GC-MS analysis and study of potential antioxidant activity of the crude ethanolic flower extract of *Hibiscus rosa sinensis* L (wild variety) by Hydrogen peroxide Scavenging assay

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Abstract:

In the present study an approach was made to know about the various phytochemical constituents of *Hibiscus rosa sinensis* ethanolic flower extract for which the GC-MS analysis was the opted method. Many phytoconstituents like dodecanoic, ethyl ester (RT: 13.84), heptadecanoic acid, ethylester (RT: 27.51), 6,6-Dimethyl-2-(4,8-dimethyl-3,7-nonadienyl)-bicyclo(3.1.1)hept-3-ene (RT: 27.51) etc. were isolated by this method. One of the other objectives of the investigation was also to check the effect of *Hibiscus rosa sinensis* ethanolic flower extract, as a potential natural antioxidant. In vitro total antioxidant capacity via hydrogen peroxide scavenging assay was performed during the investigation. For this purpose ascorbic acid (ASA) which is known to be a potent was used as a reference. The crude flower extract showed a concentration dependent hydrogen peroxide scavenging activity.

**Keywords:** *Hibiscus rosa sinensis; Hibiscus rosa sinensis* flower extract ;Antioxidant activity; Free radical Scavenging activity;Ascorbic acid; Hydrogen peroxide

1. Introduction:

*Hibiscus rosa sinensis* L (Malvaceae) is an ornamental plant admired for its bright coloured flowers and is often planted as a hedge plant. It is a common plant in India. The ethanolic extract of shoot of *H. rosa sinensis* was reported for its use in constipation and diarrhoea both. ( Gilani et al.,2005 ). Numerous research articles and ancient texts have shown that the flowers of this plant possess antifertility activity, like antiimplantation, abortifacient actions in rodents (Batta et al., 1970). Fresh root juice of the plant is given for gonorrhea and powder of the roots is beneficial in menorrhagia (The Wealth of India, 1959).

Besides, the alcoholic extract of flowers of *H. rosasinesis* has been proved to have anticonvulsant property (Kasture et al., 2000). In Ayurveda, the leaves of the plant are used in fatigue and dermatological disorders. Flowers of the plant have also proved to be beneficial in diabetes with hyperlipidemic conditions (Ghosh et al., 2017).
Also, cellular metabolism continuously produces hydrogen peroxide which is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups (Nagulendran et al., 2007). Under normal cellular conditions, the free radicals are detoxified by the antioxidants present in the body and there is equilibrium between the Reactive oxygen species (ROS) generated and detoxified by the antioxidants present. However, overproduction of ROS and sometimes inadequate antioxidants in the body can easily affect and lead to oxidative damage to cellular proteins, lipids and even DNA. Such alterations in the biomolecules lead to chronic diseases, such as cancer, diabetes, and other degenerative diseases in humans. Following one of the present trends in research which focuses on finding potent biomolecules with minimal side effects from natural sources a GC-MS analysis of the H. rosa sinensis ethanolic flower extract to check the phytoconstituents composition was performed. In addition, an in-vitro hydrogen peroxide scavenging assay was also performed during the research to check the H2O2 scavenging potential of the flowers of this plant.

2. Materials and Method:

3. Plant material:
Flowers of Hibiscus rosa sinensis(L) of wild variety were collected in the month of July, 2013 around local area of Hatia, Ranchi which falls in the Chotanagpur region of JharkhandState, India. The flower sample was authenticated by Prof. Dr. Anjani Shrivasatva, Botanist, University Department of Botany, Ranchi University, Ranchi. The collected flowers were shade dried, powdered and stored in air tight containers for extraction.

4. Preparation of alcoholic Hibiscus rosa sinensis flower extract (HRSEFE):
The flower powder obtained (100gm) was extracted each time in a soxhlet apparatus installed at the Department of Zoology Ranchi University, Ranchi with petroleum ether (60-80° C) till complete extraction. The defatted plant material so obtained was then extracted with 95% ethanol. The ethanolic Hibiscus rosa sinensis flower extract (HRSEFE) obtained was dark brown in colour which was then concentrated under reduced pressure using a concentrator to get a semisolid mass of the crude extract. The extract so obtained was kept under refrigeration below 10°C.

