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Phytochemical Analysis and *in vitro* Antioxidant Activity of Fractions of Methanol Extract of *Polyalthia longifolia* var. Pendula Leaf

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Authors' contributions

This research work was carried out in collaboration among all authors. The author BA designed the study, performed the statistical analysis and wrote the first draft of the manuscript. All authors contributed to the analysis of samples, collection of data and development of the final manuscript. All authors also read and approved the final manuscript.

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ABSTRACT

This study was aimed to determine the antioxidant capacity of various fractions of crude methanol extract of *Polyalthia longifolia* leaves. The various fractions were obtained by successive liquid-liquid partitioning of the crude methanol extract into n-hexane, chloroform and ethyl acetate. The flavonoid and phenolic contents as well as the 2, 2- diphenyl-1-picrylhydrazyl scavenging activity and total reducing power of the crude methanol extract of *Polyalthia longifolia* leaves and its fractions were analyzed using standard methods. From the results obtained, ethyl acetate fraction

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had the highest flavonoid (571.30 mgQE/g) and phenolic (278.45 mgGAE/g) contents and the lowest IC50 values for 2, 2- diphenyl-1-picrylhydrazyl scavenging activity (< 1 µg/ml) and total reducing power (0.24 µg/ml). However, the water soluble fraction showed least values for flavonoid content (7.22 mgQE/g), phenolic content (48.66 mgGAE/g) and highest IC50 values for 2, 2-diphenyl-1-picrylhydrazyl scavenging activity (505.87 µg/ml) and total reducing power (47.99 µg/ml). The flavonoid content, phenolic content and total reducing power were in the order; ethyl acetate fraction > chloroform fraction > methanol extract > n-Hexane fraction > water soluble fraction. However, the inhibition of 2, 2- diphenyl-1-picrylhydrazyl free radical followed the order; ethyl acetate fraction > methanol extract > chloroform fraction > n-Hexane fraction > water soluble fraction. In general, the antioxidant activity of the ethyl acetate fraction was the most outstanding when compared with the crude methanol extract and other fractions. Having established the presence of significant concentrations of pharmaceutically important phytochemicals in the fractions of the methanol extract of the leaves especially the ethyl acetate, more evidence based studies such as analysis of specific chemical composition and *in-vivo* pharmacological potential of each fraction is imperative in order to expound more on the medicinal usability of the various fractions of the leaves.

Keywords: Polyalthia longifolia; fractions; antioxidant; phytochemicals; phenolic; flavonoid.

1. INTRODUCTION

The contribution of plants to the sustainability of other living organisms such as man and animals cannot be over-flogged due to their use for food and medicine. Medicinal plants contain a broad spectrum of phyto-chemicals which not only make many of them suitable for food but also make them helpful in combating infectious as well as chronic life threatening illnesses. Plant-based therapies have become crucial components of traditional medicinal practices and also serve as a crucial source of inspiration for many major pharmaceutical drugs used in the defense against numerous diseases. Many plants and plant-derived products contain loads of micro and macro minerals, fats, sugars, antioxidant and antimicrobial substances; many of which make them essential for sustainability of life. Several modern scientific studies have confirmed the efficacy of traditionally used medicinal plants for combating wide spectrum of life threatening diseases ranging from all forms of infectious diseases to lifestyle diseases.

P. longifolia var. angustifolia Thw. (Family: Annonaceae) also known as Asoka is one plant which has gained prominence not only in traditional medicine but also in beautification. It is one of the ornamental plants prominently known for road side planting and beautification due to its aesthetic characteristics, drought resistance, dust scavenging capacity and most importantly; its pollution tolerance and alleviation ability [1]. *Polyalthia longifolia* is one of the most widely used indigenous medicinal plants found in tropical parts of Africa, India and Malaysia in gardens, roadsides and various campuses for their beautiful appearances [2,3]. It is a plant with records of outstanding medicinal properties ranging from antioxidant, antimicrobial, anti-inflammatory, cytotoxic, antitumor and hepatoprotective potentials amongst others. It is commonly used in traditional medicine as febrifuge, tonic [4] and in the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis [5].

Many bioactive compounds have been isolated from various parts of this plant and these isolated compounds as well as various extracts from the plant have been reported for numerous biological activities which include antitumor and antioxidant activity [6], anti-inflammatory, cytotoxic, antibacterial and antifungal activities [7]. In Alloxan induced experimental diabetic rats, [8] evaluated the hypoglycemic and antihyperglycemic activities of various solvent extracts of *P. longifolia* leaves. [9] have reported the ability of the aqueous extract of this plant to lower the blood pressure and respiration rate in experimental animals. The antiulcer and anti-inflammatory ability of ethanol and aqueous extracts of *Polyalthia longifolia* have also been reported [10]. Diterpenes, alkaloids, steroid and miscellaneous lactones have been isolated from its bark [11]. The stem bark extracts have also been found to have various biological activities like antibacterial, cytotoxicity, antifungal and antioxidant activity [3,11-13]. Virtually all parts of this plant are used in Indian traditional system to combat various ailments [5].

