MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

A COMPREHENSIVE EVALUATION OF PHYTOCHEMICAL PROFILE, ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF *Clidemia capitellata* (Bonpl.) D.Don LEAVES EXTRACT

Siti Norazura Jamal[a]* and Mohd Firdaus Ismail[b]

[a]*Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Cawangan Negeri Sembilan, 72000 Kuala Pilah Negeri Sembilan, Malaysia*
[b]*Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*

*Corresponding Author: norazura6775@uitm.edu.my

History
Received: 7th May 2021
Accepted: 19th July 2021

Keywords:

Medicinal plant, Clidemia capitellata, antibacterial, antioxidant, phytochemical profile

Abstract

In Malaysia, medicinal plants are widely used daily as a major component of society's restorative therapies. Malaysia's environment is recognized for its wide range of species and a rich source of medicinal herbs. *Clidemia capitellata* (Bonpl.) D.Don is a shrub belonging to the family of the Melastomataceae locally known as "Senduduk bulu". The current study aims to determine the antibacterial, antioxidant, and chemical profile of *Clidemia capitellata* (Bonpl.) D.Don leaf extract. Antimicrobial activity was determined by disc diffusion in agar and microdilution in broth (MIC-g/mL). Antioxidant activity was determined using the DPPH free radical capture assay, the presence of phytochemical profile was determined by thin-layer chromatography (TLC), while total phenols (EGA/g) and flavonoids (EQ/g) were quantified using spectrophotometry. The results indicated that the extract of *Clidemia capitellata* was active against *Escherichia coli* (ATCC 25922) with a minimum inhibitory concentration (MIC) of 62.5 g/mL. The extract was the most effective at sequestering DPPH free radicals (28.60 1.54 %). Total phenolic contents obtained were 80.78 ±0.06 and total flavonoid contents obtained were 64.64±0.10 of the extract, respectively. The phytochemical profile revealed the presence of condensed tannins, terpenes, steroids, and polyphenols but no alkaloids. As a result of the positive results obtained, it is anticipated that the active component of extracts will continue to be extracted and that new chemical-pharmacological assessments will be conducted in the future.

INTRODUCTION

Plants produce a diverse spectrum of bioactive chemical compounds through secondary metabolism, and some of these molecules may possess therapeutic characteristics that are beneficial to both humans and animals. The use of plant-derived substances as medications and nutritional supplements to support the body's normal physiological processes has a long history in the history of mankind, both as medicines and as nutritional supplements [1]. However, most wild plants found in forests and used by traditional healers have not been thoroughly investigated for their bioactivities to find the bioactive chemicals that are responsible for their therapeutic effects [2]. Plants have been exploited as medicines [3,4] since thousands of years ago, and plant-derived natural compounds continue to be an important component in the search for medicinal remedies to treat a wide range of infectious diseases today [5].

Secondary metabolites, which are naturally formed in plants, are responsible for producing bioactive chemicals [6]. The fact that plants are in a stationary state notwithstanding, they must cope with a variety of obstacles

such as pathogen infections, coexistence with herbivores, and fluctuations in the supply of basic nutrients that they require for the synthesis of their food [7]. It is possible that the secondary metabolites are unique to a particular species or genus and that they do not contribute in any way to the plants' primary metabolic requirements. They are critical to their existence as well as their ability to overcome local challenges [8]. Plant secondary metabolites can be divided into three main families based on their metabolic pathway, which are phenolics, terpenes and steroids, and alkaloids [8].

Many bioactive compounds are employed in a variety of applications such as antibiotics, agrochemicals, flavors and fragrances, biopesticides, food colorants or pigments, food additives, and plant growth stimulants, among other things [9]. Apart from being a rich source of novel therapeutic agents, secondary metabolites from plants have been used in traditional medicine for centuries due to their broad range of biological activity [2]. Since then, various strategies have been extensively researched with the goals of improving the production of secondary plant compounds and in the hope that the isolated novel and valuable compounds or molecules will be able to cure a variety of diseases and all of the natural compounds found in plants have been strongly associated with their ethnomedicinal values [10].

