ABSTRACT
Medicinal plants play an important role in the discovery of novel drugs used in medicine. The Mussaenda frondosa was screened for phytochemical, antimicrobial and cytotoxic activities of the methanol, hexane, petroleum ether and chloroform crude extracts were studied in this work. The preliminary screening of the various extracts revealed the presence of tannin, alkaloids, carbohydrate, saponin, terpenoids, flavonoids and cardiac glycosides. The antimicrobial screening was Carried out using the following organisms; Bacillus subtilis, Klebsiella pneumonia, Bacillus thuringiensis, Proteus vulgaris, Agrobacterium rhizogenes, Lactobacillus acidophilus, Bacillus megaterium, Candida tropicalis, Aspergillus niger, Aspergillus aculeatus, Rhizomucor miehei and Saccharomyces cerevisiae. Phytochemical analysis of aqueous extract showed that the presence of tannin, alkaloids, carbohydrate, saponin, terpenoids, flavonoids and cardiac glycosides. Hexane extract showed highest antibacterial activity against Lactobacillus acidophilus and highest antifungal activity against Candida tropicalis. An invivo cytotoxicity test using Test on Extraction method was performed with test sample Mussaenda frondosa and based on ISO 10993-5. This study offers a scope for developing medicines.

KEYWORDS: Mussaenda frondosa, phytochemical analysis, antimicrobial activity, cytotoxicity.

INTRODUCTION
Plants have been used for the treatment of diseases for thousands of years. Terrestrial plants have been used as medicine in China, India and Egypt from ancient time for number of modern drugs are developed.[1] In recent years, herbal medicines have become an integral part of the primary health care system of many nations.[2] In 300 BC man was fully aware of the medicinal value of plants.[3] Mussaenda (Rubiaceae) are increasingly popular for their different colour. Many species of this plant have been found to be biologically active.[4-6] Flavonoids and flavonoids are more common in this plant.[7-9] The variety of Mussaenda is a large shrub with medium sized oval leaves. It has large velvety sepals surrounding small star shaped leaves.[10] The leaves and flowers of Mussaenda frondosa was used in external applications for ulcers. A weak decoction of dried shoots is given to children to relieve cough. The bitter root is used in the treatment of leprosy and eye troubles. Mussaenda frondosa is distributed in central Nepal, India and Sri Lanka.[11]

MATERIALS AND METHODS
Preparation of plant extract
Fresh leaves of Mussaenda frondosa was collected were washed under running tap water, air-dried at room temperature and then reduced to coarse powder using an electric blender. The powders obtained were stored in airtight containers prior to extraction. The dried powder of leaves was successively extracted using Soxhlet apparatus involving different solvents. The soxhlet extractor was placed on to a flask containing 250ml of solvent. Solvents such as methanol, hexane, petroleum ether and chloroform separately for extraction. They were run in a soxhlet apparatus for 12-24 hours after 5 cycles the extracts were collected from the distillation flask. The extract obtained was evaporated completely for dryness. 2g of dried sample were mixed with 10ml of DMSO and stored for further studies.[12]

Screening of phytochemicals
Qualitative chemical tests were carried out using the extracts obtained from plant in solvents namely methanol, hexane, petroleum ether and chloroform using standard procedures to identify the constituents.[13-17]

Antimicrobial screening
Antibacterial screening of plant extracts
The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method. Pathogenic bacteria (Gram positive and Gram negative) were obtained from Microbial Type Culture Collection (MTCC), Institute of
Microbial Technology and Chandigarh. The bacterial strains were Bacillus subtilis, Klebsiella pneumonia, Bacillus thuringiessis, Proteus vulgaris, Agrobacterium rhizogens, Lactobacillus acidophilus, Bacillus megaterium are spread over the medium. Filter paper disc of uniform size (4mm) are impregnated with specified concentrations of plant extract and then placed on the surface of Muller Hinton agar plates that has been seeded with organism to be tested. Label the each plate with the name of the test organism to be inoculated. Bacterial colonies were allowed to grow overnight at 37°C, then the inhibition zone around the disc was measured.\(^{[18]}\)

