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Research Article

The important biological activities and phytochemistry of *Acalypha indica*

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ABSTRACT

Different plant extracts of *Acalypha indica* leaves, stems and roots were investigated for their antioxidant capacity, antibacterial activity, antidiabetic activity, cytotoxicity and phytochemicals. Plant extracts were prepared using three extraction techniques including maceration, Soxhlet extraction and sonication using five different solvents which are hexane, acetone, ethyl acetate, methanol and water. Antioxidant capacities were investigated using three assays and they are Folin-Ciocalteu assay, DPPH radical scavenging assay and FRAP assay. According to Folin-Ciocalteu and FRAP assays, the highest antioxidant capacities were shown by the methanolic Soxhlet extract of leaves. The methanolic macerated stem extract indicated the highest radical scavenging capacity in the DPPH assay. Antibacterial assay was carried out using Kirby-Bauer disk diffusion assay using four bacterial strains. Several *A. indica* extracts indicated antibacterial activities against *Staphylococcus aureus* and *Bacillus cereus*, whereas no significant antibacterial activity was observed against *Escherichia coli* and *Salmonella typhimurium*. Highest antidiabetic activity was observed for the hexane Soxhlet extract followed by the macerated ethyl acetate extract of leaves.Brine shrimp lethality assay was carried out for selected fractions which gave a significant biological activity in order to determine their cytotoxicity levels at higher concentrations.Phytochemical analysis of the bioactive extracts of *A. indica* indicated the presence of tannins, alkaloids, terpenes, steroids and phenols.

Key words: *Acalypha indica; antioxidant; antidiabetic; antibacterial; Folin-Ciocalteu.*

INTRODUCTION

Wild plants which grow naturally and aggressively are known as weeds. They inhibit the growth of useful plants in agriculture, gardens etc[1].They compete with other plants for water, nutrients, space and light. They are one of the main reasons for the reduction of crop yield and quality.[2] However; some weeds have certain beneficial characteristics as well. For an example, weeds such as *Centella asiatica* (Indian pennywort), *Alternanthera sessilis* (Dwarf copper leaf) are edible, *Abutilon indicum* (Indian abutilon) and *Leucas zeylanica* (Ceylon slitwort) are used to treat haemorroids and worm infestations respectively. In some countries like India and Sri Lanka, some of the weeds are used in their traditional medicine.[3]Different parts of the plant (leaves, stems, roots) or the entire plant is used in various preparations to treat different diseases.[4] *Acalypha indica*, also known as Indian acalypha1is a weed that can be found in Sri Lanka, India, tropical Africa, Pakistan and many other countries. It has been reported to be used in Sri Lankan and Indian indigenous medicine to treat bronchial asthma, constipation, skin disorders, snake bites, etc.[1,3]Due to the important medicinal properties reported to be associated with this weed, different parts of *Acalypha indica* plant were extracted with maceration, Soxhlet extraction and sonication techniques using a range of solvents and these plant extracts were investigated for their antioxidant capacity, antibacterial activity, antidiabetic activity, cytotoxicity and phytochemistry.

MATERIALS AND METHODS

Plant identification

Acalypha indica was collected from the areas of Western province of Sri Lanka. Herbarium specimen was

authenticated by the Department of Plant Sciences, University of Colombo.

Plant extracts preparation

Collected *Acalypha indica* plants were thoroughly washed with running water. Leaves, stems and roots were separately air dried and were coarsely powdered using a domestic grinder. Plant extractions were prepared usingthree extraction methods including maceration, Soxhlet extraction and sonication for leaves, stems and roots separately. Hexane, ethyl acetate, acetone, methanol and water were used as the extraction solvents.

