iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 11 **Issue:** XII **Month of publication:** December 2023

DOI: https://doi.org/10.22214/ijraset.2023.57325

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 11 Issue XII Dec 2023- Available at www.ijraset.com

Comparison of Antioxidant Activity of Some Antidiabetic Traditional Medicinal Plants of Madhya Pradesh, India

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Abstract: ***Traditional antidiabetic drugs are in use by a majority of patients having type II diabetes, due to their belief in traditional medicines. Here, we report the additional benefits of using such traditional plants in the diabetes therapy, so that these locally available medicines are promoted over synthetic drugs. Ten medicinal plants were chosen for the study, selected based on the traditional knowledge. The plant parts were extracted with water, methanol, ethyl acetate and petroleum ether. Antioxidant activity was determined by analyzing the potential of these extracts to scavenge superoxide, hydroxyl, nitrate, peroxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, under in vitro conditions as compared to the positive control; ascorbic acid (4 mg ml^{-1}). Highest antioxidant activity was shown by extracts of Gymnema sylvestre, followed by Withania somnifera against all free radicals tested.***

Keywords: Diabetes, traditional Indian herbs, antioxidant activity, DPPH, Gymnema sylvestre.

I. INTRODUCTION

India is a prominent nation with a significant portion of its people afflicted by type II diabetes. Scientists attribute the prevalence of diabetes mellitus among Indians to their dietary patterns, lifestyle choices, and insufficient physical activity, in addition to genetic factors. Diabetes is a longstanding disease that has been documented in ancient Indian literature as well. The old Indian medicine system recommended numerous medicinal plants for their therapeutic properties against diabetes. This traditional knowledge specifically utilised medicinal plants that were readily accessible in the local environment. Many Indians continue to adhere to the principles of Indian Ayurveda and choose to use traditional plant-based remedies, either on their own or in conjunction with modern medications, to regulate their blood glucose levels (1).

Free radicals contribute significantly to the oxidative stress that causes damage to multiple organs in diabetes. Elevated levels of free radicals, reduced antioxidant defence, and alterations in redox potential are the primary factors involved in diabetes, leading to subsequent tissue damage (2). The superoxide radical has been identified as a causative factor in diabetic retinopathy (3). Nitrate radicals have been found to decrease insulin sensitivity (4). Hydroxyl radicals are known to play a critical role in endothelial dysfunction induced by diabetes (5). Peroxide radicals are associated with lipid peroxidation (6). Therefore, addressing the free radicals can potentially mitigate the secondary complications associated with diabetes, such as nephropathy, neuropathy, and retinopathy.

We have demonstrated a relationship between the total phenolic content of traditional Indian herbs and their ability to inhibit α-amylase, a key enzyme involved in diabetes. This research aims to assess the effectiveness of these herbs in treating diabetes. This research investigates the antioxidant properties of various plants to gather comprehensive evidence supporting their utilisation as traditional remedies.

II. MATERIALS AND METHODS

A. Collection and Extraction of Traditional Medicinal Plants

During the study, we picked 10 herbs namely juice of Anar (*Punica granatum*), root powder of Ashwagandha (*Withania somnifera*), fruit juice of Karela (*Momordica charantia*), fruit powder of Peepal (*Ficus religiosa*), dried leaf powder of Tulsi (*Ocimum sanctum*), seed powder of Methi (*Trigonella foenum*), dry leaf powder of Gudmar (*Gymnema sylvestre*), fruit juice of Aamla (*Embellica officinalis*), fresh gel of *Aloe vera* and leaf powder of Aam (*Mangifera indica*). The plant materials were dried till constant weight and extracted with ethanol using Soxhlet extractor for 3 hours. The extract was concentrated *in vacuo* to get a concentration of 4 mg ml^{-1}.

B. Antioxidant Activities

1) Determination of Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity was measured by the reduction of NBT. Plant extracts were incubated in a reaction mixture (150 µL) containing phosphate buffer (0.2 M, pH 7.4), nicotinamide adenine dinucleotide (NADH) (73 µM), nitroblue tetrazolium (50 µM) and phenazine methosulfate (15 µM). After incubation for 60 min at room temperature, the absorbance of the reaction mixture was measured at 560 nm against blank. Ascorbic acid (4 mg ml^{-1}) served as positive control. The antioxidant activity was calculated by comparing the changes in absorbance with respect to the positive control (7).

