Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

In Vitro Cytotoxic Activity And Antioxidant Potential Of Different Extracts From *Pittosporum Eugenioides*

Manal M. Hamed,[a]* Laila A. Refahy,[a] Azza A. Shafei,[b] Eman G.I. Abd El Aziz[c]

[a]*Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt*
[b]*Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt*
[c]*Sheben Elkom Hospital, Menofiya, Egypt*

Abstract

Extracts of different polarity from *Pittosporum eugenioides* leaves, were investigated for their antioxidant and cytotoxic activity. Two different bioassays were used, namely scavenging of the diphenylpicrylhydrazyl (DPPH) radical and total antioxidant capacity (TAC). The butanol extract of *P. eugenioides* leaves record the strongest free radical scavenging one among the tested extracts with SC_{50} value of 26.38 µg/ml. The total flavonoid content (TFC) of the plant extracts was ranged from 212.95 to 540.85mg RE / g dry extract and the total phenolic content (TPC) of the tested extracts was ranged from 292.12 to 934.25 mg gallic acid equivalent (GAE)/g dry extract. Positive correlations were found between total phenolic content in the extracts and antioxidant activity, (R^2 = 0.9998, DPPH and R^2 =0.9302, TAC). Considering LC_{50}, the most promising cytotoxic fraction against brine shrimp (*Artemia salina* L.) was the *n*-butanol extract (34µg/ml) followed by 85% methanol extract (45µg/ml). In conclusion, *P. eugenioides* exhibit great potential for antioxidant and cytotoxic activity and may provide good source of natural antioxidant and thus inhibit unwanted oxidation processes, so it may be useful for medicinal functions.

Keywords; *Pittosporum eugenioides*; antioxidant; brine shrimp; extracts

1. Introduction

Today natural medicines have maintained its popularity in all regions of the developing world, and its use is rapidly spreading in industrialized countries. It has long been realized that the therapeutic effects of medicinal plants are due to certain chemical compounds produced by plants [1,2]. Plants produce a high diversity of secondary metabolites used for defense and survival, in response to various biotic and abiotic stresses. Currently identified secondary plant metabolites exceed 100000 substances, belonging to a variety of chemical classes, including terpenoids, phenolics and alkaloids [2]. Some saponin glycosides are used as cardiotonics while others are contraceptives and precursors for other sex hormones [3]. According to Tada *et al*., [4], terpenes have antimicrobial and antiviral properties. Phenolic compounds are characterized by the antioxidant, antiinflammatory, anticarcinogenic and other biological properties which may protect from oxidative stress and various other diseases [5]. Simple phenolics show bactericidal, antiseptic and anthelmintic properties [6]. Reactive oxygen species (ROS) are chemically reactive chemical species containing oxygen. For example peroxides, superoxide, hydroxyl radical, and singlet oxygen [7]. Exogenous ROS can be produced from pollutants, tobacco, smoke, drugs, xenobiotics, or radiation. ROS can damage lipid, DNA, RNA, and proteins, which, in theory, contributes to the physiology of aging and cancer [8,9]. Cancer remains one of the leading causes of morbidity and mortality globally. Amongst the non-communicable diseases, cancer is the second leading cause of death, after cardiovascular disease [10-14]. Globally, the number of cancer deaths is projected to increase from 7.1 million in 2002 to 11.5 million in 2030 [12]. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue injury. Also, many other plant species have been investigated in the search of novel antioxidants [15], but there is still a demand to find more effective antioxidants from plant species. It has been mentioned

*Corresponding author e-mail: manalayman90@yahoo.com.; (Manal M. Hamed).
Receive Date: 01 January 2022 **Revise Date:** 11 January 2022 **Accept Date:** 17 January 2022
DOI: 10.21608/EJCHEM.2022.113405.5189

that antioxidants activity of plants might be due to their phenolic compounds [16-18]. Flavonoids and other plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks [19]. They are important in the plant for normal growth development and defense against infection and injury. The study of antimicrobial capacity of plant phenolics is well known [20,21]. The development of resistance to various commercial anti-microbial drugs drives the increased use of these natural polyphenols in recent years. Polyphenols are major dietary constituents of many food items and beverages. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities [22]. Therefore, brine shrimp bioassay is a more convenient procedure for general toxicity screening. The lethality assay using *Artemia salina* was used in this study because it has been proven to effectively biomonitor the isolation of insecticidal, cytotoxic, antineoplastic, antimalarial, and antifeedant compounds from plant extracts [9,23]. In our study, *P. eugenioides* plant extracts were investigated for its 2,2'-diphenyl-1-picryl-hydrazyl free radical scavenging potential (DPPH), total phenolic contents TPC; total flavonoid contents TFC, total antioxidant capacity (TAC) & cytotoxic activities.

