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Full Length Article

Chemical composition, lethality and antifungal activities of the extracts of leaf of *Thaumatococcus daniellii* against foodborne fungi

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ABSTRACT

The consumers' interest in natural preservatives seemed to have increased because of the apathy and negative effect of synthetic preservatives. This study explored the use of *Thaumatococcus daniellii* leaves as potential preservatives against fungi responsible for the spoilage of orange juice and corn jell-o. The leaves of the plant were extracted with different solvents – acetone, aqueous, ethanol and hexane – and assayed against test fungi using disc diffusion method. The minimum inhibitory and fungicidal concentrations were determined. The qualitative and quantitative phytochemical analyses of leaves of the plants were carried out, the most active extract of the fresh and dried leaves was assayed via GC–MS for the essential oils and HPLC fingerprinting was used to determine the phytochemical constituents of the most active extracts. The toxicity test of the extracts against Brine shrimp was carried out after 24 hours of exposure. The ethanol extracts of the plants were the most active based on the antifungal assay. The toxicity test revealed that the extracts of the plants were non-toxic on the Brine shrimps at LD_{50} (592.95 µg/ml and 281.12 µg/ml for aqueous and ethanol extracts). The qualitative phytochemical screening revealed the presence of alkaloid, tannin, saponins, flavonoids, steroids and terpenoids in the leaf of the plant. The quantitative phytochemical analysis of the most active extract in the plants revealed alkaloids with the highest content of 107.48 mg/100 g. The GC–MS analyses of the fresh leaves of the plants revealed the presence of isodecane at 15.16%. The GC–MS analyses of the dried leaves revealed 1,2,3-trimethylbenzene in *T. danielli* with the highest percentage of 15.48%. The HPLC analysis revealed the presence of various phytochemical constituents in the dried leaves of the plant. This study has been able to ascertain the potency of *T. daniellii* leaves on fungi responsible for spoilage of sweet *Citrus sinensis* (orange) juice and Corn Jell-O ('Eko') which contribute to continuous drive towards the use and total acceptance of natural antimicrobials.

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1. Introduction

Food products are known to be susceptible to spoilage during storage. The spoilage of food products can be attributed to the effect and action of micro-organisms and extracellular activities of enzymes on them (Barth et al., 2009; Ledenbach and Marshall, 2009). The action of microorganisms changes the sensory properties and reduces the economic values of the food products. There are documented evidence in several literature that filamentous fungi and yeast are noticeable organisms associated with spoilage of food products (Adebayo et al., 2014; Aoudou et al., 2010). The malaise arisen due to microbial contaminations of food products signals the need to explore available control methods to reduce the impact of microbes on food and enhance the shelf-life of food products (Aponiene et al., 2015; Jorgen, 2005).

The appreciable interest in search of food preservative as control method from natural products has since been gathering considerable attention basically because of the negative health implications of synthetic preservatives and the safe health status of most natural products from plants. Plants have been used for ages by many cultures to enhance the flavour and aroma of foods. Early cultures have assessed the value of these plants since time immemorial as preservative agents (Erturk, 2006).

In sub-Saharan Africa with less technologies and lack of access to refrigerating equipment, the natives employ the use of leaves to wrap food to enhance its shelf-life. *Musa sapientum*, *Musa paradisiaca*, *Cola nitida*, *Cola acuminata*, *Piliostigma reticulatum*, *Theobroma cacao*, *Colocasia esculenta*, *Canna indica*, *Megaphrynium macrostachyum* and *Thaumatococcus daniellii* leaves (Adegunloye et al., 2006) are used to package and present foods to clientele. The use of these leaves is a very ancient tradition of the people; the basis of which cannot be easily ascertained, but a cursory look at these leaves reveals that they all have large surface areas, i.e. can and are used to hold/package/wrap large volumes of food (Ojekale et al., 2007). The leaf extracts of these plants have been documented to possess antimicrobial properties (Adegunloye et al., 2006; Erturk, 2006; Ojekale et al., 2007; Yazdani et al., 2011) and essential oils from plants have been generally recognized being safe and the toxicity on food might be quite inconsequential (Barbosa et al., 2014; Mith et al., 2014).

Thaumatococcus daniellii (Benn.) Benth., commonly known as Katempe, belongs to family Marantaceae. It grows throughout the hot, humid tropical rainforest and seaward zone of West Africa (Burkill, 1997). The natural habitat is the undergrowth of forest trees. *T. daniellii* is particularly found in some parts of Ghana, Cote d'Ivoire, Nigeria, Sierra Leone, Central African Republic, Uganda and Indonesia (Burkill, 1997).

Katemfe is a rhizomatous, perennial and monocotyledonous herb, propagating itself by rhizomes (Onwueme et al., 1979). The inflorescence of *T. daniellii* usually arises from the lowest node and may be simple or forked with spikes about 8–10 cm in length and bracts, usually umbricate, about 3–4 cm in length (Onwueme et al., 1979). The flowers that may be as long as the bracts form in short spikes close to the ground at the base of a swollen petiole. The plant flowers most of the year but is most prolific from July until late October, followed by fruit formation, maturing and ripening from January until mid-April (Onwueme et al., 1979).

The leaf of *T. daniellii* is used extensively in cooking and wrapping food both for domestic use and commercial enterprise. Its uses transcend the confines of the rural dwellers as evidenced by its uses for special packaging of rice by a prominent and well spread urban-based fast food restaurant (Aiyeloja and Ajewole, 2005).

This study was aimed to determine *in-vitro* antifungal assessment of essential oils and organic extracts on foodborne fungi isolated from Corn Jell-O and fruit juices and also determine the *in-vivo* lethality status on brine shrimps for toxicity appraisal of the leaf extracts.

2. Material and methods

2.1. Glasswares, consumables, equipment and solvents

The glasswares were products of Thermo Fisher Scientific and the consumables were obtained from local scientific stores in Nigeria. The rotary evaporator used was from laboratory and analytical supplies (PTY), Durban, South Africa, platform shaker from Labcon laboratory consumables (PTY), Durban, South Africa and the solvents were of high analytical grade from Merck chemicals (PTY), Wadeville, South Africa. The GC–MS system used was Hewlett Packard and HPLC system used was Schimadzu Products, Japan.

2.2. Source of specimens

2.2.1. Plant material

T. daniellii leaves were collected from Ilogbo in Otta local government (Nigeria, N06°43.296′, E003.09.403) in May, 2014. After the taxonomical identification of the plant species, a voucher specimen (LUH 6087) was deposited at Lagos University Herbarium for repository purposes.

