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## Phytochemical screening, antibacterial, antioxidant, and anti-diabetic properties of *Persicaria chinensis* leaves

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### Abstract

The main purpose of our experimental study was to identify the phytochemical components, *in vitro* antioxidant, antibacterial, and anti-diabetic potentials of *Persicaria chinensis* leaves extracts. Initially, a variety of compounds were found in different *P. chinensis* leaves extracts, however, ethyl acetate extract substantiated the presence of tannins, alkaloids, terpenoids, phenol, flavonoids, carbohydrate, glycoside, and protein, as compared to hexane and methanolic extract. Similarly, among three extracts tested, ethyl acetate extract showed maximum content of tannins ( $31.27 \pm 0.68$  mg GAE/g of extract), phenols ( $31.41 \pm 1.56$  mg GAE/g of dry extract), and flavonoids ( $36.37 \pm 1.1$  mg QE/g of dry extract). Further, the extracts were determined for their antibacterial properties against various gram-negative and gram-positive bacterial organisms using the agar well diffusion assay, which unfold ethyl acetate extract as comparatively better antibacterial agent with pronounced zone of inhibition. The antioxidant activity of extracts *in vitro* was assessed using standard protocols. Ethyl acetate extract exhibited a substantial dose-dependent scavenging of DPPH, NORSA, HRSA, ABTS and HPRSA with their  $IC_{50}$  values of  $26.88 \pm 0.04$ ,  $106.42 \pm 0.56$ ,  $137.04 \pm 0.33$ ,  $117.86 \pm 0.49$ , and  $17.04 \pm 0.49$   $\mu$ g/mL, respectively. The extracts antidiabetic properties were evaluated using the *in vitro* suppression of  $\alpha$ -amylase and  $\alpha$ -glucosidase. Ethyl acetate extract demonstrated a promising dose dependent inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase with their  $IC_{50}$  values of  $4.05 \pm 0.30$  and  $6.81 \pm 1.11$   $\mu$ g/mL, respectively. In conclusion, ethyl acetate extract of *P. chinensis* leaves suggested to be a potential bioactive agent with remarkable ethnopharmacological significance based on our present findings.

**Keywords:** Antibacterial, antioxidant, anti-diabetic, phytochemicals, *P. chinensis* leaves

### Introduction

Most countries employ conventional drug to cure numerous diseases, with over 80% using a wide range of plant components like bark, leaves, seeds, roots, flowers and fruits (WHO, 2022) [32]. About 70% of the rural citizens in India depend on herbal remedies to meet their medical care (Rahman *et al.*, 2011; Pandey *et al.*, 2013) [27, 22]. Phytochemicals are bioactive molecules derived from plants that have a number of health advantages. These phytoconstituents include dietary fibres, phytosterols, isoprenoids, carotenoids, and polyphenols, which have an impact on immunity, regulate genes, and help lower the risk of getting infectious and non-infectious diseases (Leitzmann, 2016; Kumar *et al.*, 2023) [19, 16]. Globally, bacterial infections are the main cause of illness and death, and the emergence of antibiotic-resistant bacteria is currently a major global concern. Plants are a viable option for treating infectious disorders because they have bioactive chemicals with antibacterial qualities. These compounds, which include flavonoids, essential oils, and phenolic compounds, support plant extracts' antibacterial properties (Álvarez-Martínez *et al.*, 2021) [9]. A wide variety of natural sources with antibacterial properties have been found through prior studies (Chassagne *et al.*, 2021) [6].

Antioxidants are substances that prevent the production of free radicals during oxidation. There has been report of antioxidant properties in plants including lignans, coumarin, flavonoids, flavones, isoflavones, catechins, anthocyanin and isocatechins (Dinesh *et al.*, 2011) [8]. Oxidative stress can be averted in cells by the action of enzyme systems like superoxide dismutase, and antioxidants like glutathione, mycithiol, and bacillithiol.

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Diabetes mellitus (DM) is a life systemic disease that can alter the metabolism of carbohydrate, fat, and proteins characterized by hyperglycemia, impacting with a frequency of 6.1%. Diabetes is currently one of the top 10 causes of death and disability worldwide. By 2050, there will likely be 1.3 billion cases of diabetes worldwide (Ong, 2023) [12]. The exploration for low-risk and more adequate hypoglycemic agents has increased since the modern anti-diabetic drugs have numerous adverse effects (Campbell, 2007) [5]. Two essential enzymes for the breakdown of carbohydrates are  $\alpha$ -amylase and  $\alpha$ -glucosidase. Reducing the likelihood of developing type 2 diabetes and managing postprandial hyperglycemia can be achieved by inhibiting them. There is a great deal of promise in using medicinal plants to treat DM. To effectively utilize the medicinal benefits of these natural resources, more research is necessary (Kooti *et al.*, 2016; Jacob and Kannan, 2019) [15, 4].

The Nagaland state of India has one of the most ethnic diversities, dependent on nature and natural products that intensifies the use and traditional practices of ethnobotanical values within the tribal populations yet, the knowledge on the principal and utilization of these plants persists unexplored. *Persicaria chinensis* belongs of the Polygonaceae family and it is among the most traditionally utilized plants in various folk medicines in Wokha, Nagaland, India. As a traditional natural plant pharmaceutical, the whole plant is used to eliminate toxins, reduce fever, to treat dysentery, eczema, corneal nebula, inflammatory skin disease, ulcer, allergies, insect sting, and snakebites. The rhizomes of the plant are utilized to remove heat, aid in detoxifying, and reduce edema (Anren, 1998) [1]. Additionally, the whole plant can be eaten as vegetable. Thus, screening of the *in vitro* techniques could provide the needed preliminary studies obligatory to elect crude extract of this plant with conclusively beneficial properties for further chemical and pharmacological analysis (Mathekaga and Meyer, 1998) [20]. In accountability of the importance of this plant, the present work was carried out.

