Extraction And Isolation Of The Alkaloids From The Samanea Saman (Acacia) Bark: Its Antiseptic Potential

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ABSTRACT: Antiseptics are being used to prevent the growth of pathogens which are major causes of diseases. Unfortunately, not everyone can use these antiseptics because of its price in the market. With this, the use of plants as sources of antiseptics gives rise to the continuous study of indigenous plants. Extraction and isolation of constituents such as alkaloid are commonly studied. It is believed that the isolated alkaloids can be formulated into drugs or antiseptics. This study aimed to investigate on the antibacterial potential of the alkaloidal extracts from the bark of *Samanea saman* which could be a raw material in the formulation of effective antiseptics that can be used to combat diseases, thus, this study was conducted. Specifically, the study sought answers to the following questions: 1) to determine the percentage yield of the crude acacia extract and alkaloid-rich fraction from the acacia bark, 2) to find out the number of alkaloids present in the acacia bark extract, 3) to test the antiseptic potential of the isolated alkaloids against the bacteria Escherichia coli, Staphylococcus aureus, and Bacillus cereus, and lastly, to determine the implications of this study to science and technology and to other areas of science. Based on the outcome of this research, similar studies should be conducted to the acacia bark using other solvent in extraction. The use of other solvent systems for the determination of the number of components of alkaloids should be encouraged. A follow-up study concerning the determination of functional groups and elucidation of isolated alkaloids are suggested. Conduct further study on the antibacterial potential of acacia tree against other bacteria, fungi and virus to extend its clinical uses. Organic chemistry classes may conduct experiments on the local production of indigenous plants that are possible sources of alkaloids. Community outreach program may involve the conservation and propagation of acacia tree to increase the source of alkaloids.

Key words: Alkaloids, Bacillus cereus, Eschirichia coli, Extraction, Isolation, Samanea saman, Staphylococcus aureus,

1 INTRODUCTION

The Philippines is endowed with many plants that could be possible sources to treat diseases. Millions of people believe the importance of these plants as herbal remedies to their diseases. Scientific study should make these remedies far safer and more effective in the future by determining the active constituents present in the plants. Global recognition of nature's green pharmacy should inspire individuals and nations to protect this extraordinary resource. Antiseptics are physical and chemical agents that prevent putrefaction, infection, and analogous changes in the living tissue by destroying the development of microorganisms *Samanea saman* (rain tree) barks are one of these plants. It is classified in the legume family (Leguminosae).

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The bark of the mature trees of acacia is gray, rough and fissured in long plates or corky ridges. On younger trees the bark is smoother and paler gray to brownish in color. The inner bark is light colored and bitter. Acacia has long been a source of timber and livestock feed for local consumption. The wood is used for carving items for sale to tourists and the seeds are strung in garlands. The boiled bark is applied as poultice to cure constipation. In the Philippines, a decoction of the inner bark and fresh leaves are used for diarrhea (Philippines Medicinal Plants, 2013) [1]. The extract from its leaves exhibit antiseptic property to gram-positive organisms such as Staphylococcus Aureus, Bacillus Subtilis and Sarcina Lutea and one gram-negative Escherichia Coli (Gonzales & Paombong, 1990) [2]. Liquid extract prepared from the bark can be administered for its astringent properties in doses of ½ to 1 fluid, but the use of both gum and bark for industrial purposes is much larger than their use in medicine. Thorough phytochemical study has to be done especially on the isolation and identification of these compounds responsible for its antibacterial activity. With these properties of acacia bark extract which is intended to be used as an antiseptic will entail low cost compared to the commercially available antiseptics sold in the market thus, it will be economically useful. In order to keep abreast with the new trends in science and technology, particularly in experiential learning in Chemistry, it is in this view of mind that the researchers were motivated to work on it and find out the structure of the alkaloids present in the bark extract. As science teachers in the General Education Department, the researchers strongly believe that the establishment of a scientific basis through chemical analysis of the commonly prescribed medicinal plants by the herbalists will greatly promote and enhance the wider acceptance of medicinal plants. The science teachers will be aware of the antiseptic potential of the extract from the bark of acacia and encourage its use in the attainment of healthful living in the community and in the society where the individuals live.