Preparation of plant extractions using maceration

A known weight of the plant sample was mixed with the solvent in the ratio of 1:10 (For 5 g of plant leaves 50 mL of the solvent was used). It was shaken for 24 hours at 37 °C in a shaker set to a speed of 150 r.p.m. Then the mixture was filtered and the solvent was evaporated, first using a rotary evaporator (35 °C) and then a nitrogen stream.[5]

Preparation of plant extracts using Soxhlet extraction

A known weight of the plant sample was added into the thimble of the Soxhlet extractor. A volume of 100 mL of the solvent was placed in the round bottom flask and the extraction was carried out for 4 hours with each solvent. Then the extract was filtered and the solvent was evaporated using a rotary evaporator (35 °C) and a nitrogen stream.[6]

Preparation of plant extracts using sonication

A known weight of the plant sample was mixed with the solvent in the ratio of 1:10. Then it was placed in the ultrasonic bath for 2 hours at a temperature of 35 °C. Then the mixture was filtered and the solvent was evaporated using a rotary evaporator (35 °C) and a nitrogen stream.[1]

Folin-Ciocalteu's test

A volume of 100 µL of the methanolic plant extracts or pyrogallol solutions were added into test tubes. Then a volume of 2 mL of sodium bicarbonate (2%) was added, mixed and was incubated for 2 minutes. A volume of 100 µL of the Folin-Ciocalteu's reagent was added. The mixture was incubated for 30 minutes. Absorbance values were measured at 750 nm. As the blank, 100 µL of methanol was used instead of the plant extract or pyrogallol. All experiments were carried out in triplicate.[7]

DPPH free radical scavenging activity

Then a volume of 1.9 mL of DPPH solution was added into a test tube and 100 µL of the plant extract was added to it. (A_T) The mixture was kept in dark for 30 minutes at room temperature. A solution containing 1.9 mL methanol and 100 µL of plant extract was taken as the test blank. A volume of 2.0 mL of DPPH solution was taken as the control (A_C) and a volume of 2.0 mL of methanol was used as the control blank. All of the samples were incubated in dark for 30 minutes at room temperature and the absorbance values of these samples were measured at 517 nm.[8] Then the percentage DPPH radical scavenging activity (SCV%) was calculated using the following equation. All experiments were carried out in triplicate.[9]

$$SCV\ \% = \frac{(A_C)-(A_T)}{(A_C)} \times 100\% \qquad (1)$$

A_C = Absorbance of the control at 517 nm
A_T = Absorbance of plant extract at 517 nm

FRAP assay

Ascorbic acid (AA) was taken as the standard and a series of ascorbic acid standards (50 ppm-500 ppm) were prepared using methanol. A volume of 50 µL of AA or plant extract was mixed with 1.0 mL of 1% potassium ferricyanide and 1.0 mL of 0.2 M phosphate buffer (pH 6.6). The reaction mixture was incubated for 20 minutes at 50 °C. Then a volume of 1.0 mL of 10% TCA was added to the reaction mixture. Then the mixture was centrifuged at 2000 r.p.m for 8 minutes. A volume of 1.0 mL of the supernatant was mixed with 1.0 mL of distilled water. Then a volume of 1.0 mL of 0.1% ferric chloride was added. Absorbance values were measured at 700 nm. The same mixture with methanol instead of the extract or AA was taken as the blank. All experiments were carried out in triplicate.[10,8]

Antibacterial activity

Kirby-Bauer disk diffusion assay was carried out against *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778) and *Salmonella typhimurium*. Gentamycin (1 mg mL^{-1}) was used as the positive control and 5% Dimethyl sulfoxide (DMSO) was used as the negative control for the disk diffusion assays. After 24 hours of incubation at 37 °C, the diameters of the inhibition zones were measured using a ruler. All experiments were carried out in triplicate.[11]

