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In Vitro Evaluation of Antioxidant and Cytotoxic Activity of Isolated Compounds From *Pittosporum Eugenioides*

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Abstract

Ten compounds were isolated from the *n*-butanol extract of *Pittosporum eugenioides* leaves and evaluated for their antioxidant properties and cytotoxic activity. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) and total antioxidant capacity (TAC) (phosphomolybdenum assay). The overall antioxidant activity of compounds 1, 2, 3 and 4 showed the strongest one among the tested compounds with values of 1.28±0.97, 3.21±0.24, 5.40±0.08 and 7.09±0.07 µg/ml, respectively compared to the SC_{50} value of the positive ascorbic acid control was 7.9±0.78 µg/ml. Also, these compounds were evaluated for their cytotoxic activity against brine shrimp (*Artemia salina* L.). The LC_{50} values ranged between 11 to 25 µg/ml, for the compounds 1-10. The present study reveals that the selected plants would exert several beneficial effects by virtue of their antioxidant and cytotoxic activity and could be harnessed as drug formulation.

Keywords*: Pittosporum eugenioides*; antioxidant activity; cytotoxic activity; (DPPH); *Artemia salina* L.

1. Introduction

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging [1,2]. Many antioxidant compounds can be found in fruits and vegetables including phenolics, carotenoids, anthocyanins, and tocopherols [3]. Approximately 20% of known plants have been used in pharmaceutical studies, impacting the healthcare system in positive ways such as treating cancer and harmful diseases [4]. Plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables [5]. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants [6]. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule [7]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [8]. Herbal plants considered as good antioxidant since ancient times [9-11], *P. tetraspermum* exhibited anticancer, antioxidant, antifungal and antimicrobial activity [12-16]. Flavonoids reportedly possesses effective cytotoxic [17,18], antioxidative, [19] anti-inflammatory [20] and neuroprotective activities [21] and can protect a variety of cells from *in vitro* and *in vivo* injuries. [22,23]. Triterpenoid compounds showed diverse biological activities such as molluscicidal activity [24], antioxidant [9]. The objective of our paper is to provide a phytochemical study that have addressed extracting bioactive compounds of *P. eugenioides* plant leaves. This study includes an overview of the

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antioxidant and cytotoxic activity of phenolic compounds.

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 was purchased from (Sigma-Aldrich Co.). For antioxidant activity; all absorbance measurements were recorded using the UV-Vis spectrophotometer Spectronic 601. Ascorbic acid was 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). Whatmann No. 1 (57 x 46 cm) was used for paper chromatography (PC) (Whatman Ltd., England) whereas, thin layer chromatography (TLC) was located manually over pre-coated silica plates or ready made one (GF_{254}, Merck). Polyamide 6S (Sigma), Silica gel (70-230 mesh) (Merck) for column chromatography (CC) SiO_2-$(CH_2)_8$-CH_3, Silica gel 60 GF_{254} (Merck) for Thin Layer Chromatography (TLC), Aluminium sheets silica gel 60 F_{254} pre-coated 20x20 cm with layer thickness 0.2 mm (Merck) for TLC and Sephadex LH-20 (Sigma Aldrich/ Germany).

Elution Systems for PC and TLC:

Table 1: Elution systems utilized in the chromatographic isolation and fractionation.

S. No.	Solvent composition	Solvent ratio (v/v)
S1	BuOH:AcOH:H_2O (BAW)	**4: 1: 5 upper phase**
S2	AcOH:H_2O (15% AcOH)	**15:85**
S3	$CHCl_3$:MeOH	**9:1**
S4	$CHCl_3$:MeOH:H_2O	**7:3:1**
S5	$CHCl_3$:MeOH:H_2O	**7:3:0.7**
S6	Ethyl acetate:Methanol	**8:2**
S7	$CHCl_3$:MeOH	**9.5:0.5**

2.2. 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.

