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Evaluation of the Sweetening Effects of *Thaumatococcus daniellii* Fruits in Metronidazole Syrup Formulations

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ABSTRACT

Organoleptic properties affect the acceptability of pharmaceuticals and patient compliance; many manufacturers argue that improving the palatability of liquid medicines with sucrose improves patient compliance. Chronic administration of sweetened liquid medicines increases the risk for dental caries and gingivitis in children and additional calories in adults. However, natural sweeteners could be non-toxic, non-caloric and non-cariogenic. This study aims at evaluating the degree of sweetness imparted by the sweet protein; Thaumatin, extracted from the arils of the fruit of *Thaumatococcus daniellii* (TD), in masking the bitter taste of Metronidazole. Syrup BP was used as a reference.

The fruits of TD was dried and pulverized. An extract was obtained from the arils of TD by aqueous extraction. Viscosity, pH and flow rate of the extract were determined. Metronidazole oral syrup was formulated using either the extract or Syrup BP as the syrup base. The formulations were evaluated based on their organoleptic properties of taste, odour, colour and clarity as well as physicochemical properties such as flow rate, pH and viscosity.

The extract was slightly acidic and has a viscosity of 16 ± 0.95 cP while syrup BP had a viscosity of 37 ± 1.65 cP at 100 rpm. The formulations containing Thaumatin extract had sweeter and longer lasting sweetness than formulations with Syrup BP. Formulation containing the extract had a faint odour that was pleasant while Syrup BP formulation was odourless.

The extract imparted better sweet taste and acceptable organoleptic properties to Metronidazole liquid formulations than Syrup BP.

Keywords: *Thaumatococcus daniellii*, Thaumatin, Sweetener, Syrup BP, Metronidazole.

Introduction

Pharmaceutical dosage forms are means of administering the active ingredient of a drug to the body to exert its effect and not cause harm to patients. Drug substances are seldom administered alone but rather combined with one or more of non-therapeutic agents referred to as excipients. These excipients include sweeteners, colourants, flavourants which are mostly used in liquid formulations.[1]

The liquid dosage form is the most preferred choice due to its ease of administration for children, elderly and patients that cannot swallow tablets or capsules. Liquid preparations are absorbed at a faster rate compared to solids, as the drug is already in solution; they are expected to have a palatable taste.

Many liquid oral medicines are formulated with sugars, such as sucrose and glucose, and some carbohydrates can directly influence the cariogenic potential of the solutions.[2,3] Liquid medicines can be part of a daily routine for children with chronic diseases.[4,5] As a result, these children are likely to take in more of the sugars from the medicines thereby increasing the possibility of health impairment.[6]

Many pharmaceutical companies argue that improving the palatability of liquid medicines with sucrose increases patient compliance. On the other hand, chronic administration of sweetened liquid medicines increases the risk of dental caries and gingivitis in children.[7] There is, therefore, the need for alternative sweetening agents for pharmaceuticals, apart from the sugars. Natural sweeteners have the advantages of being non-toxic, non-cariogenic and less caloric compared to the sugars.

The fruit of *Thamatococcus daniellii* Benth**.** Family: Marantaceae contains a sweet-tasting substance; the plant is also known as sweet prayers plant[8] and referred to as "ewe moinmoin" in Yoruba language. The fruit has been employed locally for sweetening drinks and foods.[9]

The arils of the fruit (Figure 1) is said to contain thaumatin which is an intensely sweet protein which on a weight basis is about 3000 times sweeter than sucrose.[10] Thaumatin is non-caloric, non-toxic natural protein sweetener which readily decomposes on hydrolysis to a normal distribution of amino acids.[11]

In this study, a comparative analysis of the sweetening effects of the extract of *Thamatococcus danielli* (containing Thaumatin) and syrup BP was made in a Metronidazole suspension formulation.

Figure 1: Dried *Thaumatococcus daniellii* Fruits

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Materials and Methods

Chemicals
Metronidazole Benzoate was a gift from Bentos Pharmaceutical Industry Ibadan, Oyo State, Nigeria. All reagents used were of analytical grade.

