Research Article

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ANTIOXIDANT ACTIVITY OF TAMRA BHASMA AND TAMRA YOGA: AN *IN VITRO* EVALUATION

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ABSTRACT

The main objective of the present study is to investigate the antioxidant activity of Tamra Bhasma and Tamra Yoga. 2,2 -Diphenyl-1-Picrylhydrazyl (DPPH) scavenging assay has been used in the determination of *In Vitro* antioxidant activity. Increasing order of Percentage of Inhibition was seen in 100 µl, 200 µl, 300 µl, 400 µl and 500 µl sample concentrations in both Tamra Bhasma and Tamra Yoga. Tamra Bhasma showed 58.67% of inhibition and Tamra Yoga showed 55.47% of inhibition in 500 µl sample concentrations respectively. The present study revealed that both the Tamra Bhasma and Tamra yoga showed good antioxidant activity. Tamra Bhasma showed relatively higher antioxidant activity when compared to Tamra Yoga.

Keywords: Tamra Bhasma, Tamra Yoga, DPPH, Antioxidant activity.

INTRODUCTION

Free radical is an atom or group of atoms that have one or more unpaired electrons. These are formed as necessary intermediates in a variety of normal biochemical reactions, but when generated in excess or not appropriately controlled, radicals can wreak havoc on a broad range of macromolecules. The main feature of radicals is that they have high chemical reactivity. Reactive oxygen species (ROS) are chemically reactive chemical species containing oxygen. ROS are formed as a natural by product of the normal metabolism of oxygen and have important role in cell signalling and homeostasis. Normally human body has a defence mechanism to neutralize the reactive oxygen species. However during conditions of excess stress caused mainly due to improper lifestyle, pollution, tobacco, alcohol etc. leads to dramatic increase of ROS. This condition is known as oxidative stress. It is defined as the imbalance between systemic manifestation of reactive oxygen species and a biological systems ability to readily detoxify the reactive intermediates or to repair the resulting damage. Oxidative stress is an important risk factor in the manifestation of many chronic diseases like atherosclerosis, neurodegenerative diseases, cataract, cancer, Parkinson's disease, Alzheimer's disease, infection, inflammation, autism and ageing[1].

Antioxidants are compounds that can delay, inhibit or prevent the oxidation of biomolecules like proteins, lipids or nucleic acids. Antioxidants help in scavenging the free radicals or break the chain reactions due to their redox properties[2]. In general, there are two types of antioxidants – natural and synthetic. Now-a-days the role of synthetic antioxidants has been replaced by natural antioxidants due to their carcinogenicity and toxicity. Many natural substances have been explored for their antioxidant activities; however there is still a need to evaluate many Ayurvedic formulations for their antioxidant potential to find out better alternative sources of treatment in conditions related to oxidative stress caused by free radicals.

Tamra Bhasma is an important metallic preparation obtained from incineration of Tamra. It is indicated in the management of various diseases like Sthoulya (Obesity), Pandu (Anaemia), Kustha (Skin diseases), Kasa (Cough), Swasa, Amlapitta (Acidity), Udara, Ama, Krimi, Arsha (Piles), Yakrut-Pliha vikaras, Grahani, prameha etc[3]. Tamra Yoga is a unique formulation mentioned in Rasa Tantra Sara va Siddha Prayoga Sangraha[4]. It contains one part of Tamra Bhasma and four parts of Yashtimadhu (*Glycyrrhiza glabra*), Chincha kshara (Alkali of Tamarind Fruit rind), Sauvarchala lavana (Black Salt), Trikatu (Shunti, Maricha, Pippali) and Hingu (*Ferula asafoetida*) each. It is indicated in the treatment of diseases like Vataja Gulma, Shula, Agnimandya etc. Since these formulations have been used successfully for various therapeutic purposes, they may also possess certain antioxidant potential. Hence in the present study an attempt has been made to evaluate the antioxidant activity of Tamra Bhasma and Tamra Yoga.

MATERIALS AND METHODS

Preparation of Tamra Bhasma and Tamra Yoga

Procurement of raw materials: Parada, Gandhaka and Tamra were obtained from the local market of Vijayawada. Chincha Phala Twak was obtained from Adilabad district of Telangana State. Hingu, Souvarchala Lavana, Yashtimadhu and Trikatu were obtained from TTD's Sri Srinivasa Ayurveda Pharmacy, Tirupati.