Although several authors have reported various pharmacological activities of the different part of *Polyalthia longifolia,* existing literature shows that no comprehensive antioxidant activities have been reported earlier for the fractions of its methanol extract. It therefore became imperative to undertake this present study which was aimed at evaluating the flavonoid content, phenolic contents and antioxidant activities of the different fractions of crude methanol extract of *Polyalthia longifolia* leaves.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh, mature and healthy leaves of *Polyalthia longifolia* were obtained from a *Polyalthia longifolia* tree within Babcock University, Ilishan-Remo Ogun State, Nigeria. The leaf was identified and authenticated by a Botanist in Botany Department of University of Ibadan, Oyo State, Nigeria and assigned an identification number; UIH-22633.

Fig. 1 represents the flowchart of the extraction and partitioning of the crude methanol extract of *P. longifolia* leaves. The leaves were oven-dried at 40±C for 24 hrs with Uniscope SM9053 Laboratory oven. About 100 grams of the oven-dried leaves was pulverized with the use of a Lexus MG-2053 Optima laboratory blender. Extraction of the pulverized leaves was carried out by maceration in 80% methanol with sample to solvent ratio of 1:7. The mixture was shaken vigorously and allowed to stand for 48 hours at room temperature. Thereafter the mixture was filtered with a Whatman No.1 filter paper and the residue was re-macerated in equal volume methanol for 24 hrs (three more times) in order to obtain adequate quantity of extract. The filtrates obtained were combined and evaporated to dryness under reduced pressure at about 40°C with the use of Eyela N-1001 vacuum rotary evaporator.

2.2 Partitioning of Methanol Extract

Some portion of the crude methanol extract of *Polyalthia longifolia* leaves was reconstituted in distilled water and then exhaustively partitioned successively into n-hexane, chloroform and ethyl acetate with the use of liquid-liquid extraction method. Each of the resulting solvent fractions; n-hexane, chloroform, ethyl acetate and the water soluble fraction was collected in separate containers and evaporated to dryness under reduced pressure at about 40°C with the use of a vacuum rotary evaporator (Eyela N-1001). The methanol extract of *Polyalthia longifolia* leaves and its different solvent fractions were immediately assayed for antioxidant activities using various standard methods.

2.3 Flavonoid Content

Flavonoid content of the samples (crude extract and fractions) was analyzed following the spectrophotometric method of [14]. Quercetin served as the standard substance. 1 ml of the sample (containing 100 µg/ml), prepared in methanol was mixed with distilled water (4 ml) in a 10 ml volumetric flask. Then, 5 % $NaNO_2$ solution (0.3 ml) was added to the flask. After 5 mins, 10% $AlCl_3$ (0.3 ml) was added and at 6th minute, 1.0 M NaOH (2 ml) was added. Distilled water (2.4 ml) was added to the reaction flask and thoroughly shaken. Absorbance of the resulting reaction mixture was then taken at 510 nm on a spectrophotometer (JENWAY 6305, Staffordshire, UK). Reagent blank; containing 1 ml methanol (instead of the extract) was concomitantly prepared and treated in the same manner as the samples. A calibration curve was prepared by repeating the same procedure for standard solutions of Quercetin (2 to 10 µg/ml, R^2 = 0.986). From the measured absorbance of the samples, the total flavonoid content was estimated from Quercetin calibration curve and results expressed as mg Quercetin Equivalent per gram (mgQE/g) of the sample on a dry weight basis. The test was carried out in triplicates.

2.4 Phenolic Content

The total phenolic content of the sample was assayed by the method of [15]. The assay is based on reduction of Folin-Ciocalteu reagent (Phosphomolybdate and phosphotungstate) by the phenolic compounds present in the extract. The reaction mixture was made by mixing 0.5 ml methanol solution of the sample (containing 100 µg/ml), 2.5 ml of 10% aqueous solution of Folin-Ciocalteu reagent and 2.5 ml of 7.5% $NaHCO_3$ solution. Blank was concomitantly prepared by mixing 0.5 ml methanol, 2.5 ml of 10% aqueous solution of Folin-Ciocalteu reagent and 2.5 ml of $NaHCO_3$ solution. The sample was incubated in a Uniscope SM801A laboratory water bath at 45°C for 45 min and thereafter the absorbance

was measured on a spectrophotometer (JENWAY 6305, Staffordshire, UK) at 765 nm. Standard solutions of gallic acid were taken through the same procedure and the absorbance values obtained were used to construct a standard calibration curve. The measured absorbance of a sample was used to extrapolate its phenolic content from the standard calibration curve. The phenolic content was then expressed as gallic acid equivalent (mg of GA/g) of the sample. Each sample was analyzed in triplicates.

