ABSTRACT
Many of the phytoconstituents were traditionally reported for dreadful ailments, are nowadays found to be exhibit interesting bioactivity and have cardinal output. One noticeable striking example is use of plant *Abutilon indicum* in various ailments. The plant parts reported to have medicinal value and used by local community in various diseased conditions. The thirst of present work is to isolate active constituent from leaves of the plant *Abutilon indicum* sweet and avail about its antipyretic activity. Extraction of leaves of plant was carried out by soxhlet apparatus. Phytochemical analysis was conducted to identify nature of chemical constituent present in the plant. Isolation and identification was conducted by chromatographic techniques. Isolated fractions were characterized using data obtained from spectral analysis. Antipyretic effect of plant extract was studied on yeast induced pyrexia. The study demonstrated the isolated fractions were betasitosterol and quercetin which hypothetically may responsible for antipyretic activity. Extract showed significant antipyretic activity as compared to standard. Novel biologically active natural products will continue to serve as
lead compounds for drug development and is biochemical probes for the discovery of pharmacological and biochemical process.

KEYWORDS: Abutilon indicum, chloroform extract, antipyretic.

1. INTRODUCTION
Natural product form plant, animal and minerals are the basis of the treatment of human disease. Today’s estimate demonstrated about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Medicinal plants are the nature’s gift to human beings to make disease free healthy life. It plays a vital role to preserve our health. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The nature has provided a complete ware house of remedies to cure ailments of mankind. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. A natural product is a chemical compound or substance produced by living organisms found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. Herbal medicine/ remedies were noticed their cardinal existence in the treatment of human diseases since the existence of human civilization. Historically, the majority of new drugs have been generated from natural origin as secondary metabolites as well as the compounds derived from natural products.

Medicinal plant *Abutilon indicum* sweet, belonging to family Malvaceae. Commonly known as karndi, Country Mallow, Atibala. The whole plant as well as parts of the plant are traditionally claimed for various ailments. The plant is used as Demulcent, Diuretic, in Chest infection, Ulcer, Leprosy. Decoction of leaves is used in toothache, tender gums and internally for inflammation of bladder. The bark is used as anthelmintic, astringent. The seeds are used in piles, laxative, expectorant, in chronic cystitis. Chemical constituents present in the plant are flavonoid, saponins, alkaloids, n-alkane, betasitosterol, vanilic acid, p-coumaric acid, caffeic acid, amino acids, gallic acid, quercetin, borneol, caryophyllene oxide, endesmol etc. The thirst of present work was to isolate active plant constituent from leaves of this plant and avail about its antipyretic activity.
2. MATERIALS AND METHODS

2.1 Collection of plant
The fresh leaves of *Abutilon indicum* sweet were collected from local region of Danoli and Waifale, State Maharashtra in the month of August 2015.

2.2 Authentication of Plant material
Authentication of the leaves of *Abutilon indicum* was done by Dr. Vadmare, Botanist, Department of Botany, Kasturbai Walchand College, Sangli.

2.3 Preparation of extract
The collected leaves were washed with tap water and air dried under the shade in house for 25-30 days. After complete drying, powdered by mixer grinder to obtain fine powder. 100 gms of the dried powder were extracted successively in soxhlet apparatus using chloroform, methanol as solvent, and the extraction was continued until the solvents in the thimble became clear. After each extraction the solvent was recovered and the extract was concentrated by using rota vapour at 70°C. Aqueous extraction was also carried out. The concentrated extract was stored in desiccator and further subjected to Preliminary phytochemical investigation, isolation and pharmacological screening.

2.4 Phytochemical analysis
All the three extract were subjected to qualitative chemical investigation to check the presence of various chemical constituents in the extract.

2.5 Thin layer chromatography
Thin layer chromatography was performed to identify and isolate chemical constituents present in extract of *Abutilon indicum* sweet. Chloroform: ethyl acetate: ethanol: glacial acetic acid (6: 2: 2: 2) was used as mobile phase.

2.6 Column Chromatography
Isolation of constituent was done by using column chromatography. Silica gel G 60-120 mesh size was used as stationary phase. Slurry of silica gel was prepared by addition of appropriate mobile phase. Chloroform: ethanol: ethyl acetate: glacial acetic acid (6:2:2:2) was used as mobile phase.
2.7 Spectroscopic analysis
Isolated fractions were subjected to spectral analysis. Fractions were characterized using data obtained from Infrared, NMR, and MASS spectroscopy.

