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RESEARCH ARTICLE

In- vitro Anti-oxidant Activity and Free Radical Scavenging Potential of roots of Malawian *Trichodesma zeylanicumm* (burm. f.)

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ABSTRACT:

Aim: To evaluate the antioxidant potential and free scavenging activity of *T. zeylanicum* powdered root extract.

Material and Methods: The plant extract (50mg) obtained by soxhlet extraction method was dissolved separately in 50ml of methanol and the resultant solution serially diluted to concentrations 0.5, 0.25, 0.125 and 0.0625 mg/ml. The antioxidant potential and free radical scavenging activity were analysed using reducing power assay and hydrogen peroxide scavenging activity methods. Phytochemical analysis was performed on the plant extract to detect the presence of phytoconstituents.

Results and Discussions: Phytochemical screening revealed the presence of phenolics, alkaloids, saponins, flavonoids and tannins. The flavonoids content were found to be 6.28±0.06 mg/gram of the dried extract. The reducing power assay showed that reducing ability of the extract were significantly increased with increasing concentration and were higher compared to the standard ascorbic acid. Methanolic extract of *T. zeylanicum* also showed good scavenging ability compared to the standard ascorbic acid. The IC_{50} values were found to be 0.122 mg/ml compared to standard ascorbic acid 0.717mg/ml. At a concentration of 1mg/ml, the scavenging percentages were 74.82 and 48.12 for *T. zeylanicum* extract and standard respectively.

Conclusion: The result indicates the potential of *T. zeylanicum* as a source of antioxidants relevant to wound treatment.

Keywords: *Trichodesma zeylanicum,* reducing power assay, hydrogen peroxide scavenging activity, antioxidant potential, Phytochemical analysis.

1. INTRODUCTION

Wound infections are one of the most common diseases in the developing countries because of poor hygienic conditions (Senthil Kumar et al., 2006). Wounds are physical injuries that results in an opening or breaking of the skin. Wound-healing is a process that comprises three phases, e.g. inflammation, proliferation and remodelling and proceeds with complicated and well-organised interaction between various tissues and cells by overlapping between successive stages. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (Jorge et al., 2010).

When wound occurs, it is generally accompanied by classical symptoms of inflammation i.e. pain, reddening and edema. The inflammation stage begins immediately after injury; first with vasoconstriction, and platelet aggregation at the injury site, and then the infiltration of leukocytes and T-lymphocytes into the wound area. The cicatrisation process proceeds naturally, since the damaged tissues attempts to re-establish hemostasis. In the inflammatory stage, the main aim is the removal of debris, damaged tissue, and bacteria by neutrophils and macrophages which have a role in anti-microbial defense and debridement of devitalized tissue by production of proteolytic enzymes and reactive oxygen species (ROS) (Jorge et al., 2010). ROS is produced in high amounts at the site of wounds as a defense mechanism against invading bacteria (Reddy et al., 2008). However, the

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presence of increased numbers of neutrophils and ROS overwhelm the antiprotease substances that normally protect the tissue cells and the extracellular matrix (Mensah et al., 2001). At high concentrations, ROS can induce severe tissues damage and even lead to neoplastic transformation decreasing the healing process by damages in cellular membranes, DNA, proteins and lipids. Fibroblasts may be killed and skin lipids will be made less flexible by excess ROS (Jorge et al., 2010).

Currently, research on wound healing agents is one of the developing areas in modern biomedical science and many traditional healers across the world have valuable information of many lesser known wild plants for treating wounds and burns (Kumar et al 2007).

In Malawi, many plants are used in the treatment of wounds, and *Trichodesma zeylanicum* leaves and roots have extensively been reported by the local communities to treat wounds. The roots are also used for treating diarrhoea while leaves are also eaten as vegetables. According to Gurib-Fakim et al 1997, *Trichodesma zeylanicum* powdered roots are applied externally on wounds and skin as analgesic. And in 1984, Msonthi isolated squalene and other known phytosterols compounds from the leaves and recommended the plant as a good source of steroidal hormone precursors because of the high yield. The plant has also been reported to contain the low toxic alkaloids supinine (O'Kelly et al., 1961)

Trichodesma zeylanicum belongs to the family Boraginaceae, and it is a densely bristly-hairy annual herbal plant that can grow up to 1 metre. Leaves are narrowly elliptic, while flowers becomes nodding, in terminal 1-sided bracteates inflorescences. Sepals are bristly hairy enlarging in fruit. Corrolla (7-9mm), are scarcely exserted from the sepals, lobes pale blue to lilac or pinkish (Hyde et al., 2013).