2. MATERIALS AND METHODS

2. 1. Plant Material

Leaves *of P. eugenioides* (Pittosporaceae Family) were collected during July 2016, from Kasr Mohamed Ali Garden, Cairo, Egypt. The plant was identified by Dr. Threase Labib consultant of plant taxonomy at the Ministry of Agriculture; formerly, The Head of Taxonomist Specialists at the garden, a voucher specimen (No. P 3) was kept at the herbarium of the Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

2. 2. Materials and Chemicals for the Biological Studies

All solvents and reagents used were of analytical grade. Trichloroacetic acid (TCA), ferric chloride, sodium carbonate, sodium phosphate, aluminum chloride, ammonium molybdate, rutin, gallic acid and ascorbic acid were purchased from (Merck Chemical Co.), all solvents and acids [petroleum ether, chloroform, ethyl acetate, *n*-butanol, acetic acid, and sulphuric acid), dimethylsulphoxide (DMSO)] were purchased from (Sigma-Aldrich Co.). 2,2'-diphenyl-1-picraylhydrazyl (DPPH) free radical and Folin–Ciocalteu's reagent (FCR) was purchased from (Sigma-Aldrich Co.). For antioxidant activity; all absorbance measurements were recorded using the UV-Vis spectrophotometer Spectronic 601. Ascorbic acid, gallic acid and rutin were purchased from (Merck Chemical Co.). Methanol (MeOH), petroleum ether, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), butanol (BuOH), (All purchased from El-Nasr Pharmaceutical Chemicals Co. Adwic; Egypt).

2. 3. Extraction and Fractionation Process

Dried leaves of *Pittosporum eugenioides* (1.2 Kg) were extracted in 85% methanol at room temperature 25±2°C, several times (5 liter x 7 times) to complete the extraction procedure and then concentrated using Rotatory evaporator at 40°C, to give 85% methanol extract residue brown sticky semi solid (490 g). About 450 g of 85% methanol extract was successively defatted with pet-ether [(60-80°C) (3 L) to eliminate the fats and oil to afford a dark residue (20.5 g)], methylene chloride [(5 L) to afford a residue (47 g)], ethyl acetate [(4 L) to afford a residue (35.2 g)], and finally macerated with *n*-butanol:distilled water (non-polar to polar) to get respective extracts [*n*-butanol (40 g), and aqueous (109 g) fractions.

2. 4. Preliminary Phytochemical Analysis

Phytochemical screening of the extracts was carried out to detect the presence of secondary metabolites using standard phytochemical methods. The qualitative screening of methanol and methylene chloride extracts showed the presence of phytochemical constituents such as saponins, terpenoids, steroids, phenols, flavonoids, carbohydrates and glycosides by using standard methods from the book Harborne and Kokate; Fransworth and Gibbs methods [24-27].

2.5. Methods for Biological Studies

2. 5. 1. Total Phenolic Content (TPC) investigation

The total phenolic contents were determined using the Folin–Ciocalteu's (FC) colourimetric method described by Dewanto *et al*., [28]. Briefly, an aliquot (125 µL) of each of the extracts described above or a standard solution was mixed with 0.5 mL of deionised water and 125 µL of the FC reagent. After 6 min, 1.25 mL of a 7% Na_2CO_3 solution was added to the mixture, followed by 1.0 mL of water to bring the final volume to 3.0 mL. After 90 min of incubation at ambient temperature in the absence of light, the absorbance at 765 nm using spectrophotometer (UV-VS spectrophotometer, Milton Roy 601, Co, USA). Gallic acid was used as the standard, and the total phenolic contents were expressed as mg gallic acid equivalents (GAE)/100 g FW. This method is based on oxidation of phenolics by F-C reagent (a molybdotungstate) in to produce a colored product (molybdenum blue) having maximum wavelength of 765 nm. As the concentration of the extract increases, the mean absorbance value also increases. The formation of the blue color is due to the electron

transfer, Folin: molybdophosphotungstate heteropolyanion reagent, in which Mo (VI) is reduced to Mo (V) with an electron donated from the antioxidant sample according to the following:

Mo (VI) (Yellow) + e^- (from A-H) → Mo (V) (Blue)
Folin reagent

At λ_{max}= 765 nm

2. 5. 2. Total Flavonoid Content (TFC) investigation

Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants, characterized by a benzo-γ -pyrone structure. Various studies have shown that flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. The mechanism of action of flavonoids is through scavenging or chelating process [29,30]. The total flavonoid content in the current study was expressed in milligram per gram dry material of extracts. The flavonoid content of the extract was measured by gravimetric method.

The flavonoids content of each extract was established according to the reported methods [31], using rutin as a control sample. An aliquot of the stock solution was transferred to a 10.0 ml volumetric flask and made to volume with methanol, resulting in the blank solution. A second aliquot of the stock solution was transferred to another 10.0 ml volumetric flask, a volume of the 2% $AlCl_3$ was added and made to volume with methanol, which was named test solution. After 40 min the absorbance of the test solution was measured at 415 nm against blank solution. The blank consists of all reagents and solvent without $AlCl_3$; and it represents the average of three-determinations.

The results were expressed as the amount of flavonoid (mg)/g of herbal material (corrected for moisture content) as mg rutin equivalents (RE)/g extract.

2. 5. 3. Antioxidant Activity

I- DPPH Free Radical Scavenging Activity

Indirect (DPPH radical scavenging assay). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assesses the capacity of an extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical which on reacting with an antioxidant compound (that can donate hydrogen) gets reduced to diphenylpicrylhydrazine (DPPHH). In the test conducted this process was evident by the switch in colour (that is, from deep violet to light-yellow) measured spectrophotometrically. The methanolic extract of the plant decolorized the purple colour of DPPH to the yellow of DPPHH with an IC_{50} value of 0.5 mg/ml. IC_{50} value is the parameter which is used to measure the radical scavenging activity of extract and is defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. The smaller IC_{50} value corresponds to the higher antioxidant activity of the plant extract [32]. The decrease in absorbance of DPPH solution (from purple to yellow) is hence attributed to intrinsic antioxidant activity and the acceleration of reaction between DPPH and antioxidant. The radical scavenging activity (%) in the samples was found to increase gradually in a dose dependent manner. The diluted working solutions of the test extracts were prepared in methanol and ascorbic acid was used as standard in 0.06, 0.12, 0.25, 0.5 mg and 1 mg/ml solution. 1 ml of 0.002% of DPPH solution prepared in methanol was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 516 nm using UV-visible Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below [33].

% Inhibition=[Absorbance of control – Absorbance of test sample] / [Absorbance of control] X 100

Each experiment was carried out in triplicate and results were expressed as mean % antiradical activity ± Standard Deviation (Hinneburg method) [34], according to the following reaction.

$(DPPH^{\cdot}) + (AH) \rightarrow DPPHH + (A^{\cdot})$

A linear regression analysis was performed from a plot of % Inhibition (%IP) against concentration, to determine the IC_{50} value (sample concentration resulting in 50% free radical inhibition) for each tested sample.

II- Total Antioxidant Capacity (TAC) Investigation

The antioxidant activity of extracts and compounds were evaluated by the green phosphomolybdenum complex formation according to the previously described method of Prieto *et al*., (1999). The absorbance of the mixture was measured at 695 nm using a spectrophotometer against blank. The results reported are mean values expressed as grams of ascorbic acid equivalents (AAE) per 100 g extract. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes containing the reaction mixture were capped and incubated in a thermal block at 95°C for 90 min and cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) with methanol. The blank consisted of all reagents and solvents without the tested sample and it also was incubated under the same conditions. All experiments were carried out in triplicate. The antioxidant activity of the tested sample was expressed as ascorbic acid (AAE) equivalent [35].