2.5 DPPH Radical Scavenging Activity

The antioxidant activity of the sample was evaluated spectrophotometrically through free radical scavenging effect on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical as described by [16]. 2.5 ml of methanol solution of the sample at various concentrations (10, 20, 40, 60, 80 and100 µg/mL) was added to 1 ml of methanolic solution of DPPH (0.3 mM) and kept in the dark at room temperature for 30 mins. The same procedure was carried out on standard gallic acid solutions at various concentrations (2, 4, 6, 8 and 10µg/ml). The absorbance of the resulting mixture was read at 518 nm and converted to percentage inhibition using the equation;

$$\% \text{ inhibition of DPPH} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

Each analysis was done in triplicate for each concentration. The control was prepared by mixing 2.5 ml methanol and 1 ml methanol solution of 0.3 mM DPPH. The IC50 value representing the concentration of the compounds that caused 50% inhibition of radical formation was obtained by interpolation from linear regression analysis [17].

2.6 Total Reducing Power

The total reducing capacity of the sample was determined according to the method described by [18]. 1 ml of various concentrations of the samples (10, 20, 40, 80 and 100 µg/ml) was mixed with phosphate buffer (500 µL 20 mM, pH 6.6) and 1% potassium ferricyanide (500 µL). It was incubated at 50°C for 20 min; after which 500 µL of 10% Trichloroacetic acid was added, and the mixture centrifuged at 2500 rpm for about 10 min. The supernatant was mixed with distilled water (1.5 ml) and 0.1% ferric chloride (300 µL) and the absorbance was read at 700 nm on a spectrophotometer (JENWAY 6305, Staffordshire, UK). Gallic acid solution of various concentrations (2, 4, 6, 8 and 10 µg/ml) were analyzed likewise. Increase in the absorbance of the reactions mixture indicated increase in the reducing power. The sample concentration which provided 0.5 of absorbance (IC_{50}) was calculated from the graph of the sample absorbance at 700 nm against sample concentration [19]. The analysis was carried out in triplicates and mean values were obtained.

3. RESULTS AND DISCUSSION

3.1 Physical Characteristics of the Extract and Fractions of *Polyalthia longifolia*

Table 1 represents some of physical characteristics of the methanol extract of *Polyalthia longifolia* leaves and its fractions. These include; percentage yield, color and state at room temperature. The crude methanol extract and n-hexane fraction were both green in color while chloroform, ethyl acetate and water soluble fractions were yellowish-green, golden-yellow and wine in color respectively. The yield of the methanol extract was about 21% of the extracted leaves while the; n-hexane, chloroform, ethyl acetate and the water soluble fractions yielded 39.24%, 28.31%, 11.98%, and 18.68% of the crude methanol extract respectively. The highest fraction yield shown by n-hexane implied that the methanol extract contained more non-polar components than semi-polar or even polar ones. At room temperature, the n-hexane fraction was oily, the methanol extract and water soluble fraction were semi-solids while both chloroform and ethyl acetate fractions were solids. Methanol like many other semi-polar solvent has been reported to be good for junk extraction owing to their ability to extract a mixture of both polar and non-polar compounds from plants. This may account for the relatively high extract yield obtained for the *Polyalthia longifolia* leaves in methanol and also the possibility of partitioning the extract into various organic solvents with different polarities.

3.2 Flavonoid and Phenolic Content

The occurrence of flavonoid and phenolic compounds in plants has been subject of many important chemical, biochemical and biological studies. They are found majorly in fruits, vegetables and beverages in varied amounts. Flavonoid and phenolic compounds are reported

to be the most important class of secondary metabolites and phytochemicals [20] with wide spectrum of remarkable pharmacological activities. Phenolic compounds are essential for plant growth and reproduction and are produced as a response to environmental factors such as light, chilling, pollution etc and to defend injured plants [21,22]. Apart from their importance in plants, flavonoids are essential for human health due to their high pharmacological activities especially as free radical quenchers [23]. In addition to their effects on mammalian metabolism, many flavonoid and phenolic compounds have been reported to possess a variety of biological activities such as antidiabetic, hepato-protective, gastro-protective, antiviral, antineoplastic, antiallergic, antiinflammatory, antiviral, antiproliferative and anticarcinogenic activities [24,25].

