SCREENING AND PARTIAL CHARACTERIZATION OF SECONDARY METABOLITE ~~SCREENING AND PARTIAL CHARACTERIZATION~~ OF *Salacia korthalsiana* Miq. (POLIPOG) ROOT DECOCTED EXTRACT

ABSTRACT

A cough is a sudden and often repetitive reflex that helps clear the air passages from secretions, irritants, and foreign particles, including microbes. Coughing can be due to a respiratory tract infection or choking, smoking, air pollution, asthma, chronic bronchitis, medications, and a variety of many other factors which becomes an emerging health problem in the globe. This study ~~aims to~~ investigated ~~if there is a~~the ~~possibility~~ role of *Salacia korthalsiana* Miq. (Polipog) root decoction as anti-coughing agent through screening and partial characterization of secondary metabolite ~~screening and partial characterization~~. The physical properties determined in the root sample were: boiling point 99.3℃, brownish red in color with a pleasant odor; pH ~~is~~ 5.86 and soluble in water and ethanol which makes the Polipog root extract polar. Phytochemical screening was used for the determination of active chemical properties for anti-cough. Further, ~~and~~ Fourier Transform Infrared spectroscopy were used to determine the active functional group for anti-cough. Among the chemical properties determined for anti-cough only steroids and saponins are present, which is an antitussive and expectorant agent ~~is~~. Fourier Transform Infrared analysis showed that the main functional groups present ~~these~~ are: – the amine and ethers, which are medically active for medicinal purposes. Therefore, Polipog roots are positively active for anti-cough.

Keywords: anti-cough, medicinal plants partial characterization, phytochemical, *Salacia korthalsiana* Miq.

I. INTRODUCTION

Coughing is one of the common symptoms related to several respiratory diseases such as asthma, bronchitis, pneumonia, etc. Though several synthetic drugs are available in the treatment of cough, the problem is that an effective therapy against coughing will inevitably bring side effects. Plants have been used in traditional medicine for several thousand years. The main advantage of using medicinal plants does not produce side effects when compared with synthetic drugs (Ragavendran et al., 2011).

Fourier Transform Infrared Spectroscopy is an analytical technique used to identify organic, polymeric and in some cases, inorganic materials showing the unique chemical bonds and the molecular structure of the sample material. This profile is in the form of an absorption spectrum which shows peaks representing components in higher concentration. Absorbance peaks on the spectrum indicate functional groups (e.g., alkanes, ketones). Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. (Rahman Gul et al., 2017).

Salacia korthalsiana Miq. is known as polipog in Northern Samar. It belongs to celastraceae family. It is generally a climbing shrub with woody stem that twine into the

surrounding vegetation and can be up to 18 meters long (Lim *et al.,* 2020; Belga *et al.,* 2021). Different studies conducted about this plant and some herbolarios that use the root parts for the remedy of cough. This encourages the researcher to investigate the anti- cough activity of the plant sample with no side effects and with higher efficiency that may contribute to the community to prevent such illness into serious problem and to have a safe and effective medication of such ailments (Alvarez *et al.,* 2020).

II. MATERIALS AND METHODS

This study was conducted at the Bio-Physical Laboratory Complex, College of Science, University of Eastern Philippines, University Town, Northern Samar. The sample of plant was collected along the coastal area of Brgy. Imelda, Mondragon, Northern Samar, Philippines.

About 300g of roots were washed thoroughly with tap water and sun dried then were placed in a 1000mL Erlenmeyer flask with a 500mL of distilled water then heated by the use of hot plate until the volume of solution reach to 250mL. Then, the root decocted was filtered using the filter paper to separate the solid particles and placed in a clean glass container with cover for prior use.

Determination of Physical Properties

Boiling point. Ten mL of polipog roots decoction was contained in a test tube and submerged in an oil bath and started to be heated by the use of hot plate. Temperature was recorded as the polipog root decoction starts to boil. The process was repeated thrice.

Color. Ten mL of the polipog root decoction was contained in a test tube and the color was determined by five evaluators, the most percieved color of their sense of sight was the resulting color of polipog root decoction.

Odor. Ten mL of the polipog root was contained in a test tube and the odor was determined by five evaluators, the most percieved odor of their sense of smell was the resulting odor of polipog root decoction.

pH. Fifty mL of the polipog root decoction was contained in 80mL beaker and a digital pH meter was used to determine the concentration of the root decoction. The digital pH meter was dipped into the polipog root decoction and reading was recorded. The process was repeated thrice. Average value were recorded.

Solubility. Two mL of the polipog root decoction was contained in three test tube separately. Each test tube were added by three selected solvents: 3mL of chloroform, 3mL of distilled water and 3mL of ethanol. Then, it was shaken vigorously and after 2 minutes, the result was recorded. The process was repeated thrice.

Determination of Chemical properties

The following methods were modified and was taken from the standard methods of Guevara (2005), Kumar *et al* (2009), Gibbs RD (1974), Dagalea *et al.* (2021), Dianito *et al.* (2022) and Dagalea *et al.* (2022). The entire test was done in three trials.

