

Antimicrobial potency of essential oil from cashew (*Anacardium occidentale* Linn.) clones

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Abstract

The increase of microbial resistance to conventional antimicrobial agents creates a need to find new antimicrobial source especially from substances of natural origin. Therefore, the aim of this study was to evaluate the antimicrobial potential of essential oil extracted from the shoot of potential cashew (*Anacardium occidentale* Linn.) clones against clinical human pathogens. Essential oils were obtained by hydrodistillation process of fresh shoots. Microbial strain-tested were *Acinetobacter anitratus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Proteus vulgaris*, *Pseudomonas aeruginosa*, *S. aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Aspergillus* sp., *Candida albicans* and *Candida tropicalis*. Antimicrobial potency was evaluated by standard disc diffusion method and minimum inhibitory concentration (MIC) test. It was found that only extract of clone F-848 had the antimicrobial activity on all the microbes tested excluding MRSA. *Acinetobacter anitratus* and *S. aureus* were sensitive to the extract of all clones tested with MIC ranging from 6250 – 12500 µg/ml and 6250 – 50000 µg/ml respectively. The lowest MIC value was 3125 µg/ml found at *C. albicans* using extract of clone F-848. From this study, it can be concluded that essential oil from cashew clone F-848 is a potential source of natural product which has broad antimicrobial effects especially against *S. aureus*, *A. anitratus* and *C. albicans*. This clone can be grown and up-scaled for the development of healthcare products such as antibacterial cream, shampoo or soap.

Keywords: essential oil, antimicrobial, disc diffusion method, minimum inhibitory concentration, clinical strain, cashew shoot

Introduction

The increase of microbial resistance to conventional antimicrobial agents such as antibiotic creates a need to find new antimicrobial source especially substances from natural origin. Recently, exploration of effective antimicrobial compound from plants has been intensified. Most plants

are medicinally useful in treating human diseases. The demand for more drugs from natural plant sources is increasing, which necessitates screening medicinal plants with promising biological activity (Sumathi and Parvathi 2010). Thus, many plants traditionally used in medication around the world have been extracted and also

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semi purified to explore their antimicrobial property individually (Prasannabalaji et al. 2012).

Anacardium occidentale (cashew) is a tree within Anacardiaceae which originally came from north eastern of Brazil. The seeds or better known as cashew nut are eaten worldwide. Locally known as *gajus*, it is a traditional vegetable that consumed by many Malaysians as *ulam*, where the young tender shoots are consumed (Mohd Shukri and Alan 2010). It is also used as homeopathy therapy in India for treatment of diseases such as blisters, itching, ulcers and warts. Cashew gum, which is the exudates from the tree, is already being used as binder and gelling agent in drug formulations (Gyedu-Akoto et al. 2007; Ofori-Kwakye et al. 2010). According to Dahake et al. (2009), the cashew nutshell liquid, a by-product of processing cashew, has been used effectively against tooth abscesses due to its lethality to gram-positive bacteria.

Major constituents such as (E)- β -ocimene, α -copaene and δ -cadinene were found in the phytochemical analysis of the leaves (Mohd Shukri and Alan 2010), while study by Maia et al. (2000) showed that the major constituents of the fruit were palmitic and oleic acids, furfural, 4-hydroxydodecanoic acid lactone, (E)-hex-enal, (Z)-hex-3-enol and hexadecanol, whereas in the flowers, β -caryophyllene, methyl salicylate and benzyl tiglate were the main constituents. This shows that *A. occidentale* is very valuable plants that should be studied to explore the potential use of its constituents and also as an antimicrobial agent. Therefore, the aim of this study was to evaluate the antimicrobial potencies of essential oil derived from the shoot of potential cashew clones (*A. occidentale* Linn.) against clinical human pathogens.

Materials and methods

All cashew clones (C-11, F-203, F-395, F-475, F-848, F-890, F-896, F-1527, M-58 and M-144) were obtained from cashew

trees cultivated at MARDI Cherating, Pahang. Fresh shoots of cashew clones were collected, washed, put in sealed plastic bags and stored at -20°C prior to the extraction of essential oils.

Essential oils were obtained by hydro-distillation process of fresh shoot (capacity 3 – 5 kg of each clone) for 4 h in a modified Clevenger-type apparatus. The oil layer was separated from the aqueous phase using separating funnel. The excess water in essential oils was removed by using anhydrous sodium sulphate. The essential oils were stored in amber bottle at -20°C until further analysis. The oil recovery was calculated on the basis of the volume of oil collected and the fresh weight of the plant material used.