Collection of Thaumatococcus danielli Fruits and Seeds
Thaumatococcus danielli fruits were collected in February 2020 from a farm in Ekiti state, Nigeria and authenticated at the Department of Botany, University of Ibadan, Nigeria with Voucher number UIH-22959. The fruits were opened to separate the seeds from the fleshy part. The arils of the fruits were dried in the oven (Model DHG-9053A, Ocean Medical, England) for 24 h at a temperature of 50^{o}C, pulverized and passed through a 0.025 mm pore-sized sieve. The powdered samples were stored in airtight containers and used to carry out the analysis.
Determination of Particle Size and Shape
The photomicrograph of the particles of the powdered fruit was observed at low, medium and high resolutions with a Digital Microscope, VJ-2005 DN MODEL BIO-MICROSCOPE®.

Determination of pH
The pH of a 1% w/v dispersion of the powder was determined using a digital pH meter (Eutech Instruments, Singapore) at room temperature (25 – 27^{o}C).

Extraction of Thaumatococcus danielli Fruits
The fruits were opened and the seeds were removed from the gel-like cover. The powdered fruits (100 g) were soaked in 200 mL of distilled water. This was agitated using a magnetic mixer. A brown syrupy liquid was obtained. The extract was then decanted and sieved. A clear brownish sweet syrupy liquid containing Thaumatin was obtained.[10,12]

Fourier Transform Infrared (FT-IR) Spectroscopy
The powder of *Thaumatococcus danielli* fruit and the extract were analyzed by the FTIR. Spectra of the powder samples were recorded using an IR spectrometer (Perkin- Elmer, Model 2000, USA). About 5 mg of each sample was dispersed in 200 mg KBr. The scanning range was 400 to 4000 cm^{-1}.

Physicochemical Properties of the Aqueous Extract Containing Thaumatin
pH
The pH of a 1% v/v (1 mL in 100 mL of distilled water) preparation of the aqueous extract was determined using a digital pH meter (Eutech Instruments, Singapore) at room temperature.

Viscosity
The viscosity of a 1% dispersion of the extract was determined using a Brookfield viscometer model RVVDV – II+P (Brookfield Eng. Inc. Middle Boro, MA, USA) at 50 and 100 rpm, using a spindle 03.

Taste
Five volunteered subjects, with informed consent were selected at random and about 1 mL of the extracted Thaumatin was given to each subject. Individual subject recorded the perceived taste.

Formulation of Metronidazole
Metronidazole syrup was prepared using either Syrup BP or the extract containing Thaumatin as the sweetener. The reference Syrup BP was prepared according to the British Pharmacopoeia.[13] The syrup base contained 66.7 g sucrose and purified water to 100 g.
A 50 mL oral syrup containing 200 mg metronidazole per 5 mL (200 mg/5 mL) was formulated (Formula I). Appropriate quantity of metronidazole benzoate was weighed into a mortar and mixed, using a pestle with about 25 mL of the Syrup BP until a smooth, uniform syrup was obtained. The syrup was transferred into a clean calibrated bottle, using a funnel. The volume was made up to 50 mL mark with Syrup BP.
A 15% dispersion of the extract was made in distilled water. The oral metronidazole syrup was formulated using the extract dispersion instead of Syrup BP (Formula II).

Formulae for Metronidazole Syrup:
Formula I (containing Syrup BP)

Metronidazole	2000 mg
Syrup BP (66.7%) to	50 mL

Formula II (containing The Extract)

Metronidazole	2000 mg
The extract (15.0%) to	50 mL

Evaluation of the Metronidazole Syrup Formulations
The formulated metronidazole syrups were evaluated for quality, stability and acceptability using the following tests:
Viscosity
The viscosity was determined using 10 mL each of the formulation containing Syrup BP and the formulation containing Thaumatin extract. The viscosity was determined using a Brookfield viscometer model RVVDV – II+P (Brookfield Eng. Inc. Middle Boro, MA, USA) at 50 and 100 rpm, with spindle number 03.

