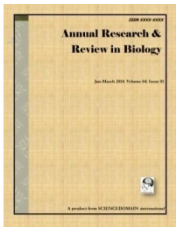

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Antioxidant Activity of Roselle (*Hibiscus sabdariffa*), Moringa (*Moringa oleifera*), Ginger (*Zingiber officinale*) and 'Ugwu' (*Telfairia occidentalis*) in the Lungs of Albino Rats (*Rattus norvegicus*) Exposed to Cement Dust

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Authors' contributions

This work was carried out in collaboration between all authors. Author TY designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JO and TA managed the analyses of the study and literature reviews. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The antioxidant effects of selected vegetables and spices were assessed in the lungs of albino rats exposed to industrial pollutants. Albino rats grouped into six containing 18 rats each were exposed to cement dust at 200 m from a cement plant in Nigeria. Group one was the control and groups two through six formed the test rats. The histology of lung tissues of the rats was examined before analysis and served as the baseline index. The test rats were then fed with 400 mg kg^{-1} extracts of roselle, moringa, ginger, 'ugwu' and a mixture of the extracts, respectively for 180 days. The control rats received only distilled water for the same duration. The rats were monitored for 180 days after which their lungs were harvested and histopathological analysis carried out again. The lungs of control rats showed severe interstitial fibrosis and cellular debris. Moderate fibrosis were observed in the lung tissues of rats treated with roselle and moringa extracts. The rats that were fed with extracts of ginger, 'ugwu' and the mixture of the extracts had mild septal fibrosis. The findings of the study demonstrated the antioxidant properties of the food plants modulated

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the effects of cement dust. Hence, the plants could be used as palliatives in polluted environments to lower the health problems associated with cement dust exposures.

Keywords: Cement dust; antioxidant; industrial pollutants; histopathological; fibrosis.

1. INTRODUCTION

Industrial Revolution started in England in the 18th century and changed the world from mainly agricultural society to a predominantly industrial society. It was a time of dramatic change, from hand tools and handmade items, to products that were made by machines. Socioeconomic activities boomed, life got easier and people migrated to the cities to work in factories. Several factories expand rapidly, and people settled around them because nobody envisaged the side effects of industrialization. Toxic wastes were discharged into the environments, and pollution became a serious problem when their effects on environments and human health became obvious.

All industries generate waste substances with health risks [1]. For example, cement and detergent industries produce pollutants that have been implicated in skin and eye defects, multi-organ injuries, blood, genetic and biochemical problems [2,3]. Air pollutants have been associated with increased risk of type 2 diabetes mellitus and hypertension [4,5]. However, as life-threatening as these pollutants were to human's health, industrialization continued to expand, it became difficult to quit because it makes life easier. Therefore, it is imperative to ameliorate these health effects.

Conventional pollution prevention and control strategies in industries have not been successful, particularly in developing countries. The problems hindering the performance of these strategies are fund constraint, strategies technicality, weak environmental protection laws and environment polluters concealing information [6]. Hence, an effective strategy is necessary. One important approach in healthcare delivery that has not been tested in pollution management is phytomedicine otherwise known as plant medicine. Phytomedicine had been used briefly in the past to detoxify the body but was forgotten with the evolution of modern medicine and synthetic drugs [7]. Modern medicine and synthetic drugs came with progress in technology as a result of industrialization. However, in the last three decades, there had been a renewed interest in phytomedicine. Plant preparations are affordable with little or no side effects compared with synthetic drugs [8]. Therefore, this study evaluates the antioxidant efficacy of selected vegetables and spices in the lungs of albino rats exposed to cement dust.

2. MATERIALS

2.1 Animal Husbandry

One hundred and fifty albino rats (*Rattus norvegicus*) weighing between 185 and 200 g were sourced from the Department of Biochemistry, University of Ibadan in August 2009.The rats were made to acclimatize to the ambient environments for seven days before commencing the research. Pellet feeds from the F. A Feeds industry, Agege-Lagos and water were given to the rats *ad libitum.*

2.2 Elemental Analysis of the Cement Dust

The elements in the cement dust were analysed by Atomic Absorption Spectroscopy (AAS), using UNICAM model 969 Spectrophotometer in the Department of Chemistry, University of Lagos.

