In-vitro assessment of cholinesterase inhibitory and thrombolytic activity of six available citrus fruits in Bangladesh: relevant for treating neurodegenerative disorder.

Abstract:

Aim:

Citrus fruits are well known for its medicinal and food value. Aim of this study is to investigate acetylcholinesterase ((AChE)) inhibitory activity, butyrylcholinesterase (BuChE) inhibitory activity, total phenolics, flavonoids, flavonols content and thrombolytic activities of crude methanol extracts of 6 citrus fruits (Citrus limon, Citrus aurantifolia, Citrus bergamia, Citrus maxima, Citrus sinensis and Citrus macroptera).

Methods:

The fruits were extracted by using methanol as solvent. Ellman’s colorimetric method was applied to determine both cholinesterase inhibitory activities, while folin-ciocalteau reagent (FCR) and aluminium chloride were used to quantify total phenolics, flavonoids, flavonol content of those fruits. Blood clot lysis method was applied for determine thrombolytic activity of those fruits.

Result:

All citrus fruits contain a good amount of phenolics, flavonoids and flavonols. C. maxima found more prominent in containing phenolics and flavonols compare to other citrus fruits, with 414.06 ± 2.87 mg Gallic Acid Equivalent/gm and 12.94 ± 1.31 mg Catechin Equivalent/gm dried extract respectively. Citrus sinensis showed the highest content in flavonoids with with 21.16± 1.37 mg Catechin Equivalent /gm dried extract. Citrus fruits are also a quality source of cholinesterase inhibitors. All the examined citrus fruits were found capable in inhibiting both acetylcholinesterase (AChE) as well as butyrylcholinesterase (BuChE). C. bergamia was most effective in inhibiting AChE with IC50 of 27.18 µg/ml where C. macroptera was best in inhibiting BuChE (IC50 32.5 µg/ml). But none of the citrus fruits were found fit for thrombolytic activity.

Conclusion:

Citrus fruits are found sound in inhibiting AChE and BuChE as well as containing Phenolics, flavonoids and flavonols. But they lack in their thrombolytic activity.

Key words:

Citrus fruit; Phenolics; Flavonoids; Flavonols; Cholinesterase inhibition

Introduction:

Citrus has long been regarded as food and medicinal plant. Due to their low cost and easy availability, Citrus fruits are offers significantly low-cost nutritional dietary supplement.[1-3] The genus Citrus belonging to the family “Rutaceae” comprises about 40 species widely distributed in the Bangladesh, India, China, Malaysia, Sri Lanka and Australia. It is one of the most important world fruit crops and
consumed freshly as fresh or as juice because of its nutritional value and special flavor.[4-6] Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as phenolic compounds, flavonoids, ascorbic acid and others. **Flavonols, flavones and flavonols** are three common types of flavonoids which occur in Citrus fruit. The main flavonoids found in citrus species are hesperidin, narirutin, naringin and eriocitrin.[7-10] Epidemiological studies on dietary Citrus flavonoids improved a reduction in risk of coronary heart disease and are attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects.[11-15] The interest in these classes of compounds is due to their pharmacological activity as radical scavengers. [16]

**Neurodegenerative disorder (ND)** is incurable conditions due to the progressive dysfunction of nervous system mainly caused by neuronal degeneration and loss of total nerve cells for reasons. The actual reasons have not yet been fully understood.[17,18] Today, a growing number of people worldwide are affected by ND, characterized by deterioration in emotional control, social behavior and social communication. ND exist in many forms, such as Multiple Sclerosis, Alzheimer’s, Parkinson’s, Huntington’s, Human prion and Motor neuron diseases.[19-22] To treat ND there are several hypothesis have be developed. Like antioxidant hypothesis, cholinergic hypothesis, tau hypothesis, Aβ hypothesis etc. Currently there is no effective treatment for ND, and the marketed drugs are mainly symptom-oriented, albeit with many side effects, limited efficacy and partial capability to inhibit disease progression.[23-28] Therefore, in order to develop novel preventive strategies or co-adjuvant therapy for ND, within the past decades, a great number of natural medicinal plants have gained attention as potential neuroprotective agents.[29] Moreover, an increasing number of studies have suggested that dietary intake of vegetables and fruits can prevent or delay the onset of ND. These properties might be due to the presence of polyphenols, an important group of phytochemicals that are abundantly present in fruits, vegetables, cereals and beverages. As Citrus fruits are rich in antioxidants, polyphenols and flavonoids, these might be a good alternative for treating ND and lowering its effects. [30-33]