Malaysia is one of the countries that encourage the use of medicinal plant prescriptions, which is an essential component to take into consideration. Malaysia has a significant advantage in terms of possible restorative plants due to the large and diverse diversity of plant species found there [11]. It has been estimated that over 12,000 flowering plants may possess potential health-promoting characteristics; however, only 1,300 species have been identified as having therapeutic potential [12]. The world is habitat to around 374,000 plant species, of which approximately 308,312 are vascular plants and 295,383 are flowering plants [13], of which 15,500 are found in Malaysia's varied biodiversity and ecosystems [14].

Clidemia is a genus of flowering plants in the Melastomataceae family that has numerous species. It contains approximately 450 species, the most well-known of which is *Clidemia hirta*, as well as others such as *Clidemia capitellata* [15]. Originally from southern Mexico to Colombia and the West Indies, *Clidemia capitellata* has naturalized in several areas of Brazil, where it is a weed in Guarana farms. It is an occasional plant in the environment with high rainfalls and interestingly growth, flowering and fruiting have been observed throughout the year [16]. Many studies have been established on Melastomataceae family, especially *Clidemia hirta* and *Clidemia rubra* on antimicrobial and antioxidant activity [6,17,18,19,10].

However, there are limited studies done on *Clidemia capitellata* [21], even though the plant has significant medicinal values. Furthermore, the purpose of this work was to examine the antibacterial and antioxidant activity, as well as the phytochemical profile, of *Clidemia capitellata* leaves extract.

MATERIALS

Clidemia capitellata (Bonpl.) D.Don were collected in UiTM Cawangan Negeri Sembilan Kuala Pilah Campus forest (2.7961950,102.2205121).

METHODS

Surface Sterilization of Plant Material

The plant specimens were recognized and the exsiccates were deposited in the Biology Laboratory, Faculty of Applied Sciences UiTM Cawangan Negeri Sembilan Kuala Pilah Campus. Fresh leaves were sterilized with a 10% sodium chloride solution and then rinsed with sterile distilled water. They were then air-dried for 48 hours at 45°C, homogenized to a fine powder, and stored in sealed bottles.

Extraction of Plant Material

Soxhlet extraction procedures were used to obtain crude extracts of the dried powder of *Clidemia capitellata*, with four different absolute solvents being used in the process (Methanol, Ethanol, Chloroform, and Hexane), based on the procedure by [22], with some modifications. Soxhlet extraction was performed by placing plant material in a thimble, which was then inserted into the Soxhlet apparatus and exposed to four different absolute solvents of different polarities, with the sample to solvent ratio being 1:10 (w/v). The extraction was carried out until the solvent in the upper extraction chamber was no longer visible in a clear solution. The extracts were then filtered twice using a Whatman No.1 under vacuum pressure to get the final product. Extracts were concentrated using a vacuum rotary evaporator, and the concentrated extracts were subsequently freeze-dried to eliminate solvents from the sample. The crude extracts were stored in glass containers and preserved at -20°C until they were subjected to further evaluation.

Disc Diffusion Method

The antimicrobial activity of the samples was evaluated using the disc diffusion method described by (Clinical and Laboratory Standards Institute, 2015), using bacteria from the American Type Culture Collection (ATCC) – *Bacillus subtilis* (B29), *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15422), *Serratia marcescens* (S381) and *Salmonella enterica* (ATCC 10708). The bacteria cultures were grown in Mueller-Hinton agar for 24 hours at 37 ° C. The plant extract was put to sterile filter-paper discs (6.0

mm in diameter) in aliquots of 20 µl (100.0 to 12.5 mg/mL). The bacteria were subsequently cultivated in DMSO with % chloramphenicol as a control. At 37°C, the bacterial plates were cultured for 48 hours. Antimicrobial activity was determined using the inhibition zone. Soxhlet extraction procedures were used to obtain the crude.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using the method described in CLSI document M7-A6 [23], with minimal modifications. The bacteria were cultured on 96-well plates for 24 hours at 37°C in the presence of various concentrations of extract (100.0 to 12.5 mg/ml) and chloramphenicol (0.1%) and DMSO (4%), which served as controls for the experiment. Without turbidity, it was presumed that wells contained active extracts.