2.3 Source of the Plant Materials

The plant materials were purchased from Ketu in Lagos and were identified by a curator, Mr. Odewo Kolawole, in the Department of Botany, University of Lagos. The voucher numbers of the authenticated samples are LUH 4394, LUH 4558, LUH 4396 and LUH 4395 for roselle, moringa, ginger, and 'ugwu', respectively.

2.4 Preparation of the Plant Materials

Fresh leaves of the plant materials were washed gently to remove impurities and air-dried under shade for a week. Dried leaves of the individual plant were milled into a powder using laboratory mill, Norris Limited, Poole, England at the Department of Pharmacognosy, University of Lagos. A mixture of the plant materials was also obtained by mixing the four parts each of the ground plant materials in the ratio 1:1:1:1. The ground plant materials were then stored in desiccators before use.

2.5 Preparation of the Plant Extracts

The bio-active compounds in the plant materials were extracted using the method of Okigbo and Ogbonnaya [9]. Fifty grams (50 g) powder of each plant material and mixture were put in 500 ml 95% cold ethanol for 72 hours. The extracts thus obtained were filtered with muslin cloth and evaporated to dryness at a temperature of $40\pm2^{o}C$. The resulting dried extracts yielded 6.6 g, 6.5 g, 6.2 g, 5.9 g, and 6.1 g of roselle, moringa, ginger, 'ugwu' and mixture, respectively. These dry extracts were reconstituted in water and were the decoctions used for the experiment.

2.6 Phytoconstituents Screening of the Plant Extracts

The phytochemical in the plant extracts were identified using standard procedures as described by Harbone [10] and Sofowora [11]. Thin layer chromatography (TLC) method as described by Meloan, [12] was used to determine the phytonutrients in the plant extracts.

2.7 Acute Toxicity Test

The acute toxicity of the plant extracts was measured using the 'Classical LD_{50}' method described by Gabriel et al. [13]. Albino rats (36) of both sexes weighing between 183 and 205 g were used for the studies. The rats were randomly distributed into six groups of 6 rats each and were made to fast for 12 hours before commencing the study. The rats in the test groups were orally administered 200, 400, 500, 700, 1500 and 2000 mg kg^{-1} doses of the crude extracts. The control rats were fed with only distilled water. The general symptoms of toxicity were monitored and recorded for each group within 24 hours.

2.8 Dosage Administered to the Rats

The acute toxicity test showed the extracts were nontoxic to the rats even at a dose of 2000 mg kg^{-1}, however, 400 mg kg^{-1} dose was chosen. This is because some of the plants have been reported by Adedapo et al. [14] to perform best at a dose of 400 mg kg^{-1}.

3. METHODS

3.1 Study Design

The albino rats (*R. norvegicus*) were grouped into six of 18 rats each. The control rats were in group one and groups two through six contained the test rat. The lungs of some of the rats were harvested and processed for histopathological examinations before exposure and served as the baseline index on which the lungs were evaluated. The entire rats were then exposed to cement dust at a distance of 200 m from a cement plant in Shagamu, Ogun state, Nigeria. The test rats were fed with 400 mg kg^{-1} ethanol extracts of roselle, moringa, ginger, 'ugwu' and a mixture of the extracts, respectively for 180 days. The control rats were given only distilled water for the same duration. The lungs of the rats were again harvested for histopathological studies.

3.2 Histopathology Studies

The lung tissues of the rats were prepared for histopathology examination using the method of Taylor et al. [15].

About 3-5 mm thick samples were cut from the lungs of the rats and the tissues were then fixed using bound's fluid. The tissues were dehydrated gradually through a series of increasingly concentrated ethanol/water mixture and finally in pure ethanol. Since alcohol does not mix with common embedding media, it was replaced with xylol. The tissues were then embedded in molten wax and allowed to set. Embedded tissues were sectioned using a microtome. Five (5) micrometers thick were cut from the wax-embedded tissues using a knife. The wax was dissolved away, and tissues partially dehydrated before staining.

The tissues were then stained using mercury oxide at low concentrations. The stained tissues on glass slides were covered tightly with cover-slips and viewed under a light microscope.

4. RESULTS

The elemental analysis of the cement dust shows it contains 57% calcium, 23% silicon, 10.5% aluminium, 8.5 % chromium and 8.0 % lead (Table 1).

