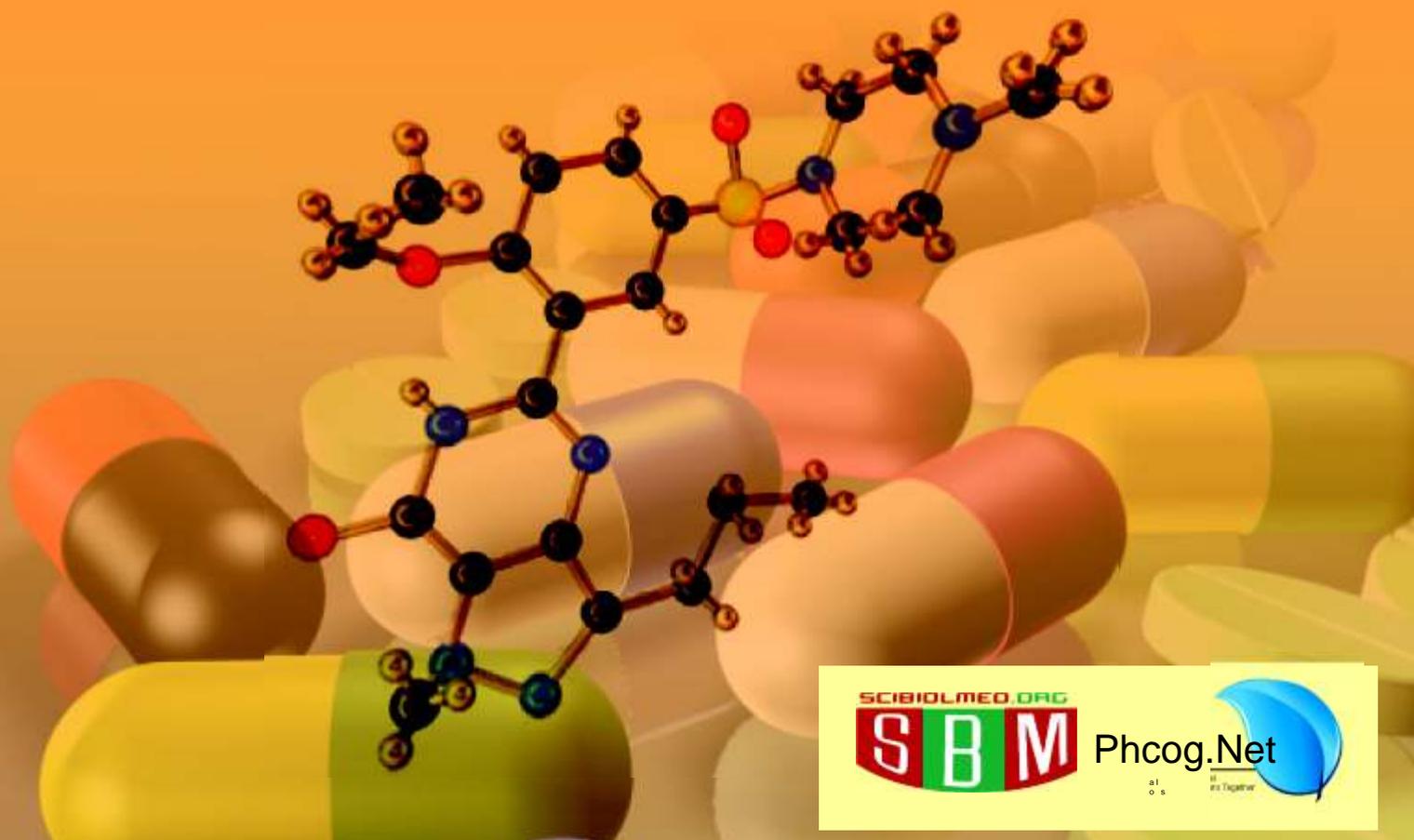


ISSN: 2231-2536

# FREE RADICALS AND ANTIOXIDANTS

Vol 6 / Issue 2 / Jul-Dec 2016 [www.antiox.org](http://www.antiox.org) | [www.journalonweb.com/fra](http://www.journalonweb.com/fra)

