Research Article

Phytochemical screening of the ethanol extract of *Gliricidia sepium* (Jacq.) Steud (Kakawate)

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Abstract

Objective: This study was conducted to determine the secondary metabolites present in *Gliricidia sepium* (Jacq.) steud (kakawate) leaf and bark extracts. **Method**: The leaf and bark extracts of kakawate were tested for the presence of secondary metabolites such as alkaloid, anthraquinone, leucoanthocyanin, phenolic compound, saponin, steroids, tannin, and terpenoids. **Results**: The results of this study suggest that the phytochemical properties of kakawate possess potential antioxidant property. **Conclusion**: This study would promote the use of kakawate leaves and barks as possible ingredient for medicinal products.

Keywords: phytochemical screening, kakawate, ethanol extract, secondary metabolites

Introduction

The importance of a country's diverse medicinal plants lies not only in their chemotherapeutic value in traditional medicine but also in their potential as sources of new chemical entities for drug discovery. Although the Philippines boast of its biodiversity and rich cultural traditions of plant use, scientific understanding of medicinal plants remains largely unexplored (Forrest, 2004).

There is a continuous and urgent need to discover plants with diverse chemical structures and novel mechanisms of actions. The wide acceptance of traditional medicine as an alternative form of healthcare and the alarming increase in the incidence of new and re-emerging infectious diseases bring about the necessity to investigate these medicinal plants. The medicinal value of plants lies in the bioactive compounds such as alkaloids, tannins, flavonoids and phenolic compounds that produce a definite physiological action on the body (Adam, 2002). The increasing use of plant extracts in the food, cosmetic, and pharmacological industries suggests that in order to extract active compounds, a systematic study of medicinal plants is very important.

This study focused on the determination secondary metabolites of the ethanol extracts of kakawate. The findings from this work may add to the overall value of the medicinal potential of the plant which is being utilized traditionally as herbal medicines.

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Materials and methods

Materials

The fresh plant of kakawate was collected from University of Eastern Philippines (UEP), Northern Samar, Philippines. The taxonomic identity was identified by an expert from the College of Science, UEP.

Preparation of the Plant Extracts

The separated parts of the plant were weighed separately and finely cut. The finely cut leaves and bark were air dried for 5 days. For the extraction procedure, about 247g of finely cut leaves and bark plant materials were soaked in 95% ethanol. This was done to prevent enzyme hydrolysis and simultaneously extract the plant constituent. The flasks were covered and the leaves and bark were soaked for 15 days with frequent agitation. After soaking, the mixtures were filtered through clean cheesecloth to separate the leaves and bark plant materials followed by Whatman No. 1 filter paper. The filtrate was concentrated and the solvent

has been removed by distillation. The percentage yield of the extracts of kakawate was determined and was stored in clean labeled airtight bottles inside the refrigerator.

Phytochemical Screening

The plant extract was subjected for chemical analysis to identify and characterize some of the secondary metabolites. The standard procedures for phytochemical screening described by Guevarra (2005) were adopted to screen the ethanolic extracts of kakawate for secondary metabolites. The different secondary metabolites of the kakawate leaves and bark extracts were determined using the following procedures.

Test for Alkaloids

In this test, the Dragendorff's reagent and Mayer's reagent are use in determining the presence of alkaloid. A positive result is indicated by the presence of orange precipitate in Dragendorff's reagent and white precipitate with the Mayer's reagent (Guevara, 2005).Take an equivalent of 20 grams of (Kakawate) leaves and bark extracts separately. This was placed it in an evaporating dish. Then, it was evaporated to a syrupy consistency over a steam bath. Five (5) mL of 2M hydrochloric acid (HCl) was added. Next, it was heated with stirring for about 5 minutes and cooled. Then, about 0.5 g of sodium chloride (NaCl) was added. Then, it was stirred and filtered. The residues were washed with enough 2MHCl to bring the filtrate to a volume of 5mL, and then the filtrate was separated into two parts. To the first part, 2-3 drops of Dragendorff`s reagent was added and to another part, add also 2-3 drops of Mayer`s reagent. **Test for Anthraquinones**

To an equivalent of 1g of (Kakawate) leaves and bark extracts separately and evaporates to incipient dryness over a steam bath. Then, add 10 mL 0.5 M potassium hydroxide and 1 ml of 5% (H_2O_2) and stir. And heat the resulting mixture over a steam bath for 10 minutes. Filter and discard the residue. Acidify the filtrate with glacial acetic acid. Extract the aqueous filtrate twice with 5ml portions of benzene. Combining the benzene extracts divide the combined benzene extracts into 2 portions, reserving one portion as the control basify the other portion with ammonia and shake (Guevara, 2005).

Test for Leucoanthocyanin

An Equivalent of (Kakawate) leaves and barks extracts and evaporate to incipient dryness over a steam bath. Then, cool to a room temperature. Defat by taking up the residue with 9 mL hexane and water or with petroleum ether. Discard the hexane or petroleum ether extract. Then dilute the defatted aqueous layer with 10 mL of 80% ethyl alcohol. Filter and divide the filtrate into two test tubes.

