ABSTRACT

Objective: Diabetic foot ulcers (DFUs) are the most common cause of non-traumatic lower extremity amputations in developing countries. The aim of this pilot study was to evaluate the medicinal plant (fruits) and herbs (seeds) to meet their primary health-care needs healing to DFUs.

Methods: Three solvents were used to acquire extracts from powdered parts of the species. The extracts were used for phytochemical screening like a standard procedure. Quantitative assay of antioxidant activity of the extracts using free radical scavenging phenomena such as a 1,1-diphenyl-2-picrylhydrazyl-assay. The antimicrobial activity of the test organisms to the 11 plant extracts was screened using the agar well diffusion method (Perez et al., 1990). Hypotonicity-induced human red blood cell (HRBC) assay performs to determine anti-inflammatory activities.

Results: The presence of maximum antioxidant activity found in Arachis hypogaea (ethyl acetate) extract followed by Piper nigrum. The comparative study of these plants and herb species (P. nigrum and A. hypogaea), where P. nigrum, contains more total tannin contents and antioxidant compounds as persistent manner compares to A. hypogaea. Ethanolic and ethyl acetate, extracts of P. nigrum were studied for the in vitro antimicrobial and anti-inflammation against DFU contamination and HRBCs. P. nigrum treated on DFU contamination and HRBC, shown with maximum inhabitation properties for DFU (800 µg/mL).

Conclusions: The finding of the present investigation demonstrated that P. nigrum significantly more suppresses the growth of DFU contamination and induces anti-inflammatory activities follow by A. hypogaea.

Keywords: Antioxidant, Diabetic foot ulcer, Anti-inflammation.
being potential drugs for the treatment of non-insulin-dependent diabetes mellitus. Tannins can improve the pathological oxidative state of a diabetic situation [9]. The phenols are dietary antioxidants include ascorbate, tocopherols, carotenoids, and bioactive plant phenols. The health benefits of fruits and seeds are largely due to the antioxidant biomolecules supported by a large number of phytochemicals, some with greater antioxidant properties. Plant phenols have not been completely studied because of the complexity of their chemical nature and the extended occurrence in plant materials [10]. The present work is to provide an overview of the findings related to the presence of antioxidant, phenols and tannin in plant and herb sources. The antimicrobial and HRBC assay performed on DFUs Sample. Certain groups of researchers have focused on the investigation of plants and microbial extracts, essential oils, pure secondary metabolites, and new synthesized molecules as potential antimicrobial agents. The in vitro antimicrobial activity of extracts or a pure compounds used by most common basic methods are the disc diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as poisoned food technique. It is important to develop a better understanding of the current methods available for screening and/or quantifying the antimicrobial effect of an extract or a pure compound for its applications in human health, agriculture, and environment. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute for bacteria and yeast testing [11].

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: The increase of vascular permeability, increase of protein denaturation, and membrane alteration [12]. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. The inflammation of tissue is due to response to stress [13]. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. The real issue in the rural community to management of inflammation related diseases and the population in these areas uses many alternative drugs such as substances produced from medicinal plants [12].

**METHODS**

**Preparation of powder**

The *P. nigrum* (100 g) and *A. hypogaea* (100 g) washed with fresh water to remove adhering dust and then dried under shade for 2 days. After shade dried, for estimation of powder was using a mixer grinder and in turn extracted with different solvents, namely, hexane (non-polar), ethyl acetate (polar), and 75% ethanol (strong polar). The extraction mixture was left untouched for a day before the extract was separated from the residue by filtration through Whatman No 1 filter paper. These extraction samples collected into jar and left its for evaporation for days.

**Phytochemical screening**

The extracts are tested for the presence of bioactive compounds (tannin, saponin, flavonoids, quinine, glycoside, cardiac glycoside, terpenoid, phenol, coumarins, steroid, alkalioids, phlobatannins, and anthraquinones) using followed by phytochemical screening standard methods.

**Quantitative assay of antioxidant activity**

Leaf extract samples of 100 µl from qualitative assay were mixed with 2.7 ml of methanol. Then, 200 µl of 0.1% methanolic 1,1-diphenyl-2-picyrilhydrazyl was added. The suspension was incubated for 30 min in dark conditions. Eventually at every 5 min interval, the absorption maxima of the solution was measured using an ultraviolet (UV) double beam spectra at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% of butylated hydroxytoluene.

