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ESTIMATION OF TOTAL ALKALOIDS, SAPONINS FLAVONOID, TANNINS AND PHENOLS IN *THAUMATOCOCCUS DANIELLI* LEAVES

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ABSTRACT

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 phytochemical screening of *Thaumatococcus danielli* was undertaken through controlled experiments. The results showed that flavonoids, alkaloids, steroids, terpenes, tannins, glycosides and saponins are present in the ethyl acetate leaf extracts. Quantitative analysis showed the order of the concentrations jn mg/g as follows tannins (4.644), flavonoid (1.830), phenols (0.756), alkaloids (0.578) and saponins (0.440). The results suggest that phytochemical properties for curing various ailments and possess potential antioxidant, anti-inflammatory, antimicrobial.

KEYWORDS: *Thaumatococcus danielli*, phytochemicals, leaves, ethyl acetate extract.

INTRODUCTION

Secondary metabolites are compounds that are not necessary for a cell (organism) to live, but play a role in the interaction of the cell (organism) with its environment and are often involved in plants protection against biotic or abiotic stresses (Pagare *et al.*, 2015). Plant products have been part of phytomedicines since time immemorial (Yakubu *et al.*, 2021). Extraction and characterization of several active phyto-compounds from these green factories have given birth to some high-activity profile drugs (Mandal *et al.*, 2007). Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available, and with lesser side effects (Labaran *et al.*, 2021). The advantages of herbal medicines are their ready availability, inexpensiveness and less or no side effects, however they are easily adulterated (Sumitra, 2014). The major drawback to the use of herbal medicine however is the lack of standardization (Namadina *et al.*, 2020). *Thaumatococcus danielli* is one of the plants which have been used in traditional medicine for many years. To the best of our knowledge little or no work has been done on the plant *T. danielli* in Taraba, Nigeria. This work is designed to enrich the available scientific data on the estimation of total alkaloids, saponins flavonoid, tannins and phenols in *T. danielli* leaves. This paper reports the estimation of total alkaloids, saponins flavonoid, tannins and phenols in *T. danielli* leaves.

MATERIALS AND METHOD

Sample Collection and Preparation

The *Thaumatococus daniellii*, leaves were collected from their natural habitat in Bekwarra Local Government Area of Cross River State, Nigeria and were air dried under shade for two weeks; the dried sample was chopped and grounded into fine powder. The extract of the leaf was 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.

Phytochemical Screening

Phytochemical examinations were carried out for the extract using standard procedures to identify the constituents. Qualitative analysis of the crude extract were carried out as described by (Ushie and Adamu, 2013, Sagayaraj *et al.*, 2015, Ushie *et al.*, 2018 and Ushie *et al.*, 2019) 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
Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

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

Detection of saponins
Froth Test: Extract was 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: Extract was 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: Extract was 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 1 ml of leaf extract 2 ml 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.

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.

Quantitative Phytochemical Analysis
Qantitative determination of some secondary metabolites (Ushie et al., 2019) was carried out to know their percentage in the *T. danielli* leaves by the methods described by AOAC (1995), Manjunath *et al.*, 2012, Vabkova and Neugebauerova (2012), Obadoni, B.O. and Ochuko, P.O. (2001), Ushie *et al*., (2018) and Ushie *et al*., (2019 a & b).

Determination of tannins
The sample (1g) was macerated with 50 ml of methanol and filtered. To the filtrate (5 ml), 0.30 ml of 0.1N ferric chloride in 0.1N HCl and 0.3 ml of 0.0008M of potassium Ferro cyanide were added and the absorbance read at 720nm.

Determination of phenols
The sample (1g) was macerated with 20 ml of 80% ethanol and then filtered. 5ml of the filtrated was added to 0.5 ml of folicio calteus reagent (FCR) and allowed to stand for 30minutes. 2 ml of 20% sodium carbonate (Na_2CO_3) was added and the absorbance read at 650nm.

Determination of saponnins
The sample (1g) was macerated with 10ml of PET ether and decanted into a beaker. Another 10ml of PET ether was added into the beaker, filtered and the filtrate was evaporated to dryness, the residue was dissolved in 6ml 0f ethanol. 2 ml of the solution was put into in a test tube, and 2 ml of chromagen solution was added into it and was left to stand for 30 minutes. The absorbance was read at 550nm.

Determination of flavonoid
The sample (1g) was macerated with 20ml of ethyl acetate for 5minutes and filtered. To 5ml of the filtrate, 5ml of the dilute ammonia (NH_3) was added and shaken for another 5minutes. The upper layer was collected and the absorbance was read at 490nm.

Determination of alkaloids
The sample (1g) was macerated with 20ml of ethanol and 20% sulphuric acid (H_2SO_4) (I.e. 1:1 v/v) and then filtered. 1 ml of the filtrate was added to 5 ml of 60% sulphuric acid. After 5 minutes, 5 ml of 0.5% formaldehyde in 60% H_2SO_4 (sulphuric acid) was mixed with the mixture (i.e. filtrate (1 ml) + 5 ml 60% H_2SO_4) and allowed to stand for 3hours. The absorbance was read at 565nm.

