In vitro and in vivo Antioxidant Properties of Extracts from the Root of Curcuma longa Linn

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors AM, MIU, AMW, AJA and SMA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AM, MIU and NAL managed the analyses of the study. Authors AM and NAL managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i630241

Received 24 January 2020
Accepted 28 March 2020
Published 03 April 2020

ABSTRACT

Many plants possess antioxidants that exhibit additive or synergistic activities. The antioxidant activities of the root of Curcuma longa Linn extracts extracted different solvents were investigated by using several established in vitro systems: α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity, hydrogen Peroxide scavenging activity (HPSA), nitric oxide radical scavenging activity (NOSA) and ferric reducing antioxidant power (FRAP). The result showed that methanol extract exhibited greater antioxidant activity in vitro which was statistically significant compared to

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the other extracts. Based on the in vitro results, the methanol extract was subjected to column chromatography. Six pooled fractions (FI-FVI) were evaluated for in vivo antioxidant activity in liver and kidney of alloxan-induced diabetic rats using a total of forty-five (45) rats which were grouped into nine (9) groups of five (5) rats. The in vivo antioxidants showed a significant decrease in superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) levels in both liver and kidney of Alloxan-induced diabetic rats. These changes were significantly reversed after treatment with methanol fraction II and the standard drug. Thus, Curcuma longa Linn may be useful in the management of diabetes and oxidative stress.

Keywords: Curcuma longa; antioxidant activity; liver; kidney; in vitro; in vivo.

1. INTRODUCTION

Antioxidants act as a defence mechanism that protect against deleterious effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system [1]. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [2]. Reactive oxygen species not only are produced naturally in cells following stress or respiration, but also have been reported to be produced by radiation, bacterial and viral toxin, smoking, alcohol and psychological or emotional stress [3]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism. The most common ROS include superoxide anion, hydrogen peroxide (H$_2$O$_2$), peroxyl (ROO$^-$) radicals and reactive hydroxyl (OH$^-$) radicals and the nitrogen derived free radicals are nitric oxide and peroxynitrite anion (ONOO$^-$) [4]. These reactive species play an important role in pathogenesis of several oxidative stress related diseases like carcinogenesis, cardiovascular diseases, rheumatoid arthritis, ulcerative colitis and neurological degenerative diseases [5]. Overproduction of ROS and/or inadequate antioxidants has been implicated in the pathogenesis and complications of diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging processes [6,7]. Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with free radicals, chelating, and catalytic metals and also by acting as oxygen scavengers [8,9]. The antioxidants can be either enzymatic or nonenzymatic in biological system. The enzymatic antioxidants include catalase, superoxide dismutase, and glutathione which catalyse neutralization of many types of free radicals [10,11], while the nonenzymatic antioxidants include vitamin C, selenium, vitamin E, carotenoids, and polyphenols. Research works have shown that antioxidants play a vital role in the prevention of heart disease, cancer, DNA degeneration, pulmonary disease and neurological disorder [12]. Recently, there has been interest in the therapeutic potential of plants as antioxidants in reducing oxidative tissue injuries [7]. Phenolic compounds such as flavonoids which are commonly found in herbs and spice have been reported to have anti-inflammatory, antiallergenic, antiviral, antiaging and anticarcinogenic activities which can be attributed to their antioxidant properties [10,12].

Turmeric (Curcuma longa), also known as Gangamau in Hausa language is a perennial plant of the ginger family also known as Zingiberaceae. Zingiberaceae grows 1.5–1.8 m high in the tropical regions of Southern Asia, with trumpet-shaped, dull yellow flowers. Its roots are bulbs that also produce rhizomes, which then produce stems and roots for new plants.