2.3. Isolation of Active Compounds from the n-Butanol Fraction by Column Chromatography Method

The *n*-BuOH fraction (40 g) was subjected to simple column chromatography (CC) using polyamide (110 X 7 cm, 220 gm) as a gradient stationary phase. The column was rinsed with distilled water (discarded), then distilled water with various concentrations of MeOH (5%, 15%, 30%, 50%, 70%, 90% and 100%, v/v) was used as mobile phase to acquire seven main fractions successively proper to the flavonoids & phenolic category. Fractions (250 ml each) were collected, evaporated and monitored by TLC and paper chromatography, and then similar fractions combined [PC (S1 & S2), TLC {S3, S4, S5, S6, S7 **(Table 1)**}, 5% $AlCl_3$ and 1% $FeCl_3$, UV light for detection to give mixture saponins]. Saponins were further separated by preparative TLC. (7 g) eluted with 5% MeOH, which was then charged into Sephadex LH-20 (CC.) to afford compound 1 (15 mg) eluted with; H_2O: MeOH (70:30/ v:v); Fraction 1 (30-48, 300 mg). (3 g) eluted with 15% MeOH, in that fraction was showing 2 spots which was dissolved in EtOAc and sonicated for 5 minutes. Even after sonication, some part of the fraction was not dissolved. Dissolved part is decanted and separated. They were dried and TLC was performed to those dissolved and undissolved portions along with initial fraction. The undissolved and dissolved parts were shown single spots on TLC to afford compound 2 (12 mg) and compound 3 (15 mg) and eluted with CH_2Cl_2: MeOH (80:20/ v:v); Fraction 2 (51-67, 220 mg). (0.8 g) eluted with 30% MeOH, this fraction was showing only 2 spots on TLC. This fraction quantity was very less and we can't charge it to the column to separate them. Preparative TLC was performed to afford compound 4 (9 mg) and compound 5 (6 mg) eluted with MeOH: H_2O (70:30/ v:v); Fraction 3 (69-83, 400 mg). (17 g) eluted with 50% MeOH, which was adsorbed over Sephadex LH-

20 (CC.) to afford compound 6 (20 mg) and eluted with MeOH: H_2O (60:40/ v:v); Fraction 4 (86-94, 270 mg). (1.7 g) eluted with 70% MeOH, which was then charged over Sephadex LH-20 (CC.) to afford compound 7 (17 mg) and eluted with MeOH: H_2O (50:50/ v:v); Fraction 5 (95-110, 311 mg). (0.8 g) eluted with 90% MeOH, which was rechromatographed over Sephadex LH-20 (CC.) two times to afford compound 8 (19 mg), compound 9 (16 mg) and compound 10 (21 mg); which eluted with MeOH: H_2O (40:60/ v:v); Fraction 6 (113-125, 430 mg).

2.4. Antioxidant Activity

2.4.1. 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 [9]. 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 [10].

% 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) [25], according to the following reaction.

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

Purple Yellow

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.

2.4.2. 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 [9, 26].

2.5. Evaluation of Cytotoxic Activity

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

Meyer method [27], 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 [17, 28]. 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.

3. 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 [29].

