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Phytochemical Analysis and Microbial Property of Parasol Leaf Tree or Elephant's Ear (Macaranga Tanarius): It's Effect on the Fermentation of Sugar Cane Vinegar

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Abstract:
:The study was conducted to perform phytochemical analysis and microbial property of parasol leaf tree or elephant's ear (MacarangaTanarius), its effect on the fermentation of sugar cane extract. This study used experimental research design in an actual laboratory set- up. There were three phases in the experimental study. Phase 1 was the preparation of the plant sample fresh leaves, dried leaves extraction using distilled water and ethyl alcohol. Phase 2 was the phytochemical analysis to determine the presence of alkaloids, sterols, glycosides. Triterpenes, flavonoids, saponinsand tannins.Phase 3 was the microbial property to test organisms. Findings shown that the parasol leaf tree (MacarangaTanarius) leaf extract contain sterols, triterpenes, flavonoids, saponins, glycosides and tannins. But it does not contain alkaloids. The samples, fresh and boiled (Samak) Parasol leaf tree or elephant's ear leaf extract produced inhibitory activity (++) with mild reactivity (2) against the test organisms, Escharichia coli, Staphylococcus aureus, and Salmonella typhimurium. Both produced complete inhibitory activity (+++) with mild reactivity (2) against the test organism, Pseudomonas aerufinosa.Amikacin 30 ug, which served as positive control for E. coli, S. typhimurium, and P. Based on the findings, the following recommendations were drawn a follow up study be conducted to quantify and identify the type of sterols, triterpenes, flavonoids, saponins, glycosides and tannins present in the leaves of samak. Other testing should be done using the samak leaves like tests for its nalgesic, antihypertensive properties.

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I. Introduction

Drugs and medicines are substances which prevent or help to cure diseases. Many drugs come from plants, as ancient cultures knew well. Many people knew and used this remedy for hundreds of years. Today, plants are grown under scientifically Controlled conditions to produce many drugs and healing substances.

. A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because of such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums and precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would be valuable in discovering the actual value

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of folkloric remedies, thus this research will be conducted.

II. Statement of the Problem

Microorganisms cause infectious diseases. Treatment requires antibiotics which are inexpensive antibiotic derived from herbal plants can be used fro therapy after an exhaustive research on its biological properties and clinical studies.

This study aimed to determine the phytochemical characteristics and anti-microbial property of Parasol leaf tree or elephant's ear (MacarangaTanarius).

Specifically, it sought to determine the following:

1. The secondary metabolites present in the leaves of the parasol leaf tree.
2. The inhibitory effects of parasol leaf tree (MacarangaTanarius) extract in terms of
   a. Type of bacteria
   b. Level of concentration
   c. Extraction solvent
3. The interaction effect of the levels of concentration of MacarangaTanarius leaves extract to the zone of inhibition of the different test organisms.

III. REVIEW OF RELATED LITERATURE AND STUDIES

Herbal medicines have been used in years. In the 20th century, herbal medicines made a comeback as people began to seek alternatives of these days. Today, herbal preparations are marketed not only in health food stores but also in pharmacies, supermarkets, department stores and even truck stops.

Most medicinal plants have been processed into dosage forms that are readily available and convenient to use.

Local names are samak (ilk.) binungan (tag) elephant's ear or parasol leaf tree (English).

IV. Local Studies

A. Phytochemical screening of the plant's extract

Phytochemical is concerned with the enormous variety of organic substance and non-nutritive bioactive that are elaborated and accumulated by plants. It deals with the chemical structure of the organic substance, their biosynthesis, turnover metabolism, their natural distribution and their biological function and considered to have beneficial effect on human health (Sanchez, 1999).

Phytochemical screening as cited by Liat (2005), is confined with the detection of important phytochemical constituents that usually exhibit biological activity like alkaloids, saponins, tannins, glycosides, and flavanoids.

In phytochemical analysis, fresh and dried plant material is finely cut and immersed into boiling alcohol. This process prevents enzyme oxidation or hydrolysis. The plant material may also be dried before extraction. It should be thoroughly dried as quickly as possible and the use of heat must be avoided. The dried material then is safely stored for long period of time.

a. Biologically active components

Alkaloids are natural steroids, physiologically active nitrogen compound derived in plants, preservatives found in animal tissue, flowering agents found in fruits and vegetables and anesthetics found in plants. Most alkaloids are colorless, crystalline; like conine, nicotine, and hygrine are liquids and some are colored like berberine which is yellow. Many alkaloids are bitter and quite a number of them possess curative properties. Morphine has a narcotic action, reserpine is a traquilizer, atropine has antispasmodica action, cocaine is a local anesthetic, and strychnine is a nerve stimulant.

