In vitro antioxidant, antimicrobial and anti-diabetic properties of polyphenols of Passiflora ligularis Juss. fruit pulp

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Abstract

In the present study, anti-radical, anti-diabetic and antimicrobial activities of different solvent extracts of Passiflora ligularis fruits were investigated. Among the various solvents, acetone extract displayed maximum total phenolic (640.70 mg GAE/g extract), tannin (214.30 mg GAE/g extract) and flavonoid contents (387.33 mg RE/g extract). Results of antioxidant studies revealed that the acetone extract of fruits possessed an efficient 2,2-diphenyl-1-picryl-hydrazyl (DPPH*) (IC50 19.13 μg/mL), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS**) (9800.94 μmol/L trolox equi/g extract), superoxide (78.27%) and nitric oxide (79.95%) radical scavenging activities, ferric reducing antioxidant power (43.06 mmol Fe (II)/mg extract), metal chelating (134.53 mg EDTA/g extract) ability. The acetone extract of P. ligularis fruits also exhibited significant (P<0.005) inhibition activities on α-amylase (82.56%) and α-glucosidase (75.36%) enzymes. Furthermore, the fruits of P. ligularis also demonstrated antibacterial activities against Gram (+) and Gram (−) bacteria and inhibited the fungal strains Candida albicans (14.85 mm) and Aspergillus niger (13.91 mm) in the disc diffusion method. Quantification of polyphenolics by HPLC showed the presence of ellagic acid, gallic acid, and rutin. Therefore, the results indicate that the fruit pulp of P. ligularis can serve as a potential antioxidative and antimicrobial agent in food and pharmaceutical industries.

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Keywords: Passiflora ligularis; Antimicrobial; α-Amylase; α-Glucosidase; Polyphenolics

1. Introduction

Currently the consumption of fruits and vegetables is gaining attention of worldwide for the health benefits which includes cardioprotective, anticancer, anti-diabetic and anti-obesity properties. The bioactive compounds such as polyphenols including flavonoids, tannins, catechins, vitamins C and E, β-carotene, etc., and several others confer these health protective benefits. The mixture of these compounds may provide better protection than single phytochemical due to their synergistic effects [1]. All these compounds have been ascribed to the scavenging of free radicals, reducing oxidative stress and preventing the oxidation of biomolecules that can break reaction chains of pathogens in the deterioration of physiological functions. Specifically free radicals produce cell damages, tissue injuries and increased levels of reactive oxygen species (ROS).

The source of oxidative stress is a cascade of ROS leaking from the mitochondria. This process has been associated with the onset of type 1 diabetes via apoptosis of pancreatic beta-cells and the onset of type 2 diabetes via insulin resistance. Furthermore, insulin deficiency also promotes β-oxidation of fatty acids, which results in increased formation of hydrogen peroxide. As such, under the diabetic condition the increased levels of ROS will damage the pancreas and liver cells. In recent diabetic treatments, α-amylase and α-glucosidase inhibitors are most warranted because they increase post-prandial hyperglycemic conditions. The antioxidant ability of phenolic compounds in fruits and vegetables could be attributed to their properties such as reducing agents, hydrogen donors, singlet hydrogen quenchers and/or metal ion-chelators [2]. Therefore, natural antioxidants can also inhibit the key enzymes α-amylase, α-glucosidase and control the post-prandial hyperglycemic conditions which are a potential approach to cure the type 2 diabetes mellitus [3].
Despite the development of numerous antimicrobial drugs in recent years, the incidence of multi drug resistance by pathogenic microorganisms has increased. Nowadays the therapeutic properties of plant polyphenols have also demonstrated antimicrobial effects by causing structural or functional damage to the bacterial cell membrane. Several studies have also shown that fruits rich in polyphenols exert both antioxidant and antimicrobial effects [4].

