Phytochemical Screening of Samak *(Macaranga tanarius)* and its Fermenting and Coloring Properties

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Abstract

This study determined the fermenting and coloring properties of "samak" (Macgranga tanariusj using leaves and barks in the production of home-made vinegar. The color, taste and aroma of the produce were compared with the commercial vinegar "Datu Puti".

In this study, phytochemical screening was limited to the leaves. In vinegar making, the barks and leaves were used separately.

This study made use of the experimental research design in an actual laboratory set-up. There were three phases: Phase I - air drying extraction process. Phase II- phytochemical screening using Mayer's Test for alkaloids. Fehling's test for glycosides, Gelatin Test for tannins, Froth Test for saponins, Color Test for flavonoids, and Lieberman-Burchard Tests for sterols; and Phase III - " samak" vinegar making as such.

The "samak" leaves are good catalyst and coloring agents for vinegar because they contain sterols, flavonoids, saponins, glyeosides, and tannins.

The bark produced the most desirable aroma in. contrast to the commercial "Datu Puti" which was categorized most sour.

A follow up study must be conducted to quantify, isolate and identify the type of alkaloids, glycosides, tannins, saponins, sterols and flavonoid content of the leaves. Phytochemical screening should likewise be done in all the other parts of the "samak" plant.

Further studies should be done to determine the differences between and among the three vinegar solutions using leaves, bark and combined leaf and hark of "samak".

Introduction

Background of the Study

The Philippines has a remarkably rich flora. Approximately 8,000 different species are known in the archipelago, and continued exploration and study will. within a few years, doubtless increase the list to more than 10,000.

New drugs originate from different sources. Accidental observations on natural products, unexpected clinical findings on known compounds, basic physiological or biochemical investigations or even test tuhe experiments have provided leads to great therapeutic discoveries.

Today, most of the new drugs are known by systematic screening methods. The processes are so designed to distinguish useful drug materials from the non-useful ones as rapidly, comprehensively and inexpensively as possible (Banez 1993).

"Samak" *(Macaranga tanarius)* is found in thickets and second-growth forests at low and medium altitudes throughout the Philippines. It has a relatively wide span of distribution – from Andaman Islands and the Malay Peninsula to Southern China, and, from Formosa southward to northeastern Australia.

It is a small dioecious tree reaching a height of 4 to 8 m. The leaves are palpate, ovate to oblong-ovate, and 10 to 25 cm long, with entire or toothed margins, rounded base 3nd pointed apex. The male flowers are small and bome on slender, branched peduncles which are shorter than the leaves. The female flowers are usually found in simple panicle spikes or racemes. The capsules are 10 to 12 mm in diameter, of 2 or 3 cocci, and covered with pale, waxy glands. and with sofl. scattered, elongated, spine like processes (Quisumbing, 1978: 519).

Objectives

This study on *Macaranga tanarius* specifically attempted to:

1. Identify the chemical substances present in the leaves:
2. Produce homenade vinegar out of the leaves and barks.
3. Differentiate the color derived vinegar with that of a commercial vinegar "Datu Puti".

4. Compare the taste and aroma of the derived vinegar with that of "Datu Puti", commercial vinegar.

Scope and Delimitation

This study was limited to the phytochemical screening of the leaf using ethyl alcohol as solvent.

The identification of the chemical substances was limited to qualitative analysis. No attempt was done to determine the amount of specific chemical constituents.

In the vinegar making, only the leaves and barks were subjected to study, the stems, roots and fruits were excluded.

The air drying and extraction processes using waterbath apparatus were done at UNP CTE Laboratory from June to July 2004. The phytochemical screening was conducted at the Industrial Technology Development Institute (ITDI), Department of Science and Technology (DOST), Bicutan. Taguig, Metro Manila from August to December 2004. The vinegar making was done in Manangat, Caoayan. Ilocos Sur.

Review of Related Literature

Macaranga tanarius belongs to the family Euphorbiaceae. It is synonymous to *Macaranga molluscala* (Kung.), *Macaranga tomentosa (Blue)* and *Mappa tanarius (L.) Blume*. In the Philippines, the tree is known as "samak" in Ilocos and "binunga" in the Tagalog regions. Other countries call it "tanpu", "loklao", "hanuva", "mara", "tata ancur", "mahong puteh", "kundoh", "hamingang", "hairy mahang" and "hu chang lek." *Macaranga tanarius* is made up of 250 to 280 species from tropical Africa, Madagascar and Pacific. It is a dioecious tree from 4 to 10 m tall. Leaves are peltate, ovate to oblong ovate ranging from 8 to 30 cm long with petiole of 6 to 25 cm (Starr, Starr and Loope, 2003).

Macaranga fanarius wood has density range from a low 300 to a high 590 kg/m³ at 15% moisture content (Soewarsono, 1990).

