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Research Article

Phytochemical Investigation and Antioxidant Screening of Crude Leaves Extract from *Epipremnum aureum*

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ABSTRACT

Objective: *Epipremnum aureum* belonging to the family Araceae is commonly known as money plant having indoor air pollution removing capacity. The aim of the present study was to investigate the presence of various phytochemical constituents which is responsible for the medical activities of the plant. Methods: The leaves were successively extracted in three different solvents viz. ethanol, acetone and chloroform. The phytochemical analysis of plant extract was performed using thin layer chromatography and preliminary screening methods. Different concentration of the crude plant extract was evaluated for antioxidant activity using DPPH scavenging activity and reducing power activity. Results: Preliminary qualitative chemical test for different extract shows the presence of steroids, terpinoids, alkaloids, saponins, tannins and flavonoids. All the three extracts were proven effective against free radicals. Ethanol extract was found to possess highest antioxidant activity compared to acetone and chloroform. Conclusion: Thus the positive results suggest that *Epipremnum aureum* extracts should be further studied to determine the bioactive chemical compounds as well as to understand the possible mechanism of action and evaluate their toxicity looking towards pharmaceutical actions.

Keywords: *Epipremnum aureum*, DPPH, Reducing power, Phytochemical, Thin Layer Chromatography

INTRODUCTION

Plants, the sources of bioactive constituents have been used traditionally to cure various ailments in Ayurveda, Unani & Siddhi. Though, during last few years, synthetic drugs occupy the position for curing various diseases, but, due to their side effects, scientists are now focusing to explore the potentiality of traditional medicines[1]. Araceae is a large family comprising of many therapeutically active medicinal plants. *Epipremnum aureum* is known by many names but most common is "Money Plant". It is a large root-climber belongs to the botanical family of Araceae and a common house plant with several cultivars and capable of removing indoor air pollutants such as xylene, formaldehyde and benzene[2]. *Epipremnum aureum* has shiny heart-shaped leaves and long slender stems, which can grow up to 3m in length. However, the stems can be wound round sticks or attached to supports to keep the plant from taking up too much space. The plant generally stands at a height of between 5m and 9m and has a total spread of 1.5m to 2.5m. *Epipremnum aureum* produces small green flowers in summer. This plant is widely known in Malaysia and Singapore and has a reputation as a traditional anticancer preparation as well as a remedy for skin diseases[3]. A decoction of the fresh leaves with meat or eggs or as tea was reported to be a common practice among the locals. Aerial roots and leaves of *Epipremnum aureum* show great potential for antimicrobial activity[4].

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Many active phytoconstituents such as Flavonoids exhibits potential antidiabetic and neuroprotective activity. Alkaloids are used as anaesthetic agents[5]. Terpenoids exhibit various important pharmacological activities such as anti-inflammatory, anticancer, antihyperlipedic, anti-viral and anti-bacterial activities. The effective identification of phytoconstituents plays an important role in determining the therapeutic activity. In this case the standardized thin-layer chromatographic procedures can be used effectively for the screening analysis as well as quality evaluation of the plant or its derived herbal products. Though the traditional system of medicine has a long history of use but they lack scientific evidence particularly in modern scientific knowledge. Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants in the form of raw extract or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress. If excess ROS are not eliminated by antioxidant system, reactive oxygen species (ROS) will exert oxidative damaging effects by reacting with mostly every molecules found in living cells including lipid, amino acids and DNA. They play important roles in aging and in the pathogenesis of age related disorders such as cancer, hypertension, atherogenesis, neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. Thus the present study aims to analyse different phytoconstituents

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Table 1: Data showing the extractive value of *Epipremnum aureum*

Plant Name	Part Used	Method of extraction	Percentage yield % Ethanol	Acetone	Chloroform
Epipremnum aureum	Arial Parts	Hot Percolation using Soxhlet Extraction	2.8g	2.1g	1.6g

Table 2: Data showing the preliminary phytochemical screening of the various extracts of *Epipremnum aureum*

Sl No.	Constituents	Ethanol extract	Acetone Extract	Chloroform extract
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Glycosides	+	+	+
4	Sterols	+	-	-
5	Terpinoids	+	+	-
6	Tannins	+	-	-
7	Fixed oils and Fats	-	+	+
8	Phytosterols	+	-	-
9	Quinones	-	-	-
10	Coumarin	-	-	-

Table 3: Flavonoid content of Epipremnun aureum plant extract

Extract	Flavonoid content
Ethanol	1.81
Acetone	1.65
Chloroform	1.47

present in the leaf extract of *Epipremnum aureum* and to evaluate its antioxidant potential.

