WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 763-768. Research Article ISSN 2277– 7105

# EVALUATION OF HYDROXYL RADICAL SCAVENGING ACTIVITY OF COMBINATION OF ETHANOLIC EXTRACTS OF SOME MEDICINAL PLANTS

**Tanuj Joshi[1]* and Vijay Juyal[2]**

[1]Ph.D. Scholar, Department of Pharmaceutical Sciences, Bhimtal, Uttarakhand.

[2]Professor, Department of Pharmaceutical Sciences, Bhimtal, Uttarakhand.

Article Received on
20 February 2018,

Revised on 12 March 2018,
Accepted on 03 April 2018,

DOI: 10.20959/wjpr20188-11841

***Corresponding Author**
**Tanuj Joshi**
Ph.D. Scholar, Department of Pharmaceutical Sciences, Bhimtal, Uttarakhand.

## ABSTRACT

As oxidative species have been indicated as main culprit in many diseases, considerable research is now directed towards finding newer antioxidants. Newer antioxidant formulations can be prepared by studying and identifying various plants with potent antioxidant activity. Plants can provide a safe and useful alternative to synthetic antioxidants. Thus, plant derived antioxidants can play an important role in oxidative stress related diseases. The present study is undertaken to evaluate the antioxidant activity of combination of extracts of aerial parts of three medicinal plants which are *Ocimum kilimandscharicum*, *Thymus serpyllum* and *Spilanthes acmella*. The extraction was done by using soxhlet's assembly using absolute ethanol. After the extracts were dried, they were combined in equal ratio and the combination was coded as TJ123. The hydroxyl radical scavenging activity of TJ123 was studied along with ascorbic acid, which was used as the standard. The results demonstrated concentration dependent inhibition of hydroxyl radicals by both TJ123 as well as ascorbic acid. TJ123 demonstrated less percentage inhibition as compared to the standard ascorbic acid. The plants used in the above study and their combination can be further explored for development of potent and safe antioxidant formulations.

**KEYWORDS:** Free radicals, oxidative stress, hydroxyl radical.

## INTRODUCTION

Oxygen is essential for the survival of various life forms on earth. Activated oxygen species are produced as a consequence of aerobic metabolism and exposure to various natural and synthetic toxicants. It is a matter of paradox that the same oxygen which is essential for the life of organisms can become dangerous to them, as conditions like oxidative stress can lead to disturbances and various pathological conditions in the body.[1] Free radicals lead to the production of very reactive metabolites. The important oxidative and nitrosative species found in the body are superoxide, nitric oxide, hydrogen peroxide, peroxynitrite and hypochlorous acid.[2] Free radicals and reactive metabolites have a dual role in the body. On one hand they play a positive role, like they have a role in phagocytosis of microbes and in several biochemical reactions like carboxylation, hydroxylation, peroxidation. Also they are involved with reduction of ribonucleotides. Recently reactive oxygen species are known to possess important biomodulating activities and regulatory activity in signal transduction processes during transduction of intracellular information. On the other hand if there is disturbance between the levels of oxidants and antioxidants it leads to oxidative stress.[3] Oxidative stress can be denoted as a disturbance in balance between pro-oxidants and antioxidants in favour of the former.[4] Oxidative stress has been associated with numerous diseases like atherosclerosis, cardiovascular diseases and neurodegenerative diseases. Thus free radicals and reactive metabolites play an important and positive role in proper functioning of the body, but if they are produced in large quantities they can be harmful to the body.[3] In normal situations thankfully we have numerous defenses to minimize oxidation. Various organisms can increase production of antioxidants and repair enzymes in order to adapt to oxidative stress. Around 30-40 genes are “turned on” in mammals, yeast and bacteria, rapidly and in a coordinated manner to deal with oxidation. Thus the responses of genes within organisms, which are responsible for production of various antioxidant enzymes can help the organism to counter oxidative stress.[5,6] Various important antioxidants in our body serve to protect the body against oxidants like superoxide dismutase and glutathione peroxidase.[1] A wide range of genetic, cellular and metabolic responses is produced by oxidative stress. Oxidative stress in cell can cause modulation of gene expression, stimulate cell growth, or may cause a protective temporary growth-arrest and transient adaptive response. This threat of oxidative stress is dealt by the various antioxidants operating in the body. The inability to cope fully with oxidative stress switches mitotic cells into a permanent growth-arrested senescence-like state, so that they can survive for long periods. As the oxidative stress becomes more severe or the level of protective enzymes and adaptive