Free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) Method

The photocolorimetric test of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by [6], with some modifications, was used to determine the quantitative evaluation of the radical scavenging activity of the extract. A diluted extract solution in ethanol (6.0 mg/ml) was made, and 50 µl of this solution was added to microplate wells previously filled with 150 µl of ethanol. Serial dilutions were used to obtain extract concentrations of 6.25–12.5, 25, 50, and 100 mg/mL. Each well contained 100 µl of a DPPH solution (0.5 mM) before being filled. After one hour of reaction time, absorbance measurements were taken using a UV-Vis spectrophotometer set to 517 nm and performed at room temperature in the absence of light. All of the tests were carried out in triplicate. L-Ascorbic acid (Vitamin C) was employed as a positive control in conjunction with the methanol as a baseline control. The following equation was used to calculate the percentage of radical scavenging activity present.

$$\text{Scavenging effect (\%)}: [(A_o - A_l) \times 100\%] / A_o$$

Where A_o is the absorbance of the control reaction, and A_l is the absorbance in the presence of the sample of the test extracts.

Quantification of Phytochemical Profile

The phytochemical profile of the extracts was assessed using chemical reactions to detect alkaloids, terpenes, and steroids. Chemical reactions were used to determine the phytochemical composition (alkaloids, terpenes, and steroids) of the extracts. The chemicals were separated using thin-layer chromatography (TLC) with a 0.2 mm silica gel (F254) in aluminum (MERCK) [24].

Quantification of Total Phenolic Compounds

The total phenolic content was determined using a modified version of the Foline Ciocalteu (Sigma) reagent method reported by [6]. The *C. capitellata* leaf extract was incubated at room temperature for 60 minutes with 0.5 mL of Foline Ciocalteu reagent and 0.05 mL of 10% (v/v) Na_2CO_3, and the absorbance at 620 nm was measured. The calibration curve for gallic acid (Figure 1) was obtained using similar procedures as for the extractions ($y=0.0517x+0.002$; $R^2 = 0.9796$). The absorbance values of the samples were interpolated against the gallic acid standard curve, which was represented in milligram of gallic acid per grams of crude extract (mg/g of extract).

