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Insights into the *in vitro* germicidal activities of *Acalypha indica*

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Abstract: Background and purpose: This study was carried out to learn more about the potential prophylactic or antibacterial activity of the plant *Acalypha indica* against selective pathogenic bacteria. **Experimental:** The test organisms were *Sarcina lutea* IFO 3232, *Bacillus subtilis* IFO 3026, *Pseudomonas denitrificans, Escherichia coli* IFO 3007, *Klebsiella pneumoniae* ATTC 10031, *Xanthomonas campestris* IAM 1671, and *Proteus vulgaris*. Leaf, stem, and bud powder of *Acalypha indica* were dissolved in various solvents, and the extracts were tested for antimicrobial activity through the disc diffusion method. GC-MS profiling was performed to characterize active chemical compounds in the essential oil of *Acalypha indica*. **Results:** The ethanol extract showed the highest activity against all bacteria, while the petroleum ether extract yielded the highest zone of inhibition against *Proteus vulgaris* (11.83 ± 1.75 mm). The minimum inhibitory concentration (MIC) of the ethyl acetate extract against *Bacillus subtilis* was 16 μg/mL. Phytochemical screening by GC-MS revealed a total of 12 bioactive compounds. **Conclusion:** Extracts of *Acalypha indica* may be useful in formulating and synthesizing new antibacterial drugs.

Key words: *Acalypha indica*, antibacterial activity, MIC, GC-MS

Abbreviations

MIC : minimum inhibitory concentration
ZOI : zones of inhibition
GC-MS : gas chromatography-mass spectrometry
EI : electron impact ionization
eV : electron volt
m/z : mass-to-charge ratio
SD : standard deviation
SPSS : statistical package for the social sciences
TIC : total ion chromatogram
NIST : national institute standard and technology
RT : retention time

1. Introduction

Pathogenic bacteria are one of the most serious threats to human health; infectious diseases caused

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by pathogenic bacteria are the second leading cause of death worldwide.[1] To protect against bacterial diseases, a large number of commercial antibiotics have been developed. However, extensive use of antibiotics has led to the emergence of multidrug resistance among pathogenic bacteria.[2] This situation has forced scientists to search for new antimicrobial substances from various sources. Screening of plant extracts and products for antimicrobial activity has shown that plants are a potential source of novel antibiotic prototypes.[3]

The use of plants as medicines is a universal phenomenon.[4] Plants are a valuable source of natural products for maintaining human health and have been studied intensively in the last decade to develop natural therapies.[5] Use of phytochemicals for pharmaceutical purposes is increasing in many countries,[6] with more than 25 % of modern medicines derived from plants.[7] About 80 % of individuals from developed countries have been estimated to use traditional medicines containing compounds derived from medicinal plants.[8] Medicines derived from plants generally have many advantages, including fewer adverse effects and/or less side effects than chemically synthesized medicines while typically being inexpensive, environmentally friendly, and clinically stable for long periods of time.[9]

Acalypha indica Linne is a member of the plant family Euphorbiaceae. It is considered a weed as it grows in fields and waste areas throughout the hotter parts of the world.[10] It is an erect, annual herbaceous plant with numerous ascending branches. The leaves have long petioles (up to 12 cm long), arranged spirally, and are simple with blades that are ovate or rhombic-ovate, acute, cuneate at the base.[11] As a traditional medicine, this herb is used to cure asthma, pneumonia, rheumatism, and several other diseases.[12] The ethanolic extract of *A. indica* was found to have wound healing activity.[13] Leaf extract has been applied to pustules and insect bites.[14] The extract of the root was shown to lower blood glucose level up to 30 %. It is also used to treat skin disease, snake bite, and jaundice.[15,16]

In the present study, we investigated the antibacterial activity of various organic extracts of *A. indica* against several human pathogenic bacteria. To this end, the chemical composition of the essential oil from *A. indica* was investigated by gas chromatography-mass spectrometry (GC-MS), with an emphasis on the possible future use of extracts of this plant as alternatives to chemically synthesized antibiotics and/or as substances for generic drug design.

