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GRADUATE RESEARCH ARTICLE

Antioxidant and Antimicrobial Properties of *Ocimum sanctum* and *Cymbopogon nardus*

Ruth Amarachi, Rahini Ramanathan*, Ng Shee Ping

School of Applied Sciences, Faculty of Engineering, Science and Technology, Nilai University, 71800 Nilai, Negeri Sembilan, Malaysia

* Corresponding Author email:

rahini@nilai.edu.my

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Student(s)

- Ruth Amarachi

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Mentor(s)

- Dr. Rahini Ramanathan
- Dr. Ng Shee Ping

ABSTRACT

Plant extracts have gained popularity recently, for their importance as potential antioxidative and antimicrobial agents. These properties have been attributed to their phytochemical content. The extraction solvent and the plant part are among the factors that influence the yield of these phytochemicals This study was therefore undertaken to investigate the antimicrobial and antioxidant activities of extracts of the leaf and stem of *Ocimum sanctum* (holy basil) and *Cymbopogon nardus* (citronella grass); two commonly occurring plants in South East Asia. The extracts were obtained by solvent extraction using water, methanol and ethanol. The percentage yield, antimicrobial activity, antioxidant activity and high-performance liquid chromatography (HPLC) analysis was investigated. The organic extracts yielded a higher percentage recovery of phytochemicals compared to the water extracts. HPLC analysis revealed the presence of chlorogenic acid in all extracts; rutin only in the leaf extracts and the citronella grass leaf extract contained both rutin and gallic acid. Antimicrobial assays were performed using the agar well diffusion method with tetracycline as positive control. Basil extracts exerted a greater inhibitory growth on both *S. aureus* and *E. coli*. *S. aureus* was found to be more susceptible to the presence of plant extracts. Water extracts did not display any zones of inhibition. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to study the antioxidant effect with Vitamin C (0.1mg/ml) as positive control. The results indicated that the Basil leaf extracts possessed greater antioxidant potential compared to the stem. The study concludes that organic extracts of *O. sanctum* and *C. nardus* possess pharmaceutical properties.

Keywords: Antioxidant, antimicrobial, *Ocimum sanctum, Cymbopogon nardus*

1 Introduction

Ocimum sanctum L., also known as *Ocimum tenuiflorum*, holy basil or Tulsi, is a popular healing herb in Ayurvedic medicine. It is a sweet-smelling plant in the family *Lamiaceae*. It is found throughout the world

and is common as a cultivated plant and an escaped weed. It is grown for religious and medicinal purposes and its essential oil. *Ocimum tenuiflorum* is utilised in various forms, aqueous extracts from the leaves (fresh or dried as powder) are utilised as a part of natural teas or blended with different herbs or nectar to improve the restorative system [1]

Cymbopogon nardus is a local therapeutic plant, generally utilised for post-partum bath. Scientific studies have demonstrated and proven that C. nardus possesses several biological activities, for example, antiviral, antibacterial, and insect repellent properties. *C. nardus*, a *Poaceae* is a therapeutic plant generally utilised for culinary purposes and in perfumery [2].

Traditionally, medicinal plants have been used as herbal remedies to prevent and cure diseases [3]. The advent of science into the search for antibiotics largely depends on some of these medicinal plants as raw materials. For many years, medicine has depended exclusively on the leaves, flowers and barks of plants. Only recently have synthetic drugs come into use and in many instances, these are carbon copies of chemicals identified in plants. According to the world health organisation (WHO), a medicinal plant is any plant which contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs in one or more of it organs [4]. At present nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and in homoeopathic or ayurvedic medicines, medicinal plants, their parts and extracts dominate the market.

The commercial value of various innumerable drugs and pharmaceuticals derived from tropical forest systems on a worldwide basis is projected at 20 billion dollars a year. Infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and governments all over the world (accounting for over 50,000 deaths every day), especially with the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the available modern drugs or antibiotics [5]. It is therefore imperative that to discover newer, and more effective antibiotic sources. Plants are the cheaper and safer alternatives for antimicrobials [6].

Sweet basil but not holy basil has been previously studied and there is limited literature on the stems of holy basil and citronella grass. Therefore, this study investigated the antimicrobial and antioxidant activity of the ethanol, methanol, and water extracts of the leaf and stem of *Ocimum sanctum* (holy basil) and *Cymbopogon nardus* (citronella grass) in the hope of finding alternative sources of natural (plant-based) pharmaceuticals.

