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Research Article

Screening of Antimicrobial and Antidiabetic Activities of Native and Cultivated Medicinal Plants of India

Pavan Kumar Mujawdiya[1,3], Pritee Pandey[1], Rishabh Singh[1], Shashwat Sharad[2] and Suman Kapur[1]*

[1]Department of Biological Sciences, Birla Institute of Technology and Science-Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad, India

[2]Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India

[3]Inochi Care Private Limited, New Delhi, India

***Corresponding Author:** Suman Kapur, Senior Professor, Department of Biological Sciences, Birla Institute of Technology and Science-Pilani, Hyderabad Campus, Hyderabad, India.

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Abstract

Background and Objective: Chronic health complications such as obesity and diabetes are associated with disrupted energy balance and abnormal glucose metabolism. Traditional and alternative medicinal systems are used to manage obesity and diabetes due to the presence of several phytoconstituents such as polyphenol and flavonoids that possess anti-obesity and anti-diabetic properties. In the present study, we have evaluated *in vitro* anti-diabetic, anti-bacterial, and anti-fungal properties of extracts of *Azadirachta indica*, *Asparagus racemosus*, *Bacopa monnieri*, *Glycyrrhiza glabra*, *Rosmarinus officinalis,* and *Rheum palmatum.*

Methods: Standard colorimetric methods were used for the estimation of total polyphenol, flavonoid, saponin, reducing sugar, glucose, and pentose sugar content. The anti-diabetic property was measured using α-glucosidase inhibition assay. Antibacterial activity was tested against gram negative bacterium *Klebsiella* sp. Antifungal activity was evaluated against common yeast, *Saccharomyces cerevisiae*.

Results: It was observed that plant extracts greatly vary in their phytoconstituent and sugar content. The highest polyphenol and saponin contents were observed in *Rosmarinus officinalis* (2312 ± 59 µg/ml and 25423 ± 0 µg/ml, respectively), whereas *Rheum palmatum* showed the highest concentration of flavonoids (91125 ± 4009 µg/ml). The concentrations of total reducing sugar, glucose, and pentose sugars in all plant extracts were measured. *Asparagus racemosus* showed the highest concentration of reducing sugars, glucose and pentose sugars (7000 ± 149 µg/ml, 154.58 ± 5 µg/ml and 49906 ± 3052, respectively). All plant extracts displayed potent antidiabetic activity as reflected by *in vitro* α-glucosidase inhibition assay. In addition, all plant extracts showed cholesterol esterase inhibitory activity, indicating their potential role in the management of dyslipidemia. Moreover, all plant extracts showed powerful antimicrobial activities with certain plants inhibiting microbial growth of up to 100%.

Conclusions: The herbal extracts of *Azadirachta indica*, *Asparagus racemosus*, *Bacopa monnieri*, *Glycyrrhiza glabra*, *Rosmarinus officinalis,* and *Rheum palmatum* possess potent α-glucosidase inhibitory potential and may aid in reducing postprandial hyperglycemia in affected individuals. Furthermore, the cholesterol esterase inhibitory properties of these plant extracts may aid in the management of dyslipidemia. Finally, the antimicrobial properties against *Klebsiella* and *Saccharomyces cerevisiae* show that these plant extracts can be useful in managing bacterial and fungal infections and may act as lead molecules for the development of potent antimicrobial agents.

Keywords: Anti-diabetic; Hyperglycemia; Antibacterial; Antifungal; Plant Extracts

Introduction

Human life expectancy has increased in recent decades. Availability of adequate food and better nutrition, improved healthcare facilities, better hygiene and reduced child mortality has ensured a higher life expectancy than our ancestors [1]. In 2015, around 617 million or 8.5% of the global population was aged ≥65 years and the numbers will increase to ~1.6 billion (17%) in 2050. Type 2 diabetes mellitus and obesity cause "accelerated aging" and significantly increase the risk for CVDs in affected individuals. Interestingly, aging is also an independent risk factor for onset and progression of T2DM and other cardiovascular ailments [2,3]. Obesity and type 2 diabetes mellitus (T2DM) are both major chronic health complications and adversely affect the quality of life of affected patients. The rising number of obese and diabetic individuals poses a major public health concern and needs immediate attention from both policy makers and medical community [4].

According to recent estimates by the World Health Organization, 1.9 billion individuals in the world were obese in 2016. Alarmingly, the prevalence of obesity is rising rapidly in children as well. The prevalence of childhood obesity has increased from 4% in 1975 to 18% in 2019 [5]. Similarly, the recent figure from the International Diabetes Federation (IDF) suggests that the number of diabetics will reach 629 million in 2050 [6]. If not controlled, diabetes may become the biggest epidemic of the 21st century [7]. India is one of the worst affected countries from diabetes and often tagged as the "Diabetes capital of the world" with projected 69.9 million diabetics by 2025 and 80 million by 2030 [8]. The total cost of medical expenditure and productivity loss due to diabetes was staggering $327 billion in 2017 as per the figures from the American Diabetes Association [9]. Post-prandial hyperglycemia (PPH) is considered as a first sign of T2DM and managing PPH has been suggested to control the progression of diabetes. Importantly, a sudden postprandial "hyperglycemic spike" is often associated with cardiac complications [10]. The use of medicinal plants to manage obesity and diabetes has been well documented and several anti-obesity and antidiabetic molecules have been reported from medicinal plants [11,12]. One of the promising options to manage PPH is to reduce intestinal absorption of glucose by inhibiting α-glucosidase. α-glucosidase is a carbohydrate metabolizing enzyme that converts complex dietary carbohydrates into glucose and causes postprandial spikes in the glucose levels [13-15]. Commercially available α-glucosidase inhibitor, acarbose, is commonly prescribed for the management of T2DM but its use is associated with adverse effects such as diarrhea, abdominal pain, cramping, bloating, and flatulence [16]. The prevalence rate of CVDs has increased substantially in recent years due to increased incidence of obesity and other metabolic disorders. Hypercholesterolemia is a major risk factor for the onset and progression of atherosclerosis and other cardiovascular disorders (CVDs), and, therefore, reducing cholesterol absorption may help in lowering the risk for CVDs [17,18]. It has been shown that hypercholesterolemia is a causative factor for atherogenesis and other associated cardiac complications and increased serum concentrations of low-density-lipoprotein (LDL) cholesterol increase the chances of coronary heart disease. Thus, lowering serum cholesterol by preventing its absorption in the gastro-intestinal tract is a promising therapeutic strategy for the management of CVDs [19].