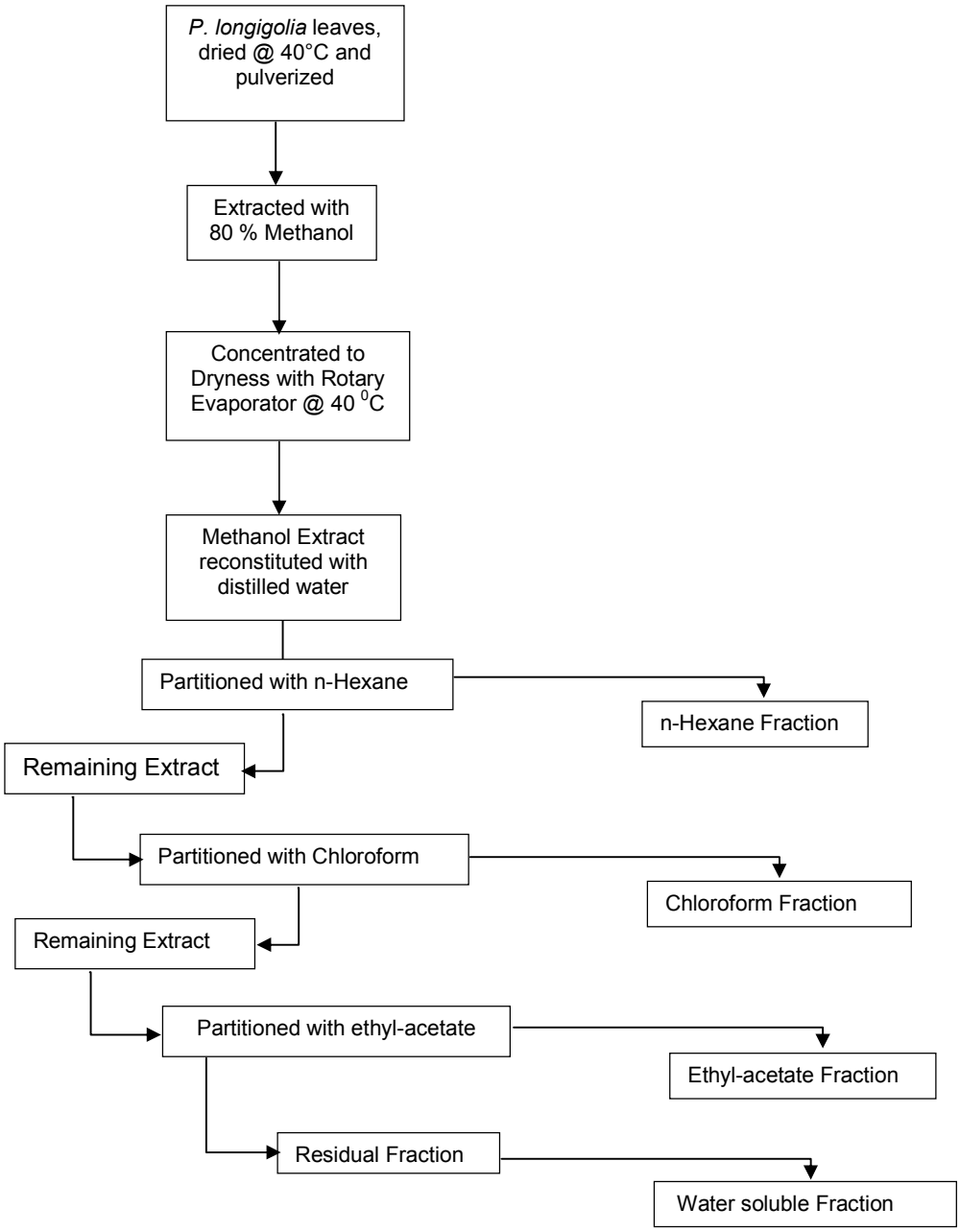

Fig. 1. Flow chat of extraction and partitioning of crude methanol extract of *P. longifolia* leaves

Table 1. Physical properties of methanol extract and fractions of *Polyalthia longifolia* leaves

Sample	Color	State at room temperature	Percentage yield
MEE	Green	Semi-solid	21.05± 0.41
NHFR	Green	Oily and viscous	39.24±0.56
CHLFR	YellowishGreen	Solid	28.31±1.22
EAFR	GoldenYellow	Solid	11.98±1.34
WSFR	Wine	Semi-solid	18.68±1.09

