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Isolation, determination and functional group examination of the crude alkaloid content of *Basella rubra* (Alugbati)

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Abstract

The research was conducted to assess the presence of crude alkaloid in *Basella rubra* and to examine the possible functional group present on the isolated crude alkaloid. The isolation made use of the typical solvent extraction method. Infrared spectroscopy and simple gravimetry was used to examine the functional groups and to determine the crude alkaloid content, respectively. Three parts of the *B. rubra* were tested namely, the stem, leaves, and roots. Results showed that the stem of *B. rubra* has the most crudealkaloid content with 375 ppm as compared to the roots and the leaves with 298 ppm, and 248 ppm, respectively. Statistical analysis showed that only the crude alkaloid content of the leaves and stem differs significantly with each other. The IR spectra of all three parts show similar vibrational frequencies. These vibrational frequencies most likely correspond to the functional groups of pyridine and pyrrole ring, imines, oximes, endocyclic systems, carboxylic acid anhydride, ether, alkenes, aromatic rings, amine, amide, carboxylic acids, esters, nitrile, nitro and alkanes.

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Introduction

Alkaloids are organic products of natural and synthetic origin which are basic in nature and contain one or more than one nitrogen compound normally heterocyclic in nature. It possesses physiological action in human and animal body when use in small quantities. The very best example of these compounds are ergot alkaloids. It was known to cause gangrene in the limbs of those who ingested infected bread. Later then, its first medicinal property as a powerful oxytocic was discovered. More recently its derivatives have been used in the treatments of migraine (Madlom, 2002). Some other examples of alkaloids are tropane alkaloids, atropine, hyoscyamine, scopolamine, caffeine and cocaine that are widely use as blockers of the parasympathetic nervous system such as anodyne and antispasmodic (Misawa, 1994). As of now, the number of structurally different alkaloids has been estimated to be 6000. Most of them occur in plants, approximately 1% in animals, and not more than 0.5% in fungi bacteria. Coccinelline (from the European ladybird beetle *Coccinela ineseptempunctata*) is an example of an alkaloid taken from animals. The deep blue pyocyanine (from *Pseudomonas aeruginosa*) exemplifies the alkaloids taken from bacteria (Guggisberg, 2009).

Due to its remarkable effects in human to cure illness when exact dose is being administered, a variety of alkaloids have been used in pharmaceuticals. Thus, the increasing demands of alkaloids as medicinal ingredients in the pharmaceutical industry lead to the synthesis of many of its kind. In Sri Lanka *Basella rubra* is one of the basic herbal medicines given for insomnia and nervous breakdown. Mensah, Okoli, Obodo and Eifediyi (2008) did a study on the phytochemical, nutritional and medicinal properties of some leafy vegetables consumed by people of Nigeria. *Basella rubra* was included and it gives a positive result in the alkaloid test. The characteristic of *B. rubra* that confirms to be an alkaloid containing plant includes its ability to be a good emollient, a hypoglycemic agent, and an analgesic.

The mucilaginous liquid obtained from the leaves and tender stalks of the plant makes a popular remedy for habitual headaches.

This study primarily examined the extent of alkaloid present in Basellarubra or Alugbati, which is truly an abundant plant here in the Philippines. To the best of the researcher's knowledge, there have been no existing studies that devote most on the isolation, quantification and functional group examination of the crude alkaloid content in *Basella rubra*. Three different parts of *B. rubra* was tested because the presence of alkaloids in plants differ accordingly due to several affecting factors. For example, the distribution of energy enabling to biosynthesis the compound may not be evenly distributed in all parts of the plants.

Materials and methods

Sample Collection

Collection of samples was done by random selection of vines in the sampling site. Enough samples that could suffice a 20 gramsample per part once dried were collected and brought to the laboratory for analysis. The samples collected were sun dried for several days and were ground. The samples were then stored in a clean and dried bottle.

