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Original Research Article

Phytochemical screening and antioxidants investigations of Ethyl Acetate and Acetone Leaf Extracts of *Thaumatococcus danielli*

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Abstract

The method of cold maceration was used in the extraction T*haumatococcus danielli.* The results obtained from qualitative phytochemical screening of the ethyl acetate and acetone extracts of *T. danielli* revealed the presence of medicinally bioactive compounds such as alkaloids, steroids, flavonoids, phenols, tannins phlobatannins and saponins. The crude ethyl acetate extract of *T. danielli* showed inhibition of DPPH radical scavenging activity at the ranges of 27.90%, 38.12%, 61.33% 65.19% and 61.60% with concentrations of 0.1, 0.3 ,0.5 ,0.7 and 1g/ml and that the crude acetone showed inhibition of DPPH radical scavenging activity at the ranges of 76.24%, 71.73%, 66.45%,62.26% and 54.96% with concentrations of 0.1, 0.3, 0.5,0.7, and 1g/ml and vitamin C showed minimum radical scavenging activity of 66.57% and maximum activity of 85.52%.

Keywords: Antioxidants, *Thaumatococcus danielli,* Phytochemicals, 1,1-diphenyl-2-picryl hydrazyl, radical scavenger.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and remarkable number of modern drugs has been isolated from natural sources. Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic, lignins, stilbenes, tannins, flavonoids, esiccat, coumarins, alkaloids, amines, betalains, and other metabolites which are rich source of free radical scavengers (Gracelin et al., 2012), Cai et al., 2003. They are also antioxidant compounds which possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities (Sala et al., 2002). The medicinal value of a plant lies in some chemical substances that produce a definite physiological action on the human body. Some of the most important of these bioactive constituents of plants include alkaloids, tannins carbohydrates, terpenoids, steroids and flavonoids (Tiwari et al., 2011; Ushie et al., 2013; Ushie et al., 2013a; Ushie et al., 2013b; Edeoga et al., 2005). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources (Lena et al., 2010). Antioxidants are substances that prevent the cells of the body against free radicals, which play a role in heart diseases, cancer and other diseases.

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation reaction can produce free radical substance to an oxidizing agent. Traditional medicines have some limitations such as lack of prescription and scientific proof. Some herbs look so common to the untrained eye that they are often mistaken for one another. For proper understanding of these plants, the medicinal value of the plant must be known, the rightful role at which the plants can play and the plant must be subjected to scientific proof. The plant kingdom holds many species of plants that contain substances of medicinal value which are yet to be discovered. This project work gives an insight on

the scientific data on the antioxidants activities of *T. danielli.* The justification for the selection of *Thaumatococccus daniellii* is that the leaf is frequently used to wrap native food and to the best of our knowledge no work has been done on the preliminary phytochemical screening and the antioxidant properties of this plant in this part of the world. This work will give information on the antioxidant activities of acetone, methanol, hexane and ethyl acetate extracts of *Thaumatococcus danielli leaves*. This will give the scientific bases for the use of plant part for medicinal purposes. The aim is to study the preliminary phytochemical screening and the antioxidant properties antioxidants activities of the ethyl acetate and acetone crude extracts leaves of *T. danielli* as a medicinal plant.

MATERIALS AND METHOD

Sample Collection, Preparation and Extraction

The *Thaumatococcus danielli* leaves were collected from their natural habitat in Wukari Local Government Area of Taraba State, Nigeria and were air dried for two weeks; the dried sample was chopped and grounded into fine powder. The extracts of the leaves were prepared by soaking 100 g of the sample in 250 ml ethyl acetate for 72 hours with frequent agitation. The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation using rotary evaporator, kept in a vacuum oven over night at room temperature to remove all the solvent and weighed. The procedure was repeated on the residue using acetone. The extracts were stored in a esiccators until required for testing.

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts using standard procedures to identify the constituents. Qualitative analysis of the crude extracts were carried out as described by (Sofowora, 1993; Edeoga et al., 2005; Tiwari, et al., 2011; Ushie and Adamu, 2013 and Sagayaraj et al., 2015) to identify the presence of the classes of Secondary Metabolites (alkaloids, flavonoids, tannins, saponins, steroids and phenols).

Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes indicates the presence of saponins.

Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of tannins

A small quantity or the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue black or brownish green indicate the presence of tannins.

Detection of Phenols

To 1ml of leaf extract 2ml of distilled water was added followed by a few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for phlobatannins

A portion of each extract was boiled with 1% aqueous HCl. The solutions were observed for a red deposit of precipitate taken as evidence for the presence of phlobatannins.

Table 1. The result obtained from qualitative phytochemical of *Thaumatococcus danielli.*

S/N	Phytochemicals	Tests	EAE	AE
1	Flavonoids	Extract + NaOH	+	+
		Extract +Lead acetate	+	+
2	Alkaloids	Extract + Mayer	+	+
		Extract +wagner	+	+
3	Phlobatannins	Extract +2% HCl	-	+
4	Steroids	Extract + H_2SO_4	-	+
5	Saponins	Froth test	-	+
		Foam test	-	+
6	Tannins	Extract +H_2O + $FeCl_3$	-	-
7	Phenols	Extract + H_2O + $FeCl_3$	-	-

Key: EAE= Ethyl acetate Extract AE= Acetone Extract

Test for steroids

5 drops of concentrated H_2SO_4 was added to1ml of each extract in a test tube. The solutions were observed for a red colouration indicating the presence of steroids in the extracts.

Antioxidant Assay using DPPH Assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

2, 2- Diphenyl -1- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

(DPPH) + (H-A) ⟶ DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Working procedure

Different volumes of the extract were taken and made up to 2ml with methanol. The following concentrations of the extract were tested (0.1, 0.3, 0.5, 0.7, and 1.0 mg/ml). Vitamin C was used as the antioxidant standard at concentrations (0.1, 0.3, 0.5, 0.7, and 1.0 mg/ml). 0.5ml of 1mM of DPPH in ethanol was added to each of the sample solutions. A blank solution was prepared containing the same amount of methanol and DPPH. The sample solutions are incubated in the dark for 3ominutes before reading the absorbance at 517nm. The radical scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

Where A = Absorption of the blank sample without extract.

B = the absorption of the extract.

RESULTS

Qualitative Phytochemical Result of *Thaumatococcus danielli*

Table 1 and Table 2

DISCUSSIONS

Preliminary Phytochemical Screening

The results obtained from the extracts *T. danielli* using ethyl acetate and acetone in the order of increasing polarity revealed the presence of medicinally bioactive compounds such as alkaloids, steroids, flavonoids, phenols, tannins, phlobatannins and saponins. The

Table 2. Antioxidant Activities of Ethyl Acetate and Acetone Extracts.

Concentration (mg)	% inhibition of EAE	% inhibition of AE	% inhibition of Vitamin C
0.1	27.90	76.24	66.57
0.3	38.12	71.73	67.41
0.5	61.33	66.45	71.31
0.7	65.19	62.26	76.60
0.9	61.60	54.96	85.52

Key: EAE= Ethyl acetate Extract AE= Acetone Extract

results obtained revealed that alkaloids were found to be present in the ethyl acetate and acetone extracts when both Wagner`s and Mayer`s reagent were used for the test.

Tannins and phenols were found to be absent in the extracts of both ethyl acetate and acetone. Alkaline test for flavonoids revealed the presence of flavonoids in both the ethyl acetate extract and acetone extract, and also flavonoids were found to be present in both the ethyl acetate extract and the acetone extract when lead acetate test was carried out. Flavonoids have been stated to possess many useful properties, containing anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity (Tapas, *et al* .,2008). Flavonoids constitute a wide range of substances that play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA (Atmani, *et al* .,2009). The extracts of ethyl acetate and acetone when tested for saponins revealed its presence in the acetone extract and its absence in the ethyl acetate extract. saponins have antitumor and anti-mutagenic activities and can lower the risk of human cancers, by prevent cancer cells from growing. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al*., 1995) .Plants make saponins has potentials to fight infections by parasites and in humans saponins serves as immune system booster and also protect against viruses and bacteria. The non-sugar part of saponins has a direct antioxidant activity which may result in reduced risk of cancer and heart diseases () [20]. Saponin is one of the useful ingredient in costmetic (Prohp and Onoagbe 2012)).