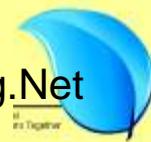


SCIBIOLMED.ORG



Phcog.Net

at  
o s



It  
is  
Together

An Official Publication of Phcog.Net & SciBioMed.Org

# Distinct Antioxidant Activity of a Common Antidepressant Drug Imipramine

Debalina Sinharoy<sup>1</sup>, Debanjan Mukhopadhyay<sup>2</sup>, Shauroseni Palchoudhuri<sup>1</sup>, Bidisha Ghosh<sup>3</sup>, Sukhen Das<sup>3</sup>, Sujata G. Dastidar<sup>1\*</sup>

<sup>1</sup>Department of Microbiology, Herbicure Healthcare Bio-Herbal Research Foundation, Saraldighi (E), Boral, Kolkata-700154, INDIA.

<sup>2</sup>Department of Molecular Biology, Herbicure Healthcare Bio-Herbal Research Foundation, Saraldighi (E), Boral, Kolkata- 700154, INDIA.

<sup>3</sup>Department of Physics, Jadavpur University, Kolkata- 700032, INDIA.

## ABSTRACT

**Background:** Free radicals are known to cause severe damage to most of the biomolecules in the human system and are responsible for various illnesses including neurodegenerative, cardiovascular and autoimmune disorders. Antioxidants can reduce effects of free radicals and are given to patients suffering from such diseases. There are certain natural antioxidants like flavonoids which possess free radical scavenging activities. The flavonoid quercetin is one such compound among naturally occurring antioxidants. The present study has been designed to determine the antioxidant activity in the synthetic antidepressant drug imipramine which is structurally similar to quercetin. **Method:** Specific standard procedures like ferric ion reducing capacity by FRAP assay, phosphomolybdenum assay and cupric ion reducing (CUPRAC) assay were carried out keeping ascorbic acid as the known standard. **Results:** Ferric ion reducing property of imipramine by FRAP assay revealed that reducing power of imipramine augmented with increasing amounts of the drug. In the phosphomolybdenum assay antioxidant capacity of imipramine increased in a dose dependent manner. In both these studies imipramine showed greater antioxidant action than ascorbic acid. In CUPRAC assay as the amount of imipramine was increased there was a definite

elevation in antioxidant activity; however, it was comparatively less active than ascorbic acid. **Conclusion:** The highly potent antioxidant property in the antidepressant synthetic compound imipramine may be recognized by physicians involved in treatment of psychosis since patients receiving this drug regularly will certainly be in an advantageous position. The parent structure of imipramine can be modified further to potentiate antioxidant property of the drug.

**Key words:** Antioxidant, Antidepressant drug, Flavonoids, Imipramine, Quercetin, Reactive Oxygen Species.

## Correspondence :

**Sujata Dastidar,**

Department of Microbiology, Herbicure Healthcare Bio-Herbal Research Foundation, Saraldighi (E), Boral, Kolkata-700154, INDIA.  
Landline No.: +913322903135, Mobile No.: +919836029744

E-mail: jumicrobiol@yahoo.co.in

DOI: 10.5530/fra.2016.2.4

## INTRODUCTION

Transfer of an electron from electron rich to electron deficient entity is termed as oxidation. The common natural oxidizing agents include metals and metallic compounds that are present naturally in all ecosystems.<sup>1</sup> A large number of studies have repeatedly proved the toxic and carcinogenic potentialities of heavy metals.<sup>2</sup> Such toxic metals have been found to interact with deoxyribonucleic acid (DNA) as well as proteins resulting in oxidative denaturation of biological macromolecules. Several heavy metals like lead, arsenic, mercury, iron, cadmium, chromium and cobalt are capable of producing reactive free radical species that may finally terminate in lipid peroxidation and oxidation of DNA and ribonucleic acid (RNA).<sup>3</sup> Prevention and slow reduction of such oxidative processes can be carried out by a variety of agents termed as antioxidants. An antioxidant is able to trap free radicals resulting in termination of a chain reaction by chelating the respective metal ion or by inhibiting the process of free radical generation and also direct scavenging of free radicals by preventing the reaction of biomolecules with the Reactive Oxygen Species (ROS). Along with ROS Reactive Nitrogen Species (RNS) is also capable of damaging biomolecules both *in vitro* and *in vivo*. Various harmful effects of ROS can be neutralized by non-enzymatic antioxidants as well as antioxidant enzymes.<sup>4</sup> Generation of free radicals is balanced by the antioxidative defense system in healthy individuals, but oxidative stress is revealed in individuals suffering from various ailments that favour free radical generation due to depletion of antioxidant levels.<sup>5</sup> Antioxidants are primarily of two types, enzymatic and non-enzymatic. Among the non-enzymatic antioxidants apart from vitamins C, E and carotenoids there is a large variety of flavonoids that are important antioxidants.