2.2.2. Sources of fungi

The fungi used for this study were isolated from orange juice and Corn Jell-O ('Eko'); they were identified by comparing their morphology with fungi descriptions of Bryce (1992) and Beveridge (2001).

The fungi were sent to CABI, Surrey, Egham, for molecular identification and confirmation of the fungi.

2.3. Preparation of inocula

The fungi for anti-fungal assays were obtained via pure culture plates of fungi devoid of contaminations. The spore suspensions of the fungi were made using sterilized normal saline water of 10 ml. The top of the fungal colonies of 3–7 days old were touched with a loop and seed into sterilized normal saline water of 10 ml in the test tube. Drops of the spore suspensions were later dropped on a sterile glass slide using a sterile dropper. The suspensions were observed under the microscope to ascertain the presence of the fungal spore; the spores were later counted using haemocytometer (Adekunle and

Ikumapayi, 2006) and then further diluted to approximately 10^6 CFU/ml (Kosanic et al., 2012).

2.4. Preparation of plant extracts for antifungal assay

2.4.1. Preparation of plant organic extracts

The leaves of *Thaumatococcus daniellii* were shade-dried for twenty two days and were ground using an industrial miller. Two hundred grams of leaves was soaked with solvents: aqueous, acetone, ethanol and hexane in different conical flasks plugged with cotton plugs respectively and observed on a shaker for seventy two hours. The extracts were filtered through a Whatman No. 1 filter paper and Muslin cloth for several times and concentrated to dryness with the aid of a rotary evaporator. The stocks were kept at 4 °C in a refrigerator until further use (Silva et al., 2014).

2.4.2. Essential oil extraction

The oil extraction was obtained with the adoption of methods described by Silva et al. (2014). The oil was obtained via hydrodistillation of 0.5 kg of fresh and dried leaves separately for 3 hours using Clevenger-type apparatus. The aqueous emulsion was concentrated and submitted to extraction with dichloromethane and the solvent was evaporated using rotary evaporator. The resulting essential oil was stored at 4 °C prior to further analyses. The essential oil was solubilized in *n*-hexane for gas chromatography and mass spectrometry analysis.

2.4.3. Determination of percentage yield of the extracts

The yields of the extracts were determined using the method of Dellavalle et al. (2011) with slight modification. The weights of the McCartney bottles of 28 ml were noted after rinsing and oven-drying. The extracts were later put into the McCartney bottles after evaporating via rotary evaporator and left devoid of any solvent on the working bench before the weights of the bottles were taken. The percentage yields of extracts were calculated, thus

$$\text{Percentage (\%) yield: } \frac{W_1 - W_2}{X_g} \times \frac{100}{1}$$

where X_g is the initial weight of dried plant samples; W_1 is the weight of McCartney bottles + Pasty extract; and W_2 is the weight of empty McCartney bottles.

2.4.4. Test for purity of the organic extracts and essential oils

Each of the extracts obtained was tested to ensure its purity via dropping on a sterile plate containing potato dextrose agar and well spread on the agar. The plates were incubated at 27 °C based on the method described by Okigbo et al. (2009). The plates were examined for possible growth of contaminants; the absence of which confirmed the purity of the test extracts.

2.4.5. Preparation of disc for anti-fungal assay

The disc preparation for anti-fungal activity involves the use of filter papers (Whatman No. 1 filter paper). They were prepared as adapted by Adekunle and Ikumapayi (2006) and Valle et al. (2016); the filter papers were perforated using a perforator. The perforated filter papers, which were the discs, were placed on an aluminum foil and wrapped, later sterilized in an autoclave and the discs were dried in an oven at 60 °C for 30 min. The discs were later soaked in the extracts prepared for 24 hours prior to use.

2.4.6. Preparation of extract concentrations for anti-fungal assay

The concentrations of plant extracts were determined after drying the plant extracts using the method adapted by Zakia et al. (2015). The concentrated extracts were reconstituted with 100 ml of dimethyl sulfoxide (DMSO) and the concentrated extracts were later varied into different concentrations: 0.1 g (10 mg/ml), 0.5 g (50 mg/ml), 1 g (100 mg/ml), 5 g (500 mg/ml) and 10 g (1000 mg/ml).

2.5. Antifungal activity of T. daniellii leaf's extracts

2.5.1. Determination of anti-fungal assay of plant extracts against isolated fungi

The disc diffusion method by Adekunle and Ikumapayi (2006) and Zakia et al. (2015) was adopted for the anti-fungal assay. The tests were conducted with authenticated pure cultures of the fungi to determine their respective tolerance of the extracts. 0.5 ml of the stocked suspension of each fungus was dropped aseptically using a dropper over the entire agar surface of a freshly prepared potato dextrose agar (PDA) plate and evenly spread using a spreader. This was done to ensure an even distribution of the spores and later dried at room temperature for 15 min prior to the application of the discs on the agar plates. The discs (4) which have been soaked in reconstituted solvents were placed at the four edges on the surface of the agar plate with the aid of a sterilized forceps. The inoculated plates were later placed in an incubator at 28 °C to ascertain if there would be inhibition or not around the extract-impregnated discs. The plates were observed after 48 hours. The extent of inhibition was determined by measuring the diameter of the inhibition zone using a transparent half ($^1/_2$) metre rule (Booths, 1971; Zakia et al., 2015). The mean zone of inhibition of the three replicated tests of the plants extracts on the test organisms was expressed in millimetres. The discs were also impregnated in equivalent volume of dimethyl sulfoxide (DMSO) as negative control and Nystatin (anti-fungal drug) as positive control.

2.5.2. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity. Modified methods of Ngonda et al. (2012) and Mishra et al. (2015) were adopted. The concentration of extract with no observable fungal growth was taken as the minimum inhibitory concentration.

2.5.3. Determination of minimum fungicidal concentration (MFC)

Minimum fungicidal concentration (MFC) was determined for each plant extract showing antimicrobial activity. Modified methods of Ngonda et al. (2012), Oro et al. (2015) and Mihai and Popa (2015) were adopted; the Petri dishes of the extracts concentrations that showed no visible fungal growth were

seeded into freshly prepared potato dextrose agar plate to assay for the fungicidal effect of the extracts. The plates containing the test organisms were incubated at 28 °C. The minimum fungicidal concentration was regarded as the lowest concentration that did not yield any fungal growth on the solid medium used.

