Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 6, 2015

Original Article

IN VITRO STUDIES ON A-GLUCOSIDASE INHIBITION, ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF *TECOMA STANS* L.

R. MARGRET EVANGELINE[1], N. MURUGAN[2], P. PRAVEEN KUMAR[3], I. V. S. NIMAL CHRISTHUDAS[4*]

[1]Department of Biotechnology, PRIST University, Yagappa Chavady, Pattukottai Road, Thanjavur, Tamil Nadu, India 614904, [2]Department of Microbiology, L&T Microbiology Research Centre, Sankara Nethralaya, Chennai, Tamil Nadu, India 600006, [3]Research Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India 600034, [4]Research and Development, Department of Phytochemistry, Biozone Research Technologies Pvt Ltd, Teynampet, Chennai, Tamil Nadu, India
Email: nimalchristhudoss@gmail.com

Received: 17 Jan 2015 Revised and Accepted: 05 May 2015

ABSTRACT

Objective: *Tecomo. stans* (*T. stans*) (L) are plants extensively used for the empirical treatment of diabetes mellitus. But the bark part of *T. stans* (L) has not been extensively studied for the anti-diabetic and antioxidant property. Hence the current study were designed to estimate the *in vitro* activity of α-glucosidase inhibition and antioxidant property from bark extracts of T stans using various solvents such as hexane, ethyl acetate and methanol.

Methods: In the current study crude extract of *T stans* L. bark part has been evaluated for *In vitro* α-glucosidase inhibition and antioxidant activity from hexane, ethyl acetate and methanol extract using standard methods.

Results: Out of three solvents studied, methanol extract of *T. stans* L. (MeTs) showed 50% α-glucosidase inhibition at the concentration of 645.20±2.79 µg/ml. The total phenolic content of MeTs was 206.81±1.11 mg of catechol equivalents/g extract. MeTs showed great scavenging activity on 2, 2-diphenyl-picrylhydrazyl (DPPH) (IC50 765.25±0.42 µg/ml), hydroxyl (IC50 245.12±1.41 µg/ml), nitric oxide (IC50 800.10±1.05µg/ml) and superoxide (IC50 595.15±0.59 µg/ml) radicals, as well as high reducing power. MeTs also showed a strong suppressive effect on lipid peroxidation (IC50 810.15±2.19 µg/ml).

Conclusion: The results obtained in this study clearly indicate that MeTs has a significant potential to use as a natural a-glucosidase inhibition, antioxidant agent.

Keywords: α-glucosidase, *T. stans,* MeTs, Antioxidant etc.

INTRODUCTION

Plants with innate ability to synthesize wide variety of chemical compounds used to perform important biological functions are designated as medicinal plants. Medicinal plants produces these compounds as an immune mechanism against wide group of attack from various predators such as insects, fungi and herbivorous mammals. The medicinal value of these plants depends on chemical substances that produce definite physiological action on the human body [1]. In India more than 62 million diabetic individuals currently diagnosed, which alarms the scientific community to search for an alternative medication, particularly antidiabetic compounds from plant derivative products. Intrusiveness from the plant sources, due to the selection of a candidate plant species with prior knowledge on the basis of long-term usage by humans termed as ethnomedicine. A kind of avenue based on the assumption that the active compounds isolated from such plants are likely to be safer than those derived from plant species with no history of human use. Screening of various bioactive compounds from plants has led to the discovery of new medicinal drug which have efficient protection and treatment roles in against various diseases [2-4].

Accumulation of free radicals can cause pathological conditions such as ischemia, asthma, arthritis, inflammation, Parkinson's diseases, mongolism, ageing process and dementia. Phenolic compounds such as flavonoids, phenolic acids, tannins etc has been identified for its potent antioxidant activity mainly derived from plant products [5, 6]. Herbal drugs containing free radical scavengers are known for their therapeutic activity [7, 8].

Hence, we have taken this study to identify α-glucosidase inhibition and antioxidant property of *T. stans* (Bignoniaceae) known as yellow elder, an erect shrub or small tree. *T. stans* whole plant has been used for a variety of medical usages in herbal medicine mainly for treating diabetes and digestive problems. Since the plant derived compounds has its own safety, effective and cheaper for larger quantity of production. We screened the whole bark of *T. stans* as a starting material for this study instead of whole plant material which is studied early by Marzouka M *et al.*, for its anticancer activity and antioxidant constituents. We extracted using various solvents such as hexane, ethyl acetate and methanol. All the extracts were evaluated for its *in vitro* α-glucosidase inhibition and antioxidant activity.

