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Original Article

EVALUATION OF *IN VITRO* ANTIOXIDANT AND α-AMYLASE INHIBITORY ACTIVITY OF *PHYLLANTHUS INDOFISCHERI* BENNET

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ABSTRACT

Objective: The present study evaluates the antioxidant and α-amylase inhibitory activity of leaf and bark extracts of *Phyllanthus indofischeri* with methanol and water as solvents. In addition to this, the total phenolic content and total flavonoid content was determined.

Methods: The total phenolic and total flavonoid content of the extracts was determined by folin ciocaletus reagent method and aluminium chloride colorimetric method respectively. The antioxidant and α-amylase inhibitory activity were measured by various assays, including α, α-diphenyl-β-dipicryl-hydrazyl (DPPH) free radical scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonicacid) (ABTS) radical scavenging, superoxide radical scavenging, total antioxidant capacity by phosphomolybdate method and porcine pancreatic α-amylase inhibitory assay. The IC_{50} values were calculated and compared with standards such as gallic acid, ascorbic acid and α-acarbose.

Results: The results illustrated that all the extracts of *Phyllanthus indofischeri* exhibit significant antioxidant and α-amylase inhibitory activity. Among the extracts, methanolic leaf extract showed high levels of activity followed by bark water extract.

Conclusion: *Phyllanthus indofischeri* extracts had shown antioxidant and α-amylase inhibitory activity. On the basis of these results, *Phyllanthus indofischeri* can be used as a natural antioxidant and hypoglycemic agent against various disorders related to oxidative stress; and the isolation of bioactive compounds was warranted.

Keywords: *Phyllanthus indofischeri*, Antioxidant activity, α-amylase inhibitory activity, Leaf and bark, Total phenolic content, Total flavonoid content

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INTRODUCTION

Oxidative stress explains the relation between the free radicals and diseases [1]. The major free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) have a dual effect on the metabolism. An imbalance of these radical species results in the damage of proteins, lipids, nucleic acids, and cell structures [2]. This is linked with the pathogenesis of various prominent diseases like cancer, fibrosis, rheumatoid arthritis, atherosclerosis, cardiovascular diseases, hypertension, and ischemia, neurodegenerative diseases like Parkinson and Alzheimer and aging [3,4]. Chronic oxidative stress due to hyperglycemia results in dysfunction of pancreatic islet β cell, furthermore these cells have low expression of oxidative enzymes and might be susceptible for ROS which leads to diabetes mellitus. The mechanism of diabetes is related to the oxidative stress and inflammation [5-8]. Antioxidants are one among the various defense mechanisms against oxidative stress [9]. The synthetic antioxidants, which were used in the food processing and preservation such as propyl gallate, tertiary butyl hydroquinone, butylated hydroxytoluene (BHT) and butylated hydroxy ani (ole (BHA) have been proved to be carcinogenic. Hence, there is an increasing demand for natural antioxidants that are safe, without any negative effects and those of plant origin such as polyphenolic compounds [10]. Plant phenolics are known to scavenge a variety of free radicals like hydroxyl, peroxyl, hypochlorous acids, and superoxide [11], and they act as protective agents [12,13]. Herbals and herbal extracts were used to cure various diseases. In India over 200 million people with limited access to primary health centers depend on traditional medicines, in the last few decades, various plants were studied for their medicinal properties. For many chronic diseases such as diabetes, Mellitus phytotherapy is considered as one of the best alternative [14]. At present nearly 400 plants were used as antidiabetic [15]. However, the plants which were scarcely used for culinary and medicinal purpose need scientific investigation. Therefore the assessment of their medicinal properties remains an interesting and useful task to find new sources of natural antioxidants and nutraceuticals.

In tropical and subtropical countries the *Phyllanthus* genus is well known for its pharmacological properties, which leads to treatment of various diseases for thousands of years in folklore medicine [16,17]. These plants were used as astringent, diuretic, antiseptic, antidiabetic, laxative, to treagonorrheaea, headache, jaundice, mammary abscess, kidney and urinary disturbances, intestinal infections, anticancer, antioxida,, nephroprotective, antitumor, and antiviral agents [18-20]. Indian gooseberry, the richest source of vitamin C next to Barbados cherry is mainly collected from *Phyllanthus emblica* and *Phyllanthus indofischeri* and is one of the heavily harvested non-timber forest products of Soligas [21].