Due to geographical consideration, a wide variety of Citrus fruit grows in Bangladesh. Among all species **six species were used** (Citrus limon, Citrus aurantifolia, Citrus bergamia, Citrus maxima, Citrus sinensis and Citrus macroptera) for test. Their physical properties are given in Table 1. The objectives of this study were to investigate and comparison of (I) Cholinesterase enzymes inhibitory activity of 6 citrus species (II) determination of their phenol, flavonoids and flavonol contents (III) comparative thrombolytic activity and (IV) analysis of correlation between them.

<table>
<thead>
<tr>
<th><em>Citrus species</em></th>
<th>Common Name</th>
<th>Color</th>
<th>Size (cm)</th>
<th>Shape</th>
<th>Taste</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. limon</td>
<td>Common Lime</td>
<td>Greenish Yellow</td>
<td>7-10</td>
<td>Oval</td>
<td>Sour</td>
<td>Fibrous</td>
</tr>
<tr>
<td>C. aurantifolia</td>
<td>Key Lime</td>
<td>Greenish Yellow</td>
<td>2.5-5</td>
<td>Round</td>
<td>Sour</td>
<td>Smooth</td>
</tr>
<tr>
<td>C. bergamia</td>
<td>Bergamot</td>
<td>Yellow</td>
<td>5-10</td>
<td>Round</td>
<td>Sour</td>
<td>Smooth</td>
</tr>
<tr>
<td>C. maxima</td>
<td>Pomelo</td>
<td>Greenish Yellow</td>
<td>15-25</td>
<td>Round</td>
<td>Sour</td>
<td>Fibrous</td>
</tr>
<tr>
<td>C. sinensis</td>
<td>Orange</td>
<td>Orange</td>
<td>5-10</td>
<td>Round</td>
<td>Sweet</td>
<td>Smooth</td>
</tr>
<tr>
<td>C. macroptera</td>
<td>Satkora</td>
<td>Green</td>
<td>5-7</td>
<td>Round</td>
<td>Sour</td>
<td>Fibrous</td>
</tr>
</tbody>
</table>
Materials and methods:

Chemicals and regents

Acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI), 5’-dithio-bis-(2-nitro) benzoic acid (DTNB), acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), eserine, galantamine, gallic acid, catechin and Streptokinase were purchased from Sigma-Aldrich (Japan). Tris.HCl buffer, sodium chloride, sodium carbonate, sodium acetate, sodium hydroxide, magnesium chloride, triton X-100, folin-ciocalteau reagent (FCR), aluminium chloride, ammonium sulphate were collected from Wako Pure Chemical Company Ltd. (Japan). **Analytical grade chemicals and solvents were used in this study.**

Preparation of fruit sample:

Fresh fruits of *C. limon, C. aurantifolia, C. bergamia, C. maxima, C. sinensis* and *C. macroptera* at the commercial mature stage were harvested from several commercial orchard in the month of October-November, from Sylhet, Hobiganj, Mymensingh, Dhaka and Rajshahi, Bangladesh. Only healthy fruits were selected randomly for their uniformity in color and shape. Fruits were then washed thoroughly with distilled water and then dried in air. Then fruits were chopped into thin slices and dried under shadow. Dried fruit slices were then grounded into finer powder using a powerful grinder. The grinded sample was sieved to get uniform particle size and kept it into air-tight container to prevent it from any photolytic degradation.

