Antibacterial properties of parasitic mistletoe - *Scurrula ferruginea* (Jack) Danser of Brunei Darussalam

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**Abstract.** Natural products especially from plants have become subject of much interest in drug discovery. *Scurrula ferruginea* is parasitic mistletoe used traditionally to treat various illnesses. The aim of this study is to investigate the potential antibacterial effect of *Scurrula ferruginea* native to Brunei Darussalam. The plants were collected from Brunei-Muara district air-dried, pulverized into powder, extracted by water maceration and freeze dried. Later, the extract was screened by disc-diffusion assay for two bacterial strains. The positive controls were Gentamycin and Ampicillin for *Staphylococcus aureus* while sulphamethoxazole was used for *Escherichia coli*. Dimethyl sulfoxide was the negative control for both strains. 100, 200 and 500 mg/ml of the extract were used for the assays. The extracts did not show any inhibition activity against *Escherichia coli*, while sulphamethoxazole showed inhibition of 24 mm. Ampicillin, Gentamycin, 100 and 500 mg/ml extract exhibited 29, 20, 8.3 and 10.7 mm inhibition zone, respectively against *Staphylococcus aureus*. There was an increase in zone of inhibition on *Staphylococcus aureus* strain with increase in extract concentration. Further investigations including Minimum Inhibition Concentration and Minimum Bactericidal Concentration methods as well as investigation on additional bacterial strains will be conducted for confirmation of antibacterial effect of the extract.

1. Introduction

Antibiotics have enormously helped in treating infectious diseases caused by bacteria and consequently aid the quality of health of the infected individual. However, these benefits are under threat because many of the common antibiotics were known to produce adverse reactions as well as the increase in emergence of the multiple drug resistance strains in microorganisms [1]. This calls for development of new drugs that produces lesser side effect and resistance.

There has been an increase of interest on developing drugs from natural product especially from traditional medicinal plants as they are recognized as important sources of therapeutically active compounds. Hence, research on medicinal plants is becoming more popular especially for the identification and isolation of bioactive compounds from natural sources. [2]

*Scurrula ferruginea* is parasitic mistletoe plant that comes under *Scurrula* genus from Loranthaceae family and is believed to have healing properties such as anticancer, antimicrobial, antioxidant as well as antihypertensive activities. [3]

Ameer *et al.* research group verified the effect of active hydrophilic extract of *Scurrula ferruginea* plant in lowering blood pressure and treating gastrointestinal ailments by experiments based on pharmacological investigations. [4]
However, antibacterial study on this plant is still in its early stage. Hence, the aim of this study is to investigate the potential antibacterial effect of *Scurrula ferruginea* plant native to Brunei Darussalam.

2. Materials and methods

2.1. Plant materials
The plant *Scurrula ferruginea* was freshly collected from the host plant, *Tabeula palleida* from Bandar Seri Begawan, Brunei Darussalam (Latitude N 4°54’57.0”, Longitude E 114°56’53.8”). The plant material was taxonomically identified and deposited in the Universiti Brunei Darussalam Herbarium.

2.2. Preparation of aqueous extract
The leaves were cleaned thoroughly with distilled water, dried with paper towel and dried under the shade (30 ± 5°C). Then, the dried leaves were pulverized into powder and stored in an airtight container until further use. Aqueous extract was prepared by maceration of the powdered leaves (20 grams) with distilled water (100 ml) and sonicated for two hours. The extract was filtered using Whatman filter paper and stored overnight in -80°C. On the following day, the extract was freeze dried using lyophiliser. The percentage yield was 3.9%. Finally, the extracts were stored in -80°C until the next experiment. Three different concentrations of aqueous extract (100, 200 and 500 mg/ml) were investigated in this study.

2.3. Growth and maintenance of Bacterial strains
The antibacterial effect of the aqueous extract was investigated on ATCC 25923 *Staphylococcus aureus* (gram positive) strain and clinical strain of *Escherichia coli* (gram negative). Both of the strains were cultured in nutrient broth and the bacterial isolates were preserved on Mueller Hinton agar at 4°C until further analysis.

2.4. Preliminary Screening: Disc diffusion method
Disc diffusion assay was carried out as a preliminary screening for antibacterial effect of the aqueous extract. Agar plates, prepared broth and other utensils were autoclaved to sterilize them prior to the test. First, the bacterial stock suspension was removed from -80°C freezer and thawed at room temperature. Inoculation from the bacterial suspension was done on a Mueller Hinton agar and incubated at 37°C overnight in the inverted position. This procedure was done to ensure the bacteria was still alive and was not contaminated by any other organisms. After 18 – 24 hours, a few colonies of the bacterial growth were cultured in nutrient broth and the bacterial isolates were preserved on Mueller Hinton agar at 4°C until further analysis.