MATERIALS AND METHODS

Collection and authentication of Plant

The fresh whole plant of *Epipremnum aureum* was collected from Kepong district, Malaysia. The plant was identified by Miss Tan Ai Lee, Research officer, Natural products, Forest Research Institute Malaysia. The voucher specimen (No. SBID: 001/15) was prepared and deposited in the Faculty of Pharmacy, Lincoln University College, Malaysia for imminent reference.

Preparation of crude drug for extraction

The authenticated leaves were washed with fresh water and dried under shade of sunlight for 5 days. The dried plant leaves were coarsely powdered with the help of mechanical grinder. The powder was stored in an airtight container for further use.

Method of extraction

The crude Continuous hot percolation process by using Soxhlet apparatus was used for the extraction of the crude leaves of *Epipremnum aureum*. The extraction was carried out as per the polarity of the solvents with chloroform, acetone and finally with ethanol[6].

Chloroform Extract

The shade dried coarsely powdered leaves of *Epipremnum aureum* (500g) was extracted with chloroform (58-62°C) until the extraction was completed. After completion extraction, the solvent was removed by distillation. Dark greenish yellow colour residue was obtained. The residue was then stored in dessicator.

Acetone Extract

The marc left after chloroform extract was dried and then extracted with acetone (55-56°C) until extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark brownish green colour residue was obtained. The residue was then stored in dessicator.

Ethanol Extract

The marc left after chloroform extract was dried and then extracted with ethanol 95% (75-78°C) until extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark brown colour residue was obtained. The residue was then stored in dessicator.

Phytochemical analysis of plant extract

Test for Glycosides

Keller Killiani Test – Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids. b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few

Table 4: Preliminary detection of various phenolic compounds against reference compounds

Extract	Compound	Rf value of Sample	Reference compound (Rf Value)
Arial parts of Epipremnum aureum	Caffeic acid	0.99, 0.75, 0.90	Caffeic acid (0.90)
	Rosmarinic acid	0.83, 0.78, 0.78	Rosmarinic acid (0.83)
	Ferulic acid	0.91, 0.95, 0.96	Ferulic acid (0.95)

Table 5: Antioxidant activities of plant extracts on DPPH free radical scavenging on UV visible Spectrophotometer at 517nm of wavelength

Sample	Concentration mg/l	Mean absorbance	%age DPPH free radical scavenging
Ascorbic acid	0.25	0.053±0.024	82%
	0.75	0.032±0.018	89%
	1.0	0.024±0.012	92%
Ethanol extract	0.25	0.179±0.071	42%
	0.75	0.152±0.011***	51%
	1.0	0.122±0.045***	60%
Acetone extract	0.25	0.216±0.027	30%
	0.75	0.210±0.024	34%
	1.0	0.179±0.017***	42%
Chloroform extract	0.25	0.213±0.021	31%
	0.75	0.188±0.011	39%
	1.0	0.157±0.018***	49%

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

drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids. b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of phytosterols

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Test for Terpenoids

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

Tests for steroids

A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids. ii. Development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

Test for Quinones

Dilute NaOH was added to the 1 ml of crude extract. Blue green or red coloration indicates the presence of quinones.

Test for Coumarin

10 % NaOH was added to the extract and chloroform was added for observation of yellow color, which shows the presence of Coumarin[7]

Qualitative analysis of phenolic acids by Thin Layer Chromatography

The TLC plates were prepared by using silica gel G coated over glass plates of 10X10 cm dimension. Silica gel plate was impregnated by dipping into 4 % solution of sodium acetate in methanol –water 3:2 for 5s followed by drying at room temperature. The plates were activated by heating the plates at 100°C to 110°C which is necessary for linear movement of solutes over stationary phase. Glass capillaries were used to spot the sample extract at distance of 1 cm at 3 track. In this case Chloroform – ethyl acetate – formic acid, 5:4:1 and ethyl acetate – methanol – water, 77:13:10 after pre-saturation with mobile phase for 20 min for development were used[8]. Solutions of standard substances caffeic acid, rosmarinic acid and ferulic acid, were prepared by dissolving of 10 mg in 1 ml of distilled water. Substances were identified using UV detection at 254 nm. Visualization was carried out by spraying with solution of iron III chloride (2% methanol) and aluminium chloride (1% in ethanol) a blue or light blue spot indicates the presence of caffeic acid, Rosmarinic acid and ferulic acid[9].

