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Phytochemical Screening, Anticancer, and Antiangiogenic Activity of Bahai (*Ormosia calavensis*) and Yakal yamban (*Shorea agsaboensis*) used as Ethnomedicine of the Manobo Tribe in Brgy. Anticala, Butuan City, Philippines

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A B S T R A C T

Ethnobotanical knowledge is an integral part of the culture of indigenous groups and plays an essential role in drug discovery. In this study, key informants of the Manobo Tribe in Brgy. Anticala, Butuan City were surveyed for their ethnomedicinal knowledge. The plant parts of frequently mentioned plants were used for phytochemical screening, MTT assay, and duck embryo chorioallantoic membrane (CAM) assay. Seventeen plant species with ethnomedicinal importance were documented to be used by the Manobos, where barks are predominantly used to treat various ailments and prepared through decoction. Yakal yamban (*Shorea agsaboensis*) and Bahai (*Ormosia calavensis*) were frequently mentioned during the survey. The barks of the *O. calavensis* and *S. agsaboensis* were then tested for their phytochemical content, anti-cancer, and anti-angiogenesis potential. Phytochemical analysis revealed the presence of alkaloids, steroids, flavonoids, saponins, and tannins in the barks of the two plant species. The bark extracts of *S. agsaboensis* (50.95±0.04% viability) and *O. calavensis* (76.97±0.18% viability) showed mild toxicity against colon cancer cell line HCT116 in the MTT assay ($P<0.05$). The bark extracts can cause dose-dependent anti-angiogenic activity in the CAM of duck embryos ($P<0.05$). The current findings suggest that the bark of the ethnomedicinal plants, *S. agsaboensis* and *O. calavensis*, used by the Manobos of Brgy. Anticala is a reservoir of bioactive compounds as potential anti-angiogenic and anti-cancer therapy sources. Further studies in identifying specific active metabolites and in-depth biological screening are recommended.

Keywords:*anti-cancer, Caraga, indigenous people*

1 Introduction

Plants have been the most common source of traditional medicine since ancient times. Traditional medicine uses plants, animals, or minerals to treat, diagnose, and even prevent illnesses or diseases. The knowledge of the use of traditional medicine is passed on to generations, and it involves exercises, manual techniques, and spiritual therapies (Zhang 2004). A specific community has local knowledge of traditional plants that would treat various ailments. Pharmaceutical companies have used the knowledge of ethnomedicine for drug discovery and development (Zolla 1980). More than 80% of the world's population depends on plants to meet their primary health care needs. Traditional medicine is widely used in developing countries where expensive orthodox treatments are (WHO

2019). The Philippines is rich in flora diversity, with the various indigenous healing practices and knowledge from ethnolinguistic groups, which is embedded in cultural practices and traditions in the country (Dela Cruz and Ramos 2006). Despite the rich ethnomedicinal knowledge in communities, inculcation of this knowledge is done only through verbal or personal experience (Omac et al. 2021). Published documentation of ethnomedicinal plants or animals is also limited to selected indigenous tribes (Quisumbing 1978).

Primary or secondary metabolites in a plant extract can be identified by phytochemical screening. Naturally, primary metabolites are found in plants and animals that participate in some metabolic pathways, while secondary metabolites are mainly used as defense and signaling needed for plant survival (Dewick 2002). Current research trends focus on identifying plant metabolites, which can be used in drug discovery.

Various assays can determine the bioactivity of plant extracts for multiple purposes. For anti-cancer, 3-(4,5-Dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide or MTT assay is a colorimetric assay that assesses the activity of living cells, which is directly related to the number of living cells (Ciapetti et al. 1993, Kumar et al. 2018, and Bahuguna et al. 2018). Briefly, MTT dye is converted into formazan when there is metabolic activity exhibited in the presence of NAD(P)H-dependent cellular oxidoreductase enzymes. Living cells produce a high reduction rate of MTT while dead cells do not. This activity can be quantified by measuring the absorbance of formazan at 540 nm wavelength (Bahuguna et al. 2018).

On the other hand, angiogenesis is a physiological process of new blood vessel development from pre-existing capillaries. However, this complex multi-step process is also a primary prerequisite for tumor growth and plays a vital role in tumor invasion and metastasis. Angiogenesis inhibitors can be used to impede the abnormal growth of blood vessels. Thus, plants with anti-angiogenesis properties are essential for diseases such as cancer (Kue et al. 2015, Li et al. 2015).

