STUDY ON *Hibiscus tiliaceus* LEAVES AS ANTIBACTERIAL AND ANTIOXIDANT AGENTS

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ABSTRACT

This study aimed to evaluate antioxidant and antibacterial activities of methanol, hexane, dichloromethane, ethyl acetate, and water crude sample fractions of H. tiliaceus leaves. Various type of samples play an important role to determining which compound that will give the positive results in antioxidant test (inhibiting free radical scavenging activity) which they probably have potency for preventing the cancer disease. Furthermore, those types of samples were also tested to the antibacterial test as their possibilities to be created as new antibiotics in the future. This research also focused on what compound groups that actually present in H. tiliaceus leaves by using the phytochemistry test. Antioxidant potency of H. tiliaceus leaves extracts for the radical scavenging activity for quantitative assay was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The antibacterial potency was measured by the Muller Hinton Agar (MHA) by applied the sample chemical crude in the plate. The result showed that all chemical crude of H. tiliaceus leaves has high antioxidant property as methanol crude (MC) 308.416%; hexane crude (HC) 232.837%; dichloromethane crude (DC) 150.837%; ethyl acetate crude (EC) 73.623% and water crude (WC) 71.777% with respected to the readings of 100% from quercetin (Q) as a positive control. From antibacterial result, entire samples had shown the positive results towards both gram positive (Staphylococcus aureus and Bacillus subtilis) and gram negative bacteria (Klebsiella pneumonia and Escherichia coli). The ranges of the inhibition zone were both at 7-10 mm and 10-15 mm. The phytochemistry test determine that the samples actually contained a lot of compounds which were proteins, carbohydrates, phenols/tannins, flavonoid, terpenoids, glycosides and also steroid. It is clearly indicated that H. tiliaceus leaves have potential to be used against the antioxidant and also as new antibacterial agents

Keywords *:H. tiliaceus, antioxidant, DPPH, antibacteria*

INTRODUCTION

Chemical constituent is a specific compound that contain in each sample. In this case, we will study about the chemical constituents that contain in the *H. tiliaceus* leaves, also distinguish which chemical constituents in it that may act as anti -bacterial and antioxidant agents [1]. Antioxidants are always related with the anticancer effects. So, the reason for detecting the antioxidant agents from the sample is to determine its ability to cure the cancer.

The antioxidant that use in this research is the free radical anti-scavenging method and as it is free from radical, it may be used to cure the cancer in human body [2]. Thus, the Hibiscus genus deserves additional evaluation as a provider of chemo-preventive agents. The importance of antibacterial test is to determine either the sample can be used as the bacterial preventer or not and it can be applied in the human life. Indeed, there is a current need for availability of new plant-derived bioactive molecules; for the development of new drugs and may provide a cost-effective mean of treating cancers and other diseases in the developing world [3].

There are some studies had already been run about this sample, however, they are using the samples from another resources and different solvent extraction method. Meanwhile, the increased cases of antibiotic resistant among pathogenic bacteria has encourage scientist to find new drugs against these pathogenic bacteria which supported by increasing number of compound with antibacterial activity extracted from botanical and animals source. Hence, extraction of antibacterial activity of medicinal plants especially from different parts of the mangrove plants is very important since vast number of medicinal plants have been used for centuries as remedies for human diseases [4].

Plants contain organic compounds that are not directly involved in the normal growth, development, or reproductions of organisms known as secondary metabolites which often play an important role in plant defenses mechanisms [5]. Secondary metabolites that were mentioned

such as alkaloids, glycosides, terpenoids, phenols, tannins, flavonoids and saponins [6], which limelight the researchers towards discovery of more effective bio-therapeutic agents [7]. However, what is the most important by using plant-derived medicines is that they are readily affordable and accessible [8].An example of *Hibiscus* genus, Malvaceae, contain several species comprises of about 275 species in the tropics and sub-tropics, many of which have been used medicinally and most *Hibiscus* species have a remarkable color pattern with the base of corolla forming a deep-coloured heart [9]. There are selected *Hibiscus* species were evaluated for antioxidant, antityrosinase and antibacterial activities from the leaves and flowers of the particular plants [10]. *Hibiscus tiliaceus* is a plant that contains many chemical constituent in it, which are needed for human being to threat so many diseases.