**Antifungal screening of plant extract**

Antifungal screening was followed by the method Cappucino et al., 2004. Fungal cultures were obtained from Microbial Type Culture Collection (MTCC), inoculated with respective fungi. The pathogenic strains include Candida tropicalis, Aspergillus niger, Aspergillus aculeatus, Rhizomucor miechei and Saccharomyces cerevisiae. The filter paper disc of uniform size (6mm) are impregnated with specified concentrations of plant extract and then placed on the surface of Rose Bengal agar plates that has been seeded with organism to be tested. Label the each plate with the name of the test organism to be inoculated. Fungal colonies were allowed to grow 7 days at 20°C and then the inhibition around the disc was measured.\(^{[19]}\)

**Cytotoxicity test**

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Test conducted</th>
<th>Chloroform</th>
<th>Petroleum ether</th>
<th>Hexane</th>
<th>Methanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannin</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>carbohydrate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Cardiac Glycoside</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavanoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

‘+’ indicates positive and ‘-’ indicates negative.

**Antimicrobial activity**

**Antibacterial activity and antifungal activity**

Ole Pederson et al., 1999 carried out invitro antibacterial and antifungal activity of plant extracts from Mussaenda. The present study the results of antibacterial activities of plant, Mussaenda frondosa were studied against human pathogenic bacteria and fungi. Among the five extracts, hexane extract showed highest antibacterial activity against Lactobacillus acidophilus and highest antifungal activity against Candida tropicalis. The results of antibacterial activity and antifungal activity screening are summarized below in figure 1 and 2.

An in vitro cytotoxicity test using Test on Extract method was performed with test sample of Mussaenda frondosa and based on ISO10993-5. Extract was prepared by incubating test materials with culture medium containing serum at 37±2 c for 24-26 hours at an extraction ratio of 0.1 g/ml. extract was acidic in nature and pH of the extract was adjusted to 7.4 with 0.1 M NAOH. 100% extracts were diluted to get concentrations of 50% and 25% with media. Different dilutions of extracts on test sample, negative control and positive control in triplicate were placed on subconfluent monolayer of L-929cells. After incubation of cells with extracts of test sample and controls at 37±2 c for 24±1hour, cell culture was examined microscopically for cellular response. Cellular responses were scored as 0, 1, 2, 3 and 4 according to none, slight, mild, moderate and severe based on USP28.

**RESULT AND DISCUSSION**

**Phytochemical analysis**

The phytochemistry of Mussaenda species has been studied extensively since 1990s. Iridoids, flavanoids and triterpenes are the common chemical ingredients distributed in Mussaenda species. The present study showed the preliminary phytochemical screening of seven compounds (tannin, flavanoids, saponin, alkaloids, carbohydrate, cardiac glycosides and terpenoids) were tested for their presence or absence in five different extracts of Mussaenda frondosa. The results were summarized in table 1.
Figure 1: Antibacterial activity of different extracts of *Musssaenda frondosa* against human pathogens.

Figure 2: Antifungal activity of different extracts of *Musssaenda frondosa* against human pathogens.

**Cytotoxicity test**
Patcharawan *et al.*, 2003 studied about twenty plants in the family rubiaceae collected from Northern Thailand were extracted and compared for their cytotoxic activities against a human cervix carcinoma cell line (KB-3-1). These plants were classified into three groups as active, moderately active and inactive plants according to their cytotoxic activity. The present study test material *Musssaenda frondosa* showed severe cytotoxic response to fibroblast cells (strain-L-929) at an extraction ratio 0.1 g/ml. The negative and positive controls used were High density poly ethylene and dilute phenol. Extracts of negative control gave none cytotoxic response and positive control gave severe cytotoxic response. The results were summarized in table 2.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Sample</th>
<th>Cytotoxic scale</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>0</td>
<td>None cytotoxic</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>4</td>
<td>Severe cytotoxic</td>
</tr>
<tr>
<td>3</td>
<td><em>Musssaenda frondosa</em></td>
<td>4</td>
<td>Severe cytotoxic</td>
</tr>
</tbody>
</table>

**CONCLUSION**
In the present study *Musssaenda frondosa* was screened for phytochemicals, antimicrobial activity and cytotoxicity. The study revealed that the plant, *Musssaenda frondosa* have severe cytotoxic activity and moderate antimicrobial activity. The phytochemical such as tannin, alkaloids, carbohydrate, saponin, terpenoids, flavonoids and cardiac glycosides are present in the plant. This study offers a scope for developing medicines.

**ACKNOWLEDGEMENT**
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REFERENCE
3. Le Strange JN. The useful plants of west tropical Africa. The crown agents, LONDON, 1977; 325.