IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2023 Vol.:28, Issue:2

Evaluation of *In-Vitro* Antioxidant and Radical Scavenging Activity of Trikatu Churna

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Babu N[1*], Indumathi R[2]

1-Student, Department of Pharmacology, College of Pharmacy, Madras Medical College, Chennai-03. India.

2- Assistant professor, Department of Pharmacology, College of Pharmacy, Madras Medical College, Ch-03. India.

Submitted: 18 August 2023
Accepted: 20 September 2023
Published: 30 September 2023

Keywords: Ayurvedic medicine, trikatu churna, DPPH assay, Hydrogen peroxide, antioxidants.

ABSTRACT

In Ayurvedic medicine "Trikatu churna" (TKC) is a combination of three herbal drugs, as per Ayurveda's Bhaisajyaratnawali is a compound herbal formulation containing three bitter herbs mixed together in equal quantities. In Sanskrit Trikatu word means 'three acrids'. Three herbs are dried fruits of *Piper nigrum* (Maricha) and *Piper longum* (Peepli) and dried rhizomes of *Zingiber officinalis* (Sunthi). Trikatu possesses immunomodulatory, antiviral, expectorant, carminative, hypolipidemic, hypoglycemic, antiemetic, anti-inflammatory potentially and etc. Antioxidant capacity is related to compounds capable of protecting a biological system against the potentially harmful effect of processes or reactions involving reactive oxygen and nitrogen species (ROS and RNS). Antioxidants exert beneficial effects against oxidative stress; many large-scale randomized controlled trials gave inconsistent and disappointing results on the prevention of chronic diseases. The three herbs of the Trikatu churna individually have an antioxidant activity due to the presence of phenolic, polyphenolic and flavonoids. Here, a combination of these three showed potential antioxidant and radical scavenging activity was evaluated by DPPH assay and hydrogen peroxide radical scavenging assay compared with the standard ascorbic acid. IC_{50} value of Trikatu churna by the methods of DPPH assay and standard ascorbic acid; hydrogen peroxide radical scavenging assay and standard ascorbic acid were founds to be 121.1 µg/ml and 80.32 µg/ml; 93.23 µg/ml and 86.76 µg/ml. the results concluded that the TKC have a potential source of antioxidant from the natural origin.

INTRODUCTION

Trikatu Churna (TKC) is an important group of formulations used by Ayurveda and Siddha physicians to treat various types of diseases and disorders. Trikatu, as per Ayurveda's bhaisajyaratnawali is a crucial herbal formulation containing three bitter herbs mixed together in equal portions. Trikatu Churna is crucial Ayurvedic formulation, is the official ayurvedic formulary of India is combination of three reputed herbs, comprised of the fruits *Piper nigrum* L. (Piperaceae), *Piper longum* L. (Piperaceae) and rhizomes of *Zingiber officinale* R. (Zingiberaceae).[1] It is prescribed in Ayurvedic and Siddha system of medicine for the treatment of digestive impairment, chronic rhinitis/sinusitis, skin diseases, asthma, cough, diuresis, obesity, inflammatory bowel disease and filariasis. Apart from traditionally known health benefits, trikatu also possesses immunomodulatory, antiviral, expectorant, carminative, hypolipidemic, hypoglycaemic, antiemetic and anti-inflammatory potential.[2] Trikatu is also added in various Ayurvedic formulations with a view to restoring the disturbed "tridoshas-vatta, pitta and kapha".[3]

Antioxidants play a vital role in food preservation by inhibiting oxidation processes and contributing to health promotion rendered by many dietary supplements, nutraceuticals and functional food ingredients.[4] When the antioxidant protection is unbalanced by a series of factors, deterioration of physiological functions may occur, and in this way, diseases and accelerated ageing can appear.[5] The wide knowledge of antioxidant the excessive reactive oxygen species (ROS) or reactive nitrogen species (RNS) induced oxidative stress will cause significant damage to cellular structure and biomolecular function, directly or indirectly leading to a number of diseases. The excessive production of ROS/RNS will balanced by nonenzymatic antioxidants and antioxidant enzymes.[6]

Different methods of *in-vitro* assays have been introduced to evaluate the antioxidant activities so as to assess an antioxidant that would be useful scientific evaluation of biological systems. Basically, two different methods are used for determining antioxidant activities: hydrogen atom transfer-based methods and single electron transfer-based methods according to their reaction mechanisms.[6]

Aim of the present study is to determine the *in-vitro* antioxidant activity of Trikatu churna by the methods of DPPH radical scavenging method and hydrogen peroxide scavenging method.