Table 1. Elemental analysis of Shagamu cement dust

Element	Cement Chemist Notation	Mass %
Calcium Oxide CaO	C	57 %
Silicon Oxide SiO_2	S	23 %
Aluminum Oxide $A1_2O_3$	A	10.5 %
Chromium Oxide	Cr	8.5 %
Lead Oxide	Pb	8.0 %

The phytochemical found in the roselle extract include alkaloids, tannins, glycosides, and reducing sugars; moringa contains all the tested phytochemicals except flavonoids and phlobatanins. Ginger extract has glycosides, reducing sugars, saponins, and flavonoids; 'ugwu' extract has all the phytochemicals except reducing sugars and phlobatanins. The mixture extract has all the tested bio-active compounds (Table 2).

Table 2. The phytochemicals present in the plant extracts

Extract	Roselle	Moringa	Ginger	'Ugwu'	Mixture
Phytochemical					
Alkaloids	+	+	-	+	+
Tannins	+	++	-	++	++
Glycosides	++	++	++	+	++
Reducing sugars	+	++	+	_	++
Saponins	-	+	+	+	+
Flavonoids	-	-	+	+	+
Phlobatanins	-	-	-	-	+

*Key: **-** = not present; + = present in moderate amount; ++ = present in abundant amount*

The phytonutrients analysis of the extracts shows roselle contains calcium, iron, zinc, magnesium, vitamins A and vitamin C. Ginger has zinc, magnesium, vitamin A and vitamin C. Moringa, 'ugwu' and mixture extracts have all the tested nutrients (Table 3).

Table 3. The phytonutrients present in the plant extracts

Extract	Roselle	Moringa	Ginger	'Ugwu'	Mixture
Phytonutrient					
Calcium	+	++	-	+	+
Iron	+	++	-	++	++
Zinc	++	++	++	+	++
Magnesium	+	++	+	++	++
Vitamin A	++	+	+	+	+
Vitamin C	++	++	+++	+	++
Protein	-	++	-	++	++

Key: - = not present; + = present in moderate amount; ++ = present in abundant amount

The results of the acute toxicity test showed the plant extracts were nontoxic to the rats even at a dose of 2000 mg kg^{-1}. The general observations showed no mortality occurred 24 hours after administering the plant extracts. The rats that received roselle extract displayed a readiness to take more, and were licking the cannula used to administer the extract. The rats that received ginger, moringa, 'ugwu', and mixture extracts did not show any signs of illness.

Plates 1-7 show the effects of the extracts in the lung tissues of albino rats exposed to cement dust. The lung tissues of the albino rats before exposure showed normal alveolus and bronchus (Plate 1). After exposure to cement dust, the control rats revealed severe interstitial fibrosis and cellular debris (Plate 2). Moderate fibrosis was observed in the lung tissues of the albino rats treated with roselle and moringa extracts (Plates 3 and 4), respectively. The rats that were fed with extracts of ginger, 'ugwu' and mixture of the extracts had mild septal fibrosis (Plates 5, 6 and 7), respectively.

