International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 758-763
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

COMPARATIVE *IN VITRO* ANTI OXIDANT ACTIVITY OF FLAVONOIDS WITH SYNERGISTIC EFFECT OF THEIR COMBINATION

Kumar Rajesh*[1] and Shailendre Sharma
***Research Scholar, Shivalik College of Pharmacy, Nangal, Dist-Ropar (Pb), Pin- 140126**
Jodhpur National University, Jodhpur
[2]Principal, Jodhpur Institute of Pharmacy, Jodhpur National University, Jodhpur

***Corresponding Author Email: samtaby@rediffmail.com**

ABSTRACT

Aim *The present study was designed to examine free radical scavenging potential (anti-oxidant activity) of some selected flavonoids and their effect in combination.* ***Method:****In the present study, free radical scavenging potential of two flavonoids, kampferol, quercetin and their combination has been investigated by DPPH, NO free radical scavenging assays.* ***Result:*** *The IC50 value of quercetin and kampferol for DPPH radicals was found to be 29.05 µg/ml and 33.07µg/ml respectively as compare to standered ascorbic acid 26.08µg/ml. The IC50 value of quercetin and kampferol for NO radicals was found to be 42.50µg/ml and 46.23µg/ml respectively as compare to standard 27.21 µg/ml. Hence quercetin can be considering a good scavenger of free radicals. The IC50 value of combination is found to be 25.60 for DPPH radical and 31.76 for NO radicals.* ***Conclusion*** *It is concluded that quercetin is powerful antioxidant than kampferol and their combination exhibit synergestic activity.*

KEY WORDS

Quercetin, Kampferol, Free radical scavenging, Antioxidant

INTRODUCTION

Biological free radicals are highly unstable molecules that react with various organic substrates such as lipids, proteins, DNA causing cellular injury. At high concentrations, they generate oxidative stress, a damaging process that can damage all cell structures. [1-4] At present, the research is focused on the use of antioxidants in preventing many diseases caused by the free radicals. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Medicinal compounds from higher plants have continued to play a leading role in the maintenance of human health. [5-6] Man was totally dependent on synthetic drugs before the availability of plant-based drugs, for their primary health care. [7] Since ancient time, natural products obtained from plants have been used as a projecting source of prophylactic agents for the prevention and treatment of diseases in humans and animals. Natural products obtained from plants, animals, minerals and marine sources have been used to treat various human disorders. [8] The study on plant products has become urgency due to their dietary health benefits as peoples suffering from different diseases increases day by day due to increased pollution, harmful waste from industries, cigarette smoking, stress, exposure of body to UV radiation and electromagnetic radiations which causes production of free radicals.[9] The hypothesis of oxygen-free radicals has been known about fifty years ago.[10] Oxidative stress plays a major role in the progress of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases.

Antioxidants are the compounds which scavenge the free-radicals and present the protection to living organisms from damage caused by uncontrolled production of these reactive oxygen species and subsequent lipid peroxidation, protein damage and DNA strand breaking. The role of antioxidants is, to counterbalance the excess of free radicals produced and to divert ROS to other reaction pathways with less reactive products. [11-12]

In recent years, the use of natural antioxidants present in food and other biological materials has attracted great interest due to their accepted safety, nutritional and therapeutic value. Many studies show that phytonutrients from fruits and vegetables may be valuable in defending the human body against damage caused by reactive oxygen and nitrogen species. Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body's ability to use the free radicals. [13]

In recent years, the use of natural antioxidants like flavonoids, present in food and other biological materials has attracted great interest due to their accepted safety, nutritional and therapeutic value. Efforts to gain wide knowledge regarding the power and potential of antioxidants from plants increasing day by day. Nutraceuticals are supposed to hold the key to a healthy society in the upcoming future. The hunt for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge.

