Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(4); 677-683

ISSN: 0975-4873

Research Article

Evaluation of Ethanolic Extract of *Pericampylus glaucus* (Lamk.) Merr for Total Phenolic, Total Flavonoids Contents and *In-vitro* Anti - Oxidant Activity

Muhammad Kifayatullah*, Pinaki Senguptha, Mohd. Shahimi Mustafa, Sreemoy Kanti Das, Mrutunjay Sisugoswomi

Faculty of Pharmacy, Lincoln University College, 47301 Petaling Jaya, Selangor, Malaysia

Available Online: 15th June, 2015

ABSTARCT

Objective: To aim of the present study was to investigate the total phenolic, total flavonoids content and antioxidant activity of crude etahnolic extracts from the leaves of *Pericampylus Glaucus (Lamk.) Merr* for possible sources of novel antioxidants in food and pharmaceutical formulations. Methods: The total phenolic contents were determined by Folin ciocalteu method using gallic acid as a standard and the total flavonoid contents were determined by Aluminum chloride method using Rutin as a standard by Uv-visible spectrophotometer with the range of 765nm and 415nm.The antioxidant activity of ethanolic extract of *Pericampylus Glaucus (Lamk.) Merr* was determined by in-vitro model i.e. DPPH (1, 1–diphenyl picrylhyrazyl) and reducing power method against Ascorbic acid as a reference drug, by Uv-visible spectrophotometer with the range of 517nm and 700nm. Results: The phytochemical screening of crude *Pericampylus Glaucus (Lamk.) Merr* leaves plant extract showed the presence of various bioactive compounds while absence of anthraquione compound. Total phenolic and total flavonoid contents in the ethanolic extract were 52.51mg/gm with percentage yield as 5.20% w/w of the extract mg gallic acid equivalents/g and 51.70mg/gm with yield was 5.17% yields w/w of the extract mg rutin equivalents/g, respectively. The result showed that *Pericampylus Glaucus (Lamk.) Merr h*as a good radical scavenging effect (8.18%) at a dose 0.125mg/ml, 20.67% at a dose 0.25mg/ml, 37.69% at a dose of 0.75 and 51.67%% at a dose 1mg/ml, while at the same concentration, the reference Ascorbic acid the effect was 87.53%, 91.86%, 92.37% and 93.64%. The reducing power of crude plant extract 0.0964±0.001, 0.3553±0.0217, 0.5506±0.0117 and 0.6660±0.015 at concentration of 100, 300, 600 and 900ug/ml respectively, while at the same concentrations the reducing power for reference ascorbic acid was0.9051±0.0009, 1.4773±0.2551, 2.3763±0.044 and2.561±0.086. Conclusion: The positive results suggest that the crude leaf extracts of *Pericampylus Glaucus (Lamk.) Merr* should be further studied to determine the bioactive chemical compounds as well as to understand the possible mechanism of action that could be used as potential sources of new antioxidant.

Key words: Antioxidants, Free radicals, *Pericampylus Glaucus (Lamk.) Merr*, DPPH, reducing power, Total phenol, Rutin, Folin ciocalteu reagent

INTRODUCTION

In worldwide about 10,000 species of plants are medicinally important in traditional systems of medicine[1]. According to (WHO), 80% of the world's population used traditional medicine for their healthcare[2]. Because of side effects of synthetic drugs, currently most of the research is focus on plants based drug for various kinds of human diseases with antioxidant potential. Reactive oxygen species are responsible for pathogenesis of more than hundred chronic disorders, such as diabetes, cancer, aging, AIDs, arthritis, inflammation, hypertension, heart attack, atherosclerosis and other degenerative diseases[3].The main source for producing free radicals in body is metabolism, nitric oxide radical, super oxide, that are in connection with oxidative damage of lipids, amino acid, DNA and protein[4]. Free radicals targets natural properties of membrane, like ion transport mechanism, fluidity and result loss in cross linking, enzyme activity, protein cross linking, inhibition of protein synthesis and DNA damage[5]. The currently available synthetic antioxidants causes negative effect on health as promoters of cancer and other defects[6]. Therefore, the searching for alternative sources of natural antioxidant is become increasing important. Plant based drug with antioxidant compounds may potentiate the body's anti-inflammatory and antioxidant defense mechanisms or may act as antioxidants[7]. Natural antioxidants reduced high risks of heart disease, arthritis, inflammation cancer, hyperglycemia and other chronic human disorder[8]. Studies have been confirmed that free radical scavenger compounds stabilize or deactivate the free radicals protecting the human body from various diseases before they attacked on biological cell[9]. The