The present study aims to evaluate the methanolic extract of *T. zeylanicum* powdered roots for antioxidant potential and free radical scavenging activity in order to understand the usefulness of the plant in the treatment of wound infections.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant material

The fresh plant of *Trichodesma zeylanicum* was collected in Zomba district and authenticated by Mr Patel of National Herbariums and Botanical Gardens of Malawi. The root parts of the plant was washed with water, shade dried powdered in a mechanical grinder and kept in air tight polythene bag till use.

2.2 Preparation of the plant extract

The powder (50gm) of the root parts were initially de-fatted with petroleum ether (60-80^{o}C), followed by 250ml methanol by Soxhlet extraction method for 72hrs. Solvent elimination under reduced pressure afforded the petroleum ether and methanol extract of which methanol extract was further used for antioxidant assay methods. The extract was dried in vacuum desiccators to obtained constant weight. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts were obtained by the solvent evaporation and used to determine the concentration in mg/ml. The extract was used directly for the reducing power assay, hydrogen peroxide radical scavenging assay, the total flavonoid content estimation.

2.3 Chemicals and standard

The following chemicals and reagents were purchased from Merck Chemicals (Pty) Ltd, RSA, SAARCHEM Ltd, RSA and BDH Chemicals, England; methanol, petroleum ether, hydrogen peroxide, phloroglucinol , 5% ferric chloride solution, lead acetate solution, 95% ethanol, concentrated hydrochloric acid, ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, hydrogen peroxide (40mM) and vanillin reagent.

2.4 Phytochemical screening

The dried methanolic extract was used to analyze qualitatively various phytoconstituents such as flavonoids, phenolic compounds and tannin using standard procedures.

2.4.1 Test for Flavonoids

5ml of 95% ethanol was added to aqueous methanol extract, followed by few drops of concentrated hydrochloric acid and 0.5g magnesium turnings. Pink colour indicated the presence of flavonoids.

2.4.2 Test for Phenolics

Neutral ferric chloride was added to the aqueous methanol extract. Appearance of bluish green colour indicated the presence of phenolic compounds.

2.4.3 Test for Tannins

2ml of aqueous methanol extract was added to 5ml of n-butanol- hydrochloric acid solution in a test tube. The mixture was warmed for 1 hour at 95^{o}C in a water bath. Appearance of red colour indicated the presence of tannin.

2.4.4 Test for Saponins

5ml of distilled water was added to 0.5g of extract. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.5 Estimation of flavonoid content

2.5.1 Preparation of test and standard solutions

The plant extract (50mg) was dissolved separately in 50ml of methanol. The solution was further serially diluted with methanol to obtain lower dilutions. Phloroglucinol (50mg) was dissolved in 50ml of distilled water. And it was also

further serially diluted with water to obtain lower dilutions.

2.5.2 Protocol for total flavonoid content

The total flavonoid content was determined by adding distilled water to 0.2ml of the extract in a test tube to the final volume of 2ml. To this solution, 4ml of vanillin reagent was added rapidly. Exactly after 15 minutes absorbance was recorded at 500nm against blank. The unknown was read from a standard curve prepared using different concentration of phosphoglucinol.

2.6 Reducing power assay method

Fe^{3+} reducing power of *T. zeylanicum* extract was determined by modified method of Oyaizu. Reducing power was determined by taking different concentration of the plant extract. Ascobic acid was used as reference standard. 50mg of methanol extract was dissolved in 50ml of methanol. From the above different concentrations (1, 2, 3, 4, 5ml) were pipetted out and made up to 10ml with methanol. 2.5ml of phosphate buffer 6.6 and 2.5ml of potassium ferricyanide were added to each of the test tubes and incubated at 40°C for 20minutes. After incubation, 2.5ml of trichloroacetic acid was added and centrifuged for 5min. To 2.5ml of this reaction mixture, 0.5ml ferric chloride and 2.5ml water were added. The absorbance was measured at 700nm using UV spectrophotometer.