2.6. Lethality test using brine shrimp

The brine shrimps lethality test using the larvae of brine shrimp nauplii, *Artemia salina* L., was carried out using the method described by Kayode and Afolayan (2015), with slight modification. 1 mg ml^{-1} concentration of the extract was prepared from the original stock solution of *T. daniellii* that was used for antifungal assays. The extracts were further diluted to final concentrations of 20, 40, 60, 80 and 100 µg ml^{-1} in different vials using DMSO. Ten nauplii were transferred into each vial using Pasteur pipettes and were not given food because hatched brine shrimp can survive up to 48 hours without food as they still feed on their yolk during this period. The control vials contain normal saline solution and dimethyl sulfoxide (DMSO) separately. The experiments were replicated three times. After 24 hours of incubation, the content of each vial was transferred into 65 mm Petri dish and examined; the number of surviving larvae was counted and the percentage of mortality was evaluated. Larvae were considered dead if they did not exhibit any form of movement during several seconds of observation. Extracts are regarded as non-toxic if its LC_{50} is greater than 100 µg ml^{-1} in brine shrimp lethality assay.

2.7. Qualitative and quantitative phytochemical screening

The acetone, aqueous, hexane and ethanol extracts of *T. daniellii* were subjected to qualitative preliminary phytochemical screening by adopting the standard methods as described by Harbone (1973), Trease and Evans (2009), Sofowora (1993), Edeoga et al. (2005), and Ashafa and Umebese (2012). They were later subjected to quantitative analysis of the phytochemical constituents based on the methods of Edeoga et al. (2005) and Prohp and Onoagbe (2012).

2.7.1. Qualitative phytochemical screening of the plant extracts

2.7.1.1. Test for alkaloids. Briefly, 0.5 g of the powdered material was stirred in 5 ml of 1% aqueous hydrochloric acid, heated on a water bath and filtered. Then, 1 ml of the filtrate was treated with a few drops of Mayer's reagent and a second portion was treated the same way only with Dragendroff's reagent. Turbidity of precipitation with either of those reagents was taken as preliminary evidence for the presence of alkaloids in the extract.

2.7.1.2. Test for tannins. In the test for tannins, 0.5 g of dried powdered sample was boiled in 20 ml of water in a test tube and filtered. Few drops of 0.1% ferric chloride were added and observed for brownish green or a blue black colouration as indication of tannins.

2.7.1.3. Test for phlobatannins. In brief, aqueous extract was boiled with 1% aqueous hydrochloric acid and observed for deposition of red precipitate as indication of phlobatannins.

2.7.1.4. Test for saponins. Approximately 2 g of powdered material was boiled in 20 ml of distilled water in a water bath and filtered. Next, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as indication of saponin.

2.7.1.5. Test for flavonoids. A portion of the powdered material was heated with 10 ml of ethyl acetate over a steam bath for three minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Development of yellow colouration is an indication of the presence of flavonoids.

2.7.1.6. Test for steroids. In this test, 2 ml of acetic anhydride was added to 0.5 g of ethanolic extract with 2 ml concentrated H_2SO_4. The colour change from violet to blue or green is indication of steroids.

2.7.1.7. Test for terpenoids (Salkowski's test). In brief, 5 ml of extract was mixed with 2 ml chloroform and 3 ml H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was indication of terpenoids.

2.7.1.8. Test for cardiac glycosides (Keller–Kiliani test). In this test, 5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

2.7.2. Quantitative phytochemical determination

2.7.2.1. Determination of alkaloids. Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The beaker was covered and allowed to stand for 4 hours. It was then filtered and the extract was concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (2 M) and then filtered. The residue if available is the alkaloid which is then dried and weighed.

2.7.2.2. Determination of tannin. Five hundred milligrams of the sample was weighed into a 50 ml plastic bottle. Fifty millilitres of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

2.7.2.3. Determination of saponin. The samples were ground and 20 g of each was put into a conical flask followed by the addition of 100 ml of 20% aqueous ethanol. They were then heated over a hot water bath for 4 hours with continuous stirring at about 55 °C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. Saponin content was calculated as percentage.

2.7.2.4. Determination of flavonoids. Ten grams of the stem bark extracts was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.8. Analysis of essential oils

The essential oils of the extracted plant samples (fresh and dried) were analysed based on the method described by Ashafa et al. (2010); one hundred and fifty grams of fresh and dried leaves of *T. daniellii* was subjected to hydrodistillation for three hours using a Clevenger-type apparatus.

The oil was analysed using Hewlett Packard 6890 Gas Chromatograph linked with Hewlett Packard 5973 mass spectrometer system which was equipped with a HP5-MS capillary column (30m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies Wilmington, DE, USA). The oven temperature was programmed from seventy to two hundred and forty degree Celsius at the rate of 5 °C min^{-1}. The ion source was set at two hundred forty degree Celsius with ionization voltage of 70 eV. Helium was used as a carrier gas. Spectra were analysed using the Hewlett Packard Enhanced Chem Station G1701 program for windows.

The components of the oils were identified by matching their spectra and retention indices with those of the Wiley 275 (Wiley, New York) in the computer library and literature. Percentage composition was calculated using the summation of the peak areas of the total oil composition.

2.9. Determination of phytoconstituents by high performance liquid chromatography

The constituents of the crude extracts of ethanol which were quite inhibitory on all organisms tested against were determined using a method employed by Ngonda et al. (2012); Schimadzu LC-10AD HPLC system (Japan), equipped with a Schimadzu SPD-10AV UV–VIS spectrophotometer detector with a thermostated flow cell and a selectable two wavelengths of 190–370 nm or 371–600 nm with SCL-10A system control. The detector signal was operated at ultraviolet wavelength detection at 254 nm. An Agilent ZORBEX SB-C18 (Agilent Technologies, USA) column (3.5 µm, 4.6 mm × 150 mm, i.d.) was used for chromatographic separation. The injection volume of 20 µl was used. The isocratic mobile phase comprised methanol:acetonitrile (60:40). Analysis was performed at a flow rate of 0.6 mL/min. Ten (10) milligrams of the plant extracts was dissolved in appropriate solvents filtered through Whatman paper No. 1 into volumetric flask and made up to 25 mL.

2.10. Statistical analysis

The data obtained were analysed using analysis of variance (ANOVA) and compare using Duncan's multiple range test (DMRT) (Leech et al., 2008).

3. Results

3.1. Plant identity

The *T. daniellii* plant was identified with voucher no. LUH 6087 at the Lagos University Herbarium.

3.2. Test isolates

The isolated test fungi are *Aspergillus aculeatus*, *Aspergillus niger*, *Aspergillus flavus* strain, *Rhizopus stolonifer*, *Issatchenkia orientalis*, *Meyerozyma guilliermondii*, *Fusarium oxysporum*, *Paecilomyces variotii*, *Penicillium crustosum*, *Trichoderma harzianum* and *Meyerozyma caribbica*.

