INTRODUCTION

Free radicals are highly reactive and oxygen-containing molecules. Various types of reactive oxygen species include hydroxyl radical (OH\(^-\)), superoxide anion radical, singlet oxygen, nitric oxide radical (NO\(^-\)), hypochlorite radical, and hydrogen peroxides [1]. Free radicals are produced by physiological/biochemical reactions or by pollution and other endogenous sources. Free radicals react with membrane lipids, proteins, nucleic acids, enzymes, and other small molecules, resulting in cellular damage [2]. The human system is protected by antioxidants, which play an important role in scavenging free radicals, and providing protection against degenerative diseases [3]. In recent years, the research is focused on natural antioxidants from medicinal plants to know their constituents and biological activity [4]. Plants are the rich source of alkaloids, phenolic acids, tannins, flavonoids, terpenoids, and other metabolites that act as primary antioxidants or free radical scavengers [5]. The imbalance between the production of reactive oxygen species and antioxidant defenses leads to oxidative stress [6]. Redox stress cause immune cells to release proinflammatory cytokines, reactive oxygen, and nitrogen species, that damages biological molecules and induce imbalances in physiological and pathological pathways [7]. α-amylase is involved in the breakdown of long chain carbohydrates, serve as the major digestive enzyme and help in intestinal absorption. α-amylase inhibitors are the potential targets for the development of lead compounds for the treatment of diabetes [8].

Acacia catechu (L.f) Willd is a moderate-sized tree, which belongs to the family of Leguminosae. It is commonly used in herbal preparations for diabetes [9]. Conventionally, A. catechu has been used as an antioxidant, anti-inflammatory, antimicrobial as well as antifungal property and also to treat diabetes and obesity [10]. Natural α-amylase inhibitors from the dietary plants can be used as an effective therapy for treating post-prandial hyperglycemia with minimal side effects. The present study was carried out to investigate the antioxidant activity and inhibitory potential of A. catechu (L.f) Willd leaf extract on α-amylase, in vitro. Hence, an attempt was made to find remedy for a prevalent clinical ailment through natural source.

METHODS

Plant collection and preparation of extract

The plant A. catechu (L.f) Willd was collected from Kanjikode, Kerala, identified and certified by a taxonomist at Botanical Survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore, (Plant Identification No. BSI/SRC/5/25/2014-2015/Tech/699). The leaves of A. catechu (L.f) Willd were shade dried and ground to a coarse powder by a mechanical device. The extract was prepared using different solvents, namely, petroleum ether, chloroform, acetone, ethanol, hydroethanol, and water by cold maceration process. The filtrate was stored in an airtight container until use.

Free radical scavenging assays

1,1-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity was assayed using the method of Brand-Williams et al. [11]. The radical scavenging activity of sample against DPPH was determined spectrophotometrically. The reaction mixture in a total volume of 3.0 ml contained 1.0 ml of DPPH, 0.5 ml of sample and made up to 3.0 ml with water. The tubes were incubated for 10 minutes at 37°C. A blue chromophore was formed, the absorbance of which was measured at 515 nm.
compound DPPH radical is used to investigate the antioxidant potential of a given DPPH radical scavenging assay.

**RESULT AND DISCUSSION**

DPPH radical scavenging assay

DPPH radical is used to investigate the antioxidant potential of a given compound in vitro. The hydroethanolic leaf extract of *A. catechu* (L.f) Willd. at 5 different concentrations (20-100 µg/ml), produced dose-dependent radical scavenging activities. The plant extract showed the maximum scavenging effect on DPPH as 62.94±0.91% inhibition at 100 µg/ml whereas ascorbic acid showed 89.42±0.82% inhibition as shown in the Fig. 1. The IC₅₀ value of DPPH radical scavenging activity was found to be 73.81±0.95 µg/ml. The radical scavenging activity indicates the antioxidant capacity of extract and its reducing properties might be associated with the presence of reductones [16]. In a similar study, Sanjay et al. reported that the methanolic heartwood extract of *A. catechu* showed promising DPH radical scavenging activity. Since the *A. catechu* extract showed DPHH radical scavenging activity, the natural antioxidants present in the extract might possess the ability to neutralize free radicals and thereby act as scavengers, helping prevent cell and tissue damage.

**OH⁻ scavenging assay**

OH⁻ are the most reactive oxygen species that can cause oxidative damage to DNA, lipids, and proteins [17,18]. Scavenging of OH⁻ is an important antioxidant activity because of very high reactivity of the OH⁻, enabling it to react with a wide range of molecules in the living cells, such as amino acids, sugars, lipids, and nucleotides [19]. The maximum scavenging effect for OH⁻ was obtained at 63.5±1.08% inhibition at 100 µg/ml of the plant extract, whereas the standard ascorbic acid showed 78.14±0.75% inhibition as shown in the Fig. 2. The IC₅₀ value of OH⁻ was found to be 69.0±0.58 µg/ml. The scavenging activity may be due to the presence of flavonoids in the extract. As the hydroethanolic extract of *A. catechu* (L.f) Willd exhibit OH⁻ activity, it can be assumed that this plant can be used as a marker for oxidative stress in cells and tissues.