The radical of sample is calculated by the following formula

\[
\text{Inhibition}\% = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100
\]

**Quantitative phytochemical analysis**

**Determination of tannin content**

The tannin content in the extracts was determined by Folin-Ciocalteu method. 0.1 ml of the sample extracts containing 1 mg were added to test tubes containing 7.5 ml of distilled water and 0.5 ml of Folin-

**Table 3:** Total tannin contains in *Arachis hypogaea* and *Piper nigrum*

| S. No. | Sample/g | Total tannin contents concentration (mg tannic acid/g dry sample) |
|-------|----------|---------------------------------------------------------------|
| 1.    | Piper nigrum | 109.75                                                       |
| 2.    | *Arachis hypogaea* | 75.85                                                        |

**Table 4:** Total phenol contains in *Arachis hypogaea* and *Piper nigrum*

| S. No. | Sample/g | Total phenol contents concentration (mg GAE/g dry sample) |
|-------|----------|----------------------------------------------------------|
| 1.    | Piper nigrum | 122.4                                                     |
| 2.    | *Arachis hypogaea* | 175.7                                                    |

**Table 5:** Formula of respective solvents and antimicrobial

| Solvent      | Formula | Activity           |
|--------------|---------|--------------------|
| Hexane       | C₆H₁₂  | Solvent            |
| Ethyl acetate| C₆H₁₂O₄ | Solvent            |
| Ethanol      | C₆H₁₀O₂ | Solvent            |
| Tetracycline | C₈H₇N₂O₃ | Antimicrobial      |
Cicalete reagent, 1 ml of 35% Na$_2$CO$_3$ and made it up to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80, and 100 µg/ml) was also used. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalence (TE) µg/mg of extract.

**Determination of total phenolic content**

Total phenolic content was determined by the Folin-Cicalete method [14]. Total phenolic content was determined by the Folin-Cicalete reagent, 1 ml of 35% Na$_2$CO$_3$ and made it up to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80, and 100 µg/ml) was also used. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalence (TE) µg/mg of extract.

**Table 6: The diameters of inhibition growth zones of samples extract with different solvent and antimicrobial agents (control) are measured in mm**

| Biotic       | Extract | Inhibition (mm) | Inhibition (mm) |
|--------------|---------|----------------|-----------------|
| Arachis hypogaea | Sample (µl) | Sample | Control (C$_6$H$_5$N$_2$O$_2$) |
| DFU ([C]$_3$H$_5$N$_2$) | 20 | 1 | 15 |
| DFU ([C]$_3$H$_5$N$_2$) | 40 | 4 | 15 |
| DFU ([C]$_3$H$_5$N$_2$) | 60 | 4.6 | 15 |
| DFU ([C]$_3$H$_5$N$_2$) | 80 | 8.2 | 15 |
| DFU ([C]$_3$H$_5$N$_2$) | 20 | 2 | 14 |
| DFU ([C]$_3$H$_5$N$_2$) | 40 | 3 | 14 |
| DFU ([C]$_3$H$_5$N$_2$) | 60 | 6.2 | 14 |
| DFU ([C]$_3$H$_5$N$_2$) | 80 | 10 | 14 |
| DFU ([C]$_3$H$_5$N$_2$) | 10 | - | - |
| DFU ([C]$_3$H$_5$N$_2$) | 80 | - | - |

| Piper nigrum | Extract | Inhibition (mm) | Inhibition (mm) |
|--------------|---------|----------------|-----------------|
| Biotic       | Sample (µl) | Sample | Control (C$_6$H$_5$N$_2$O$_2$) |
| DFU ([C]$_3$H$_5$N$_2$) | 20 | 2 | 15 |
| DFU ([C]$_3$H$_5$N$_2$) | 40 | 5 | 15 |
| DFU ([C]$_3$H$_5$N$_2$) | 60 | 8 | 15 |
| DFU ([C]$_3$H$_5$N$_2$) | 80 | 10 | 15 |
| DFU ([C]$_3$H$_5$N$_2$) | 20 | 1 | 14 |
| DFU ([C]$_3$H$_5$N$_2$) | 40 | - | - |
| DFU ([C]$_3$H$_5$N$_2$) | 60 | - | - |
| DFU ([C]$_3$H$_5$N$_2$) | 80 | - | - |
| DFU ([C]$_3$H$_5$N$_2$) | 20 | 3 | 10 |
| DFU ([C]$_3$H$_5$N$_2$) | 40 | 5 | 10 |
| DFU ([C]$_3$H$_5$N$_2$) | 60 | 8 | 10 |
| DFU ([C]$_3$H$_5$N$_2$) | 80 | 11 | 10 |