*Author for Correspondence

Table 1: Phytochemical analysis of ethnolic extract of *Pericampylus Glaucus (Lamk.) Merr*

Sr. No	Chemical compounds	Test name	Observations	Results
1	*ALKALOIDS*	Mayer's test	Pale ppt formed	Alkaloids present
2	*SAPONINS*	Froth test froth present	Stable persistent	Saponins
3	*REDUCING SUGARS*	Fehling test present	Brick red ppt	Reducing sugars
4	*PHENOLS*	Ferric chloride test	Bluish color formed	Phenols present
5	*TANNINS*	Lead acetate test	Red ppt observed	Tannins present
6	*FLAVANIODS*	Alkaline reagents tests	Reddish pink colors observed	Flavonoids presents
7	*RESINS*	Acetone water test	Clear solutions observed	Resins absent
8	*TERPENOIDS*	Salkowaski test observed	Reddish brown coloration	Terpenoids presents
9	*ANTRAQUIONONES*	Bontrager's test	No pinks colors observed	Anthraquionone absent (-ve)
10	*STEROLS*	Liebermann-Burchard test	dark pink	Sterol presents

Table 2*:* Quantity of Phenol contents in *ethanolic extract of Pericampylus glaucus (Lamk.) Merr at 765nm*

Concentration mg/ml	Means absorbance of Gallic acid ± SEM
0.01	0.0710 ± 0.010
0.05	0.899 ± 0.054
0.25	1.91773 ± 0.015
1.25	4.6297 ± 0.212

Value expressed as mean ± SEM. N=3

Table 3: Determination of total Quantity of Flavonoids content at 415nm wavelength

Concentration (mg/ml)	Absorbance of extract (Mean ± SEM) of Rutin
0.001	0.0436 ±0.0018
0.003	0.063±0.0009
0.009	0.038 ± 0.004
0.027	0.2241± 0.001
0.081	0.230 ± 0.002
0.24	0.4175 ± 0.002
0.720	1.202 ± 0.058

Value expressed as mean ± SEM. N =3

plants based products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment[10]. The plants of the Menispermaceae family are known to be rich source of bioactive compounds that have significant role in in aliment of various diseases. The *Pericampylus Glaucus (Lamk.)Merr is* a climber shrubs belongs to the family of menispermacea that is commonly found in ground and forest area of Malaysia. It also occurs in Thailand, India, China, Indonesia, Myanmar, Taiwan, Philippine, Vietnam[11]. In Malaysia, traditional name commonly is Akar chuping[12]. Traditionally *Pericampylus* species have reported to possess active pharmacological properties out of which some have established scientific data but the active constituents still needs to be explored. It is possible that exploration of the other parts such as leaves can lead to new evidences regarding to free radical scavenger. The plant *Pericampylus Glaucus (Lamk.) Merr* is used in traditional medicinal Asian system, and since a long period of time most of these in connection with inflammation, arthritis, sore throat, abdominal pain, productive cough, headache, wheezing, abdominal distention, stomachache, dispels chills, antiasthmatic, antitussive and snake biting[11]. Despite the traditional uses of the plant, there is little in-vitro investigations have been published against Hepatitis B and HIV Virus[13]. Triterpenes isolated from plant have been reported in-vitro anticancer activity[14].Therefore, the aim of this present work is to investigate the antioxidant activity of *Pericampylus Glaucus (Lamk.) Merr*.

MATERIALS AND METHODS

Chemical and Reagents

Lead (II) acetate trihydrate, potassium iodide, potassium sodium tartrate, hydrochloric acid, copper sulphate pentahydrate, Ibuprofen, 1, 1-diphenyl-2- picryl hydrazyl (DPPH), Gallic acid, Rutin, tricholoroacetic acid, potassium feericyanide, vitamin C (ascorbic acid), methanol, ethanol, absolute ethanol, ferric chloride, sodium carbonate anhydrous. All the other chemical reagents were used Merck (Darmstadt, Germany), astral laboratory chemicals R/M chemicals, loba chemicals, Alpha chemika and Sigma Aldrich Co. (UK).