2 Research Methodology

2.1 Preparation of plant extracts

Fresh leaves and stems of *Ocimum sanctum* and *Cymbopogon nardus,* with no apparent physical, insect or microbial damage, were collected from three different locations around Nilai, Malaysia (Nilai University, Desa Cempaka, Desa Melati). Samples were washed with tap water, rinsed and air-dried for 7-14 days at room temperature of about 23^{0}C. Upon drying, the samples were grinded using a household blender, after which they were stored in Schott bottles at 4˚C for further periodical use.

The preparation of crude extract was carried out as described by [7] with slight modifications. The powdered leaf and stem samples were extracted with different solvents (water, 80% ethanol and 80% methanol). 4g of the samples was diluted with 100ml of solvent in a conical flask and covered with aluminium foil. The flask was placed in an orbital shaker at 120-150 rpm at room temperature. After 24 hours, the solution was filtered using Whatman filter paper No 2 and the filtrate was evaporated to dryness in the fume hood. The dried extracts were reconstituted in 80% DMSO (Dimethyl sulfoxide) to give a stock concentration of 100mg/ml and the solutions were kept in the fridge (4^{0}C) until further use.

2.2 Phytochemical screening

The phenolic acid screening was conducted using High-Performance Liquid Chromatography (HPLC). The samples (2 mL) were transferred into HPLC vials and the HPLC system was conditioned to specific parameters as follows:

Mobile phase A: Deionised water (18.2 MΩcm conductivity unit for ultra-pure water) with 3% of acetic acid, Mobile phase B: Acetonitrile (BDH HPLC grade), Flow rate of mobile phase: 0.8 mL/ minutes and sample injection volumes: 10 µL. The detector was set at 280 nm; wavelength at which phenolic acid is commonly absorbed. All samples and standards were run using the same conditions before the comparison of retention time were made. Finally, the retention time of the signals from basil and citronella grass leaves and stem extracts were compared with signals from rutin, quercetin, chlorogenic and gallic acid standards at 280 nm.

2.3 Test Microorganisms

Stock cultures of all experimental bacteria were obtained from the laboratory of the School of Applied Science, Nilai University. *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were the two microorganisms used for this study.

2.4 Preparation of Stock Culture

1.3 g of nutrient agar was added to 100 ml of distilled water and stirred using a magnetic stirrer until dissolved. The media was then autoclaved for 20 mins, after which it was poured into sterile plastic petri dishes and the microorganisms were subculture using the streak plate technique. The microorganisms were incubated at 37^0C until confluent and then stored in the refrigerator at 4°C until needed.

2.5 Cell Number Determination

The test bacteria *S. aureus* and *E. coli* were standardised using a spectrophotometer at a wavelength of 600nm, until a reading of was 0.8 to 1.0 was obtained. The suspension was used within 15-20 minutes of preparation.

2.6 Assay for antibacterial activities

The antibacterial screening using agar well diffusion method was carried out according to [8] with slight modifications. 0.1ml each of the test bacteria was inoculated onto the Mueller Hinton agar (MHA) plates by the spread plate method. Each plate contained 20ml MHA. The solutions were allowed to dry for a few minutes and then wells were made into each quarter of the agar using a cork borer (6mm). 0.1ml (100ul) extract of each sample was introduced into the well (6mm) and allowed to dry. DMSO was used as a negative control and tetracycline was used as the positive control. The culture plates were incubated at 37°c for 24hrs. After 24hrs, antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) around each sample.

2.7 DPPH (Diphenyl-picryl-hydrazyl) free scavenging activity

Radical scavenging abilities of the samples were evaluated using DPPH assay as described by [9] with slight modifications. 1.5ml samples of varying concentrations (2, 4, 6, 8, and 10mg/mL) were made up with DMSO. 0.5ml of 0.1mM DPPH was added to the samples and the tubes were incubated in the dark for 30min.DMSO was used as a negative control and Vitamin C (0.1mg/ml) was used as the positive control. Absorbance was measured using a spectrophotometer at a wavelength of 517nm. Percentage scavenging was calculated using the formula below.

$$\text{DPPH scavenging effect (\%)} = \frac{Absorbance\ control - Absorbance\ text}{Absorbance\ control} \text{ x } 100$$