Caraga Region, Philippines, is rich with ethnolinguistic groups like Butuanon, Mamanwa, Higaonon, and Manobo. The Manobo tribe mainly inhabits the Province of Agusan del Sur (Dapar et al. 2020), but they are also present in Sitio Iyao, Barangay Anticala, Butuan City. Since this group has thrived in the region for several generations already, their indigenous knowledge must be given importance. In this study, the ethnomedicinal knowledge of key informants of the Manobo Tribe in Barangay Anticala, Butuan City, Philippines, is documented. Of the plants the informants mentioned, Yakal yamban (*Shorea agsaboensis*) and Bahai (*Ormosia calavensis*) were selected to further investigate these species' bioactivity. Crude ethanolic extracts of the bark of *S. agsaboensis* and *O. calavensis* were tested for angiogenic activity and cytotoxic activity using CAM and MTT assay, respectively.

2 Materials and Methods

Description of Study Area

The study was conducted in Sitio Iyao, Barangay Anticala, Butuan City (9°00'46.8" N and 125°40'11.7" E) (Figure 1). It is a mountainous area where the Taguibo Watershed is located and characterized by its moist temperate climate with a wide array of vegetation, including grasses, shrubs, herbs, and trees. The inhabitants of this area are mainly of the Manobo Tribe.

There is no formal marketing of medicinal plant or plant products in Sitio Iyao, implying that the middleman benefits from the trade. The modern medical facility is far from the area; hence, plants are vital sources of medicines for ailments. With this, it is essential to document the knowledge of locals on medicinal plants used by their community.

Ethnomedicinal Survey and Selection of Respondents

The survey of ethnomedicinal plants was conducted in June 2019 within the domain of the Manobo tribe. Reconnaissance surveys and permits from the National Commission for Indigenous Peoples (NCIP) Caraga Region were first secured before the actual study. The village chieftain, local healer, and two local residents knowledgeable in using plants as medicine were identified to participate as key informants for the survey. The ethnomedicinal data about the plants were collected using an open-ended questionnaire. The ethnobotanical species, the local name, the method of preparation in ethnomedicine, and the ailments it could cure were asked during the survey.

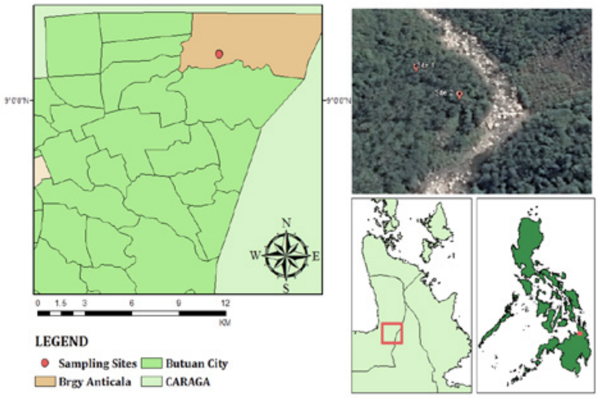

Figure 1. Map of Sitio Iyao, Barangay Anticala, Butuan City Agusan Del Norte, Philippines

Collection, Identification, and Preparation of Plants

Since *O. calavensis* and *S. agsaboensis* were frequently mentioned as useful medicinal plants, samples of these species were collected for identification. The collected plant samples were wrapped with newspaper and stored in a plastic bag to avoid dehydration. Plant samples were immediately brought to the Forestry Department, Caraga State University, for verification.

Based on the knowledge of the key informants, barks of *O. calavensis* and *S. agsaboensis* are used for phytochemical screening, antibacterial activity, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and chorioallantoic membrane (CAM) assay. For sample preparation, plant barks were washed under running tap water and allowed to stand for a couple of minutes to remove excess water. The sliced barks were placed on paraflex ultra sheets and were then processed further to extract the water content in the bark using a dehydrator (Excalibur food dehydrator) for 6 hours and at 100°F.

Extraction and Phytochemical Screening

Dried barks were ground and submerged in separate Erlenmeyer flasks containing 80% ethyl alcohol for 48 h. The soaked samples were then filtered, and filtrates were concentrated in vacuo using a rotary evaporator at 50ºC. The concentrated ethanolic extracts were then transferred into a beaker and stored in a refrigerator at 4ºC. Crude extracts were prepared by placing the plant extract in an evaporating dish over a steam bath until a syrupy consistency was achieved. Phytochemical screening was carried out to identify the presence of alkaloids, steroids, flavonoids, saponins, and tannins based on Aguinaldo et al. (2005) protocol. Test for alkaloids was done using Dragendroff's test, Liebermann-Burchard test to detect steroids, Base-Smith and Metcalf Method to test flavonoids, Froth test for saponins, and ferric chloride test to detect tannins.