One of the most important properties of flavonoids is their protection against oxidative stress.<sup>6</sup> Flavonoids are polyphenolic compounds possessing 15 carbon atoms, two benzene rings which are joined by a linear three carbon atom chain. The flavonoid quercetin is known to be present in many fruits, vegetables, olive oil, red wine and tea.<sup>7</sup> During the past few years presence of quercetin has been reported in different parts of several higher plants.<sup>8-13</sup> Mishra and Flora observed further that administration of quercetin could successfully protect arsenic induced oxidation in experimental animals.<sup>14</sup> Based on these studies, the tricyclic antidepressant drug imipramine which is structurally similar to quercetin and a member of the dibenzazepine group of compounds was selected to determine its antioxidant potentiality.

## MATERIALS AND METHODS

**Drug:** The drug imipramine (Sigma Aldrich, USA) was received as a gift from Dr. J. Christensen, University of Copenhagen, Copenhagen, Denmark.

## Chemicals

All the chemicals and reagents used in this study were of analytical grade. Ascorbic acid was purchased from Sisco Research Laboratory (SRL, India). For ferric ion reducing assay ethanol, hydrochloric acid, potassium ferrocyanide and ferric chloride were obtained from Merck, India; and sodium dodecyl sulphate was from SRL, India. For phosphomolybdenum assay the chemicals ammonium molybdate and phosphoric acid were received from Merck, India. For cupric ion reducing assay cupric chloride, trisodium citrate and neocuproine were procured from Loba Chemie, India.

### Assay procedures for detection of antioxidant property of imipramine

In all the tests antioxidant capacity of imipramine is expressed as ascorbic acid equivalent (AAE). Analyses of all the data in the following tests were recorded statistically with the help of Graph-pad prism version 5.0 software. All values were mean  $\pm$  SD.

### Ferric ion reduction by assay of ferric reducing power (FRAP)

The method described by Prieto *et al.* was followed.<sup>15</sup> In this process an antioxidant compound is able to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by donating an electron. To the tubes containing 0.1 ml volume of increasing concentrations of imipramine (25, 50, 100, 200, 500  $\mu\text{g/ml}$ ) were added to 0.5 ml of deionized water plus 0.09 ml of 95% ethanol. This was followed by addition of 0.15 ml each of 1M HCl, 1% potassium ferrocyanide, 0.05 ml of 1% sodium dodecyl sulfate, 0.1 ml of 0.2% ferric chloride. All the tubes were vortexed for 20 minutes. Readings were taken at 700 nm in a spectrophotometer (Evolution 201 UV-VIS Spectrophotometer, Thermo Fisher). By this process the amount of  $\text{Fe}^{2+}$  can be monitored by measuring the formation of Prussian blue colour. The antioxidant capacity of imipramine was evaluated from the standard curve of ascorbic acid concentrations against O.D. at 700 nm (Figure 1).

### Phosphomolybdenum assay

The method described by Prieto *et al.* was followed.<sup>15</sup> Tubes containing 0.2 ml of imipramine at varying concentrations (25, 50, 100, 200, 500  $\mu\text{g/ml}$ ) were mixed with 1.8 ml of distilled water and 2 ml of phosphomolybdenum reagent in labeled centrifuge tubes. The tubes were then kept at 95°C in a water bath for 90 minutes. The mixtures were cooled down to room temperature and readings were taken at 695 nm in the same spectrophotometer. In this assay formation of bluish green colour is recorded to prove reduction of phosphate-Mo (VI) to phosphate-Mo (V). The total antioxidant capacity of imipramine was evaluated from the standard curve of ascorbic acid concentrations against O.D. at 695 nm (Figure 2).

