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Phytochemical Screening and Evaluation of Angiogenesis Activity of *Hyptis Capitata* Jacq. Leaf Ethanolic Extract

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ABSTRACT

Plants are valuable compounds that can be used to develop medicines against various diseases. *Hyptis capitata* Jacq. is a shrub commonly found on roadsides and is used to treat ailments by local people. In this study, the ethanolic leaf extract of *H. capitata* was subjected to phytochemical screening to determine the secondary metabolites present in the plant leaves. Chorioallantoic membrane assay (CAM) was used to evaluate the plant extract's antiangiogenic activity. Concentrations of 0.1 ppm, 1 ppm, 10 ppm, 100 ppm, 1000 ppm, and 10000 ppm from the plant's leaf extract, distilled water as the negative control, and retinoic acid as the positive control were treated on the CAM. Results revealed that the *H. capitata* leaves contain steroids, flavonoids, saponins, and tannins. The plant's leaf extract has antiangiogenic potential as 1000 ppm and 10000 ppm concentrations significantly decreased the blood vessels ($P<0.05$) with percent inhibition of 26.78 % and 35.49 %, respectively. Studies exploring other assays for angiogenesis and toxicity tests to support the plant's potential as an angiogenesis inhibitor are recommended.

Keywords: *Ethnomedicine, Chorioallantoic Membrane Assay*

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1 Introduction

Angiogenesis is a physiological process of forming new blood vessels from preexisting vascularization. This process is necessary for wound healing growth, development, and granulation tissue formation. However, it also plays a fundamental role in pathological conditions such as chronic inflammation, tumor growth, and metastasis (Salas and Totaan 2015). Thus, angiogenesis inhibitors can be a potential approach to the abnormal growth of the blood vessels that may lead to these pathological diseases (Camposano et al. 2016).

Chorioallantoic membrane (CAM) assay has been utilized to perform *in vivo* angiogenesis or anti-angiogenesis studies (Naik et al. 2018). It is a well-established model that provides a vascular structure that can be used to study tumor cell invasion, angiogenesis/anti-angiogenesis, and metastases (Mousa et al. 2017). CAM model is advantageous because it is relatively simple, low cost, highly reproducible, and is a closed system composed of multilayer ectoderm with extracellular matrix proteins that mimic the physiological cancer cell environment (Ribatti 2017; Lokman et al. 2012). Besides angiogenesis and tumor invasion, CAM assay can also be used to study bone regeneration (Moreno-Jimenez et al. 2016) and avian body growth (Rosal et al. 2020; Gamallo et al. 2016), among others.

Plants are a valuable source of medication

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and play a significant role in global health (Sofowora et al. 2013). It is estimated that 80% of the world's population relies on herbal medicine as primary health care, especially in developing countries. Incorporating herbs in traditional medicine practices is an intrinsic part of the culture in those communities (Ekor 2014). According to Oladeji (2016), plant-derived medications have been identified through recent studies of curative, therapeutic, traditional remedies, and most notably, indigenous people's folk knowledge. Despite recent advances in science and technology, some of these claims and beliefs remain irreplaceable.

Hyptis capitata Jacq., is a slightly aromatic herbaceous plant belonging to the family Lamiaceae and is a common weed found in open fields and along roadsides (Sulistyaningsih et al. 2017). The decoction of leaves of *H. capitata* has been used for cough, malaria, diarrhea, stomachache, newborn baby care, fever, gas pain, and flatulence. Crushed leaves are used for cuts and wounds by Manobo of Agusan (Dapar et al. 2020). Despite the various ethnomedicinal uses of *H. capitata*, this plant needs more scientific studies, particularly on its antiangiogenic activity.

This study aims to determine some of the active classes of secondary metabolites and evaluate the effects of *H. capitata* leaf crude ethanolic extract on the angiogenesis and morphometrics of the duck *Anas platyrhynchos* embryos. The result of this study will be additional knowledge on *H. capitata* and serves as a scientific basis for alternative and natural angiogenesis inhibitors.

2 Materials and Methods

Plant collection and extraction

The *H. capitata* plant samples were collected from Buenavista, Agusan del Norte (8°57'43.54" N, 125°23'51.09" E), and species authentication was done the Biology Department of Caraga State University (CSU), Ampayon, Butuan City. The plant extraction was carried out following the standard method described by Guevarra et al. (2005). The collected leaves were washed thoroughly and air-dried for one week. The dried *H. capitata* leaves were cut into smaller pieces and placed in ziplock bags for further extraction. The cut pieces of the air-dried plant were subjected to ethanolic extraction in the CSU Chemistry Laboratory. Briefly, the 300 grams of the cut-dried leaves were soaked in 1500 mL of 95% aqueous ethanol for 48 hours at room temperature with intermittent shaking. The extracts were filtered through an ordinary filter paper and concentrated using a rotary evaporator to yield crude ethanolic crude extract.

Phytochemical screening

The phytochemical screening for secondary metabolite determination was done following the methods described by Guevarra et al. (2005). Briefly, the test for alkaloids was done using Dragendroff's test, the test for steroids using Liebermann-Burchard test, the test for flavonoids using Based-Smith and Metcalf Method, the Froth test for saponins, and ferric chloride test for tannins.

Preparation of test treatments and CAM assay

The chorioallantoic membrane assay was performed thrice following the protocol described by Ribatti (1997) and Vergara et al. (2021) with few modifications. Working concentrations of the *H. capitata* leaf ethanolic extract were set to explore the concentration-dependent angiogenic effects on duck embryos. In this study, concentrations of the leaf extract were 0.1, 1, 10, 100, 1000, and 10000 ppm, respectively. Since distilled water was utilized as the negative control and solvent to dissolve the stock solution of the leaf extracts, retinoic acid was used as the positive control. Both negative and positive controls were used to compare the effects of leaf extract solutions in the *in ovo* experiment.

Eight-day-old fertilized duck (*A. platyrhynchos*) eggs were bought from a local supplier in Ampayon, Butuan City, Philippines. Eggs were thoroughly cleaned by removing dirt and excrement and were sanitized by wiping 70% ethanol to the surface of the eggs. All the eggs that were used were incubated at 37°C. Three fertile eight-day duck eggs were used for each experimental group, and the experiment was conducted thrice.

Maintained sterile conditions, and appropriate labeling was strictly followed. To ensure that the eggs were fertilized, egg candling was performed to observe if there was a germ spot on the yolk. Unfertilized eggs were discarded, and the fertilized ones were used for downstream analyses.

A window in the eggshell, about 1 x 1 cm, was made to expose the chorioallantoic membrane for experimental manipulation. A 100 µL of *H. capitata* leaf solutions and controls was topically applied on the CAM of each egg and sealed using sterile parafilm tape. Eggs were incubated for 72 h at 37°C.

Visual assessment and photography

On day 11 post-fertilization, eggs were harvested by reopening the sealed portion, and shells were removed to expose the CAM widely. Each CAM was photographed twice, which was used for counting the blood vessel vascularity. Branch points were manually calculated using Fiji software (https://fiji.sc). The CAM inhibition was expressed as a percentage of the control as follows:

$$Vascular\ Inhibition = \frac{BP_c - BP_t}{BP_c} x100\%$$

Where BPc is the number of branch points of the negative control while BPt is the number of branch points of the treatment.

Morphometric analysis

Weight and morphometry of the embryos of eggs treated with the test chemicals were measured following the method described by Vergara et al. (2021) to test if the test concentrations of the plant extract may play a role in the development of the duck embryo. The same concentration of extracts and control treatments in the CAM assay was used in the morphometric analysis. The following indices measured were the eye diameter (ED), crown-to-rump length (CRL), head-beak length (HBL), forelimb length (FL), and hindlimb length (HL). The weight of the embryos was measured using a digital weighing scale, and morphometric indices were measured using a Vernier caliper.

Statistical analysis

The results were presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by the Tukey post-hoc test was utilized to compare between groups. Differences with $P<0.05$ between experimental groups were considered statistically significant. The statistical test used Statistical Package for the Social Sciences (SPSS) version 23.

3 Results and Discussion

Phytochemical constituents in H. capitata leaf extract

Phytochemicals play an essential role in the growth and development of plants (Martinez et al., 2017). It helps protect plants from unfavorable environmental conditions; hence, it attracts interest and has been utilized in various studies to evaluate its beneficial effects on acute and chronic human diseases (Kadioglu et al. 2013). In this study, the leaf ethanolic extract of *H. capitata* was subjected to phytochemical screening to determine the secondary metabolites. *H. capitata* contained steroids, flavonoids, saponins, and tannins. No alkaloids were observed in the test (Table 1).

Steroids are significant medicinally active organic compounds. It was reported to have cholesterol-reducing properties, which can lower up to 15% of cholesterol levels, act as cancer preventative, and has significantly been marketed as a dietary supplement (Sultan 2015). Flavonoids are a group of phenolic compounds mainly found in fruits and vegetables and have strong antioxidant properties against oxidative stress (Rodríguez-García et al. 2019; Sahib et al. 2010). It also exhibited other biological activities, including antibacterial, antiviral, anti-allergic, anti-inflammatory, and anticancer (Chaves et al. 2020; Kopustinskiene et al. 2020; Abotaleb et al. 2018). According to Ravishankar et al. (2013), flavonoids in the process of tumorigenesis or carcinogenesis interfere with cancer progression by modulating the signal transduction pathways limiting the proliferation, angiogenesis, and metastases.

Saponins are primarily seen in plants and have been studied for various properties (Faizal and Geelen, 2013) because of their pharmacological activities such as anti-inflammatory, antibacterial,

Table 1. Result of phytochemical screening of *Hyptis capitata* leaf ethanolic extract.

Phytochemicals	Presence/ Absence
Alkaloids	absent
Steroids	present
Flavonoids	present
Saponins	present
Tannins	present

antiviral, antifungal, anticancer, and cytotoxic activity (Ashour et al. 2019). Tannins, another phytoconstituent abundant in plant new leaves and flowers, possess various biological functions. Hydrolyzable tannins were reported to have anticancer, antiangiogenic, antioxidant, anti-inflammatory, and anti-ulcer activities (Amarowicz and Janiak 2019). The absence of alkaloids may be associated with the environment where the plant was collected, affecting the plant's phytochemicals (Kusuma et al. 2020).

Antiangiogenic activity

The antiangiogenic potential of leaf ethanolic crude extract of *H. capitata* was evaluated using the CAM assay (Figure 1). CAM assay revealed a significant decrease in vascular density (Figure 2A) and a substantial increase in vascular inhibition (Figure 2B) as the concentration of the plant extract increased (P<0.05). However, post-hoc analysis showed that only the positive control (P=0.013), 1000 ppm (P=0.04), and 10000 ppm (P=0.002) have a significant difference compared to the negative control.

The highest concentration, 10000 ppm, has the highest vascular inhibition of 35.49%. It was followed by 1000 ppm with 26.78 % inhibition, while 0.1 ppm had the least percent inhibition of 16.75%. The result of the CAM is consistent with the study of Mamutuk & Usman (2017) on the antiangiogenicity of *Hyptis suaveolens*, which revealed a dose-dependent effect on angiogenesis. However, the IC50 revealed higher than the highest concentration, 10000 ppm, applied on the CAM.

The inhibition of angiogenesis is possibly done by suppressing the expression of vascular endothelial growth factor or VEGF, matrix metalloproteinases, and inhibiting the migration and proliferation of endothelial cells (Subbaraj et al. 2021). VEGF is considered one of the most important pro-angiogenesis factors. It enhances the blood vessels' permeability while altering the extracellular matrix-degrading enzyme production, which causes the vascular system to expand. It also activates endothelial cells through its receptors, leading to the secretion of metalloproteinases that will allow the migration of endothelial cells (Hoseinkhani et al. 2020; Niu and Chen 2010). Notably, plant compounds such as flavonoids and tannins have been reported to target angiogenesis by inhibiting the expression of VEGF (Kadioglu et al. 2013). The presence of these phytochemicals may explain the anti-angiogenic effect of *H. capitata* ethanolic leaf extracts.

Morphometric analysis of the embryos

Avian embryos can be used for the toxicity assessment of compounds (Smith et al. 2012).

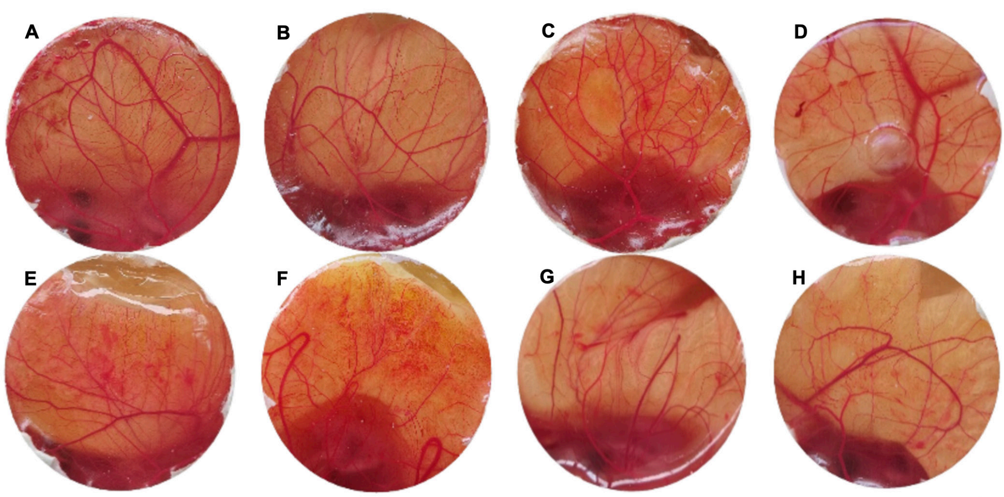

Figure 1. Representative of duck CAM treated with distilled water (a) and retinoic acid (b) control treatments and *Hyptis capitata* leaf ethanolic extract with the concentrations: 0.1 ppm (C), 1 ppm (D),10 ppm (E),100 ppm (F), 1000 ppm (G), and 10000 ppm (H).

Morphometric analysis was done to determine the effect of *H. capitata* leaf ethanolic extract on the development growth of the duck embryos. Morphological parameters such as CRL, HBL, FL, HL, and ED were measured in the analysis.

There were no abnormalities in the gross appearance and form of the embryos treated with controls and different concentrations of the plant's leaf ethanolic extract. No significant effect was found on the weight, CRL, HB, FL, HL, and ED upon treatment with the various concentrations of the plant extract (Table 2).

Treatments administered topically to the CAM may reach systemic circulation and alter the embryo's development (Ribatti 2017). However, the current result contradicts prior short-term (48-72 h) teratogenicity studies conducted to explore the effects of compounds on the avian system's embryonic development (Al-Qahdi et al. 2019; Gamallo et al. 2016; Baharara et al. 2014). The resistance of the morphometries to the inhibitory effects may be due to the small number of bioactive compounds exerted by the plant extract (Mamutuk and Usman 2018). This result may suggest that the *H. capitata* leaf ethanolic extract may not be potent enough to disrupt the normal development of the duck embryos.

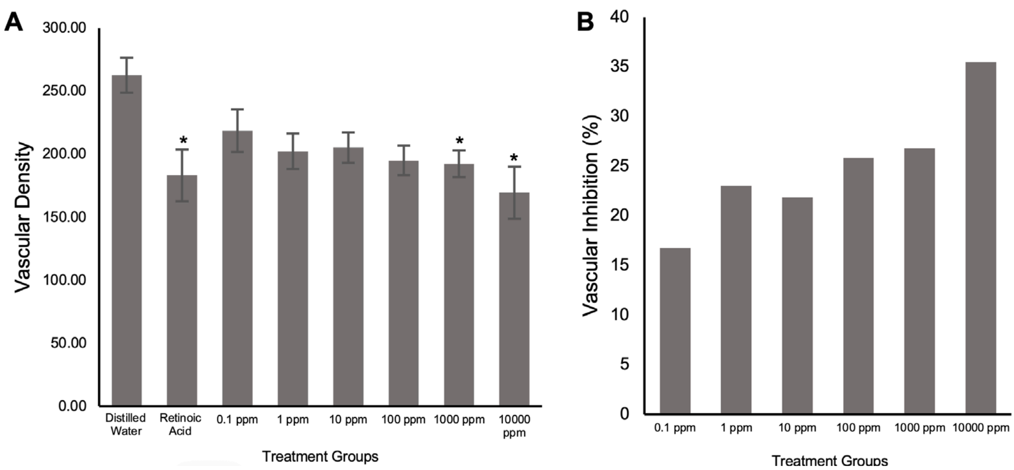

Figure 2. Blood vessel density (A) and vascular inhibition (B) of CAM treated with control treatments and different concentrations of *Hyptis capitata* leaf ethanolic extract.

**Significantly different from the negative control based on Tukey's posthoc test (P<0.05)*

Table 2. Means (± SEM) of weight, crown-rump (CRL), head-beak (HBL), forelimb (FL), hind limb (HL) length, and eye diameter (ED) duck embryos treated with controls and different concentrations of *Hyptis capitata* leaf ethanolic extract.