The species of Passifloraceae are very popular for their passion fruits wherein almost 294 volatile compounds of interest have been isolated from them [5]. Passiflora ligularis, commonly called as Sweet granadilla, grow in the cool highlands in Indonesia, New Guinea, Jamaica, Sri Lanka, India and several other tropical regions of the world. Morton [6] have previously reported that the P. ligularis fruit pulp consists of considerable amount of nutrients like protein, carbohydrates, amino acids, vitamin C and crude fiber. The peel possesses high molecular weight polysaccharides like xylose, glucose, galactose, galactosamine, and fructose [7]. Several types of fruits of Passifloraceae have been found to possess antioxidant and antimicrobial effects. However, the fruits of P. ligularis have not been exposed to antioxidant and pharmacological investigations. Therefore the present study aimed to evaluate the in vitro antioxidant and antimicrobial activity of the pulp extract of the P. ligularis fruit.

2. Materials and methods

2.1. Chemicals and reagents

All the antioxidant, anti-diabetic, antimicrobial chemicals and standards were obtained from Himedia (Mumbai, India) and Sigma–Aldrich (St. Louis, MO, USA). Petroleum ether, chloroform, acetone, methanol and all the culture media were purchase from Himedia. All other reagents used were of analytical grade.

2.2. Preparation of extracts

P. ligularis fruits were collected from Western Ghats of Tamil Nadu, India. The size of fruits slightly varies from one to the other, with an average weight of 65.34 g. The peel was orange in color and average length of the fruit was 7.14 cm and width of the fruit was 5.78 cm. The fruit pulps were lyophilized (4KBTXL-75; Vir Tis Benchtop K, New York, USA) and the fruit pulp powder was extracted by percolation using Soxhlet extractor (3840; Borosil Glass Works Ltd., Mumbai, India) with different organic solvents including petroleum ether, chloroform, acetone and methanol. Different solvent extracts were concentrated using a rotary vacuum evaporator (Yamato BO410, Yamato scientific Co., Ltd, Tokyo, Japan) and then dried. The extracts thus obtained were used directly to assess the antioxidant potential through various in vitro assays.

2.3. Quantification of total phenolics, tannins and flavonoid contents

The total phenolic and tannin contents of P. ligularis fruit pulp extracts were determined using the method described by Sidduraju and Manian [8]. One hundred microliters of each plant extract was taken into test tubes and made up to 1 mL with distilled water. Then 0.5 mL Folin-ciocalteu phenol reagent (1:1 with water) and 2.5 mL sodium carbonate solution (20%) were added sequentially in each tube. After that the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. Using the same extract, the content of tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP). One hundred milligrams of PVPP was weighed into a 100 mm × 12 mm test tube and 1.0 mL distilled water and 1.0 mL of tannin containing phenolic extract were added. The content was vortexed and incubated at 4 °C for 4 h. Then the sample was centrifuged (3,000 × g for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics without tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured as mentioned above. From the above results, the tannin content of the sample was calculated as the difference between total phenolics and non-tannin phenolics [9]. These analysis were performed in triplicates and the results were expressed as the gallic acid equivalents (GAE).

The flavonoid content was determined according to the method described by Zhishen et al. [10]. 0.5 mL of extract aliquot (1 mg/mL) was mixed with 2 mL distilled water and subsequently with 0.15 mL of 5% NaNO₂ solution. After 6 min, 0.15 mL of 10% AlCl₃ was added and the mixture was allowed to stand for 6 min, and then 2 mL of 4% NaOH solution was added to the mixture. Distilled water was immediately added to bring the final volume to 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm against the water blank. Rutin was used as the standard compound for the quantification of total flavonoids and the results were expressed as rutin equivalents (RE).

2.4. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant capacity of different solvent extracts of P. ligularis fruit pulp was estimated by the method described by Pulido et al. [11]. FRAP reagent (900 µL), prepared freshly and incubated at 37 °C, was mixed with 90 µL of distilled water and 50 µL test sample (1 mg/mL). The test samples and the reagent blank were incubated at 37 °C for 30 min in a water bath. At the end of incubation, the absorbance was taken immediately at 593 nm using a spectrophotometer. Methanolic solutions with known Fe (II) concentration, ranging from 100 to 2000 µmol/L (as FeSO₄·7H₂O), were used for the preparation of the calibration curve. The FRAP values were expressed as mmol/L Fe (II) equivalent/mg extract.