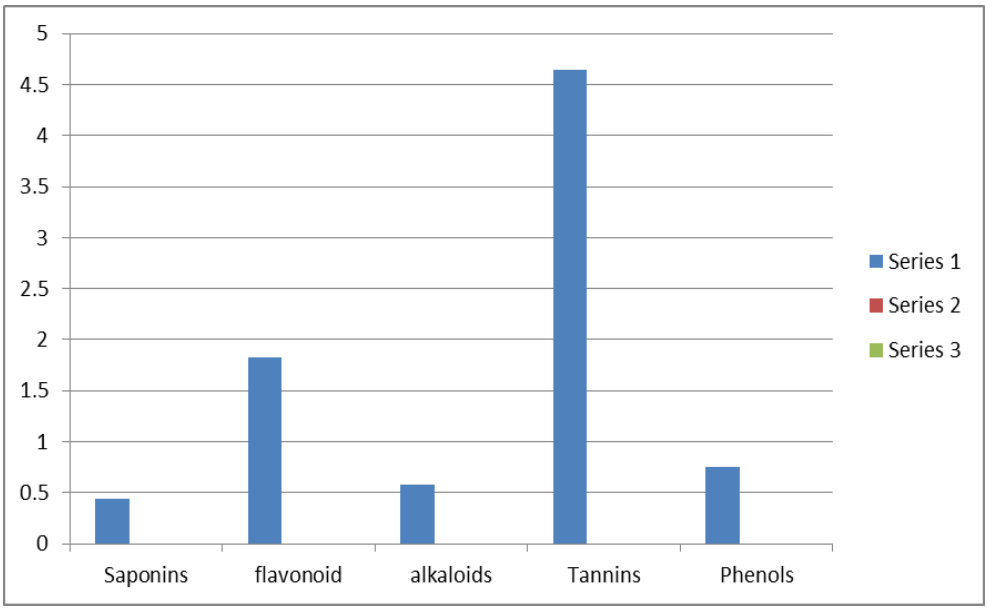
RESULTS AND DISCUSSION

Table 1: The ethyl acetate extract of *Thaumatococcus danielli* was screened for the presence of some phytochemicals such as alkaloids, flavonoids, anthraquinone, steroids, phlobatannins, tannins, phenol, terpenoids, saponnins and presented in table 1.

S/N	Phytochemicals	Tests	Acetone Extract
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 + $FeCl_3$	+

Table 2: The result obtained from the quantitative phytochemical results of *Thaumatococus daniellii* is presented in Table 2.

Phytochemicals	Concentration (mg/g)
Saponins	0.440
Flavonoid	1.830
Alkaloids	0.578
Tannins	4.644
Phenols	0.756



DISCUSSIONS

The ethyl acetate extract of the leaves of *Thaumatococus daniellii* was screened for the presence of some phytochemicals such as alkaloids, anthraquinones, saponnins, steroids, terpenes, flavonoids, tannins, phenols, glycossides and phlobatannins. The ethyl acetate extract of *Thaumatococcus danielli* was screened for the presence of some phytochemicals such as alkaloids, flavonoids, anthraquinone, steroids, phlobatannins, tannins, phenol, terpenoids, saponnins. The total content of tannins was found to be maximum in *Thaumatococus daniellii*, i.e. 4.644 mg/g. Flavonoids are present in appreciably quantity which is 1.830 mg/g. phenol is the next (0.756 mg/g) in the series followed by alkaloids and the least is the saponnins (0.578 and 0.440 respectively).

Tannins contribute property of astringency i.e. fasten the healing of wound and inflamed mucous membrane and have receives considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory properties (Savithramma *et al*., 2013). Tannins are complex moieties produced by

majority of plants as protective substances; they have wide pharmacological activities and have been used since past as tanning agents and they posses astringent, anti diarrhoea, anti-inflammatory activities (Killedar and More 2010). Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent, the flavonoids show antioxidant activity and their effects on human nutrition and health is considerable (Savithramma *et al*., 2013). The mechanisms of action of flavonoids are through scavenging or chelating process (Cook and Samman 1999).

Traditionally saponins have been extensively used as detergents as pesticides and molluscides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects (Okwu and Okwu 2004). Saponin has relationship with sex hormones like oxytocin, oxytocins is a sex hormone involved in controlling the onset of labour pains in women and the subsequent release of milk (Shi *et al.*, 2004). Saponin causes complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection (Francis *et al.;* 2002). *T. daniellii* is important in pharmacy because it contains steroidal compounds which are of importance and interest in pharmacy due to their relationship with sex hormones (Okwu 2001). These are known to effect the development and control of the reproductive tract in humans and molt insects. Saxena 2013 pointed out that recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as acquired immune deficiency syndrome (AIDS) and various cancers (Blytt *et al*., 1988). Alkaloids were detected in all the extract. Hence, *T. daniellii* has the potential to be used as an analgesic or anaesthetic since it contains alkaloids. The presence of terpenoids that have carboxylic acid groups could also be responsible for the activity of the organic extracts (Njoku and Obi 2009). *T. daniellii* can potentially be used in the treatment of certain illnesses because it contains glycosides. Glycosides contain steroid as aglycone component in combination with sugar molecules. They are important in medicine because of their action on heart and are used in cardic insufficiency (Balch and Balch 2000). Thus, cardiac glycosides are drugs and can be used in the treatment of congestive heart failure and cardiac arrhythmia (Savithramma *et al*., 2013).

CONCLUSION

The bioactive components extracted from the leaves of *Thaumatococus daniellii* include; flavonoid, alkaloids, steroids, terpenes, glycosides, saponins and tannin were detected in the ethyl acetate extract, This medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments : bactericidal, pesticidal or fungicidal in nature thus confirming the anti-microbial property to plants.

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