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Phytochemical Analysis and Antioxidant Potential of Ethylacetate Extract of *Tamarindus Indica* (Tamarind) Leaves by Frap Assay

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Abstract
Oxidative stress is characterized by an imbalance in the generation of free radicals and their subsequent elimination by endogenous antioxidants. It is a characteristic of several diseases, especially during the progression stage, which can lead to fatal effects. This study aims to investigate the phytochemical components and antioxidant capability of *Tamarindus indica* and assess its capability as a candidate for managing diseases associated with oxidative stress. The gravimetric method detected and quantified phytochemicals, while the reducing power assay determined the antioxidant potential. Saponins, steroids, and flavonoids were detected in 6.83 ±0.44, 4.30 ±0.60, and 10.17% ±0.60, respectively, without alkaloids, glycosides, and terpenoids. The antioxidant test showed a concentration-dependent increase in absorbance of both the extract and standard (Ascorbic acid). However, Ascorbic acid had higher absorbance. At 100% concentration, the sample had an absorbance of 0.388 ±0.022, which was lower than the absorbance of Ascorbic acid (0.411 ±0.009) at 40% concentration. It can be concluded that Tamarind leaves could be utilized to manage diseases associated with oxidative stress, evidenced by their antioxidant potential credited to the phytochemical content of the leaves. However, there is a need for further studies to ascertain the exact compounds and their modes of action.

Keywords: antioxidant; ethylacetate; oxidative stress; phytochemical; *Tamarindus indica*

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INTRODUCTION

Oxidative stress is a state with a high level of reactive oxygen species (ROS) accompanied by low levels of endogenous antioxidants due to disturbance in the balance for production and neutralization of the ROS by the antioxidants.[1] Several diseases, including diabetes, neurodegenerative, and cancer, are associated with oxidative stress, notably during the progression of the diseases where there is continuous damage to DNA, lipids, and proteins.[2-4] In proteins, oxidative damage goes through carbonylation, modification of the side chain, and integrity of the protein molecule. In contrast, in DNA damage, 8-hydroxydeoxyguanosine is formed, which binds to thymidine instead of cytosine,

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causing mutagenesis.[5,6] Some antioxidants in the form of enzymes found within cells are crucial in maintaining cellular homeostasis, including catalase, superoxide dismutase, and glutathione peroxidases. However, non-enzymatic forms of antioxidants also exist, which include ascorbic acid, glutathione, and vitamin E.[7]

In type 2 diabetes, a generation of ROS causes the development and progression of diabetes by disrupting the β-cell signaling and regulation pathway, subsequently causing β-cell dysfunction and insulin resistance.[8] Generation of ROS is a characteristic of cancer cells due to the rapid cellular division. However, strategies are adopted by these cells to stay beneath a threshold for activation of apoptosis, leading to their proliferation. During cancer progression, there is an overexpression of antioxidant enzyme genes and the production of NADPH, thus emphasizing the effects of oxidative stress on cancer progression.[2] In cardiovascular diseases, ROS is an important part of signaling in the heart, acting as a second messenger. However, oxidative stress sets in when they are in excess, leading to cardiac dysfunction, hypertrophy, apoptosis, and heart failure.[9] In neurodegenerative disease, oxidative stress causes target macromolecules and the oxidation of these molecules; proteins, lipids, and nucleic acids, disturbance in proteasome and mitochondrial function, production of cytokines and inflammatory responses, formation of amyloid β deposition, plaque, and advanced glycation end products, and cell death.[10]

Plant and their products are applied in managing diseases through several mechanisms, sometimes attributed to their antioxidant potential.[11-13] Phytochemicals from plants are implicated with therapeutic roles of ailments associated with oxidative stress, which is credited to their several pharmacological effects acting individually or synergistically.[14] For example, phytochemicals were previously implicated in managing cancer progression by suppressing DNA damage caused by oxidative stress and modulating signaling pathways leading to carcinogenesis due to their antioxidant effects.[15] Considering the background mentioned above, this study aims to investigate the phytochemical components and antioxidant potential of Tamarind (*Tamarindus indica*) leaves to assess their capability for managing diseases linked to oxidative stress. It considers the effects of oxidative stress in the progression of several diseases, including diabetes, cardiovascular and neurodegenerative diseases, and cancer.