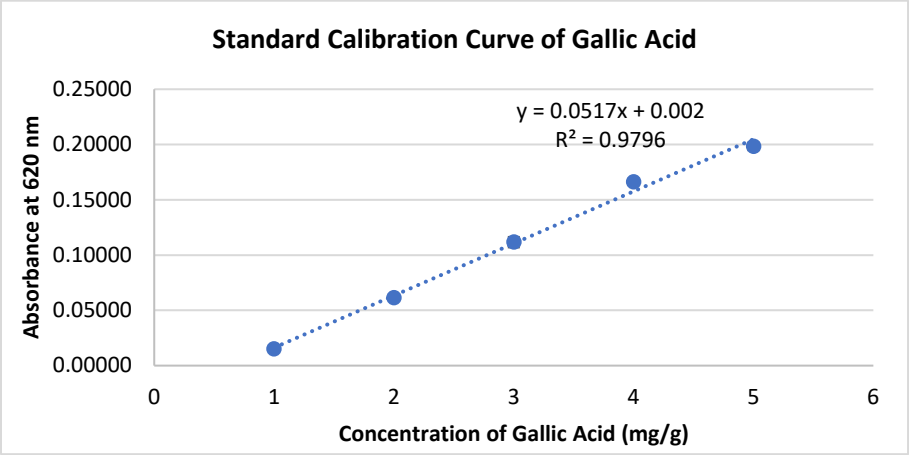

Figure 1. Standard Calibration Curve of Gallic Acid

Quantification of Total Flavonoids

The total flavonoids were measured using an aluminum chloride ($AlCl_3$) reaction [6]. To obtain a final concentration of 10% (w/v), 0.5 mL of *C. capitellata* leaf extract was mixed with 0.1 mL of 10% (w/v) $AlCl_3$, 0.1 mL of 1 M potassium acetate, and 2.8 mL of double-distilled water. After 30 minutes of incubation at room temperature, the absorbance at 415 nm was measured. The results were expressed as milligrams of quercetin per grams of crude extract (mg/g extract), with a higher value indicating greater quercetin activity. Calibration curve for quercetin (Figure 2), which was obtained under the same process parameters (y = 0.0551 - 0.0019, R2 = 0.983).

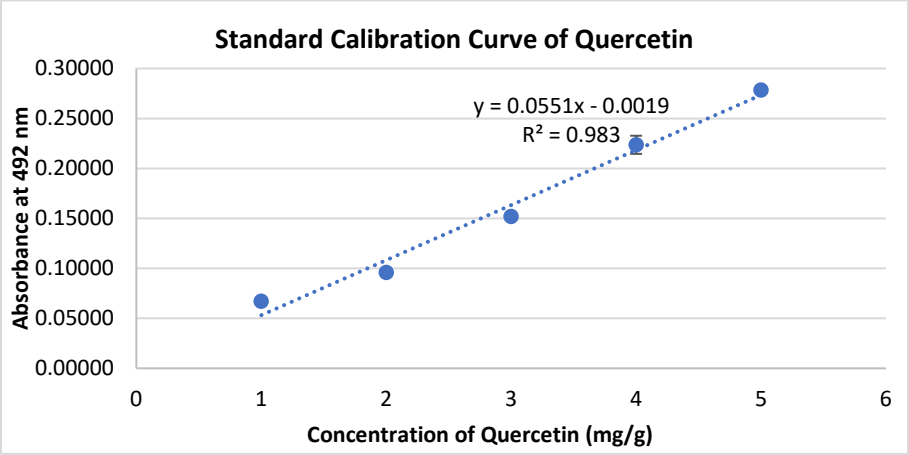

Figure 2. Standard Calibration Curve of Quercetin

Statistical Analysis

The results of this research were collected in triplicates for verification purposes. The statistical significance and standard errors of the means were determined using SPSS 27.0. The variances of the mean were evaluated using one-way ANOVA, with a p-value of 0.05 for the mean comparison.

RESULTS AND DISCUSSION

Plant extracts were extracted using various solvents with differing polarities. These solvents were employed in Soxhlet extraction procedures. The protocol adopted from (Albayrak et al., 2017) was followed for the preliminary plant extractions. The yields of crude extract % (w/w), are represented in the following Table 1.

Table 1. Total extracts yields of 50 grams of *C. capitellata* leaves crude extract

Solvents	% (w/w)
Methanol	14.60±0.2
Ethanol	11.23±0.15
Chloroform	3.4±0.1
Hexane	0.87±0.05

Values are expressed as the Mean ± Standard Deviation; n=3 in each group.

The highest yield was obtained from methanolic extracts, whereas the lowest yields were obtained from hexane extracts. The methanolic extract produced the highest yield of all the extracts (7.40 g), whereas the hexane extract produced the lowest yield of all the Soxhlet extracts (0.40 g). The color of the plant residues that remained once extracted was nearly identical to the color of the starting mixture that was used in the extraction. The ethanolic and methanolic extracts had a crusty form, whereas the viscosity of the hexane and chloroform extracts was thick and greasier in appearance, and the viscosity of the ethanolic extract was crusty in appearance as well as in appearance.