3.3. Percentage yield of T. daniellii extracts

The yield of the extracts obtained from 200 g of leaves of *T. daniellii* was deduced as mentioned in Sections 2.4.2. and 2.4.3; it was observed that the aqueous extract had the highest yield among the solvents used for extraction as depicted in Table 1.

3.4. Purity of the extract

The observable activity of the extract on medium as shown in Plate 1 indicated that the extract has not been contaminated prior.

3.5. Antifungal assays of T. daniellii on fungi isolated from orange juice and Corn Jell-o ('Eko')

The concentration of acetone, ethanol, hexane and aqueous extracts from *T. daniellii* increased with an increase in diameter of zone of inhibition as depicted in Table 2. Analysis of variance revealed that the rate of inhibition differs significantly ($p < 0.05$) along the varied concentrations of the extracts against each test isolate. The concentrations of some of the

Table 1 – Yield of plants after extraction.

Solvent	Yield of extract (g)	Yield of extract (%)
Acetone	9.6	4.7
Ethanol	24.1	12.1
Hexane	21.6	10.8
Aqueous	19.8	39

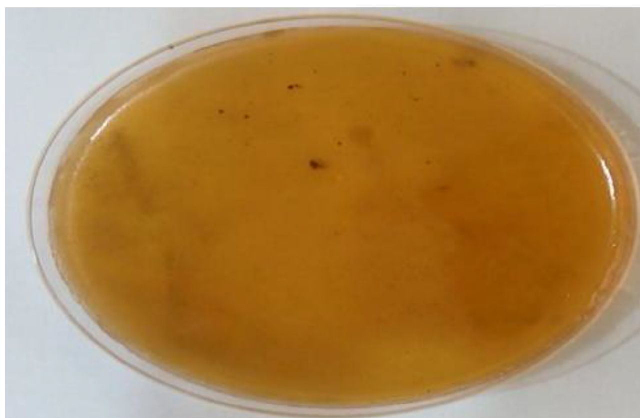

Plate 1 – Purity of the extract on potato dextrose agar.

test fungi such as *M. guilliermondii* increased from 10 ± 0 mm at 0.1 g to 14 ± 0.6 mm at 10 g while *M. caribbica* increased from 9.7 ± 0 mm at 0.1 g to 13.7 ± 0.3 mm at 10 g and this applies to other test isolates with different extracts from *T. daniellii* leaves. The comparative activities of antifungal drug (Nystatin) on test isolates seemed to be less potent compared to the test extracts from *T. daniellii* leaves. The alphabets (a, b, c, d, e, f and g) were used as distinguishing character for their significant differences. Table 2 also shows that the activities of ethanol extract were more pronounced with higher activities across all tested isolates compared with the activities of aqueous, acetone and hexane extracts. Table 3 shows the minimum inhibitory concentration of the extracts from *T. daniellii* leaves. It was observed based on Table 3 that the ethanol extract had the lowest minimum inhibitory concentration among the test extracts on test isolates; the range of the MIC of ethanol extract is between 0.5 g and 1.0 g. The minimum fungicidal concentration exhibited the potency of the extracts to kill the test fungi at a range of 0.1 g–10.0 g as seen in Table 2 and it was also manifested that the rate of the fungicidal activities of ethanol was higher than other test extracts of *T. daniellii* leaves with values that range between 0.1 g and 5.0 g on most of the test isolates.

3.6. *Brine shrimps lethality assay after 24 hours of exposure*

The lethality dose of extracts required to kill fifty percent of the population of Brine shrimps is depicted in Table 4. It shows that the ethanol and aqueous extracts of *T. daniellii* of 281.12 µg/ml and 592.95 µg/ml are non-toxic because they were above the general toxicity test agreement of LD_{50} being above 100 µg/ml for toxic-free substances.

3.7. *Phytochemical screening*

The results of screened qualitative and quantitative phytochemical constituents of different extracts of *T. daniellii* leaves are shown in Table 5a and b.

3.7.1. *Qualitative phytochemical screening*

The qualitative phytochemical constituents of *T. daniellii* as shown in Table 5a signify the presence of alkaloids and saponins in all the test extracts of *T. daniellii* leaves; steroids were present in acetone and aqueous extracts and flavonoids were present in ethanol and aqueous extracts.

3.7.2. *Quantitative phytochemical determination*

The result of quantitative phytochemical constituents of ethanol extract of *T. daniellii* leaves which was the most active result was depicted in Table 5b.

3.8. *Essential oil analysis of fresh and dried leaves of* T. daniellii

Table 6a and b shows the percentage of essential oil composition of the dried and fresh leaf samples of *T. daniellii* respectively.

3.9. *HPLC determination of the phytoconstituents*

3.9.1. *HPLC determination of the ethanol extract of* T. daniellii

The analytical HPLC used to analyse the phytochemical compounds of *T. danielli* revealed the presence of 19 phytochemical compounds as shown in Table 7.

4. Discussion

The consumer interest towards naturally derived antimicrobials for the enhancement of food products has created attention because of the documented side-effects of synthetic preservatives. Several authors have examined the potency of essential oils and other plants' antimicrobials for food enhancement (Hintz et al., 2015; Hyldagaard et al., 2012; Regnier et al., 2012; Tarek et al., 2014; Weli et al., 2014). This study has been able to ascertain the potential of *T. daniellii* as an antimicrobial agent against food spoilage fungi isolated orange juice and Corn Jello.

The extracts of *T. daniellii*'s leaves obtained from acetone, aqueous, ethanol and hexane were assayed for this study. The percentage yield of the aqueous extract of the extract was the highest among the four solvents used which agrees with the work of Dhanani et al. (2013) and corroborate earlier work by Adekunle and Ikumapayi (2006) in which the yield of the aqueous extract was higher than the yield of ethanol extract of *Funtumia elastica* and *Mallotus oppositifolius*. Francois et al. (2015) posited that higher yield of the aqueous extracts compared with the ethanol, benzene and chloroform extracts suggest higher proportion of water-soluble plant components. The yield of the hexane extracts shows the presence of some non-polar compounds in the three plants which agrees with the works of Okwori et al. (2006), Patel and Kumar (2008), Adeshina et al. (2012), Ekwealor et al. (2012), Souryadeep et al. (2012), and Sardashti and Adhami (2013). The substantial yield of the ethanol extract can be attributed to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds in the plants (Anukworji et al., 2012; Onifade, 2002). The yield of acetone extracts was momentous because of the polarity and non-polarity status of the solvent which encourages absorption of

Table 2 – Concentration and zones of inhibition of *Thaumatococcus daniellii* leaf extracts against the test fungi.