MATERIALS AND METHODS

Chemicals and reagents

DPPH (1, 1-diphenyl, 2-picrylhydrazyl), NBT (nitro blue tetrazolium), NADH (nicotinamide adenine dinucleotide phosphate reduced), PMS (phenazine methosulphate), TCA (trichloro acetic acid), ferric chloride and BHT (butylated hydroxyl toluene) were obtained from Sigma chemical co., USA. Ascorbic acid was obtained from SD fine chem. Ltd., Biosar, India. β-Carotene, ferrozine, folin–phenol reagent and Tween 40 were purchased from Hi-Media Pvt. Ltd. Mumbai, India. All other chemicals used were analytical grade.

Collection of *T. stans*

T. stans whole bark was collected from Loyola College campus and taxonomical identity of plant was confirmed by Dr. G. Jeyajothi, Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamil Nadu, South India.

Extraction of *T. stans*

The bark was shade dried and powdered. The powder (1 kg) was extracted three times by cold percolation method with 3L of hexane, ethyl acetate and methanol at room temperature for 72 h. The filtrates were concentrated under reduced pressure at 40 °C and stored in a refrigerator at 2–8 °C for use in subsequent experiments.

The concentrations of extracts for *in vitro* α-glucosidase inhibition and antioxidant assays were fixed based on the previous studies [11, 12].

Determination of *in vitro* α-glucosidase inhibition and Antioxidant assays of *T. stans*

***In vitro* α-glucosidase inhibition**

In order to investigate the inhibition activity of *T. stans* hexane, ethyl acetate and methanol extracts, an *in vitro* α-glucosidase inhibition test was performed. The inhibitory effect was measured using the method slightly modified from [13]. After fasting for 20 h, the small intestine between the part immediately below duodenum and the part immediately above the cecum was cut, rinsed with ice-cold saline, and homogenized with 12 ml of maleate buffer (100 mM, pH 6.0). The homogenate was used as the **α-**glucosidase solution. The assay mixture consisted of 100 mM maleate buffer (pH 6.0), 2% (w/v) each sugar substrate solution (100 µl), and the sample extract (200–1000 µg/ml). It was pre incubated for 5 min at 37 °C, and the reaction was initiated by adding the crude a-glucosidase solution (50 µl) to it, followed by incubation for 10 min at 37 °C. The glucose released in the reaction mixture was determined with kit described above. The rate of carbohydrate decomposition was calculated as the percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of prevention was calculated by the following formula:

Inhibition (%) = [(amount of glucose produced by the positive control)-(amount of glucose produced by the addition of sample)/(amount of glucose produced by the positive control)] X 100.

Antioxidant assays

Determination of total phenolic content

Total phenolic content of *T. stans* hexane, ethyl acetate and methanol extracts were evaluated according to the Folin–Ciocalteau method [14] with some modifications. Briefly, 0.1 ml of extracts (200–1000 µg/ml), 1.9 ml distilled water and 1 ml of Folin–Ciocalteau reagent were seeded in a tube, and then 1 ml of 100 g/l Na_2CO_3 was added. The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and calibration curve with six data points for catechol was obtained. The results were compared to a catechol calibration curve and the total phenolic content of *T. stans* was expressed as mg of catechol equivalents per gram of extract.

DPPH radical scavenging assay

DPPH quenching ability of *T. stans* hexane, ethyl acetate and methanol extracts were measured according to [15]. The methanol DPPH solution (0.15%) was mixed with serial dilutions (200–1000 µg/ml) of the extracts and after 10 min, the absorbance was read at 515 nm. The antiradical activity was expressed as IC50 (lg/ml), (the antiradical dose required to cause a 50% inhibition). Vitamin C was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = $(A_0 - A_1)/ A_0 \times 100$ …… (1)

Where A_0 is the absorbance of the control at 30 min, and $A_1$is the absorbance of the sample at 30 min. All samples were analysed in triplicate.