2 RESEARCH METHODOLOGY

2.1 Collection and Preparation of Samples

The barks of acacia were randomly collected from Alangilan, Batangas City on May 15, 2008. The barks were air dried and crushed into powdered form using Wiley mill, and was kept in a dry, clean container ready for the investigation and extraction of the active constituents.

2.2 Extraction of Alkaloids

The extraction of the alkaloid was done using the continuous extraction method using the Soxhlet apparatus. Four hundred grams (400 g) of ground acacia bark (Figure 1) was weighed and packed in a cheesecloth bag which served as an extraction thimble. The thimble was then placed into a suitable jar with cover. The sample was moistened with sufficient amount of 95% ethanol. This was made alkaline with sufficient quantity of ammonia T.S. and mixed thoroughly. The sample in the thimble was macerated overnight (Figure 2), and then placed in the Soxhlet extractor on the next day. Sufficient amount of 95% ethanol was placed in the solvent flask (4.8 liters). The sample was extracted for about 3 – 4 hours. The ethanol extract was filtered and was concentrated in a Soxhlet distilling apparatus at 60^{o}C (Figure 3). The crude alkaloid extract was further treated with 1.0 N hydrochloric acid. This was filtered and the filtrate was collected. The filtrate was alkalinified with ammonia T.S. and placed in a separatory funnel. Measured quantities of chloroform was added into the separatory funnel, mixed and shaken for about five times and allowed to separate into two layers. The lower layer of chloroform contained the alkaloids and the upper layer the aqueous portion. The upper layer was extracted until the last chloroform extract was found negative to Dragendorff's reagent. The combined chloroform extract was concentrated in Soxhlet distilling apparatus at 60 ^{o}C and evaporated in water bath maintained at that temperature until semi-dry. The residue was weighed and percentage yield was calculated using the formula:

$$\% \text{ yield} = \frac{\text{weight of the alkaloidal residue} \times 100}{\text{weight of ground acacia barks}}$$

Figure 1
Ground *Samanea saman* (Acacia) Bark

Figure 2
Maceration Set-up of Ground Acacia Bark

Figure 3
Extraction Using Soxhlet Apparatus

2.3 Isolation and Partial Purification of Alkaloid

Silica gel 60F254 precoated in TLC plate was used as a stationary phase with toluene: acetone: ethanol: ammonia (40:40:6:2) as the mobile phase. The thin-layer chromatography (TLC) chamber (9" x 4 ½") was lined with filter paper. The solvent system used was prepared in a separate flask and sufficient amount was poured into the TLC chamber. The residue (alkaloid rich-extract) was dissolved in chloroform and spotted to about 1 ½ cm apart on a silica gel G coated glass plate with the use of capillary tubing until the spot point

was visibly clear. A 10 centimeter mark above the spot was placed. The plate was now introduced into the TLC developing chamber. The solvent was allowed to reach the lower edge of the adsorbent, but the spot points were not allowed to be immersed. The cover was placed and the system was maintained until the solvent ascended to the point 10 cm above the initial spots. The TLC plate was then viewed under the long wave UV light (366 nm). The distance of each spot from the point of origin was measured and then recorded. The spots were sprayed with Dragendorff's reagent and then observed. Orange spots indicated the presence of alkaloids. The Rf values of the sample were computed as follows:

$$Rf = \frac{\text{distance traveled by the solute}}{\text{distance traveled by the solvent}}$$

Thin-layer chromatography can now be repeated and the spots identified as alkaloid can now be isolated from the TLC plate. Alkaloid containing spots can be scraped and dissolved in chloroform for isolation of the semi-pure alkaloid. Then, this was filtered to remove silica gel and the filtrate was placed in water bath for evaporation of the solvent.