Flow Rate
The time taken for 1 mL sample of each formulation to completely drain out of a pipette was determined. The flow rate was calculated using the equation below:

$$\text{Flow rate} = \frac{V}{T} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where V is the volume of the formulation, and T is the time taken for complete flow out from the pipette.

pH Determination
The pH of a 1% v/v (1 mL formulation in 100 mL of distilled water) of the formulated syrups was determined using a digital pH meter at room temperature.

Organoleptic Properties
a. Taste: Using Hedonic scale method of measuring preferences, the five subjects selected with informed consent were requested to taste the preparations. About 0.5 mL of each preparation was given to each subject. The ability of each sweet base to mask the bitter taste of metronidazole was then recorded based on which formulation tasted sweeter. The preferences of each subject were recorded.
b. Colour: Little quantity (0.5 mL) of each formulation was poured into different transparent containers, and the colour exhibited by each preparation was noted.
c. Clarity: Little quantity (0.5 mL) of each of the formulation was poured into different transparent containers, and clarity of each preparation was compared with water and recorded.
d. Odour: Little quantity (0.5 mL) of each formulation was poured into different transparent containers, and the odour characteristic exhibited by each formulation was perceived and compared to the sharp odour of peppermint oil.

Statistical Analysis
The results obtained were subjected to statistical analysis using ANOVA, followed by a posthoc Tukey's test to determine the level of significance (p-value) of an effect or the difference between means. Parameters were considered significantly different at $p < 0.05$ at 95% confidence interval.

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Results and Discussion

Yield
A 45% yield of the aqueous extract, containing Thaumatin was obtained from *Thaumatococcus daniellii* fruits. This is quite a high yield and it indicates that the plant can be a good source of pharmaceutical excipient. [14,15]

Physicochemical Properties
The pH of the aqueous extract of *Thaumatococcus daniellii* fruits was 6.28 making it to be slightly acidic (Table 1). This suggests the compatibility of the extract with formulations whose absorption is either in the stomach or small intestine.

Particle Shape
The Photomicrographs obtained showed irregular shapes and sizes of the powdered fruit of *Thaumatococcus daniellii* (Figures 2). Particle shape and size have been found to affect the packing properties of materials.[16]

FT-IR Analysis
Fourier transform infrared spectroscopy (FT-IR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. The FT-IR of *Thaumatococcus daniellii* powdered fruit and the aqueous extract had the same absorbance (Figure 3). The various functional groups present were alcohols, carboxylic acid, vinyl ethers, and alkyl halides with wavenumbers of 3615 cm^{-1}, 1528 cm^{-1} 2929 cm^{-1}, and 667 cm^{-1}, respectively. This indicates that all the functional groups present in the aril of TD fruits are retained in the aqueous extract.

Evaluation of the Metronidazole Formulations
pH
There was a similarity between the pH of the Metronidazole syrup formulated with the aqueous extract and Syrup BP (Table 1). It has been reported that sugars can maintain the pH values of medicines for a long period of time. [17] The similarity and consistency observed in pH of the metronidazole formulations suggest that Thaumatin would also maintain the pH in liquid formulations.

Flow Rate
The flow rate of the Metronidazole syrup formulated with the aqueous extract containing Thaumatin was lower than that formulated with Syrup BP (Table 1). However, the difference between the flow rates was not statistically significant ($p > 0.05$). This suggests that Thaumatin is capable of imparting flow rate and easy pourability similar to that of Syrup BP on liquid formulations.

Viscosity
The viscosity of Metronidazole syrup formulated using Syrup BP was higher at 100 rpm as compared with the syrup containing the extract (Table 1). This is attributable to the viscous nature of Syrup BP; which could be an advantage in suspending poorly soluble drugs better than a less viscous vehicle.

Organoleptic properties
Taste
From the results obtained using the Hedonic scale, the Metronidazole syrup formulated with the aqueous extract containing Thaumatin had a sweeter and long-lasting sweet taste compared to the syrup formulated with Syrup BP (Table 2). This suggests that Thaumatin is capable of masking the bitter and unpleasant tastes of medicaments to a level that is higher than that of Syrup BP. This property will increase formulation acceptability and lessen the difficulty experienced by parents in administering bitter tasting drugs to their children. It is worthy of note that the sweetening principle in Thaumatin is non-caloric and devoid of cariogenic effects of the sugars.[17] This indicates that the extract could be used as a sweetener in adult liquid preparations with no fear of increasing blood sugar, especially in diabetic patients.

