



Research article

Assessment of antioxidant potential of selected roadside trees leaves in Kumasi Metropolis, Ghana

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Abstract: The roadsides of the Kumasi Metropolis, Ghana are lined with the several species of trees, such as, *Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia*. The people use them for their health care needs. The vehicle emissions results in oxidative injury in these plants, due to the production of reactive oxygen species. The present study assessed the antioxidant potential of leaves of these tree species subjected to vehicular pollutants. The free radical scavenging activity of leaf extracts of the four tree species were measured using 1, 1-diphenyl-2-picryl hydrazyl (DPPH). The total phenolic content (TPC) of the extract was determined by a spectrophotometric assay using the Folin-Ciocalteu's reagent. Total antioxidant capacity (TAC) was measured using Phosphomolybdate assay. In this study, the medicinal properties of leaves of *Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia* sampled from the control sites showed better medicinal properties. DPPH scavenging activity at concentration 2.7 ug ml⁻¹ was lower at the arterial road sites in all the four tree species. A higher DPPH percentage inhibition was recorded at the control sites. The IC₅₀ values were higher for the leaf sample extracts from the arterial road sites and lower for the Control site. The total phenolic content of leaf samples of all the four tree species at the arterial road sites were lower than and significantly different from those at the Control site (p=0.000). The TAC values were lower at the arterial road sites in comparison to the control sites. There was a significant difference among the arterial road sites and also when compared with the control (P<0.05). It could be suggested from this study that variability exists in the antioxidant activities of plants due to a decrease in the medicinal properties of plants subjected to constant auto vehicular pollution.

Keywords: Arterial roadside tree species - 1, 1-diphenyl-2-picryl hydrazyl (DPPH) - Total Antioxidant Capacity - Total Phenolic Compounds.

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INTRODUCTION

Plants are essential features of biological diversity and of great usefulness to mankind. In the entire world, there is growing interest in the usage of herbal preparations from medicinal plants in health care (Jassim & Naji 2003). It has been reported that 80% of the African population uses traditional medicine for primary health care (Weeks & Strudsholm 2008). According to WHO (2002) report, 60% of Children with fever resulting from malaria are treated with herbal medicine at home. It has been reported that plants rich in antioxidants play a protective role in health and diseases (Milner 1999). An exploratory study has noted that pollutants not only change the morphology, physiology and anatomy of plants but also affect their medicinal characteristics (Panda 1989, Trivedy & Singh 1990). Vehicles plying the circulatory facilities have been identified as the single largest source of air pollution in Kumasi Metropolis, Ghana (Agyemang-Bonsu *et al.* 2007) and the magnitude of vehicular air pollution in the Metropolis is on the increase resulting from the rising number of vehicles. The resultant effect can lead to respiratory infections in humans and affect animals as well as plants health severely.

Antioxidants are molecules that without harm are in contact with free radicals and stop chain reactions

before vital molecules are destroyed. Free radicals are atoms with the unpaired number of electrons that are formed when oxygen interacts with certain molecules. On formation, these highly reactive radicals can start a chain reaction that wreaks havoc on cells. Free radicals can also be caused by environmental sources such as tobacco smoke, oil fumes, chemical pollutants, radiation and ozone. Free radicals are the foremost cause of some health problems like ageing, cancer and atherosclerosis. Antioxidants freely give up on encountering a free radical an electron of its own stops the unprecedented damage. This electron damage makes antioxidants free radical. On the other hand, the chain reaction is stopped the new free radical arising from the Antioxidants is weak and may not do further harm. Antioxidant compounds like Phenolic acid, Polyphenols and flavonoid scavenge free radicals such as Peroxide, hydroperoxide, thus inhibiting the oxidative mechanism that lead to degenerative diseases. The roadsides of Kumasi metropolis, Ghana is majorly adorned with *Terminalia catappa* L., *Mangifera indica* L., *Ficus platyphylla* Del. and *Polyalthia longifolia* (Sonn.) Thwaites and residents use them for their health care needs. Since the vehicular emissions result in oxidative injury in plants by the production of reactive oxygen species. Therefore, this study assesses the antioxidant capacity of leaves of *Terminalia catappa*, *Mangifera indica*, *Ficus platyphylla* and *Polyalthia longifolia* subjected to vehicular pollutants in the Kumasi metropolis, Ghana.

MATERIALS AND METHODS

Ethical considerations

In our Institution ethical consideration is only sought when it involves the use of human and animal participants.

