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# Effect of Antimicrobial Activities on the Various Solvents Extracts of Leaves of *Scurrula Ferruginea* (Jack) Danser (Loranthaceae)

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## ABSTRACT

*Scurrula ferruginea* is widely distributed in Southeast Asian countries and has commonly been used as a medicinal plant to treat many diseases caused by microbes. This study was conducted to evaluate the effect of using various solvents extractions on *S. ferruginea* leaves and their antimicrobial activities. Oven dried (60°C) leaves of *S. ferruginea* were extracted with aqueous and organic solvents. Antimicrobial activities of the extracts were tested against *Staphylococcus aureus* S261, *Escherichia coli* E57, *Candida albicans* C205 and *Trichophyton rubrum* T62 using Disc Diffusion Method, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) respectively. The ideal solvent was 80% methanol with values of the zone of inhibition ranging from 7.98 to 9.71 mm and 450 to 900µg/mL (MIC and MBC) for *S. aureus* and *E. coli*, respectively. The present findings revealed that the leaves of *S. ferruginea* have inhibitory effects on several pathogenic microbes and can be suggested as a potential source of natural antimicrobial compounds.

*Keywords:* MBC, MFC, MIC, Mistletoes, *Scurrula ferruginea*

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## INTRODUCTION

The complexity of plant phytochemical due to the various chemical structures and properties in different polarity, makes it an unorthodox solvent for extracting bioactive compound for each plant species (Khoddami, Wilkes, & Roberts, 2013). Universal solvent extraction has been used to investigate the best solvent for extracting

healing properties in plant materials. Traditionally, the aqueous solvent was used in ethnobotany practices and is found to be the most polar solvent. Meanwhile, organic solvents and their combination with water are considered modern and widely accepted as a result of positive outcomes in extracting more bioactive metabolites (Garcia-salas, Morales-soto, Segura-carretero, & Fernandez-gutierrez, 2010). The use of varying polarity solvents is crucial for a systematic approach in obtaining the finest solvent that shows optimum antimicrobial activity.

Medicinal plants that have been used since ancient times have therapeutic properties for numerous human diseases caused by pathogens. Bioactive compounds comprise phenolic compounds, alkaloids, flavonoids and tannins are notable sources of anti-infective agents that contribute to human health (Hassan, Sirat, Yagi, Koko, & Abdelwahab, 2011; Farjana, Zerin, & Kabir, 2014). Currently, several studies have shown that natural products possess more than one biological effects such as antioxidant and antimicrobial (Ahmad, Anwar, Hameed, & Boyce, 2011; Bano, Girmay, & Tan, 2012; Ghasemzadeh, Jaafar, Rahmat, & Ashkani, 2015; Norziah, Fezea, Bhat & Ahmad, 2015; Syukriah, Liza, Harisun, & Fadzillah, 2014). These make medicinal plants interesting sources of natural antimicrobial properties as they give numerous beneficial effects to human well-being.

Nowadays, antimicrobial resistance is rapidly increasing and has become a worldwide concern (Oldfield & Feng, 2014). This has prompted a deep awareness on the need for exploration of novel antimicrobial compounds from medicinal plants for alternative antimicrobial as it does not offer negative effects to human health like the way antibiotics do. O'Neill, (2016) reported that an approximate of 10 million lives will be lost globally by the year 2050 as a result of antibiotic resistance by microbes. This figure is more than the death that occurs from cancer annually. As such, a novel product of antimicrobial compound derived from plants needs to be explored and studied extensively.

*Scurrula ferruginea* (Jack) Danser is a hemiparasitic plant that is mainly distributed in Malaysia, Singapore, Indonesia, Vietnam, Thailand, Myanmar, the Philippines, Cambodia, China and Laos (Huaxing & Gilbert, 2003). In Malay, it is locally known as "dedalu api merah" and has been used in folk medicine in Southeast Asia. In traditional practices, leaves of *S. ferruginea* are used in the treatment of shingles, malaria, high blood pressure, wound healing, snake-bites, easing urination pain, hypertension, gastrointestinal conditions and protective medicine after childbirth (Burkill, 1996; Lemmens & Bunyapraphatsara, 2003; Mat-Salleh & Latiff, 2002; Werner, 2002). Thus, a systematic approach on the effect of solvent extraction on antimicrobial activity of *Scurrula ferruginea* is studied to determine the finest solvent that shows optimal inhibitory effects on selective microbes. To date, no literature has been reported on solvent effects on antimicrobial activities of this mistletoe.