2.7 Hydrogen peroxide scavenging activity

The scavenging activity of extract towards hydrogen peroxide radicals was determined by the modified method of Dehpour. Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560nm using UV spectrophotometer. 0.1mg/ml of the extract was added to hydrogen peroxide solution and absorbance measured at 560nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula:

Percentage scavenged [H_2O_2] = 1- Abs (standard)/Abs (control) x100

where, Abs control was the absorbance of the control (without extract) at 560nm; Abs sample was the absorbance in the presence of the extract at 560nm. The experiment was repeated in triplicate.

2.8 Calculation of IC_{50}

Various concentrations (0.625-1mg/ml) of methanolic extracts of *T. zeylanicum* were taken for the study and IC_{50} values which shows 50% inhibition was calculated using regression analysis in MS excel.

2.9 Statistical analysis

All experimental measurements were carried out in triplicate and were expressed as average of three analyses ±standard deviation. Statistical analyses were performed by one sample t-test and p-values were done by one way ANOVA. The p-value<0.05 were regarded as significant.

3. RESULTS

3.1 Preliminary phytochemical analysis

Preliminary phytochemical screening of the methanolic extracts of *T. zeylanicum* revealed the presence of flavonoids, saponins, tannins and phenolic compounds.

Phytocompounds	Colour change	Presence
Flavonoids	Pink	+
Tannins	Red	+
Phenolic compounds	Bluish green	+
Saponins	Emulsion formed	+

Table 1: Preliminary phytochemical screening of the methanolic extracts of *T. Zeylanicum*

3.2 Total flavonoid content

The flavonoid content was found to be 6.28±0.06 mg/gram of dried extract equivalent to phloroglucinol. The total flavonoids content shows a linear relation in both standard and sample extract.

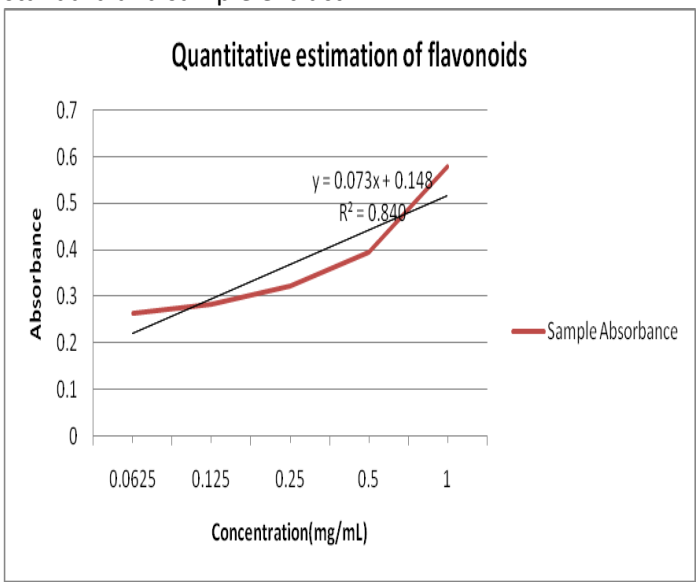

Figure 1: Quantitative estimation of flavonoids

3.3 Reducing power assay

The reducing power of the extract compared to ascorbic acid is shown in Fig 1. In the reductive ability measurement, Fe^{3+} - Fe^{2+} transformation in the presence of extract sample was investigated. Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. In this investigation, Table 2 and Figure 1 shows the reductive capabilities of methanolic extract of *T. zeylanicum* when compared to the standard ascorbic acid. The reducing power increased significantly ($p<0.05$) with increasing concentration of the extract. At 0.625 mg/ml, the absorbance of plant extract and standard were 0.45 and 0.425 respectively, while at 1mg/ml, the absorbances were 0.9 and 0.7 respectively. Good correlation between concentration and absorbance of both standard and methanol extract were observed in the study (0.885 and 0.9494 respectively).