Data are expressed as mean ± standard error of three replicates. MEE= Methanol extract, NHFR= n-Hexane fraction, CHLFR= Chloroform fraction, EAFR= Ethyl acetate fraction, WSFR= Water Soluble fraction

The results of the flavonoid and phenolic contents of fractions of methanol extract of *Polyalthia longifolia* leaves are represented in Fig 2 and are shown to be at varied concentrations. From the results obtained, ethyl acetate fraction showed the highest concentrations of both flavonoids and phenolics while the least concentrations of both phytochemicals were obtained for the water soluble fraction. Our result is in consonant with the findings of [26] who reported that the concentrations of flavonoids and flavonols were highest in the ethyl acetate fraction of methanol extracts of both leaves and seeds of coriander and least in the water soluble fractions for both. The presence of the least flavonoid and phenolic contents in the water soluble fraction may be due to the fact that the partitioning solvents (i.e n-hexane, chloroform and ethyl acetate) have extracted the bulk of the phytochemicals originally present in the crude methanol extract. This may also suggest poor solubility of these phytochemicals in water. The trend of occurrence of both flavonoids and phenolics however was; ethyl acetate fraction > chloroform fraction > methanol extract > n-Hexane fraction > water soluble fraction. It was also shown that the phenolic contents of crude methanol extract and its hexane, chloroform and water soluble fractions were higher than their flavonoid contents. However, ethyl acetate fraction was the only fraction that showed flavonoid content that was higher in more than double folds than its phenolic content. This implied that in comparison with other solvents used for the partitioning, ethyl acetate was most suitable for the extraction of both flavonoids and phenolics from the methanol extract of *Polyalthia longifolia* leaves. This could also imply that most flavonoids present in the methanol extract have the highest solubility in ethyl acetate or better still have the most agreeable chemistry with ethyl acetate. This however did not correlate with the yield or product recovery of the fractions of the extract as obtained in this study. This is because the lowest fraction yield was reported for ethyl acetate (Table 1) which showed the highest concentration of flavonoids and phenolics. In order words, the concentration of both flavonoid and phenolics in the fractions is independent on the yield of the fractions but rather on the level of extractability of these phytochemicals by the partitioning solvent from the crude methanol extract. The varied concentrations of flavonoid and phenolics in the fractions showed that extraction solvent has an influence on the total flavonoid and total phenolic content of the plant extracts.

3.3 DPPH Scavenging Activity

The antioxidant capability of the methanol extract of *Polyalthia longifolia* and its fractions was analyzed by determining their ability to scavenge or quench standard DPPH free radical and the results of this assay are presented in Table 2. DPPH is prominent for its use in determining the capacity of compounds for scavenging free radicals and the antioxidant activity of various plant extracts and isolated phyto compounds. It is a stable and standard free radical molecule with an immense purple coloration in alcohol. In the presence of substances such as pure antioxidant compounds, plant extracts, fruits and other food materials with electron donating capability, DPPH becomes decolorized and the extent of this decolorization is a function of the concentration of electron-donating antioxidant phytochemicals present in the plant extract. The results showed that the DPPH scavenging capacity of the extract and its fractions were concentration dependent and the methanol extract and its chloroform and ethyl acetate fractions showed considerably good antioxidant activity particularly at 100 µg/ml concentration. However, the ethyl acetate fraction showed the most remarkable antioxidant activity with an IC_{50} value of less than 1 µg/ml while the water soluble fraction showed the least scavenging activity with an IC_{50} of 505.87 µg/ml. The results indicated the presence of more antioxidant compounds in the ethyl acetate fraction than every other fraction of the crude methanol extract. The trend of the

DPPH scavenging activity of the extract and its fractions was; ethyl acetate fraction > methanol extract > chloroform fraction > n-hexane fraction > water soluble fraction. This showed a slight correlation between the concentrations of flavonoid and phenolics and the DPPH scavenging activity of the samples in that, the ethyl acetate fraction with the highest flavonoid and phenolic contents also showed the highest antioxidant activity while the water soluble fraction with the least flavonoid and phenolic contents also showed the lowest antioxidant activity. Although flavonoids and phenolic compounds are renowned for having remarkable antioxidant activities; phytochemicals other than these two may be present which also contributed to the DPPH scavenging activity of the test samples. Some other phytochemicals apart from these two which have been reported to also confer antioxidant capacity on plant extracts

Table 2. Percentage inhibition of DPPH by methanol extract and fractions of *Polyalthia longifolia* leaves

