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An evaluation of *Syzygium lineatum (DC.) Merr. & L.M. Perr*. Extracts as functional foods

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Abstract
Indigenous plants have been attracting attention from the food industry because of their unique appearance, flavor and nutritional value. *Syzygium lineatum (DC.) Merr. & L.M. Perry (S. lineatum*) is an indigenous plant and has recently been used for processed foods in the Province of Apayao and Lallo, Cagayan. In the Philippines, *S. lineatum* berry (malibado) is one of the fruits that it is native to Luzon originating from primary forests at low to medium elevation. *S. lineatum* berries are believed to contain antioxidants. It is also known as an excellent source of Vitamin C, a very good source of dietary fiber, and a good source of the essential dietary mineral and may contain phytonutrients that brings varieties of health benefits. *S. lineatum* is now rarely found in its natural habitat, a fact that drives the need to implement conservation measures. The best way to do this is to promote its usefulness as a highly nutritious food source which could be provided by data on its functionality as well as the phytochemicals or secondary metabolites it contains. With better appreciation, demand will come leading to its wider propagation in the country. The functionality of *S. lineatum* leaves and bark extract was evaluated on their biological activities. The results obtained indicated that the crude extract of *S. lineatum* contain substances with antioxidant property, cytotoxicity property using brine shrimps and substances with anthelmintic property against the worm eggs from the sheep and earthworms. Functionality of *S. lineatum* could be attributed to the presence of bioactive constituents that can provide health and wellness through its therapeutic and pharmacological benefits.

Keywords: S. lineatum, indigenous plant, phytochemicals, functional foods

1. Introduction

Plants naturally contain phytochemicals which possess health-protective or disease-preventing properties. Phytochemicals are bioactive compounds that naturally occur in plants and which are involved in health promotion and disease prevention. Edible plants providing health-promoting phytochemicals on top of conventional nutrition are called *functional foods*. Functional foods are foods or dietary components that may provide a health benefit beyond basic nutrition [12]. It may help to prevent some diseases, enhance health or reduce the risks of developing certain diseases. Malibado, (*Myrtaceae* Family) a Philippine berry is one of the fruits that can be found in the Philippines. This indigenous plant can be considered as source of essential nutrients for good nutrition and other chemical substances potential for disease prevention and treatment as they possess medicinal properties. The best way to do this is to promote its usefulness as a highly nutritious food source which could be provided by data on its functionality as well as the phytochemicals or secondary metabolites it contains. With better appreciation, demand will come leading to its wider propagation in the country. Thus, it would be of great interest to carry out a phytochemical analysis in order to evaluate their utilization as a resource of functional foods. What makes a food "*functional*" is its potential ability to positively affect one's health. Thus, the findings of this study would give baseline information on the most promising plant species that could be used as a basis of new tools of great therapeutic importance.

2. Materials and methods

2.1. Plant Materials

S. lineatum (malibado) plant were collected at Annafunan, Echague, Isabela. These were selected because of their availability of plant species in the town that could be tapped for the food supplementing and nutritional values. The identity of the plant was authenticated by a forester from the Community Environment and Natural Resources Office, San Isidro, Isabela, Philippines.

2.2. Extraction of Sample

The dried leaves and bark of *S. lineatum* were cut into small pieces and air dried at room temperature in a well-ventilated room. The dried leaves and bark were ground into a fine powder, placed in a conical flask and soaked with 95% Ethanol. The extraction was repeated three times and the combined extracts were then filtered through Whatmann No. 1 paper and evaporated in a vacuo using a rotary evaporator (Stuart RE 301).

2.3. Functionality Determination

2.3.1. Antioxidant Activity

The antioxidant activity of malibado leaves and bark were determined using the rapid thin layer chromatography screening assay. The concentrated extract was spotted on TLC strips (Merck: silica gel 60 F_{254} in aluminum sheets) and developed in a solvent system. For the leaf extract the solvent system is Ethyl acetate: Butanol (7:3) while for bark, the solvent system is Ethanol: Methanol (7:3). The

TLC strips were allowed to dry and were sprayed with potassium-ferricyanide-ferric chloride spray reagent. The appearance of blue spot in the TLC strips indicated the presence of antioxidant in the plant extract [10].

2.3.2. Anthelminthic Activity
The anthelminthic activity of *S. lineatum* leaves and bark extract were evaluated using the parasitic worm eggs in fecal sample of sheep (*Ovis aries*) and against earthworm (*Pheretima posthuma*) as the test organism.

