International Journal of Agricultural Technology 2016 Vol. 12(7.1):1635-1643
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Evaluation of The Phytochemical, Antioxidant and Cytotoxic Properties of Tungog (*Ceriops Tagal)*, A Philippine Mangrove Species

May V. Basco*, Jo Aileen Marie R. Mallare, Sandrah Lynne A. Ruiz, James Kennard S. Jacob and Cynthia C. Divina

Department of Biological Sciences, College of Arts and Sciences,Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

Basco, M. V. , J. A M.R. Mallare, S. L. A. Ruiz, J. K. S. Jacob and C.C. Divina (2016). Evaluation Of The Phytochemical, Antioxidant And Cytotoxic Properties Of Tungog (*Ceriops tagal)*, A Philippine Mangrove Species. International Journal of Agricultural Technology 12(7.1):1635-1643.

Ceriopstagal or tungog is a species of mangrove that gives Philippine local coconut wine (*tuba)* its maroon-orange color and bitter taste.. This current research was conducted to evaluate the phytochemical content, antioxidant and cytotoxic activities of crude extracts of barks of *C. tagal*. Phytochemical analysis was carried out using the standard protocols and antioxidant activities was done using the DPPH radical scavenging assay using the 98% standard antioxidant absorbance. The cytotoxic activities of *C. tagal* was assessed using brine shrimp lethality assay. Tannins, flavonoids and cardiac glycosides were found present in C. tagal barks, while 93% absorbance was detected against the 98% standard for the test of antioxidant activities conducted. Moreover, *C. tagal*exhibited moderately cytotoxic activities against *A. salina* at 425 ppm.

Keywords: antioxidant, *Ceriopstagal*, phytochemicals, brine shrimp, mangrove, bark

Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro *et al.,* 2000). According to the World Health Organization (WHO) in 20- 08, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. In the Philippines, the importance of the country's diverse medicinal plants lies not only in their chemotherapeutic value in traditional healthcare but also in their potential as sources of new chemical entities for drug discovery. Although the country boasts of its remarkable biodiversity and rich cultural traditions of plant use, scientific understanding of

* **Coressponding Author:** May V. Basco; **E-mail:** mei.basco@yahoo.com

medicinal plants remains largely unexplored and pharmacological investigation of the Philippine flora gained momentum only recently (Vital and Rivera, 2009).

Mangrove plants remain underutilized in the Philippines. The dried bark from *C. tagal* (tungog) is traditionally used for coconut wine, locally known as tuba, because it is effective in delaying fermentation. Extract from *C. tagal* is also used to color rice, to dye thick leather, cotton, nylon, mats, etc. and to prevent scales from forming in water boilers. Essentially, this group of plants has been used in traditional medicine and has been proven to contain inhibitory properties against several human and animal pathogens. Several species of mangroves are able to produce bioactive compounds with control microbial growth. Mangrove extracts can also be the possible sources of antifungal and antioxidant.

The use of medicinal plants for primary health care is a substantial help to developing countries like the Philippines in meeting their drug requirements. Medicinal plant use however, has been based mostly on empirical grounds. There is a need for scientific validation of such empirical knowledge (Philippine Council for Health Research and Development, 1991).

Thus, this study was conducted to evaluate the phytochemical contents, cytotoxic and antioxidant properties of *C. tagal*.

Materials and Methods

Extract Preparation. Barks of *C. tagal* (tungog) was collected from the Leyte, Southern Philippines. The bark were sundried for 48 hours prior storage and were oven dried for six (6) hours at 70℃ and powdered using mortar and pestle. For the preparation of ethanol extracts, 50g grams of powdered *C. tagal* was soaked in 250 ml 95% ethanol for 48 hours at room temperature. The suspension was filtered and filtrate was subjected to a rotary evaporator at 200rpm. For the preparation of aqueous extracts, 50g of powdered *C. tagal*barks were suspended in a 250ml sterilized distilled water for 24 hours. Suspension was filtered using Wattman's filter paper No. 01 and filtrate was stored in sterile amber bottles and kept refrigerated prior to use.

Qualitative Analysis of Phytochemical Composition. The ethanol extract of *C. tagal* was assayed qualitatively for its phytochemical properties. Standard protocols for test for alkaloids, saponins, flavonoids, tannins, cardiac glycosides, steroids, terpenoids and resins were done.

*Test for Alkaloids***.** Five (5) grams of dried ethanol extract of *C. tagal*was prepared in a beaker and 200 ml of 10% CH3CO2H in C_2H_5OH was added.

The mixture was covered and allowed to stand for 4 hours. The mixture was then filtered and the extract was allowed to concentrate in a water bath until it reached ¼ of the original volume. Concentrated NH_4OH was then added. For the presence of alkaloids, the mixture was observed for the formation of white precipitate or turbidity.