MTT assay

Crude bark extracts were dissolved to dimethyl sulfoxide (DMSO) to get the 100 µg/mL final concentration used in the MTT assay. The cytotoxicity test was performed using the Promega CellTiter 96® Non-radioactive Cell Proliferation Assay (Promega 2004). Digitonin was used as a positive control for its known cytotoxic activity against cancer cells (Eid et al. 2011).

Briefly, HCT116 cancer cell lines were cultured and grown to 80% confluency. A volume of 90 µL of 20,000 cells was pipetted into the 96-well plates and incubated overnight at 37°C and 5% CO_2. Crude bark extracts were then added the next day and incubated. A dye solution was added to the plates and incubated at 37°C and 5% CO_2 atmosphere. A solubilization solution was added and incubated at room temperature. Absorbance values were measured using a plate reader at 570nm. Four trials were conducted for each bark sample (Morgan 1998, Plumb 2004, and Meerloo et al. 2011).

Corrected absorbance values were calculated by subtracting the average blank absorbance from the individual absorbance value. The percent viability of the absorbance values was computed following Wahab et al. (2014).

$$Percent\ (\%)\ Viability = \frac{Average\ Corrected\ Absorbance}{Average\ Absorbance\ of\ Negative\ Control} x100$$

The cytotoxicity of extracts against HCT116 cells was based on the cytotoxic index, where 0.1-10% indicate high toxicity, 10-50% viability means moderate toxicity, and 50-100% viability suggests mild toxicity. The assay was repeated four times to ensure the validity of the result.

CAM Assay

Mallard duck (*Anas platyrhynchos*) eggs, approximately 60 g, were cleaned using 95% ethanol and were incubated at 37ºC. Before treatment administration, egg candling was done to check viability and locate the egg's air space. Under-developed embryos were discarded (Ribatti et al. 2006). Six eggs were used in each treatment group. Distilled water and vitamin A (NCPC, Ltd.) was used as the negative and positive control, respectively, while three concentrations of the bark extracts (250 µL/mL, 500 µL/mL, and pure extracts) were prepared.

A 5 mm x 5 mm window was opened at the surface of the day eight eggs, just above the embryo. A volume of 100 µL of each treatment was pipetted on the surface of the chorioallantoic membrane. Eggs were then sealed with parafilm and incubated for 72 h at 37ºC. After the 72-h incubation, they were harvested by re-opening the sealed parts and removing other shells to expose the CAM widely. CAM of each egg was photo-documented for further analysis. Determination of vascular density was done following the protocol of Ribatti et al. (2006). Briefly, branch points of blood vessels were manually counted with the aid of TPS software (Rohlf 2008, & Rohlf 2009).

Statistical Analysis

The normality and homogeneity of variances of the data were first checked using the Shapiro-Wilk test and Levene's test, respectively. Tukey's pairwise comparison determined significant differences in the CAM assay's duck embryo CAM vascular density. Statistical analysis of the percent viability of the HCT116 cell line obtained from the absorbance value was calculated by using the One-way Analysis of Variance (ANOVA) at a significance level of P<0.05 through SPSS software version 16.0.

3 Results and Discussion

Ethnomedicinal Survey

Seventeen plant species belonging to 13 families were documented to be used by the Manobo tribe in Barangay Anticala, Butuan City. The most represented family is Fabaceae, with three species. *S. agsaboensis* or yakal yamban (13.19) and *O. calavensis* or bahai (9.89) were the most mentioned (Table 1).

The most frequently used plant part were barks, roots, and leaves, although stem, fruit, and the whole plant are still valuable parts used for treatment. The key informants have listed four ways of preparing medicinal plants depending on how they apply them to the patient. Decoction is the most common way of making medicinal plants, followed by ingestion, topical application like pasting the medicinal plant in the affected area, and steam bathing. Common health problems that are believed to be cured by these plants include fever, cough, headache, wounds, diarrhea, flu, and stomachache. During the survey, pregnancy-related health problems such as relapse and miscarriage and kidney problems like urinary tract infection were also mentioned.

