

## The Antibacterial Properties of *Euphorbia Tirucalli* Stem Extracts against Dental Caries-Related Bacteria

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### ABSTRAK

*Euphorbia tirucalli* dilaporkan mempunyai aktiviti antibakteria terhadap pelbagai mikroorganisma. Kajian *in vitro* ini bertujuan untuk menilai ciri-ciri antibakteria ekstrak (metanol, etanol dan ekstrak akueus) batang *Euphorbia tirucalli* terhadap bakteria yang berkaitan dengan karies gigi, iaitu *Streptococcus mutans* (*S. mutans*) dan *Streptococcus sobrinus* (*S. sobrinus*). Sifat-sifat antibakteria telah ditentukan menggunakan ujian resapan agar berlubang pada kepekatan ekstrak yang berbeza (10, 20 dan 30 mg/ml). Komersial amoxicillin (10 µg) telah digunakan sebagai kawalan positif manakala pelarut yang sesuai telah digunakan sebagai kawalan negatif. Ekstrak metanol dan ethanol daripada batang *Euphorbia tirucalli* didapati berkesan terhadap *S. mutans* dan *S. sobrinus*. Walau bagaimanapun, ekstrak akueus batang *Euphorbia tirucalli* tidak menunjukkan aktiviti terhadap kedua-dua strain bakteria. Perbezaan dalam ciri-ciri antibakteria dalam perbezaan ekstrak *Euphorbia tirucalli* mungkin disebabkan oleh perbezaan dalam juzuk fitokimia.

Kata kunci: *Euphorbia tirucalli*, ciri-ciri antibakteria, *Streptococcus mutans*, *Streptococcus sobrinus*

### ABSTRACT

*Euphorbia tirucalli* are reported to possess antibacterial activity against various microorganisms. This *in vitro* study aimed to evaluate the antibacterial properties of *Euphorbia tirucalli* stems extracts (methanol, ethanol and aqueous extracts) against dental caries-related bacteria, i.e. *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*). The antibacterial properties were determined using agar-well diffusion method at different extract concentrations (10, 20 and 30 mg/ml). Commercially available amoxicillin (10 µg) was used as positive control while the appropriate solvent served as negative control. The methanolic and

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ethanolic extracts of *Euphorbia tirucalli* stem were found to be effective against *S. mutans* and *S. sobrinus*. However, the aqueous extract of *Euphorbia tirucalli* stem showed no activity against both bacterial strains. The differences in the antibacterial properties in different extracts of *Euphorbia tirucalli* may be due to the differences in phytochemical constituents.

Keywords: antibacterial properties, *Euphorbia tirucalli*, *Streptococcus mutans*, *Streptococcus sobrinus*

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## INTRODUCTION

Dental caries are multifactorial diseases involving complex biological interactions of acidogenic bacteria, fermentable carbohydrates and host factors such as the teeth and saliva (Selwitz et al. 2007). The acidogenic bacterial species, *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) have been considered as the main causative agents of dental caries. Higher incidences of dental caries are recorded when these two bacteria co-exist (Okada et al. 2005; Nurelhuda et al. 2010).

The interest on botanical medicine as a source of antimicrobial agents are increasing globally. The emergence and re-emergence of infectious diseases, the increase of chemotherapeutic failure and antibiotic resistance exhibited by pathogenic infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Kumar et al. 2015). *Euphorbia tirucalli* or known as Tetulang, belongs to *Euphorbiaceae* family and it is one of the most important trees known worldwide for its multiple uses. It is an evergreen shrub or a small tree endemic to tropical areas with pencil-

like branches containing white latex (Mwine & Van Damme, 2011).

Various studies indicated that this plant is a valuable source of medicinal compounds. Researches showed that active compound such as alkaloids, tannins and phenols in *Euphorbia tirucalli* was contributed their effectiveness in medicinal treatment (Sugumar et al. 2010). Researchers found that flavonoids in *Euphorbia tirucalli* effectively inhibited the bacteria growth due to its ability to form complex with extracellular proteins of cell wall and disrupt microbial membrane, whereas phenolic and polyphenols was toxic to micro-organisms. They also found that tannins in *Euphorbia tirucalli* able to inhibit bacteria by inactivate microbial adhesion, enzymes and cell envelope transport proteins (Upadhyay et al. 2010). Traditionally, Euphorbias are used as purgative, analgesic, anti-oxidant, anti-inflammatory, antipyretic, anti-microbial, anti-parasitic, anti-arthritis in the treatment of cough, asthma, rheumatism, cancer and other maladies as folk remedies (Prabha et al. 2008; Upadhyay et al. 2010; Sugumar et al. 2010; Jahan et al. 2011; Priya & Rao 2011; Rathi et al. 2012; Gupta et al.

