INTRODUCTION

Medicinal plants are used since ages as remedies for human diseases as they contain numerous phytochemicals with high therapeutic value and are considered to be more natural and safe when compared to synthetic drugs [1]. One of the most useful medicinal plants is Abutilon indicum. A. indicum belongs to family Malvaceae, which is commonly called as country mallow (English), Kanghi (Hindi), and Atibala (Sanskrit) [2]. This plant is extensively grown in India, Pakistan, and Bangladesh [3]. The phytochemical analysis of A. indicum leaves demonstrated a significant antibacterial activity in the well diffusion assay, S. aureus 17 mm and E. coli 15 mm, with a MIC value of 1.11 mg/mL. Comet assay demonstrated no DNA damage.

Methods: CSE was subjected for gas chromatography-mass spectrometry (GC-MS) analysis to identify its components. Antioxidant potential was analyzed using agar well diffusion method and minimum inhibitory concentration (MIC) was detected using 96-well plate method, against Staphylococcus aureus (MTCC: 3160) and Escherichia coli (MTCC: 443). DNA damage study was performed using comet assay. Antioxidant capability was studied using 2,2-diphenyl-1-picrylhydrazyl scavenging assay.

Results: GC-MS analysis suggested a library match to benzene-1,4-bis(phenylmethyl), with a molecular weight of 258 g/mol to be the major component in the CSE at 21.25 RT. CSE demonstrated 96.16% free radical scavenging activity at 2.5 mg/mL concentration. CSE demonstrated a significant antibacterial activity in the well diffusion assay, S. aureus 17 mm and E. coli 15 mm, with a MIC value of 1.11 mg/mL. Comet assay demonstrated no DNA damage.

Conclusion: These results conclude that CSE of A. indicum leaves possesses promising antibacterial and antioxidant potential.

Keywords: Abutilon indicum, Saponin, Escherichia coli, Staphylococcus aureus, 2,2-diphenyl-1-picrylhydrazyl, Antibacterial assay.
column (HP5) was fused silica 50 m×0.25 mm. Analysis conditions were 20 minutes at 100°C, 3 minutes at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 μl) was evaporated in a splitless injector at 300°C. Run time was 30 minutes. The compounds were identified by GC coupled with MS. The molecular weight and structure of the compounds were ascertained by matching with reference compounds available in the National Institute Standard and Technology [14].

**Agar well diffusion assay (antimicrobial susceptibility test)**

Antimicrobial activity of the sample was determined using agar well diffusion method. 20 ml of sterile nutrient broth was prepared and dispersed into four different test tubes, such that each containing 5 ml of broth. To each of the test tube, 0.1 ml of four different strains- *Staphylococcus aureus* (MTCC: 3160) and *Escherichia coli* (MTCC: 443) was inoculated and incubated overnight. Meanwhile, 150 ml of Mueller-Hilton Agar was prepared and is it carefully poured into four different Petri plates. After solidification, four different pathogen cultures were uniformly spread using cotton swab (lawn culture). Wells of about 6 mm in diameter was punched using cork borer. Then, the well was loaded with 100 μl extract (which was resuspended in the dimethyl sulfoxide [DMSO] solvent). DMSO was used as negative control and streptomycin was used as positive control. The inoculated plates were then, incubated at 37°C for about 24 hrs. The plates were further observed for the presence of the zone of clearance around the wells. The size of the zone obtained was measured and the antimicrobial activity obtained was measured in term of the average diameter of zone of inhibition in millimeter. This enables to compare with the standard antibiotics [15].

**Minimum inhibitory concentration (MIC)**

MIC of the CSE was identified using the 96-well plate method. Pathogenic microbes were grown in 96-well ELISA plates and were treated with serially diluted CSE. The growth of microorganisms was observed in ELISA reader by checking the turbidity. The microorganisms used in this study are *E. coli* (MTCC: 443) and *S. aureus* (MTCC: 3160).