## MATERIALS AND METHODS

### Preparation of Plant Material

The leaves of *Scurrula ferruginea* were collected at the full flowering stage and the plant was taxonomically authenticated by Prof. Dr. Rusea Go. The plant's voucher specimen (RG4664), was deposited at the Biology Department Herbarium, Universiti Putra Malaysia (UPM). The leaf samples were oven dried at 60°C for 24 hours. The dried leaves were ground using a mill, and the powdered samples were then packaged in nylon linear low-density polyethylene pouches and stored in the dark at an ambient temperature.

### Crude Extracts Preparation

The method described by Obeidat et al. (2012) was adopted with slight modification. For extraction of the dried powder, aqueous, organic and aqueous-organic solvents were used. About 10 grams of dried powder of *S. ferruginea* leaves were soaked in each of deionised water, 80% methanol, 80% acetone, and benzene solvents (1:10 w/v) respectively and extracted for 24 hours at 28±2°C with vigorous shaking at 200 rpm. The samples were then filtered through Whatman No. 1 filter paper before the filtrated aqueous extracts were lyophilised. The extracts were evaporated using a rotary evaporator at 40±1°C. The dried crude extracts were weighed and stock solution (100mg/ml) was prepared by diluting it according to their solvent before keeping at -20°C freezer.

### Growth and Maintenance of Microbes

Four species of pathogenic microbes comprising *Staphyloccocus aureus* S261, *Escherichia coli* E57, *Candida albicans* C205 and *Trichophyton rubrum* T62 were obtained from Institute of Medical Research, Kuala Lumpur, Malaysia. Bacterial strains were maintained in nutrient agar plates and fungi strains in potato dextrose agar plates respectively in biosystematics plant and Microbe Laboratory, Biology Department, Faculty of Science, UPM. Malaysia. All microbes were kept in a chiller, at 4°C.

### Antibacterial Activity

Antibacterial activity was determined using modification protocol by Hussain, Khan, Hussain and Habib (2011). Using disc diffusion method, an inoculum containing bacterial cells from agar plate stock was subcultured in 5 ml of Mueller-Hinton Broth (MHB) and incubated for 24 hours. Bacterial inoculum was prepared in 10 ml MHB that contained 1 x $10^8$ (CFU $ml^{-1}$). A volume of 400 microliter 1 x $10^8$ (CFU $ml^{-1}$) was poured evenly over the surface of 9 cm diameter petri dishes containing Mueller Hinton Agar (MHA).

Sample disc was prepared by using 6 mm antibiotic assay discs (Whatman Grade AA disc). Following this, 20 microliters (100 mg/ml) were pipetted from the stock sample and dropped to the sample discs. The discs were placed on a prepared bacterial MHA plates. Ciprofloxacin (100 µg/ml) and water were used as positive control and negative control respectively.

Zone of inhibition (mm) of bacteria was examined after incubation for 24 hours at 37°C. All experiments were carried out in triplicates. Extracts that showed positive response or susceptibility to the strains were selected for further analysis.

**Antifungal Activity**

Antifungal activity was carried out using the method of Hussain et al. (2011) with modifications. *Candida albicans* and *Trichophyton rubrum* were cultivated in Saboraud Dextrose Agar (SDA) for 72 hours and 14 days at 28±2°C respectively. Fungal spores were collected using 0.05% between 80 and centrifuged at 3000 rpm for five minutes. Subsequently, the fungal inoculum was prepared in 10 ml Sabouraud Dextrose Broth (SDB) containing $1x10^6$ spore $ml^{-1}$ using supernatant solution. About 400 microliter $1x10^6$ spore $ml^{-1}$ were poured into SDA plates.

