

Anticariogenic Properties of *Solanum ferox* L. Ethanol Extract

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Ann Dent UM 2019, 26:22-29
DOI: 10.22452/adum.vol26no4

KEYWORDS

Antibiofilm, Anticariogenic, *Solanum ferox* L., *Streptococcus pyogenes*, *Staphylococcus aureus*

ABSTRACT

Solanum ferox L. is a plant species which belongs to the Solanaceae family and the genus Solanum. The Solanum genus was found to exhibit anticariogenic activity and was traditionally used to treat oral diseases. However, there is no scientific study done specifically for *Solanum ferox* L. Hence the aim of the study is to determine the anticariogenic properties of flesh and leaf of ethanolic extract of *Solanum ferox* L. Alkaloids, flavonoids and tannins were detected in the leaf ethanolic extract via preliminary phytochemical screening. The presence of these phytochemicals may contribute to the anticariogenic activity. Treatment with different concentrations of flesh and leaf of ethanolic extract were used against *Streptococcus pyogenes* and *Staphylococcus aureus* via the method of agar well diffusion to indicate zones of inhibition. The antibiofilm activity of the flesh and leaf ethanolic extracts was tested. The flesh and leaf ethanolic extracts possess antimicrobial activity dose-dependently and positive antibiofilm activity against respective pathogens. The flesh ethanolic extract has stronger anticariogenic activity compared to leaf ethanolic extract against respective pathogens. *Streptococcus pyogenes* exhibited higher susceptibility as compared to *Staphylococcus aureus*. In conclusion, it has been shown that the ethanolic extract of *Solanum ferox* L. exhibit anticariogenic properties against *Streptococcus pyogenes* and *Staphylococcus aureus*.

INTRODUCTION

Anticariogenic is a term used to describe foods, chemicals, or other agents that tend to contribute favorably to dental health by remineralizing teeth and discouraging the acid that causes dental caries [1]. There are some medicinal plants that show antimicrobial and anticariogenic effects [2]. Genus Solanum belongs to the family Solanaceae and comprises more than 1700 species, found in the tropical and temperate zones. *Solanum ferox* L. is a herb plant that can grow up to 2.5m, has white flowers and fruits with colours ranging from green, yellow to purplish-yellow according to their maturity (Figure 1).

Solanum ferox L. is locally known as terung asam, terung dayak or tiyung daya' [3]. The genus

Solanum has been claimed to traditionally treat oral disease. The fruit of *Solanum elaeagnifolium* D., *Solanum nigrum* L., *Solanum surateense* L., *Solanum virgatum* L., *Solanum xanthocarpum*, as well as seeds of *Solanum khasianum* and *Solanum torvum* are claimed to have oral medicinal properties traditionally used by tribes in India [4]. *Solanum surateense* is also found to have antimicrobial activity [2]. The leaf extract of *Solanum nigrum* L. and *Solanum myriacanthum* Dunal contain polyphenol which give them the antimicrobial and anticariogenic effects [5]. Polyphenols such as flavonoid and tannins contributed to the anticariogenic activity by inhibiting the oral bacterial growth or the glucotransylferase activity [6,7].

The present study was aimed to determine the anticariogenic properties of ethanol extract from flesh and leaf of *Solanum ferox* L. against 2 oral pathogens including gram positive bacteria

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Streptococcus pyogenes (*S. pyogenes*) and *Staphylococcus aureus* (*S. aureus*). Biofilm-associated diseases caused by gram-positive bacteria including caries, gingivitis, periodontitis, endocarditis and prostatitis. Coral-associated bacteria (CAB) has antibiofilm activity against biofilm formed by *S. pyogenes* [8]. *S. aureus* is also a putative pathogen involved in many oral diseases such as oral mucositis, periodontitis, peri-implantitis, endodontic infections and even dental caries [9]. *S. aureus* was involved in giving the yellow pigmentation in teeth that is considered pathognomonic of dental caries [10].



Figure 1. Shrub of *Solanum ferox* L.

MATERIALS AND METHODS

Preparation of ethanol extract

The fruit and leaf of *Solanum ferox* L. used in this study is originated from Bau, Sarawak (Figure 1). The crude ethanol extract preparation was prepared according to the method of Fazil and Ali (2014) [11] with slight modification. Initially, the sliced flesh and leaf of *Solanum ferox* L. were dried at 45°C. Following that, the samples were grounded into fine particles. The samples were macerated in 95% of undenatured ethanol with the ratio of weight to volume of 1 to 10. The samples were filtered and concentrated using rotary evaporator, 40°C. The crude was stored at 4°C for further use. The percentages of yield for crude extracts were also determined.