Collection and extraction of the plant sample

The whole plant of *Pericampylus Glaucus (Lamk.) Merr* was collected in the month of September 2014 from village Kampung Jeram Kedah, Negeri Sembilan, Malaysia and was authenticated by Ms. Tan Ai Lee at Forest Research Institute Malaysia (FRIM), Malaysia where voucher specimen Herbarium with number (SBID:014/14) was deposited at the Faculty of Pharmacy. After Washing with running water leaves were separated and dried in shade for 20 days and converted into coarse of powder with blender. The coarse powder was extracted by continuous hot extraction using the soxhlet apparatus at a temperature of 78°C for 48hr using 95% ethanol. The extract was then concentrated under reduce pressure through rotary evaporator. The extracts were collected and preserved in a desiccator until used for further studies.

Phytochemical screening of the Plant Extract

The ethanol crude extracts of *Pericampylus Glaucus*

Table 4: Antioxidant activities of plant extracts on DPPH free radical scavenging On UV visible Spectrophotometer at 517nm of wavelength

Sample	Con mg/ml	Mean absorbance at 517nm	%age DPPH free radical scavenging
	Control	0.3932±0.008	
Ascorbic acid	0.125	0.049±0.029	87.53%
	0.25	0.032±0.015	91.86%
	0.750	0.030±0.088	92.37%
	1	0.025±0.012	93.64%
Pericampylus Glaucus	0.125	0.361±0.011***	8.18 %
	0.25	0.312±0.015***	20.65 %
	0.750	0.245±0.024***	37.69%
	1	0.190±0.025***	51.65%

Data are expressed as Mean ±SEM. Values are considered as significant at ***p<0.001, When compared to control N= 3

Table 5: Effect of extract *Pericampylus Glaucus Lamk Merr* on reducing power method for antioxidant activity at wavelength of 700nm.

Sample	Mean absorbance at 700 nm	Mean absorbance at 700 nm	Mean absorbance at 700 nm	Mean absorbance at 700 nm
Concentration µg/ml	900	600	300	100
Ascorbic acid	2.561± 0.0394	2.3763± 0.2568	1.4773± 0.1472	0.9051±0.0005
Pericampylus Glaucus	0.6660±0.0087***	0.5506±0.0067***	0.3553±0.0125****	0.0964±0.0011****

(Lamk.) Merr was tested by standard method for the presence of various bioactive compounds[15].

Determination of total Phenolic contents

The total phenol contents in the ethanol extract of *Pericampylus Glaucus (Lamk.) Merr* was determined by Folin ciocalteu method, calculated as gallic acid equivalence (GAE)[16]. An amount of 0.5ml of sample (1mg/ml of extract in 1ml of ethanol) was mixed with 2.5ml of dilute Folin ciocalteu reagent in a test tube and was then agitated on a vortex, placed in a dark for 3mintue. Then 2ml of 7.5% of sodium carbonate was added into the mixture and again vortex for 3 minute. Finally the mixture was placed in dark for 1 hrs. The absorbance was measured at 765nm on UV visible Spectrophotometer. Same procedure was repeated for blank and standard. All experiment was performed in triplicates. For standard curve, gallic acid in concentration 0.01mg/ml, 0.05mg/ml, 0.25mg/ml and 1.25mg/ml were prepared in ethanol. GAE was calculated from the graph based on linear regression analysis of data and equation of straight line was obtained. The total quantity of phenol was determined by the following formula:

Total phenolic content = GAE X V/M where, GAE was gallic acid equivalents or the concentration (mg/ml) of gallic acid obtained from the calibration curve(mg/ml) and V is the volume of extract (ml), and M is the weight of pure plant extract (g).