CONCENTRATION (mg/ml)	WAVELENGTH (nm)	ABSORBANCE(nm)	
		Methanol extract±SD	Ascorbic acid±SD
0.0625	700	0.45±0.013	0.425±0.0013
0.125	700	0.7±0.013	0.55±0.01
0.25	700	0.75±0.06	0.6±0.04
0.5	700	0.8±0.001	0.65±0.014
1	700	0.9±0.04	0.7±0.03

Table 2: Results of reducing power assay

CONCENTRATION (mg/ml)	Percentage Inhibition of	
	Methanol extract±SD	Ascorbic acid±SD
0.0625	16.89±0.009	16.11±0.017
0.125	43.45±0.013	17.61±0.008
0.25	61.72±0.008	23.265±0.006
0.5	71.78±0.015	38.415±0.005
1	74.82±0.04	48.12±0.01
IC50	0.122mg/ml	0.717mg/ml

Table 3: Hydrogen peroxide scavenging activity

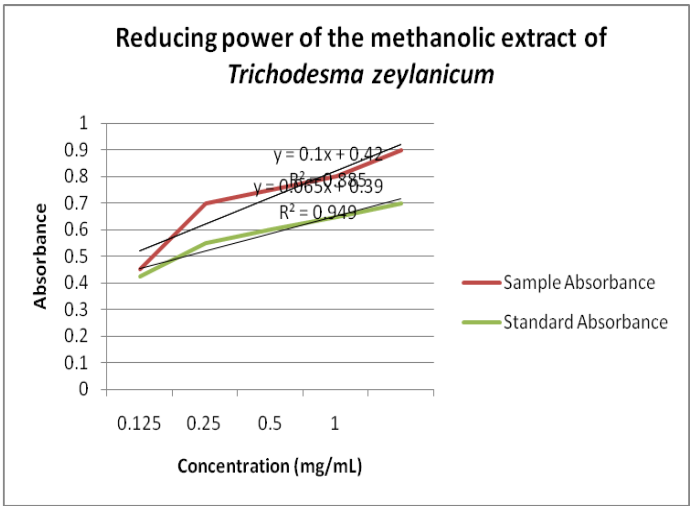

Figure 2: Reducing power of the methanolic extracts of *T. Zeylanicum*

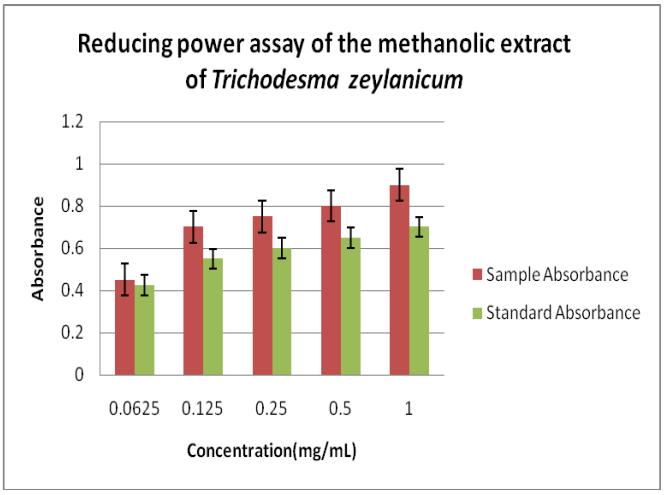

Figure 3: Reducing power assay

3.4. Hydrogen peroxide scavenging activity

Table 3 shows hydrogen peroxide scavenging activity of the methanol extract and standard. *T. zeylanicum* extract caused a strong dose-dependent inhibition of hydrogen peroxide. The methanol extract showed good scavenging ability compared to the standard compound. The IC_{50} values for the extract were found to be 0.122 mg/ml compared to standard ascorbic acid 0.717mg/ml. At a concentration of 1mg/ml, the scavenging percentages were 74.82 and 48.12 for methanol extract and standard respectively. P values were found to be significant ($p \leq 0.05$) for both standard and extracts. Regressions coefficient (R^2 was found to be 0.9609 and 0.8458) for the methanolic extract and standard respectively.