Phytochemical Screening

Alkaloids, steroids, flavonoids, saponins, and tannins were present in the crude extract of the barks of the bark of the two plants (Table 2). Qualitative phytochemical analysis revealed also that primary and secondary alkaloids were present in *S. agsaboensis* and *O. calavensis*, respectively.

MTT Assay

The MTT assay was performed to investigate the cytotoxic ability of *O. calavensis* and *S. agsaboensis* bark extracts against colon cancer cell line HCT116. The results indicate that 100 µg/mL of outer bark extracts of *S. agsaboensis* (50.95± 0.04% viability) and *O. calavensis* (76.97±0.18% viability) have mild toxicities against colon cancer cell line HCT116 (P<0.05). Nevertheless, the bark extract of *S. agsaboensis* showed a relatively higher anti-proliferative property than the bark extract of *O. calavensis* (Figure 2). Although there were mild cytotoxic effects on HCT116, characteristics of apoptotic cells like disoriented plasma membrane disintegrated nuclei and cell shrinkage were observed in the bark extract-treated setup (Figure 3).

Table 1. List of ethnomedicinal plants used by the Manobo tribe of Brgy. Anticala, Butuan City, Philippines

No.	Family	Scientific name	Local Name	Plant part used	Treatment process	Disease treated	Mode of Preparation	Frequency of citation (%)
1	Anacardiaceae	*Dracontomelon dao*	dao	bark, fruit, leaf	ingestion, paste	headache, wounds	Chew fruit or leaf, scrape the bark and apply in the affected area	3.30
2	Annonaceae	*Annona muricata*	rabana	leaf,	decoction	cough	Boil leaf with water	6.59
3	Araucariaceae	*Agathis philippinensis*	almasiga	root and bark	decoction, steam bath	fever	Boil root and bark; while hot, perform the steam bath in a close area	3.30
4	Asteraceae	*Blumea balsamifera*	gabon/ sambong	whole plant	decoction, ingestion	fever, cough, kidney, UTI	Boil with water drink when warm	5.49
5		*Artemisia vulgaris*	hilbas	leaf	decoction	fever, spasm & flatulence	Add a few leaves to the water and boil	3.30
6	Bombakaceae	*Ceiba pentandra*	gapas	bark	decoction	fever, wounds, diarrhea, flu, relapse	Boil bark with water, soak cotton in a glass of water, and drink	6.59
7	Boraginaceae	*Cordia dichotoma*	anonang	bark,	ingestion	relapse	Chew a few barks for a minute	3.30
8	Dipterocarpaceae	*Shorea agsaboensis*	Yakal yamban	bark, leaf, roots	decoction, paste	fever, wounds, diarrhea, flu, relapse	Boil with water, crushed	13.19
9	Fabaceae	*Pterocarpus indicus*	narra	bark,	paste	wounds and mouth sores	Scrape the bark and apply it to the affected area	6.59
10		*Cassia alata*	sunting	root, bark	decoction	UTI, headache	Boil root with water	6.59
11		*Ormosia calavensis*	bahai	bark, leaf, roots	decoction, paste	fever, wounds, diarrhea, flu, relapse	Boil with water, crushed	9.89
12	Lamiaceae	*Vitex negundo*	lagundi	whole plant	decoction	cough, fever	Boil plant	2.20
13		*Vitex parviflora*	togas/ molave	bark, leaf, root, wood, stem	ingestion	miscarriage	Boil a specific plant part or two or more plant parts	6.59
14	Lygodiaceae	*Lygodium* sp.	nito	root	decoction	cough, headache, stomachache, fever	Boil roots with water	6.59
15	Moraceae	*Artocarpus macrophyla*	langka	leaf	ingestion	headache	Chew few parts of young leaf	6.59
16	Myristacaceae	*Myristica simiarum*	duguan	bark,	ingestion	anemia, rashes, bleeding	Get enough bark and chew	6.59
17	Rubiaceae	*Coffea arabica*	kapi	root	decoction	relapse	Boil root part with water	3.30

Mallard Duck CAM Assay

CAM assay was conducted to evaluate the angiogenic activity of *O. calavensis* and *S. agsaboensis* bark crude extracts (Figure 4). All eggs were viable until the harvest of the eggs. Gross morphologic observations of the CAM of duck embryos showed many pathologies in the vascular density. Qualitative deviations in the vasculature of the treated duck embryos were observed, such as disrupted growth, irregular branching