Hydroxyl radical scavenging assay

The assay was performed as described by the method of [16] with minor changes. All solutions were prepared freshly. One millilitre of the reaction mixture contained 100 µl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500 µl solution of various concentrations of *T. stans* hexane, ethyl acetate and methanol extracts (200–1000 µg/ml), 200 µl of 200 µM FeCl3 and 1.04 mM EDTA (1:1 v/v), 100 µl H2O2 (1 mM) and 100 µl ascorbic acid (1 mM). After incubation period of 1 h at 37 °C, the extent of deoxyribose degradation was measured by TBA reaction. The absorbance was read at 532 nm against blank solution. Vitamin C was used as positive control. The scavenging activity was calculated using formula (1).

Nitric oxide radical Inhibition assay

Sodium nitroprusside is an aqueous solution at physiological pH spontaneously generates nitric oxide; it interacts with oxygen to produce nitrite ions, which can be estimated using GriessIllosvoy reaction [17]. In the present investigation, GriessIllosvoy reagent was modified using naphthyl ethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and different concentration of *T. stans* hexane, ethyl acetate and methanol extracts (200-1000 µg/ml) or standard solution (0.5 ml) were incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylenediamine dihydrochloride (1%) was added, mixed and allowed to stand for 30 min. A pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank. Vitamin C was used as positive control. The scavenging activity was calculated using the formula (1).

Superoxide anion radical-scavenging activity

Superoxide scavenging activities of *T. stans* hexane, ethyl acetate and methanol extracts were determined by monitoring the competition of those with NBT for the superoxide anion generated by the PMS–NADH system [18]. Superoxide radicals were generated in 1 ml of 20 mM Tris–HCl buffer pH 8.0 containing 0.05 mM nitroblue tetrazolium (NBT), 0.01 mM phenazine methosulphate (PMS) and different concentration of extracts (200–1000 µg/ml) were pre incubated for 2 min. The reaction was initiated by the addition of 0.078 mM NADH. Blue chromogen, formed due to NBT reduction was read at 560 nm. Results were expressed as percentage of inhibition of superoxide radicals. Vitamin C was used as a positive control. The scavenging activity was calculated using the formula (1).

Determination of reducing power

The reducing power of *T. stans* hexane, ethyl acetate and methanol extracts were evaluated according to the method of [19]. Different amounts of the extracts (200–1000 µg/ml) were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50 °C for 20 min; 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated the ability of reducing power. Butylated hydroxyl toluene (BHT) was used as standard.

Lipid peroxidation inhibition

The inhibition effect of *T. stans* hexane, ethyl acetate and methanol extracts on lipid peroxidation was determined according to the thiobarbituric acid method. $FeCl_2$–H_2O_2 was used to induce liver homogenate peroxidation [20]. In this method, 0.2 ml of different concentration of extracts (200–1000 µg/ml) was mixed with 1 ml of 1% liver homogenate (each 100 ml homogenate solution contains 1 g rat liver); then 50 µl of $FeCl_2$ (0.5 mM) and H_2O_2 (0.5 mM) was added. The mixture was incubated at 37 °C for 60 min; then 1 ml of trichloroacetic acid (15%) with thiobarbituric acid (0.67%) was added and the mixture was heated in boiling water for 15 min. The absorbance was recorded at 532 nm. Vitamin C was used as positive control. The percentage of inhibition was calculated using the formula (1).

RESULTS

***In vitro* α-glucosidase inhibition of *T. stans* Bark**

The results for α-glucosidase inhibition assay are shown in table 1 and all the concentration of standard exhibited above 50% of inhibition for MeTs and no inhibition was found in Ethanol and Hexane extract.

Table 1: α-Glucosidase inhibition of extracts of *T. stans.* Each value represents the mean±SEM of triplicate experiments

Sample	Conc(µg/ml)	% of α-Glucosidase inhibition	IC_{50} µg/ml	% of acarbose	IC_{50} µg/ml
	200	20.15±2.93		40.91±1.54	
	400	49.93±2.99	645.20±2.79	61.14±0.29	550.20±0.29
MeTs	600	54.05±2.79		81.75±0.29	
	800	62.41±1.00		92.76±0.29	
	1000	71.58±1.65		93.93±0.50	

Antioxidant assays

Determination of total phenolic content *T. stans*

The total phenolic content of hexane, ethyl acetate and methanol extracts of *T. stans* was found to be 86.89±0.46, 106.16±0.57 and 206.81±1.11 mg catechol equivalent/gram extract respectively.

