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PHYTOCHEMICAL CONTENT AND TOXICOLOGICAL POTENTIALS OF *Musa textilis*, *Agathis philippinensis* AND *Cinnamomum mercadoi* LEAF EXTRACTS FROM MAT-I, CLAVERIA, PHILIPPINES

MA. VANESSA O. BALANGIAO [a,b*] AND ANGELO MARK P. WALAG [b,c]

[a] Department of Physical Sciences and Mathematics, Mindanao State University – Naawan, Naawan, Philippines.

[b] Department of Science Education, University of Science and Technology of Southern Philippines, Cagayan de Oro City, Philippines.

[c] Center for Advanced Plant Science and Phytotechnologies, University of Science and Technology of Southern Philippines, Cagayan de Oro City, Philippines.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Plants have been well-known to be an excellent source of potentially bioactive compounds. Even with this great importance, modernization and industrialization continually threaten our knowledge of using plants as traditional medicines. In this study, ethanol and ethyl acetate crude extracts of *Musa textilis, Agathis philippinensis,* and *Cinnamomum mercadoi* leaves were investigated for its phytochemical content and toxicological potentials. These plants have been known to be used by indigenous people of Mat-I, Claveria Philippines, for various ailments. Standard methods were utilized to determine the qualitative presence of different phytochemicals while Brine Shrimp Lethality Assay was used to evaluate toxicological potentials. From the results, *A. philippinensis* ethyl acetate extracts and *C. mercadoi* ethanol and ethyl acetate extracts are considered active or slightly toxic at prolong administration. Results of phytochemical screening revealed presence of alkaloids, flavonoids, amino acids, triterpenoids, steroids and terpenoids in *A. philippinensis* ethyl acetate extracts and *C. mercadoi* crude ethanol and crude ethyl acetate extracts. While *A. philippinensis* ethanol extracts is observed to contain flavonoids, amino acids, triterpenoids, steroids and terpenoids. *M. textilis* ethanol extracts is observed to contain flavonoids, amino acids and steroids, while results revealed the presence of

**Corresponding author: Email: mvanolinanbalangiao@gmail.com;*

flavonoids, triterpenoids, steroids and terpenoids in its ethyl acetate extracts. *A. philippinensis* ethyl acetate extracts and *C. mercadoi* ethanol and ethyl acetate extracts showed to be active and contain bioactive components that could be potential for further studies. Further studies are suggested to isolate and characterize these natural products for in-depth bioassay studies.

Keywords: Bioactive compounds; herbal medicine; natural products; secondary metabolites; toxicity.

1. INTRODUCTION

Plants are one of the groups in which living things are classified. They are very useful and important organisms on earth and other living organisms depend on them. Traditionally, plants have been used to sustain human lives and as part of human cultures; as shelter, source of foods and in treating wounds and illness. It brings a great variety of components with relevant biological activities, which are produced as final products of their metabolism [1]. These plants contain many active components and essential oils making it a prospect for more scientific studies. Essential oils make a major contribution to the plants biological activity [2]. Plants have been a valuable source of natural products for maintaining human health [3] thus it became the basis for further pharmaceutical research and developments. It is for this reason, plants have been used traditionally for various purposes, especially in treating mild diseases and disorders.

The use of herbal medicines in the treatment of wounds and diseases has been a practice for centuries [4]. This practice occupy a distinct position right from the primitive period to the present day [5]. The medicinal value of the plants is due to the substances that it contains [6] as plants contain many active components and essential oils that have a physiological effect on the human body. Essential oils in plants play an important role in their biological activity [2]. Amounts of different plant-derived bioactive compounds offer various health benefits [7] and are used to treat illnesses [8]. Knowledge of the utilization and efficacy of plants is an important part of the culture and is the basis of pharmaceutical research and advancement. Due to the growing recognition of the medical importance of herbal medicines [9] and their affordability, the demand for herbal medicines is increasing [7]. Although herbal medicines are used since antiquity, there needs to be solid convincing evidence concerning their clinical safety and efficacy, thus the need for exploratory studies like this [10].

