DPPH SCAVENGING ACTIVITIES OF SELECTED FLAVONOIDS AND THEIR MIXTURES

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1.INTRODUCTION

Flavonoids belong to the group of polyphenols. They are the most common in human diet and in many plants. They are found in almost all fruits such as citrus species, black and green tea and onions. Daily intake of flavonoids fluctuates from 70 to 170mg per day(Erlund,2004). This fluctuation is mainly because of the different dietary habits among countries. Increased consumption of foods containing flavonoids is observed in countries with Mediterranean climate because it more likely for those plants containing flavonoids to grow. Not to forget, food preparation usually decreases flavonoid content even to its half.

In vitro studies have shown that flavonoids have:

- High anti-oxidant activity
- Anti-inflammatory activity
- Anti-cancer activity
- Anti-allergic activity
- Anti-bacterial activity
- Antiviral activity
- Cardiovascular effect

However, the in vivo mechanisms are yet unknown mainly because most of the studies have focused on in vitro tests and the concentrations or doses are much higher than in human investigations. Due to imbalance between ROS/RNS and the anti-oxidants, there is a phenomenon called oxidative stress. Oxidative stress is linked with many diseases such as cancer, neurodegenerative disorders and aging. The anti-oxidative activity of flavonoids is based on their ability to donate a hydrogen or an electron and to delocalize the unpaired electron inside the aromatic structure. Synergy and other interactions of herbal mixtures seem to have positive effect from the point of view of side effects because compounds with high toxicity when combined with others may be administered in lower doses.

2. OBJECTIVE

The aim of our study was the identification of the anti-oxidant activity of selected flavonoids (quercetin, luteolin and galangin) and their mixtures using DPPH method including their synergistic or antagonistic interactions.

3. METHODS

- Chemicals used: quercetin, luteolin and galangin 98% pure all solvents were distilled before use.
- Apparatus used: TECAN M200 and UV-Star-96-well.
- Method: Using DPPH (2,2-diphenyl-1-picrylhydrazyl).
- Software used: CompuSyn 1.0.1

Drug synergy in our study was based on the Chou-Talalay method in which the median effect equation based on the mass-action law principle is used, giving us the common link between single compounds and multiple ones and first or higher dynamics. The resulting combination index (CI) indicates CI=1 for additive effect, CI<1 for synergism and CI>1 for antagonism in drug combinations (Chou et al, 2006).

DPPH enzyme reaction

. The calculation is based on the following formula:

% = 100 × (ADPPH – Asample) / ADPPH

Note: The mixtures of flavonoids were prepared in equimolar ratio: 1:1 or 1:1:1.

4. RESULTS AND DISCUSSION

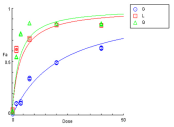

Figure 2. Dose-effect drug

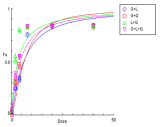

Figure 3. Dose-effect combos

Fa/ Fa=0.5	IC50 (μM)	r	CI
G	20.5271	0.98128	-
L	3.64320	0.90754	-
Q	3.02745	0.88422	-
G-L	6.03707	0.96879	0.97559
G-Q	6.23764	0.78525	1.18212
L-Q	3.09835	0.87928	0.93693
G-L-Q	4.21137	0.93788	0.91739

IC50, r, CI values for each solution and their mixtures r is the correlation coefficient (ideal value: r = 1)

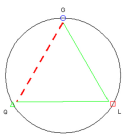

Figure 4. Polygonogram at Fa = 0.5

By comparing the IC50 values we observe that the best is quercetin's with 3.02745 μM and after comes luteolin and galangin respectively. It seems that the presence of 4'-OH group is important for the DPPH scavenging activity. As we observe from the results in the combination IC50s in equimolar solutions, luteolin-quercetin has the highest activity of all combinations with 3.09835 Mm (two most potent scavengers) and after that the triple combination of quercetin+ luteolin+galangin. According our results quercetin is the most active flavonoid. The combination index indicates that the best combination is the triple with a value of 0,91739 and it is characterized as nearly additive. The combinations of luteolin with quercetin and luteolin with galangin are categorized as nearly additive as well. On the other hand, the combination of quercetin with galangin is slightly antagonistic. A more qualitative than quantitative observation is presented in the polygonogram indicating nearly additive effect with a thin green line and slightly antagonistic with a dashed red line. These results denote that in our study no synergy was observed. Another inference extracting about the efficacy of compounds concerning dose-efficacy and cost-evaluation in real life, for optimum solution using a DPPH scavenger is the sole quercetin the best to use, which was the cheapest flavonoid in our study.

5. CONCLUSION

Flavonoids have many beneficial effects. Checking the scavenging activity and synergy of quercetin, luteolin, galangin and their combinations led as to conclude that no synergy was found but only nearly additive effect was observed. All results for flavonoid combination as determined for DPPH radical scavenging are described for the first time.

6. REFERENCES

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