Preliminary phytochemical screening

The crude extracts were subjected to preliminary phytochemical screening for detection of alkaloids, flavonoids, tannins and saponins.

Test for Alkaloids

Crude extract (0.5 g) was diluted with 10 ml of 10% (v/v) acetic acid in ethanol, boiled and filtered while hot. Two ml of 10% (v/v) dilute ammonia and 5 ml of chloroform was added to 5 ml of filtrate. The filtrate was shaken gently to extract the alkaloid base. The chloroform layer was extracted with 5% of HCl. The filtrate was treated with a few drops of Mayer's reagent. Formation of white precipitates indicated the presence of alkaloids [12,13].

Test for Flavonoids

Crude extract (1g) was added with 5 ml ethanol, boiled and filtered. A few drops of concentrated HCl and magnesium tape ribbon (1-2 cm) was added. Colours ranging from orange to red indicated flavones, red to crimson indicated flavonols and crimson to magenta indicated flavonones [14].

Test for Saponins

Crude extract (1g) was boiled in 10 ml of distilled water in a water bath and filtered. The filtrate was shaken vigorously (1-2 min) for a stable and persistent froth (for 15 min) to determine of saponins [12,13,15].

Test for Tannins

Crude extract (0.5 g) was boiled in 20 ml of water and then filtered. A few drops of 2 ml of 10% ferric chloride were added [16]. An intense blue black colour was taken as an evidence for the presence of hydrolysable tannins, while brownish green indicated that of condensed tannins [12,13,15].

Antimicrobial Assay

Agar Well Diffusion Method

S. pyogenes (ATCC 700698) and *S. aureus* (ATCC 19615) were grown on blood agar and Mueller-Hinton agar (MHA) respectively. Bacterial culture was spread on the agar plates. Well of 7 mm diameter was punched off onto agar medium. The wells were filled with respective treatment against *S. pyogenes* or *S. aureus*. Experimental design was expressed as in Table 1. Concentration of 320, 640, 1280, 256 mg/ml against *S. aureus* was chosen according to the ineffective results with the used of initial dosage range 20, 40, 80, 160 mg/ml. The plates were incubated at 37°C for 24hrs until appearances of zone of inhibition [2]. The experiment was conducted in triplicate. ANOVA was performed for significance determination comparing to positive control. A significant difference was considered as $p < 0.05$.

Data were expressed as means \pm standard error of the mean (SEM). Turkey's multiple comparison test was performed to determine significant differences between positive control and treatment group.

Table 1. Experimental design of antimicrobial and antibiofilm assay with the treatment of flesh or leaf of *Solanum ferox L.* against *S. pyogenes* or *S. aureus*.

Treatment group	Dosage diluted with 2% DMSO (mg/ml)			
Control	Normal saline			
Against <i>S. pyogenes</i>	20	40	80	160
Against <i>S. aureus</i>	320	640	1280	2560
Ampicillin	100			

Antibiofilm assay

Tube Method

An assessment of biofilm formation was determined by tube method with slight modification [17]. The test tube with normal saline was inoculated with 100 μ l of *S. pyogenes* or *S. aureus* from overnight culture plates and added with respective treatment. Experimental design was expressed as in Table 1. The test tubes were incubated for 24hrs at 37°C. The test tubes were decanted and washed with PBS (pH 7.3) and dried tubes were then stained with crystal violet. Excess stain was removed and the tubes were washed with deionized water. The tubes were then dried in inverted position and observed for biofilm formation. Biofilm formation was considered positive or exist when there is a visible film on wall and bottom of the tube. The experiment was done thrice. A well-functioning anticariogenic should not produce biofilm.

RESULTS

Percentage of yield

The yield percentage for flesh and leaf crude extracts were 23.58% and 41.93% respectively with the initial weight of 40 mg.

Phytochemical Screening

The results for phytochemical screening of ethanol extracts of flesh and leaf of *Solanum ferox*

L. are shown on Table 2. Alkaloids were detected with weak precipitation and strong precipitation for flesh and leaf of *Solanum ferox L.*, respectively. Flavonoids were detected with red to crimson color (probably flavonols) for both flesh and leaf of *Solanum ferox L.* Tannins were detected with a brown-green color (condensed tannins) for both flesh and leaf of *Solanum ferox L.* Saponin was not detected in the crude extracts.