2.5. Metal chelating activity

The chelation of metal ions by various extracts of fruit pulp of P. ligularis was done by the method of Dinis et al. [12]. One hundred microliters plant extracts was added to 50 µL 2 mmol/L FeCl₂. The reaction was initiated by addition of 200 µL of 5 mmol/L ferrozine and the mixture was shaken vigorously and
left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank. The chelating activity of the extracts was evaluated using EDTA as a standard. The results were expressed as mg EDTA equivalent/g extract.

2.6. DPPH radical scavenging activity

Radical scavenging activity of different solvent extracts of *P. ligularis* fruit pulp was measured by DPPH radical scavenging method, described by Blois [13]. Plant extracts at various concentrations were taken and the volume was adjusted to 100 μL with ethanol. 5 mL of 0.1 mmol/L ethanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm. IC₅₀ values of the extract, i.e., the concentration of extract necessary to decrease the initial concentration of DPPH by 50%, was calculated. A lower IC₅₀ value indicates higher activity.

2.7. ABTS cation radical scavenging activity

ABTS cation radical decolorization assay was measured according to the method described by Re et al. [14]. ABTS⁺⁺ was produced by reacting 7 mmol/L ABTS aqueous solution with 2.4 mmol/L potassium persulfate in the dark for 12–16 h at room temperature. Prior to the assay, this solution was diluted in ethanol (about 1:89, V/V) and equilibrated at 30 °C to give an absorbance at 734 nm of 0.700 ± 0.02. After the addition of 1 mL diluted ABTS solution to 10 μL sample or trolox standards (final concentration 0–15 μmol/L) in ethanol, absorbance was measured at 30 °C exactly 30 min after the initial mixing. Solvent blanks were also run in each assay. The total antioxidant activity (TAA) was expressed as the concentration of trolox having equivalent antioxidant activity in terms of μmol/L/g sample extract.

2.8. Superoxide radical scavenging activity

The assay was based on the capacity of the plant extract to inhibit formazan formation by scavenging superoxide radicals generated in riboflavin–light–nitro blue tetrazolium (NBT) system [15]. Each 3 mL reaction mixture contained 50 mmol/L sodium phosphate buffer (pH 7.6), 20 μg riboflavin, 12 mmol/L EDTA, 0.1 mg NBT and 1 mL sample solution (50–250 μg/mL). Reaction was started by illuminating the reaction mixture with different concentrations of sample extracts for 90 s. Immediately after illumination, the absorbance was measured at 590 nm. Identical tubes with reaction mixture kept in dark served as blanks. The inhibition rate of superoxide anions generation was calculated as

\[
\text{Inhibition/\%} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100
\]

2.9. Hydroxyl radical scavenging activity

The scavenging activity of different solvent extracts of fruit pulp of *P. ligularis* and the reference standard rutin and BHT was measured according to Klein et al. [16]. 100 μg/mL of extract in different test tubes were mixed with 1.0 mL iron-EDTA solution (0.13% ferrous ammonium sulphate, 0.26% EDTA disodium salt), 0.5 mL of EDTA solution (0.018%, pH 8.0), and 1.0 mL dimethyl sulfoxide sequentially. The reaction was initiated by adding 0.5 mL ascorbic acid (0.22%) and incubated at 80–90 °C for 15 min in a water bath. After incubation, the reaction was terminated by addition of 1.0 mL ice cold trichloroacetic acid (17.5%, m/V). Subsequently, 3.0 mL Nash reagent (75.0 g ammonium acetate, 3.0 mL glacial acetic acid, and 2 mL acetyl acetone were mixed and raised to 1 L with distilled water) was added to each tube and left at room temperature for 15 min. The reaction mixture without the sample was used as a control. The intensity of the solution was measured spectrophotometrically at 412 nm against the reagent blank. The inhibition rate of hydroxyl radical scavenging activity was calculated as per formula (1).

