**Short communication** **Bimonthly published scientific journal**

INTERNATIONAL JOURNAL of Indigenous Herbs and Drugs

ISSN-2456-7345

# Antioxidant activity of different extracts of fruits of *Morus alba*

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**Abstract**

Objective: The current scrutiny was designed to investigate the antioxidant activity of aqueous and ethanol extracts of *Morus alba*.

Methods: Antioxidant evaluation was done by screening of hydroxyl radical scavenging through deoxyribose assay and estimatedlipid peroxide level in liver and brain homogenate of rat's tissues by thiobarbituric acid methods.

Results: The overall antioxidant activity of *Morus alba* was found to be considerable by diminishing in hydroxyl radical activity and level of lipid peroxidation. $IC_{50}$ values of aqueous and ethanol extracts of *Morus alba* was found to be 766 µg/ml and 539 µg/ml respectively for hydroxyl radical scavenging activity. $IC_{50}$ values of aqueous and ethanol extracts of *Morus alba* was found to be 791 µg/ml and 724 µg/ml respectively for lipid peroxidation assay in liver homogenates. Fifty percentage of inhibition concentration was perceived to be 592 µg/ml and 478 µg/ml for aqueous and ethanol extracts of *Morus alba* respectively for lipid peroxidation in brain homogenates.

Conclusion: This investigation recommends that *Morus alba* extracts display potential antioxidant activity which can be useful in herbal formulation as nutritional supplements and it can help fight against ageing, cancer, and chronic diseases like cardiovascular disease.

**Keywords:** Antioxidant activity, hydroxyl radical scavenging, lipid peroxidation, *Morus alba*

One of most crucial pathogenesis factors of heart failing, cancer, atherosclerosis, inflammatory and cognitive deficit disease can be oxidative stress which is initiated by reactive nitrogen species and reactive oxygen species [1]. Oxidative stress is explicated as imbalance between oxidants and antioxidants which lead to damage biomolecules such as protein, nucleic acid, DNA, and RNA [2]. Antioxidant compounds can be defined as substance which has ability to scavenge of free radicals for instance superoxide, hydrogen peroxide, lipid peroxide and etc [3]. Nowadays major investigation programs are being undertaken for antioxidant activity focus on the consumption of natural antioxidants as food supplements and traditional medicines [1]. Consequently, present research was planned to assess the antioxidant activity of aqueous and ethanol extracts of fruits of *Morus alba* by screening of hydroxyl radical scavenging through deoxy ribose assay and estimating of lipid peroxide level in liver and brain homogenate of rats tissues. The fruits of *Morus alba* were collected from Chennai, Tamil Nadu, India and authenticated by Green Chem, Bangalore, Karnataka, India, a voucher specimen (MAT-SIP-501) were preserved for future references. For preparation of extracts, the fruits materials (1 kg) were dried, powdered and extracted with water and ethanol (60-80 ºC) using soxhlet methods. The filtrate was evaporated at 70 ºC in a vacuum dryer to give final yield 40.5 g. All chemicals were analytical up grade and acquired from local store of Visveswarapura Institute of Pharmaceutical Sciences. Female albino wistar rats (180-220 gm) were obtained from the NIMHANS animal house, Bengaluru and were housed at room temperature in a well-ventilated animal house under 12 hrs light / dark cycle in polypropylene cages (29"x 22"x 14") with stainless steel grill top, bedded with paddy husk. The animals were maintained under standard conditions in an animal house as per the guidelines of "Committee for the Purpose of Control and Supervision on Experiments on Animals" (CPCSEA) for at least one week prior to use. The rats had free access to standard rat chow and water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), Visveswarapura Institute of Pharmaceutical Sciences, Bangalore (Registration No: 152/1999, renewed in 2012). Hydroxyl radical scavenging activity was determined by using the deoxyribose assay. A portion (20 µl) of various concentration of extract sample (50, 100, 200, 400, 800, 1000 µg/ml) was mixed with 35 µl of following solution: ascorbate (1.2 mM), EDTA (1.2 mM), $FeCl_3$ (300 µM), $H_2O_2$ (33.6 mM) and phosphate buffer (120 mM). Added 35 µl of deoxy ribose to above mixture then centrifuged at 3200 rpm for 1 min. Incubated at 37 ºC for 1 min and allowed them to cool and again centrifuged at 3200 rpm for 1 min. The centrifuged tubes were placed in boiling water for 20 min and then cooled for 20 min. Added 1120 µl of 1-butanol and consequently centrifuged for 3200 rpm for 6 min. The upper layer was collected and measured absorbance at 532 nm using 1-