METHODS AND MATERIALS

Plant materials

Trikatu Churna was procured from the reputed Siddha manufacturer; Indian Medical Practitioner's Co-Operative Society (IMPCOPS) and was authenticated by comparing with the in-house standards of Botany/Pharmacognosy department, Siddha Central Research Institute, Arignar Anna Government Hospital Campus, Arumbakkam, Chennai – 600 106. The churna was stored in an airtight container.

Preparation of extracts

The 50g of TKC and its plant ingredients were extracted with 100 ml ethanol at 50-60^{o}C in a soxhlet apparatus. The different extracts were filtered, evaporated in vacuum and dried by rotary evaporator at 60^{o}C. Dried extracts were stored in air tight container at 4^{o}C and later used for *in-vitro* study and further processing[7, 8].

In-vitro antioxidant activity

a) DPPH radical scavenging assay

The DPPH assay is widely used in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. DPPH is one of the few stable and commonly available organic nitrogen radicals. The antioxidant effect is directly proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its accuracy and reproducibility. DPPH produces a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH due to the absorption of hydrogen ions from an antioxidant. This reaction is stoichiometric based on the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily validated by following the decrease of UV absorption at 517 nm.

Briefly, prepared 0.1 mM of DPPH solution in methanol and added 100 µl of this solution to 300 µl of ethanolic extracts of sample Trikatu churna (TKC) at different concentrations (500, 250, 100, 50 and 10 µg/mL). The mixtures were shaken vigorously and allowed to stand for 30 minutes at room temperature. Then the absorbance was measured at 517 nm by using a UV-VIS spectrophotometer. (Ascorbic acid could be used as the reference). Lower

absorbance values of the reaction mixture indicated higher free radical scavenging activity[9-12]. The capability of scavenging the DPPH radical was calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibit)} = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

The antioxidant activity of TKC extract was expressed as IC50 and compared with the standard.

b) Hydrogen peroxide scavenging assay

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic reactions. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate together.

A solution of hydrogen peroxide (43 mM) was prepared in phosphate buffer (1 M pH 7.4). Different concentration of sample RS1 (500, 250, 100, 50 and 10 µg/ml) was added to a hydrogen peroxide solution (0.6 ml, 43 mM). Take absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard[13-17].

$$\text{\% Scavenged} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The antioxidant activity of TKC extract was expressed as IC_{50} and compared with the standard.

RESULTS

In-vitro confirmation of their antioxidant of Trikatu churna on two different methods of DPPH method and hydrogen peroxide scavenging assay. These TKC were screened for their antioxidant activity at different concentrations to determine the IC_{50} value of % inhibition of TKC.

DPPH radical scavenging assay

Table no. 1: DPPH free radical scavenging assay of TKC

S. No	Concentration of sample (µg/ml)	The absorbance of sample (In triplicates)			%Inhibition of the sample
1.	Control	1.072	1.032	1.048	------
2.	**500 µg/ml**	0.475	0.493	0.49	**53.7143**
3.	250 µg/ml	0.616	0.617	0.628	40.9206
4.	100 µg/ml	0.751	0.746	0.757	28.4444
5.	50 µg/ml	0.855	0.825	0.839	20.0317
6.	10 µg/ml	0.963	0.932	0.928	10.381
7.	**Ascorbic acid**	0.15	0.095	0.132	**88.0317**