Phyllanthus indofischeri Bennet is a woody tree species endemic to Deccan plateau and certain drier forest pocket of Southern India locally known as Ittu nelli. It differentiates from *Phyllanthus emblica* by monoecious nature, marble green fruit with alternate, subsessile and oblong leaves. Emblica is well known for its medicinal values and widely used in various medicines due to the presence of bioactive compounds [22]. In folk medicine, these plant species were used for anemia, asthma, dropsy, earache, dyspepsia, and jaundice [23,24]. The plant has been reported to contain various bioactive compounds, possess antimicrobial and hepatoprotective activities [25,26]. To the authors' knowledge, there was no report on the antioxidant activity and α-amylase inhibitory activity of *Phyllanthus indofischeri*. Therefore, the present study aims to investigate the antioxidant and α-amylase inhibitory activities of *Phyllanthus indofischeri* along with the determination of total phenolic content [TPC] and total flavonoid content [TFC].

MATERIALS AND METHODS

Plant material

Plant material was collected from the Nallamala forest, near to Bramhamgari matam located in Kadapa district, Andhra Pradesh, India.

The plant was identified and deposited, voucher specimen (No: 48757) in the Department of Botany, SriKrishnadevaraya University, Anantapur, Andhra Pradesh, India, as herbarium for future reference.

Preparation of extracts

Pulverized plant materials, leaf and bark of *Phyllanthus indofischeri* (50g) were successively extracted by using a soxhlet apparatus (Quickfit, England) with methanol and water for 6-8 hr until the solvent is colorless to ensure complete extraction. After soxhlation, methanolic leaf extract (LME), leaf water extract (LWE), bark methanolic extract (BME) and bark water extract (BWE) marc were concentrated under vacuum. The extracts were stored separately in airtight containers in the refrigerator at 4 °C for further use.

Chemicals, reagents and solvents

α-amylase, 3,5-dinitrosalicilic acid (DNS), α-acarbose, folin ciocaltaeus reagent, gallic acid, quercetin, DPPH, ABTS, phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), were acquired from Sigma-Aldrich (St. Louis, Mo, USA). Na2CO3, AlCl3, potassium acetate, potassium persulfate, ascorbic acid, KH2PO4, NADH, H2SO4, sodium phosphate, ammonium molybdate, were purchased from Hi-Media, Mumbai. Starch, sodium, potassium tartrate, NaOH were obtained from SRL Pvt. Ltd., Mumbai. All other unlabeled chemicals were of analytical grade.

Determination of total phenolic content

The total phenolic content was determined by using folin ciocaltaeus reagent method described by Singleton and Rossi [27] with slight modifications. An aliquot (1 ml) of approximately diluted extracts and standard solutions of gallic acid at various concentrations ranging from 20-100 mg/l were added to a 25 ml volumetric flask containing 9 ml of double distilled water. 1 ml of folin and ciocaltaeus phenol reagent was added to the mixture and shaken. After 5 min, 4 ml of 20% W/V Na_2CO_3 was added with stirring. Immediately, the solution was then diluted to 25 ml with double distilled water and mixed thoroughly. A reagent blank with distilled water was prepared simultaneously. After 90 min of incubation at room temperature, the absorbance was recorded by using UV-visible spectrophotometer (Evolution 201, Thermo scientific, USA.) at 750 nm. Percentage of total phenolic content was calculated from the calibration curve of gallic acid standard and total phenolic content was expressed as mg gallic acid equivalents (GAE)/g dry weight of the extract. All samples were analyzed in triplicate.

Determination of total flavonoid content

The total flavonoid content was determined by aluminium chloride colorimetric method described by Lin and Tang [28]. Briefly, 0.5 ml (1 mg/ml) of extracts and standard solution of quercetin, concentration ranging from 20-100 mg/l was mixed with 1.5 ml of 95 % alcohol, 0.1 ml of 10 % $AlCl_3$. $6H_2O$, 0.1 ml of 1 M potassium acetate and 2.8 ml of double distilled water were added later. After incubation for 40 min at room temperature, the absorbance of the mixture was observed at 415 nm against the blank. Total flavonoid was expressed as mg quercetin equivalents [QE]/g dry weight of the extract.

