorophylls a and b at 2 mg/mL were analyzed in a UV/VIS spectrophotometer at 653 and 666 nm.

$$\text{Chlorophyll a (mg/L)} = 15.65\ Abs_{666} - 7.340\ Abs_{653}$$

$$\text{Chlorophyll b (mg/L)} = 27.05\ Abs_{653} - 11.21\ Abs_{666}$$

Determination of Total Phenolic Content

The Total Phenolic Content (TPC) was determined using Folin-Ciocalteau Reagent. New samples of pine pollen were used and the powdered sample was extracted with 95% ethanol at room temperature for 48 hours. The extract was separated from the residue by filtering through an ordinary filter paper. The crude extract was collected and kept in dark glass bottles and stored in refrigerator for future use.

The determination of TPC was carefully done following the existing protocol [23]. About 400 µL (0.4 mL) of each sample extract was added with 0.8 mL distilled water and 1.0 mL diluted Folin-Ciocalteau reagent (1:10). It was allowed to stand for 5 minutes before the addition of 1 mL of 7.5 % sodium carbonate (w/v). After 2 hours, the absorbance of sample was taken at 765 nm wavelength using Unico spectrophotometer 1200. Calibration curve of gallic acid was set-up to estimate the TPC of the samples. The result was

expressed in milligram of Gallic acid equivalent per gram of dry weight sample.

Evaluation of Antioxidant Property of Pine Pollen using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Method

The 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity assay was adopted with modifications [22]. Different dilutions of the extracts (1000 ppm, 100 ppm, 10 ppm, 1 ppm, 0.1ppm and 0.01ppm) were prepared. DPPH solution was also prepared by dissolving 6.0 mg DPPH in 100 mL methanol. A volume of 1.5 mL of extract from each dilution was added into the test tube containing 2.5 mL of DPPH solution. Butylated hydroxylanisole (BHA) and ascorbic acid were used as comparators. The mixture was shaken vigorously and left to stand in a dark area for 30 minutes. The absorbance of the resulting solution was measured using spectrophotometry at 517 nm. Triplicate samples were prepared and the scavenging activity of each extract of DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging activity} = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \times 100$$

The scavenging activities of the extracts were expressed as EC_{50} (Effective concentration) values. The EC of the extract required to scavenge 50% of DPPH radical was also calculated from the curves obtained after applying non-linear regression (sigmoidal dose-response) to the data using Graph Pad Prism 7.0.

Determination of Vitamin A and C

Dried *P. kesiya* pine pollen **s**ample of 500 g was packed aseptically and sent through courier to SentroTek Corporation in Mandaluyong City, Philippines for the evaluation of vitamin A and C contents.

RESULTS AND DISCUSSION

Pigment Analysis of *P. kesiya*

This is a preliminary information on total carotenoid content of *P. kesiya* pine pollen. The result of analysis for pigments such as total carotenoid (TC), β-carotene, chlorophyll a, and chlorophyll b contents of pine pollen were presented in Table 1.

Small amount of carotenoids at 2.9 µg/g was observed, β-carotene has mean levels at 0.06 µg/g. Chlorophyll a content was recorded as 409.41 mg/L and chlorophyll b at 704.68 mg/L.

Table 1: Analysis of pigments in *P. kesiya*

PIGMENTS	LEVELS OF PIGMENTS
Total Carotenoid	**2.90 µg/g**
B-carotene	**0.06 µg/g**
Chlorophyll a	**409.41 mg/L**
Chlorophyll b	**704.68 mg/L**

Carotenoids are secondary metabolites commonly found in fruits and vegetables with many medical applications [9]. Carotenoids are reportedly effective inhibitor of heart disease and risk-reducer to some types of cancer [10]; improve eye health [11] and have potential antimicrobial activity [7]. Carotenoids are plant pigments responsible for bright red, yellow and orange hues in fruits and vegetable and one of the carotenoid families include β-carotene [12] and as source of nutrients [13].

The β-carotene content of Scots Pine (*Pinus sylvestris* L.) and found out that it contained 0.015 to 0.027 ug/g of the said carotenoid. This shows that *P. kesiya* (0.06µg/g) has higher β-carotene content than *P. sylvestris*L[14].