The medicinal plants used in this study were Abaca (*Musa textilis),* Almaciga *(Agathis philippinensis)* and Kalingag *(Cinnamomum mercadoi).* The sap of abaca is used on wounds to induce blood clotting; the decoction of the young shoot is used for cough and diarrhea. The methanol extract of fruits of *Musa textilis* significantly reduces the blood glucose level of a person with high blood sugar [11]. Boiling of the roots, bark and resins of Almaciga, locally termed as *salumayag*, is used in treating relapse or locally termed as *'bughat'*. The steam of the burning resin is used in treating asthma. Kalingag is used to treat various diseases, including relapses, bloating, flatulence, diarrhea, indigestion, vomiting, rheumatism, colds, fever, headache, sinusitis, asthma, injuries. The bark extract of kalingag showed a reduction of diarrheal feces that is comparable to the standard drug [12]. Methanolic extracts from the bark and leaves of kalingag appear to be a potential source of anti-oxidants [13].

There are a limited number of studies done on these three herbal plants. Studies on abaca include the characterization of fibre, evaluation of flexural properties and strength of the fibre, isolation of the p-hydroxycinnamate esters from the acetone leaf extracts of abaca, glucose tolerance test of the methanolic extracts of fruits of abaca, which result, reported that there is a significant decrease of the blood-glucose levels in glucose-loaded mice [11]. The crude methanol extracts of the bark of kalingag, *Cinnamomum mercadoi Vidal*, was studied for its toxicity against albino mice, phytochemical content, analgesic and antibacterial activity. Study on the antidiarrheal activity of the crude methanol extracts of the bark of kalingag showed pronounced reduction of diarrheal feces of the mice with castor oil induced diarrhea [12]. The ethanol extracts of the leaves is reported to contain high amount of total phenolics and strong scavenging activity [14]. Water-distillation of almaciga resins is found to contain great amount of limonene [15]. Fresh leaves of *Agathis dammara*, which is a suggested synonym species of almaciga, *Agathis philippinensis*, was studied for its phytochemical content and reported contain monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpene, limonene, b-bisabolene, b-myrcene, germacrene and abisabolol and the essential oil is observed to have antibacterial activity [16].

The present study aims to evaluate the toxicity potentials and phytochemical components of the crude ethanol and crude ethyl acetate leave extracts of Abaca (*Musa textilis),* Almaciga *(Agathis philippinensis)* and Kalingag *(Cinnamomum mercadoi).*

2. MATERIALS AND METHODS

2.1 Plant Preparation

Enough amount of the leaves of abaca (*Musa textilis*), Almaciga (*Agathis philippinensis*) and kalingag (*Cinnamomum mercadoi*), were collected in Mat-I, Claveria, Misamis Oriental, Philippines. The leaves were washed with running water, air-dried under the shade and the dried plant materials were pulverized into fine powder [17]. About 100 g of each powdered plant materials were extracted with each of the solvents- ethanol and ethyl acetate. The crude extracts were collected after 48 hours. Ethanol and ethyl acetate were used as solvent to ensure that polar and slightly polar bioactive components are extracted from the sample [17].

2.2 Sample Preparation

Stock solutions of the crude ethanol extracts and crude ethyl acetate extracts of *M. textilis*, *A. philippinensis* and *C. mercadoi* were prepared by dissolving separately 50 mg from each crude extracts into their respective solvents. Different concentrations were then prepared from each of the stock solutions and were subjected for phytochemical screening and toxicity test.

2.3 Phytochemical Qualitative Analysis

Qualitative tests for alkaloids, amino acid, anthraquinones, flavonoids, saponins, steroids, triterpenoids, and terpenoids were performed using standard methods [17-19].

2.4 Brine Shrimp Lethality Assay

The brine shrimp lethality assay is a useful and convenient method for testing the biological activity of the plant extracts [20,7,21,22]. The method used in this test was by Meyer et al. (1982) with minor modifications. In hatching the brine shrimps, a shallow rectangular dish was filled with sterilized filtered seawater. A plastic divider punched with several 2 mm holes was placed to divide the dish into two compartments. The minute brown brine shrimp eggs were sprinkled into one of the compartment. Upon being placed in Filtered Sea Water, the eggs of the brine shrimps (*Artemia salina*) were aerated within 48 hours in order to hatch and provide large numbers of larvae (nauplii) for experimental use.