2.10. Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity of different solvent extracts of *P. ligularis* fruit pulp was calculated according to the method of Sreejayan and Rao [17]. Sodium nitroprusside (10 mmol/L) in phosphate buffered saline, was mixed with fruit pulp extracts of different concentrations and incubated at room temperature for 150 min. Griess reagent (0.5 mL containing 1% sulphanilamide, 2% H₃PO₄ and 0.1% N (1-naphthyl) ethylene diamine dihydrochloride was added to the mixture after incubation time. The absorbance of the chromophore formed was read at 546 nm. BHT, rutin and the same mixture of the reaction without plant extracts were employed as the positive and negative control. The inhibition percentage of nitric radical generation was calculated as per formula (1).

2.11. α-Amylase inhibition assay

Different concentrations of the fruit extracts and 500 μL of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) containing porcine pancreatic α-amylase enzyme (0.5 mg/mL) were incubated at 25 °C for 10 min. After the incubation, 500 μL of 1% starch solution in 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) was added to the reaction mixture. Subsequently, the reaction mixture was incubated at 25 °C for 10 min, followed by addition of 1.0 mL of dinitrosalicylic acid (DNS). Finally the reaction was stopped by incubation in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 10 mL distilled water, and the absorbance was measured at 540 nm [18]. The mixture of all other reagents and the enzyme except the test sample was used as a control and the results of α-amylase inhibition activity were expressed in terms of inhibition percentage.
2.12. α-Glucosidase inhibition assay

Various amounts of fruit extracts (50–250 μL) and 100 μL α-glucosidase (0.5 mg/mL) in 0.1 mol/L phosphate buffer (pH 6.9) solution were incubated at 25 °C for 10 min. Then, 50 μL of 5 mmol/L 3-nitrophenyl-α-D-glucopyranoside in 0.1 mol/L phosphate buffer (pH 6.9) solution was added. Reaction mixtures were incubated at 25 °C for 5 min and the absorbance was taken at 405 nm by a spectrophotometer [19]. The mixture of all other reagents and the enzyme except the test sample was used as a control and the results of α-glucosidase inhibition activity were expressed in terms of inhibition percentage.

2.13. Antimicrobial activity

Different solvent extracts of <i>P. ligularis</i> fruit pulp were separately tested against a group of pathogenic micro-organisms including <i>Streptococcus fecalis</i>, <i>S. pyogenes</i>, <i>Bacillus subtilis</i>, <i>Klebsiella pneumoniae</i>, <i>Salmomella paratyphi</i>, <i>S. typhi</i> A, <i>S. typhi</i> B and pathogenic fungi <i>Candida albicans</i> and <i>Aspergillus niger</i>. The pure bacterial and fungal strains were obtained from the PSG Medical College and Hospital, Coimbatore, Tamil Nadu, India. Bacterial strains were cultured for 24 h at 37 °C on nutrient agar (NA, Himedia, India), while the fungal strains were cultured for 24–48 h at 37 °C using potato dextrose agar (PDA, Himedia, India).