Available antidiabetic drugs are associated with several side effects such as nausea, vomiting, diarrhea, heartburn, and abdominal pain and they have diminishing response over long term use, necessitating dose escalation and or polypharmacy. These challenges offer an opportunity to discover new anti-diabetic and anti-obesity molecules with fewer side-effects and enhanced efficacy from natural plant sources. Thus, the present work aims to study the α-glucosidase inhibitory potential of extracts of *Azadirachta indica*, *Asparagus racemosus*, *Bacopa monnieri*, *Glycyrrhiza glabra*, *Rosmarinus officinalis,* and *Rheum palmatum.* Herbs and herb-based products form a major proportion of alternative and traditional medicinal systems. According to an estimate, approximately 61% of total drugs developed between 1981-2002 were based on plant based phytoconstituents and natural products. Natural products also show potent antimicrobial properties and several plant extracts and phytoconstituents have been tested for their antibacterial properties. The antibacterial properties of plants are primarily attributed to several secondary metabolites such as polyphenols, flavonoids, terpenes, alkaloids, and glycosides [20]. The rising prevalence of antibiotic-resistant bacterial species is a global health issue of major concern and alternative drugs to combat antibacterial resistance are urgently required. Several plant-derived agents can be promising antibiotics and their use to control multi-drug resistant bacterial infections may provide a future treatment strategy to kill antibiotic resistant bacteria, often referred to as "superbugs" [21].

Diabetics are more prone to develop microbial infections and infectious disorders due to hyperglycemic conditions that reduce the body's immune response by lowering the functions of immune cells (neutrophils), and reducing antioxidant mechanisms of the body. Therefore, diabetics often develop foot infections, rhinocerebral mucormycosis, gangrenous cholecystitis and otitis [22]. Diabetes is also a well-known risk factor for *Klebsiella* infections such as *Klebsiella pneumoniae* liver abscess (KPLA). A poorly managed diabetes and uncontrolled hyperglycemia (HbA1c ≥ 7%) increases the risk for gas-forming liver abscess compared with patients with controlled hyperglycemia (HbA1c < 7) [23]. Baker's yeast or *Saccharomyces cerevisiae* is generally a commensal organism in humans but may cause infections in certain cases [24]. *Saccharomyces cerevisiae* is an opportunistic pathogen and individuals with cancer, chronic disorders, and immunosuppression develop fungemia, skin infections, urinary tract infection, esophagitis and laryngitis [25]. In another observation, a 51-year-old woman suffering from chronic kidney disease (CKD) and diabetes developed acute pyelonephritis caused by *Saccharomyces cerevisiae*. The study demonstrated that *Saccharomyces cerevisiae* can become virulent in certain immunocompromised patients under favorable conditions [26]. Therefore, the present study aims to study antimicrobial action of extracts of *Azadirachta indica*, *Asparagus racemosus*, *Bacopa monnieri*, *Glycyrrhiza glabra*, *Rosmarinus officinalis,* and *Rheum palmatum* against both bacterial (*Klebsiella* sp.) and fungal (*S. cerevisiae*) agents.

Materials and Methods

Procurement of plant extracts

The herbal extract of *Azadirachta indica*, *Asparagus racemosus*, *Bacopa monnieri*, *Glycyrrhiza glabra*, *Rosmarinus officinalis,* and *Rheum palmatum* were purchased from GMP certified manufacturers and used in the supplied form without any further processing.

Chemicals and reagents

Yeast α-Glucosidase, extra pure (100-150 units/mg) was purchased from SRL Laboratories, India. Substrate for α-Glucosidase, p-nitrophenyl α-d-glucopyranoside (PNPG) obtained from Alfa Aesar, USA. Sodium Carbonate and Gallic acid were purchased from SD Fine Chemicals, India. Catechin and Saponin were purchased from Sigma chemicals, USA. Luria-Bertani and Yeast Potato Dextrose Agar were purchased from HiMedia Laboratories (Mumbai, India).

Determination of total polyphenol content

The estimation of total polyphenols was carried out using the Folin-Ciocalteu colorimetric method. The chemical reaction between the polyphenol and the F-C reagent yields a blue color complex and the color is read at 760 nm. Briefly, 50 µl plant extract was homogeneously mixed with 50 µl of Folin-Ciocalteu reagent and the reaction mixture was incubated at room temperature for 2 minutes. The reaction was stopped after the incubation period by adding 500 µl of 5% Sodium Carbonate (w/v). Finally, 400 µl distilled water was added to make up the volume to 1 ml and the reaction mixture was subsequently heated at 45°C for 30 minutes followed by cooling at room temperature [27]. A gallic acid standard curve was prepared for concentrations ranging from 0-300 µg/ml (R^2 = 0.998). All experiments were done in triplicates and the results are expressed as mean ± SD.