Sample	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	100 g/ml	IC50(µg/ml)
MEE	8.10±0.10	2.33±0.15	29.40±0.10	55.23±0.15	64.10±0.10	73.78±0.16
NHFR	5.55±0.05	9.40±0.10	15.50±0.10	16.70±0.10	18.03±0.06	348.37±3.45
CHLFR	9.40±0.10	6.40±0.10	27.27±0.15	46.03±0.15	54.37±0.15	89.12±0.30
EAFR	45.67±0.16	5.27±0.15	81.77±0.15	85.33±0.15	87.57±0.06	< 1
WSFR	2.47±0.15	7.30±0.10	9.10±0.10	9.90±0.10	13.27±0.15	505.87±1.05

Data are expressed as mean ± standard error of three replicates. MEE= Methanol extract, NHFR= n-Hexane fraction, CHLFR= Chloroform fraction, EAFR= Ethyl acetate fraction, WSFR= Water Soluble fraction

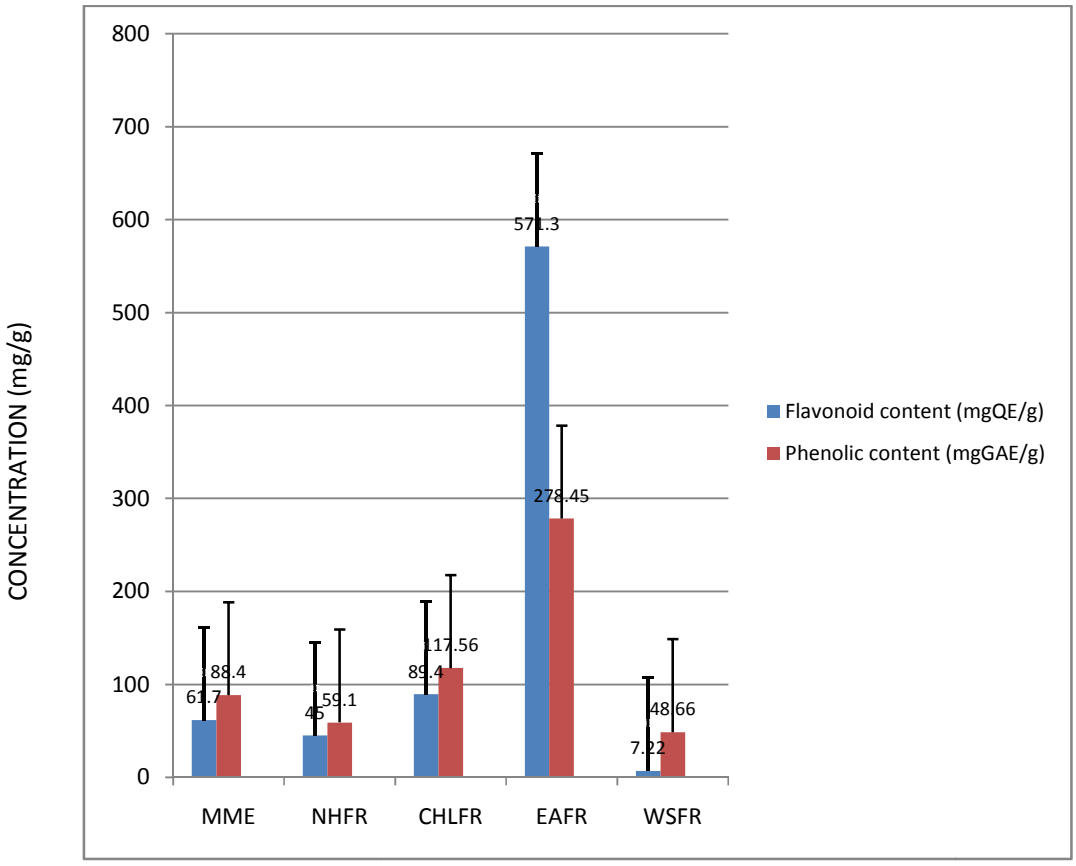

Fig. 2. Flavonoid and phenolic contents of methanol extract and fractions of *Polyalthia longifolia* leaves. data are expressed as mean of three replicatses

Table 3. Total reducing power (Absorbance) of methanol extract and fractions of *Polyalthia longifolia* leaves

Sample	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	100 µg/ml	IC50(µg/ml)
MEE	0.717±0.001	0.736±0.002	0.781±0.001	0.962±0.001	1.115±0.001	13.11±0.16
NHFR	0.687±0.001	0.706±0.002	0.726±0.002	0.803±0.002	0.852±0.001	17.18±0.10
CHLFR	0.761±0.001	0.786±0.001	0.912±0.002	1.09±0.001	1.117±0.002	6.81±0.13
EAFR	0.833±0.001	1.050±0.002	1.251±0.001	1.631±0.002	1.785±0.001	0.24±0.03
WSFR	0.502±0.002	0.542±0.001	0.591±0.002	0.601±0.001	0.625±0.001	47.99±0.06