Turmeric has a somewhat bitter taste; individual plants grow up to 1m tall with long oblong leaves and requires a temperature of between 20°C and 30°C (68°F and 86°F) and high rainfall to flourish [13]. Plants are gathered annually for their rhizomes, and are reseeded from some of the collected rhizomes in the following season [1]. The rhizome from which the turmeric is derived, is tuberous, with a rough and segmented skin which mature beneath the foliage in the ground, and are yellowish brown with a dull orange interior. The main rhizome is pointed or tapered at the distal end and measures 2.5–7.0 cm (1–3 inches) in length and 2.5 cm (1 inch) in diameter, with smaller tubers branching off. When the turmeric rhizome is dried, it can be ground to a yellow powder with a bitter, slightly acrid, yet sweet, taste [6]. The known active compounds in Turmeric include curcuminoids, a family of curcumin and related compounds and the volatile oil fraction, characterized by turmerones. The aim of this research is to establish if whole plant turmeric extracts are more effective than isolated.
2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The root of *Curcuma longa* Linn was collected from Toro Local Government Area of Bauchi State, Nigeria. It was identified and authenticated by a taxonomist from the Department of Plant Biology, Faculty of Sciences, Bayero University Kano and was given a voucher number of (BUK/HAN/0188). The root was air dried at room temperature.

2.2 Aqueous Extract Preparation

The dried *Curcuma longa* Linn root was ground using pestle and mortar and the powdered root was kept in air tight container until required for analysis. Exactly 500 g of the dried sample was soaked in 1 litre of distilled water in a conical flask. The suspension was shaken vigorously and left to stand at room temperature for 24 hours with intermittent vigorous shaking. The extract was then filtered with muslin cloth and filtered by passing through Whattman’s No 1 filter paper. The filtrate obtained was concentrated by evaporation of the solvent (distilled water) using a rotary evaporator at 50ºC and dried in a water bath to yield an aqueous extract [14].

2.3 Methanol and n-Hexane Extracts Preparation

Five hundred grams of the powder was weighed and soaked in one litre each of methanol and n-hexane. The solution was shaken vigorously and left to stand at room temperature for 24 hours. The suspension was transferred into a separatory funnel for separation and the extracts were concentrated and evaporated using rotary evaporator at a temperature of 40ºC and dried in a water bath to yield methanol extract and n-hexane extract [15].

2.3.1 In vitro antioxidant activity

2.3.1.1 DPPH radical scavenging assay

Free radical scavenging activity of different extracts was tested against a methanol solution of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) by a method reported by Pank et al. [16].

2.3.1.2 Hydrogen peroxide scavenging assay

The capacity of each extract to scavenge superoxide radicals was examined by a pyrogallol auto-oxidation system [17].

2.3.1.3 Nitric oxide radical scavenging assay

The ability of *Curcuma longa* root extracts to scavenge hydrogen peroxide was estimated according to the method reported by [18].

2.3.1.4 Ferric reducing antioxidant power assay

The reducing power of *Curcuma longa* root extracts was determined by the method of Vijayalakshmi and Ruckmani [19].

2.3.2 In vivo antioxidant activity

2.3.2.1 Screening of column chromatography fractions for hypoglycemic activities

Forty-five (45) rats were used and grouped into nine (9) groups of five (5). Fractions were administered to animals for a period of three weeks. Diabetes was induced using the method described by Muhammad et al. [20] for the extracts.

2.3.2.2 Determination of super-oxide dismutase activity

The activity of SOD was measured by water-soluble tetrazoliumWST-1 method as reported by Kakkar et al. [21].

2.3.2.3 Determination of catalase activity

The ELISA kit uses Sandwich-ELISA as the method [22].

2.3.2.4 Determination of glutathione peroxidase activity

The ELISA kit uses Sandwich-ELISA as the method [22].

3. RESULTS

Table 1 presents the in-vitro radical scavenging activities of *Curcuma longa* Linn aqueous, methanol and n-hexane root extracts. The results showed that methanol extract exhibited significantly greater (<0.05) radical scavenging activity, compared to aqueous and n-hexane extracts.