### Assay by cupric ion reducing antioxidant capacity (CUPRAC)

The method of Apak *et al.* was followed.<sup>16</sup> For this purpose to each of the labeled centrifuge tubes were added 0.6 ml each of distilled water, 0.01 M of cupric chloride and 25% solution of sodium citrate and mixed thoroughly. Then 0.6 ml of increasing amounts of imipramine (25, 50, 100, 200, 500  $\mu\text{g/ml}$ ) were added one by one to the tubes and again mixed thoroughly. Finally to each tube 0.6 ml neocuproine (0.0075M) was added, vortexed and allowed to react at room temperature for 30 minutes. The readings were taken in the same spectrophotometer at 450 nm. The antioxidant capacity of imipramine was evaluated from the standard curve of ascorbic acid concentrations against O.D. at 450 nm (Figure 3).

## RESULTS

### Determination of ferric ion reducing power by following FRAP assay

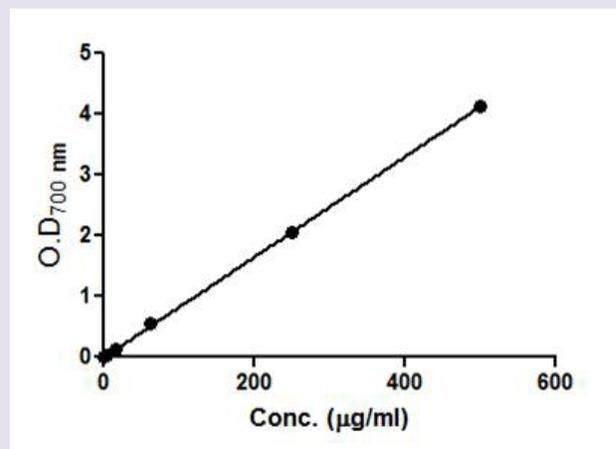
FRAP measures the reducing potency of imipramine. Since an antioxidant can reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by donating an electron, the resultant  $\text{Fe}^{2+}$  forms a complex with ferrocyanide that was monitored by measuring the formation of Prussian blue coloured complex which absorbed light at 700 nm. Our study revealed that the reducing power of imipramine increased with the increase of their concentrations ranging from 25 $\mu\text{g/ml}$  to 500  $\mu\text{g/ml}$  (Figure 4). The obtained ascorbic acid equivalent (AAE) value demonstrated that imipramine is a better antioxidant than ascorbic acid.

### Evaluation of total antioxidant capacity of imipramine by phosphomolybdenum assay

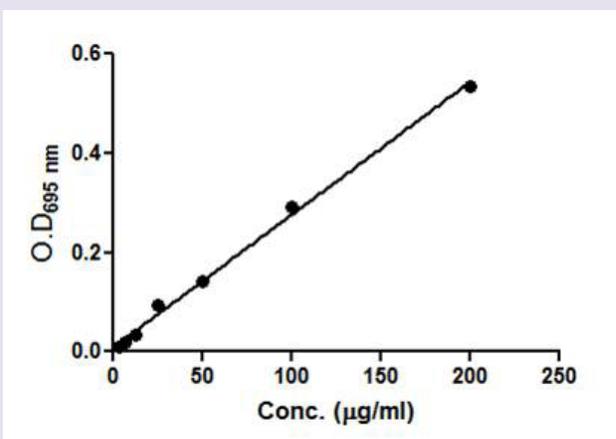
Our study showed that the antioxidant capacity of imipramine increased in a dose dependent manner ranging from 25 $\mu\text{g/ml}$  to 500  $\mu\text{g/ml}$  (Figure 5). The obtained AAE value proved that imipramine is a better antioxidant than ascorbic acid and moreover, this assay showed better molybdenum ion reducing power than Fe ion reducing power.