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Saponins

Saponins have the property to of reducing the interfacial surface tension of heterogenous system; the presence of hydrophilic and hydrophobic molecular parts is responsible for this phenomenon.

Saponins are not used as detergent anymore.

Anthraquinone

Anthraquinone is an aromatic organic compound. It is a derivative of anthracene. It has the appearance of yellow to light gray green solid crystalline powder. Its other names are 9, 10 anthracenedione, anthradione, 9, anthrachinon, anthracene 9, 10 quinone and trade name hoelite, morkit, corbit. It is insoluble in water but dissolve in alcohol, nitrobenzene and aniline. It is chemically fairly stable under normal conditions. Anthraquinone naturally occurs in some plants(e.g. aloe, senna, rhubarb, and casearubucktorn), fungi, lichens, and insects where it serves as a basic skeleton of their pigment.(Http.//en.Wikepedia.org/wiki/anthraquinone)

B. The test organisms

a. Escherichia Coli

Escherichia is a simple celled organism which occurs widely in nature. The body fluid of warm blooded animals including man will support their growth. They also occur in soil, on plants, in air, and in water. In fact, very few places if investigated fail to reveal their presence. (Microsoft Encarta Reference Library, 2004)

Food borne diseases currently causing major concern in developed countries is caused by a particular variant of the common intestinal bacterium E. coli. Although E. coli produces toxins, that cause bloody diarrhea and in some cases for more severe problems including kidney failure.

b. Staphylococcus aureus
c. Enterobacteraerogene
d. Salmonella enteric
e. Pseudomonas aeruginosa

Foreign Studies

S. Phommart (2005). Cited in his study the decoction of the root of this plant is drunk as an antipyretic and also as an antitussive. The dried root is used as an anti-inflammatory.

Godofredo Stuart. In the Philippine Herbal Medicine, his illustrated compilation with the botanical information, macarangatanarius has chemical properties and folkloric uses.

K Matsunami (2006). There are four new megastigmane glucosides, named macarangaiosides A-D (2-5) together with mallophenol B, lauroside E, methyl brevifolin carboxylate, and hyperin and isoquercitrin as a mixture were isolated from leaves of macarangatanarius.

J. Nat. Prod (2008).the chemical content from the leaves of macarangatanarius are macaflavanones A-G, prenylated Flavanones.

V. RESEARCH METHODOLOGY

This section presents the different materials and the different experimental procedures that was used in the study.

I. *Materials* :

The following materials was used in the study: sterile forceps, petria dishes, aluminum foil, Whatman paper discs, incubator, nutrient agar stopper, rotary evaporator, Buchner funnel, filter Paper, Erlenmeyer flask, macarangaTanarius leaves, Bunsen burner, test tubes, pipettes, filter Paper auto clave, 2 tubes Eschirichia coli, 2 tubes staphylococcus aureus, 2 tubes EnterobacterAerogenes, 2 tubes salmonella enteric.

The reagents and chemicals that will be used are the following: alcohol extract, magnesium Turnings, ferric chloride, 0.5 M potassium hydroxide, and acetic acid.

II. Experimental Procedure:

A. Gathering of Samples

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Matured leaves of MaracangaTanarius were gathered at Tumauini, Isabela. They washed thoroughly and air dry for about two weeks. The air- dried leaves wascutted into pieces and pulverized using a blender. Finally the product was kept in a dry container at room temperature.

B. Phytochemical Analysis

B. 1 Test for Alkaloids

Five mi. of the plant extract was measured and stir while heating for about 5 minutes over a water bath and cooled at room temperature. Powered NaCI was added and then stir and filter. Enough freshly prepare 2M HC1 was added and washed the filtrate and brought the filtrate to final volume of 5 ml to 1 ml, of aliquot, 2-3 drops of Meyer's reagent or Wagner's reagent was added. This was observed and the result was recorded. Any turbidity that was observed is considered preliminary evidence alkaloids were present.

B.2 Test for Tannins

The dried alcoholic extract was extracted with hot water and aqueous extract was filtered. Seven drops of furic acid indicated the presence of Tannins.