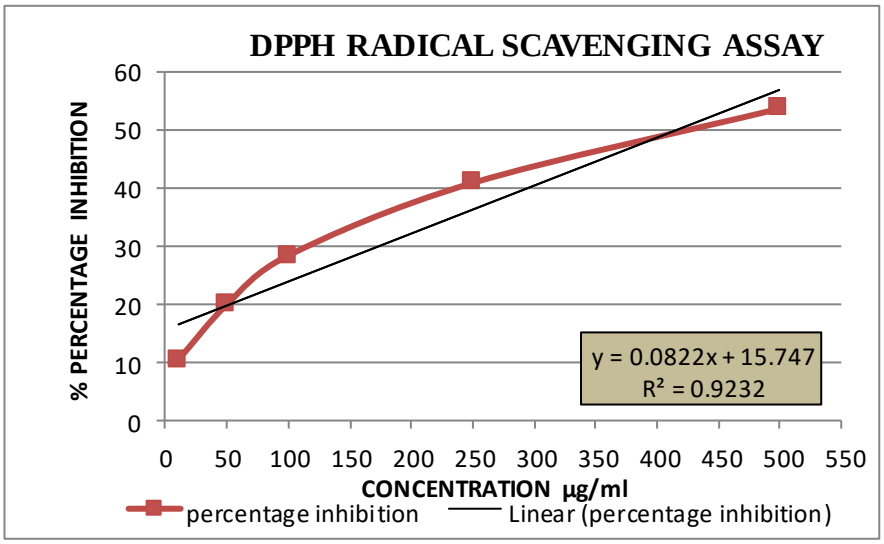

Figure 1: Effect of TKC on DPPH radical scavenging activity

From the results, the IC_{50} value of TKC was found to be **121.2 µg/ml** and IC_{50} value of Ascorbic acid was found to be **80.32 µg/ml.**

Hydrogen peroxide radical scavenging assay

Table no. 2: H_2O_2 radical scavenging assay of TKC

S. No	Concentration of sample (µg/ml)	The absorbance of sample (In triplicates)			%Inhibition of the sample
1.	Ascorbic acid	1.53	1.505	1.506	**89.2936**
2.	500 µg/ml	0.233	0.211	0.255	**84.5695**
3.	250 µg/ml	0.285	0.304	0.324	79.8455
4.	100 µg/ml	0.428	0.46	0.469	70.0442
5.	50 µg/ml	0.634	0.633	0.629	58.1457
6.	10 µg/ml	0.728	0.738	0.721	51.7219

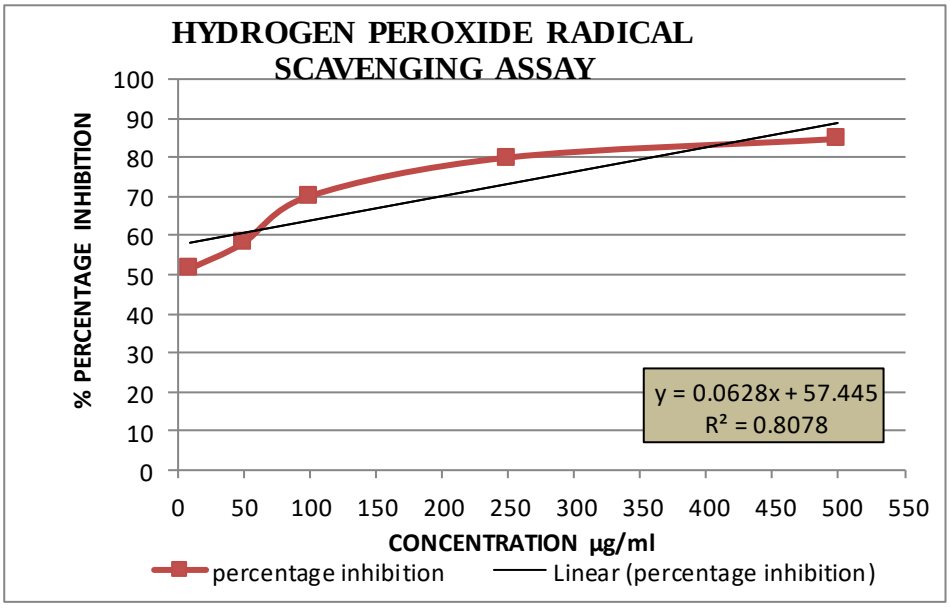

Figure 2: H_2O_2 scavenging activity of TKC

From the results, the IC50 value of TKC was found to be **93.23µg/ml** and IC50 value of Ascorbic acid was found to be **86.76 µg/ml.**