2013). The stem of *Euphorbia tirucalli* is used to treat whooping cough, asthma, and infections of the spleen. The alcoholic extract of the stem of *Euphorbia tirucalli* was reported to possess broad-spectrum antimicrobial activity against *Escherichia coli*, *Proteus vulgaris*, *Salmonella enteritidis*, *Bacillus subtilis*, *Staphylococcus aureus*, *S.epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Xanthomonascitri*, *Candida albicans*, *C. tropicalis*, *C.glabreta*, *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Fusarium oxysporum* (Jadhav et al. 2010; Prasad et al. 2011; Rathi et al. 2012; de Araújo et al. 2014; Muthukumar et al. 2014).

Hence, the present study was conducted to evaluate the in vitro antibacterial activity of *Euphorbia tirucalli* stem extracts (methanolic, ethanolic and water) against dental caries-related bacteria (*S. mutans* and *S. sobrinus*) using the agar-well diffusion method.

## MATERIALS AND METHODS

### BACTERIAL STRAINS

Two oral bacterial strains used in this study, i.e. *S. mutans* (ATCC 25175) and *S. sobrinus* (ATCC 33478) were commercially obtained from the American Type Culture Collection (ATCC, USA). The bacteria were grown anaerobically at 37°C with 5% CO<sub>2</sub> in Brain Heart Infusion broth (Oxoid, UK) for 48 hrs. All bacterial growths were confirmed by microscopy and gram staining.

### COLLECTION OF PLANT MATERIAL

Fresh stems of *Euphorbia tirucalli* were collected from the local area of Kubang Kerian, Kelantan Malaysia. The taxonomic identity of the plant was confirmed by botanist from the Forest Biodiversity Department, Forest Research Institute Malaysia (FRIM) and the voucher number was PID 440915-23. The stem was thoroughly washed under running tap water, air-dried and subsequently dried in hot air oven (Protech, Malaysia) at 60°C for 48 hrs.

### PREPARATION OF EUPHORBIA TIRUCALLI STEM EXTRACTS

*Euphorbia tirucalli* stem extracts (methanol, ethanol and aqueous) were used for evaluation of antimicrobial properties. Dried stems of *Euphorbia tirucalli* were homogenized to a fine powder using a blender (Panasonic, Malaysia) and stored in an air tight bottle. One gm of the powdered stem was mixed with 30 ml of each solvent in an incubator shaker (Jeio Tech, Korea) at 37°C for 24 hrs. The mixture was then centrifuged for 1400 x g for 5 mins. The resulting extracts were filtered using filter paper (Whatman No. 1, USA) and each filtrate was concentrated with a rotary evaporator (Heidolph, Germany) at 60°C under vacuum condition. The concentrated supernatant was then deep frozen at -20°C for 24 hrs and freeze-dried to powder. All extracts were kept at 4°C until use. The final concentrations of 10, 20 and 30 mg/ml were prepared by dissolving the powder in the same solvent.

## AGAR-WELL DIFFUSION METHOD

The dried plant extracts were dissolved in the same solvents, to final concentrations of 10, 20 and 30 mg/ml and were sterilized by filtration through 0.45 µm Millipore filters (Nalgene, UK). The antibacterial activity of various extracts of *Euphorbia tirucalli* stem was analysed in vitro by an agar-well diffusion method according to Jain et al. (2015) with some modifications. Using a sterile cotton swab, an overnight bacterial suspension was swabbed on the surface of sterile Mueller Hinton Blood agar (MHBA) (Thermo scientific, USA). The plates were dried for 5 mins to allow any excess moisture to be absorbed. Five wells of about 6.0 mm diameter were aseptically punched on MHBA plates by the sterile cork borer. Fixed volumes (50 µl) of different concentrations of methanolic, ethanolic and aqueous extract were added into each well. Commercially available amoxicillin (10 µg) was used as positive control while appropriate solvent was served as negative control. These plates were allowed to stand for 5 mins for the diffusion of extract to take place. The plates were then incubated at 37°C for 48 hrs. Antibacterial activity was evaluated by measuring the zones of inhibition (clear zone around each well) in millimeter (mm) using a digital calliper (Mitaka, Japan). The experiment was done in triplicates for each concentration and organism.