Different concentrations were prepared from each of the ethanol and ethyl acetate stock solutions of *M. textilis*, *A. philippinensis* and *C. mercadoi* by transferring 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL and 1.0 mL into separate vials. The solution in each vial was air-dried then diluted to 5 mL by adding sterilized filtered sea water. Then ten nauplii were transferred into each sample vial. The surviving brine shrimps were first counted after 6 hours (acute) then after 24 hours (chronic). The recorded number of deaths in the 24 hour count was used in the calculation of the LC_{50} using Reed-muench method.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Content

Alkaloids, Flavonoids, Amino Acids, Triterpenoids, Terpenoids, Steroids, Saponins and Tannins were the preliminary phytochemicals identified in the plant extracts of *Musa textilis*, *Agathis philippinensis* and *Cinnamomum mercadoi*. Result of the phytochemical analysis of the ethanol and ethyl acetate extracts of the selected plants is presented in Table 1.

The phytochemicals found in the plants used in this study are also observed in some plants that are proven to have pharmacological and biological activities. Alkaloids which are observed in *A. philippinensis* ethyl acetate extracts and *C. mercadoi* ethyl acetate and ethanol extracts are considered the most efficient therapeutic agent [23] as it have analgesic, antispasmodic and antibacterial properties [24,25]. Alkaloids are also observed to have antiasthma, anticancer, antimalarial, antiarrhythmic, antihyperglycemic activities [26].

Steroids and Flavonoids which were present in all plant extracts used in this study are group of secondary metabolites which were found to have various pharmacological applications. Plants containing steroids have notable pharmacological activities such as anabolic, antidiabetic, anti-inflammatory and anthelmintic [27]. Flavonoids which were usually present in fruits and vegetables are found to be beneficial to human body [25]. Flavonoids and terpenoids were among the secondary metabolites that potentially provide health benefits, as flavonoids shown to prevent platelet aggregation, reduced hearth disease and estrogen related cancer [28]. Flavonoids are found to have antioxidant, anti-cancer and anti-inflammatory activities [25] while, Terpenoids which are usually found in green plants and whole grain were found to reduce risks of cancer [28] and exhibit anti-inflammatory, anticancer, anti-malarial, anti-viral and anti-bacterial activities [29].

Presence of saponins were detected in ethanol extracts of *M. textilis* and ethyl acetate extracts of *A. philippinensis* and *C. mercadoi.* Saponins are also known to have pharmacological activities such as

antibiotic, antifungal, antiviral, anti-ulcer, anti-inflammatory, expectorant and hepatoprotective [25 & 26]. Tannins which were present in all plant extracts except *M. textilis* ethanol extracts were also known as treatment for inflammation, leucorrhoea, burn and diarrhea [25] and have been accounted for the antidiarrheal activities of medicinal plants [12].

3.2 Toxicity Potentials

The different concentrations of *Musa textilis, Agathis philippinensis* and *Cinnamomum mercadoi* crude ethanol and crude ethyl acetate leaf extracts were tested for its toxicity potential against brine shrimp nauplii. The results of the tests were summarized in Table 2 and Table 3.

Results after 24 hours showed increasing percent mortality for all plant extracts, showing maximum percent mortality at 2000 ppm, the highest concentration used. *C. mercadoi* extracts showed more toxicity than *M. textilis* and *A. philippinensis*, recording 89.47% for its ethanol extracts and 97.78% for its ethyl acetate extracts, compared to *M. textilis* (ethanol=60.87% and ethyl acetate=62.00%) and *A. philippinensis* (ethanol=82.46% and ethyl acetate=93.24%).

