EVALUATION OF ANTIOXIDANT CONTENTS AND PROXIMATE COMPOSITIONS OF PURSLANE (*Portulaca oleracea* L.) AT DIFFERENT GROWTH STAGES

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ABSTRACT

Purslane (*Portulaca oleracea* L.) is an attractive candidate as a useful vegetable crop and as a cosmetic ingredient containing elevated quantity of nutrients, highly rich in antioxidant properties. Due to limited information to date on nutritional quality of purslane, experiment was conducted at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah to evaluate the antioxidant content and proximate composition of purslane (*Portulaca oleracea* L.) at different growth stages. The experiment was conducted in polybags filled with top soil in the rainshelter structure of Faculty of Sustainable Agriculture, UMS, campus Sandakan. The soil had electrical conductivity of 1.15 dS m^{-1} and pH of 5.23 and 22.02% of organic carbon. Plant samples were harvested at interval of 15 day for two months which were at day 15, 30, 45 and 60. The experimental design was conducted using Completely Randomized Design (CRD) with six replications. The treatment means were compared by Least Significance Differences (LSD) at 5 % significant level. Physical characteristic of *P. oleracea* at each stage of maturity showed significant differences ($p<0.05$) in plant height, leaf length, leaf width, stem diameter, shoot fresh and dry weight. Total phenolic content of purslane was found to be highest at 15 days with the value 4.54 mg GAE/g while 45 days purslane has the highest total flavonoid content which was 22.22 mg QE/g. Highest ash content was found at 15 day old purslane which was 20.50 % while highest crude protein and crude fat was found at 30 day purslane plant with the value 32.46 % and 7.67 % respectively. The level of ash, crude protein and crude fat was found to decrease ($p<0.05$) with advanced plant maturity. While crude fibre in *P. oleracea* increased significantly ($p<0.05$) with plant maturity.

PENILAIAN KANDUNGAN ANTIOKSIDAN DAN KOMPOSISI PROKSIMAT DALAM PURSLANE (Portulaca oleracea L.) PADA TAHAP PERTUMBUHAN YANG BERBEZA

ABSTRAK

Purslane (Portulaca oleracea L.)merupakan tumbuhan yang berguna sebagai tanaman sayur-sayuran dan juga sebagai bahan kosmetik yang kaya dengan nutrien dan antioksidan. Satu eksperimen telah dijalankan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah, Kampus Sandakan untuk menilai kandungan antioksidan dan komposisi proksimat purslane (Portulaca oleracea L.) pada tahap pertumbuhan yang berbeza. Kajian ini dijalankan dengan menanam purslane dalam polibeg yang diisi dengan tanah dan diletak dalam struktur pelindung hujan Fakulti Pertanian Lestari, UMS, Kampus Sandakan. Tanah tersebut mempunyai 1.15 dS m^{-1} kekonduksian elektrik, 22.02 % karbon organic dan pH 5.23. Sampel tumbuhan telah dinilai setiap 15 hari selama dua bulan iaitu pada hari ke- 15, 30, 45, dan 60. Kajian ini dianalisi menggunakan reka bentuk rawak lengkap dengan enam replikasi. Keputusan kajian telah diuji dengan beda nyata terkecil pada aras signifikasi 5%. Ciri- ciri fizikal purslane termasuk ketinggian tumbuhan, panjang daun, lebar daun, diameter batang, berat kering dan basah pucuk, dan kandungan air pucuk menunjukkan perbezaan yang signifikan ($p<0.05$) pada tahap pertumbuhan yang berbeza. Jumlah kandungan fenolik purslane didapati tertinggi pada hari ke- 15 dengan nilai 4.54 mg GAE/g, manakala purslane pada hari ke- 45 mempunyai jumlah kandungan flavonoid yang paling tinggi iaitu 22.22 mg GE/g. Kandungan abu yang paling tinggi dalam purslane didapati pada hari ke -15 dengan nilai 20.50 % manakala purslane pada hari ke- 30 mempunyai kandungan protein mentah dan lemak mentah yang paling tinggi iaitu 32.47 % dan 7.67%. Tahap abu, protein mentah dan lemak mentah dalam purslane didapati berkurangan ($p<0.05$) dengan kematangan tumbuhan manakala kandungan serat mentah dalam purslane meningkat ($p<0.05$) dengan kematangan tumbuhan.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

