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Pharmacognostical, Phytochemical, Antioxidant and Antimicrobial Activity of Costus woodsonii

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Abstract: Background: Herbal medicine mostly contains wide range of chemical compounds responsible for medicinal therapeutic use. Costus woodsonii is commonly called as Red Button Ginger and synonyms of the botanical name are Costus spiralis, Alpinia spiralis and Costus pisonis. In Malay, it is known as Setawar Halia Merah. In Chinese, it is known as Hong Bi Qiao Jiang. Objective: This research was conducted to study the pharmacognostical, phytochemical, antioxidant and antimicrobial activity of C. woodsonii leaf extracts. Method: Macroscopy, microscopy, phytochemical analysis, thin layer chromatography, antioxidant activity and antimicrobial activity of C. woodsonii leaf were carried out. Total flavonoids were estimated in the leaf extract. The total phenolic content of C. woodsonii leaf was determined using Folin-Ciocalteu reagent. The antioxidant activity of leaf extract of C. woodsonii was determined by performing DPPH radical scavenging. The microbial activity was determined by Well diffusion test, MIC (Minimum Inhibitory Concentration) test and MBC (Minimum Bactericidal Concentration) test. Result and Discussion: C. woodsonii belongs to costaceae with elliptical green leaves. Till now are no extensive studies on C. woodsonii. Preliminary phytochemical analysis revealed the presence of flavonoid, steroid, fat, phenol, tannin and mucilage in leaf extract. Physicochemical studies further revealed the ash value of leaf as 8.7%. Among the three extractions, alcohol extractive values showed the highest as 13%. Loss on drying at 105 degree Celsius in leaf was found to be 12.67%. The plant extract showed total phenolic content of 7.941 mg GAE/g at concentration of 5μg/ml. As for flavonoids content, plant extract showed 21.7 mg RE/g at concentration of 200μg/ml and 43.4 mg RE/g at concentration of 400μg/ml. For antioxidant activity, the plant extract showed weak antioxidant activity in DPPH scavenging activity assay. For antimicrobial test, the leaf extract of C. woodsonii showed weak antimicrobial activity. Conclusion: From this study, it can conclude that C. woodsonii leaf extract possess weak antioxidant activity and weak antimicrobial activity which need to be further validated by using more antioxidant assays and antimicrobial tests.

Keywords: *Costus woodsonii, Antioxidant activity and Antimicrobial activity.*

1. Introduction

Herbal medicines have been utilized throughout written history, and probably even longer. Archaeological evidence suggests the use of herbal medicines for various conditions as early as 60,000 years ago [1]. Herbal medicines are plant-based medicines which are complex mixture that made from different combinations of plant parts such as leaves, flowers, stems, fruits or roots. Each part can have different medicinal uses and the many types of chemical compounds needed different extraction methods. Both fresh and dried plant matter are used and it is depending on the herb [2]. The plant that I have selected for my research project is C. woodsonii. It is commonly called as Red Button Ginger, Scarlet Spiral Flag, Red Cane, Panamanian Candle Ginger, Indian Head Ginger, Dwarf French Kiss and Dwarf Cone Ginger. The synonyms of the botanical name are C. spiralis, Alpinia spiralis and C. pisonis [3]. In malay, it is known as Setawar Halia Merah. In chinese, it is known as Hong Bi Qiao Jiang (红闭鞘姜). The classification of C.woodsonii is as follow [3-5]. Nehete J 2010 studied C. Speciosus in vitro antioxidant activity of different extracts. It was revealed that benzene extract had the maximum phenolic content 4.38% and showed good correlation coefficient (r2) for all antioxidant methods [6]. Khan MM 2019 used the Bulb Extract of C. woodsonii for Phytogenic Synthesis of Band Gap-Narrowed ZnO Nanoparticles. C. woodsonii was used as a green approach to synthesize zinc oxide (ZnO) nanoparticles from its bulb (flower). These studies confirmed the synthesis of ZnO nanoparticles with a lower band gap [7]. Until now there are less research done on Costus family and there were no extensive studies on C. woodsonii.

Figure 1.
C. woodsonii collected from Lakeview Horticulture Nursery in Sungai Lalang, Malaysia.

A study was conducted to further discover this plant. The objective of this research was to evaluate the phytochemical constituents, antioxidant and antimicrobial properties of the plant, with the aim to discover their medicinal value and standardizing this plant with its potential benefit.