Determination of antioxidant activity

Evaluation of DPPH radical scavenging activity

The DPPH radical scavenging activity of the extracts was determined by Liu *et al.* [29]. Stock solutions (10 mg/ml) of plant extracts were prepared in their respective solvents, and various concentrations (30-300 µg/ml) were taken as samples. 1 ml of plant extract was mixed with 1 ml of DPPH in methanol. The mixture was shaken vigorously and incubated in the dark for 30 min. simultaneously control was maintained by replacing the sample with methanol. The absorbance was measured at 517 nm. Gallic acid was used as positive control. The DPPH radical scavenging activity was determined by using the formula,

$$\text{DPPH\%} = \frac{A_c - A_s}{A_c} \times 100.$$

DPPH % is the percentage of DPPH radical scavenging activity, A_c is the absorbance of control, and A_s is the absorbance of the sample. The inhibition curve was plotted and IC_{50}, the concentration necessary for 50 % reduction of DPPH radical was determined.

Evaluation of radical scavenging activity by ABTS

The ABTS radical scavenging assay was performed by the method described by Re *et al.* [30]. ABTS radical ($ABTS^{\bullet}$) was generated by mixing equal proportions of 7 mmol/l ABTS and 2.45 mmol/l potassium persulphate via incubation for a period of 12-16 h in the dark at room temperature. 1 ml of $ABTS^{\bullet}$ solution was diluted with 60 ml of methanol to obtain absorption of 0.708±0.001 units at 745 nm using a spectrophotometer. Then 1 ml of ABTS* solution was added to 1 ml of plant extract and incubated for 15 min at room temperature, and the absorbance was measured at 745 nm. ABTS* solution was freshly prepared for each extract. The disappearance of ABTS* determines the decrease in absorbance. The absorbance was taken within 6 min. Then the final absorbance was noted. Ascorbic acid was used as a standard. The percentage of ABTS free radical scavenging activity was calculated by the formula,

$$\text{ABTS\%} = \frac{1 - A_s}{A_c} * 100.$$

ABTS % is the inhibition percentage of ABTS radical, A_s is the absorbance of the sample, A_c is the absorbance of control. The antioxidant capacity of plant extracts was expressed as IC_{50}, the concentration necessary for 50% reduction of ABTS radical.

Superoxide radical scavenging activity

The superoxide radical scavenging activity was evaluated using an improved method described by Chouhan and Singh [31]. 0.1 ml of PMS (0.1 mmol), 0.1 ml of NBT (1 mmol) was mixed with different concentrations of plant extracts (30-300 µg/ml). The volume was finally made to 0.9 ml with 0.05 M KH_2PO_4 buffer (pH 7.4). By addition of 0.1 ml of 2 mmol NADH, superoxide radicals were generated in the reaction mixture and were incubated for 10 min at 25 °C. The absorbance was measured at 570 nm. Ascorbic acid was used as a standard. The percentage of generated superoxide anion scavenging activity was calculated by using the formula,

$$\text{SOD \%} = \frac{1 - A_s}{A_c} * 100.$$

SOD % is the percentage of superoxide scavenging activity, A_s is the absorbance of sample, and A_c is the absorbance of control. IC_{50} values were calculated to determine the 50 % inhibition of superoxide radicals.

Evaluation of total antioxidant capacity

The total antioxidant capacity was evaluated by phosphomolybdate assay described by Jan *et al.* [32]. 0.1 ml of plant extract at various concentrations (30-300 µg/ml) was mixed with 1 ml of phosphomolybdate reagent solution (0.6 mol H_2SO_4, 28 mmol sodium phosphate and 4 mmol ammonium molybdate). The test tubes were covered and incubated at 95 °C for 90 min in a water bath. Test tubes were removed from the water bath and cooled to room temperature. The absorbance of the reaction mixture was measured at 695 nm. Ascorbic acid was used as a standard. Calibration graph was plotted between absorbance and concentration of ascorbic acid. Total antioxidant capacity of plant extracts was calculated from the graph and expressed as milligrams of ascorbic acid equivalent (AAE)/g dry weight of the extract.

