Antibacterial and Antioxidant Activities of *Synedrella nodiflora* (L.) Gaertn. (Asteraceae)

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Abstract

The hexane, ethyl acetate, ethanol and water extracts of *Synedrella nodiflora* (L.) Gaertn. (Asteraceae) were assessed for their antibacterial and antioxidant capacities. The antioxidant capacities were evaluated using the ferric reducing antioxidant power (FRAP) and β-carotene bleaching assays. The antimicrobial activities were assessed using the classical pour-plate disc diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and death kinetic assays, against six bacterial strains. The ethanol extract displayed significant antioxidant capacities both in the FRAP and β-carotene bleaching assays. The ethanol extract abrogated the growth of all the bacteria tested. Folin-Ciocalteu and aluminium chloride spectrometry assays indicated the presence phenolic compounds, including flavonoids in the ethanol extract.

KEYWORDS: antibacterial, antioxidant, Asteraceae, Synedrella nodiflora

1. Introduction

The Asteraceae family consists of herbs which are known to accumulate substantial amount of flavonoids and to display anti-inflammatory, antioxidant, antimicrobial, analgesic and antipyretic properties (Odom, et al., 2000). *Synedrella nodiflora* (L). Gaertn. (Asteraceae) or Cinderella weed, is native to tropical America and grows in Malaysian plantation crops (Holm, et al., 1997).

Traditionally, the leaves are used as poultice for rheumatism and the juice of the leaves is used for earache in India (Rathi & Gopalakrishnan, 2006). In Ghana, this weed is used for the treatment of epilepsy and pain (Idu & Onyibe, 2007). In Nigeria, the plant is used to heal putrefied wounds (Burkill, 1985). In Malaysia and Indonesia, the plant is used for headaches, earaches, stomachaches and rheumatism (Woode, et al., 2010).

Extracts of *Synedrella nodiflora* (L). Gaertn. displayed anti-inflammatory, analgesic, antinociceptive, antipyretic and insecticidal properties (Abad, et al., 1996; Forestieri, et al., 1996; Rathi & Gopalakrishnan, 2006; Woode, et al., 2010). The aim of the present study is to demonstrate that the antiseptic and anti-inflammatory uses of *Synedrella nodiflora* (L). Gaertn. are owed to antibacterial and antioxidant phenolic compounds including flavonoids.

Epidemiological studies indicate that dietary antioxidants are highly associated with reduced risks of chronic diseases such as heart disease, cancer, diabetes, neurological disease, immune diseases, and eye diseases (Willcox, et al., 2004; Temple, 2000; Slavin, et al., 1997; Grundman, 1997; Block, et al., 1992). According to Sanchez-Moreno et al. (1999), natural antioxidants from plants have become a prominent area of scientific research, as plants offer a wide range of secondary metabolites with high antioxidant potential.

According to the World Health report of infectious diseases 2000, overcoming antibiotic resistance is one of the major issues of the WHO for the present millennium. Hence, the last decade witnessed an increase in the investigation of plants as a source of human disease management (Prashanth, et al., 2001). Plant synthesizes a broad array of secondary metabolites, which play a key role in the natural defence mechanism against microorganisms and insects (Cowan, 1999). Traditional medicine derived from plants source are still used to treat various infectious diseases (Rios & Recio, 2005).

2. Materials and Methods

2.1. Plants collection and extraction

Synedrella nodiflora (L.) Gaertn. was collected in Broga, Selangor, Malaysia. A Voucher specimen (UNMC51W) was deposited in the herbarium of the School of

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Pharmacy, Faculty of Science, University of Nottingham Malaysia Campus. The air-dried and finely milled sample (300 g) was extracted by hexane (the extract yield was 4.05 %), ethyl acetate (1.21 %), ethanol (3.66 %) and water (3.51 %) sequentially. The extracts were concentrated using rotary evaporator (Buchi, USA) under reduced pressure at 40° C. Dried extracts were kept at -20° C until further tests. For stock solutions, 100 mg/mL of each extract was dissolved in DMSO (dimethyl sulfoxide).

