Antidiabetic, antioxidant and antimicrobial activities of extracts of *Tephrosia bracteolata* leaves

Godshelp Osas Egharevba a,c,*, Omotayo O. Dosumu b, Stephen O. Oguntoye d, Ngaitad S. Njinga e, Samuel Olatunde Dahunsi f, A. Abdulmumeen Hamid g, Ajay Anand c, Zehra Amtul c, Priyanka Ujjukuri c

a Industrial Chemistry Programme, Department of Physical Sciences, College of Pure and Applied Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria
b Department of Industrial Chemistry, Faculty of Physical Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria
c Medicinal Chemistry and Pharmacology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India
d Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria
e Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria
f Department of Microbiology, College of Pure and Applied Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria

A R T I C L E   I N F O

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A B S T R A C T

Aims: Plant extracts have long been used for the ethnomedical treatment of diabetes, microbial infections and as a source of antioxidant. This study was aimed at investigating the antidiabetic, antioxidant, and antimicrobial activities of the n-hexane and ethyl acetate extract of *Tephrosia bracteolata* leaves (TBL) as associated with the ethnomedical knowledge of the local people of Nigeria.

Main methods: The phytochemical composition of the n-hexane and ethyl acetate extract of the leaves of *T. bracteolata* were determined following standard procedures in literature, and it’s in vitro inhibitory activities against α-glucosidase enzyme, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS -) and 1,1-diphenyl-2-picrylhydrazyl (DPPH -) antioxidant activities were also examined. Well diffusion method was employed in evaluating the antimicrobial property of the extracts.

Key findings: The ethyl acetate extract of *T. bracteolata* leaves had the greatest inhibitory effect on α-glucosidase, followed by the n-hexane with IC₅₀ 43.95 μg/ml and IC₅₀ 50 μg/ml respectively. The ethyl acetate also exhibited significant DPPH - and ABTS - antioxidant activity with IC₅₀ of 24.96 μg/ml and 6.48 μg/ml as compared to Ascorbic acid and Trolox (12.24 μg/ml and 5.91 μg/ml) respectively. The zones of inhibition of the ethyl acetate extract of *T. bracteolata* leaves ranges from 10 – 25 mm at a concentration of 6.25–200 mg/ml, and it showed a greater antibacterial activity than the n-hexane extract, having a zone of inhibition from 10 – 20 mm at concentration of 12.5–200 mg/ml when compared to the standard Gentamycin. Similarly, the ethyl acetate extract of *T. bracteolata* showed a better anti fungi activity at concentration range 12.5–200 mg/ml than the n-hexane extract at concentration range of 25–200 mg/ml with reference to Tioconazole. These results indicated for the first time that the ethyl acetate extract of *T. bracteolata* leaves extracts exerted potent inhibitory effects against α-glucosidase, actively scavenge DPPH - and ABTS - free radicals and successfully inhibits the proliferation of Gram positive and Gram negative microorganism.

Significance: TBL is an important source of antidiabetic, antimicrobial and antioxidant agent.

1. Introduction

Pathogenic micro-organisms have built great resistance against several synthetic antibiotics; as a result, much attention is being paid to isolate biologically active compounds from plant species used in primitive medicine [1]. Thus, offering a new lead towards drug discovery [2]. Extracts from plants have been used since ancient times for the traditional treatment of diabetes, microbial infections, stress related disorder, sources of antioxidant, etc., and now they are widely accepted as supplementary alternative for orthodox drugs [3]. Plants represents majorly the useful dietary supplements for improving blood glucose control and preventing long-term complications in type 2 diabetes mellitus (T2DM).
2. Materials and methods

2.1. Reagents

Potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, glucose, BSA (Bovine serum albumin), sodium phosphate buffer, ascorbic acid, ethanol, Acarbose Ascorbic acid, 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), para-nitrophenol-alpha-D-glucopyranoside, etc, were purchased from Sigma-Aldrich chemicals, USA, Merck Limited, India and S.D. Fine Chemicals Limited India.

2.2. Materials

Gram-negative bacteria: Salmonella typhii (UCH 4801), Escherica coli (UCH 1102) and Klebsiellae pneumoniae (UCH 2894) while Bacillus subtilis (UCH 74230) and Staphylococcus aureus (UCH 2473) are Gram-positive, clinical strains from the Medical Microbiology unit, University College Hospital, Ibadan, were screened at the Department of Pharmaceutical Microbiology laboratory, University of Ibadan, Ibadan, Nigeria. Solvent of n-hexane and ethyl acetate were used as negative controls in the assays. Antimicrobial agents: Gentamycin (10 μg/ml) and Ticonazole (0.7 μg/ml) were included as standard reference drugs in the study.