The tree can be used as firewood, dyes, shades, timber and most especially in fermented drinks from its leaves and barks.

Macaranga tanarius is an evergreen tree and a dioecious plant with no petal. Buds are in light green color. It flourishes only under sufficient sunshine

and usually grows in open and damaged lands. *Macaranga* is often seen growing naturally on the ridge of fields and fanns. When its stem is broken off, the cleft will oxidize and become red in color. A white latex will run slowly out of the cleft. It is not noticeable at first, but after a day the cut will be wrapped by a blood-like succus. This is the origin of the name 'sietong'.

A decoction of the bark is applied against dysentery and a decoction of the root against fever and haemoptysis. Powdered leaves are used as poultices for healing wounds. Such are practiced in India, Myanmar, Malaysia and the Philippines.

Definition of Terms

The following terms were defined for a better understanding of the study,

Alkaloids. Group of mildly alkaline compounds, mostly of plant origin and of moderate molecular complexity, Even in very small amounts, the alkaloids produce strong physiological effects on the body. All contain nitrogen atoms that are structurally related to those of ammonia. Some alkaloids are used in medicine while others are highly poisonous.

Extraction. The process of obtaining the plant constituents by using ethyl alcohol as solvent and by refluxing the system with a sensitive apparatus, the "Rotavap".

Fehling's Test. The test for the presence or absence of glycosides in the ethanol extract, using anhydrous sodium carbonate and Fehling's solution. An increase in the amount of brick red precipitate in the hydrolyzed sample indicates the presence of glycosides.

Fermenting Property. The catalytic attribute of "samak" *Macaranga tanarius* in the local production of "samak" vinegar.

Flavonoids. Derivatives of glycosides usually occurring in plants where one or more of phenolic hydroxyl groups are combined with sugar residue (Capal, 1992:6). Its presence in a sample is tested by the formation of a red color with acidified magnesium.

Gelatin Test. The test which is used for the determination of the presence or absence of tannins in the ethanol extract using gelatin solution. The formation of a very heavy precipitate suggests the presence of tannins.

Glyosides. Compounds that are soluble in water and are obtained from lants by water extraction. They are mostly colorless crystalline solids with a bitter taste. Simple glycosides have been synthesized in the laboratory, and several hundred glycosides have been extracted from plants and used for many purposes. Among the important glycosides are *indican* used for dyeing, *digitalin* used in medicine; and, *saponins,* foaming agents used in industry and medicine.

Liebermann-Buchard Test. The test for the presence or absence of triterpenes and sterols in the ethanol extract using acetic anhydride and sulfuric acid as reagents. A pink to red color is indicative of triterpenes, while a pink to blue color is indicative of sterols.

Mayer's Test. The test used to determine the presence or absence of alkaloids in the ethanol extract using mercuric chloride and potassium iodide in distilled water as reagents. The formation of precipitate upon the addition of Mayer's reagent in ethanol extract is suggestive of the presence of alkaloids.

Pbytocbemical. The chemical constituents or substance found in plants i.e. in the leaves of *Macaranga tanarius* considered in this study: alkaloids, glycosides. tannins, flavonoids, triterpenes and saponins.

Saponins. Group of naturally occurring glycosides that foam freely when shaken with water. They occur in a wide variety of plants. ' Saponins have been, and sometimes are still, used as cleaning agents and as foam producers, notably in fire-extinguishing fluids. They have a bitter taste and when ingested orally are non-poisonous to warm-blooded animals. When injected directly into the bloodstream, however, they are dangerous as they quickly dissolve red blood cells. Hydrolysis of a saponin, brought about by acids or by enzymes, gives a sugar and a sapogenin, the latter being either a triterpenc or a steroid. Some sugars and saponins are raw materials for synthesis of steroid hormones.

"S2mak" yinegar. Locally produced acetic acid made of *Macaranga tanarius* leaves and stems used as sour tasting condiment and preservatives.

Sterols. Large group of naturally occurring and synthetic lipids, or fat-soluble chemicals, with a great diversity of physiological activity. Included among the steroids are certain alcohols (sterols), bile acids, many important hormones, some natural drugs, and the poisons found in the skin of some toads. Various sterols found in the skin of human beings are transformed into vitamin D when they are exposed *to* the ultraviolet rays of the sun.

Tannins. Also called tannins acid with the chemical formula $CHO_{,4}$. Exposure to light deepens the color. They are used as mordant for cloth, as sizes for paper on silk and as coagulants for rubber.

Triterpenes. Substance found in essential oils of many plants and have carbon skeletons made up of isoprene units joined in a regular, head *to* tail way. These compounds can be sources of Vitamin A. In the Liebermann-Burchard Test, a pink to red color was indicative of the presence of triterpenes.