	Weight (g)	CRL (mm)	HBL (mm)	FL (mm)	HL (mm)	ED (mm)
Distilled Water	1.36 ± 0.17	34.62 ± 3.43	14.97 ± 1.12	8.96 ± 1.19	10.93 ± 0.77	5.45 ± 0.26
Retinoic Acid	1.29 ± 0.17	34.13 ± 2.13	13.56 ± 1.81	8.62 ± 1.24	10.96 ± 0.84	5.75 ± 0.37
0.1 ppm	1.27 ± 0.34	33.34 ± 3.75	13.31 ± 2.19	8.68 ± 2.01	11.00 ± 1.87	5.09 ± 2.12
1 ppm	1.40 ± 0.17	34.57 ± 1.38	14.48 ± 1.32	8.997 ± 1.31	11.85 ± 1.00	5.92 ± 0.53
10 ppm	1.35 ± 0.18	34.67 ± 1.53	14.50 ± 1.30	9.72 ± 1.27	11.80 ± 1.18	5.85 ± 0.65
100 ppm	1.30 ± 0.25	34.23 ± 2.64	14.39 ± 2.07	9.59 ± 1.57	12.09 ± 1.59	5.96 ± 0.42
1000 ppm	1.35 ± 0.12	34.82 ± 2.27	13.62 ± 1.20	9.27 ± 1.49	11.85 ± 1.36	6.01 ± 0.42
10000 ppm	1.27 ± 0.13	32.95 ± 2.42	13.48 ± 1.49	8.62 ± 1.34	11.05 ± 1.29	5.74 ± 0.57

4 Conclusions and Recommendations

The current findings revealed the presence of steroids, flavonoids, saponins, and tannins in the leaf ethanolic extract of *H. capitata*. The plant extract can cause significant concentration-dependent inhibition on the blood vessels of the *A. platyrhynchos* chorioallantoic membrane. However, the extract did not significantly affect the embryo's weight, CRL, HBL, FL, HL, and ED. With this, it is recommended for future research to use higher concentrations of the plant extract and explore the effects of long-term exposure to the extract in other higher animal model organisms.

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5 Statement of Conflict of Interest

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

6 Literature Cited

Abotaleb, M., Samuel, S. M., Varghese, E., Varghese, S., Kubatka, P., Liskova, A., & Büsselberg, D. (2018). Flavonoids in Cancer and Apoptosis. *Cancers*, **11**(1), 28.

Al-Qahdi, S. S., Alzohari, N., Alsaid, A. Y., Ashour, A. A., Aboulkassim, T., Vranic, S., Moustafa A.A., & Khalil, A. A. (2019). *Teucrium polium* plant extract provokes substantial cytotoxicity at the early stage of embryonic development. *Bosnian Journal of Basic Medical Sciences,* **19**(1).

Amarowicz, R., & Janiak, M. (2019). Hydrolysable Tannins. *Encyclopedia of Food Chemistry,* 337–343.

Ashour, A. S., el Aziz, M. M. A., & Gomha Melad, A. S. (2019). A Review on Saponins from Medicinal Plants: Chemistry, Isolation, and Determination. *Journal of Nanomedicine Research,***7**(4), 282–288.

Baharara, J., Namvar, F., Mousavi, M., Ramezani, T., & Mohamad, R. (2014). Anti-angiogenesis effect of biogenic silver nanoparticles synthesized using saliva officinalis on chick chorioallantoic membrane (CAM). *Molecules*, **19**(9).

Camposano, J., Torre, G.L., Laxamana, J., & Larcia, L.L. (2016). Screening for Anti-angiogenic Activity of Selected Philippine Medicinal Plants using Chorioallantoic Membrane Assay. *Mahidol University Journal of Pharmaceutical Sciences*, **43**, 173-182.

Chaves, J. O., de Souza, M. C., da Silva, L. C., Lachos-Perez, D., Torres-Mayanga, P. C., Machado, A. P. D. F., Forster-Carneiro, T., Vázquez-Espinosa, M., González-de-Peredo, A. V., Barbero, G. F., & Rostagno, M. A. (2020). Extraction of Flavonoids from Natural Sources Using Modern Techniques. *Frontiers in Chemistry*, **8**.

Dapar, M. L. G., Meve, U., Liede-Schumann, S., & Alejandro, G. J. D. (2020). Ethnomedicinal plants used for the treatment of cuts and wounds by the Agusan Manobo of Sibagat, Agusan del Sur, Philippines. *Ethnobotany Research and Applications*, **19**.

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, **4**.

Faizal, A., & Geelen, D. (2013). Saponins and their role in biological processes in plants. *Phytochemistry Reviews*, **12**(4), 877–893.

Gamallo, J. P. M., Espere, G., Carillo, D. M. C., Blanes, D. N., Abuda, F. G., Labarda, H. J., Madelo, X. M., & Jumawan, J. C. (2016). Evaluation of antiangiogenic property of *Ocimum basilica* ethanolic leaf extract by using duck embryo chorioallantoic membrane (cam) assay and its morphometric analysis. *International Journal of Herbal Medicine*, **4**(4)

Guevara, B. Q., Claustro, A. C., Madulid, R.S., Aguinaldo, A. M., Espeso, E. L., Nonato, M. G., Quinto, E. A., Santos, M. A. G., de-Castro-Bernas, G., Gonzalez, R. E., del Catillo Salevilla, R.C., and Ysrael, M. C., (2005). A Guide to Plant Screening: Phytochemical and Biological Research Center for Natural Science. University of Santo Tomas, Manila, and UST Publishing House

Hoseinkhani, Z., Norooznezhad, F., Rastegari-Pouyani, M., & Mansouri, K. (2020). Medicinal Plants Extracts with Antiangiogenic Activity: Where Is the Link? *Advanced Pharmaceutical Bulletin,* **10**(3), 370–378.

Kadioglu O, Seo EJ, & Efferth T (2013). Targeting Angiogenesis By Phytochemicals. *Medicinal & Aromatic Plants*, **2**, 134.

Kopustinskiene, D. M., Jakstas, V., Savickas, A., & Bernatoniene, J. (2020). Flavonoids as Anticancer Agents. *Nutrients*, **12**(2), 457.

Kusuma, I.W., Rahmini, R., Arung, E.T., Pramono, A., Erwin, E., & Supomo, S. (2020). Biological activities and phytochemicals of *Hyptis capitata* grown in East Kalimantan, Indonesia. *Journal of Applied Biology & Biotechnology*, **8**(2), 58–64.

Lokman, N. A., Elder, A. S. F., Ricciardelli, C., & Oehler, M. K. (2012). Chick Chorioallantoic Membrane (CAM) Assay as an In Vivo Model to Study the Effect of Newly Identified Molecules on Ovarian

Cancer Invasion and Metastasis. *International Journal of Molecular Sciences*, **13**(8), 9959–9970.

Mamutuk, R. L., & Usman, C. M. (2017). Anti-Angiogenicity And Teratogenicity Of *Hyptis Suaveolens* Leaf Ethanolic Extract In Mallard Duck (*Anas Platyrhynchos*) Embryos. *Science International,* **29**(4), 817-817.

Martinez, K. B., Mackert, J. D., & McIntosh, M. K. (2017). Polyphenols and Intestinal Health. *Nutrition and Functional Foods for Healthy Aging,* 191–210.

Moreno-Jiménez, I., Hulsart-Billstrom, G., Lanham, S. A., Janeczek, A. A., Kontouli, N., Kanczler, J. M., Evans, N. D., & Oreffo, R. O. (2016). The chorioallantoic membrane (CAM) assay for the study of human bone regeneration: a refinement animal model for tissue engineering. *Scientific reports*, **6**(1).

Mousa, S. A., Yalcin, M., & Davis, P. J. (2017). Models for Assessing Anti-Angiogenesis Agents: Appraisal of Current Techniques. *Anti-Angiogenesis Strategies in Cancer Therapeutics,* 21–38. https://doi.org/10.1016/b978-0-12-802576-5.00002-4

Naik, M., Brahma, P., & Dixit, M. (2018). A Cost-Effective and Efficient Chick Ex-Ovo CAM Assay Protocol to Assess Angiogenesis. *Methods and Protocols*, **1**(2), 19.

Niu, G., & Chen, X. (2010). Vascular Endothelial Growth Factor as Anti-Angiogenicnic Target for Cancer Therapy. *Current Drug Targets,* **11**(8), 1000–1017.

Oladeji, O. (2016). The Characteristics and Roles of Medicinal Plants: Some Important Medicinal Plants in Nigeria. *Natural Products: An Indian Journal,* **12**.

Ravishankar, D., Rajora, A. K., Greco, F., & Osborn, H. M. (2013). Flavonoids as prospective compounds for anticancer therapy. *The International Journal of Biochemistry & Cell Biology*, **45**(12), 2821–2831.

Ribatti, D. (2017). The chick embryo chorioallantoic membrane (CAM) assay. *Reproductive Toxicology*, **70**,97101.

Ribatti, D., Gualandris, A., Bastaki, M., Vacca, A., Iurlaro, M., Roncali, L., & Presta, M. (1997). New Model for the Study of Angiogenesis and Antiangiogenesis in the Chick Embryo Chorioallantoic Membrane: The Gelatin Sponge/ Chorioallantoic Membrane Assay. *Journal of Vascular Research*, **34**(6), 455–463.

Rodríguez-García, C., Sánchez-Quesada, C., & Gaforio, J. J. (2019). Dietary Flavonoids as Cancer Chemopreventive Agents: An Updated Review of Human Studies. *Antioxidants*, **8**(5), 137.

Rosal, J., Solania, C., Agan, M. Q., Mondea, D., Villa, B., & Sanchez, D. (2021). Effects of Prenatal Exposure to Urea Fertilizer on the Angiogenesis, Body Growth, and Liver Structure of Duck (*Anas platyrhynchos*) Embryos. *Pollution*, **7**(2).

Sahib, H., Harchan, N., Atraqchi, S., & Abbas, A. (2010). The Role of Medicinal Herbs in Angiogenesis Related Diseases. *International Journal of Pharmacology,* **6**(5), 616–623.

Salas, G. M., & Totaan, E. V. (2015). Selected Philippine Herbal plant extracts as Angiogenesis inhibitors using Chick Chorioallantoic Membrane (CAM) assay. International *Research Journal of Biological Sciences*, **4**(9).

Smith, S. M., Flentke, G. R., & Garic, A. 2012. Avian models in teratology and developmental toxicology. *Developmental Toxicology*, **85**.

Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicines,* **10**(5).

Subbaraj, G. K., Kumar, Y. S., & Kulanthaivel, L. (2021Anti-angiogenic role of natural flavonoids and their molecular mechanism: an update. *The Egyptian Journal of Internal Medicine,* **33**(1).

Sultan, A. (2015). Steroids: A Diverse Class of Secondary Metabolites. *Medicinal Chemistry*, **5**(7).

Sulistyaningsih, Y., Ratnadewi, D., & Darius, R. (2017). Identification of Secretory Structure, Histochemistry and Phytochemical compounds of Medicinal Plant *Hyptis capitata* Jacq. *Biotropia*, **24**(2).

Vergara, J., Demetillo, M., Ombat, L., & Rosal, J. (2021). *Neonauclea formicaria* (Rubiaceae) Leaf Extract Inhibits Vascularization in the Chorioallantoic Membrane of Duck Embryos. *International Letters of Natural Sciences,* **83**, 22–31.

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Biochemical Characterization and Bioactivity of the Tape Seagrass *Enhalus acoroides (L.f.) Royle* from Gosoon, Carmen, Agusan Del Norte, Philippines

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ABSTRACT

Seagrasses have been used for food and traditional medicine for centuries, but only a few studies report their bioactivities and nutritional and medicinal values, particularly in Caraga Region, Philippines. Thus, crude ethanolic extracts of the tape seagrass *Enhalus acoroides* collected from Gosoon, Carmen, Agusan Del Norte, Philippines, were evaluated for biochemical constituents and bioactivity. Proximate analysis of *E. acoroides* yields the following results: moisture: 18.3%, Ash: 16.50%, crude fiber: 11.49%, crude protein: 5.52%, crude fat: 0.91%, and total sugar: 2.06%. Fourier-transform infrared (FT-IR) spectroscopy analysis identified the presence of hydroxyl groups, alkanes, esters, and carbohydrates. *Enhalus acoroides* showed active inhibition against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* (25±0.00 mm and 23.67±0.58 mm, respectively) using the Disk-diffusion method. Furthermore, cytotoxicity against *Artemia salina* nauplii revealed high toxicity of *E. acoroides* after 18 (LC_{50} value of 0.31 ppm) and 24-hour (LC_{50} value of 4.5x10-3 ppm) treatment with complete mortality at 24 hours. The present findings suggest the potential of *E. acoroides* for nutritional and medicinal purposes.

Keywords: *FT-IR, Cytotoxicity, Proximate Composition, Antibacterial Activity, Nutritional, Medicinal*

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1 Introduction

In developing countries, plant materials have been used to treat many infections and diseases due to a lack of access to healthcare (Compean and Ynalvez 2014). Coastal communities rely on marine natural products because they have limited access to terrestrial plants. Interestingly, local communities have little knowledge about the utility of seagrasses (Newmaster et al. 2011). Among marine natural products, seagrasses are not thoroughly studied in relation to drug discoveries (De la Torre-Castro and Rönnbäck 2004; Orno et al. 2020). Despite the efforts exerted on seagrass biodiversity for conservation, the conditions of seagrass still face challenges (Capin et al. 2021).

Enhalus acoroides is a large seagrass found widespread in subtidal zones of the Indo-Pacific region and is commonly known as tape seagrass (Short and Waycott 2010). It is distinctive among seagrass species due to its large size and rhizome, which is densely clothed with numerous stiff black fibrous strands that are remnants of previous leaves (Klangprapun et al. 2018). It serves as foraging and breeding grounds for fish, waterfowl, dugongs, manatees, sea turtles, and many other animals that are of commercial importance (Fajardo et al. 2016) and has also been exploited for feedstuffs (Newmaster et al. 2011).

Several communities in the Philippines and South Sulawesi, Indonesia, have utilized *E. acoroides* seeds for food consumption (Syed et al.

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2019; Gatta et al. 2020). The seeds of *E. acoroides* are highly nutritious as they contain carbohydrates, protein, and fat (Klangprapun et al. 2018). In traditional medicine, the rhizome, roots, fruit, and leaves treat different ailments and, more importantly, diseases such as hypertension (Newmaster et al. 2011). Furthermore, *E. acoroides* displays phytochemical compounds responsible for various bioactivities such as anti-larvicidal, antibacterial, antioxidant, and anti-cancer (Qi et al. 2008; Kannan et al. 2013).

Studies on seagrass species in the Philippines have focused more on its diversity despite its potential biological uses. Studies regarding the nutritional value, biochemical constituents, and bioactivity of *E. acoroides* are scant (Tangon et al. 2019; Tangon et al. 2021), especially within the bounds of the Caraga Region. Additionally, Pradheeba et al. (2011) noted significant seasonal variations of nutritional value in seagrass species. Gosoon, located in the Municipality of Carmen, in the province of Agusan del Norte, is abundant in the seagrass species *E. acoroides.*

Evaluation of the biochemical composition of *E. acoroides* can help educate seagrass in local communities and eventually address it in conservation efforts through a multifaceted and interdisciplinary perspective. Also, as the need for a constant search for potential drugs emerges, seagrasses pose the potential to be a rich source of novel bioactive compounds, allowing for significant bioactivities that will contribute to developing curative agents against emerging diseases. Thus, this study evaluated the biochemical composition of *E. acoroides* from Gosoon, Carmen, Agusan Del Norte and tested its antibacterial activity and cytotoxicity as baseline data.

2 Materials and Methods

Sampling Conditions

Samples of tape seagrass *E. acoroides* were collected between low and high tides in August on the coastal zones of Gosoon, Carmen, Agusan Del Norte, specifically on GPS waypoints of 09°04' 13.04" N, 125°13' 18.8" E (Figure 1). Water parameters such as salinity, temperature, pH, oxidation-reduction potential, and electric conductivity were measured under partly cloudy weather using an Oakton PC 450 Waterproof Portable Meter. Samples were photographed in situ (Figure 2).

Sample Collection and Extract Preparation

Enhalus acoroides samples were identified, and 2 kg samples of the whole plant were placed in a polythene bag and transported to the laboratory

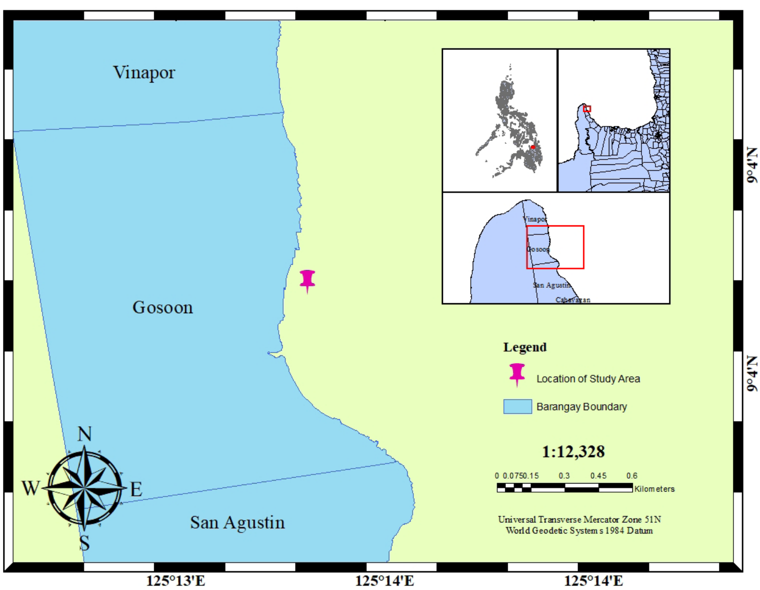

Figure 1. Collection Site for *Enhalus acoroides* at Gosoon, Carmen, Agusan del Norte

Figure 2. Freshly collected samples of *Enhalus acoroides* showing the (A) rhizome and roots and (B) whole plant. Scale Bar= 30cm.

for drying. Fresh samples were thoroughly washed with tap water to remove extraneous contaminants, shade-dried at room temperature (25°C) until brittle, and ground into powder using a mortar and pestle. Extraction was performed by the Department of Science and Technology XIII- Regional Standards and Testing Laboratory (DOST-RSTL). The powder was soaked in ethanol, and plant extraction was conducted as described by Guevarra et al. (2005).

