Determination of Antioxidant activity of Mandura Bhasma and Baladi Manduram

[1]Dr. Kishore Kumar H, [2]Dr. Sridurga CH, [3]Venkata Subbaiah K

[1]PG Scholar final year, Department of Rasa Shastra and Bhaishajya Kalpana, S. V. Ayurvedic College, Tirupati.

[2]Associate Professor and HOD, Department of Rasa Shastra and Bhaishajya Kalpana, S. V. Ayurvedic College, Tirupati.

[3]Research Scientist, Department of Science and Technology, PURSE centre, S V University, Tirupati.

Corresponding author E-mail: kishorekumarbams@gmail.com

Mobile No: 9966773324.

ABSTRACT

Objective: Mandura Bhasma is an important preparation obtained by the incineration of Mandura (Rust iron). Baladi Manduram is a herbo-mineral formulation described in Rasakamadhenu Amlapitta Rogaadhikara containing Mandura Bhasma as the major ingredient along with herbal ingredients like Balamula, Satavarimula, Erandamula, Yava, Pippali, Jiraka, Twak, Ela, Patra, Nagakesara and Guda. In the present study, an attempt has been made to find out the antioxidant activity of Mandura Bhasma and Baladi Manduram.

Methods: 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) scavenging assay has been used in the determination of In Vitro antioxidant activity.

Results: Increasing order of Percentage of Inhibition was seen in 100 µl, 200 µl, 300 µl, 400 µl and 500 µl sample concentrations in both Mandura Bhasma and Baladi Manduram. Mandura Bhasma showed 60.27% of inhibition and Baladi Manduram showed 72.14% of inhibition in 500 µl sample concentrations respectively.

Conclusion: Baladi Manduram showed higher antioxidant activity when compared to Mandura Bhasma.

Keywords: Mandura Bhasma, Baladi Manduram, DPPH, Anti-oxidant activity.

INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals. The free radicals cause a state of increased oxidative stress which mainly leads to chain reactions that may damage cells. Antioxidant plays a vital role in inhibiting and scavenging free radicals, thus protecting the humans against infection and degenerative diseases.[1] Now the attention of the modern research fraternity is focussed towards the natural antioxidants due to the safe therapeutic approach.

Ayurveda is the oldest medical system in the world that uses processed metals / minerals in the form of Bhasma for therapeutic purposes. Most of the preparations of Rasa Shastra are Herbo-mineral-metallic in nature, as they contain minerals and metals as an integral part of their formulations along with specified herbs. The use of metals and minerals for therapeutic purpose has been in vogue since Vedic period.

Mandura Bhasma is an important metallic preparation used in the management of various diseases like anaemia specially iron deficiency anaemia, jaundice, spleenic disorders, weakness and digestive disorders like loss of appetite, anorexia etc [2]. Baladi Manduram is a unique formulation described in classics like Rasa Kamadhenu [3] and Rasa yoga sagara- II Pakaradi Rasa [4] for the management of Amlapitta, which contains Mandura Bhasma as the chief ingredient along with the herbal drugs like Balamula (Sida cardifolia), Satavarimula (Asparagus racemosus), Erandamula (Ricinus communis), Yava (Hordeum vulgare), Guda (Jaggery), Jiraka (Cuminum cyminum), Pippali (Piper longum), Twak (Cinnamomum zeylanicum), Ela (Elleteria cardomum), Patra (Cinnamomum tamala) and Nagakesara (Mesua ferrua).

Depending on the available information obtained from the classics regarding the therapeutic uses of Mandura Bhasma and Baladi Manduram, the present investigation was undertaken to investigate and confirm the antioxidant activity of these preparations using in-vitro antioxidant activity evaluation.

MATERIALS AND METHODS

Preparation of Mandura Bhasma and Baladi Manduram:

- Procurement of raw materials- Mandura and Triphala were obtained from local market of Chennai, Tamil Nadu. Balamula, Satavarimula, Erandamula, Yava, Pippali, Jiraka, Twak, Ela, Patra, Nagakesara, Guda and Kumari were obtained from TTD's

Sri Srinivasa Ayurveda Pharmacy, Tirupati. Gomutra was collected from the Goshala TTD, Tirupati.