2. Materials and Methods

Plant of study which is C. woodsoniiwas collected in 16th October 2018 from Lakeview Plant Nursery in Bedong, Sungai Petani, Kedah, Malaysia. The leaves of plant were dried at 45 degree Celsius in hot air oven for 4 days. The leaves were continued to dry until it was able to crush into small pieces and blended into fine powder using blender. Then the blended powder was passed through sieve to get the evenly smaller size fine powder and separated it from the plant fiber that was unable to be reduced in size. A herbarium voucher specimen of C. woodsonii was prepared and then submitted to Faculty of Pharmacy of AIMST University, Malaysia. The selected plant was examined for its size, shape, color, texture, surface and macroscopic characteristics. In microscopic study, leaf of the selected plant and its powder were studied for various micro chemical test and microscopically parameters using standard methods.In physicochemical analysis, extractive values with different solvents, loss on drying, total ash value, foreign organic matter, fluorescence analysis and thin layer chromatography was carried out using the procedures mentioned in reference text. Extraction was done by using Soxhlet extraction. After extraction, the solvent was removed by using rotavapour and dried extract was preserved for further studies. Phytochemical Screening of the plant extract was carried out to detect various phytochemicals present in plant by following reference methods as cited in pharmacopoeial standards.

2.1. Total Phenolic Content

For the standard stock solution, gallic acid was used and was prepared in concentration of 1000 μg/ml by dissolving 10mg of gallic acid in 10 ml of 95% ethanol. Mixing of 95 ml of absolute ethanol with 5 ml of distilled water produced 95% ethanol. Then serial dilution was performed from the stock solution. Several dilution of standard gallic acid was prepared which include 2, 4, 6, 8 and 10μg/ml. The total phenolic contents of the ethanolic extracts of sample were estimated using the Folin Ciocalteu reagent. 2.5% sodium bicarbonate was prepared by dissolving 2.5g in 100ml of distilled water. After serial dilution, 0.2ml of prepared Folin-Ciocalteu reagent and 4ml of sodium carbonate was added according to 0.2ml of stock

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solution of various concentrations. The sample was then incubated at room temperature for 1 hour. The absorbance of each concentration was measured at 750nm using UV spectrophotometer and subsequent calibration curve was constructed [8].

In order to measure the absorbance of various concentrations, two sets of blank were prepared by mixing in each set with 0.2 ml of 95% ethanol and 0.2 ml of Folin-Ciocalteu reagent and 4 ml of sodium carbonate. These blank was also incubated as same manner as done with gallic acid. As for the extract sample, stock solution was prepared for a concentration of 1000 µg/ml similar to gallic acid preparation, which is 10mg of ethanolic leaves extract were dissolved in 10 ml of 95% ethanol. 3 solutions were made through serial dilution for concentration of 5, 10 and 20 µg/ml. Similarly, 0.2ml of Folin-Ciocalteu reagent and 4ml of sodium carbonate was added to each prepared extract dilution. After preparation, they were incubated at room temperature for 1 hour. The phenolic content was determined for ethanolic leaves extract by using the formula,

Total Phenolic Content, C = (A/B) x Dilution Factor.

Where

C = Expressed as mg GAE/g dry weight of the extract.

A = the equivalent concentration of gallic acid established from calibration curve (mg).

B = Dry weight of the extract (grams).

2.2. Total Flavonoid Content

Rutin was used as standard stock solution. Initially stock solution was prepared for concentration of 1000 µg/ml by dissolving 10 µ 10 mg of rutin in 10 ml of 95% ethanol. Serial dilution was performed from stock solution to form differences concentrations which are 10, 20, 40, 60, 100, 200 and 300 µg/ml. Then, 2% AlCl3 solution was prepared by dissolving 2 g in 100 ml of ethanol. 0.5 ml of sample from each dilution was added with 1.5ml of ethanol, 0.1 ml of 10% AlCl3 solution, 0.1 ml 1 M potassium acetate solution and 2.8 ml of distilled water and incubated at room temperature for 1 hour accordingly. Meanwhile, stock solution for leaf extract was prepared by dissolving 10 mg in 10 ml of 95% ethanol to make a concentration of 1000 µg/ml. It was then diluted to 200 µg/ml and 400 µg/ml respectively. Also, 1.5 ml of ethanol, 0.1 ml of 10% AlCl3 solution, 0.1 ml 1 M potassium acetate solution and 2.8ml of distilled water and incubated at room temperature for 1 hour accordingly. In order to measure the absorbance of various concentrations, two sets of blank were prepared by mixing in each set with 1.5ml of ethanol, 0.1 ml of 10% AlCl3 solution, 0.1 ml 1 M potassium acetate solution and 2.8 ml of distilled water [9]. The blank was incubated as the same manner done for rutin and leaf extract. After incubation, absorbance of various concentrations of rutin stock solution and ethanolic extract was determined at maximum wavelength of 415 nm. Calibration curve was constructed and flavonoid content was calculated as follow,

Total Flavonoid content, C = (A/B) x dilution factor

Where

C = expressed as mg RE/g dry weight of extract.