### Detection of antioxidant capacity by CUPRAC assay method

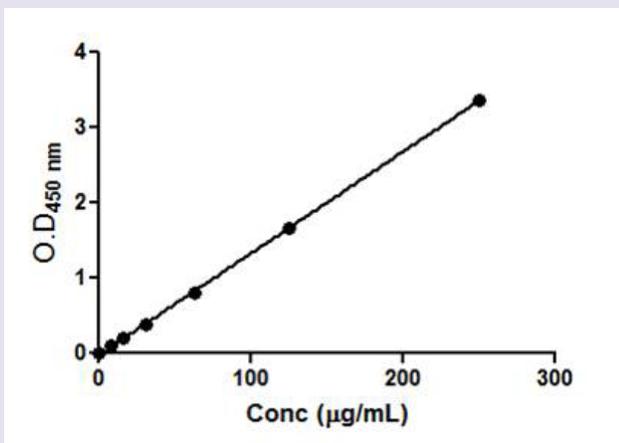
Antioxidants especially the hydroxyl groups of phenolic compounds are converted to their respective quinines [oxidized form] in the presence of cupric chloride ( $\text{Cu}^{+2}$ ) which, in turn, can get reduced to  $\text{Cu}^+$ . The formed  $\text{Cu}^+$  then reacts with the chromogen, neocuproine (2, 9 dimethyl-1, 10 phenanthroline) to form a yellow coloured complex which can be spectrophotometrically measured at 450 nm. Study revealed that the reducing power of imipramine although increased with the increase in their concentrations ranging from 25 $\mu\text{g/ml}$  to 500  $\mu\text{g/ml}$ , it is less active than well known antioxidant ascorbic acid (Figure 6).



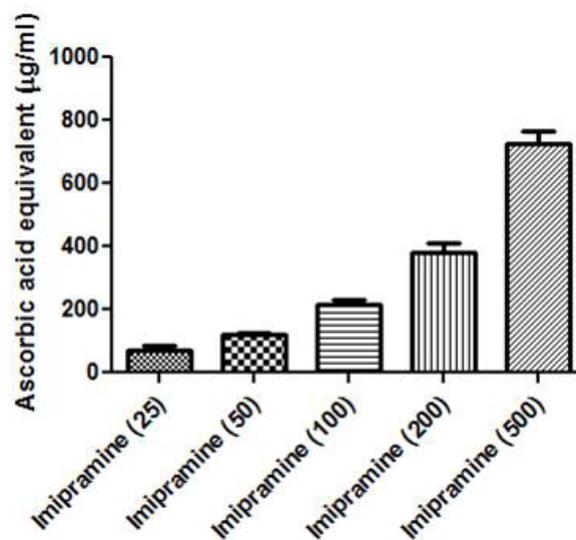
**Figure 1:** Standard curve of ascorbic acid taking 200, 400 and 600  $\mu\text{g/ml}$  of the same to determine the ferric ion reducing power of imipramine.



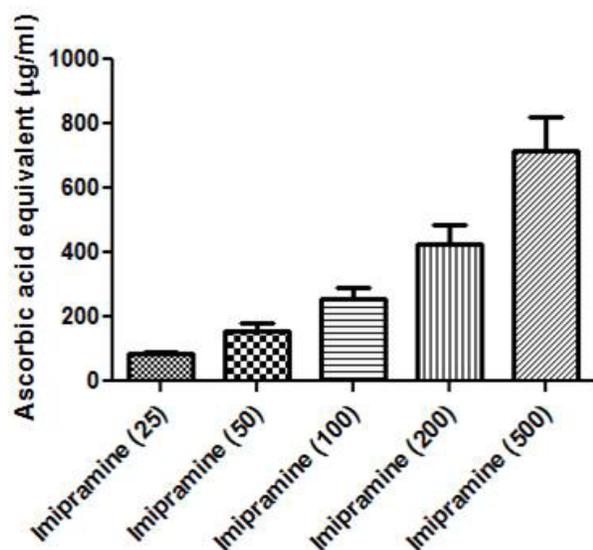
**Figure 2:** Standard curve of ascorbic acid taking 50, 100, 150, 200 and 250  $\mu\text{g/ml}$  of the same to determine the molybdenum ion reducing power of imipramine.