3 Results and Discussion

The chemical nature of phytochemicals, the extraction method used, sample particle size and the solvent used are all factors that influence the efficiency in extracting plant compounds. The higher yield indicates that more phytochemicals have been extracted by the extraction solvent. According to the results shown above (Table 1), water yielded the lowest percentage recovery (4.14% -6.51%) of phytochemicals for all

samples. This indicates that water is a poor choice of extraction solvent. The organic solvents (80% methanol and 80% ethanol) were able to extract more phytochemicals with values ranging from 10.07 - 16.9%. Methanolic and ethanolic extracts of Basil leaves yielded a higher percentage recovery compared to the stem extracts. While for citronella grass, the percentage recovery in the organic extracts was comparable for both the stem and the leaves.

Table 1*: Percentage Recovery*

Extracts	% Recovery
Basil leaf water	6.51±0.58
Basil leaf methanol	14.48±2.79
Basil leaf ethanol	15.73±3.22
Basil stem water	4.28±2.83
Basil stem methanol	10.07±2.82
Basil stem ethanol	10.13±2.46
Citronella grass leaf water	4.89±0.93
Citronella grass leaf methanol	13.23±6.27
Citronella grass leaf ethanol	12.08±7.60
Citronella grass stem water	4.14±1.06
Citronella grass stem methanol	16.9±27.00
Citronella grass stem ethanol	12.59±6.59

The SD values indicate that there is a large variation in the phytochemical content of the plant samples obtained from the three locations. The phytochemical composition of plants varies with species, variety, cultivation, region, weather and soil conditions [10] showed that location, as a result of different climatic conditions (eg. temperature, radiation), affects the amount of bioactive compounds synthesised by white cabbage grown in Spain. Since climatic conditions throughout Malaysia are fairly constant, the variation in the yield of phytochemicals could be attributed to the soil and environmental (pollution) conditions, in which the plants were grown.

Table 2 is tabulated from the results obtained from the HPLC assays conducted on the ethanol extracts of the stem and leaf of Basil and Citronella grass. The results show that all samples contained chlorogenic acid. Chlorogenic acid is an ester of caffeic acid and quinic acid. It has been found in coffee beans and the seeds and leaves of many dicotyledonous plants. Chlorogenic acid is a common polyphenol and has been reported to have potent antioxidative and free radical-scavenging activities in vitro [11]. Chlorogenic acid also increases the resistance of LDL to lipid peroxidation and inhibits DNA damage [12]. The antioxidative and anti-inflammatory effects suggest that chlorogenic acid could aid in the prevention of cardiovascular diseases**.**

Table 2: *HPLC Analysis*

Sample	Rutin	Quercetin	Gallic acid	Chlorogenic acid
Basil leaf	+	-	-	+
Basil stem	-	-	-	+
Citronella grass leaf	+	-	+	+
Citronella grass stem	-	-	-	+

"+" indicates presence of compound and "-" indicates absence of compound

Rutin is present in the leaf samples of both Basil and Citronella grass, while gallic acid is only found in the citronella grass leaf extract. Therefore, it can be seen that the leaf extracts contain a greater variety of

polyphenols compounds compared to the stem extracts. The citronella grass leaf extract possesses the greatest variety of bioactive compounds (rutin, gallic acid and chlorogenic acid). Quercetin was not present in any of the samples tested.

Table 3: *Free radical scavenging effect of plant extracts*

Extracts	EC_{50} values (mg/mL)
Basil leaf water	5.83±0.32
Basil leaf methanol	2.30±0.10
Basil leaf ethanol	2.07±0.32
Basil stem water	5.77±0.25
Basil stem methanol	5.30±3.05
Basil stem ethanol	6.43±4.18
Citronella grass leaf water	5.00±1.04
Citronella grass leaf methanol	5.50±1.56
Citronella grass leaf ethanol	2.07±0.12
Citronella grass stem water	5.27±1.10
Citronella grass stem methanol	2.47±0.99
Citronella grass stem ethanol	2.27±0.23

