INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 21, No. 1, p. 31-36, 2022
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Flavonoid & steroid composition of twelve selected Malaueg medicinal plants

Blancas Pamela Joy S.[*1], Diesta Jocelyn S.[2], Ramirez Ramelo Jr. B.[3]

[1]*Natural Product Research and Innovation Center, Cagayan State University, Andrews Campus, Tuguegarao City, Province of Cagayan, Northern Philippines*

[2]*College of Allied Health Sciences, Cagayan State University, Andrews Campus, Tuguegarao City, Province of Cagayan, Northern Philippines*

[3]*Natural Product Research and Innovation Center, Cagayan State University, Tuguegarao City, Province of Cagayan, Northern Philippines*

Article published on July 15, 2022

Key words: Qualitative analysis, Quantitative analysis, Steroid content, Flavonoid content, Indigenous plants.

Abstract

The search for alternative sources of medicines includes a test for the phytochemical composition. This study aimed to determine the qualitative and quantitative flavonoid and steroid composition of twelve selected Malaueg medicinal plants generally. Specifically, it aimed to determine the leaves of the medicinal plants if it contains the composition of flavonoids and steroids. The presence of flavonoids may indicate its protective effects against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases. Its presence indicates antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties, free radical scavenging capacity, hepatoprotective, capacity to modulate key cellular enzyme functions, and possible inhibition of several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase. The presence of steroids points to the following potentials of plant steroids: medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, anti-helminthic, cytotoxic and cardiotonic activity. This study highlights the amount and presence of flavonoids and steroids of 12 medicinal plants. It is recommended that the aqueous extract be studied for the flavonoid presence. It is further recommended that other solvents be utilized in the extraction process.

***Corresponding Author:** Blancas, Pamela Joy ✉ pjblancas22@gmail.com

Introduction

The Malaueg community of Rizal, Cagayan is one of the identified indigenous groups in the Province of Cagayan. They live in a mountainous area and in the early days utilized plants as sources of cure for ailments. These twelve plants used as medicines have a folkloric basis. The twelve plants are Bicarud *(Tabernaemontana pandacaqui*), Biga (*Alocasia macrorrhiza*), Gabut (*Imperata cylindrica L.),* Kunig *(Curcuma sp),* Liw-liw *(Ficus septica),* Lubigan *(Cymbopogon citratus),* Matalagdaw *(Hyptis brevipes Poit.),* NPA *(Chromolaena odorata),* Pansit-pansitan *(Peperomia pellucida),* Papait *(Mollugo sp.),* Tarattut *(Eleusine indica),* and Wild tea *(Ehretia microphylla).* Scientific confirmation of these folkloric uses is important for the possibilities of creating products like herbal formulations for metabolic disorders, home and personal care products, and other products. Such scientific confirmation is needed to ensure that products from these indigenous medicinal plants are safe for human consumption.

Proper qualitative and quantitative analysis of extracts will point out the efficacy of the biological property thus the production of safe and potent herbal formulations and other products that are utilized by human beings. Resulting phytochemical analysis may show what other capacities or abilities of these plant may be used for aside from their folkloric usage. Moisture content can affect the extraction procedures involved in obtaining the bioactive components. The presence of pesticide deposits and overwhelming metals within the soil reflect on the extricates and can cause the extracts to be risky. There's subsequently a got to decide the presence of the folkloric movement through a subjective and quantitative assurance of the plants' chemical composition. Similarly, significant is the dampness content which influences the measure of phytochemicals extricated and the assurance of the presence of contaminants like pesticide deposits and overwhelming metals will affect in the security of concentrates.

According to Kumar S, Pandey AK. (2013) study, Flavonoids are omnipresent in plants and are the central essential kind of polyphenolic compound found within the human diet. Flavonoids have ability to induce human guarded protein structures. The number of considers has proposed cautious effects of flavonoids against various compelling (bacterial and viral ailments) and degenerative ailments, such as, cardiovascular diseases, cancers, and other age-related infections. They also go about as a helper cell reinforcement resistance structure in plant tissues revealed to various abiotic and biotic anxieties. They are also growth regulator factors in plants such as auxin. Biosynthetic qualities have been gathered in a few microscopic organisms and fungi for improved production of flavonoids. Studies by Patel SS, Savjani JK. (2015), plant steroids have many fascinating therapeutic, pharmaceutical and agrochemical exercises like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant development hormone controller, sex hormone, anti-helminthic, cytotoxic and cardiotonic action. In news article from medicine Net (2020), Plant sterols is a type of plant steroid that are taken by mouth to lower cholesterol levels and help prevent heart disease and heart attacks. Plant sterols are also utilized for a few cancers such as stomach cancer, colon cancer, and rectal cancer. Plant sterols are also used for weight loss.

