IN VITRO ANTIOXIDANT ACTIVITY OF LEAVES EXTRACTS OF *CASELPINIA BONDUCELLA*

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Summary

The objective of the present investigation is to assess the most potent antioxidant dicholromethane extract from the leaf of *Caesalpinia bonducella.* The *in-vitro* methods used to predict the antioxidant effect were DPPH radical scavenging assay, reducing power ability, Superoxide radical scavenging assay, nitric oxide scavenging assay & the estimation of total phenolic contents using gallic acid as standards. The % scavenging activity at different concentrations was determined and the IC 50 value of the extracts was compared with that of standard, ascorbic acid. The dichloromethane leaf extract gave an IC 50 value of 100.381 ug/ml. The reducing power was investigated by Fe3+ _ Fe2+ transformation in the presence of extracts tested using ascorbic acid as standard. The dichloromethane leaf extract showed increase reducing ability with increase in concentration at 700nm. The total antioxidant capacity by super oxide scavenging method and nitric oxide scavenging method is expressed as ascorbic acid and curcumin equivalents, respectively. The content of total phenolics (71.6 µg gallic acid equivalent/mg) in dichloromethane leaf extract was found. Based on the above results, the higher the phenolic content, the higher the antioxidant capacity was very well observed with dichloromethane leaf extract. Hence the dichloromethane extract of *Caesalpinia bonducella* dried leaf could be considered for preparation of nutraceuticals with potent antioxidant effect suitable for prevention of human disease.

Keywords: *Caesalpinia bonducella*, Antioxidant activity, Dichloromethane extract (DCM), Leaf.

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Introduction

Natural Products have been our single most successful source of medicines. Each plant is like a chemical factory capable of synthesizing unlimited number of highly complex and unusual chemical substances whose structures could otherwise escape the imagination forever.

Although clinical trials and experiments involving whole animals are important in natural product screening but because of financial, ethical and time limitations, importance of *in-vitro* screening is gaining popularity [**1**].

Free radicals easily react with macro-molecules of crucial biological significance (DNA, lipids, protein) and destroy their structure and function what accelerates ageing and might lead to degenerative diseases, including cancer [**2,3**]. Certain portion of reactive oxygen species (ROS) is generated in normal human metabolism and the production rate is precisely controlled by specialized system of antioxidant defense [**4**]. This well-balanced ROS synthesis is impaired by inflammatory events, where activated macrophages and neutrophils upon contact with proinflammatory stimuli; release substantial amounts of aggressive oxygen and nitrogen-centered radicals [**5**].

Natural antioxidant defense system involves enzymes (superoxide dismutase, catalase, and glutathione peroxidase), other proteins (albumin, ferritin, ceruloplasmin) and numerous smaller molecules (e.g. reduced glutathione, α-tocophrol, β-carotene, bilirubin, uric acid) of various modes of action. Antioxidant molecules counteract ROS and diminish their deleterious effects [**6,7**]. This protective barrier can be enhanced by the use of antioxidant micronutrient (vitamins C, E, β –carotene) and non-nutrient ingredients of edible plants, like polyphenols. Polyphenol subgroup of chemicals, flavanoids, is the extensively examined group of antioxidants [**8,9**].

Caesalpinia bonducella roxb. (Caesalpinia crista), Caesalpinaceae, is widely distributed throughout india. Antimalarial Activity of Cassane- and Norcassane-Type Diterpenes from *Caesalpinia crista* and Their Structure–Activity Relationship **[10]** and Wound healing activity of the seed kernels **[11]** and anthelmentic activity **[12]** have been reported in journal. Seed kernel is reported to have antipyretic, antidiuretic, antibacterial, anthelmintic **[13]**, antianaphylactic and antidiarrhoeal **[14]**, antiamoebic and antiestrogenic **[15]** and antiviral **[16]** properties. They reported the hypoglycaemic properties of the seeds of *C. bonducella*, in normal as well as streptozotocin diabetic rats **[17]**. The ethanolic and aqueous extracts have also been observed to slightly lower blood pressure in dogs **[18]**. The leaf juice is used for asthma treatment and has an antifilarial effect **[19]**. Leavs of *Caselpinia bonducella* traditionally used in diabetes. The aim of this study is to prove traditional use scientifically.