A = the equivalent concentration of rutin established from the calibration curve (mg).

B = dry weight of extract (g).

2.3. DPPH (1,1-Diphenyl-2-Picryl Hydrazyl) Radical Scavenging Assay

In DPPH assay, Butylated Hyroxytoluene (BHT) was used as standard stock solution. It was prepared by dissolving 10ml of BHT in 10 ml of 95% ethanol to make a concentration of 1000 µg/ml. Serial dilution was done from the stock solution to make various concentration which are 10, 20, 40, 60, 80 and 100 add until 400 µg/ml respectively. The DPPH scavenging activity of the ethanolic extracts of samples were estimated using the DPPH reagent. Thus, the 0.1mM DPPH reagents were prepared by dissolving 3.94 mg in 100ml of 95% ethanol. 3 ml of DPPH reagent prepared was added into 2.5ml of each dilution of stock solution. Since DPPH reagents are extremely photosensitive, each test tube was wrapped with aluminum foil after addition of DPPH reagent. In addition the whole process was carried out in a dark room. The mixture was then well shaken and allowed to stand for incubation in dark at room temperature for 30 minutes. In order to measure the absorbance of various concentrations, two sets of blank were prepared by mixing in each set with 2.5 ml of 95% ethanol and 3 ml of DPPH reagent. The blank was incubated as the same manner done for BHT. As for the extract sample, stock solution was prepared for a concentration of 1000 µg/ml similar to BHT, which is 10mg of ethanolic leaves extract were dissolved in 10ml of 95% ethanol. Serial dilution was made into various concentrations which include 20, 40, 60, 80 and 100 µg/ml. Similarly, 3 ml of DPPH reagent was added to 2.5ml of each prepared ethanolic leaves extract and then test tube was wrapped with aluminum foil accordingly [10]. The mixture was then well shaken and allowed to stand for incubation in dark at room temperature for 30 minutes. Absorbance for both BHT and ethanolic extract was determined and calibration curve was constructed. Antioxidant activity usually expressed as IC50 which can be calculated from standard graph plotted. Percentage scavenging activity of ethanolic extract can be calculated as follow,

Percentage scavenging activity = [(Ac-As)] x 100

Where,

Ac = absorbance of control reaction.

As = absorbance of extract samples.

2.4. Antimicrobial Study

Agar well diffusion method: In this study, Bacteria strains of Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Salmonella typhi, Acinetobacter baumannii, Bacillus pumilusand Micrococcus luteus were cultured using nutrient agar broth in 6 different universal bottles. The bacterial strains stored in the universal bottles were left shaking incubator for 24 hours at 37°C at 180 rpm. The next days, the bacteria strain which has grown in nutrient agar broth is then cultured into nutrient agar plate. 15% Dimethyl Sulfoxide (DMSO) solution was prepared by dissolving 15 ml of DMSO in 100ml of sterilized distilled water. 15% DMSO solution and penicillin was used as a positive control and an agar plate without culture growth was used as a negative control. Marker pen was used to label the bottom of the prepared petri dish. 100 µl of bacterial strain (Pseudomonas aeruginosa) was spread onto the surface of agar by using spreader. 4 different spot corresponding to the label around 6 to 8 mm were punched aseptically by using cork-borer. Each hole was filled with 10.0 mg/ml, 20.0 mg/ml, 40.0 mg/ml, 60.0 mg/ml, 80.0 mg/ml, 100.0 mg/ml, of extract respectively using 100µl micropipette. The procedure was repeated for another 5 bacterial strains (Escherichia coli, Staphylococcus aureus, Salmonella typhi, Acinetobacter baumannii, Bacillus pumilusand Micrococcus luteus). After that, the agar plates were covered and subjected to incubate at 37°C for 24 hours. Determination of antimicrobial activity was determined and zone of inhibition was measured [11].

2.5. Minimum Inhibitory Concentration (MIC) Test

Preparation of standard McFarland bacteria culture: 4-5 loops of the bacterial strains were cultured in each sterile nutrient broth. Incubated the prepared nutrient broth at 37 degree Celsius for 24 hours. After the incubation, allow the incubated culture to centrifuge at 3000 rpm for 15 minutes to obtain the cell mass (pellet). After 15 minutes, the supernatant was discarded and the resulting cell mass was re-suspended in another new sterile nutrient broth. The suspension was standardized by ensuring an absorbance reading range is around 0.08-0.1 using a spectrophotometer at 625nm.