Andriani *et al.* [11] stated that *H. tiliaceus* had good and strong antioxidant properties with the IC_{50} value at 86.5 µg/mL and 15.00 µg/mL compared to Ascorbic acid (IC_{50}=15.00 µg/mL) and Quercetin (Q), respectively. Furthermore, the ethanol extract of *H. tiliaceus* also showed activity against three strains of bacteria *S. aureus* (gram positive), and *E. coli* [12]. However, there isn't enough information about antioxidant and antibacterial of *H. tiliaceus* leaves in its various fractions. Hence, this current study was determined the antibacterial and antioxidant activitiesof various fractions of *H. tiliaceus* leaves.

A research regarding the leaves of *H. sabdariffa,* which at 1 mg extract/disc, it able to inhibit Gram-positive bacteria of *Bacillus cereus, Micrococcus luteus* and *Staphylococcus aureus* [13]. In addition to that, at 2mg extract/disc, leaves of that particular same plant able to inhibited both Gram-positive and Gram-negative bacteria of *Escherichia coli, Pseudomonas aeruginosa, Salmonella choleraesuis* [14]. According to Ghaffar and Elaimy reported that the level of nitric oxide was significantly reduced by particular *Hibiscus* plants extract, *H. rosa* whereas it scavenged up to 36.3% nitric oxide radicals at a concentration of 500 µg/mL [15]. The research might contribute knowledge on the antioxidant and bioactivities of selected some species of *Hibiscus.*

There are also previous research regarding antioxidants of total phenolic content, radical-scavenging activity expressed as ascorbic acid equivalent antioxidant capacity, and ferric-reducing power of selected *Hisbiscus* genus plant, *H. tiliaceus,* were high [16]. Moreover, Vijay and Rajendra [17] revealed the phytochemical study of *H. tiliaceus* with positive result of carbohydrates, proteins, steroids, alkaloids, saponins, tannins, glycosides, and Amino acids from parts of wood and the leaves. Other report using the leaves of *H. tiliaceus* using brine shrimp assay as a guide led to the isolation of five compounds: β-amyrin, lupeol, p-methoxycinnamic acid, gossypol and vanillic acid [18].

RESEARCH METHODOLOGY

Hisbiscus tiliaceus leaves collection and preparation

The sample of *H. tiliaceus* leaves (Figure 1) for this experiment was collected at the Universiti Malaysia Terengganu (UMT) area since it is located nearby the beaches. Coastal areas do get greater UV radiation due to the reflection of sunlight from sand and sea surface. With greater UV radiation in higher altitudes and in coastal areas, one would expect highland and coastal plants to have greater antioxidant properties [19].

The *H. tiliaceus* leaves sample was then been air dried before grounded into fine particle size, it was stored in a plastic bag and the dry mass of sample was measured and recorded. Right after that, this sample was soaked in methanol solvent for 5-7 times to yield methanol extract. Then, all the supernatants were combined and collected and solvent contain was dried by using Rotary Evaporator Vacuum Controller V-850 (Buchi, Switzerland).

Figure 1: *Hibiscus tiliaceus* leaves

The crude that remains in the flask after the extraction done was then collected and dried. After the crude collected was dried enough, it was then weighted and labeled properly. Furthermore, Solvent-solvent partitioning was used to obtain some solvent fractions using hexane, chloroform and ethyl acetate. Totally 6 samples were collected, namely methanol extract and 5 solvent fractions (hexane, dichloro methane (DCM), ethyl acetate (EA) and water. Quercetin was used as a control.

Preparation of reagents

First of all, the DPPH solution was taken as much as 2.37 mg in 100 mL of methanol to prepare the reagents. The sample stock was taken as much as 10 mg in 1 mL of dimethyl Sulfoxide (DMSO),while for standard, quercetin 1 mg/mL (dissolve 1 mL of quercetin in 1 mL of DMSO) was used. All of these reagents were prepared accurately and mixed well before they were stored at the dark and safe place.