## Materials and Methods

### Plant material

Collection of Fresh and healthy leaves of *P. chinensis* was done at Wokha, Nagaland, India. It was further subjected for identification and authentication at Siddha Central Research Institute (SCRI), Chennai, India. The individual herbarium voucher specimen with the code P20122305C was deposited in Department of Pharmacognosy, SCRI, India. The collected leaves were washed, shade-dried, ground into coarse granulate, and stored at room temperature for further experimental analyses.

### Extracts preparation

In accordance with the polarities, 1 kg of the coarse powdered leaves was extracted with 3 L of hexane, followed by ethyl acetate and methanol. The solvent from the filtrate was evaporated at room temperature after being filtered using Whatman No. 1 filter paper. The yield of the extracts was recorded and stored for further experiments at 4 °C.

### Phytochemical assay

The extracts were subjected to detect the presence of varied phytoconstituents using standard protocols (Roghini and Vijayalakshmi, 2018) [28].

### Total tannins content (TTC) determination

The TTC was estimated according to the method of Peri and Pompei (1971) [24]. The absorbance of the sample was determined at 725 nm, and TTC were given as mg GAE/g of dry extract.

### Determination of Total phenol content (TPC)

According to the method of Lefahal *et al.* (2018) [8], the TPC was calculated. The absorbance of the sample was measured at 765 nm and TPC was reported as mg GAE/g of dry extract.

### Determination of Total flavonoid content (TFC)

The TFC was carried out according to the technique of Lefahal *et al.*, (2018) [8]. The absorbance of the sample was measured at 510 nm and calculated as mg QE/g of dry extract.

### Antibacterial activity

With using the method of the agar well diffusion assay, the antibacterial activities of extracts were assessed against gram-positive (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, and *Staphylococcus aureus*) and gram-negative (*Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella flexneri*, and *Salmonella typhi*) bacterial cultures. On sterile Mueller Hinton agar medium plates, test cultures that had grown overnight were swabbed, and wells were made to pour the extracts (100  $\mu$ L; 50-100  $\mu$ g/mL), and incubated at 37 °C for 24 h. The antibacterial activity of the extracts was revealed by measuring the inhibition zone (in mm).

### In vitro antioxidant activity

#### DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

After combining 2.5 mL of 0.1 mM DPPH solution with several concentrations of extracts (5-320  $\mu$ g/mL), the mixture was incubated for 30 minutes at room temperature in a dark space. At 517 nm, the mixture's optical density, or absorbance, was measured. The standard utilized was ascorbic acid (Perumal and Saravanabhavan, 2018) [25]. The DPPH inhibition (%) was calculated as mentioned below:

$$\% \text{ inhibition} = [(\text{OD of test-OD control} / (\text{OD control})) \times 100]$$

#### ABTS (2,2-axino-bis (3-rthylbenzothiazoline-6-sulphonic acid) assay

Extracts ranging in concentration from 5 to 320  $\mu$ g/mL were combined with a solution of ABTS (7 mM) and K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (140 mM). The mixture was measured at 680 nm after 30 minutes of incubation at 37 °C. The standard utilized was ascorbic acid (Perumal and Saravanabhavan, 2018) [25]. The ABTS inhibition (%) was calculated as mentioned below:

$$\text{ABTS inhibition} = [(\text{OD of test-OD control} / (\text{OD control})) \times 100]$$

#### Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) assay

Different concentrations (5-320  $\mu$ g/mL) of the extracts were added into H<sub>2</sub>O<sub>2</sub> solution (2mM). Following a 10-min incubation time, at 230 nm, the OD was determined with a blank solution. The standard utilized was ascorbic acid (Ruch *et al.*, 1989) [29]. The H<sub>2</sub>O<sub>2</sub> inhibition (%) was calculated as mentioned below:

$\text{H}_2\text{O}_2$  inhibition = [(OD of test-OD control)/ (OD control)]  $\times 100$

### Nitric oxide (NO) assay

Different concentrations (5-320  $\mu\text{g/mL}$ ) of the extracts were added into 10 mM solution of sodium nitroprusside. The mixture was incubated at room temperature for about 2h. It was added 0.5 mL of Griess reagent after the incubation period, and the OD was measured at 546 nm (Sreejayan and Rao, 1997) [30]. The NO inhibition (%) was calculated as mentioned below:

NO inhibition = [OD of test-OD control]/ (OD control)]  $\times 100$

### Hydroxyl radical scavenging assay (HRSA)

The extracts were diluted with 0.5 mL of 0.018% v/v EDTA solution, 1 mL of 0.85% v/v DMSO, and 0.5 mL of ascorbic acid (0.22%) at varying doses (5-320  $\mu\text{g/mL}$ ). The mixture was then incubated for 15 minutes at 80-90  $^\circ\text{C}$  in a water bath. After the incubation period, 1 mL of low-temperature pre-cooled TCA at a concentration of 17.5% (w/v) was added to terminate the reaction. Once added, 3 mL of Nash reagent was allowed to sit at room temperature for 15 minutes. Using a reagent blank as a reference, the color intensity was measured at 412 nm (Klein *et al.*, 1991). To determine hydroxyl radical scavenging, the following formula was used:

HRSA inhibition = [(OD of test-OD control)/ (OD control)]  $\times 100$

### In vitro antidiabetic assay

#### $\alpha$ -amylase inhibition activity

With some slight modifications, the  $\alpha$ -amylase inhibition assay was carried out using Unuofin *et al.* (2018) [31] methodology. After adding 100  $\mu\text{L}$  of  $\alpha$ -amylase (0.1 mg/mL) solution, various doses (10-320  $\mu\text{g/mL}$ ) of the crude extracts were incubated for 15 minutes at room temperature. It was then further filled with 100  $\mu\text{L}$  of starch solution and allowed to sit at room temperature for one hour. It was then mixed with 100  $\mu\text{L}$  of iodine reagent and 10  $\mu\text{L}$  of 1M HCl. The standard was acarbose. The mixture was measured at 565 nm and  $\alpha$ -amylase inhibition (%) was calculated as mentioned below:

$\alpha$ -amylase inhibition = [(OD of test-OD control)/(OD control)]  $\times 100$

#### $\alpha$ -glucosidase assay

The  $\alpha$ -glucosidase inhibition assay was conducted adopting Apostolidis *et al.* (2007) [2] methodology. The 100 mM phosphate buffer (pH 6.8) was used to prepare the pNPG substrate solution. For about 10 minutes, 200  $\mu\text{L}$  of  $\alpha$ -glucosidase solution was combined with extracts at varying concentrations (10-320  $\mu\text{g/mL}$ ). Subsequently, 400  $\mu\text{L}$  of the prepared pNPG solution was added, and it was left to incubate for a further 20 minutes at room temperature. 1 mL of 0.1 M  $\text{Na}_2\text{CO}_3$  solution was added, and the release of 4-nitro phenol—a yellow-colored reaction mixture resulting from the degradation of pNPG—was measured by reading

the optical density at 405 nm. Voglibose was employed as positive control. To calculate  $\alpha$ -glucosidase inhibition, use this formula:

$\alpha$ -glucosidase inhibition = [(OD control-OD of sample)/ (OD control)]  $\times 100$

### Statistical analysis

All the experimental data in this study was carried out by mean  $\pm$  standard deviation (mean  $\pm$  SD).

## Results and Discussion

### Phytochemical screening

Phytochemicals have drawn a lot of interest since they can expand and shield sophisticated restorative pharmacophores (Patra *et al.*, 2020) [23]. Therefore, for the identification of the chemical status of active substances of the test plant, phytochemical screening was performed with particular attention to their antioxidant and anti-diabetic activity. Various species of *Persicaria* have been widely used as a traditional ethnomedicinal system of therapeutics in many countries. From the qualitative findings, it was perceived that ethyl acetate extract of *P. chinensis* leaves substantiated the presence of tannins, alkaloids, terpenoids, phenol, flavonoids, carbohydrate, glycoside, and protein; whereas saponins was completely absent. In the case of hexane and methanol extracts, saponins were found completely absent (Table 1). Different phytochemicals of various extracts contain several compounds, including triterpenoids, saponins, gums and mucilage, and flavones and flavanones (Lai *et al.*, 2012) +.

**Table 1:** Qualitative phytochemical analysis

| S.no | Phytochemical constituent | Hexane | Ethyl acetate | Methanol |
|------|---------------------------|--------|---------------|----------|
| 1    | Tannins                   | +      | +             | +        |
| 2    | Alkaloids                 | +      | +             | +        |
| 3    | Terpenoids                | +      | +             | +        |
| 4    | Phenol                    | +      | +             | +        |
| 5    | Flavonoids                | +      | +             | +        |
| 6    | Carbohydrate              | -      | +             | +        |
| 7    | Glycoside                 | +      | +             | +        |
| 8    | Proteins                  | +      | +             | +        |
| 9    | Saponins                  | -      | -             | -        |

**Note:** '+' represents present; '-' represents absent.

The total content of tannins, phenols and flavonoids is tabulated (as shown in Table 2). Among three extracts tested, total content of tannins, phenolic, and flavonoids were found higher in ethyl acetate extract which showed maximum TTC, TPC, and TFC of 31.27 $\pm$ 0.68 mg GAE/g of extract, 31.41 $\pm$ 1.56 mg GAE/g dry extract, followed by 36.37 $\pm$ 1.1 mg QE/g dry extract respectively. Methanol extract depicted TTC, TPC, and TFC of 23.02 $\pm$ 0.23 mg GAE/g dry extract, 26.56 $\pm$ 0.42 mg GAE/g dry extract, and 27.33 $\pm$ 1.08 mg QE/g dry extract, respectively; while hexane extract revealed TTC, TPC, and TFC of 17.63 $\pm$ 1.12 mg GAE/g of extract, 23.47 $\pm$ 0.56 mg GAE/g dry extract, followed by 21.20 $\pm$ 0.65 mg QE/g dry extract respectively.

**Table 2:** Quantitative phytochemical assay

| S. No | Phytochemical constituents (mg/g) | Hexane     | Ethyl acetate | Methanol   |
|-------|-----------------------------------|------------|---------------|------------|
| 1     | Total Tannins (mg GAE/g dw)       | 17.63±1.12 | 31.27±0.68    | 23.02±0.23 |
| 2     | Total Phenols (mg GAE/100g dw)    | 23.47±0.56 | 31.41±1.56    | 26.56±0.42 |
| 3     | Total Flavonoids (mg QE/g dw)     | 21.20±0.65 | 36.37±1.00    | 27.33±1.08 |

**Note:** values are mean of triplicate determination±standard deviation

### Antibacterial assay

Plethora of studies has reported the different medicinal plant extracts of the antibacterial potential in the past, and our present investigation supported the earlier reports by Astal *et al.* (2005) [3] and Khan *et al.* (2007) [13]. Our results illustrated the effectiveness of leaves extracts from *P. chinensis* in inhibiting all the test bacterial organisms, except for the hexane and ethyl acetate extract against *S.*

*flexneri* at 50 µg/mL, and methanol extract at the same concentration, which showed no activity against *S. typhi*. At a concentration of 100 µg/mL, ethyl acetate extract illustrated a maximum antibacterial activity of 26.66±2.08 mm against *S. aureus*. The present study revealed that ethyl acetate extract exhibited a strong bactericidal effect in comparison to hexane and methanol extracts (Table 3A-C).