Quercetin (3,3',4',5,7-pentahydroxyflavon) a well-known plant pigment, belongs to the flavonols class of polyphenolic flavanoids. The majority of flavonoid intake includes up to 60-75% of quercetin and its glucosylated forms. [14] Apples, berries, Brassica vegetables, capers, grapes, onions, shallots, tea, red wine, kale, and tomatoes, as well as many seeds, flowers and bark are the enriched dietary sources of quercetin. It also characterizes a main component of various medicinal plants including *Ginkgo biloba*, *Hypericum perforatum* (St. John'swort), *Solanum Trilobatum* and *Sambucus Canadensis* (elder) and manyothers.[15 Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one)is a natural flavonol belonging to the family of secondary metabolites called flavonoid. Kaempferol enriched dietary sources involve tea, broccoli, cabbage, kale, beans, endive, leek, tomato, strawberries, cucumbers, spinach, apple, potato and grapes. Many plants having medicinal importance like *Ginkgo biloba, Euphorbia pekinensis, Moringa oleifera, Sophora japonica, Aloe vera, Coccinia grandis, Cuscuta chinensis, Glycine max, Rosmarinus officinalis, Sambucus nigra, and Toona sinensis, Hypericum perforatum, Tilia spp* and *Equisetum spp* have kaempferol as one of their important constituent.[16-17] Sugars such as glucose, rhamnose, galactose and rutinose are usually bound to kaempferol to form glycosides like kaempferol-3-O-glucoside, also called astragalin and kaempferol-3-(p-coumaroyltriglucoside).

MATERIAL AND METHODS

Chemical and reagents

Quercetin, Kampferol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, sodium nitroprusside, naphthyl ethylenediamine dihydrochloride, sulphanilamide, phosphoric acid, were obtained from SD fine chemicals, Himedia or Sigma loba chemicals. All other reagents used were of analytical grade.

DPPH Radical Scavenging Activity [18]

The antioxidant activity of the quercetin,rutin was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to method of Dnyaneshwar M. Nagmoti, et al309 A methanol solution of the sample at various concentrations (30-60µg/ml) was added to 0.5 ml of 0.1 mM methanolic solution of DPPH and allowed to stand for 30 min at 25°C. The absorbance of the sample was measured at 517 nm. A 0.1 mM solution of DPPH in methanol was used as control, whereas ascorbic acid was used as reference standard. All tests were performed in triplicate. Radical scavenging activity is expressed as the inhibition percentage of free radical by the sample and standard was calculated using the formula:

% inhibition = [(Abs (control) – Abs (test)]/ (Abs (control)] × 100

Nitric oxide (NO•) scavenging activity [19]

Nitric oxide scavenging activity quercetin and rutin was determined in terms of NO• generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction.[19] One milliliter of sodium nitroprusside (10 mM) in phosphate-buffered saline (pH 7.4) was mixed with 1 ml of test solution at

various concentrations (30-60µg/ml) dissolved in methanol and a control without a test compound, but with an equivalent amount of methanol. The mixture was incubated at 25°C for 30 min. After 30 min, 1 ml of the incubated solution was withdrawn and mixed with 1 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the pink chromophore formed during the diazotization of the nitrite with sulphanilamide and the subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured at 546 nm. All the tests were performed in triplicate. Percentage inhibition was calculated using Equation:

% inhibition = [(Abs (control) – Abs(test)] / (Abs (control)] × 100

RESULTS AND DISCUSSION

DPPH radical scavenging activity

This method has been widely used to determine the free radical scavenging activity of antioxidants. The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant. When an odd electron of DPPH radical paired with hydrogen, it reduces to DPPH-H which leads to change in colour depending upon the number of electrons taken up. Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. Prasad et al. reported that phenolics and flavonoids reduce the DPPH radicals by their hydrogen donating ability. [20]

The effect of quercetin and kampferol on 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging activity is shown in Table 1 and Figure 1. The quercetin and kampferol showed the DPPH radical scavenging activity in a concentration dependent manner. Both the drugs under test shows significant effect when compared to standard ascorbic acid. The quercetin and rutin showed IC50 value of 29.05µg/ml and 33.07µg/ml. This shows that quercetin has more scavenging power than kampferol. Whereas the combination of both drugs shows IC50 value is 22.31µg/ml as shown in Table 3 and Figure 3. Less the IC50 value means more scavenging power, hence shows synergestic effect.