*Test for Saponins***.** Ethanol extract of *C. tagal* (2 grams each) was boiled together with 20ml distilled water in a water bath and then filtered. Filtered sample (10ml) was mixed with 5 ml distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion.

Test for Flavonoids. A few drops of 1% NH_3 solution was added to the ethanol extract of *C. tagal*in a tube. A yellow coloration indicated the presence of flavonoids.

*Test for Tannins***.** Dried ethanol extract (0.5 grams) was boiled into 20ml of distilled water in a test tube and then filtered in a conical flask using filter paper. 0.1% Fe was added CL_3 to the filtered samples and observed for a brownish green or a blue black coloration for positive result.

*Test for Cardiac Glycosides***.** One (1) ml of concentrated H_2SO_4 was prepared in a test tube. Then 5 ml of ethanol extract of the samples was mixed with 2 ml of glacial acetic acid (CH_3CO_2H) containing 1 drop of $FeCl_3$.The mixture was carefully added to a prepared 1 ml of concentrated hydrogen sulfate so that the H_2SO_4 will be underneath the mixture. The test tube was observed for the presence of a brown ring.

Test for Steriods**.** 0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Terpenoids**.** Using Salkowski's test,0.2 g of the extracts was dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase indicated the deoxy sugar characteristics of cardenolides.

Test for Resins**.** Test for resins 5 ml of copper acetate solution was added to 5 ml of the extracts. The resulting solution was shaken vigorously and allowed to separate. A green coloured solution is an evidence of the presence of resin

Assessment of Antioxidant Activities of *C. tagal.* To determine the antioxidant property of *C. tagal*, the Free-Radical Scavenging Activity using DPPH Scavenging Assay was conducted. The DPPH radical scavenging activity assay of Chan, Lim and Omar (2007) was modified. Different

concentration of extracts (1.5 mL) were mixed to DPPH solution (2.5 mL) prepared by dissolving 6 mg DPPH in 100 mL methanol to afford 1, 10, 100, 300, and 1000 ppm. The control was prepared by adding methanol instead of the extract. Ascorbic acid was used as positive control. The mixture was vigorously shaken and was left in the dark for 30 min before reading its absorbance at 517 nm in a visible spectrophotometer (Cole Parmer Spectrophotometer 1200). The DPPH radical scavenging activity was calculated using the following formula:

$$\%\ scavenging\ activity\ = (1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ control}) \times 100$$

The EC50 was estimated using nonlinear regression curve fitting function, sigmoidal dose-response of GraphPad Prism 6.0.

Assessment of Cytotoxicity using Brine shrimp lethality assay. The cytotoxic property of *C. tagal* bark ethanol extract was monitored using the brine shrimp lethality test described by Syahami *et al.* (2010) with some modifications. Hatched eggs of *Artemia salina* were obtained from Bureau of Fisheries and Aquatic Resources, Central Luzon State University, Science City of Muñoz, Nueva Ecija. Ten newly hatched nauplii were introduced in plates with 5 ml of artificial sea water, hence, 10 shrimps per dilution. The treatments were done in triplicates. The extract concentrations tested were left uncovered under a lamp. The number of death naupli was monitored 6^{th}, $12^{th,}$ 18^{th} and 24^{th} hours. Percent mortality was documented and LC_{50} was determined through Probit Analysis.

Results

Phytochemical Screening

Phytochemicals are naturally occurring constituents of plants and various researches have been done to prove the bioactivities of such constituents. The result obtained on phytochemical analysis of the *C. tagal* bark is shown in Table 1. Results revealed that among the eight (8) phytochemicals tested, flavonoids, cardiac glycosides and tannins were detected in traceable and appreciable amounts in its bark.

Table 1. Phytochemical composition of *Ceriops tagal* (Tungog) bark using ethanol extract

Phytochemicals	Makabuhay
Alkaloids	Absent
Saponins	Absent
Flavonoids	Present
Tannins	Present
Cardiac Glycosides	Present
Steroids	Absent
Terpenoids	Absent
Resins	Absent

Antioxidant Properties of C. tagal

DPPH is a stable radical that has been used to evaluate the antioxidant activity of plant and microbial extracts (Ka ħko ïnen, 1999). In the current study, DPPH scavenging activity was found in *C. tagal* bark extract. The radical scavenging activity of the sample was close to that of the Ascorbic Acid, which was used as the standard for this test. The result showed the *C. tagal* sample with 93% scavenging activity at effective concentration (EC_{50}) of 7.05 ppm versus 98% for the standard Ascorbic acid at effective concentration (EC_{50}) of 2.55 ppm (Figure 1)