4. Results And Discusion

4.1. In Vitro Antioxidant Activities of the Pure Isolates

The pure isolates was 3-O-[α - L- arabinofuranosyl - (1→3) - α - L - rhamnopyranosyl (1→2) - α - L- arabinopyranosyl] hederagenin ***(*1)**, 3 β - 4β - 15, 16, 22 - trihydroxy olean 12-en-17 oic acid **(2)**, rutin **(3)**, isoquercitrin **(4)**, Eudesmine **(5)**, (2S,3S,4E,6S,7R,8R,9S,11E,13S,14S,15R)-7,8,9,14,15-pentaacetoxy-3-(benzoyloxy)-6-hydroxyjatropha-4,11-diene **(6)**, β- Amyrin **(7)**, limonene **(8)**, α pinene **(9)** and kanesulone A **(10)**. Two methods and modifications have been proposed to determine the in vitro antioxidant activity; radical scavenging (DPPH) effects (SC_{50} values) and total antioxidant capacity (TAC) (phosphomolybdenum assay). In this study, the scavenging effect of pure compounds on the DPPH radical expressed as SC_{50} values. In particular, compounds 1, 2, 3 and 4 showed the strongest activity. Consequently, this study shows that the DPPH inhibitory concentration SC_{50} values of [(3-O-[α - L- arabinofuranosyl - (1→3) - α - L - rhamnopyranosyl (1→2) - α - L- arabinopyranosyl] hedragenin)], [(3-O-β-Glucopyranozyl (1→4) - [O-β-glucopyranozyluronic acid] 15,16,22-trihydroxy - 4,4,20,20-heptamethyl 1,2,3,4,5,6,7,8,9,10,11,14,15,16,18, 19, 21,22 octadecahydro-2H-pic-12-ene-carboxylic acid)], rutin and isoquercitrin compounds were 1.28±0.97, 3.21±0.24, 5.40±0.08 and 7.09±0.07 µg/ml, respectively. Whereas, compounds 7, 8 and 9 exhibited moderate free radical activity with SC_{50} value of 9.70±0.54, 17.26±0.05 and 18.41±1.09 µg/ml, respectively. But compounds 5, 6 and 10 showed the lowest DPPH radical scavenging activity with SC_{50} value of 27.60±0.54, 38.50±2.0 and 29.44±0.15 µg/ml, respectively (Table 2) compared to the SC_{50} value of the positive ascorbic acid control was 7.9±0.78 µg/ml (Table 2). Moreover, the total antioxidant capacity (TAC) values of the pure compounds were, 707.68±0.42; 699.00±1.20; 595.36±3.05; 540.73±1.8; 174.55±1.3; 186.36±0.19; 490.09±1.5; 522.39±2.0; 510.32±0.50 and 195.04±0.17 mg ascorbic acid equivalent AAE /g compound, respectively for the compounds 1-10 (Table 2). From the structural activity relationship (SAR) point of view, the pure isolated compounds showed a different antioxidant activities within variety effects in both of scavenging activities and total antioxidant capacity values, these results may be attributed to the presence or absence of the characteristic structural criteria for effective free radical scavenging activities including; the presence of ortho di-hydroxy groups, an 2, 3 unsaturated double bond, 4-oxo group for (ring-C), and 5-OH (A-ring) for flavonoids system. Moreover, mono and di glycosides were less active than their aglycones, which appears may be due to the steric hindrance occurred by the bulky glycosidic molecules [10,30].

Cytotoxicity of the methanol extract is due to its strong cytotoxic constituents such as compound **1** (3-*O*-[α - L- arabinofuranosyl - (1→3) - α - L - rhamnopyranosyl (1→2) - α - L- arabinopyranosyl] hedragenin) which exhibited anticancer activity against human colon carcinoma H-116, human lung carcinoma A-549 and human lung carcinoma HT-29 cell lines with IC_{50} 5.0, 2.5 and 2.5µg/ml respectively [31]. Furthermore, the isolated compounds from *P. eugenioides* were evaluated for their cytotoxic activity against brine shrimp (*Artemia salina* L.) and thus the results showed that; the cytotoxic effects were at (LC_{50} 11, 12, 13, 14, 24, 28, 19, 22, 23 and 25 µg/ml, respectively) for the compounds 1-10 **(Table 3, Figures 1, 2)**. The conversion of the carboxyl group at C-17 on the beta-side increases the activity [32]. A follow-up chemical investigation to methanol extract of *Conyza dioscoridis* (Family Asteraceae) led to the isolation of rutin which showed significant cytotoxicity with LC_{50} value of 10 µg/ml against brine shrimp larva and against colon carcinoma cells (HCT-116) with IC_{50} value of 30 µg/ml [17]. Rutin exhibited strong DPPH radical scavenging activity. At the concentration of 0.05 mg/ml, ascorbic acid (Vc), butylated hydroxytoluene (BHT) and rutin showed 92.8%, 58.8%, and 90.4% inhibition, respectively. In addition,