Nitric oxide scavenging activity

Nitric oxide (NO) is a free radical involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases. Nitric oxide is a very unstable species under aerobic conditions. It reacts with O_2 to produce stable product nitrate and nitrite. The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Suppression of NO release may be attributed to a direct NO scavenging effect of the extracts decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro* as shown in Table 2 and Figure 2. The results show that scavenging activity in a concentration dependent (25-100µg/ml) The quercetin and kampferol showed IC_{50} value of 42.05µg/ml and 46.23µg/ml. Whereas the combination of both drugs shows IC50 value is 31.76 µg/ml as shown in Table 4 and Figure 4. Less the IC50 value means more scavenging power, hence shows synergestic effect.

Table 1: DPPH radical scavenging activities of quercetin, Kampferol and standards. Each value is expressed as a mean ± S.E.M (n = 3).

S.No.	Concentration	Percentage scavenging activity		
		Quercetin (test)	Kampferol (test)	Ascorbic acid (std.)
1	30	50±1.83	45±1.25	53±1.04
2	40	62±1.04	58±1.30	66±0.60
3	50	79±2.40	77±2.43	82±1.25
4	60	83±2.71	82±0.60	86±1.04

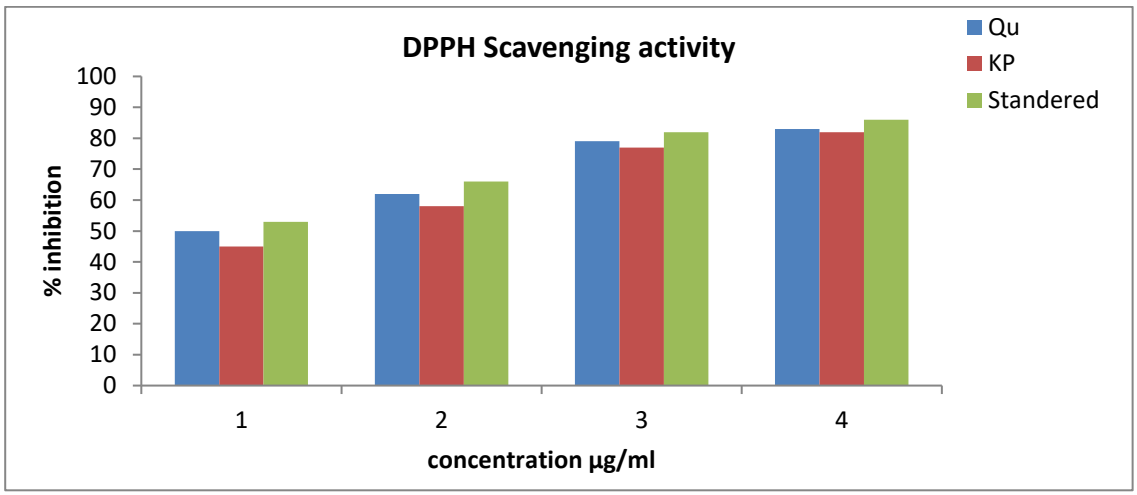

Figure 1: DPPH radical scavenges activities of quercetin, Kampferol and standards. Each value is expressed as a mean ± S.E.M (n = 3).

Table2:NO radical scavenging activities of quercetin, Kampferol and standards. Each value is expressed as a mean ± S.E.M (n = 3).