Protocol of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The methodology was modified from Hu *et al.*[20]. After the samples were loaded inside the wells in column A and B, 20 µL micropipette was used to perform 2 folded dilutions of the samples, from column B to G continuously.

Column A was act as the original sample stock loaded in the wells, as 2 folded dilutions was begins at column B. After column G was reached, the solution was then diluted and discarded. Column H was not be diluted with sample, and it was leaves only DMSO inside the well to act as negative control of this experiment. Meanwhile, quercetin was placed in the wells of row 10, 11 and 12 with 3 replicates to act as positive control. Quercetin was used as positive control.

Later, 200 µL micro pipette was used to add methanolic DPPH solution into each well. DPPH solution was induced fluorescence of the sample stock containing antioxidant component under Elisa Reader. Later, the 96 well-plates were incubated in the dark room for 30 minutes.

Then, the absorbance value (Abs) of the stock sample were measured at 517 nm in an automated microplate reader Multiskan Ascent (Thermo Electron Corporation, USA).

The antioxidant capacity was calculated by using the following equation:

$$\textbf{\% Inhibition} = \frac{([\text{Abs control} - (\text{Abs sample} - \text{Abs blank})]}{(\text{Abs control})} \times \mathbf{100}$$

Where the Abs control was the absorbance of the control (DPPH without sample), the Abs sample was the absorbance of the test sample (the sample test and DPPH solution), and the Abs blank was the absorbance of the sample blank (Sample without the DPPH solution).

The half-maximal inhibitory concentration (IC_{50}) was calculated by linear regression analysis and expressed as mean of three determinations.

Antibacterial test

This research were used the well test and paper disc test (Kirby Bauer test). Twenty milligram of crude extract was diluted in 1 mL of dimethyl sulfoxide (DMSO). Then it wastested for antibacterial activity by using four target bacteria, which were
E. coli, *K. pneumonia*, *S.aureus*, and *B. cereus.* All the bacterial were obtained from Institute of Marine Biotechnology, Universiti Malaysia Terengganu.

For agar well diffusion methods, all bacteria were cultured in appropriate broths at 30°C for overnight and the concentration was adjusted using by a spectrophotometer (λmax 600nm) to 10^5-10^6 colony forming units (CFU) per mL. Agar cultures were prepared as described by Jorgensen [21] and bacterial test was performed by the Mueller Hinton agar well diffusion method (modification method of Andriani *et al*. [22].

The bacteria suspension was used to inoculate 90 mm diameter Petri dishes with a sterile non-toxic cotton swab on a wooden applicator. The wells were cut from the agar using 6 mm cork borer. The cut agar disks were carefully removed by the use of forceps. All these processes were done in sterile condition.

To each well were introduced different concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.625 mg/mL) of crude extract. Sample of crude extracts (20µL) was loaded onto each well. Then, antibiotic gentamycin (xx) was located on the middle of agar surface as a positive control. While the solvent that used to dilute the samples (DMSO) was used as negative control. The plates were incubated for 24 hours at 37°C. Clear

inhibition zones around the well were measured after the incubation period.

All the determinations were performed in triplicates. The clear inhibition zones around the well indicated the presence of antimicrobial activity [23].

Phytochemical Screening

Phytochemical screening refers to extraction, screening and identification of medicinally active substances found in plants. Medicinal plants were contain some organic compounds which provide definite physiological action on the human body. For the *H. tiliaceus* leaves samples, numbers of test were run in the phytochemical test were as follow [24]:

Test for proteins. (Millon's test), Crude extract when mixed with 2 mL of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein. (*Ninhydrin test),* crude extract when boiled with 2 mL of 0.2% solution of Ninhydrin, violet color appeared suggesting the presence of amino acids and proteins.

Test for carbohydrates.(Fehling's test), equal volume of Fehling A and Fehling B reagents were mixed together and 2 mL of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars. (*Benedict's test),* crude extract when mixed with 2 mL of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Test for phenols and tannins. Crude extract was mixed with 2 mL of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

For Alkaline reagent test, Crude extract was mixed with 2 mL of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

For Test for saponins, Crude extract was mixed with 5 mL of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

For Test for glycosides. Salkowski's test: Crude extract was mixed with 2 mL of chloroform. Then mL of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

RESULTS AND DISCUSSION

H. tiliaceus sample

In this study, the dry sample of *H. tiliaceus* obtained after the grounded process was1728.0 g from 14 kg of fresh leaves.

