



## Potential Effect of *Citrus decumana* Extract on Stress Induced Peptic Ulcer in Rat

Shailja SOOD <sup>1\*</sup>, Arunachalam MUTHURAMAN <sup>1</sup>, Bhawna ARORA <sup>1</sup>, Stuti BANSAL <sup>1</sup>,  
Manoj BALI <sup>2</sup> & Pritam D. SHARMA <sup>3</sup>

<sup>1</sup> Rayat Institute of Pharmacy, Nawanshahr District, Railmajra, Near Ropar, Punjab-144533 (India).

<sup>2</sup> Rayat Institute of Engineering and Technology, Ropar Campus, Railmajra, Punjab-144533 (India).

<sup>3</sup> University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh (India).

**SUMMARY.** The present study was designed to investigate the antiulcer activity of ethyl acetate extract of *Citrus decumana* (grapefruit) peels. The antiulcerogenic activity was evaluated in water immersion and hypothermic restraint stress models at different doses (150, 250 and 350 mg/kg). The antiulcer potential of the extract was assessed by determining and comparing the ulcerative index and biochemical estimation was carried out using various oxidative stress markers i.e., TBARS, GSH, SOD and CAT in the blood and tissue samples. The highest dose (350 mg/kg) of the extract showed significant decrease in the ulcerative index and TBARS level, whereas there was increase in the GSH, SOD and CAT levels. Whereas the lowest and medium dose (150 mg/kg and 250mg/kg) did not produce any significant results. Therefore, our study indicate that the *Citrus decumana* peel extract may be used as a natural therapeutic agent in the treatment of peptic ulcers.

### INTRODUCTION

Phytogenic agents have traditionally been used by herbalists and indigenous healers for the prevention and treatment of peptic ulcer <sup>1</sup>. There are various plant-originated "gastroprotectors" with different composition that have been used in clinical and folk medicine by many countries due to their beneficial effects on the mucosa of GIT <sup>2</sup>. Peptic ulcers are a deep gastrointestinal erosion disorder that involve the entire mucosal thickness, penetrating the muscular mucosa <sup>3</sup>. Peptic ulcers were caused by an imbalance between the aggressive factors either exogenous aggressive factors such as spicy and fatty foods, smoke, anti-inflammatory drugs, alcohol, stress etc or endogenous aggressive factor such as reactive oxygen and nitrogen species (ROS/RNS), hydrochloric acid, *Helicobacter pylori*, pepsin etc. and a number of known defense mechanisms <sup>4-6</sup>. Therefore, treatment with antioxidants and synthetic drugs such as H<sup>+</sup>K<sup>+</sup>-

ATPase pump inhibitors, histamine H<sub>2</sub>-receptor blockers can decrease gastric mucosal damage <sup>7,8</sup>. But these synthetic drugs have various side effects such as diarrhoea, headache, drowsiness, fatigue, and muscular pain <sup>9</sup>. Hence these days natural compounds are being explored so that they could replace these synthetic drugs. Clinical research has confirmed the efficacy of several plants for the treatment of gastrointestinal diseases like *Momordica charantia*, *Garcinia indica*, *Carpolobia lutea*, etc. <sup>10-13</sup>.

In recent years there is a growing interest in citrus fruits (family rutaceae) because their consumption decreases the risk of cancer, inflammation, heart disease, ulcers etc. Citrus juices are considered to be a rich source of antioxidants including vitamin C, phenolic compounds and carotenoids which are responsible for their health benefits <sup>14,15</sup>. However, most people throw away the peels after enjoying citrus fruit. Even during the processing of citrus fruit or