METHOD

Reagent

All the reagents utilized in this study were of AnarlaR.

Plant material

Tamarind leaves were collected from the Yolde Pate area of Yola south Local Government, Adamawa State, Nigeria. It was identified by a Forest Technologist from the Forestry Technology Department of Adamawa State Polytechnic, Yola. A voucher specimen was maintained in the departmental herbarium with voucher number ASP/FT/118. The leaves were air-dried and ground to powder using a blender.

Extraction

The plant sample was extracted by maceration of 300 g of the leave powder in 1L of 70% ethyl acetate for 48 h, followed by filtration and concentration to dryness under reduced pressure yielding 9.2 g extract.[16]

Qualitative phytochemical Analysis

Phytochemical tests and identification in ethyl acetate extract of Tamarind leaves (EETL) were carried out to detect alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids, as previously reported.[16]

Quantitative phytochemical Analysis

Saponins content

Saponins were quantified by the gravimetric method.[17]

Steroids content

Steroids were quantified by the gravimetric method.[18]

Flavonoids content

Flavonoids were quantified according to a method described previously.[18]

Reducing power assay

The reducing power of EETL was carried out by a previously reported method.[19] The plant extract varying concentrations of 20, 40, 60, 80, and 100% were prepared in distilled water. 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide were added. It was then incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added, then centrifugated for 10 min at 3000 rpm, and the upper layer was collected. Lastly, 2.5 mL of distilled water was added to 2.5 mL of the upper layer solution, followed by adding 0.5 mL of 0.1% $FeCl_3$ solution. The absorbance was measured at 700 nm against a blank using a UV-Vis spectrophotometer (752 UV-VIS Spectrometer, Shanghai Yoke Instruments Co., Ltd, China). Ascorbic acid was used as standard.

Statistical Analysis

Data obtained in the study were expressed as mean ± standard error of triplicate determinations' mean (± SEM) evaluated with Statistical Package for the Social Sciences (SPSS) version 22 Software.

RESULTS AND DISCUSSION

Phytochemical Analysis of EETL

The results of the quantitative determination of the phytochemical composition of the ethylacetate extract of Tamarind leaves are presented in Table 1. Saponins, steroids, and flavonoids were detected, while alkaloids, glycosides, and terpenoids were absent.

Table 1: Phytochemicals detected in EETL

Phytochemical	Inference
Alkaloids	Absent
Saponins	Present
Steroids	Present
Glycosides	Absent
Terpenoids	Absent
Flavonoids	Present

The phytochemical reported in this study correlates with a previous report on an ethyl acetate extract of Tamarind leaves. However, in their study, alkaloids were detected as absent.[20-22] A similar study on the ethanol extract of Tamarind reported the absence of saponins, although alkaloids were detected.[23] Besides, in the other study, saponins, steroids, and flavonoids were detected in the ethanol extract of Tamarind leaves at the moment alkaloids were also found.[24] In the same study, vitamin E was detected as a good antioxidant. The present study partially agreed with this study for detecting saponins, steroids, and flavonoids. The polarity of ethyl acetate was lower than that of ethanol; thus, the difference in solvent might be the reason for not being able to detect alkaloids.[25]

The phytochemicals quantified in the ethylacetate extract of Tamarind leaves are shown in Table 2. Flavonoids were quantified in the highest (10.17% ±0.60) concentration, followed by saponins which were present in a concentration of 6.83% ±0.44. Meanwhile, steroids were quantified in the least concentration (4.30% ±0.60).

Table 2: Quantitation of phytochemical analysis of EETL

Phytochemical	Concentration (%)
Saponins	6.83 ±0.44
Steroids	4.30 ±0.60
Flavonoids	10.17 ±0.60

Values are in triplicate determinations ± SEM.