All microbial strains used in this study were clinical strain except for *Aspergillus* sp. and provided by The Microbial Culture Collection Unit, Institute of Bioscience, Universiti Putra Malaysia. Microbial strain tested were *Acinetobacter anitratus*, *Aspergillus* sp., *Candida albicans*, *C. tropicalis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia marcescens*, methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus* and *S. epidermidis*. The growth of *A. anitratus*, MRSA, *P. vulgaris*, *P. aeruginosa*, *S. aureus*, *S. epidermidis* and *S. marcescens* were performed in nutrient agar while *Aspergillus* sp., *C. albicans* and *C. tropicalis* were grown in Sabouraud dextrose agar.

Antimicrobial screening was performed by standard disc diffusion method (Bauer et al. 1966). The microbial cultures were standardised to 0.5 McFarland standard turbidity which is equivalent to 10^8 cfu/ml. Then, they were smeared onto the growth medium by spreading 0.1 ml of the microbial suspension with a sterile cotton swab. Paper disc with 6 mm diameter was impregnated with 50 μl of the extract and placed onto the microbial inoculated plate. Nystatin was used as positive control for fungi, while Ampicillin and Streptomycin were used as positive control for gram-

negative and gram-positive bacteria respectively. Bacteria were incubated at 30 – 37 °C for 16 – 24 h while fungi were incubated for 24 – 48 h or until they reached sufficient growth. The inhibition zone was measured after incubation hour and the experiment was performed in triplicates. Inhibition zones with diameter less than 12 mm were considered as having low antimicrobial activity. Diameters between 12 and 16 mm were considered moderately active and those with more than 16 mm were considered highly active.

The minimum inhibitory concentration (MIC) test was performed according to Campos et al. (2012). A 24 h grown microbial strain was inoculated and adjusted to 0.5 McFarland standard turbidity which was equivalent to 10⁸cfu/ml in the appropriate media (Muller-Hinton broth for bacteria and Sabouraud broth for fungi) with the extract concentrations ranging from 391 – 50000 µg/ml. Samples were incubated for 24 h at 37 °C and the MIC was determined by observing the lowest concentration that completely inhibited macroscopic growth of the microorganisms. All assays were performed in triplicate.

Statistical analysis

Analysis was carried out using ANOVA and multiple comparisons of means using Tukey's Honest Significance Difference test. All statistical analyses were performed with SAS (9.1) 2002 – 2003 for Windows.

Results and discussion

Each clone produced similar oil recovery between 1 – 2% (v/v) from each distillation. The colour of the oils is yellowish-green. Based on previous study, 100% of oil extracts showed very strong antimicrobial effect against several bacteria mainly MRSA (Mat Ali 2008). In addition, the oil was also prepared in several dilutions in dimethyl sulfoxide to determine the lowest concentration that inhibited the bacteria.

Antimicrobial screening

The antimicrobial activity was determined based on inhibition zone formed around the disc impregnated with the extract over the lawn of microbial culture. From the screening, it was found that only extract of clone F-848 showed antimicrobial activity on all the microbes tested (Table 1). Santos et al. (2013) reported that the presence of phytochemical such as phenols, tannin, flavonoids, catechins and alkaloids in *A. occidentale* leaves may contribute to the

Table 1. Antimicrobial activity of different *Anacardium occidentale* clones by disc diffusion method (mm). All assays were done in triplicate. Mean separations were carried out using Tukey's Honest Significance Difference test

	F-848	F-203	F-395	C-11	F-890	F-1527	M-144	F-896	M-58	F-475
<i>Acinetobacter anitratus</i>	20.00	14.33	16.00	17.33	16.67	16.00	14.67	14.00	11.00	17.00
<i>Aspergillus</i> sp.	10.33	0.00	7.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Candida albicans</i>	17.33	9.67	10.00	10.00	9.00	9.00	9.00	8.33	7.33	9.50
<i>Candida tropicalis</i>	14.33	8.67	10.00	10.00	9.00	19.00	20.00	12.50	14.67	17.67
MRSA	11.33	0.00	0.00	0.00	0.00	7.67	8.00	8.50	9.00	8.33
<i>Proteus vulgaris</i>	24.67	19.33	20.33	21.00	19.33	0.00	0.00	0.00	16.00	0.00
<i>Pseudomonas aeruginosa</i>	12.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Serratia marcescens</i>	13.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Staphylococcus aureus</i>	35.33	20.00	23.00	20.00	19.00	20.00	21.33	16.33	16.00	26.67
<i>Staphylococcus epidermidis</i>	22.00	17.67	17.67	16.00	17.67	14.67	15.33	15.67	14.00	17.00

antimicrobial activity. The most prominent antimicrobial activity showed by F-848 extract was against *S. aureus* (35.33 mm). These were followed by *P. vulgaris* (24.67 mm), *S. epidermidis* (22.00 mm), *A. anitratus* (20.00 mm) and *C. albicans* (17.33 mm). Prior study by Chabi Sika et al. (2014) found that ethanol extracts of leaves and bark of *A. occidentale* showed antibacterial activity against *S. aureus* which verified our findings. Antimicrobial activity against *C. albicans* concurs with previous study by Dahake et al. (2009) on antimicrobial screening by different extract of *A. occidentale* leaves.