2.13.1. Disc diffusion method

<i>P. ligularis</i> fruit pulp extracts were dissolved in relevant solvents, to a final concentration of 300 mg/mL. The antimicrobial test was carried out by national committee for clinical laboratory standards NCCLS disc diffusion method [20]. Briefly, 100 μL of suspension containing approximately 10<sup>8</sup> colony form units (CFU)/mL of bacterial cells and 10<sup>4</sup> cells/mL of fungal cells were spread on to NA and PDA medium, respectively. For the antimicrobial test, paper discs (6 mm diameter) were separately impregnated with 15 μL of the 300 mg/mL plant extracts (4500 μg/disc) and placed on the inoculated agar. For the positive control, paper discs were impregnated with 15 μL of ampicillin (4500 μg/disc) dissolved in both types of solvents used. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. For the antifungal test, <i>P. ligularis</i> fruit pulp extracts were dissolved in their respective solvents to a final concentration of 300 mg/mL. Paper discs were impregnated with 30 μL of 10 mg/mL extracts (300 μg/disc) and placed onto the inoculated agar. As a positive control, fluconazole was prepared by dissolving it in both solvents used to a final concentration of 1 mg/mL. Paper discs for the positive control were impregnated with 30 μL fluconazole (30 μg/disc). Negative controls were prepared again using the same solvents employed to dissolve the plant extracts. Plates were incubated at 37 °C for 18–24 h for bacterial strains and for 24–48 h for fungal strains. Antimicrobial activity was assessed by measuring the diameter of the growth inhibition zone (IZ) in millimeters (including the disc diameter of 6 mm) for the test organisms compared to controls.

2.14. HPLC analysis

The acetone extract of <i>P. ligularis</i> fruit pulp were subjected to HPLC analysis along with standard chemical markers on High Performance Liquid Chromatography system (Beckman, USA) equipped with UNIPoint system software and the chromatographic separations were performed using a C<sub>18</sub> Kromiacil Column (250 mm × 4.6 mm), with a flow rate of 1 mL/min. 20 μL phenolic standards (gallic acid, caffeic acid, rutin, ellagic acid, quercetin and kaempferol) and the plant extracts (2 mg/mL in methanol) were prepared to obtain a final concentration of 100 μg/mL in methanol. They were loaded, injected by the auto sampler and eluted through the column with a mobile phase system consisting of water-0.4% acetic acid-methanol-acetonitrile (70:20:5:5). The sample was monitored by UV detector at 340 nm at ambient temperature. Peak purity was checked by the software contrast facilities and the quantification of phenolic compounds was calculated using the following equation:

\[
C_c = \frac{A_c}{A_{st} \times C_{st}}
\]

where \(C_c\) is the concentration of the compound in the sample; \(A_c\) is the peak area of the compound in the sample chromatograms; \(C_{st}\) is the concentration of the standard in the reference solution and \(A_{st}\) is the area of the peak for the standard in the reference chromatograms [21].

Statistical analysis was carried out by analysis of variance (ANOVA) followed by Duncan’s test. \(P < 0.05\) was considered as indicative of significance, as compared to the control group using the SPSS (version 17.0).

3. Result and discussion

3.1. Total phenolics, tannin and flavonoid contents

The results of total phenolics, tannin and flavonoid content of <i>P. ligularis</i> fruit pulp are shown in Table 1. The acetone extract of fruit pulp recorded the highest total phenolic content (640.70 mg GAE/g extract) compared to other solvent extracts. In addition to it, the acetone extract also recorded the maximum contents of tannin (214.30 mg GAE/g extract) and flavonoid (387.33 mg RE/g extract). Phenols from food plants constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators [22]. Calderon [23] reported that the fruits of <i>P. mollissima</i> also contained higher amounts of phenolics (635 ± 2.71 mg GAEs/100 g extract). It has also been reported that several other species of <i>Passiflora</i> leaves/fruit/roots also have higher amounts of phenolics/flavonoids in the acetone extracts [25].

3.2. Ferric reducing antioxidant power (FRAP) assay

The results of FRAP assay exhibited by the different solvent extracts of <i>P. ligularis</i> are shown in Table 2. All the solvent extracts possessed the ferric reducing ability. However, the acetone extract showed the maximum degree of reduction (43.06 mmol Fe (II)/mg extract).
3.4. Total phenolics, tannin and flavonoid contents of *P. ligularis* fruit pulp.