**Table 3A:** Antibacterial activity (Zone of inhibition in mm) and Au of *P. chinense* hexane extract

| Bacteria                | Antibacterial activity Hexane extract (in mm) |            |             |                 |
|-------------------------|-----------------------------------------------|------------|-------------|-----------------|
|                         | 50(µg /mL)                                    | 75(µg /mL) | 100(µg /mL) | S <sup>10</sup> |
| <i>B. subtilis</i>      | 11.33±0.57                                    | 11.66±1.15 | 12.33±0.57  | 26.33±4.04      |
| <i>E. faecalis</i>      | 11.66±1.52                                    | 12.33±1.15 | 14.66±1.52  | 14.66±2.51      |
| <i>S. aureus</i>        | 13.66±1.15                                    | 15.33±1.52 | 16.33±2.51  | 18.33±2.08      |
| <i>S. saprophyticus</i> | 12.33±1.52                                    | 14.00±1.00 | 13.33±1.52  | 22.66±2.30      |
| <i>E. coli</i>          | 11.66±1.52                                    | 13.66±1.52 | 14.33±1.5   | 24.00±4.00      |
| <i>K. pneumonia</i>     | 10.33±0.57                                    | 11.66±0.57 | 13.66±0.57  | 16.33±1.52      |
| <i>S. flexneri</i>      | NA                                            | 11.66±0.57 | 12.33±1.15  | 15.33±2.51      |
| <i>S. typhi</i>         | 10.66±0.57                                    | 10.66±1.52 | 12.00±1.73  | 10.66±1.52      |
| <i>S. typhimurium</i>   | 11.66±0.57                                    | 14.66±0.57 | 14.00±2.00  | 16.66±1.52      |
| <i>P. aeruginosa</i>    | 10.00±1.00                                    | 10.33±0.57 | 12.53±0.59  | 18.66±1.52      |

Values and mean of experiments were performed in triplicate and data are expressed as Mean ± SD. NA= no activity

**Table 3B:** Antibacterial activity (Zone of inhibition in mm) of *P. chinensis* ethyl acetate extract

| Bacteria                | Antibacterial activity Ethyl acetate extract (in mm) |            |             |                 |
|-------------------------|------------------------------------------------------|------------|-------------|-----------------|
|                         | 50(µg /mL)                                           | 75(µg /mL) | 100(µg /mL) | S <sup>10</sup> |
| <i>B. subtilis</i>      | 13.33±1.52                                           | 14.33±1.15 | 14.66±0.57  | 14.66±1.52      |
| <i>E. faecalis</i>      | 13.66±1.15                                           | 16.00±1.73 | 17.33±1.52  | 15.33±0.57      |
| <i>S. aureus</i>        | 15.33±1.15                                           | 19.66±1.52 | 26.66±2.08  | 22.33±1.15      |
| <i>S. saprophyticus</i> | 13.00±1.73                                           | 14.66±1.52 | 14.66±1.52  | 24.00±2.00      |
| <i>E. coli</i>          | 12.66±0.57                                           | 16.33±1.52 | 16.66±1.15  | 24.33±2.08      |
| <i>K. pneumonia</i>     | 13.66±1.52                                           | 14.33±1.52 | 21.66±1.52  | 20.66±1.52      |
| <i>S. flexneri</i>      | NA                                                   | 13.66±1.52 | 14.33±1.15  | 20.33±2.08      |
| <i>S. typhi</i>         | 12.66±0.57                                           | 13.33±0.57 | 15.33±1.52  | 18.33±0.57      |
| <i>S. typhimurium</i>   | 13.66±1.15                                           | 15.33±0.57 | 17.66±1.52  | 20.33±2.51      |
| <i>P. aeruginosa</i>    | 12.33±0.57                                           | 15.33±1.15 | 16.33±0.57  | 21.00±2.64      |

Values and mean of experiments were performed in triplicate and data are expressed as Mean ± SD. NA= no activity

**Table 3C:** Antibacterial activity (Zone of inhibition in mm) of *P. chinensis* methanol extract

| Bacteria                | Antibacterial activity Methanol extract (in mm) |            |             |                 |
|-------------------------|-------------------------------------------------|------------|-------------|-----------------|
|                         | 50(µg /mL)                                      | 75(µg /mL) | 100(µg /mL) | S <sup>10</sup> |
| <i>B. subtilis</i>      | 12.33±1.15                                      | 13.33±2.08 | 12.00±2.64  | 10.66±1.52      |
| <i>E. faecalis</i>      | 10.66±2.08                                      | 13.33±2.08 | 13.66±2.51  | 13.66±1.15      |
| <i>S. aureus</i>        | 12.00±1.00                                      | 11.66±2.51 | 14.33±2.08  | 17.00±2.64      |
| <i>S. saprophyticus</i> | 12.33±0.57                                      | 11.33±1.52 | 11.66±2.08  | 14.33±1.15      |
| <i>E. coli</i>          | 12.33±2.08                                      | 14.33±0.57 | 13.66±2.08  | 24.66±3.51      |
| <i>K. pneumonia</i>     | 10.66±0.57                                      | 10.66±1.52 | 11.00±2.00  | 14.66±0.57      |
| <i>S. flexneri</i>      | 10.00±1.00                                      | 10.66±1.15 | 12.66±0.57  | 14.66±0.57      |
| <i>S. typhi</i>         | NA                                              | 11.33±0.57 | 11.33±1.52  | 13.66±2.51      |
| <i>S. typhimurium</i>   | 11.33±0.57                                      | 12.66±0.57 | 11.66±1.15  | 16.66±2.51      |
| <i>P. aeruginosa</i>    | 10.66±1.52                                      | 12.00±2.64 | 12.66±2.51  | 15.33±1.52      |

Values and mean of experiments were performed in triplicate and data are expressed as Mean ± SD. NA= no activity