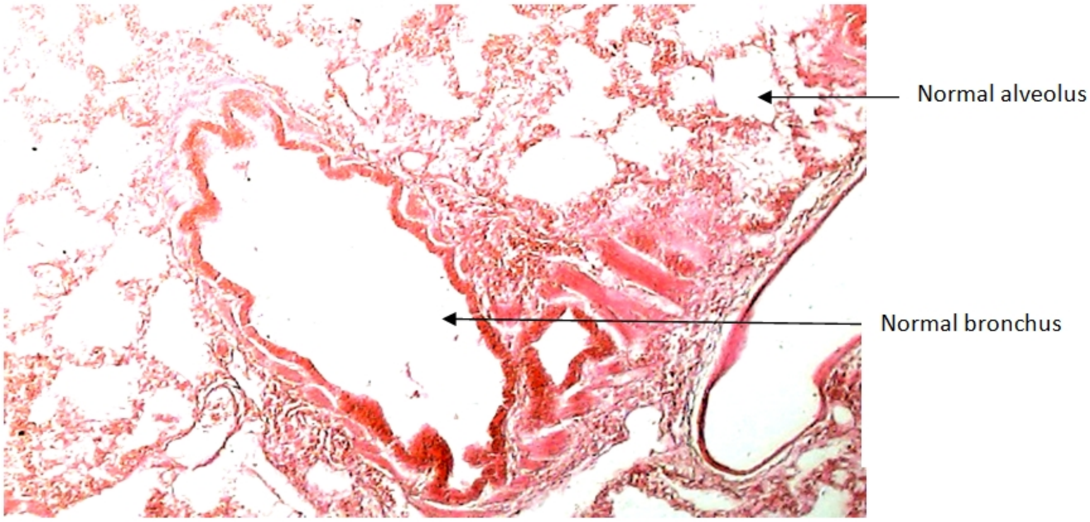

Plate 1. Photomicrograph of the lung tissues of the albino rats before exposure (X 400)

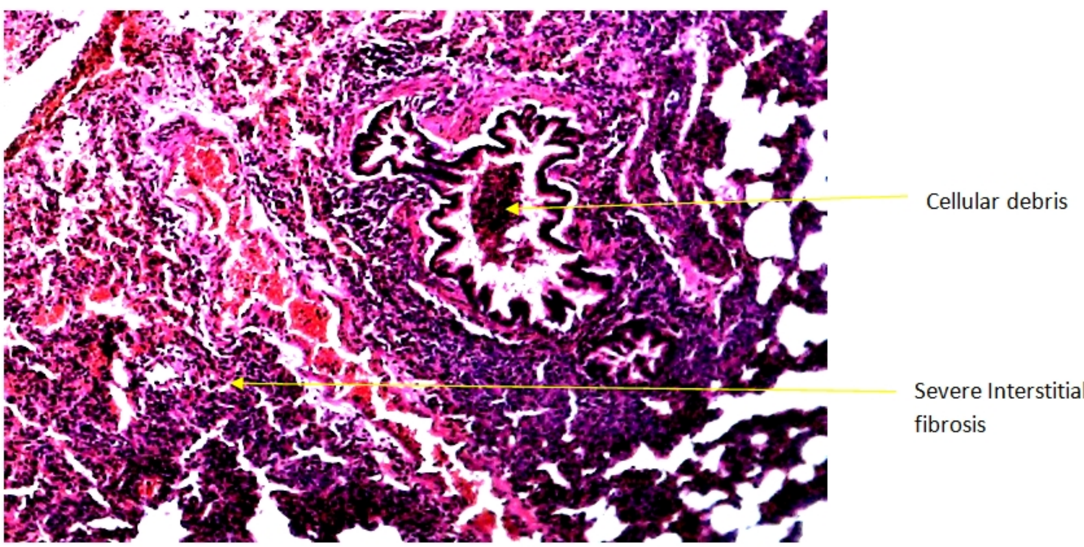

Plate 2. Photomicrograph of the lung tissues of the control albino rats after exposure to cement dust (X400)

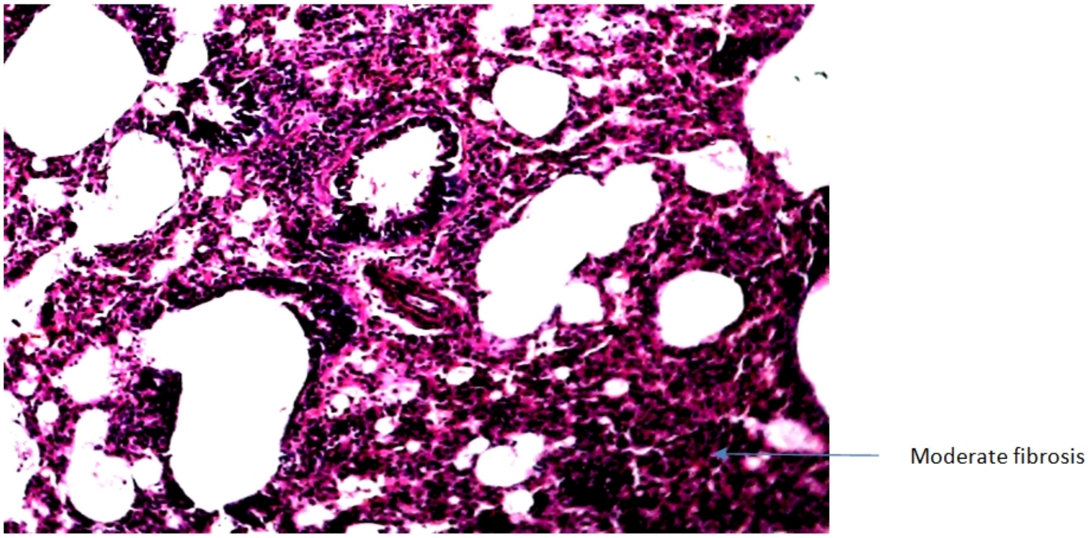

Plate 3. Photomicrograph of the lung tissues of the exposed albino rats fed with roselle extract (X 400)

