International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume: 3 | Issue: 4 | May-Jun 2019 Available Online: www.ijtsrd.com e-ISSN: 2456 - 6470

Anti-Microbial Activities and Phytochemical Screening of the Premna Odorata Blanco (Alagaw) Leaf Extract

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How to cite this paper: Bernadette C. Mollejon | Charito V. Mollejon "Anti-Microbial Activities and Phytochemical Screening of the Premna Odorata Blanco (Alagaw) Leaf Extract" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-3 | Issue-4, June 2019, pp.343-345, URL: https://www.ijtsrd.com/papers/ijtsrd23751.pdf

IJTSRD23751

ABSTRACT

The researcher focused mainly to determine the antimicrobial properties and phytochemical screening of the Alagaw leaf extract. Specifically, this study was conducted to determine the percent yield, antimicrobial activities and the secondary metabolites of Alagaw leaf extract which was analyzed and it include alkaloid, anthraquinone, saponins and steroid. Findings of the study showed that the Alagaw leaf extract has a percent yield of 11.5%. Anti-Microbial Activity was tested by petri disk on a plate Nutrient Agar streaked with the E. coli bacteria, the plates were incubated for 24hrs and 37oC. Results were observed for the presence of zone inhibition (clear area) around the test disk. Results from this study showed that the antimicrobial activity on E. coli as indicated as negative by the presence of Alagaw leaf extract. Furthermore, the result suggests that the Alagaw leaf extract did not suppress the growth of the E. coli bacteria, hence it indicates that it has no anti-microbial effect to the test organism. While the secondary metabolites such as alkaloid, anthraquinone, saponins and steroid is found negative. It is therefore recommended that further study of the chemical properties of alagaw leaf extract, barks and roots should be conducted

INTRODUCTION

To achieve mastery over the powerful forces of nature, man has always turned to plants. It gives shelter, food, materials to make our clothing etc. Our environment is surrounded by different kinds of plants. These served as our refuge in different kinds of pollution. Plants used for natural healing is a common practice in specially areas. Substances that are being metabolized in the seed, root, items, fruits and leaves are used as active ingredients. The country needs medicinal plants. With proper nutrition, disease prevention and control measures help provide adequate health care to the population and contribute to the improvement of the quality of life. Medicinal plants are need for fresh plants materials and must always be observed in the use.

Premna odorata Blanco was one of the healing plants for Worldwide, cancer causes more deaths than AIDS, tuberculosis and malaria combined although several anti-cancer drugs already commercially available, a number of adverse effects sometimes occur during chemotherapy. To reduce this unwanted effect many chemotherapeutic agents in the clinic are derived from natural products or designed on the basis of original compounds found in natural product. In the search new cancer chemo preventive agents from natural sources, This research was directed towards the screening of plants with antimutagenic activity in the micronucleus test (MT) based on the method of schmid, MT is highly sensitive in vivo test designed to detect carcinogenic effects. In addition, this study also focused on the isolation and characterization of the antimutagenic expressions of the plant identified with highest activity using a bioassay directed scheme. As a part of systematic investigation of identifying bioactive compounds from Philippine plant extract was subjected to different investigator. Initiated screens at the institute of chemistry and cell Biology (ICCB) Screening Facility at Harvard Medical School. The selected plants that were selected to the preliminary screening for bioactivity in MT included *Premna Odorata Blanco*. These plants were chosen since they were among the few plant extract gave positive results in various assays from the Philippine plant extract libraries deposited at the ICCB. The literature search revealed that iridoid glycosides were isolated from *Premna Odorata Blanco*.

METHODOLOGY

Calculate the percent yield of the mass of crude extract of leaf over the volume of the leaf extract multiply by one hundred (100) of *Premna Odorata Blanco* (Alagaw) leaf extract using the formula:

$$\% \text{ yield} = \frac{mass\ of\ extract}{volume\ of\ extract} \times 100$$

Test for the presence of alkaloid

In this test the Dragendorff's reagent and Mayer's reagent was used to test the presence of alkaloid in leaf extract of *Premna Odorata Blanco*. From cold extract it was separated

and took 5ml of leaf extract of *Premna Odorata Blanco* and was separated in evaporating dish. It was evaporated over a steam bath and added 5 ml of 2 M HCl, heated while stirring for 5 minutes and let was cooled. Then added about 0.5 g NaCl stir and filtered, washed the residue with enough 2 M HCl to bring the filtrate to a volume of the leaves filtrate and treated with Mayer's reagent. The result was recorded. A positive result indicated by orange precipitate with draggendorff's reagent and white precipitate with the Mayer's reagent.