Therefore, the aim of this study is to determine the presence of the folkloric activity through a qualitative and quantitative determination of the plants' chemical composition. Equally important is the moisture content, which affects the amount of phytochemicals extracted and the determination of the presence of contaminants like pesticide residues and heavy metals will impact on the safety of extracts. As a result, we can create cosmetic products containing these plant extracts to treat skin irritations, illnesses, and other bacterial and viral diseases.

Materials and methods

A. Plant Collection Procedures

Mature leaves will be collected for extraction purposes. Separate at least five leaves each plant sample for moisture determination purposes. Wiped clean and stored in Ziplock bags. Store away from sunlight.

B. Preparation of Plants for Extraction

Plant leaves should be brushed briskly to remove visible soil and dust particles by deionized water as quick as possible. Plant parts are placed on a spin drier and blot off into filter paper then weigh and place it in oven. Plant parts are oven dried at 40^{0}C for 12 to 72 h. (drying times depends on type and amount of samples in the oven).

Samples are grinded finely in a heavy-duty grinder. Samples are kept in a tightly capped glass jars or sealed polyethylene bags, labeled properly and stored prior to analysis. Containers should be kept in a cool dry place. For long term storage samples should be thoroughly dried, sealed and placed under refrigerated conditions (4^{0}C) until required analysis is completed.

Extraction process using 2 solvents (ethanol and methanol).

Weigh 10 grams of dried material with constant weight and soak it with 90ml of solvent (70% ethanol and 80% methanol). Place it in a hot plate at 50^{o}C for 2 hrs. with constant stirring. After 2 hrs., place it overnight in a refrigerator at 4^{o}C. Then place it in a platform shaker for 2 hrs. at constant stirring. Collect extract.

Qualitative analyses of Secondary Metabolites

a) Test for Flavonoids (Edeoga 2005 and N. Morsy et al. (2014).

Boil 0.5mL of the extract with 2.5mL of distilled water for 5 min. Filter while mixture is hot. Add a few drops of 20% sodium hydroxide solution to the cooled filtrate. The change from yellow to colorless solution upon the addition of acid (10% HCl) indicates the presence of flavonoids.

b) Test for Steroids (Edeoga 2005, Kumae et al., IJPSR 2010).

Dissolve 0.5mL of the extract in 2.5mL chloroform. Equal volume of concentrated sulfuric acid is added to the side of the test tube. The presence of steroids is detected by the upper layer turning red and the sulfuric acid layer shows yellow with green fluorescence.

Quantitative analyses of Secondary Metabolites

a) *Determination of total flavonoid content (Stancovic MS, et. al, Park et al. (2008) and Saikaew et al).*

A 0.1mL extract is diluted with distilled water in test tube to a volume of 5mL. 0.3mL 5% $NaNO_2$ is added, after 5 mins. 3mL 10% $AlCl_3$ is added. 2.0mL 1 M NaOH is added after 5 minutes followed by addition of distilled water. After 15 minutes, absorbance is measured against blank at 510nm. Total flavonoid content is expressed as mg quercitin per g of dry weight.

b) Determination of steroids (Hemalatha S. et al. 2017).

A 1.0mL of test extract of steroid solution was transferred into 10mL volumetric flasks. Sulphuric acid (4N, 2mL) and iron (III) chloride (0.5% w/v, 2mL) are added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5mL). The mixture is heated in water bath maintained at 70±2^{0}C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780nm against reagent blank. Total steroid content is expressed in mg cholesterol per g of dry weight.

Results and discussions

The method used to determine the secondary metabolites present in plants was carried out in standard methods for all extracts, using different solvents for extraction and examination.

Table 1. Qualitative Phytochemical Analyses of twelve Malaueg Plants.

Plants	Flavonoids		Steroids	
	Ethanolic Extract	Methanolic Extract	Ethanolic Extract	Methanolic Extract
Bicarud	-	-	+++	+++
Biga	-	-	+++	++
Gabut	+++	++	+++	+++
Kunig	+	+	+++	+++
Liw-liw	++	-	+++	+++
Lubigan	+++	++	++	+++
Matalagdaw	++	++	++	++
NPA	+++	-	+++	+++
Pansit-pansitan	+	-	+++	+++
Papait	++	-	++	+++
Tarattut	++	+	++	+++
Wild tea	++	+++	++	++