Materials and Methods

Plant Material

Leaves of *Caselpinia bonducella* was procured from local region of Rajkot, Gujarat, India. The plant was identified and authenticated by Prof. Vishal Muliya, Christ College, Rajkot and a voucher specimen was deposited.

Chemicals

All chemicals were analytical grade and chemicals required for all biochemical assays were obtained from Sigma Chemicals Co., USA.

Extraction:

The leaves of *Caselpinia bonducella* were dried at room temperature and reduced to a coarse powder. The powdered materials (leaves) were subjected to qualitative tests for the identification of various phyto constituents like alkaloids, glycosides, steroids, terpenoids and flavanoids. Then the powder was subjected to soxhlet extraction with dichloromethane, benzene, chloroform and alcohol (90%) and water separately for 72 hours at a temperature of 50-60_C. The extracts were concentrated and the solvent was completely removed. They were freeze dried and stored in the vacuum dessicator. Further, the dichloromethane extract of leaf was used for the in vitro antioxidant studies.

Methodology:

DPPH radical scavenging assay

The free radical scavenging activity of the extracts was measured *in vitro* by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay **[20]**. About 0.3 mM solution of DPPH in methanol was prepared and 150 ul of this solution was added to different volume of the extract dissolved in methanol at different concentrations (50-250 μg/ml). The mixture was shaken and allowed to stand at room temperature for 15 min and the absorbance was measured at 517 nm using a UV-visible spectrophotometer shimadzu, UV-1700, Japan. The % scavenging activity at different concentrations was determined and the IC50 value of the extracts was compared with that of ascorbic acid, which was used as the standard.

Reducing power ability

The reducing power capacity of the extracts was assessed as described **[21]**. The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. One ml of the extract (500-3500 μg/ml), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50°C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000g. About 2.5 ml of the supernatant was diluted with 2.5 ml of water and is shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Ascorbic acid was used as the standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Nitric oxide scavenging method

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent by the method **[22]**. Various concentration of the extract and sodium nitroprusside (10m M) in phosphate buffer saline and 150 ul of each dose level by dilution with methanol was incubated at room temperature for 150 min. After the incubation period, 5 ml of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid, 0.1% napthyl ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm using UV-visible spectrophotometer, shimadzu, UV-1700, Japan. The inhibiton of nitric oxide generation was estimated by comparing the absorbance values of control with that of treatments. Curcumin was used as standard values are reported as mean $\pm$ sd of three determinations.

Superoxide scavenging method

Super oxide generated from DMSO (Dimethyl sulphoxide) was measured **[23]**. To the reaction mixture containing 0.1 ml of NBT (1 mg/ml solution in DMSO) and 0.3 ml of extract, standard compound in DMSO, 1 ml of alkaline DMSO (1 ml DMSO containing 5 mM NaoH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560 nm using UV-visible spectrometer shimadzu, UV-1700, Japan.

Estimation of total phenolic content

Total soluble phenolics of the extract were determined with Folin-Ciocalteu reagent using Gallic acid as the standard **[24]**. An aliquot of 0.1 ml suspension of 1 mg of the extracts in water was totally transferred to a 100 ml volumetric flask and the final volume was adjusted to 25 ml by the addition of distilled water. Folin-Ciocalteu reagent (1 ml) was added to this mixture, followed by 4 ml of 20% sodium carbonate 5 min later. Subsequently, the mixture was shaken for 30 min at room temperature and the absorbance was measured at 760 nm using UV-visible spectrometer shimadzu, UV-1700, Japan. The concentration of total phenolic compounds in the extracts was determined as µg gallic acid equivalent by using the standard gallic acid graph.