**NO⁺ scavenging assay**

NO⁺ causes DNA fragmentation, cell damage, neurotoxicity, and lipid peroxidation [20]. The maximum scavenging effect of nitric oxide was measured by the method of Halliwell et al. [12]. The incubation mixture in a total volume of 1.0 ml contained 0.1 ml of buffer, 0.5 ml of ferric chloride, 0.1 ml of ascorbic acid, 0.1 ml of ethylenediaminetetraacetic acid (EDTA), 0.1 ml of hydrogen peroxide, and 0.2 ml of 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 minutes, and then, added 1.0 ml of thiobarbituric acid and 1.0 ml of trichloroacetic acid. All the tubes were kept in a boiling water bath for 30 minutes. The absorbance at 535 nm with reagent blank containing water in place of the extract.

**Superoxide radical (SO) scavenging assay**

The SO scavenging activity was studied using the method of Winterbourn et al. [14]. The assay mixture contained (0.2-1.0 ml) sample with 0.1 ml of Nitroblue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1 mM EDTA), 0.1 ml riboflavin (0.12 mM), and 2.55 ml of phosphate buffer (0.67 M). The control tubes were also setup, wherein dimethyl sulfoxide was added instead of the sample. The reaction mixture was illuminated for 30 minutes and the absorbance at 560 nm was measured against the control sample. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples using the formula:

\[
\text{Percentage inhibition} = \frac{(\text{Absorbance (control)} - \text{Absorbance (sample)})}{\text{Absorbance (control)}} \times 100
\]

**α-amylase inhibition assay**

α-amylase inhibition was studied using the method of Narbhe et al. [15]. The enzyme solution was prepared by dissolving α-amylase in 20 mM phosphate buffer (pH=6.9) at the concentration of 0.5 mg/ml. 1.0 ml of the extract at various concentrations (20, 40, 60, 80, and 100 µg/ml) and 1.0 ml of enzyme solution were mixed together and incubated at 25°C for 10 minutes. After incubation, 1.0 ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10 minutes. The reaction was then stopped by adding 2.0 ml of dinitrosalicylic acid (DNS, color reagent), heated the reaction mixture at 100°C for 5 minutes. After cooling, the absorbance was measured colorimetrically at 565 nm. The inhibition percentage was calculated using the formula:

\[
\text{Percentage inhibition} = \frac{(\text{Absorbance (control)} - \text{Absorbance (sample)})}{\text{Absorbance (control)}} \times 100
\]

**Statistical analysis**

Data were expressed as mean ± standard deviation.

**Fig. 1:** 1,1-diphenyl-1-picryl hydrazyl radical scavenging assay of *Acacia catechu* (L.f) Wildl. leaf extract. Values are represented in mean ± standard deviation of triplicates.

**Fig. 2:** Hydroxyl radical scavenging assay of *Acacia catechu* (L.f) Wildl. leaf extract. Values are represented in mean ± standard deviation of triplicates.
reported that the 70% methanolic heartwood 
−
showed maximum scavenging effect for SO at 51.50±0.86% inhibition at 
factors for the pathogenesis of various diseases [23]. The plant extract 
the living cells [22]. Superoxide anions are well-characterized etiological 
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SO scavenging assay
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100 μg/ml and 61.11±1.13% was recorded for ascorbic acid as shown in the 
Fig. 4. The IC₅₀ value of SO was found to be 83.10±1.00 μg/ml. The 
antioxidant property of the secondary metabolites may be attributed 
to the scavenging of SO. Since the A. catechu (L.f) Willd showed promising NO scavenging activity.

α-amylase inhibition assay
The maximum inhibitory effect for α-amylase by the plant extract was 
obtained at 62.95% inhibition at 100 μg/ml, whereas the standard 
maltose showed 77.77% inhibition as shown in the Fig. 5. The IC₅₀ 
value of α-amylase was found to be 71.17 μg/ml. Phenolic compounds 
are reported to be effective human α-amylase inhibitors [24]. 
Previous studies on α-amylase inhibitors identified from medicinal 
herbs recommend that a number of capability inhibitors belong to 
flavonoid class that has features of inhibiting α-amylase activity [25]. 
The presence of such inhibitors in food stuff is useful in the control of 
hyperglycemia as they delay carbohydrate digestion by reducing the 
rate of glucose absorption, thereby retard the post-prandial plasma 
glucose rise [26,27].

CONCLUSION
The results obtained from the present study suggested that the hydroethanolic 
leaf extract of A. catechu (L.f) Willd has a significant antioxidant and 
antihyperglycemic activities and it can be utilized as a potential source of 
therapeutic agent in the management of diabetes mellitus.

ACKNOWLEDGMENT
Financial support by University Grants Commissions, in the form 
of Minor Research Project (No.F MRP: 6281/15 [SERO/UGC]) is 
acknowledged.

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