<table>
<thead>
<tr>
<th>Solvent extract</th>
<th>Total phenolics (mg GAE/g extract)</th>
<th>Tannin (mg GAE/g extract)</th>
<th>Flavonoids (mg RE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>102.40 ± 1.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>38.60 ± 1.89&lt;sup&gt;e&lt;/sup&gt;</td>
<td>218.33 ± 1.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chloroform</td>
<td>125.70 ± 2.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>65.23 ± 2.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>178.00 ± 2.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetone</td>
<td>640.70 ± 2.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>234.30 ± 4.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>387.33 ± 1.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol</td>
<td>137.90 ± 1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.90 ± 3.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>233.33 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Note: Values are expressed as mean ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference (P < 0.05). Similarly herein after.*

### Table 2

<table>
<thead>
<tr>
<th>Solvent extract</th>
<th>FRAP (mmol Fe (II)/mg extract)</th>
<th>Metal chelating activity (mg EDTA/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>20.51 ± 0.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61.32 ± 0.55&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chloroform</td>
<td>22.82 ± 0.18&lt;sup&gt;e&lt;/sup&gt;</td>
<td>79.25 ± 0.78&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetone</td>
<td>43.06 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.53 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol</td>
<td>35.30 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>113.79 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### 3.3. Metal chelating activity

Iron, in nature, can be found as either ferrous (Fe<sup>2+</sup>) or ferric ion (Fe<sup>3+</sup>), with ferric ion predominating in foods. Ferrous ions (Fe<sup>2+</sup>) chelation may render important antioxidative effects by retarding metal-catalyzed oxidation. Ferrous ions (Fe<sup>2+</sup>) are the most powerful pro-oxidants among the various species of metal ions. Minimizing ferrous (Fe<sup>2+</sup>) ions may afford protection against oxidative damage by inhibiting production of ROS and lipid peroxidation. The metal chelating activity of fruit pulp of *P. ligularis* is shown in Table 2. The fruit pulp extracts were assessed for their ability to compete with ferrozine for ferrous iron in the solution. In this assay, the fruit pulp interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are capable of capturing ferrous iron before ferrozine. The acetone extract reduced the red color complex immediately and showed the highest chelating activity (134.53 mg EDTA/g extract) followed by the methanol extract.

### 3.4. Free radical scavenging activity

Radical scavenging activities of different solvent extracts of fruit pulp was investigated in DPPH<sup>•</sup>, ABTS<sup>••</sup>, O<sub>2</sub>•, •OH and NO<sup>•</sup> scavenging assays and the results are depicted in Figs. 1–3. The result suggested that the acetone extract of fruit pulp of *P. ligularis* exhibited the highest activity (IC<sub>50</sub> 19.13 µg/mL), followed by the methanol extract (IC<sub>50</sub> 23.71 µg/mL) (Fig. 1). The efficiency of ABTS cation radical scavenging activity of different solvent extracts of fruit pulp of *P. ligularis* revealed that the acetone extract of fruit pulp possessed the utmost activity (9800.94 µmol/L trolox equi/g extract) (Fig. 2). Furthermore, the results of O<sub>2</sub>•, •OH and NO<sup>•</sup> scavenging activities also demonstrated that the acetone extract of fruit pulp had the highest scavenging activity, being 78.27%, 77.9% and 79.95%, respectively.
DPPH• and ABTS** scavenging activities involve hydrogen atoms transfer and electrons transfer [24]. The results of the present investigation explains that the acetone extract of fruit pulp of P. ligularis may contain enormous amount of hydrogen donor molecules which may reduce the production of radicals and the decolorization in the DPPH• and ABTS** assays. Earlier study had also reported that the fruit peel, flower, leaves and seeds in the species of P. foetida exhibited good DPPH• and ABTS** scavenging activities [25].

Superoxide is also one of the most harmful radicals and its scavenging is necessary because it is a precursor for other major ROS, like hydrogen peroxide, hydroxyl and singlet oxygen. Numerous biological reactions generate superoxide radicals [26]. The hydroxyl radical is an exceedingly deleterious free radical produced in biological systems, being able to damage almost every molecule found within living cells. It has been considered as a vastly destructive group in the free radical pathology. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins [27]. Besides, nitric oxide plays an important role the production of radicals and inflammatory process in animal cells. The results make it evident that all the solvent extracts of P. ligularis fruit pulp were able to scavenge hydroxyl radicals at all concentrations tested. However, the higher amount of phenolic content present in the acetone extract of fruit pulp of P. ligularis may be the reason for its high O2•−, •OH and NO• scavenging properties.