**KEY WORDS:** Antiulcer activity, *Citrus decumana* peels, oxidative stress markers.

\*Author to whom correspondence should be addressed. E-mail: soodshalurayat@gmail.com

juice in food industries, peels are the primary byproducts. Recently, citrus peels have attracted the attention of researchers as they were found to be an interesting source of phenolic compounds, which include phenolic acids and flavonoids<sup>16</sup>. The peels of many citrus species, like *Citrus sinensis*, *Citrus paradisi* and *Citrus reticulata* have been evaluated for antioxidant activity due to presence of flavonoids and other phenolic compounds<sup>17</sup>. Hydroxylated polymethoxyflavones and methylated flavonoids have also been isolated from *Citrus sinensis* peel extract<sup>18</sup>. Many studies show that flavonoids present in the citrus peel possess strong antioxidant, anti-atherogenic, antiviral, antiaggregatory, antimutagenic, antiulcer and antitumor effects<sup>19-21</sup>. It is also observed that oxidative stress plays a role in ulcer formation<sup>22</sup>. So these flavonoids can be used to treat gastrointestinal disorders, including gastric ulcer<sup>23</sup>. The present study was undertaken to study the antiulcer potential of *Citrus decumana* (grapefruit) peel extract in stress induced peptic ulcer in rats.

## MATERIALS AND METHODS

### Plant material

For the present investigation the fruits of *Citrus decumana* were collected from northern region of India. The plant material was authenticated and the voucher specimen no. 0353 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar. The fruits were washed and dried properly before removing peels. The peels were then dried under shade at room temperature. The dried peels were grounded into a coarse powder in a mixer. The powder was sieved through a 1mm metal sieve to obtain a standard particle size.

### Animals

The wistar albino rats of either sex were obtained from Sanjay Biologicals, Amritsar. They were kept at standard laboratory diet, environmental temperature and humidity. A 12 h light-dark cycle was maintained throughout the experimental protocol. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg No. 874/ac/05/CPCSEA).

### Extraction

The dried peel powder of plant material was extracted by maceration process using solvents of increasing polarity; hexane, chloroform, ethyl acetate and methanol. The powdered material was extracted with each solvent three times at room temperature over a period of 24 h. The material was kept for 24 h between each successive solvent for proper drying. The extracts were filtered and concentrated under vacuum on a rotary evaporator at 40 °C and stored in a refrigerator for further analysis.

### Phytochemical screening

The crude extracts were analyzed for alkaloids, tannins, saponins, flavonoids, steroids, terpenoids and phenolic acids using standard procedures of analysis<sup>24</sup>. The ethyl acetate extract showed the presence of flavonoids and phenolic acids. Shinoda test and ferric chloride test were carried out for the confirmation of flavonoids and phenolic acids. The crude extracts were also evaluated for their *in vitro* antioxidant activity using DPPH and hydrogen peroxide methods. Among all four extracts ethyl acetate extract of *Citrus decumana* peels (EtCD) showed maximum antioxidant activity. Hence EtCD was further used for antiulcer studies.

### Methods for induction of peptic ulcer in animal models

#### Water immersion- induced stress (WIS)

The rats were fasted for 24 h before inducing the ulcer and test samples were administered 1 h prior to stress induction. These were kept immobilized in a stress cage and then immersed to the level of the xiphoid process in a water bath at  $23 \pm 0.2$  °C for 7 h<sup>25</sup>. The blood samples were collected by the retro-orbital sinus puncture for the estimation of biomarker components. The animals were sacrificed. The stomach of each animal was removed and the extent of gastric damage was assessed by estimating ulcerative index.

#### Hypothermic restraint stress (HRS)

The rats were fasted for 36 h before inducing the ulcer and test samples were administered 1 h prior to stress induction. These were kept immobilized in a restraint cage at 4 °C for 3 h<sup>26</sup>. The blood samples were collected by the retro-orbital sinus puncture for the estimation of biomarker components. The animals were sacrificed. The stomach of each animal was removed and extent of gastric damage was assessed by estimating ulcerative index.