Antidiabetic assay

A volume of 200 µL of the plant extract was placed in a test tube. Then a volume of 200 µL of α-amylase in 0.02 M phosphate buffer (pH 6.9) was added. The mixture was kept for 15 minutes at room temperature. Then a volume of 200 µL of 1% starch solution in phosphate buffer was added. Again the mixture was incubated for 15 minutes at room temperature. Then a volume of 400 µL of 3,5-Dinitrosalicyclic acid (DNS) reagent was added. (DNS reagent was prepared by dissolving 0.1 g of DNS, 2.99 g of sodium potassium tartarate and 0.16 g of sodium hydroxide in 10.0 mL of phosphate buffer) Then the test tube was kept in a boiling water bath for 5 minutes and was cooled to room temperature. The reaction mixture was diluted with 1 mL of distilled water and the absorbance was measured at 540 nm. This test sample was blanked with the same mixture where 400 µL of buffer was used instead of the DNS reagent. Same mixture where 200 µL of DMSO was used instead of the plant extract was taken as the control sample. Control sample was blanked with the same mixture where 400 µl of buffer was used instead of the DNS reagent. The percentage α-amylase inhibition was calculated using the following equation. All of the extracts which were used in this assay were of 20 mg mL-1

concentration. All experiments were carried out in triplicate.[12]

$$Percentage\ inhibition\ (\%) = \left\{\frac{(A_C)-(A_T)}{A_C}\right\} \times 100\ \% \quad (2)$$

A_C = Absorbance of the control sample at 540 nm
A_T = Absorbance of the test sample at 540 nm

Brine shrimp lethality assay

Ten living nauplii were added to each of the test tubes containing a volume of 5 mL 3.8% of sodium chloride. To each test tube 50 µL of plant extracts were added. This assay was carried out for certain plant extracts which indicated an interesting biological activity. A volume of 50 µL of 5% DMSO was taken as the blank. Number of nauplii in each test tube was counted hourly and percentage mortality was calculated. All of the extracts which were used in this assay were of 100 mg mL-1 concentration. All experiments were carried out in triplicate.[13]

Phytochemistry analyses

Phytochemistry analyses were carried according to previously published procedures.[6,14,15]

RESULTS AND DISCUSSION

Percentage Yield

Since the compounds being extracted could depend on the extraction technique, three extraction techniques were used including maceration (M), Soxhlet extraction (S) and sonication (SN). Hexane (H), ethyl acetate (EA), acetone (A) and methanol (MeOH) were used as the solvents to prepare leaf, stem and root extracts. Additionally, aqueous extracts of the *A. indica* leaves were also prepared using the three extraction methods. Compared to the stems and roots, leaves resulted higher percentage yields regardless of the extraction method. The highest percentage yields were obtained by the Soxhlet extraction method for leaves (water - 13.4%), stems and roots (methanol - 6.0% and 5.5% respectively). The lowest percentage yields were observed for the sonication method. Therefore, among the three extraction techniques Soxhlet extraction is more suitable as an extraction technique to obtain a higher percentage yield of the plant material.

Antioxidant Capacity (AOC)

Antioxidant capacity of *Acalypha indica* was investigated using the Folin-Ciocalteu assay, DPPH radical scavenging assay and a modified version of the Ferric Reducing Antioxidant Power assay (FRAP assay).

Folin-Ciocalteu's antioxidant capacity test (FC assay)

FC assay has been more commonly utilized to measure the total phenolic content of plant extracts. However, it has been recently reported to be more appropriate to the total AOC of a plant extract due to the reagents ability to react with a wide range of antioxidants in addition to the phenolic derivatives.[16] The FC assay is based on the ability of the antioxidant materials in the extract to reduce the yellow coloured Mo (VI) complex to the blue coloured Mo (V) complex. According to the results obtained for the FC assay (Table 1), the highest AOC was observed for the methanolic Soxhlet extract of leaves (100 µg PGE/mg). A comparable AOC was also observed for the leaf extract obtained by sonication in acetone (94 µg PGE/mg). Compared to the stems and roots, leaves displayed a significant AOC.