Phytochemicals exert different pharmacological effects, including antioxidant activities through several mechanisms of action. Saponins exert their antioxidant effect by raising the concentration of antioxidant enzymes, decreasing the release of malondialdehyde or lactate dehydrogenase, and restoring Glutathione homeostasis. Thus, they were implicated in repairing oxidative damage in cells and inhibiting cell death.[26] Saponins were previously reported to exert an antioxidant effect on Alzheimer's disease (AD) by preventing DNA damage due to the formation of 8-hydroxydeoxyguanosine and increasing the expression of endogenous antioxidant enzymes in the brain of mice, making it a potential therapeutic in the therapy of AD.[27] Saponins were also reported to possess hepatoprotective effects on alcohol induced-liver injury by reducing the level of ethanol-induced oxidative stress.[28] Polymyxin E-induced nephrotoxicity was previously reported to be decreased by saponins, and a possible mechanism of action was postulated to be inhibiting oxidative stress and cell death through the mitochondrial pathway.[29]

Furthermore, flavonoids were previously postulated as a crucial metabolite for managing central nervous system disorders by acting as gamma-aminobutyric acid (GABA) receptors.[30] The flavonoid naringenin exerts an antioxidant effect by neutralizing ROS and promoting the activities of endogenous antioxidant enzymes in diabetes, cardiovascular and neurodegenerative diseases.[31] In a similar study, flavonoids were reported to exert antioxidant and antiradical effects against ROS and were postulated for application in managing oxidative stress.[32] Flavonoids were previously reported to exert an antioxidant effect on streptozotocin-induced diabetic rats by direct antiradical effects.[33] Hepatoprotective effects of flavonoids against oxidative stress induced by high glucose were previously reported to regulate antioxidant enzymes.[34]

Reducing the power of EETL

The reducing power of the EETL is presented in Table 3. A concentration-dependent increase in absorbance by the ethyl acetate extract of Tamarind leaves was observed, with the lowest absorbance at 20% concentration (0.235 ±0.008), while the highest was observed at 100% concentration (0.388 ±0.022). However, the absorption of Ascorbic acid was higher than the extract, even at 40% concentration (0.411 ±0.009).

Table 3: Reducing power of EETL

Concentration (%)	Absorbance	
	Tamarind leaves	Ascorbic acid
20	0.235 ±0.008	0.243 ±0.012
40	0.245 ±0.014	0.411 ±0.009
60	0.259 ±0.008	0.644 ±0.016
80	0.298 ±0.017	0.815 ±0.026
100	0.388 ±0.022	1.131 ±0.018

Values are in triplicate determinations ± SEM.

Several studies reported different pharmacological effects of Tamarind, which were attributed to the antioxidant potential of the plant. Tamarind leaves revealed a high radical scavenging effect *in vitro* by DPPH and ABTS. They were suggested to be a natural antioxidant with anti-diabetic properties.[35] The antioxidant activity of Tamarind was reported in a similar study and was attributed to the phenolic compounds detected due to an observed correlation between the total phenolic compounds and DPPH radical scavenging inhibition.[36] A previous study on the antioxidant potential of Tamarind leaves using FRAP reported a concentration-dependent increase in absorbance, although it was lower compared to Ascorbic acid, which aligns with the result of our study.[37] In this study, the absorbance of EETL increased along with concentration. However, the absorbance of the extract was lower than that of ascorbic acid. Besides, several studies reported similar results for FRAP of Tamarind leaves.[38-42]

CONCLUSION

This research investigated the phytochemical components and antioxidant potential of ethylacetate extract of Tamarind leaves for possible application in diseases associated with oxidative stress. Based on the result of this study, Tamarind leaves might be able to be utilized in diseases associated with oxidative stress, evidenced by its established antioxidant potential attributed to the phytochemicals detected in the leave. However, there is a need for further studies to ascertain the exact compounds and their mechanisms of action.

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