2.2. Antioxidant activity

2.2.1. Ferric reducing power antioxidant (FRAP) assay

The antioxidant activity of the extracts was estimated by the FRAP method of Benzie & Strain (1996) with slight modifications. The working FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio. Briefly, 180 µL of the FRAP reagent was mixed with 20 µL of the test sample, so the final dilution of the test sample in the reaction mixture was 1/10. Readings were taken after 90 minutes (at 600 nm) using spectrophotometer (Dynex MRX-Revelation, USA). Fe(II) concentrations in the range 1 µM – 125 µM ($FeSO_4.7H_2O$) were used for calibration. Trolox and quercetin were used as positive controls. FRAP value was calculated as Ferrous Equivalents, the concentration of trolox/quercetin or extracts which produced an absorbance value equal to that of 1 mM $FeSO_4$.

2.2.2. β-Carotene bleaching assay

β-Carotene bleaching assay was conducted according to Miller (1971) with some modifications. A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL chloroform. Two mL of this solution was pipetted into a 100 mL round-bottom flask. After the chloroform was removed at 40° C under vacuum, 40 mg of linoleic acid, 400 mg of tween 80, and 100 mL of distilled water were added to the flask with vigorous shaking. A 96-wells microtiter plate was used for this method. As soon as the emulsion was added to each well, the zero time absorbance was measured at 490 nm using spectrophotometer (Dynex MRX-Revelation, USA). Absorbance readings were recorded at 20 min intervals for 240 minutes. A blank, devoid of β-carotene, was prepared for background subtraction. Percentage of antioxidant activity (AA) was calculated using the following equation (Mayachiew & Devahastin, 2008): % AA = ((DR control-DR sample) / DR control) x 100, where DR is degradation rate of sample (DR = ln (initial absorbance (490 nm) at time zero) / (absorbance at 240 minutes) / t (time in minutes)). EC_{50} (the concentration exhibiting 50 % of the percentage

antioxidant activity) of samples were calculated from the graph of antioxidant activity percentage against concentration of the extracts.

2.3. Antibacterial properties

Six strains of human pathogenic bacteria were used in this assay. Two Gram-negative: *Citrobacter freundii* ATCC 8090 and *Escherichia coli* ATCC 8739 and four Gram-positive: *Staphylococcus aureus* ATCC 11632, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 11778 were used.

2.3.1. Pour-plate disc diffusion method

The antibacterial activity was carried out using the pour-plate disc diffusion method. The soft Mueller Hinton Agar (MHA) inoculated with 100 μL of inocula (1×10^8 microorganism/mL) was spread on MHA. Sterile filter paper discs (6 mm) were impregnated with plant extracts (1 mg and 3 mg) before they were placed on the surface of the inoculated agar plates. The plates were incubated at 37°C for 24 hours (Mayachiew & Devahastin, 2008). The results were obtained by measuring the inhibition zone diameter around the paper disc with a calliper. The experiment was carried out in duplicate in three independent experiments and the results were presented as diameter zone of inhibition ± SD (standard deviation). Streptomycin (1 μg), tetracyclin (1 μg) and ampicillin (1 μg) were used as antibiotic controls.

2.3.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

This assay was conducted according to Otzurk & Ercisli (2006). The extracts were prepared at the concentration ranging from 23 μg/mL to 3000 μg/mL in a 96-well micro plate. Each well were inoculated with 5×10^5 microorganism/mL. The micro plate wells were incubated on an incubator shaker at 180 rpm at 37°C for 24 hours. The turbidity of the well indicated the level of the growth of bacteria. The turbidity was measured by reading the absorbance at 600 nm using spectrophotometer (Dynex MRX-Revelation). The MIC of each extract was recorded as the lowest concentration that showed no growth. The extracts tested on this study were done in three independent experiments for each microorganism. Serial dilution of streptomycin (0.78125-100 μg/mL), ampicillin (0.78125-100 μg/mL) and tetracycline (0.15625-20 μg/mL) were used as standard drugs.