2. Experimental

2.1. Plant materials

The plant material used in this study was collected from the Jessore region of Bangladesh during April 2013. It was identified and authenticated by Bushra Khan, Principal Scientific Officer, National Herbarium, Mirpur, Dhaka 1216, Bangladesh; a voucher (DACB 38572) of the corresponding work has been deposited.

2.2. Preparation of extracts

Fresh leaves were collected, cleaned with running tap water and distilled water, chopped into small pieces, and air dried under shade at room temperature for 15 to 20 days. The dried leaves were pulverized into fine powder using a grinding machine, and the powder was stored in a dark, airtight container. Then, powdered leaves (10 g) were suspended in each of the following solvents – methanol, ethanol, ethyl acetate, *n*-hexane, petroleum ether, and dichloromethane - inside a conical flask and shaken for 24 h at room temperature. The resulting solutions were subject to filtration using Whatman No 1 filter paper. Finally, all extracts were concentrated to create 4 mg/mL stock solutions and stored in a refrigerator at 4 °C in sterile containers for further use.[17]

2.3. Test organisms

Seven pathogenic bacterial strains were used for the current research: *B. subtilis* IFO 3026, *S. lutea* IFO 3232, *X. campestris* IAM 1671, *E. coli* IFO 3007, *K. pneumoniae* ATTC 10031, *P. vulgaris* MTCC 321, and *P. denitrificans* KACC 32026. These strains were collected from the Microbiology Laboratory of the Department of Biotechnology and

Genetic Engineering, Islamic University, Kushtia, Bangladesh.

2.4. Antibacterial assay

In vitro antimicrobial activity was determined using the disc-diffusion method.[18] Whatman No. 1 filter paper discs with a diameter of 5.5 mm were prepared by infusing the discs with 300 mg of extract. To determine minimum inhibitory concentration (MIC), blank filter paper discs were infused with different concentrations of extracts: 16, 32, 64, 128, 256, 512, 1024, and 2048 mg/mL.[19] Test bacteria were grown on petri dishes containing nutrient agar media, and dried paper discs were placed on them. Standard antibiotic discs were used as positive controls, while the respective solvents were used as negative controls. Plates were incubated at 37 °C for 24 hours. After incubation, the size of the zone of inhibition (ZOI) was recorded in millimeters.

2.5. GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out by the Bangladesh Council of Scientific & Industrial Research, Dhaka. Agilent Technologies 7890A (GC Model) and Agilent Technologies 5975C (MS model) inert XL EI/CI MSD with a triple axis detector were used to perform GC-MS analysis. One microliter of extract was injected in splitless mode into the injection port of the GC system. The inlet temperature was set at 250 °C, and the oven temperature was programmed as 60 °C for 0 min, followed by ramping to 240 °C min^{-1} at 5 °C min^{-1} for 4 min. Total run time was 40 min. Helium gas was used as the carrier gas at a constant flow rate of 1.0 ml/min. The interface transfer line temperature was set at 280 °C.

MS detection was set in scan mode. Quadrupole analyzer temperature was 230 °C; ion source temperature was 150 °C. Ions were obtained by electron ionization (EI) mode at 70 eV. The scan time and mass range were 1 s and 50–550 *m*/*z*, respectively.[20]

2.6. Statistical analysis

Each experiment was performed in triplicate, and mean values and standard deviations (SD) were calculated using SPSS software package (version 11.0; SPSS Inc., Chicago, IL).