µg	Microgram
µL	Microliter
AAC	Ascorbic acid
AEAC	Ascorbic acid equivalent antioxidant activity
Ca	Calcium
ca.	Circa
CoQ10	Coenzyme Q10
CRD	Completely Randomized Design
DM	Dry matter
EC	Electrical conductivity
EPO	Erythropoietin
Fe	Iron
FRAP	Ferric-reducing antioxidant power
FW	Fresh weight
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
Hb	Hemoglobin
HCT	Hematocrit
HDL	High density lipoprotein
IC_{50}	DPPH scavenging capacity
IgG	Immunoglobulin G
IgM	Immunoglobulin M
K	Potassium
Kcal	Kilocalorie
LD_{50}	Lethal dosage
LDL	Low density lipoprotein
LSD	Least significant difference
M	Molarity
Mg	Magnesium
mg	Milligram
mol	Mole
mRNA	Messenger RNA
Na	Sodium
RBC	Red blood cell
ROS	Reactive oxygen species
SAS	Statistical analysis system
SOD	*Superoxide* dismutases
TFC	Total flavonoid content
TPC	Total phenolic content
TW	Turgor weight
VLDL	Very low density lipoprotein
w/v	Weight per volume
Zn	Zinc

LIST OF FORMULAE

Formula **Page**

3.1 $Y = 9.0571x - 0.0012, R^2 = 0.9998$ 23

Where:

Y= Absorbance obtained from spectrophotometer

X= mg mL^{-1} GAE

3.2 $\text{Total Phenolic Content} = \frac{GAE \times V \times DF}{M}$ 23

Where:

C = Total phenolic content in mg GAE/g dry sample

GAE = Concentration of gallic acid from the calibration curve in mg mL^{-1}

V = Volume of the sample in mL

DF = Dilution factor

M = Weight of dry sample extract in g

3.3 $Y = 3.82x - 0.0027, R^2 = 0.9996$ 24

Where:

Y = Absorbance obtained from spectrophotometer

x = mg mL^{-1} QE

3.4 $\text{Total flavonoid content} = \frac{QE \times V \times DF}{M}$ 24

Where:

C = Total flavonoid content in mg QE/g dry sample

GAE= Concentration of quercetin from the calibration curve in mg mL^{-1}

V = Volume of the sample in mL

DF = Dilution factor

M = Weight of dry sample extract in g

3.5 $\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of original sample}} \times 100\%$ 25

3.6 $\text{Crude fibre content (\%)} = \frac{(m_3 - m_1 - m_4 - m_5)}{m_2} \times 100\%$ 26

Blank value, $m_5 = m_7 - m_6$

Where:

m_1 = Fibrebag (g)

m_2 = Initial sample weight (g)

m_3 = Incinerating crucible and dried fibrebag after digestion (g)

m_4 = Incinerating crucible and ash (g)

m_5 = Blank value of the empty fibre bag (g)

m_6 = Incinerating crucible (g)

m_7 = Incinerating crucible and ash of the empty fibrebag (g)

3.7 $\text{Crude fat content(\%)} = \frac{\text{Weight of flask with fat - weight of clean flask}}{\text{Weight of original sample}} \times 100\%$ 26

CHAPTER 1

INTRODUCTION

1.1 Introduction

Today, medicinal plants play an important role and have great demand in both developed and developing country as a source of primary health care. Medicinal plants have wide biological and medicinal activities, high safety margins and are less costly to be consumed. According to Balick and Coz (1996), a number of important modern drugs have been derived from plants used by indigenous people even with the advent of modern and allopathic medicine. The herbal molecules in medicinal plants are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell *et al.*, 2006)

Purslane (*Portulaca oleracea* L.) is one of the important medicinal plants which can be easily found in Malaysia. Purslane is also known as Pigweed, Little Hogweed, Pursley, and Verdolaga. It is an annual succulent plant under the family of *Portulacaceae* and able to reach 40cm in height. It is a native of India and the Middle East, but is naturalized elsewhere and was considered as invasive weed in some countries. *P. oleracea* is an edible plant at which its stem and leaves are slightly acidic and salty taste. It contains high nutritive and antioxidant properties as human food, animal feed and medical utilization (Uddin *et al.,* 2012).

P. oleracea contains higher nutritive value than other common vegetables found on the market due to its omega-3 fatty acid, α-tocopherol, ascorbic acid, β-carotene and glutathione rice shoots (Uddin *et al.*, 2012,; Wenzel *et al.*, 1990). A research done had shown that *P. oleracea* is a rich source of omega-3 fatty acids which can be used in preventing heart attack and strengthen the immune system (Simopoulus, 2004).