2) Determination of Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity assay was based on quantification of the degradation product of 2-deoxy-2-ribose sugar by condensation with 2-thiobarbituric acid. The reaction mixture contained 240 µL 2-deoxy-2-ribose (3 mM), phosphate buffer (20 mM, pH 7.4), $FeCl_3$ (0.1 mM), ethylenediamine tetra-acetic acid (0.1 mM), H_2O_2 (2 mM), 1 ml of ascorbic acid (4 mg ml^{-1}) or the plant extract. The reaction mixture was incubated for 45 min at 37°C before addition of 40 µL of 2.8% (v:v) trichloroacetic acid, and 40 µL of thiobarbituric acid (0.5% (v:v) in 0.025 N NaOH). The reaction mixture was again incubated at 95 °C for 15 min. After cooling to room temperature, the pink colour developed was measured at 532 nm against blank solution (8).

3) Determination of Nitrite Oxide (NO) Radical Scavenging Activity

In this assay, the solution of sodium nitroprusside (10 mmol L^{-1}) in phosphate buffered saline (pH 7.4) was mixed with plant extracts, and incubated at 37 °C for 60 min in light. An aliquote of this mixture was then mixed with equal quantity of the Griess reagent (10 g L^{-1} sulphanilamide, 20 mL L^{-1} phosphoric acid and 1 g L^{-1} naphthyl ethylene diamine dihydrochloride, and the mixture was incubated at 25 °C for 30 min in dark. The absorbance of the reaction mixture was recorded at 546 nm against an approrpiate blank. Ascorbic acid (4 mg ml^{-1}) served as positive control. The antioxidant activity was calculated by comparing the changes in absorbance with respect to the positive control (9).

4) Determination of Peroxide radical Scavenging Activity

Peroxide radical scavenging activity was determined using the The Fe^{3+}/ascorbic acid dependent non-enzymatic lipid peroxidation. The reaction mixture contained 50 µL of 5 mg mL^{-1} bovine brain phospholipids, 1 ml of 1 mM $FeCl_3$, 1 ml of 20 mM phosphate buffer (pH 7.4) and 1 ml of with a final volume of ascorbic acid (positive control) or the plant extract. This reaction mixture was incubated at 37°C for 1 h. The hydroxyl radicals generated in the reaction initiated the lipid peroxidation, which in turn produces malondialdehyde (MDA). The amount of MDA produced was measured by addition of 40 µL of 2.8% (v:v) trichloroacetic acid, and 40 µL of thiobarbituric acid (0.5% (v:v) in 0.025 N NaOH). The reaction mixture was warmed at 95 °C for 15 min. After cooling to room temperature, the absorbance was measured at 532 nm against blank solution (10).

5) Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical Scavenging Assay

DPPH radical scavenging activity was determined by incubating 1 ml of ethanolic extracts of various plants with 1 ml of 0.004% DPPH free radical (Sigma, USA) in ethanol. The mixture was incubated at 37 °C in dark for 30 min with constant shaking at 150 rpm min^{-1}. Decrease in absorbance at 517 nm was recorded using a spectrophotometer (EI, India) against DPPH blank. Ascorbic acid (4 mg ml^{-1}) served as positive control. The antioxidant activity was calculated by comparing the changes in absorbance with respect to the positive control (11).

III. RESULTS

The present study compares the antioxidant potential of traditional Indian medicinal plants against a variety of reactive oxygen species, as well as against a synthetic free radical; DPPH. The antidiabetic activity of these plants have been established (12).

Against the superoxide free radical, ascorbic acid at 4 mg ml^{-1} concentration was able to scavenge 97.92 ±1.96% of free radical. Among the tested plants, highest antioxidant activity was recorded with *G. sylvestre* (84.31 ± 1.96 %), followed by *W. somnifera* (71.24 ± 4.08 %) and *T. foenum* (69.93 ± 3.00 %).

Ascorbic acid was able to scavenge hydroxyl free radicals at the rate of 92.16 ± 1.12%, while in comparison, plant extracts of *G. sylvestre* could scavenge 79.90 ± 1.53 % hydroxyl free radicals, followed by *F. religiosa* (66.67± 2.25 %) and *A. vera* (63.73 ± 1.85 %).

Against the nitrite free radicals, the positive control; ascorbic acid was able to scavenge 91.60 ± 1.53% of free radicals from the reaction mixture, while among the plant extracts, *G. sylvestre* could scavenge 80.41 ± 1.17 %, followed by *W. somnifera* (79.64 ± 1.17 %) of nitrite free radicals.

Ascorbic acid could also scavenge 89.89 ± 0.37% peroxide free radicals. Among the plant extracts, highest peroxide scavenging activity was shown by *G. sylvestre* (87.74 ± 2.58 %), followed by *W. somnifera* (86.24 ± 1.62 %).