2.4 Identification Test for the Presence of Alkaloids

Crude ethanol extract of acacia bark (Figure 4) was tested for the presence of alkaloid using Dragendorff's and Mayer's reagents, respectively. A portion of the extract was allowed to evaporate to a syrupy consistency over a steam bath. Five milliliters of 2N HCl was added and heated with stirring in water bath for about 5 minutes and cooled. About 0.5 g of NaCl was added to prevent a false positive result. This was stirred, filtered and the residue was washed with enough 2N HCl to bring the filtrate to a volume of 5 mL. In one test tube, 1 mL of the filtrate was treated with 2-3 drops of Mayer's reagent. In another test tube, 1 mL of the filtrate was treated with 2-3 drops of Dragendor'ffs reagent and in the third test tube 1 ml of filtrate was used as a control.

Figure 4
Extracts from Ground *Samanea saman* (Acacia) Bark

2.5 Antiseptic Test

The test bacteria were subcultured in nutrient broth and incubated at 37°C overnight. One loopful (10µL) of organism was added to 5 mL nutrient broth and mixed vigorously. One hundred µL was added to each plate, overlaid with soft nutrient agar, swirled to mix and allowed to solidify. The filter paper discs were immersed into extract with finely pointed forceps for saturation. The excess liquid was drained off by touching it to the sides of container. The forceps used was flamed, washed, reflamed and cooled between dilutions for asepsis. The three separate discs were dipped into the test samples and placed in proper distances upon the surface of the agar to allow the development of inhibition zones. A fourth disc was dipped in the control and placed upon the agar; arranged at equal distances with disc containing the test sample liquid or extract. All the test plates were incubated upside down at the temperature of 37°C for 24 – 48 hours. After the incubation, the presence or absence of circular zones of inhibitions were examined depending on the activity of the sample being tested. Within limits the diameter zones of inhibition which may not be knife-edged were noted. Clear and well-defined zones of inhibitory zones around the disc were observed if the sample tests possessed antibacterial activity while failure of the test disc to exhibit zones of inhibition indicate the absence of antibacterial effects. The diameter zone of inhibition produced by each test sample was measured in terms of a celluloid ruler through direct light or with the aid of magnifier (Figures 5, 6 and 7).

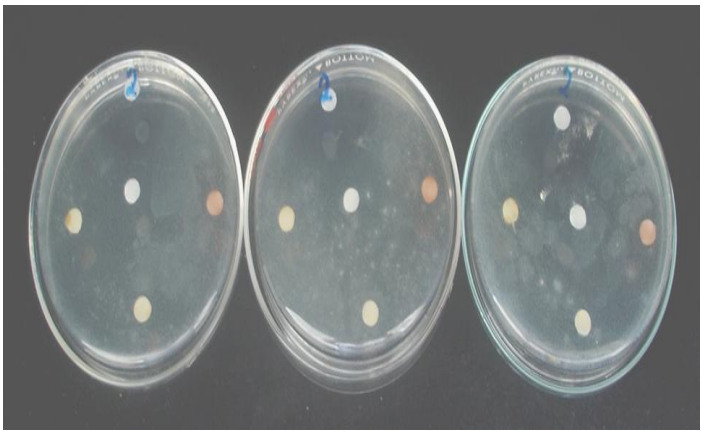

Figure 5
Antiseptic Test of Isolated Alkaloids from Acacia Bark with Escherichia Coli

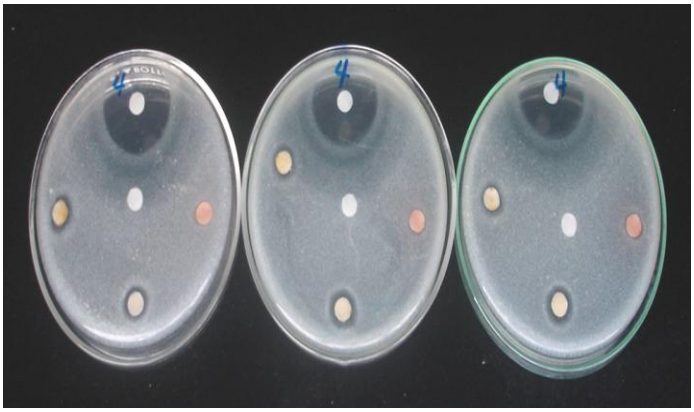

Figure 6
Antiseptic Test of Isolated Alkaloids from Acacia Bark with Staphylococcus aureus