From the methanol extractionand solvent-solvent partitioning process,the weight of methanol extract and solvent fractions were shown in Table 1.

From the data obtained, it showed the *H. tiliaceus* leaves contained highest quantity of hexane crude (non-polar compounds), followed by water crude (polar compounds) and then ethyl acetate and lastly dichloromethane (semi-polar compounds).

Table 1. The weight of methanol extract and fractions obtained

No.	Sample	Weight
1.	Methanol extract (MC)	255.013g
2.	Hexane solvent fraction (HC)	72.631g
3.	Dichloromethane solvent fraction (DCM)	19.568g
4.	Ethyl Acetate solvent fraction (EA)	20.268g
5.	Water solvent fraction (WC)	32.795g

The compounds were then being tested for its anti bacterial and antioxidant properties to check their activeness towards the test.

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Antioxidant activity

After the test was done by following the procedure in 3.5, the quercetin (Q) as a positive control was added one more which become Q1 and Q2 to ensure the test was not contaminate and to assure the samples were not contaminated by anything. The data that collected from the antioxidant analysiswas stated in Table 2 and Figure 2, all antioxidant activities were compared to the antioxidant standard, namely quercetin (Q).

From figure 2 we can see that the *H. tiliaceus* leaves were had highest antioxidant properties as its IC_{50} value of the antioxidant test higher than the quercetin, positive control. The quercetin was used as positive control. The reason of using two quercetin samples was to reassure the control used is accurate and does not contaminated by other substances or impurities.

The methanol crude (MeOH) gives the highest readings of the antioxidant value which 308.416 % compare to the average readings of Quercetin (Q1 and Q2) which only 90.3175 %.

The reason was the entire compound of the *H. tiliaceus* leaves were contained in the MC since methanol was the universal extraction solution. So, the value obtained cannot be sure which crude were actually contributed to the high readings of the antioxidant. But the readings of MC can be as a bench mark to clarify that the *H. tiliaceus* leaves really had the antioxidant compounds in it.

The reading showed that HC was had 232.837 % and hold the second highest readings from all crude Besides, if compare to the quercetin's readings was just 90.3175 %, HC also shown good result for the antioxidant test. HC was a non-polar solvent. It was use in this experiment to absorb all of the non-polar compounds that contained in the *H. tiliaceus* leaves which from the graph shown it have highest quantity of them compare to the other semi-polar and polar compounds.

DCM crude (DC) gives the readings value as 150.837 % which compare to the quercetin (Q) which only 90.3175 %. DCM solvents used to absorbed semi-polar compounds that contain in the *H. tiliaceus*leaves and it was run after HC because the needs to use the increases of polarity ingredients to get the exact value and also to ensure all of the compounds were already being extracted.

The meaning here, the steps should be started from the non-polar solvents first, then followed by the semi-polar solvents and lastly for polar solvents. The readings shown for semi-polar compounds of the *H. tiliaceus* leaves not as much as the non-polar compounds (HC) but was still bigger than the control (Q) value and it also shown that the semi-polar compounds in the *H. tiliaceus* leaves may use in the anticancer drugs.

The other semi-polar solvents that used in this experiment were EA crude (EC). The reason of using the other semi polar solvents after the DCM was to reassure all of the semi polar compounds already been extract before moved to the next solvents which was polar solvents. The readings shown by the EC was quite bigger as it was 73.623 % compare to the control (Q) which only 90.3175 %.

The reason why the readings was quite lower that the DC because most of the semi-polar compounds had already absorbed by the DCM solvent in the steps before. Although the readings were quite low compare to the other readings, but it was actually still high if compare to the control (Q). In the antioxidant test, the only matter here was the readings with compare to the control (Q) only. Since its value not varies too far from the control (Q), the EC also had been considered as good antioxidant crude.