Table 1: AOCs of the leaf, stem and root extracts of *Acalypha indica* according to the FC assay

Solvent[a]	Method of extraction[b]	Antioxidant capacity in µg PGE/mg[c]		
		Leaves	Stems	Roots
H	M	10 ± 0	3 ± 0	10 ± 1
	S	38 ± 3	17 ± 1	5 ± 1
	SN	9 ± 1	39 ± 0	8 ± 1
EA	M	30 ± 1	15 ± 0	11 ± 1
	S	63 ± 2	65 ± 4	41 ± 6
	SN	38 ± 1	35 ± 2	45 ± 0
A	M	61 ± 1	32 ± 2	24 ± 0
	S	41 ± 1	25 ± 3	37 ± 1
	SN	94 ± 1	22 ± 1	19 ± 1
MeOH	M	41 ± 1	62 ± 2	69 ± 3
	S	100 ± 5	27 ± 2	48 ± 1
	SN	31 ± 1	40 ± 1	45 ± 0
W	M	15 ± 0	-	-
	S	63 ± 4	-	-
	SN	14 ± 1	-	-

Note: [a]H-Hexane, EA-Ethyl acetate, A-Acetone, MeOH-Methanol, W-Water. [b]M- Maceration, S- Soxhlet extraction, SN- Sonication.[c]PGE – Pyrogallol equivalents

DPPH radical scavenging activity

Ability of the antioxidants to reduce the DPPH radical is measured during this assay. Depending on the plant part (Table 2), the highest DPPH radical scavenging percentages were observed for the aqueous Soxhlet extract of leaves (92%), methanolic macerated extract of stems (95%) and methanolic Soxhlet extract of roots (93%) at the tested concentrations. In general, the leaf extracts of *A. indica* displayed higher DPPH radical scavenging activity compared to that of stems and roots.

Table 2: DPPH radical scavenging percentages of leaf, stem and root extracts of *Acalypha indica*

Solvent[a]	Method of Extraction[b]	Percentage DPPH SCV (%)		
		Leaves (6.25 mg mL^{-1})	Stems (10 mg mL^{-1})	Roots (10 mg mL^{-1})
H	M	81 ± 0	11 ± 1	14 ± 1
	S	43 ± 3	27 ± 1	16 ± 1
	SN	71 ± 5	22 ± 0	8 ± 1
EA	M	90 ± 5	20 ± 1	7 ± 0
	S	86 ± 1	70 ± 4	70 ± 3
	SN	75 ± 2	20 ± 1	43 ± 3
A	M	90 ± 6	50 ± 1	29 ± 0
	S	69 ± 2	47 ± 2	45 ± 4
	SN	45 ± 2	17 ± 3	35 ± 1
MeOH	M	75 ± 9	95 ± 0	92 ± 1
	S	22 ± 7	61 ± 0	93 ± 1
	SN	49 ± 1	46 ± 1	86 ± 2

W	M	13 ± 1	-	-
	S	92 ± 0	-	-
	SN	6 ± 1	-	-

Note: [a]H- Hexane, EA- Ethyl acetate, A- Acetone, MeOH- Methanol, W-Water. [b]M-Maceration, S- Soxhlet extraction, SN- Sonication. [c]SCV-radical scavenging

FRAP assay

In this assay the ability of the antioxidants in the plant extracts to reduce Fe^{3+} to give Fe^{2+} is monitored through a colour change observed from yellow to blue green. The highest ferric reducing antioxidant power among the leaf extracts was observed for the methanolic Soxhlet extract (36 µg AAE/mg) whereas the methanolic macerated extracts indicated the highest ferric reducing antioxidant power among the of stem and root extracts (34 and 29 µg AAE/mg respectively**)**.(Table 3)The overall results indicate that the compounds which are capable of reducing the Fe^{3+} ions in the ferricyanide complex are getting extracted into polar solvents more easily compared to the nonpolar solvents such as hexane.