From MIC result, 10 µL were taken and spread on MHA. The number of colony was counted after 18 hours - 24 hours of incubation at 37° C as a result for MBC value.

2.3.3. Death kinetic assay

From the stock solution, a required volume of plant extracts was pipetted into MHB to obtain a concentration range of 0.01 mg/mL up to 10 mg/mL. Readings of the absorbance were taken at 0 min and every 30 minutes for 16 hours. EC_{50} values of the plant extracts, which is the concentration of sample (plant extracts or antibiotics) that kills 50 % of the population of microorganism, was calculated from the graph of dose against percentage of survival index (SI) (Noga, et al., 1994).

$$SI = \frac{OD_{600} \text{ of test sample at corresponding time point}}{OD_{600} \text{ at mid-log of control bacterial growth}} \times 100$$

2.4. Phytochemical screening

Phytochemical analysis was carried out according to Odebiyi & Sofowora (1978) and Trease & Evans (1989).

2.5. Total phenolic contents

The total phenolic contents of *Synedrella nodiflora* (L.) Gaertn. was determined using Folin-Ciocalteu assay, as described by Slinkard and Singleton (1977). Briefly, 20 µL of diluted extracts (1 mg/mL) were mixed with 1.58 mL of water and 100 µL of Folin-Ciocalteu reagent. After standing for 5 minutes at room temperature, 300 µL of sodium carbonate (20 % w/v) were added. The solutions were mixed and allowed to stand for 30 minutes at 40° C. Changes in absorbance were determined at 765 nm against the blank using a UV-Vis Spectrophotometer (Biochrom Libra S12, USA). Gallic acid (50, 100, 150, 250 mg/mL) was used as a calibration standard curve. Results were expressed in mg gallic acid equivalents/g of sample.

2.6. Total flavonoid contents

Estimation of total flavonoid contents in the plant extracts was carried out using the method described by Froehlicher et al. (2009). The flavonoid amount was expressed in mg of quercetin/g of plant extracts.

2.7. Statistical analysis

All data were expressed as mean ± standard deviation. Data were analyzed using one way anova followed by Tukey test using GraphPad Prism5 software. A significant difference was considered at the level of $P < 0.05$.

3. Results

3.1. Antioxidant properties

The reducing ability of the *Synedrella nodiflora* (L.) Gaertn. extracts was in the range of 1.21 mg/mL – 18.88 mg/mL. The water extract exhibited the strongest reducing ability, with a value of 1.24 mg/mL (Table 1). The rank order of potency observed in the β-carotene bleaching assay was trolox > quercetin > ethanol > water > ethyl acetate > hexane extract.

Table 1

Antioxidant properties, total phenolic contents (TPC), and total flavonoid contents (TFC) of plant extracts

Sample	FRAP assay	β-carotene bleaching assay
	FRAP value (mg/mL)	EC_{50} (mg/mL)
Hexane	18.88 ± 0.01[e]	170.2 ± 0.83[c]
Ethyl acetate	3.94 ± 0.02[d]	16.68 ± 0.44[d]
Ethanol	1.21 ± 0.06[cb]	0.29 ± 0.15[a]
Water	1.89 ± 0.08[c]	6.15 ± 0.34[b]
Trolox	1.14 ± 0.02[ca]	0.05 ± 0.17[a]
Quercetin	1.31 ± 0.02[c]	0.13 ± 0.17[a]

Data were obtained from three independent experiments, each performed in triplicates (n=9) and represented as mean ± SD. Data with different letters in the individual plant extracts are significantly different (< 0.05) according to Tukey multiple comparison test

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3.2. Antibacterial properties