Then, 20 microliters (100 mg/ml) of plant extracts were pipetted and dropped to the sample discs. The sample discs were subsequently placed on a prepared fungal SDA plates. Nystatin (50 µg/mL) and water were used as positive control and negative control respectively. Zone of inhibition (mm) of bacteria was measured after incubation of 72 hours at 28±2°C. All experiments were carried out in triplicates. The extracts which showed positive response or susceptibility to the strains were selected for further analysis.

**Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)**

MIC, MBC and MFC tests were performed in sterile 96-well microplates as described by Marvibaigi et al. (2014) and Al-Hussaini and Mahasneh (2011) with slight modification. All extracts were properly prepared and transferred to each microplate well in order to obtain a twofold serial dilution of the original extract (from the concentration, 1800 to 14.06 µg/ml). Experiments were performed with 100µl of stock sample added in the first three rows and 100 µl Mueller-Hinton broth (bacteria) and Sabouraud Dextrose Broth (fungi) used as diluents followed. Then, 30 microliters of bacteria $1 \times 10^8$ (CFU $ml^{-1}$) and fungi $1x10^6$ spore $ml^{-1}$ samples were added to all rows. The plates were incubated at 37°C for 24 hours and 28±2°C for 72 hours for bacteria and fungi respectively, after which they were examined for the presence or absence of growth. The MIC was determined as the lowest concentration of extracts at which no colony was observed after incubation (no macroscopic visible growth).

In order to determine MBC and MFC, a small amount of sample from microplates with no visible growth were streaked gently on agar plates using a sterile cotton swab and incubated at 37°C for 24 hours and 28±2°C for 72 hours for bacteria and fungi respectively. MBC and MFC were defined as the lowest concentration of extract that inhibited 99.9% of the bacteria and fungi growth. Each experiment was repeated in triplicates.

### Statistical Analysis

Results were expressed as the mean ± standard deviation of triplicate and analysed using SPSS software (version 22). One-way analysis of variance (ANOVA) with Duncan's test was carried out to test significant difference between levels of treatment. $P < 0.05$ was considered significant and $P < 0.01$ as very significant.

## RESULTS

### Antimicrobial Activities

The analysis showed that extraction solvents significantly affected antimicrobial activity (disc diffusion method) of *S. ferruginea* leaves ($p<0.05$), as shown in Table 1. The zone of inhibition (mm) based on extraction solvents *S. aureus* ranged from 7.91 mm to 9.63 mm. Methanol offered the highest inhibition zones (9.63 mm), followed by 80% methanol (9.55 mm) while the least was in acetone (7.91 mm). However, the zone of inhibition in *E. coli* varied from 7.10 mm to 8.26 mm. The highest zone of inhibition belonged to 80% methanol (8.26 mm), followed by methanol (8.20 mm) while the least was in acetone (7.10 mm). As expected, the negative controls showed no activity against any of the bacterial or fungal strains. The standard antibiotic ciprofloxacin exhibited the highest zone of inhibition (mm) in *S. aureus* (14.65 mm) and *E. coli* (12.43 mm) while nystatin gave 11.26 mm for *C. albicans* but there was no activity against *T. rubrum*.

Table 1
*Antimicrobial activities (disc diffusion method) on leaf extracts of* S. ferruginea.

| *Samples* | *Inhibition zones (mm ± SD)*[1] | | | |
|---|---|---|---|---|
| | *Staphylococcus aureus S261* | *Escherichia coli E57* | *Candida albicans C205* | *Trichophyton rubrum T62* |
| deionized water | na | na | na | na |
| 100% methanol | 9.63±0.13$^{c}$ | 8.20±0.02$^{c}$ | na | na |
| 80% methanol | 9.55±0.16$^{c}$ | 8.26±0.28$^{c}$ | na | na |
| 100% acetone | 7.91±0.15$^{a}$ | 7.10±0.04$^{a}$ | na | na |
| 80% acetone | 8.86±0.03$^{b}$ | 7.93±0.08$^{b}$ | na | na |
| benzene | na | na | na | na |
| Ciproflocaxin[2] | 14.65±0.11$^{d}$ | 12.43±0.16$^{d}$ | — | — |
| Nystatin[3] | — | — | 11.26±0.10$^{a}$ | na |