The whole plant part of *P. oleracea* is considered antiphlogistic, bactericide, antidiabetic, anaphrodisiac, emollient, calmative, diuretic and is a refreshing agent (Boulos, 1983). Purslane is used as a gastric sedative, to allay excessive heat and pain, and applied to the eyes in order to remove inflammation (Quisumbing, 1978). Purslane has no cytotoxic or gemotoxic effects due to the presence of various bioactive and phenolic antioxidant. Hence, it is certified safe for daily consumption as vegetable (Yen *et al.*, 2001).

It was found that oxidative stress is one of the major causative factors which lead to many chronic and degenerative diseases such as atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative disease and others (Young and Woodside, 2001). Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress (Zheng and Wang, 2001). Antioxidants are substances that when present in low concentration, compared to those of an oxidisable substrate significantly delay or prevent oxidation of the substances (Halliwell and Gutteridge, 1989).

Proximate analysis provide the most extensive information about the composition and nutrition value of food. Throughout the world, there is increasing interest in the importance and knowledge of nutrition value in the prevention of several diseases. Both antioxidant and nutrients plays an important role in maintaining human health. Therefore, there is a need to compare the antioxidant content and proximate composition in *P. oleracea* at different growth stages.

1.2 Justification of Study

This research was conducted to evaluate the total phenolic content, total flavonoid content and mineral content in purslane (*Portulaca oleracea* L.) at different growth stages. *Portulaca oleracea* was chosen for this study due to its high nutritive value at which it is contain high levels of vitamin E, C, and beta carotene. In fact, it is also recognized as weeds in the crop field of Malaysia hence it is easily available for this study.

Portulaca oleracea has long been present in Malaysia, but it had been considered as invasive weed in the crop field. In fact, *P. oleracea* is claimed to have high antioxidant properties and mineral content. It deserves more attention from the agriculturalists and nutritionists. There are limited information regarding nutritional quality of *P. oleracea* at different maturity stages have been published. Therefore, this research was carried out in Sabah to evaluate the antioxidant content and proximate composition of *P. oleracea* at different growth stage.

Besides that, the quantitative analytical method was used for the evaluation of total phenolic content, total flavonoid content and proximate composition of *P. oleracea* at different growth stage. A simple, rapid and high degree of sensitivity test was used in this research which is total phenolic content assay, total flavonoid assay to test for phenolic and flavonoid content respectively. Apart from that, a simple and rapid test was carried out to test on the proximate composition of *P. oleracea*.

Through this research, more information related to the antioxidant content and proximate composition in *P. oleracea* can be greatly increased. We would able to know the benefits of planting *P. oleracea* and it can be widely used as important medicinal plant for human health and also as animal feed for livestock in the future.

1.3 Objective of Study

The objective of this research study was

(i) To determine and evaluate the antioxidant contents and proximate compositions in purslane (*Portulaca oleracea* L.) at different growth stages.

1.4 Hypothesis of Study

H_0: There is no significant difference between the antioxidant contents and proximate compositions in purslane (*P. oleracea* L.) at different growth stages.

H_A: There is significant difference between the antioxidant contents and proximate compositions in purslane (*P. oleracea* L.) at different growth stages.

CHAPTER 2

LITERATURE REVIEW

2.1 Purslane

Portulaca oleracea is one of the members from purslane, Family "portulacaceae (Hyam and Pankhurst, 1995), the genus Portulaca contains about 40 topical and warms climate species. The plants are characterized by its taller upright growth habit and larger leaves and seeds (Gorske *et al.,* 1979; Gledhill, 1985).