Panda et al. (2019) claimed that the solvent used in solid-liquid extraction is an essential component influencing the effectiveness of the process [23]. The type of solvent used in the extraction technique has a significant impact on determining physiologically active chemicals from plant material in the laboratory. The solvents employed in plant extraction must be non-toxic, easy to evaporate at cold temperatures, promote quick physiologic absorption of the extract, have preservation properties, and be incapable of causing the extract to be complex or dissociate during the extraction process.

In our research, polar solvents such as methanol and ethanol demonstrated much greater antibacterial activity than less polar solvents such as chloroform and hexane. Several published research confirmed this study's findings; these studies tested different solvents to see if they could solubilize antibacterial compounds extracted from plants.

Lopez et al. (2016) and Albayrak et al. (2017) investigated the potential of a range of solvents to solubilize antimicrobial from Clidemia sp. in a variety of conditions [6, 21]. The extraction solvents employed in these experiments were methanol, ethanol, and chloroform. As a result, even at low concentrations of up to 50 µg/ml, ethanol and methanol were efficient against Gram-positive and Gram-negative bacteria.

The antimicrobial activity of the plant extracts was first determined using disc diffusion, followed by the determination of the minimum inhibitory concentrations (MICs) (Table 2).

Table 2. Antimicrobial activity (zone of inhibition) of methanol crude extracts of *C. capitellata*

Microorganisms	-Ve Control	+ Ve Control	Concentration (mg/ml)		
			10	5	2.5
B. subtilis	NA	31.18±0.0	15.13±0.15	13.2±0.20	11.47±0.15
E.coli	NA	34.85±0.0	26.84±0.0	24.16±0.0	20.87±0.0
P. aeruginosa	NA	14.36±0.0	9.45±0.0	8.56±0.0	6.75±0.0
S. aureus	NA	26.84±0.0	15.1±0.10	13.07±0.12	10.03±0.06
S. marcencens	NA	24.44±0.0	12.43±0.12	10.27±0.21	9.03±0.06
S. choleraesuis	NA	32.06±0.0	14.55±0.0	13.59±0.0	13.26±0.0

NA (not applicable)
± Standard deviation of triplicate readings.
(-Ve) Control = 10 % DMSO
(+Ve) Control = Chloramphenicol 20 µg/ml

The findings of the disc diffusion experiment revealed that the concentration of the extracts had a statistically significant increasing effect on bacterial growth inhibition ($P<0.05$) as the concentration of the extracts increased (Figure 3). Among methanolic extracts, *E.coli* showed the greatest inhibitory activity (26.84±0.0 mm), with the largest inhibition zones being recorded in *E.coli*. 6.75±0.0 mm was the smallest inhibitory zone measured in *P. aeruginosa* and was the lowest recorded in any other bacteria.

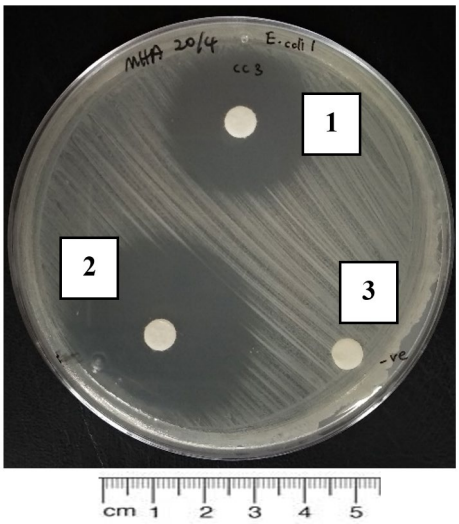

Figure 3. Disc diffusion assay of *Clidemia capitellata* methanolic extract in *E. coli*. 1=10 mg/ml *C. Capitellata* methanolic extract, 2=0.1% chloramphenicol, 3=10 % DMSO

Data from the disc diffusion assay of the plant extracts revealed that methanolic extracts at concentrations ranging from 10 to 2.5 mg/ml had potential antimicrobial activity. As a result, these extracts were tested on the same bacterial species again to identify the minimum inhibitory concentrations (MICs). The minimum inhibitory concentration test result demonstrated that MICs varied according to the microorganism strain tested. The greatest activity was shown in methanolic extracts of *C. capitellata* against the bacteria *E. coli* (MIC 62.5 µg/ml) (Table 3).