Extraction

Powdered fruits (500g) were placed into an amber coated bottle and soaked into 1000 ml of methanol and contents were sealed into bottle for 10 days with occasionally stirred and shaken. After 10 days, the whole mixtures were filtered by Whitman No. 1 filter papers, and the filtrated solutions were concentrated under reduced pressure, heating below 50°C. Finally, near about 20g of crude methanolic extracts (CMEs) of fruits were obtained.

**Determination of total phenolics:** [36]

The total content of phenolics in fruits was measured by using substrate FCR, where gallic acid used as a standard. In a reaction mixture 0.5 mL CME of fruits, 2.5 ml of FCR and 2 ml of sodium carbonate (7.5%) were added. The tubes were mixed and let to stand for 2 hours. At 760 nm absorbance was measured.

**Estimation of total flavonoids:** [37]

Total content of flavonoids was measured according to the method of Zhishen et al. [37]. Fruit extract was added with 0.5 mL in 0.15 mL of 5% sodium nitrite and well mixed. After 5 min of incubation, 0.3 mL of 10% aluminum chloride solution was added. After 6 min of interval, 1 mL of 1M sodium hydroxide was added to the mixture and the volume was made up to 10 mL with distilled water. The absorbance was taken at 510 nm with UV–vis spectrophotometer. Total content of flavonoids were calculated from a Catechin standard curve and expressed as mg Catechin equivalents/gm (mg CE/gm).

**Determination of total flavonols:** [38]

Total amount flavonol was determined by using aluminum chloride as a substrate and standard Gallic acid as a standard. 300μ/L MLE CME were placed in a10mL test tube & methanol was added up to 1 mL. Then, 1 mL of aluminum chloride solution (2%) is added to it. Finally 1.5 mL of 5% w/v sodium acetate was added in the test tube which is then incubated at room temperature for two and half hours.
Absorbances were taken at 440 nm. Total Flavonol amounts were expressed as Gallic acid equivalents/g (mg GAE/gm) dry matter. All samples were analyzed thrice and result averaged.

**Determination of AChe Inhibitory Activity:** [39]

Modified Ellman’s colorimetric method was applied to run *in-vitro* AChE inhibitory assay and ATCI used as a substrate. AChE hydrolysis rate was monitored spectrophotometrically. Each fruit extract or standard (various concentrations) was mixed with 200 μL of enzyme solution (5.21 x 10⁻³ U) and incubated at 37°C for 30 min. After that, Ellman’s reaction mixture (400 μL of 0.35 mM ATCI, 200 μL of 0.7 mM DTNB) was placed in an extraction buffer saline (50 mM Tris.HCl buffer, 50 mM MgCl₂, 50mM NaCl, 1% Triton X-100, pH 8.0) to adjust it 3 ml of final volume. Absorbance at 412 nm was taken after 30 min incubated this mixture at 37°C. The blank reaction was measured by substituting buffer saline for the enzyme. Eserine was used as a standard drug. Percentage of inhibition of AChE enzymes were determined by comparison of reaction rates of samples related to blank using the formula of (E-S)/E x 100, where E is the activity of enzyme without test sample and S is the activity of the enzyme with test sample.

**Determination of BuChE Inhibitory Activity:** [39]

BuChE inhibitory assay was performed by modified Ellman’s colorimetric method, where BTCI acts as a substrate. BuChE hydrolysis rate was spectrophotometrically examined to run this test. Each fruit extract or standard (various concentrations) was mixed with 50μL enzyme solution (4.16 x 10⁻³ U) and incubated at 37°C for 30 min. After adding Ellman’s reaction mixture (400 μL of 0.35 mM BTCI, 200 μL of 0.7 mM DTNB) in a buffer saline (50 mM of Tris.HCl buffer, 50 mM of MgCl₂, 50 mM of NaCl and 1% Triton X-100, pH 8.0) to the above reaction mixture, to adjust final volume of 3 ml. To verify the result, all reading was repeated 3 times. The blank reaction was measured by substituting buffer saline for the enzyme. Galantamine was used as a reference standard. Percentage of inhibition of BuChE enzymes was determined by comparison of reaction rates of samples related to blank using the formula of (E-S)/E x 100, where E is the activity of enzyme without test sample and S is the activity of the enzyme with test sample.