Purslane (*Portulaca oleracea*) is a nutritious vegetable which can be consumed by human being, and it was mentioned in Egyptian texts from the time of the Pharaohs (Mohamed and Hussein, 1994). *P. oleracea* can be eaten raw as a salad, eaten cooked as a sauce in soups or eat as green vegetables. *P. oleracea* provides a rich plant source of nutritional benefits (Sudhakar *et al.,* 2010). According to Simopoulos and Salem (1986), *P. oleracea* is one of the green plants which is rich in omega-3 fatty acids and alinolenic acid. Low incidence of cancer and heart disease were reported in the areas where *P. oleracea* is eaten, possibly due to *P. oleracea's* naturally occurring omega-3 fatty acids (Simopoulos, 1991). *P. oleracea* has been used as an antiseptic, antidiuretic, vermifuge in oral ulcer and urinary disorders. Recent research reported that *P. oleracea* shows a wide range of biological effects, including skeletal muscle relaxant effect (Parry *et al.,* 1993), analgesic and anti-inflammatory effects (Chan *et al.,* 2000), antifungal activity (Oh *et al.,* 2000) and antifertility effect (Verma *et al.,* 1982). Other than these, it also shows other beneficial effects such as anti-diabetic (Gong *et al.,* 2009) and wound healing properties (Rashed *et al.,* 2003). Besides that, *P. oleracea* may have a protective effect against oxidative stress caused by vitamin A deficiency (Arruda *et al.,* 2004). In addition, *P. oleracea* consists of active molecules which can be used for the treatment of some parasitic infectious diseases such as leishmaniasis and trypanosomiasis (Costa *et al.,* 2007).

Skulski (2010) reported that *P. oleracea* has freshy leaves with slightly sour taste, it is rich in iron content and has significant amount of Omega-3 fatty acids which is commonly found in seed. Hence, *P. oleracea* is used widely in Chinese medicine as an herb which is able to clear heat toxin and antibiotic and antifungal effect increases uterine contraction and prevention and treatment of dysentery.

2.2 Botanical Description of *Portulaca oleracea*

Portulaca oleracea is a succulent annual herb. Stems of *P. oleracea* are sometimes flushed red or purple, not articulated, prostrate or decumbent, less often erect, diffuse, much branched; leaf axils with a few inconspicuous stiff bristles. The leaves are alternate or occasionally subopposite, with short petiole and flat leaf blade, obovate, 10-30 × 5-15 mm, base cuneate, apex obtuse, rounded, and truncated. The flowers of *P. oleracea* are in clusters of three to five, 0.4-0.5 cm in diameter, surrounded by involucre of two to six bracts. Sepals are green, helmeted, ca. 4 mm, apex acute, and keeled. Petals 5, yellow, obovate, 3-5 mm, slightly connate at base, apex retuse. Stamens 7-12, circa 12mm; anthers are yellow. The ovary is glabrous with four to six lobed stigma. Capsule ovoid, ca. 5 mm. Seeds are glossy black when mature, never iridescent, obliquely globose-reniform, 0.6-1.2 mm; testa cells stellate, usually with central peg like tubercle, sometimes without and then surface granular (Lu and Michael, 2003). Seed production of these plants is very high (one plant can introduce up to 10,000 seeds to the environment. It has a slightly sour and salty taste. The stems, leaves and flower buds are all edible (Wiersema and Leon, 1999).