- Mandura (Rust iron) was taken and subjected to Shodhana (purification) by Niravapa (quenching) in Gomutra Triphala Kashaya for 7 times.
- Then the Shodhita Mandura was triturated with Kumari Swarasa and subjected to Marana (incineration) by Gaja puta for 7 times. Very fine Mandura Bhasma having all the Bhasma lakshanas was obtained after 7^{th} puta.
- Then Guda was taken and made into paka and fine powders of all the herbal ingredients and Mandura Bhasma was added one by one and heated on moderate flame. After self-cooling, the mixture was dried under sunlight in a tray. Homogenous mixture of Baladi Manduram was filled in capsules of 500mg.
- Entire preparation of Baladi Manduram was carried out in TTD's Sri Srinivasa Ayurveda Pharmacy and Department of Rasa Shastra and Bhaishajya Kalpana, S.V.Ayurvedic College, Tirupati.

Table No. 1. Showing contents of Baladi Manduram:

S.No	Name of content	Quantity
1	Bala mula	100g
2	Satavari mula	100g
3	Eranda mula	100g
4	Yava	100g
5	Pippali	50g
6	Jiraka	50g
7	Twak	8g
8	Ela	8g
9	Patra	8g
10	Naga kesara	8g
11	Guda	100g
12	Mandura bhasma	1264g

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

Test samples were prepared by taking 1 mg of Mandura Bhasma and Baladi Manduram and mixing it in 10 ml of distilled water, centrifuged and the supernatant solutions

were used for the procedure. Total five dilutions were prepared for evaluation of antioxidant activity. 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 min incubation period at room temperature, de-colourization of DPPH was determined by measuring the absorbance against blank at 517 nm. The percentage of inhibition of free radical production from DPPH was calculated by the following equation:

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

A control = absorbance of the control reaction (containing all reagents expect the test compound), A sample = absorbance of the test compound.

Table No.2 Showing the 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 No. 3 Showing the result of preparation of Mandura bhasma

Weight of Mandura taken	Weight of Mandura Bhasma obtained	Loss in Weight
1800g	1575g	225g

Table No. 4 Showing the result of preparation of Baladi Manduram

Weight of ingredients taken	Weight of Baladi Manduram obtained	Loss in Weight
1382 g	1380g	2g

Table No.5 Showing the result of Anti- oxidant activity of Mandura Bhasma

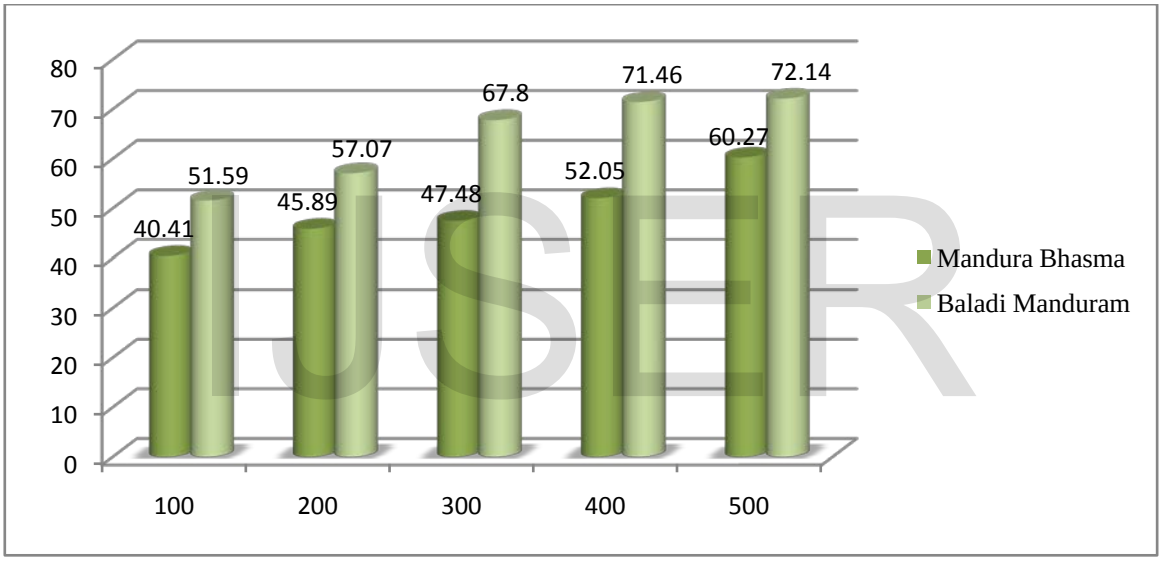
Sample concentration	Sample OD	Inhibition %
A	0.261	40.41
B	0.237	45.89
C	0.230	47.48
D	0.210	52.05
E	0.174	60.27