The last fraction that was tested in the antioxidant test was the water crude (WC). The value of the antioxidant shown is 71.777 %. The reading was quite similar value with the EC. The Water was actually a polar solvent so the WC was the polar crude which contains all the polar compounds in the *H. tiliaceus* leaves. Since the readings were also varies not too far from the control (Q) value, WC was also been considered as a good antioxidant crude.The graph clearly showed the antioxidant for all of the crude extracted from *H. tiliaceus* leaves gives really high and even more than four times bigger than the control (Q) value.

This was due to the chemical constituents in the *H. tiliaceus* leaves which proven that it can be used as anticancer drug if more studies were conducted on it since this test was using the free radical anti-scavenging method for the antioxidant test.

Antibacterial

The antibacterial test was done with very precaution to prevent any mistakes happen in order to get the precise data as possible as stated in the Table 4, and the data of antibacterial can be simplified to easily understand them just like in Table 3.

The *K. pneumonia* and the *E. coli* were categorized as the Gram negative bacteria, while the *S.aureus* and *B.sublitis* were categorized as Gram positive bacteria. From the data obtained, the methanol, hexane and DCM crude were all active in Gram positive and Gram negative bacteria, but the water crude was only active to react with the Gram positive bacteria. However, all of the compounds from the *H. tiliaceus* leaves were all active towards the antibacterial test which giving chance to conduct more study about the *H. tiliaceus* leaves.

The reason why three antibiotics used in this experiment was to ensure the samples were actually active and they does not contaminated by other impurities. Since all of the antibiotics were shown positive results, it's clearly shown that the data obtained from the antibacterial test were absolutely true. Even the measuring of the halo area from this test was only done by using the ruler and naked eyes, but the focus on works may give the best results. The antibacterial test for

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Table 2.The average value of the antioxidant activity of samples collected

Concentration	MC	HC	DC	EA	WC	Q (1)	Q(2)
10	308.416	232.837	150.837	73.623	71.777	89.700	90.935
5	243.672	203.652	147.271	75.061	77.243	88.424	90.784
2.5	170.553	156.394	84.227	55.756	78.998	88.418	90.784
1.25	144.703	119.713	64.302	40.468	78.978	88.574	91.968
0.625	118.064	70.755	38.529	27.862	71.956	88.424	81.411
0.313	85.117	41.407	37.622	17.917	47.343	84.674	74.008
0.156	44.236	13.321	7.284	9.360	32.369	43.014	64.229
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000

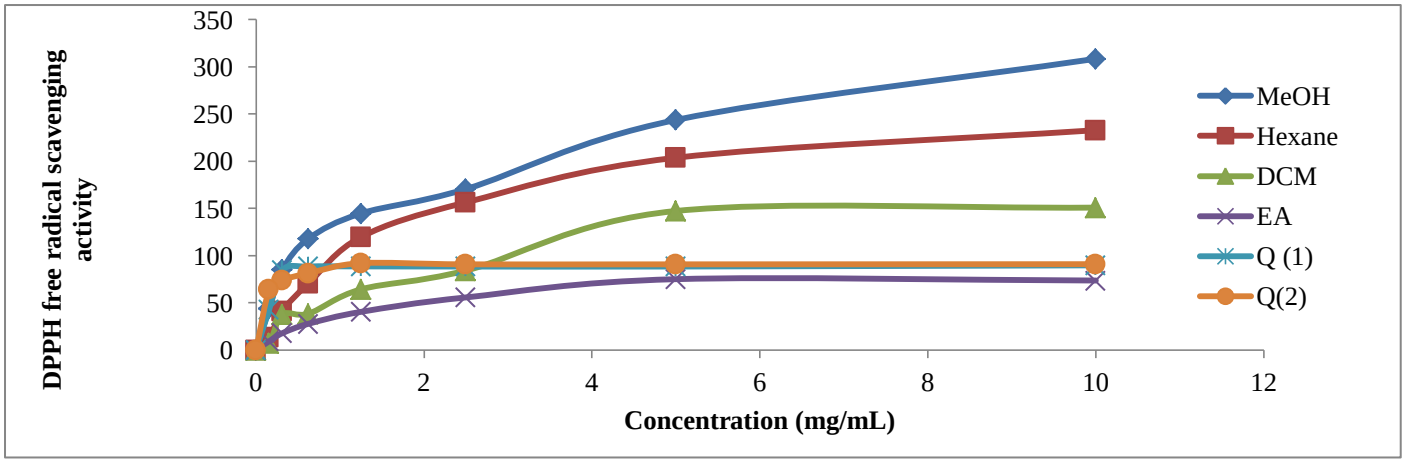

Figure 2.The DPPH free scavenging activity of samples.