Effect of flesh ethanolic extract of *Solanum ferox L.* against *S. pyogenes*

Antimicrobial activity is presented in Figure 2(a). It illustrated by bar chart of Figure 2(b). Antibiofilm assay is presented in Figure 2(c). The flesh of ethanolic extract possess antimicrobial activity against *S. pyogenes* significantly ($p < 0.05$) from dosage of 40mg/ml to 160mg/ml compared to positive control, 100 mg/ml ampicillin. None of the dosage possess the same antimicrobial activity compare to ampicillin. The zone of inhibition value of flesh ethanolic extracts increased significantly ($p < 0.05$) in a dose dependent-manner. No biofilm formation was detected in the test tubes treated with dosage of 40, 80 and 160 mg/ml of flesh ethanolic extract. Hence, antibiofilm properties were exhibited against *S. pyogenes* at a dosage starting from 40 mg/ml.

Effect of leaf ethanolic extract of *Solanum ferox L.* against *S. pyogenes*

Antimicrobial activity is presented in Table 5 and Figure 3(a) respectively. It illustrated by bar chart of Figure 3(b). Antibiofilm assay is presented in Figure 3(c). The leaf ethanolic extract possess antimicrobial activity against *S. pyogenes* significantly ($p < 0.05$) from dosage of 80mg/ml to 160mg/ml compared to positive control, 100mg/ml ampicillin. None of the dosage possessed the same antimicrobial activity compare to ampicillin. The zone of inhibition value of leaf ethanolic extracts increased significantly ($p < 0.05$) in a dose dependent-manner. No biofilm formation was detected in the test tubes treated with dosage of 80 and 160 mg/ml of leaf ethanolic extract. Hence, antibiofilm properties were exhibited against *S. pyogenes* at a dosage starting from 80 mg/ml.

Table 2. Phytochemical constituents of flesh and leaf of *Solanum ferox* L. ethanolic extract.

Constituents	Flesh	Leaf
Alkaloids	++	+++
Flavonoids	++	++
Tannins	+	+
Saponins	-	-

Note:Alkaloids: + negligible amount of precipitation; ++ weak precipitate; +++ strong precipitate
 Flavonoids, tannins: + weak colour; ++ mild colour; +++ strong colour
 Saponins: + 1-2cm froth; ++ 2-3cm froth; +++ >3cm froth

Effect of flesh ethanolic extract of *Solanum ferox* L. against *S. aureus*

Antimicrobial activity is presented in Figure 4(a). It illustrated by bar chart of Figure 4(b). Antibiofilm assay is presented in Figure 4(c). The flesh ethanolic extract possess antimicrobial activity against *S. aureus* significantly ($p < 0.05$) from 1280 mg/ml to 2560 mg/ml dose compared to positive control, 100 mg/ml ampicillin. None of the dosage possess the same antimicrobial activity compare to ampicillin. The zone of inhibition value of ethanolic extracts increased significantly ($p < 0.05$) in a dose dependent manner.

No biofilm was formation detected in test tubes treated with dosage of 1280 mg/ml and 2560 mg/ml of flesh ethanolic extract. Hence,

antibiofilm properties were exhibited against *S. aureus* at a dose starting from 1280 mg/ml

Effect of leaf ethanolic extract of *Solanum ferox* L. against *S. aureus*

Antimicrobial activity is presented in Figure 5(a). It illustrated by bar chart of Figure 5(b). Antibiofilm assay is presented in Figure 5(c). The leaf ethanolic extract possess antimicrobial activity against *S. aureus* significantly ($p < 0.05$) from dosage of 1280 mg/ml to 2560 mg/ml compared to positive control, 100mg/ml ampicillin. None of the dosage possess the same antimicrobial activity compare to ampicillin. The zone of inhibition value of ethanolic extracts increased significantly ($p < 0.05$) in a dose dependent manner. No biofilm formation was detected in the test tubes treated with dosage of 2560 mg/ml leaf ethanolic extract. Hence, antibiofilm properties were exhibited against *S. aureus* at the dosage of 2560 mg/ml.