**Thrombolytic Activity Test:** [40]

For thrombolytic activity test for the fruits human blood was used. Blood was withdrawn from healthy human volunteers (n=10) having no history of blood related disorder, oral contraceptive pills administration or ongoing anticoagulant therapy. 1.0 ml of venous blood from each volunteer was transferred to the sterilized eppendorf tubes (volume 1.5 ml) and incubated for 45 min at 37°C and was allowed to form clot. Fruits extracts (100 mg) were suspended into 10 ml of distilled water. After clot formation, the serum was completely removed from eppendorf tube. Blood clot was again weighed to determine the weight of clot. For each eppendorf tube with the pre-weighed clot, 100 μl aqueous solution of the crude extract was added separately. 100 μl of SK (30,000 IU) were added to the positive control and 100 μl distilled water were added to negative control tubes, respectively. All tubes were then again incubated for 90 min at 37°C to observe clot lysis. Then, the released fluid was removed and tubes were again weighed. Difference obtained in weight taken before and after clot lysis by the extract, positive control and negative control, was expressed as percentage of clot lysis and the equation is shown below:

\[
\text{% of Clot lysis} = \frac{\text{Weight of clot after release of fluid}}{\text{Weight of clot before release of fluid}} \times 100\%
\]

**Statistical Analysis**

Values in this experiment are expressed as mean of triplicate determination ± Standard Deviation. All data used are subjected to one way analysis of variance (ANOVA) and the significant difference between means was determined by Duncan’s Multiple Test (P<0.05) using Statistical Package for the social science version 13.0 (SPSS Inc., Chicago, IL, USA).
Results and discussion:

Total phenolic content

Phenolic compounds, as secondary metabolites, are excellent antioxidant due to their ability to donate electron or hydrogen from phenolic hydroxyl groups, which possesses ideal structure for scavenging free radicals generated in the body. These are major class of bioactive molecules. So, regular consumption of these chemicals from dietary supplement can be beneficial by inhibiting carcinogenesis and mutagenesis.[41-43] Among all Citrus fruit C. maxima contain maximum amount of phenolics compare to other citrus fruits whereas C. macroptera contain least, 414.06 ± 2.87 mg GAE/gm and 146.44 ± 1.55mg Gallic acid equivalent per gram (GAE/gm) respectively. Next to C. maxima, key lime and common lime contain greater phenolics with 377.45 ± 2.64mg GAE/gm, 318.61 ± 2.23 mg GAE/gm of dry extract respectively. Orange and C. bergamia were found moderate in phenolics content with 268.81 ± 1.83mg GAE/gm, 221.13 ± 1.82mg GAE/gm of dry extract respectively. According to our result all Citrus fruit contain significant amount of total which increase antioxidant and free radical scavenger in daily human diet.

Total flavonoids content

The flavonoids are one of the most prominent group of secondary metabolites in Citrus fruit with enormous biological activity like anti-microbial, anti-inflammatory, anti-oxidant and anti-carcinogen. They are also strong free radical scavengers.[44-46] Total flavonoids content of Citrus fruits are expressed as mg catechin equivalent/gm. Our research shows that these citrus fruits are different in their flavonoid content. C. sinensis was the most prominent in flavonoid content with 21.16 ± 1.37mg CE/gm compare to other citrus fruits. C. maxima and C. macroptera comes next with 18.40 ± 1.61 mg CE/gm and 17.44 ± 1.18 mg CE/gm respectively. The remaining 3 citrus contain flavonoids between 11 to 15 mg CE/gm.