Values are means of three replicates (N=3 ± SD). Samples with similar letters superscript are significantly similar ($p>0.05$) checked by Duncan test. [1]Inhibition zones including the diameter disk (6 mm); [2]ciprofloxacin at 100 µg/ml; [3]nystatin at 50 µg/ml; (na): not available; (—): not evaluated

Antimicrobial activity (MIC) of *S. ferruginea* leaves influenced by extraction solvents are presented in Table 2, while the effect of extraction solvents of antimicrobial activity (MBC and MFC) on *S. ferruginea* leaves is presented in Table 3. Extraction solvent significantly affects antimicrobial activity of *S. ferruginea* leaves ($p<0.05$). From this result, the best extraction solvent that showed lowest MIC and MBC values for *S. aureus* (450 µg/ml) and *E. coli* (750 µg/ml) respectively was 80% methanol. Although, there was no significant difference between 80% methanol and other extracts solvent in MIC values, yet it showed a significant difference ($p<0.05$) in MBC values in *S. aureus* but not in *E. coli*. As predicted, standard antibiotic (ciprofloxacin) yielded superior MIC (1.57 µg/ml) and MBC (3.13 µg/ml) in *S. aureus*. For *E. coli*, the MIC values were much higher with 4.17 µg/ml and MBC 6.25 µg/ml. Yet, there was no indication of MFC values in all extracts solvents as the extract present inactivity. Standard antifungal (nystatin) gave MIC (12.50 µg/ml) and MBC (25.00 µg/ml) values in *C. albicans*. However, no MIC and MBC values were determined in *T. rubrum*.

Table 2
*Antimicrobial activities (mic) on leaf extracts of* S. ferruginea.

| *Samples* | *MIC (µg/mL ± SD)*[1] | | | |
|---|---|---|---|---|
| | *Staphylococcus aureus S261* | *Escherichia coli E57* | *Candida albicans C205* | *Trichophyton rubrum T62* |
| deionized water | nt | nt | nt | nt |
| 100% methanol | 450±0.00[b] | 900±0.00[b] | nt | nt |
| 80% methanol | 450±0.00[b] | 450±0.00[b] | nt | nt |
| 100% acetone | 600±259.81[b] | 900±0.00[b] | nt | nt |
| 80% acetone | 450±0.00[b] | 900±0.00[b] | nt | nt |
| benzene | nt | nt | nt | nt |
| Ciproflocaxin[2] | 1.57±0.00[a] | 4.17±1.80[a] | — | — |
| Nystatin[3] | — | — | 12.50+0.00[a] | nt |

Values are means of three replicates (N=3 ± SD). Samples with similar letters superscript are significantly similar ($p>0.05$) checked by Duncan test. [1]Minimum Inhibitory Concentration (MIC); [2]ciprofloxacin (antibiotic); [3]nystatin (antibiotic); (nt): not tested cause inactive extract; (—): not evaluated

Table 3
*Antimicrobial activities (mbc and mfc) on leaf extracts of* S. ferruginea.

| *Samples* | *MBC (μg/mL ± SD)*[1] | | | |
|---|---|---|---|---|
| | *Staphylococcus aureus S261* | *Escherichia coli E57* | *Candida albicans C205* | *Trichophyton rubrum T62* |
| deionized water | nt | nt | nt | nt |
| 100% methanol | 750±259.81[c] | 900±0.00[b] | nt | nt |
| 80% methanol | 450±0.00[b] | 900±0.00[b] | nt | nt |
| 100% acetone | 900±0.00[c] | 900±0.00[b] | nt | nt |
| 80% acetone | 900±0.00[c] | 900±0.00[b] | nt | nt |
| benzene | nt | nt | nt | nt |
| Ciproflocaxin[2] | 3.13±0.00[a] | 6.25±0.00[a] | — | — |
| Nystatin[3] | — | — | 25.00+0.00[a] | nt |

Values are means of three replicates (N=3 ± SD). Samples with similar letters superscript are significantly similar ($p$>0.05) checked by Duncan test. [1]Minimum Bactericidal Concentration (MBC); [2]ciprofloxacin (antibiotic); [3]nystatin (antibiotic); (nt): not tested cause inactive extracts; (—): not evaluated