Table 2. Phytochemicals present in the bark of *Ormosia calavensis* and *Shorea agsaboensis*

Parameter	*Ormosia calavensis*	*Shorea agsaboensis*
Alkaloids Confirmatory Tests: (+) Primary Alkaloid (++) Secondary Alkaloid (+++) Tertiary Alkaloid	++	+
Steroids Keller-Killiani Test (for 2-deoxysugars) Liebermann-Burchard Test (for unsaturated steroids)	+ +	+ +
Flavonoids Bate-Smith & Metcalf Method (for Leucoanthocyanins)	+	+
Saponins Froth Test	+	+
Tannins Ferric Chloride Test (hydrolysable tannins)	+	+

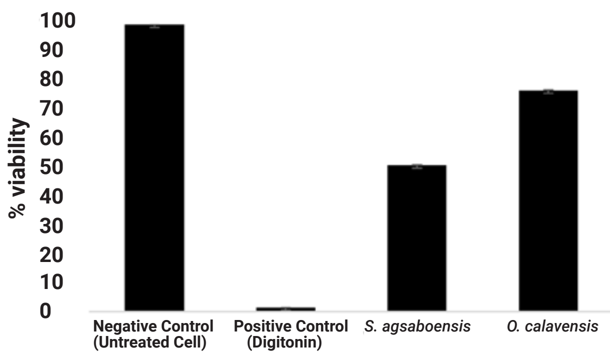

Figure 2. Percent viability of colon cancer cell lines HCT116 treated with controls and bark crude extracts of *Shorea agsaboensis* and *Ormosia calavensis*

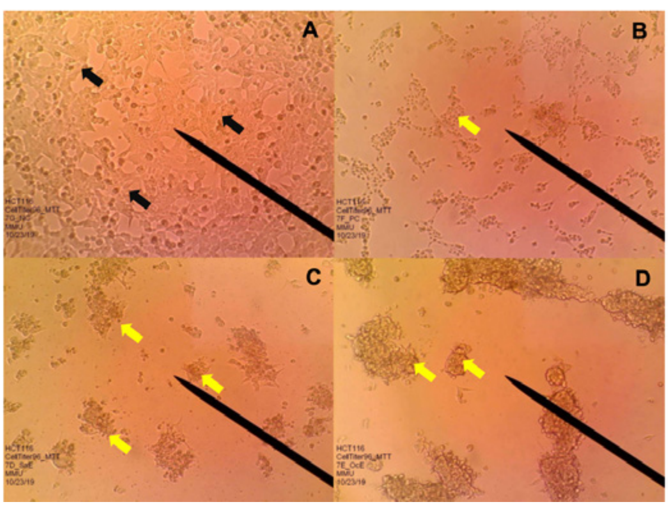

Figure 3. Images showing untreated colon cancer cells HCT116 (A), treated digitonin (B), and with 100 µg/mL concentrations of *Shorea agsaboensis* (C) and *Ormosia calavensis* (d). Note the disoriented plasma membrane, disintegrated nuclei, and shrinkage of cells in the treated groups (yellow arrow). Cancer cell proliferation was observed in the untreated group (black arrow)

of capillaries, and thin veins. Duck embryos treated with the negative control showed the usual branching pattern characterized by extensive junctional complex with multiple membrane contact points (Gamallo et al. 2016). In contrast, duck embryos treated with retinoic acid as positive control showed less irregular vascularization.

Compared to the control, inhibition of angiogenesis in the CAM of duck embryos showed a statistical difference upon treatment of *O. calavensis* (P=0.001) and *S. agsaboensis* (P=0.000) bark extracts. Anti-angiogenic activity increases as the concentration of the bark extract increases (Figure 5). However, the vascular density of the lowest concentration (250 µL/mL) for *O. calavensis* and *S. agsaboensis* was not statistically different compared to the negative control (P>0.05). All concentrations of *O. calavensis* bark extracts have higher vascular density than the retinoic acid. The higher concentrations of *S. agsaboensis* were lower than the retinoic acid, although it did not show a statistical difference (P>0.05). Generally, higher concentrations of bark extracts from the two plant species displayed anti-angiogenic effects on the blood vessel of the duck CAM compared to the negative control (P<0.05).