Legend: +++ *Highly present,* ++ *moderately present,* + *trace,* - *Negative*

Phytochemical analyses revealed the presence of steroids and flavonoids in methanolic and ethanolic extracts. The number of traces of each result is the intensity of their presence. Table 1 shows that the ethanolic extracts of the plants showed the presence of flavonoids in 10 plants, except for bicarud and biga. The methanolic extracts yielded flavonoids in 6 plants, and no flavonoids were detected in the methanolic extracts of bicarud, biga, liwliw, NPA, pansit-pansitan, and papait. The presence of more flavonoids was qualitatively detected in the ethanolic extracts of gabut, liwliw, lubigan, NPA, pansit-pansitan, papait, and tarattut. The methanolic extract of wild tea showed more flavonoids. The same amounts of flavonoids were detected in both extracts of bicarud, biga, kunig, and matalagdaw. As given results in qualitative analysis (Table 1), ethanolic extract gave dominant results for most of the plants in the detection of flavonoids, while in the detection of steroids, it was better to use methanol solvent.

Table 2. Quantitative Analyses of Twelve Malaueg Plants using two Solvents.

Plant	Flavonoid		Steroid	
	Ethanol	Methanol	Ethanol	Methanol
Bicarud	361.64	340.09	68.50	67.87
Biga	596.09	545.27	57.78	36.08
Gabut	278.64	280.64	41.01	30.89
Kunig	434.36	608.55	67.80	62.03
Liwliw	124.64	314.36	54.98	53.05
Lubigan	187.82	280.82	48.20	41.09
Matalagdaw	124.91	344.64	63.46	42.38
NPA	500.27	704.64	53.17	60.34
Pansit – Pansitan	212.64	608.09	71.19	73.06
Papait	89.00	245.45	36.58	32.69
Tarattut	68.45	237.32	36.28	51.66
Wild Tea	54.64	381.55	25.28	44.32

Legend: Flavonoid expressed in mg quercetin/gram sample
Steroids expressed in mg cholesterol/gram sample

Table 2 Quantitative analysis is much more tangible. It entails looking at hard data, or actual numbers. The flavonoids were expressed in mg of quercetin per gram of sample, and the steroids were expressed in mg of cholesterol per gram of sample. More flavonoids were detected quantitatively in methanolic extracts, while detection of steroids was higher in ethanolic extracts.

The 12 Malaueg Medicinal Plants tested qualitatively and quantitatively in this study were found to have steroids and flavonoids in both extracts, almost. According Kumar, S., & Pandey, A. K. (2013) and Patel, S. S., & Savjani, J. K. (2015), these two secondary metabolites are important factors in the prevention of many infectious and degenerative diseases, including cancer and cardiovascular disease. Their presence indicates antioxidative, anti-inflammatory, anti-mutagenic, anti-carcinogenic properties, anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, anti-helminthic, cytotoxic, and cardiotonic activity. In line with this, we can produce products focused in curing skin diseases at low cost for indigenous people who need treatments. For the overall results, we will possibly conduct a study where we can produce products focused on curing skin diseases and cancer treatments using these plants at a low cost for indigenous people who need treatments.

Conclusions

The detection of flavonoids and steroids in qualitative and quantitative analyses yielded impressive results. However, when we used methanolic extract, these parameters improved noticeably. Based on the findings, the methanolic extract performed exceptionally well in terms of steroid and flavonoid detection. Through quantitative and qualitative analyses, the twelve malaueg medicinal plants were identified for this study, and they have industrial potential, particularly for therapeutic formulations.

Recommendation

It is recommended that the aqueous extract be studied for flavonoid presence. It is also recommended that other solvents be used for extraction purposes. And other parts of the plant sample will be studied.

Acknowledgement

The researchers would like to express gratitude to Dr Maria Nilda M. Muñoz for her guidance and suggestions for the development of this research

work. Her willingness to give so generously of her time has been highly appreciated. We'd also like to thank the Natural Product Research and Innovation Center at Cagayan State University for conducting the whole study.

References

Aska AS, Kubmarawa D, Nkafamiya IA, Shagal HM, Oladosu PO. 2020. Quantitative phytochemical analysis and Anti-tuberculosis activity of some selected medicinal plants in some Northern parts of Bauchi state, Nigeria. Medicine Net. Plant Sterols Supplement: Benefits, Uses, Side Effects, Dosage & Interactions. Retrieved July **16.**

Du F, Zhang F, Chen F, Wang A, Wang Q, Yin X, Wang S. 2011. Advances in microbial heterologous production of flavonoids. African Journal of Microbiology Research **5(18),** 2566-2574.

Edeoga HO, Okwu DE, Mbaebie BO. 2005. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology **4(7),** 685-688.