Against the synthetic free radical; DPPH, ascorbic acid was able to scavenge 77.45 ± 2.42% DPPH free radicals. Among the traditional Indian plants with antidiabetic activity, *G. sylvestre* was most potent antioxidant against DPPH free radicals with 74.04 ± 1.89 % activity, followed by *W. somnifera* (66.81 ± 2.67 %) (Fig 1).

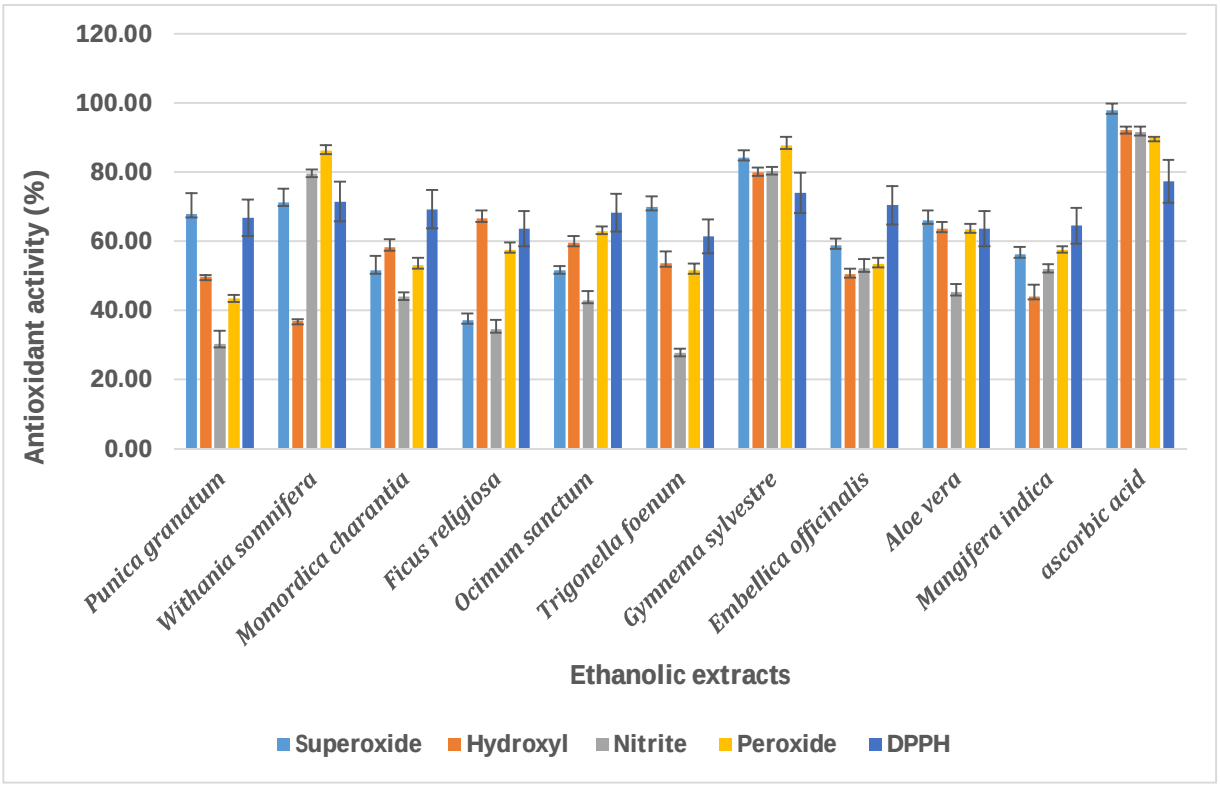

Fig 1: Comparison of antioxidant activities of traditional Indian medicines against a variety of free radicals. Ascorbic acid served as positive control. Mean values from three replicate tests are presented, while error bars represent standard deviations.

IV. DISCUSSION

The current study demonstrates that while traditional Indian plants with antidiabetic properties are capable of neutralising free radicals, not all plants exhibit significant antioxidant activity. Gymnema sylvestre and Withania somnifera exhibited antioxidant properties that were similar to ascorbic acid when evaluated. Nevertheless, nearly all of the plants examined shown the capability to diminish the burden of free radicals by a minimum of 50% in the mixture of reactions. This indicates that these plants can offer supplementary assistance if incorporated into their antidiabetic treatment.