Table 2
Inhibition zone diameter of *Synedrella nodiflora* (L.) Gaertn. extracts

Sample	Dose (mg)	Inhibition zone (mm)					
		Ec	Cf	Sa	Se	Bs	Bc
Hexane	1	6.00 ± 0.00	6.58 ± 0.19	8.55 ± 1.19	6.48 ± 0.49	6.65 ± 1.04	7.41 ± 0.67
	3	7.25 ± 1.45	6.55 ± 0.47	8.43 ± 1.77	6.98 ± 0.30	9.17 ± 1.59	7.75 ± 1.71
Ethyl acetate	1	6.00 ± 0.00	6.45 ± 0.53	9.46 ± 1.29	6.68 ± 0.42	6.90 ± 0.98	7.82 ± 1.32
	3	6.00 ± 0.00	6.68 ± 0.31	7.45 ± 0.97	7.15 ± 1.05	7.30 ± 1.01	6.98 ± 0.33
Ethanol	1	6.30 ± 0.36	6.54 ± 0.36	9.20 ± 1.95	6.90 ± 0.54	6.58 ± 0.76	7.15 ± 0.85
	3	7.75 ± 2.18	6.78 ± 0.31	11.65 ± 1.21	7.28 ± 1.76	9.37 ± 1.10	7.80 ± 1.43
Water	1	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.43 ± 0.85	7.53 ± 1.39
	3	7.13 ± 0.90	6.58 ± 0.15	7.90 ± 1.69	6.00 ± 0.00	7.47 ± 0.75	7.88 ± 1.59
S	0.1	11.40 ± 0.66	12.70 ± 0.26	6.60 ± 1.41	6.00 ±0.00	16.44 ± 0.54	14.47 ± 1.25
A	0.1	7.07 ± 0.62	6.00 ± 0.00	10.45 ± 0.69	9.42 ± 0.45	10.77 ± 1.24	6.00 ± 0.00
T	0.1	15.89 ± 1.68	21.21 ± 1.38	20.64 ± 1.39	6.00 ± 0.00	24.15 ± 0.97	20.20 ± 1.85

Data were obtained from three independent experiments where each experiment done in duplicate
Ec: *Escherichia coli*; Cf: *Citrobacter freundii*; Sa: *Staphylococcus aureus*; Se: *Staphylococcus epidermidis*; Bs: *Bacillus subtilis*; Bc: *Bacillus cereus*; S: Streptomycin; A: Ampicillin; T: Tetracycline

Table 3
Minimum inhibitory concentrations and Minimum bactericidal concentration
value of *Synedrella nodiflora* (L.) Gaertn. extracts

Sample	[a]MIC and [b]MBC value (mg/mL)					
	Ec	**Cf**	**Sa**	**Se**	**Bs**	**Bc**
HE	n.a	n.a	3	n.a	1.5	3
EAE	6	n.a	n.a	n.a	6	0.38
EE	6	6	6	6	6	0.75
WE	n.a	n.a	n.a	n.a	n.a	n.a
S	6.25×10^{-3}	$3.13 \times \times 10^{-3}$	2.5×10^{-2}	0.1	3.13×10^{-3}	3.13×10^{-3}
A	0.1	0.1	3.13×10^{-3}	0.05	6.25×10^{-3}	0.1
T	2.5×10^{-3}	2.5×10^{-3}	1.25×10^{-3}	2×10^{-2}	2.5×10^{-2}	3.1×10^{-4}

[a]MIC: Minimum inhibitory concentration
[a]MBC: Minimum bactericidal concentration
Data were obtained from three independent experiments
Ec: *Escherichia coli*; Cf: *Citrobacter freundii*; Sa: *Staphylococcus aureus*; Se: *Staphylococcus epidermidis*;
Bs: *Bacillus subtilis*; Bc: *Bacillus cereus*; HE: Hexane extract; EAE: Ethyl acetate extract; EE: Ethanol
extract; WE: Water extract; S: Streptomycin; A: Ampicillin; T: Tetracycline; n.a: not active.