ACETONE	0.1	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	9 ± 0^{b,c}	10 ± 0^{c}	5 ± 0^{a}	6 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	9.7 ± 0.3^{c}
	0.5	6.3 ± 0.3^{b}	6 ± 0^{b}	5 ± 0^{a}	5 ± 0^{a}	9.3 ± 0.3^{c}	10.3 ± 0.3^{c}	5 ± 0^{a}	9 ± 0^{b,c}	9.7 ± 0.3^{c}	9.3 ± 0.3^{c}	10.7 ± 0.3^{c}
	1.0	10 ± 0.6^{d}	9 ± 0.3^{d}	8.7 ± 0.3^{b}	9 ± 0^{c}	11.3 ± 0.9^{d}	12.7 ± 0.3^{d}	8.3 ± 0.3^{b}	9.7 ± 0.3^{c}	11 ± 0.6^{d}	11.3 ± 0.3^{d}	12 ± 0.6^{d}
	5.0	10 ± 0.6^{d}	9.3 ± 0.3^{e}	10 ± 0^{c}	10 ± 0.6^{d}	13.3 ± 0.7^{e}	12.7 ± 0.3^{d}	9 ± 0^{c}	11 ± 0.6^{d}	12.7 ± 0.3^{e}	11.3 ± 0.7^{d}	13 ± 0^{d,e}
	10.0	12.7 ± 0^{e}	13 ± 0.3^{f}	12 ± 0.6^{d}	14.3 ± 0.3^{e}	14.7 ± 0.9^{e}	14 ± 0.6^{e}	11 ± 0^{d}	13 ± 0.6^{e}	13.3 ± 0.3^{e}	13 ± 0.6^{e}	13.7 ± 0.3^{e}
NYSTATIN	PO	8 ± 0^{c}	7.3 ± 0.3^{c}	8 ± 0^{b}	8 ± 0^{b}	7.3 ± 0.3^{b}	8 ± 0^{b}	8 ± 0^{b}	8.3 ± 0.3^{b}	8 ± 0^{b}	7.7 ± 0.3^{b}	7.7 ± 0.3^{b}
HEXANE	0.1	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	7.6 ± 0.7^{b}	10 ± 0^{c}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	10.3 ± 0.3^{c}
	0.5	8 ± 0^{b}	8 ± 0^{b}	6.7 ± 0.3^{a}	9.7 ± 0.7^{b,c}	9 ± 0.3^{c}	11.3 ± 0.3^{d}	8.3 ± 0.3^{b}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	11.3 ± 0.3^{d}
	1.0	9.3 ± 0.3^{d}	9 ± 0^{b}	8.7 ± 0.3^{c,d}	9.7 ± 0.3^{b,c}	9.7 ± 0.3^{c}	12 ± 0^{e}	9.3 ± 0.3^{d}	5 ± 0^{a}	5 ± 0^{a}	8.3 ± 0.7^{b,c}	12 ± 0^{d}
	5.0	10.7 ± 0.3^{d}	9 ± 0.6^{b}	9.3 ± 0.3^{d,e}	9.7 ± 0.7^{b,c}	9.7 ± 0.3^{c}	12.3 ± 0.3^{e}	9 ± 0.3^{c,d}	9 ± 0.3^{c}	9.3 ± 0.3^{c}	9 ± 0^{c}	12 ± 0^{d}
	10.0	12 ± 0.6^{e}	6.3 ± 0^{a,b}	10 ± 0.6^{e}	10 ± 1^{c}	12 ± 0.6^{d}	13 ± 0^{f}	9 ± 0.6^{c,d}	9.3 ± 0.3^{c}	9.3 ± 0.3^{c}	9 ± 0^{c}	11.3 ± 0.3^{d}
NYSTATIN	PO	8 ± 0^{c}	7.3 ± 0.3^{a,b}	8 ± 0^{c}	8 ± 0^{b}	7.3 ± 0.3^{b}	8 ± 0^{b}	8 ± 0^{b}	8.3 ± 0.3^{b}	8 ± 0^{b}	7.7 ± 0.3^{b}	7.7 ± 0.3^{b}
ETHANOL	0.1	8 ± 0^{b}	7 ± 0.6^{c}	7 ± 0^{b}	10 ± 0^{c}	9 ± 0^{c}	9.3 ± 0.3^{c}	8 ± 0.6^{b}	10 ± 0.3^{c}	8.7 ± 0.3^{b,c}	9 ± 0^{c}	10 ± 0.5^{c}
	0.5	9 ± 0^{c}	10 ± 0^{c}	7.7 ± 0.3^{b}	10 ± 0.3^{c}	9.3 ± 0.3^{c}	9.3 ± 0.3^{d}	8.7 ± 0.3^{b}	10 ± 0.6^{c}	9.7 ± 0.3^{b,c,d}	9.7 ± 0.3^{c}	10.7 ± 0.3^{c}
	1.0	10.3 ± 0.7^{d}	10.3 ± 0.3^{c}	10 ± 0.6^{c}	11 ± 1^{c,d}	10.7 ± 0.6^{d}	11.3 ± 0.3^{d}	10 ± 0^{c}	10.3 ± 0.3^{c}	10.3 ± 0.8^{c,d}	9.7 ± 0.7^{c}	11.3 ± 0.3^{d}
	5.0	10.7 ± 0.3^{d}	10.7 ± 0.3^{c}	11 ± 0^{d,e}	11.7 ± 0.7^{c,d}	11.7 ± 0.7^{d,e}	11.7 ± 0.3^{d,e}	10.7 ± 0.3^{c}	11.3 ± 0.7^{d}	11.3 ± 0.9^{d}	11.7 ± 0.7^{c}	11.7 ± 0.3^{d,e}
	10.0	11.7 ± 0.33^{e}	10.7 ± 0.3^{c}	12 ± 0.6^{e}	12.3 ± 0.3^{d}	12.3 ± 0.3^{e}	12.7 ± 0.6^{e}	10.3 ± 0^{c}	13.7 ± 0.3^{e}	12.3 ± 0.7^{e}	12.3 ± 0.3^{d}	12 ± 0^{e}
NYSTATIN	PO	8 ± 0^{b}	7.3 ± 0.3^{b}	8 ± 0^{b}	8 ± 0^{b}	7.3 ± 0.3^{b}	8 ± 0^{b}	8 ± 0^{b}	8 ± 0^{b}	8 ± 0^{b}	7.7 ± 0.3^{b}	7.7 ± 0.3^{b}
WATER	0.1	5 ± 0^{a}	5 ± 0^{a}	7 ± 0^{c}	6 ± 0^{b}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	7.7 ± 0.9^{b}	6 ± 0^{b}
	0.5	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	11.3 ± 0.3^{c}	7 ± 0^{c}
	1.0	5 ± 0^{a}	5 ± 0^{a}	6 ± 0^{b}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{b}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	14.7 ± 0.3^{e}	7.3 ± 0.3^{c}
	5.0	5 ± 0^{a}	5 ± 0^{a}	6.7 ± 0.3^{c}	5 ± 0^{a}	5 ± 0^{a}	8 ± 0^{b}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	13 ± 0.6^{c}	8.7 ± 0.3^{d}
	10.0	9.7 ± 0.7^{c}	10 ± 0^{c}	7 ± 0^{c}	10 ± 0.6^{d}	10 ± 0.7^{c}	11.3 ± 0.7^{c}	10 ± 0^{c}	10 ± 0.6^{c}	10 ± 0^{c}	11.3 ± 0.9^{c}	10.7 ± 0.3^{e}
NYSTATIN	PO	8 ± 0^{b}	7.3 ± 0.3^{b}	8 ± 0^{c}	8 ± 0^{c}	7.3 ± 0.3^{b}	8 ± 0^{b}	8 ± 0^{b}	8.3 ± 0.3^{b}	8 ± 0^{b}	7.6 ± 0.3^{b}	7.7 ± 0.3^{c}
NO	DMSO	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}