Aspergillus sp. and MRSA appeared to be resistant towards F-848 extracts, with minor inhibition zone of 10.33 mm and 11.33 mm respectively. *Staphylococcus aureus* was susceptible to the extracts of all clones. The susceptibility was indicated by bigger inhibition zone against extract of all clones except for clone M-58, which was moderately active. This result is in line with those reported by Dahake et al. (2009), Agedah et al. (2010) and Chaithra et al. (2013). The extract of clone F-848, F-203, F-395, F-890 and F-475 also showed high antimicrobial activity against *S. epidermidis* while activity of other clones was moderate. In addition, a gram-negative bacterium, *A. anitratus* was susceptible to the extracts of all clones except M-58. All the gram-positive bacteria tested (*S. aureus* and *S. epidermidis*) except MRSA were more sensitive towards *A. occidentale*. This could be explained by the lack of an outer membrane in their cell walls which may be responsible for the differences in the degree of sensitivity. According to Agedah et al. (2010), gram-negative bacteria have an outer membrane which may prevent a substantial amount of the extract having contact with the cell wall.

Aspergillus sp. and MRSA were resistant to the extracts of all clones tested. These were revealed when only two clone extracts showed very low antimicrobial activity against *Aspergillus* sp., while for

MRSA there were six clone extracts that showed very low antimicrobial activity. The result for MRSA is in agreement with Olugbuyiro et al. (2013) who used *A. occidentale* stem bark extract for antimicrobial activity. However, it is contrary with earlier report by Parasa et al. (2011) who showed the potential antimicrobial activity against clinical isolates of MRSA using cashew nut shell liquid, whereas in this study, the leaves of cashew were used. Different plant part used in both studies may probably influenced the results. It is well known that cashew nut shell liquid has anacardic acid as the main component, which demonstrated promising antimicrobial activity.

On the other hand, *P. aeruginosa* and *S. marcescens* were moderately sensitive to the extract of clone F-848 while no inhibition zone was detected when they were tested against other clones. A greater resistance of *P. aeruginosa* and *S. marcescens* was anticipated since they are gram-negative bacteria. Gram-negative bacteria have a wall composed of several layers of peptidoglycans, which differ in their chemical composition and consequently more complex than the wall of gram-positives bacteria (Gobbo-Neto 2007; Gonçalves and Gobbo 2012). These contribute to an efficient permeability barrier that limits the penetration of antimicrobial phytochemical compounds as compared with gram-positives bacteria that only have a single membrane which made it more accessible to antimicrobial phytochemical compound (Chanda and Kaneria 2011).

MIC test

Antimicrobial potency of the clone extract against the tested bacterial and fungal strains was expressed in MIC as presented in Table 2. The values studied from the range of 391 – 50000 µg/ml. *Acinetobacter anitratus* and *S. aureus* were sensitive to the extract of all clones tested with MIC ranging from 6250 – 12500 µg/ml and 6250 – 50000 µg/ml respectively. Our result

Table 2. Minimum inhibitory concentration of *Anacardium occidentale* clones extract ($\mu\text{g/ml}$). All assays were done in triplicate. Mean separations were carried out using Tukey's Honest Significance Difference test

	F-848	F-203	F-395	C-11	F-890	F-1527	M-144	F-896	M-58	F-475
<i>Acinetobacter anitratus</i>	6250	12500	12500	12500	6250	6250	12500	12500	12500	12500
<i>Aspergillus</i> sp.	12500	nd	nd	nd	nd	nd	nd	nd	nd	nd
<i>Candida albicans</i>	3125	25000	25000	25000	nd	nd	25000	nd	12500	25000
<i>Candida tropicalis</i>	6250	nd	12500	12500	nd	12500	12500	12500	nd	12500
MRSA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<i>Proteus vulgaris</i>	6250	25000	25000	25000	12500	nd	nd	nd	50000	nd
<i>Pseudomonas aeruginosa</i>	50000	nd	nd	nd	nd	nd	nd	nd	nd	nd
<i>Serratia marcescens</i>	50000	nd	nd	nd	nd	nd	nd	nd	nd	nd
<i>Staphylococcus aureus</i>	6250	50000	50000	50000	12500	50000	25000	50000	50000	50000
<i>Staphylococcus epidermidis</i>	50000	50000	50000	nd	50000	nd	nd	nd	nd	nd

*nd = not detected

is in agreement with Ifesan et al. (2013), who demonstrated antimicrobial activity of ethanol extract from *A. occidentale* leaf when subjected to *Acinetobacter* spp. and *S. aureus*. The lowest MIC value was 3125 $\mu\text{g/ml}$ by *C. albicans* using extract

of clone F-848. This is in line with study done by Dahake et al. (2009), who found significant antifungal activities against *C. albicans*. In addition, latter study found that acetyl acetate extract of *A. occidentale* leaf has lowest MIC against *S. aureus* and *C. albicans* (Chabi Sika et al. 2014).