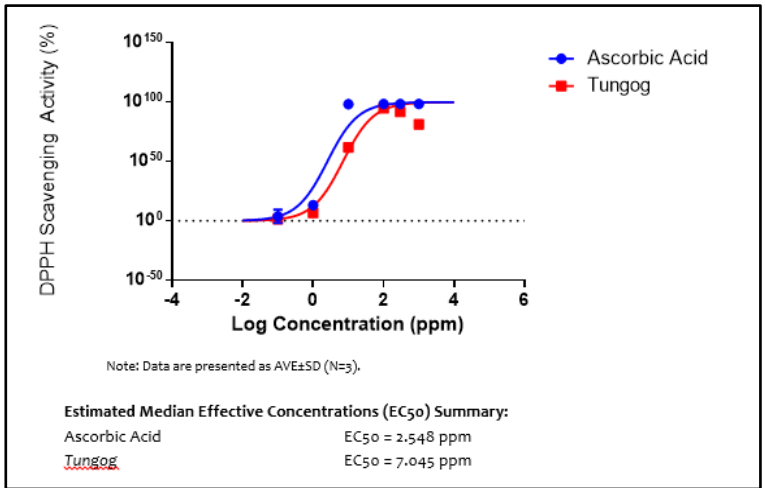

Figure 1. Free-radical scavenging activity of *C. tagal*bark extract measured using the DPPH assay

Cytotoxic Effect of C. tagal using Brine Shrimp Lethality Assay

The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials. (McLaughlin *et al.*, 1991). Concentrations of *C. tagal* extract used ranged from 10 – 10000 ppm with pure artificial sea water was used as control. Figure 2 shows the mortality rate of nauplii exposed to different concentrations of *C. tagal* extracts. It was observed that the highest mortality rate was displayed at 10,000 ppm which makes it lethal to 63.33% of the test organism after 24 hours of observation. On the other hand, 10 ppm exuded 20% lethality while 100 ppm showed 26.67% lethality. The linearity of the results shows that the higher the concentration of *C. tagal*, the higher the mortality rate. Computed LC_{50} using linear equation is at 409.52 ppm wherein, according to McLaughlin and Rogers (1998), LC50 of ≤ 249 µg/mL as highly toxic; LC50 of 250 - 499 µg/mL is moderately toxic; and LC50 of 500-1000 µg/mL is mildly toxic and values above 1000 µg/mL are non-toxic, therefore, the computed LC_{50} value 409.52 ppm is considered moderately toxic.

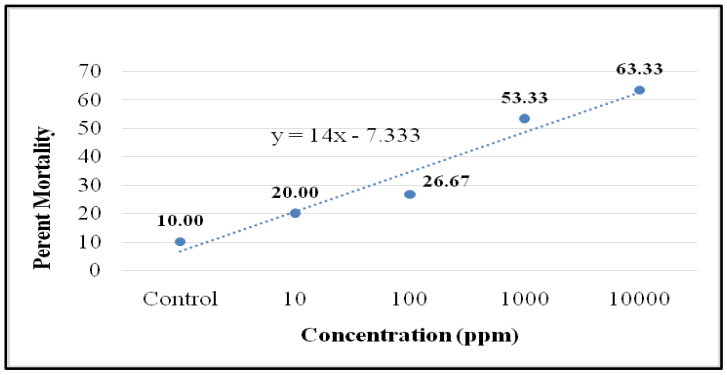

Figure 2. Cytotoxic Lethality Assay of *C. tagal* ethanol extract to *A. salina*

Discussion

Although the knowledge of how these phytochemicals provide medicinal value to humans reveals recent scientific understanding, the use of plants and plant extracts to heal, relieve pain and promote good health dates back to before the beginnings of medical science. Phytochemical screening of *C. tagal* barks was conducted in the present study to provide a strong

foundation on the existing practice of *C. tagal* bark being used by locals to treat various ailments. These phytochemicals exhibit significant human healthful benefits. For instance, tannins which is a broad class of compounds fight cavities, diarrhea, and some even protect heart diseases and cancer. Tannins are well known for their astringent property thus, they had been used as a base for several herbal treatments. They act by iron deprivation, hydrogen bonding or specific interaction with proteins such as enzymes, cell envelopes and complex formation with polysaccharides (Dharmananda, 2003; Hisanori *et al.,* 2001). Herbs with tannins as their component are used for treating intestinal disorders such as diarrhea and dysentery (Just *et al.,* 1998). On the other hand, therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure. Although in the study of Jacob and David (2016), it was stated that the presence of tannins in plants can also cause negative effect on productivity, reduced nutrient availability, reduced digestibility, impaired digestive physiology and maybe mucosal perturbations for those who will intake such plants.