Methodology

The experimental research design in actual laboratory set-up was utilized in the study. Three phases were included: Phase I - gathering of fresh leaves of *"Macaranga tanarius"*, air drying and extraction; Phase II - the phytochemical screening *to* determine the presence of alkaloid, glycoside, tannins, saponins. flavonoids, triterpenes and sterols in the stems; and, Phase III - vinegar-making using "samak" leaves and barks.

Phase I - **Preparation of extract.** Fresh leaves of "samak" were gathered in Manangat, Caoayan, Ilocos Sur. They were washed thoroughly and air dried for a week then cut into small pieces. Five hundred grams of the finely cut materials were placed in an Erlenmeyer flask and were weighed in a balance. The material was stoppered and soaked for 24 hours, then filtered through a glass funnel.

The flask and the plant material were rinsed with 95% ethyl alcohol. Extraction was done in Rotavap Apparatus. The filtrates were concentrated under vacuo to about 50 ml. The exact volumes of the concentrated extracts were measured. Then, the extracts in tightly stoppered containers were stored inside a refrigerator ready for the phytochemical screening and pharmacological testing.

Phase II - **Phytochemical screening.** Phytochcmical screening determined the presence of alkaloids, glycosides, tannins, saponins, flavonoids, triterpenes and sterols in the leaves of "samak" *(Macaranga tanarius)*. These methods and procedures were based on the methods available at the Chemistry and Pharmacological Division, Department of Science and Technology, Bicutan, Taguig, Metro Manila.

I. Alkaloidal test using the leaves. Ten (10) ml of the leaf extract was evaporated to a syrup consistency on an evaporating dish over **a** water bath. To the concentrated extract, 5 ml of hydrochloric acid solution was added while heating.. The solution was stirred for about five minutcs and then cooled to room temperature. To this was added about 0.5 gm of NaCl powder. It was stirred and enough fresh hydrochloric acid solution was added to wash and bring the filtrate to a final volume of 3 ml.

A few drops of Mayer's reagent were added to one milliliters of liquid. The formation of precipitate upon the addition of the Mayer's reagent was suggestive of the presence of alkaloids. Precipitation that was fonned upon the addition of Mayer's reagent indicated the presence of alkaloids.

2. Test for glycosides (Fehling's Test). Ten (10) milliliters of leaf extract was dissolved in hot water and filtered. The filtrate was used for the test. Two (2) ml each sample was placed in two test tubes. To sample 1, 1 ml diluted HCL was added. To sample 2, nothing was added. Then the two test tubes were placed in a boiling water bath for 5 minutes. Then the test tubes were cooled. Both were neutralized with anhydrous sodium carbonate until no more effervescence was produced. Fehling's solution was added to the two test tubes which were heated over a water bath for two minutes. An increase in the amount of brick red precipitate in the hydrolyzed sample as compared to the other sample indicated the presence of glycosides. The same procedure was done using the stem extract.

3. Test for tannins (Gelatin Test). Ten milliliters of the ethanol extract of the leaves was evaporated to dryness over a water bath and then cooled. The residue was extracted with twenty milliliters of hot distilled water, cooled, then to it, five drops of 10 % sodium chloride solution was added to salt out undesirable constituents and then the residue was filtered.

The filtrate was divided into two test tubes A and B. Test tube A was kept as the control. To test tube B, 3 drops of I% gelatin solution was added. The same procedure was done for the stem extracts. The formation of precipitation suggested the presence of tannins.

4. Test for saponins l Froth Test). Ten (I 0) ml of the leaf extract was dissolved in hot water. The aqueous extract was shaken vigorously for about 30 seconds and was allowed to stand and was observed over a period of 30 minutes. The formation of honeycomb froths at a height of 3 cm indicated positive results.

5. Test for flavonoids (Color Test). Two (2) milliliters of the leaf extract was treated with 2 ml 10% hydrochloric acid and magnesium turnings. Formation of red color indicated a positive result.

6. Test for triterpenes and sterols (Libermann-Burchard Test). Two (2) milliliter of leaf extract was dissolved in acetic anhydride. The soluble portion was decanted and to this, 1-2 drops of concentrated sulfuric acid were added. A pink to red color was indicative of triterpenes, while a pink to blue color was indicative of sterols.

Phase III - **"Samak" vinegar making**. The leaves and barks were tested separately for the production of vinegar. Vinegar was produced using the following

procedure. a) thoroughly wash the involved plants parts – leaves and barks separately to be free from dirt; b) cut into small pieces; c) measure 4 cups of small pieces of the materials and place in a glass jar; vinegar should be placed in a glass jar; a glass container allows visual monitoring of the process but does not impart any flavor to the vinegar; d) add 4 cups of water and 1 cup of brown sugar; e) cover it with white cloth; f) stir everyday for almost a month; g) after a month squeeze the plant parts drain with cloth; and, h) boil for an hour.