Discussion

The ethnomedicinal survey revealed that Family Fabaceae and Dipterocarpaceae species were the most frequently mentioned plants. According to Molares et al. (2011), Fabaceae has great ethnomedicinal importance to the world's indigenous communities and urban communities. The group is widely distributed and is the third-largest land plant family in terms of several species

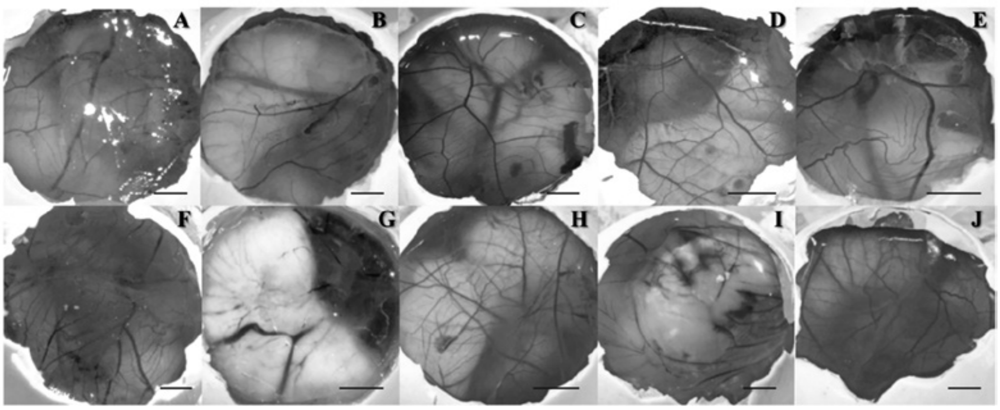

Figure 4. Chorioallantoic membrane (CAM) images upon harvest after 72-h. Negative control (A,F); positive control (B,G); *Ormosia calavensis* bark ethanolic extract with concentration of 250 µL/mL (C), 500 µL/mL (D), and pure extract (E); *Shorea agsaboensis* bark crude extract with concentration of 250 µL/mL (H), 500 µL/mL (I), and pure extract (J). Scale bars represent 10 mm

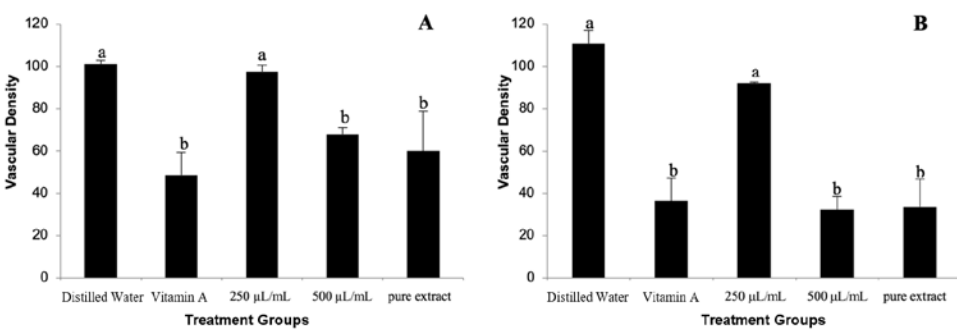

Figure 5. Vascular density in the chorioallantoic membrane (CAM) of duck embryos treated with *Ormosia calavensis* (A) and *Shorea agsaboensis* (B) bark extracts. Mean values with different letters (a-b) are significantly different (P<0.05) based on Tukey's pairwise comparison

(Rahman et al. 2014). Hence, its accessibility could be a huge factor in its usage in ethnomedicine. On the other hand, Family Dipterocarpaceae is solely represented by *S. agsaboensis*, where the bark is used for treating ailments. In this study, barks were the most mentioned plant part used to treat health problems. A study in Johor, Malaysia, listed the bark of *Dipterocarpus sublamellatus* as one of the treatments for tuberculosis, while other species of Dipterocarpus bark are also used to treat ailments such as rheumatism, respiratory, and digestive system-related diseases, and even sexually transmitted diseases (Aslam et al. 2015, & Sabran et al. 2016). Although the root is the second most utilized plant part in this study, the usage of roots should be regulated since harvesting of this part might hamper plants' growth and survival (Tantengco et al. 2018). This study also revealed that decoction is the most widely used process to prepare ethnomedicinal plants. Decoction involves boiling plant material for a particular time to soften the plants and extract its active compounds (Morilla et al. 2014, & Balangcod and Balangcod 2015).