Evaluation of α-amylase inhibitory activity

α-amylase inhibitory assay method was performed by using the method described by Wan *et al.* with slight modifications [33]. Briefly, 500 µl of plant extracts of various concentrations (30-300 µg/ml) were mixed with 500 µl of α-amylase (E. C 3.2.1.1) and pre-incubated in 20 mmol sodium phosphate buffer (pH 6.7) at 37 °C for 5 min. To the above mixture, 1 ml of 0.2% (W/V) starch dissolved in buffer was added and finally made up to 2 ml. The whole reaction mixture was incubated at 37 °C for 5 min. After the incubation period, 1 ml of DNS colour reagent (96 mmol (20 ml), 5.3 mmol sodium potassium tartrate in 2 M NaOH (8 ml) and deionized water (12 ml) was added and placed in water bath at 85 °C for 5 min, the

mixture was cooled to room temperature and 6 ml of deionized water was added. α-amylase inhibitory activity was determined by measuring the absorbance at 540 nm. α-acarbose, a known α-amylase inhibitor was used as a positive control. The inhibitory activity was calculated by using the formula,

$$\% \text{ inhibition} = \frac{Ac - As}{Ac} * 100.$$

% inhibition is the percentage of α-amylase inhibitory activity, A_c is the absorbance of control, A_s is the absorbance of the sample.

Statistical analysis

All the experiments were performed in triplicate, and the data were expressed as mean±SD Results were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism version 6.07 for Windows, Graph Pad Software, San Diego, CA, USA. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The extraction yield, total phenolics, and total flavonoids of various extracts of *Phyllanthus indofischeri* are shown in table 1. Extraction yield ranges from 18.9 to 28.7%. The highest yield was observed for LME, whereas the lowest for the BWE. The type of solvent used for extraction plays an important role in the isolation of bioactive compounds. Polar solvents are able to extract more polar compounds than nonpolar solvents. Methanol being a volatile and organic solvent is able to degrade plant cell wall, inhibit plant polyphenol oxidases and is able to extract a greater amount of endocellular materials than water [34,35]. The total phenolic content was ranged from 53.6 mg to 86.2 mg GAE/g dry weight of the extract. LME showed highest phenolics, whereas BME was much smaller. *Phyllanthus* species contain various phenolic compounds such as gallic acid, ellagic acid, pedunculagin, emblicannins and various low molecular weight compounds which are known for its biological activities [36,37].

The total flavonoid content of these extracts ranges from 70.39 mg to 104.21 mg QE/g dry weight of the extract. LME contains highest, followed by BME, and LWE contains the smallest fraction of flavonoids. The flavonoids are one type of polyphenols which are able to scavenge free radicals, chelate metal ions (Fe, Cu). Recently, it was shown that the intake of flavonoids to be inversely related to the coronary heart disease and reduces lipid levels [38,39]. Leaf extracts exhibit more total phenolic and total flavonoid content than the bark due to the fact that leaf which is the primary site of secondary metabolism possess more polyphenolic content than the bark. These results suggest that the antioxidant and α-amylase inhibitory activity of *Phyllanthus indofischeri* may be due to the presence of phenolic and flavonoid compounds.

Table 1: Percentage of yield, total phenolic content and total flavonoid content of *Phyllanthus indofischeri* extracts

Plant extract	Percentage of yield	TPC (mg GAE/g dry weight of extract)	TFC (mg QE/g dry weight of extract)
LME	28.7±1.6	68.93±0.05	104.216±0.71
BME	23.6±2.9	42.34±2.11	76.53±2.62
LWE	21.2±3.1	51.12±3.62	70.393±3.21
BWE	18.9±2.3	53.60±1.91	84.21±1.84

Each value in the table is represented as mean±SD (n=3), means not sharing the same letter are significantly different (LSD) at P ≤0.05 probability level in each column. TPC= total phenolic content, TFC= total flavonoid content, GAE= gallic acid equivalent, QE= quercetin equivalent, LME= leaf methanolic extract of *P. indofischeri*, BME= bark methanolic extract of *P. indofischeri*, LWE= leaf water extract of P. indofischeri, BWE= bark water extract of *P. indofischeri*

The radical scavenging activity of *Phyllanthus indofischeri* was measured by several assays such as DPPH, ABTS, superoxide scavenging, and total antioxidant capacity by phosphomolybdate method. These assays differ in terms of substrates, probes, reaction conditions and quantification methods. The activity carried under specific reaction conditions such as pressure, temperature, reaction media, co-reactants, and reference point specifies the particular reaction. Plant extract which contains different chemical compounds reflects its potential at various conditions; hence it is appropriate to use various assays to evaluate the mechanism of scavenging [40].