Determination of total flavonoid content

Total flavonoid content in the plant extracts was measured using Aluminum Chloride method [27]. Briefly, 50 µl plant extract was homogeneously mixed with 100 µl of 2% $AlCl_3$ and the reaction mixture was incubated in the dark for 1 hour at room temperature. Absorbance of the reaction mixture was measured at 405 nm in an ELISA reader (BioTek, Elx 800, USA). A standard calibration curve of Catechin was plotted for concentrations of 0 to 1000 µg/ml with a linear fit (R^2 = 0.9896). All samples were analyzed in triplicates and the results are expressed as mean ± SD.

Estimation of total saponin

Vanillin-sulfuric acid assay was used to measure the total saponin content of plant extracts [28]. In brief, 10 µl of each plant extract was homogeneously mixed with 20 µl of Vanillin reagent (8%, w/v in 99.9% ethanol) followed by addition of 200 µl of 72% (v/v) Sulphuric acid to each tube. The reaction mixture was vortexed and heated in a water bath at 60°C for 10 minutes. After completion of the reaction, the final reaction mixture was then cooled. An appropriate blank (tube without saponin) was run and a standard calibration curve at various concentrations of saponin (10-80 µg/ml) was obtained with a linear fit (R^2 = 0.9916). The total saponin content of all plant extracts was expressed as saponin equivalents (µg/ml). All samples were analyzed in triplicates and the results are expressed as mean ± SD.

Determination of total reducing sugar

The total reducing sugar in the plant extracts was measured using the DNS method after some modifications [29]. Briefly, 500 µl of each plant extract was mixed with 500 µl of DNS reagent. The sample was tightly covered to avoid any loss of the sample. The reaction mixture was subsequently heated in a water bath at 90º C for 5-15 minutes to develop the red-brown color. Finally, the absorbance was recorded at 575 nm. Glucose standard curve (R^2 = 0.996) with various concentrations of glucose was plotted to measure the content of reducing sugar in plant extracts. All samples were analyzed in triplicates and the results are expressed as mean ± SD.

Determination of glucose

Total glucose in the extracts was estimated using the glucose estimation kit (Accurex, India) as per the standard protocol supplied by the manufacturer. Briefly, 3 µl of herbal extract was mixed with 300 µl glucose reagent and incubated at room temperature for 10 minutes. Absorbance was recorded at 490 nm in an ELISA reader (BioTek, Elx 800, USA). All samples were analyzed in triplicates and the results are expressed as mean ± SD.

Determination of pentoses

Total ketoses were estimated using the Bial's test [30]. Briefly, 50 µl of herbal extract was mixed properly with 250 µl Bial's reagent and heated at 90º C for 15 minutes. The tubes were properly capped to avoid any loss of reaction mixture. After completion of the incubation period, the tubes were cooled to room temperature and absorbance was measured at 630 nm against a blank. A xylose standard curve with different concentration points was plotted (R^2 = 0.994). All samples were analyzed in triplicates and the results are expressed as mean ± SD.

Quantitative hemolytic activity assay

Quantitative hemolytic activity assay was performed with all plant extracts as per the method described by Bondoc., *et al.* with slight modifications [31]. Briefly, human blood from a volunteer was collected in ethylene di amine tetraacetic acid (EDTA), as anticoagulant, containing vial and centrifuged at 800 x g for 5 minutes to remove the plasma. Next, the red blood cell pellet was gently washed twice with cold 1X phosphate-buffered saline (1X PBS; pH 7) to remove hemoglobin released from lysed RBCs, and the RBC pellet was suspended into 1X PBS to make 0.5% solution of RBCs. Quantitative hemolytic assay was performed in a 96-well plate in triplicate. 1X PBS acted as a negative control and 1% saponin (final concentration) acted as a positive control (100% lysis). Initially, 45 µl of 0.5 % RBC solution was added to each well and subsequently 5 µl of 1X PBS/10% saponin/plant extract was added to the RBC solution and mixed gently. After 30 seconds, 200 µl 1X PBS was added to each well and the plate was centrifuged to separate the supernatant. Finally, absorbance was recorded in an ELISA reader at 450 nm. (BioTek, Elx 800, USA).

α-glucosidase inhibition assay

The α-glucosidase inhibition assay was performed to evaluate the antidiabetic potential of plant extracts using a method reported previously with slight modifications [32]. Briefly, 10 µl of α-glucosidase (1.5 units/ml) was mixed with 120 µl 0.1 M phosphate buffer (pH 6.9) followed by addition of appropriate volumes of plant extract (2 µl-32 µl) and the complete reaction mixture was pre-incubated for 15 min at 37°C. Reaction mixture without plant extract was used as a positive control (100% enzyme activity). After the incubation period, enzymatic reaction was initiated by adding 5mM PNPG and the reaction was incubated at 37°C for another 30 min. After the incubation period, enzymatic reaction was terminated by adding 80 µl of 0.2 M Sodium Carbonate solution and absorbance was recorded on an ELISA reader at 405 nm (BioTek, Elx 800, USA). Each experiment was conducted in triplicate and data is presented as mean ± SD. Enzyme inhibitory activity was calculated as per the following formula.

$$\text{Enzyme inhibitor activity} = \frac{A_{\text{control}} - A_{\text{Sample}}}{A_{\text{control}}} \times 100\%$$