Equal quantities of Shodhitha Parada and Gandhaka were taken and made into Kajjali. Tamra Patras were taken and subjected to Samanya shodhana and Visesha shodhana. Equal quantities of Kajjali and Shodhitha Tamra Patras were triturated in a khalwa

yantra using Nimbu Swarasa. Chakrikas of uniform size were prepared and placed in a Sharava and subjected to Sharava samputikarana. This was subjected to Laghu puta and the entire procedure was performed for 18 times. Tamra Bhasma having all the Bhasma laxanas was attained. Then the obtained Tamra Bhasma was triturated with Kumari Swarasa and subjected to Amrutikarana procedure by Laghu puta for 7 times.

Chincha phala twak was converted to ash by heating in a mesh placed over the hearth. To the ash obtained four parts of water was added and kept overnight. Then the supernatant water was collected and heated in moderate flame. Chincha Kshara was obtained. Raw drugs of Yashtimadhu, Sauvarchala lavana, Trikatu and Hingu were made into fine powder. Then one part of Tamra Bhasma and 4 parts each of Chincha Kshara, Yashtimadhu churna, Sauvarchala lavana churna, Trikatu churna and Hingu churna were added and mixed together to prepare Tamra Yoga.

Entire preparation of Tamra Bhasma and Tamra Yoga was carried out in Department of Rasa Shastra and Bhaishajya Kalpana, TTD's S.V. Ayurvedic College and Sri Srinivasa Ayurveda Pharmacy, TTD, Tirupati.

Determination of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity

Test samples were prepared by adding 1 mg of Tamra Bhasma and Tamra yoga by dissolving it in 10 ml of distilled water, centrifuged and the supernatant solutions were used for the procedure. The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple coloured methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the test compounds (100,200,300,400 and 500 µg/mL) in methanol was added to 4 ml of 0.004% (w/v) methanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation:

$$\%\ \text{of scavenging} = \frac{(\text{A control} - \text{A sample})}{(\text{A control})} \times 100$$

A control = absorbance of the control reaction (containing all reagents expect the test compound) and A sample = absorbance of the test compound. Tests were carried in triplicate.

Table 1: Volume of samples taken in the procedure

	Blank	Control	A	B	C	D	E
Sample concentration	-	-	100 µl	200µl	300 µl	400 µl	500 µl
Concentration of sample in µg/ml	-	-	10	20	30	40	50
Volume of Methanol	1000µl	1000µl	990µl	980 µl	970µl	960µl	950µl
DPPH	-	1ml	1ml	1ml	1ml	1ml	1ml

RESULTS

Table 2: Preparation of Tamra Bhasma

Weight of Tamra patra and Kajjali	Weight of Tamra Bhasma obtained	Loss in Weight
200 g	167 g	33g

Table 3: Preparation of Tamra Yoga.

Initial Weight of ingredients	Final Weight after mixing	Loss in Weight	Final Weight
2100 g	2094 g	6 g	0.28%

Table 4: Anti- oxidant activity of Tamra Bhasma

Sample concentration	Sample OD	Inhibition %
A	0.239	45.43
B	0.228	47.94
C	0.208	52.50
D	0.198	54.79
E	0.181	58.67

Table 5: Anti- oxidant activity of Tamra Yoga

Sample concentration	Sample OD	Inhibition %
A	0.262	40.10
B	0.245	44.06
C	0.231	47.26
D	0.217	50.45
E	0.195	55.47