Table 2: Antioxidant and α-amylase inhibitory activity of *Phyllanthus inofischeri*

	IC_{50} (µg/ml)				
Plant extracts/standard	DPPH radical scavenging assay	ABTS radical scavenging assay	Superoxide radical scavenging assay	Total antioxidant capacity (mg of AAE/g of extract)	α-amylase inhibitory assay
LME	148.18±1.23	111.98±2.31	178.29±1.22	153.44±1.86	175.57±0.43
BME	340.22±0.98	241.23±0.67	212.90±1.75	105.89±1.36	252.26±1.34
LWE	272.48±1.76	166.42±0.55	215.61±0.67	94.72±1.22	209.93±1.76
BWE	184.69±0.88	260.21±1.78	251.07±1.34	55.16±0.54	250.24±2.01
Gallic acid	107.07±1.09	-	-	-	-
Ascorbic acid	-	38.12±1.54	53.52±0.97	-	-
α acarbose	-	-	-	-	84.73±0.49

Each value in the table is represented as mean±SD (n=3), Means not sharing the same letter are significantly different (LSD) at P≤0. 05 probability level in each column. LME= leaf methanolic extract of. *P. indofischeri*, BME= bark methanolic extract o. *P. indofischeri*, LWE= leaf water extract of *P. indofischeri*, BWE= bark water extract of *P. indofischeri*

DPPH radical scavenging assay

DPPH is a stable chromogen radical which was in purple color, upon reduction by donating hydrogen atom converted to pale yellow hydrazine which is generally evaluated in organic media by measuring the decrease in absorbance at 515-528 nm [41]. Fig. 1A depict the results of DPPH radical scavenging activity of *Phyllanthus indofischeri*. These extracts show a concentration-dependent response of scavenging. The LME has highest scavenging ability followed by BWE, LWE and BME with an IC_{50} value of 148.18, 184.69, 272.48, 340.22 µg/ml, respectively, whereas, the gallic acid which was used as a standard shows an IC_{50} value of 107.77 µg/ml. These results are in agreement with other similar reports of strong DPPH radical scavenging activity of *Phyllanthus* species [42-44].

ABTS radical scavenging assay

This method is applicable for both hydrophilic and lipophilic antioxidants. It is a decolourization technique in which radical is generated directly into a stable form prior to reaction with putative antioxidants. The ABTS* is a blue/green color chromophore was developed by the reaction between ABTS and persulphate, with absorption maxima at 745 nm. Percentage of inhibition of ABTS* is directly proportional to decolourisation and radical scavenging activity

of the extract [30]. Fig. 1B shows that the extracts of *Phyllanthus indofischeri* have good antioxidant potential against ABTS radical with respect to IC_{50} values. LME (111.98 µg/ml) and LWE (166.42 µg/ml) exhibited highest antioxidant activities compared to BME (241.23 µg/ml) and BWE (260.21 µg/ml). Ascorbic acid, which was used as a positive control had shown an IC_{50} value of 38.12 µg/ml, which is very effective when compared to the extracts. Our results are in concordance with similar reports of *Phyllanthus niruri* [43] which has potential ABTS radical scavenging activity. Prieto *et al.* [45] concluded that phenolic compounds were responsible for scavenging of ABTS*. Therefore, the antioxidant capacity of the extracts can be attributed to the polyphenols which play a vital role as antioxidant in living systems due to the para and ortho positions of hydroxyl groups.