Table 0. Minimum inhibitory concentration (µg/mL) of methanolic extracts of *C. capitellata*

Bacteria	Minimum concentration (µg / mL)	(+) control
E. coli	62.5	15.7
S. aureus	125	31.5
B. subtilis	125	31.5
P. aeruginosa	125	31.5
S.choleraesuis	500	31.5
S. marcencens	125	31.5

For antioxidant activity, the leaves extract of *C. capitellata* was most effective (28.60±1.54). The leaves extract tested positive for total phenols, total flavonoids, tannins, terpenes, and steroids but negative for alkaloids. Total phenolic contents obtained were 80.78±0.06 of the extract and total flavonoid contents obtained were 64.64±0.10 of the extract, respectively. The results indicate that an extract having significant free radical scavenging also included a greater concentration of total flavonoids and polyphenols, implying a direct relationship between their presence and antioxidant activity. The presence of substances known to have pharmacological and physiological actions in plant extracts was discovered through the phytochemical examination of the extracts [24].

Plant extracts were discovered to include a variety of previously undiscovered phytochemicals, including phenols, flavonoids, tannins, terpenes, and steroids. The phenolic compounds are the most common and extensively distributed families of plant metabolites, accounting for approximately one-third of all plant metabolites [25]. The chemicals compounds exhibit anti-aging, anti-apoptotic, anti-inflammatory, anti-carcinogenic, cardioprotective, endothelial function enhancing effects, anti-atherosclerosis, and as well as the ability to suppress vasculature and cell growth [26]. A thorough investigation has been carried out to study the antioxidant effects of medicinal plants that contain high concentrations of phenolic chemicals [6,21].

Phytochemicals such as flavonoids, phenolic acids, tocopherols, and others are found in abundance in plants, and they account for the vast majority of natural antioxidants [27]. Bacterial growth is prevented by tannins, which bind to proline-rich proteins and hinder their production. A class of phenolic compounds known as flavonoids is produced by plants in response to pathogenic microorganisms infection. Flavonoids exhibit antibacterial properties against a wide variety of pathogens in vitro. These bacteria' tendency to develop complexes with proteins and other macromolecules, as well as with the bacterial cell membrane, is most probably the cause for their proliferation [28].

Apart from that, they are potent antioxidants with anticancer potential [29-31]. Because of their interactions with other molecules, such as sex hormones, steroids have been proven to have antibacterial properties. They are extremely important compounds because of their antibacterial properties [32].

Table 4. Screening of phytochemical profile and free radical sequestration of *C. capitellata* extracts

Total phenols (mg EGA/g)	Total flavonoids (mg EQ/g)	Alkaloids	Tannins	Terpenes/Steroids	Scavenging effect (%)
80.78 ±0.06	64.64±0.10	-	+	+	28.60 ± 1.54

(+) = *Present*
(-) = *Not Present*

CONCLUSION

The *C. capitellata* extracts evaluated in this study are a very promising source of substances with antioxidant and antimicrobial properties. This plant must be emphasized for all tests carried out to demonstrate its activity. There must be a strong emphasis on this plant in all tests conducted to demonstrate its functionality. New chemical, pharmacological assessments and the separation of the active compounds from the extracts should be conducted to demonstrate the links and mechanisms behind their antibacterial and antioxidant properties.