butanol as blank. The % scavenging and $IC_{50}$ was calculated [4]. Evaluation of lipid peroxidation produced by $Fe^{2+}$/ ascorbate system in rat liver and brain homogenate was done by thiobarbituric acid method (TBA) method. The rat liver/ brain homogenate 0.1 ml (25% w/v) was mixed with potassium chloride (30 mM), Tris–HCL buffer (20 mM, pH 7.0), ascorbate (0.06 mM), ferrous ammonium sulphate (0.16 mM) and various concentrations of aqueous and ethanol extracts of *Morus alba* (50, 100, 200, 400, 800, 1000 µg/ml) in a final volume of 0.5 ml. Incubated for 1 h at 37 ºC. Then a portion (0.4 ml) of mixture was removed and combined with 1.5 ml glacial acetic acid (20%, pH 3.5), 0.2 ml sodium dodecyl sulphate (SDS) (8.1%) and 1.5ml TBA (0.8 %). The total volume was made up to 4 ml with distilled water and then kept in a water bath at 95-100 ºC for 1 h. Added 0.5 ml of n-butanol, pyridine mixture (15: 1, v/v) and 1.0 ml of distilled water and mixture was shaken vigorously and was centrifuged at 4,000 g for 10 min. Removed the organic layer and measured absorbance at 532 nm. The % inhibition and $IC_{50}$ was calculated [5]. Antioxidants have potential protective activity against free radical induced oxidative stress in the body. The antioxidant potential of aqueous and ethanol extracts of *Morus alba* was appraised in the screening for new bioactive compounds from natural sources [3]. Phenolic compounds of plants can act as antioxidant and reducing agent by hydrogen donating property of their hydroxyl group which can lead to scavenge the free radical or express metal chelating activity which causes to deactivate free radicals [6]. Table 1 shows the hydroxyl radical scavenging activity of aqueous and ethanol extract of *Morus alba*. Degradation of deoxyribose mediated by hydroxyl radicals which is generated by $Fe^{3+}$/ ascorbtae/ EDTA/ $H_2O_2$ system was found to be prohibited by aqueous and ethanol extract of *Morus alba*. Both extracts displayed hydroxyl radical scavenging activity with different concentration of 50, 100, 200, 400, 800 and 1000 µg/ml in dose dependent manner. Fifty percentage of inhibition concentration values ($IC_{50}$) of aqueous and ethanol extracts of *Morus alba* was found to be 766 µg/ml and 539 µg/ml respectively for hydroxyl radical scavenging activity. Hence, ethanol extract of exhibited great hydroxyl radical scavenging activity compared with aqueous extract of *Morus alba*. Table 2 and 3 reveal the effect of aqueous and ethanol extracts of *Morus alba* on percentage inhibition of lipid peroxidation level in liver and brain homogenates of rat tissues respectively. Lipid peroxides generated by induction of ferrous/ascorbate on rat liver/brain homogenates was found to be inhibited by aqueous and ethanol extracts of *Morus alba*. The extracts manifested better lipid peroxide inhibition activity in brain homogenate compared with liver therefore it indicated that it was more effective in brain. Ethanol extract of *Morus alba* exhibited good lipid peroxide inhibition activity with $IC_{50}$ value of 724 µg/ml and 478 µg/ml for liver and brain homogenate respectively compared to $IC_{50}$ value of 791 µg/ml and 592 µg/ml of aqueous extract of *Morus alba* for liver and brain homogenate respectively. From data obtained from current investigation it is evidence that aqueous and ethanol extracts of *Morus alba* have greatness hydroxyl radical scavenging and lipid peroxidation inhibition activity. The previous phytochemical screening of *Morus alba* revealed the presence of phenolic compounds such as flavonoids (quercetin, rutin), tannin which could be expected to be responsible for antioxidant activity [7]. The current research manifested that aqueous and ethanol extracts of *Morus alba* have antioxidant activity due to scavenging of hydroxyl radical and inhibition of lipid peroxidation. In addition, ethanol extract of *Morus alba* exhibited superiority antioxidant activity as compared to aqueous extract of *Morus alba*.

**Table 1: Hydroxyl radical scavenging activity of aqueous and ethanol extract of *Morus alba***

| S. No. | Concentration (µg/ml) | % inhibition | |
|---|---|---|---|
| | | Aqueous extract | Ethanol extract |
| 1 | 50 | 5 | 7 |
| 2 | 100 | 9 | 13 |
| 3 | 200 | 19 | 21 |
| 4 | 400 | 36 | 45 |
| 5 | 800 | 52 | 73 |
| 6 | 1000 | 60 | 86 |
| | $IC_{50}$ | 766 µg/ml | 539 µg/ml |



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**Table 2: Effect of aqueous and ethanol extracts of *Morus alba* on percentage inhibition of lipid peroxidation level in liver homogenates of rat tissues**

| S. No. | Concentration (µg/ml) | % inhibition | |
|---|---|---|---|
| | | Aqueous extract | Ethanol extract |
| 1 | 50 | 3.69 | 4.06 |
| 2 | 100 | 6.86 | 8.03 |
| 3 | 200 | 12.45 | 16.89 |
| 4 | 400 | 24.67 | 33.56 |
| 5 | 800 | 48.78 | 51.75 |
| 6 | 1000 | 65.64 | 69.56 |
| | $IC_{50}$ | 791 µg/ml | 724 µg/ml |

**Table 3: Effect of aqueous and ethanol extracts of *Morus alba* on percentage inhibition of lipid peroxidation level in brain homogenates of rat tissues**

| S. No. | Concentration (µg/ml) | % inhibition | |
|---|---|---|---|
| | | Aqueous extract | Ethanol extract |
| 1 | 50 | 8.61 | 8.66 |
| 2 | 100 | 15.54 | 16.36 |
| 3 | 200 | 25.73 | 30.53 |
| 4 | 400 | 48.56 | 54.53 |
| 5 | 800 | 59.42 | 76.45 |
| 6 | 1000 | 78.67 | 89.54 |
| | $IC_{50}$ | 592 µg/ml | 478 µg/ml |

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**Acknowledgement**
The authors are grateful to Green Chem Company, Bangalore, Karnataka, India for providing extracts for this investigation.

Conflict of Interest: None declared
Received: 20 November 2017, Revised: 02 December 2017, Accepted: 20 December 2017
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