3. Results

3.1. Antibacterial assay

Various organic extracts of *A. indica* had antibacterial activity against Gram-positive and Gram-negative pathogenic bacteria. Activity was assessed by the presence or absence of inhibition, and this zone, when present, was measured (see *Fig.* 1). Ethanol extract of *A. indica* exhibited good antibacterial activity against all bacteria (zone ranging from 7 to 12 mm), while the dichloromethane extract showed antibacterial activity against *S. lutea*. Petroleum ether, *n*-hexane, and methanol extracts had moderate activities against *S. lutea* and *X. campestris* (6.5 to 9 mm). The ethyl acetate extract also showed moderate activity against *P. vulgaris* (8.5 mm). The highest zone of inhibition exhibited by petroleum ether extract was against *P. vulgaris* (13.5 mm). Minimum inhibitory concentration (MIC) values of various extracts against the tested pathogenic bacteria are reported in *Fig.* 2. The lowest MIC value of the ethyl acetate extract was 16 μg/mL against *B. subtilis*. Ethyl acetate and petroleum ether extracts had the second lowest MIC values against *S. lutea, X. campestris*, and *P. vulgaris.*

3.2. Chemical composition of the essential oil of *A. indica* by GC-MS

GC-MS analysis of the essential oil led to reliable identification of 12 different compounds. The

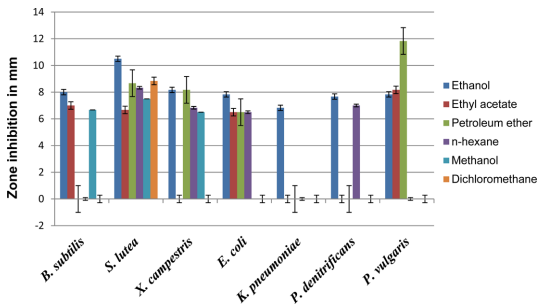

Fig. 1. Zone inhibition by various extracts of *A. indica*. Values are given in mm as mean ± SD (n=3).

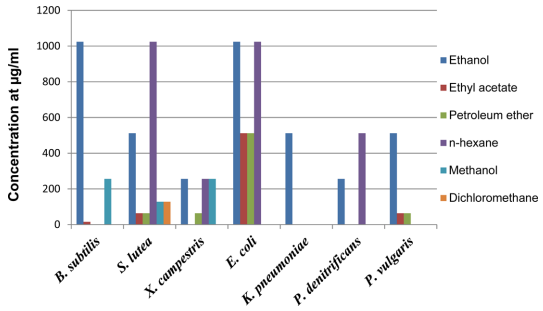

Fig. 2. MIC values of various extracts of *A. indica*in µg/mL.

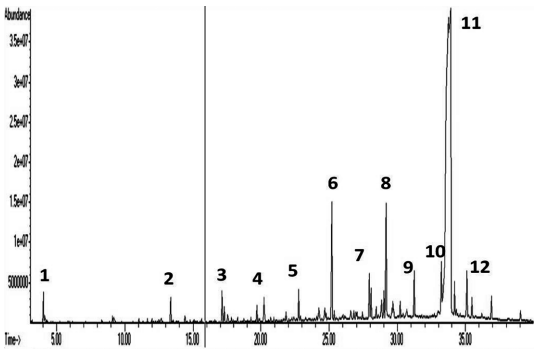

Fig. 3. Total ion chromatogram of essential oil extract of *A. indica* (1 µL of sample).

compounds extracted from *A. indica* are shown in *Table* 1. A typical total ion chromatogram (TIC) of the oil extract is shown in *Fig.* 3; 12 characteristic peaks were present. The major compounds detected in oil were phytol isomer (35.5 %), palmitaldehyde (3.74 %), heneicosan (1.83 %), docosane (1.51 %), and eicosane (1.24 %). The relative composition, as shown in brackets in *Fig.* 3, was computed as the relative proportion of the GC-MS peak areas on column: the peak area of each target was divided by the summed area of all targets and multiplied by 100 %.[21] Trace amounts of 9,12,15-octadecatrienoic acid, neophytadiene, and tetradecanal were also detected.