Superoxide radical scavenging assay

Fig. 1C indicates that the extracts of *Phyllanthus indofischeri* had good inhibitory potential against Riboflavin-NBT-light system *in vitro*. The scavenging potential of extracts were in the following order LME>BME>LWE>BWE with an IC_{50} value of 178.29, 212.90, 215.61, 251.07 µg/ml respectively in dose-dependent manner. The positive control ascorbic acid had an IC_{50} value of 53.52 µg/ml, which is more effective than that of extracts. Such crude extract superoxide radical scavenging properties are shown by some other plants [46,47]. This assay was originally reported by Beauchamp and Fridovich, the sensitivity and specificity of this assay are quite remarkable. In the presence of oxidizable substances, the riboflavin undergoes reduction, which on reoxidation in air reduces flavins to generate O_2^-, and reduces the colorless NBT to blue formazon [48].

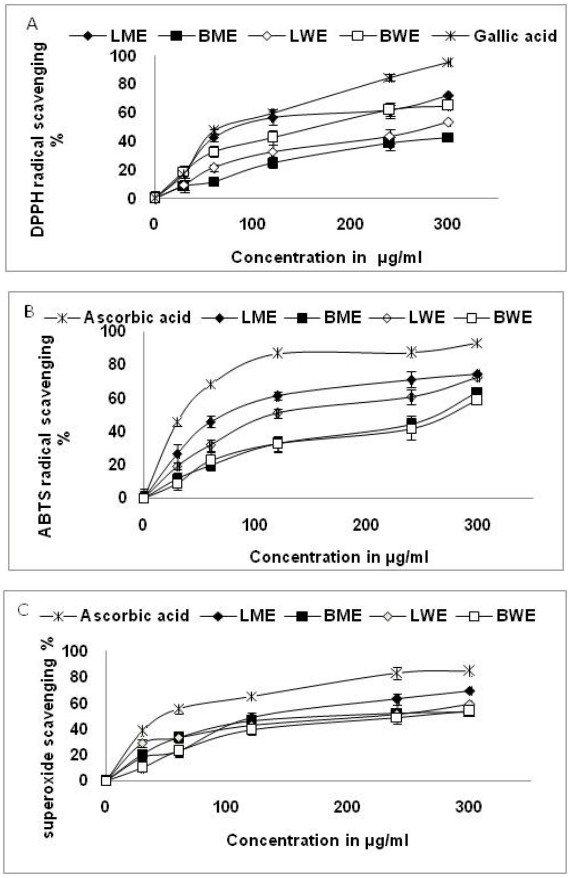

Fig. 1: Antioxidant activities of *Phyllanthus indofischeri* with various extracts of leaf and bark at different concentrations. Each value represents a mean±SD (n=3);A. DPPH radical scavenging activity B. ABTS radical scavenging activity C. superoxide radical scavenging activity. LME= leaf methanolic extract of *P. indofischeri*, BME= bark methanolic extract of *P. indofischeri*, LWE= leaf water extract of P. indofischeri, BWE= bark water extract of *P. indofischeri*

Total antioxidant capacity by phosphomolybdate assay

The total antioxidant capacity was stated in terms of AAE/g of dry extract; fig 2 shows the total antioxidant capacity of *Phyllanthus indofischeri* extracts by phosphomolybdate method. The antioxidant capacity ranges from 55.16 to 153.44 AAE/g dry weight of the extract. LME had shown the highest, whereas BWE was least among the extracts. Phosphomolybdate assay is very simple and quantitative in nature, hydrogen and electron transfer occurs from oxidant to molybdenum (VI) to convert it into molybdenum (V) green phosphate, which is measured spectrophotometrically. In general, this method uses to detect antioxidants such as vitamin C, E and some specific phenols [45,49,50].