According to Table 3 above, water extracts of both Basil and Citronella grass have poor antioxidant effects with EC_{50} values ranging from 5.00 to 5.83 mg/mL. Since the pharmacological effects of plant extracts are attributed to the amount of phytochemicals extracted, water extracts because of their poor percentage recovery (Table 1) exerted poor antioxidant effects (high EC_{50} values). The methanolic and ethanolic Basil leaf extracts had lower EC_{50} values (2.30 ± 0.10mg/mL and 2.07± 0.32mg/mL respectively) compared to the stem extracts (5.30 ± 3.05mg/mL and 6.43 ± 4.18mg/mL respectively. This indicates that the leaves possess greater antioxidant potential than the stem. This fact could be attributed to the greater variety of polyphenols detected in the leaf extract (Table 2)

However, with the Citronella grass samples, the EC_{50} values of the ethanolic stem and leaf extracts were comparable (2.07 ±0.12 and 2.27 ± 0.23mg/mL respectively). While with the methanolic extracts, the leaves stem was more effective at scavenging free radicals than the stem (EC_{50} values 2.47 ± 0.99mg/mL vs 5.30 ± 3.05mg/mL).

The percentage recovery of phytochemicals of the stem and leaf ethanolic extracts were similar (around 12%) and although the leaf extract contained the most variety of bioactive compounds (Table 2), the antioxidative effects of the stem and the leaf extracts were similar. The reason for this could be the presence of other bioactive compounds not detected in the HPLC analysis that contributed to the antioxidant activity of the extract.

The phytochemicals present and thus extracted from each sample contributed to the antioxidant effect of the extracts. The large difference in standard deviations (SD) of some of the extracts might be due to the varied chemical composition of the plants obtained from the different locations [13]. Other factors such as geographical and stage of harvest could contribute to the differences as well.

According to results of the antimicrobial assay (Table 4), Basil extracts inhibited the growth of both *S. aureus and E. coli* more effectively than the Citronella grass extracts. Both extracts were more effective in inhibiting the growth of *S. aureus.* With *S. aureus,* the average zone of inhibition was 15mm for Basil and 10mm for Citronella grass as compared to *E. coli*, with an average zone of inhibition was of 10mm for Basil and 6mm for Citronella grass. The Gram-positive bacterium was more susceptible to the effects of the extracts, due to the absence of an outer protective wall.

Table 4: *Antimicrobial assay against S.aureus and E.coli (mm)*

Extracts	*S.aureus*	*E. coli*
Basil leaf water	0	0
Basil leaf methanol	15.6±17.4	11.2±1.11
Basil leaf ethanol	15.2±0.5	9.7±0.4
Basil stem water	0	0
Basil stem methanol	16.8±0.6	9.8±1.8
Basil stem ethanol	15.7±0.7	10.2±0.9
Citronella grass leaf water	0	0
Citronella grass leaf methanol	12.0±3.1	8.2±1.1
Citronella grass leaf ethanol	8.3±6.4	4.7±3.4
Citronella grass stem water	0	0
Citronella grass stem methanol	8.7±1.1	8.3±1.6
Citronella grass stem ethanol	11.0±2.6	6.5±4.6
+ Tetracycline (Basil)	19.0±0.8	15±0.0
+ Tetracycline (Citronella grass)	16.0±1.4	14±0.0
- DMSO	0	0

Water extracts due to their low percentage recovery of phytochemicals, did not exhibit any zones of inhibition on either of the microorganisms tested.

4 Conclusion

The organic extracts (methanol and ethanol) exerted greater antioxidative and antimicrobial activities compared to the water extracts. Their potency can be attributed to the fact that the organic extraction solvents were able to extract more phytochemicals (greater percentage recovery). HPLC analysis detected the presence of polyphenols which are known to possess good pharmacological properties. The study therefore concludes that extraction solvent plays a crucial role in the determining the pharmacological potency of plant extracts. In the future study, demonstration of the antibacterial activity of *O. sanctum and C. nardus* against *S.aureus and E.coli* is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents. The tested extract can also be tested on various microorganisms like *S. pyogenase, S. pneumoniae, S. typhi, p. mirabilis, S. flexnerri, E. faecalis, P. aeruginosa, B. cereus* and many more. Also, further studies on which free radical species (i.e. superoxide anion, reactive nitrogen species) that the extracts will be most effective on can help in narrowing the specific radicals that can be used to minimize or prevent lipid oxidation in pharmaceutical products, maintain nutritional quality and prolong shelf-life of food and pharmaceuticals.

5 Declarations

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5.2 Funding source

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5.3 Competing Interests

Authors declare no conflict of interest.

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