IDENTIFICATION OF PHYTOCOMPONENTS FROM HEXANE SOLUBLE FRACTION OF WILD ALOE VERA (L.) BURM. F. GEL COLLECTED FROM UNIVERSITY OF NORTH BENGAL CAMPUS THROUGH GC-MS ANALYSIS

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ABSTRACT
Gas Chromatography Mass Spectrometry is an advanced technique to recognize individual compounds from a mixture of compounds. Recently this technique has been extensively used in the identification of herbal constituents from potential medicinal plant materials. Aloe vera (L.) Burm. F. (Family Xanthorrhoeaceae) is a naturalized plant found in all over India. This plant is very commonly used by the ethnic people as food and medicinal resource for amelioration of various diseases. In this study, GC-MS of the selected wild plant from the University of North Bengal campus has revealed 61 major compounds with at least 16 compounds with biological activities. Aloe gel was collected and sample was prepared using hexane as solvent. Qualitative detection of phytochemical components were done in Thermo Scientific Trace 1300 GC and ISQ Mass spectrophotometer equipped with an AI/AS 1310 auto sampler in a 30 meter long TG5ms column. Some major compounds isolated are ethyl iso-allocholate, octadecane, geldaramycin, astaxanthin, picrotoxinin etc. All these compounds have documented biological activities in different diseases conditions. This study re-establishes the importance of Aloe vera as a potent medicinal plant by exploring the phytoactive compounds of the plant.

Key Words- Aloe vera gel, hexane, GC-MS.

INTRODUCTION
Aloe vera (L.) Burm. F. (Family Xanthorrhoeaceae) is a very well known plant found in the sub-Himalayan region. Aloe vera is a short-stemmed or stemless succulent plant which grows up to 100 cm in height and spreads by offsets. The leaves are thick, filled with parenchymal gel and are fleshy. Colour varies from green to grey-green. Some varieties have white spots and flecks on their upper and lower stem surfaces. This plant is largely used in Ayurveda and as a traditional dermal care product for its phytochemical properties. Aloe vera contains different phytochemical agents which are able to cure different disease symptoms (Sujushe et al., 2008). The plant is used widely in dermal diseases and is a good laxative agent (Eshun and He, 2004). It also represents compounds responsible for anti-diabetic (Tanaka et al., 2006), anti-oxidant (Hu et al., 2003), anti-microbial (Arunkumar and Muthuselvan, 2009), wound healing (Davis et al., 1989) and anti-inflammatory activities (Paul et al., 2014). Topical use of the gel in dermal care and in wound healing is also very accepted. This plant is reported to have more than 200 phytochemical compounds. In this study, the plant was collected from the adjacent regions of University of North Bengal and the phytochemicals were identified using Gas Chromatography Mass Spectrometry.

Materials and Methods
Collection of the sample: Naturaly growing wild
**Aloe vera** (L.) Burm. F. (Class Magnoliopsida, Order Asparagales, Family Xanthorrhoeaceae) was collected from the adjacent regions of the University of North Bengal campus. The sample was identified by Prof. A. P. Das, plant taxonomist in the Department of Botany, University of North Bengal [Accession no. 09884 (NBU)].

**Preparation of plant extract:** Plant extract was prepared in HPLC grade n-hexane which is a non-polar solvent. The plant was collected, washed thoroughly and the leaf was cut open to collect the gel with the help of a sterilized scalpel. The gel was then properly homogenized to prepare a slimy end product. This slimy end product is then mixed with n-hexane in 1:5 (w: v) ratio. The mixture was kept in magnetic stirrer for 24 hours at room temperature. Next day, the supernatant was collected, filtered through Whatman No 1 filter paper and the filtrate was dried completely using nitrogen flow as it is an inert non-reactive gas. The dried end product was re-suspended in hexane and was used for GC-MS analysis.

**Instrumentation and Chromatographic conditions:** The GC-MS analysis of n-hexane fraction of **Aloe vera** leaf gel was performed in Thermo Scientific Trace 1300 GC associated with ISQ Mass spectrophotometer. The machine was equipped with an AI/AS 1310 auto sampler. The experiment was done in TG 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 in film thickness. The column oven temperature was kept 80°C with of 5°C/min to 300°C gradual increase in steps; injection temperature was set at 250°C at a pressure of 5 kPa, with total flow and column flow of 10 ml/min and 1 ml/min, respectively. The purge flow rate was 3.0 ml/min. The GC program ion source and interface temperature were 220°C and 300°C, respectively, with solvent cut time of 5 min. The MS program starting time was 5 min which ended at 46.00 min with event time of 0.50 s, mass range 50 650. The sample volume of **Aloe vera** n-hexane fraction was 1 l (split ratio 10:1). The samples were repeatedly used to find the best result.

**Data interpretation:** The analysis of GC-MS fragments and data interpretation was done using Xcalibur software version 2.0.1.3 with the help of the NIST Mass Spectral Search Programme for the NIST/EPA/NIH Mass Spectral Library version 2.0g, built May 19, 2011.

**RESULT**

**GC-MS analysis:** The GC-MS analysis of n-hexane fraction of **Aloe vera** has shown the presence of 61 major peaks each corresponding to a single compound. We have found some principle compounds which are already documented as molecules with potent biological properties. The principle anti-inflammatory and/or anti-arthritic compounds from the plant are listed below in the Table 1. The total chromatogram is presented in the Figure 1.