Data are expressed as mean ± standard error of three replicates. TRP= Total Reducing Power MEE= Methanol extract, NHFR= n-Hexane fraction, CHLFR= Chloroform fraction, EAFR= Ethyl acetate fraction, WSFR= Water soluble fraction

Table 4. Percentage inhibition of DPPH and total reducing power of gallic acid

Gallicacid standard	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml	IC50(µg/ml)
Percentage inhibition of DPPH	32.56±0.02	61.55±0.03	85.50±0.03	90.1±0.01	94.91±0.04	3.01±0.01
Total reducing power (Absorbance)	0.599±0.15	0.674±0.03	.774±0.21	0.989±0.01	1.075±0.01	1.00±0.02

Data are expressed as mean ± standard error of three replicates

include; ascorbic acid, carotenoids, tannins and α-tocopherol [27,28]. From the results of this study, it was also noted that even though ethyl acetate fraction gave the least fraction yield (Table 1), it showed the most outstanding antioxidant activity. This suggests that the biological functionality of a plant extract or fraction of an extract is not necessarily dependent of yield or amount extracted but rather could be attributed to the pharmacological strength of the different classes of compounds extracted by either the extracting or partitioning solvent as the case may be. However, the classes of phyto-compounds as well as their concentrations that are obtainable from a plant material either by extracting directly from the plant tissues or by partitioning of the crude plant extract is dependent on their solubility of these compounds in the solvent.

3.4 Total Reducing Power

Reductive strength of plant extracts could serve as a significant indicator of their potential antioxidant activities [29]. In the reducing power assay, the antioxidant compounds convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}) which enable the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of the phytochemicals [30]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [31]. The results of the total reducing power of the methanol extract of *Polyalthia longifolia* and its fractions are shown in Table 3. The results showed that the total reducing power of the extract as well as its fractions were also concentration dependent as depicted in DPPH scavenging activity. The trend of the total reducing power of the extract and fractions was; ethyl acetate fraction > chloroform fraction > methanol extract > n-hexane fraction > water soluble fraction. This followed the trend shown in the concentrations of flavonoid and phenolic of the extract and its fractions. This may imply that the reducing power of the fractions had a direct relationship with the concentration of both flavonoid and phenolic compounds they contain. Several reports have stated the correlation of phytochemicals; especially flavonoid and phenolic compounds with various antioxidant activities of plant extracts [32-34].

4. CONCLUSION

From the results of this study, it is concluded that different fractions of the methanol extract of *P. longifolia* leaves possess significant antioxidant properties. Moreover, the results also indicated that ethyl acetate fraction exhibited the highest antioxidant property when compared with all the other fractions and even the crude methanol extract while the water soluble fraction