## DETERMINATION OF RELATIVE PERCENTAGE INHIBITION

The relative percentage inhibition of the *Euphorbia tirucalli* stem extracts

with respect to positive control was calculated by using the following formula (Ajay et al. 2003):

$$\frac{100 \times (X - Y)}{(Z - Y)}$$

Where,

X: total area of inhibition of the test extract

Y: total area of inhibition of the solvent

Z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using  $\text{area} = \pi r^2$ ; where, r=radius of zone of inhibition.

## STATISTICAL ANALYSIS

Results were expressed as Mean ± standard deviation. Statistical analysis was done using a statistical package, IBM SPSS Statistics version 22 (IBM, Chicago, IL, USA) by applying mean values using non-parametric Kruskal-Wallis test followed by Mann-Whitney test to determine if there was a significant difference among different *E. tirucalli* extracts. A *p* value of less than 0.05 was considered significant.

## RESULTS

The results of the antibacterial activities of *Euphorbia tirucalli* extracts are shown in Figure 1. The methanolic and ethanolic extracts of *Euphorbia tirucalli* showed inhibitory effects against the growth of *S. mutans* and *S. sobrinus* at a concentration of 20 mg/ml and 30 mg/ml. Methanolic extract of *Euphorbia tirucalli* exhibited slightly higher inhibitory effect against *S. mutans* and *S. sobrinus* compared to

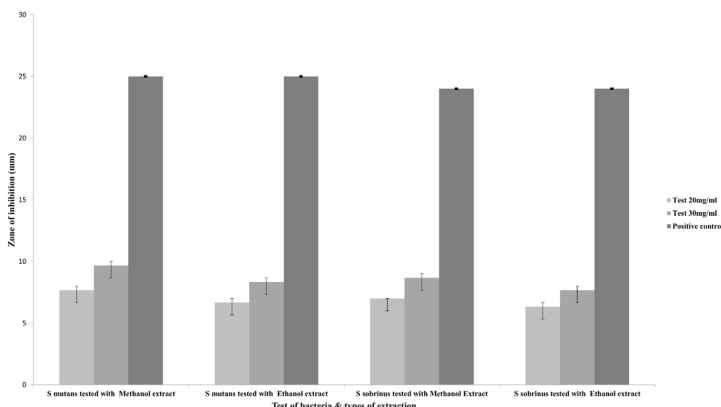


Figure 1: Inhibitory growth activity of methanol and ethanol extract of *Euphorbia tirucalli* against *S.mutans* and *S.sobrinus* using agar-well diffusion test.

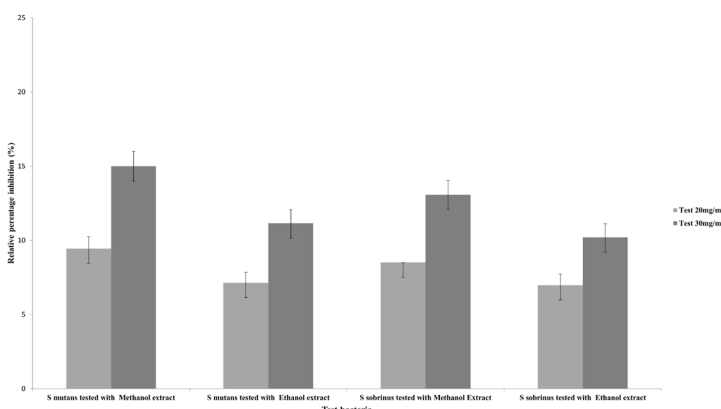


Figure 2: Relative percentage inhibition of methanol and ethanolic extracts of *Euphorbia tirucalli* stem compared to amoxicillin.

ethanolic extract of *Euphorbia tirucalli*. The aqueous extract of *Euphorbia tirucalli* did not show any inhibitory effect against *S. mutans* and *S. sobrinus* at all concentrations tested.

The antibacterial activity of *Euphorbia tirucalli* methanol, ethanol and aqueous extracts were compared with the positive control amoxicillin (standard drugs) for evaluating their relative percentage inhibition (Figure 2). *E. tirucalli* methanolic stem extract exhibited relative percentage inhibition

against *S. mutans* (9.44%) and *S.sobrinus* (8.51%) at 20 mg/ml while relative percentage inhibition against *S. mutans* (14.96%) and *S.sobrinus* (13.5%) at 30 mg/ml. While the ethanolic stem extract of *Euphorbia tirucalli* showed relative percentage inhibition against *S. mutans* (7.14%) and *S. sobrinus* (6.96%) while relative percentage inhibition at 30 mg/ml extraction concentration against *S. mutans* (11.10%) and *S.sobrinus* (10.21%).