The letters denote significance difference based on Duncan Multiple Range Test. The letters when they are the same, it indicates no significance and when the letters are different, it connotes significance.

Table 3 – Minimum inhibitory concentration and minimum fungicidal concentration of test extracts on isolated fungi.

Isolates	Acetone		Hexane		Ethanol		Aqueous	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
ANS	1.0	10	1.0	10	0.5	10	10	–
AN	1.0	10	5.0	–	0.5	0.5	10	10
AFS	1.0	10	5	–	0.5	10	10	–
AF	5	10	5	–	0.5	5	10	–
MCR	5	10	0.5	–	0.1	5	10	–
YST	0.1	1.0	0.5	10	0.1	1.0	10	–
YSTA	0.1	1.0	0.1	1.0	0.1	1.0	10	–
YSTB	1	1.0	0.1	1.0	0.5	1.0	10	–
FSR	5	–	1.0	–	1.0	1.0	10	–
YB	0.5	5.0	5.0	–	0.1	5.0	10	–
PENU	0.5	5.0	5.0	–	0.5	5.0	10	–
TRI	0.5	1.0	5.0	–	0.1	5	0.5	1.0

ANS: *Aspergillus aculeatus*; AN: *Aspergillus niger*; AFS: *Aspergillus flavus* Strain A; AF: *Aspergillus flavus* strain; MCR: *Rhizopus stolonifer*; YST: *Issatchenkia orientalis*; YSTA: *Meyerozyma guilliermondii*; YSTB: *Meyerozyma caribbica*; FSR: *Fusarium stolonifer*; YB: *Paecilomyces variotii*; PENU: *Penicillium crustosum*; TRI: *Trichoderma harzianum*.

polar compounds and non-polar compounds and this assertion uphold earlier reports of Ellof (1998), Sheela et al. (2012), Sivasankaridevi et al. (2013), and Dzoyem et al. (2014).

The ascertained pure extract devoid of contamination agrees with the work of Cheesbrough (2000), Okigbo et al. (2009), and Anukworji et al. (2012). This study also revealed that preservative and fungicidal compounds were present in the leaves of *T. daniellii*, since they were able to inhibit the growth of the test fungi; this revelation is in consonance with earlier reports on several studies but on different fungal organisms (Ajayi and Ojelere, 2013, 2014; Awosan et al., 2014; Grillo and Lawal, 2010). Hence the four extracts used have the potential application in

Table 4 – The effect of *Thaumatococcus daniellii* leaf extracts on brine shrimps.

Conc. (μg/ml)	Total No.	Control		Ethanol	Hexane	Acetone	Aqueous
		DMSO	Saline water				
20	10	0	0	1	5	4	0
40	10	0	0	3	6	5	0
60	10	0	0	2	8	7	2
80	10	0	0	2	9	8	0
100	10	0	0	4	9	8	1
LD_{50}				281.12 μg/ml	22.77 μg/ml	31.27 μg/ml	592.95 μg/ml

Table 5 – (a) Qualitative phytochemical screening of the plant' leaf. (b) Quantitative phytochemical screening of the plants' leaf.

(a)

S/No.	Compound	*Thaumatococcus daniellii*			
		Acetone extract	Ethanol extract	Hexane extract	Aqueous extract
1	Alkaloid	+	+	+	+
2	Tannins	–	+	–	+
3	Phlobatannins	–	–	–	–
4	Saponins	+	+	+	+
5	Flavonoids	–	+	–	+
6	Steroids	+	–	–	+
7	Terpenoids	–	–	–	+
8	Cardiac glycosides	–	–	–	–

(b)

Plants	Phytoconstituents (mg/100 g)			
	Alkaloids	Tannin	Saponins	Flavonoids
Thaumatococcus daniellii	98.96	81.77	22.92	35.03

+, Presence; –, Negative.

Table 6 – (a) Essential oil analysis of dried leaves of *Thaumatococcus daniellii*. (b) Essential oil analysis of fresh leaves of *T. daniellii*.

(a)

Peak No.	Dried leaves (*Thaumatococcus daniellii*)			
	Plant constituents (essential oils)	RT	RI	Composition (%)
1	2,2,5,5-tetramethylhexane	8.20	0	1.78
2	2-Fluoro-3,3-dimethylbutanol	8.30	0	0.99
3	1-Aza-thiapentane	8.64	813	0.68
4	Isodecane	8.70	0	1.66
5	Ethyl benzoyl pyruvate	8.83	0	1.63
6	3,3-dimethylbutyrophenone	8.92	0	2.69
7	6-Iodo-1-heptene	9.21	0	0.53
8	1,3,5-Trimethylbenzene	9.40	0	11.80
9	1,2-Dineopentyldisulfane	9.83	1824	0.98
10	2,3-Dimethyl-3-phenybutan-2-ol	9.91	0	3.58
11	8-(Phenylsilyl)-3-phenyoct-1-ene	10.18	0	0.55
12	(E)-Ethyl 5-N-benzamido-2-pentenoate	10.41	0	1.33
13	5,9,9-Trimethylspiro(3–5) nona-5,7-dien-1-one	10.51	0	3.11
14	1-Methylbutyl nitrite	10.58	0	0.55
15	1,4,5,8-tetrathiadelin	10.67	0	1.07
16	o-Diethylbenzene	10.84	0	0.98
17	(z)-Lyratyl acetate	10.90	0	1.01
18	1-methyl-3-isoprophylbenzene	11.61	0	2.17
19	Hendecane	11.18	0	4.73
20	Perilla acetate	11.55	0	1.41
21	*n*-Nonane	12.78	0	2.98
22	Dodecane,1,1-difluoro	14.26	0	1.19
23	Bibenzene	15.60	0	1.33
24	1-methylbutyl nitrite	16.95	0	0.57
25	Octyl disulfide	19.34	0	0.52
26	4-Hydroxycarbonyl-2-oxobutyltriphenylphosphonium bromide	24.39	0	2.41
27	1,3-Diphenyl-1-(trimethylsilyl)oxy)-1(E) heptene	31.63	0	0.56
28	:(E)-1-[(1′,1′′dimethylethyl)diphenylsilyl]-2-(trimethylsilyl)ethylene	33.99	0	0.61
29	2-(N-methyl-N-acetylamino)-4,4-diphenyl-4-ethylcarbonylbutane	34.35	0	0.52
30	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	34.35	0	0.54
31	4,4-Dimethyl-2-oxo-tetrahydrofur-3-yl 2-[Tris(trimethylsilyl)silyl]-4-phenyl-but-3-enoate	34.66	0	0.50