The result suggests that there is a strong relation on the biochemical components of *C. tagal* barks with its scavenging ability using DPPH assay. Previous studies have reported that phenolic compounds including tannin possess antioxidant activity (Hatano *et al.*, 1989; Satoshi & Hara, 1990; Wang *et al.,* 1999). Furthermore, on the study of Aziz *et al.* (2014) on the evaluated capacity of guava (*P. guajava*) to scavenge DPPH free radicals, it was proven that while the concentration of tannin increases the capacity to scavenge free radical also increases and vice versa. *C. Tagal* bark extract can then be considered as a good source of natural antioxidant for medicinal uses although, further studies are necessary to isolate active principles responsible for the overall antioxidant activity of the extract.

Brine shrimp nauplii have been previously utilized in various bioassay systems. Among these applications have been the analyses of pesticidal residues, mycotoxins, stream pollutants, anesthetics, dinoflagelate toxins, morphine-like compounds, carcinogenicity of phorbol esters and toxicants in marine environment. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay (Meyer *et al.,* 1982; Sam TW, 1993). According to National Cancer Institute (NCI, USA), this bioassay is significantly used as a pre-screening tool for antitumor drug development due to the significant correlation between the brine shrimp assay and in vitro growth inhibition of human solid tumor cell lines (Anderson *et al*., 1991).

To conclude, *C. tagal* extract was found to have flavonoids, cardiac glycosides and tannins. It was also found to have good antioxidant property and is moderately toxic to brine shrimps. Investigation of acute toxicity and

phytochemical analysis is very important in the toxicological analysis of any medicinal plant since a toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. Toxicity results from animals will be crucial in definitively judging the safety of *C. tagal* extracts if they are found to have sufficient potential for development into pharmacological products. Furthermore, the phytochemical components, antioxidant and cytotoxicity activity possessed by *C. tagal* in this study suggests that bark from this mangrove, when further investigated, can lead to the development of a potential drug that could treat various kinds diseases and could be beneficial for full utilization by the local community and may also be of interest to research institutes in the development of new drugs.

Acknowledgement

The researchers would like to thank Sir Fitz Gerard Mamuad and Agricultural Technology and Business Incubator for allowing the researchers to use their facilities during the study period. Gratitude is given to Department of Science and Technology- SEI, Philippines for their support and assistance.

References

Anderson JE, Goetz CM, McLaughlin JL and Suffness M (1991). A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumor prescreens. *Phytochem Analysis 2*: 107-11.

Dharmananda, S. (2003). Gallnuts and the uses of tannins in Chinese medicine. Proceedings of Institute for Traditional Medicine, Portland, OR, USA.

Hatano, T., H. Kagana, T. Yasuhara, T. and Okuda. (1989). Two new flavonoids and other constituents in licorice root, their relative astringency and radical scavenging effects. Chem. Pharm. Bull 1988;36:2090-7.

Hisanori A, Kazuyasu F, Osamu Y, Takashi O and Keiji I. (2001) Antibacterial action of several tannins against *Staphylococcusaureus.* Journal of Antimicrobial Chemotherapy. 48:487–491.

Jacob, J.K.and David, E. (2016). Qualitative Phytochemical Analysis and Microbial Inhibitory Activities of Pacific Rain Tree (Samaneasaman (Jacq.) Merr.) Pods

Just MJ, Recio MC, Giner RM, Cuellar MJ,Manez S, Bilia AR and Rios JL (1998). Anti-inflammatory activity of unusual lupinesaponins from *Bupleurumfruticescens.* Pl. Med. J. 64:404–407.

Ka ħko ïnen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauuha, J. P.; Pihlaja, K.; Kujala, T. S.;and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* 1999, *47*, 3954-3962.

McLaughlin JL, Rogers LL, Anderson JE, (1998). The use of biological assay to evaluate botanicals. Drug Inf J, 32:513 - 524.

McLaughlin J, Chang C and Smith D (1991). Proceedings of the 18 th National Seminar and Unesco Workshop on Natural Products. Institute of Advance Studies, University of Malaysia, Malaysia.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE and McLaughlin JL (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med 45*: 31-34.

Nostro A, Germano MP, DÁngelo V and Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol. 30: 379-384

Pierangeli G. (2009). Vital andWindell L. Rivera Journal of Medicinal Plants Research Vol. 3(7), pp. 511-518, July, 2009 . Antimicrobial activity and cytotoxicity of Chromolaenaodorata (L. f.) King and Robinson and Uncariaperrottetii (A. Rich) Merr. extracts

Polistico, E. (2011). Tungog (Barok in Southern Luzon and in Waray). Retrieved from http://pinoyfoodillustrated.blogspot.com/2011_09_01_archive.html