Table No.6 Showing the result of Anti- oxidant activity of Baladi Manduram

Sample concentration	Sample OD	Inhibition %
A	0.212	51.59
B	0.188	57.07
C	0.141	67.80
D	0.125	71.46
E	0.122	72.14

*OD- Optical Density

Graph No. 1 Showing the Anti – oxidant activity of Mandura Bhasma & Baladi Manduram.



DISCUSSION

In the recent years there is an increase in the incidence of many diseases like carcinoma, diabetes mellitus, hypertension, arthritis, anaemia, neuromuscular diseases etc. Majority of these diseases have been linked to oxidative stress due to free radicals. In the treatment of these diseases antioxidant therapy has gained utmost importance. The need to replace synthetic antioxidants due to their carcinogenicity has diverted the attention towards natural antioxidants. Majority of herbo-mineral, metallic and herbal preparations mentioned in Ayurveda were used in the management of various diseases. The health benefits of these preparations may be due to their antioxidant activity. However, due scientific attention has not been given to evaluate the antioxidant potential of Ayurvedic drugs. Hence to analyse the

antioxidant potential of Mandura Bhasma and Baladi Mandura, the present study has been carried out.

The results of evaluation of antioxidant activity of Mandura Bhasma and Baladi Manduram indicated significant antioxidant activity in both the preparations. Baladi Manduram showed relatively higher antioxidant activity when compared to Mandura Bhasma. Increasing order of Percentage of Inhibition was seen in 100 µl, 200 µl, 300 µl, 400 µl and 500 µl sample concentrations in both Mandura Bhasma and Baladi Manduram. With the increase in the sample concentrations, the percentage of inhibition of free radicals was also seen increasing. This indicates that the antioxidant activity of both Mandura Bhasma and Baladi Manduram are dose dependent. Mandura Bhasma showed 60.27% of inhibition and Baladi Manduram showed 72.14% of inhibition in 500 µl sample concentrations respectively. Higher antioxidant activity of Baladi Manduram may be due to the addition of other herbal ingredients to the Mandura Bhasma. All the herbal ingredients present in the Baladi Manduram have been proved for their antioxidant activity.

Research studies conducted to find the comparative antioxidant potential of ethanolic extracts of *Sida cardifolia* leaf, stem, root and whole plant showed effective reducing power and free radical scavenging activity. The highest antioxidant activity was observed in root extract.[5] The methanolic extracts of roots of *Asparagus racemosus* tested in vitro using DPPH showed moderate free radical scavenging activity [6]. Methanolic extracts of *Ricinus communis* Linn. studied in wistar albino rats showed significant free radical scavenging activity by inhibiting lipid peroxidation initiated by carbon tertrachloride and ferrous sulphate in rat liver and kidney homogenates[7]. Water extracts of barley showed higher antioxidant activity when compared with water extracts of roasted barley prepared under different roasting temperature [8]. Methanolic extracts of *Piper longum* seeds has shown significant antioxidant activity by in vitro DPPH assay [9]. Polyphenol rich methanolic extract of cumin showed efficient free radical scavenging and metal chelating activity to protect biomolecules like proteins, lipids and DNA against oxidative stress[10]. Etheric, methanolic and aqueous cinnamon extracts showed antioxidant property by inhibition of oxidative stress in 68%, 95.5% and 87.5% respectively [11]. Leaves extract of *Cinnamomum tamala* evaluated by using DPPH free radical scavenging assay and ascorbic acid as a standard showed good antioxidant and cytotoxic properties[12]. Methanolic extracts from the seeds and pods of *Elleteria cardomum* showed antioxidant activity and antimutagenic potential[13]. Ethanolic extracts of *Mesua ferrea* showed good antioxidant property by in vitro method[14]. Jaggery and other

sugars namely white, refined and brown were evaluated for cytoprotectivity on NIH 3T3 fibroblasts and erythrocytes, DPPH radical scavenging activity and DNA protection. The analysis revealed higher cytoprotective and antioxidant activity when compared to other sugars [15]. Based on the results of the above research activities, we can confirm the reason for the higher antioxidant activity of Baladi Manduram compared to Mandura Bhasma.