*****OD- Optical Density

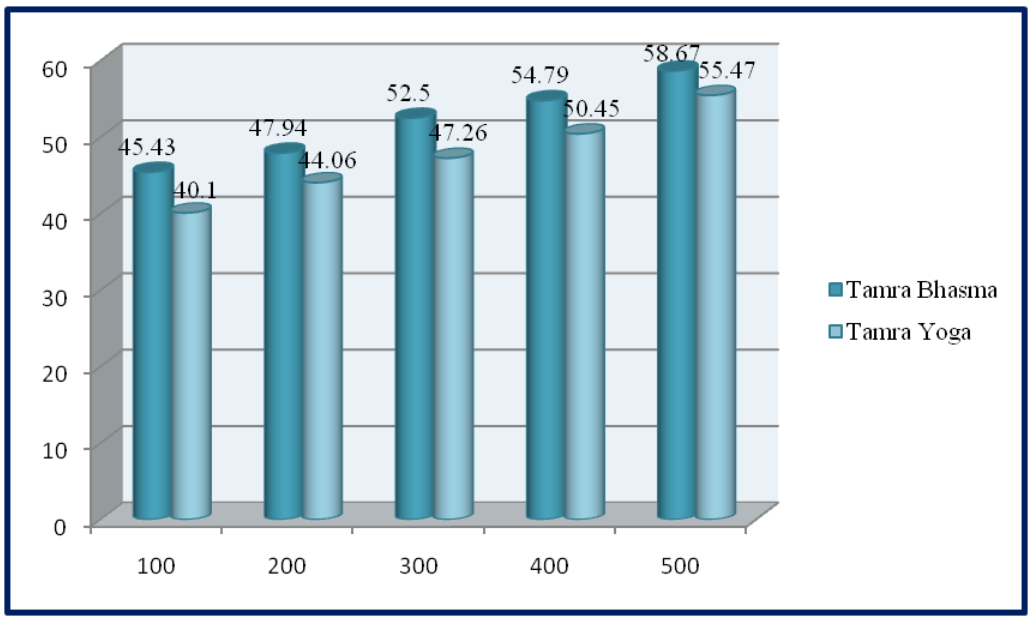

Figure 1: Anti – oxidant activity of Tamra Bhasma & Tamra Yoga.

DISCUSSION

Ayurveda is the oldest medical system in the world that uses processed metals / minerals in the form of Bhasma for therapeutic purposes. Use of metals in medicine is often associated with toxicity, but Ayurveda made them into biocompatible form by certain detoxification processes like Shodhana (Purification), Marana (Incineration), Bhavana (Trituration) etc. which removes the toxic potential from metals and imparts them with therapeutic efficacy of a high grade. Many herbal drugs have been investigated for their antioxidant potential, but very less scientific research has been done on the metallic and herbo-metallic preparations which were used in the management of various chronic diseases caused due to oxidative stress. Hence this present investigation has been undertaken in studying the antioxidant potential of the Tamra Bhasma and Tamra Yoga.

The results of antioxidant activity of Tamra Bhasma and Tamra Yoga showed good antioxidant activity. Tamra Bhasma showed relatively higher antioxidant activity when compared to Tamra yoga. Ascending order of Inhibition percentage was seen in 100 µl, 200 µl, 300 µl, 400 µl and 500 µl sample concentrations in both Tamra Bhasma and Tamra Yoga. With the increase of the concentration, there is a decrease of absorbance value and increase of inhibition percentage. Tamra Bhasma showed 58.67% of inhibition and Tamra Yoga showed 55.47% of inhibition in 500 µl sample concentrations respectively. This shows that Tamra Bhasma has relatively higher free radical scavenging capacity when compared to Tamra Yoga.

A research study conducted to find the toxicology and free radicals scavenging property of Tamra Bhasma in albino rats showed strong antioxidant activity with no detectable adverse effects. It was also proved that Tamra Bhasma inhibits lipids peroxidation (LPO), prevents the rate of aerial oxidation of reduced glutathione (GSH) content and induces the activity of superoxide dismutase (SOD) [5]. Moreover all the herbal drugs present in the Tamra yoga have been proved for their antioxidant activity. Methanolic extract of *Glycyrrhiza glabra* showed potent cytotoxic, anti-microbial and antioxidant activities[6]. A study conducted on methanolic extract of *Zingiber officianale* rhizome (ZOME) showed that ZOME inhibited the proliferation and colony formation in HeLa and MDA-MB-231 cells, in a dose and time dependent manner and induced typical changes in nuclear morphology, chromatin condensation and fragmentation, membrane shrinkage and blebbing in both the cells indicated apoptotic property. ZOME exhibited potent antiradical activity against DPPH[7]. Methanolic extracts of *Piper longum* seeds has shown significant antioxidant activity by *In Vitro* DPPH assay[8]. Piperine a pungent alkaloid present in *Piper nigrum* showed many pharmacological activities like antioxidant, anti-hypertensive, anti-tumour, anti-diarrhoeal, immunomodulatory, hepato-protective analgesic and anti-inflammatory activity[9]. Ferulic acid and umbelliferone present in asafoetida showed strong antioxidant property. Aqueous and ethanolic extracts of leaf, stem and flower have been proven for antioxidant activity[10]. Tamarind fruit showed antioxidant, anti-venomic, anti-hyperlipidemic, anti-malarial, anti-microbial and anti-diabetic activity[11]. This justifies the natural free radical scavenging mechanism present in these preparations.

CONCLUSION

It can be ascertained from the present study, that both the Tamra Bhasma and Tamra Yoga possess good antioxidant activity. Hence these metallic preparations mentioned in Ayurveda can be safely used in the management of various chronic and degenerative diseases by considering them as potent natural antioxidants. Further research can be carried out in finding the antioxidant potential of these formulations by various other *In-vitro* studies.

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