MIC test: 30 mg of the ethanolic plant extract was dissolved in 3 ml of 15% DMSO to obtain concentration of 1000 µg/ml. Two fold serial dilutions were made to get 5 different concentration which was 1000, 500, 250, 125, and 62.5 µg/ml. 0.2ml of extract solution was transferred into a cleaned assay tube by micropipette and 1.8ml of standard McFarland bacterial culture was then added into the tube containing extract solution by micropipette.

1ml of the mixture was then transferred into a second cleaned assay tube already containing 1 ml of standard McFarland bacteria culture by micropipette to obtain 500 µg/ml. The steps were repeated until 62.5 µg/ml concentrations were achieved. Each time transfer, the micropipette tip should be changed. This is to prevent the mixing of concentration or increase in concentration. The process was repeated for each bacterial strain and duplicate sets were made. Two positive tubes containing only the bacterial culture suspension were prepared respectively. One negative tube containing the sterilized nutrient broth was also prepared. The tubes were then incubated at 37 degree Celsius for 24 hours. The MIC activity was observed and determined.

2.6. Minimum Bactericidal Concentration (MBC) Test

After 24 hours incubation, the tube which showed clear solution was chosen as test sample for each bacterial strain. The agar plate was labeled with name, Nutrient agar, name of bacteria and date. 0.1 ml of incubated nutrient broth of selected tube was transferred onto an agar plate and spread evenly. 0.1ml of each clear solution of bacterial strain was transferred onto an agar plate and spread evenly. The plate was incubated at 37 degree Celsius for 24hours. The result was observed [12-15].

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3. Results and Discussion

Morphology of leaves as follows: Mature Foliage Color(s): Green, Mature Foliage Texture(s): Smooth, Glossy/Shiny, Prominent Young Flush Color(s): Green, Young Flush Texture(s): Smooth, Foliar Type: Simple/Unifoliate, Foliar Arrangement along Stem: Spiral, Foliar Attachment to Stem: Sessile, Foliar Shape(s): Non-Palm Foliage (Elliptical), Foliar Venation: Parallel, Foliar Margin: Entire, Foliar Apex/Tip: Acute Herbarium voucher specimen of Hedyotis hedyotidea was prepared and submitted to the Herbaria of Faculty of Pharmacy (FOP), AIMST University, Malaysia. Herbarium voucher specimen accession number AIMST/FOP/41 was assigned to the specimen. Visualization by using naked eyes and with the help of magnifying glasses, morphology of the plant was observed. Each and every external structures of plant leaf were noted in accordance with the reference of literature reviews. The microscopic features of the leaf were observed with and without staining agents by using Binocular compound microscope. When the leaf was stained with Ruthenium Red, it showed mucilage containing cells in pinkish red color. When the leaf stained with 60% sulfuric acid, it showed needle shaped crystals in lamina and midrib of the leaf. When it stained with dilute iodine, it showed starch grain in dark brown color. When it stained with Sudan Red III, it showed red color protein crystals. From the observation, the type of stomata was identified to be tetracytic stomata. Microscopic studies showed the presence of many microscopic cellular structures and pictures of some of the structures are cited below in (Figure 2 to Figure 16).

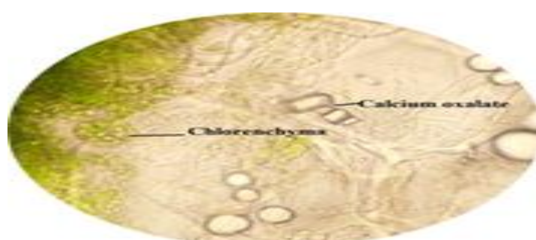

Figure 2.
Calcium oxalate and chlorenchyma in lamina of leaf.

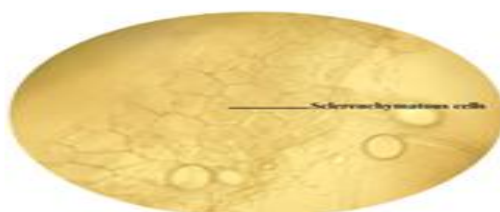

Figure 3.
Sclerenchymatous cells in lamina of leaf.

Figure 4.
Needle shaped crystals in lamina of leaf.