Hemalatha S, Bhaskar Reddy K, Parepalli. 2017. Invitro Antipsoriatic and Inhibitory Effects Of Ethyl Acetate And Methanol Fraction Of Cassia Sophera and Mallotus Philippinensis On Inflammatory Cytokines. International Journal of Phytopharmacology.

Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q. 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pacific Journal of Tropical Biomedicine **3(9),** 705-710. https://doi.org/ 10.1016/s2221-1691(13)60142-2

Iqbal E, Salim KA, Lim LBL. 2015. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. Journal of King Saud University - Science **27(3),** 224-232.

Kumar S, Pandey AK. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal 1-16 https://doi.org/10.1155 /2013/162750

Kumar U, Kumar B, Bhandari A, Kumar Y. 2010. Phytochemical investigation and comparison of antimicrobial screening of clove and cardamom. Int J Pharm Sci Res **1(12),** 138-47.

Mangesh S Kharate, Narayan B Pandhure. 2017. Quantitative Estimation of Phytochemicaland Antimicrobial Properties of Crude Leaf Extract, Mimusopselengi L. International Journal for Research in Applied Science & Engineering Technology (IJRASET) ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor :6.887 Volume 5 Issue XII December

Marinova D, Ribarova F, Atanassova M. 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the university of chemical technology and metallurgy **40(3),** 255-260.

Onuh J, Idoko G, Yusufu P, Onuh F. 2017. Comparative Studies of the Phytochemical, Antioxidant and Antimicrobial Properties of Cashew Leaf, Bark and Fruits Extracts. American Journal of Food and Nutrition **5(4),** 115-120. https://doi.org/ 10.12691/ajfn-5-4-1

Patel SS, Savjani JK. 2015. Systematic review of plant steroids as potential antiinflammatory agents: Current status and future perspectives. The Journal of Phytopharmacology **4(2),** 121-125.

Pratim Sarma M. 2017. Phytochemical Analysis of Traditional Medicinal Plants and their Antimicrobial Activity: An Experience from North East India. Open Access Journal of Pharmaceutical Research **1(1).** https://doi.org/10.23880/oajpr-16000104

Robertson Sally. 2018. What are Flavonoids?. News-Medical. Retrieved on July 16, 2020 from https://www.news-medical.net/health/What-are-Flavonoids.aspx.

Roghini R, Vijayalakshmi K. 2018. Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of citrus paradisi. International journal of pharmaceutical sciences and research **9(11),** 4859-4864.

Saikaew K, Lertrat K, Ketthaisong D, Meenune M, Tangwongchai R. 2018. Influence of variety and maturity on bioactive compounds and antioxidant activity of purple waxy corn (*Zea mays* L. var. ceratina). International Food Research Journal **25(5),** 1985-1995.

Shah MD, Hossain MA. 2014. Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant Merremia borneensis. Arabian Journal of Chemistry, **7(6),** 1034-1038. https://doi.org/10.1016/j.arabjc. 2010.12.

Stankovic S, Zia-Ul-Haq M, Bojovic BM, Topuzovic MD. 2014. Agricultural Academy, total Phenolics, flavonoid content and antioxidant power of leaf, flower and fruits from cornelian cherry (*Cornus mas* L. M.) Bulgarian Journal of Agricultural Science **20 (No 2),** 358-363.

Sultan A, Raza AR. 2015. Steroids: A diverse class of secondary metabolites. Med chem **5(7),** 310-317.

Sultan A. 2015. Steroids: A Diverse Class of Secondary Metabolites. Medicinal Chemistry **5(7).** https://doi.org/10.4172/2161-0444.1000279

Suryawanshi S. 2019. Chemistry and Health benefits to humans of Some Flavonoids: An Overview. Current Pharma Research **9(2),** 2799-2804.

Teresa M, Bandiola B. 2018. Extraction and qualitative phytochemical screening of medicinal plants: A brief summary. International Journal of Pharmacy **8(1),** 137-143.

Thao NP, Luyen BTT, Kim EJ, Kang JI, Kang HK, Cuong NX, Nam NH, Kiem PV, Minh CV, Kim YH. 2015. Steroidal Constituents from the Edible Sea Urchin Diadema savignyi Michelin Induce Apoptosis in Human Cancer Cells. Journal of Medicinal Food **18(1),** 45-53. https://doi.org/ 10.1089/jmf.2013.3105

VK D, VC, SS K. 2020. Phytochemical Studies and In Vitro Evaluation of Antioxidant & Antihyperglycemic Activities of *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson (Kiwifruit). Journal of Biochemistry and Analytical Studies **4(1).** https://doi.org/10.16966/2576.