exhibited the lowest properties in all of the assays. The results also showed positive correlation between flavonoid content, phenolic content and the antioxidant capacity of the various fractions. This suggests that the presence of more antioxidant phytochemicals such as flavonoid and phenolic compounds in the ethyl acetate fraction contributed significantly to the fraction's most outstanding antioxidant activity. Further evidence based studies on the specific chemical composition and *in-vivo* pharmacological activities of the various fractions of methanol extract of *P. longifolia* leaves need to be carried out. This would expound more on the medicinal usability of the various fractions of the leaves.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar SR, Arumugam T, Anandakumar CR, Balakrishnan S, Rajavel DS. Use of plant species in controlling environmental pollution- a review. Bulletin of Environment, Pharmacology and Life Science. 2013;2(2):52-63.
2. Bose TK, Das P, Maiti GG. Trees of the World, (Regional Plant Resource Centre, Bhubaneswar, India). 1998;I:370.
3. Njoku UO, Joshua PE, Omeh, OV. Antioxidant properties of *Polyalthia longifolia*. New York Science Journal. 2011;4(6):83-87.
4. Anonymous, The Wealth of India. New Delhi: Publication and Information Directorate, CSIR. 1969;3(1):287.
5. Subramanion LJ, Yee SC, Dharmaraj S, Subramanian D, Lachimanan YL. et al. *Polyalthia longifolia* Sonn: An ancient remedy to explore for novel therapeutic agents. Research Journal Pharmaceutical, Biological and Chemical Sciences. 2013; 1:714-730.
6. Manjula N, Mruthunjaya,VP, Nitesh K, Challamudi MR. Antitumor and antioxidant activity of stem bark ethanol extract of *Polyalthia longifolia.* Pharmaceutical Biology. 2010;48(6):690- 696.
7. Malairajan P, Geetha G, Narsasimhan S, JessiKala VK. Evaluation of anti-ulcer activity of *Polyalthia longifolia* (Sonn.). Indian Journal of Pharmacology. 2008; 40(3):126 -128.
8. Nair R, Shukla V, Chanda S Assessment of *Polyalthia Longifolia* Var. Pendula for hypoglycemic and antihyperglycemic activity. Journal Clinical Diagnostic Research. 2007;1:116-121.
9. Saleem R, Muhammad A, Iqbal AS, Mohammad A, Khan AR, Rasool N, Saleem H, Noor F, Faizi S. Hypotensive activity and toxicology of constituents from root bark of *Polyalthia longifolia* var. pendula. Phytotherapy Research. 2005; 19:881-884.
10. Sharma RK, Mandal S, Rajani GP, Gupta N, Srivastava DP. Antiulcer and antiinflammatory activity of fresh leave extracts of *Polyalthia longifoliai* in rats. International Journal of Drug Development Research. 2011;3(1):351-359.
11. Ghosh G, Durga MK, Bharata BS, Sagar KM. Anti-hyperglycemic and antioxidant activity of stem bark of *Polyalthia longifolia* var. angustifolia. Der Pharmacia Lettre. 2010;2(2):206-216.
12. Rao RAK, Rehman F. Use of *Polyalthia longifolia* seeds (seeds of indian mast tree) as adsorbent for the removal of cd (II) from aqueous solution. Journal of Dispersion Science and Technology. 2012;33(4):472-481.
13. Subramanion LJ, Azlan A, Sreenivasan S. International Conference on Nutrition and Food Science. 2012;226-230.
14. Dewanto V, Wu X, Adom KK and Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry. 2002;50: 3010-3014.
15. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymology. 1999;299: 152-178.
16. Mensor LL, Menezes FS, Leitao GG, Reis AS, Santos TS, Coube CS. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical

method. Phytotherapy Research. 2001;15: 127–130.
17. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of a ginger extracts (*Zingiber officinale*). Food Chemistry. 2007;102:764-770.
18. Premanath R, Lakshmidevi N. Studies on Anti-oxidant activity of *Tinospora cordifolia* (Miers.) Leaves using *in vitro* models. Journal of American Science. 2010;6(10): 736-743.
19. Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chemistry. 2007;103:413-419.
20. Kim D, Jeond S, Lee C. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry. 2003;81:321-326.
21. Valentine IK, Maria VK, Bruno B. Phenolic cycle in plants and environment. Journal of Molecular Cell Biology. 2003;2:13-18.
22. Massimiliano Tattini, Lucia Guidi, Laura Morassi-Bonzi, Patrizia Pinelli, Damiano Remorini, Elena Degl'Innocenti, Cristiana Giordano, Rossano Massai, Giovanni Agati. On the role of flavonoids in the integrated mechanisms of response of *Ligustrum vulgare* and *Phillyrea latifolia* to high solar radiation. New Phytologist. 2005;167:457-470.
23. Cook NC, Samman S. Review: Flavonoids—chemistry, metabolism, cardioprotective effects and dietary sources. Journal of Nutritional Biochemistry. 1996;7:66–76.
24. Zand RSR, Jenkins DJA, Diamandis EP. Flavonoids and steroid hormone-dependent cancers. Journal of Chromatography B. 2002;777:219– 232.
25. Ren W, Qian Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. Medicinal Research Review. 2003;23(4): 519–534.
26. Rajeshwari CU, Shobha RI, Andallu B. Antihemolytic activity of various fractions of methanolic extract of coriander (*Coriandrum sativum* L.) leaves and seeds: A comparative study. Pakistan Journal of Food Science. 2012;22(1):1-6.
27. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chemistry. 2003;83: 547–550.
28. Amarowicz R, Tannins: The new natural antioxidants? European Journal of Lipid Science and Technology. 2007;109:549–551.
29. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. Journal of Agricultural and Food Chemistry. 1995;43:1813–1819.
30. Mahmood RM, Soheila M, Saeid A. Radical Scavenging and Reducing Power of *Salvia mirzayanii* subfractions. Molecule. 2008;13:2804-2813.
31. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry. 1995;43(1):27-32.
32. Sahreen S, Khan MR, Khan RA. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. Food Chemistry. 2010;122(4):1205–1211.
33. Erkan N, Ayranci G, Ayranci E. Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chemistry. 2008;110(1):76–82.
34. Arash Khorasani Esmaeili, RosnaMat Taha, Sadegh Mohajer, Behrooz Banisalam, Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (Red Clover), Bio Med Research International. 2015; 2015:1-11.

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