## DISCUSSION

This preliminary investigation showed that the quality of *S. ferruginea* crude extracts was influenced by the solvents used. Extracted solvents play a key role to recover miscellaneous therapeutic properties and boost the synergistic effects of the antimicrobial constituents, thus reducing the growth of bacteria. This is because each of the solvents used is inimitable, has diverse solubility to cell matrix and differs in relative polarities. Previous studies showed that extracted solvents reduce the activity of microbial growth based on the solvents solubility and relative polarities (Cowan, 1999; Garcia-salas, Morales-soto, Segura-carretero, & Fernández-gutiérrez, 2010; Neenah & Ahmad, 2011; Shobowale, Ogbulie, Itoandon, Oresegun & Olatope, 2015). These studies were in agreement with this study. Therefore, the variety of solvents polarity and solubility is recommended for plant materials extraction to select the finest desired biological substances.

The findings of this study revealed that 80% methanol extract has optimum antimicrobial properties in *S. ferruginea* leaves. The phenolic compounds in *S. ferruginea* leaves contribute to several antimicrobial mechanisms (Marvibaigi et al., 2014). Basically, antimicrobial mechanisms are known for targeting cell wall synthesis, protein synthesis, RNA synthesis, DNA synthesis, and intermediary metabolism (Cowan, 1999; Hooper, 2001). Phenolic compounds that have abundant and complex biochemical structures might have more than one antimicrobial mechanisms which have synergistic effects

against infectious microbes compared to synthetic antibiotics. However, this study does not fully clarify the potency against a range of microorganisms and which of the mechanisms are employed.

The varieties of chemical structure and function in plants secondary metabolites contribute to different potency against vast pathogenic microbes. Antifungal compounds embattled the formation or the function of ergosterol, which is an important component of fungal cell membrane, and form pores in the membrane that leads to $K^+$ leakage, acidification, cellysis and death of fungus (Ghannoum & Rice, 1999; Hammond, 1977). Yet, the results from this study show no available antifungal compound that might be present in the *S. ferruginea* leaves extract that can be suggested as having inhibitory effects on *C. albicans* and *T. rubrum*.

Previous studies by Srinivasan, Nathan, Suresh and Perumalsamy, (2001) showed that only eight out of 50 plant crude extracts have antimicrobial activity against pathogenic fungi. The result of our study also support the findings by Hussain et al., (2011) who showed that *Viscum album* extracts (acetone, petroleum ether, ethyl acetate, chloroform, ethanol, methanol and water) did not have antifungal activity against *S. cerevisiae* and *A. flavus*. Similarly, another literature reported non-activity in water extract against most bacterial strains (Igbinosa, Igbinosa, & Aiyegoro, 2009).

Additionally, the results also display anthropophilic dermatophyte, where *T. rubrum* turned into 'superbug' which shows resistance to antifungal compound (nystatin). This is in disagreement with the previous study reported by Al-Janabi, (2006). It can be postulated that *T. rubrum* developed resistance to ergosterol biosynthesis inhibitors in nystatin. The antifungal compound becomes inactive and degraded when the biochemical reaction process which is binding the ergosterol is not completed. This is caused by proteolytic enzymes secreted into the extracellular medium (Chen et al., 2010; Ghannoum & Rice, 1999). As a result, the pathogenic fungi *T. rubrum* evolved to become resistant against nystatin.

This study also presents the case that Gram-positive bacteria are more susceptible than Gram-negative bacteria against antimicrobial extracts and antibiotics. This is due to the dissimilarity of a morphological component of the bacterial cells. It is noted that Gram-negative bacteria are protected by an outer membrane that acts as an impermeable membrane for many small molecules. On the other hand, Gram-positive bacteria are only protected by a thick layer of peptidoglycan (Wendakoon & Gagnon, 2012).

## CONCLUSIONS

Malaysian mistletoe, *S. ferruginea* leaves extracts exhibit moderate antibacterial activity against Gram positive and Gram negative bacteria. However, no antifungal

activity is present against *C. albicans* and *T. rubrum*. It is found that 80% methanol extract offers the optimum degree of antimicrobials activity (disc diffusion method, MIC and MBC). These findings conclude that *S. ferruginea* leaves extract is an unprecedented source of antimicrobial compound.

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