Statistical analysis

All results are expressed as mean ± S.E.M. Linear regression analysis (Origin 6.0 version) was used to calculate the IC_{50} values.

Results

Phytochemical Investigations

Preliminary phytochemical tests of dichloromethane leaf extract shows the presence of Alkaloids, phytosterol, saponin and flavonoids as predominant active constituent.

DPPH assay

The leaf extract demonstrated H-donor activity. The highest DPPH radical scavenging activity was detected in the dichloromethane extract with the IC50 value of 100.381 µg/ml (Table 1). These activities are less than that of ascorbic acid (9.9982 ug/ml).

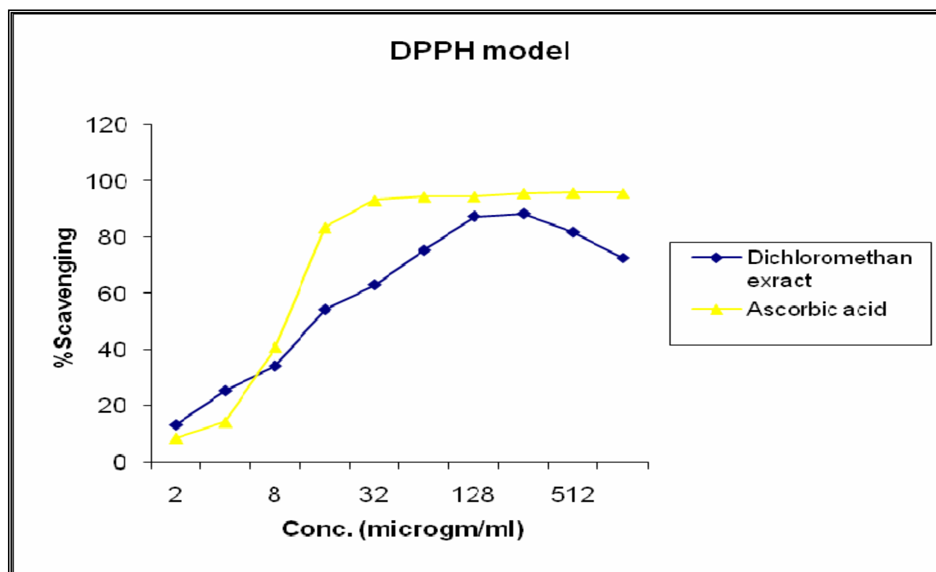

Fig. 1 Antioxidant activity of different concentrations of dichloromethane extract and ascorbic acid in DPPH radical scavenging method. Each value represents mean ± SEM

Table 1: Comparison of IC_{50} values of extract with standard

Sr no.	Model	IC_{50} value of Dichloromethane extract (ug/ml)	IC_{50} value of standard (ug/ml)
1.	DPPH radical scavenging activity	100.381	9.9982
2.	Nitric oxide scavenging method	21.77	38.68
3.	Super oxide scavenging method	15.91	16.33

Reducing power ability

Figure 4 shows the reductive capabilities of the extract when compared to the standard, Ascorbic acid. The reducing power increased with increasing amount of the extract. The leaf extract showed the highest reducing ability. However, the activity was less than the standard, ascorbic acid.