DISCUSSION

Preliminary phytochemical screening of the ethanol extract of flesh and leaf of *Solanum ferox* L. showed the presence of bioactive compounds alkaloids, flavonoids and tannins. This is consistent with the findings of Parashurama, Parinitha, Mallikarjunaswamy, and Shivanna (2013) [18] and Esteves-Souza et al. (2002) [19] which found the bioactive compound alkaloids and flavonoids in the genus *Solanum*. According to

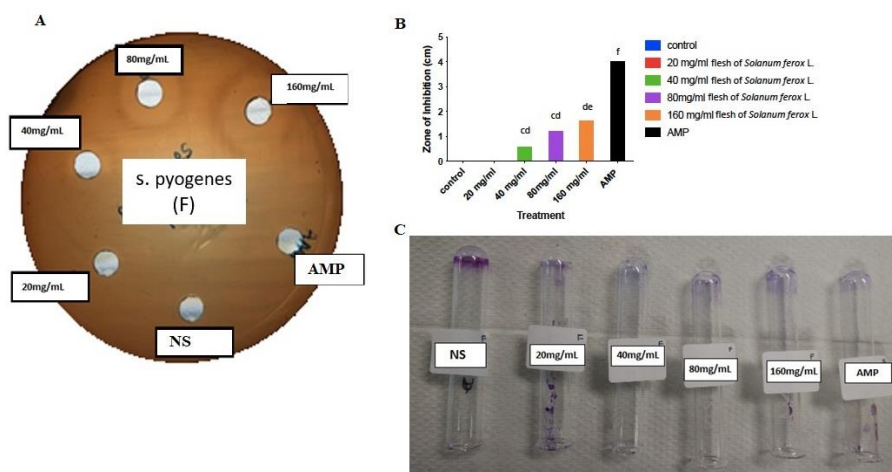


Figure 2. (a) Zone of inhibition (cm) of flesh ethanolic extract of *Solanum ferox* L. against *S. pyogenes*. **(b).** Zone of inhibition (cm) in presence of flesh ethanolic extracts of *Solanum ferox* L. against *S. pyogenes*. **Note:** Values presented as mean \pm S.E.M of minimum 3 repetitions per group cdef comparison of means between column significant at $p < 0.05$. **(c).** Antibiofilm assay of flesh ethanolic extract of *Solanum ferox* L. against *S. pyogenes*

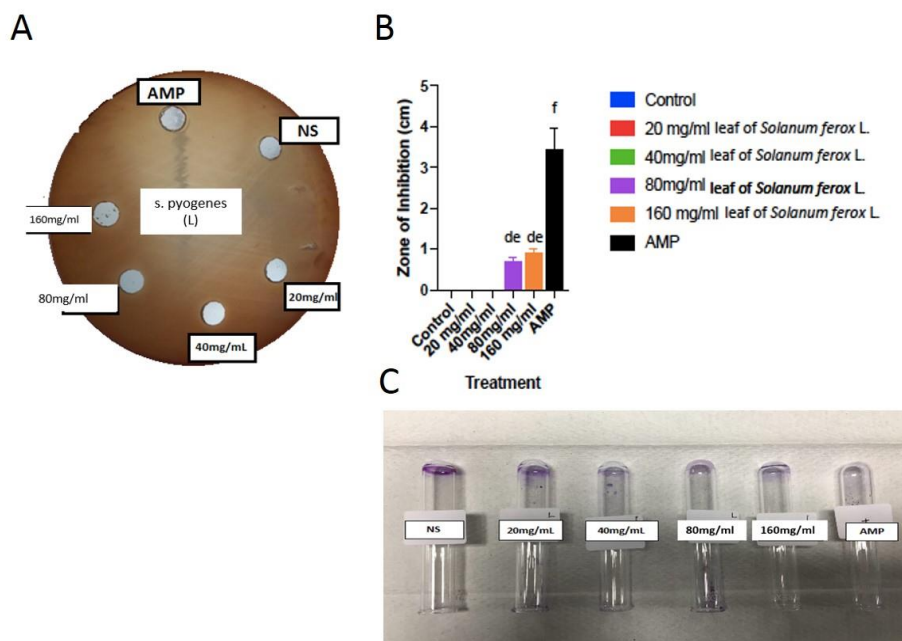


Figure 3.(a) Zone of inhibition (cm) of leaf ethanolic extract of *Solanum ferox* L. against *S. pyogenes* (b) Zone of inhibition (cm) in presence of leaf ethanolic extracts of *Solanum ferox* L. against *S. pyogenes*. **Note:** Values presented as mean \pm S.E.M of minimum 3 repetitions per group def comparison of means between column significant at $p < 0.05$) (c) Antibiofilm assay of leaf ethanolic extract of *Solanum ferox* L. against *S. pyogenes* (Note: Values presented as mean \pm S.E.M of minimum 3 repetitions per group def comparison of means between column significant at $p < 0.05$)