DISCUSSION

The *in-vitro* antioxidant activity of TKC was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and H_2O_2 (hydrogen peroxide) radical scavenging assays. In the DPPH radical scavenging assay, the IC_{50} (half maximal inhibitory concentration) of TKC was

determined to be **121.2 mcg/ml.** As a reference, the IC_{50} value for the standard ascorbic acid was found to be **80.32 mcg/ml.** This indicates that TKC exhibits antioxidant properties, but its efficacy in scavenging DPPH radicals is slightly lower compared to ascorbic acid. Similarly, in the hydrogen peroxide radical scavenging assay, the IC_{50} of TKC was measured to be **93.23 mcg/ml,** while the standard ascorbic acid exhibited an IC_{50} value of **86.76 mcg/ml.** These results demonstrate that TKC possesses significant antioxidant activity by effectively scavenging hydrogen peroxide radicals. Although the IC_{50} values for TKC are slightly higher than those of ascorbic acid, indicating a comparatively lower potency, the antioxidant potential of TKC suggests its potential use in combating oxidative stress-related conditions.

CONCLUSION

The results obtained in the present study indicate that ethanolic extract of TKC exhibits free radical scavenging activity and reducing power. TKC has a three different plant mixture is *P.nigrum, P.longum, Z.officinale*. They individually have an anti-oxidant activity due to the presence of phenolic, polyphenolic, flavonoids, and other phytochemicals. A combination of these three is an effective source of antioxidant activity and reducing power. The findings of the present study suggested that TKC could be a potential source of natural antioxidants and that could have great importance as therapeutic agents in preventing or slowing the progress of aging and oxidative stress-related degenerative diseases and disorders.

REFERENCES

1. Thakur M, *et al*. Development of standardization of trikatu churna: A spectroscopic approach. International journal of pharmacy & life sciences (2012); 3(12): 2254-2258.
2. Kaushik R. Trikatu – A combination of three bioavailability enhancers. International journal of green pharmacy (2018); 12(03).
3. Rahul K, *et al*. Trikati: Transforming food into medicines. Innovations in food technology: Current perspectives and future goals (2020); 501-508.
4. Shahidi F, *et al*. Measurement of antioxidant activity. Journal of functional foods (2015); 18: 757-781.
5. Bunaciu AA, *et al*. Recent applications for in-vitro antioxidant activity assay. Critical reviews in analytical chemistry (2015); 46(5): 389-399.
6. Wang J, *et al*. Reviews on mechanisms of *In-vitro* antioxidant activity of polysaccharides. Oxidative medicine and cellular longevity (2015); 2016: 13 pages.
7. Dahikar SB, *at al*. Evaluation of the antibacterial potential of Trikatu churna and its ingredients: An *in-vitro* study. International Journal of Phytomedicine (2010); 2: 412-417.
8. Uma Reddy B, *et al*. Antimicrobial and analgesic activities of Trikatu churna and its ingredients. Pharmacologyonline (2009); 3: 489-495.
9. Athavale A, *et al*. Evaluation of *In-vitro* antioxidant activity of Panchagavya: A traditional ayurvedic preparation. International journal of pharmaceutical sciences and research (2012); 3(8): 2543-2549.

10. Naik GH, *et al*. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry (2003); 63: 97-104.
11. Makhija IK, *et al*. *In-vitro* antioxidant studies of sitopaladi churna, a polyherbal ayurvedic formulation. Free radicals and antioxidants (2011); 1(2).
12. Rajesh PM, *et al*. In-vitro antioxidant activity of coumarin compounds by DPPH, super oxide and nitric acid-free radical scavenging methods.
13. Gulcin I, *et al*. Determination of *in-vitro* antioxidant and radical scavenging activities of propofol. Chemical and pharmaceutical billetin (2005); 53(3): 281-285.
14. Guddadarangavvanahally K, *et al*. Antioxidant activities of flavidin in different *in-vitro* model systems. Bioorganic and medicinal chemistry (2004); 12(19): 5141-5146.
15. Oyedemi SO, *et al*. *In-vitro* and *–vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr J Pharm Pharmacol (2010); 4(2): 70-78.
16. Kose K, *et al*. In-vitro antioxidant effect of Ginkgo biloba extract (EGb 761) on lipoperoxidation induced by hydrogen peroxide in erythrocytes of Behcets patients. The Japanese journal of pharmacology (1997); 75(3): 253-258.
17. Kumaran A, *et al*. In-vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. Food science and technology (2007); 40(2): 344-352.