5. Processing of the extract for GC-MS Analysis:
The HRSEFE obtained by the above process was then dissolved in 95% ethanol for 12 hours. The extract was then filtered through Whatman filter paper No. 41 along with 2 g sodium sulphate which efficiently removed the traces of water from the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. Further, the filtrate concentrated by bubbling nitrogen gas into the solution. This ethanolic extract contained both polar and non-polar phytoconstituents of the flower. 2µL of this solution was subjected to GC/MS analysis (Merlin et al., 2009).

6. GC-MS Analysis:
GC-MS analysis was carried out on a GC clarus 500 Perlin Elmer system gas chromatograph with to a mass spectrophotometer (GC – MS) instrument. The instrumentation comprised of column Elite – 1 fused silica capillary column (30 x 0.25 mm ID x 1 EM df, composed of 100% Dimethyl polysiloxane). The electron impact mode was set at 70 eV and helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1 injector temperature 250 C; ion-source temperature 280 C. The oven temperature was programmed from 110 C (isothermal for 2 min) with an increase of 10° C/min to 200 °C then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV. The scan interval of 0.5s and fragments from 40 to 550 Da were preferred.

7. Identification of Phytoconstituents:
The mass spectrum obtained post GC-MS was compared using the database of National Institute Standard and Technology (NIST) patterns stored in the NIST library. The name and molecular weight of the components of the test sample were recorded.

8. Hydrogen peroxide scavenging assay:
Principle:
Hydrogen peroxide is a weak oxidizing agent. It oxidizes an essential sulphydryl (-SH) group in many key enzymes. H2O2 can also cross the plasma membrane of cells very easily and probably react with Fe2+ to form hydroxyl radical (OH•). The generation of OH• Probably leads to many toxic effects in biological systems. Therefore, the cells do control the amount of hydrogen peroxide that
accumulates over time (Free Radicals In Biology and Medicine, 1993).

Hydrogen peroxide scavenging activity by the flower extract was determined by the method prescribed by (Ruch et al., 1989). The flower extract (4 ml) was prepared in distilled water at various concentrations and was mixed with 0.6 ml of 4 mM \( \text{H}_2\text{O}_2 \) solution prepared in phosphate buffer (0.1 M, pH 7.4) and was incubated for 10 min. The absorbance of the solution was recorded at 230 nm against blank solution containing the flower extract without \( \text{H}_2\text{O}_2 \). Ascorbic acid was used as a positive control. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using following equation.

\[
S\% = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100
\]

Where,

- \( A_{\text{control}} \) = absorbance of the blank control (containing all reagents except the flower extract solution)
- \( A_{\text{sample}} \) = absorbance of the test sample.

9. Statistical Analyses:

All values are expressed as mean ± S.D. Statistical analyses were performed by Student’s t-test. The values of \( p \) lower than 0.05 were considered significant where \( p \) stands for probability.

10. Results and Discussion:

Free radicals play a key role in the development of tissue damage. Any substance which has the capability to remove these will protect the cell system from cytotoxic damage caused due to the free radicals generated from hydrogen peroxide (van et al., 2008). GC-MS method used for the analysis of the Hibiscus rosa sinensis ethanolic flower extract can be an interesting tool for testing the quantity of some active compounds and their principles in herbs used in pharmaceutical drugs, cosmetic etc. It is evident from the table 1 that the ethanol fraction has a complex chemical composition. Few GC-MS peaks remained unidentified, because of the lack of authentic samples and library data of corresponding compounds.

These results of GC-MS indicated The GC-MS analysis of Hibiscus rosa sinensis flowers has shown many phytochemicals which add to the medicinal properties of the plant (Table 1). The major constituents present in the flowers are dodecanoic, ethyl ester (RT: 13.84), heptadecanoic acid, ethyl ester (RT: 27.51), 6,6-Dimethyl-2-(4,8-dimethyl-3,7-nonadienyl)-bicyclo (3.1.1) hept-3-ene (RT: 27.51) and 1-Dotriacontanol (RT: 29.6). These bioactive compounds present in HRSEFE belong to various classes of phytochemicals such as tannins, glycosides, alkaloids, flavonoids, steroids, etc.