**Fig. 1: 1,1-diphenyl-2-picrylhydrazyl free radical scavenging percentage of Arachis hypogaea**

**Antimicrobial activity screening tests**

The antimicrobial activity of the test organisms to the 11 plant extracts was screened by using the agar well diffusion method (Perez et al., 1990). Swabbed Uniformly of suspension inoculum on solidified 20 ml Mueller-Hinton Agar for bacteria and Sabouraud dextrose agar for fungi and the inoculum was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using sterile cork borer. Take 50 µl of aliquot from each plant crude extract (500 mg/mL) was added into each well on the seeded medium and allowed to stand on the bench for 60 min for proper diffusion and thereafter incubated at 37°C for 24 h. The resulting inhibition zones were measured in millimeters. The sample procedure was followed for the fungus Candida albicans but incubated at 30°C. Negative controls using 50 µl tetracycline were also run in the same manner and parallel to the treatments. These studies were performed in triplicate. The minimal microbial concentration and minimum inhibitory concentration were determined for the active plant extracts that showed the widest spectrum of antimicrobial activity against test microorganisms.

**Hypotonicity-induced human red blood cell (HRBC)**

1.0 mL of test sample of different concentrations (50–200 µg) in 1 ml of 0.2 M phosphate buffer and 0.5 ml of 10% HRBC suspension incubated at 37°C for 30 min, with 0.5 ml of 0.25% hyposaline and centrifuged at 3000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and control was prepared by distilled water instead of hypo saline to produce 100% hemolysis without samples. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated using the following formula:

Percentage of hemolysis = (Optical density of test sample/optical density of control)×100

Percentage protection = 100−[(Optical density of test sample/optical density of control)×100] [15].

**RESULTS AND DISCUSSION**

**Qualitative phytochemical analysis of P. nigrum and A. hypogaea, fruits, and seeds extract**

The phytochemical screening of the plant studied showed the presence of tannin, saponin, flavonoids, quinine, glycoside, cardiac glycoside,
terpenoids, phenol, coumarins, steroids, alkaloids, phlobatannins, and anthraquinones. On *P. nigrum* (Table 1) ethyl acetate fruits extract was found the highest positive response followed by other solvents while in *A. hypogaea* (Table 2) ethanol seeds extract was found the highest positive response followed by other solvents such as hexane and ethyl acetate of fruits and seed extracts, respectively.

**Quantitative phytochemical screening**

The tannin content was expressed in terms of mg of TE µg/mg of the extract. While *P. nigrum* exhibited the maximum content of Tannin (Table 3), whereas *A. hypogaea* exhibited the maximum content of phenol (Table 4) compounds comparatively together, hence greatest antioxidant profiles these two compounds of plant and herb contains.

In vitro antioxidant activity: Percentage: Among these, two species of herb and plant are used for maximum antioxidant activity found in *A. hypogaea* (ethyl acetate: Fig. 1) extract followed by *Piper nigrum* (Fig. 2). Table 5 shows formula of respective solvents and antimicrobial.

We found that in this study, the plant and herb extracts by ethanol and ethyl acetate of *P. nigrum* and *A. hypogaea*, respectively, provided more consistent antimicrobial activities for DFU contaminations, compared to those extracted by other solvents (Table 6).

**Antimicrobial assay**

The inhibition zones of the Antimicrobial assay were measured in millimetres shown in table 6. The *Arachis hypogaea* and the *Piper nigrum*, crude extract of samples with [C₆H₅O₂] and [C₆H₁₀OH] respectively shown maximum antimicrobial activities.

**CONCLUSION**

The study clearly indicates that the extract possesses antioxidant, antimicrobial, and anti-inflammatory substances. At the specific space and time of abiotic factors induces more ability to suppress growths of DFU contamination and anti-inflammatory activities comparison to underground abiotic factors.

These findings justify the traditional uses of this plant’s fruit in the treatment of DFU, inflammatory.

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**Authors’ Contributions**

Dr. Sharmila KJ, supervised my project work and reviewed the manuscript; and Dr. R. Caroline Jeba provided the chemical details and the samples.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.
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