### Experimental Design

In the present antiulcer studies ten groups, each comprising of six rats, were used: Group 1 - WIS control group; Group 2 - WIS + Ranitidine 50 mg/kg, p.o. treated group; Group 3 - WIS + EtCD 150 mg/kg, p.o. treated group for 10 days; Group 4 - WIS + EtCD 250 mg/kg, p.o. treated group for 10 days; Group 5 - WIS + EtCD 350 mg/kg, p.o. treated group for 10 days; Group 6 - HRS control group; Group 7 - HRS + Ranitidine 50 mg/kg, p.o. treated group; Group 8 - HRS + EtCD 150 mg/kg, p.o. treated group for 10 days; Group 9 - HRS + EtCD 250 mg/kg, p.o. treated group for 10 days; Group 10 - HRS + EtCD 350 mg/kg, p.o. treated group for 10 days.

### Measurement of ulcerative index

Ulcerative index was measured <sup>27</sup>. Briefly, the stomach was opened and washed with running tap water. Then it was placed on a flat glass plate to count the ulcerative area. Standardization was made with a 10x10cm squared glass plate. Opened stomach, overlaid squared flat glass plate, exposing the mucous, showing the counting methodology of the injuries per square mm. The ulcer index was determined by using the formula, Ulcer Index = 10/X Where, X = Total mucosal area / Total ulcerated area.

### Biochemical estimation

#### Estimation of tissue and plasma TBARS

Lipid peroxide content was determined in terms of thiobarbituric acid reactive species (TBARS) in tissue <sup>28</sup> and in plasma using tetramethoxypropane as standard <sup>29</sup>. The results were expressed as nmol/g protein in tissue and nmol/ml in plasma.

#### Estimation of tissue and plasma GSH

The reduced glutathione level in different samples were determined by the enzymatic method <sup>30</sup>. The results were expressed as  $\mu\text{mol/g}$  of protein in tissue and  $\mu\text{mol/ml}$  in plasma.

#### Estimation of Superoxide dismutase (SOD) and Catalase (CAT) activity in tissue and serum

The activities of SOD and CAT in tissue and serum were determined using commercially available kits. (Span Diagnostics, Gujarat, India). The SOD and CAT activities were expressed as units U/mg protein in tissue and U/ml in serum

#### Estimation of protein content

Protein concentration was determined using bovine serum albumin as a standard <sup>31</sup>. The re-

sults were expressed as mg/g of tissue and mg/ml in plasma.

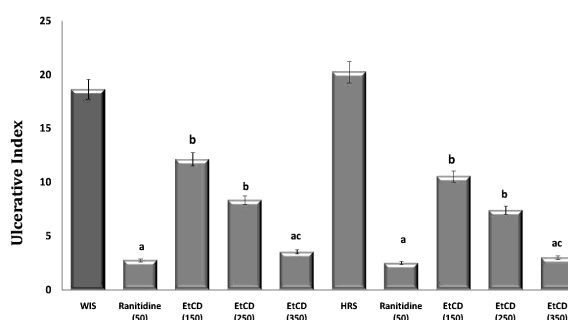
### Statistical analysis

All the results were expressed as mean  $\pm$  standard error of means (S.E.M). The data was statistically analyzed by one way analysis of variance (ANOVA) followed by Tukey's multiple range tests by using Sigmastat Version-2.0 Software. The p-value < 0.05 was considered to be statistically significant.

### RESULTS

The results of ethyl acetate extract of *Citrus decumana* peels on ulcerative index in both WIS and HRS model had been depicted in Figure 1. The decrease in the ulcerative index in rats treated with dose of 150 and 250 mg/kg EtCD was found to be insignificant as compared to that of ranitidine (50 mg/kg) treated group. However, higher dose (350 mg/kg) showed significant reduction in ulcerative index similar to that of ranitidine treated group.

The Tables 1 and 2 show the tissue and plasma biomarker changes in WIS and HRS model respectively. There was an increase in the TBARS level and a decrease in the level of ROS scavenging enzymes i.e SOD and CAT in the control groups. GSH level also decreases. Further, pretreatment with EtCD (150, 250 and 350 mg/kg) and ranitidine (50 mg/kg) showed reversible changes in the above parameters. However, only higher dose (350mg/kg) showed statistically significant ( $p < 0.05$ ) results similar to that of ranitidine (50 mg/kg) treated group.