DPPH radical scavenging assay of *T. stans*

Methanol extract exhibited a significant dose dependent inhibition of DPPH activity compared to hexane and ethyl acetate extracts, with a 50% inhibition (IC_{50}) at a concentration of 765.25±0.42µg/ml. The results are presented in [fig. 1]. The IC_{50} value of vitamin C was 690.25±2.36 µg/ml.

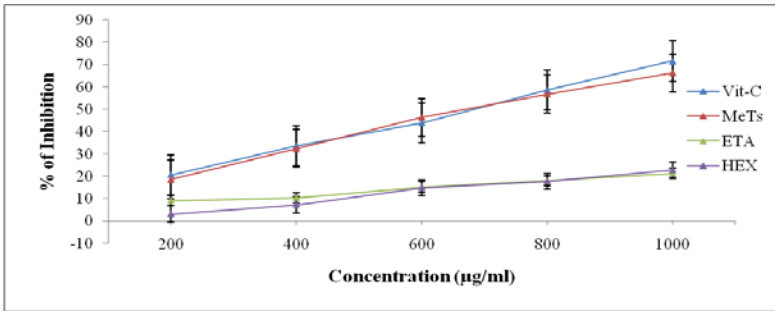

Fig. 1: DPPH scavenging effect of different concentrations (200–1000 µg/ml) of *T. stans* Methanol (MeTs), Ethanol (ETA), Hexane (Hex) extracts and Vitamin C. Each value represents the mean±SEM of triplicate experiments

Hydroxyl radical scavenging assay of *T. stans*

To attack the substrate deoxyribose hydroxyl radicals were generated by reaction of Ferric-EDTA together with H_2O_2 and ascorbic acid. When the plant extracts were incubated with above reaction mixture, it could prevent the damage against sugar. The results for hydroxyl scavenging assay are shown in [fig. 2]. The concentrations for 50% inhibition were found to be 245.12±1.41and 215.12±0.19µg/ml for the MeTs and vitamin C respectively. ETA and Hex showed less than 50% inhibition effect.

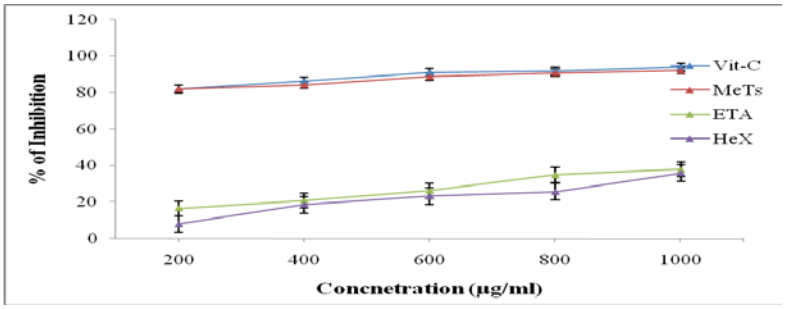

Fig. 2: Hydroxyl radical scavenging effect of different concentrations (200–1000 µg/ml) of *T. stans* Methanol (MeTs), Ethanol (ETA), Hexane (Hex) extracts and Vitamin C. Each value represents the mean±SEM of triplicate experiments

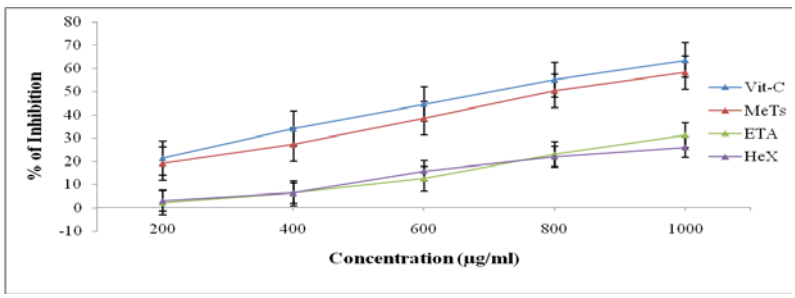

Fig. 3: Nitric oxide scavenging effect of different concentrations (200–1000 µg/ml) of *T. stans* Methanol (MeTs), Ethanol (ETA), Hexane (Hex) extracts and Vitamin C. Each value represents the mean±SEM of triplicate experiments