## DISCUSSION

Recent interest in exploring the antimicrobial potential of wide variety natural source has encouraged more studies done in this area. This study was aimed to evaluate and compare the efficacy of different *Euphorbia tirucalli* stem extracts against oral pathogens implicated in dental caries.

Methanolic and ethanolic extracts, more polar extracts exhibited inhibitory effects against *S. mutans* and *S. sobrinus* at a concentration of 20 and 30 mg/ml. At a concentration of 10 mg/ml, none of the tested strains were sensitive. This finding showed that high amount of bioactive antimicrobial compounds in the *Euphorbia tirucalli* was extracted with these solvents. Our findings are in agreement with the previous study that most of the bioactive antimicrobial compounds were extracted with methanol (Parekh & Chanda 2007; Sugumar et al. 2010). A methanolic extract of *Euphorbia tirucalli* showed slightly higher inhibitory activity against the selected bacterial strains than that of ethanolic extract. In our study, aqueous extract of *Euphorbia tirucalli* did not exhibit any antimicrobial effect against both bacteria. Our findings are in agreement with the previous reports (Parekh & Chanda 2007; Jadhav et al. 2010). Organic solvents were more effective compared to aqueous extract. This may be due to dissolution of plant metabolites in organic solvents as compared to aqueous solvent (Ahmad et al. 2001).

The differences in the antimicrobial properties in different extracts of *Euphorbia tirucalli* may be due to the

biologically active phytochemical constituents. Some of the extracts may contain potential antibacterial constituents (Siddhartha et al. 2007). The presence of various phytochemicals such as alkaloids, tannins, polyphenol and triterpenes in *Euphorbia tirucalli* stems extracts which possess antimicrobial property may contribute to the formation of inhibition zone (Sugumar et al. 2010). Mechanism to fight bacteria was different, depending on the types of active compound. Alkaloid has been reported able to inhibit nucleic acid synthesis of bacteria, whereas the tannins able to give toxic to bacteria by increased their hydroxylation processes (Cushnie et al. 2014; Min et al. 2008). Another study found that polyphenol disturbs the growth of bacteria by inhibition of c-di-AMP that controls various functions in bacteria (Opoku-Temeng & Sintim 2016).

In this study, *Euphorbia tirucalli* extracts exhibited low percentage of inhibition as compared to control antibiotic at the tested concentrations, probably due to the differences in the bactericidal mechanism. While effective combinations between single natural products, with chemosynthetic or antibiotics have been reported, this phenomenon could also be applied to *Euphorbia tirucalli*. Although *Euphorbia tirucalli* extracts alone exhibited low antibacterial activity, drug synergism between antibiotics and bioactive plant extracts could be beneficial (synergistic or additive effects). On the other hands, cytotoxicity analysis of this compound need to be undertaken in order to expand its therapeutic indication so

that better understanding of their characteristics and potential can be gained. Furthermore, this experiment was conducted *in vitro*, which is considered a static system compared to *in vivo* tests, which may reflect the influence of various dynamic factors like systemic conditions, salivary flow, diet, and dental anatomy (Castro et al. 2000).

To the best of our knowledge, this is the first study to investigate the antibacterial activity of *Euphorbia tirucalli* stems extracts on oral bacteria. This *in vitro* study shows that *Euphorbia tirucalli* stems extracts have effect as an antimicrobial agent and implied that there are potential usages of this extract in the mouthwash and toothpaste.

## CONCLUSION

In conclusion, methanolic and ethanolic stem extracts of *Euphorbia tirucalli* possessed antibacterial activity against *S. mutans* and *S. sobrinus*. Further phytochemical and pharmacological studies are required to identify the active principles of the extracts and their mechanism of actions.

## ACKNOWLEDGEMENT

The authors wish to thank the staff of the Craniofacial Science Laboratory, School of Dental Sciences for their technical assistance and facilities provided. The authors declare that none of them has any conflict of interest in this study. The study was supported by Universiti Sains Malaysia Short Term Grant (304/PPSG/61312115).

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Received: 29 June 2016

Accepted: 11 January 2017