High percent mortality of the crude ethanol and crude ethyl acetate extracts of *C. mercadoi* can be accounted from the presence of phytochemical compounds. In the preliminary evaluation of its phytochemical contents, moderate to strong presence of Alkaloids, flavonoids, amino acids, triterpenoids, terpenoids, steroids, tannins and saponins were detected in *C. mercadoi* ethyl acetate extracts and ethanol extracts (except saponins). These compounds are also been recorded to have various biological and pharmacological activities such as anti-inflammatory, anti-diabetic, anabolic, anthelmintic, antioxidant, anti-cancer, anti-malarial, anti-viral and anti-bacterial and antidiarrheal. Results from other studies reported that methanolic extracts of *Cinnamomum mercadoi* have an antidiarrheal property [12] and possess antibacterial activity [30]. These findings supports the claim about the medicinal value of the plant. Traditionally, the bark of *C. mercadoi* have been used as remedy for colds, fevers, sinus infections, bronchitis, toothache, headache, rheumatism, dysentery stomach troubles, tuberculosis, diarrhea, menorrhagia, dysmenorrhea and neuralgic pains, yeast infections, diabetes, indigestion, flatulence, bloating, loss of appetite and vomiting and treatment of scabies and lice [31,14]. The crude methanolic extracts of *C. mercadoi* exhibited toxicity against male mice resulting to decrease in motor activity, respiratory rate, ptosis, hyperemia, diarrhea and mortality [31].

High percent mortality was also observed in the crude ethanol and crude ethyl acetate extracts of the leaves of *A. philippinensis*. Moderate to strong presence of phytochemical compounds were detected in the *A. philippinensis* crude ethyl acetate extracts and ethanol extracts (except alkaloids and saponins). Least toxic activity was observed in *M. textilis* which phytochemical evaluation also showed absence of some phytochemical compounds. The toxic activity of the plant extracts can be due to the compounds they contain and which can be accounted for their medicinal value.

It is observed that the percent mortalities of the crude extracts of the plants used are directly proportional to the concentration, as the number of death increased as the concentration increased. It is evident that in the evaluation for cytotoxicity using Brine shrimp nauplii, the degree of lethality of *M. textilis, A. philippinensis* and *C. mercadoi* leaf extracts are directly proportional to the concentration of the extracts and that the crude ethanol and crude ethyl acetate leaf extracts of the plants used exhibited increasing activity especially at higher concentrations.

Table 1. Phytochemical content of *M. textilis, A. philippinensis* and *C. mercadoi* crude ethanol and ethyl acetate leaf extracts

Phytochemical	*M. textilis*		*A. philippinensis*		*C. mercadoi*	
	Ethanol	**Ethyl acetate**	**Ethanol**	**Ethyl acetate**	**Ethanol**	**Ethyl acetate**
Alkaloid	-	-	-	++	+++	+++
Flavonoid	++	++	+++	+++	+++	+++
Amino Acid	++	-	+++	+++	+++	+++
Antraquinone	-	-	-	-	-	-
Triterpenoids	-	+	+++	+++	+++	+++
Steroids	++	++	+++	+++	+++	+++
Terpenoids	-	++	+++	+++	+++	+++
Saponins	+	-	-	++	-	+++
Tannins	-	+	++	++	+	++

Legend: +++ strong presence, ++ moderate presence, + trace presence and – absence

Table 2. Toxicity of the crude ethanolic leaf extracts of *M. textilis, A. philippinensis* and *C. mercadoi* after 24 hours

Dose (ppm)	Dead	Alive	Accumulated dead	Accumulated alive	% Mortality
M. textilis					
2000	12	18	28	18	60.87
1000	6	24	16	42	27.59
800	5	25	10	67	12.99
600	3	27	5	94	5.05
400	2	28	2	122	1.61
200	0	30	0	152	0
A. philippinensis					
2000	20	10	47	10	82.46
1000	9	21	27	31	46.55
800	6	24	18	55	24.66
600	5	25	12	80	13.04
400	4	26	7	106	6.19
200	3	27	3	133	2.21
C. mercadoi					
2000	22	8	68	8	89.47
1000	15	15	46	23	66.67
800	12	18	31	41	43.06
600	10	20	19	61	23.75
400	6	24	9	85	9.57
200	3	27	3	112	2.61