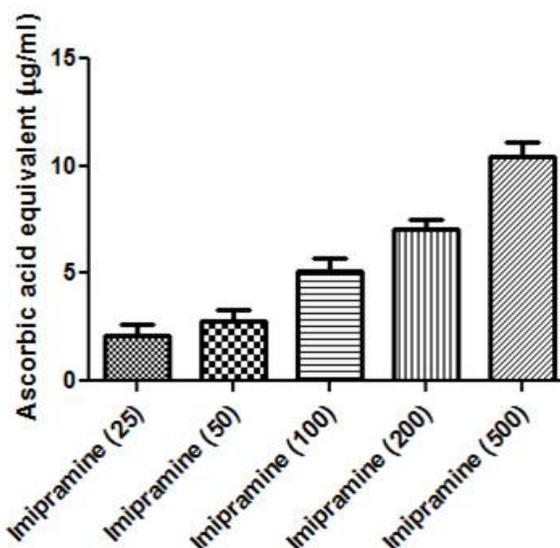
**Figure 3:** Standard curve of ascorbic acid taking 100, 200 and 300 µg/ml of the same to determine the cupric ion reducing capacity of imipramine.



**Figure 5:** Graphical representation of determination of total antioxidant capacity of imipramine by phosphomolybdate assay.



**Figure 4:** Graphical representation of determination of ferric ion reducing antioxidant capacity of imipramine by FRAP assay.



**Figure 6:** Graphical representation of determination of cupric ion reducing antioxidant capacity of imipramine by CUPRAC assay.

## DISCUSSION

The antipsychotic and antidepressant drug imipramine was found to possess powerful antioxidant property. The methods used for this study were primarily based on action of imipramine with respect to the known antioxidant compound ascorbic acid.<sup>17</sup> In the first two tests antioxidant capacity of imipramine was distinctly higher than the competent antioxidant ascorbic acid; however, in CUPRAC assay imipramine was less active than ascorbic acid although the reducing power of imipramine gradually increased with higher amounts of the compound. The drug imipramine is structurally similar to flavonoids, whose antioxidant capacity has been evaluated by various researchers.<sup>8-13</sup> The common feature of flavonoids is hydroxyl group substituted flavan moiety. Such characteristic chemical entities are essential for the flavonoids to scavenge free radicals and prevent oxidation of biological molecules by converting the ROS into inactive forms.<sup>18</sup> Imipramine being structurally similar to

flavonoids its antioxidant property could be beneficial in the prevention and treatment of oxidative stress induced disorders and diseases.

Imipramine is prescribed for the treatment of major depression associated with agitation and anxiety and is also used to treat enuresis. All such disorders are found to occur among aged men and women. Imipramine is administered to such patients on a long term basis. Therefore the patients who are treated with such a drug are doubly benefited since imipramine is now proved, without any doubt, to be a highly potent antioxidant. As this drug is in routine therapeutic usage satisfying human toxicity tests, imipramine may, in course of time, be developed as one of the most active antioxidants. Thus the present study

opens up among many other possibilities a structural modification of the parent molecule to form a more competent antioxidant for routine intake by ailing elderly patients, whose requirement for antioxidant is possibly far more than an adult normal human being. Future studies will focus on the mechanism of action of imipramine on specific free radical scavenging abilities such as hydrogen peroxide,<sup>17</sup> superoxide, hydroxyl radical<sup>19</sup> and such studies are underway.

## CONCLUSION

An elaborate analysis on the presence of antioxidant property in natural products revealed that the flavonoid quercetin possesses such an activity. Efforts were then followed to find out structurally identical pharmaceutical compounds. Imipramine was found to contain the benzene rings much similar to the quercetin. With the help of standard assay procedures imipramine showed remarkable antioxidant property. Such an action was repeatedly observed in all the test procedures. Moreover, imipramine proved to be more potent than the known antioxidant ascorbic acid. Therefore with this study a new avenue has come up to obtain many more such active antioxidants among conventionally used drugs and develop them further for human use.

## CONFLICT OF INTEREST

We have no conflict of interest.