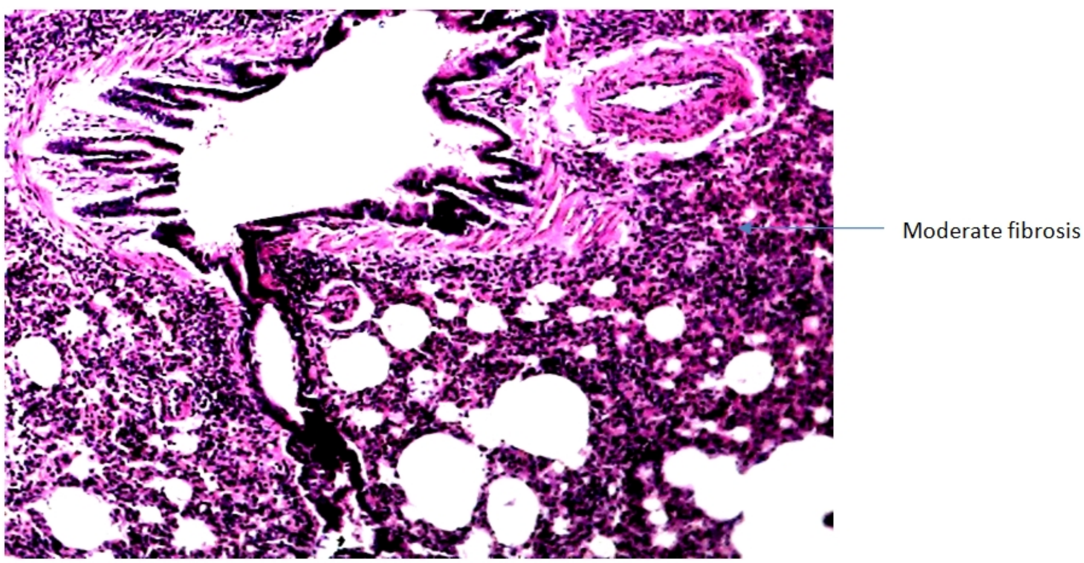

Plate 4. Photomicrograph of the lung tissues of the exposed albino rats fed with moringa extract (X 400)

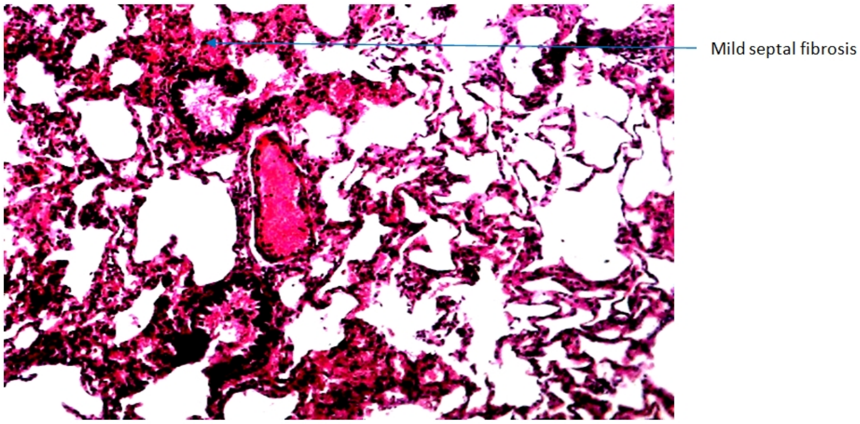

Plate 5. Photomicrograph of the lung tissues of the exposed albino rats fed with ginger extract (X400)