Test for the presence of anthraquinones

Test for the presence of anthraquinone was done using the procedure provided by Guevarra 2005. The modified Bontragers test was used in determining the presence of Anthraquinone. A pink color indicated a positive of Anthraquinone.

Equivalent of 1g extract was evaporated to incident dryness over a steam both, and then 10ml 0.5M potassium hydroxide and 1ml of 1% (H_2O_2) were added and stirred. The resulting mixture was heated over a steam bath for 10 minutes. The residue was filtered and discarded. The filtrate was acidified with glacial acetic acid. The aqueous filtrate was extracted twice in 5ml portions of benzene (caution: carninogenic !). Combining the benzene extracts and divided the extracts into 2 portions as the control and the other portions was treated with ammonia solutions. The tube was shaken and compared with the control tubes.

Test for the presence of saponin

The capillary test was used to determine the presence of saponin if the level of the plant extract in capillary tube is half in the other tube containing water, the presence of saponin may be inferred. A capillary tube was loaded with the plant extract by immersing the tube to a height 10mm in the plant. Likewise load another capillary tube was loaded with distilled water, the lift capillary tubes and keep both in a vertical position to allow the liquid inside to flow out freely after sometimes the height of the liquid in the two tubes was compared.

Test for the presence of steroid

The lie berman-burchard test was used to detect the presence of steroid especially the unsaturated one. The positive result gave the color of ranging from blue to green, red, pink, or violet because of the presence of steroid skeleton.

About 10g of the plant material from the prepared plant extract was used and evaporated to incipient dryness over stream bath and cooled to room temperature to defats the material by taking up the residue with 6ml of hexane and 3ml of water and was gently shaked the mixture in a test tube then pipette out the upper layer, after that it was repeated the treatment with hexane after most of the colored pigment has been removed, discarded all the hexane extract properly, there treated the aqueous layer with 10ml chloroform and gently shake the mixture. It was allowed to stand and pipette the chloroform then dry the chloroform extract by filtering the mixture though about 100mg anhydrous sodium sulfate hold over dry filter paper , divided it by two portion. One was used for control, and then the other portion was treated with 3 drops of acetic anhydrides then one drop of concentrated sulfuric acid. It was observed for any immediate color change and was let it stand for an hour and was observed if further color was changed.

Preparation of the Bacteria

The non-pathogenic strain of the *E. coli* bacteria was used in this study. The bacteria was cultured using an agar in nine (9) petri dishes. It was placed in a dark and warm place.

Get 9.5g of Nutrient Agar was distilled into 250ml of water it was boil into 15minutes while stirring in 50°c then cool, after the preparation of agar it was divided into nine (9) portions using in petri dish and streak a soup of bacteria stir and then put into the petri dish and swirled to spread overly. The extract was distilled with the solvent to get the pure Alagaw extract, distilled in the water as negative control and dissolved 500mg at clarithromycin capsule in 10ml water as positive control. It was submerged at the paper disk to the extract positive control and negative control. Putted the paper was put in the disk to the prepared cultured bacteria and then placed in the dark and warm room for 24hrs. at inoculation. After the period the zone of inhibition was measured by centimeters, that is the clearing of the bacteria in the petri dish. All possible result was recorded.

RESULTS AND DISCUSSION

Table1. Summary on Phytochemical screening Tests

Physical Properties	Leaf Extract	Interpretation
Alkaloid	No Orange precipitate form when treated with Dragendorff's reagent and no white precipitate with Mayer's reagent	Negative
Anthraquinone	All trials has no pink color observed	Negative
Saponin	Lower than in the other water tube containing water	Negative
Steroid	No production of range in colors from blue to green, red, pink, violet or purple	Negative

It is implicit that all trials in the secondary metabolites such as alkaloid, anthraquinone , steroid and saponin and steroid in alagaw extract is negative.

Table2. Summary on Anti-Microbial Tests

	Negative Control	Positive Control	Alagaw Extract	Interpretation
E. Coli	No Inhibition	Trial 1 & 3 is 20mm Inhibition and Trial 2 is 22 Inhibition	Trial 1 & 3 is 6mm Inhibition and Trial 2 is no inhibition	Negative

The Anti-Microbial Property for the *E. Coli* bacteria tested was found negative in alagaw leaf Extract since the zone of inhibition was found to be less than 20mm as stated by Lalitha MK (2004).

CONCLUSIONS

Based on the results of the study, the following conclusions were drawn by the researcher: Alagaw leaf has 11.5% percent yield. Alagaw leaf extract does not contains secondary metabolites called alkaloid, steroid saponin,athraquinone. Even though there is a zone of inhibition, the alagaw extract has no antimicrobial resistance against E. coli.

References

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