2.3.2.1. Activity of the plant extracts against the worms eggs of sheep
Coprological (fecal) examination of the experimental animal (sheep) was done to determine the presence of parasitic worm eggs in fecal sample of sheep. Fecal samples were collected directly from the rectum of the sheep. Gastrointestinal parasitic worm eggs were counted using Modified Wisconsin Sugar Flotation Method.
Fifteen ml was filled with 10 ml Sheater's solution. 3.0g of feces was weighed and placed into a cup. Sheater's solution was poured from the test tube into the cup and was mixed well. The fecal-sugar solution was poured into a test tube with funnel and a strainer. Using tongue depressor, the liquid was squeezed out of the feces that is left in the strainer. The solution was centrifuged for 2 to 4 minutes and filled with Sheater's solution. The glass slide was put on top of the tube and was left to stand for 5 minutes. The glass slide was removed and examined for the presence of parasitic worm eggs. The number of parasitic worm eggs was recorded. The fecal solution was placed in a petri dish containing the malibado leaves and bark extract. Observations were made for the anthelmintic activity of the malibado plant extract in terms of the decrease in the number of parasitic worm eggs.

2.3.2.2. Activity of the plant extracts against earthworms
The anthelmintic assay was evaluated using earthworm (*Pheretima posthuma*) as test organism; owing to its resemblance in terms of anatomy and physiology with the intestinal roundworm parasite of human beings [3, 13, 17]. The worms were collected from moist garden soil and washed with saline solution then normal water to removed soil particles and fecal matter. Three actively moving worms were placed in petri dishes containing 5ml of ethanolic plant extract of malibado leaves and bark. Observations on behavioral responses of the earthworms were recorded.

2.3.3. Cytotoxicity Activity
The cytotoxicity of *S. lineatum* crude extracts was evaluated against brine shrimps (*Artemia salina*). The brine shrimp eggs were hatched in a small seawater jar (3.8 g sodium chloride with 100 mL distilled water). This was covered with aluminum foil, completely aerated and illuminated at room temperature. The eggs were incubated for 48 hours to produce large quantities of brine shrimp larvae or nauplii. The concentrations of the crude extracts were prepared employing tenfold serial dilution with 10,000 ppm (10 mg/mL) to 1 ppm (0.001 mg/mL) in distilled water. There were 5 vials with different concentrations of crude extracts (1000 ppm, 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm) were prepared in triplicate to have 15 vials for each test samples. There were 10 brine shrimps in each vial with 100 µL seawater. A 900 µL of each crude aqueous extract were added to each of the vials producing a total of 1000 µL or 1mL per test tube. The brine shrimp mortality was counted and recorded with an interval of 3 hours for 24 hours. The % mortality for each sample extract was determined with the equation:

% mortality = Dbs/Lbs x 100

where Dbs refers to the number of dead brine shrimps and Lbs refers to the initial number of live brine shrimps. To determine the concentration needed to obtain 50% lethality of brine shrimps (LC_{50}), Probit analysis was conducted [9]. LC_{50} values below 100 ppm (or 100 µg/mL) were considered significantly potent or lethal [11].

3. Results and Discussions
3.1. Functionality Determination
3.1.2. Antioxidant Activity
The antioxidant property of the crude ethanolic extract of malibado leaves and bark were evaluated using thin layer chromatographic technique. Blue colored spots developed on the chromatograms (Figure 1) when sprayed with Potassium ferricyanide-ferric chloride reagent. It was observed that the blue colored spot of malibado bark was darker than the malibado leaves extract. The results indicated that the extract from leaves and bark of malibado exhibited antioxidant property. Examining the spots however, showed difference in the intensity of the color reflecting the difference in the antioxidant property. The darker color of the blue spot that developed in the chromatogram containing the bark extract indicated strong antioxidant activity. These results conforms with the data reported by Bizimenyera [6] that the bark and root extracts of *Peltophorum Africanum* Sond. (Fabaceae) had higher antioxidant activity than the leaf and in the study of Sultanaa et. al. [16]. when an investigation of the extracts from barks of four trees: *Azadirachta indica, Terminalia arjuna, Acacia nilotica*, and *Eugenia jambolana Lam*. gave results indicative of the higher antioxidant activity of the bark samples.

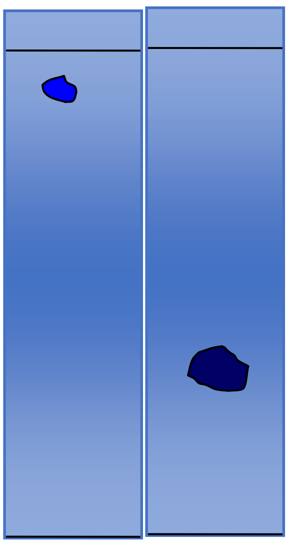

Fig 1: Result of TLC Antioxidant Activity of *S. lineatum* extract

3.1.2. Anthelmintic Activity
In the evaluation of anthelmintic activity, the test organisms

used were worm eggs from sheep and the earthworm. The worms from sheep can be easily determined by fecal egg counting. The number of worms eggs present in the sheep is the level of infestation [19]. The worms from sheep are very prolific egg layer thus worm numbers can build up very rapidly.