B.3 Test for Sapon

The dried alcoholic extract was dissolved in hot water, the aqueous extract was shaked for about 30 minutes and was allowed to stand and itwas observed over a period of 30 minutes. Formation of "honey comb" forth greater than 3 cm above the surface of the liquid and persisted for more than 30 minutes was indicated the presence of saponins.

B.4 Test for Anthraquinone

Five nil from the plant extracts was taken and evaporated to incipient dryness over a water bath. 10 ml. of 05M KOH was added to 10 ml of 5% hydrogen peroxide. The mixture was heated to 10 minutes. The filtrate was filtered and acidified with glacial acetic acid. Five ml of benzene was extracted twice and divided in two portions. One portion was reserved.

Another portion of benzene basified with ammonia and compared it with the control. A pink color indicated a positive result.

C. Microbiological Screening

C. 1 Venue for the Microbiological Activity Test

The experiment was conducted at the Department of Science and Technology, Regional Office, Tuguegarao City.

C.2 General Procedure for experimental Microbiological Test

C.2. 1. Sterilization of Equipment

C.2.2 Preparation of Plant materials

D. Preparation of Test Organisms

The test organisms was obtained at the Department of Science and Technology, Regional Office, Tuguegarao City.

The organisms wereEschevichia coli; Staphylococcus aureus; Salmonella Enteric; enterobacteraerogenes; and Pseudomonas aeruginosa.

After acquiring the test organisms, 0.1 ml of nutrient broth containing the bacterial species was placed in each petri dish using the spread plate method, the inoculums was spread evenly with a sterile glass L-rod to obtain a level of bacterial lawn. This was stored in an incubator at 37 degrees Centrigrade within 24 hours.

E. Paper Disc Diffusion Method

E. I Preparation of Filter Paper Disc

Prepared filter paper disc from what man antibiotic Assay disc (AA discs). A metal pluncher was used in cutting the paper disc into a diameter of 0.6 cm and wrapped it in sets of 10 in aluminum foil and was sterilized.

E.2 Preparation of Sample for Assay

The paper disc agar diffusion assay was used to determine the antibacterial properties of the sample. 10 ml of the aqueous plant extract, ethanolic extract, and commercial antibacterial drug was dispensed into sterile paper discs and was performed in five trials. The discs were sceptically applied and pressed into the seeded nutrient agar in an equidistant manner.

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F. Testing of the Anti-Microbial Property

The petri dishes were labelled for anti-microbial property. Sets of petri dishes were arranged in a wooden tray. The petri dishes were incubated at 35 to 37 degrees Centrigrade for 24 hours.

G. Experimental Design

The Randomized Complete Block Design in Factorial experiment was used in the study. The treatments were replicated 5 times to have more precise results. Test for hypotheses were the Fischer's test (F-test). Replicates were the petri dishes. The total number is 5 Factor A treatments x 5 Factor B (bacteria) x 5 replications.

Two — way ANO VA was used to test the hypotheses at 0.01 level of significance.

VI. RESULTS AND FINDINGS

Phytochemical Analysis

Results on the phytochemical analysis of the samakleaves (MacarangaTanarius) as presented:

Table 1. Results of the phytochemical analysis of Samak Leaves (MacarangaTanarius).

Note: (+) Traces, (++) moderate, (+++) abundant
(-) absence of constituents
Reference: Pharmacognosy, 15th edition, 2002, Trease& Evans

It can be gleaned from the table that Tannins on the theSamak leaves ethanolic extract and samak leaves water extractsamples 1, indicated (+++), it means that the two samples have abundant tannins.

Sterols, triterpenes, saponins, samples as shown indicated (++), it means that three samples contain moderate sterols, triterpenes, and saponins. But the samak leaves in water extract contain abundant saponins.

Glycosides and flavonoids, both samples contain glycosides and flavonoids. But in the samak leaves in water extract has no flavonoids.

Alkaloids, the two samples have no alkaloids.

VII. CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. TheSamak leaves ethanolic extract and samak leaves water extract contain abundant tannins. Therefore the samak leaves is effective in fermentation of wine and vinegar.
2. Both samples contain moderate. But the samak leaves in water extract contain abundant saponins.
3. Glycosides and flavonoids, both samples contain glycosides and flavonoids. But in the samak leaves in water extract has no flavonoids.

The two samples have no alkaloids.

VIII. RECOMMENDATIONS

1. A follow up study should be conducted to quantify, isolate, and identify the type of alkaloids, glycosides, flavonoids, and tannins present in the leaves of samak.
2. Other pharmacological testing should be done using the samak leaves like tests for its analgesic and anti- hypertensive properties.

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