Sampling of tree species and collection of samples

Sampling of the four tree species was done at three arterial roads of the Metropolis namely: Accra road (Arterial road I), Offinso road (Arterial road II) and Mampong road (Arterial road III), whilst Kwame Nkrumah University of Science and Technology campus which served as the Control. At each sampling site, from each tree species replicate 25 physiologically active leaves, third from the tip of the apical bud, were harvested from the side of the tree facing the road for antioxidant properties determination. The harvested leaves were placed in a self-sealing polythene bags and labelled. Samples were washed thoroughly and dried overnight in an oven at 40°C to obtain the dry weight. It was pulverised coarsely, stored in paper bag and kept in the laboratory at room temperature.

Preparation of leaf extract

For each of the four tree species, 200 g of the coarsely milled leaf samples were added to 750 ml of methanol and allowed to stand at room temperature (28°C) for 3 days with frequent shaking. It was sieved through Whatman No.1 filter paper. The filtrate was evaporated in a steam bath to dryness. The recovered dried extracts were placed in sterilized screw- capped bottles and stored at 4°C for DPPH radical scavenging analysis.

DPPH radical scavenging assay

The free radical scavenging activity of leaf extracts of the four tree species were measured using 1, 1-diphenyl-2-picryl hydrazyl (DPPH). DPPH radical was determined according to (Liyana-Pathirana & Shahidi 2005). A solution of DPPH in methanol (0.135 mM) was prepared and 5 ml of the solution was mixed with 1 ml of leaf extract in methanol at different concentrations (1.495, 1.80, 2.10, 2.40 and 2.70 µg ml⁻¹). Ascorbic acid in distilled water was used as standard. After incubation for 30 minutes in the dark, absorbance was recorded at 517 nm. The experiment was performed in triplicates. The IC₅₀ values of the samples, which is the concentration of the sample needed to inhibit 50% of DPPH free radical was calculated using Log-Dose inhibition curve. Lower absorbance values of reaction mixture indicate higher free scavenging activity (Koleva *et al.* 2002). The capability of DPPH radical scavenging was calculated as follows: -

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A₀ is the control reaction absorbance and A₁ is all the extract samples and reference absorbance. All the tests were performed in triplicates and the results averaged.

Determination of total phenolic content

The total phenolic content of the extract was determined by spectrophotometric assay using the Folin-Ciocalteu's reagent as according to (Singleton *et al.* 1999). Plant extract solution (1 ml) of varying concentrations (31.25–500 µg ml⁻¹) was added to 1 ml Folin-Ciocalteu's reagent in a test tube. The content of the test tube was mixed and allowed to stand for 5 min at 25°C in an incubator. A solution (1 ml) of sodium

bicarbonate (2%) was added to the mixture. The reaction mixture was allowed to stand for 2 hours with shaking at 25°C in an incubator. The mixture was then centrifuged at 3000 rpm for 10 min and absorbance of the supernatant determined at 760 nm. For each concentration of tannic acid and extracts, three replicates were prepared. Distilled water (1 ml) was applied to Folin-Ciocalteu's reagent (1 ml) pulverised in the same way as the test drugs and performed as blank. Tannic acid was used as reference. Four concentrations of tannic acid were used to construct a calibration curve and the total phenols expressed as mg of tannic acid equivalents (TAE)/g of extract.

Total antioxidant capacity (TAC) assay

The evaluation of total antioxidant capacity was according to the method described by (Prieto *et al.* 1999). An aliquot of 3 ml each of the extract was placed in a test tube. The reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate and 4 mM Ammonium molybdate) (1 ml) was then added and the resulting mixture incubated at 95°C for 90 minutes. After the mixture has cooled to room temperature, the absorbance of each solution was measured in triplicates using the UV-Visible spectrophotometer at 695 nm against a blank. The standard antioxidant drug used was Ascorbic acid. The total antioxidant capacity was expressed as Ascorbic Acid Equivalents (AAE $\mu\text{g ml}^{-1}$).

Data analysis

The data generated was subjected to descriptive statistics (Mean and Standard error) and one way Analysis of Variance (ANOVA) using SPSS. Each time ANOVA reveals significance difference ($P < 0.05$), a multiple comparison of the means by least significant difference (Turkey HSD) test was performed.

RESULTS AND DISCUSSION

In herbal medicines, toxic agents at high levels can occur when they are obtained from highly contaminated areas, such as areas near roadways, industrial areas, oil refineries or metal mining sites (Vasudevan *et al.* 2009). An exploratory study has noted that pollutants not only change the morphology, physiology and anatomy of plants but also affect their medicinal characteristics (Panda 1989, Trivedy & Singh 1990). In recent decades, the use of herbal medicines and herbal nutrition has grown considerably, such that 65–80 percent of people worldwide use herbal medicines as therapeutic choices for many diseases (Ayodele *et al.* 2013).