Solvent	Method of extraction	Ferric Reducing Antioxidant Power (µg AAE/mg)[c]		
		Leaves	Stems	Roots
H	M	15 ± 4	8 ± 2	3 ± 0
	S	3 ± 1	8 ± 1	3 ± 1
	SN	6 ± 1	10 ± 2	6 ± 0
EA	M	11 ± 0	10 ± 4	4 ± 0
	S	18 ± 3	27 ± 5	21 ± 0
	SN	18 ± 1	25 ± 2	17 ± 4
A	M	10 ± 0	18 ± 2	6 ± 0
	S	29 ± 2	22 ± 1	20 ± 1
	SN	21 ± 4	16 ± 2	8 ± 2
MeOH	M	17 ± 3	34 ± 1	29 ± 1
	S	36 ± 4	17 ± 2	28 ± 1
	SN	32 ± 2	5 ± 2	23 ± 2
W	M	9 ± 1	-	-
	S	33 ± 3	-	-
	SN	16 ± 2	-	-

Note: [a]H-Hexane, EA-Ethyl acetate, A-Acetone, MeOH-Methanol,W-Water. [b]M- Maceration, S- Soxhlet extraction, SN- Sonication. [c]AAE – Ascorbic acid equivalents

Antibacterial activity

Disk diffusion assay was carried out for all the prepared plant extracts of *A. indica*. However, only few plant extracts were active against *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778) at the tested concentrations and none of them showed any inhibition zones for *Escherichia coli* (ATCC 35218) and *Salmonella typhimurium* (ATCC 14028).(Table 4- Only the data for antibacterial active extracts are shown). Previous studies carried out in different countries have reported *A. indica* plant extracts to have higher antibacterial activity against *S. aureus* and *B. cereus*, even at lower concentrations.[17] The differential antibacterial activity of the extracts seen during different studies might be due to a regional effect or due to slight differences in the extraction procedures. However, in the previous studies also, antibacterial activity was not observed against *E. coli* and *S. typhimurium* for most of the *A. indica* extracts similar to this study.

Table 4: Antibacterial activity of leaf, stem and root extracts of *Acalypha indica*

Solvent	Method of extraction	Part of the plant	Diameter of the inhibition zones (mm)		
			Concentration mg mL^{-1}	*B. cereus*	*S. aureus*
H	S	Leaf	200	7.0 ± 0.0	7.0 ± 0.0
MeOH	M		50	8.2 ± 0.3	NI
W	M		190	7.0 ± 0.0	7.0 ± 0.0
	S		250	NI	7.0 ± 0.0
MeOH	M	Stem	800	NI	7.0 ± 0.0
A	S	Root	75	NI	7.0 ± 0.0
Positive control			1	25.3 ± 0.9	21.0 ± 0.0
Negative control				NI	NI

Note: NI – No inhibition

Antidiabetic activity (α-amylase enzyme inhibitory assay):

The ability of the *α*-amylase inhibitors to prevent the degradation of starch to glucose is monitored through a colourimetric reaction. Most of the leaf extracts indicated varying amounts of *α*-amylase inhibitory activity. The highest amount of *α*-amylase inhibitory activity was displayed by the hexane Soxhlet (78 %) and ethyl acetate macerated (75 %) extracts. (Table 5)

Table 5: Percentage of *α*-amylase inhibition of the leaf extracts (20 mg mL^{-1}) of *Acalypha indica*

Solvent[a]	Method of extraction[b]	Percentage *α*-amylase inhibition (%)
H	M	23 ± 0
	S	78 ± 1
	SN	25 ± 2
EA	M	75 ± 6
	S	66 ± 2
	SN	57 ± 13
A	M	69 ± 5
	S	NI
	SN	15 ± 2
MeOH	M	46 ± 11
	S	71 ± 10
	SN	NI
W	M	18 ± 2
	S	NI
	SN	

Note: NI - No inhibition.[a]H- Hexane, EA- Ethyl acetate, A- Acetone, MeOH- Methanol, W-water. [b]M- Maceration, S- Soxhlet extraction, SN- Sonication

Cytotoxicity (Brine shrimp lethality assay)

Brine shrimp lethality assay can be used to investigate the cytotoxic effects of plant extracts as such data could be important when incorporating these plant extracts into medicinal preparations.[18] The least cytotoxicity was given by the sonicated ethyl acetate extract and followed byacetone Soxhlet extract (Table 6 and Figure 1). This suggests that this extract might be a better option for direct usage of the plant extracts.