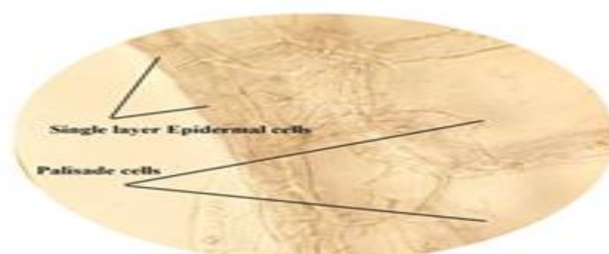

Figure 5.
Single layer epidermal cells and palisade cells in lamina of leaf.

Figure 6.
Starch grain in lamina of leaf in lamina of leaf.

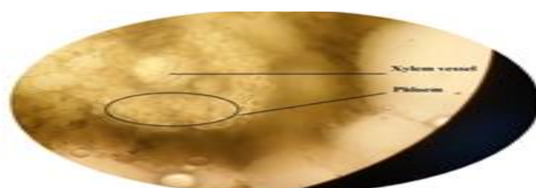

Figure 7.
Xylem vessel and phloem in mid rib of leaf.

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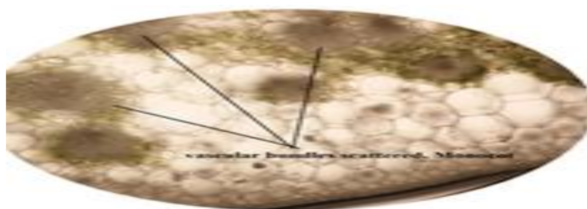

Figure 8.
Vascular bundles scattered, monocot type in mid rib of leaf.

Figure 9.
Chloroplast containing parenchyma in mid rib of leaf.

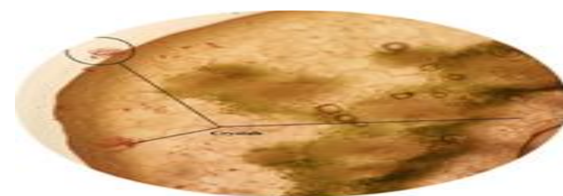

Figure 10.
Crystals in mid rib of leaf.

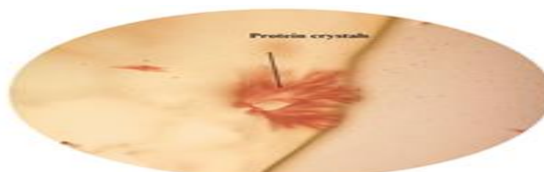

Figure 11.
Protein crystal crystals in midrib of leaf.

Figure 12.
Monocot type vascular bundles in petioles of leaf.

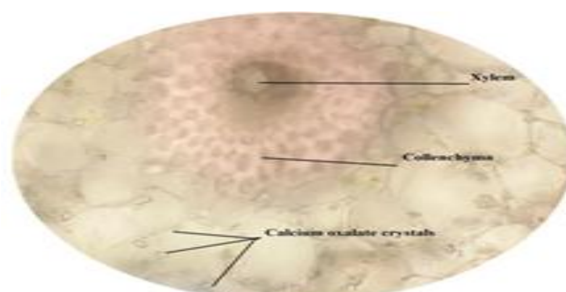

Figure 13.
Xylem, collenchyma and calicum oxalate crystal in petiole of leaf.

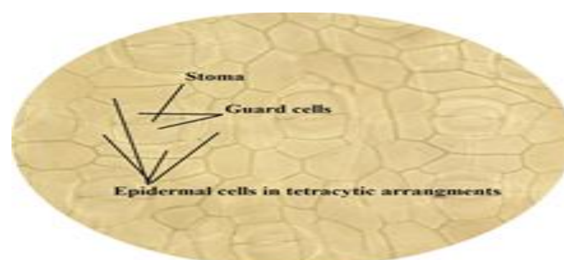

Figure 14.
Stoma, guard cells and epidermal cells in tetracytic arrangements in stomata of leaf.

Figure 15.
Spiral xylem in powdered leaf.

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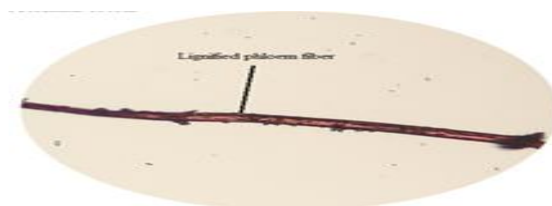
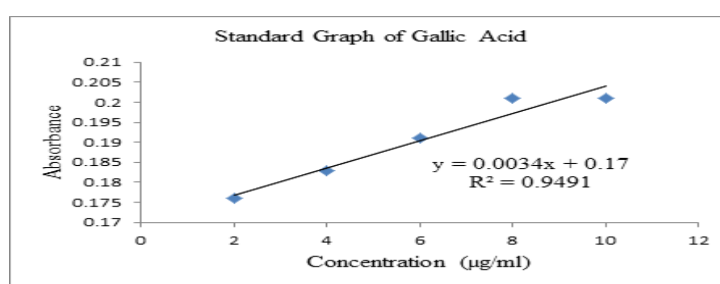


Figure 16.
Lignified phloem fiber in powdered leaf.