Proximate Analysis

The proximate composition of crude protein, crude fiber, crude fat, ash, moisture, and total sugar (as glucose) was performed at the BIOTECH Central Analytical Service Laboratory at the University of the Philippines and determined following the methods described by the Association of Official Analytical Chemists (AOAC): (1) moisture was determined following AOAC 925.45 B; (2) Ash- AOAC 923.03; (3) Crude fat- AOAC 2003.05; (4) Crude Fiber- AOAC 978.10; (5) Crude Protein- AOAC 081.10; (6) Total Sugars- Phenol Sulfuric Acid Method. The extraction and antibacterial activity were performed by DOST Caraga-RSTL.

Fourier-transform Infrared Spectroscopy

Functional groups were determined via Fourier-transform Infrared (FTIR) spectroscopy at the Chemistry Laboratory of Caraga State University. Peaks were characterized using a PerkinElmer Spectrum Two FT-IR Spectrometer- Attenuated Total Reflectance (ATR).

Antibacterial Assay

Enhalus acoroides samples were subjected to an antibacterial assay following Guevarra et al. (2005). Paper disk diffusion was carried out using a 6mm diameter disc. *Staphylococcus aureus* (BIOTECH 152) and *Escherichia coli* (BIOTECH 1634) sensitivity to samples were tested in three replicates. Antibacterial activity was determined by measuring the clear or bland zones surrounding the disc.

Cytotoxic Activity

The brine shrimp lethality assay described by Guevarra et al. (2005) was adopted to assess the cytotoxicity of *E. acoroides* extract with modifications. Briefly, Artemia salina eggs were hatched in 2 L artificial seawater (38 g rock salt per 1 L distilled water: 38 ppt) for 48 h under constant aeration and illumination (3 watts). Sterile vials containing 4.5 mL artificial seawater and 0.5 mL *E. acoroides* extract with varying concentrations of 100, 10, and 1 ppm were loaded with ten nauplii using a micropipette and kept under illumination for 24 h. Artificial seawater without extract was used as a control. The number of surviving nauplii was determined following a series of 6 h interval observations for 24 h of exposure. The LC_{50} was

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determined by Probit analysis (Finney, 1952). The criterion for cytotoxic activity (LC50) was according to Clarkson et al. (2004) and Meyer et al. (1982) toxicity index, where; 0-100 ppm (Highly Toxic); 101-500 ppm (Moderately Toxic); 501-1000 ppm (Slightly Toxic); and 1000 ppm and above (Non-toxic).

Statistical Analysis

Descriptive statistics utilized IBM SPSS Statistical Analysis Software to analyze variations in the antibacterial activity, proximate composition, and water conditions. Meanwhile, Microsoft Excel 2016 was used for Brine Shrimp Lethality Test statistical data and probit analysis (Finney 1952).

3 Results and Discussion

Sampling Conditions

Temporal and spatial changes have been recorded to affect seagrass cover, species distribution (Ahmad-Kamil et al. 2013), and biochemical composition (Kolsi et al. 2017; Pradheeba et al. 2011). Several studies have noted variations in secondary metabolites, bioactivity, and biochemical composition of seagrass, including *E. acoroides*, from different sampling locations (Natrah et al. 2015; Pradheeba et al. 2011; Sidi et al. 2018; Gatta et al. 2020; Tangon et al. 2021). In the present study, water salinity, temperature, pH, oxidation-reduction potential, and electrical conductivity were 38.7 ppt, 32.3°C, 7.73 pH, 66.1 mV, and 78.1 mS/cm, respectively. These parameters are prominent in seagrass viability (Arumugam et al. 2013). Therefore, physicochemical properties should be documented to evaluate the factors required for biochemical characterization.

Proximate Composition

The moisture content (Table 1) of *E. acoroides* crude ethanolic extracts was 18.3±0.42%. Determination of seagrass moisture content is necessary since it affects the product's natural stability and encourages microbial contamination and chemical degradation by providing a substrate for various processes (Rohani-Ghadikolaei et al. 2012).

The amount of ash in plants measures their mineral content preserved in food materials; variations in ash content are likely related to ambient environmental conditions, which may affect the mineral transfer process (Gatta et al. 2020). Ash content in this study was found to be relatively low compared to ash values of *E. acoroides* from previous research (Rengasamy et al. 2013; Klangprapun et al. 2018; Tangon et al. 2019) but is similar to the ash content of *Cymodocea nodosa* from the Coast of Chebba (Kolsi et al. 2017).

The fiber content in the present study was comparable with previous findings for the seagrasses *Thalassia testudinum* (Coria-Monter and Durán-Campos 2015) and *C. nodosa* (Rengasamy et al. 2013). The high fiber content in food aids digestion and colon cancer prevention. Seed and seed pods of *E. acoroides* also showed high fiber content, 2.38% and 15.48%, respectively (Ratnawati et al. 2019), which supports the utilization of *E. acoroides* as a remedy to ease indigestion and become a basis for drug formulation (Newmaster et al. 2011).

The fat and lipid content of seagrass is generally in the range relevant to the fat content in the present study. Interestingly, Taba et al. (2019) investigated *E. acoroides* and found 0.27% fat content while identifying 11 fatty acids, with palmitic acid, linolenic acid, and oleic acid having the highest % content.

Every living cell's primary structural component, protein, is crucial for growth and development. It is a complex molecule composed of amino acids, nine of which the body cannot synthesize and must be obtained from food sources. Seagrass typically contains less protein than animals, so it cannot be considered a viable source of protein (Immaculate et al. 2018). In the present study, protein content was relatively low compared to the value reported by Rengasamy et al. (2013) for *E. acoroides*.

Carbohydrate is one of the essential components of metabolism, and it supplies the energy needed for respiration and other necessary processes. The body breaks down carbohydrates into simple forms called monosaccharides, which are used as the primary source of energy for cells, tissues, and organs. Most seagrass species contain soluble products, such as sucrose, as major storage carbohydrates (Pradheeba et al. 2011). In the present study, the total sugars of *E. acoroides* estimated as glucose were 2.06±0.18% which is low compared to the carbohydrate content reported for seagrass species *Zosteria marina* (50.9%) and *Cymodocea serrulata* (19.8%), whose large rhizomes act as reservoirs for food storage (Pradheeba et al. 2011; Tangon et al. 2021).

Accordingly, Tangon et al. (2019) recorded a high carbohydrate content of *E. acoroides* from the coast of Carmen, Agusan del Norte.

Variations in biochemical constituents are most probably due to geographic distribution and ambient environmental conditions (Pradheeba et al. 2011; Cristianawati et al. 2019; Gatta et al. 2020). The findings suggest that nutrient concentrations in sediments and dissolved nutrients in seawater in Gosoon, Carmen, Agusan Del Norte may be deficient due to lower values obtained than in other studies (Table 1).

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy is an essential tool researchers use to discern the chemical constituents present in a sample (Kannan et al. 2011). It is presently used to discover novel chemical and molecular components in unknown compounds (Sumayya and Murugan 2017). The FT-IR spectrum (Figure 3) of the *E. acoroides* ethanolic extract showed the presence of both a broad absorption peak at 3361 cm-1 and a weak signal at 2973 cm-1 assigned as the O-H stretching in the hydroxyl group and CH stretching vibration (Gómez-Ordóñez and Rupérez 2011; Kannan et al. 2011; Kannan 2014), respectively. This spectrum, which depicts the presence of alcohols, may indicate the extraction solvent ethanol (Sumayya and Murugan 2017; Rasheed et al. 2019; Pharmawati and Wrasiati 2020).

The presence of a weak band at 1647 cm-1 is a characteristic of a hydroxyl compound; according to Nandiyanto et al. (2019), the broad absorption band at 3600-3250 indicates a hydrogen bond, which in turn confirms the presence of a hydroxyl group that should usually be followed by the presence of additional spectra at frequencies in the ranges of 1600-1300, 1200-1000, and 800-600. Similarly, Pharmawati and Wrasiati (2020) reported a peak at 1654 cm-1 that is believed to be due to chlorophyll and protein content in *E. acoroides*. Stretching bands between 1500 and 1000 cm-1 are complex regions of ethers and alcohol, making it hard to assign the peaks of these regions. However, this C-O stretching region is a characteristic band

Table 1. Proximate analysis of *Enhalus acoroides.*

Biochemical composition	Amount (%)
Moisture	18.3±0.42
Ash	16.50±0.14
Crude fiber	11.49±0.62
Crude protein	5.52±0.08
Crude fat	0.91±0.03
Total sugar (as glucose)	2.06±0.18

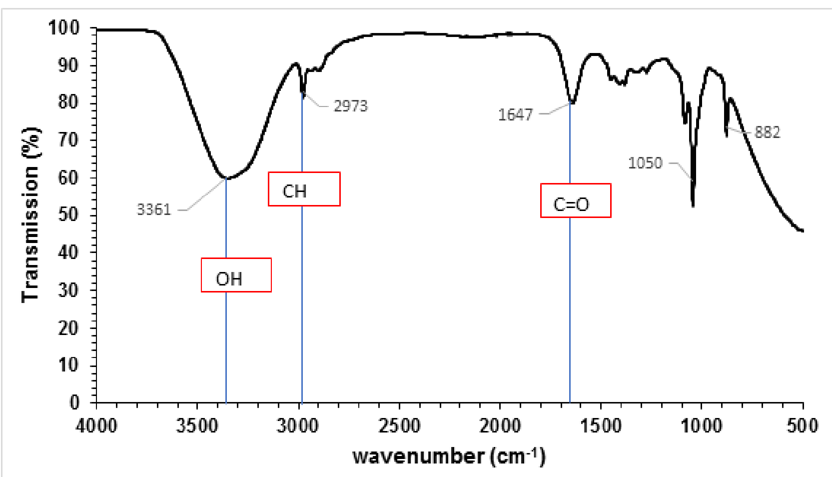

Figure 3. FTIR spectrum of *Enhalus acoroides* ethanolic extract

typical for carbohydrates (Svečnjak et al. 2011). Because seagrass has a high carbohydrate content, the strong peak at 1050 in the fingerprint region might be attributed to the carbohydrates in the samples. Also, A weak-medium peak at 820-920 cm-1 was observed. It could indicate the presence of out-of-plane C-H bending of glucose (Kannan 2014) or C-C skeletal vibration of alkanes, which was confirmed by TLC analysis in a recent study by Pharmawati and Wrasiati (2020) on *E. acoroides* dried leaf powder.

Antibacterial Activity

Seagrasses are rich in secondary metabolites that are effective antibacterial agents (Pradheeba et al. 2011; Tangon et al. 2019; Windyaswari et al. 2019), such as Alkaloids, tannins, and flavonoids, which they produce to survive the harsh marine environment (Qi et al. 2008; Kannan et al. 2013). Plant secondary metabolites target the microbial cell in several ways, including interaction with membrane proteins, disruption of cytoplasmic membrane function and structure, prevention of enzyme synthesis, and interruption of DNA/RNA synthesis and function (Gorlenko et al. 2020). This study's crude ethanolic extract of tape seagrass *E. acoroides* exhibited very active inhibition (Guevarra et al. 2005). Zones of inhibition of extracts showed comparable results between gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* (Table 2). However, *E. coli* exhibited slightly higher clear zones (25±0.00) than *S. aureus* (23.67±0.58). The gram-positive bacteria possess a thick and rigid peptidoglycan in their cell wall but lack an outer membrane.

In contrast, gram-negative bacteria possess a thin peptidoglycan and lipid-rich outer membrane, which consist of a major component called lipopolysaccharide (LPS); this outer membrane is an additional protective layer in gram-negative bacteria. However, this membrane contains channels called porins, which allow the entry of various molecules, such as drugs (Kapoor et al. 2017). Vanitha et al. (2017) determined the phytochemicals in *E. acoroides* using different extracts and found that the ethanol extract of *E. acoroides* yielded quinones and flavonoids. Flavonoids render the membrane more permeable and disrupt it by interacting with membrane proteins found in bacterial cell walls (Gorlenko et al. 2020). Compean and Ynalvez (2014) stated that flavonoids display astounding inhibition against gram-negative bacteria compared to gram-positive bacteria. The susceptibility of gram-negative bacteria against *E. acoroides* showed promising activity (Manikandan and Kolanjinathan 2016), suggesting that *E. acoroides* in the present study exhibits antibacterial activity against gram-negative and gram-positive bacteria.

Cytotoxic Activity

Cytotoxic activity of *E. acoroides* was carried out using the brine shrimp lethality test (BSLT). BSLT provides baseline information for the anti-cancer potential or safety of bioactive compounds in samples (Premarathna et al. 2020). There are alternative methods for testing a sample's cytotoxicity, but BSLT is inexpensive, reliable, and convenient (Vinayak et al. 2011).

Enhalus acoroides ethanol extracts showed cytotoxic activity to *Artemia salina* nauplii following a series of 6 h interval observations for 24 h for all three replicates. Cytotoxicity was expressed as % mortality and LC_{50} values. The 18 hour and 24 hour exposures of *A. salina* nauplii to *E. acoroides* ethanol extract showed a high toxic interpretation. They had complete mortality at 24 hour exposure (4.5x10-3 ppm), while 6 hour (LC_{50} 2951.20 ppm) and 12 hour (LC_{50} 245.8 ppm) exposures were interpreted as non-toxic and moderately toxic, respectively (Table 3).

There are a few studies of the cytotoxic activity of seagrass, e.g., Kannan et al. (2013) recorded lesser cytotoxicity in *E. acoroides* compared to other seagrass species. In another investigation by Orno et al. (2020), different plant parts (e.g., leaf, stem, and rhizome) of *E. acoroides* were found to inhibit the growth of *A. salina* nauplii, categorizing them as non-toxic and moderately toxic. The cytotoxic activity of three novel secondary

Table 2. Antibacterial activity of *Enhalus acoroides* Ethanol Extract.

Bacteria	Zone of Inhibition (mm)	Remarks
Staphylococcus aureus	23.67±0.58	very active
Escherichia coli	25±0.00	very active

Mean ± Standard Deviation.

Table 3. *Enhalus acoroides* brine shrimp lethality assay results.

Conc (ppm)	6H			12 H			18 H			24 H		
	%M	LC_{50}	R	%M	LC_{50}	R	%M	LC_{50}	R	%M	LC_{50}	R
100	19	2951.20 ppm	NT	44	245.89 ppm	MT	99	0.31 ppm	HT	100	4.5x10-3 ppm	HT
10	8			24			88			100		
1	2			14			59			94		

Note: %M- % mortality (mean), Conc- concentration, R- remarks: NT- non-toxic; MT- moderately toxic; HT- highly-toxic.

metabolites, apigen, luteolin 3'-glucuronide, and p-hydroxy-benzaldehyde, isolated from *E. acoroides* against *Spodoptera litura* cells was found to be highly toxic (Qi et al. 2008). The inhibitory effect of the extract is attributed to several cytotoxic compounds such as flavonoids, saponins, and alkaloids that are stated to possess anti-cancer, antiproliferative, and antitumor properties and are reported to be abundant in seagrass (Widiastuti et al. 2021). These compounds inhibit the feeding power of *A. salina* nauplii by disrupting the digestive tract and inhibiting taste receptors, failing to detect food and eventually causing starvation and death (Orno et al. 2020).

The cytotoxic activity exhibited by *E. acoroides* in the present study establishes the presence of potent bioactive compounds. The findings indicate that *E. acoroides* ethanol extracts are dosage dependent since fatality was strongly correlated with the concentration of the ethanol extract. Differing plant organs may contribute to the diversity of cytotoxic activity (Orno et al. 2020). Each organ is an organized group of specialized tissues that perform a specific function. Thus, it may contain its unique bioactive compounds, considering different parts of *E. acoroides* are used for different ailments in local communities (Newmaster et al. 2011).

4 Conclusions and Recommendations

The biochemical characterization of *E. acoroides* reported in this study indicates that seagrass is a good source of nutrients for human consumption and feed formulation. With the rising threat of antimicrobial resistance, new classes of antimicrobial drugs are urgently required. The active inhibition of *E. acoroides* suggests its potential as a center for research and development of new antibacterial drugs. Meanwhile, the high toxicity value of *E. acoroides* found in this study exhibits prospective potential anti-cancer, antitumor, or antiproliferative activity.

In contrast to prior studies, the current research found that the biochemical composition of seagrass species varies according to their geographic distribution. Hence, the findings of this study recommend that we describe and compare the biochemical composition of seagrass found within the bounds of the Caraga Region. Also, the results of the bioactivity of *E. acoroides* warrant further studies to elucidate the phytocompounds contained in them responsible for their bioactivity. Specifically, the researchers recommend that *E. acoroides* be tested for antibacterial activity using different solvents against a broader range of pathogens and tested for their anti-cancer and antitumor action.