Steroids were found to be absent in the ethyl acetate extract but present in the acetone extract. Steroidal compounds are of importance and interest in pharmacy due to their relationship with compounds such as sex hormones (Okwu, 2001). Consequently, the leaves of *Thaumatococcus danielle* may be useful as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance, has being used in some countries, since steroidal structure could serve as potent starting material in the synthesis of these hormones (Edeoga, and Eriata, 2001). Phlobatannins were found to absent in the ethyl acetate extract but present in the acetone extract. Hence, *Thaumatococcus danielle* can be used for wound healing properties, these are anti-inflammatory and Analgesic (Ayinde et al., 2007) and antioxidant (Okwu and Okwu, 2004). Alkaloids are known to have muscle relaxant property and can be utilized for their analgesic, antispasmodic and bactericidal effects (Stray, 1998; Okwu and Okwu, 2004). Alkaloids has been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine andanti-hypertensive antifungal, anti-inflammatory, anti-fibrogenic effect (Ghosal et al., 1996). Some alkaloids are useful against HIV infection as well as intestinal infection associated with AIDS (McDevitt et al., 1996). The presence of alkaloids in the six medicinal plants makes them recommendable for patient as alkaloids possess a significant pharmacological property.

To determine the antioxidant activity of a specific solution, there will be a significant decrease in the absorbance for sample which contain antioxidant company (purple colour vanishing coupled with the yellow colour build up clearly noticed by naked eye) the intensity of the yellow colour was directly proportional with the antioxidant activity in the tested solution, the higher the scavenging the higher the activity (Sagare and singh, 2011). The free radical scavenging activity was evaluated by accessing its discolouration of 2,2 diphenyl-1-picrylhydrazyl radical (DPPH) in methanol by a slightly modified method . The following concentrations of the extract were tested (0.1, 0.3, 0.5, 0.7 and 0.9 mg/ml). the decrease in absorbance was monitored at 517nm. Vitamin C was used as the antioxidant standard at a concentrations (0.1, 0.3, 0.5, 0.7 and 0.9mg/ml) (Figure 1).

The crude ethyl acetate extract of *Thaumatococcus danielli* displayed inhibition of DPPH radical scavenging activity at the range of 27.90%, 38.12%, 61.33%, 65.19% and 61.60% with concentration of 0.1, 0.3, 0.5, 0.7 and 1 g/ml respectively while vitamin C showed minimum radical scavenging activity of 66.57% and maximum activity of 85.52% (Figure 2).

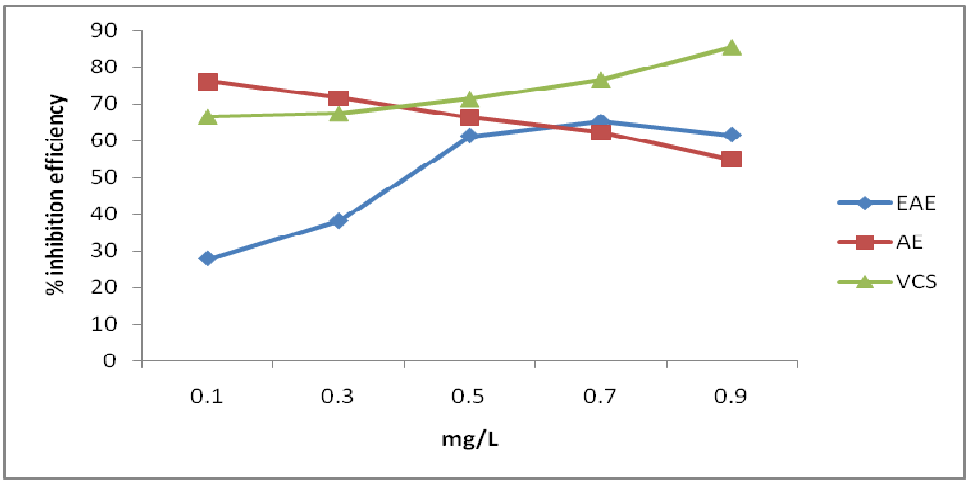

Figure 1. Graph of Antioxidant Activities of Ethyl Acetate, Acetone and Vitamin C.