2.5. Statistical analysis
All the data obtained were presented as the mean ± standard deviation. One-way ANOVA test was done and subjected to Scheffe post hoc test where values p < 0.05 was considered as significant. All statistical analysis was processed using SPSS, version 21, IBM Corporation, NY, USA.
3. Results

Table 1. Anti bacterial effect of aqueous extract of *Scurrula ferruginea* leaves on *Staphylococcus aureus* and *Escherichia coli*

| Bacterial strains         | Zone of inhibition (mm) | Mean ± standard deviation |
|---------------------------|-------------------------|---------------------------|
|                           | DMSO 10 μg              | Amp 10 μg                 | Gen 25 μg | SXT 500 | 200 | 100 | *Scurrula ferruginea* Aqueous Extract (mg/ml) |
| *Staphylococcus aureus*   | 0 ± 29.33 ±             | 20 ± 1.41                | NT        | 10.67 ± | 9.33 ± | 8.33 ± |
| ATCC 25923                | 0.00                    | 3.79                     | 1.41 NT   | 0.58*** | 0.58*** | 0.58*** |
| *Escherichia coli*        | 0 ± 24.0 ±              | 1 ± 0.00                 | 0 ± 0.00  | 0 ± 0.00 |
|                           | 0.00                    | 1.00                     | 0 ± 0.00  | 0 ± 0.00 |

Values are represented as mean of inhibition (mm) ± standard deviation of triplicates; NT = Not Tested; DMSO = Dimethyl Sulfoxide; Amp = Ampicillin; Gen = Gentamycin; SXT = Sulphamethaxazole * = P <0.05; ** = P < 0.01 and *** = P < 0.001 significantly lower when compared with Ampicillin.

Table 1 shows the results of the antibacterial effect of aqueous extract of *Scurrula ferruginea* leaves on *Staphylococcus aureus* and *Escherichia coli*. Dimethyl Sulfoxide was used as the negative control for both strains which did not exhibit any zone of inhibition. Ampicillin and Gentamycin (10 μg each) were used as positive control and exhibited 29.33 ± 3.79 and 20 ± 1.41 mm zone of inhibition respectively against *Staphylococcus aureus*. Additionally, 25 μg of Sulphamethaxazole was used as positive control and exhibited 24.0 mm zone of inhibition against *Escherichia coli*. The concentrations of the aqueous extract tested were 100, 200 and 500 mg/ml. The aqueous extracts suggest minimal antibacterial action against *Staphylococcus aureus* because the zones of inhibitions were significantly lower when compared with Ampicillin (P < 0.001) and Gentamycin (P < 0.01). Nonetheless, the study has shown that when the aqueous extract was tested against *Staphylococcus aureus* strain, the zone of inhibition increases from 8.33 to 10.67 mm as the concentration of aqueous extract increase from 100 mg/ml to 500 mg/ml. However, the aqueous extract did not show any inhibition activity against *Escherichia coli*.

4. Discussion

There has been an increase in reports of antimicrobial research on medicinal plants from different parts of the world. About 80% of the world population used plant extract or their active compounds as traditional medicine as estimated by the World Health Organization. [5] Few studies particularly on antibacterial activity has been done on mistletoe *Scurrula ferruginea* which belongs to Loranthaceae family. Marvibaigi et al., investigated the antibacterial study of different parts such as leaf, stem and flower of *Scurrula ferruginea* found in Malaysia against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas putida* and *Bacillus subtilis*. The difference is that the plant materials were macerated using Acetone and water (8:2 v/v) although the specific concentration of extracts being used were not mentioned. The study demonstrated extract from stem possessed highest antibacterial activity on *Pseudomonas putida*. The other crude extracts also capable of inhibiting the growth of *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* at minimal effect. [6]

*Staphylococcus aureus* belongs to gram positive bacteria whereas *Escherichia coli* to gram negative bacteria. The main difference between gram positive and negative bacteria is the existing peptidoglycan cell wall in gram positive bacteria and unique outer membrane in gram negative bacteria. [7]
In this study, aqueous extract of leaves of *Scurrula ferruginea* was found to possess antibacterial effect on *Staphylococcus aureus*. However, the aqueous extract did not show any antibacterial effect on *Escherichia coli*. As a preliminary explanation, this could be because of the presence of the phytochemical constituents present in the aqueous extract of *Scurrula ferruginea* leaves which may inhibit only the growth of gram positive bacteria instead of gram negative bacteria by targeting the peptidoglycan cell wall.