rutin had effective inhibition of lipid peroxidation. Those various antioxidant activities were compared to standard antioxidants such as BHT and Vc [33]. isoquercitrin was the less efficient chloramine scavenger by presenting low inhibition even with high concentrations (24% inhibition on 0.401 mM). These results may be due the block of the hydroxyl at position 3 of the ring C on isoquercitrin by linking to a sugar moiety; these data revealed the importance of the free hydroxyl group on position 3 of C ring and two hydroxyl groups on B ring as important sites for scavenging free radical [34]. Isolated isoquercitrin from *A. marina* leaves showed cytotoxicity against SiHa cell line at IC_{50} of 980 µM [35]. Jatrophane diterpenes isolated from *Euphorbia mellifera* exhibited apoptosis-inducing activity on COLO 320 cells, using the annexin-V/propidium iodide assay [36]. *β*-amyrin isolated from *Prunus africana* showed cytotoxic activity on the Caco-2 cell line was determined using the MTT cell viability assay and gave significant cytotoxic activity with IC_{50} values of 81 µg mL^{-1} [37]. Moreover, *β*-Amyrin showed very good scavenging effects on 2,2-diphenyl-picrylhydrazyl (DPPH) (IC_{50} 89.63 ± 1.31 µg/ml, AAI = 1.11), hydroxyl (IC_{50} 76.41 ± 1.65 µg/ml, AAI = 1.30), nitric oxide (IC_{50} 87.03 ± 0.85 µg/ml, AAI = 1.14) and superoxide (IC_{50} 81.28 ± 1.79 µg/ml, AAI = 1.23) radicals as well as high reducing power and strong suppressive effect on lipid peroxidation [38]. Limonene significantly enhanced the cytotoxicity to DU-145 cells which causes apoptotic effect was significantly blocked on pretreatment with N -acetylcystein, indicating that antitumor effect is initiated by ROS generation, and caspase cascades contribute to the cell death **[**39**]**. The cytotoxicity of the positive enantiomers of pinene to murine macrophages was evaluated and 250 µg/mL of (+)-α-pinene reduced the cell viability to 66.8% [40]. Both *in vitro* and *in vivo* studies have shown that *β*-amyrin acetate also has important biological functions and *β*-amyrin acetate derivatives proved to be the most potent [41].

Table 2. DPPH scavenging, total antioxidant capacity of the pure compounds isolated from *P. eugenioides*.

Compound	[a]DPPH SC_{50} (µg/ml)	[b]Total antioxidant capacity (mg AAE/g sample)
Compd. 1	1.28±0.97	707.68±0.42
Compd. 2	3.21±0.24	699.00±1.20
Compd. 3	5.40±0.08	595.36±3.05
Compd. 4	7.09±0.07	540.73±1.8
Compd. 5	27.60±0.54	174.55±1.3
Compd. 6	38.50±2.0	186.36±0.19
Compd. 7	9.70±0.54	490.09±1.5
Compd. 8	17.26±0.05	522.39±2.0
Compd. 9	18.41±1.09	510.32±0.50
Compd. 10	29.44±0.15	195.04±0.17
Ascorbic acid	7.9±0.78	

[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.).

α-Pinene inhibited the radicle growth of all the test species. Exposure of *C. occidentalis* roots to alpha-pinene enhanced solute leakage, and increased levels of malondialdehyde, proline and hydrogen peroxide, indicating lipid peroxidation and induction of oxidative stress. Activities of the antioxidant enzymes SOD, CAT, GPX, APX and GR were significantly elevated, thereby indicating the enhanced generation of reactive oxygen species (ROS) upon alpha-pinene exposure. Increased levels of scavenging enzymes indicate their induction as a secondary defense mechanism in response to alpha-pinene [42].