H. tiliaceus leaves were successful and the data obtain was also good. It is hard to get the positive results towards the gram negative bacteria, hence if the samples used were contribute to it, the sample should be studied further [25]. Referring to the Vijay & Rajendra [26], the *H. tiliaceus* leaveswas a good choice for the sample used in the project because it's clearly shown the positive results to the Gram negative bacteria and also to the Gram positive bacteria.

The antibacterial test was successful and the data obtain is very good which make the *H. tiliaceus* leaves was a good samples not only as the antioxidant agents but also as the antibacterial agents too.The positive results towards Gram positive and also Gram negative bacterial makes the *H. tiliaceus* leaves place itself in its own class.

H. tiliaceus leaves can be applied to other products as its antibacterial ability is good.

Phytochemical Screening

The phytochemical test was used to get the information about what compounds group that actually appear in the sample as showed in Table 4 .It is shows that the *H. tiliaceus* leaves contained most of the compounds that usually have in the plant except two, which were saponins and alkaloids. Other than that, all of them were happen to be inside the *H. tiliaceus* leaves, and they were proteins, carbohydrates, phenols/tannins, flavonoid, terpenoids, glycosides and also steroid. Seven out of nine groups of compound that usually existed in the plants that had used to treat so many diseases wereavailable in the *H. tiliaceus* leaves which make it as a good sample and can be applied in so many medicine or other applications

Proteins from natural products can also use in the therapeutics products to increase the bodies figure and beautiful skin. Since *H. tiliaceus* leaves also contains protein in it, it means it can also been applied to the therapeutics products. *H. tiliaceus* leaves also contains carbohydrates which important to human to build the DNA structure.

The present of the carbohydrates in the *H. tiliaceus* leaves was showing the good results because it may be used in the drugs or medicine for human used in order to cure some diseases which was related to the functions of the carbohydrates. Meanwhile, the importance of phenols/tannins that also present in the *H. tiliaceus* leaves was important in the dietary of human.

Table 4. Phytochemicals screening of *H. tiliaceus* leaves

No.	Test	Result
1.	Proteins: millon	+
2.	Carbohydrates: Fehling	+
3.	Phenols/tannins	+
4.	Flavonoids – alkaline reagent	+
5.	Terpenoids	+
6.	Glycosides – Salkowski's	+
7.	Steroid	+
8.	Saponins	-
9.	Alkaloids	-

Flavonoids also happen to be in the *H. tiliaceus* leaves. Flavonoids is an important compounds that extracted from the natural products to use in many medicine now a days such as in the anticancer, inflammatory and allergies. Besides that, flavonoids also often used to get its beautiful orange reddish colour in the tattoo formation and also in the food.

Terpenoids was one more compounds that contains in the *H. tiliaceus* leaves. Terpenoids can be used in the therapeutics medicine and also as the chemopreventive agents. Terpenoids were already used world wide since it had huge ability to treat many diseases other that its ability as a chemopreventive agents. The usage of terpenoids in the therapeutics was similar with the proteins in the therapeutics products.

Glycosides was also another compounds that often found in the natural products as a curing diseases agents and it happen to be appeared in the *H. tiliaceus* leaves also. Glycoside is the sugar in the human body, and its role in the human body was to supply energy for them. Last but not least, steroids.

Steroid was an important compound that uses to protect the critical body organs such as heart in human body. Steroids also presented in the *H. tiliaceus* leaves, which makes it can be used more in the medicine for curing human organ diseases.

From all the compounds that determined in the phytochemical test, it was now proven that the *H. tiliaceus* leaves was very good sample not only for the anticancer agents but also for curing so many other diseases such as allergies and inflammatory.