Cholesterol esterase inhibition assay

Cholesterol esterase inhibition assay was performed in a 96-well plate in triplicates according to a protocol described by Pietsch and Gutschow with slight modifications [33]. Briefly, 10 µl cholesterol esterase (2.5 µg/ml) was mixed with 150 µl sodium phosphate buffer, pH 7.0 and incubated with various volumes of plant extract (2 µl - 32 µl) for 10 minutes at 25°C. After incubation, 20 mM p-nitrophenyl butyrate (prepared in 100 mM sodium phosphate buffer) was added to the reaction mixture and incubated for further 5 min at 25°C. Finally, the absorbance of the reaction mixture was recorded at 405 nm. The reaction mixture without plant extract was used as a positive control. Reaction mixture without enzyme was used as a negative control.

$$\text{Enzyme inhibitor activity} = \frac{A_{\text{control}} - A_{\text{Sample}}}{A_{\text{control}}} \times 100\%$$

Antibacterial activity of plant extracts

The antibacterial activity of all extracts was performed using standard microbiology protocols and modified method reported previously [34]. In brief, 10^3 cells/ml *Klebsiella* cells (*K. pneumoniae* subsp *Ozaenae*, MTTC number: 2653, IMTECH, Chandigarh, India), were mixed with plant extract to make a final dilution of 1:05, 1:10, 1:20, 1:40 and 1:80 in a final volume of 190 µl of Luria-Bertani broth. The cells were incubated at 37ºC for 10 minutes and 50 µl mixture was spread on an LB Agar plate and incubated overnight at 37ºC (18 hrs incubation). Colonies were counted using a digital colony counter. 50 µl herbal extract alone was placed on a separate plate to check the sterility of the herbal extract. Autoclaved water (50 µl) was placed on a separated plate to check the sterility of the overall handling process.

Antifungal activity of plant extracts

The antifungal activity of all extracts was performed using standard protocols and modified method reported previously [35]. For determining the antifungal activity, 50 mg Baker`s Yeast (*Saccharomyces cerevisiae*) was purchased from local market and revived in 500 µl lukewarm water and kept in dark at 37ºC water for 15 minutes. Thereafter, 50 µl of revived culture was added to 5 ml Yeast Potato Dextrose Agar (YEPD) broth and kept overnight (14-16 hrs) at 37ºC in an orbital shaker at 120 rpm. Next morning, the absorbance of the yeast culture was taken at 600 nm and the cell numbers were adjusted to 10^3 cells/ml. To test the antifungal activity, different dilutions of plant extract (1:05, 1:10, 1:20, 1:40, and 1:80) were prepared in yeast culture medium and the final culture volume for all concentrations was 200 µl. The mixture was incubated at 37ºC for 20 minutes and after incubation 50 µl culture was poured on YEPD Agar plates, spread uniformly, and the plates were incubated at 30ºC for 16 hrs. Colonies were counted using a digital colony counter. 50 µl herbal extract alone was placed on a separate plate to check the sterility of the herbal extract. Autoclaved water (50 µl) was placed on a separate plate to check the sterility of the overall handling process.

Results

Relative concentration of various phytochemicals and sugars

Total polyphenol, flavonoid, saponin, reducing sugar, glucose, and pentose content were measured for all six extracts using standard colorimetric methods. The relative concentrations of all phytoconstituents and sugars are presented in Table 1 (Figure 1). It was observed that plant extracts greatly vary in their phytoconstituent and sugar content. The highest polyphenol and saponin contents were observed in *Rosmarinus officinalis* (2312 ± 59 µg/ml and 25423 ± 0 µg/ml, respectively), whereas *Rheum palmatum* showed the highest concentration of flavonoids (91125 ± 4009 µg/ml). We also measured the concentrations of total reducing sugar, glucose, and pentose in all plant extracts. *Asparagus racemosus* showed the highest concentration of reducing sugars, glucose, and pentose sugars (7000 ± 149 µg/ml, 154.58 ± 5 µg/ml and 49906 ± 3052 µg/ml, respectively).

α-glucosidase inhibition assay

The α-glucosidase inhibitory potential of all plant extracts was evaluated using in vitro enzyme inhibition assay. All six extracts displayed strong inhibitory activity against α-glucosidase but the inhibition was non-linear with increasing concentration. The range of α-glucosidase inhibition was 19.84%-62.95% for A*zadirachta indica,* 73.2%-95% for *Asparagus racemosus*, 41.42%-94.6% for *Glycyrrhiza glabra*, 6.3%-56% for *Bacopa monnieri*, 65%-95% for *Rheum palmatum,* and 28.3%-97.1% for *Rosmarinus officinalis*. The inhibition showed concentration dependent but nonlinear behavior and the *in vitro* findings demonstrate that these plant extracts can be used to manage postprandial hyperglycemia due to their inhibitory potential on α-glucosidase enzyme (Figure 2).

Cholesterol esterase inhibition assay

All plant extracts used in the present study also showed inhibitory potential against cholesterol esterase, an enzyme involved in cholesterol metabolism and hydrolyzes dietary cholesterol esters into cholesterol and free fatty acids. Like α-glucosidase, the inhibition was concentration-dependent but non-linear. The inhibitory potential against cholesterol esterase can be useful in management of dyslipidemia, one of the characteristic features of metabolic syndrome. The range of cholesterol esterase inhibition was 22.32%-63.56% for A*zadirachta indica,* 9.92%-51.71% for *Asparagus racemosus*, 3.93%-66.86% for *Glycyrrhiza glabra*, 11.96%-82.2% for *Bacopa monnieri*, 9%-86.72% for *Rheum palmatum,* and 43.75%-86.2% for *Rosmarinus officinalis* (Figure 3). Importantly, all plant extracts showed inhibition of both α-glucosidase and cholesterol esterase, indicating that each extract can help in managing both hyperglycemia and dyslipidemia, two

major symptoms observed in obese patients, and has devastating consequences on various organs such as the liver, pancreas and the adipose tissues.