2.3. Collection of plant

The plant was collected from Jalala Estate, University of Ilorin, Tanke Oke-Odo, Ilorin, Kwara State, Nigeria. The plant materials were taxonomically identified and verified at the Department of Plant Biology, University of Ilorin, Ilorin, where a voucher specimen (UIH004/1189) was deposited.

2.4. Preparation of plant extracts

Fresh leaves of T. bracteolata were harvested, weeded, washed, and air-dried under shade. The air-dried leaves were gradiently extracted in n-hexane and ethyl acetate respectively. The n-hexane and ethyl acetate crude extracts were concentrated and stored at 4 °C.

2.5. Preliminary phytochemical screening of T. bracteolata leaves extract

The n-hexane extract (TbHHL) and the ethyl acetate extract (TbEAL) of the leaves of T. bracteolata were each screened for various bioactive pharmaceutical constituents such as anthraquiones, steroids, cardiac glycosides, tannins, saponins, phlobatannins, terpenoids, flavanoids, and alkaloids using standard methods [14, 15].

2.6. Antidiabetic study

The antidiabetic Studies was carried out using Alpha-Glucosidase Inhibition Assay [16]. Briefly, in a 96-well microplate, 20 μL of each extract was incubated with 50 μL of crude intestinal α-glucosidase for 5 min and 50 μL of substrate (5 mM, p-nitrophenyl-α-glucopyranoside, prepared in 100 mM phosphate buffer, pH 6.8) was added. The pale yellow colour due to the release of p-nitrophenol from α-linkage of glucopyranoside by the action of enzyme α-glucosidase was measured spectrophotometrically at 405 nm (BioTek synergy4, BioTek Instruments Inc, Winooski, VT, USA) after incubation for 10 min. An individual blank for each extract was prepared to counterbalance absorbance due to the colour of samples, where, in lieu of enzyme, 50 μL of normal saline was added. Percentage of enzyme inhibition was obtained applying the following formula:

\[
\text{% Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of extract}]}{\text{Absorbance of control}} \times 100
\]

The antidiabetic Studies was carried out using Alpha-Glucosidase Inhibition Assay [16]. Acarbose was used as the reference standard.

2.7. Free radical scavenging assay

2.7.1. DPPH free radical scavenging assay

Decolorisation of DPPH radical was determined by reported standard method [11]. In brief, 25 μL of various dilutions of the extracts (n-hexane and ethyl acetate), 100 μL of Tris HCl buffer (0.1 M, pH 7.4) and 125 μL DPPH solution (0.5 mM in methanol) were added in a 96-well micro plate and incubated in the dark for 15 min. Absorbance was recorded at 517 nm, and the percentage of DPPH scavenging by extracts was calculated applying the formula:

\[
\text{% Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of extract}]}{\text{Absorbance of control}} \times 100
\]

Several serial dilutions of respective extracts were prepared and analysed [17]. Ascorbic acid was used as standard. The percentage of DPPH scavenging was calculated as above. The IC50 of the extracts were calculated applying a suitable regression analysis.

2.7.2. ABTS+ scavenging assay

Scavenging of 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS+ cation radical was performed with suitable modifications. Briefly, 100 ml stock solution of ABTS+ (0.5 mM) was prepared by addition of 1 ml potassium persulfate (6.89 mM in Phosphate-buffered saline (PBS) (pH 8.0)). Mixture was stored in dark for 16 h. 10 μL of each of the different extracts (5 μg/ml in PBS) were added to 190 μL of ABTS+ in 96-well microplate. Percentage scavenging of ABTS+ by test samples were calculated as follows using:

\[
\text{% Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of extract}]}{\text{Absorbance of control}} \times 100
\]

Necessary calculations were applied to obtain the IC50 [18].

2.8. Antimicrobial assay

Microorganism’s cultures of six human pathogenic bacteria made up of four Gram negative and two Gram positive were used for the antibacterial assay. The Antifungal assay was tested on four fungi: Candida albicans; Aspergillus niger; Rhizopus stolon; and Penicillium notatum.