Determination of total Flavonoid

For determination of total flavonoid contents in ethanol extract of *Pericampylus Glaucus (Lamk.) Merr*, aluminum chloride method was incorporated, using Rutin as a reference compound[17]. The stock solution was prepared by dissolving of 1mg of extract in 1ml of the absolute ethanol. From stock solution 0.3ml of the extract was taken in a test tube and mixed with 0.5ml of D/W and 90µl of 5% sodium nitrate solution. The mixture was then placed for six minutes at room temperature. Six minutes later, 180µl of 10% Aluminum chloride solution was added to the mixture and was again allowed to stand for further 5min. Then 0.5ml of 1M NaOH solution was added to the mixture, and final volume of the mixture was made upto 3ml with distilled water. The mixture was then agitated on vortex for complete mixing. The prepared solution was run in triplicates for each observation and mean value of absorbance was obtained. Repeated the same protocol for standard (rutin), replacing the extract with 0.3ml of rutin. For calibration curve, rutin in concentration 0.001mg/ml, 0.003mg/ml, 0.009mg/ml, 0.027mg/ml, 0.081mg/ml, 0.24mg/ml and 0.72mg/ml was prepared. Absorbance was measured against the blank at 415nm of wavelength on U V visible Spectrophotometer. Ethanol in place of extract was as blank and rutin were used as a reference solution. Rutin equivalents were obtained on linear regression observation.

The total amount of flavonoids in the ethanolic extracted of *Pericampylus Glaucus (Lamk.) Merr* was determined by following formula.

T F=RE x V/M where RE was Rutin equivalents expressed as (mg/ml), V was the volume of extract expressed in (ml) and M was the weight of extract expressed in gram.

Anti-oxidant activity

DPPH radical scavenging Assay

The antioxidant potential of ethanolic extract of *Pericampylus Glaucus (Lamk.) Merr* was assessed on the basis of radical scavenging effect of the stable 1, 1-diphenyl-2- picryl hydrazyl (DPPH) free radical[18]. An amount of 0.2mM DPPH solution was prepared by

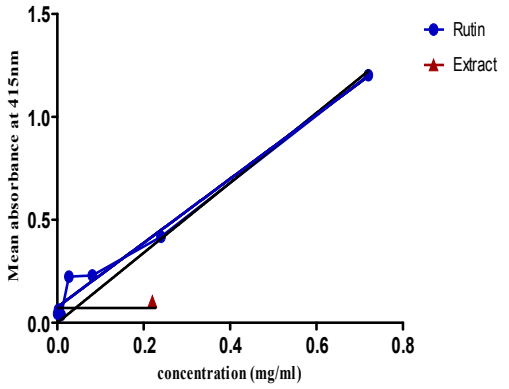

Figure 1: Total Flavonoids contents

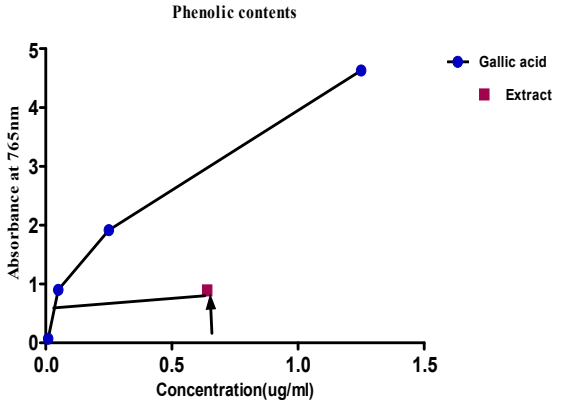

Figure 2: Total Phenol contents

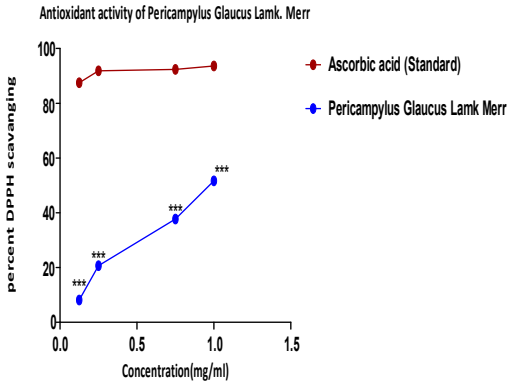

Figure 3: DPPH radical scavenging Activity
Percentage DPPH free radicals scavenging of extracts from Pericampylus Glaucus Lamk Merr compared with ascorbic acid at different concentration and extract at 517nm for antioxidant activity. Mean ± SEM. P<0.001 as compared to control