Alkaloid Screening

The dried sample was decocted with distilled water. It was filtered and about 5 mL was pipetted out and placed in an evaporating dish. It was concentrated and 5 mL of 2 M HCl was added. It was boiled for 5 minutes, cooled and about 2.5 g of NaCl was added. The solution was filtered, and the filtrate was added with 2 M HCl to obtain 5 mL solution. About 1 mL of the filtrate solution was tested for the presence of alkaloid by adding drops of Mayer's reagent on one part and Hager's reagent on another 1mL.A formation of yellow precipitate signify the presence of alkaloid in the sample.

Extraction and Isolation of Crude Alkaloids

Powdered part of the plant of about 20 grams was weighed and added with 15 mL of NH_3 solution (25%).

Then solvent extraction was performed with 300 mL of ethyl acetate for 72 hours. The extract was filtered and the solvent was evaporated in a rotary evaporator under reduced pressure at 40˚C. The residue was dissolved in water and acidified with H_2SO_4 to pH 3-4, and was extracted with diethyl ether to remove lipophilic, acidic and neutral material. After adjusting the aqueous solution to pH 9-10 with NH_3 solution (25%), it was extracted with chloroform. The extract was washed with distilled water, dried with anhydrous Na_2SO_4 powder, filtered and concentrated to dryness under reduced pressure to obtain crude alkaloids.

Quantitation

Direct weighing of the isolated alkaloid was done to determine the alkaloid content of *B. rubra* in parts per million (ppm).

IR Spectroscopy Analysis of Functional Groups

The isolated crude alkaloid was dissolved with chloroform. It was injected in the sample cell and run in a Fourier Transform Infrared Spectrophotometer. The functional groups present were examined in the printed spectrum.

Statistical Analysis

The study made use of the analysis of variance (ANOVA) to determine the significant differences on the crude alkaloid contents of the different parts of *Basella rubra*. For post hoc analysis, Tukey HSD test was used to evaluate which of the three parts differ significantly with each other.

Results

Alkaloid Screening

A qualitative test for the alkaloid content of the leaves, stem, and roots of *Basella rubra* was examined using two different methods, the Mayer's test and the Hager's test. Table 1 shows that all three specified parts of *B. rubra* contain alkaloid.

Table 1. Screening of Alkaloid of *B. rubra*.

Parts	Mayer's Test	Hager's Test
Leaves	+	+
Stem	+	+
Roots	+	+

+ = positive result.

The screening gives yellow precipitate for both tests upon the addition of the reagent which is an indication of a positive result.

Crude Alkaloid Content Determination The extent of the amount of alkaloid on each three parts was determined using an extraction technique of isolation and quantification. The results are summarized in table 2 and the statistical test by ANOVA and Tukey HSD test are presented in table 3 and 4, respectively.

Table 2. Crude Alkaloid Content of the Three Parts of *B. rubra*.

Parts	Crude Alkaloid Content, ppm
Leaves	248 (±32)
Stem	375 (±40)
Roots	298 (±18)

Functional Group Examination

The IR spectra of the three specified parts, the leaves, the stem, and the roots, are shown in Figure 1, 2, and 3, respectively.

Discussion

Alkaloid Screening

The data shows that the stem contains the highest amount of crude alkaloid followed by the roots and

the leaves. The leaves were the most varied result base on the standard deviation.

The analysis of variance implies that there is a significant difference on the crude alkaloid content among the three parts of *B. rubra*. It was found out on the Tukey test that the crude alkaloid content of the leaves and the stem differs significantly. A higher concentration of crude alkaloid content was found in the stem than the other two parts of *B. rubra* implies that the nitrogen absorbed by the *B. rubra* is utilized mainly at the stem.

Table 3. Analysis of Variance of the Crude Alkaloid Content of *B. rubra*.