3.5. α-Amylase inhibition activity

Pancreatic α-amylase, as a key enzyme in the digestive system, is involved in the breakdown of starch into disaccharides and oligosaccharides and finally liberating glucose which is later absorbed into the blood circulation. Inhibition of α-amylase would diminish the breakdown of starch in the gastro-intestinal tract. Therefore, the postprandial hyperglycemia level may also be reduced [28]. The inhibition of α-amylase enzyme activity of P. ligularis fruit pulp and standard drug acarbose is presented in Fig. 4. Obtained results revealed that the acetone and methanol extracts inhibit α-amylase enzyme in a dose-dependent manner (75–125 µg/mL). Compared with the standard acarbose, the acetone extract of plant sample showed significant inhibition activity (82.56%). P. nitida leaf extract also inhibited α-amylase enzymes (63%) [29]. Shobana et al. [30] reported that the phenolic-rich plant extracts have higher ability to inhibit α-amylase enzyme. Plant-derived phenolics and natural antioxidants are recently warranted because of their less severe side effects [31]. Therefore, the high phenolic content and antioxidant potential of the acetone extract of P. ligularis fruit pulp might be the reason for α-amylase inhibition activity.

3.6. α-Glucosidase inhibition assay

High concentration of blood sugar can cause diabetic complications which affects the internal organ functions. Hence the diabetic treatment is mainly focused on the reduction in the oscillations of blood sugar levels and complications. In the current scenario the anti-diabetic treatment is concerned toward the inhibition of α-glucosidase. Generally, the drugs acting as α-glucosidase inhibitors are considered as oral hypoglycemic agents owing to their role of inhibiting the degradation of disaccharides to monosaccharides, thereby maintains the blood sugar level normal (UK prospective diabetes study) [32]. Fig. 5 shows α-glucosidase inhibitory activity of different extracts of P. ligularis fruit pulps. The results revealed that all the extracts of P. ligularis fruit pulps had potential inhibitors of α-glucosidase. The acetone extract showed the most significant inhibition activity of 75.36% in a concentration-dependent manner (75–125 µg/mL), which is comparable with the standard acarbose (79.87%). Montefusco-Pereira et al. [29] reported that the P. nitida inhibited α-glucosidase with an IC50 value of (6.78 ± 0.31) µg/mL. (72%). Compared with the P. nitida leaf, P. ligularis fruit pulp exhibited higher α-glucosidase inhibition activity, which may be due to its high phenolic, flavonoid contents and antioxidant activity of fruit pulp. Phenolic compounds are a naturally occurring compounds. Most of these compounds possess rich sources of antioxidant and free radical scavenging properties as well as medicinal properties and have long been used as anti-diabetic drugs. Flavonoids are natural phenolic compounds with several biological activities. These phytochemicals with strong antioxidant properties have been reported to be good inhibitors of α-glucosidase, and also regulators of hyperglycemia and other diabetic complications arising from oxidative stress [29].

3.7. Antimicrobial activity

The in vitro antimicrobial activities of the different solvent extracts of P. ligularis fruit pulp against the tested
Table 3
Antimicrobial activity of *P. ligularis* fruit pulp.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibition zone/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ampicillin</td>
</tr>
<tr>
<td><em>S. fecalis</em></td>
<td>15.45 ± 0.23 +</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>16.87 ± 0.12 +</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>17.76 ± 0.21 +</td>
</tr>
<tr>
<td><em>S. paratyphi</em></td>
<td>28.01 ± 0.50 +</td>
</tr>
<tr>
<td><em>S. typhi A</em></td>
<td>31.01 ± 0.12 +</td>
</tr>
<tr>
<td><em>S. typhi B</em></td>
<td>32.30 ± 1.00 +</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>33.21 ± 0.65 +</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>–</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>–</td>
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Note: + Significantly different (*P* < 0.05) compared to the positive control.