**Figure 1.** Effect of *Citrus decumana* on UI in WIS and HRS induced peptic ulcer. Values are mean  $\pm$  SEM of 6 animals. **a** =  $p < 0.05$ , pretreatment groups as compared to water immersion stress (WIS) and hypothermic restraint stress (HRS) control group respectively; **b** =  $p < 0.05$ , as compared to ranitidine treated group; **c** =  $p < 0.05$ , as compared to ethyl acetate extract of *Citrus decumana* peels (EtCD) 150 and 250 mg/kg pretreated group, in parenthesis indicated the dose in mg/kg.

| Groups<br>(mg/kg) | TBARS                         |                               | GSH                           |                                | SOD                           |                               | CAT                           |                               |
|-------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                   | Tissue<br>(nmol/g)            | Plasma<br>(nmol/ml)           | Tissue<br>( $\mu$ mol/g)      | Plasma<br>( $\mu$ mol/ml)      | Tissue<br>(U/mg)              | Plasma<br>(U/ml)              | Tissue<br>(U/mg)              | Plasma<br>(U/ml)              |
| WIS               | 5.05 $\pm$ 0.21               | 5.19 $\pm$ 1.01               | 0.72 $\pm$ 0.05               | 8.72 $\pm$ 1.21                | 2.75 $\pm$ 0.67               | 1.43 $\pm$ 0.51               | 2.47 $\pm$ 1.03               | 0.26 $\pm$ 0.01               |
| Ranitidine (50)   | 3.56 $\pm$ 0.31 <sup>a</sup>  | 3.75 $\pm$ 0.91 <sup>a</sup>  | 1.55 $\pm$ 0.03 <sup>a</sup>  | 16.52 $\pm$ 1.9a               | 4.97 $\pm$ 1.04 <sup>a</sup>  | 2.15 $\pm$ 0.43 <sup>a</sup>  | 6.76 $\pm$ 1.05 <sup>a</sup>  | 0.46 $\pm$ 0.02 <sup>a</sup>  |
| EtCD (150)        | 4.36 $\pm$ 0.46 <sup>b</sup>  | 4.46 $\pm$ 0.67 <sup>b</sup>  | 0.93 $\pm$ 0.02 <sup>b</sup>  | 10.13 $\pm$ 1.76 <sup>b</sup>  | 3.30 $\pm$ 1.01 <sup>b</sup>  | 1.57 $\pm$ 0.56 <sup>b</sup>  | 3.25 $\pm$ 1.01 <sup>b</sup>  | 0.28 $\pm$ 0.01 <sup>b</sup>  |
| EtCD (250)        | 4.31 $\pm$ 0.21 <sup>b</sup>  | 4.33 $\pm$ 0.94 <sup>b</sup>  | 1.07 $\pm$ 0.04 <sup>b</sup>  | 13.46 $\pm$ 1.54 <sup>b</sup>  | 4.01 $\pm$ 1.07 <sup>b</sup>  | 1.66 $\pm$ 0.65 <sup>b</sup>  | 5.09 $\pm$ 1.37 <sup>b</sup>  | 0.32 $\pm$ 0.03 <sup>b</sup>  |
| EtCD (350)        | 3.60 $\pm$ 0.65 <sup>ac</sup> | 3.81 $\pm$ 0.77 <sup>ac</sup> | 1.46 $\pm$ 0.06 <sup>ac</sup> | 16.24 $\pm$ 1.74 <sup>ac</sup> | 4.85 $\pm$ 1.11 <sup>ac</sup> | 2.03 $\pm$ 0.77 <sup>ac</sup> | 6.70 $\pm$ 1.46 <sup>ac</sup> | 0.46 $\pm$ 0.01 <sup>ac</sup> |