2.5. 4. Evaluation of Cytotoxic Activity

Toxicity Tests Using the Method of Brine Shrimp Lethality Test (BSLT)

Meyer method [36], is used to study the toxicity of the general sample using shrimp eggs (*A. salina* Leach). Brine Shrimp Lethality Test (BSLT) is one of the methods bioactive compounds present in natural materials using shrimp larvae (*A. salina*). Known toxicity properties based on the number of larvae mortality [37]. An extract is said to be toxic to *A. salina* if it has a value of LC_{50} (lethal concentration to 50% larval shrimp) less than 1000 µg/ml. The hatching of Shrimp larvae prepared shrimp vessel for hatching eggs which have been filled with sea water 1,500 ml, with pH of 7.7 and 87.6% salinity levels, place the lamp to warm temperatures in vessel of hatching and fed air by using the aerator. Inserted into the sea water of 0.3 g shrimp eggs for hatching. Vessel hatching eggs covered with aluminum foil, and the lights turned on for 48 hours to incubate the eggs. After 48 hours of shrimp eggs will hatch into larvae and ready for use. Shrimp larvae that will be used for testing were taken using a pipette. Preparation of sample solution that will be tested. Methanol extract of *P. eugenioides* that will be tested each made in concentrations of 0, 10, 100, 200, 500 and 1000 ppm in sea water. When the methanol extract insoluble added 2 drops of DMSO (dimethyl sulfoxide).

Procedure of Toxicity Test Methods Using BSLT:

Pipette 100 µL seawater containing as many as 20 larvae shrimp, then put into a test tube. Added solution of methanol extract of the sample concentrations of 10, 100, 200, 500 and 1000 µg/mL and performed 3 repetitions. To control performed without the addition of methanol extract. Test tube and placed under light irradiation was left for 24 hours, then counted the number of larvae that die and are still alive and then used to determine the level of toxicity (LC_{50}) and toxicity categories.

2.6. Statistical analysis

Experiment data were subjected to an analysis of variance (ANOVA) using the procedure in the SPSS software package (SPSS v. 16 Inc. USA) for the analyses; our data were presented as mean ± S.D. and done in triplicates (n=3), where applicable according to Annegowda *et al.*, 2010 [38].

3. Results and Discussion

From **(Table 1)**, our study revealed the absence of tannins and alkaloids in the methanol extract.

Total phenolic Content (TPC) and Total Flavonoid Content (TFC)

Phenolic compounds are major plant secondary metabolites that have important roles in plants and are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including the main antioxidant resource found in horticultural crops. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolic are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects [39]. Flavonoids constitute an important group of polyphenolic compounds in plants, which are secondary metabolites that have strong antioxidant activities and many health promoting properties [40,41].

The results shown in **(Table 2)** revealed that the total phenolic and flavonoid contents of different extracts/fractions of *P. eugenioides* were arranged in the order (TPC/TFC): 85% MeOH (840.73±1.22/310.15±0.53);BuOH (934.25±2.01/540.85±0.70);EtOAc (754.62±2.44/400.20±0.53);CH_2Cl_2 (418.51±1.22/257.51±0.88) and pet. ether (292.12±1.52/212.95±0.53) mg GAE / g dry extract/ mg RE / g dry extract, respectively.

Table 1: A brief summary of phytochemical screening of secondary metabolites from *Pittosporum eugenioides* leaves dry powder extracts.

	Solvents					
Phyto- constituent	Petroleum ether	Methylene chloride	Ethyl acetate	Methanol	Aqueous	Butanol
Saponins	-	+	-	+	+	+
Glycosides	-	+	-	+	+	+
Steroids	-	+	+	+	-	+
Carbohydrates	-	+	-	+	+	+
Alkaloid	-	-	-	-	-	-
Sterols	+	+	-	+	+	+
Teriterpens	+	+	+	+	+	+
Tannins	-	-	+	-	+	+
Flavonoids	+	+	+	+	-	+