(b)

Peak No.	Fresh leaves (*Thaumatococcus daniellii*)			
	Plant constituents (essential oils)	RT	RI	Composition (%)
1	2,6-Dimethyloctane	8.18	887	1.58
2	5,6-dimethylundecane	8.57	1185	0.68
3	2,3,4-Trimethylheptane	8.70	0	1.70
4	1,3,5-Trimethylbenzene	8.77	0	4.61
5	3-Ethyltoluene	8.83	0	1.47
6	3-Methylnonane	8.90	0	2.26
7	1-Ethyl-2-methylbenzene	9.10	1006	1.83
8	1-Isobutyl-3-methylcyclopentane	9.20	956	1.19
9	Isodecane	9.43	0	15.27
10	2-Phenylbutane	9.68	0	0.86
11	2-Butyloctanol	9.76	1393	1.05
12	4-Methyldecane	9.83	0	3.91
13	(1,3-dimethylbutyl) cyclohexane	10.006	0	0.71
14	3,7-Dimethylnonane	10.01	0	0.79
15	(1-Propyldecyl)cyclohexane	10.12	1909	2.13
16	Octadecyl hexanoate	10.19	2574	0.75
17	*p*-Ethyethylbenzene	10.35	0	1.06
18	1,3-Hexanedione, 1-phenyl-2,5-dimethyl-	10.42	1633	2.70
19	1,4-Diethylbenzene	10.51	0	3.77
20	2-Methyl-decane	10.57	0	2.21
21	(1,3,3-Trimethylnonyl)benzene	10.68	1738	3.84
22	1,2-Dimethyl-3-ethylbenzene	10.85	0	1.37
23	Thymene	10.90	0	1.80
24	*p*-isopropyl-Benzaldehyde	11.01	0	1.80
25	1-Methylindan	11.06	0	0.52
26	2-Propyl-2-heptenal	11.10	0	0.58
27	Hendecane	11.18	0	8.94
28	*p*-Cumic aldehyde	11.27	1230	0.83
29	(1,1,4,6,6-pentamethylheptyl) Benzene	12.35	0	0.65
30	Nonan	11.44	0	0.60
31	*p*-Cymol	11.61	1042	1.12
32	(+)-(R)-p-Mentha-1,8-dien-4-ol	12.14	0	0.99
33	Hexadecyl chloride	12.22	0	0.65
34	*n*-Tridecane	15.65	1413	1.49
35	Isododecane	12.78	0	0.57
36	*n*-Tetradecane	15.65	1413	1.49
37	*n*-Octadecane	18.18	0	1.12
38	2-Keto-butyric-acid	20.44	0	0.80
39	*n*-Hexadecoic acid	22.12	0	0.60
40	9,12,15-Octadecatrienal	23.93	0	1.36
41	Di-(2-ethylhexyl)phthalate	30.12	0	1.81

Table 7 – HPLC determination of the ethanol extract of *Thaumatococcus daniellii*.

Spectrum Max Plot

Pk # name	Retention time	Start time	Stop time	Area	Area percent
1	11.774	11.11	12.07	25,716,659	8.68
2	12.363	12.09	12.85	38,686,413	13.06
3	13.152	12.85	13.74	11,859,356	4.01
4	14.283	13.74	14.61	38,402,254	12.97
5	14.709	14.61	15.11	16,543,520	5.59
6	15.456	15.11	15.58	5,930,881	2.00
7	15.947	15.58	16.32	11,253,413	3.80
8	16.597	16.32	16.85	2,555,085	0.86
9	17.291	16.85	17.64	12,976,393	4.38
10	18.080	17.64	18.33	10,591,257	3.58
11	18.720	18.33	19.25	4,658,620	1.57
12	19.723	19.25	20.47	8,264,670	2.79
13	20.800	20.47	21.12	2,565,391	0.87
14	21.611	21.12	22.55	29,746,553	10.05
15	24.651	22.83	25.69	29,364,214	9.92
16	26.219	25.69	26.74	7,885,506	2.66
17	27.339	26.74	28.74	36,272,676	12.25
18	28.907	28.74	29.34	1,872,750	0.63
19	29.781	29.42	29.98	965,989	0.33

the inhibitory activity against the test fungi isolated from orange juice and spoilt Corn Jell-O ('Eko'). However the efficacy of the extracts differed based on the concentration of the extract, solvent of extraction and with each test fungus.