S.No.	Concentration	Percentage scavenging activity		
		Quercetin	Kampferol)	Ascorbic acid (std.)
1	25	37±0.16	34±0.13	48±0.27
2	50	58±0.09	55±0.94	62±0.27
3	75	70±010	72±0.20	75±0.13
4	100	83±0.09	77±0.19	85±0.55

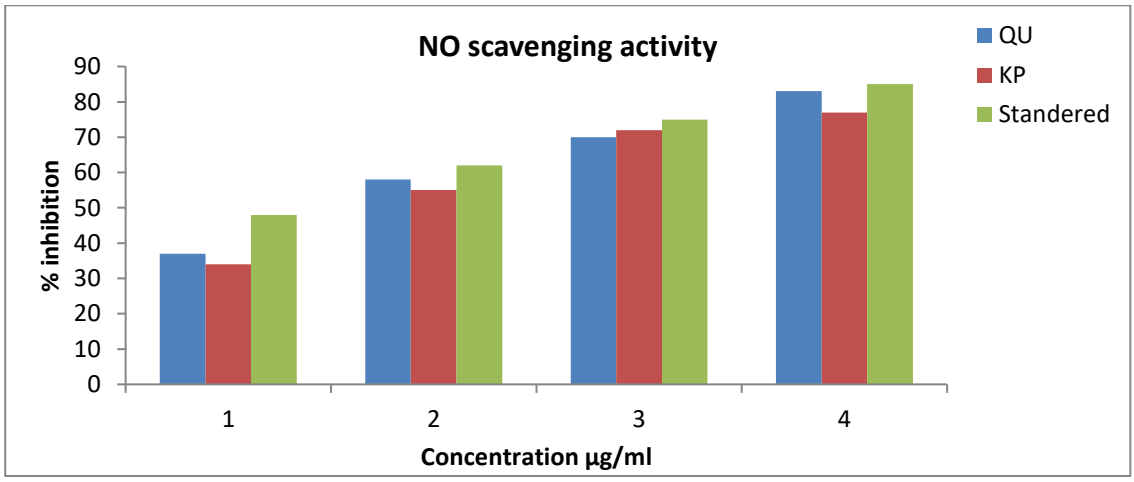

Figure2:NO radical scavenging activities of quercetin, Kampferol and standards. Each value is expressed as a mean ± S.E.M (n = 3).

Table 3: DPPH radical is scavenging activities of quercetin+ Kampferol and standards. Each value is expressed as a mean ± S.E.M (n = 3).

S.No.	Concentration	Absorbance	Percentage scavenging activity	
			Qu+KP (test)	Ascorbic acid (std.)
1	30	0.47	53±0.332	53±1.040
2	40	0.35	65±0.353	66±0.636
3	50	0.22	80±0.436	82±1.252
4	60	0.16	83±0.758	86±1.043