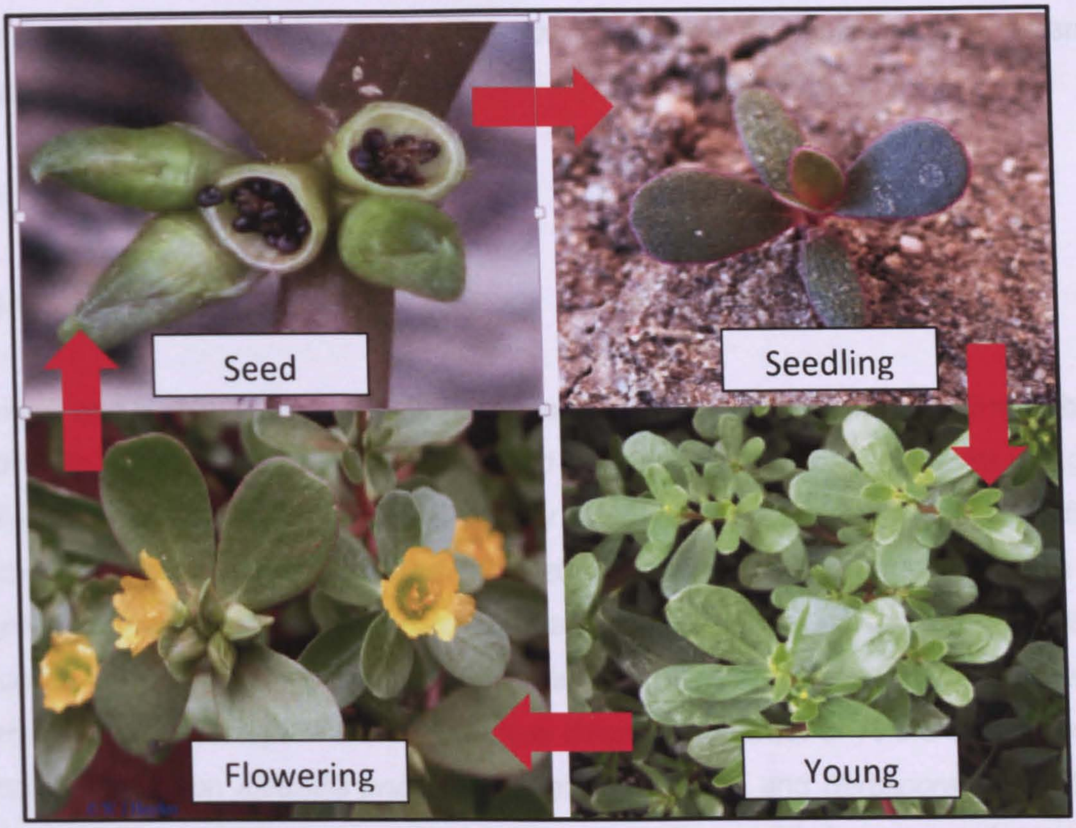

Figure 2.1 Life cycle of *Portulaca oleracea* from seeds to flowering
Sources: Amirul *et al.*, 2014

2.3 Chemical Constituents of *Portulaca oleracea*

Aberoumand (2009) reported that the leaves and stems of *P. oleracea* contained ashes (22.66%), crude protein (23.47%), lipid (5.26%) and fibers (40.67%). The stems and leaves also have high energy values which is 303.9 Kcal/100g dry weight. Mineral contents (mg/100gm DM) were K (14.71), Na (7.17), Ca (18.71), Fe (0.48) and Zn (3.02).

According to Chowdhary *et al.* (2013), *P. oleracea* contains more omega-3 fatty acids, alpha-linolenic acid than other leafy vegetables plant available in the market. *P. oleracea* has 0.01mg/g of eicosapentaenoic acid (EPA) which is an Omega-3 fatty acids found mostly in fish, some algae and flax seeds. *P. oleracea* also contains vitamin as well as dietary minerals such as magnesium, calcium, potassium and iron. It also contains two types of betalain alkaloid pigments which are the reddish betacyanins visible in the coloration of stem and the yellow betaxanthis that can be seen from the flowers. These two pigments are potent antioxidants and have been found to have anti-mutagenic properties. Many types of chemical compound has been found present in *P. oleracea* including alkaloids, terpenoids, organic acids, coumarins, flavonoids, volatile oil and polysaccharides (Prashanth *et al.*, 2005)

2.4 Pharmacological Study

2.4.1 Anti-microbial Activity

Ramesh and Hanumantappa (2011) had reported the phytochemical and anti-microbial activity in the aerial parts of chloroform and ethanolic extracts of *P. oleracea* by agar diffusion method. The antimicrobial activity in *P. oleracea* are used in against five bacteria for example *Bacillus cereus* and *Klebisilla pneumonia* and three fungi such as *Aspergillus fumigates* and *Nerospora crassa*. Ethanolic crude extract of *P. oleracea* showed maximum effect on organism such as *Staphylococcus aureus, Klebisilla pneumonia* and *Nerospora crassa*. Whereas chloroform extract of *P. oleracea* showed moderate effect on *Klebisilla pneumonia, Aspergillus niger,* and *Nerospora crassa.* The result of this research supported the folklore usage of the studied plant and shows that the extract of this studied plant contains compounds which have anti-microbial agent in the form of drugs for the therapy of infectious diseases caused by pathogens.

Based on the previous study of Bae (2004), antimicrobial effect of *P. oleracea* extracts on food borne pathogens was assessed. His study had found that ethyl acetate extract was having highest antimicrobial activity against *Staphylococcus aureus* and *Shigella dysentrica* compared to petroleum ether, chloroform and methanol extracts. Strong antimicrobial activity was found from the ethyl acetate extract of *P. oleracea* to against *Staphylococcus aureus* at 4000 ppm concentration. This concentration able to slow down the growth of *S. aureus* by more than 24 hours and *S. dysentrica* up to 12 hours at 37°C.