ACKNOWLEDGMENT

This work was supported by the Malaysia Toray Science Foundation (File No. 200602STRG0211). The authors would like to thank the Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Cawangan Negeri Sembilan, and Faculty of Agriculture, Universiti Putra Malaysia, for their support during the laboratory work.

CONFLICT OF INTEREST

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

REFERENCES

1. Alam, A., Ferdosh, S., Ghafoor, K., Hakim, A., Juraimi, A.S., Khatib, A., and Sarker, Z.I. (2016) *Clinacanthus nutans*: A review of the medicinal uses, pharmacology and phytochemistry. Asian Pacific Journal of Tropical Medicine 9(4), 402-409.
2. Mushtaq, S., Abbasi, B.H., Uzair, B., and Abbasi, R. (2018) Natural products as reservoirs of novel therapeutic agents. EXCLI J 77,420-451.
3. World Health Organization (WHO) (2009) The Canon of Medicine. World Health Organization (WHO), 1–30.
4. World Health Organization (WHO) (2013) WHO Traditional Medicine Strategy 2014-2023. World Health Organization (WHO), 1–76.
5. World Health Organization (WHO) (2019) WHO global report on traditional and complementary medicine.
6. Lopez, T., Corbin, C., Falguieres, A., Doussot, J., Montguillon, J., Hagège, D., Hano, C., and Lainé, É. (2016) Secondary metabolite accumulation, antibacterial and antioxidant properties of in vitro propagated *Clidemia hirta* L. extracts are influenced by the basal culture medium. Comptes Rendus Chimie 19(9), 1071-1076.
7. Rehab A.H., and Amira A.E. (2019) Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. Herbal Medicine, pp. 11-27.
8. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., and Lightfoot, D. A. (2017) Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6(4), 1-23.
9. Das, K., and Gezici, S. (2018) Review article Plant secondary metabolites, their separation, identification and role in human disease prevention. Annals of Phytomedicine: An International Journal 7(2), 13-24.
10. Baling, J., Noweg, G. T., Sayok, A. K., Wadell, I., and Ripen, J. E. (2018) Medicinal Plants Usage of Jagoi Bidayuh Community, Bau District, Sarawak, Malaysia. Journal of Borneo-Kalimantan 3(1), 67-87.
11. Jamal, P., Barkat, A. A., and Amid, A. (2010) Distribution of phenolics in various Malaysian medicinal plants. Journal of Applied Sciences, 10(21), 2658–2662.
12. Maarten J.M., and Christenhusz, J.W.B. (2017) The number of known plants species in the world and its annual increase. Phytotaxa, 3(261), 201–217.
13. Saw, L.G., and Chung, R.C.K. (2015) The flora of Malaysia projects. Rodriguesia, 66(4), 947-960.
14. Quattrocchi, U. (2017). Common Names, Scientific Names, Eponyms, Synonyms, and Etymology.CRC World Dictionary of Plant Names (1st edition), pp. 728.
15. Miléo, L.J., Silva, J.F., Bentes, J.L.S., and Christoffoleti, P.J. (2007) Alternative host weeds of Colletotrichum guaranicola in guarana crops in the State of Amazonas. Weed, 25(4), 771-782.
16. Azahar, N.F., Gani, S.S.A., Zaidan, U.H., Bawon, P., and Halmi, M.I.E. (2019). Melastomataceae family species (*Melastoma Malabathricum* L. Smith, *Melastoma malabathricum* var. alba and *Clidemia Hirt*a (L.) D.Don: A review of their ethnomedicinal, chemical constituents and pharmacological properties. Journal of Advanced Research in Dynamical and Control Systems, 11(8), 1105-1114.
17. Dianita, R., Ramasamy, K., and Ab Rahman, N. (2011) Antibacterial Activity of Different Extracts of *Clidemia hirta* (L.) D. Don leaves. Planta Medica, 77(12), 1069-1227.