Media: Nutrient agar, Sabouraud dextrose agar, nutrient broth and broth...
tryptone soya agar were used in this study.

2.8.1. Antimicrobial activity determination

Agar diffusion-pour plate method (bacteria) [19, 20]. An overnight culture of each organism was prepared by taking two wire loop of the organism from the stock and inoculated each into the sterile nutrient broth of 5ml, each incubated for 18–24 h at 37 °C. From overnight culture, 0.1 ml of each organism was taken and put into the 9.9 ml of sterile distilled water to obtained 10^{-2} inoculum concentration of the organism. From the diluted organism (10^{-2}), 0.2 ml was taken into the prepared sterile nutrient agar cooled to about 40–45 °C, then poured into sterile Petri dishes and allowed to solidify for about 1 h. Using a sterile cork-borer of 8 mm diameter, the wells were made accordingly including the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2 h to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated for 18–24 h at 37 °C.

Agar diffusion-surface plate method (fungi): A sterile sabouraud dextrose agar was prepared accordingly, and aseptically poured into the sterile plates in triplicates and solidified properly. 0.2 ml of the 10^{-2} inoculum concentration of the organism was spread on the surface of the agar using a sterile Petri-dish to cover the surface of the agar. Eight wells were bored using a sterile cork-borer of 8 mm diameter. The graded concentrations of the extracts were put into the wells accordingly including the controls. All the plates were left on the bench for 2 h to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated at 25 °C for 72 h [19,20].

2.9. Statistical analysis

Results were presented as mean ± standard error of mean (SEM). Statistical analysis was carried out with Graph Pad Prism 6 software, Incorporated, USA. There were significant differences between the extracts and the positive control. It may be less because its a crude extract.

3. Results and discussion

3.1. Phytochemical screening

The results of the phytochemical screening carried out on the TbHL and TbEAL extracts of T. bracteolata leaves is shown in Table 1 below. The result revealed the presence of alkaloids, steroids, tannins, flavonoids and terpenoids. The analysis revealed the presence of constituents which are known to exhibit medicinal activities (Lwande et al., 1985).

3.2. Antidiabetic activity

The results of the in vitro α-glucosidase enzyme inhibition assay (Fig. 1) of the TbHL and TbEAL extracts of T. bracteolata leaves revealed moderate antidiabetic activities. TbHL extract of T. bracteolata showed a lower α-glucosidase enzyme percentage inhibition activity (57%), when compared to the TbEAL (67%) extract and the standard, Acarbose (92.95%). Between these two plant extracts, TbEAL extract of T. bracteolata exhibited IC_{50} 43.95 μg/ml as compared to Acarbose 11.31 μg/ml though not statistically significant (p < 0.05) compared to the standard of (Fig. 2). Rajaram and Suresh [21] reported the ethyl acetate stem extract of Tephrosia tinctoria to have shown significant inhibitory activity (IC_{50} 94.33 ± 3.65 μg/ml) compared to standard (Acarbose - IC_{50} 38.92 ± 3.52 μg/ml). The aqueous seed extract of Tephrosia purpurea was reported to have demonstrated significant in vivo antihyperglycemic activity in streptozotocin induced diabetic rats [22]. Likewise, the ethanol extract of Tephrosia villosa leaves showed reduction in glucose level and pancreatic cell regeneration in alloxan induced diabetes in rats [23]. These results also lend credence to the fact that TbEAL extract of T. bracteolata demonstrates anti diabetic activity.

3.3. In vitro antioxidant activities

The results of the in vitro antioxidant activity of TbHL and TbEAL extracts of T. bracteolata presented in Figs. 3 and 4 showed moderate activity respectively as revealed by the DPPH and ABTS + free radical scavenging activity. The TbHL extract of T. bracteolata showed a lower percentage inhibition of DPPH antioxidant activities of (39.29 ± 0.07) when compared to that of the extract of TbEAL (80.12 ± 0.07). The percentage inhibition of TbHL extract was not significantly different from that of the standard compound, Ascorbic acid (94.23 ± 0.00). Between these two plant extracts, the TbEAL extract of T. bracteolata

![Fig. 1. α - Glucosidase % Inhibition of TbHL and TbEAL](image)

![Fig. 2. IC_{50} Value of TbHL and TbEAL Extracts on α - Glucosidase Inhibitory Assay.](image)
exhibited significant inhibition of IC50 24.69 μg/ml as compared to the standard compound, Ascorbic acid 12.24 μg/ml. Similarly, the TbHL extract of T. bracteolata showed a lower percentage inhibition of ABTS + antioxidant activities of (11.14 ± 0.36) as compared to that of TbEAL extract (96.46 ± 0.00). The percentage inhibition of TbEAL extract was not significantly different from that of the standard compound, Trolox (98.73 ± 0.30). The IC50 also of TbEAL extract 6.48 μg/ml as compared to Ascorbic acid and Trolox (12.24 μg/ml) respectively.