Hu, Takahashi, and Yamada (2000) [20], alkaloids possess bactericidal activity against oral bacteria. Flavonoids are known to contribute to the anticariogenic activity by inhibiting the oral bacterial growth or the glucotransylferase activity [6,7].

Tannins also known to exhibit cytotoxic effects and are effective in treating swelling as well as hemorrhage of the oral mucosa [21]. Higher polyphenol content will exhibit more anticariogenic properties and are more effective

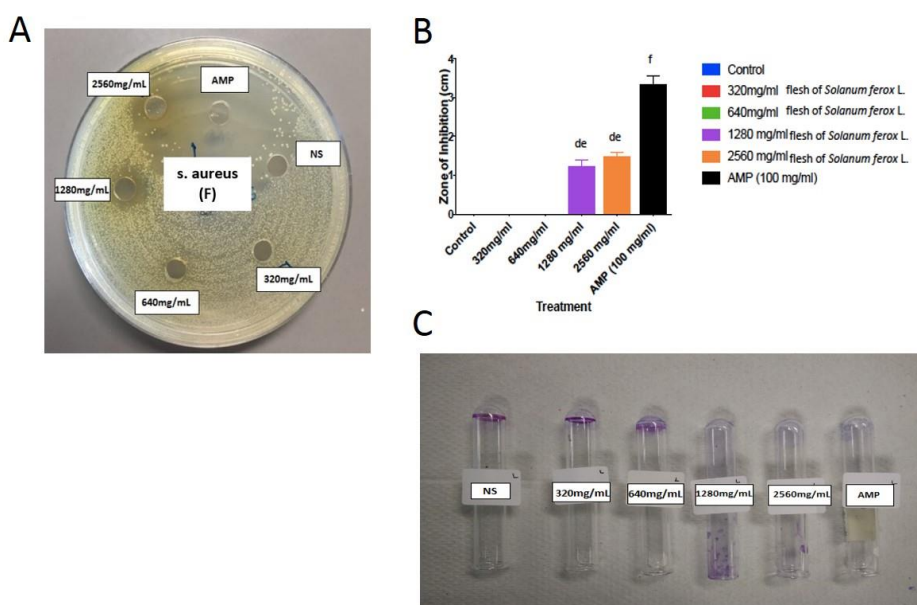


Figure 4. (a) Zone of inhibition (cm) of flesh ethanolic extract of *Solanum ferox* L. against *S. aureus*. (b) Zone of inhibition (cm) of flesh ethanolic extract of *Solanum ferox* L. against *S. aureus* (Note: Values presented as mean \pm S.E.M of minimum 3 repetitions per group def comparison of means between column significant at $p < 0.05$) (c). Antibiofilm assay of flesh ethanolic extract of *Solanum ferox* L. against *S. aureus*.