+ Signified the presence of the constituents

- Signified the absence of the constituents

Also, Mani and Thomas, 2014, [42] investigated that for *Pittosporum dasycaulon*; the highest TPC was observed in aqueous extracts [182.2±0.015 mg of GAE/g of extract] and the aqueous extract (215.5±0.2 mg of TAE/g of extract) had a greater tannin content than the methanol extract (100±0.10 mg of TAE/g of extract) and the aqueous extract had the highest DPPH scavenging activity with an IC_{50} value of 151.6 µg. The IC_{50} value of the positive ascorbic acid control was 30.5 µg [42]. In a similar study, the methanol extract of *P. manni* (synonym; viridiflorum) derived from Cameroon was shown to be very rich in phenolic derivatives (having 314 ± 4 mg/g gallic acid equivalent) and showed an excellent inhibitory activity of 68.82% against DPPH radical at a concentration of 250 µg/ml [43,44]. Therefore, the total antioxidant activity of the methanol extract was not only due to phenolics and lignans, but also to other metabolites, such as mono-, di-, and triterpenoidal saponins [45]. Thus, although only the phenolic compounds were given special attention in terms of antioxidant properties, it is possible that their antioxidant characteristics work synergistically with other groups of phytochemical compounds. The DPPH radical scavenging is considered to be a good *in vitro* model for assessing antioxidant activity within a short time. The deep purple color of the DPPH radical changes to yellow when reduced to a stable diamagnetic molecule by an antioxidant compound. This is taken as an indication of the hydrogen donating ability of a tested sample [46]. However, extracts of *P. eugenioides* leaves exhibited scavenging activities and there was a strongest connection between the DPPH radical scavenging activity and phenolic contents. DPPH free radical scavenging effect of *P. eugenioides* extracts was in this order: BuOH > MeOH > EtOAc > CH_2Cl_2> pet. ether, so higher level of antioxidant activity is observed in the butanol leaves extract when compared with other tested extracts and antioxidant potential of pet. ether extract being the lowest. Therefore, butanol extract was observed to be the best solvent for the extraction of the phenolic compounds from the *P. eugenioides* powder. The compounds studied here are also well known for their antioxidant potential and therapeutic benefits. Nevertheless, the possibility exists that novel antioxidant compounds may be present in the extracts which require extensive characterization procedures. Following this, whether these novel compounds possess any therapeutic potential also requires further elucidation through *in vitro* and *in vivo* studies.

In our study, a significant linear correlation between the total phenolics content and (total antioxidant capacity and DPPH radical scavenging activities values) were, (R^2 = 0.9302, TAC), (R^2 = 0.9998, DPPH), the total phenolic content was observed to be a good indicator of the presence of antioxidant compounds for the solvent extracts (Figure 1 and 2). Our findings now provide a basis for developing a valuable food additive to enhance human nutrition via their phenolic composition and antioxidant activity.

However, these results indicate that these extracts and/or pure compounds can serve as free radical inhibitors or scavengers, possibly acting as primary antioxidants [46]. Phenolics are the major plant compounds with natural antioxidant activities, mainly due to redox properties that play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides [47,48]. The fact that none of the plant samples evaluated showed an activity that was as strong as that of vitamin C was attributed the fact that the additive or synergistic effects of polyphenols make the antioxidant activity of the extracts weaker than that of the isolated bioactive compounds [49]. In addition, the total phenolic content in the crude extracts does not incorporate all the antioxidants. The phenolic compounds present in the extracts could be responsible for the observed DPPH radical scavenging activity, since they can readily donate hydrogen atom to the radical [50].

Flavonoids and tannins are polyphenolic compounds and both exhibit antioxidant activities. Their effects on human nutrition and health are potentially considerable [51]. In the present study, the antiradical effect of *P. eugenioides* leaf extracts were positively connected with the total phenolics and flavonoid contents, but other metabolites also contributed to the total antioxidant activity. *P. eugenioides* leaf is a promising source of potential antioxidants. Further investigation of this medicinal plant for discovery of new natural bioactive compounds will continue. This is the first report on the antioxidant properties of *P. eugenioides*.

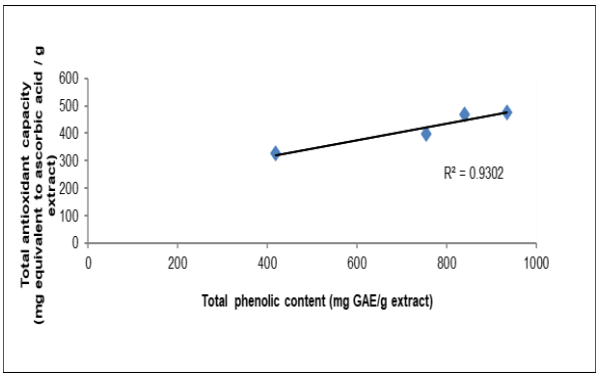

Fig. 1. Correlation between the total antioxidant capacity of different extracts from *P. eugenioides* and their total phenolic contents.