2.4.2 Anti-oxidant Activity

In 2012, Uddin *et al.* had reported the antioxidant activity of *P. oleracea* over different plant maturity stages by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP) assays. Iodine titration was used in this study to determine the ascorbic acid content (AAC). From the study, he found that DPPH scavenging (IC_{50}) capacity ranged from 1.30±0.04 to 1.71±0.04 mg/mL. For ascorbic acid equivalent antioxidant activity (AEAC), the values were 229.5±7.9 to 319.3±8.7 mg AA/100g. It was found that the total phenolic content (TPC) in *P. oleracea* varied from 174.5±8.5 to 348.5±7.9 mg GAE/100g, ascorbic acid content (AAC) ranged from 60.5±2.1 to 86.5±3.9mg/100g and FRAP ranged from 1.8±0.1 to 4.3±0.1mg GAE/g.

The study found that there was a good correlation between the results of TPC and AEAC, and between IC_{50} and FRAP assays ($r^2>0.9$). This study also reported the concentration of Ca, Mg, K, Fe, and Zn increased with plant maturity. Calcium (Ca) showed negative relationship with sodium (Na), Chloride (Cl), but showed positive relationship with magnesium (Mg), potassium (K), iron (Fe) and Zinc (Zn). It was concluded that mature plants of *P. oleracea* has higher total phenolic content and antioxidant activities than plants at immature stages.

2.4.3 Anti-atherogenic, and Immunomodulatory Activity

Rasha *et al* (2011) had reported the efficiency of *P. oleracea* (components of ω -3 and ω-6) on hyperlipidemia, kidneys function and as immunomodulators in rats which fed with high cholesterol diets. In this study, 40 male albino rats were divided into four groups which are control group, hypercholesterolemic rats, fed the balanced diet supplemented with cholesterol at a dose level of 2g/100g diet. The other two groups of rats were fed with the same as previous hypercholesterolemic diet supplemented with *P. oleracea* (ω -3 and ω-6). The study showed that there was a significant increase in total cholesterol, total lipids, and triacylglycerol in both serum and liver caused by the 2% cholesterol administration. Serum phospholipids, LDL-C, and atherogenic index (AI) also showed significantly increased compared to the control groups of rats. Cholesterol-enriched diet significantly increased serum urea, creatinine, sodium, potassium levels and also serum IgG and IgM compared to the control group. A significantly decrement in lipid parameters and significant improvement in IgG and IgM levels was found in the hypercholesterolemic rats fed with *P. oleracea*. This result showed that *P. oleracea* had anti-atherogenic hypolipidemic and immunmodulator effects which were probably mediated by unsaturated fatty acids present in seed mixture.

2.4.4 Anti hyperlipidemic Activity

In the previous study of Sankara *et al.* (2012), anti-hyperlipidemic activity of ethanolic extract of leaves of *P. oleracea* were reported. Significant inhibition against dexamethasone induced hyperlipidemia in adult wistar rats were shown from the test extracts (200 and 400mg/kg) treatment for 8 days. Biochemical parameters such as total cholesterol, total triglycerides, phospholipids, high density lipoproteins (HDL), low density lipoproteins (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol, atherogenic index levels were measured and compared with standard gemfibrozil. A significant decrease in triglycerides ($p<0.01$), LDL ($p<0.001$), VLDL ($p<0.01$), HDL ($p<0.01$), and cholesterol ($p<0.001$) were shown in the ethanolic extract of *P. oleracea*. This study shows that the ethanolic extract of *P. oleracea* shows good anti-hyperlipidemic activity.

2.4.5 Anti-arthritic Activity

Jagan *et al.* (2012) reported the anti-arthritic activity of Petroleum-ether extract of *P. oleracea* Linn by Fruends Adjuvant arthristis model in male wistar rats. The extracts of *P. oleracea* were at the dose of 100, 200 and 300 mg/kg/p.o and standard as Indomethacin at a dose of 100mg/kg. Maximum of inhibition which is about 77.82% was observed on 21st day. This study revealed the anti-arthritic activity of aqueous extract of *P. oleracea*.