**Table 1.** Effect of *Citrus decumana* on biomarker changes in WIS model. Values are mean  $\pm$  SEM of 6 animals. <sup>a</sup> =  $p < 0.05$ , pretreatment groups as compared to water immersion stress (WIS) and hypothermic restraint stress (HRS) control group respectively; <sup>b</sup> =  $p < 0.05$ , as compared to ranitidine treated group; <sup>c</sup> =  $p < 0.05$ , as compared to ethyl acetate extract of *Citrus decumana* peels (EtCD) 150 and 250 mg/kg pretreated group, in parenthesis indicated the dose in mg/kg.

| Groups<br>(mg/kg) | TBARS                        |                                | GSH                           |                                | SOD                           |                               | CAT                           |                               |
|-------------------|------------------------------|--------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                   | Tissue<br>(nmol/g)           | Plasma<br>(nmol/ml)            | Tissue<br>( $\mu$ mol/g)      | Plasma<br>( $\mu$ mol/ml)      | Tissue<br>(U/mg)              | Plasma<br>(U/ml)              | Tissue<br>(U/mg)              | Plasma<br>(U/ml)              |
| HRS               | 6.02 $\pm$ 0.72              | 6.025 $\pm$ 0.61               | 0.71 $\pm$ 0.05               | 7.49 $\pm$ 1.26                | 2.43 $\pm$ 0.39               | 1.43 $\pm$ 0.01               | 2.21 $\pm$ 1.01               | 0.27 $\pm$ 0.02               |
| Ranitidine (50)   | 4.58 $\pm$ 0.51 <sup>a</sup> | 4.98 $\pm$ 0.34 <sup>a</sup>   | 1.63 $\pm$ 0.07 <sup>a</sup>  | 16.07 $\pm$ 1.57 <sup>a</sup>  | 4.79 $\pm$ 0.41 <sup>a</sup>  | 2.08 $\pm$ 0.09 <sup>a</sup>  | 6.7 $\pm$ 1.31 <sup>a</sup>   | 0.5 $\pm$ 0.04 <sup>a</sup>   |
| EtCD (150)        | 5.7 $\pm$ 0.63 <sup>b</sup>  | 5.973 $\pm$ 0.27 <sup>b</sup>  | 0.76 $\pm$ 0.01 <sup>b</sup>  | 9.95 $\pm$ 1.31 <sup>b</sup>   | 2.59 $\pm$ 0.54 <sup>b</sup>  | 1.52 $\pm$ 0.06 <sup>b</sup>  | 2.45 $\pm$ 1.21 <sup>b</sup>  | 0.26 $\pm$ 0.02 <sup>b</sup>  |
| EtCD (250)        | 4.71 $\pm$ 0.21 <sup>b</sup> | 5.658 $\pm$ 0.36 <sup>b</sup>  | 1.11 $\pm$ 0.06 <sup>b</sup>  | 13.29 $\pm$ 1.56 <sup>b</sup>  | 4.45 $\pm$ 0.62 <sup>b</sup>  | 1.77 $\pm$ 0.03 <sup>b</sup>  | 6.35 $\pm$ 1.04 <sup>b</sup>  | 0.35 $\pm$ 0.01 <sup>b</sup>  |
| EtCD (350)        | 4.6 $\pm$ 0.90 <sup>ac</sup> | 5.011 $\pm$ 0.12 <sup>ac</sup> | 1.55 $\pm$ 0.03 <sup>ac</sup> | 16.02 $\pm$ 1.97 <sup>ac</sup> | 4.68 $\pm$ 0.31 <sup>ac</sup> | 1.98 $\pm$ 0.02 <sup>ac</sup> | 6.63 $\pm$ 1.07 <sup>ac</sup> | 0.46 $\pm$ 0.01 <sup>ac</sup> |

**Table 2.** Effect of *Citrus decumana* on biomarker changes in HRS Model Values are mean  $\pm$  SEM of 6 animals. <sup>a</sup> =  $p < 0.05$ , pretreatment groups as compared to water immersion stress (WIS) and hypothermic restraint stress (HRS) control group respectively; <sup>b</sup> =  $p < 0.05$ , as compared to ranitidine treated group; <sup>c</sup> =  $p < 0.05$ , as compared to ethyl acetate extract of *Citrus decumana* peels (EtCD) 150 and 250 mg/kg pretreated group, in parenthesis indicated the dose in mg/kg.

## DISCUSSION

In the present study, the ethyl acetate extract of *Citrus decumana* peels was evaluated for its *in vivo* antiulcer activity in both water immersion and hypothermic restraint stress models. Grapefruit juices are known to possess antioxidant potential may be due to the presence of flavanone naringin and its aglycone, naringenin. But literature reveal that these constituents are present in more abundant in peel part of *Citrus decumana*<sup>14,32,33</sup>. Hence, in our study we tried to explore the effect of its peel extract on stress induced peptic ulcer. The induction of stress generates free radical, which causes mucosal damage and change in antioxidant enzymes<sup>34</sup>. The excess free radical generation causes enhanced lipid peroxidation which was indicated by an increase in the levels of TBARS. Due to increased TBARS level antioxidant defense mechanisms fail to prevent the formation of excess free radicals and leads to tissue injury<sup>35</sup>. GSH acts as an important endogenous defense substance against the reactive oxygen species

(ROS). This GSH level is reduced in rats subjected to increased stress levels<sup>36</sup>. Various antioxidant enzymes like SOD, CAT prevent the accumulation of ROS. Stress results in the imbalance in the activity of these enzymes which leads to faulty disposal of free radicals and their accumulation<sup>37</sup>.

Our studies showed that EtCD has dose dependent antiulcer effect in both water immersion and hypothermic restraint stress model. Low and medium dose was not found to be significant in treating ulcer as compared to ranitidine treated group but higher dose (350 mg/kg) showed significant effect. Phytochemical screening revealed the presence of flavonoids and phenolic acids in the EtCD extract, which are known to possess antioxidant activity<sup>38</sup>. Thus the action of extract may be through free radical scavenging mechanism. The decrease in ulcerative index and lipid peroxidation i.e. TBARS level and increase in the activities of free radical scavenging enzymes and glutathione in the extract treated group compared to ulcerated group

suggests the ability of extract to protect the gastric mucosa against free radical mediated tissue injury.

WIS and HRS stress models cause potential alteration of physiological antioxidant status and imbalance in the free radical defense enzymatic system. Hence, in both the models, EtCD showed potential amelioration of ulcerative and oxidative stress marker changes in blood and tissue.

## CONCLUSION

Therefore the above studies showed that EtCD possess gastroprotective effect at a dose level of 350 mg/kg in both water immersion and hypothermic restraint stress models. Thus, EtCD may be a potent herbal therapeutic agent for the treatment of peptic ulcer disorders.

**Acknowledgements.** Thanks to Professor A. C. Rana and all faculty members of Rayat Institute of Pharmacy for their encouragement and support. We are also grateful to Rayat & Bahra Educational and Research Trust for their unconditional helps to carry out this project.