The other aspect of our present study included the Hydrogen peroxide assay (Table 2). \( \text{H}_2\text{O}_2 \) acts as a weak oxidizing agent and can penetrate biological membranes easily. It can probably react with \( \text{Fe}^2+ \) and \( \text{Cu}^2+ \) ions to form hydroxyl radical which destabilises cellular machinery and proves toxic to the cells (Halliwell et al., 1993). The HRSEFE extract effectively scavenged \( \text{H}_2\text{O}_2 \) from the cells though the scavenging action was less when compared to that of ASA. The flower extract upon quantification by colorimetric methods were found to be rich in phenolic compounds (tannins and flavonoids) and therefore exhibited very good scavenging activity against \( \text{H}_2\text{O}_2 \) free radicals. The antioxidant activity performed was compared to the standard antioxidant, ascorbic acid (ASA) (Table 2).

Based on the results, it can be concluded that HRSEFE could be used as a natural source of antioxidants and its regular consumption in diet could provide health benefits to humans by the protection against oxidative stress. Further detailed in vitro and in vivo studies on along with the isolation of active compounds is much needed to develop natural and safe treatment strategies for free radical induced diseases.

<table>
<thead>
<tr>
<th>RT (min)</th>
<th>Name of the compound</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.84</td>
<td>Dodecanoic, ethyl ester</td>
<td>228</td>
</tr>
<tr>
<td>25.99</td>
<td>Heptadecanoic acid, ethyl ester</td>
<td>298</td>
</tr>
<tr>
<td>27.51</td>
<td>6,6-Dimethyl-2-(4,8-dimethyl-3,7-nonadienyl)-bicyclo(3.1.1)hept-3-ene</td>
<td>272</td>
</tr>
<tr>
<td>28.13</td>
<td>Pentacontanoic ethyl ester</td>
<td>760</td>
</tr>
<tr>
<td>29.6</td>
<td>1-Dotriacontanol</td>
<td>466</td>
</tr>
</tbody>
</table>

Table 1: Phytocompounds identified in the ethanolic extract of the flowers of H. rosa sinensis by GC-MS.

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**Table 2: Hydrogen Peroxide scavenging activity:**
( Mean±SEM)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition (mean ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (Ascorbic Acid- ASA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12.5</td>
<td>27±0.47</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>40.34±1.09</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>63.34±0.78</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>76.33±1.25</td>
</tr>
<tr>
<td><em>Hibiscus rosa sinensis</em> ethanolic flower extract (HRSEFE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>96±2.35</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>90.67±1.57</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>82.66±1.41</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>80±1.88</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>73.34±1.41</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
<td>65±1.09</td>
</tr>
</tbody>
</table>

**Figure 1: Hydrogen Peroxide scavenging activity of Ascorbic acid and HRSEFE**

**Figure 2: A section of GC-MS chromatogram of *H. rosa sinensis* flower extract.**
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11. Conclusion:
The present study confirms the presence and identification of a variety of phytoconstituents present in *Hibiscus rosa sinensis* (wild variety) flower extract. In addition the results of in vitro antioxidant potential of crude extract of the flower of *H. rosa sinensis* were comparable with the standard antioxidant i.e. ascorbic acid. The phytochemicals in the flower makes it a pharmacologically effect antioxidant. However, we are now focussing on the active principle present in the extract which adds to the H$_2$O$_2$ scavenging antioxidant activity. The current findings suggest that *H. rosa sinensis* flower could be a potential source of natural antioxidant that could have great importance as therapeutic agent in the near future.

12. Acknowledgement:
The authors would like to thank Dr. Manish Kumar, Department of Biotechnology, Birla Institute of Technology, Mesra, Ranchi, for the GC-MS analysis. The authors are also grateful to INSPIRE-DST Fellowship Division, Govt. of India, New Delhi for providing the monetary aid for the research work.

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13. References:

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