## REFERENCES

- Bargagli R. Trace metals in Antarctica related to climate change and increasing human impact. *Rev Environ Contam Toxicol.* 2000;166(1):129-73.
- Flora SJS. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxi Med Cell Longev.* 2009;2(4):191-206.
- Kalia K, Flora SJS. Strategies for safe and effective treatment for chronic arsenic and lead poisoning. *J Occup Hlth.* 2005;47(1):1-21.
- Halliwel B. Antioxidant in human health and disease. *Ann Rev Nutr.* 1996;16(1):33-50.
- Kratchanova M, Denrv P, Ciz M, Lojek A, Mihailov A. Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. *Acta biochim Polonica.* 2010;57(2):229-34.
- Polovka M, Brezova V, Stasko A. Antioxidant properties of tea investigated by EPR spectroscopy. *Biophys Chem.* 2003;106(1):39-56.
- Lee K, Kim Y, Kim D. Major phenolics in apple and their contribution to the total antioxidant capacity. *J Agric Food Chem.* 2003;51(22):6516-20.
- Mishra US, Chakraborty P, Dasgupta A, Dastidar SG, Martins M, Amaral L. Potent bactericidal action of a flavonoid fraction isolated from the stem bark of *Butea frondosa*. *In vivo.* 2009;23(1):29-32.
- Paria S, Chaki S, Dastidar SG, Mookerjee M. Evaluation of antimicrobial activity and antioxidant property of *Vitex. negundo* leaf extract. *Int J Drug Form and Res.* 2011;2(4):337-45.
- Maji HS, Maji S, Bhar K, Chandra J, Baral M, Chakraborty P, *et al.* Anthelmintic, free-radical scavenging property and potent antibacterial activity of flavonoid fraction isolated from the whole plant of *Oldenlandia corymbosa*. *Int J Biomed Pharma Sci.* 2011;5(1):43-8.
- Mahapatra SK, Mookerjee M, Sinharoy D, Karak P, Das S, Dastidar SG. Evaluation of antimicrobial potentiality of a flavonoid, isolated from the leaf of the plant *Colebrookea oppositifolia*. *Int J Biol Pharm Res.* 2013;4(4):225-30.
- Dasgupta A, Jeyaseeli S, Mukhopadhyay A, Palchoudhuri S, Sinharoy D, De B, *et al.* Possibilities of developing new formulations for better skin protection from a traditional medicinal plant having potent practical usage. *Brit J Pharm Res.* 2014;4(7):870-9.
- Chaki S, Ghosh B, Bandyopadhyay S, Mookerjee M, Das S, Dastidar SG. Detection of various phytochemical compounds from seeds of *Acacia. auriculiformis* for possibilities of obtaining potent antimicrobial agents. *Int J Biol Pharma Res.* 2015;6:120-8.
- Mishra D, Flora SJS. Quercetin administration during chelation therapy protects arsenic induced oxidative stress in mouse. *Biol Trace Elem Res.* 2008;122(2):137-47.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem.* 1999;269(2):337-41.
- Apak R, Guclu K, Ozyurek M, Karademir SE. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim Acta.* 2008;160(4):413-9.
- Mukhopadhyay D, Dasgupta P, Sinharoy D, Palchoudhuri S, Ali S, Dastidar SG. A sensitive *in vitro* spectrophotometric hydrogen peroxide scavenging assay using 1,10-phenanthroline. *Free Rad Antiox.* 2016;6(1):123-31.
- Elliott MJR, Chithan K, Theoharis CT. The effects of plant flavonoids on mammalian cells: implication for inflammation heart disease and cancer. *Pharmacol Rev.* 2000;52(4):673-751.
- Mukhopadhyay D, Rahman KA, Roy D, Dasgupta P. Evaluation of in-vitro antioxidant activity and phytochemical constituents of kulekhara (*Hygrophilia spinosa*). *Int J Pharmacog Phytochem Res.* 2015;7(5):984-90.

## SUMMARY

- Antidepressant synthetic compound imipramine selected for determination of antioxidant activity for its structural similarity with known natural antioxidant quercetin.
- Imipramine possesses highly significant antioxidant property, even greater than the known antioxidant ascorbic acid.
- Ailing patients receiving imipramine are doubly benefitted.
- Structural modification of imipramine may produce a new generation of antioxidants.

## ABOUT AUTHOR



**Debalina Sinha Roy:** M.Sc in Microbiology, is a Research Fellow at the Department of Microbiology, Herbicare Healthcare Bio-Herbal Research Foundation, Kolkata, India. At present she is pursuing her Ph.D degree at the Jadavpur University, Kolkata, India. Since tricyclic pharmacological compounds are known for their antimicrobial potentiality as "Non-antibiotics", Debalina selected a structurally similar compound, the antidepressant imipramine. She has experimentally proved antimicrobial property both *in vitro* and *in vivo* in this compound. A further extension of the property of imipramine as an antioxidant is expected to make imipramine as a unique pharmacological compound.