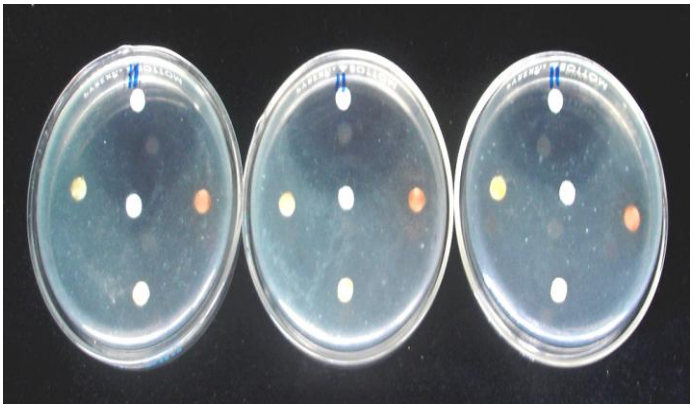

Figure 7
Antiseptic Test of Isolated Alkaloids from Acacia Bark with Bacillus cereus

3 RESULTS AND DISCUSSION

3.1 Determination of the Percentage Yield of Crude Extract and Alkaloid-Rich Fraction from the Ground Acacia Bark

The weight and percentage yield of the crude extract and alkaloid-rich fraction from the ground acacia bark are discussed in this study. These are shown in the succeeding tables.

3.1.1 Percentage Yield of Crude Extract from Ground Acacia Bark

Table 1.1 presents the percentage yield of crude extract from ground acacia bark.

Table 1.1: Percentage Yield of Crude Extract from Ground Acacia Bark

Trial	Weight of Ground Acacia Bark (grams)	Weight of Crude Acacia Extract (grams)	Percentage Yield (%)
1	400	143.60	35.90
2	400	143.56	35.89
Average	**400**	**143.60**	**35.90**

It can be gleaned from the table that in Trial 1, 400 grams of ground acacia bark yielded a crude extract of 143.60 grams and had a percentage yield of 35.9 percent. In Trial 2, out of 400 grams of ground acacia bark gave a crude extract of 143.56 grams and had a percentage of 35.89 percent. The average weight of ground acacia bark used was 400 grams with an average weight of crude extract of 143.60 grams and an average percentage yield of 35.90 percent. This can be deduced from the data that less than 50 percent can be yielded from the first crude acacia extract.

3.1.2 Percentage Yield of Alkaloid-Rich Fraction from Ground Acacia Bark

The percentage yield of the alkaloid-rich fraction from ground acacia bark is reflected in Table 1.2

Table 1.2: Percentage Yield of Alkaloid-Rich Fraction from Ground Acacia Bark

Trial	Weight of Ground Acacia Bark (grams)	Weight of Alkaloid-Rich Fraction (grams)	Percentage Yield (%)
1	400	2.85	0.71
2	400	2.84	0.71
Average	**400**	**2.85**	**0.71**

Table 1.2 shows that 400 grams of ground acacia bark in Trial 1 gave 2.85 grams of alkaloid-rich fraction of acacia extract and had a percentage yield of 0.71 percent. On the other hand, Trial 2 reveals that in 400 grams of the sample produced 2.84 grams of alkaloid-rich fraction and a percentage yield of 0.71 percent. Its average was 400 grams of ground acacia bark yielded 2.85 grams of alkaloid-rich fraction and percentage yield of 0.71 percent. The percentage yield acquired in this study is comparatively higher than the 0.4 to 0.5 percent alkaloid obtained from the dried bark of Acacia *phlebophylla* (Roux & Tyndale 1996) [3]. It is much higher compared to the studies made by Smoke (1994) [4] wherein only 0.2 percent of the alkaloids were produced in the flowers of Acacia *longifolia.* On the other hand, Dimaandal (2003) [5] who used *Samanea saman* leaves gave 0.25 percent alkaloids. Moreover, in the study conducted by Delima (1993) [6] two different species of acacia were used namely *Samanea saman (Jacq.)* and Acacia *concinna Willd. D.C.* yielded 2.55 percent and 0.3 percent were extracted respectively. The data signifies that the specie *Samanea saman* in general has the highest percentage yield. Dimaandal (2003) cited that the lowest yield by weight of any medicinally useful alkaloid ever produced on a commercial basis is 0.003 percent which was extracted from the *Catharanthus roseus*. Hence, the 0.71 percent yield on the bark of *Samanea saman* in this study is more enough that can be used commercially.