**2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction assay**

DPPH solution of 1 mg/ml concentration dissolved in methanol was used for this study. 200 μl of DPPH solution was added to all the test tubes. 100 μl of methanol was used as blank. 100 μl of ascorbic acid (1 mg/ml) was used as standard. 100 μl of CSE at varying concentrations (0.25 mg/0.1 ml to 2.5 mg/0.1 ml) was used as test. These tubes were incubated then incubated in the dark region for about 30 minutes. Then, absorbance was observed in an ultraviolet spectrophotometer at 517 nm. The radical scavenging activity (inhibition of DPPH free radical in percent) was calculated using the following formula: % inhibition=\([\text{Ac}-\text{At}] / \text{Ac}\) *100. Where Ac: Absorption of the blank sample; At: Absorption of the test sample. Percentage of inhibition concentration was calculated from the graph plotting inhibition percentage against extract concentration [16].

**Comet assay**

About 0.5% agarose was prepared and uniformly spread on glass slide and allowed to form gel at 4°C. 40 μl of bacterial suspension was mixed with 40 μl of 0.1% low melting agarose. The mixture was loaded onto the well punched in the gel in glass slide. Lysis was carried out in 2.5 M NaCl, 10 mM Na2EDTA (ph 8), 10 mM Tris-HCl (ph 8), 1% of N-lauroylsarcosine sodium salt, 1% of Triton X-100, and 10% DMSO for 5 minutes. Electrophoresis was performed. The slides were then dehydrated with 70% of ethanol for 5 minutes and dried at room temperature.

**RESULTS**

**GC-MS**

GC-MS analysis results are tabulated in Table 1. Total of 11 different components were detected and tabulated. The major saponin component present in the mixture was identified as benzene, 1,4-bis(phenylmethyl), found at 21.25 retention time, with a molecular weight of 258 g/mol.

**Agar well diffusion**

The investigation was carried on two Gram-negative pathogens, i.e., *S. aureus* and *E. coli*. CSE produced a zone of inhibition of 17 mm (*S. aureus*) and 15 mm (*E. coli*). Fig. 1 shows that 2 mg of CSE produced 17 mm ZoI against *S. aureus* which is almost equal to the positive control streptomycin (10 mg).

![Fig. 1: Zone of inhibition for *Staphylococcus aureus*](image1)

**Fig. 1: Zone of inhibition for *Staphylococcus aureus***

![Fig. 2: 2,2-diphenyl-1-picrylhydrazyl assay for antioxidant activity of crude saponin extract](image2)

**Fig. 2: 2,2-diphenyl-1-picrylhydrazyl assay for antioxidant activity of crude saponin extract**

![Fig. 3 Comet assay results (a: Negative control, b: Sample)](image3)

**Fig. 3 Comet assay results (a: Negative control, b: Sample)**
Minimal inhibitory concentration (MIC)
CSE demonstrated a significant MIC of 1.11 mg/ml concentration against S. aureus and E. coli.

Antioxidant assay (DPPH reduction assay)
CSE demonstrated increasing percentage of antioxidant activity, with lowest inhibition of 15.7% at 0.25 mg/ml concentration, to a maximum of 96.17% inhibition at 2.5 mg/ml concentration. Fig. 2 shows the graphical representation of the DPPH assay results.

Comet assay
Comet assay showed no formation of comet tail, indicating that the CSE did not cause any DNA damage at MIC concentration (1.11 mg/ml). Fig. 3 shows the gel image of comet assay for control and test group.

CONCLUSION
Based on the results obtained from this work, it is evident that the CSE from A. indicum leaves has potential for biological applications as an antibacterial agent and also as an antioxidant agent. Although further in vivo studies and toxicity studies are required to confirm this report, a significant antibacterial activity similar to that of the standard antibiotic, with a significant MIC value at 1.11 mg/ml, and also an excellent antioxidant activity at 2.5 mg/ml concentration are strong proof of its biological activity of CSE from A. indicum leaves.

REFERENCES

Table 1: GC-MS analysis of crude saponin extract

<table>
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<tr>
<th>RT</th>
<th>Reference similarity</th>
<th>Name of the compound</th>
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<th>Observed molecular weight</th>
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<td>17.86</td>
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GC-MS: Gas chromatography-mass spectrometry