18. Gordon, A., Schadow, B., Quijano, C. E., and Marx, F. (2011) Chemical characterization and antioxidant capacity of berries from *Clidemia rubra* (Aubl.) Mart. (Melastomataceae). Food Research International, 44(7), 2120-2127.
19. Narasimham, D., Bindu, Y.H., Cheriyamundath, S., Raghavan, R., Kumari, M.K., Chandrasekhar, T., and Madassery, J. (2017) Evaluation of in Vitro Anticancer and Antioxidant Activities From Leaf Extracts of Medicinal Plant *Clidemia Hirta*. International Journal of Pharmacy and Pharmaceutical Sciences, 9(4), 149-153.
20. Ellen, M.S.B., Tamires, G. dos S., Adriele, S. de O.C., Marcos, da C.S., Edson, de J.M., and Vera, L.C.V. (2020) Antimicrobial, antioxidant and cytotoxic activites and chemical profile of species of *Miconia ruiz* Pav., *Clidemia* D. Don and *Tibouchina* Aubl. (Melastomataceae). Journal of Medicinal Plants Research, 14(1), 1–6.
21. Albayrak, S., Atasagun, B., and Aksoy, A. (2017) Comparison of phenolic components and biological activities of two *Centaurea* sp. obtained by three extraction techniques. Asian Pacific Journal of Tropical Medicine, 10(6), 599-606.
22. Clinical and Laboratory Standards Institute. (2015) Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M07 A6. Wayne (PA): The Institute; 2003. CLSI, 35(2).
23. Panda, S. K., Mohanta, Y. K., Padhi, L., & Luyten, W. (2019) Antimicrobial activity of select edible plants from Odisha, India against food-borne pathogens. LWT, 113,1-10.
24. Altiok, E., Bayçin, D., Bayraktar, O., and Ülkü, S. (2008) Isolation of polyphenols from the extracts of olive leaves (*Olea europaea* L.) by adsorption on silk fibroin. Separation and Purification Technology, 62(2), 342–348.
25. Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. Journal of Alternative and Complementary Medicine, 2(3), 365-372.
26. Sytar, O., Hemmerich, I., Zivcak, M., Rauh, C., and Brestic, M. (2018) Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. Saudi Journal of Biological Sciences, 25(4), 631-641.
27. Tungmunnithum, D., Thongboonyou, A., Pholboon, A., and Yangsabai, A. (2018) Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines, 5(93), 1-16.
28. Ayoub, Z., and Mehta, A. (2018) Medicinal plants as a potential source of antioxidant agents: A review. In Asian Journal of Pharmaceutical and Clinical Research, 11(6), 50-56.
29. Mahato, T. K., and Sharma, K. (2018) Study of Medicinal Herbs and its Antibacterial Activity: A Review. Journal of Drug Delivery and Therapeutics, 8(5), 47-54.
30. Ali, S., Nasreen, S., Safeer, S., Andleeb, S., Ejaz, M., Bano, S., and Shakir, H.A. (2016) Medicinal plants as therapeutic agents for cancer treatment. In Punjab University Journal of Zoology, 31(2), 295-305.
31. Alves-Silva, J. M., Romane, A., Efferth, T., & Salgueiro, L. (2017) North African Medicinal plants traditionally used in cancer therapy. In Frontiers in Pharmacology 8(383), 1-24.
32. Nadin M.A., Xiaolei Z., Lihe J., Amy R., Deborah K., Shuo W., Fan W., Jingquan Y., Elliot A., Ying G., Jianhua M. (2018) Evaluation of extracts prepared from 16 plants used in Yao ethnomedicine as

potential anticancer agents. Journal of Ethnopharmacology, 211 (2018), 224–234.

33. Epand, R.F., Savage, P.B., and Epand, R.M. (2007) Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). Biochimica et Biophysica Acta - Biomembranes, 1768(10), 2500–2509.