Table 2: DPPH scavenging, total antioxidant capacity, total phenolic content, total flavonoid content of 85% methanolic extract of *P. eugenioides* as well as its different extracts.

Sample	[a]DPPH SC_{50} (µg/ml)	[b]Total antioxidant capacity (mg AAE/g sample)	[c]Total phenolic content) (mg GAE/g ext.)	[d]TFC (mg RE / g ext.)
Methanol ext.	30.49±0.37	467.33±3.52	840.73±1.22	310.15±0.53
Ethyl acetate ext.	34.04±0.84	397.33±1.76	754.62±2.44	400.20±0.53
CH_2Cl_2 ext.	49.26±0.38	326.66±2.40	418.51±1.22	257.51±0.88
Butanol ext.	26.38±1.92	471.33±2.90	934.25±1.22	540.85±0.70
Pet. ether ext.	82.25±0.19	185±1.74	292.12±1.52	212.95±0.53

Results are expressed as mean values ± standard deviation (*n*=3).
[a]DPPH results are expressed as µg compound/ml (µg/ml)
[b]Total antioxidant capacity values are expressed as mg ascorbic acid equivalent/g extract (mg AAE/g ext.).
[c]TPC (total phenolic content) values are expressed as mg gallic acid equivalent/g extract (mg GAE/g ext.).
[d]TFC (total flavonoid content) values are expressed as mg rutin equivalent/g extract (mg RE/g ext.).

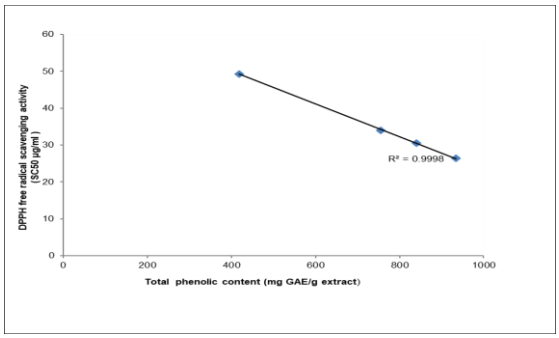

Fig. 2. Correlation between the DPPH radical scavenging activities of different extracts from *P. eugenioides* and their total phenolic contents.

Antioxidant Activities

The total antioxidant capacity measure was considered appropriate for assessing the cumulative antioxidant properties of plant foods [52,53]. However, the impossibility of comparing results obtained with different methodologies has seriously limited understanding of the role of total antioxidant capacity in disease prevention [54]. Furthermore, Total antioxidant capacity of extracts and compounds were expressed as the number of gallic acid equivalents. Phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex. The highest TAC value was observed in BuOH extract (471.33±3.52) followed by 85% Methanol extract (467.33±2.90), then EtOAc extract (397.33±1.76), then CH_2Cl_2 extract (326.66±2.40) and pet. ether extract (185±1.74) mg ascorbic acid equivalent/g dry extract. Further complementing the results from Table 2 ascertained butanol to be the better solvent for the extraction of antioxidant compounds from the *P. eugenioides* leaves. The antioxidant activity of a triterpenoid hederagenin glycoside; isolated from the berries of *Hedera colchica*, an ivy species endemic in Georgia, was investigated at a 30 microg/mL concentration, the inhibitory effects of OGH on the peroxidation of linoleic acid emulsion was found to be 95.3%, whereas alpha-tocopherol and trolox exhibited 88.8% and 86.2% inhibition of peroxidation in the system, respectively. In addition, OGH had effective DPPH scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, total reducing power and metal chelation of ferrous ions activities. These various antioxidant activities were compared with alpha-tocopherol and trolox [55].

The antioxidants which were characterized to be present in *P. eugenioides* leaves have been identified to possess functional properties and health benefits.

Total flavonoid content of *P. eugenioides* leaves extracts with different solvents concentrations is shown in table 2. Total flavonoid content was expressed as mg rutin equivalent/g extract (mg RE/g ext.). It was observed that flavonoid activity in all samples were solvent concentration dependent with the highest activity at 90% (v/v) concentration; this means that flavonoids contents is directly proportional to solvent concentration. Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo* [56,57]. As this is the first report on the antioxidant activity of *P. eugenioides*. The amount of the total flavonoid content ranged from 212.95±0.53 to 540.85±0.70 rutin equivalents (RU mg/g) of dry weight of extract (Table 2).