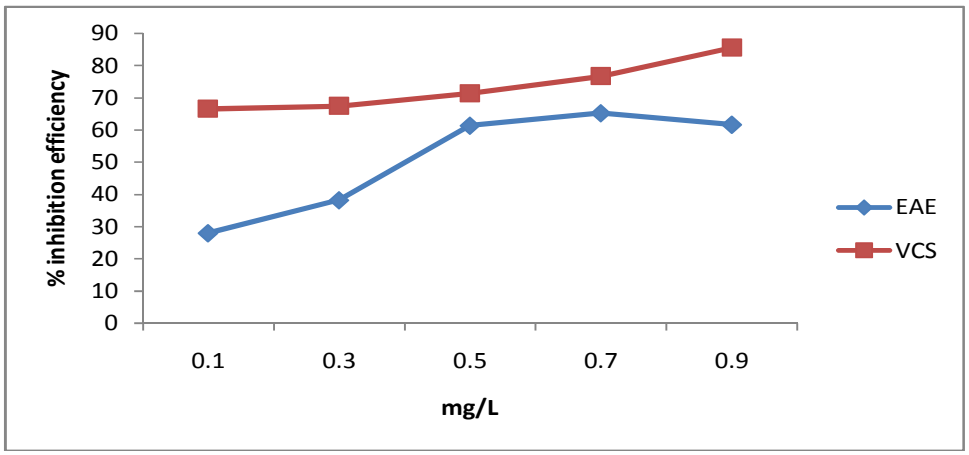

Figure 2. Graph of Antioxidant Activities of Ethyl Acetate.

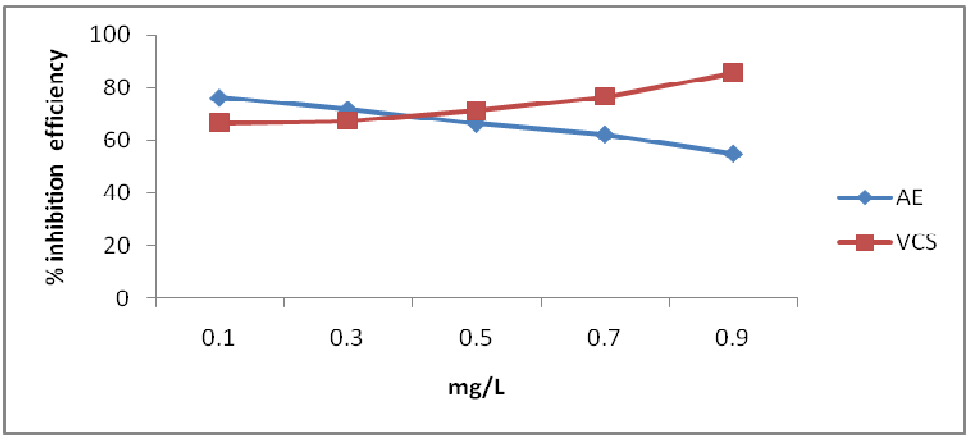

Figure 3. Graph of Antioxidant Activities of Acetone.

The crude acetone extract of *Thaumatococcus danielli* displayed inhibition of DPPH radical scavenging activity at the range of 76.24%, 71.73%, 66.45% 62.26% and 54.96% with the concentration of 0.1, 0.3, 0.5, 0.7 and 1 g/ml respectively while vitamin C showed minimum radical scavenging activity of 66.57% and maximum activity of 85.52%. (Figure 3).

CONCLUSION

The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. The ethyl acetate and acetone extracts of *Thaumatococcus danielli* has been found to exhibit antioxidant properties and the extract of the plant can be said to contain same compound that can be used to slow the oxidative reaction

REFERENCES

Ajiboye BO, Ibukun EO, Edobor GI, Ojo AO, Onikanni SA (2013). Qualitative and quantitative analysis of phytochemicals in senecio biafrae leaf. Int. J. Inventions Pharm. Sci. 1(5)2013;428-432.

Atmani D, Nassima C, Dina A, Meriem B, Nadjet D, Hania B (2009). Flavonoids in Human Health: From Structure to Biological Activity. Current Nutrition & Food Science, 5: 225-237.