Table 3. Toxicity of the crude ethyl acetate leaf extracts of *M. textilis, A. philippinensis* and *C. mercadoi* after 24 hours

Dose (ppm)	Dead	Alive	Accumulated dead	Accumulated alive	% Mortality
M. textilis					
2000	11	19	31	19	62
1000	8	22	20	41	32.79
800	7	23	12	64	15.79
600	3	27	5	91	5.21
400	2	28	2	119	1.65
200	0	30	0	149	0
A. philippinensis					
2000	25	5	69	5	93.24
1000	14	16	44	21	67.69
800	13	17	30	38	44.12
600	8	22	17	60	22.08
400	6	24	9	84	9.68
200	3	27	3	111	2.63
C. mercadoi					
2000	28	2	88	2	97.78
1000	22	8	60	10	85.71
800	16	14	38	24	61.29
600	11	19	22	43	33.85
400	8	22	11	65	14.47
200	3	27	3	92	3.16

Table 4. LC_{50} of different crude leaf extracts of *M. textilis*, *A. philippinensis*, and *C. mercadoi* after 24 hours

Species	LC_{50} (ppm)	Remarks	p-value
Ethanol			
M. textilis	1678.80	Non-toxic	< 0.000*
A. philippinensis	1071.52	Non-toxic	< 0.000*
C. mercadoi	833.78	Toxic	< 0.000*
Ethyl Acetate			
M. textilis	1519.91	Non-toxic	< 0.000*
A. philippinensis	834.50	Toxic	< 0.000*
C. mercadoi	718.21	Toxic	< 0.000*

**Significant at 0.05 level*

3.3 Median Lethal Concentration (LC_{50})

The primary determinant of the safety of a substance is the dose. Toxicological studies for all herbal medicines including the determination of their median lethal dose (LD_{50}) and other such parameters essential for a proper dosage are desirable and necessary [32]. LC_{50} is the concentration required to kill 50% of the population. In this study the 24 hour count was used to determine the LC_{50}, using Reed- muench method. Based on the calculated values, *A. philippinensis* ethyl acetate extracts which LC_{50} value is 834.50 ppm and *C. mercadoi* ethanol and ethyl acetate extracts, with LC_{50} of 833.78 ppm and 718.21 ppm respectively are considered active or slightly toxic at prolong administration. As reported, the plant crude extract is toxic (active) if it has an LC_{50} value of less than 1000 ppm while non-toxic (inactive) if it is greater than 1000 ppm [23]. The calculated LC_{50} values also indicated that *A. philippinensis* ethyl acetate extracts and *C. mercadoi* ethanol and ethyl acetate extracts contain bioactive components that could be accounted for its pharmacological or toxicological effects. The bioactivity of the plant is an indicative of the presence of potent toxic components which warrants for further investigation [33]. Though results some study presents that the methanol extracts of the fruit of *M. textilis* significantly reduced glucose level of person with high blood pressure [11], in this study *M. textilis* showed least toxic activity among the plants used and LC_{50} of its ethanol and ethyl acetate extracts are found to be above 1000 ppm and considered non-toxic.

Results also show a clear significant difference ($p<0.05$) of the mortality rate of brine shrimp exposed to the different concentrations of *M. textilis*, *A. philippinensis*, and *C. mercadoi* ethanol and ethyl acetate crude leaf extracts.