Table 2: Total Phenolics, Flavonoids and Flavonol content of Citrus fruits

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Phenolics*</th>
<th>Flavonoids**</th>
<th>Flavonols**</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. limon</td>
<td>318.61 ± 2.23</td>
<td>14.98± 1.67</td>
<td>10.68 ± 1.78</td>
</tr>
<tr>
<td>C. aurantifolia</td>
<td>377.45± 2.64</td>
<td>11.44± 1.49</td>
<td>8.16± 0.74</td>
</tr>
<tr>
<td>C. bergamia</td>
<td>221.13± 1.82</td>
<td>13.31± 1.02</td>
<td>9.60± 1.06</td>
</tr>
<tr>
<td>C. maxima</td>
<td>414.06± 2.87</td>
<td>18.40± 0.61</td>
<td>12.94± 1.31</td>
</tr>
<tr>
<td>C. sinensis</td>
<td>268.81± 1.83</td>
<td>21.16± 1.37</td>
<td>10.86± 1.82</td>
</tr>
<tr>
<td>C. macroptera</td>
<td>146.44± 1.55</td>
<td>17.44± 1.18</td>
<td>12.52± 1.35</td>
</tr>
</tbody>
</table>

Values are means of triplicate determination ± Standard Deviation.

* mg GAE/gm of dried sample

** mg CE/gm of dried sample
Total flavonol contents

Flavonols are one of the classes of flavonoids containing 3-hydroxy-2-phenylchromen-4-one ring in it. Biologically they play an important role in neuroprotection, as they can re-establish the redox regulation of proteins, transcription factors and signaling cascades that are otherwise inhibited by elevated oxidative stress. The final survival or death of the neuron depends on flavonol concentrations, time of exposure as well as metabolic and oxidative neuronal circumstances.

Citrus fruits are always been a very eminent source of flavonols. In our study we can see those Citrus fruits contain almost similar amount of flavonols, ranges between 8-13 mg Catechin equivalent/gm of dried extracts. *C. maxima* was found leader in holding flavonols with 12.94 ± 1.31 mg CE/gm of dried extracts beating *C. macroptera* (12.52 ± 1.35 mg CE/gm) and *C. sinensis* (10.86 ± 1.82 mg CE/gm). *C. aurantifollia* was least in containing this bioactive molecule, with 8.16 ± 0.74 mg CE/gm.

Other Citrus fruits *C. limon* and *C. bergamia* contains 10.68 ± 1.78 mg CE/gm and 9.60 ± 1.06 mg CE/gm respectively.

Acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE), a hydrolase, plays a crucial role in cholinergic transmission by catalyzing acetylcholine (ACh), vital neurotransmitter for cognition. In several neurodegenerative disorder the expression of AChE increases enormously, causes breakdown of ACh as a greater extent, which leads to deficit in cognitive function. Beside this in several types of dementia and Alzheimer’s disease the number of neuron decreases. This get more worsen when limited neuron released neurotransmitter (especially ACh) breaks apart with AChE. Inhibiting AChE found beneficial for these patients.

Inhibitory activities of the fruits are demonstrated in Table 3. All most all citrus fruit that we were used in our experiment found active against inhibiting AChE and *C. bergamia* found most active with the IC50 of 27.18 µg/ml. Next to *C. bergamia; C. limon, C. sinensis* and *C. aurantifollia* found similar in inhibiting AChE with IC50 of 38.21 µg/ml, 35.92 µg/ml and 40.52 µg/ml respectively. *C. maxima* and *C. macroptera* have moderate activity in inhibiting AChE with IC50 of 59.16 & 100.62 µg/ml respectively. IC50 of these fruits may be higher than the standard but as a dietary supplement it can be a potential source of AChE inhibitor.

Butyrylcholinesterase inhibitory activity

Butyrylcholinesterase is a nonspecific cholinesterase enzyme that hydrolyses many different choline-based esters. It not only breakdown both choliesterases (Ach and BuCh), but also synergists function of AChE enzyme. In neurodegenerative disorder, like AD, the expression of BuChE also increases enormously. So, inhibiting this enzyme can also be found very effective in AD and other types of Dementia.