capacity decline as a result of aging, cell may undergo apoptosis in order to protect other healthy tissues surrounding them from further damage. Necrotic death is only shown by the cells under the most severe oxidative stress conditions.[1] Oxidative stress is involved in the mechanisms of several diseases like aging, psychological diseases, inflammatory diseases, diabetes mellitus, cancer, Parkinson's disease, Alzheimer's disease and atherosclerosis. Oxidative stress has a destructive effect on the various biomolecules, organs and the whole body. Thus, in normal circumstances free radical and oxidative species do not cause much harm to the body but if their level exceed a certain level they become highly damaging to the body.[3] The present study deals with the evaluation of antioxidant activity of combination of ethanolic extracts of aerial parts of three medicinal plants which are *Ocimum kilimandscharicum* (Lamiaceae), *Thymus serpyllum* (Lamiaceae) and *Spilanthes acmella* (Asteraceae).

## MATERIAL AND METHODS

### Plant Collection and Identification

The aerial parts of *Ocimum kilimandscharicum*, *Thymus serpyllum* and *Spilanthes acmella* were collected from the the herbal garden of Defence Institute of Bioenergy research, Pithoragarh and identified by Botanical Survey of India, Dehradun.

### Preparation of the Extract

The aerial parts of the plants were dried with the help of oven and extracted with absolute ethanol (99.9%) using soxhlet's assembly. Rotatory vaccum flash evaporator was used to dry the extracts obtained after extraction.[7]

### Hydroxyl Radical Scavenging Assay

The extracts so obtained were sent to Deshpande laboratories private limited, Bhopal, Madhya Pradesh, India (An ISO 9001:2008 Certified Drug Testing Laboratory) for analyzing hydroxyl radical scavenging activity for evaluation of antioxidant activity by the hydroxyl radical scavenging assay. The extracts were combined in equal ratios and coded as TJ123. In the method used reaction mixture in a final volume of 1 ml contained 100µl of 2-deoxy 2-ribose (28 mM in 20 mM $KH_2PO_4$ buffer, pH 7.4), 500 µl of the extract at various concentrations (0.0001-50µg/ml) in buffer, 200 µl of 1.04 mM EDTA and 200µM $FeCl_3$ (1:1, v/v), 100µl of 1.0 mM hydrogen peroxide ($H_2O_2$) and 100 µl of 1.0 mM ascorbic acid. Test samples were kept at 37°C for 1 hour. The free radical damage imposed on the substrate, deoxyribose was measured using the thiobarbituric acid test. One ml of 1% thiobarbituric

acid (TBA) and 1.0 ml 2.8% trichloroacetic acid (TCA) were added to the test samples and incubated at 100ºC for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Ascorbic acid was used as the standard antioxidant.[8,9] The plate were read on BMG Fluostar (GERMANY) and the % inhibition data was analyzed on MARS software BMG (Germany).

## RESULTS AND DISCUSSION

### Results

The percentage inhibition produced by the combined ethanolic extracts of *Ocimum kilimandscharicum*, *Thymus serpyllum* and *Spilanthes acmella* (TJ123) and ascorbic acid were studied at various concentrations. The percentage inhibition of hyroxyl radicals increased as the concentration of TJ123 increased. The follwing results were obtained after performing hydroxyl radical scavenging method (Figure. 1.).