Cytotoxic Activity via Preliminary Brine Shrimp Assay (BSLT)

Artemia salina, commonly known as the brine shrimp, is a small crustacean, which has been the subject of many studies. The brine shrimp lethality assay is considered one of the most useful tools for the preliminary assessment of general toxicity. It has been established as a safe, practical and economic method to determine the bioactivity of plant products [9,58,59]. The aim of this method is to provide a front-line screen that can be backed up by more specific and more expensive bioassays once the active compounds

have been isolated. In the current research work, the cytotoxicity of extracts, fractions and compounds of *Pittosporum eugenioides* was evaluated by *Artemia salina* lethality test according to the procedure described by Meyer *et al*., (1982) [36]. The findings in (Table 3, Figure 4) showed that the BuOH extract was the strongest cytotoxic extract with LC_{50} value of 34 µg/ ml, followed by 85% methanol extract with LC_{50} value of 45 µg/ ml, EtOAc extract with LC_{50} value of 52 µg/ ml, CH_2Cl_2 extract with LC_{50} value of 68 and pet. ether extract with LC_{50} value of 109 µg/ ml; which represents the lowest cytotoxic effect.

Wanyoike *et al.*, [60] have been evaluated the brine shrimp cytotoxicity of *Pittosporum lanatum* leaves and roots, it showed significant toxicity against brine shrimp with LC_{50} value 27.4 ± 0.3 and 17.8 ± 0.5 µg/ ml, respectively [60]. Furthermore, All *Pittosporum phylliraeoides* extracts displayed toxicity in the *Artemia franciscana* bioassay. The only significant increase in mortality above that of the control was seen for the ethyl acetate, chloroform and hexane extracts, inducing less than 50% mortality at 72 h [61]. The acetone extract of *P. viridiflorum* possess a significant cytotoxicity against the cancer cells (MCF7, Caco-2, A549 and Hela) with LC_{50} values ranging from 3.1-626.87 µg/ml [62].

Table 3. Cytotoxic activity of different extracts of *P. eugenioides* screened against brine shrimp (*Artemia salina* L.).

Extract	LC_{50} (µg/ ml)
Methanol	45
Petroleum ether	109
CH_2Cl_2	68
Ethyl acetate	52
BuOH	34

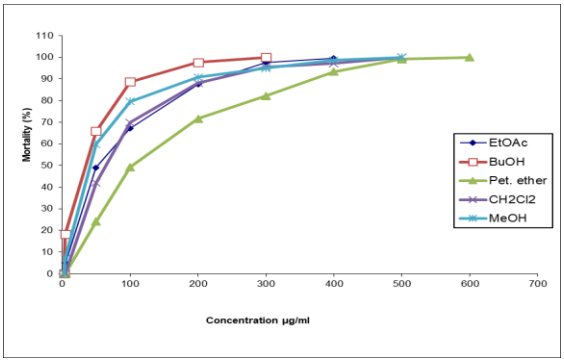

Fig. 3: Brine shrimp lethality larvae mortality bioassay against different concentrations of different *P. eugenioides* extracts.

4. Conclusions

The medicinal properties of plants have been the center of attraction for researchers in recent scientific developments throughout the world, due to their potent antioxidant properties and economic viability. Based upon the results of the present investigation it can be concluded that butanol and methanol are the most effective solvents for recovering antioxidant components from *Pittosporum eugenioides*. It is further recommended to optimize the antioxidant extraction efficacy of these solvents using different extraction techniques. The medicinal properties of *P. eugenioides* extract may be due to the presence of above-mentioned phytochemicals rendering them as a potential source for the isolation of compounds for development of diseases. Based on the previous results, pharmacognostical studies are suggested to confirm the antioxidant ability before going for commercialization. In conclusion, the free radical-scavenging activities for *P. eugenioides* extracts were evaluated by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, providing to be effective for the selection of this plant with strong antioxidant activities with potential use in medicinal preparations.

5. Conflicts of interest

There are no conflicts of interest between authors.

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