It was observed that the scavenging activities of the methanolic extract of the tree leaf samples from the arterial road sites were lower than the control site, but higher than the reference standard ascorbic acid except for *Ficus platyphylla* and *Polyalthia longifolia* (Table 1). The DPPH scavenging activity in a concentration dependent manner ranging from 1.5–2.7 $\mu\text{g ml}^{-1}$ are presented in tables 2–5. The Methanolic extracts of the *Terminalia catappa* tree leaf samples at concentration of 2.7 $\mu\text{g ml}^{-1}$ recorded percentage inhibition of 98.80% at the Control site and arterial road sites (97.79%) (Table 2). At the same concentration, *Mangifera indica*

Table 1. Effect of vehicular air pollution on DPPH free radical scavenging activity of methanolic leaf extract of tree species at various sampling sites.

	% Inhibition				
	<i>Terminalia catappa</i> L.	<i>Mangifera indica</i> L.	<i>Ficus</i> Del.	<i>platyphylla</i>	<i>Polyalthia longifolia</i> (Sonn.) Thwaites
Control	98.80 ± 0.17	98.63 ± 0.23		76.98 ± 1.72	92.78 ± 0.65
Ascorbic acid	83.76 ± 0.45	83.76 ± 0.45		83.76 ± 0.45	83.76 ± 0.45
Arterial road sites	97.79 ± 0.53	96.74 ± 0.61		73.02 ± 2.13	83.45 ± 3.95

Note: 50% and above of DPPH radical is considered as significant for scavenging activity.

Table 2. Effect of vehicular air pollution on DPPH radical scavenging of leaves of *Terminalia catappa* L.

	% Inhibition				
	Concentration ($\mu\text{g ml}^{-1}$)				
	1.50	1.80	2.10	2.40	2.70
Control	53.18 ± 0.82	77.84 ± 1.61	93.30 ± 1.47	97.51 ± 0.43	98.18 ± 0.17
Ascorbic acid	27.15 ± 0.52	32.65 ± 1.21	44.16 ± 1.94	98.80 ± 0.34	83.76 ± 0.45
Arterial road sites	55.84 ± 3.10	77.61 ± 7.02	93.41 ± 0.07	96.10 ± 0.50	97.79 ± 0.53

Note: 50% and above of DPPH radical is considered as significant for scavenging activity.

recorded 98.63% at the Control site, while the arterial road sites had lower values (96.74%) (Table 3). The DPPH percentage inhibition of *Ficus platyphylla* methanolic leaf extract was 76.98% at the Control site and the arterial road sites (73.02%) at concentration of 2.7 $\mu\text{g ml}^{-1}$ (Table 4). The Methanolic extracts of the *Polyalthia longifolia* tree leaf samples at concentration of 2.7 $\mu\text{g ml}^{-1}$ recorded percentage inhibition of 92.78% at the

Control site and arterial road sites (83.45%) (Table 5). In this study, the medicinal properties of leaves of *Terminalia catappa*, *Ficus platyphylla* and *Polyalthia longifolia* sampled from the control sites showed better medicinal properties. DPPH scavenging activity at concentration 2.7 $\mu\text{g ml}^{-1}$ was lower at the arterial road sites in all the four tree species. A higher DPPH percentage inhibition was recorded at the control sites. A higher percentage inhibition indicates better scavenging activity or antioxidant potential. The IC_{50} values were higher for the leaf sample extracts from the arterial road sites and lower for the Control site (Table 6). The IC_{50} is the quantity of extract required for 50% inhibition of DPPH free radical. A better scavenging ability of the sample is shown by lower IC_{50} value. The higher IC_{50} values showcased by the methanolic leaf extract of the selected tree species at the experimental sites when compared to the control sites and the standard is an indication that it has less DPPH scavenging activity. DPPH scavenging activity was at concentration 2.699 $\mu\text{g ml}^{-1}$ was lower at the polluted sites in all the four tree species. This implied that the studied tree species at the polluted sites has less ability to protonate or scavenged less of the DPPH free radical. A higher DPPH percentage inhibition was recorded at the control. A higher percentage inhibition indicates better scavenging activity or antioxidant potential.

Table 3. Effect of vehicular air pollution on DPPH radical scavenging of leaves of *Mangifera indica* L.