Quantitative hemolytic activity assay

Quantitative Hemolytic Activity Assay was performed to evaluate the membrane lytic activity of plant extracts. 1% saponin was used as a positive control and lysis induced by 1% saponin was considered 100%. It was observed that extracts of *Glycyrrhiza glabra*, *Azadirachta indica*, and *Asparagus racemosus* were less effective than saponin in inducing erythrocyte membrane lysis, whereas *Bacopa monnieri*, *Rosmarinus officinalis,* and *Rheum palmatum* were more effective in inducing erythrocyte membrane lysis. The highest erythrocyte membrane lysis was observed with *Rheum palmatum* (138% or 38% more than 1% saponin) (Figure 4).

Antibacterial activity of plant extracts

All plant extracts displayed potent antibacterial activity against *Klebsiella* species. The control well (without plant extract) showed the highest number of colonies. However, all plant extract displayed a concentration dependent antibacterial response as reflected by significant reduction in bacterial survival and significantly lower CFUs observed for plant extract treated cells. At certain dilution, plant extracts completely inhibited *Klebsiella* growth, indicating a potent inherent antibacterial property (Table 2 and Figure 5, Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12).

Antifungal activity of plant extracts

Antifungal activity of various plant extracts was evaluated against yeast (*Saccharomyces cerevisiae*). The control plate (without plant extract) showed the highest number of colonies. Like antibacterial activity, all plant extracts displayed a concentration-dependent antifungal response. The extract showed powerful antifungal activity as reflected by absence of yeast colonies by certain plant extracts even at lower dilutions (Table 2 and Figure 6, Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12).

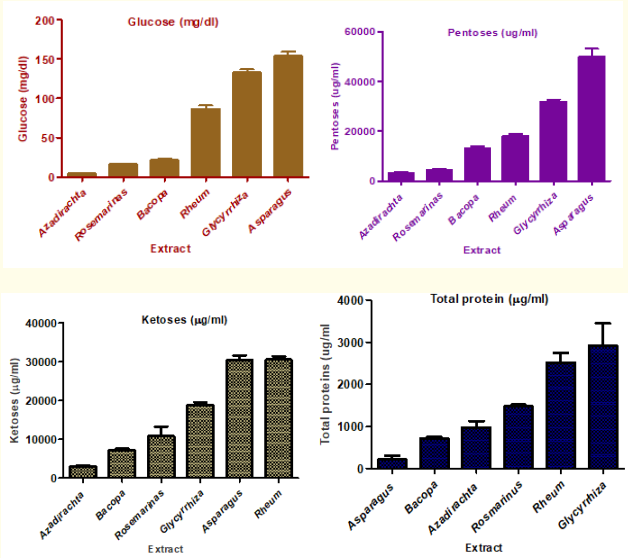
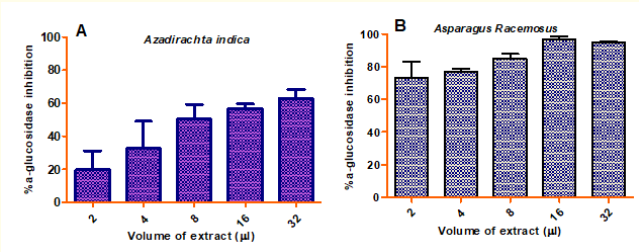


Figure 1: Bar charts representing total polyphenol, flavonoids, saponin, total sugars, glucose, pentoses, ketoses and total protein content in various plant extracts.



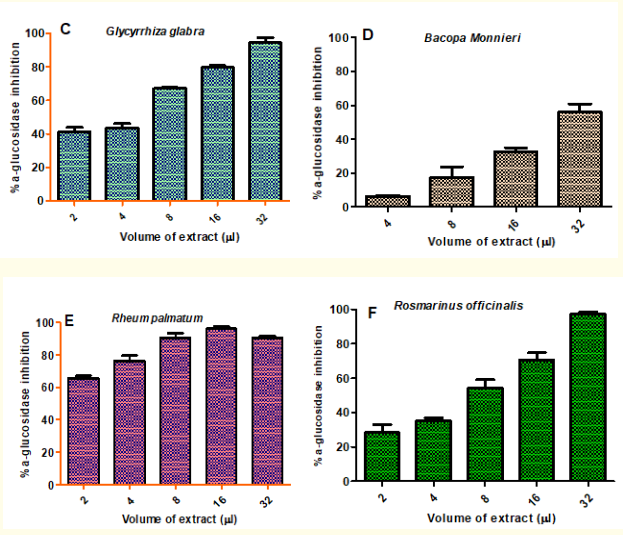


Figure 2: Bar charts representing alpha-glucosidase inhibitory potential of various plant extracts. A: *Azadirachta indica*; B: *Asparagus racemosus*; C: *Bacopa monnieri*; D: *Glycyrrhiza glabra*; E: *Rheum palmatum*; F: *Rosmarinus officinalis.*