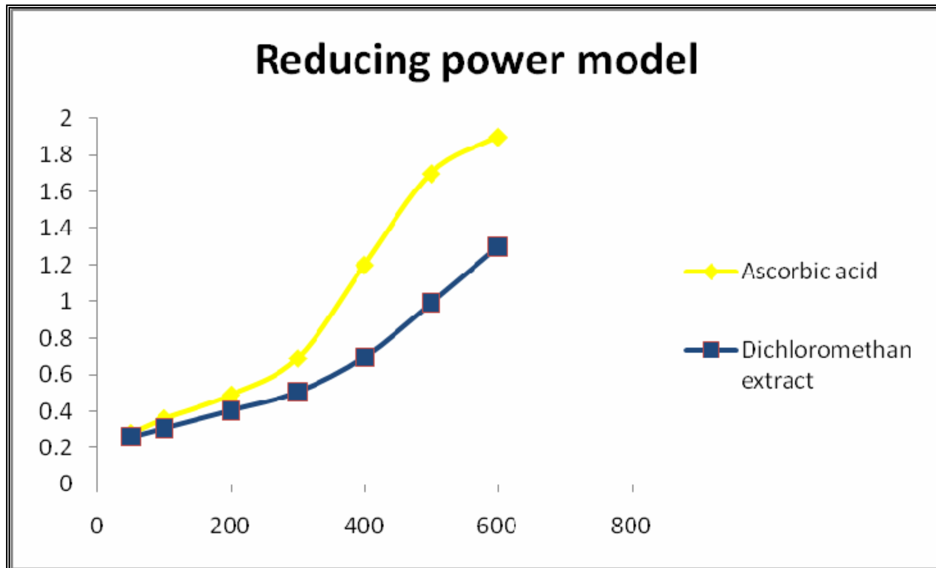

Fig. 4 Antioxidant activity of different concentrations of dichloromethane extract and ascorbic acid in reducing power method. Each value represents mean ± SEM

Nitric oxide scavenging method

The nitric oxide scavenging method showed moderate scavenging activity compare to standard, curcumin. The IC_{50} value of leaf extract was found to be 21.77 $\pm$ 2.1 ug/ml (Table 1).

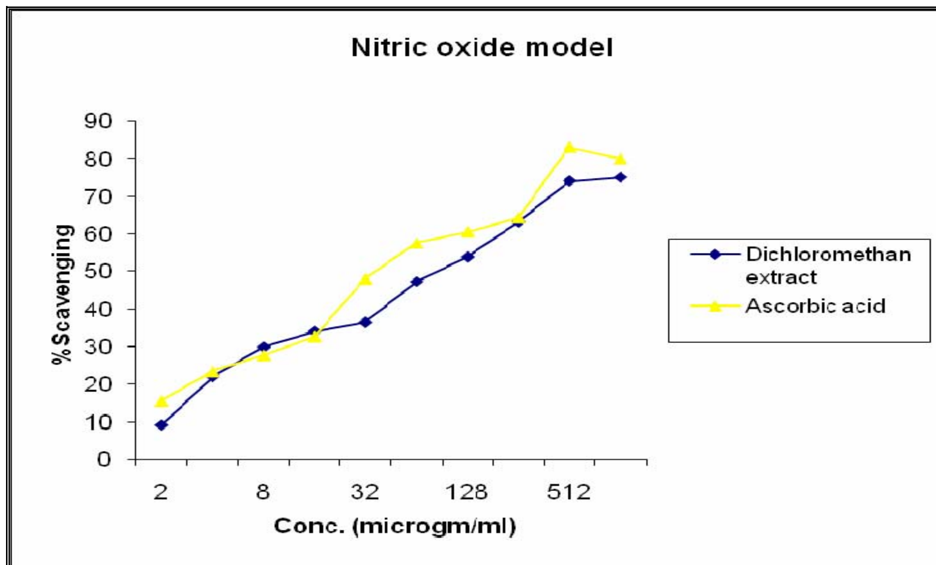

Fig. 2 Antioxidant activity of different concentrations of dichloromethane extract and ascorbic acid in nitric oxide scavenging method. Each value represents mean ± SEM

Superoxide scavenging method

The dichloromethane extract of *Caselpinia bonducella* leaf was found to be a scavenger of super oxide generated by alkaline DMSO. The IC_{50} value of leaf extract was 15.91 ± 11.2 ug/ml (Table 1).

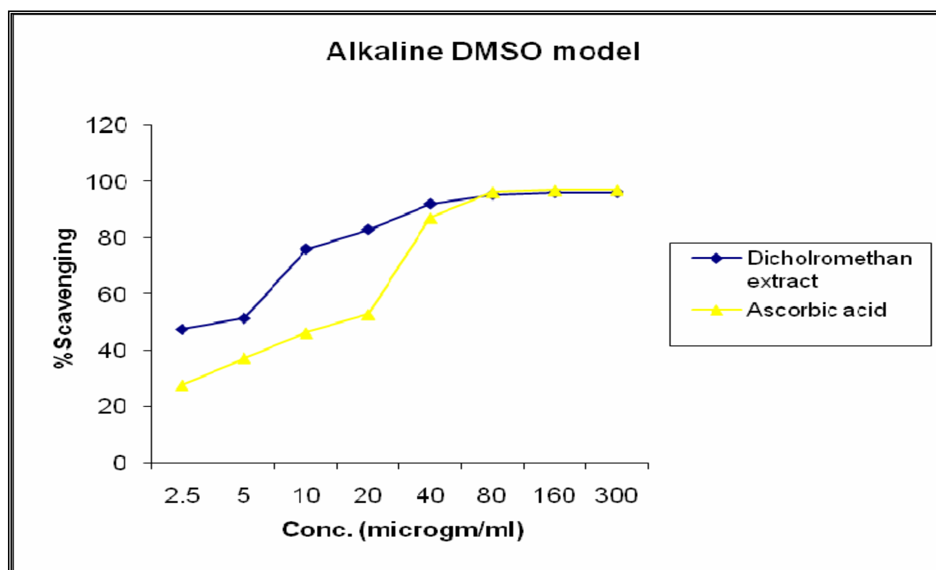

Fig. 3 Antioxidant activity of different concentrations of dichloromethane extract and ascorbic acid in super oxide scavenging method. Each value represents mean ± SEM

Total phenolic content

Total phenolic content was estimated by using Folin-Ciocalteu reagent. Total phenolic content of the extract was solvent dependent and expressed as µg gallic acid equivalent. The content of the total phenolics in the extract was 71.6 µg gallic acid equivalent/mg. concentration sufficient to obtain 50% of a maximum effect estimate in 100%. All values given are mean of triplicate experiments at S.D (5%) for the above tables.

Discussion

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, and inflammation and neurodegenerative diseases. Many flavanoids may help to provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body. In this connection, the phytochemical results also indicate the predominant active constituent flavanoid is present in dichloromethane extract of *Caselpinia bonducella*.

The results obtained from DPPH radical scavenging assay reveals that the dichloromethane extract gave an IC50 value of 100.381μg/ml when compared with ascorbic acid (IC50 value = 9.9982 μg/ml). Further more, the results from reducing power ability, nitric oxide scavenging capacity, super oxide scavenging capacity and total phenolic content in comparison with corresponding standards ascorbic acid, curcumin and gallic acid also clearly demonstrates that the leaf extract showed markedly high antioxidant activity and free radical scavenging activity. (Table1). The protective role of flavanoids involves several mechanism of action: direct antioxidant effect, inhibition of enzymes of oxygen – reduction pathways and sequestration of transient metal cations **[8,9,25]**.

Some homoIsoflavones and other flavanoid act as antioxidants through the inhibition of enzyme aldose reductase of oxygen-reduction pathway **[26]**. Since the plant possesses the isoflavone active constituent, the dichloromethane extract of *Caselpinia bonducella* exert their mechanism probably by modulating the enzyme aldose reductase action in mammals. Further investigation is needed in this aspect. The above research studies suggest that *Caselpinia bonducella* leaf possess a good antioxidant property which supports the literature that it maintains good health by boosting the immune system and reducing inflammation and allergies.

Therefore, it can be concluded that the dichloromethane extract of *Caselpinia bonducella* could be considered for preparation of nutraceuticals with potent antioxidant activity suitable for prevention of human disease.