2.4.6 Anti-diabetic Activity

The effects of crude polysaccharide from *P. oleracea* on blood glucose, body weight, total cholesterol, high density lipoprotein cholesterol, triglyceride and serum insulin levels in diabetes mellitus mice were reported by Gong *et al.* (2009). A significant decrease in the concentrations of fasting blood glucose, cholesterol and triglyceride in mice were found from the treatment with crude polysaccharide from *P. oleracea* (200, 400 mg/kg bw) for 28 days. The concentration of HDL-c, body weight and serum insulin level in the mice were significantly increased by this polysaccharides. Besides that, it did not produce any physical or behavioural signs of toxicity. The data demonstrated best effects at the dose of 400 mg/kg bw. These results shows that crude polysaccharide from *P. oleracea* can be used to control blood glucose and modulate the metabolism of glucose and blood lipids in diabetes mellitus mice.

A study was done by Gao *et al.* (2010) revealing the effects of polysaccharide from *P. oleracea* on alloxan-induced diabetic rats and its mechanisms. The polysaccharide treatment shows significant decreases in fasting blood glucose, total cholesterol and triglycerides. Polysaccharide also showed a tendency of improvement body weight gain on diabetic rats. In addition, the diabetic control group had low serum insulin level comparing with that of normal control group, at the same time, the insulin levels were dose-dependently raised in the polysaccharide treated groups than that of diabetic control group. According to the result get from single cell gel electrophoresis and LD51 analysis, polysaccharide was proved to be nontoxic to the animals. The result shows that polysaccharide would alleviate the blood glucose and lipid rising associated with diabetes. It also improved the abnormal glucose metabolism and increase insulin secretion by restoring the impaired pancreas cells in alloxan-induced diabetic rats, which suggest that polysaccharide has the hypoglycemic potential and could be useful on the diabetes therapy.

2.4.7 Hepatoprotective Activity

Based on the previous study of Prabhakaran *et al.* (2010), the suspensions of methanol and petroleum ether extracts of whole plant parts of *P. oleracea* in carboxy methyl cellulose were evaluated for hepatoprotective activity in Wister albino rats by inducing hepatic injury with D-galactosamine (400 mg/kg). At the dose levels of 200 and 400 mg/kg, altered biochemical parameters were significantly restored when compared to d-galactosamine and Silymarin treated groups. Histology of the liver sections of albino rats also showed to significantly prevent the d-galactosamine toxicity as revealed by the hepatic cells with well-preserved cellular architecture. Both biochemical and histological data had confirmed significant hepatoprotective activity of these extracts from *P. oleracea*.

2.4.8 Nephro-protective Activity

Karimi *et al.* (2010) reported the aqueous and ethanolic extract of *P. oleracea* against cisplatin induced acute renal toxicity in rats. Treatment with aqueous and ethanolic extracts in the highest dose (0.8 and 2 g/ kg), 6 and 12 hour before cisplatin injection reduced blood urea nitrogen and serum creatinine. Tubular necrotic damage was also not observed. Meanwhile in another group rats treated with aqueous and ethanolic extract, 6 and 12 hours after cisplatin injection also had blood urea nitrogen and serum creatinine levels significantly lower compared to those receiving cisplatin alone but mild to moderate cell injury was observed.

2.4.9 Neuronal Activity

The neuronal activities of aqueous extract of stem and leaves of *P. oleracea* with a dose of 1.5ml/kg in adult rats for 12 days were reported by Abdel *et al.* (2012). This study showed significant decrease in the Ca^{2+} level in cerebral cortex by about -25.2% at $p<0.05$. There was significantly decrease in dopamine content in spinal cord but significantly increase in dopamine content in cerebellum, cerebral cortex, thalamus and hypothalamus of rats. This study concluded that *P. oleracea* has the potential as neurotransmitters, which plays an integral part of many neurodegenerative disorders.

2.4.10 Neuroprotective Activity

In 2005, Li *et al.* had reported the effect of flavones extracted from *P. oleracea* on ability of hypoxia tolerance in mice. The survival time of mice in hypoxic conditions in flavones-treated group was found to be significantly longer compared to the untreated group. The RBC, Hb concentration, HCT, plasma EPO level and the relative values of EPO mRNA in renal tissue and pallium of mice were significantly higher in the flavones treated group compared to the untreated group

2.4.11 Anti-inflammatory Activity

According to Chan *et al.* (2000), *P. oleracea* sub sp. sativa was evaluated for further work because of its abundant availability from reliable sources. The 10% ethanolic extract of the aerial parts showed significant anti-inflammatory activity in the carrageenan-induced hind paw oedema and the cotton pellet-induced granuloma models in rats. Besides that, significant analgesic activity were also found in the hot-plate and tail flick models (in mice and rats, respectively) after intraperitoneal administration.

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