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5 Statement of Conflict of Interest

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

6 Literature Cited

Ahmad-Kamil, E.I., Ramli, R., Jaaman, S.A., Bali, J. & Al-Obaidi, J.R. (2013). The effects of water parameters on monthly seagrass percentage cover in Lawas, East Malaysia. *The Scientific World Journal,* **13**, 1-8. http://dx.doi.org/10.1155/2013/892746

Arumugam, R., Kannan, R.R.R., Saravanan, K.R., Thangaradjou, T., & Anantharaman, P. (2013). Hydrographic and sediment characteristics of seagrass meadows of the Gulf of Mannar Marine

Biosphere Reserve, South India. *Environmental monitoring and assessment,* **185**(10), 8411-8427. https://doi.org/10.1007/s10661-013-3183-6

Capin, N.C., Pototan, B.L., & Delima, A.G.D. (2021). Distribution and Abundance of Seagrasses in the Southwest Coast of Davao Oriental, Philippines. *Philippine Journal of Science*, **150**(S1), 383-394. https://doi.org/10.56899/150.S1.28

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P.J., & Folb, P.I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *Journal of Ethnopharmacology,* **92** (2-3), 177-91. https://doi:10.1016/j.jep.2004.02.011

Compean, K.L., & Ynalvez, R.A. (2014). Antimicrobial activity of plant secondary metabolites: A review. *Research Journal of Medicinal Plant*, **8**(5), 204-213. https://doi:10.3923/rjmp.2014.204.213

Coria-Monter, E., & Durán-Campos, E. (2015). Proximal analysis of seagrass species from Laguna de Términos, Mexico. *Hidrobiológica*, **25**(2), 249-255.

Cristianawati, O., Sibero, M.T., Ayuningrum, D., Nuryadi, H., Syafitri, E., Riniarsih, I., & Radjasa, O.K. (2019). Screening of antibacterial activity of seagrass-associated bacteria from the North Java Sea, Indonesia against multidrug-resistant bacteria. *Aquaculture, Aquarium, Conservation & Legislation*, **12**(4),1054-1064.

De la Torre-Castro, M., & Rönnbäck, P. (2004). Links between humans and seagrasses—an example from tropical East Africa. *Ocean & Coastal Management,* **47**(7-8), 361-387. https://oi:10.1016/j.ocecoaman.2004.07.005

Fajardo, D.R.M., Magdugo, R.P., & Seronay, R.A. (2016). Diversity and taxonomic assessment of marine benthic macrophytes near mining situated area in Cagdianao, Claver, Surigao Del Norte, Philippines. *Journal of Biodiversity and Environmental Sciences,* **8**(6), 49-56. https://innspub.net/diversity-and-taxonomic-assessment-of-marine-benthic-macrophytes-near-mining-situatedarea-in-cagdianao-claver-surigao-del-norte-philippines/

Finney, D. (1952). Probit Analysis: *A Statistical Treatment of the Sigmoid response curve.* Cambridge University Press.

Gatta, R., Nessa, N., & Jompa, J. (2020). Spatial variation of nutritional content in *Enhalus acoroides* (L.f.) royle seeds and seed pods. *Future of Food: Journal on Food, Agriculture and Society,* **8**(1), 1-12. https://doi:10.17170/kobra-202003241096

Gorlenko, C.L., Kiselev, H.Y., Budanova, E.V., Zamyatnin, A.A. Jr., Ikryannikova, L.N. (2020). Plant Secondary Metabolites in the Battle of Drugs and Drug Resistant Bacteria: New Heroes or Worse Clones of Antibiotics?. *Antibiotics*, **9**(4), 170. https://doi:10.3390/antibiotics9040170.

Gómez-Ordóñez, E., & Rupérez, P. (2011). FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds. *Food hydrocolloids,* **25**(6), 1514-1520. https://doi:10.12691/jfnr-3-7-5

Guevarra, B., Clanstro, A., Madulid, R., Aguinaldo, A., Espeso, E., Nonato, M., Quinto, M., Santos, M., Bernas, G., Gonzales, R., Sollevilla, R., & Ysreal, M. (eds) (2005). *A guide book to plant screening, phytochemical and biological*. Manila, Philippines: University of Sto. Tomas Press.

Immaculate, J.K., Lilly, T.T., Patterson, J. (2018). Macro and micronutrients of seagrass species from Gulf of Mannar, India. *MOJ Food Process Technology*, **6**(4), 391-398.https://DOI:10.15406/mojfpt.2018.06.00193

Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance of antibiotics: A guide for clinicians. *Journal of Anesthesiology Clinical Pharmacology,* **33**(3), 300-305.

Kannan, R.R.R., Arumugam, R., & Anantharaman, P. (2011). Chemometric studies of multi-elemental composition of few seagrasses from Gulf of Mannar, India. *Biological trace element research,* **143**(2), 1149-1158. https://DOI:10.1007/s12011-010-8911-y

Kannan, R.R.R., Arumugam, R., Lyapparaj, P., Thangaradjou, T., & Anantharaman, P. (2013). In vitro antibacterial, cytotoxicity and hemolytic activities and phytochemical analysis of seagrasses from the Gulf of Mannar, South India. *Food chemistry,* **136**(3-4), 1484-1489. https://doi:10.1016/j.foodchem.2012.09.006

Kannan, S. (2014). FT-IR and EDS analysis of the seaweeds *Sargassum wightii* (brown algae) and *Gracilaria corticata* (red algae). *International Journal of Current Microbiology and Applied Sciences*, **3**(4), 341-351.

Klangprapun, S., Buranrat, B., Caichompoo, W., & Nualkaew, S. (2018). Pharmacognostical and physicochemical studies of *Enhalus acoroides* (L.f.) Royle (Rhizome). *Pharmacognosy Journal,* **10**(6), s89-s94. https://DOI:10.5530/pj.2018.6s.17

Kolsi, R.B.A., Salah, H.B., Saidi, S.A., Allouche, N., Belghith, H., & Belghith, K. (2017). Evaluation of nutritional value, characteristics, functional properties of *Cymodocea nodosa* and its benefits on health diseases. *Lipids in health and disease,* 16(1), 1-13. https://DOI: 10.1186/s12944-017-0626-z

Manikandan, C. & Kolanjinathan, K. (2016). Antibacterial activity of seagrass therapeutic plants against Gram-negative bacterial pathogens. Indo-Asia *Journal of Multidisciplinary Research,* **6**(2), 930-936. https://DOI:10.21276.iamjmr.2016.2.6.14

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., & McLaughlin, J.L. (1982). Brine Shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, **45**(5), 31-34. https://DOI: 10.1055/s-2007971236

Nandiyanto, A.B.D., Oktiani, R., & Ragadhita, R. (2019). How to read and interpret FTIR spectroscope of organic material. Indonesian *Journal of Science and Technology,* **4**(1), 97-118. https://DOI:10.17509/ijost.v4i1.15806

Natrah, F.M.I., Harah, Z.M., Sidik, B.J., Izzatul, N.M.S., & Syahidah, A. (2015). Antibacterial activities of selected seaweed and seagrass from port Dickson coastal water against different aquaculture pathogens. *Sains Malaysiana,* **44**(9), 1269–1273. https://DOI:10.17576/jsm-2015-4409-08

Newmaster, A.F., Berg, K.J., Ragupathy, S., Palanisamy, M., Sambandan, K., & Newmaster, S.G. (2011). Local knowledge and conservation of seagrasses in the Tamil Nadu State of India. *Journal of Ethnobiology and Ethnomedicine,* **7**(1), 1-17.https://DOI:10.1186/1746-4269-7-37

Orno, T.G., & Rantesalu, A. (2020). In vitro cytotoxicity assays of seagrass (*Enhalus acoroides*) methanol extract from soropia coastal waters southeast sulawesi regency. *Indonesian Journal of Medical Laboratory Science and Technology,* **2**(1), 27-33. https://DOI:10.33086/ijmlst.v2i1.1463

Premarathna, A.D., Ranahewa, T.H., Wijesekera, S.K., Harishchandra, D.L., Karunathilake, K.J.K., Waduge, R.N., Wijesundara, R.R.M.K., Jayasooriya, A.P., Wijewardana, V., & Rajapakse, R.P.V.J. (2020). Preliminary screening of the aqueous extracts of twenty-three different seaweed species in Sri Lanka with in-vitro and in-vivo assays. *Heliyon*, **6**(6). e03918.https://DOI:10.1016/j.heliyon.2020.e03918

Pharmawati, M., & Wrasiati, L.P. (2020). Phytochemical screening and FTIR spectroscopy on crude extract from Enhalus acoroides leaves. *Malaysian journal of analytical science,* **24**(1), 70-77.

Pradheeba, M., Dilipan, E., Nobi, E.P., Thangaradjou, T., & Sivakumar, K. (2011).Evaluation of seagrasses for their nutritional value. *Indian journal of geo-marine sciences*, **1**(40), 105-111.

Qi, S.H., Zhang, S., Qian, P.Y., & Wang, B.G. (2008). Antifeedant, antibacterial, and antilarval compounds from the South China Sea seagrass *Enhalus acoroides*. *Botanica Marina,* **51**(1), 441-447. https://DOI:10.1515/BOT.2008.054

Rasheed, I., Tabassum, A., Khan, U. & Rehman, A. (2019). Fourier Transform Infrared (FT-IR) Spectroscopy of Agar from Red Seaweeds of Karachi Coast. *International Journal of Biology and Biotechnology,* **16**(1), 59-63.

Ratnawati, Nessa, N., Jompa, J., & Rappe, R.A. (2019). Fruits of Enhalus acoroides as a source of nutrition for coastal communities. In IOP Conference Series: *Earth and Environmental Science,* **235**(1), 012073. https:// DOI 10.1088/1755-1315/235/1/012073.

Rengasamy, R.R.K., Radjassegarin, A., & Perumal, A. (2013). Seagrasses as potential source of medicinal food ingredients: Nutritional analysis and multivariate approach. *Biomedicine & Preventive Nutrition,* **3**(4), 375-380.https://doi.org/10.1016/j.bionut.2013.06.011

Rohani-Ghadikolaei, K., Abdulalian, E., & Ng, W.K. (2012). Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. *Journal of food science and technology,* **49**(6), 774-780. https://DOI: 10.1007/s13197-010-0220-0

Short, F.T. & Waycott, M. (2010). *Enhalus acoroides*. The IUCN Red List of Threatened Species 2010: e.T173331A6992567. Downloaded on 13 May 2021.

Sidi, N., Aris, A.Z., Yusuff, F.M., Looi L.J., & Mokhtar, N.F. (2018). Tape seagrass (*Enhalus acoroides*) as a bisoindicator of trace metal contamination in Merambong shoal, Johor Strait, Malaysia. *Marine pollution bulletin,* **126**,113-118.https://doi.org/10.1016/j.marpolbul.2017.10.041

Sumayya, S., & Murugan, K. (2017). Phytochemical screening, RP-HPLC and FTIR analysis of *Kappaphycus alvarezii* (Doty) Doty EX PC Silva: Macro red algae. *Journal of pharmacognosy and phytochemistry,* **6**(1), 325-330.

Svečnjak, L., Biliškov, N., Bubalo, D., & Barišić, D. (2011). Application of infrared spectroscopy in honey analysis. *Agriculturae Conspectus Scientificus*, **76**(3), 191-195.https://doi.org/10.1051bioconf/20181002008

Syed, F., Zakaria, M.H., Bujang, J.S., Ramaiya, S.D., & Hayashizaki, K. (2019). Physicochemical properties of starches from seed and rhizome of *Enhalus acoroides*. *Philippine Journal of Natural Sciences*, **24**, 27-33.

Taba, P., Nafie, N.L., Noor, A., & Muzakkar, M.Z. (2019). Fatty acid map of various species seagrasses on the Donggala Beach. In IOP Conference Series: *Earth and Environmental Science,* **382**(1), 012-018. https://doi:10.1088/17551315/382/1/012018.

Tangon, E., Canencia, O.P., & Del Rosario, R.M. (2019). Phytochemical screening and proximate composition of the seagrasses *Enhalus acoroides* and *Thalassia hemprichii* in the Coastal Waters of Carmen, Agusan Del Norte. *Philippine International Journal of Biosciences,* **15**(1), 1-7. http://dx.doi.org/10.12692/ijb/15.1.1-7

Tangon, E. (2021). Phytochemical screening and proximate analysis of seagrass *Halodule pinifolia* of the coastal waters of Carmen, Agusan Del Norte, Philippines. *International Journal of Modern Pharmaceutical Research,* **5**(2), 75-80.

Vanitha, V., Amudha, P., Bharathi, N., Jayalakshmi, M. & Mohanasundaram, S. (2017). Phytochemical analysis and in-vitro antioxidant screening of sea grass

Enhalus acoroides. International Journal of Research in Pharmaceutical Sciences, **8**(2), 251-258.

Vinayak, R.C., Sabu, A.S., & Chatterji, A. (2011). Bio-prospecting of a few brown seaweeds for their cytotoxic and antioxidant activities. *Evidence-based complementary and alternative medicine,* **2011**, Article ID 673083, 9 pages. https://doi:10.1093/ecam/neq024

Widiastuti, E. L., Rima, K., & Busman, H. (2021). Anticancer Potency of Seagrass (*Enhalus acoroides*) Methanol Extract in the HeLa Cervical Cancer Cell Culture. *Proceedings of the International Conference on Sustainable Biomass*, **202**, 38-42.https://doi.org/10.2991/aer.k.210603.007

Windyaswari, A.S., Purba, J.P., Nurrahmah, S.S., Ayu, I.P., Imran, Z., Amin, A.A., Kurniawan, F., Pratiwi, N.T.M., & Iswantari, A. (2019). Phytochemical profile of sea grass extract (*Enhalus acoroides*): A new marine source from Ekas Bay, East Lombok. In IOP Conference Series: *Earth and Environmental Science,* **278**(1), 012081. https://DOI:10.1088/1755-1315/278/1/01

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Ethnomedicinal study of animals and plants used by Agusanon Manobo in La Paz, Agusan del Sur, Philippines

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ABSTRACT

This study documented the ethnomedicinal use of plants and animals by the Agusanon Manobo in La Paz, Agusan del Sur. A semi-structured questionnaire was used to gather information from 50 informants between the ages of 25-80 years old from two different barangays. The most commonly used medicinal animals were determined using fidelity level (FL) and informant consensus factor (ICF). The medicinal plant species having local importance was determined using the relative frequency of citation (RFC). Ten species of medicinal animals from ten families have been documented during the survey. The most commonly used animals are *Malayophyton reticulatus* (baksan) was used to cure digestive system related ailments, the most preferred animal part to be used is the bile. A total of 39 species with medicinal values were documented for ethnomedicinal plants, there were 27 plant families noted, and mostly from Lamiaceae. Leaves were widely used plant parts and decoction was the most common way of preparation that is administered orally. The ethnomedicanal plants reported having highest RFC values is *Artemisia vulgaris* (hilbas) used for treating respiratory related ailments. It was also mentioned by the Agusanon Manobo that there was no side effects on taking medicinal animals and plants thus, advance scientific investigation is needed to further validate the pharmacological effects and active components present in these medicinal animals and plants.

Keywords: *Artemisia vulgaris, baksan, hilbas, Malayophyton reticulatus*

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1 Introduction

Ethnomedicine examines and translates health-related knowledge and theories that people inherit and learn by living in a culture. Each society has a particular medical culture or "ethnomedicine," which forms the culture's medical common sense, or logic. An ethnomedical system has interrelated notions about the body, the causes and prevention of illnesses, diagnosis and treatment (Quinlan 2011). The information and folk knowledge regarding the medicinal and therapeutic uses of these indigenous animals and plant materials have been handed down from generation to generation through verbal communication. Traditional medicine is used globally and has a rapidly growing economic importance. In developing countries, traditional medicine is often the only accessible and affordable treatment available (Bussmann & Sharon 2006).

The traditional medicinal knowledge of indigenous people across the globe has played an important role in identifying living organisms which are endowed with medicinal values important for treating human and livestock health problems (Kendie et al. 2018). The treatment of human diseases using animals and animal-derived treatments is known as zootherapy (Costa-Neto 1999). Using animals and their products to treat patients suffering from various health problems has a long tradition. It is still prevalent in many parts of the world, even when medical science has achieved great heights (Jugli et al. 2019). Animals and their products had been used traditional treatments, playing significant roles in healing practices, magic rituals, and religious practices amongst various cultures and communities. The use of ethnomedicinal animals is believed to treat various types of ailments (Gomez et al. 2021). Animals are not the only source of ethnomedicine, but also the plants around us. The use of medicinal plants was discovered a long time ago. This is most affordable and easily accessible source of treatment as traditional medicines remained in the primary healthcare system (Omac et al. 2021; Belayneh et al. 2014). An indigenous medicinal plant in the Philippines is in need of assessment in the different vegetation types in terms of species richness, diversity, and ecological status. Plant-base medicines are the beginning of ethnomedicine where pharmaceuticals were developed (Blasco et al. 2014). Tuklas Lunas, a program that gave birth to the *Vitex negundo* (lagundi) and *Blumea balsamifera* (sambong) as medicinal products, continues to pursue drug discovery and development by leveraging on the country's very own biodiversity.

On the other hand, the Manobo tribe is one of the populous indigenous group of people in the island of Mindanao, Philippines and that includes the Agusanon Manobo tribe in the province of Agusan del Sur. Manobo, the name may come from Mansuba from man (person or people) and suba (river), meaning river people. An indigenous community seems to hold the habitual knowledge of herbal remedies for different minor to chronic diseases (Abbasi et al. 2009). Approximately 80% of the population depends exclusively on animals and plants for their health and healing (Majumdar et al. 2006).