4. CONCLUSION

The Brine Shrimp Lethality Assay of the crude ethanol and crude ethyl acetate leaf extracts of *M. textilis*, *A. philippinensis* and *C. mercadoi* showed increasing mortality as concentrations of the extracts increased. Among the extracts used *A. philippinensis* ethyl acetate extracts and *C. mercadoi* ethanol and ethyl acetate extracts exhibited cytotoxicity activities. These extracts also exhibited strong presence of alkaloids, flavonoids, amino acids, triterpenoids, steroids and terpenoids compared to the other extracts used. *A. philippinensis* ethyl acetate extracts and *C. mercadoi* ethanol and ethyl acetate extracts showed to be active and contain bioactive components that could be potential for further studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mtewa AG, Walag AMP, Muhumuza A, Kharwar RN, Ahmed O. Phytochemicals, plant toxins, and their influence in military strategies. In Phytochemistry, the Military and Health. Elsevier. 2021;13-25.
2. Judzentiene A, Buzelyte J. Chemical composition of essential oils of *Artemisia vulgaris L.* (mugwort) from plants grown in North Lithuania. Chemija. 2006;17(1):12-15.
3. Dhanavade M, Jalkute C, Gosh J, Sonawane K. Study antimicrobial activity of lemon (*Citrus lemon L.*) peel extracts. British Journal of Pharmacology and Toxicology. 2011;2(3):119-122.
4. Asare GA, Bugyei K, Sittie A, Yahaya ES, Gyan B, Adjei S, Addo P, Wiredu EK, Nyarko AK. Genotoxicity, cytotoxicity and toxicological evaluation of whole plant extracts of the medicinal plant *Phyllanthus niruri* (Phyllanthaceae). Genetics and Molecular Research. 2012;11(1):100-111. Available:http://197.255.68.203/handle/123456789/3519

5. Egbuna C, Gupta E, Ezzat SM, Jeevanandam J, Mishra N, Akram M, Onyekere PF. Aloe species as valuable sources of functional bioactives. In Functional Foods and Nutraceuticals. Springer, Cham. 2020;337-387.
6. Olowa LF, Nuňeza O. Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan City, Philippines. International Research Journal of Biological Sciences. 2013;2:74-77.
7. Hossain S, Tuly FA, Arman SI, Aziz A, Mahmud E, Das P, Babu MH, Islam M. Determination of Antiemetic, Antimicrobial, Anti-Radical and Cytotoxic Activity of Methanolic Extracts of *Centella asiatica*. Plant Journal. 2018;6(1):1-7.
DOI: 10.11648/j.plant.20180601.11
8. Morilla L, Sumaya N, Rivero H, Madamba M. Medicinal plants of the Subanens in Dumingag, Zamboanga del Sur, Philippines. International Conference on Food, Biological and Medical Sciences. 2014;38-43.
DOI:http://dx.doi.org/10.15242/IICBE.C0114577
9. Baleta FN, Donato JG, Bolaños JM. Awareness, utilization and diversity of medicinal plants at Palanan, Isabela, Philippines. Journal of Medicinal Plants Studies. 2016;4(4):265-269.
10. Ezzat SM, El Bishbishy MH, Walag AMP, Mtewa AG. FDA drug candidacy acceptance criteria and steps: problems and way forward. In Phytochemicals as Lead Compounds for New Drug Discovery. Elsevier. 2020;39-63.
11. Rahmatullah M, Lopa AF, Jannat K, Hamid A. Oral glucose tolerance tests with methanolic extract of fruits of *Musa textilis Nee*. Journal of Medicinal Plants Studies. 2018;6(5):130-132.
12. Gorgonio SR, Fuentes R. Antidiarrheal Activity of *Cinnamomum mercadoi* Methanolic Leaf and Bark Extracts. Phil J of Nat Sci. 2011;16(1):43-47.
13. Fuentes RG, Diloy FN, Tan IL, Balanquit BR. Antioxidant and antibacterial properties of crude methanolic extracts of *Cinnamomum mercadoi Vidal*. Phil J of Nat Sci. 2010;15:9-15.
14. Latayada F, Uy M. Screening of the antioxidant properties of the leaf extracts of Philippine medicinal plants Ficus *nota* (Blanco) Merr, *Metroxylon sagu* Rottb, *Mussaenda philippica* A. Rich, *Inocarpus fagifer*, and *Cinnamomum mercadoi* Vidal.. Bulletin of Environment, Pharmacology and Life Sciences. 2016;5(3):18-24.
Available:http://www.bepls.com
15. Lassak E, Brophy J. The steam-volatile oil of commercial "Almaciga" resin (*Agathis philippinensis Warb*.) from the Philippines. Journal of Essential Oil Bearing Plants. 2013;11(6):634-63.
DOI: 10.1080/0972060X.2008.10643679
16. Chen Z, He Z, Deng J, Zhu J, Mao Q. Chemical composition and antibacterial activity of the essential oil from *Agathis dammara* (Lamb.) Rich fresh leaves. Natural Product Research; 2015.
DOI:http://dx.doi.org/10.1080/14786419.2015.1022544
17. Walag AMP, Cepeda ABM, Galenzoga AS, Sambaan SMJ. Initial phytochemical screening of the different parts of *Mansoa alliacea L*.(Garlic Vine). International Journal of Biosciences. 2017; 11(3):227-31.
18. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020;8(2):603-608.
DOI: 10.22271/chemi.2020.v8.i2i.8834
19. Walag AMP, Del Rosario RM, Canencia OP. Zoochemical composition of selected sea stars collected from the Coastal Waters of Carmen, Agusan Del Norte, Philippines, Asian Journal of Biological and Life Sciences. 2019;8(2):25-33.
20. Carballo JL, Inda Z, Pérez P, Grávalos M. A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC Biotechnology; 2002.
Available:
http://www.biomedcentral.com/1472-6750/2/17
21. Krishanaraju AV, Rao TN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. International Journal of Applied Science and Engineering. 2005;3(2):125-134.
22. Walag AMP, Del Rosario RM. Proximate biochemical composition and brine shrimp lethality assay of selected sea stars from Goso-on and Vinapor, Carmen, Agusan del Norte, Philippines. Malaysian Journal Biochemistry and Molecular Biology. 2018;21(3):11-8.
23. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Journal of Medicinal Plant Research. 1982;45: 31-34.
24. Pradeep A, Dinesh M, Govindaraj A, Vinothkumar D, Ramesh Babu NG. Phytochmical analysis of some important medicinal plants. International Journal of Biological & Pharmaceutical Research. 2014; 5(1):48-50.