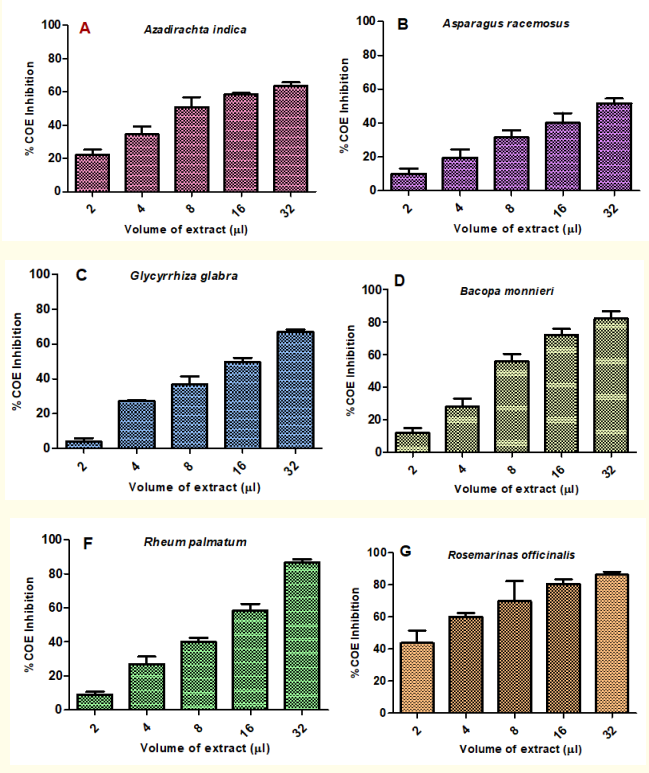


Figure 3: Bar charts representing cholesterol esterase inhibitory potential of various plant extracts. A: *Azadirachta indica*; B: *Asparagus racemosus*; C: *Bacopa monnieri*; D: *Glycyrrhiza glabra*; E: *Rheum palmatum*; F: *Rosmarinus officinalis.*

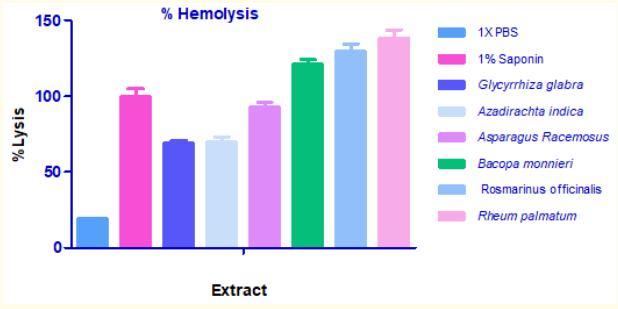


Figure 4: Bar charts representing % hemolytic activity of various plant extracts. 1X PBS acted as a negative control and lysis caused by 1% saponin was used as positive control, assigned 100% lysis. The hemolytic activity of plant extracts was evaluated against 1% saponin.

Figure 5: Antibacterial potential of (A) *Azadirachta indica* (B) *Asparagus racemosus* (C) *Glycyrrhiza glabra* (D) *Bacopa monnieri* (E) *Rheum palmatum* and (F) *Rosmarinus officinalis* extract against Klebsiella species. Control: Control well (without treatment);I = 1:80; II = 1:40; III: 1:20; IV: 1:10; V: 1:5; VI: Only extract; VII: Water blank.

Figure 6: Antifungal potential of (A) *Azadirachta indica* (B) *Asparagus racemosus* (C) *Glycyrrhiza glabra* (D) *Bacopa monnieri* (E) *Rheum palmatum* and (F) *Rosmarinus officinalis* extract against *Saccharomyces cerevisiae*. Control: Control well (without treatment); I = 1:80; II = 1:40; III: 1:20; IV: 1:10; V: 1:5; VI: Only extract; VII: Water blank.

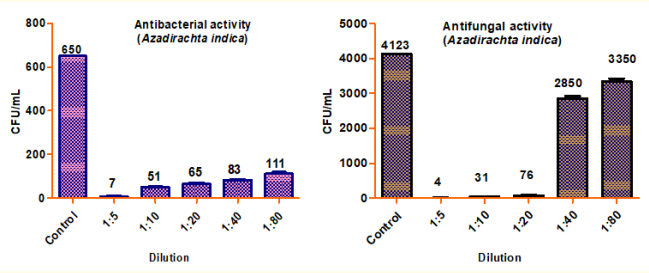


Figure 7: Bar graphs representing the antibacterial and antifungal potential of *Azadirachta indica* extract.

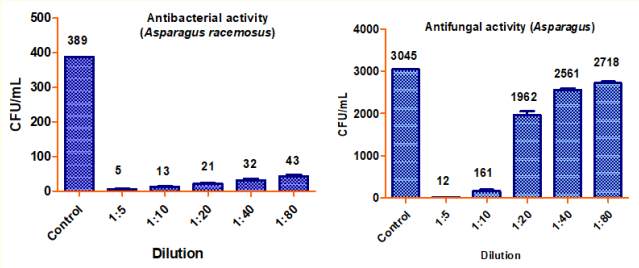


Figure 8: Bar graphs representing the antibacterial and antifungal potential of *Asparagus racemosus* extract.

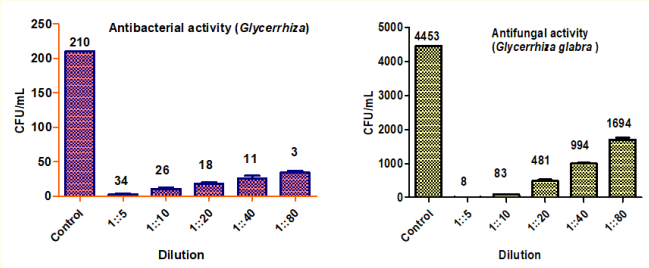


Figure 9: Bar graphs representing the antibacterial and antifungal potential of *Glycyrrhiza glabra* extract.

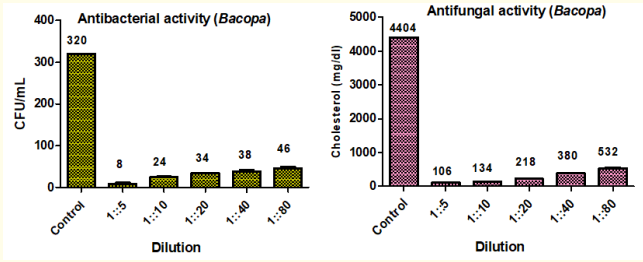


Figure 10: Bar graphs representing the antibacterial and antifungal potential of *Bacopa monneri* extract.

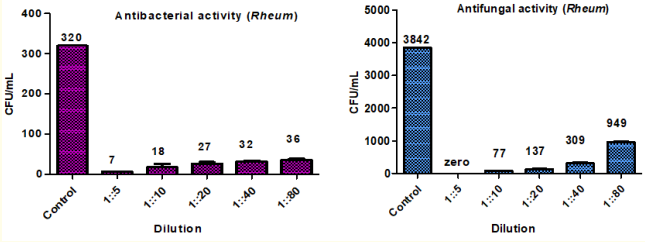


Figure 11: Bar graphs representing the antibacterial and antifungal potential of *Rheum palmatum* extract.

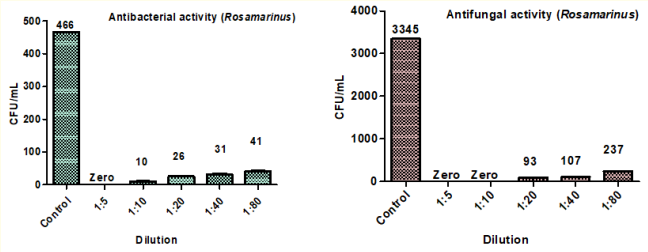


Figure 12: Bar graphs representing the antibacterial and antifungal potential of *Rosmarinus officinalis* extract.

Constituent (µg/ml)	**Mean ± SD**					
Plant	AI	AR	BM	GG	RP	RO
Polyphenol	1122 ± 59	363 ± 50	1050 ± 62	926 ± 107	1943 ± 53	2312 ± 59
Flavonoid	2900 ± 0	2200 ± 0	6275 ± 176	18000 ± 70.71	91125 ± 4009	8275 ± 176
Saponin	15771 ± 187	50447 ± 223	24179 ± 1294	38885 ± 902	21666 ± 2219	38333 ± 2313
Reducing sugar	709 ± 56	7000 ± 149	2992 ± 100	3609 ± 22	5053 ± 406	1209 ± 205
Glucose	4.54 ± 0.35	154.58 ± 5	21.8 ± 1.14	133 ± 4	86.76 ± 4.24	16.32 ± 0.93
Pentoses	3306 ± 228	49906 ± 3052	12928 ± 862	31882 ± 728	18105 ± 936	4460 ± 407
Total protein	982 ± 148	217 ± 95	715 ± 43	2917 ± 530	2521 ± 218	1480 ± 38
Ketoses	3073 ± 174	30403 ± 1179	7142 ± 477	18738 ± 815	30487 ± 830	10765 ± 2465

Table 1: Concentration of various phytoconstituents and sugars in plant extracts.

AI: *Azadirachta indica;* AR: *Asparagus racemosus*; BM: *Bacopa monnieri;* GG: *Glycyrrhiza glabra*; RP: *Rheum palmatum; RO: Rosmarinus officinalis.*

		% Inhibition against the positive control				
Plant extract		**1:5**	**1:10**	**1:20**	**1:40**	**1:80**
Azadirachta indica	Antibacterial	98.9	92.1	90%	87.2	82.9
	Antifungal	99.9	99.2	98.1	30.9	18.8
Asparagus racemosus	Antibacterial	98.7	96.6	94.6	91.8	87.4
	Antifungal	99.6	94.7	35.5	15.8	10.7
Bacopa monnieri	Antibacterial	97.5	92.5	89.3	88.1	85.6
	Antifungal	97.5	96.9	95	91	87.9
Glycyrrhiza glabra	Antibacterial	98.6	94.7	91.4	87.61	83.8
	Antifungal	99.8	98.1	89.1	77.7	61.9
Rosmarinus officinalis	Antibacterial	97.8	94.3	91.5	90	88.7
	Antifungal	100	98	96.4	92	75.3
Rheum palmatum	Antibacterial	100	98	94.5	93.3	91.3
	Antifungal	100	100	97.2	96.8	92.8

Table 2: Antibacterial response of plant extracts against *Klebsiella* species.