The traditional knowledge has been passed on verbally from one generation to another (Bora et al. 2012). The knowledge on the use of different animals and plants in traditional medicine by different ethnic communities is generally passed orally from one generation to another, and this knowledge is sometimes lost with the death of the elderly knowledgeable person. Nowadays, traditional knowledge system is fast eroding due to urbanization. Also, given that most medicines available in the country are developed abroad and are distributed by multinational companies, these products are usually offered at higher prices which in turn becomes a barrier for Filipinos to access treatment. To develop drugs that are sourced locally makes them more accessible and affordable to communities. So, it is vital to study and document the ethnobiological information regarding the therapeutic use of different animals and plants in traditional medicine among different ethnic communities before the traditional cultures are completely lost (Trivedi 2002). The findings of this research would be of great help to all fields of sciences and in future studies. It will also provide insights on the utilization, management, and conservation of medicinal animals and plants in the area. Thus, the main objective of the study was to assess and identify the ethnozoological and ethnobotanical usage as a medicinal source in treating different diseases. This paper also provides insights on the treatment process of each animal and plant species with notes on the frequency of usage, its effectivity, and dosage which is a vital information that are rarely been presented in this type of study. The informant consensus factor, relative frequency of citation, fedility level of the species and internetwork analysis were also presented to increase the confidence level of the most important organisms with medicinal values used by the Agusanon Manobo tribe in La Paz, Agusan del Sur.

2 Materials and Methods

Description of the study Area

La Paz is a municipality in the landlocked province of Agusan del Sur. The municipality has a land area of 1,481.12 square kilometers or 571.86 square miles which constitutes 14.83% of Agusan del Sur's total area (Figure 1). Its population as determined by the 2015 Census was 28,217. This represented 4.03% of the total population of Agusan del Sur province, or 1.09% of the overall population of the Caraga region. La Paz is composed of 15 barangays, the municipal center of La Paz is situated at approximately 8° 17' North, 125° 49' East, in the island of Mindanao. Elevation at these coordinates is estimated at 25.2 meters or 82.7 feet above mean sea level.

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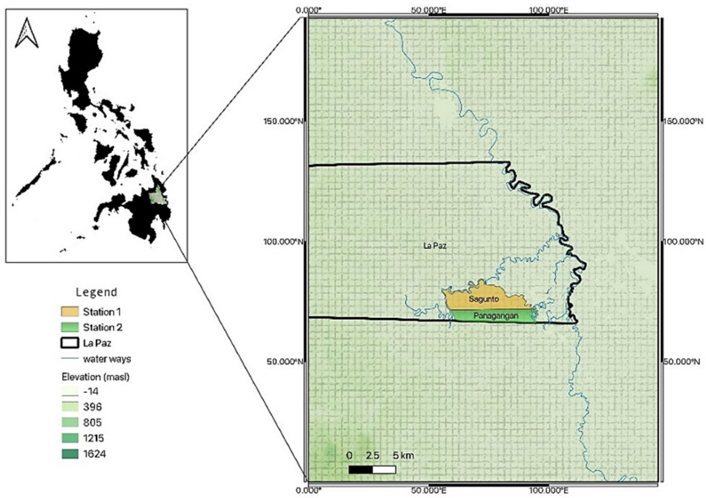

Figure 1. Map of the Philippines showing the location of the study area, La Paz, Agusan del Sur.

Data Collection

Surveys were done in Barangay Sagunto and Barangay Panagangan in La Paz, Agusan del Sur. Respondents (n=50) from both Barangays participated in the ethnomedicinal survey. The respondents were selected using the following criteria: (a) 25 years old and above; (b) availability of the respondents; (c) willingness to participate; (d) being a member of the Agusanon Manobo tribe; and (e) the accessibility of the participant's area (Gomez et al. 2021).

A semi-structured questionnaire on medicinal animal and plant utilization was utilized. This questionnaire was adapted from Gomez et al. (2021) with few modifications. The survey was performed from January to February 2022. The local name, usage, preparation, and specific part of the animal and plant species used for treatment were included in the questionnaire. Also, the frequency of usage, and effectiveness of the medicine in terms of its fast-acting properties when applied were noted. With the help of a local guide, the questions were translated orally into the Agusanon Manobo dialect.

The ethnomedicinal data about the use of animals and plants were collected using participatory rural appraisal (PRA) methods, where the informants also sometimes become the investigator themselves. It involved an informal interview/meetings, open and group discussion among family members (Gomez et al. 2021; Omac et al. 2021).

Ethical consideration

In keeping the views of the local community's cultural values, the data collected were handled with care. The free and prior informed concent was utilized in this study and was based on the NCIP Administrtive Order No. 3 series of 2012. The respondents were informed that the study was carried out for academic reasons and not for commercial purposes. A mayor's permit and barangay's permit was secured before the conduct of the study. An individual informed consent was also obtained from the respondents before the face to face interview, allowing the researcher to collect data for the study (Gomez et al. 2021).

Species identification

Animal identification

The local name of the animals and their associated medical attributes were recorded in this study. Local names of the animals was translated by the local guide into common name. A photo of an animal was shown to the respondents for verification of species they have mentioned. Species identification was done using the published journal article (i.e. Weinell et al. 2019; Diesmos et al. 2015).

Plant identification

During interview, common plants mentioned were observed around the area and were photo-documented for further identification. Plants were initially identified with the help of availabe taxonomic literature (i.e. Fernando et al. 2004; Merrill 1903; Pelser et al. 2011- onwards) and further verified with the help of plant experts in Caraga State University.

Relative frequency of citation (RFC)

Relative frequency of citation index shows the local importance of each species. The RFC value was calculated using the formula RFC=FC/N; where FC is the number of informants mentioning the use of species and N is the number of informants participating in the survey (Vitalini et al. 2012). This RFC index varies from 0 to 1, where RFC index is 0, it means that 0 refers to the animals as useful and when RFC index 1 it indicates that all informants in the survey refer to animals as useful (Mohomodly et al. 2014).

Fidelity level (FL)

The most commonly used animals in the treatment of a particular disease category by informants of the study area were also determined. Fidelity level (FL) was used to identify the residents most preferred species used for treating specific ailments. The FL was calculated using the formula follows:

$$FL=Np/N \times 100$$

where Np is the number of informants that claim as a use of a specific animal species to treat a particular ailment and N is the total number of the informants who utilized the medicinal plant/animal to treat any given disease (Freedman et al. 1986).

Informant consensus factor (ICF)

Informant consensus factor was calculated to estimate the relationships between the numbers of use reports in each category (Nur) minus the number of species used (Nt) and the number use reports in each category. The formula is ICF= Nur – Nt/ Nur – 1, the values of ICF ranges 0 to 1. The high values close to 1 indicates relatively few taxa (usually species) are used by the large proportion of the people, whereas a low value indicates that the informants disagree on the taxa to be used in the treatment within a category of illness (Logan 1986; Heinrich et al. 1998).

Data analysis

Collected data were analyzed using microsoft excel for entering, calculating and analyzing data. The SPSS version 14 was used for statistical computing as well as for graphics and the significance level was considered at alpha = 0.05. To compare the species variation and abundance, the ANOSIM or the analysis of similarity was used (Table 1).

The inter-network analysis was done in this study to gain more network information between the animal and plant species used, the organisms parts used, treatment process and applications, and disease treated. These were analyzed using Gephi software version 0.9.4.

3 Results and Discussion

Medicinal animals recorded

Ten species belonging to 10 families were reported by the Agusanon Manobo to have ethnomedicinal benefits (Table 2). The Manobo of Eastern Mindanao believe that animals such as goats, deer, turtles, monkey, snakes (python and cobra), electric eel, chicken, cat, lizard, and even part of the newly born baby have ethnomedicinal benefits (Gomez et al. 2021). This finding indicate that traditional medicinal practitioners and indigenous people are mostly dependent on the wild sources which might be related to the availability of animals in their area as source of medicine and food. The present investigation shows that various indigenous communities use animals in their traditional medicines, pythons were the most used animal by the Agusanon Manobo. Skin, meat, blood, and marrow are among the python body parts utilized in medicine, in addition to bile. Individuals who consume python bile utilize it by ingesting it directly or drying it, then

Table 1. ANOSIM coefficient interpretation (Sop et al. 2012).

Range	Verbal Interpretation
0.00 to 0.25	No difference/Similar
0.26 to 0.75	Some separation/Some Dissimilarities
0.75 to 0.99	Well Separated/Wdissimilarities
1	Totally Dissimilar

cutting it into pieces and ingesting it with water or putting it in an empty capsule and drinking it (Gomez et al. 2021; Zulkarnain et al. 2021).

Animal parts and its derivatives used

Results indicate that different parts of medicinal animals and derivatives are used for healing values. It shows that bile (26%) is the most commonly used animal part, followed by blood (18%), feces and egg (11%), liver (10%), flesh (8%), horn (7%), skin (4%), and honey an animal derivatives (2%) (Figure 2).

The snakes that are usually used in Agusanon Manobo are cobras and pythons. Pythons or 'sanca' have been known as domesticated snakes, although not as famous as the cobra. The Agusanon Manobo tribe used the bile of phyton as thier primary source of medicine. Bile is a yellow, orange, or slightly green aqueous fluid that is the "exocrine" secretion of the liver. It forms first in bile canaliculi enclosed between parenchymal cells of the liver and flows continuously into ever enlarging ducts to exit the liver via two hepatic ducts (David et al. 2014). Agusanon Manobo use the python bile by swallowing it directly or drying it, then cut it into pieces and swallowed with water or put in an empty capsule and drunk. The python bile is believed to be able to overcome colds/ runny nose, malaria fever, fever, cough, shortness of breath, aches, and increase body immunity.

Treatment process of medicinal animals

The Agusanon Manobo have six ways of preparing ethnomedicinal animals to treat ailments. This includes ingestion of animal parts (39%), followed by decoction (28%), ointment (20%), topical patching (8%), roasting (3%) and offering (1%) (Figure 3).

Results of this study is also comparable to the reports of Gomez et al. (2021) in the Manobo

Table 2. List of ethnomedicinal animals used by the Agusanon Manobo in La Paz, Agusan del Sur.

Animal family	Scientific name	Local name	Common name	Animal part used	Treatment process	Disease/ ailment treated	Mode of preparation
Anatidae	*Anas platyrhynchos* Linnaeus, 1758	pato	domesticated duck	blood	ingestion	leukemia	Blood drained in the snake body and drink fresh.
Apidae	*Apis dorsata* Fabricius, 1793	putyukan	giant common bee	honey	ingestion	teething	Drink the honey
Bovidae	*Capra hircus* Linnaeus, 1758	kanding	goat	feces	grilled, fried, crushed, decoction	measles, fatigue	Grilled feces is crushed into a powder, boiled with hot water & served as a tea.
Cervidae	*Rusa marianna* Desmarest, 1822	usa	deer	horn	crushed & decoction, paste	wounds, swelling, bites	Horn is boiled with water and served as a tea.
Elapidae	*Ophiophagus hannah* Cantor, 1836	banakon	cobra	blood, skin, flesh, bone	chewing, ingestion	headache, stomachache, diarrhea, spasm, swelling, bites, high blood, diabetes, rheumatism	Blood drained in the snake body and drink fresh.
Gekkonidae	*Hemidactylus frenatus* Dumeril & Bibron, 1836	tuko	common house gecko	bone, flesh, internal organs, skin, tail, blood	grilled, fried, crushed, decoction	headache, fatigue	Grilled & crushed into powder & water added. Served as a tea
Geoemydidae	*Siebenrockiella leytensis* Taylor, 1920	bao	turtle	bone, flesh, internal organs (liver), shell,	decoction, ingestion, steam, bath, fried, ointment	stomachache, asthma, rheumatism, malaria, cough	Turtle's body part is boiled with water and served as a tea.
Hirudinea	*Hirudo medicinalis* Lamarck, 1818	linta	leech	jaw	topical patching	rheumatism	Attached /paste in the affected area
Phasuanidae	*Gallus domesticus* Linnaeus, 1758	manok	domesticated chicken	blood, egg	ingestion, offering	fever, measles	Blood from the chicken is used as an offering
Pythonidae	*Malayophyton reticulatus* Schneider, 1801	baksan	reticulated python	bone, flesh, internal organ (bile), skin, fats, liver	decoction, ingestion, ointment, steam bath	headache, stomachache, fever, diarrhea, asthma, high blood, diabetes, malaria	Decocted bile is taken orally, fats is used as ointment.

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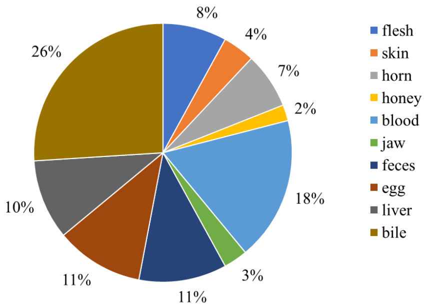

Figure 2. Percentage compostion of animal parts and its derivatives used as medicine by the Agusanon Manobo tribe in selected barangay in La Paz, Agusan del Sur.

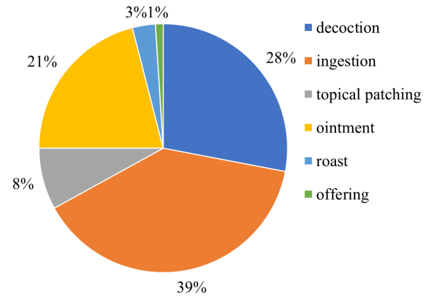

Figure 3. Percentage compostion of treatment process of animal medicine by the Agusanon Manobo tribe in selected barangay in La Paz, Agusan del Sur.

Umayamnon where 11 ways of preparing ethnomedicinal animals. Offering of animals to cure certain diseases was also mentioned in this study which is also unique among Agusanon Manobo tribe. Different indigenous tribal groups also sacrifice animals for different rituals and religious purposes in keeping with their mythological myths and beliefs. The Manobo of Eastern Mindanao believed that epidemics are attributed to sea-demons' malignancy, and by way of appease and lure to this plague-spirits to hurry off with their outbreak, offering was performed in the nearest rivers (Garvan 1927). For the Agusanon Manobo they made offerings like fresh chicken blood to cure a specific ailment caused by the spirit.

Informant consensus factor (ICF) of animals

The Agusanon Manobo have indicated that some diseases are cured through treatment using their identified medicinal animals and these diseases were grouped into different categories. The highest recorded ICF values indicated the best level of agreement among the informants in terms of the use of the medicinal animals species reported. The digestive system related ailments got the highest computed ICF (0.94); (Table 3).

Digestive system disorders are one of the most common types of ailments affecting humans. Several ethnomedicinal studies revealed that the use of medicinal animals by traditional people against digestive system disorders is a common practice throughout the world (Tangjitman et al. 2015). Digestive system disorders, particularly diarrhea, was the fifth leading cause of global mortality, as approximately 100 million people died worldwide in 2012 from these types of disorders. Moreover, in South-East Asia, diarrhea has been the cause of 10% of deaths among children below the age of 5 years. Few investigators have reported on the pharmacological relevance of the animals used in digestive system disorders (Tangjitman et al. 2015).

Fidelity level of animals

Many indigenous people were found to lack formal education, but they have knowledge about the use of local animal resources for traditional medicines. Fidelity levels (FL) demonstrate the percentage of respondents claiming the use of a certain animal or its product for the same ailments. A higher FL of or close to 100 for a specific animal indicates that all of the used reports mentioned the same method for using the animals as medicine for the same diseases. Six animal species with medicinal purposes have FL of 100 (Table 4).

The honey of bee species (*Apis mellifera*) is known to relieve wart, asthma, diarrhea, throat pain, stomachache, cough, and tuberculosis and achieves the highest fidelity level (Kendie et al. 2018). On the other hand, Jaroli et al. (2010) reported that blood of pigeon (*Columba livia*) is used to treat paralysis, in this study the Agusanon Manobo tribe used blood of chicken to treat fever and measles.

Relative frequency of citation (RFC) of animals

Relative frequency of citation (RFC) index was calculated to determine the local importance of each species. The family Pythonidae had the highest number of citations (62%), while the Apidae yielded the lowest RFC value (4%); (Figure 4).

Snakes are among the animals that have most influenced the human psyche since ancient times. In Mexico, the Aztecs made extensive use of the ophidiofauna: several species were consumed, offered to the gods and used as medicines (Fita et al. 2010). Mardiastuti et al. (2021) reported that reptile species indicated that the local people believe that reptiles, in general, were excellent as traditional medicine and able to cure various diseases. The use of reptiles for medicinal purposes has been commonly practiced in many parts of the world, not only in Indonesia. Global reviews on wildlife use by local people elsewhere revealed that at least 284 species of reptiles have been used as traditional medicine (Alves et al. 2013).

Frequency of use, effectivity and animals dosage

The result on the frequency of the use and effectivity of animals to cure diseases, where also determined in this study. *Malayophyton reticulatus* was considered the most widely used animal and the most effective (Table 5). Different people have different ways and basis in utilizing ethnomedine. This study also shows of how much dosage of animal based medicine use to cure certain diseases and how often it was taken. Agusanon

Table 3. Informant Concensus Factor values of the diseases cured by the medicinal animals used by the Agusanon Manobo tribe in selected barangays in La Paz, Agusan del Sur.