Table 2 is showing the effect of oral administration of 200 mg/kg body weight of fractions of methanol extract of *Curcuma longa* Linn on catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels
Table 1. Free radical scavenging activity of aqueous, methanol and n-Hexane root extracts of Curcuma longa Linn

| Solvent     | DPPH (%)       | HPSA (%)       | NOSA (%)       | FRAP (µg dried sample) |
|-------------|----------------|----------------|----------------|------------------------|
| Aqueous     | 62.12±1.12a    | 44.68±3.35a    | 48.22±2.00a    | 58.04±3.35a            |
| Methanol    | 68.32±1.18a    | 54.82±1.88a    | 52.38±2.38a    | 67.82±1.82a            |
| n-Hexane    | 22.48±0.48b    | 10.42±1.00b    | 12.48±2.48b    | 10.42±1.00b            |

Results are expressed as mean ± SD (n=3). Values in the same column bearing the same letter indicate no significant difference (p>0.05). DPPH-radical scavenging activity, HPSA- Hydrogen Peroxide scavenging activity, NOSA- Nitric oxide radical scavenging activity, FRAP- Ferric reducing antioxidant power.

Table 2. Liver antioxidants of alloxan-induced diabetic rats orally administered with 200 mg/kg body weight of fractions of methanol root extract of Curcuma longa Linn after 21 days

| Group      | CAT (µg protein) | SOD (µg protein) | GPx (µg protein) |
|------------|------------------|------------------|------------------|
| Normal     | 86.16±3.24a      | 8.61±1.83a       | 8.08±1.32a       |
| Diabetic   | 42.14±2.63b,c    | 3.62±0.82b,c     | 4.01±1.70b,c     |
| Metformin  | 78.16±2.98a      | 7.84±0.87c       | 7.52±1.48c       |
| F1         | 52.63±2.67d      | 5.48±0.78d       | 5.86±0.96d       |
| F2         | 79.05±4.26a      | 7.15±0.56c       | 7.12±1.37c       |
| F3         | 44.47±2.08b      | 4.08±0.83b,c     | 4.98±0.79b,c     |
| F4         | 48.09±2.56d      | 4.96±0.74c       | 5.05±0.42c       |
| F5         | 44.98±2.57d      | 3.89±0.66g       | 4.58±0.97g       |
| F6         | 42.98±2.33d      | 3.72±0.68h       | 4.49±0.67h       |

Values are expressed as mean ± SD, n= 5 for each group. Values in the same column bearing the same letter are not significantly different at P<0.05.

Table 3. Kidney antioxidants of alloxan-induced diabetic rats orally administered with 200 mg/kg body weight of fractions of methanol root extract of Curcuma longa Linn after 21 days

| Group      | CAT (µg protein) | SOD (µg protein) | GPx (µg protein) |
|------------|------------------|------------------|------------------|
| Normal     | 34.63±2.84a,b,c,g,h | 16.96±2.97a,b,c,g,h | 6.02±1.26a,b,c,g,h |
| Diabetic   | 19.36±1.64a,b,c   | 7.48±1.49a,b,c   | 3.13±0.72a,b,c   |
| Metformin  | 34.07±1.49b       | 16.05±2.07b,c    | 5.76±0.64b,c     |
| F1         | 26.65±1.84d       | 13.27±1.56d      | 4.04±0.79d       |
| F2         | 33.41±2.57c       | 15.80±2.73c      | 5.96±2.24c       |
| F3         | 21.64±1.09g       | 9.94±1.42f       | 3.89±0.08g       |
| F4         | 23.67±1.57f       | 10.56±1.06f      | 3.92±0.72f       |
| F5         | 20.85±1.68c       | 8.77±1.34g       | 3.33±0.08g       |
| F6         | 18.89±1.78b       | 8.06±1.58h       | 3.38±0.16h       |

Values are expressed as mean ± SD, n= 5 for each group. Values in the same column bearing the same letter are not significantly different at P<0.05.

in liver of alloxan-induced diabetic rats. Significant decreases (p<0.05) were found in liver levels of CAT, SOD and GPx in the diabetic control group (Group II) compared to the normal control (Group I). Significant increases (p<0.05) in liver levels of CAT and GPx of groups administered with standard drug and fraction F2 when compared with diabetic control group (group II), liver levels of SOD when administered with standard drug, fraction F1 and fraction F2 as compared with diabetic control group (Group II).