### In vitro antioxidant assay

DPPH test is frequently used to assess the antioxidative potency of natural products (Oyaizu, 1986; Prior *et al.*, 2005) [21, 26]. In our present study, the *P. chinensis* solvent extracts of hexane, ethyl acetate and methanol were assessed for their capacity to scavenge radicals using the DPPH radical scavenging test (Fig. 1A), and it was observed that ethyl acetate extract exhibited a substantial dose-dependent scavenging of DPPH, in contrast to hexane and methanol extracts. Furthermore, the IC<sub>50</sub> values of the extracts and the control ascorbic acid were calculated as 35.26±0.15 µg/mL (hexane), 26.88±0.04 µg/mL (ethyl acetate), 57.15±0.34 µg/mL (methanol), and 3.54±0.40 µg/mL (ascorbic acid) respectively. The findings of the ABTS assay revealed that ethyl acetate extract demonstrated the most potent antioxidant properties at higher concentrations (Fig. 1B). The IC<sub>50</sub> values of the extracts and the control ascorbic acid were estimated as 157.39±0.04 µg/mL (hexane), 106.42±0.56 µg/mL (ethyl acetate), 144.12±0.33 µg/mL (methanol), and 3.54±0.40 µg/mL (ascorbic acid) respectively. The results for HRSA are shown in Fig. 1C. The IC<sub>50</sub> values for hexane, ethyl acetate, methanol extracts, and ascorbic acid were estimated as 226±0.51, 137.04±0.33, 173.10±0.61, and 3.54±0.40 µg/mL, respectively. Hydroxyl radical scavenging results depicted IC<sub>50</sub> values as 192.59±0.36 µg/mL (hexane), 117.86±0.49 µg/mL (ethyl acetate), 145.78±0.45 µg/mL (methanol), and 3.54±0.40 µg/mL (ascorbic acid), respectively (Fig. 1D). On the other hand, in terms of nitric oxide radical scavenging, the IC<sub>50</sub> values for hexane, ethyl acetate, methanol extracts, and ascorbic acid were estimated as 80.43±0.78, 17.04±0.49, 26.58±0.64, and 3.54±0.40 µg/mL, respectively (Fig. 1E). The amount of phenolic hydroxyl groups has a considerable impact on the free radical scavenging activity. The

substantial abundance of phenolic compounds in the extract of plants has attracted considerable interest owing to their

powerful antioxidant capabilities (Isik, 2020) <sup>[11]</sup>.

**Table 4:** The IC<sub>50</sub> values in antioxidant assays of different solvent leaves extracts of *P. chinensis*

| S. No. | Antioxidant assays | Hexane      | Ethyl acetate | Methanol    | Standard  |
|--------|--------------------|-------------|---------------|-------------|-----------|
| 1      | DPPH               | 35.26±0.15  | 26.88±0.04    | 57.15±0.34  | 3.54±0.40 |
| 2      | ABTS               | 157.39±0.04 | 106.42±0.56   | 144.12±0.33 |           |
| 3      | HPRSA              | 226±0.51    | 137.04±0.33   | 173.10±0.61 |           |
| 4      | HRSA               | 192.59±0.36 | 117.86±0.49   | 145.78±0.45 |           |
| 5      | NORSA              | 80.43±0.78  | 17.04±0.49    | 26.58±0.64  |           |

Each value was obtained by calculating the average and data are expressed as mean ± SD

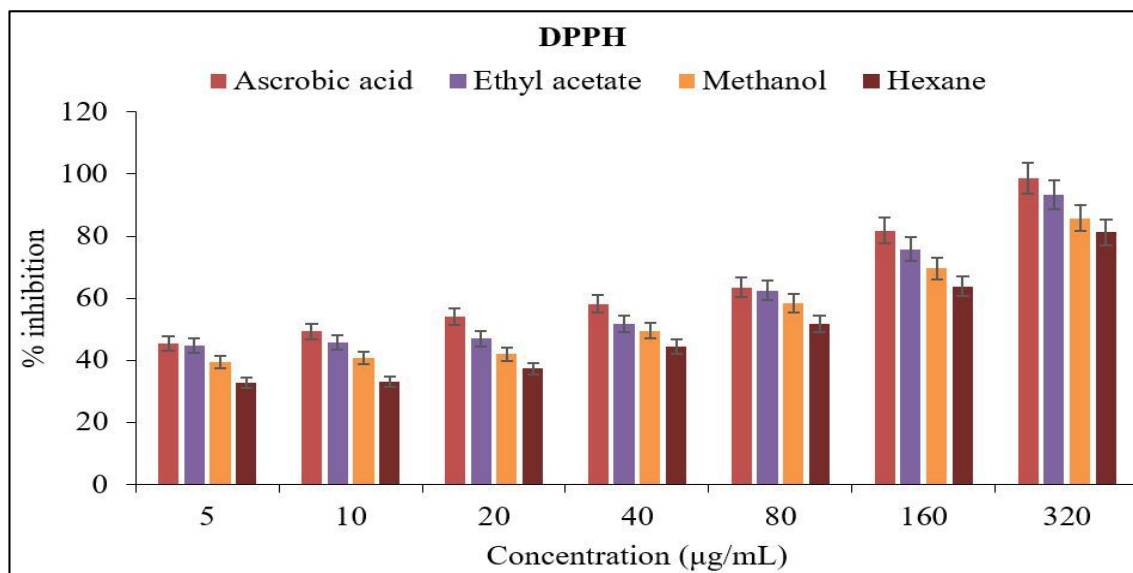


**Fig 1A:** DPPH scavenging activity of hexane, ethyl acetate and methanol leaves extract of *P. chinensis* and ascorbic acid at different concentration. Each value was obtained by calculating the average and data are expressed as mean ± SD

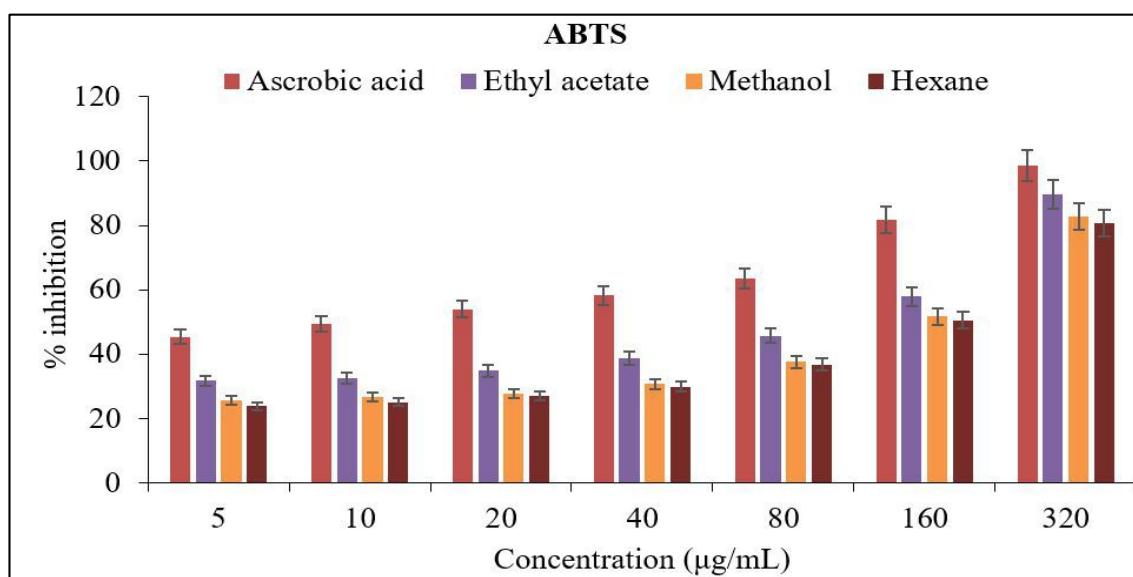


**Fig 1B:** ABTS scavenging activity of hexane, ethyl acetate and methanol leaves extract of *P. chinense* and ascorbic acid at different concentration. Each value was obtained by calculating the average and data are expressed as mean ± SD

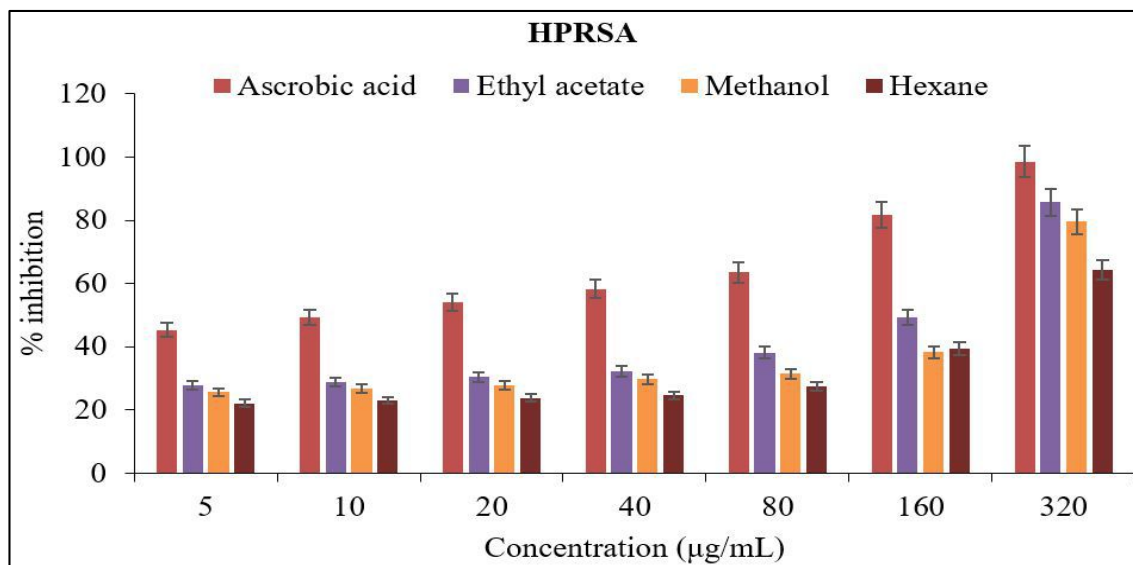


**Fig 1C:** HPRSA scavenging activity of hexane, ethyl acetate and methanol leaves extracts of *P. chinensis* and ascorbic acid at different concentration. Each value was obtained by calculating the average and data are expressed as mean  $\pm$  SD

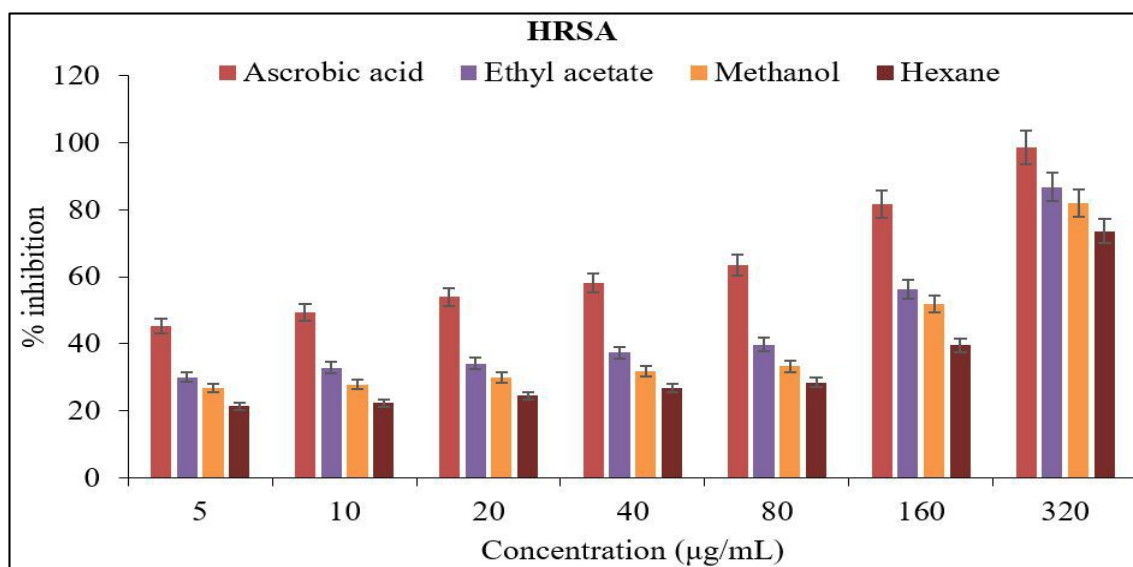


**Fig 1D:** HRSA scavenging activity of hexane, ethyl acetate and methanol leaves extracts of *P. chinensis* and ascorbic acid at different concentration. Each value was obtained by calculating the average and data are expressed as mean  $\pm$  SD

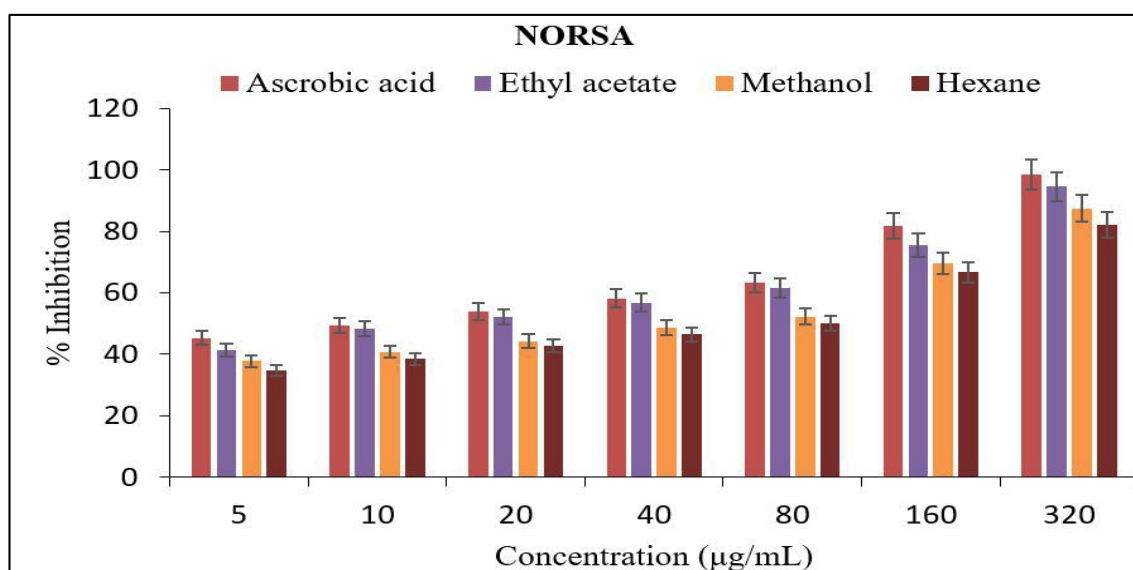


**Fig 1E:** NORSA scavenging activity of hexane, ethyl acetate and methanol leaves extracts of *P. chinensis* and ascorbic acid at different concentration. Each value was obtained by calculating the average and data are expressed as mean  $\pm$  SD

**In vitro antidiabetic assay**

Inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase can be a major strategy for managing postprandial blood glucose levels in patients with diabetic type 2. This is because a mixed carbohydrate diet can greatly lower the subsequent increase in blood sugar levels due to the enzymes that regulate the digestion of carbohydrate (Gong *et al.*, 2020) [10]. The present study demonstrated that the  $IC_{50}$  value of hexane, ethyl acetate and methanol extracts were calculated as  $8.17 \pm 0.73$ ,  $4.05 \pm 0.30$ , and  $6.52 \pm 1.06$   $\mu\text{g/mL}$ , respectively. Acarbose demonstrated potential inhibitory with an  $IC_{50}$  value of  $2.94 \pm 0.03$   $\mu\text{g/mL}$  (Fig. 2A).

$\alpha$ -glucosidase inhibitor delays the eupepsia of carbohydrate and decelerates the adsorption.  $\alpha$ -glucosidase inhibiting prospect of all the solvent leaves extracts were ascertained and the outcomes are illustrated in Fig. 2B. Previous studies had shown that miglitol and acarbose inhibit  $\alpha$ -glucosidase competitively, reducing the absorption of starch and disaccharides (Davis and Granner, 2001) [7]. However, in the

present study, Voglibose is employed as a standard drug for  $\alpha$ -glucosidase inhibitor. In this context, all the extracts revealed prominent  $\alpha$ -glucosidase inhibitory activity according to a dosage. However, ethyl acetate extract of *P. chinensis* displayed potent inhibitory activity with an  $IC_{50}$  value of  $6.81 \pm 1.11$   $\mu\text{g/mL}$ . The  $IC_{50}$  value of the control Voglibose was observed as  $4.05 \pm 0.05$   $\mu\text{g/mL}$ .

**Table 5:** The  $IC_{50}$  values in antidiabetic assays of different solvent leaves extracts of *P. chinensis* in comparison with standard drug Acarbose for  $\alpha$ -amylase and Voglibose for  $\alpha$ -glucosidase

| S. No. | Anti-diabetic assays        | Hexane           | Ethyl acetate   | Methanol        | Standard        |
|--------|-----------------------------|------------------|-----------------|-----------------|-----------------|
| 1      | $\alpha$ -amylase assay     | $8.17 \pm 0.73$  | $4.05 \pm 0.30$ | $6.52 \pm 1.06$ | $2.94 \pm 0.03$ |
| 2      | $\alpha$ -glucosidase assay | $11.95 \pm 1.01$ | $6.81 \pm 1.11$ | $9.31 \pm 1.52$ | $4.05 \pm 0.05$ |

Each value was obtained by calculating the average and data are expressed as mean  $\pm$  SD