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References

1. Shinde V, Dhalwal K. Pharmacognosy: The changing scenario. Pharmacognosy Reviews 2007; 1:2-4.
2. Ishizaki T, Kishi T, Sasaki F, et al. Effect of probucol, an oral hypocholesterolemic agent, on acute tobacco smoke inhalation in rats. Clin Sci. 1996; 90:517-523.
3. Kehrer JP. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol. 1993; 23:21-48.
4. Ignatowicz E, Rybczynska M. Some biochemical and pharmacological aspects of free radical-mediated tissue damage. Pol. J Pharmacol. 1994; 46:103-114.
5. Halliwell B. Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: measurement, mechanism and effects of nutrition. Mutat Res. 1999; 443:37-52.
6. Halliwell B, Gutteridge JMC. The antioxidants of human extra cellular fluids. Arch Biochem Biophys. 1990; 280:1-3.

7. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev. 1994; 74:139-162.
8. Cotelle N. Role of flavanoids in oxidative stress. Curr Top Med Chem. 2001; 1:569-590.
9. Rice-Evans C. Flavanoid antioxidants. Curr Med Chem. 2001; 8:797-807.
10. Kalauni S, Awale S, Tezuka Y, Banskota A, Zaw Linn T, Kadota S. Methyl Migrated Cassane-Type Furanoditerpenes of *Caesalpinia crista* from Myanmar. Chem. Pharm. Bull. 2005*;* 53(10):1300—1304.
11. Patil KS. Wound healing activity of the seed kernels of *Caesalpinia crista* Linn. Journal of Natural Remedies 2005; 5(1):26-30.
12. Hördegen P, Cabaret J, Hertzberg H, Langhans W, Maurer V. *In-vitro* screening of six anthelmintic plant products against larval *Haemonchus contortus* with a modified methyl-thiazolyl-tetrazolium reduction assay. Journal of Ethnopharmacology 2006; 108(1, 3):85-89.
13. Neogi NC, Nayak KP. Biological investigation of *Caesalpinia bonducella* Flem. Ind J Pharm. 1958; 20:95–100.
14. Iyengar MA. Pendse GS. Anti-diarrhoeal activity of the nut of *Caesalpinia bonducella*. Flem. Ind J Pharm. 1965; *27*:307–308.
15. Raghunathan K, Mitra R. *Caesalpinia bonducella*. In: Raghunathan K, Mitra R, eds., *Pharmacognosy of Indigenous Drugs*. New Delhi, *Central Council for Research in Ayurveda and Siddha* 1982;484–487.
16. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Roy C. Screening of Indian plants for biological activity. Ind J Exptl Biol. 1968; 6:232–247.
17. Sharma SR, Dwivedi SK, Swarup D. Hypoglycaemic, antihyperglycaemic and hypolipidemic activities of *Caesalpinia bonducella* seeds in rats. J Ethnopharmacol. 1997; 58: 39–44.
18. Bouquet A, Debray M. Plantes Me´dicinales de la Coˆ te d'Ivoire. *ORSTOM*, Paris. 1974; 56–60.
19. Bellomaria B. Kacou P. Plantes et me´decine populaire d'Agboville (Coˆ te d'Ivoire). Fitoterapia 1995; 65:126–130.
20. Mensor LL, Menezes FS, Leitao GG, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 2001; 15:127-130.
21. Oyaizu M. Studies of products browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. Jap.J.Nut.1986; 44:307-315.
22. Sreejayan Rao MNA. Nitric oxide scavenging by curcuminoids. Journal of Pharmacy and Pharmacology 1997; 49:105-107.
23. Elizabeth K, Rao MNA. Oxygen scavenging activity of curcumin. International journal of Pharmaceutics 1990; 58:237-240.
24. Gulcin I, Oktay M, Kufrevioglu I, et al. Determination of antioxidant activity of lichen cetraria islandica (L) Ach. J. Ethnopharmacol. 2002; 79:325-329.
25. Robak J, Gryglewski RJ. Bioactivity of flavanoids. Pol. J Pharmacol. 1996; 48:555-564.
26. End DW, Look RA, Shaffer NL, et al. Non selective inhibition of mammalian protein kinases by flavanoids invitro. Res. Commun. Chem. Pathol. Pharm. 1987; 56:75-86.