Nitric oxide radical inhibition assay of *T. stans*

The scavenging of nitric oxide by MeTs was increased in a dose-dependent manner as illustrated in [fig. 3]. At concentration of 800.10±1.05µg/ml of extract 50% of nitric oxide generated by incubation was scavenged. The IC_{50} value of vitamin C was 750.40±0.49µg/ml. But ETA and Hex showed no scavenging activity.

Superoxide scavenging activity of *T. stans*

The superoxide anion derived from dissolved oxygen by phenazine methosulphate/NADH coupling reaction reduces nitroblue tetrazolium. The decreased absorbance at 560 nm with plant extract indicates the consumption of superoxide anion in the reaction mixture. As mentioned in [fig. 4]. The MeTs as well as vitamin C showed the scavenging activity; IC_{50} values, 595.15±0.59 and 490.50±2.08µg/ml, respectively. But ETA and Hex extract showed no scavenging activity.

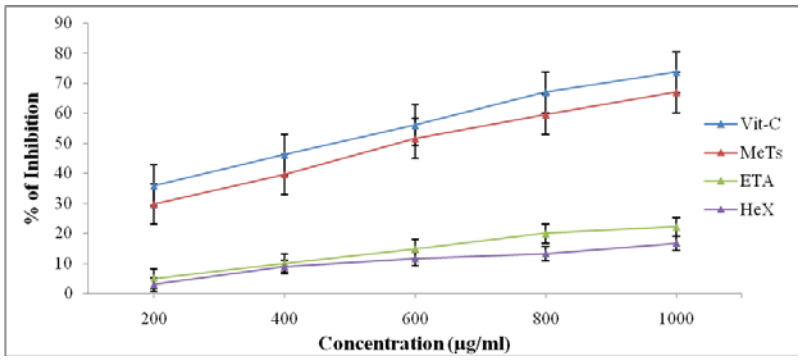

Fig. 4: Super oxide scavenging effect of different concentrations (200–1000 µg/ml) of *T. stans* Methanol (MeTs), Ethanol (ETA), Hexane (Hex) extracts and Vitamin C. Each value represents the mean±SEM of triplicate experiments

Reducing ability assay of *T. stans*

Reductive capabilities of MeTs of *T. stans* compared to butylated hydroxytoluene shown in [fig. 5]. The plant extract could reduce the most Fe3+ions, which had a lesser reductive activity than the standard of butylated hydroxy toluene. ETA and Hex showed lesser reductive capability than MeTs.

Lipid peroxidation in rat liver homogenate by *T. stans*

Activity of extracts on lipid peroxidation is shown in [fig. **6**]. Addition of Fe2+/ascorbate to the liver micro somes cause increase in lipid peroxidation. MeTs showed inhibition of peroxidation effect in all concentrations, which showed 50% inhibition effect at 810.15±2.19 µg/ml. The IC_{50} value of vitamin C was 780.10±2.76µg/ml. ETA and Hex showed less than 50% inhibition effect.

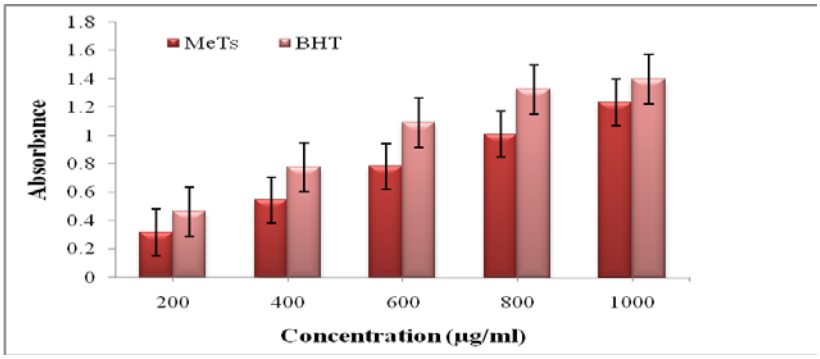

Fig. 5: Reductive ability of different concentrations (200–1000 µg/ml) of *T. stans* Methanol (MeTs), Ethanol (ETA), Hexane (Hex) extracts and Butylated hydroxy toluene(BHT). Each value represents the mean±SEM of triplicate experiments