In this study, alcohol-soluble extractive value (13%) was found to be the highest among the 3 type of solvents. This shows that the constituents of the drug are more extracted and soluble in alcoholic solvent as compared to water, chloroform and petroleum ether as solvent. In this study, the Loss on drying of leaves of C. woodsonii was estimated to be 12.67%, which showed that leaves contain large amount of water content. This might due to the time for the collection of the leaf of the sample was during morning and the surrounding temperature was low. The higher the surrounding temperature, the more the amount of water lost due to evaporation [15]. It was found to that the recommended moisture content value for powder production using dried leaves is 10% and below [16].

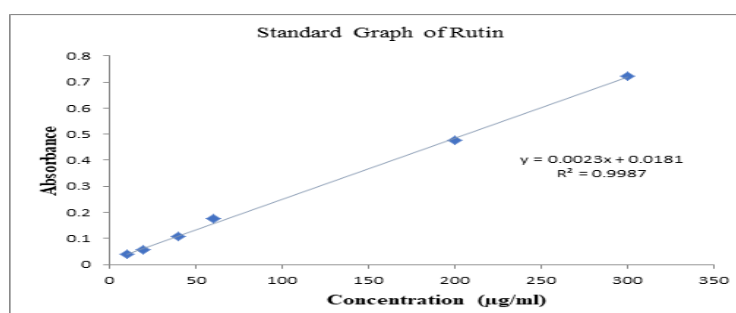
The ash content of the crude drug is residue remaining after incineration that all the water and organic matter in the plant cell have been completely removed by heating. Ash commonly contains inorganic radicals like carbonates, calcium, potassium, magnesium and many more. After complete burning, ash value obtained for leaves of C. woodsonii was 8.7%. A high ash value is the indication of consequences contamination, adulteration, substitution or carelessness in preparing the drug. It was found that the ash value for individual drug is between the ranges of 4.18% to 14.47% w/w [17]. In this study, the percentage showed a normal ash value, thus the present of inorganic matter content in the leaves of plant is within the normal range. From this study, foreign organic matter was extremely low which was found to be 0.301%. Foreign matter that present were mostly sand or dust adhere on the surface of the leaves. The fluorescent studies of dried leaf powder of C. woodsonii with different chemical do not show fluorescence under UV light with wavelength of 254 µm and 365 µm. phytochemical analysis results showed the presence of carbohydrates, amino acid, flavonoid, alkaloids, tannins and phenolic compounds.

In Thin Layer Chromatography (TLC) solvent development, first solvent system was tried was Toluene: Ethyl Acetate: Formic Acid, TEaF (5:4.5:0.5) and Butanol: Ethanol: Water BEW (5:1:1.1). However these 2 solvent systems did not show clear separation. Thus referring to the Phytochemical Methods by J.B Harbone, some other solvent systems were tried such as Butanol: Acetic Acid: Water, BAW (4:1:0.5) and BEW with different ratio (3:1:3.3). These two solvent systems have shown some separation of component under ultraviolet light. After comparison BAW (4:1:0.5) and BEW (3:1:3.3) were selected. TLC plates were observed under UV light with wavelength 254 nm and 365 nm. Spots if separations were marked accordingly and the retention factor (Rf value) was calculated using formula. A total of 6 components found in C. woodsonii leaves which Rf values are 0.183, 0.521, 0.583, 0.634, 0.761, 0.817. In Total phenolic content (TPC), Standard calibration curve was plotted with an equation of $y = 0.0034x + 0.17$, $R^2 = 0.9491$. The absorbance of each concentration of the extract was recorded. It showed that the absorbance value increase as the concentration (µg/ml) increase. C. woodsonii extract showed total phenolic content of 7.941 mg GAE/g at concentration of 5 µg/ml. This indicate that leaf contain large amount of polyphenol content (Graph1).



Graph 1.
Standard Graph of Gallic Acid.