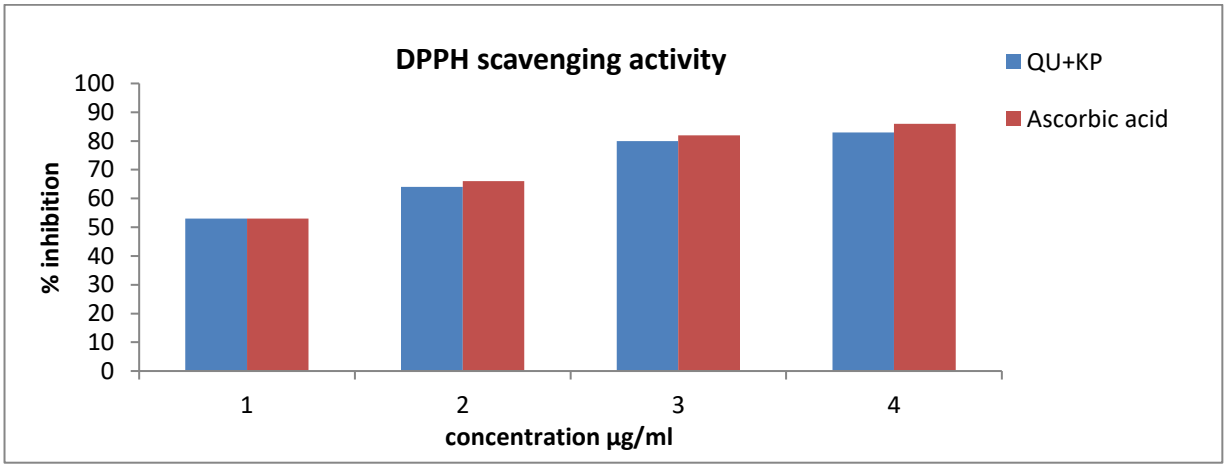

Figure 3: DPPH radical scavenging activities of quercetin+ Kampferol and standards. Each value is expressed as a mean ± S.E.M (n = 3).

Table 4: NO radical scavenging activities of quercetin+ kampferol and control standards. Each value is expressed as a mean ± S.E.M (n = 3).

S.No.	Concentration	Absorbance	Percentage scavenging activity	
			Qu+kp (test)	Ascorbic acid (std.)
1	25	0.51	45±1.772	48±0.237
2	50	0.45	60±0.293	62±0.270
3	75	0.34	74±0.224	75±0.103
4	100	0.17	81±0.084	85±0.220

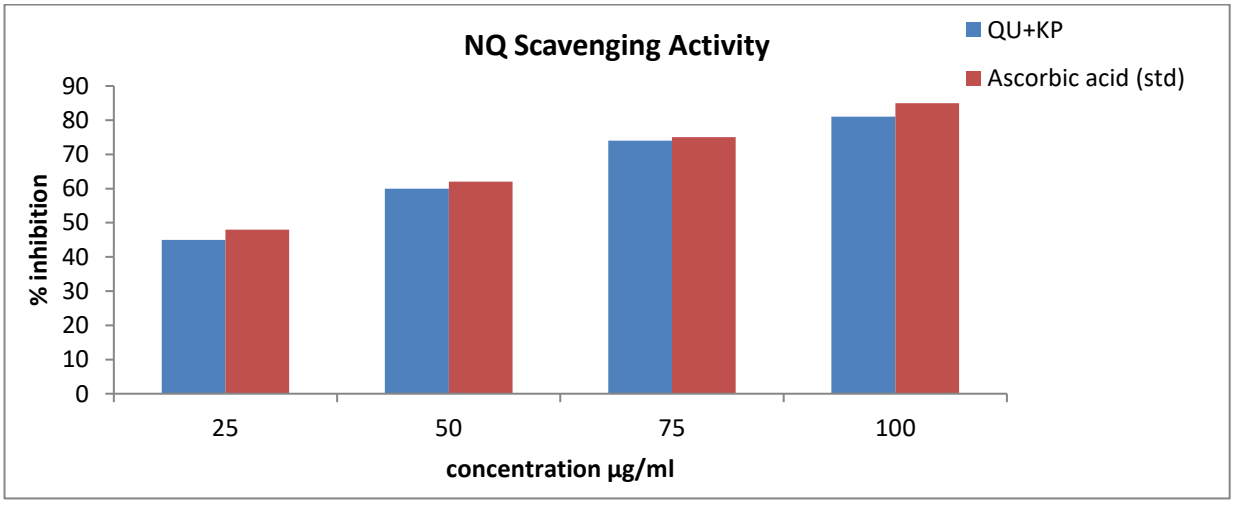

Figure4:NO radical scavenging activities of quercetin + kampferol and control standards. Each value is expressed as a mean ± S.E.M (n = 3).

CONCLUSION

From the result obtained in this study, it is concluded that both quercetin and rutin exhibit antioxidant activity. These *in vitro* assays indicate that quercetin is stronger anti-oxidant than kampferol. The data shown in Table 3, 4 and Figure 3, 4 is due to synergistic effect of both above mentioned compounds. The present study indicates that quercetin and kampferol have strong anti-oxidant potential and their combination shows synergestic effect, therefore, can be used as promising natural source of anti-oxidants for application in nutritional and pharmaceutical field, in prevention of diseases caused by free radicals.