3.2 Determination of Alkaloid Components from Ethanol Extract from Acacia Bark

The isolation and partial purification of alkaloid-rich fraction of acacia bark were done using Thin-Layer Chromatography. The mobile phase used was toluene:acetone:ethanol:ammonia (40:40:6:2). Table 2 reveals the Rf values of the separated alkaloids from ethanol extract from acacia bark.

Table 2: Rf Values of Alkaloid-Rich Fraction from Ethanol Extract from Acacia Bark

Component	Rf Value	Observation
1	0.178	Produced orange-brown spot with Dragendorff"s reagent; fluoresced blue light at U.V. 366 nm
2	0.840	Produced orange-brown spot with Dragendorff"s reagent; fluoresced blue light at U.V. 366 nm

As shown in the table, Thin-layer Chromatography (TLC) on precoated silica gel 60F254 plate with toluene:acetone:ethanol:ammonia (40:40:6:2) solvent system afforded two alkaloids. Alkaloid 1 has a smaller Rf value which is 0.178. It exhibited the lesser affinity toward the mobile phase. On the other hand, alkaloid 2 spot has Rf value of 0.840. It has a higher Rf value compared to the alkaloid 1. It shows greater affinity toward the eluent. Both alkaloids produced orange-brown spots when sprayed with Dragendorff's reagent. These spots fluoresced blue at U.V. light at 366 nm indicating the presence of alkaloids. Corollary to the studies of Dimaandal and Delima, this study also isolated alkaloids from Samanea saman. Using the same solvent system Dimaandal was able to isolate six alkaloids from the leaves. But, this study yielded only two alkaloids from the bark. In the study conducted by Delima, four alkaloids were produced using methanol:acetic acid:distilled water (8:1:1) as solvent system. The results showed that using *Samanea saman* there are more components of alkaloids that can be isolated from the leaves compared to its bark.

3.3 Antiseptic Potential of Alkaloids from Ethanol Extracts of Acacia Bark

The antiseptic potential of the two acacia extracts namely: crude extract and alkaloid-rich fraction were analyzed using the Disc agar diffusion method. Table 3 reflects the zone of inhibition exhibited by the acacia extracts against test organisms.

Table 3: Antiseptic Potential of Acacia Extracts

	Zone of Inhibition (diameter in mm)								
	E. Coli (002)			**S. areus (004)**			**B. cereus (011)**		
Sample	**Trial**	**Trial**	**Trial**						
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Gentamicin Sulfate	40	40	40	30	30	31	35	35	35
Crude acacia Extract	-	-	-	10	10	10	-	-	-
Alkaloid-rich fraction	-	-	-	11	11	11	-	-	-