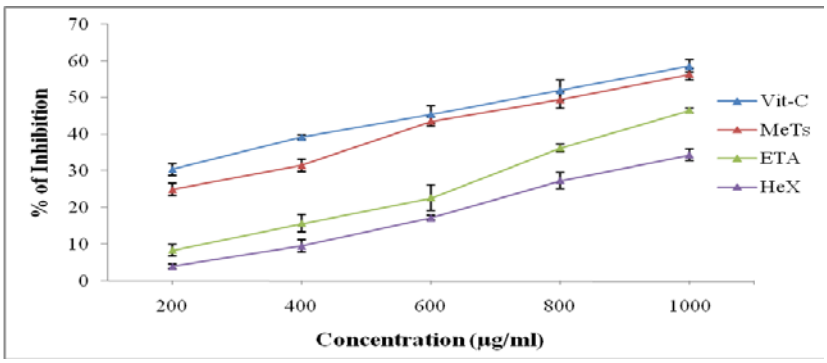

Fig. 6: Anti lipid peroxidation effect of different concentrations (200–1000 µg/ml) of *T. stans* Methanol (MeTs), Ethanol (ETA), Hexane (Hex) extracts and Vitamin C. Each value represents the mean±SEM of triplicate experiments

DISCUSSION

Diabetic scenario of the past two decades necessitates the need for oral hypoglycaemic agent. Hence, the current study on α-glucosidase inhibitory activity can be useful as a one of the source for oral hypoglycemic agent to control hyperglycemic condition in diabetic patients. Recent findings suggests that the prevention of excessive postprandial blood glucose level rise by α-glucosidase inhibition from natural resources is effective in real life as well.

The current study evaluated the total phenolic content of MeTs and estimation showed higher amount of polyphenols were obtained from methanol extract. Polyphenols are the major plant compounds with antioxidant property mainly due to their redox properties, which plays an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

DPPH test is commonly used as substrate to evaluate antioxidative activity of antioxidants [19]. The evaluation is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating antioxidant from the extract by which the formations of the non-radical form DPPH-H [21]. MeTs has the ability to reduce the stable radical DPPH to the yellow-coloured diphenyl picryl hydrazine. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [22].

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity [23]. MeTs inhibited free radical-mediated deoxyribose damage remarkably. Nitric oxide plays an important role in various types of inflammatory processes in the animal body. Nitric oxide radical inhibition study showed that the extract was potent scavenger of nitric oxide. MeTs inhibited nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [24].

In the PMS–NADH–NBT system, superoxide anion derived from the dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease in the absorbance at 560 nm with antioxidants indicates the consumption of generated superoxide anion in the reaction. Superoxide (one-electron reduced form of molecular oxygen) is a precursor of other ROS such as hydrogen peroxide, hydroxyl radical, and singlet oxygen that have the potential of reacting with biological macromolecules and thereby inducing tissue damages [25]. These results clearly indicated that MeTs is a potent scavenger of superoxide radicals in a dose-dependent manner.

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products. Malondialdehyde (MDA), one of the products of lipid peroxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress [26]. MeTs showed a strong inhibition of lipid peroxidation from this study.

For the measurements of the reductive ability, we studied the $Fe^{3+}$to Fe^{2+}transformation in the presence of *T. stans* extracts using the method followed by Oyaizu M [19]. The reducing power increased with increasing concentration of extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [27]. We believe that our current study results indicates that higher activity with antioxidant and α-glucosidase inhibition property is mainly due to the presence of increased phenolic compound concentration as estimated by Folin–Ciocalteau method [28].

CONCLUSION

This study clearly indicates, MeTs extract of *T. stans* have the potential for α-glucosidase inhibition and antioxidant activity which may help in inhibiting or reducing the growth of numerous oxidative stress-related diseases. Further exploration on the isolated element on α-glucosidase inhibition and antioxidant activity may lead to chemical entities for clinical use.

CONFLICT OF INTERESTS

Declared None

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