	% Inhibition				
	Concentration ($\mu\text{g ml}^{-1}$)				
	1.50	1.80	2.10	2.40	2.70
Control	57.73 \pm 2.61	71.31 \pm 4.37	81.87 \pm 1.35	95.70 \pm 0.87	98.63 \pm 0.23
Ascorbic acid	27.15 \pm 0.52	32.65 \pm 1.21	44.16 \pm 1.94	78.18 \pm 0.17	83.76 \pm 0.45
Arterial road sites	66.26 \pm 2.28	81.04 \pm 2.28	87.11 \pm 0.60	92.04 \pm 2.20	96.74 \pm 0.61

Note: 50% and above of DPPH radical is considered as significant for scavenging activity.

Table 4. Effect of vehicular air pollution on DPPH radical scavenging of leaves of *Ficus platyphylla* Del.

	% Inhibition				
	Concentration ($\mu\text{g ml}^{-1}$)				
	1.50	1.80	2.10	2.40	2.70
Control	47.42 \pm 2.23	60.14 \pm 2.85	69.42 \pm 1.59	74.14 \pm 0.23	76.98 \pm 1.72
Ascorbic acid	27.15 \pm 0.52	32.65 \pm 1.21	44.16 \pm 1.94	78.18 \pm 0.17	83.76 \pm 0.45
Arterial road sites	41.75 \pm 4.25	50.29 \pm 3.41	59.62 \pm 1.96	65.92 \pm 2.53	73.02 \pm 2.13

Note: 50% and above of DPPH radical is considered as significant for scavenging activity.

Table 5. Effect of vehicular air pollution on DPPH radical scavenging of leaves of *Polyalthia longifolia* (Sonn.) Thwaites.

	% Inhibition				
	Concentration ($\mu\text{g ml}^{-1}$)				
	1.5	1.8	2.1	2.4	2.7
Control	54.04 \pm 1.89	60.22 \pm 1.74	69.07 \pm 1.79	80.76 \pm 4.84	92.78 \pm 0.65
Ascorbic acid	27.15 \pm 0.52	32.65 \pm 1.21	44.16 \pm 1.94	78.18 \pm 0.17	83.76 \pm 0.45
Arterial road sites	42.73 \pm 4.43	51.63 \pm 1.71	61.71 \pm 1.09	71.19 \pm 4.42	83.45 \pm 3.95

Note: 50% and above of DPPH radical is considered as significant for scavenging activity.

Table 6. IC_{50} values of DPPH free radical scavenging activity of selected tree species at various Sampling sites.

	IC_{50} ($\mu\text{g ml}^{-1}$)				
	<i>Terminalia catappa</i> L.	<i>Mangifera indica</i> L.	<i>Ficus platyphylla</i> Del.	<i>Polyalthia longifolia</i> (Sonn.) Thwaites	
Control	7.83	8.73	16.00	100.70	
Ascorbic acid	13.03	13.03	13.03	13.03	
Arterial road sites	20.28	33.95	72.49	153.07	

The total phenolic content of leaf samples of all the four tree species at the arterial road sites were lower than and significantly different from those at the Control site ($p=0.000$) (Table 7). The total phenolic content of leaf samples at the arterial road sites ranged between 58.41 and 172.90 mg g^{-1} GAE whilst those at the Control site were ranged from 173.10 and 202.30 mg g^{-1} GAE. The total phenolic content in *Terminalia catappa* leaf samples was significantly different among the arterial road sites except for Arterial road I and Arterial road II at $p=0.189$ as well as with the control ($p=0.00$). *Mangifera indica* total phenolic content in the leaf samples were significantly different among the arterial road sites except for Arterial road I and Arterial road III at $p=0.64$ and when compared with the control ($p=0.00$). The total phenol content of *Ficus platyphylla* leaf samples was not significantly different among the arterial road sites, but when compared with the control site, it was significant ($p=0.00$). In *Polyalthia longifolia*, total phenol content of the leaf samples among the arterial road sites and the control were significantly different ($p=0.00$). However, there was no significant difference in total phenol

content of the leaf samples collected from Arterial road III and the Control site ($p = 0.71$). In this study, leaf extracts of the four tree species studied from the arterial road sites recorded lower contents of phenolics, while the control had a higher phenolic content. A total phenolic assay using Folin ciocalteau reagent is used consistently in studying phenolic antioxidants. Phenolic compounds are carried in plant defense process against bacteria and other environmental stress (Weeks & Strudsholm 2008).

Table 7. The effect of vehicular air pollution on total phenolic content of studied tree species at various sampling sites.