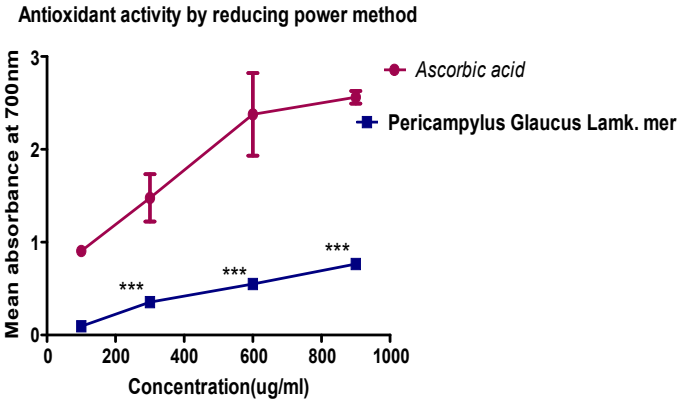

Figure 4: Antioxidant activity by reducing power method
Reducing power of crude ethanolic extract of Pericampylus Glaucus Lamk Merr and ascorbic acid, as measured by change in absorbance at 700nm. Mean ±SEM significant p< 0.001 as compared to control

dissolving 7.8mg of DPPH powder in 100ml of methanol. Ascorbic acid was used as reference. Different concentration of extract and standard were prepared like 0.125mg/ml, 0.25mg/ml, 0.50mg/ml, 0.75mg/ml and 1mg/ml in methanol. 2ml of extract and ascorbic acid was mixed with 1ml of 0.2nM of DPPH separately and then was shaken on vortex and kept in dark at 37C° for 30 min and optical absorbance was recorded on UV visible Spectrophotometer at wave length 517nm. The same procedure was repeated for ascorbic acid. The method was run in triplicate for both plant extract and reference ascorbic acid. Methanol was served as blank which contain only methanol and negative control contain 1ml of extract and 3ml of 0.2nM of DPPH. The percentage of scavenging of DPPH free radical was calculated by following equation:

%age scavenging activity =

$$\frac{A\ (control) - A\ (s)}{A\ (control)} \times 100$$

Where A (c) = Absorbance of control (solution without extract)

And A (s) = Absorbance of the samples (extract + standard)

Reducing power assay

The reducing power of the crude plant extract of *Pericampylus Glaucus (Lamk.) Merr* was determined according to standard procedure[19]. Vitamin C compound was used as standard. 1% of vitamin C solution was made by dissolving the vitamin C in d/w. Different concentration of extract and vitamin C were prepared (100, 300, 600, 900ug/ml) in 1ml of distilled water. The extract was mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium feericyanide solution. The resulting mixture was placed in incubator at 50c° for 20 minutes and then adds 10% of 2.5ml of tricholoroacetic acid. The mixture was then centrifuge at 3000 rpm for 10 min to collect the upper layer of solution (2.5ml), mixed with 2.5ml distilled water and 0.5 ml of freshly prepared 0.1 %(w/v) ferric chloride solutions and then the absorbance was measured at 700nm of wave length on U V visible Spectrophotometer. All measurements were run in triplicate. Same procedure was repeated for phosphate buffer (pH 6.6), used as a blank solution.

Statistical analysis

Statistical analysis were performed by two way ANOVA test using graph pad prism version 5.0 and the standard error p <0.001was regarded to be statistically significant

RESULTS

The present study was carried out on the leaves of crude plant extract of *Pericampylus Glaucus (Lamk.)Merr* for total phenolic, total flavonoids and antioxidant activity. After converting the plant into coarse powder, 12gm of extract was obtained though soxhlet apparatus.The phytochemicals analysis on crude ethanolic extract of plant *Pericampylus Glaucus (Lamk.) Merr* leaves showed the presence of alkaloids, saponins, reducing sugars, tannins, flavonoids, sterol, phenols, terpenoids, while absence of anthraquionones. The results are presented in Table 1.

Total Phenolic contents

The total phenolic contents in ethanolic extract of *Pericampylus Glaucus (Lamk.) Merr* was manifested as mg/g Gallic acid equivalent using the standard curve equation (Y=mx+b) where Y=3.316±0.5354x.30withr^2=0.9504. Absorbance was measured on U-V visible spectrophotometer at 765nm of wave length. The total quantity of phenol in dry extract was 52.51mg/gm with percentage yield as 5.10% w/w of the extract. Results are shown in Table 2, Fig2.

Total Flavonoid contents

The total quantity of flavonoid in ethanol extract of *Pericampylus Glaucus (Lamk.) Merr* was evaluated by method of Aluminum chloride using rutin as reference compound on UV visible Spectrophotometer at wave length of 415nm. The total flavonoids content was measured as rutin equivalents (mg/gm.) using equation based on the calibration curve y = 1.557 X + 0.07653 with r^2=0.9767, where y is rutin equivalents (RE) and x is the absorbance. The total contents of flavonoid (TFC) in dry extract were 51.70mg/gm with yield was 5.17% yields w/w of the extract.