Colour
Colourants are added to formulations for aesthetic purposes or to differentiate them from other formulations.[18] The Metronidazole syrup formulated with the aqueous extract containing Thaumatin had a brown colour while that formulated with simple sugar had an off-white colour (Table 3). The brown colour is due to the colour of the extract and this characteristic brown colour could be an advantage. However, the brown colour could be masked by a colourant if a better colour is desired.

Clarity
The Metronidazole syrup formulated with the aqueous extract containing Thaumatin was clearer than that of the Syrup BP (Table 3). Clear homogeneous syrup was obtained in the formulation containing Thaumatin while the formulation with Simple Syrup had the metronidazole particles dispersed in the vehicle. This indicates that the extract produced a more elegant formulation which could give better dosing uniformity and patient acceptability.

Odour
The Metronidazole syrup formulated with the aqueous extract containing Thaumatin had a faint odour, while that formulated with simple syrup was odourless (Table 3). The faint odour was not however unpleasant.
Results of the organoleptic properties suggest Thaumatin be able to impart properties that are suitable for the formulation of liquid dosage forms with good acceptability by patients.

Table 1: Comparative Analysis of the Extract Containing Thaumatin and Syrup BP

Properties	Extract	Syrup BP
Viscosity (cP) at 100rpm	16 ± 0.95	37 ± 1.65
pH	6.28 ± 2.35	6.88 ± 2.70
Flow rate	0.20 ± 2.15	0.14 ± 0.75

Table 2: Showing the Degree of Sweetness and the Ability of Each Sweet Base to Mask the Bitter Taste of Metronidazole

Subjects	Metronidazole in Aqueous Extract containing Thaumatin	Metronidazole in Simple Syrup BP
S1	++	+
S2	++	+
S3	++	+
S4	+	++
S5	++	+

Legend: Sweeter = ++; Sweet = +

Table 3: showing other organoleptic properties of the metronidazole syrup formulations.

Properties	Metronidazole in Aqueous Extract containing Thaumatin	Metronidazole in Simple syrup BP
Colour	Brown	Off-white
Odour	Faint odour	Odourless
Clarity	Clear	Less clear

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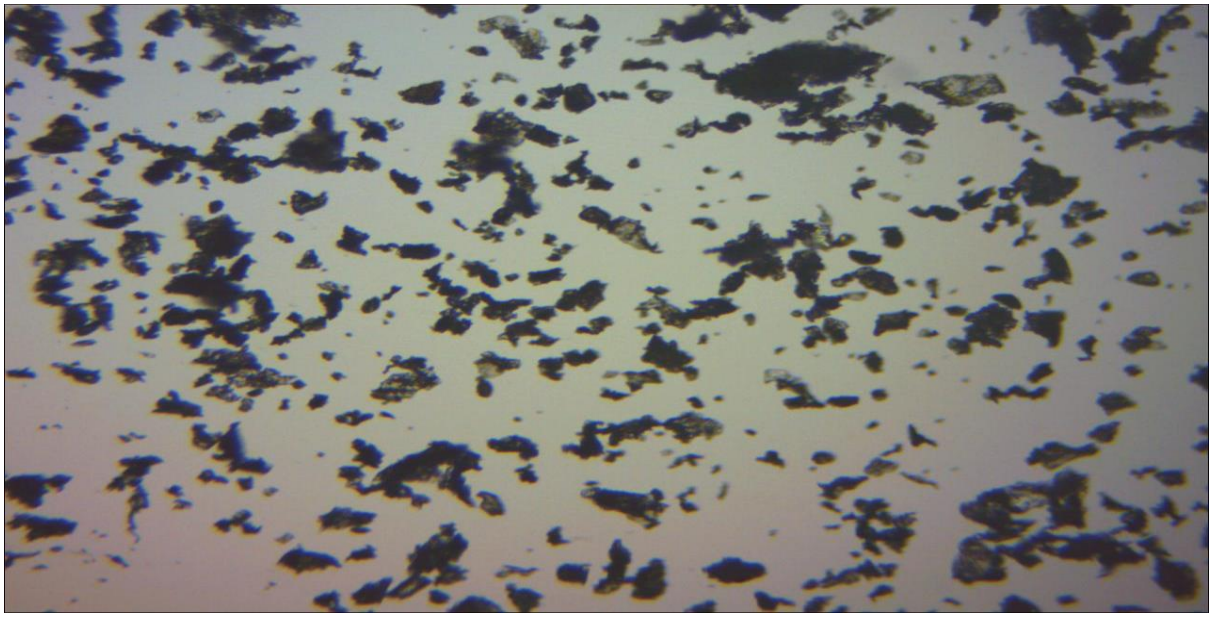