Sampling sites	Total Phenolic Content (mg g ⁻¹ GAE)			
	<i>Terminalia catappa</i> L.	<i>Mangifera indica</i> L.	<i>Ficus platyphylla</i> Del.	<i>Polyalthia longifolia</i> (Sonn.) Thwaites
Control	173.10 ± 7.43 ^b	202.30 ± 10.57 ^c	146.50 ± 11.71 ^b	181.40 ± 2.37 ^c
Arterial Road I	58.41 ± 1.17 ^a	61.01 ± 5.65 ^a	67.74 ± 0.52 ^a	62.68 ± 2.37 ^a
Arterial Road II	72.53 ± 0.74 ^a	150.20 ± 6.35 ^b	74.47 ± 0.28 ^a	94.64 ± 7.02 ^b
Arterial Road III	165.50 ± 4.65 ^b	72.61 ± 0.40 ^a	75.71 ± 0.52 ^a	172.90 ± 6.20 ^c

Note: Mean ± SE in the same column with different letters in superscript differ significantly ($P < 0.05$).

Total Phenolics are also free radical scavengers and possess antioxidative property. The enhancement of health by antioxidants from plants stems from their counteracting reactive oxygen species. According to (Theodora *et al.* 2013), Phenolic compounds are antioxidants agents that act as free radical terminators. In this study, the polluted sites recorded higher contents of phenolics in leaf extracts of the four tree species studied. Similar report was given by (Sharma *et al.* 2012). It could be due to increased level of free radicals emanating from pollutants derived from auto vehicular emissions. It has been posited that antioxidant potential of medicinal plants could be related to the concentration of their phenolic compounds (Djeridane *et al.* 2006).

Table 8. The effect of vehicular pollution on total antioxidant capacity of selected tree species at various sampling sites.

Sampling sites	Total Antioxidant capacity (mg/AAE)			
	<i>Terminalia catappa</i> L.	<i>Mangifera indica</i> L.	<i>Ficus platyphylla</i> Del.	<i>Polyalthia longifolia</i> (Sonn.) Thwaites
Control	912.24 ± 158.93 ^b	1170.45 ± 31.15 ^d	748.50 ± 79.23 ^c	1439 ± 27.34 ^c
Arterial Road I	394.02 ± 53.44 ^a	83.03 ± 9.08 ^a	63.24 ± 2.86 ^a	98.02 ± 0.30 ^a
Arterial Road II	515.18 ± 20.60 ^a	637.84 ± 13.40 ^c	220.38 ± 8.06 ^{ab}	468.09 ± 10.65 ^b
Arterial Road III	399.12 ± 21.50 ^a	274.66 ± 31.23 ^b	283.06 ± 2.75 ^b	110.30 ± 1.38 ^a

Note: Mean ± SE in the same column with different letters in superscript differ significantly ($P < 0.05$).

The Total antioxidant capacity TAC values were lower at the arterial road sites in comparison to the control sites. There was significant difference among the arterial road sites and also when compared with the control ($P < 0.05$) (Table 8). Total antioxidant capacity content of *Terminalia catappa* was highest (912.24 mg/AAE) in the Control site and while lowest (394.02 mg/AAE) content was observed at Arterial road I. The values showed no statistically significant differences among the arterial road sites, but when compared with the control using one way ANOVA, it was significant ($p = 0.08$). The total antioxidant capacity content of *Mangifera indica* was highest (1170.45 mg/AAE) at the control site and the lowest (83.03 ± 9.08) was recorded at the Arterial road I. There was a significant difference between the arterial road sites and the control as determined by one way ANOVA ($p = 0.00$). In *Ficus platyphylla*, highest total antioxidant capacity content of 748.50 mg/AAE was noted in the control site, while the lowest amount of 63.24 mg/AAE was observed in Arterial road I. There was no significant difference among the arterial roadsides and when compared with the control at $p = 0.00$. *Polyalthia longifolia* showed highest content of total antioxidant capacity (1439 mg/AAE) in control site and lowest (98.02 mg/AAE) content was observed at Arterial road I. There was a significant difference among the arterial road sites except for Arterial road I and Arterial road III ($p = 0.932$). However, there was a significant difference when compared to the Control site ($p = 0.00$). Total antioxidant capacity is defined as a measure of the ability of the herbal matrix to delay oxidation in a controlled system (Zeneli *et al.* 2013). This study displayed lower antioxidant capacity in the polluted sites, thus an indication that vehicular emissions results in oxidative injury in plants by the production of reactive oxygen species (Zeneli *et al.* 2013).

It could be suggested from this study that variability exists in the of antioxidant activities of plants due to a decrease in the in the medicinal properties of plants subjected to constant auto vehicular pollution.

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