The table shows that the three test organisms namely Escherichia coli, Staphylococcus aureus and Bacillus cereus were all susceptible to gentamicin sulfate with diameters of 40 mm, 30 mm and 35 mm, respectively. Gentamicin sulfate exhibits complete inhibition in the organism E. coli since the halo zone appears wider as compared to the other two organisms. In this test, gentamicin sulfate is used as a reference substance for such inhibition. Moreover, the two alkaloid extracts have negative activity against the organisms E. coli and B. cereus. No zone of inhibition was observed. The test organism S. aureus was susceptible to the two alkaloids extracts as shown in Figure 9. In the crude acacia extract the diameters in three trials are 10 mm while in alkaloid-rich fraction the diameters in the three trials are 11 mm. The susceptibility of the organisms to the alkaloids is directly related to the size of the zone of growth inhibition around the disc containing the extracts. The more susceptible the organisms to the alkaloid extracts the wider are the zone (diameter) of the growth inhibition. This finding attests the statement of Gonzales and Paombong (1990) that the extracts of acacia exhibited complete activity against gram-positive organisms (Staphylococcus aureus, Sarcina lutea and Bacillus subtilis) and partial activity against gram-negative organism (E. coli). This study is different in the sense that they used extract from leaves while the researchers in this study made use of the bark of acacia. The extracts from the bark of this study exhibits positive activity against the bacteria Staphylococcus aureus. S. aureus produces many toxins that contribute to the bacterium's pathogenity by increasing its ability to invade the body or damage tissue. It produces the toxin responsible for toxic shock syndrome, a severe infection characterized by high fever and vomiting sometimes even death. S. aureus also produces an enterotoxin that causes vomiting and nausea when ingested. It is one of the most common causes of food poisoning (Tortora G. J.,Funke, B.R.& Case, C.L. 2005) [7]. The result of the study implies that the extracts of acacia bark such as its crude extract and the alkaloid-rich fraction can be used as a bacterial agent.

3.4 Implications to Science and Technology

As a whole, the result of this study would yield a significant contribution to the Philippine plants with medicinal use. This would likewise help Philippine economy especially the local manufacturers of drugs from the indigenous plants. *Samanea saman* locally known as acacia bark would be an alternative organic medicine in which the Filipinos would depend on instead of buying expensive medicinal products from multinational drug companies in treating wounds and other diseases. In the field of medicine, alkaloids are part of every medicinal scientist's resources and play an important role in treating diverse diseases. They are also a vital part of the successful regimens that have led to major therapeutic triumphs in chemotherapy. In pharmaceutical sciences, they serve as raw materials in the formulation of new and effective drugs. This study would serve as a guide for the science teachers and students in their researches on the use of indigenous materials in their community. Likewise, this study would help the students in working on their scientific activities, thereby preparing and enabling them to participate in school, regional, national and even international science fairs. Moreover, the research procedures employed in this study would give an avenue to facilitate an appreciation and application of the topic in science. Lastly, this study would

serve as an input to consider the profitable use of materials under investigation. This further enhances promotion of science and technology that would arouse the interest of teachers, students and lay people on how the principles of science will be converted to technology.

4 CONCLUSION

The bark of *Samanea saman* contains a higher percentage yield of alkaloid as it exceeds the lowest yield of any medicinally useful alkaloid ever produced on a commercial basis. Two alkaloids were isolated from the ground bark of *Samanea saman* using toluene:acetone:ethanol:ammonia as solvent system. The crude acacia extract and alkaloid-rich fraction exhibit complete inhibition against Staphylococcus aureus. Bark of *Samanea saman* may be used as a source of alternative medicine.

REFERENCES:

[1]. Philippines Medicinal Plants. Medicinal Uses for Samanea Saman/National Tropical Botanical Garden, August, 2013. available at http://www.stuartxchange.com/Acacia.html.com

[2]. Gonzales, Ma. V. M. & Paombong, A. A. (1990). The antiseptic activity of the extract from Samanea saman (Acacia) leaves. Unpublished Master's Thesis. BSU, Batangas City.

[3]. Tyndale & Roux (1994-1996). Phytochemical Studies (1996) from: www.muga.yage

[4]. Smoke (1994). Acacia longifolia from: www.shamanshop.net/store/proddetail.cfm[2001].

[5]. Dimaandal, L. G. (2003). Antiseptic property of the alkaloid content from ethanol Extract of Samanea saman (Acacia) leaves. Unpublished Master'sThesis. BSU, Batangas City.

[6]. Delima, L.S. (1993). Separation and comparison of the physical and chemical characteristics of the total alkaloids of the leaves of Samanea saman (Jacq.) Merr. and Acacia Concinna Willd. D.C. (Family Leguminosae). Unpublished Master's Thesis. CEU, Manila.

[7]. Tortora, Gerald J. et.al. (2005). Microbiology an introduction (8th ed.). San Francisco: Pearson Education, Inc.