The Rf value of the sample was calculated and compared with the Rf value of the standard compounds. The identified compounds with the respective Rf values were depicted in table no.3.

Determination of total flavonoids by colorimetric method

All the three crude extracts were used to determine the total flavonoids contents. The total flavonoids contents of different crude extracts were estimated by aluminium chloride colorimetric method[10]. Sodium nitrate (2.5 g) was taken in a volumetric flash (50 mL) and added water upto the mark that was 5% sodium nitrate. Sodium hydroxide (2.5 g) was taken in another volumetric flash (50 mL) and added water upto the mark that was 4% sodium hydroxide. Then 10% aluminium chloride solution was prepared the same procedure. The different crude extracts (0.25 mg)

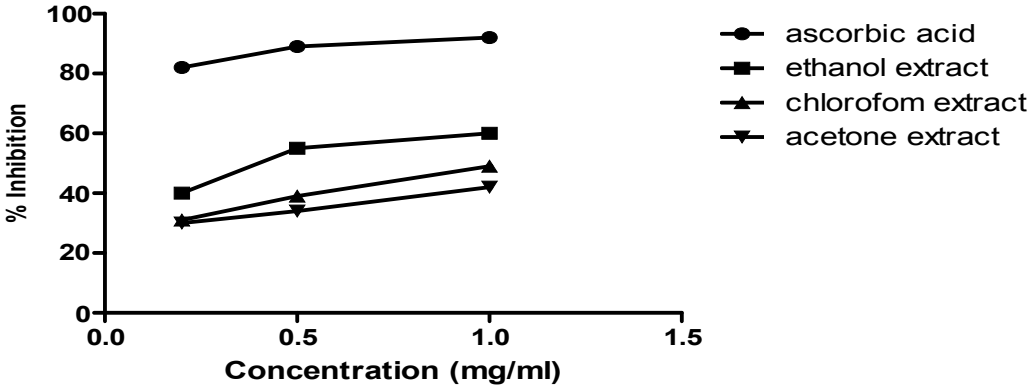

Figure 1: DPPH radical scavenging activities of the various extracts of Epipremnum aureum and ascorbic acid. Result represents means of triplicates of different concentrations analyzed.

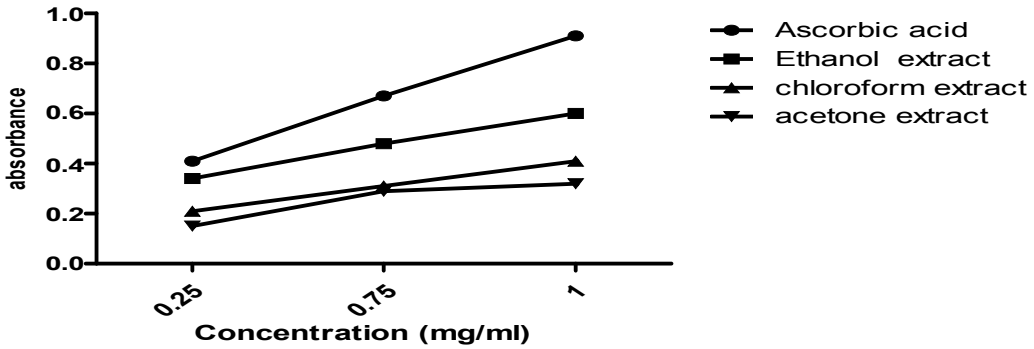

Figure 2: Reducing power activities of the various extract of Epipremnum aureum comparison with ascorbic acid.

were taken in a test tube and added water (1.25 mL) and sodium nitrate (0.75 µL) then mixed together. All the test tubes were kept in the dark place for 6 min. Then 10% aluminium chloride (0.150 µL) was added into the test tube and wait for 5 min in the dark for complete reaction. Finally, 5% sodium hydroxide (0.5 mL) and water (0.275 mL) were added to the test tube. The absorbance was measured of all samples at a fixed wavelength 510 nm using UV spectrophotometer. Quercetin standard was used for the calibration curve. The estimation of total flavonoids contents in the crude extracts was carried out in triplicate and the results were averaged. The total flavonoid was calculated by the following formula:

X= (A. mo)/(Ao. m)

Where "X" is the flavonoid content, mg/g plant extract, "A" is the absorption of plant crude extract solution, "Ao" is the absorption of standard quercetin solution, "m" is the weight of crude drug extract in mg and "mo" is the weight of quercetin in the solution in mg.