Category	Diseases/ ailment	Animal family most used	Used citation	Animal taxa used	ICF
Digestive system ailments	Stomachache and diarrhea	Pythonidae	68	5	0.94
Fever ailments	Fever	Phasuanidae	16	2	0.93
Dermatological problems	Wounds, bites, and measles	Bovidae	37	4	0.92
Muscle-skeletal ailments	Spasm and rheumatism	Bovidae	35	6	0.85
Blood-related ailments	High blood, diabetes, and malaria, luekemia	Elapidae	55	9	0.85
Nervous system ailments	Headache	Elapidae	13	3	0.83
Inflammation and body pain	Swelling and teething	Cervidae	9	3	0.75

Table 4. List of fidelity level (FL) for ethnomedicinal animals in treatment of certain ailment used by the Agusanon Manobo tribe in selected barangay in La Paz, Agusan del Sur.

Scientific name	Common/ local name	Disease/ ailment	Frequency of citation	Total number of citation	Fidelity level
Anas platyrhynchos Linnaeus, 1758	duck/ pato	leukemia	4	4	100
Apis dorsata Fabricius, 1793	bee/ putyukan	dental ailment due to teething	2	2	100
Capra hircus Linnaeus, 1758	goat/ kanding	measles spasm	12 10	13 13	92 77
Gallus domesticus Linnaeus, 1758	chicken/ manok	fever measles	8 12	13 13	62 92
Hemidactylus frenatus Dumeril & Bibron, 1836	gecko/ tuko	headache asthma	2 5	5 5	40 100
Hirudo medicinalis Lamarck, 1818	leech/ linta	rheumatism	4	4	100
Ophiophagus hannah Cantor, 1836	banakon/ cobra	headache stomachache diarrhea spasm highblood diabetes malaria	10 6 1 3 13 14 11	17 17 17 17 17 17 17	59 35 6 18 77 82 65
Malayophyton reticulatus Schneider, 1801	phyton/ baksan	headache stomachache fever diarrhea asthma rheuma highblood	1 29 10 22 1 4 2	31 31 31 31 31 31 31	3 94 32 71 3 13 7
Rusa marianna Desmarest, 1822	deer/ usa	wounds swelling bites	8 5 5	8 8 8	100 63 63
Siebenrockiella leytensis Taylor, 1920	turtle/ bao	stomachache asthma diabetes	10 12 1	12 12 12	83 100 8

Manobo recommends a daily dosage of 1-10 grams for *Malayophyton reticulatus* (baksan) and *Ophiophagus hannah* (banakon), and 1-20 grams for *Siebenrockiella leytensis* (bao) (Table 5). Baksan and banakon are taken once a day, while bao is taken twice a day.

In the ancient medicine, every part of the turtle is consumed, such as their meat, as well as their skin, heads, eggs, shells and even their blood, urine, and bile. Turtle meat is also believed to enrich your blood and cool your body, causing turtle soup to be recommended by alternative medicinal practitioners like Sheng-Nong for menopausal symtoms such as night sweats hot flashes and irritability (Crandall 2014).

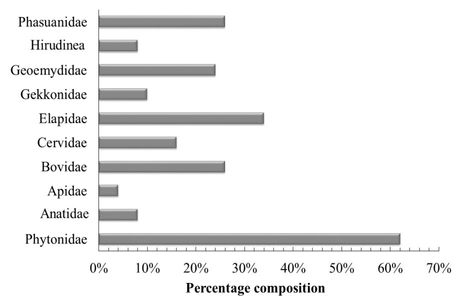

Figure 4. Relative Frequency of Citations of Animals mentioned by the Agusanon Manobo tribe in selected barangay in La Paz, Agusan del Sur.

Analysis of similarity (ANOSIM) and internetwork analysis (INA) of medicinal animals

The analysis of similarity in animals between Barangay Panagangan and Sagunto shows that there are some similarities in animals used (ANOSIM R= 0.26). The findings of this ethnozoology study suggested that the traditional zootherapeutic remedial measures followed and practiced by the Agusano Manobo tribe of La Paz, Agusan del Sur plays an important role in their primary healthcare. The documentation of this indigenous information on animal-based medicines should be very helpful in the formulations of strategies for sustainable management and conservation of bio-resource as well as providing potential for novel drug discoveries.

The data obtained from the respondents were further analyzed to have an overview of what animals (orange node coded with A) having different internetwork relationships in terms of: (a) animal parts used (yellow nodes coded with AP); (b) treatment process and application (green nodes coded with T); and (c) disease treated (blue nodes coded with D). Internetwork analysis shows that the animal having great medicinal importance is the *Malayophyton*

Table 5. Frequency of use, effectivity of the mentioned ethnomedicinal and animal dosage used by the Agusanon Manobo tribe in selected barangay in La Paz, Agusan del Sur.

Scientific name	Local name	Frequency of Use		Effectivity			Animal Dosage: Gram		
		Always	Sometimes	Fast (1 - 12 hrs)	Moderate (13 - 24 hrs)	Slow (Above 24 hrs)	No Idea	1 to 10	11 to 20
Malayophyton reticulatus Schneider, 1801	baksan	29	2	30	1	-	2	29	-
Hirudo medicinalis Lamarck, 1818	linta	-	4	2	2	-	4	-	-
Siebenrockiella leytensis Taylor, 1920	bao	11	1	10	1	-	2	-	12
Anas platyrhynchos Linnaeus, 1758	pato	-	4	-	1	3	-	4	-
Apis dorsata Fabricius, 1793	putyukan	-	2	-	-	2	-	2	-
Gallus domesticus Linnaeus, 1758	manok	10	3	12	1	1	1	10	2
Rusa marianna Desmarest, 1822	usa	4	4	8	-	-	-	8	-
Ophiophagus hannah Cantor, 1836	banakon	5	12	14	1	1	2	15	-
Capra hircus Linnaeus, 1758	kanding	12	1	1	10	1	1	12	-
Hemidactylus frenatus Dumeril & Bibron, 1836	tuko	-	5	-	4	1	2	2	3

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reticulatus (A10) having variuos unique interconnections (Figure 5).

Medicinal plants species

Thirty nine species from 27 families were documented to have ethnomedicinal uses (Table 6). In the Philippines, indigeneous people passed their knowledge about the use of plants as medicine to their next generation through oral tradition that is why until now the practice is still present especially to the tribe living outside the city (Gruyal et al. 2014; Ahmad et al. 2011).

Majority of plant family recorded in this study belongs to Lamiaceae (12%), followed by Malvaceae (10%), Asteraceae and Poaceae (8%), Convolvulaceae and Euphorbiaceae (5%) (Figure 6). These results are in agreement with the study of Fiscal (2017) on medicinal plants in Laguna, which also showed Lamiaceae as the botanical family with the highest number of plant species.

Plant part used

Different parts of the plants were used by the Agusanon Manobo tribe to cure various diseases. Among the recorded parts, leaves (50%) are the most commonly used for Agusanon Manobo tribe in La Paz followed by fruit (14%), stem (10%), wood (7%), bark (6%), whole plant (5%), flower (3%), sap and, root crop (2%), and seeds (1%) (Figure 7).

Leaves are found to be the most commonly used part of the plant for herbal medication. One reason for this is that leaves are the easiest to take and they preserve the wholeness of the plants as they are easily regenerated, unlike stems and roots. Moreover, important chemical compounds such as tannins, essentials oils, and flavonoids are stored in the leaves at high concentrations (Morilla et al. 2014). Many ethnobotanical studies not only in the Philippines documented similar results in which leaves are the most commonly used plant parts. Because leaves are easy to gather and not destructive to the plants thus, helping maintaining its sustainability for future use (Tantengco et al. 2018; Bhatta & Datta 2018).

Treatment process of medicinal plants

The Agusanon Manobo has six (6) ways of preparing medicinal plants depending on how

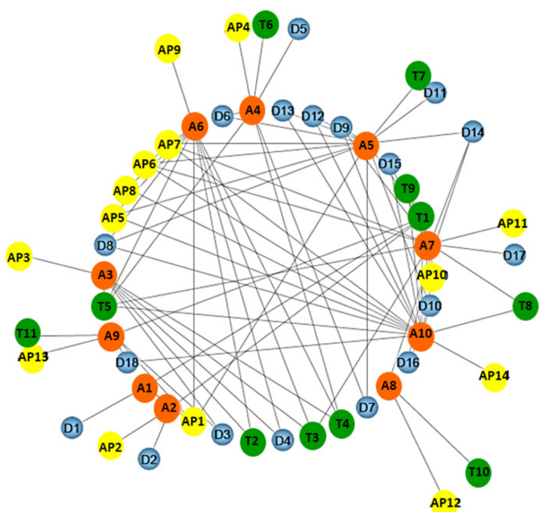

Figure 5. Internetwork relationship between disease treated, animal parts and derivative used as medicine, its treatment process and applications, and disease/ailment treated and ethnomedicinal animals used by the Agusanon Manobo tribe of La Paz Agusan del Sur.

Animal species coding:A1. *Anas platyrhynchos* Linnaeus, 1758; **A2**. *Apis dorsata* Fabricius, 1793; **A3**. *Capra hircus* Linnaeus, 1758; **A4**. *Rusa marianna* Desmarest, 1822; **A5**. *Ophiophagus hannah* Cantor, 1836; **A6**. *Hemidactylus frenatus* Dumeril & Bibron, 1836; **A7**. *Siebenrockiella leytensis* Taylor, 1920; **A8**. *Hirudo medicinalis* Lamarck, 1818; **A9**. *Gallus domesticus* Linnaeus, 1758; **A10**. *Malayophyton reticulatus* Schneider, 1801; **Animal parts used coding: AP1.** blood; **AP2**. honey; **AP3**. feces; **AP4**. honey; **AP5**. skin; **AP6**. flesh; **AP7**. bone; **AP8**. internal organs; **AP9**. tail; **AP10**. liver; **AP11**. shell; **AP12**. jaw; **AP13**. egg; **AP14**. fats; **Treatment process and applications coding: T1.** ingestion; **T2**. grilled; **T3**. fried; **T4**. crushed; **T5**. decoction; **T6**. paste; **T7**. chewing; **T8**. steam; **T9**. ointment; **T10**. topical patching; **T11**. offering; **Disease treated coding: D1**. leukemia; **D2**.dental ailment due to teething; **D3**. measles; **D4**. fatigue; **D5**. wounds; **D6**. swelling; **D7**. bites; **D8**. headache; **D9**. stomachache; **D10**. diarrhea; **D11**. spasms; **D12**. high blood; **D13**. diabetes; **D14**. rheumatism; **D15**. asthma; **D16**. malaria; **D17**. cough; **D18**. fever; **D19**. UTI, **D20**. toothache; **D21**. relapse; **D22**. dengue; **D23**. *Tinea versicolor*; **D24**. Anti-tetanus; **D25**. anti-bacterial; **D26**. ulcer; **D27**. inflammation; **D28**. flatulence; **D29**. muscle spasms; **D30**. schistosomiasis ; **D31** weight loss

Table 6. Plants with ethnomedicinal uses according to the Agusanon Manobo in La Paz, Agusan del Sur.

Plant Family	Scientific name	Local name	Distribution status	Part Used	Treatment Process	Diesease/ ailment Treated	Mode of Preparation
Acanthaeae	*Graptophyllum pictum* (L.) Griff	atay-atay	cultivated	leaf	decoction	Cough, Fever	Boiled with water and serve like a tea
Annonaceae	*Annona muricata* Linn.	rabana	wild, cultivated	leaf	decoction	Diarrhea, UTI	Boiled with water and serve like a tea
Arecaceae	*Cocos nucifera* Linn.	lubi	wild, cultivated	leaf, root, flower, fruit	decoction, ingestion, paste	Wounds, toothache, UTI	Coconut water is taken orally
Asteraceae	*Artemisia vulgaris* Linn.	hilbas	wild, cultivated	leaf	decoction, paste	Cough, Fever	Boiled with water and serve like a tea
Asteraceae	*Blumea balsamifera* Linn.	sambong	wild, cultivated	leaf	decoction	Cough, fever, headache	Boiled with water and serve like a tea
Asteraceae	*Chromolaena odorata* Linn.	hagonoy	wild	leaf	paste, crushed	Wound	Crushed leaf and apply leaf extract directly to the open wound
Bixaceae	*Bixa orellana* Linn.	suwetis	wild, cultivated	leaf	decoction	Diarrhea	Boiled with water and serve like a tea
Cannabaceae	*Cannabis* sp. Linn.	wild mariwana	wild, cultivated	sap/ juice	paste, ingestion, ointment	Wound, Anti-tetanus,	Filtrate drop on the wound
Caricaceae	*Carica papaya* Linn.	kapayas	wild, cultivated	sap/ juice, root	paste, decoction	Wound, Relapse	Drop on the wound, Boiled
Clusiaceae	*Garcinia mangostana* Linn.	mangoestin	cultivated	fruit	decoction	Diarrhea, UTI, Highbood,	Boiled with water and serve like a tea
Convolvulaceae	*Cordia dichotoma* Forst. f.	anunang	wild, cultivated	bark, leaf, wood	decoction	Cough	Boiled with water and serve like a tea
Convolvulaceae	*Ipomoea batatas* (L.) Lam.	ganas	cultivated	leaf, stem	decoction	Diarrhea	Boiled with water and serve like a tea
Crassulaceae	*Kalanchoe pinnata* (Lam.) Pers.	hanlilika	wild, cultivated	leaf, flower	paste, crushed	Wound, Toothache	Paste on the wound or painful teeth
Cucurbitaceae	*Momordica charantia* Linn.	paliya	cultivated	leaf, fruit	decoction	Diarrhea	Boiled with water and serve like a tea
Ebenaceae	*Diospyros seychellarum* Hiern	sagay	wild, cultivated	leaf, stem	decoction	UTI	Boiled with water and serve like a tea
Euphorbiaceae	*Euphorbia hirta* Linn.	tawa- tawa	wild	whole plant	decoction	Cough, Relapse, Dengue	Boiled with water and serve like a tea
Euphorbiaceae	*Jatropha curcas* Linn.	tuba- tuba	wild, cultivated	leaf, bark	decoction	Headache, Stomachache, Fever	Boiled with water and serve like a tea
Fabaceae	*Senna alata* Linn.	asunting	wild, cultivated	leaf	paste, crushed	Tinea versicolor	Paste on the affected area
Lamiaceae	*Origanum vulgare* Linn.	kalabo	cultivated	leaf	decoction	Cough	Boiled with water and serve like a tea
Lamiaceae	*Mentha arvensis* Linn	herbuena	wild, cultivated	leaf	decoction	Cough, Fever	Boiled with water and serve like a tea
Lamiaceae	*Vitex negundo* Linn.	lagundi	wild, cultivated	leaf	decoction	Cough, fever	Boiled with water and serve like a tea
Lamiaceae	*Coleous amboinicus* Lour.	vicks	wild, cultivated	leaf	paste	Wound, anti-bacteria	Paste on the affected area
Malvaceae	*Kleinhovia hospita* Linn.	bitan-ag	wild, cultivated	wood, fruit	decoction	Cough	Boiled with water and serve like a tea
Malvaceae	*Corchorus olitorius* Linn.	saluyot	wild, cultivated	leaf	decoction	Ulcer	Boiled with water and serve like a tea
Malvaceae	*Allium sativum* Linn.	ahos	cultivated	root crop	decoction	Stomachache, Highblood	Boiled with water and serve like a tea
Marantaceae	*Rauvolfia serpentina* (L.) Benth.	lalat chena/ serpentina	wild, cultivated	whole plant	decoction	Arthrites, Flatulence (Panuhot), Muscle spasms (Pamaol)	Boiled with water and serve like a tea

Table 6. Plants with ethnomedicinal uses according to the Agusanon Manobo in La Paz, Agusan del Sur.

Plant Family	Scientific name	Local name	Distribution status	Part Used	Treatment Process	Diesease/ ailment Treated	Mode of Preparation
Meliaceae	*Swietenia macrophylla* King	mahugani	wild	leaf	decoction	Stomach ache	Boiled with water and serve like a tea
Menispermaceae	*Tinospora rumphii* Boerl	panyawan	wild, cultivated	stem, leaf	decoction	Diarrhea	Boiled with water and serve like a tea
Moraceae	*Artocarpus heterophyllus* Lam.	langka	wild, cultivated	fruit	ingestion	Shistosomiasis, Relapse	Mixed with alcohol and drink
Moringaceae	*Moringa oleifera* Lam.	kamungay	cultivated	leaf, wood	decoction	Cough, headache, stomach ache	Boiled with water and serve like a tea
Myrtaceae	*Psidium guajava* Linn.	bayabas	wild, cultivated	leaf	decoction, paste, chewing, crushed	Wound	Boiled with water, Crushed and paste on the wound
Poaceae	*Paspalum conjugatum* (P.J. Berguis) Roxb.	sagbot/ carabaw grass	wild	whole plant	decoction	Diarrhea, UTI, Diabetes	Boiled with water and serve like a tea
Poaceae	*Saccharum offinarum* Linn.	tubo	cultivated	stem	decoction, ingestion	Cough, Malaria	Boiled with water and serve like a tea
Poaceae	*Cymbopogon citratus* (DC.) Stapf	tanglad	cultivated	whole plant	decoction	Hypertension/ highblood	Boiled with water and serve like a tea
Rubiaceae	*Neolamarckia cadamba* (Roxb.) Bosser	kadamba	wild, cultivated	leaf	decoction	Wound, malaria, anti-bacterial	Boiled with water and serve like a tea
Rutaceae	*Citrus microcarpa* (Bunge) Wijnands	agri	cultivated	fruit	ingestion	Cough	Whole fruit is taken orally
Solanaceae	*Capsicum frutescens* Linn.	sili	wild, cultivated	fruit	ingestion	Weight loss	Whole fruit is taken orally
Zingiberaceae	Curcuma longa Linn.	dulaw	wild, cultivated	root crop	decoction	Stomachache, arthrites, UTI	Boiled with water and serve like a tea

they apply it to the patient. Decoction is the most common way of preparing medicinal plants which (65%), followed by paste (15%), crushed (13%), ingestion (4%), ointment (2%), and lastly chewing (1%) (Figure 8).