2.8 Pharmacological activity
India has century’s old and rich heritage of medicinal and aromatic plant due to diversity in environment for curing human illness. The most common illness is fever which is pharmacologically known as pyrexia characterized by elevation of temperature above the normal range of 36.5°C to 37.5°C. Antipyretic effect of extract on yeast induced pyrexia in rat was studied.

3 RESULTS AND DISCUSSION
3.1 Physicochemical evaluation

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Total ash</th>
<th>LOD</th>
<th>Acid insoluble ash</th>
<th>Water soluble ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.12</td>
<td>1.44</td>
<td>1.05</td>
<td>0.75</td>
</tr>
</tbody>
</table>

3.2 Phytochemical analysis
Phytochemical analysis reveals that only chloroform extract showed presence of carbohydrate, glycosides, steroids, flavonoids. Among which steroids and flavonoids were hypothetically found to be responsible for pharmacological activity. So only chloroform extract was used for further study.

3.3 Chromatographic Analysis
Identification and isolation of constituents from chloroform extract was carried out by using thin layer and column chromatography. Two fractions obtained from column were collected, dried, and used for further analysis.

3.4 Spectral data
Isolated compound 1- IR V$_{\text{max}}$ (neat cm$^{-1}$) 3371.92-OH, 2851- CH, 1650- C=C, 3231.15- Cyclic olefinic, 1020- Cycloalkane. 7.782, $^1$H NMR (CDCl$_3$, 400 MHz): δ 7.782 s-aromatic proton, δ 0.879-0.859 (d) - aliphatic CH$_2$ terminal, δ 1.254 (s) angular CH$_3$ at C$_{18}$, δ 4.053 (s)-OH, δ 2.291-2.339 (t) - aliphatic CH$_3$ Mass, molecular ion peak 412.
Fig. 1: IR spectra of isolated compound 1.

Fig. 2: NMR spectra of isolated compound 1.

Fig. 3: MASS spectra of isolated compound 1.
**Isolated compound 2**- IR $V_{\text{max}}$ (neat cm$^{-1}$) 3415.31-OH, 1736.50- C=O, 1612.2 C=C, 674.963- aromatic C-H bending $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 2.74 OH proton, $\delta$ 7.282 Aromatic proton, Mass, molecular ion peak 286.

![Fig. 4: IR spectra of isolated compound 2.](image1)

![Fig. 5: NMR spectra of isolated compound 2.](image2)
3.5 Pharmacological Activity

Antipyretic activity

The effect of *Abutilon indicum* chloroform extract at dose of 200mg/kg and 400mg/kg on yeast induced pyrexia was studied and it was found that chloroform extract at dose of 400mg/kg caused significant lowering of body temperature up to 5 hr following its administration. Subcutaneous injection of baker’s yeast suspension markedly elevated the rectal temperature 3 hrs. after administration. The result showed that chloroform extract at dose of 400mg/kg of *Abutilon indicum* possess significant antipyretic effect in the yeast provoked elevation of body temperature in rats than extract at dose of 200mg/kg extract. The standard drug paracetamol at the dose of 150mg/kg significantly reduced the yeast provoked elevation of body temperature. The result obtained for both the standard drug treated and Abutilon extract treated rats were compared with the control (2% Gum acacia) group and significant reduction in yeast elevated rectal temperature was observed in plant extract.

Table no. 1: Data for antipyretic activity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose Mg/kg</th>
<th>Rectal temp. in °C after 3 hrs of yeast injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-3</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>35.8±0.05</td>
</tr>
<tr>
<td>Paracetamol (standard)</td>
<td>150</td>
<td>36.3±0.2</td>
</tr>
<tr>
<td>Chloroform extract (test 1)</td>
<td>200</td>
<td>35.43±0.15</td>
</tr>
<tr>
<td>Chloroform extract (test 2)</td>
<td>400</td>
<td>35.51±0.2</td>
</tr>
</tbody>
</table>

*The results are mean ±SEM, n=6 p>0.10*
Fig. 7: Effect of test compound and paracetamol on yeast induced pyrexia.

Betasitosterol

Flavonoids
CONCLUSION
Phytochemical analysis reveals that chloroform extract showed presence of steroids and flavonoids which were hypothetically found to be responsible for pharmacological activity. From phytochemical, chromatographic and spectral data it was concluded that Beta-sitosterol and Quercetin are the possible structure of isolated compounds 1 & 2 respectively. The selected extract was subjected to pharmacological screening and evaluated for antipyretic activity. Pyrexia was induced and effect of extract was studied. Chloroform extract at dose of 400mg/kg showed significant antipyretic activity.

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