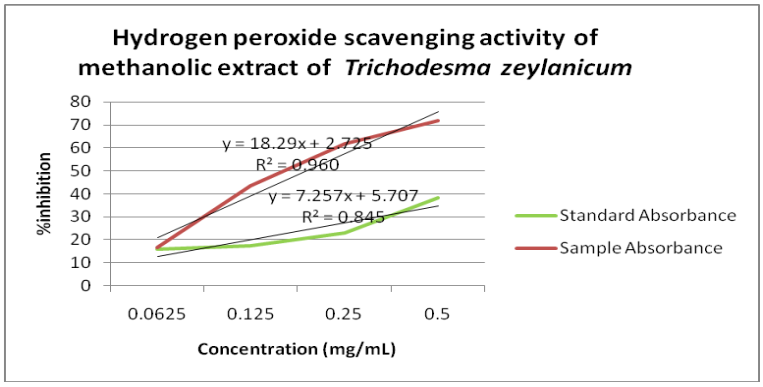

Figure 4: Hydrogen peroxide scavenging activity of the methanolic extracts of *T. Zeylanicum*

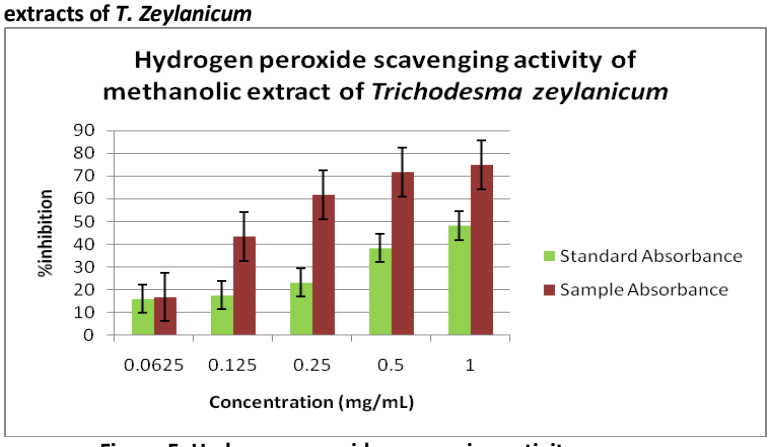

Figure 5: Hydrogen peroxide scavenging activity

4. DISCUSSION

Methanolic extract of *T. zeylanicum* roots demonstrated significant in vitro antioxidant potential and free radical scavenging activity which is relevant to wound treatment. The investigations showed that phytochemical constituents such as flavonoids and other phenolic compounds which have been reported to have multiple biological effects such as antioxidant activity, anti-inflammatory actions, inhibition of platelets aggregation and antimicrobial activities (Venkatanarayan et al., 2010). Antioxidant reduces the adverse effect of wounds by removing products of inflammation. They counter the excess proteases and ROS often formed by neutrophil accumulation in the injured site and protect protease inhibitors from oxidative damage (Houghton et al., 2005)). The most likely mechanism of antioxidant protection is direct interaction of the extract (or compounds) and the hydrogen peroxide rather than altering the cell membranes and limiting damage (Annan and Houghton,

2008). Compounds with high radical-scavenging capacity have shown to facilitate wound healing. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects (Kumaran et al., 2007).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging (Yildirim et al., 2000).

The results indicate that *T. zeylanicum* methanolic extract contain significant amounts of phytoconstituents such as flavonoids, saponins, phenolic compounds). Flavonoids and phenolic compounds have good antioxidant potentials and mechanism of action of flavonoids is through scavenging or chelation (Yildirim et al., 2000), while phenolic compound are important because of their hydroxyl groups which confer scavenging ability (Cook and Samman 1996).

5. CONCLUSION

Based upon the results obtained in the present study, it is concluded that methanolic extract of *Trichodesma zeylanicum* contains considerable amount of flavonoids and phenolic compounds, exhibits high antioxidant and free radical scavenging activities relevant to wound treatment. It also chelates iron and has reducing power. These indicate that the plant is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses and treating wound infections. However, further isolation of bioactive compounds would assist to ascertain its potency and safety as a lead candidate of antioxidant for pharmaceutical uses.

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Conflict of Interest: None Declared

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