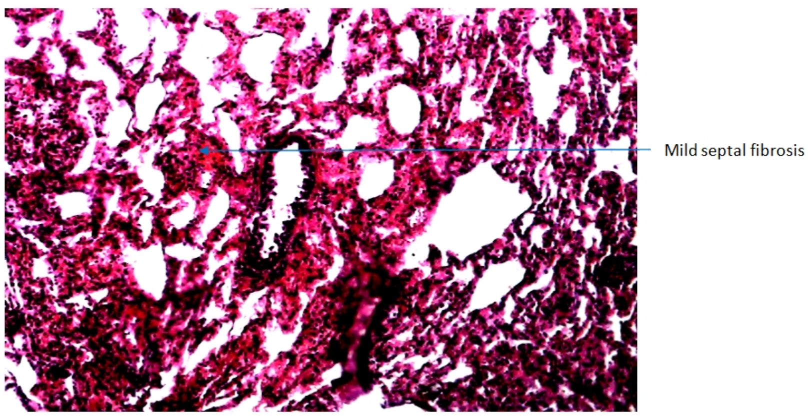

Plate 6. Photomicrograph of the lung tissues of the exposed albino rats fed with 'ugwu' extract (X400)

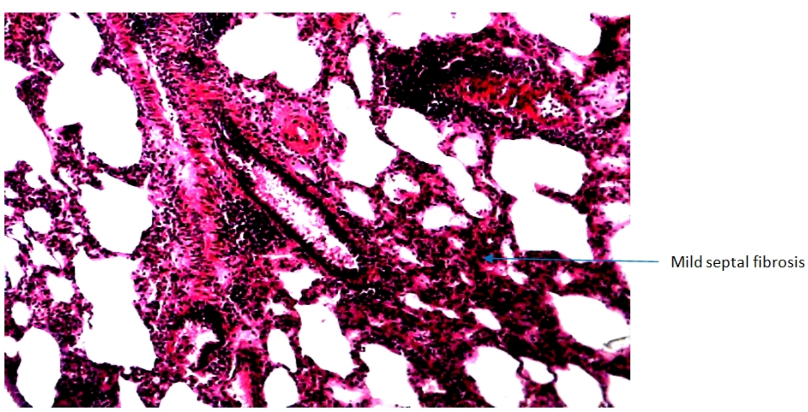

Plate 7. Photomicrograph of the lung tissues of the exposed albino rats fed with a mixture of the extracts (X400)

5. DISCUSSION

Environmental pollutants produce free radicals in exposed individuals causing cell and tissue damage. However, some herbs, vegetables and spices, have been reported to remove free radicals or prevent their oxidative process. These plants have antioxidants with therapeutic effects.

The damage observed in the lungs of exposed rats could be the effects of toxic elements in the cement dust. Calcium was found in the cement dust in high concentration, which might have induced toxicity. Fan *et al*. [16] stated that although calcium is important in metabolism, excess amounts may cause brain damage. Silicon, aluminium and chromium compounds present in the dust in high concentrations have been implicated in silicosis, increased risk of cancer and tissue damage by Hughes et al. [17] and ATSDR [18]. Yahaya and Okpuzor [19] observed high levels of calcium, silicon, aluminium, lead and chromium in the damaged lung tissues of rats exposed to cement dust. The moderate to normal lung tissues of the treated rats compared with the control rats may be the antioxidant effects of phytochemical and phytonutrients in the extracts. Flavonoids, glycosides, tannins, saponins and ascorbic acid found in the extracts have been reported by Krishnaiah et al. [20] to prevent or neutralize free-radicals and toxic elements. Saponins in plant extracts have been observed by Barakat et al. [21] to help humans prevent or fight tumor cells, particularly lung and blood cancers caused by toxic substances. Ascorbic acid protects and removes toxic substances from the body and intervenes in the regeneration of damaged tissues [22]. Cell regeneration and tissue healing of *Hibiscus Rosa-sinensis* have been attributed to the presence of tannins and terpenoids [23,24].

6. CONCLUSION

The food plants used in this study contain antioxidants with protective properties that could prevent cell and tissue damage in cement dust polluted environments. By extension, these plants may also produce protective effects in various cells and tissues of people living near industries that emit pollutants similar to cement manufacture. People living near cement plants should include these plants in their diets. Government at various levels should also incorporate phytomedicine in pollution control measures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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