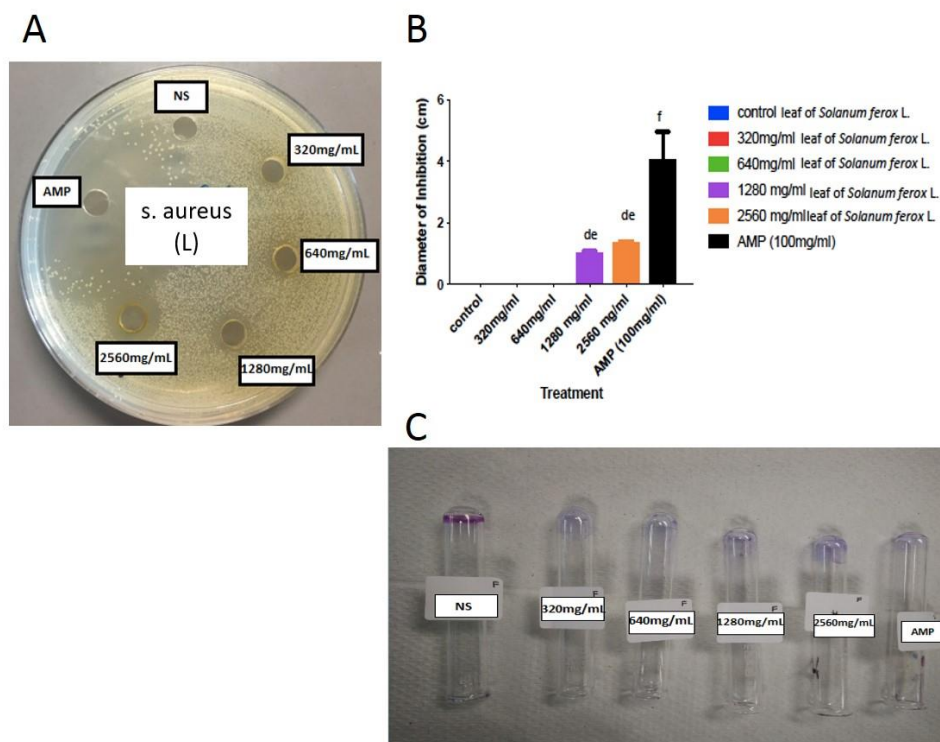


Figure 5. (a) Zone of inhibition (cm) of leaf ethanolic extract of *Solanum ferox* L. against *S. aureus*. **(b)** Zone of inhibition (cm) in presence of leaf ethanolic extracts of *Solanum ferox* L. against *S. aureus*. **Note:** Values presented as mean \pm S.E.M of minimum 3 repetitions per group def comparison of means between column significant at $p < 0.05$). **(c)** Antibiofilm assay of leaf ethanolic extract of *Solanum ferox* L. against *S. aureus*

in reducing the incidence of dental caries [22]. According to Xu et al. (2014) [23], polyphenols has the ability to eradicate *S. aureus* biofilm by causing cell lysis and therefore compromise the integrity of the bacterial membrane. In summary, the anticariogenic properties of the flesh and leaf ethanolic extracts are more likely due to the presence of alkaloids, flavonoids and tannins either singly or in combination.

According to the results pattern against *S. pyogenes*, flesh ethanolic extract has stronger antimicrobial activity (starting dosage of 40 mg/ml) compared to leaf ethanolic extract (dosage required is 80 mg/ml). Antibiofilm activity is stronger in flesh ethanolic extract (required low dosage level which is 40 mg/ml) compared to leaf ethanolic extract (required higher dosage level which is 80 mg/ml). Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) are recommended for further investigation to quantifying the concentration of active compounds which may contribute to the differentiation of dosage level required to possess significant anticariogenic activity by different part of the plant.

According to the results pattern against *S. aureus*, flesh and leaf ethanolic extracts possessed equal

level of antimicrobial activity with starting dosage of 1280 mg/ml to inhibit zone significantly ($p < 0.05$) in comparison to positive control, 100 mg/ml ampicillin and starting dosage of 1280 mg/ml and 2560 mg/ml respectively required to possess antibiofilm activity. According to the summary of results above, *S. aureus* needs higher concentration to possess anticariogenic activity in comparison to *S. pyogenes*. The respective results pattern might be due to high resistance value of *S. aureus* than *S. pyogenes* with the treatment of fresh and leaf ethanolic extracts.

CONCLUSIONS

Based on the results of the preliminary phytochemical screening, the presence of alkaloids, flavonoids and tannins detected in flesh and leaf of *Solanum ferox* L. might contribute to its anticariogenic activities. Flesh and leaf of ethanol extracts exhibit the significant anticariogenic properties against *S. pyogenes* and *S. aureus* by observing antimicrobial and antibiofilm activity. *S. pyogenes* seems to be more susceptible to the flesh and leaf ethanolic extract of *Solanum ferox* L. at a range of dose of 40-160mg/ml compared to *S. aureus* at a range of

dose of 1280-2560mg/ml. The plant extracts of *Solanum ferox* L. could therefore be used as a potential source of useful treatment in medicine.

ACKNOWLEDGEMENTS

The authors would like to thank the Sarawak Biodiversity Centre, Institute of Borneo Studies, UNIMAS, the Department of Agriculture Sarawak

and the Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak for the support.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible with the content of this article.

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