Chlorophyll a functions mainly as conversion of light energy to chemical energy while Chlorophyll b serves as accessory pigments in photosynthesis [15]. Chlorophyll is the green pigments in plants and algae which is essentially used in photosynthesis by which two types of chlorophyll, a and b, have been described to produce energy [16]. Chlorophyll-a is recognized as the main pigments which convert light energy into chemical energy [15] while Chlorophyll-b acts as accessory pigments indirectly in photosynthesis by transferring the light it absorbs to chlorophyll-a [17]. Chlorophyll, along with carotenoids, plays an important role in providing nutrients.

Total Phenolic Content of Pine Pollen Expressed as Gallic Acid

The total phenolic content (TPC) of the Benguet pine (*Pinus kesiya*) pollen expressed in terms of Gallic acid equivalent (GAE) was presented in Table 2.

Table 2: Total phenolic content of *P. kesiya* pine pollen in comparison with the other pine pollen

Sample	Concentration (mg GAE/g Dry Weight)
Benguet pine pollen (*P. kesiya*)	**3.548**
Masson pine pollen*	**0.05**

*Source: Cong et al. (2015)

It was found out that pine pollen (*P. kesiya)* contains 3.548 mg gallic acid equivalent/g dry weight (mg GAE/g DW). Composition analysis for total phenolic contents has been undertaken in Masson pine pollen [18] in which it has lower total phenolic content of 0.05 mg GAE/g dry weight compared to the total phenolic content of Benguet Pine (*P. kesiya*) pollen.

Antioxidant Property of *P. kesiya*

The antioxidant activity of pine pollen was also evaluated and measured in terms of the DPPH radical scavenging

activity done in parallel with other antioxidants like BHA and Ascorbic acid. Table 3 shows that the antioxidant activity of pine pollen was significantly higher than those of BHA and Ascorbic acid.

Table 3: Antioxidant activity of pine pollen in comparison with other antioxidants

SAMPLE EXTRACT	EC_{50} (mg/L)
Pine pollen	**271±1.70[a]**
BHA	**1.66±0.12[b]**
Ascorbic acid	**1.42±0.03[b]**

Means with the same letter superscript was not significantly different

Antioxidant activities were evaluated by its median effective concentration (EC_{50}) which is defined as the concentration of the extract needed to scavenge 50% of the radicals in a solution. Pine pollen has high antioxidant activity compared to BHA and Ascorbic acid.

This observation is related to the relatively large amount of pine pollen (271.10 mg/L) required to exert its antioxidant activity and scavenge radicals compared to either BHA (1.66 mg/L) and Ascorbic acid (1.42 mg/L) to exert their activity. This is the preliminary data that reveals the anti-oxidant activity of pine pollen.

However, similar study conducted on antioxidant activity of pine pollen in vitro revealed that other pine pollen has an EC_{50} of 500mg/L compared to the results of this study with 271.1 mg/L. This proves that Benguet pine (*P. kesiya*) pollen has higher antioxidant activity compared to other pine pollen [19].

Vitamin Analyses of *P. kesiya*

Result of analysis for Vitamin A and C contents of Benguet (*P. kesiya*) pine pollen is shown in Table 4. Vitamins A and C are important compounds in boosting the immune system.

Table 4: Vitamin composition of pine pollen (*P. kesiya)* in reference to other pine pollen

VITAMIN	AVERAGE
A	**0.103 µg/g**
C	**0.500 µg/g**

Vitamin A content of *P. kesiya* pine pollenis recorded at 0.103 µg/g and Vitamin C content was0.500 µg/g. Differences in vitamin contents may happen due to the reported quantitative variation of values of vitamins in different variety and level of maturity [20].

Vitamins in the pine pollen exist in a biologically natural form in the cells, with full bioactivity and good absorptivity and they work together in the body of human. Vitamins A, C and E protect one another from being oxidized and the best effect is obtained when they supplement each other. Vitamin C can help the absorption of iron, Vitamin E and selenium supplement each other [21].

CONCLUSION

Benguet (*P. kesiya*) pine pollen contained 2.90 µg/g total Carotenoid, 0.06 µg/g β-carotene, 409.41 µg/g Chlorophyll A and 704.68 µg/g Chlorophyll B, 0.103 µg/g Vitamin A and 0.500 µg/g Vitamin C and displayed an antioxidant component with 271.10 mg/L that can scavenge 50% of radicals. This study is of great potential remedy to reduce antibiotic resistance in fish and provide a plant-based anti-oxidant.

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