Antioxidant evaluation

DPPH scavenging activity

The free radical scavenging activity of the fractions was measured in vitro by 2, 20 - diphenyl-1-picrylhydrazyl (DPPH) assay[11]. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with methanol and the absorbance obtained was 0.3098±0.02 at 517 nm using the spectrophotometer which was taken as absorbance of control[12]. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (0.25, 0.75 and 1 mg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition was calculated by the following:

Scavenging (%) = $[A_1 – A_2 / A_1]$ X 100

A1 = Absorbance of Control

A2 = Absorbance of sample

Reducing power

The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the solvent fractions[13]. The Fe (II) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Various concentrations of the sample (2 ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/l). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml from each of the mixture earlier mentioned was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power.

RESULTS AND DISCUSSION

The phytoconstituents were extracted by using different solvents of increasing polarity like chloroform, acetone and finally with ethanol. The extractives value was given in Table 1.

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities. The qualitative phytochemical screening revealed the ethanolic extract richness in tannins, saponins, terpinoids, flavonoids, phytosterols, glycosides and alkaloids. It was found that the chloroform extract contains fixed oils and fats which was absent in ethanolic extract. Moreover quinones and coumarin were undetected in any of the extracts. The phytochemical characteristics of the aerial plant extract of *Epipremnum aureum* investigated are summarized in table 2.

Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids. Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. Flavonoids are hydroxylated phenolics and are potent water-soluble antioxidants which help in radical scavenging and prevention of oxidative cell damage. Moreover they are found to be effective in scavenging free radicals as a result of their redox properties that allow them to act as reducing agents. In the present study among the three crude extracts from *epipremnum aureum*, ethanol extract was found be containing highest amount of flavonoids content compounds followed by acetone and chloroform extract.

The result of total flavonoid contents of the three crude extracts of Epipremnum aureum is given in table 3. Among the three crude extracts, ethanol extract contained the highest (1.81 mg/g) amount of flavonoids content compounds followed by acetone (1.65 mg/g), chloroform (1.47 mg/g). The variation may be due environmental conditions, which can modify the constituents of the plant. The qualitative determination of constitutes by TLC analysis showed the presence of three different phenolic compounds caffeic acid, ferulic acid and rosmarinic acid in the investigated extracts. The phenolic compounds may contribute directly to antioxidative effect[13]. The Rf values of the obtained spots were compared with the Rf values of the standard compounds. The Rf values of the sample extract was found to be (0.90, 0.83, 0.95) which proves the presence of caffeic acid, rosmarinic acid and ferulic acid respectively which are shown in table 4. In the present study the presence of phenolic compounds in the plant extract also supports the antioxidant property of *Epipremnum aureum.*

Antioxidant activity is evaluated by different methods but the most widely used methods are those that generate free radical species which are then neutralized by antioxidant compounds. In the present study the antioxidant activity test was found to be positive for each extract which can be attributed to the presence of phenols and flavonoids as shown in the phytochemical screening test. The comparative study shows the higher antioxidant activity of ethanolic extract as compared to chloroform and acetone extract. The highest antioxidant activity of ethanolic extract may be attributed due to the presence of phenolic acids which were confirmed by TLC profiling. In comparison to the standard ascorbic acid the ethanolic extract showed more than 60% of free radical scavenging efficacy; whereas acetone soluble fraction shows least antioxidant efficacy of about 30% free radical scavenging activity. The ethanolic extract showed the highest percentage of inhibition at a concentration of 1mg/l whereas the lowest inhibition percentage of 42% as shown in (figure 1) was observed at a concentration of 0.025 mg/l. The acetone and chloroform extract also followed the concentration dependent activity as shown in (table 4). Reducing power is associated with antioxidant activit y and may serve as a significant reflection of the ant ioxidant activity. Compounds with reducing power ind icate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxi dants. It was observed that the reducing ability of the extract was dose dependent and all the three extract showed an increase in absorbance with the increase in dose. The ethanolic extract showed an absorbance of 0.603 which was higher acetone and chloroform extract. The results of reducing power activity reveal that ethanolic extract has higher antioxidant potential as compared to acetone and chloroform extract. In case of both DPPH scavenging activity and reducing power activity the three extracts were found to dose dependent which was directly proportional to concentration. The results strongly suggest that phenolic compounds are important components of this plant, and some of its pharmacological effects could be attribute to the presence of these valuable constituents.