The result of the IC50 value of DPPH and ABTS + assay shown on Fig. 4.

Fig. 4. IC50 value of DPPH and ABTS + assay.

Table 2: Antibacterial activities of the n-hexane and ethyl acetate extract of T. bracteolata leaves.

| Plant Extract     | Concentration (mg/ml) | Zone of inhibition of Bacteria (mm) | Mean ± Standard deviation |
|-------------------|-----------------------|-------------------------------------|---------------------------|
|                   |                       | S. aureus                          | E. coli                   | B. subtilis               | P. aeruginosa              | K. pneumonae               | S. typhi                  |
| n-Hexane          | 200                   | 20.0 ± 0<sup>ab</sup>              | 20.0 ± 0<sup>ab</sup>     | 18.0 ± 0<sup>ab</sup>    | 14.0 ± 0<sup>ab</sup>    | 14.0 ± 0<sup>ab</sup>    | 14.0 ± 0<sup>ab</sup>    |
|                   | 100                   | 18.0 ± 0<sup>ab</sup>              | 18.0 ± 0<sup>ab</sup>     | 16.0 ± 0<sup>ab</sup>    | 12.0 ± 0<sup>ab</sup>    | 12.0 ± 0<sup>ab</sup>    | 12.0 ± 0<sup>ab</sup>    |
|                   | 50                    | 14.0 ± 0<sup>ab</sup>              | 14.0 ± 0<sup>ab</sup>     | 14.0 ± 0<sup>ab</sup>    | 10.0 ± 0<sup>ab</sup>    | 10.0 ± 0<sup>ab</sup>    | 10.0 ± 0<sup>ab</sup>    |
|                   | 25                    | 12.0 ± 0<sup>ab</sup>              | 12.0 ± 0<sup>ab</sup>     | 12.0 ± 0<sup>ab</sup>    | -                       | -                       | -                        |
|                   | 12.5                  | 10.0 ± 0<sup>ab</sup>              | 10.0 ± 0<sup>ab</sup>     | 10.0 ± 0<sup>ab</sup>    | -                       | -                       | -                        |
|                   | 6.25                  | -                                  | -                       | -                           | -                       | -                       | -                        |
| Ethyl Acetate     | 200                   | 25.0 ± 1.4<sup>ab</sup>            | 24.0 ± 0<sup>ab</sup>     | 25.0 ± 1.3<sup>ab</sup>  | 20.0 ± 0<sup>ab</sup>    | 19.0 ± 1.5<sup>ab</sup>  | 19.0 ± 1.4<sup>ab</sup>  |
|                   | 100                   | 22.0 ± 2.8<sup>ab</sup>            | 19.0 ± 1.4<sup>ab</sup>   | 20.0 ± 0<sup>ab</sup>    | 18.0 ± 0<sup>ab</sup>    | 16.0 ± 2.8<sup>ab</sup>  | 16.0 ± 2.8<sup>ab</sup>  |
|                   | 50                    | 19.0 ± 1.4<sup>ab</sup>            | 17.0 ± 1.3<sup>ab</sup>   | 16.0 ± 0<sup>ab</sup>    | 14.0 ± 0<sup>ab</sup>    | 13.0 ± 1.5<sup>ab</sup>  | 13.0 ± 1.4<sup>ab</sup>  |
|                   | 25                    | 16.0 ± 2.8<sup>ab</sup>            | 13.0 ± 1.4<sup>ab</sup>   | 14.0 ± 0<sup>ab</sup>    | 12.0 ± 0<sup>ab</sup>    | 11.0 ± 1.2<sup>ab</sup>  | 11.0 ± 1.5<sup>ab</sup>  |
|                   | 12.5                  | 13.0 ± 1.5<sup>ab</sup>            | -                       | 12.0 ± 0<sup>ab</sup>    | 10.0 ± 0<sup>ab</sup>    | 5.0 ± 0<sup>ab</sup>     | 5.0 ± 0<sup>ab</sup>     |
|                   | 6.25                  | 10.0 ± 0<sup>ab</sup>              | -                       | 10.0 ± 0<sup>ab</sup>    | -                       | -                       | -                        |
| Control           | n-hexane              | -                                  | -                       | -                           | -                       | -                       | -                        |
|                   | Ethyl Acetate         | -                                  | -                       | -                           | -                       | -                       | -                        |
|                   | Gentamycin (10 mg/ml) | 40.0 ± 0                           | 39.0 ± 1.4              | 38.0 ± 0                     | 39.0 ± 1.39             | 39.0 ± 1.41             | 40.0 ± 0                  |