Fig. 6. HPLC profiles of mixed phenolic standards (A) and acetone extract of *P. ligularis* fruit pulp (B).

Microorganisms were qualitatively assessed by the inhibition zones. According to the results in Table 3, the acetone extract of *P. ligularis* fruit pulp exhibited a potent inhibitory effect against all bacteria especially *S. paratyphi* (28 mm), *S. typhi A* (31 mm), *S. typhi B* (32 mm) and *K. pneumonia* (32 mm). The acetone extract showed more inhibition against *C. albicans* (14.85 mm) and *A. niger* (13.91 mm). The greater inhibition zone, indicate the greater antibacterial and antifungal activity the extract has. The results from the disc diffusion method indicated that the tested *P. ligularis* fruit pulp extracts has higher inhibition of antibacterial and antifungal effects against both Gram-positive and Gram-negative bacteria and fungal strains. The results were comparable with the standard drug, ampicillin and fluconazole.
3.8. Quantification of phenolic compounds by HPLC

The polyphenolic profiles of *P. ligularis* fruit are shown in Fig. 6. The quantification of polyphenolic compounds by HPLC showed that *P. ligularis* fruit contained gallic acid (21.22 mg/g extract), caffeic acid (26.22 mg/g extract), rutin (33.89 mg/g extract), ellagic acid (62.44 mg/g extract) and kaempferol (3.05 mg/g extract) by comparing the chromatogram of the reference standards. Retention time for the acetone extract of *P. ligularis* at 4.346, 8.769, 14.848, 16.164 and 20.235 min corresponded well with the standard chromatogram peaks of gallic acid, caffeic acid, rutin, ellagic acid and kaempferol at 4.479, 9.434, 14.571, 16.078 and 20.834 min, respectively. From the results, the presence of ellagic acid in higher concentration could contribute to the excellent antioxidant, anti-inflammatory, chemopreventive and antiatherogenic properties of the plant extracts [33]. Earlier literatures have also reported that the leaf extracts of *P. alata* and *P. edulis* were rich in polyphenols, especially C-glycosyl derivatives of apigenin and luteolin, such as vitexin, isovitexin, orientin and isoorientin [34].

Phenolic compounds are a category of phytonutrients with strong antioxidant properties and the relationships between phenolic content and antioxidant activity have been reported earlier [35]. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations. The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity, and this is referred to as structure–activity relationships (SAR). In the case of gallic acid, the higher degree of hydroxylation, *i.e.* the trihydroxylated condition, the higher antioxidant activity it shows. With regard to kaempferol, a double bond between C-2 and C-3, combined with a 3-OH, in ring C enhances the active radical scavenging capacity of this flavonoids [23]. Synergism has been demonstrated in various combinations of flavones and flavonols in antimicrobial activity. For instance, the flavonoids quercetin and naringenin significantly inhibited bacterial motility [36] and epicatechin gallate along with their analog was effective against methicillin-resistant *Staphylococcus aureus* [37]. Hence, it could be concluded that synergism between naturally occurring polyphenolic compounds is in no way inferior to a single compound of biological interest. Therefore, the presence of these compounds may be the reason for the excellent antioxidant, anti-diabetic, antibacterial and antifungal activity seen in the fruit pulp of *P. ligularis*.

4. Conclusion

From the observed results we concluded that the acetone extract of fruit pulp of *P. ligularis* is rich in polyphenolic compounds, such as ellagic acid, gallic acid, rutin, kaempferol and caffeic acid contents, which demonstrated potent antioxidant, anti-diabetic and antimicrobial properties. Therefore, the direct intake of fruits can supply the natural antioxidants for humans, in addition to the enormous amounts of sugars, proteins and fatty acids, favoring their nutraceutical properties for consumption.

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References