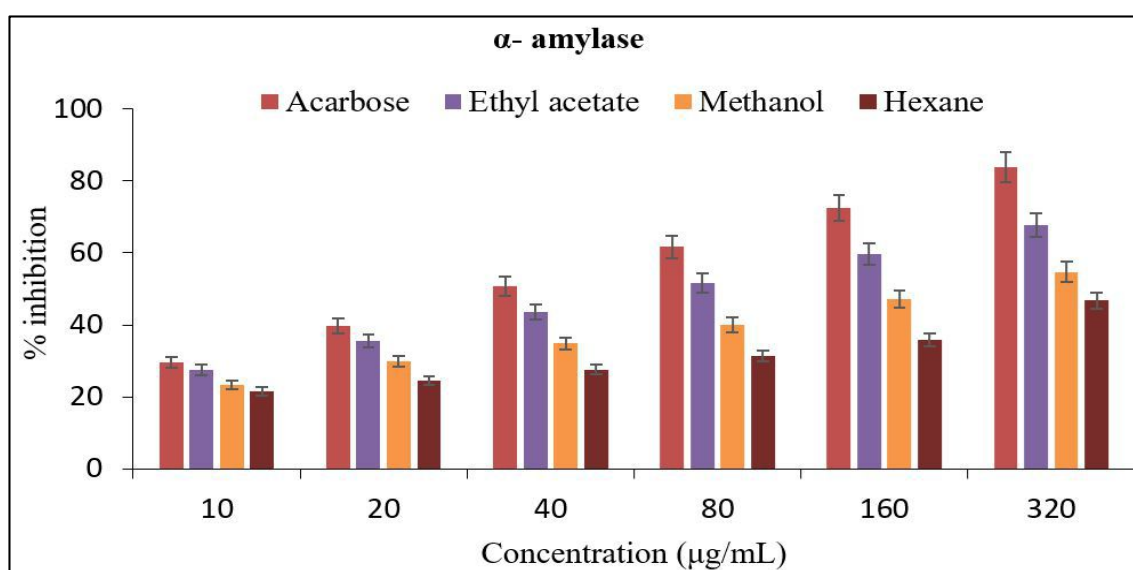


**Fig 2A:**  $\alpha$ -Amylase inhibitory activity of different concentration (10-320  $\mu\text{g/mL}$ ) of *P. chinensis*. (Hexane, ethyl acetate and methanol extracts and standard acarbose). Each value represents the mean  $\pm$  SEM of triplicate experiments

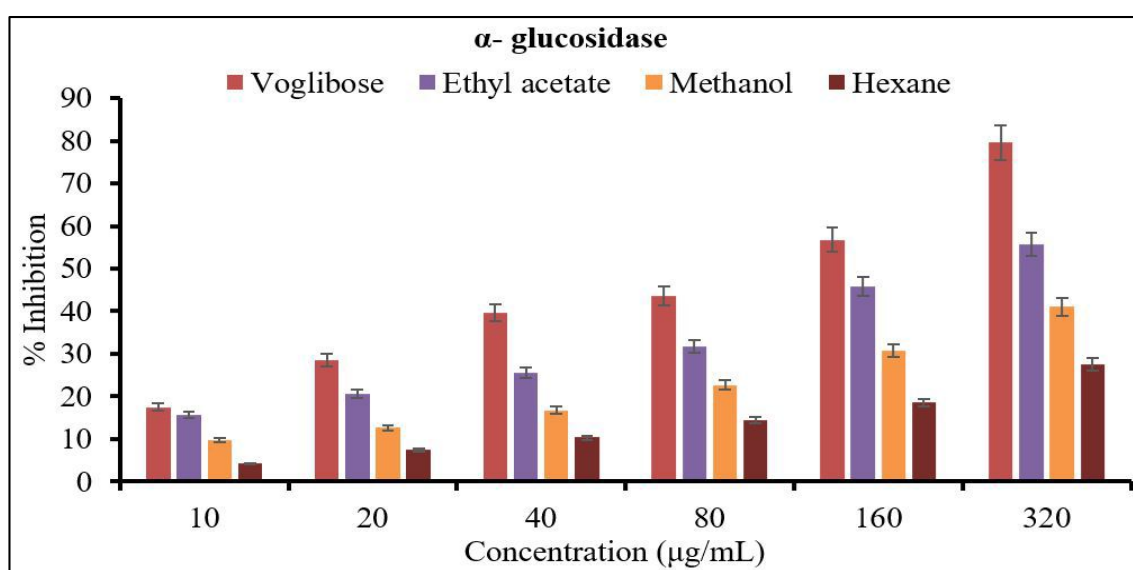


**Fig 2B:**  $\alpha$ -glucosidase inhibitory activity of different concentration (10-320  $\mu\text{g/mL}$ ) of *P. chinensis*. (Hexane, ethyl acetate and methanol extracts and standard Voglibose). Each value represents the mean  $\pm$  SEM of triplicate experiments

## Conclusion

In summary, *P. chinensis* leaves extracts indicated the existence of varied phytochemicals. Among these three extracts, ethyl acetate extract depicted maximum TTC, TPC, and TFC of  $31.27 \pm 0.68$  mg GAE/g of extract,  $31.41 \pm 1.56$  mg GAE/g dry extract, followed by  $36.37 \pm 1.00$  mg QE/g dry extract respectively. Ethyl acetate extract displayed a pronounced antibacterial effect against tested pathogens in comparison to hexane and methanol extracts. In addition, ethyl acetate extract showed promising antioxidant activities by scavenging free radicals significantly reduced IC<sub>50</sub> values in a dose-dependent manner. Likewise, ethyl acetate extract depicted satisfactory anti-diabetic activities by inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase in a dose dependent manner. This study will take further research to identify and isolate active bioactive compounds from ethyl acetate extract of *P. chinensis* leaves and suggest its potent traits for extensive pharmacological applications in future.

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