## REFERENCES

- Borrelli, F. & A.A. Izzo (2000) *Phytother. Res.* **14**: 581-91.
- Zayachkivska, O.S., S.J. Konturek, D. Drozdowicz, P.C. Konturek, T. Rzozowski & M.R. Ghogotsky (2005) *J. Physiol. Pharmacol.* **56**: 219-31.
- Tarnawski, A.S. (2005) *Digest. Dis. Sci.* **50**: 24-33.
- Kaunitz, J.D. & Y. Akiba (2004) *Curr. Opin. Gastroenterol.* **20**: 526-32.
- Bandyopadhyay, D., K. Biswas, M. Bhattacharyya, R.J. Reiter & R.K. Banerjee (2001) *Curr. Mol. Med.* **1**: 501-13.
- Konturek, P.C. & S. Konturek (1994) *J. Physiol. Pharmacol.* **45**: 333-50.
- Salim, A.S. (1994) *J. Surg. Res.* **56**: 45-52.
- Waldum, H.L., B. Gustafsson, R. Fossmark & G. Qvigstad (2005) *Dig. Dis. Sci.* **50** Suppl 1: S39-44.
- Zimmerman, T.W. (1984) *Am. J. Med.* **77**: 51-6.
- Kanner, J. & T. Lapidot (2001) *Free Radic. Biol. Med.* **31**: 1388-95.
- Gurbuz, I., C. Akyuz, E. Yesilada & B. Sener (2001) *J. Ethnopharmacol.* **71**: 77-82.
- Yamaguchi, F., M. Saito, T. Ariga, Y. Yoshimura & H. Nakazawa (2000) *J. Agric. Food Chem.* **48**: 2320-5.
- Nwidi, L.L. & P. A. Nwafor (2009) *Afr. J. Biotechnol.* **8**: 12-9.
- Gattuso, G., D. Barreca, C. Gargiulli, U. Leuzzi & C. Caristi (2007) *Molecules.* **12**: 1641-73.
- Proteggente, A.R., A. Saija, A.D. Pasquale & C.A. Rice- Evans (2003) *Free Radic. Res.* **37**: 681-7.
- Allesandra, B., M.E. Cuvelier, H. Richard & C. Berset (1998) *J. Agric. Food Chem.* **46**: 2123-9.
- Alicia, M.R., V.A. Marina & C.P. Fanny (2005) *Archi. Latinoam. Nutr.* **55**: 305-10.
- Li, S., C.Y. Lo & C.T. Ho (2006) *J. Agric. Food Chem.* **54**: 4176-85.
- Benavente-Garcia, O., J. Castillo, F.R. Marin & A. Ortuno (1997) *J. Agric. Food Chem.* **45**: 4505-15.
- Rehman, Z. (2006) *Food Chem.* **99**: 450-4.
- Parmar, H.S. & A. Kar (2008) *J. Med. Chem.* **11**: 376-81.
- Das, D., D. Bandyopadhyay, M. Bhattacharjee & R.K. Banerjee (1997) *Free Radic. Biol. Med.* **23**: 8-18.
- Izzo, A.A., G.D. Carlo, N. Mascolo, F. Capasso & G. Autore (1994) *Phytother. Res.* **8**: 179-81.
- Harborne, J.B. (1973) "Phytochemical methods", Chapman and Hall, Ltd., London, pp. 49-188.
- Hayase, M. & K. Takeuchi (1986) *Dig. Dis. Sci.* **31**: 166-71.
- Brodie, D.A. (1962) *Gastroenterology* **43**: 107-9.
- Takagi, K., S. Okabe & R. Saziki (1969) *Jpn. Pharmacol.* **19**: 418-26.
- Niehaus, W.G. & B. Samuelsson (1968) *Eur. J. Biochem.* **6**: 126-30.
- Yagi, K. (1998) *Mol. Biol.* **108**: 107-10.
- Tietze, F. (1969) *Anal. Biochem.* **27**: 502-22.
- Lowry, O.H., N.J. Rosenbrough, A.L. Farr & R.J. Randall (1951) *J. Biol. Chem.* **193**: 265-75.
- Wang, Y.C., Y.C. Chuang, H.W. Hsu (2008) *Food Chem.* **106**: 277-84.
- Koca, U., B. Rathinasabapathi, G.A. Moore (2003) *Proc. Fla. State Hort. Soc.* **116**: 197-200.
- Das, D & R.K. Banerjee (1993) *Mol. Cell. Biochem.* **125**: 115-25.
- Vendemiale, G., I. Grattagliano & E. Altomare (1999) *Int. J. Clin. Lab. Res.* **29**: 49-55.
- Sahin, E. & S. Gumuslu (2004) *Int. J. Biometeorol.* **48**: 165-71.
- Halliwell, B. (1981) *Bull. Eur. Physiopathol. Respir.* **17**: 21-9.
- Rice-Evans, C.A., N.J. Miller, P.G. Bolwell, P.M. Bramley & J.B. Pridham (1995) *Free Radic. Res.* **22**: 375-83.