Decoction was the most common and widely used method of concentrated brew extracted from simmered plants in boiling water (Israel & Youngkin 1997). The decoction was reported to be beneficial for it extracts its bioactive constituent when heated or boiled (Blasco et al. 2014). According to the study of Tandon et al. (2008), decoction allows to dissolute of the plant material to extract the components that are active in water-soluble like volatile organic compounds and other various chemical substances.

Informant consensus factor (ICF) of plants

The documented ethnomedicinal plants were used to treat 22 different ailments which were grouped into 8 different categories. The ICF values ranged from 0.80 to 0.89. The highest ICF value was for digestive system ailments (0.89) while the lowest ICF value was (0.80) for inflammation and pain (Table 7).

Similar of Ghorbani et al. (2011) found that digestive system disorders had the highest ICF value, whereas Juárez-Vázquez et al. (2013) noted this as their second highest observed ICF value. This ranking might be due to a lack of adequate knowledge about the pathogenicity of disease and drinking polluted water. This is one fact that although there is now access to the government healthcare system, the people living in this part of the Philippines did not lose their values and traditions in using plants as the primary source of medicine (Ramalingam et al. 2016). The ICF values reported will guide medical practioners to focus and prioritize treating diseases in this category for this is what the tribe and the communinty needed more of the medical attentions.

Relative frequency of citation (RFC) of Plants

The relative frequency of citation computation also revealed that *Artemisia vulgaris* has the highest RFC (54%) values, followed by *Diospyros seychellarum* and *Kleinhovia hospita* (40%), *Euphorbia hirta* (34%), *Origanum vulgare* (32%), and *Jatropha curcas* (26%) (Table 8). *Artemisia vulgaris, Euphorbia hirta,* and *Origanum vulgare*

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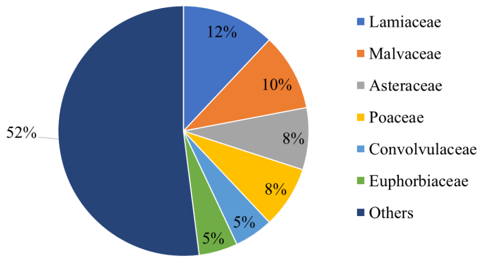

Figure 6. Percentage composition of plant families mentioned by the Agusanon Manobo tribe in selected barangay in La Paz, Agusan del Sur.

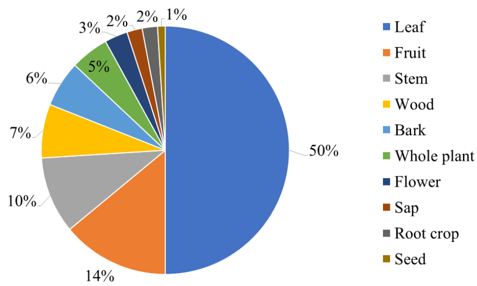

Figure 7. Percentage composition of plant parts used for medical purposes used by the Agusanon Manobo tribe in selected barangay in La Paz, Agusan del Sur.

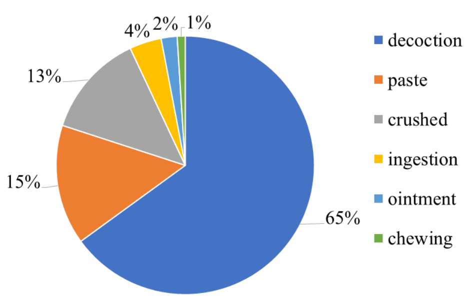

Figure 8. Percentage composition of the treatment process of the medicinal plants used by the Agusanon Manobo of La Paz, Agusan del Sur.

are used as treatments for cough and fever, abdominal pain, and body pains according to the study of Ong and Kim (2014).

Relative frequency of citation (RFC) index was calculated to determine the local importance of each species. The highest value of RFC index was scored by *Artemisia vulgaris* which demonstrate the importance of plant species to the Agusanon Manobo in La Paz, Agusan del Sur, as it is mentioned by a higher number of informants.

Frequency of use, effectivity and plant dosage

Among the ethnomedicinal plants mentioned, *Artemisia vulgaris* has been reported to have the the fastest activity to cure cough and cold (Table 9). It is one of the best known species of this genus, which has a widespread distribution in the natural habitats worlwide (Europe, Asia, North and South America, and Africa). For many centuries, this species has been mainly used for treating gynecological ailments and gastrointestinal diseases (Wichtl 2004). This study also shown the result of how much dosage of plants was to cure diseases and how often it was taken. It shows that plant dosage varies from each respondents. The *Klieinhovia hospita* (bitan-ag) has the dosage of 1-10 g and was taken thrice a day, while *Artemisia vulgaris* (hilbas) has a dosage of 11-20 g and was taken thrice a day. Interestingly wild mariwana was also mentioned in this report and it can be taken once, twice and thrice (Table 9).

Most of the Agusanon Manobo in La Paz, Agusan del Sur believed that there is no side effects on taking medicinal plants. The dose depends on the users, the one who prepares the herbs for medicine, similar to the study of Omac et al. (2021). While this study did not uncover any apparent side effects associated with the use of the medicinal plants investigated, further research is required to investigate their long-term effects on human health. It is therefore crucial to regulate the consumption of these plants as medicines and examine their active metabolic properties to ensure their safe and effective use.

Analysis of similarity (ANOSIM) and Internetwork analysis (INA) in medicinal plants

ANOSIM analysis of medicinal plants used by Agusanon Manobo in La Paz Agusan del Norte showed no significant difference in plant usage between Barangay Panagangan and Barangay Sagunto with an ANOSIM R coefficient of 0.014. ANOSIM compares dissimilarities between and within groups, with R values close to one indicating significant dissimilarities within groups and R values close to zero indicating no significant difference within and between groups (Clarke and Gorley 2001).

There are many factors for these similarities of plants species used as medicinal plants. Indigenous information of healing using plants was easily pass

Table 7. Informant Consensus Factor (ICF) values of the diseases cured by medicinal plants reported by the Manobo Agusanon tribe of La Paz, Agusan del Sur.

Category	Diseases/ ailment	Plant most used	Numbersed citation	Animal taxa used	ICF
Digestive system ailments	diarrhea, stomachache, shistosomiasis, ulcer	*Tinospora rumphii* Boerl	132	16	0.89
Respiratory ailments	cough	*Artemisia vulgaris* Linn.	106	14	0.88
Urological ailments	urinary tract infection	*Diospyros seychellarum* Hiern	41	6	0.87
Blood related ailments	malaria, dengue, diabetes, highblood	*Euphorbia hirta* Linn.	65	9	0.87
Nervous system ailments	headache	*Jatropha curcas* Linn.	67	10	0.86
Fever ailments	fever	*Kleinhovia hospita* Linn.	73	11	0.86
Dermatological ailments	wound, Rashes,anti-tetanus, tinea versicolor, bite	*Neolamarckia cadamba* (Roxb.) Bosser	95	15	0.85
Inflammation and pain	toothache,muscle pain,athrites, swelling	*Rauvolfia serpentina* (L.) Benth.	58	12	0.80

Table 8. Relative Frequency of Citation of medicinal plants mentioned by the Agusanon Manobo tribe in La Paz, Agusan del Sur.

Scientific name	Local name	RFC	Scientific name	Local name	RFC
Artemisia vulgaris Linn.	Hilbas	0.54	*Cymbopogon citratus* (Bunge) Wijnands	Tanglad	0.12
Kleinhovia hospita Linn.	bitan-ag	0.40	*Momordica charantia* Linn.	Paliya	0.12
Diospyros seychellarum Hiern	Sagay	0.40	*Allium sativum* Linn.	Ahos	0.12
Euphorbia hirta Linn.	tawa-tawa	0.34	*Cocos nucifera* Linn.	Lubi	0.10
Origanum vulgare Linn.	Kalabo	0.32	*Paspalum conjugatum* (P.J. Berguis) Roxb.	Karabaw grass	0.08
Jatropha curcas Linn.	tuba-tuba	0.26	*Hibiscus rosa-sinensis* Linn.	Gumamela	0.08
Blumea balsamifera Linn.	Sambong	0.24	*Coleous amboinicus* Lour.	Vicks	0.08
Neolamarckia cadamba (Roxb.) Bosser	Kadamba	0.24	*Saccharum offinarum* Linn.	Tubo	0.08
Mentha arvensis Linn.	Herbuena	0.20	*Garcinia mangostana* Linn.	Mangoestin	0.08
Rauvolfia serpentina (L.) Benth.	lalat chena	0.20	*Artocarpus heterophyllus* Lam.	Langka	0.06
Chromolaena odorata Linn.	Hagonoy	0.18	*Tinospora rumphii* Boerl	Panyawan	0.06
Cordia dichotoma Forst. f.	Anunang	0.16	*Curcuma longa* Linn.	luyang dilaw	0.06
Cannabis sp. Linn.	wild mariwana	0.16	*Kalanchoe pinnata* (Lam.) Pers.	Hanlilika	0.06
Annona muricata Linn.	Rabana	0.14	*Corchorus olitorius* Linn.	Saluyot	0.04
Vitex negundo Linn.	Lagundi	0.14	*Carica papaya* Linn.	Kapayas	0.04
Moringa oleifera Lam.	Kamungay	0.14	*Capsicum frutescens* Linn.	Sili	0.04
Swietenia macrophylla King	Mahugani	0.14	*Citrus microcarpa* (Bunge) Wijnands	Agri	0.04
Graptophyllum pictum (L.) Griff	atay-atay	0.12	*Ipomoea batatas (L.)* Lam.	Ganas	0.04
Psidium guajava Linn.	Bayabas	0.12	*Bixa orellana* Linn.	Suwetis	0.02
Senna alata Linn.	Asunting	0.12			

Table 9. Frequency of use, effectivity of cited ethnomedicinal plants, and dosage of medicinal plants mentioned by the Agusanon Manobo tribe in La Paz, Agusan del Sur.

Scientific name	Local name	Frequency of Use		Effectivity Category			Plant Dosage: Grams		Daily Dosage		
		Always	Sometimes	Fast (1 - 12 hrs)	Moderate (13 - 24 hrs)	Slow (Above 24 hrs)	1 to 10	11 to 20	Once	Twice	Thrice
Kleinhovia hospita Linn.	bitan-ag	15	5	2	4	14	16	4	3	5	12
Cordia dichotoma Forst. f.	anunang	6	2	-	4	4	8	-	-	4	4

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Table 9. Frequency of use, effectivity of cited ethnomedicinal plants, and dosage of medicinal plants mentioned by the Agusanon Manobo tribe in La Paz, Agusan del Sur.

Scientific name	Local name	Frequency of Use		Effectivity Category			Plant Dosage: Grams		Daily Dosage		
		Always	Sometimes	Fast (1 - 12 hrs)	Moderate (13 - 24 hrs)	Slow (Above 24 hrs)	1 to 10	11 to 20	Once	Twice	Thrice
Origanum vulgare Linn.	kalabo	10	6	-	6	10	10	6	-	6	6
Graptophyllum picyum (L.) Griff	atay-atay	5	1	-	1	5	1	5	2	1	3
Mentha arvensis Linn.	herbuena	8	2	5	2	3	3	-	-	-	3
Euphorbia hirta Linn.	tawa- tawa	15	2	15	2	15	10	6	1	10	3
Diospyros seychellarum Hiern	sagay	20	-	1	15	4	15	-	3	5	10
Artemisia vulgaris Linn.	hilbas	22	5	17	5	4	15	12	7	9	11
Corchorus olitorius Linn.	saluyot	2	-	-	-	2	2	-	-	-	-
Cannabis sp. Linn.	wild mariwana	1	7	1	2	5	2	-	2	2	2
Jatropha curcas Linn.	tuba- tuba	13	-	-	3	10	4	9	-	3	10
Carica papaya Linn.	kapayas	-	2	-	-	2	2	-	2	-	-
Paspalum conjugatum (P.J. Berguis) Roxb.	Karabaw grass	4	-	-	2	2	2	2	1	1	2
Artocarpus heterophyllus Lam.	langka	-	3	-	-	3	1	1	-	-	2
Tinospora rumphii Boerl	panyawan	-	3	-	-	3	1	2	1	-	2
Rauvolfia serpentina (L.) Benth.	lalat chena	8	2	2	1	7	8	2	2	2	6
Blumea balsamifera Linn.	sambong	11	1	-	8	4	12	5	1	2	16
Hibiscus rosa-sinensis Linn.	gumamela	1	3	-	1	3	3	1	1	-	2
Neolamarckia cadamba (Roxb.) Bosser	kadamba	10	2	-	-	12	2	10	1	1	10
Curcuma longa Linn.	luyang dilaw	3	¬-	-	3	-	2	1	-	1	2
Annona muricata Linn.	rabanna	7	-	-	4	3	6	1	2	1	4
Psidium guajava Linn.	bayabas	6	-	-	6	-	4	2	1	3	2
Vitex negundo Linn.	lagundi	7	-	-	7	-	1	4	1	-	4
Coleous amboinicus Lour.	vicks	4	-	-	-	4	3	1	-	2	2
Saccharum offinarum Linn.	tubo	4	-	-	3	1	1	3	1	2	1
Kalanchoe pinnata (Lam.) Pers.	hanlilika	4	-	-	-	4	1	3	-	-	4
Garcinia mangostana Linn.	mangoestin	4	-	-	2	2	2	2	-	-	4
Senna alata Linn.	asunting	6	-	-	2	4	3	3	1	2	3
Capsicum frutescens Linn.	sili	1	-	1	-	-	1	1	-	-	2
Bixa orellana Linn.	suwetis	1	-	-	-	1	1	-	-	-	1
Chromolaena odorata Linn.	hagonoy	7	2	-	5	4	1	3	4	-	-
Cymbopogon citratus (Bunge) Wijnands	tanglad	5	1	-	1	5	3	-	-	-	3

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Scientific name	Local name	Frequency of Use		Effectivity Category			Plant Dosage: Grams		Daily Dosage		
		Always	Sometimes	Fast (1 - 12 hrs)	Moderate (13 - 24 hrs)	Slow (Above 24 hrs)	1 to 10	11 to 20	Once	Twice	Thrice
Citrus macrocarpa (Bunge) Wijnands	agri	2	-	-	-	1	2	-	1	-	1
Cocos nucifera Linn.	lubi	5	-	-	4	1	2	2	3	1	-
Ipomoea batatas (L.) Lam.	ganas	¬-	2	-	0	2	1	1	1	1	-
Moringa oleifera Lam.	kamungay	5	-	-	2	-	2	2	2	1	1
Momordica charantia Linn.	paliya	2	-	-	-	2	2	2	-	-	4
Allium sativum Linn.	ahos	4	2	-	2	4	3	2	-	2	3
Swietenia macrophylla King	mahugani	5	2	-	2	5	2	5	2	2	3

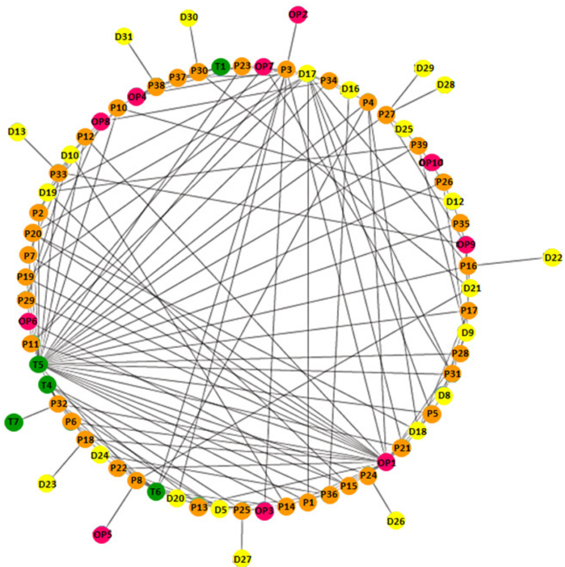

Figure 9. Internetwork relationship between disease treated, plant parts used as medicine, its treatment process and applications, and disease/ ailment treated and ethnomedicinal plants used by the Agusanon Manobo tribe of La Paz Agusan del Sur.