Source of Variation	SS	DF	MS	F_{calc}	F_{crit}
Between groups	24, 304.70	2	12, 152.35	12.01	5.14
Within groups	6, 071.30	6	1, 011.88		
Total	30, 376.00	8			

This is consistent with the fact that the process of nitrogen reduction is extremely energy dependent. The energy dependency is best illustrated when one take into account that the triple bond energy in molecular nitrogen is 225 kcal/mol and the industrial production of ammonia requires temperatures of 500˚C and a pressure of 300 atm.

Even if the roots are susceptible for nutrient intake, it does not necessarily follow that it contains great amount of nitrogenous matter. This is because of the distance from the energy supplier which is the leaves. In case of the leaves being far from the nutrient intake and being the one supplying the energy needed is the least to contain nitrogenous matter.

Table 4. Tukey HSD Test of the Crude Alkaloid Content of *B. rubra*.

	μ_{leaves}	μ_{roots}	μ_{stem}	HSD
μ_{stem}	127	77		79.71
μ_{roots}	50			
μ_{leaves}				

Functional Group Examination

All absorption in the IR spectrum are considered to be relevant. The vibrational frequencies revealed the possible presence of some functional groups present in the crude alkaloid of the three parts of *B. rubra*. The absorption of 1647 cm^{-1} corresponds to pyridine and pyrrole ring stretch, imines and oximes.

The 1617 cm^{-1} band is in accord to C=C stretch in endocyclic systems. Frequency at 1676.47 cm^{-1} gives rise to the C=C alkenes in the aromatic rings, this confirmed by the band existence between 793.75 cm^{-1} and 965.44 cm^{-1} and some overtones at 1852.94 cm^{-1} and 1941.17 cm^{-1}.

The asymmetric stretch of anhydride and C–C stretch for ether appear at 1794.12 cm^{-1} and 1117.65 cm^{-1}, respectively. Amine C–N stretch is confirmed at absorption band between 1235 cm^{-1} and 1343.14 cm^{-1}. The presence of carboxylic acids and ester C=O stretch is observed at 1735.29 cm^{-1} and 1779.41 cm^{-1}.

Confirmation of the presence of carboxylic acid is reflected by the absorption of the acidic OH stretch band 1029.41 cm^{-1} and 1058.82 cm^{-1} corresponds to amide C–N stretch. Bands between 2264.70 cm^{-1} and 2274.50 cm^{-1} indicate a nitrile.

The symmetric nitro stretch and CH_3 bending at 1352.94 cm^{-1} and 1382.35 cm^{-1} confirms the existence of a nitro and alkane groups, respectively. The presence of the last five unidentified bands is considered for now as overlaps due to influences of the mixture of several alkaloids.