CONCLUSION

The present study clearly indicates that the plant Epipremnum aureum is a rich source of active phytoconstituents responsible for pharmacological activities. The results of the present study suggest that tested plant extracts have moderate to potent antioxidant activity and or free radical scavenging activity. Nevertheless, the great antioxidant potential will be of immense benefit from the consumption of these medicinal plant extracts. The qualitative TLC analysis of the three different plant extract reveals the presence of phenolic compounds which contributes the antioxidant potential of the extracts. Hence, it is proved that Epipremnum aureum contains effective phytoconstituents which needs to be explored on the basis of pharmacological importance.

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REFERENCES

1. Saswati R, Dutta M, Paul SB. In vitro antibacterial activity of Alocasia decipiens schott, International

Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5(1): 155-57
2. Chetan SS, Deepali MJ, Sushama DP, Leena JP, Vilasrao JK. Phytochemical screening and in vitro antimicrobial activity studies of Epipremnum aureum Linn. leaves extracts, Der Pharmacia Sinica, 2011; 2 (5):267-72
3. Chan MJ. Turner, I.M. The use of *Epipremnum pinnatum* (*Araceae*) in Singapore in the treatment of cancer: an unreported application of a herbal medicine, *Economic Botany*, 1998; *52*(1): 108.
4. Srivasatava N, Shwarupa S, Bhagyawant SS. Comparative Study on the Anti Termite, Antimicrobial and Antioxidant Activity of Leaf and Root Extracts of Pothos Aurea, Journal of Pharmaceutical Research & Clinical Practice, 2011; 1(2):1-11
5. Pandey M, Debnath M. Gupta S, Chikara SK. Phytomedicine: An ancient approach turning into future potential source of therapeutics, Journal of Pharmacognosy and phytotherapy,2011; 3(1): 113-17
6. Palanisamy P, Jayakar B, Kumuthavalli MV, Kumar Y, Kashi S. Preliminary phytochemical evaluation of whole plant extract of *Dipteracanthus prostrates nees*, International research journal of pharmacy, 2012; 3(1): 150-53
7. Kiran KSP, Sridevi V, Chandana LMVV. Studies on Phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants, Journal of chemical, biological and physical science, 2012;2(3):1326-332.
8. Hossain MA, Muhammad MD, Charles G, Muhammad I. In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetrastigma from Sabah. Asian Pacific Journal of Tropical Biomedicine, 2011; 4(9): 717-21.
9. Leonard VM, Tungamirai M, John MB. Determination of ferulic acid and related compounds by thin layer chromatography, AJB, 2006; 5(13):1271-273.
10. Olubunmi AW, Anthony JA. Phytochemical constituents and antioxidant activities of the whole leaf extract of Aloe ferox Mill, Pharmacognosy magazine, 2011; 7(28):325-33.
11. Abbas S, Wink M. Epigallocatechin gallate from green tea (Camellia sinensis) increases lifespan and stress resistance in Caenorhabditis elegans. Planta Med. 2009; 75:216–21.
12. Dehshahri S, Wink M, Afsharypuor S, Asghari G, Mohagheghzadeh A. Antioxidant activity of methanolic leaf extract of Moringa peregrina (Forssk.) Fiori. Res Pharm Sci. 2012; 7(2): 111–18.
13. Rosalind TH, Dutta B K, and Paul S B. Evaluation of in vitro antioxidant activity, estimation of total phenolic and flavonoid content of leaf extract of eurya japonica thunb. AJPCR, 2013; 6(1):152-55
14. Hossain MA, AL-Raqmi KA, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents andphytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris, APJTB, 2013; 3(9):705-10.
15. Radomir VM, Eva SL, Ljiljana AK. TLC analysis of some phenolic compounds in kombucha beverage, APTEFF, 2004; 35: 1-280.