The observation of the activities of the extracts against test fungi showed that the activity of the aqueous and hexane extracts against the fungi were quite ineffective at any concentration except in fringes among test organisms which agrees with Alo et al. (2012) who reported that aqueous extract of *Ocimum gratissimum* did not inhibit the growth of test organisms. The work of Oluremi et al. (2010) corroborates Alo et al. (2012) that some organisms are not susceptible to the extract's activity and the resistance to antimicrobial agents cannot be eliminated but curtailed since some organisms are intrinsically resistant. The inactivity of hexane and aqueous extracts also agrees with earlier works by El-Mahmood (2010). The absence of activity in the hexane and aqueous extracts of *T. daniellii*'s leaves might either be due to the more solubility of the active principles in other solvents than hexane and aqueous solvents (Francois et al., 2015; Parekh and Chanda, 2007) or the presence of active components in insufficient quantities in the crude extracts to show the activity with the dose levels employed (Taylor et al., 2001).The activity of the acetone and ethanol extracts was quite inhibitory on all test fungi and these occur as the concentration increases which agrees with Ekwenye and Elegalem (2005). At higher concentration wider zone of inhibition was produced and it reduced as the concentration reduces. Cowan (1999) reported that the ethanolic extracts showed better results as compared to aqueous because of the ability to extract organic compounds and this encourages the release of greater amount of active antimicrobial components. The high activity of the acetone extract against the test fungi agrees with Vaghasiya and Chanda (2010) and Abraham and Thomas (2012). Minhas et al. (2013) acknowledged the ineffectiveness of acetone extract against *Aspergillus flavus* but recognized the activeness of the acetone extract against some bacteria species. The predominance of ethanol extracts as against other solvents in exhibiting antifungal activities against the test fungi might not be unconnected with the solubility of the plant in the different extracting solvents; more inhibiting effect observed with ethanol extracts than the other extracts could be explained by the fact that ethanol is acidic and in solution donates a proton which makes the medium acidic (Nwankwo and Amaechi, 2013), any organism in the solution will accept the proton as a base (Nwankwo and Amaechi, 2013). The increase in concentration of the hydrogen ions inhibits the activities of the microorganism thereby resulting in their death (Uruquiaga and Leighton, 2000). The apparent reduction in the spectrum of activity in other extracts may signal a possible loss of potency in the event of extraction and further purification of the plant components.

The activities of the various extracts were gauged by determining their minimum inhibitory and fungicidal concentrations. The minimum inhibitory and fungicidal concentrations are tests of *in-vitro* susceptibility that is predictive of *in-vivo* therapeutic efficacy. The minimum inhibitory concentration, which is the lowest concentration of potency of the extract that inhibited the visible growth of fungi after incubation as shown in Table 3, signalled acetone and ethanol extracts at 0.1 mg/ml against *I. orientalis*, *M. caribbica* and *Meyerozyma guilliermondii* among the test fungi with lowest minimum inhibitory concentration needed for the inhibition. The minimum inhibitory concentrations in hexane and water were quite infinitesimally low, observed only with *M. caribbica* and *M. guilliermondii* when their growth was hindered with hexane extract of *T. daniellii*. The minimum inhibitory concentration was also low in *T. harzianum* when inhibited with water extract of *T. daniellii*. The minimum inhibitory concentration activity of the extracts against the test fungi agrees with several works by Okigbo et al. (2009), Grillo and Lawal (2010), Alo et al. (2012), Ashafa and Umebese (2012), and Haruna et al. (2013).

The minimum fungicidal concentration, which is the concentration of the extracts with no observable growth of fungi after incubation as shown in Table 5, indicated *T. daniellii* at

0.5 mg/ml against *I. orientalis*, *M. caribbica* and *M. guilliermondii* among the test fungi with lowest minimum fungicidal concentration needed for the inhibition. The minimum fungicidal concentration of hexane and water extracts of *T. daniellii* was quite miniscule, observed only with *M. caribbica* and *M. guilliermondii* when their growth was hindered with hexane extract of *T. daniellii*. The minimum fungicidal concentration was also low in *Trichoderma harzianum* when inhibited with water extract of *Thaumatococcus daniellii*. The minimum fungicidal concentration activity of the extracts against the test fungi agrees with several works by Okigbo et al. (2009), Grillo and Lawal (2010), Alo et al. (2012), Ashafa and Umebese (2012), and Haruna et al. (2013).

The *in-vivo* lethality in a simple zoological organism, such as brine shrimp test (BST), might be used to guide screening and fractionation of physiological active plant extracts, where one of the simplest biological responses to monitor is lethality, since there is only one criterion; either dead or alive (Ashafa, 2013; Olorunnisola et al., 2011; Pimentel-Montanher et al., 2002). In this study, extracts from acetone, ethanol, hexane and aqueous of *T. daniellii* leaves displayed significant lethality and non-lethality against brine shrimp nauplii with LC_{50} of 31.27 μg/ml, 281.12 μg/ml, 22.77 μg/ml and 592.95 μg/ml respectively. There is a general toxicity test agreement that LC_{50} required to kill 50% of the population of brine shrimp above 100.00 μg/ml is non-toxic while below 100.00 μg/ml is indicative of toxicity. The significant lethality and non-lethality of the extracts of *T. daniellii* are indications that there is presence of cytotoxic compounds and also less harmful compounds in the extracts which can be ascribed to the solvents used in extraction.

The presence of bioactive compounds has been responsible for the various activities of the leaf extracts; this confers activity of the extracts against the test fungi and it therefore explains the demonstration of anti-fungal and preservative activity by the extracts used in this study. As a result the antifungal property of these plant extracts is due to the presence of phytochemical constituents which are anti-microbial agents and they are inhibitory to the growth of the test fungi (Adekunle and Ikumapayi, 2006; Adekunle and Uma, 2005; Anukworji et al., 2012; Haruna et al., 2013). Qualitative phytochemical screening of the extracts extracted with acetone, ethanol, hexane and aqueous revealed the presence of all the phytochemicals tested, namely alkaloids, tannins, saponins, flavonoids, steroids and terpenoids. The quantitative phytochemical determination of the ethanol extracts of *T. daniellii* leaves revealed high yield of alkaloids, flavonoids, saponins and tannins in *T. daniellii*. The bioactivity of the constituents in the plants laid credence to several works that have identified the role of plant constituents in the inhibition of food spoilage fungi by plant (Efterpi et al., 2012; Lucera et al., 2012; Rasooli, 2007; Settanmi and Corsetti, 2008). The compounds obtained via GC/MS and HPLC fingerprinting revealed avalanche of constituents in the plants at the crude level of the plant and Russo et al. (2013) showed the complexity of the essential oils' chemical composition with dozens of compounds. This makes the identification process for the component that is responsible for the antimicrobial activity very difficult. They also observed that the antimicrobial activity result from the synergism or antagonism between several components. Daferera et al. (2003) postulated that the antifungal activity of essential oils is mainly attributable to their main components although there is possibility of other phenomena, such as synergy or antagonism with minor components.

5. Conclusions

This study reported the preservative potential of *T. daniellii* leaves on fungi responsible for spoilage of sweet *Citrus sinensis* (orange) juice and Corn Jell-O ('Eko'). The *in-vitro* assessment of the plant extracts against the test fungi and the phytochemical compounds present in the plants shows good inhibitory activity of the leaves on isolated fungi and is quite indicative of the potential of the plants as preservative agents. The study further portends that the purification and isolation of responsible compounds for inhibition via biomonitoring will provide us the privilege of having natural compounds that are safe and without health consequence compared to established synthetic preservatives that have been documented to have side effect.

The contributory effect of this study towards management of postharvest spoilage will spell improvement in sustainable food production and food security programmes of developing countries of the world with nearly 1 billion severely hungry people.

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