Table 4
EC_{50} value of *Synedrella nodiflora* (L.) Gaertn. extracts

Sample	EC_{50} (mg/mL)	
	***Staphylococcus aureus* (Gram +)**	***Escherichia coli* (Gram -)**
HE	1.98	1.60
EAE	8.13	1.59
EE	1.84	1.47
WE	1.86	3.03
S	1.15×10^{-3}	3.13×10^{-3}
A	2.25×10^{-3}	9.52×10^{-2}
T	0.34×10^{-3}	$1.35 \times \times 10^{-3}$

EC_{50} is the concentration of sample (plant extracts or antibiotics) that kills 50% of the population of
microorganism.Data were obtained from two independent experiments.
HE: Hexane extract; EAE: Ethyl acetate extract; EE: Ethanol extract; WE: Water extract; S: Streptomycin; A:
Ampicillin; T: Tetracycline

The largest inhibition zones were obtained with the ethanol extract (Table 2).
MIC and MBC values for all the extracts tested were in the range 0.38 mg/mL – 6
mg/mL (Table 3). The ethanol extract abrogated the survival of 5 of the 6
bacterial strain tested and was mostly active against *Bacillus* species.
Staphylococcus aureus (Gram-positive) and *Escherichia coli* (Gram-negative)
were used for death kinetic assay. In this assay, the ethanol extract gave the
lowest EC_{50} value 1.84 mg/mL against *Staphylococcus aureus* and 1.47 mg/mL
against *Escherichia coli*, respectively (Table 4).

3.3. Phytochemical screening

Table 5

Phytochemical screening of *Synedrella nodiflora* (L.) Gaertn. extracts

Sample	Alkaloid	Flavonoid	Phenol	Tannin	Steroid	Triterpenoid	Saponin
HE	-	+	-	+	-	+	-
EAE	-	+	+	+	-	-	-
EE	-	+	+++	-	-	-	-
WE	-	+	++	-	-	-	-

HE: Hexane extract; EAE: Ethyl acetate extract; EE: Ethanol extract; WE: Water extract; S: Streptomycin; A: Ampicillin; T: Tetracycline

-: Negative (absent); +: Positive (Slightly present); ++: Positive (Moderately present); +++: Positive (Considerably present)

Phytochemical screening of *Synedrella nodiflora* (L.) Gaertn. extracts revealed the presence of phenolic compounds (tannins and flavonoids) and triterpenoids (Table 5). The total phenolic contents assay showed that the ethanol extract had the highest phenolic contents with the value of 521 mg/g gallic acid equivalent (Table 6). The standard curve of gallic acid obtained was $y = 0.0011x + 0.0018$ ($R^2 = 0.9996$), with x as the concentration of gallic acid (mg/mL). The flavonoid contents was measured by the aluminium chloride spectrometry method, using quercetin as a standard ($y = 0.0207x - 0.0202$; $R^2 = 0.9993$, with x as the concentration of quercetin in mg/mL). Most flavonoids were found in the ethanol extract (Table 6).

Table 6

Total phenolic (TPC) and total flavonoid content (TFC) of *Synedrella nodiflora* (L.) Gaertn. extracts

Sample	TPC	TFC
	[Gallic acid Equivalent (mg/g)]	[Quercetin Equivalent (mg/g)]
Hexane	69 ± 0.00^a	0.98 ± 0.00^a
Ethyl acetate	272 ± 0.01^a	1.62 ± 0.00^a
Ethanol	521 ± 0.01^a	2.18 ± 0.00^a
Water	486 ± 0.00^a	0.98 ± 0.00^b

Data were obtained from three independent experiments, each performed in triplicates (n=9) and represented as mean ± SD.

[a] Values with the same superscript letter are significantly different ($P<0.05$) according to Tukey multiple comparison test.