The Total Flavonoids Content (TFC) can be determined using 2 calorimetric methods, which uses either aluminum chloride or DNP (2, 4-Dinitrophenylhydrazine). In this study aluminum chloride was used. The TFC in the leaf extracts using the aluminum chloride was expressed in term of rutin equivalent (mg RE/g). Standard calibration curve was plotted with an equation of $y = 0.0023x+0.0181$, $R^2 = 0.9987$. It showed that the absorbance value increases as the concentration (µg/ml) of the plant extract increase. By comparing the absorbance of the standard rutin absorbance, it was found that the C. woodsonii showed total flavonoids content of 21.7 mg RE/g at concentration of 200 µg/ml and 43.4 mg RE/g at concentration of 400µg/ml. This indicated that the leaf extract contain less amount of flavonoids (Graph 2).



Graph 2.
Standard Graph of Rutin.

In this study, DPPH (2, 2-Diphenyl-1-Picryl-Hydrazyl) was used to determine the antioxidant capacity of leaf extract. DPPH remain as stable free radical on room temperature and appears as violet molecule. When undergoes reduction which caused by antioxidant molecule, it decolorize from deep purple to yellow color [18]. The evaluation of the antioxidant activity of sample was determined based on the scavenging activity against the free radical DPPH through the calculation of IC50, which represents the concentration of the material required for inhibit 50% of the free radicals. Thus, a lower IC50 value in a particular sample indicates a greater ability to neutralize free radical. In this study, both the BHT standard and the plant extract were determined at 518nm. Standard calibration curve was plotted with an equation of $y = 0.1963x+5.7545$ $R^2 = 0.9806$. It showed that the absorbance decreases as the concentrations (µg/ml) increase. However, the increase in concentration results in increase of scavenging percentage which results in the linear graph. The results of IC50 were calculated based on the standard curve of BHT, the value of standard BHT was 225.4µg/ml. The IC50 value of standard BHT plant extract was much higher compare to the plant extract as the scavenging activity for the plant extract on 5000 µg/ml was only 33.75% (Graph 3 and Graph 4). It indicated that ethanolic extract of C. woodsonii had very less antioxidant capacity [19].

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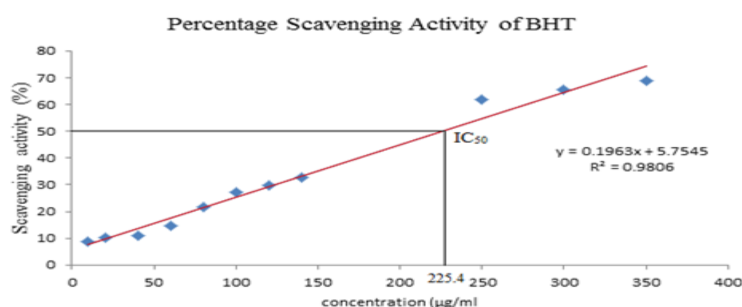
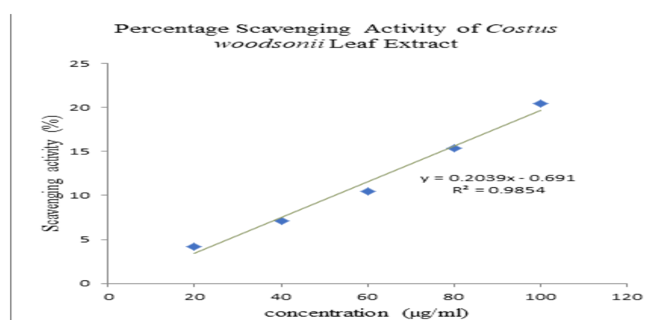


Graph 3.
Percentage scavenging activity of BHT.

IC50 of BHT was found to be IC50=225.4 µg/ml



Graph 4.
Percentage scavenging activity of C. woodsonii leaf extract.

In Antimicrobial Test, there was no zone of inhibition for Escherichia coli and Staphylococcus aureus. Most probably this is because of the minimum inhibition concentration of leaf extract of C. woodsonii for Escherichia coli and Staphylococcus aureus was higher or it does not shown antimicrobial activity to Escherichia coli and Staphylococcus aureus. The ethanolic extraction of C. woodsonii with the concentration of 80mg/ml showed the largest zone of inhibition (3.98 cm) among other 5 concentration (10, 20, 40, 60 and 100 mg/ml) on Acinetobacter baumannii. For Bacillus pumilus, 40mg/ml of the ethanolic extraction of C. woodsonii showed the largest zone of inhibition (3.02 cm) among other 5 concentration. For Micrococcus luteus, 80mg/ml showed the largest zone of inhibition (2.98 cm) among the other 5 concentrations.