Test for the Presence of Alkaloids. In this test, the Dragendorff's reagent and the Mayer reagent was used in determining the presence of alkaloids. A positive result indicates an appearance of orange precipitate in Dragendorff's test and a white precipitate in Mayer's test. Ten

mL of the Polipog root decoction was contained in an evaporating dish. Then it was evaporated to a syrupy consistency over a steam bath. A 5mL of 2M HCl was added. Then, the solution was heated in a hot plate with stirring for about 5 min using stirring rod. Then, the solution was cooled down. Plus, 0.5g of NaCl was added with stirring the solution and then filtered. The residue was washed with enough 2M HCl until the filtrate reached to a volume of 5mL. Then the filtrate was divided into two parts. The first part was added of Dragendorff's reagent for about 2-3 drops and the second part was added of Mayer's reagent for about 2-3 drops. Then, the result was recorded.

Test for the Presence of Flavonoids. In this test, the alkaline reagent was used to determine the presence of flavonoids. The positive result indicates the appearance of an intense yellow color and becomes colorless. Five mL of the Polipog root decoction was contained in an evaporating dish. Then, it was treated with few drops of sodium hydroxide solution. The result was observed as further addition of dilute acid. The result was recorded.

Test for the Presence of Saponins. Froth test: In this test a formation of 2cm foam height indicates the presence of saponins. About 5mL of root decoction was mixed with 20mL of distilled water and then agitated in a graduated cylinder for 15 minutes. The result was recorded.

Test for the Presence of Steroids. In this test, the upper layer turns red and sulfuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids. One mL of the Polipog root decoction was dissolved in 5mL of chloroform and an equal volume of concentrated sulfuric acid was added by sides of the test tube. The result was recorded.

Determination of Functional Groups

The polipog root samples were brought to Research Center for the Natural & Applied Sciences, University of Santo Tomas for FTIR analysis. The polipog roots were powdered. It was oven dried for about 70℃ then grinded using the mortar and pestle. The polipog root powder was kept in a zip lock bag with label.

III. RESULT

Physical Properties

The result showed in Table 1 that polipog root decoction has a boiling point of 99.3℃. The color is brownish red, and the odor is pleasant. The pH is 5.86. For solubility test, the polipog root decoction is miscible in water and ethanol which are polar solvent while immiscible in chloroform which is also a polar solvent.

Chemical Properties

The active chemical properties for anti-cough determined for polipog root decoction were alkaloids, flavonoids, saponins and steroids. Among the four active chemical properties for anti-cough only steroids and saponins were found positive, Table 2.

Functional Group Analysis

Fourier Transform Infrared spectroscopy results fo ~~T~~the functional groups active for anti-cough ~~was determined using Fourier Transform Infrared spectroscopy. Result showed the FTIR~~

~~spectra (in Figure 1) of the functional groups active for anti-cough~~are presented in Figure 1. ~~It~~ Result showed ~~appears there are~~ many functional groups ~~presents~~ in the root sample. Each vibrations of the molecules represents the functional groups. The most intense bands occuring at 2376.30cm^{-1}, 2314.58cm^{-1} ,3589.53cm^{-1}, 3307.92cm^{-1}, and 1735.93cm^{-1} corresponding to O-H/N-H and C=O stretching vibrations respectively indicates the presence of carbonyl compounds, amine and ethers in Polipog root sample. The observed very strong absorption band between 2500cm^{-1} - 3300cm^{-1} indicates the presence of carbonyl compounds (O-H stretching). Alkene group are absorbed at the region 675-1000cm^{-1}. The root sample containes 817.82cm^{-1} and 709. 80cm^{-1} (=C-H bending). The band occuring between 1400cm^{-1} -1600cm^{-1} indicates the presence of aromatic, C=C stretching (Table 3).