4. Discussion

Medication without side-effects is rare. Herbal medicines can be, however, exceptional in this regard. They might be the best alternatives to antibiotics for treating severe pathogens. We assessed the germicidal activities of extracts of *Acalypha indica*, which is a member of the Euphorbiaceae family. Numerous reports have shown that plants in this family possess anti-microbial activity.[22,23] Consistent with the results of the currently study, water and ethanol extracts of *Bridelia ferruginea* (Euphorbiaceae) showed antimicrobial activities against many pathogenic bacteria *in vitro*.[24] The ethanolic extract of *A. indica* had the greatest antimicrobial activity against all investigated pathogens. Other researchers have also assessed the antibacterial efficacy of ethanolic extract of *A.* indica.[25,26] Assessment has also been performed on the prophylactic activities of *A. indica* with several

Table 1. Chemical composition of essential oil of *A. indica* (spectrum was interpreted based on the National Institute Standard and Technology (NIST) library).

Peak no.	Retention time (RT)	Area %	Name of compound	Formula
1	4.014	0.64	(Z)-3-Hexen-1-ol,	$C_6H_{12}O$
2	13.375	0.56	(E)-3,7-dimethyl-2,6-Octadien-1-ol	$C_{10}H_{18}O$
3	17.134	0.73	10-(Acetylmethyl)-(+)-3-carene	$C_{13}H_{20}O$
4	20.236	0.76	Tridecanal	$C_{13}H_{26}O$
5	22.759	0.75	Tetradecanal	$C_{14}H_{28}O$
6	25.208	3.74	Palmitaldehyde	$C_{16}H_{32}O$
7	27.920	1.14	Neophytadiene	$C_{20}H_{38}$
8	29.173	0.81	9,12,15-Octadecatrienoic acid	$C_{18}H_{32}O$
9	31.245	1.24	Eicosane	$C_{20}H_{42}$
10	33.213	1.83	Heneicosane	$C_{21}H_{44}$
11	33.745	35.48	Phytol isomer	$C_{20}H_{40}O$
12	35.101	1.51	Docosane	$C_{22}H_{46}$

extracts such as methanol, acetone, chloroform, petroleum ether, and *n*-hexane.[27] Findings in this previous study were similar to those in the present study. Moreover, findings were common for strains such as *E. coli, B. subtilis, K. pneumonia,* and *P. vulgaris.* The MIC values of *A. indica* found here were similar to those reported in a previous study.[28]

The components of volatile essential oils can be identified by GC-MS analysis. Organic extracts of *A. indica* were subjected to GC-MS study to identify their components. Major components (in terms of GC-MS peak areas) of *A. indica* essential oil were phytol isomer and palmitaldehyde. Previous studies have shown that these components have antimicrobial activity.[29,30] Minor compounds (in terms of GC-MS peak areas) like heneicosane, docosaneare, eicosane, and neophytadine also have some antibacterial activity. The antibacterial effects of these minor compounds may be conferred by associating with other active compounds.

5. Conclusions

This study was performed to determine the phytochemical composition of *A. indica* essential oil and to evaluate the antibacterial efficacy of various organic extracts of this plant. The results of our GC-MS profiling indicated a total of 12 bioactive compounds. Ethanol and petroleum ether extracts of *A. indica* showed good antibacterial activity against all tested organisms. The data from this pilot study indicate that extracts of this plant can potentially be used to formulate and synthesize novel antibacterial drugs, although toxicity testing will have to be performed to assess the safety of these extracts.

6. Conflict of Interest Statement

None of the authors has any conflicts of interest to declare.

7. Competing Interests

None of the authors have any competing interests to declare.

8. Author Contributions

MSR, FKS, SMR, JH, SKS, and KHK designed the study. RH and MSR carried out all the analysis and *in vitro* tests. All authors coordinated the preparation of the manuscript, contributed to data analysis, and read and approved the final manuscript.

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