Conclusions

By the end of this research, it was concluded that the *H. tiliaceus* leaves compounds were giving the positive results towards antioxidant and antibacterial activities. For antioxidant test, all five samples were giving positive results which make them possible to be used in the anticancer medicine.

For the antibacterial test, all of the *H. tiliaceus* leaves samples were showed very good activity against all gram positive and gram negative bacterial tested.

H. tiliaceus leaves were could be applied in so many things such as therapeutics and chemo-preventive agents. From the phytochemistry test, it

showed that the compounds content in the *H. tiliaceus* leaves were proteins, carbohydrates, phenols/tannins, flavonoid, terpenoids, glycosides and also steroid.

Further study will be needed, especially on isolating the bioactive compounds which could be have responsible to the both antioxidant and antibacterial activities.

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REFERENCES

[1] Chan, E.W.C., S.K. Wong, H.T. Chan, A Review on the Phytochemistry and Pharmacology of two *Hibiscus* Species with Spectacular Flower Colour Change: *H. tiliaceus* and *H. Mutabilis*, *International Journal of Pharmacognosy and Phytochemical Research* , 2016 : 8(7) : 1200-1208.

[2] Rosa R. M, Melecchi M.I, da Costa Halmenschlager . R, Abad F. C, Simoni C.R, Caramão E. B, *et al*, Antioxidant and antimutagenic properties of *Hibiscus tiliaceus* L. methanolic extract, *J Agric Food Chem.*, 2006 : 20(54): (19):7324-7330.

[3] Andriani, Y. Effendy, E.W.M. Tengku Muhammad, T.S. and Mohamad, H. Antibacterial, radical scavenging activities and cytotoxicity properties of *Phaleria macrocarpa* (Scheff.) Boerl.)leaves in HepG2 cell lines. *International Journal of Pharmaceutical Sciences and Research*, 2011: 2: 1700-1706.

[4] Musa ,N. S, Nadia Madiha Ramli, Jaznizat Saidin,Yosie Andriani, Antioxidant And Cytotoxicity Propertise Of Ethyl Acetate Fractions Of *Pandanus tectorius* Fruit Against HELA Cell Line, *Alotrop* , 2017: 1(2): 106-112.

[5] Pagare, S., Manila Bhatia, Niraj Tripathi, Sonal Pagare and Y.K. Bansal., Secondary Metabolites of Plants and their Role : Overview , *Current Trends in Biotechnology and Pharmacy*, 2015 : 9(3): 293-304

[6] Herbert Baxter, J.B. Harborne, Gerald P. Moss, *Phytochemical Dictionary: A Handbook of Bioactive Compounds from Plants, Second Edition* , 1998, CRC Press ISBN 9780748406203

[7] Andriani, Y, Habsah Mohamad, M.N.I, Kassim., N.D. Rosnan., D.F. Syamsumir., J.Saidin., *et al* , Evaluation on *Hydnophytum formicarum Tuber* from Setiu Wetland (Malaysia) and Muara Rupit (Indonesia) for Antibacterial and Antioxidant activities and anti-cancer Potency against MCF-7 and HeLa Cell. *Journal of Applied Pharmaceutical Science*, 2017: 7(9):30-37.

[8] Ekor, M., The growing use of herbal medicines: issues relating to adversere actions and challenges in monitoring safety, *Front. Pharmacol*. , 2014: 4 (177) : 1-10

[9] Salem, M.Z.M., J. Olivares-Pérez, A.Z.M. Salem, Studies on biological activities and phytochemicals composition of *Hibiscus* species-A review, *Life Science Journal*, 2014; 11 (5) : 1-8.

[10] S.K. Wong, Y.Y. Lim , E.W.C. Chan, Evaluation of antioxidant, anti-tyrosinase and antibacterial activities of selected Hibiscus species, *Ethnobotanical Leaflets*, 2010 : 14: 781-96.

[11] Lim, T.K., *Edible Medicinal and Non Medicinal Plants: Volume 8, Flowers* , 2014 Edition, Springer, ISBN-13: 978-9401787475 , and 10: 9401787476 .