Table 6: Results of the brine shrimp lethality assay carried out with selected leaf extracts of *Acalypha indica*

Solvent	Method of extraction	Percentage mortality with time (%)						
		1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour	7th hour
EA	M	0 ± 0	7 ± 6	13 ± 3	17 ± 3	27 ± 7	43 ± 3	97 ± 3
	SN	0 ± 0	0 ± 0	3 ± 3	7 ± 2	7 ± 2	10 ± 0	23 ± 3
A	S	0 ± 0	0 ± 0	3 ± 3	7 ± 2	13 ± 3	17 ± 2	30 ± 0
MeOH	S	3 ± 3	10 ± 5	20 ± 0	27 ± 6	37± 6	63 ± 12	77 ± 2
W	S	0 ± 0	0 ± 0	27 ± 2	30 ± 6	37 ± 2	50 ± 2	63 ± 3
	SN	0 ± 0	0 ± 0	3 ± 3	13 ± 7	23 ± 3	27 ± 7	37 ± 7
Control		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Note: [a]EA-Ethyl acetate, A-Acetone, MeOH-Methanol, W-Water. [b]M-Maceration, S- Soxhlet extraction, SN- Sonication.

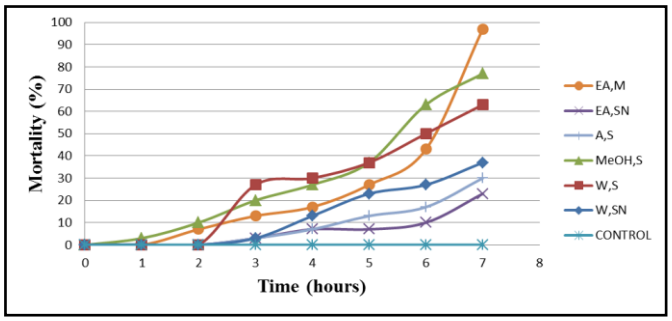

Figure 1: Graphical representation of the results of the brine shrimp lethality assay carried out with selected bioactive leaf extracts of *Acalypha indica*

Note: The figure legends indicate the solvent followed by the extraction method. EA- Ethyl acetate, A- Acetone, MeOH-Methanol, W-water, M- Maceration, S- Soxhlet extraction, SN-Sonication

Phytochemical analyses

Phytochemical analysis of the bioactive fractions displayed the presence of important secondary metabolites such as tannins, phenols, alkaloids, terpenes and steroids. (Table 7) Some of the plant extracts displayed positive results for reducing sugars. Both methanolic and aqueous Soxhlet extracts which displayed higher AOCs indicated the presence of phenols. Different bioactivities observed from these extracts might be due to the diversity of the secondary metabolites present in these extracts.

Table 7: Phytochemical analyses of the selected leaf extracts of *Acalypha indica*

Phytochemical test		H, M	EA, SN	A, S	MeOH, M	MeOH, S	W ,S	W, SN
Tannins and phenols	(a)	-	-	-	+	+	+	+
	(b)	-	-	-	+	+	+	+
Saponins		+	+	+	+	+	+	+
Flavanoids		-	-	-	-	-	-	-
Terpenes and steroids		-	-	-	+	+	+	+
Alkaloids		-	-	-	-	+	-	+
Reducing sugars		-	-	+	-	+	+	-

Note:
(+) ⟶ positive (-) ⟶ negative
(a) $FeCl_3$ test (b) Lead acetate test
H-Hexane, EA-Ethyl acetate, A-Acetone, MeOH-Methanol, W-water. M- Maceration, S- Soxhlet extraction, SN- Sonication

CONCLUSION

Based on the results of this study, it is possible to conclude that *Acalypha indica* consists of numerous important biological activities including antioxidant, antidiabetic and antimicrobial activities. The extraction technique and the solvent should be carefully chosen according to the desired bioactivity.

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