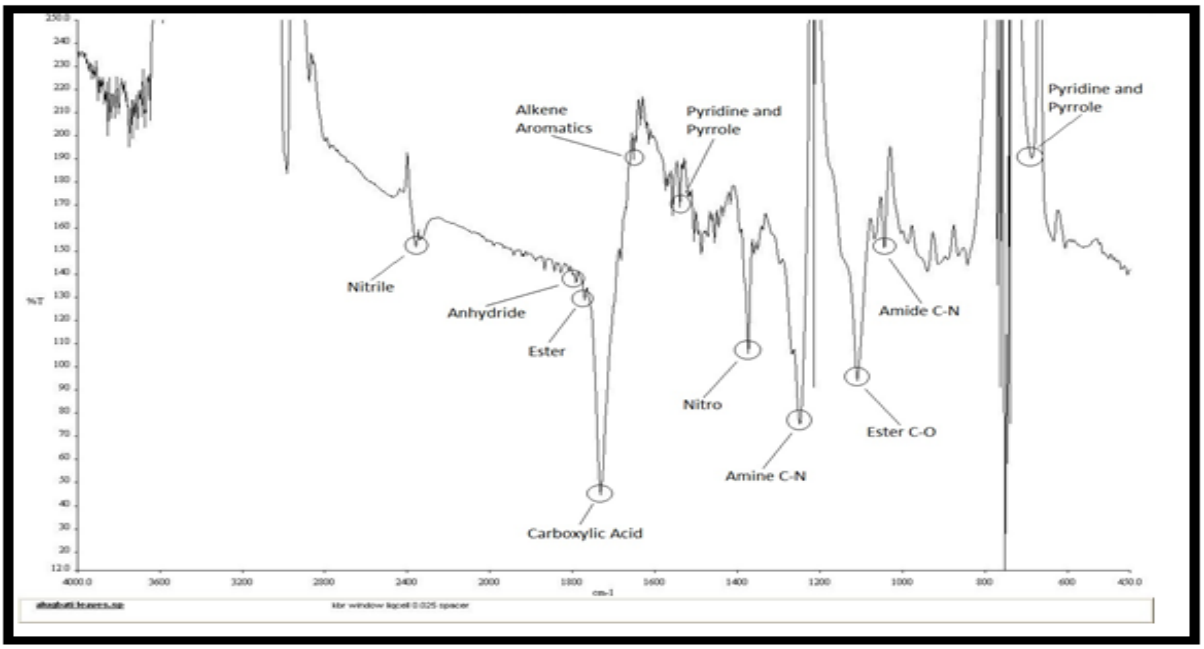

Fig. 1. IR spectrum of the crude alkaloid of the leaves of *B. rubra*.

Special cases absorption bands for pyridine and pyrrole rings are also shown on some IR spectra of several alkaloids. Caffeine for example shows peaks at 717 cm^{-1} and 904 cm^{-1} correspond to the out of plane bending of the C–H bond of mono substituted pyridinic cycle.

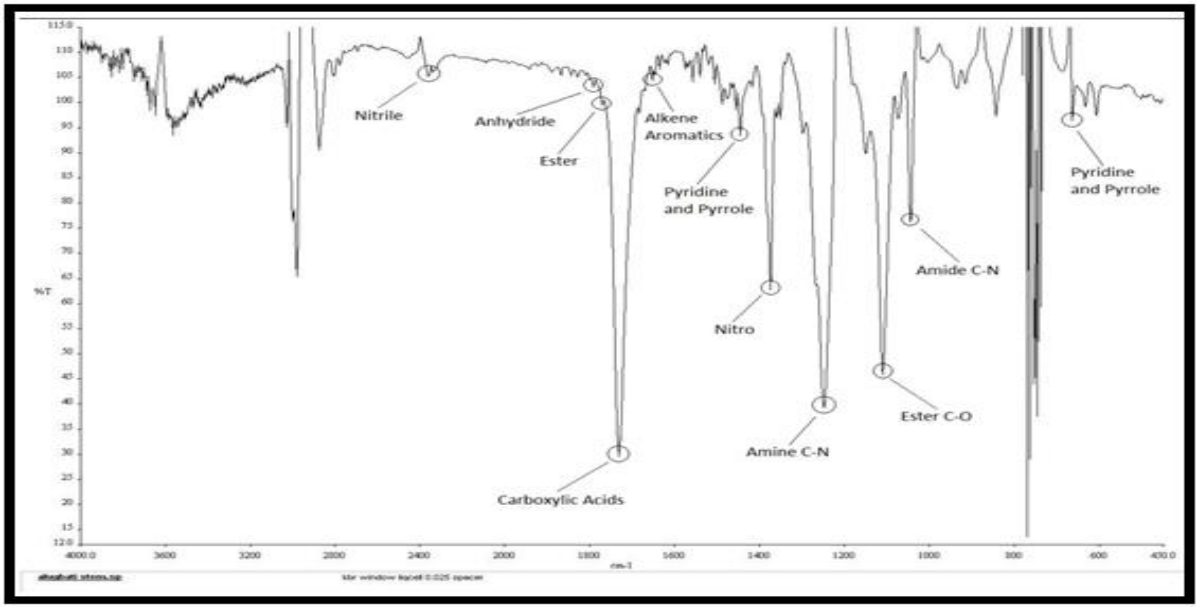

Fig. 2. IR spectrum of the crude alkaloid of the stem of *B. rubra*.