BuChE have structurally similarity with AChE, so sometimes AChE inhibitors can inhibits BuChE. Citrus fruit are capable to inhibit this enzyme, more or less. In our study we found that *C. macroptera* and *C. bergamia* were most effective fruits that inhibit BuChE at lower concentration with IC50 of 32.50 and 34.74 µg/ml compare to the rest. *C. maxima* had shown least activity against this enzyme. Remaining fruits (*C. limon, C. sinensis* and *C. aurantifollia*) gave moderate activity. (Figure no. 4)
From all six citrus fruit *C. limon*, *C. aurantifolia*, *C. bergamia* and *C. maxima* found more prominent in inhibiting AChE enzyme compare to their BuChE inhibitory activity. But *C. sinensis* and *C. macroptera* had shown their potentiality in inhibiting BuChE than AChE.

Table 3: Acetylcholinesterase inhibitory activity of Citrus fruits

<table>
<thead>
<tr>
<th>Citrus Species</th>
<th>20 µg/ml</th>
<th>50 µg/ml</th>
<th>100 µg/ml</th>
<th>200 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. limon</em></td>
<td>31.71 ± 1.57</td>
<td>57.50 ± 1.24</td>
<td>72.92 ± 1.69</td>
<td>78.21 ± 1.58</td>
</tr>
<tr>
<td><em>C. aurantifolia</em></td>
<td>28.19 ± 1.29</td>
<td>52.73 ± 1.54</td>
<td>63.06 ± 1.82</td>
<td>69.26 ± 1.09</td>
</tr>
<tr>
<td><em>C. bergamia</em></td>
<td>42.44 ± 1.63</td>
<td>62.90 ± 0.93</td>
<td>78.02 ± 1.41</td>
<td>83.12 ± 1.11</td>
</tr>
<tr>
<td><em>C. maxima</em></td>
<td>27.75 ± 1.48</td>
<td>44.55 ± 1.32</td>
<td>55.09 ± 0.65</td>
<td>64.17 ± 1.12</td>
</tr>
<tr>
<td><em>C. sinensis</em></td>
<td>32.11 ± 0.89</td>
<td>60.40 ± 1.14</td>
<td>74.28 ± 1.29</td>
<td>80.02 ± 0.73</td>
</tr>
<tr>
<td><em>C. macroptera</em></td>
<td>24.37 ± 1.08</td>
<td>38.51 ± 0.77</td>
<td>49.88 ± 1.22</td>
<td>55.63 ± 1.19</td>
</tr>
<tr>
<td>Eserine (Std.)</td>
<td>72.34 ± 0.83</td>
<td>88.12 ± 1.47</td>
<td>91.44 ± 1.50</td>
<td>92.14 ± 1.74</td>
</tr>
</tbody>
</table>

Values are means of triplicate determination ± Standard Deviation.

Figure 1: Acetylcholinesterase inhibitory activity of Citrus fruits
### Table 4: Butyrylcholinesterase inhibitory activity of Citrus fruits

<table>
<thead>
<tr>
<th>Citrus Species</th>
<th>% of Butyrylcholinesterase Enzyme inhibition (µg/ml)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 µg/ml</td>
<td>50 µg/ml</td>
<td>100 µg/ml</td>
<td>200 µg/ml</td>
<td></td>
</tr>
<tr>
<td>C. limon</td>
<td>20.92±1.47</td>
<td>44.94±1.66</td>
<td>54.61±1.89</td>
<td>58.11±1.16</td>
<td></td>
</tr>
<tr>
<td>C. aurantifolia</td>
<td>27.54±1.35</td>
<td>48.23±1.79</td>
<td>56.09±1.04</td>
<td>61.02±1.29</td>
<td></td>
</tr>
<tr>
<td>C. bergamia</td>
<td>32.67±1.61</td>
<td>55.23±0.56</td>
<td>62.63±1.93</td>
<td>68.11±1.62</td>
<td></td>
</tr>
<tr>
<td>C. maxima</td>
<td>14.16±1.42</td>
<td>29.18±1.23</td>
<td>42.76±1.71</td>
<td>61.98±1.49</td>
<td></td>
</tr>
<tr>
<td>C. sinensis</td>
<td>17.67±1.18</td>
<td>30.22±1.70</td>
<td>46.24±1.55</td>
<td>55.08±1.47</td>
<td></td>
</tr>
<tr>
<td>C. macroptera</td>
<td>39.43±0.71</td>
<td>58.07±1.31</td>
<td>67.86±1.35</td>
<td>74.43±0.64</td>
<td></td>
</tr>
<tr>
<td>Galantamine(Std.)</td>
<td>72.43±1.64</td>
<td>86.17±1.32</td>
<td>88.42±1.40</td>
<td>91.41±1.72</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of triplicate determination ± Standard Deviation.