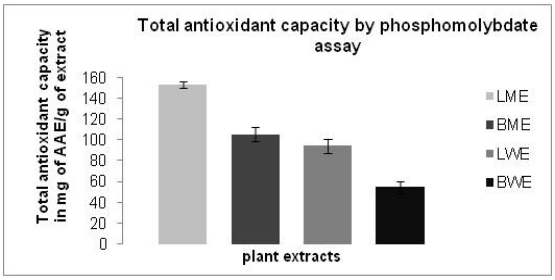

Fig. 2: Total antioxidant capacity of *Phyllanthus indofischeri* by Phosphomolybdate assay. Each value represents a mean±SD (n=3); LME= leaf methanolic extract of *P. indofischeri*, BME= bark methanolic extract of *P. indofischeri*, LWE= leaf water extract of P. indofischeri, BWE= bark water extract of *P. indofischeri*

α-amylase inhibitory assay

All the extracts tested were positive for porcine pancreatic amylase inhibition in dose-dependent manner. Fig. 3 shows the inhibitory percentage of extracts of *Phyllanthus indofischeri* in comparison with the reference drug α-acarbose. The α-amylase inhibitory activities are in following order LME>LWE>BWE>BME with an IC_{50} value of 175.57, 209.93, 250.24, 252.26 µg/ml and inhibitory percentages of 46.64, 38.98, 35.92, 32.94 respectively. The standard drug acarbose had an IC_{50} value of 84.73 µg/ml and inhibitory percentage of 58.21, which was much greater than the plant extracts. Still the extracts the *Phyllanthus indofischeri* revealed their potential hypoglycemic activity.

The retardation of the breakdown of glucose and absorption by α-amylase is one of the therapeutic approaches available for diabetes mellitus. Most of the drugs available had side effects such as abdominal distention, flatulence, meteroism, possibly diarrhea. The standard drug α-acarbose used in the study as a positive control was a strong competitive inhibitor of α-amylase due to the presence of pseudoring and glycosidic nitrogen linkage. It mimics the transition state of enzymatic cleavage of glycosidic bond [51]. The α-acarbose directly interacts with the active site (Glu 233, Asp 300 and Asp 197) of α-amylase to inhibit its activity [52]. The plant phenolic compounds are known to be potential α-amylase inhibitors due to the fact that the phenolic compounds bind to the reactive sites of the enzymes and alter its catalytic activity [53]. Some fruits and vegetables are considered as antihyperglycemic agents due to the presence of phenolic compounds [54]. The *Phyllanthus* species are well known for its medicinal importance, and plants such as *P. amarus, P. niruri, P. emblica, P. maderaspatensis, P. urinaria* are reported for its α-amylase inhibitory activity [55-59].

In the present study, correlations between the antioxidant and total phenolic content, and α-amylase inhibitory activities and total phenolic content were observed. They were in agreement with other such reports [60,61]. The antioxidant activity of polyphenolic compounds was due to the redox properties, which can play an important role in absorbing, neutralizing, and quenching of singlet, triplet oxygen or decomposing peroxides and other radicals [62-65].

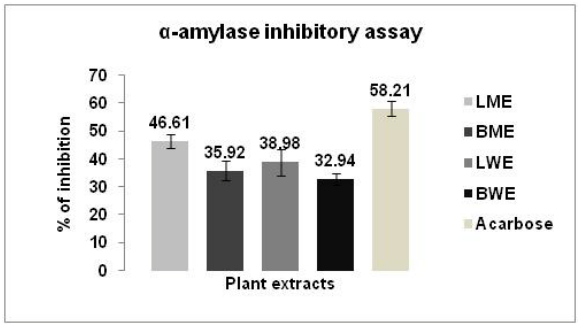

Fig. 3: α-amylase inhibitory activity of *Phyllanthus indofischeri*. Each value represents a mean±SD (n=3); LME= leaf methanolic extract of *P. indofischeri*, BME= bark methanolic extract of *P. indofischeri*, LWE= leaf water extract of P. indofischeri, BWE= bark water extract of *P. indofischeri*

CONCLUSION

In conclusion, the present study reveals that the extracts of *Phyllanthus indofischeri* exhibited significant antioxidant, free radical scavenging, and α-amylase inhibitory activities. Leaf methanolic extract had the highest activity followed by bark water extract which are in correlation with the total phenolic content. Therefore, *Phyllanthus indofischeri* can be suggested as a potential natural source as an antioxidant and hypoglycemic agent. Natural source of antioxidant and hypoglycemic agents in *Phyllanthus indofischeri* can be attributed to its phenolic and flavonoid contents. The presence of phenolic and flavonoid contents also validates its ethnomedicinal use. Further studies are warranted for the isolation, identification, and characterization of active principles.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest

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