Treat one portion of the above alcohol filtrate with 0.5 mL conc. HCl, observe for any color change. Warm the solution for 15 minutes in a water bath. Then, observe for further color change within an hour and compare with the control. Record your result.

Test for Phenolic Compound

Dilute the extract with 100 ml of distilled water and then 2 ml of the extract. Then take an equivalent of 2g (Kakawate) leaves and bark extracts separately and add 2 drops of 3% $FeCl_3$. Formation of green or blue color indicates the presence of phenolic.

Test for Saponin

An equivalent of 2g of (Kakawate) leaves and bark extracts separately and load to a capillary tube by immersing the tube to a height of 10 mm in the plant extract. Likewise, load another capillary with distilled water. Then, lift the capillary tubes and keep both in a vertical position to allow the liquid inside to flow freely. After sometime, compare the height of the liquids in the two tubes. If the level of the plant extract in the capillary tube is half or less than the in the other tube containing water, then the presence of saponin may be inferred.

Test for Steroids

Take an equivalent of 2g of (Kakawate) leaves and bark extracts separately and evaporate the extract to incipient dryness over a water bath. Then, cool to room temperature, and defat by taking up the residue with 6 mL of hexane and water, 2:1 v/v- partition by gently shaking the mixture in a test tube and pipette out the upper hexane layer, repeat the treatment with hexane until most of the colored pigments have been removed. And discard all the hexane extract properly. Then, heat the defatted aqueous layer over a water bath to remove the residual hexane and cool to room temperature.

To another portion of the defatted aqueous layer free from hexane add 10 mL of dichloromethane and stir the mixture with a glass rod for a few min. allow standing. Pipette off the lower dichloromethane extract. And dry the dichloromethane extract by passing this extract through about 100 mg anhydrous sodium sulfate (Na_2SO_4) placed over dry filter paper in a funnel. Then, divide the filtrate into two portions, use one for the control. The other portion is treated with 3 drops of acetic anhydride then one drop of conc. sulfuric acid. Let it stand for an hour for further color change. Compare with the control. The positive results give colors ranging from blue to green, pink, red, purple, or violet, because of the steroid/ triterpenoid skeleton.

Test for Tannins

Two (2) mL of (Kakawate) leaves and bark extracts was

added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Test for Terpenoids

Two (2) mL of (Kakawate) leaves and bark extracts was added to 2 ml of acetic anhydride and concentration of H_2SO_4. Formation of blue, green rings indicates the presence of terpenoids.

Results and discussion

The phytochemical screening showed that the leaves and bark extracts of kakawate do contain secondary metabolites such as alkaloids and saponin. The positive result for alkaloid was indicated by the presence of an orange precipitate in Draggendorff's reagent and white precipitate the Mayer's reagent. Positive result for saponin was determined by its level in the capillary tube which is half or less than in other tube containing distilled water. Saponins have been used as detergents, pesticides, molluscicides, apart from its industrial applications as foaming and surface active agents which show beneficial health effects. The secondary metabolites that are not present in the leaves and bark extracts of kakawate are the anthraquinone, leucoanthocyanin, phenolic compounds, steroids, tannins, terpenoids. The results are presented in Table 1.

Phytochemical screening of plants is very important in identifying new sources of therapeutically and industrially important compounds. The present work attempt to assess the status of phytochemical properties of leaves and barks of kakawate to improve the health status of people particularly in Northern Samar and also to use in pharmaceutical and Nutraceutical products of commercial value.

Conclusions

In this work, we have reported the secondary metabolites present in the leaves and bark of kakawate. This research presents idea about the possible uses of the various parts of this plant sample. The analyzed physicochemical characteristics and secondary metabolites of kakawate include useful metabolites which can be extracted for the

Table 1. Summary of the Phytochemical Screening done for the Leaves and Bark Extracts of Kakawate

Secondary Metabolites	Test Results		Interpretation
	Leaves	Bark	
Alkaloids	Dragendorff's reagent orange precipitate formed	Dragendorff's reagent orange precipitate formed	Positive
	Mayer's reagent white precipitate formed	Mayer's reagent white precipitate formed	Positive
Confirmatory	No precipitate formed	No precipitate formed	Negative
Anthraquinone	Two layers were formed; the upper layer was clear and the lower layer has a light yellow	Two layers were formed; the upper layer was cloudy and the lower layer has a cloudy yellow	Negative
Leucoanthocyanin	There is no different between the control	Orange color and has a small precipitate formed	Negative
Phenolic compound	Clear yellow	Clear yellow	Negative
Saponin	Lower than distilled water capillary tube	Lower than distilled water capillary tube	Positive
Confirmatory	Greater than 2 cm high of the honeycomb's	Greater than 2 cm high of the honeycomb's	Positive
Steroids	Cloudy	Cloudy	Negative
Tannins	Green precipitate formed	Green precipitate formed	Negative
Terpenoids	Violet ring	Maroon ring	Negative

purpose of utilizing it in medicinal products. The results of this study might be the basis that wild plant of kakawate can be used as an ingredient that promotes health advantage.

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