Ayinde BA, Omogbai EK, Amaechina FC (2007). Pharmacognosy and hypotensive evaluation of Ficus exasperata Vahl (Moraceae) leaf. Acta Pol Pharm 64: 543-546.

Cai YZ, Sun M, Corke H (2003). Antioxidant activity of betalains from plants of the Amaranthaceae**.** J Agric Food Chem. Pp. 51(8): 2288-2294.

Edeoga HO, Eriata DO (2001). Alkaloid, tannin and saponin contents of some Nigeria medicinal plants. J. Med. Aromatic Plant Sci. 23:344-349.

Edeoga HO, Kwa D, Mbaebia BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol.;(7): 685-688.

Ghosal S, Krishna-Prasad BN, Laksmi V (1996). Antiamoebic activity of Piper longum London, pp. 12-16

Gracelin DH, Britto AJ, Kumar BJR (2012). Qualitative and quantitative analysis of Phytochemicals in five Pteris species. Int. J. Pharm. Pharm. Sci. Vol 5 Sppl. Pp: 105-107.

Islam T, Mamun AA, Rahman H, Rahman AM, Ashraf S (2016). Qualitative and Quantitative Analysis of Phytochemicals in Some Medicinal Plants in Bangladesh Qualitative J. Chem. Biol. Phys. Sci. JCBPS; 6(2); 530-540.

Lena G. (2010). Phenolic compounds, antioxidant activity and invitro inhibitory potential against key and enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs pieces in Latin America Bio Resour Technol,; 10: 4676-4689

McDevitt JT, Schneider DM, Katiyar SK, Edlind FS (1996). Berberina: a candidate for the treatment of diarrhea in AIDS patients abstract. In program and Abstracts of the 36th Interscience conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D. C.

Okwu DE (2001). Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J. Pure Appl. Sci. 7(3): 455-459.

Okwu DE and Okwu ME (2004) Chemical composition of SpondiasmombinLinn. Plants parts. J. Sust. Agric. Environ. 6: 140-147.

Roa RR, Babu RM, Rao MRV (1995). Saponins as anti-carcinogens. The J. Nutr. 125: 717-724.

Sagare BK, Singh RP (2011). Genesis and Development of DPPH Method of Antioxidant assay. Food Science Technol. 48 (4): 412-422.

Sagayaraj MI, Britto SJ, Arulappan MT, Krishnakumar J, Thomas S, George M (2015). Antimicrobial studies and Phytochemical screening of the leaves of Stephanla japonica (Thunb), Mier and Cocculus hirsutus (L) Diels (Menispermaceae). Euro. J. Biomed. Pharm. Sci. 2(1); 201-210.

Sala A, Recio MD, Giner RM, Manez S, Tournier H, Schinella G, Rios JL (2002). Antiinflammatory and antioxidant properties of Helichrysum italicum. J. Pharm. Pharmacol. 54(3): 365-371.

Sofowora A (1993). Medicinal plants and traditional medicine in Africa. 2nd Edn. Spectrum Books Limited, Ibadan Nigeria. 1-153.

Stray F (1998). The natural guide to medicinal herbs and plants. Tiger Books International, London, pp. 12-16.

Tapas B, Sakarkar AR, Kakde RB (2008). Flavonoids as Nutraceuticals: A Review. Tropical J. Pharm. Res., 7: 1089-1099.

Tiwari P, Kumar B, Kaur G, Kaur H (2011). Photochemical screening and extraction: A Review. Int. J. Pharm. Sci.1(1), 98-106.

Ushie OA, Adamu HM, Ntui DAO, Gunda HJ (2013). Phytochemistry of Borreria verticillata stem Bark. Int. J. Trad. Natural Med., 2 (2): 175-178.

Ushie OA, Egwaikhide PA, Longbap BD (2016). Phytochemical screening and Antimicrobial Activity of Tamarindus indica. Int. J. Trad. Complementary Med.

Ushie OA, Neji PA, Nsor GE (2013). Phytochemical screening and Antimicrobial activities of Phyllanthus amaratus stem bark extracts. Int. J. Modern Bio. Med., 3(3): 101-112.