CONCLUSION

From the results obtained from the present study, it can be confirmed that Mandura Bhasma and Baladi Manduram have significant antioxidant activity. Hence a wider research must be carried out to find out the efficacy and potential of the Ayurvedic formulations in the management of several diseases mainly caused due to increased oxidative stress.

REFERENCES

1. Helliwell B. Free radicals, antioxidants and human diseases curiosity, cause and consequence. Lancet. 1994:344:721-724.
2. Pandit Kasinath Shastry, Sri Sadananda sharma Virachitha Rasa Tarangini, Taranga 20 Verse no. 132-134, Varanasi: Motilal Banarasidas, 2014; p-517.
3. Acharya Sri Gulraj Sharma Mishra and Vaidya Santhosh Kumar Sharma, Rasa Kamadhenuh compiled by Sri Chudamani Mishra, Fourth Chikitsapada, Chapter 11, Verse no.41-44 , Varanasi: Choukhambha Orientalia, 2014; p-214
4. Vaidya Pandit Hariprapanna Sharma, Rasa Yoga Sagara, Volume II, Pakaradi Rasa, Verse no. 1594-1597, Varanasi: Choukhambha Krishnadas Academy, 2010, p-107.
5. K. Dhalwal, Y.S. Deshpande, A.P. Purohit, S.S. Kadam. Evaluation of the antioxidant activity of sida cardifolia, Journal of pharmaceutical biology, 2005: 43(9):754-761.
6. Senahlatha dohare, Mohd. Shuaib, Kamran J. naquvi, In- Vitro antioxidant activity of asparagus recemosus roots, International journal of biomedical research, 2011(4):228-235.
7. Ilavarasan R, Mallika M, Venkataraman S, Anti-inflammatory and free radical scavenging activity of Ricinus communis root extract, journal of ethnopharmacology, 2006, feb (20); 103 (3): 478-480.
8. Omwamba M, Hu Q, Antioxidant activity in barley (Hordeum vulgare L.) grains rosted in a microwave oven under conditions optimized using response surface methodology, Journal of food science, 2010; 75(1): C66-73.

9. Adwaid manu k, Ambili narikot, Lokesh ravi and Sarveswari s, Anti haemolytic and antioxidant activity of piper longum seeds, Advances in biotechnology and microbiology, 2017;4(4); 001-003.
10. Anita dua, S K Gupta, A Mittal and RituMahajan, A study of antioxidant properties and antioxidant compounds of cumin (Cuminum cyminum), internal journal of pharmacy and biological archives, 2012;3(5):1110-1116.
11. Mancini-Filho J, Van-Koiij A, Mancini DA et.al, antioxidant activity of cinnamon (Cinnamomum zeylanicum, Breyne) extracts, Bolletino chimico farmaceutico, 1998,dec; 137 (11):443-7.
12. Salma Akter, Md. Ashraf Ali, Ranjan Kumar barman, Bytul Mokaddesur rahman and Mir Imam Ibne Wahed, Invitro antioxidant and cytotoxic activity of ethanolic extract of cinnamomum tamala leaves, international journal of pharma sciences and research, 2015;6 (3):532-6.
13. Asma saeed, bushra sultana, farooq anwar, mohammad mushtaq, Khalid m. alkharfy, Antioxidant and Antimutagenic potential of seeds and pods of green cardomum (Eletteria cardomum), International of jornal of pharmacology, 2014; 10(8);461-469.
14. Narendra Prasad D, B Ganga rao, E Sambasiva Rao, T Mallikarjuna Rao, VS Praneeth D, Quantification of phytochemical constituents and in vitro antioxidant activity of Mesua ferrea leaves, Asian Pacific journal of tropical biomedicine, 2012; 2(2): S539-542.
15. M.A.Harish Nayaka, U. V.Satisha, M.P. Manohar, K.B. Chandrashekhar, Shylaja M Dharmesh, Cytoprotective and antioxidant activities of jiggery sugar, Food chemistry;2009;115 (1);113-118.