Table 3. Cytotoxicity using brine shrimp (*Artemia salina* L.) lethality of isolates from *P. eugenioides*.

Compound	LC_{50}
1	11
2	12
3	13
4	14
5	24
6	28
7	19
8	22
9	23
10	25

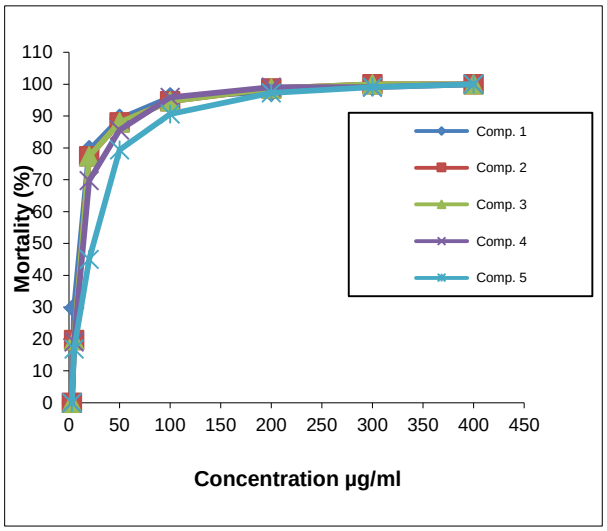

Fig. 1. Brine shrimp lethality larvae mortality bioassay against different concentrations of compounds (1-5) isolated from *P. eugenioides*.

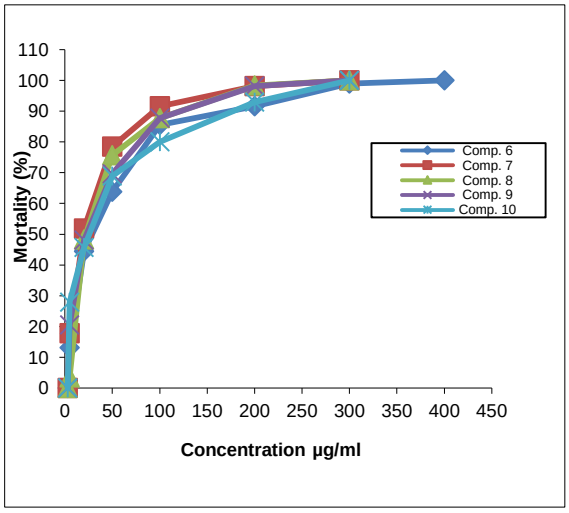

Fig. 2. Brine shrimp lethality larvae mortality bioassay against different concentrations of compounds (6-10) isolated from *P. eugenioides*.

The antioxidant and cytotoxic properties of the methanol extract may be attributed to the synergistic effects of its diverse major and minor components. From our study, it is interesting to show that the cinnamoyl substituent which can be found in oleanane type structure triterpenoid that exhibit antitumor activity. The results suggested that, the attached glycoside moiety to C-3 of the aglycone will play important roles in cytotoxicity, thus it was essential for anticancer drug discovery.

5. Conclusions

In conclusion, we evaluated the *in vitro* antioxidant and cytotoxic activities of various isolated compounds from *P. eugenioides* leaves; they exhibited potent antioxidant and cytotoxic activities. Importantly, the current study has addressed the cytotoxic effects against brine shrimp of chemical constituents isolated from the *P. eugenioides* for the first time. This highlights the need for further investigations of the isolated compounds from this species for potential use as complementary cancer therapy. An additional mechanism of cancer treatment might be the scavenging and blocking of free radical that is associated with cancer cell development. Therefore, future studies should be performed to investigate the *in vitro* and *in vivo* mechanisms of action of these important compounds.

6. Conflicts of interest

The authors declare no conflict of interests.

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