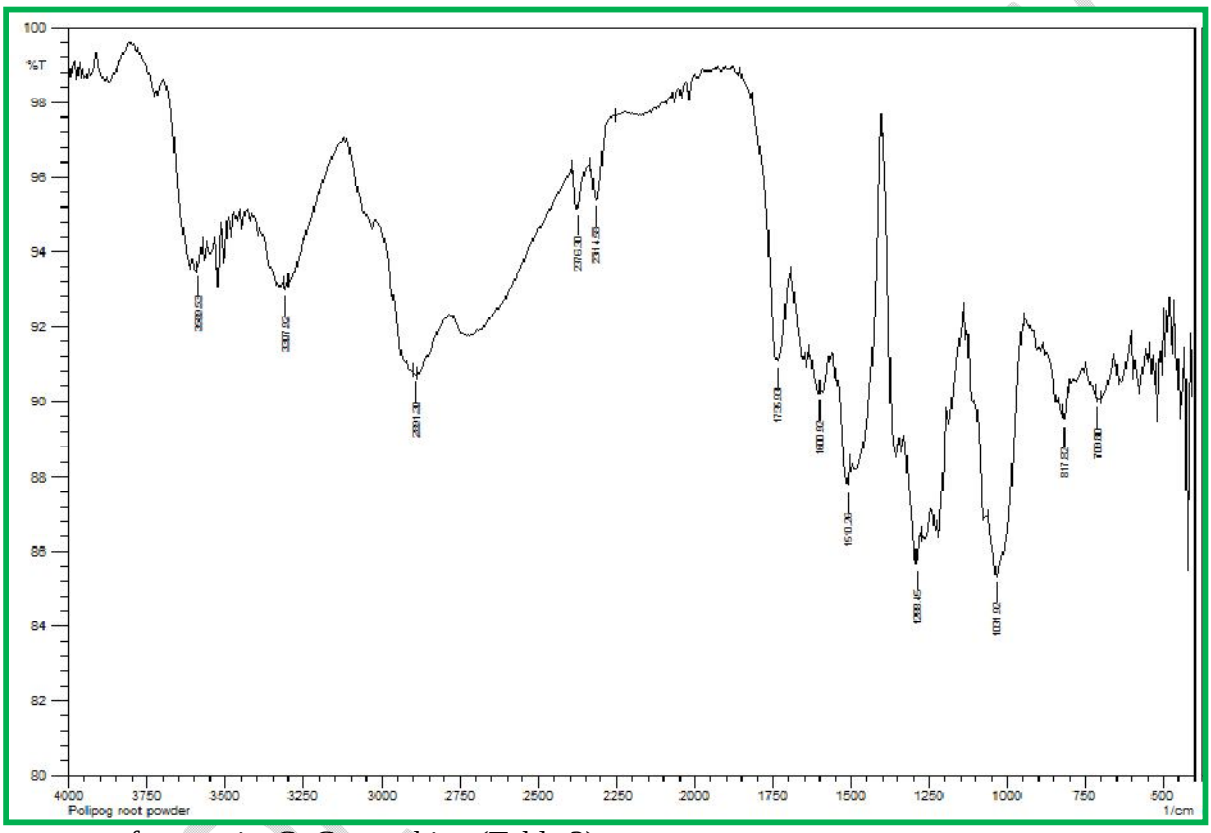

Figure 1. FTIR spectrum of Polipog root

Table 1. Physical Properties of Polipog root decoction

Physical properties	Polipog root decoction	Interpretation
Boiling point	99.3℃	Lower boiling point than water
Color	Brownish red	Brownish red
Odor	Pleasant	Pleasant
pH	5.86	Weakly acidic

Solubility		
Ethanol	Miscible	Polar
Water	Miscible	Polar
Chloroform	Immiscible	Non-Polar

Secondary Metabolites	Treatment	Results	Interpretation
Alkaloids	Dragendorff's reagent Mayer's reagent	No orange precipitate No white precipitate	Negative Negative
Flavonoids	Alkaline reagent	Light red color appeared	Negative
Steroids	Salkowski test	A green fluorescence occurred	Positive
Saponins	Froth test	2cm height of foam	Positive

Table 2. Chemical Properties of Polipog root decoction

IV. DISCUSSION

Result showed that polipog root extract has a lower boiling point than water. It is browish red and has a pleasant odor. The extract is weakly acidic. It is miscible in polar solvent which indicates the polar nature of the polipog root extract (Table 1). The active chemical properties for anticough (Table 2) showed only the positive result of saponin and steroid. Saponins are a class of chemical compounds found abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions. They are also immunostimulatory, hypocholesterolemia, anti-carcinogenic, anti-inflammatory, anti-microbial, anti-protozoan, molluscicide and have anti-oxidant properties. According to Gairola 2010, saponins are glycosides that employed expectorant and antitussive agent which is a cough-suppressing activity. Steroid is composed of seventeen carbon atoms, bonded in four "fused" rings: three six-member cyclohexane rings and one five-member cyclopentane ring. Steroids vary by the functional group attached to this four-ring core and by the oxidation state of the rings. Steroids help to stimulate metabolism and immune function. Steroid, these secondary metabolites will be the ones responsible for the antitussive and other activities of the plant sample (Dapaah et al., 2016).

Among the IR spectra of Polipog root (Table 3), it is shown that the main functional group of *Salacia korthalsiana Miq.* are amine and ethers which responsible for various medicinal properties (Ashokkumar and Ramaswamy, 2014).

Based on the findings of this study the researcher concluded that *Salacia korthalsiana* Miq. (Polipog) root decoction has active chemical properties for anti-cough ~~these are~~like

saponins and steroids which are agent for cough suppressing activity. The functional groups present~~s~~ are also active for medicinal properties.

The researcher ~~highly~~ strongly recommends conducting more possible application of the plant sample to ~~. D~~determine other chemical constituents of the plant sample to e~~. Enn~~hance the application of current study ~~using~~ through even using other analytical method.

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