Members of this group of biochemicals are cholesterol, dehydrocholesterol and ergosterol. In the blood, it plays an important role in transporting fatty acids in the form of cholesterol esters. Ergosterol is responsible for the low incidence of rickets because of the supply of vitamin D, thus the plant could be a source of vitamin D.

Results and Discussion

Phytochemical Tests

The chemical components present in "samal" leaves are presented in Table 1.

Sterols are moderately present in the "samak" leaves. The presence was indicated by the formation of a blue color with the Liebermann-Burchard reagent. Sterols are derivatives of the steroid nucleus.

The Liebermann-Burchard test for triterpenes gave a negative result on the leaves. This means that the plant is not a good source of vitamin A. Traces by flavonoids were observed in the leaves as evidenced by the formation of red precipitates in the color test. This implies that the plant has antiviral, antifungal. anti-inflammatory and cytotoxic properties (Capal, 1992).

Table 1. Qualitative constituents of "samak" leaves.

COMPONENT	QUALITATIVE TESTS	RESULTS
Sterols	Liebermann Burchard Test	Moderate amounts
Triterpenes	Liebermann Burchard Test	Negative
Flavonoids	Color Test	Traces
Alkaloids	Mayer's Test	Negative
Saponins	Froth Test	Traces
Glycosides	Fehling's Test	Moderate amounts
Tannins	Gelatin Test	Abundant

The Mayer's test for alkaloids gave a negative result in the plant. This implies that the plant has no anti-hypertensive, anti-neoplastic and does not demonstrate cncolytes and toxicity properties.

The froth test for saponins showed that the leaves of "samak" have traces of these components. This implies that the leaves are emulsifying agent and can be used as detergent. The Fehling's test for glycosides showed a positive result, moderate amounts were present and this shows that the plant could be used for dyeing. It is a good source of glucose and could be used in making wine and vinegar.

The gelatin test showed a positive result in the leaves. Abundant amounts of tannins were present in the leaves. This implies that the leaves could be used as mordant for dyeing as evidenced in the color produced in making the "samak" vinegar.

Table 2. Level of acceptability of "samak?" leaf and bark vinegar.

VARIABLE	DATU PUTI		SAMAK LEAF		SAMAK BAR	
	5	Level	5	Level	5	Level
Aroma	1	Desirable	2	More desirable	3	Most Desirable
Taste	3	Most sour	1.2	Sour	1.8	More Sour

Samak bark vinegar ranked first in aroma with a mean rating of 3 described as most desirable.

As regards the taste of the vinegar, "Datu Puti" ranked first and claimed by the ten evaluators as 'most sour' (5= 3) followed by "samak" bark vinegar more sour (5= 1.8) and "samak" leaf vinegar as 'sour'(= 1.2).

Evaluators claimed that the "samak" leaf and bark vinegars were the same in aroma and taste with that of "basi", an Ilocano wine made from processed sugarcane juice. "Samak" leaf vinegar ranked second with a mean rating of 2 described as more desirable in aroma than the commercial, "Datu Puti" which ranked third.

Color Test

The "samak" leaf vinegar gave-off a darker color (brown) than the "samak" bark. This was supported in the phytochemical test that "tannins" are abundant in the leaf as shown in the qualitative test, specifically "Gelatin Test". Therefore "samak" has a dyeing/coloring properties. The commercial "Datu Puti" vinegar is

whitish in color which implies that the organic substances used have no dyeing coloring properties.

Conclusion

The *samak (Macaranga tanarius)* leaves contain chemical constituents such as sterols, flavonoids, saponins. glycosides and tannins. It has antiviral. antifungal. anti-inflammatory and cytotoxic activities. It is a good emulsifying agent. It can be used as mordant for dyeing, coagulants for rubber and can be used in making wine and vinegar.

The Liebermann-Burchard test for triterpenes showed a negative result. Likewise, Mayer's test showed negative results in alkaloids.

Samak leaves and barks can be made into vinegar. This is economical and is possible for commercial purposes.

As to aroma, *samak* bark vinegar is the most desirable and "Datu Puti" vinegar is the most sour.

Samak leaves are better coloring agents than *samak* barks because of more tannin content of the former.

Recommendations

1. A Follow-up study shp.uld be conducted to perform the phytochemical screening of the other parts of the "samak" plant like stems, roots, fruits, and flowers. Types of alkaloids, glycosides, tannins, saponins, sterols and flavonoids present in all parts of the plant should he quantified, isolated and identified.

2. A follow-up study should be done to further determine , the difference among the three vinegar solutions: "samak" leaf. *samak* bark and mixed *samak* left and bark.

3. *Samak* is recommended for microbiological and other phannacological screening.

4. The plant is recommended for tanning leathers, coloring foods and other materials.

5. The *samak* vinegar can he a better alternative to commercial vinegar.

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