Plants are known to contain abundant bioactive secondary metabolites, such as flavonoids, tannins, and terpenoids, which are polyphenolic chemicals. We have previously demonstrated the association between phenolic chemicals and their ability to reduce blood sugar levels in individuals with diabetes (12). In addition to their strong antioxidant properties, these phenolic compounds have been demonstrated to decrease glucose absorption, making them potentially beneficial in mitigating postprandial hyperglycemia (13). Oxidative stress caused by oxygen and nitrogen free radicals leads to a disruption in the equilibrium of redox reactions, which in turn acts as a catalyst for poor sugar metabolism (14). Additionally, oxidative stress has been demonstrated to disrupt the process of glucose absorption by muscle cells, leading to decreased insulin sensitivity and exacerbating the symptoms of diabetes (15). This research strongly promotes the utilisation of traditional Indian botanicals for the management and regulation of type II diabetes. The utilisation of these plants will not only aid in managing hyperglycemia, but also offer further protection against the co-morbidities associated with diabetes mellitus.

V. ACKNOWLEDGEMENT

Authors are grateful to the Managing Director, Excellent Bio Research Solutions Pvt. Ltd., Jabalpur (MP) for the technical support.

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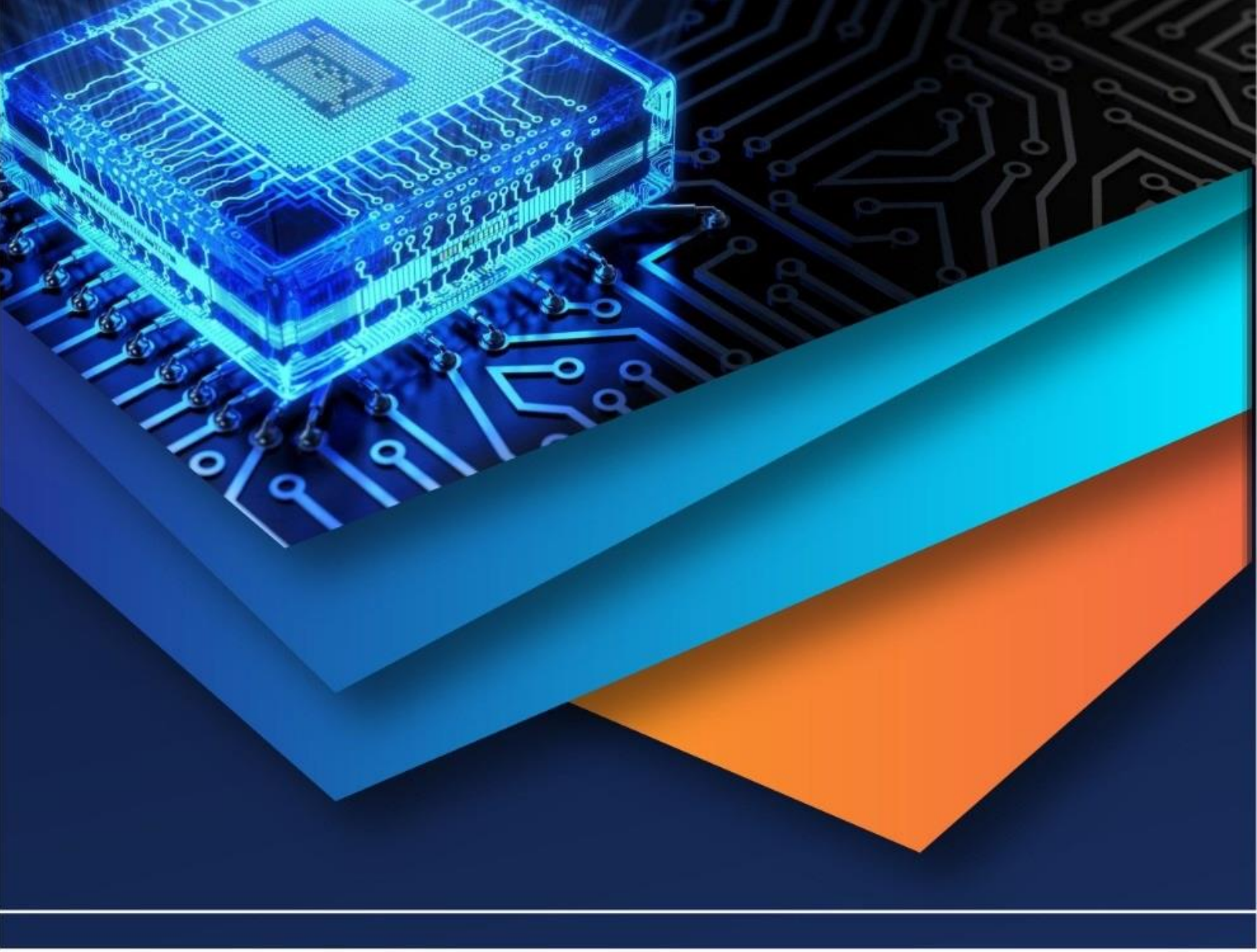

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