REFERENCES

1. Willcox, J. K.; Ash, S. L.; Catignani, G. L. Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr, 44: 275-95, (2004)
2. Genestra, M. Oxyl radicals, redox-sensitive signaling cascades and antioxidants. Cellsig, 19: 1807-1819: (2007)
3. Halliwell, B. Biochemistry of oxidative stress. Biochem. Soc. Trans., 35: 1147-115, (2007).
4. Young, I.; Woodside, J. Antioxidants in health and disease. J. Clin. Pathol, 54, 176–186. (2001)
5. Crag, G. Newman, D. J. Medicines for the millennia. Ann. NY Acad. Sci, 95(3), 3-25, (2001).
6. Farombi, E.O. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. Afr. J. Biotech., 662-671. (2003)
7. Singh, A.; Malhotra, S.; Subban, R. Anti-inflammatory and analgesic agents from Indian medicinal plants. Int. J. Integr. Biol 3, 57, (2008)
8. Gill, N.S.; Sharma, P.; Bajwa, J.; Sood, S.; Sharma, P.D.; Bali, M. Study on cucumis melo var. utilissimus seeds for their therapeutic potential. J. Plant. Sci, 5(3), 248-255, (2010)
9. Halliwell, B.; Gutteridge, J. M. C. *Free radicals in biology and medicine. Clarendon Press*: Oxford, UK, 2007.
10. Willcox, J. K.; Ash, S. L.; Catignani, G. L. Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr.,44, 275-95, (2004)
11. Iris, F. F. Evolution of dietary antioxidants. Comparative Biochemistry and Physiology 136, 113-126, (2003)

Received:08.05.18, Accepted: 07.06.18, Published:01.07.2018

12. Lekameera, R. P.; Somasundaram, S. T. Evaluating antioxidant property of brown ALGA *Colpomenia sinuosa*. Afr. J. Food Sci., 2: 126-130, (2008)
13. Wolfe, K.; Xianzhong, W. U.; Liu, R. H. Antioxidant activity of apple peels. J. Agricul. Food Chem. 51: 609–614, **(**2003)
14. Bouktaib Metal. Regio- and stereoselective synthesis of the major metabolite of quercetin, quercetin-3-O-b-D-glucuronide. Tetrahedron Letters, 43: 6263-6266. (2002)
15. Kleemann R, Verschuren L, Morrison M, Zadelaar S, Van Erk MJ, Wielinga PY and Kooistra, T. Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models. Atherosclerosis.; 218: 44-52, (2011)
16. Calderon-Montano JM, Burgos-Moron E, Perez-Guerrero C and Lopez-Lazaro M. A review on the dietary flavonoid kaempferol. Mini Rev Med Chem. 11(4): 298–344, **(**2011)
17. Liu RH. Health-promoting components of fruits and vegetables in the diet. Adv Nutr. **2013**; 4(3): 384S–392S.
18. Dnyaneshwar M. Nagmoti, et al.: Antioxidant activity and free radical-scavenging potential of Pithecellobium dulce Free Radicals and Antioxidants 2 (2): 39-40, (2012)
19. Ganapaty S, Chandrashekhar VM, Chitme HR, Lakashami NM. Free radical scavenging activity of gossypin and nevadensin: An in vitroevaluation. Indian J Pharmacol. 39(6):281-3, (2007)
20. Shirwaikar, K. S. Prabhu, and I. S. R. Punitha, "In vitro antioxidant studies of Sphaeranthus indicus (Linn)," Indian Journal of Experimental Biology, 44, (12), 993-996, (2006)

***Corresponding Author:**
Kumar Rajesh*
Email: samtaby@rediffmail.com