Table 3 lists the effect of oral administration of 200 mg/kg body weight of fractions of methanol extract of Curcuma longa Linn on catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels in kidney of alloxan-induced diabetic rats. Significant decreases (p<0.05) were found in kidney levels of CAT, SOD and GPx in diabetic control group (Group II) compared to the normal control (Group I). Significant increases (p<0.05) in kidney levels of CAT and GPx of groups administered with standard drug and fraction F2 when compared with diabetic control group (group II), kidney levels of SOD when administered with standard drug, fraction F1 and fraction F2 as compared with diabetic control group (Group II).
4. DISCUSSION

The results in this study suggests that the methanol extract of *Curcuma longa* Linn contains the most active antioxidants. The action of the methanol extract could be ascribed to the effects of phytochemicals like flavonoids, cardiac glycosides, tannins and phenolic compounds present in the extract [23]. These may serve as antioxidants which scavenge free radical species generated by alloxan, thus retarding/reversing the destruction of pancreatic beta cells and to maintain physiological functions of body organs [19]. The findings are similar to studies which showed that antioxidants offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and thus prevent the disease progression [2,24].

The present research showed that 21 days' administration of methanol fractions of *Curcuma longa* Linn resulted in a marked reduction in hyperglycaemia in alloxan-induced diabetic rats and also improved the bodyweight [24]. Therefore, after showing the improvement in glucose homeostasis in the treated animals, the metabolic consequences of this treatment in liver and kidney were further examined. Under hyperglycaemic conditions, there is increase in the production of free radicals that in turn cause lipid peroxidation in lipoproteins [25].

The activities of some major antioxidant enzymes were measured in control and experimental rats in which diabetic rats showed altered levels of the antioxidant enzymes studied in liver and kidney, thus generating oxidative stress in these organs. Under normal physiological condition there is a critical balance in the generation of oxygen free radicals and its antioxidant defence systems used by organism to deactivate and protect themselves against free radical toxicity [26]. Disorder in the oxidant/antioxidant equilibrium in favour of the former incites a situation of oxidative stress which is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases which leads to the pathogenesis of diabetic complications [26]. Different tissues are more prone to oxidative damage and could result in complications in long-term diabetes, restoration of antioxidant status is an important parameter to check the effect of antidiabetic compound. In the present study, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were studied to evaluate the antioxidant status in liver and kidney of methanol fractions of *Curcuma longa* Linn treated diabetic rats which showed increased activity. Although studies have shown that the mechanism responsible for the decreased activity of these enzymes in diabetic state is not fully known, the results obtained may be because of phosphorylation and decreased expression of these enzymes [27].

Superoxide anion (O$_2^-$) dismutation into molecular oxygen and water is as a result of SOD oxide-reductase group. In this study, SOD activity was higher in the kidney homogenates of alloxan-induced diabetic rats than that of normal control rats which may have been in response to greater hepatic oxidative stress as suggested in a study carried out by Tagang et al. [28]. In addition, SOD activity was lower in liver homogenates of the diabetic non-treated group, compared to diabetic treated group. Izabela et al., [25] showed that, in cultured hepatocytes, MnSOD (mitochondrial SOD) and CuSOD (cytosolic SOD) are differentially expressed when exposed to high glucose concentrations (40 mM). Although high-glucose induces low MnSOD expression, it also leads to high CuSOD expression. These differences could be explained by potential differences in SOD concentration between liver and kidney homogenates. Treatment with methanol fraction II increases SOD activity in liver and kidney which may lead to the protection against oxidative injury. Furthermore, the GPx activity has been shown to be higher in liver homogenates than kidney [29]. The main intracellular antioxidant mechanism is the glutathione system which is dependent on GSH concentration and the GSH/GSSG ratio, normally regulated by de novo synthesis of glutathione in a redox cycle mediated by reactions controlled by GPx and GR. Additionally, low GSH levels have been considered as the reason for oxidative stress [29]. Furthermore, high GPx activity in diabetic animals is related to high oxidative stress in the liver. The decreases in GPx activity for the diabetic group in the present study corroborate the findings of Izabela et al. [25].

5. CONCLUSION

The present study showed that *Curcuma longa* Linn root extracts possessed antioxidant activity *in vitro*, with methanol extract showing the strongest antioxidant activity among the extracts. Fractions II obtained from methanol extract also
demonstrated strong antioxidant activity in vivo. Additional studies are in progress to possibly elucidate fraction II to better understand the specific component that confer these antioxidant properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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