25. Hussain I, Ullah R, Ullah R, Khurram M, Ullah N, Baseer A, Khan FA, Khattak MR, Zahoor M, Khan J, Khan N. Phytochemical analysis of selected medicinal plants. African Journal of Biotechnology. 2011;10(38):7487-7492.
DOI: 10.5897/AJB10.2130
26. Awuchi CG. The biochemistry, toxicology, and uses of the pharmacologically active phytochemicals: alkaloids, terpenes, polyphenols, and glycosides. Journal of Food and Pharmaceutical Science. 2019;7(3):131-150.
27. Rahman SU, Ismail M, Khurram M, Ullah I, Rabbi F, Iriti M. Bioactive steroids and saponins of the genus *Trillum*. Molecules. 2017;22(12):2156.
DOI: 10.3390/molecules22122156
28. Prakash D, Gupta C, Sharma G. Importance of phytochemicals in nutraceuticals. Journal of Chinese Medicine Research and Development. 2012;1(3):70-78.
29. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R, Asnad. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochemistry & Analytical Biochemistry. 2013;2(4).
DOI: 10.4172/2161-1009.1000144
30. Marcellano JP, Collanto AS, Fuentes RG. Antibacterial activity of endophytic fungi isolated from the bark of *Cinnamomum mercadoi*. Pharmacognosy Journal. 2017;l 9(3):405-409.
DOI: 10.5530/pj.2017.3.69.
31. Torres RC, Sison FM, Ysrael MC. Phytochemical screening and biological studies on the crude methanol extract of *Cinnamomum mercadoi*, vidal. Philippine Journal of Science. 2003;132(1):27-32.
32. Abere TA, Okoto PE, Agoreyo FO. Antidiarrhoea and toxicological evaluation of the leaf extract of *Dissotis rotundifolia triana* (Melastomataceae). BMC Complementary and Alternative Medicine. 2010;10(71).
DOI:https://doi.org/10.1186/1472-6882-10-71
33. Otang WM, Grierson DS, Ndip RN. Assessment of potential toxicity of three South African medicinal plants using the brine shrimp (*Artemia salina*) Assay. African Journal of Pharmacy and Pharmacology. 2013;7(20): 1272-1279.