Results are shown in Table 3, Fig 1.

Antioxidant activity

DPPH Scavenging assay

The result showed that radical scavenging of the plant extract of *Pericampylus Glaucus (Lamk). Merr* and the standard are concentration dependent (increased with increasing concentration). In vitro, antioxidant study of the plant extract, the amount of DPPH radical scavenging at different concentration (0.125- 1mg/ml) of plant extract on UV visible spectrophotometer at 517nm of wavelength compared with standard ascorbic acid was (8.18%) at a dose 0.125mg/ml, 20.67% at a dose 0.25mg/ml, 37.69% at a dose 0.75mg/ml and 51.67% at a dose 1mg/ml, while for that of the standard ascorbic acid 87.53%, 91.86%, 92.37% and 93.64%. The results showed that plant extract has scavengers against DPPH radicals but were less than those of ascorbic acid standard. Results are shown in Table 4, Fig 3.

Reducing power assay

The results showed that plant extract exhibit increase in reducing power as the concentrations of extract increased (concentration dependent). The reducing powers of crude plant extract of *Pericampylus Glaucus (Lamk). Merr* was0.0964±0.001, 0.3553±0.0217, 0.5506±0.0117 and0.6660±0.015 at concentration of 100, 300, 600 and 900ug/ml respectively, while at the same concentrations the reducing power for standard vitamin C was0.9051±0.0009, 1.4773±0.255, 2.3763±0.044 and 2.561±0.086. Thus increased absorbance of the reaction mixture indicates an increased reducing power. Results are shown in Table 5, Fig 4.

DISCUSSIONS

The present research was carried out on crude leaves extract of *Pericampylus Glaucus Lamk Mer*. After extraction with 95% absolute ethanol by soxhlet apparatus, 12gm of extract was produced showing a percentage yield as 8.5%. The dried extract was placed in an air tight sterilized bottle within a desiccator containing silica gel, in order to protect from moisture and contamination. Study on crude plant extract was positive for the presence of alkaloids, tannins, reducing sugars, phenols, flavonoids, terpenoids, sterol and saponins and was negative for anthraquionones. The studied had confirmed that the largest group of plant metabolites are phenolic compound protecting the body against aging (especially in skin), inflammations, cancers, in addition with inhibition of rapidly producing cells and also reduce the risk of many disease like heart diseases, arthrosclerosis, stroke and blood pressure[20]. Tannins provide protection against oxidation, bacteria, virus and diabetes[20].In the field of medicines, pharmacy and food industries the role of saponins and flavonoids are preservative, antioxidant, flavoring agents and potent anti-oxidant against super oxide radicals[21]. Research has been reported that inhibition of inflammation, coagulation and precipitation of red blood cells are also the property of saponins[22]. Phenol acts as free radical scavengers or antioxidants. Therefore it was necessary to determine the total contents of phenol in the extracts. The Folin Ciocalteu method was used to determine the total quantity of phenols in the extracts using gallic acids as a standard. Phenol contents was determined in terms of gallic acid equivalents using a standard equations (Y=mx+b) with Y=3.316±0.5354x.30 with r^2=0.9504. Folin ciocalteu reagent is a mixture of phaspotungstate and phosphomolybadate used for determination of total quantity of phenol[23]. The total phenol compounds in plant extract were 52.51mg/gm and the percentage yield was 5.20%. The total contents of flavonoid was determined in terms of rutin equivalents using a standard curve equation y= 1.557 X + 0.07653 with r^2=0.9767. It was observed that flavonoid contents were 51.70mg/gm in plant extracts with 5.1 %age yields was observed. Flavonoids posses a wide range of activity against microbes, insects, herbs, virus and cancers[24]. Flavonoid and phenolic compounds has multiple biological importance including antioxidants, anti-inflammatory and also prevents the initiation, promotion and development of cancer[25]. Medicinally flavonoids protecting LDL from oxidation prevents platelet aggregation and relaxes cardiovascular smooth muscles. Flavonoids have positive effect on inhibiting reverse transcriptase enzyme and have beneficial effect against