Alkaloids, steroids, and flavonoids were identified in the barks of *O. calavensis* and *S. agsaboensis*. Alkaloids are naturally used by plants to attract pollinators and are considered part of an elaborate system of chemical defense against some animals and microbes (Roberts 2013). Similarly, it was reported that alkaloids play a significant role in the defense system against animals and pathogen, and it also possesses both antimicrobial and anti-parasitic activities (Bouyad et al. 2011). Steroids were also detected in the phytochemical screening. Steroids are restricted only to a few plant families (Heftmann 1975). The plant produces a wide array of steroid molecules with physiological roles such as growth-promoting phytohormones or pheromones and may display protective actions towards phytophagous animals or parasitic fungi (Dinan et al. 2001).

Flavonoids exhibit biological effects, including antibacterial, antiviral, anti-inflammatory, anti-allergic, and vasodilatory actions (Cook and Samman 1996). Saponins and tannins were also detected during the phytochemical screening. Saponins are primarily seen in plants and plant products important in nutrition (Olawa et al. 2013). They play a significant role in immunological, physiological, and pharmacological aspects. Also, saponins have increasing evidence of their health benefits, such as cholesterol-lowering and anti-cancer properties (Ozlem et al. 2007). Tannins help treat external diseases like inflammatory injuries and chronic diseases (Sieniawska and Baj 2017). As also reported in the study of Cagauan et al. (2011), tannins influence anti-inflammatory and hypoglycemic activity. It also possesses antimicrobial properties and is used to treat wounds, hemorrhoids, and diarrhea (Doughari 2006). With this diversity of phytochemicals found in the bark of *O. calavensis* and *S. agsaboensis*, these species may have a potential application in drug discovery.

Cancer is one of the common causes of death worldwide (WHO 2019). However, plants are good reservoirs for anti-cancer drug discovery. This study tested *O. calavensis* and *S. agsaboensis* bark ethanolic extracts for their cytotoxic activity using an MTT assay. The mean percent viability of the HCT116 cancer cell line falls within 50-100%, which means that both bark extracts have mild toxicity against the colon cancer cells. However, apoptotic and necrotic cells are still observed in the wells containing *O. calavensis* and *S. agsaboensis*. Apoptosis and necrosis include lost integrity of plasma membrane, the disintegration of the nucleus, cell fragments engulfed by neighboring cells, chromatin condensation, or shrinkage of cells. On the other hand, necrosis is characterized by increased cell volume, organelle swelling, or disorganized cellular content (Galluzzi et al. 2009).

Angiogenesis plays an essential role in tumor and cancer progression since blood carries the needed nutrients for its development (Fryer and Field 2005). Identification of growth factors responsible for regulating angiogenesis has been studied since the 1800s. However, in the 1980s, the vascular endothelial growth factor (VEGF) isolated from tumor cell lines was the principal biomarker for endothelial cell migration and progression. CAM offers advantages over other assays in investigating angiogenesis activities of potential antitumor drugs since it is a low-cost method, and blood vessels can be directly observed and quantified (Kue et al. 2015, & Li et al. 2015). The current study observed inhibition of vascularity in the CAMs treated with *O. calavensis* and *S. agsaboensis* bark crude extracts commonly used by the Manobo tribe. It follows a concentration-dependent manner where pure extracts have the highest potential to reduce the growth of blood vessels. Anti-angiogenic agents from the bark of these trees could inhibit the activities of growth-promoting factors like VEGF for angiogenesis. Alkaloids, flavonoids, saponins, steroids, and tannins were phytochemical compounds present in the bark of *O. calavensis* and *S. agsaboensis*. These plant metabolites induce cellular stress by increasing reactive oxygen species, thus inducing a toxic cellular environment which may lead to programmed cell death or apoptosis in endothelial cells (Gaziano et al. 2016).

4 Conclusion and Recommendations

Medicinal plants are an essential part of the culture of the Manobo tribe in Sitio Iyao, Brgy. Anticala, Butuan City, Philippines. Among the 16 plant species mentioned by the key informants, the bark of *O. calavensis* and *S. agsaboensis* were utilized for multiple phytochemical and biological screening. Crude bark extracts of these species revealed essential compounds. The crude bark extracts also have mild cytotoxicity against HCT116 but could prevent angiogenesis at high concentrations. This study still suggests that *O. calavensis* and *S. agsaboensis* have medicinal value, and further studies for identifying specific active metabolites and in-depth biological screening are recommended.

5 Acknowledgement

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Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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