Quorum sensing is a system of cell-to-cell communication between bacteria involved in regulation of genes expression of disease causing traits, such as motility, biofilm information, sporulation, and expression of many virulence determinants. The signalling molecules known as autoinducers involved in quorum sensing found in gram positive and gram negative bacteria are different. For instance, gram negative bacteria use N-acyl homoserine lactones (AHLs) while gram positive use the post-translationally modified oligopeptides as signalling molecules. [8] Numerous researches have published the ability of plant extracts and phytochemicals to interfere the quorum sensing system. The difference of signalling molecules involved between gram positive and gram negative bacteria could be one of the reasons why the aqueous extract of *Scurrula ferruginea* only inhibit the gram positive bacteria. The most common inhibition mechanisms are due to the similarity of their chemical structure to the signalling molecules or the ability to degrade the signal receptors[8]. However, further in vivo studies need to be done to validate if the results are truly based on quorum sensing inhibition.

Moreover, another factor that is often reported is the higher resistance of gram negative bacteria to the plant- based extracts and essential oils. This is because gram negative bacteria have hydrophilic cell wall structure which constitutes mainly of lipo-polysaccharides (LPS). The LPS structure will prevent any hydrophobic agent from penetrating and accumulating in the target cell membrane of the bacteria. [9] Hence, this could be another possible factor for inhibition of gram positive bacteria only by the aqueous extract of *Scurrula ferruginea*.

Further studies which include investigation of methanolic extract of *Scurrula ferruginea* extracted using Soxhlet apparatus is still ongoing in order to validate the overall potencies of antibacterial effect of *Scurrula ferruginea* and the outcome of the studies will be published at a future date when the results are available.

As reported previously, the phytochemical compounds that have been isolated so far from *Scurrula ferruginea* were quercitrin, quercetin and 4'-O-acetylquercitrin which are common flavanols. [10] Further in 2010, Terpenoids were discovered by Ameer et al. [11]. Several molecules of the secondary metabolites from phytochemical constituents such as alkaloids, flavonoids, saponins and tannins were found to be active against pathogenic microorganisms. [12]

Moreover, many researchers have consistently mentioned inhibition potential of these metabolites which significantly reduce the growth of bacteria; thus, flavonoids and terpenoids have been found to be an effective antimicrobial substance against various microorganisms. [13]

This study has also exhibited the proportionate increase of the zone of inhibition with increase in the concentration of the aqueous extract. This could also mean a higher concentration of extract contains more of the active phytochemical compounds therefore displaying larger zone of inhibition.

In another study, Tripathi et al., investigated antibacterial effect of *Scurrula atropurpurea* found in South West Bengal which comes from the same *Scurrula* genus against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Vibrio Cholerae*. They have prepared two types of macerated extracts namely, 70% crude methanolic extract and aqueous extract. The study demonstrated the effectiveness of crude methanolic extract possessing antibacterial effect against all of the bacterial strains mentioned previously. However the aqueous extract did not exhibit antibacterial effect against any of the bacterial strains including *Escherichia coli*. [14] From their study, it is possible that aqueous extract from *Scurrula* genus does not have any antibacterial effect on *Escherichia coli*. However, to confirm this statement, further investigation needs to be done specifically on the other *Scurrula* genus.
It could be also the aqueous extract of *Scurrula ferruginea* does not have any antibacterial effect on gram negative bacteria. This also needs to be confirmed by screening more bacterial strains especially on gram negative bacteria.

5. Conclusion

This is the first study reported on investigation of antibacterial effect of aqueous extract from *Scurrula ferruginea* leaves found in Brunei against *Staphylococcus aureus* and *Escherichia coli*. In summary, this study has shown that aqueous extract of *Scurrula ferruginea* leaves found in Brunei has a potential of antibacterial effect on *Staphylococcus aureus* ATCC 25923 strains but shows no inhibition against *Escherichia coli*. Further investigation which includes Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) has been initiated to confirm the antibacterial effect. In addition, investigation of *Scurrula ferruginea* against more bacterial strains with different types of extracts will also be preferred.

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