### Figure 2: Butyrylcholinesterase inhibitory activity of Citrus fruits
Thrombus formation in the blood vessels can obstructs blood flow through the circulatory system leading hypertension, stroke to the heart, anoxia, and so on.[63-65] If it occurs in the brain it can also lead to neurodegeneration.[66]Thrombolytic drugs are mainly prescribed for controlling thrombosis patients. According to our test we found Citrus fruits are not very potential in clot dissolving manners. They reported very minor thrombolytic activity ranges from 0.3 to 7 % in total. *C. macroptera* can dissolve $6.908 \pm 1.702$ % of total blood clot, which was the highest among all citrus fruits. *C. aurantifollia* and *C. bergamia* have almost similar types of thrombolytic property with $5.453 \pm 0.896$ % and $5.942 \pm 1.179$ % clot lysis. Similar to other citrus fruits, rest of the fruit extracts are also not so good in dissolving blood clots. As *C. sinensis* can clot $4.798 \pm 0.806$ % clot, *C. maxima* can break $1.785 \pm 0.478$ % clot and *C. limon* can lysis only $0.369 \pm 0.148$ % clot. So treating thrombus and clotting disorder by using citrus fruit is found impossible. Platelets play a significant role in blood clotting by the development of thrombosis atherothrombosis, which in mainly initiated from damage the regions of endothelial surface by reactive oxygen species (ROS). The stimulated platelets enhance platelet-platelet bonding.[67-69] This binding can also traps other blood cells which accelerate
process of plaque development and progression. As citrus fruits are highly effective against ROS by scavenging them, they lacks in thrombolytic property.[70,71]

Table 5: Thrombolytic activity of Citrus fruits

<table>
<thead>
<tr>
<th>Citrus Species</th>
<th>% of clot lysis (Con. 100µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. limon</td>
<td>0.369 ± 0.148</td>
</tr>
<tr>
<td>C. aurantifolia</td>
<td>5.453 ± 0.896</td>
</tr>
<tr>
<td>C. bergamia</td>
<td>5.942 ± 1.179</td>
</tr>
<tr>
<td>C. maxima</td>
<td>1.785 ± 0.478</td>
</tr>
<tr>
<td>C. sinensis</td>
<td>4.798 ± 0.806</td>
</tr>
<tr>
<td>C. macroptera</td>
<td>6.908 ± 1.702</td>
</tr>
<tr>
<td>Streptokinase (Std.)</td>
<td>87.016 ± 2.253</td>
</tr>
</tbody>
</table>

Values are means of triplicate determination ± Standard Deviation.

Figure 4: percentage of clot lysis by Citrus fruits

Conclusion:

All six citrus fruits are rich in phenolics, flavonoids and flavonols, while C. maxima contain maximum. In enzyme inhibitory capabilities, C. bergamia was found most capable in inhibiting AChE (IC50 27.18 µg/ml) and C. macroptera was most active in inhibition of BuChE (IC50 32.50µg/ml). According to the study, citrus fruit are not that much suitable for thrombolysis. So, on the basis of cholinesterase inhibitory activity and chemical contents, this study provides information
that, citrus fruit can improve ACh. Further study is needed to find actual molecule that is responsible for their specific action.

References:


