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Evaluation of Phytochemical and *in vitro* Antimicrobial Effects of *Solanum lycopersicum* Linn. (Tomato) on Oral Thrush and Human Cariogenic Pathogens

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Authors' contributions

This work was carried out in collaboration between all authors. Author MU designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AZ and JOO managed the analyses of the study. Authors HSH and AAA characterized and authenticated the tomato plant and author AS performed the statistical analysis. Author IBM performed plant extraction and managed the literature searches. Authors IMA and AAY interpreted the data. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Study on the evaluation of phytochemical and antimicrobial effects of tomato *Solanum lycopersicum* (L.) on oral thrush and human cariogenic pathogens was designed to determine the antimicrobial effect of tomato extracts, and to identify the common phytochemical constituents of tomato that may be inhibitory to oral pathogens.

Place and Duration of Study: Fresh tomato fruit samples were collected from Samaru market, Zaria in the month of November and December 2015, and analyzed at Microbiology Laboratory, Nigerian Institute of Leather and Science Technology Zaria, Kaduna.

Methodology: Cold maceration extraction using methanol and water was adopted. All the extracts were subjected to standard phytochemical qualitative screening for the presence or absence of various primary and secondary metabolites. Antimicrobial susceptibility was carried out by agar-well diffusion technique. The antimicrobial susceptibility test of *Solanum lycopersicum* (tomato) fruit extracts against selected bacterial (*Streptococcus mutans*, *Bacillus subtilis*) and yeast (*Candida albicans*) pathogens capable of causing oral thrush and dental caries was carried out. Ciprofloxacin and Econazole were used as positive controls. The Minimum Inhibitory Concentrations (MICs) were determined in three concentrations; 100 mg/ml, 50 mg/ml and 25 mg/ml of each extract. Mean zone of inhibition was used to measure the antimicrobial potential of tomato fruit extracts against the test organisms. The microbial isolates were obtained from the Department of Medical Microbiology, Ahmadu Bello University, Zaria.

Results: Alkaloids, flavonoids, glycosides, saponins, tannins, steroids, phlabotannins, terpenoids and tannins are present in tomato fruit extract, but, anthraquinones and phlabotannins are absent in methanolic extract. While, the only phytochemical that was not detected in the aqueous extract is anthraquinones. Highest antibacterial activity was recorded on *Bacillus subtilis* at the concentrations of 100 mg/ml and 50 mg/ml for aqueous and methanolic extracts respectively. *Candida albicans* and *Streptococcus mutans* showed resistance to the various extracts at various concentrations used. The MIC of 100mg/ml was recorded on *Bacillus subtilis*, whereas all other test organisms showed relative resistance to various concentrations of the extracts used.

Conclusion: Tomato fruit contains phytochemicals that showed promising antimicrobial effect on oral thrush and cariogenic pathogens. The tomato extract recorded antimicrobial effect against *Bacillus subtilis* with MIC of 100 mg/ml. The methanolic extract was the most active that could compete favourably with the conventional antibiotic (ciprofloxacin) at higher concentrations, which suggests that possible new drug candidates can be harnessed from the tomato fruit against oral thrush and cariogenic pathogens.

Keywords: Extraction; lycopene; minimum inhibitory concentration; phytochemistry; Solanum lycopersicum.

1. INTRODUCTION

Tomato, *Solanum lycopersicum* (Linn), previously known as *Lycopersicon esculentum* (Mill) is a red berry fruit [1]. It belongs to the plant family solanaceae and genus solanum [2]. Tomato is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components [3]. It is used as juice, soup, ketchup or paste [4]. Although tomatoes are often closely associated with Italian cuisine, they are actually originally native to the Western side of South America in the region occupied by Columbia, Ecuador, Peru, Chile and the western half of Bolivia. Tomato is very rich in vitamins, minerals, essential amino acids, sugars, dietary fibers and lycopene, an antioxidant that reduce the danger related to many cancers and neurodegenerative diseases [5]. Today tomatoes are enjoyed worldwide to the tune of 130 million tons per year by the largest tomato producing country, which is China (with approximately 34 million tons of production) followed by United States, Turkey and India [6]. The plant requires relatively cool weather for optimum yield [7], it typically grows up to 1 to 3 meters (3 − 10 feet) in height and have a weak stem that often sprawls over the ground. It is a perennial in its native habitat, and grown as an annual in temperate climates. The leaves are 10 – 25 cm long, odd pinnate, with five to nine leaflets on petioles; each leaflet is up to 8 cm long with a serrated margin [8].

Tomato contains variety of phytonutrients such as carotenoids (including beta-carotene, lutein, and zeaxanthin); flavonoids (including naringenin, chalconaringenin, rutin, kaempferol, and quercetin); hydroxycinnamic acids (including caffeic, ferulic, and coumaric acid); glycosides (including esculeoside A); and fatty acid derivatives (including 9-oxo-octadecadienoic acid). Lycopene, being the major carotenoid in tomato fruit, is the common antioxidant that reduces the danger related to many cancers and neurodegenerative diseases. It is a powerful antioxidant, anti-inflammatory and also has an antimicrobial property [5,9,10].

Some studies have shown that tomatoes have cancer prevention effect. The effect of tomato in the eradication of cancer is still unknown, but it was believed that lycopene and the newly discovered bioflavonoids in tomatoes are responsible cancer fighting agents [9]. Tomato is an excellent fruit vegetable for rapid skin cell replacement. Its juice can be used for healing sunburn because of its unique vitamin C. Tomato juice is a good sport drink used to restore one's body from fatigue and sleepiness [11].

The ever increasing demand for safer and cheaper herbal recipes in the developed countries has led to the extraction and development of several drugs and chemotherapeutic agents from plants as well as from traditional herbal remedies [12]. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal spices for the treatment of various diseases [13].

Majority of chemically synthesized drugs have serious adverse effect to the recipients, which may lead to temporary or permanent disability and incapacitations. Also, oral blisters, oral thrush, dental caries and oral ulcers are very serious infections that can lead to inability to take food or water as a result of dysphagia. The oral thrush disorder is a serious disease that can affect many people at various stages of their lives causing distress and discomfort. Sometimes, the disorder can lead to hospitalization as a result of starvation and dehydration following the victim's inability to ingest food or water [14,15].

Direct topical application of raw tomato in combination with salt has been used for long in many African tribes to treat oral thrush, blisters, dental caries and ulcers of varying degrees. Therefore this research was designed to determine the antimicrobial effect of tomato, and to identify the common phytochemical constituents of tomato that may be inhibitory to oral pathogens. The research findings can further be used by the dental, pharmaceutical industries, complementary medicine and other medical sectors in tackling the menace of microbial oral disorders.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh physically undamaged fruits of *Solanum lycopersicum* (tomato) were collected from Samaru market, Zaria based on the guidelines of convention on Biological Diversity, and taxonomically characterized as Roma VFN, at the herbarium sections of Biological Science Department, Ahmadu Bello University, Zaria and National Horticultural Research Institute, Bagauda Station, Kano with the aid of botanical keys [16], ensuring that the tomato fruit is not among the endangered plant species. The tomato fruits were collected fresh, healthy and free from organic contaminants that may interfere with the substances of interest. The fruits were thoroughly washed through running water, cut and dried under shade for 4-16 days, after which the dried fruits were pounded into a fine powder using a sterile mortar and pestle [17]. The tomato fruit powder was stored and sealed in labeled sterile reagent bottles for further use. The bioactive components were extracted using the method of Akerele et al. [18].

2.2 Preparation of Plant Extract

Plant extracts were prepared by the method of Alade and Irobi [19] with minor modifications. To study the antibacterial potential of *Solanum lycopersicum* (tomato), non-polar solvent such as methanol, and polar solvent such as water were used. A mass of 30 g of dried powder was weighed on a weighing balance (Mettle 166®, USA) and was extracted by 100 ml of each solvent until the aqueous content evaporated completely as adopted by Lin et al. [20]. At the end of extraction, the extracts were filtered through Whatman No. 1 filter paper and concentrated over a water bath using a rotary-vacuum evaporator to recover the solvents. The dried extracts were collected and weighed in varying concentrations and kept in airtight containers at 4℃.

2.3 Preliminary Phytochemical Screening

The preliminary phytochemical investigation was carried out for aqueous and methanolic extracts of *Solanum lycopersicum* for the detection of various phyto-constituents by using standard procedures to identify the constituents [20,21].

2.3.1 Test for the presence of alkaloids (Wagner's test)

Wagner's reagent was prepared by dissolving 2 g of iodine and 6 g of KI in 100 ml of water. The plant extract was prepared by using 500 mg of plant extract, which was treated with few drops of Wagner's reagent. A reddish brown coloured precipitate indicates the presence of alkaloids [22].

2.3.2 Test for the presence of anthraquinones (Borntrager's test)

About 0.5 g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of rose-pink colour indicated the presence of anthraquinones [23].

2.3.3 Test for the presence of flavonoids (Ethyl acetate test)

The crude powder of dried plant was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution, after which a yellow colouration was observed [24].

2.3.4 Test for the presence of phlobatannins (HCl test)

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl) to observe the deposition of red precipitate [25].

2.3.5 Test for the presence of glycosides (Fehling's test)

The crude plant powder of 0.5 g was dissolved in 5 ml of methanol. Exactly 2 ml of this solution was dispensed into a test tube, and to it 10 ml of HCl was added. The mixture was heated in a boiling water bath for 30 minutes, and to the mixture 5ml of Fehling's solution was added and the mixture was boiled for another 5 minutes to observe a brick red precipitate as an indication for the presence of glycosides [26].

2.3.6 Test for the presence of saponins (Frothing test)

About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy layer of small bubbles) showed the presence of saponins [24].

2.3.7 Test for the presence of steroids (Salkowski test)

A volume of 1 ml of plant extract was dispensed into a test tube, and to it few drops of concentrated sulphuric was added. The presence of red colouration indicated the presence of steroids [27].

2.3.8 Test for the presence of terpenoids

A mass of 0.2 g of the plant extract was mixed with 2 ml of chloroform and filtered. Acetic anhydride was added to the filtrate, and 3 ml concentrated sulphuric acid (H_2SO_4) was added carefully to form a layer. A pink coloration of the interface was formed to indicate positive results [27].

2.4 Bacterial Pathogens

Characterized isolates of overnight cultures of oral thrush and dental caries-causing test organisms such as *Candida albicans*, *Streptococcus mutans* and *Bacillus subtilis* were collected from the Dental Section, Medical Microbiology Laboratory, Ahmadu Bello University, Teaching Hospital, Zaria. The isolates were kept in fresh broths to maintain their viability.

2.5 Antimicrobial Susceptibility Screening

Screening of the antibacterial activity of the extracts was carried out using agar-well diffusion method. Nutrient agar (N70123, Sigma-Aldrich® Co., Germany) for bacterial isolates and Sabouraud's Dextrose agar (RODAC™, Becton-Dickson and Co., USA) for *Candida albicans* were prepared according to manufacturer's specifications, sterilized, and poured into Petri dishes and allowed to set. Wells were made in the inoculated media using sterile cork-borer (6 mm diameter, NC-11269, Science Company®, New Jersey, USA) after which a little molten media was used to seal the base of the wells.

The characterized and 0.5 scale McFarland standardized test organisms were inoculated on the media by spread plate method using a sterile rod spreader to obtain uniform microbial growth. A volume of 0.1 ml each of the prepared extracts (100 mg/ml, 50 mg/ml and 25 mg/ml) were transferred into the corresponding wells with a sterile micropipette and were appropriately labelled, while 0.1 ml of 20% Dimethyl sulfoxide (DMSO) (DMS, Gaylord Chemical Co., Louisiana, USA) (free of extract) was dispensed into a separate well to serve as the negative control. Ciprofloxacin (30 µg) was used as the positive control for bacterial isolates, while Econazole (30 µg) was used as positive control for *Candida albicans*. The plates were incubated for 48 hours at 37℃. Antibacterial and antifungal activities were evaluated by measuring the diameters of zones of growth inhibition in millimeters (Plate 1) after 48 hours incubation. Oxoid [28] standard susceptibility range was used to classify zones of inhibition as either sensitive (> 10 mm) or resistant (≤ 10 mm).Each experiment was conducted twice, and the mean of two results was taken for both the test and control as adopted by Irobi et al. [29].

Plate 1. Antimicrobial susceptibility setup of tomato extract

2.6 Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) was determined as the least concentration that showed an inhibitory effect on test organism using the tube method. Two fold serial dilutions were made using nutrient broth (N70123, Sigma-Aldrich® Co., Germany). The 5 ml of a solution of the extract (250 mg/ml) was added aseptically to 5 ml of double strength medium and mixed by shaking. Using a fresh pipette, 5 ml of the mixture was transferred to the second test tube which contained 5 ml of the single strength medium. These two components were mixed by shaking and from it 5 ml will be taken into third test tube aseptically and mixed by shaking. The 9th tube containing no test compound served as control. Finally, to each tube 0.2 ml inoculums of the test organisms were added aseptically. The test tubes were covered with cotton wool and incubated at 37℃ for 24 hours and then observed for turbidity. The lowest concentration that inhibited growth of test organism was noted as the MIC [30].

3. RESULTS AND DISCUSSION

Table 1 showed the phytochemical screening of tomato fruit extract. Phytochemicals such as alkaloids, flavonoids, phlabotannins, glycosides, saponins, tannins, steroids and terpenoids were detected in aqueous extracts analyzed. While, anthraquinone was not detected in either of the extracts analyzed. Whereas, all the phytochemicals tested were present in the methanolic extract except anthraquinones and phlabotannins.

Table 1. Qualitative phytochemical screening of tomato fruit extracts

Phytochemicals	Aqueous extract	Methanol extract
Alkaloids	+	+
Flavonoids	+	+
Phlabotannins	+	−
Glycosides	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+
Anthraquinones	−	−
Steroids	+	+

**+ = Present; − =Absent*

Table 2 showed the antimicrobial effects of tomato aqueous extract against bacterial isolates. Highest antibacterial activity was recorded on *Bacillus subtilis* at the concentration of 100 mg/ml. All other test organisms showed resistance to the various extracts at various concentrations used.

Table 3 showed the antimicrobial effects of tomato methanol extract against bacterial isolates. Highest antibacterial activity was recorded on *Bacillus subtilis* at the concentration of 100 mg/ml and a slight sensitivity at 50 mg/ml. All other test organisms showed resistance to the various extracts at various concentrations used.

Table 2. Antibacterial effect of aqueous tomato fruit extract

Test organism	Concentration/ Zones of growth inhibition						
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Ciprofloxacin	Econazole	DMSO
Bacillus subtilis	10 mm	0 mm	7 mm	0 mm	32 mm	–	0 mm
Candida albicans	0 mm	0 mm	0 mm	0 mm	–	36 mm	0 mm
Streptococcus mutans	0 mm	0 mm	0 mm	0 mm	35 mm	–	0 mm

DMSO= Dimethyl sulfoxide

Table 3. Antibacterial effect of methanol tomato fruit extract

Test organism	Concentration/ Zones of growth inhibition						
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Ciprofloxacin	Econazole	DMSO
Bacillus subtilis	25 mm	15 mm	0 mm	0 mm	32 mm	–	0 mm
Candida albicans	0 mm	0 mm	0 mm	0 mm	–	35 mm	0 mm
Streptococcus mutans	0 mm	0 mm	0 mm	0 mm	36 mm	–	0 mm

DMSO= Dimethyl sulfoxide

Table 4. Minimum inhibitory concentration (MIC) of tomato fruit extracts

Test organisms	Concentration		
	Aqueous	Methanol	Control
Bacillus subtilis	100 mg/ml	50 mg/ml	None
Candida albicans	None	None	None
Streptococcus mutans	None	None	None

Table 4 showed the minimum inhibitory concentration of the tomato fruit crude extract. The MIC of 100 mg/ml was recorded on *Bacillus subtilis*, whereas all other tests organisms showed relatively resistance to the extract, and hence their MICs were not determined.

Based on the phytochemical analysis findings of the research study, it was found out that phytochemicals such as alkaloids, flavonoids, glycosides, saponins, tannins, steroids, phlabotannins and terpenoids and tannins were present in both aqueous and methanolic extracts of tomato fruit which have been associated with antimicrobial activities [31,32]. Anthraquinone is absent in aqueous extract, while anthraquinone and phlabotannins are absent in methanolic extract. This might be attributed to the difference in the method of extraction employed, polarity of solvents used and also pointing to the fact that the use of solvents during extraction of medicinal plant might have some advantages to individual application. Sukhdev et al. [33] also reported the effect of temperature on phyto-components due to the fact that some of these components are thermo-labile in nature. The result reveals the presence of alkaloids which are a large group of secondary chemical constituents of plants made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, mostly containing oxygen and they are intensely bitter in solutions.

The results for the antimicrobial screening have shown that at the concentration of 100 mg/ml, all the extracts have antibacterial activity on *Bacillus subtilis*. Effectiveness factor of the tomato is due to the presence of Lycopene that possesses antibacterial and antifungal properties [34,35]. The results of the inhibition of bacterial growth have shown that the extracts are active at high concentration and inactive at very low concentrations. Thus the study suggests that the inhibition of bacterial growth activity of the extracts is dose dependent. The aqueous and methanolic extracts had no inhibitory effect on the *Streptococcus mutans* and *Candida albicans* but showed inhibitory effects on the Gram-positive rod bacterium, *Bacillus subtilis*, with activity increasing with increase in concentration of extracts. This concurs with the findings of Omodamiro and Amechi [36] who reported antibacterial activity of tomato extract on some of pathogenic microbes. But, resistance of *Candida albicans* to both extracts is contrary to the findings of Sung et al. [37] and Pavlovic et al. [38] who reported that tomatoes proved to be effective against microorganisms such as

Staphylococcus aureus, *Proteus*, *Bacillus* and antifungal to *Candida albicans* and *Aspergillus niger*.

This may be due to the better solubility of the bioactive agents in the non-polar solvents. This conforms to the work of El-Mahmood et al. [39] who reported that the extractability of phyto-constituents differs from solvent to solvent depending on their polarities. It also reveals that the methanolic extract was more active against the Gram-positive bacteria. Since the traditional herbal remedy preparation use water as the extractant, it is a paradox that the aqueous extracts were less active in this study. It is possible that the aqueous extracts may contain antimicrobial constituents insufficient for efficacy in this study, which may explain why large amounts of the decoctions must be drunk by the patients. Jigna and Chanda [40] and Kitonde et al. [41] found out that, aqueous extracts showed little or no antimicrobial activity in contrast to those made using organic solvents. Yinegar et al. [42] reported that the success in traditional medicines may be due to administration of the extracts in large quantities and over a long period of time.

The Minimum Inhibitory Concentration (MIC) of the aqueous extract was highest for *Bacillus subtilis* at the concentration of 100 mg/ml with no activity in all other extract concentrations used. While, the MIC of the methanolic extract was least for *Bacillus subtilis* at the concentration of 50 mg/ml, suggesting that the methanol extract is more efficacious on *Bacillus subtilis* than the aqueous extracts. This is contrary to the studies of Omodamiro and Amechi [36] who reported MIC of 31.25 mg/ml for *Proteus mirabilis* and *Pseudomonas aeruginosa*. The antimicrobial effect of tomato in the treatment of oral microbial diseases can probably be enhanced when tomato is used in combination with table salt. The synergy between raw tomato and salt or honey might inhibit diverse types of oral pathogens. Previous studies by Al-Oqaili et al. [43] reported correlation between synergistic combination of tomato and honey with high antimicrobial therapeutic effects.

4. CONCLUSION

The secondary metabolites detected in the tomato fruit extracts include alkaloids, flavonoids, glycosides, saponins, tannins, steroids, phlabotannins, terpenoids and tannins. Anthraquinone and phlabotannins are absent in methanolic extract. While, the only phytochemical that was not detected in the aqueous extract is anthraquinones.

Bacillus subtilis was susceptible to all the extracts at the concentrations of 100 mg/ml, but *Streptococcus mutans* and *Candida albicans* were not susceptible to the aqueous and methanolic extracts within the concentration range for this study. The methanolic extract was the most active that could compete favourably with the conventional antibiotic (ciprofloxacin) at higher concentrations. The activity of the extracts on the test organisms increased with increase in concentration. The methanol extract exhibited bacteriostatic effects on the *Bacillus subtilis* at lower concentrations and bactericidal effects at higher concentrations, with minimum inhibitory concentration of 100 mg/ml.

This research may serve as a scientific basis and lend credence to the claim by the traditional medicine practitioners that the tomato fruit is a useful herbal remedy to various microbial oral infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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