[12] Sumardi , Mohammad Basyuni , Ridha Wati , Antimicrobial activity of polyisoprenoids of sixteen mangrove species from North Sumatra, Indonesia, *BIODIVERSITAS* , 2018: 19 (4): 1243-1248

[13] Chavez , P.R.,Jordy Perez–Gonzalez, Rafael Salgado–Garciglia, Enrique Ramirez- Chavez, Jorge Molina–Torres, Miguel Martinez-Trujillo, *et al* Antibacterial and cytotoxicity activities and phytochemical analysis of three ornamental plants grown in Mexico, *Anais da Academia Brasileira de*

Ciências , 2019: 91(2): 1-13.
[14] Al-Snafi AE. Pharmacological and therapeutic importance of *Hibiscus sabdariffa* -Areview. *International Journal of Pharmaceutical Research* 2018 : 10(3): 451-475.
[15] Ghaffar, F.R., El-Elaimy,I.A., In vitro, Antioxidant And A scavenging Activities Of *Hibiscus Rosa sinensis* Crude Extract. *Journal of Applied Pharmaceutical Science* , 2012 : 2:51-58.
[16] Sunil Kumar, Dinesh Kumar and Om Prakash, Evaluation Of Antioxidant Potential , Phenolic And Flavonoid Contents Of *Hibiscus Tiliaceus* Flowers , *EJEAFChe*, 2008: 7 (4) : 2863-2871.
[17] Vijay, T , Rajendra, B. Phytochemical Screening And Anthelmintic Activity Of Wood And Leaves Of Hibiscus tiliaceus Linn. *World Journal Of Pharmacy And Pharmaceutical Science*, 2014: 3(10) : 880-889.
[18] Shamsuddin, A.A., M. Najiah, A. Suvik, M.N. Azariyah,B.Y, Kamaruzzaman, A. W. Effendy , *et al* , Antibacterial Properties of Selected Mangrove Plants Against Vibrio Species and its Cytotoxicity Against Artemiasalina, *World Applied Sciences Journal*, 2013: 25(2), 333-340
[19] Wong,S.K, Lim,Y.Y., Chan, E.W.C. Anti -oxidant Properties Of Hibiscus Species Variation, Altitudinal Change, Coastal Influence And Floral Colour Change. *Journal of Tropical Forest Science*, 2009: 21(4), 307–315.
[20] Hu FL, Lu RL, Huang B, Ming L.Free Radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. *Fitoterapia*. 2004: 75(1):14-23.
[21] Jorgensen J.H., Mary Jane Ferraro , Anti -microbial Susceptibility Testing: General Principles and Contemporary Practices , *Clinical Infectious Diseases* , 1998: 26(4): 973-980
[22] Andriani, Y., Mohamad, H., Kesaven, B., Abdullah, M.I., Amir, H., Phytochemical, antibacteria and antibiofilm activities against *Pseudomonas aeruginosa* of various extracts and fractions of mangrove-associated, *Hibiscus tiliaceus*, *Journal of Sustainability Science & Management* (JSSM), 2017: 12(2): 45-51
[23] Ramproshad , S, T. Afroz, B. Mondal, A. Haque, S.Ara, R. Khan, S. Ahmed, Antioxidant And Antimicrobial Activities Of Leaves Of Medicinal Plant *Hibiscus Tiliaceus* L. *Pharmacology online*, 2012 : 3: 82 - 87
[24] Abdul-Awal, S.M., Sonia Nazmir, Sonia Nasrin, Tauhidur Rahman Nurunnabi, Shaikh Jamal Uddin, Evaluation of pharmacological activity of *Hibiscus tiliaceus*, *Springer Plus* , 2016: 5:1209
[25] Elisha, I.L., Francien S. Botha, Lyndy Joy McGaw, Jacobus Nicolaas Eloff, The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts, *BMC Complementary and Alternative Medicine* , 2017: 17(133): 1-10.
[26] Abdul-Awal, S.M., Sonia Nazmir, Sonia Nasrin, Tauhidur Rahman Nurunnabi , Shaikh Jamal Uddin, Evaluation of pharmacological activity of *Hibiscus tiliaceus*, *SpringerPlus* , 2016: 5 (1209): 1-6.

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