The zone of inhibition should increase as the concentration of plant extract increase. The factors that cause these might be the difference in amounts of microorganism presence in the nutrient agar plates as the volume of each inoculations of bacteria were not standardised. If the inoculation of the bacteria is too much, it required more extract to inhibit the growth of the bacteria. In the other way, the ethanolic extraction of C. woodsonii had larger zone of inhibition at lower concentration of extract for Acinetobacter baumannii, Bacillus pumilus and Micrococcus luteus. This might due to lesser amount of bacteria inoculated on that particular plate so lower concentration and lesser amount of extract is required to inhibit the growth of the bacteria, thus showing larger zone of inhibition at lower concentration of extract used. Repetition and further investigation is required to determine the error and the causes. For Salmonella typhi, only 40mg/ml of the ethanolic extraction of C. woodsonii showed the zone of inhibition (1.88 cm) among other 5 concentration. The absent of zone of inhibition for other 4 concentration may due to contamination of the extract solution for those 4 concentration (10, 20, 60, 80 and 100 mg/ml).

Another reason might be inadequate amount of extract added to the well of the agar plate. 10 µg/ml penicillin and 15% DMSO were used as the positive control for each type of bacteria test and a nutrient agar plate without culture of bacteria was used a negative. The penicillin showed no zone of inhibition on Micrococcus luteus, this may cause by the improper diffusion of penicillin or insufficient amount of penicillin added into the well of the agar plate (Figure 17 to figure 20). By comparing the zone of inhibitions for each concentrations of plant extract on the 4 bacteria species, the plant extract showed weak antimicrobial activity. Repetition of the test or further investigation should be carried out for the validation of the results.

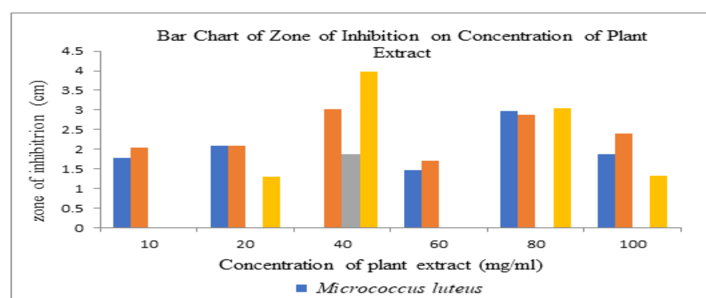


Figure 17.
Bar Chart of Zone of Inhibition on Concentration of C. woodsonii.

Figure 18.
Zone of inhibition on Micrococcus luteus.

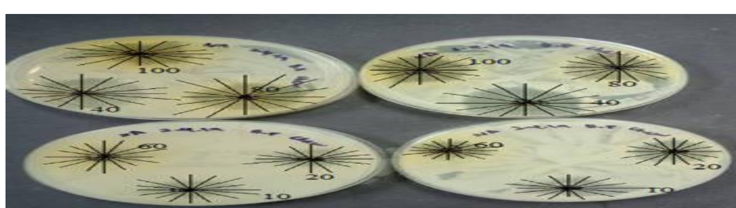


Figure 19.
Zone of inhibition on Bacillus pumilus.

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Figure 20.
Zone of inhibition of Acinetobacter baumannii.

In Minimum Inhibitory Concentration (MIC) Test, Based on the result shown, there was no significant of MIC in the test. So MBC Test was not carried out. The absence of MIC might due the contamination of nutrient broth or contamination during any process of MIC or the MIC for ethanolic extract of leaf of C. woodsonii is higher than 1000 µg/ml. Repetition and further investigation are required for the validation of the results.

4. Conclusion

C. woodsonii was chosen for this study as there was no research done on it before. Soxhlet extraction method was used for the extraction of leaf of Costus woodsonii. All standardization parameter of leaf of the plant were done according to pharmacopoeia standards. The results of these parameters were found within the limits. The phytochemical analysis of C. woodsonii revealed the presence of carbohydrate, amino acids, fat and oils, flavonoids, alkaloids and phenolic compounds. Besides, antioxidant activity studies such as DPPH radical scavenging activity were carried out for the leaf extract. Based on the results, it can be concluded that ethanolic leaf extract of C. woodsonii possess weak antioxidant activity and thereby required validation by more antioxidant assays. Antimicrobial test such as Well Diffusion Test and MIC Test were carried out and the result showed that ethanolic extract of leaf of C. woodsonii has weak antimicrobial activity and need more antimicrobial test for the validation of the result. Furthermore, C. woodsonii required more extensive studies such as pharmacological activities as it might have other potential which have not been explored yet.

Abbreviations: MIC-Minimum Inhibitory Concentration, MBC-Minimum Bactericidal Concentration, DPPH-1,1-Diphenyl-2-Picryl Hydrazyl, BHT- Butylatedhyroxytoluene and DMSO-Dimethyl Sulfoxide.

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