**DISCUSSION**

Use of plant-derived drugs has got an increased acceptance in the recent years throughout the World. There is a continuous search of natural-products for bioactive phyto-constituents that could serve as drug leads for treatment of various human ailments world-wide (Kalimuthu and Prabakaran, 2013). In the GC-MS analysis, 61 compounds are identified by the NIST library search in which at least 9 molecules are documented as potent agents for different biological activities. Ethyl iso-allocholate is a steroid derivative which has important antibacterial, anti-oxidant, anti-tumor, cancer preventive, chemopreventive and pesticidal activity. Fatty acids like 9, 12, 15-Octadecatrienoic acid and Octadecane are documented to have potent anti-arthritic and anti-microbial activities. Antibiotic like geldaramycin is anti-tumor antibiotic used in medicinal biology. (5α)Pregnane-3,20α-diol which is a neuroactive steroid, is involved in the neuromodulatory activity in different model animal systems. Astaxanthin, a
Table 1: List of major phytocompounds isolated from Aloe vera gel and their biological activities with references.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Name of the Compound</th>
<th>RT</th>
<th>Mol. Formula</th>
<th>Biological Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethyl iso-allocholate</td>
<td>5.64</td>
<td>C_{20}H_{44}O_{5}</td>
<td>Antibacterial, Antioxidant, Anti-tumor, Cancer preventive, Chemo preventive and Pesticide.</td>
<td>Saravanan et al., 2014</td>
</tr>
<tr>
<td>2.</td>
<td>9,12,15-Octadecatrienoic acid</td>
<td>5.87</td>
<td>C_{21}H_{38}O_{4}</td>
<td>Omega 3 fatty acid, Anti-RA activity, heart disease.</td>
<td>Online: University of Maryland Medical System online database</td>
</tr>
<tr>
<td>3.</td>
<td>Octadecane</td>
<td>6.07</td>
<td>C_{18}H_{36}</td>
<td>Antioxidant and Antimicrobial</td>
<td>Saravanan et al., 2014</td>
</tr>
<tr>
<td>4.</td>
<td>Geldaramycin</td>
<td>9.18</td>
<td>C_{20}H_{40}N_{2}O_{5}</td>
<td>Anti-tumor antibiotic</td>
<td>Jilani et al., 2013</td>
</tr>
<tr>
<td>5.</td>
<td>Astaxanthin</td>
<td>10.15</td>
<td>C_{20}H_{30}O_{4}</td>
<td>Alzheimer’s disease, Parkinson’s disease, Brain stroke, AMD.</td>
<td>Nakao et al., 2010</td>
</tr>
<tr>
<td>6.</td>
<td>9,10-Secocholesta-5,7,10-(19)-triene-3,24,25-triol</td>
<td>10.79</td>
<td>C_{27}H_{46}O_{3}</td>
<td>Calcitriol derivative (Chempider), treat hyperparathyroidism, Vitamin D3.</td>
<td>Online: ChemSpider online database</td>
</tr>
<tr>
<td>7.</td>
<td>(3α,5α,14α)-Pregnane-5,20α-diol</td>
<td>11.27</td>
<td>C_{29}H_{48}NO_{6}</td>
<td>Neuroactive steroid antagonist to the glycine receptor in the nervous system</td>
<td>Lan and Gee, 1994</td>
</tr>
<tr>
<td>8.</td>
<td>Picrotoxinin</td>
<td>11.39</td>
<td>C_{15}H_{10}O_{6}</td>
<td>A non-competitive channel blocker for the GABA&lt;sub&gt;A&lt;/sub&gt; receptor chloride channels.</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td>9.</td>
<td>1,2-dihydro-2,2,4-trimethyl-Quinoline</td>
<td>15.78</td>
<td>C_{12}H_{13}N</td>
<td>Preparation of hydroxyquinoline sulfate and miacin, used as dye.</td>
<td>Collin and Hoke, 2005</td>
</tr>
</tbody>
</table>

Figure 1: Chromatogram of Aloe vera gel hexane fraction showing major peaks and their relative abundance. 9,12,15-Octadecatrienoic acid, with reported anti-rheumatoid arthritis activity has been shown (inset).
remedy for diseases like Alzheimer’s disease and Parkinson’s disease is also found from our GC-MS analysis.

In Ayurveda, synergy is thought to be a principle way of action that involves a mixture of herbal products containing different bioactive principles in natural proportion. In this study, the data clearly states that the Aloe vera gel contains different bioactive compounds which can cure different diseased conditions and hence, it is very good as a dietary supplement. Many ethnic populations emphasize on limited regular consumption of such plants in their medicinal practices. Studies on Aloe vera phytocompounds by other workers have also revealed similar compounds from the plant. Similar fatty acids and steroidal compounds are found from the analysis of other workers as well (Lakshmi and Rajalakshmi, 2011).

CONCLUSION
The presence of various bioactive principles in Aloe vera crude gel extract is an indication that the plant could be used as a herbal drug of pharmacological significance. However, the isolation of individual constituents and their biological activities will be of advanced medical significance.

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GC-MS analysis of *Aloe vera* gel hexane fraction