Key: - = no inhibition; <sup>a</sup>b = values significantly different when compared to negative control using t test; alphabetic superscript other than that of Gentamycin means significantly different (P < 0.05) when compared to positive control.

Fig. 3. % inhibition of DPPH and ABTS + assay respectively.
The observed biological effects are suggested. Further studies to investigate the bioactive compounds responsible for the observed biological effects are suggested.

### Table 3

| Extract         | Concentration (mg/ml) | C. albicans Mean ± S. Dev. (mm) | A. niger Mean ± S. Dev. (mm) | R. stolon Mean ± S. Dev. (mm) | P. notatum Mean ± S. Dev. (mm) |
|-----------------|-----------------------|---------------------------------|-----------------------------|-----------------------------|--------------------------------|
| n-Hexane        | 200                   | 17.0 ± 1.31a b                  | 15.0 ± 1.38a b              | 15.0 ± 1.21a b              | 17.0 ± 1.11a b                  |
|                 | 100                   | 14.0 ± 0.6a                    | 13.0 ± 1.50a b              | 12.0 ± 0.6b                 | 13.0 ± 1.39a b                 |
|                 | 50                    | 12.0 ± 0.6a                    | 10.0 ± 0.6b                 | 10.0 ± 0.6b                 | 10.0 ± 0.6b                    |
|                 | 25                    | 10.0 ± 0.6b                    | -                           | -                           | -                              |
|                 | 12.5                  | -                               | -                           | -                           | -                              |
|                 | 6.25                  | -                               | -                           | -                           | -                              |
| Ethyl Acetate   | 200                   | 19.0 ± 1.37a b                  | 17.0 ± 1.43a b              | 17.0 ± 1.35a b              | 20.0 ± 0.6b                    |
|                 | 100                   | 17.0 ± 1.4a b                   | 14.0 ± 0.6a                 | 14.0 ± 0.6b                 | 17.0 ± 1.4a b                  |
|                 | 50                    | 14.0 ± 0.6b                     | 12.0 ± 0.6b                 | 12.0 ± 0.6b                 | 14.0 ± 0.6b                    |
|                 | 25                    | 12.0 ± 0.6b                     | 10.0 ± 0.6b                 | 10.0 ± 0.6b                 | 12.0 ± 0.6b                    |
|                 | 12.5                  | 10.0 ± 0.6b                     | -                           | -                           | 10.0 ± 0.6b                    |
|                 | 6.25                  | -                               | -                           | -                           | -                              |
| Control         | n-hexane              | -                               | -                           | -                           | -                              |
|                 | Ethyl Acetate         | -                               | -                           | -                           | -                              |
|                 | Ticonazole (70%)      | 28.0 ± 0.00a                    | 28.0 ± 0.0a                 | 28.0 ± 0.0a                 | 26.0 ± 0.0a                    |

**Key:** a = no inhibition; b = values significantly different when compared to negative control using t test; alphabetic superscript other than that of Ticonazole means significantly different (P < 0.05) when compared to positive control.

### Declarations

**Author contribution statement**

Godshelp Osas Egharevba: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Omotayo O. Dosumu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Stephen O. Oguntoye: Conceived and designed the experiments; Analyzed and interpreted the data.

Ngaait S. Njiga, Samuel Olatunde Dahunsi: Analyzed and interpreted the data; Wrote the paper.

A. Abdulummeen Hamid: Analyzed and interpreted the data.

Ajay Anand, Zehra Amtul: Performed the experiments; Analyzed and interpreted the data.

Priyanka Ujjukuri: Performed the experiments.

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### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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