Plant species coding: P1. *Graptophyllum pictum* (L.) Griff; **P2**. *Annona muricata* Linn.; **P3**. *Cocos nucifera* Linn.; **P4**. *Artemisia vulgaris* Linn.; **P5**. *Blumea balsamifera* Linn.; **P6**. *Chromolaena odorata* Linn.; **P7**. *Bixa orellana* Linn.; **P8**. *Cannabis* sp. Linn.; **P9**. *Carica papaya* Linn.; **P10**. *Garcinia mangostana* Linn.; **P11**. *Cordia dichotoma* Forst. f.; **P12**. *Ipomoea batatas* (L.) Lam.; **P13**. *Kalanchoe pinnata* (Lam.) Pers.; **P14**. *Momordica charantia* Linn.; **P15**. *Diospyros seychellarum* Hiern; **P16**. *Euphorbia hirta* Linn.; **P17**. *Jatropha curcas* Linn.; **P18**. *Senna alata* Linn.; **P19**. *Origanum vulgare* Linn.; **P20**. *Mentha arvensis* Linn.; **P21**. *Vitex negundo* Linn.; **P22**. *Coleous amboinicus* Lour.; **P23**. *Kleinhovia hospita* Linn.; **P24**. *Corchorus olitorius* Linn.; **P25**. *Hibiscus rosa-sinensis* Linn.; **P26**. *Allium sativum* Linn.; **P27**. *Rauvolfia serpentine* (L.) Benth.; **P28**. *Swietenia macrophylla* King; **P29**. *Tinospora rumphii* Boerl; **P30**. *Artocarpus heterophyllus* Lam.; **P31**. *Moringa oleifera* Lam.; **P32**. *Psidium guajava* Linn.; **P33**. *Paspalum conjugatum* (P.J. Berguis) Roxb.; **P34**. *Saccharum offinarum* Linn.; **P35**. *Cymbopogon citratus* (Bunge) Wijnands; **P36**. *Neolamarckia cadamba* (Roxb.) Bosser; **P37**. *Citrus microcarpa* (Bunge) Wijnands; **P38**. *Capsicum frutescens* Linn.; **P39**. *Curcuma longa* Linn.; **Plant parts used coding: OP1**. leaves; **OP2**. roots; **OP3**. flower; **OP4**. fruit; **OP5**. sap; **OP6**. bark; **OP7**. wood; **OP8**. stem; **OP9**. whole plant; **OP10**. root crop; **Treatment process and applications coding: T1**. ingestion; **T2**. grilled; **T3**. fried; **T4**. crushed; **T5**. decoction; **T6**. paste; **T7**. chewing; **T8**. steam; **T9**. ointment; **T10**. topical patching; **T11**. offering; **Disease treated coding: D1.** leukemia; **D2**. teething; **D3**. measles; **D4**. fatigue; **D5**. wounds; **D6**. swelling; **D7**. bites; **D8**. headache; **D9**. stomachache; **D10**. diarrhea; **D11**. spasms; **D12**. high blood; **D13**. diabetes; **D14**. rheumatism; **D15**. asthma; **D16**. malaria; **D17**. cough; **D18**. fever; **D19**. UTI, **D20**. toothache; **D21**. relapse; **D22**. dengue; **D23**. *Tinea versicolor*; **D24**. anti-tetanus; **D25**. anti-bacterial; **D26**. ulcer; **D27**. inflammation; **D28**. flatulence; **D29**. muscle spasms; **D30**. shistosomiasis; **D31** weight loss through several generations. The geographical location of the two barangay is near with each other in which the sharing of knowledge about the usage and other information about medicinal plants has been easily passed through by indigenous communities for several decades (Olowa et al. 2012). Also the usage of plants as medicine are easily being shared compared to animals because plants are more common and diverse in the area and easily be determine its locations.

The internetwork analysis of plants species, plant parts used, its treatment process and applications, and disease treated was also analyzed (Figure 9). Each orange nodes (coded P) is represnted by 39 plants species having medicinal uses. Each nodes is connected with other nodes creating an internetwork relationship to: (a) plant parts used (pink nodes coded with OP); (b) treatment process and applications (green nodes coded T); and (c) common diseases (yellow nodes coded with D). For plant parts most used as depicted by the different interconnections is the leaves (OP1). For treatment process most nodes having variuos connection is decoction (T5) and cough (D17) is the most treated diseases in Agusanon Manobo tribe in La Paz Agusan del Sur.

4 Conclusions and Recommendations

This study shows that there is a prevailing knowledge and usage of traditional medicinal animals and plants treating certain ailments and health problems among the Agusanon Manobo in La Paz, Agusan del Sur . Ten animals were used for medicinal purposes, with *Malayophyton reticulatus* (baksan) being the most versatile in treating various diseases. *Artemisia vulgaris* (hilbas) was the most frequently cited medicinal plant among the 39 species documented. Overcollection and usage of these commonly used animals and plants could lead to population decline, highlighting the need for proper regulation. This documentation provides a catalog of useful animals and plants of the Agusanon Manobo in La Paz, Agusan del Sur and will also serve as a physical record of their culture. This may also be beneficial for those people who are still relying on the usage of medicinal animals and plants as their primary source of healthcare. This study may lead somehow to discover new and effective medicinal animals and plants that are urgently needed by the world today, such as the cure for COVID-19.

It is crucial to document, preserve, and manage indigenous knowledge as modern treatment methods advance, risking the loss of important traditional medicine knowledge with the passing of elders and experts. Community-based management and public awareness are essential to sustainably conserve medicinal species, particularly those with commercial value. Furthermore, further research should investigate the potential medicinal properties of various animal and plant species.

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5 Statement of Conflict of Interest

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

6 Literature Cited

Abbasi, A.M., Khan, M.A., Ahmad, M., Zafar, M., Khan, H., & Muhammad, (2009). Medicinal plants used for the treatment of jaundice and hepatitis based on socio-economic documentation. *African Journal Biotechnology,* **8** (8): 1643-1650.

Ahmad C., Shapi, K., Matengu H., & Ashekel E., (2011). Ethnobotanical Study of Indigenous Knowledge on Medicinal Plants Used by Traditional Healers in Oshkoto Region, Namia. *Journal of Ethnobiology and Ethnomedicine,* **7**:10.

Alves, R.R.N., Souto, W.M.S., Barboza, R.R.D., & Bezerra, D.M.M. (2013). Animals in Traditional Folk Medicine. Implications for Conversation, *Springer*, Berlin, Heidelberg, p.493.

Belayneh, A., & Bussa, N. F., (2014). Ethnomedicinal plants used to treat human ailments in the prehistoric place of Harla and Dengego valleys, eastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **10**(1): 18.

Bhatta, R., & Datta, S., (2018). Ethnomedicinal Survey of Plants among khasia Tribes residing in Kamalganj, Maulvibazar, Bangladesh. *Journal of Pharmacy Practice and Education*, **2**:3.

Blasco, F.A., De Guzman, G.Q., & Alejandro, G.J.D., (2014). A survey of ethnomedicinal plants in Surigao del Sur Mountain Range, Philippines. *International Journal Pure Applied Biosciences,* **2**, 166-172.

Bora, A., Devi, P., & Borthakur, S.K., (2012). Phyto-remedies of jaundice-A traditional approach on Majuli, Special reference to Satra culture people, Assam. *Asian Journal Plant Science Research,* **2**(6): 664-669.

Bussmann, R.W., & Douglas-Sharon, D., (2006). Traditional medicinal plant use in Northern Peru: tracking two thousand years of healing culture. *Journal of Ethnobiology* and Clarke KR, Gorley RN (2001). Primer v5: user manual/tutorial. Primer-E Ltd: Plymouth. p91

Christakis, N.A., Fowler, J.H., (2013). Social contagion theory: examining dynamic social networks and human behavior. *Statistics in Medicine,* **32**:556–577.

Clarke KR, Gorley RN (2001). Primer v5: user manual/ tutorialPrimer-E Ltd: Plymouth.Coasta-Neto Eraldo, (2003). Animal-based medicines: biological prospection and the sustainable use of zootherapeutic resources. *Annals of the Brazilian Academy of Sciences,* **77**(1): 33-43

Cleland, E. E., Collins, S. L., Dickson, T. L., Farrer, E. C., Gross, K. L., Gherardi, L.A., & Suding, K. N. (2013). Sensitivity of grassland plant community composition to spatial vs. temporal variation in precipitation. *Ecology*, **94**(8): 1687-1696.

Costa-Neto, E.M., (1999). Healing with animals in Feira de Santana city, Bahia, Brazil, *Journal Ethnopharmacol,* **65**: 225-230.

Crandall, E.,. (2014). The threat of traditional medicine: China's boom may mean doom for turtles. *Mongabay Natures Frontline,* **23**(2): 4–29.

David, Q.H., Wang, Martin, C., & Carey, (2014). Therapeutic uses of animal biles in traditional Chinese medicine: An ethnopharmacological, biophysical chemical and medicinal review. *World Journal of Gastroenterology,* **20**(29): 9952–9975.

Dickson, G., Ghazali, M., Ogden, R., Brown, R., & Auliya, M., (2017). Phylogeography of the reticulated python (*Malayopython reticulatus* ssp.): Conservation implications for the worlds' most traded snake species. *PlosOne*, 1-28.

Diesmos, A.C., Watters, J.L., Huron, N.A., Davis, D.R., Alcala, A.C., Crombie, R.I., Afuang, L.E., Gee-Das, G., Sison, R.V., Sanguila, M.B., Penrod, M.L., Labonte, M.J., Davey, C.S., Leone, E. A., Diesmos, M.L., Sy, E.Y., Welton, L.J., Brown, R.M., & Siler, C.D., (2015). Amphibians of the Philippines, Part I: Checklist of the Species, *Proceedings of the California Academy of Sciences,* Series 4. **62**(20): 457–539.

Fernando, E.S., B.Y. Sun, M. H. Suh, H.Y. Kong & K.S. Koh, (2004). Flowering plants And ferns of Mt. Makiling. Korea: ASEAN-Korea *Environmental Cooperation Unit (AKECU).* (https://agris.fao.org/agrissearch/search.do?recordID=XF2015039509). Retrieved on May 2021.

Fiscal, R., (2017). Ethnomedicinal Plants used by Traditional Healers in Laguna, Philippines. *Asia Pacific Journal of Multidisciplinary Research,* **5**(4): 132–137.

Fita, D.S., Costa Neto, E.M., & Schiavetti, A., (2010). 'Offensive' snakes: cultural beliefs and practices related to snakebites in a Brazilian rural settlement. *Journal of Ethnobiology and Ethnomedicine*, **6**: 1-13.

Freedman J., Dafni, A., Pelewitch, D., & Yaniv, Z., (1986). A Preliminary classification of the healing potential of medicinal plants based on rational analysis of an ethnopharmacological field survey among bedouins in the Negev Desert israel. *Journal of Ethnopharmacology,* **16**:275-287.

Garvan, J.M., (1927). A Survey of the Material and Sociological Culture of the Manobo in Eastern Mindanao, *AnthroSource*, **29**:4.

Ghorbani, A., Langenberger, G., Feng, L., & Sauerborn, J. (2011). Ethnobotanical study of medicinal plants utilised by Hani ethnicity in Naban River Watershed National Nature Reserve, Yunnan, China. *Journal Ethnopharmacol*, **134**, 651.

Gomez, E.D., Gamalinda, E.F., Along, A.A., Ombat, L. A., & Almadin, F.J.F. (2021) "Ethnozoological Study of Traditional Medicinal Animals and Their Products Used by the Manobo Umayamnon Tribe in the Southern Philippines." *Journal of Ecosystem Science and Eco-Governance,* **3**(1):25–36.

Gruyal, G.A., del Rosario, R., & Palmes, N.D., (2014). Ethnomedicinal Plants used by Residents in Northern Surigao del Sur, Philippines. *Natural Products Chemistry & Research,* **2**:4.

Heinrich, M., Ankli, A., Frei, B., Weimann, C., & Sticher, O., (1998). "Medicinal plants in Mexico: healers' consensus and cultural importance," *Social Science and Medicine,* **47**:11, 1859–1871.

Israel, D., & Youngkin, E.Q., (1997). "Herbal therapies for perimenopausal and menopausal complaints." *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy,* **17**.5: 970-984.

Jaroli, D.P., Mahawar, M.M., & Vyas, N., (2010). An ethnozoological study in the adjoining areas of Mount Abu wildlife sanctuary, *Indian Journal Ethnobiology Ethnomedicine*, **6**:6.

Juárez-Vázquez, M. D. C., Carranza-Álvarez, C., Alonso-Castro, A. J., González- Alcaraz, V. F., Bravo-Acevedo, E., & Chamarro-Tinajero, F. J., (2013). Ethnobotany of medicinal plants used in Xalpatlahuac, Guerrero, México. *Journal Ethnopharmacol*. **148**,521–527.

Jugli S, Chakravorty J, & Meyer-Rochow VB. 2019. Zootherapeutic uses of animals and their parts: an important element of the traditional knowledge of the Tangsa and Wancho of eastern Arunachal Pradesh, North-East India. *Environment, Development and Sustainability,* **22**, 4699–4734

Kendie, F., Mekuriaw, S., & Dagnew, M., (2018). Ethnozoological study of traditional medicinal appreciation of animals and their products among the indigenous people of Metema Woreda, North Western Ethiopia, *Journal of Ethnobiology and Ethnomedicine,* **14**:37, 1-12.

Logan, M. H., (1986). "Informant consensus: a new approach for identifying potentially effective medicinal plants," in Plants in Indigenous Medicine and Diet, *Biobehavioral Approaches, ed N. L. Etkin (Bedford Hills, NY: Redgrave publishers),* 91–112.

Majumdar, K., Saha, R., Datta, B.K., & Bhakta, T., (2006). Medicinal plants prescribed by different tribal and non-tribal medicine man of Tripura State. *Indian Journal Traditional Knowledge,* **5**(4): 559-562.

Mardiastuti, A., Masy'ud, B., Ginoga, L.N., Sastranegara, H., & Sutopo, (2021). Short Communication: Wildlife species used as traditional medicine by local people in Indonesia. *Biodiversitas*, **22**:1, 329-327.

Merrill, E.D., (1903). A dictionary of the plant names of the philippines islands. Manila Bureau of Public Printing.(https://www.biodiversitylibrary.org/item/42176#Page/8/mode/1up). Retrieved on December 2020.

Mohomodly, M.F., & Mootsamy, A., (2014). Quantitative Ethnozoological assessment of Traditional used animals based therapies in the island of Mauritus. *Journal in Ethnopharmacology,* **154**(3):847-857

Morilla, L.J., Sumaya, N.H., Rivero, H., & Madamba, R.S., (2014). Medicinal Plants of the Subanen in Dumingag Zamboanga del Sur Philippines. Proceedings of the International Conference on Food, *Biological and Medical Sciences,* 28–29.

Olowa, L.F., Torres, M.A.J., Aranico, A.C., & Demayo, C.G., (2012). Medicinal Plants used by the Higaonon, Illigan City, Mindanao, Philippines. *Advances in Environmental Biology,* **6**(4):1442-1449.

Omac, M. G., Along, A. A., Ligalig, R. J., Rosal, J. J., & Almadin, F. J. F. (2021). "Medicinal plants used by the local communities of Sitio Lomboyan, Barangay Guinabsan, Buenavista, Agusan del Norte, Philippines." *Annals of Studies in Science and Humanities*, **3**(1):1–14.

Ong, H.G., Kim, Y.D. 2014. Quantitative ethnobotanical study of the medicinal plants used by the Ati Negrito indigenous group in Guimaras Island, Philippines. *Journal of Ethnopharmacology,* **157**: 228–242.

Pelser P.B., Barcelona J.F., & Nickrent (eds) D.L., (2011-onwards). Co's Digital Flora Of the Philippines. www.philippineplants.org

Quinlan, M.B., (2011). Ethnomedicine, in A Companion to Medical Anthropology. 1st edition. *Merril Singer & Pamela I. Erickson. Blackwell Publishing Ltd.* 381-403.

Ramalingam P., Vijayakumar, S., Prabhu, S., & Morvin Yabesh, J. G. E. (2016). Quantitative traditional knowledge of medicinal plants used to treat livestock diseases from Kudavasal taluk of Thiruvarur district, Tamil Nadu, India. *Brazillian Journal of Pharmacognosy,* **26**(1), 109–121

Sop, T.K., Oldeland, J., Bognounou, F., Schmieldel, U., & Thiombiano, A., (2012). Ethnobotanical knowledge and valuation of woody plants species: a comparative analysis of three ethnic groups from the sub-Sahel of Burkina Faso. Environment, Development and Sustainability: *A Multidisciplinary Approach to the Theory and Practice of Sustainable Development,* **14**:627–649.

Tandon, Sudeep, & Rane (2008). "Decoction and hot continuous extraction techniques." *Extraction technologies for medicinal and aromatic plants*, **93**

Tangjitman, K., Wongsawad, C., Kamwong, K., Sukkho, T., Trisonthi, C., (2015). Ethnomedicinal plants used for digestive system disorders by the Karen of northern Thailand. *Journal Ethnobiology Ethnomedicine,* **11**, 27.

Tantengco, O.A., Condes, M.L., Estadilla, H.T & Ragragio, E.M., (2018). Ethnobotanical survey of medicinal plants used by Ayta communities in Dinalupihan, Bataan, Philippines. College of Medicine, University of thePhilippines Manila. *A Multifaceted Journal in the field of Natural Products and Pharmacognosy,* **10**(5):859-870.

Trivedi, P.C., 2002. Ethno-medicinal Plants of Rajasthan State India. In: Ethnobotany, Trivedi, P.C. (Ed.). *Aavishkar Publishers and Distributors, Jaipur*, **412**.

Vitalini, S., Iriti, M., Ciuchi, D., Puricelli, C., Segali, A., & Fico, G., (2012). Traditional knowledge on medicinal and food plants used in Valsa Giacomo (Sondrio, Italy) An Alphine ethnobotanical study. *Journal in Ethnopharmacology,* **145**:517-529.

Weinell, J.F., Hooper, E., Leviton, A.E., & Brown, R,M., (2019). Illustrated key to the snakes of the Philippines. *Procedings of the California Academy of Sciences,* **4**(66): 1-49.

Wichtl, M. (2004). Herbal drugs and phytopharmaceuticals: a handbook for practice on a scientific basis 2004, No.Ed.3, 704

Zulkarnain, Z., Sholikhah, I.Y.M., & Dewi, T.F., (2021). Efficacy and safety in consuming python bile: a literature study, IOP Conference Series: *Earth and Environmental Science,* **637**:1-8.

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