Also, a study on the crude alkaloid extract of *Ipomea fistulosa* conducted by Cholich, Bogado, Jorge, Acosta and Castro (2007) that is composed of four different alkaloids which is found to be Calistegina A_3, B_1 and B_2 and Swansonina reveals asymmetric stretching of the ring of heterocyclic, pyridine and pyrrole appears at 1634.91 cm^{-1} and within the range between 752.83-702.30 cm^{-1} appear the characteristic groups enabling to identify to the alkaloids and they are deformation of the ring of pyrrole, deformation of the pyridine ring tetra and trisubstituted and deformation out the plane of C–H and O–H groups.

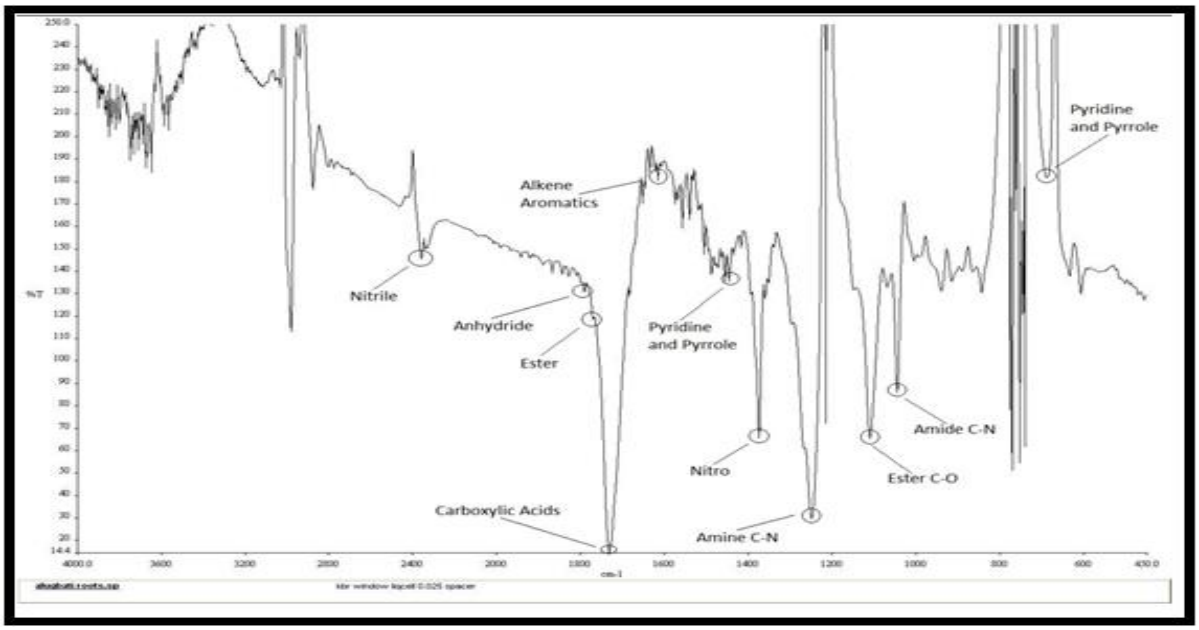

Fig. 3. IR spectrum of the crude alkaloid of the roots of *B. rubra*.

Recommendations

There should be a study on the crude alkaloid of *B. rubra*. Out of all parts of the plant only the fruits are not included in the scope of the study. In that way, there is a more discreet comparison on the presence of alkaloids in all the parts.

A study that identifies how many individual alkaloid compounds are present in each part will also be very valuable. Through this, clearer information on how many possible types of alkaloids will be established. A study on the separation of the mixture will then follow to isolate these alkaloids and elucidate their structures if it is a new compound.

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