3.1.2.2. Anthelmintic Activity Fecal Sample of Sheep

Samples were collected directly from the rectum of the sheep using gloves and were assessed through coprological (fecal) examination methods using the Modified Wisconsin Sugar Flotation Technique. The samples in the glass slide were examined through an electric microscope and the presence of parasitic worm eggs were confirmed. Eggs found in the feces indicates the presence of live worms in the animal from which a stool sample was examined. The parasitic worm eggs identified was the *Haemonchus* or the Barbers pole worm numbering to 987.

It was observed that there is reduction of 68.19% in the number of live worm eggs counted after treatment with the leaf extract. For fecal sample treated with bark extract, it was observed that the parasitics worm eggs were totally immobile indicating death of parasites (Table 1). The result further showed that the bark has a higher anthelmintic activity than the leaves. These results conform with the study of Badar et. al. [4] that bark extract of *Acacia (A.) nilotica (L.) Willd. ex Delile* proved to be a better ovicidal in egg hatch test *in vivo* and it was observed to be more effective in reducing the eggs per gram of feces compared with the leaf extract.

Table 1: The number of parasitic worm eggs before and after the application of the extract

S. lineatum crude extract	Number of parasitic worm eggs		
	Before	After	Mortality Rate
Leaves	987	673	68.19 %
Bark	987	0	100.00 %

3.1.2.2. Anthelmintic Activity Against Earthworm

At the start of the evaluation, earthworms that served as test organisms were observed to be moving actively, they were soft and slimy and their color was brown. Five minutes after the application of the malibado leaf extract, the earthworms were observed to be moving slowly. It was observed that there was a marked decrease in vigorous wriggling movement of the earthworms indicating paralysis after 10 minutes of application [15]. The fleshy part of the earthworms was pricked with a pin to observe possible reaction and it was observed that the earthworms move very slowly and secreted sticky clear mucus. The test organisms became inactive and no movement was observed after 15 minutes of application of the leaf extract (Table 2). The leaf extract might have caused paralysis that led to loss of motility to loss of response to stimuli, which eventually progressed to death in a longer time [14].

Response of the bark extract-treated earthworm differed from that of the leaf extract treatment. Wriggling and movement forward and backward was observed after 5 minutes of the treatment. The earthworms tried to escape from the petri dish and secreted sticky clear mucus. The earthworms became inactive and no movement was observed 10 minutes after the extract application. The fleshy part of the earthworms was pricked with a pin to observe possible reaction; however, the earthworms did not respond to the touch stimulus applied. The earthworms then were considered dead and no further movement was noted. The loss of motility followed with fading of the body color, eventually by death of the earthworms.

Table 2: Responses of earthworms when treated with *S. lineatum* extract

S. lineatum Extract	Observations			
	0 min.	5 min.	10 min.	15 min.
Leaves	Actively moving	Slowly moving	Paralysis	No response
Bark	Actively moving	Wriggling	No response	-----

The results of the current investigation indicate that the bark extract of *S. lineatum* have caused responses on the earthworms requiring less time than the leaf extract. From the observation, both of malibado extracts showed anthelmintic activity with the crude extract of malibado bark showing paralytic effect much earlier and the time to death was shorter.

3.1.3. Cytotoxic Activities of *S. lineatum*

The cytotoxic activities of the leaves and bark extract are shown in Table 3. The results revealed that the percent mortality of the brine shrimp increased with increasing concentration. Both *S. lineatum* samples exhibited high cytotoxic activities after a day. However, of the two samples, the bark extract exhibited a highly significant cytotoxic activity than the leaves extract (t (4) = 3.835, p < 0.01).

Table 3. Cytotoxic activity of the crude extracts against brine shrimp after 24 hours

S. lineatum	Mean % mortality at different concentrations (ppm)					LC50, 24h
	62.5	125	250	500	1000	ppm
Leaves	10	26.7	36.7	43.3	93.3	340.4
Bark	96.7^a	96.7^a	96.7^a	96.7^a	100^a	62.5^b

Legend: a % mortality after 3h b LC_{50} after 3h

The *S. lineatum* cytotoxic activity may be due to the existence of three forms of triterpenes compared to the leaves with a single form of triterpene. Several studies linked the presence of triterpenes with cytotoxic properties [2, 18] and chemopreventive activities [7]. Cytotoxic activities of plant extracts are essential for anticancer drug development [1, 5]. Several cytotoxic studies were conducted against lung adenocarcinoma cancer cell line [8] and human breast cancer cells [1].

4. Conclusion

From the biological activities conducted on the *S. lineatum* leaves and bark extract, the plant could be a potential source of natural antioxidant, a source of anticancer agents and the plant showed anthelmintic activity against the worm eggs from sheep and earthworms. The bioactivity results in this study validates *S. lineatum* leaves and bark as a potential source of useful drugs and also to improve the health status of the consumer as a result of the presence of various Compounds that are vital for good health.

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