4. Discussion

In recent years, much attention has been devoted to natural antioxidants and their association with health benefits (Arnous, et al., 2001). Antioxidants are one of the primary defence mechanisms employed to combat the damaging effects of free radicals (Bunger, et al., 2006). In this study, the antioxidant properties of *Synedrella nodiflora* (L.) Gaertn. extracts were determined using the FRAP and β-carotene bleaching assays. The ethanol extracts displayed significant antioxidant activities. According to the "polar paradox" lipid oxidation occurs at the water/oil interface, where lipophilic antioxidants are located, the more hydrophilic of the sample would have shown a lower antioxidant activity. However, in our study, using β-carotene bleaching assays the crude ethanol extract exhibited higher antioxidant activity than the ethyl acetate and hexane extracts. This might be due to the value of total phenolic and total flavonoid contents in the ethanol extract which is higher than the hexane and ethyl acetate extracts.

Infectious diseases represent a serious health problem worldwide today, mostly due to the resistance of antibiotics. In this study, two doses (1 mg and 3 mg) of *Synedrella nodiflora* (L.) Gaertn. extracts were used in the pour-plate disc diffusion assay. Increasing doses showed increasing antibacterial activity. The ethanol extract was the most active and exhibited inhibition zone diameters superior to 7 mm. According to Gislene et al. (2000), any chemicals that have antibacterial activity with zones of inhibition of 7 mm and above can be considered as potential antimicrobial candidates. Most extracts of *Synedrella nodiflora* (L.) Gaertn. were active against *Staphylococcus aureus* and *Bacillus cereus* but were inactive against *Citrobacter freundii*.

According to Fabry, et al (1998), crude extracts are considered as potentially useful therapeutically if they have MIC values < 8 mg/mL. The MIC and MBC values of *Synedrella nodiflora* (L.) Gaertn. ethanol extract were equal and in the range 0.75 mg/mL – 6 mg/mL. In the death kinetic assay, the ethanol extract had the lowest EC_{50} against *Escherichia coli* and *Staphylococcus aureus*. Overall, our results indicate that the crude extracts were more effective against Gram-positive bacteria than Gram-negative bacteria (Greenberg, et al., 2008). Among them, the ethanol extract negated the growth of all the bacteria tested.

To explore the correlation between secondary metabolites and the biological activities, phytochemical testing of the extracts were conducted. Phenolic compounds (tannins and flavonoids) and triterpenoids were detected. The high concentration of phenolic compounds in the ethanol extract explains the antioxidant and antibacterial activities recorded.

Several reports emphasize on the fact that there is a positive relationship between total phenols and antioxidant activity (Robards, et al., 1999; Oktay, et al., 2003; Ferreira, et al., 2007). In agreement to previous reports, our study revealed

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the existence of a positive correlation between the total phenolic contents and FRAP value (y = -0.0364x + 18.755, r^2 = 0.8383) and total phenolic contents and β-carotene bleaching value (y = -0.3446x + 164.46, r^2 = 0.7867).

Flavonoids have strong antioxidant activities due to their ability to scavenge free radical and terminate radical chain reactions (Bors, et al., 1990). This ability allows them to interfere with the pathophysiology of inflammation (Conner & Grisham, 1996). Flavonoids have been reported to possess antibacterial properties (Havsteen, 2002). Their mode of action is based on the inhibition of the macromolecular (DNA, RNA and protein) or lipid synthesis in the bacteria (Mori, et al., 1987). Flavonoids are most efficient against Gram-positive bacteria than Gram-negative bacteria because of the lipid content of Gram-negative cell wall is greater than Gram-positive bacteria (Klancnik, et al., 2009; Cushnie & Lamb, 2005). The present study suggests that the ethanol extract of *Synedrella nodiflora* (L.) Gaertn. has antioxidant and antibacterial properties on account of phenolic compounds, of which flavonoids. The present results substantiate the antiseptic and anti-inflammatory traditional uses of the plant.

5. Conclusion

Extracts of *Synedrella nodiflora* (L.) Gaertn. displayed promising antimicrobial and antioxidant activities. Total phenolic and flavonoid contents showed a positive correlation with the antioxidant activities exhibited. The present study demonstrates that the ethanol extract of *Synedrella nodiflora* (L.) Gaertn. has antioxidant and antibacterial properties on account of phenolic compounds, of which flavonoids.

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