The MIC test showed that *Aspergillus* sp., *P. aeruginosa* and *S. marcescens* were only susceptible to the extract of clone F-848 as compared to other clones. The MIC values for *Aspergillus* sp., *P. aeruginosa* and *S. marcescens* were 12500, 50000 and 50000 $\mu\text{g/ml}$ respectively. Furthermore, extract of clone F-848 was effective against all microbial strains tested excluding MRSA. A broad range of antimicrobial activity on the extract of clone F-848 could be influenced by the production of active component such as phenol compounds. The absence of MIC showed that all clones were not capable of inhibiting MRSA at the tested concentrations. Perhaps the concentration of active constituents such as tannins was lower in the leaves as compared to other parts of *A. occidentale*. Furthermore, the extraction technique could also influence the active constituents that involved in antimicrobial activity as displayed by Leitaoa et al. (2013), who found that more functional compounds in *A. occidentale* by using Supercritical Fluid Extraction. Earlier reports by Parasa et al. (2011) and Campos et al. (2012) showed that cashew nut shell liquid and cashew tree gum had an inhibitory effect against MRSA. Recent MIC study also showed that MRSA was susceptible to cashew gum-based silver nanoparticles (Quelemes et al. 2013).

Besides clone F-848, extract of clone F-395 was effective against six of the microbial strains tested (*A. anitratus*, *C. albicans*, *C. tropicalis*, *P. vulgaris*, *S. aureus* and *S. epidermidis*). On the other hand, extract of clone F-896 and F-1527 had the lowest number of MIC value with only three microbes that were sensitive (*A. anitratus*, *C. tropicalis* and *S. aureus*). The variation in antimicrobial

activity between *A. occidentale* clones could be influenced by the amount and nature of active constituents in the leaves. Seasonality, age or development stage, temperature, water availability and mechanical stimulation could be the factors that influenced the active constituents in a plant as described by Gobbo-Netto and Lopes (2007). Furthermore, Santos et al. (2013) reported that seasonal variations can virtually change the content of virtually all classes of secondary metabolites such as essential oils, phenol acids, flavonoids, saponins, alkaloids and tannins.

Conclusion

It can be concluded that essential oil from cashew clone F-848 is a potential source of natural product which has broad antimicrobial effects especially against *S. aureus*, *A. anitratus* and *C. albicans*. These antimicrobial characteristics of cashew clone F-848 are prospectively precious for the future as this clone can be grown and up-scaled for the development of healthcare products such as antibacterial cream, shampoo or soap.

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Abstrak

Keperluan untuk mencari sumber antimikrob yang baru terutamanya daripada bahan semula jadi berlaku berikutan peningkatan rintangan mikroorganisma terhadap ejen antimikrob konvensional. Oleh itu, matlamat kajian ini adalah untuk menilai potensi antimikrob daripada minyak pati pucuk klon gajus yang berpotensi (*A. occidentale* Linn.) terhadap patogen manusia. Minyak pati diperoleh daripada pucuk gajus segar yang melalui proses 'hydrodistillation'. Strain mikroorganisma yang diuji ialah *Acinetobacter anitratus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Proteus vulgaris*, *Pseudomonas aeruginosa*, *S. aureus*, *S. epidermidis*, *Serratia marcescens*, *Aspergillus* sp., *Candida albicans* dan *C. tropicalis*. Potensi antimikrob dinilai melalui kaedah 'standard disc diffusion' dan ujian 'Minimum Inhibitory Concentration' (MIC). Melalui kajian ini, didapati bahawa hanya ekstrak daripada klon F-848 mempunyai aktiviti antimikrob yang tinggi terhadap semua mikroorganisma yang diuji kecuali MRSA. *Acinetobacter anitratus* dan *S. aureus* adalah sensitif terhadap ekstrak semua klon dengan nilai MIC masing-masing daripada 6250 – 12500 µg/ml dan 6250 – 50000 µg/ml. Nilai MIC terendah ialah 3125 µg/ml oleh *C. albicans* menggunakan ekstrak klon F-848. Dapat disimpulkan bahawa minyak pati daripada klon F-848 mempunyai potensi sebagai bahan semula jadi yang mempunyai kesan antimikrob yang meluas terutamanya terhadap *S. aureus*, *A. anitratus* and *C. albicans*. Klon ini boleh ditanam secara skala yang besar bagi penghasilan produk kesihatan yang sesuai seperti krim antibakteria, sabun atau syampu.