Figure 2: Photomicrograph of *Thaumatococcus daniellii* Fruit Powder Particles (x100)

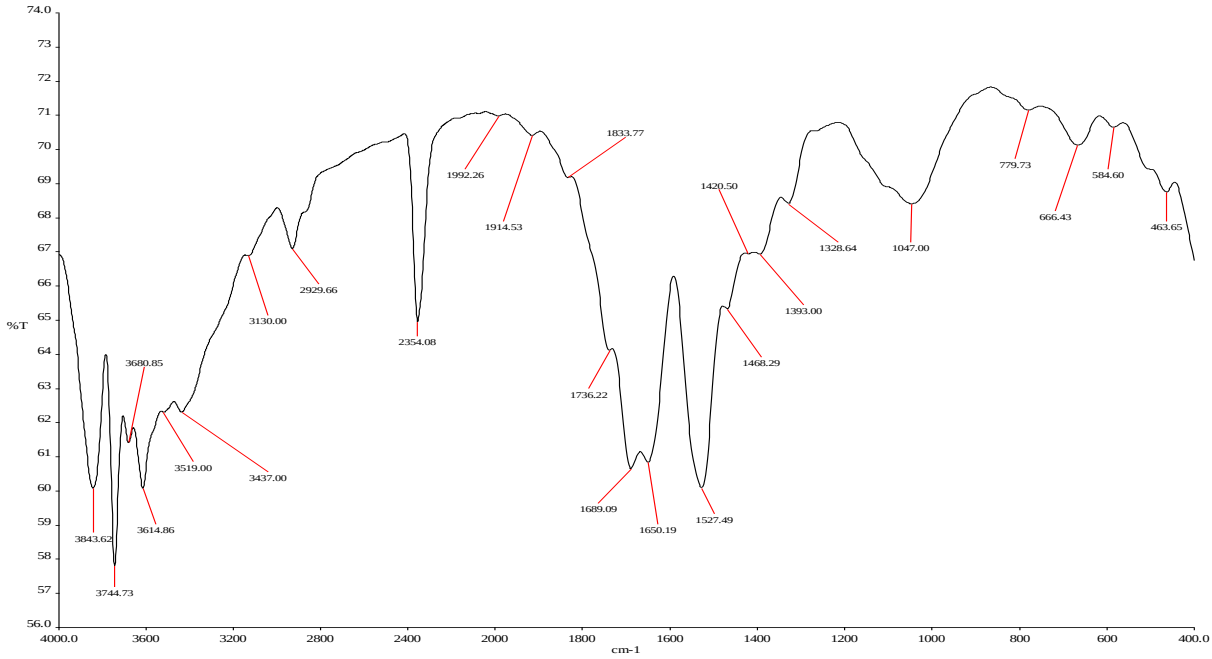

Figure 3: FT-IR Spectra for *Thaumatococcus daniellii* Powdered Fruit and Aqueous Extract (Containing Thaumatin)

Conclusion

The aqueous extract of the arils of the fruits of T*haumatococcus daniellii* contained a sweet protein which imparted better organoleptic properties of sweet taste, acceptable odour, and clarity to metronidazole oral syrup when compared with Syrup BP.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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