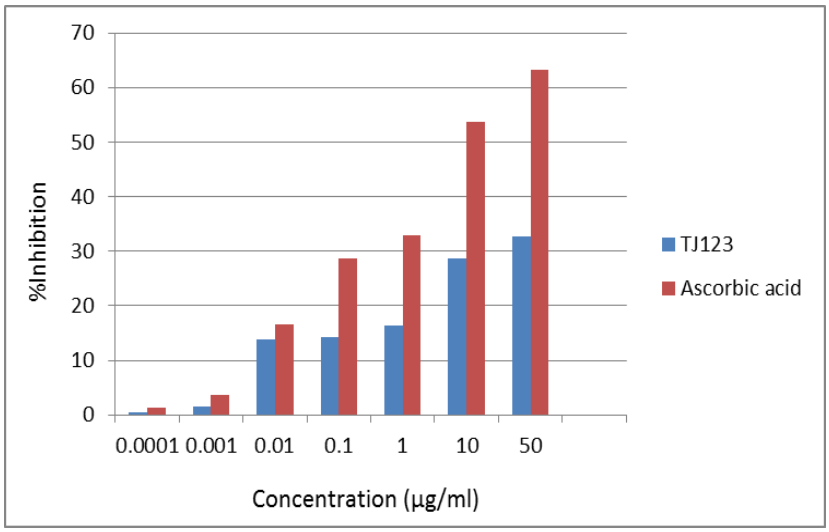


**Figure 1: Percentage inhibition produced by seven different concentrations of ascorbic acid and combined extracts of Ocimum kilimandscharicum, Thymus serpyllum, Spilanthes acmella (TJ123) using hydroxyl radical scavenging method.**

## DISCUSSION

As stated above oxidative species can have a positive protective effect on the body in normal circumstances as well as a damaging effect on the body. Oxidative stress leads to many diseases and is a cause of major concern in today's life. Under normal circumstances the antioxidant defence mechanisms of the body are sufficient to handle the formation of oxidative species and free radicals but when they become excess treatment is required.[3] Since use of synthetic antioxidants can be harmful to the body, current research is now

directed towards herbal drugs for their antioxidant activity, since most herbal drugs are considered safe. Herbal drugs and medicinal plants can provide answer for oxidative stress.[10] In the present study ethanolic extracts of aerial parts of three important medicinal plants were combined in equal ratio and the antioxidant activity of the combined extract (TJ123) was evaluated using hydroxyl radical scavenging method. In the present study TJ123 showed 0.47%, 1.45%, 13.89%, 14.25%, 16.45%, 28.74% and 32.67% inhibition at concentrations of 0.0001 µg/ml, 0.001 µg/ml, 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml and 50 µg/ml respectively. Thus a concentration dependent effect was shown by TJ123. Percentage inhibition of hydroxyl radicals increased as the concentration of TJ123 increased. The percentage scavenging shown by ascorbic acid was found to be higher at all concentrations as compared to TJ123. The inhibition of hydroxyl radicals produced by the TJ123 may be due to the presence of a specific phytochemical or phytochemicals that are present in the ethanolic extract of the above mentioned plants.

## CONCLUSION

In the present study it has been stated that increased oxidative stress can produce damaging effects on the body. Synthetic antioxidants have been developed over years but they carry risks of causing cancers and are not good for health. Plants and vegetables have always been good sources of antioxidants. Many vegetables and fruit we eat are good antioxidants. Various medicinal plants can be explored so that we can find better, safer and more effective antioxidants. In the present study TJ123, which is a combination of ethanolic extract of three medicinal plants was explored for antioxidant activity. Though ascorbic acid demonstrated good antioxidant activity still we can say that TJ123 showed a concentration dependent antioxidant effect at very low concentrations. The medicinal plants used in the study and their combinations can be explored for development of antioxidant formulations, which can be helpful in a variety of oxidative stress related diseases.

## REFERENCES

1. Davies KJA. Oxidative Stress, Antioxidant Defenses, and Damage Removal, Repair, and Replacement Systems. IUBMB Life, 2000; 50: 279-289.
2. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc., 1998; 75: 199-212.
3. Ďuračková Z. Some Current Insights into Oxidative Stress. Physiol Res., 2010; 59: 459-469.

4. Sies H. Oxidative stress: Introductory remarks: In: Sies H (ed.). Oxidative stress, London; Academic Press, 1985; 1-8.
5. Davies KJA. Oxidative stress: the paradox of aerobic life. Biochem Soc Symp, 1995; 61: 1–31.
6. Davies KJA, Ursini F. The Oxygen Paradox. Padova, Italy; CLEUP University Press, 1995.
7. Kokate CK, Purohit AP, Gokhale SB: Pharmacognosy. 45th ed., Pune; Nirali Prakashan: 2010.
8. Gutierrez RMP, Ahuatzi DM. Investigating Antioxidant Properties of the Diterpenes from Seeds of *Phalaris canariensis*. J Nutr Food Sci., 2015; 5: 1-9.
9. Klein SM, Cohen G, Cederbaun AI. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. Biochemistry, 1981; 20: 6006-6012.
10. Nandhakumar E, Parameswaran I. *In vitro* Antioxidant Activities of Methanol and Aqueous Extract of *Annona squamosa* (L.) Fruit Pulp. J Acupunct Meridian Stud, 2013; 6(3): 142-148.