Discussion and Conclusion

The global misuse of existing antibiotics has led to the emergence of multi-drug resistant bacterial species, and promoted researchers worldwide to explore novel and highly effective antimicrobial agents from plants and other natural resources. Literature review shows that plant extracts and plant-derived phytochemicals can be a promising and prospective resource for not only novel antimicrobial agents with broad-spectrum antiviral, anti-bacterial, anti-fungal, anti-helminthic agents [21,27], but also simultaneously provide distinct metabolic benefits for preventing age-related chronic diseases. *Klebsiella* is a major pathogenic bacterium for humans and cause several disorders including sepsis, urinary tract infection, pneumonia, and community-acquired infections. Importantly, *Klebsiella* can cause severe infections in neonates, elderly, and immunocompromised individuals, and hyper virulent strains of *Klebsiella* are associated with increased mortality and morbidity. Due to the highly pathogenic nature of *Klebsiella*, it is regarded as an 'urgent threat to human health [36]. Alarmingly, several *Klebsiella* species have become multi-drug resistant and pose a public health challenge [37]. S*accharomyces cerevisiae* is a frequent colonizer of human skin and mucosal surfaces and usually non-pathogenic in nature. However, S*accharomyces cerevisiae* causes infections in immuno-compromised patients, ICU-admitted patients and individuals using antibiotics and probiotics [38]. Several cases of *Saccharomyces cerevisiae*-induced fungemia have been reported [39]. We characterized the plant extracts for phytoconstituents such as total polyphenol, flavonoid, saponin, reducing sugar, glucose, and pentose sugar content, and *in vitro* evaluated the anti-diabetic and anti-hyper-cholesterolemic properties using α-glucosidase and cholesterol esterase inhibition assays, respectively. Our analysis showed that *Rosmarinus officinalis* contained highest concentrations of polyphenol and saponin contents, while *Rheum palmatum* was richest in flavonoids. *Asparagus racemosus* was found to be richest in reducing sugars, glucose and pentose sugars. In addition, all plant extracts displayed significant hemolytic activity with *Rheum palmatum* showed the highest hemolytic activity in the assay. Interestingly, the membrane-disrupting properties of the saponin fraction may provide antiviral properties to the extracts and may be explored for antiviral effects against enveloped viruses, such as SARS-CoV-2 [40]. We observed that all plants studied for antibacterial activity against *Klebsiella* showed highly promising antibacterial activity with >97% reduction in colony forming units (CFU) at 1:5 dilution. Interestingly, *Rheum palmatum* was found to be the most potent antibacterial and antifungal agent in our study with 100% reduction in CFU of both *Klebsiella* and S*accharomyces cerevisiae* at 1:5 dilution (Table 2). Other plants that show 100% reduction in CFU against S*accharomyces cerevisiae* are *Azadirachta indica* and *Rosmarinus officinalis* (Table 2). A gradual but non-proportional reduction in antibacterial and antifungal activity was observed at higher dilutions (1:20, 1:40, and 1:80). However, the reduction was not the same for all extracts and varied based on the plants. For instance, *Azadirachta indica* showed 83% reduction in CFU against *Klebsiella* at 1:80 dilution, indicating that 1:80 dilution can also kill 83% *Klebsiella* cells. In contrast, *Azadirachta indica* showed only 19% reduction in CFU against *Saccharomyces* cerevisiae, indicating that 81% cells survived at 1:80 dilution (Table 2). This trend is also observed for *Asparagus racemosus*, where higher antibacterial activity was observed for 1:80 dilution compared to antifungal activity. In our study, only *Bacopa monnieri* displayed a similar trend for antibacterial and antifungal activity (Table 2). We believe that a higher concentration of polyphenols and flavonoids of *Rheum palmatum* may be responsible for the best antimicrobial action of this plant, and identifying the major bioactive component/fraction from *Rheum palmatum* may be of interest to scientists working in this area.

Glucose metabolism plays a central role in maintaining metabolic homeostasis in the system with insulin, a hormone secreted by the pancreatic β-cells, which is crucial in maintaining normal glucose levels in the system [41]. Importantly, a gradual decline in the insulin activity is observed with aging, leading to imbalanced glucose metabolism in the aging population [42]. In addition, lifestyle triggers such as lower physical activity, poor dietary habits, and obesity further exacerbate high glucose levels in the body [43]. The worldwide prevalence of impaired glucose tolerance and type 2 diabetes mellitus continues to rise, and increasing emphasis, resources, and amounts of gross domestic product of nations are used in the services of lowering circulating plasma glucose levels to prevent CVD and other diabetes mellitus–related outcomes [44,45]. We here demonstrate that all plant extracts used in the present study are potent inhibitors of α-glucosidase and may aid in reducing postprandial hyperglycemia and higher cholesterol levels observed in diabetics (Figure 3A- Fig 3F). as all plant extracts also displayed inhibitory activity against

cholesterol esterase (Figure 4A- Figure 4F), thus making these extracts useful in the management of hypercholesterolemia and dyslipidemia. The causality of the relationships between elevated plasma glucose, type 2 diabetes mellitus, and CVD is of great importance to global public health and cannot be overstated. Chia., *et al*. concluded, based on results from major randomized controlled trials., that risk for CVD can be reduced by appropriate glycemic control, an advantage offered by all the plants used in the present study [3]. It is noteworthy that all the six plant extracts tested herein are not associated with side effects like flatulence, bloating, and stool changes as can be concluded on the basis of their long-term use by AYUSH professionals. Taken together, outcomes of the present study demonstrate that plants extracts of *Azadirachta indica*, *Asparagus racemosus*, *Bacopa monnieri*, *Glycyrrhiza glabra*, *Rosmarinus officinalis,* and *Rheum palmatum* possess powerful antibacterial, antifungal, and antidiabetic activities and hence can be used in the development of novel antibiotics, antifungal, and antidiabetic agents. Moreover, the plant extract can be used in managing bacterial and fungal infections in diabetics with the added advantage of managing blood glucose levels. Finally, large lifestyle intervention trials have shown that appropriate dietary measures are of benefit in reducing postprandial glucose and postprandial glucose variability. Most observational studies show that diets decreasing postprandial glycaemia are associated with a lower risk of diabetes. Interest in the relationship between diet and health has increased the demand for functional foods (foods with specific beneficial properties beyond their basic nutritional contribution). The plant extracts tested in the present study can well be used as additives in functional foods to address the risk for age-related chronic disorders. The relationship between postprandial glucose, diabetes and CVD is a continuum, indicating that early intervention through nutraceuticals may prove helpful in normalizing postprandial glucose to address the growing health burden due to obesity, T2DM and CVD.

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