AIDS[26]. The medicinal importance of *Pericampylus Glaucus Lamk Merr* lies in the presence of the photochemical constituents. Phenolic compounds acts a radical scavenger terminating free radicals and chelating metal ion that catalyze formation of ROS which promotes the process of oxidation[27]. Based on mechanism of action, two main types of antioxidants namely primary which donate electrons and scavenge free radicals or a hydrogen atom to make the free radicals more stable and secondary which concealed the formation of radicals[28].The antioxidant potential of the ethanolic extract of *Pericampylus Glaucus (Lamk). Merr* was determined by two method i-e DPPH free radical scavenging assay and reducing power method. 1,1-Diphenyl ,2 picryl hydrazyl is an accurate and frequently use method to generate free radical compounds which determine radical scavenging effect of extracts[29]. The plant extract posses a scavenging capacity of 8.18%, 20.67%, 37.69% and 51.67%% with respected concentration of 0.125, 0.25, 0.75 and 1.25mg/ml on DPPH radicals. The ascorbic acid (positive control), showed maximum scavenging effect even at very low concentration. Though the DPPH radical scavenging ability of the test extract was less than standard, but may be useful for treating radical related pathological diseases. For evaluation the ability of an antioxidant to donate electron, reducing power method was used. The reducing power method is used to test the reducing ability of the extracts to convert Fe^{3}+ (potassium feericyanide) to Fe^{2}+ (potassium ferrocyanide), Fe^{2}+ then reacts Fecl2 (ferric chloride) and results the formation of complex ferrous[30]. The reducing capacities at 700nm for plant extracts were 0.096, 0.236, 0.348 and 0.3950 at dose of 100, 300, 600 and 900ug/ml respectively. Reducing power of extracts increased with the concentration of extracts.

CONCLUSIONS

The result obtained in present study showed that the extract of *Pericampylus Glaucus (Lamk.) Merr* contain significant amount of bioactive compounds and thus exhibited antioxidant activity. The radicals scavenging property of plant might be due phenolic, flavonoids and other phytochemicals constituents that provide the necessary component as radical's scavengers. The present study also provides scientific basis of the use of plant extract in traditional health system. The present study suggested that *Pericampylus (Glaucus.) Lamk Merr* plant is a significant source of natural antioxidants, that might be helpful in preventing or slowing the progress of various oxidative stress induced diseases.

ACKNOWLEDGMENTS

The authors are grateful to the Dean Faculty of Pharmacy, Lincoln University College, Malaysia for providing the necessary laboratory facilities.

REFERENCES

1. Sheng-Ji,P.,*Ethnobotanical approaches of traditional medicine studies: some experiences from Asia.* Pharmaceutical biology, 2001. 39(s1): p. 74-79.
2. Mukherjee, P.K. and A. Wahile, *Integrated approaches towards drug development from Ayurveda and other Indian system of medicines.* Journal of ethnopharmacology, 2006. 103(1): p. 25-35.
3. Sen, S., Raja Chakraborty, *Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect.* International Journal of Pharmaceutical Sciences Review and Research, 2010. 3(1): p. 91-100.
4. Suprava Sahoo,Goutam Ghosh, Debajyoti Das, and Sanghamitra Nayak., *Phytochemical investigation and In vitro antioxidant activity of an indigenous medicinal plant Alpinia nigra BL Burtt.* Asian Pacific journal of tropical biomedicine, 2013. 3(11): p. 871-876.
5. Sharma, P.,Bhushan Jha, A., Shanker Dubey R., et al., *Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions.* Journal of Botany, 2012. 2012.
6. Kahl, R. and H. Kappus, *Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E.* Zeitschrift für Le0bensmittel-Untersuchung und-Forschung, 1993(196): p. 329-38.
7. Surveswaran, S., Cai Yi-Zhong, Corke , Mei Sun H,.*Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants.* Food Chemistry, 2007. 102(3): p. 938-953.
8. Misra, B.B. and S. Dey, *Biological Activities of East Indian Sandalwood Tree, Santalum album*, 2013, PeerJ PrePrints.
9. Saeed, N., M.R. Khan, and M. Shabbir, *Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L.* BMC complementary and alternative medicine, 2012. 12(1): p. 221.
10. Ehsan, B., A. Vital, and N. Bipinraj, *Antimicrobial activity of the ethanolic extract of Bryonopsis laciniosa leaf, stem, fruit and seed.* African Journal of Biotechnology, 2009. 8(15).
11. Wiart, C., *Medicinal plants of Asia and the Pacific*. 2006: CRC Press.
12. Ong, H., S. Chua, and P. Milow, *Ethno-medicinal plants used by the Temuan villagers in Kampung Jeram Kedah, Negeri Sembilan, Malaysia.* Ethno Med, 2011. 5(2): p. 95-100.
13. Yan, M.-H., *Periglaucines A−D,Anti-HBV and-HIV-1 Alkaloids from Pericampylus glaucus.* Journal of natural products, 2008. 71(5): p. 760-763.
14. Zhao, w.-q. and c.-b. cui, *Triterpenoidal constituents of Pericampylus glaucus and their antitumor activity in vitro.* Chinese Journal of Medicinal Chemistry, 2009. 3: p. 012.
15. Ayoola, G., Coker HAB, Adesegun SA, Adepoju-Bello et al., *Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria.* Tropical Journal of Pharmaceutical Research, 2008. 7(3): p. 1019-1024.
16. Dudonne, S., *Comparative study of antioxidant properties and total phenolic content of 30 plant*

extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of Agricultural and Food Chemistry, 2009. 57(5): p. 1768-1774.
17. Stankovic, M.S., Niciforovicet N., Topuzovic M., al., *total phenolic content, flavonoid concentrations and antioxidant activity, of the whole plant and plant parts extracts from teucrium montanum l. var. montanum, f. supinum (l.) reichenb.* Biotechnol. Biotechnol, 2011. 25(1): p. 2222-2227.
18. Mahmoudi, M., Ebrahimzadeh, M. A., Ansaroudi, F., Nabavi, S. Fet al., *Antidepressant and antioxidant activities of Artemisia absinthium L. at flowering stage.* African Journal of Biotechnology, 2009. 8(24).
19. Patil, S., V. Kadam, and R. Ghosh, *In vitro antioxidant activity of methanolic extract of stem bark of Gmelina arborea roxb.(Verbenaceae).* Intern. J. Pharm. Tech Res., 2009; 1 (4): 1480, 2009. 1484.
20. Serrano, J., et al., *Tannins: current knowledge of food sources, intake, bioavailability and biological effects.* Molecular nutrition & food research, 2009. 53(S2): p. S310-S329.
21. Soetan, K. and O. Aiyelaagbe, *The need for bioactivity-safety evaluation and conservation of medicinal plants-a review.* Journal of Medicinal Plants Research, 2009. 3(5): p. 324-328.
22. Ebrahimzadeh, M., S. Nabavi, and S. Nabavi, *Essential oil Composition and Antioxidant Activity of Pterocaryafivaxinifolia.* Pakistan Journal ofBiological Sciences, 2009. 12(13): p. 957-963.
23. Alo, M., et al., *Antibacterial activity of water, ethanol and methanol extracts of Ocimum gratissimum, Vernonia amygdalina and Aframomum melegueta.*
24. Jayanthi, P. and P. Lalitha, *Reducing power of the solvent extracts of Eichhornia crassipes (Mart.) Solms.* Int J of Pharm and Pharm Sci, 2011. 3: p. 126-128.
25. Ndhlala, A.R., M. Moyo, and J. Van Staden, *Natural antioxidants: fascinating or mythical biomolecules?* Molecules, 2010. 15(10): p. 6905-6930.
26. Hue, S.M., A.N. Boyce, and C. Somasundram, *Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato ('ipomoea batatas').* Australian Journal of Crop Science, 2012. 6(3): p. 375.
27. Hue, S.-M., A.N. Boyce, and C. Somasundram, *Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato ('ipomoea batatas').* 2012.
28. Irshad, M., Md.Zafaryab,[1] Man Singh, et al., *Comparative Analysis of the Antioxidant Activity of Cassia fistula Extracts.* International Journal of Medicinal Chemistry, 2012. 2012.
29. Shirwaikar, A., K.S. Prabhu, and I. Punitha, *In vitro antioxidant studies of Sphaeranthus indicus (Linn).* Indian journal of experimental biology, 2006. 44(12): p. 993.
30. Arulpriya, P., P. Lalitha, and S. Hemalatha, *Invitro antioxidant testing of the extracts of Samanea saman (Jacq.) Merr.* Der Chemica Sinica, 2010 ,1(2): p. 73-79.