

In vitro Studies on Antioxidant Potential of Aqueous Extract of *Bridelia ferruginea* Stem Bark in Brain and Liver of Wistar-albino Rats using Sodium Nitroprusside as Pro-oxidant

Adesola Oluwaseun Adeleye^{1*}

¹Department of Chemical Sciences, Olusegun Agagu University of Science and Technology, P. M. B. 353, Okitipupa, Nigeria.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/AJBGMB/2020/v3i330089

Editor(s):

(1) Dr. Mangala Kohli, VMMC & SJH New Delhi, India.

Reviewers:

- (1) Ochieng O. Anthony, Sumait University, Tanzania.
(2) Pipat Chooto, Prince of Songkla University, Thailand.
(3) Lukman Muslimin, Bangladesh.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55967>

Received 09 February 2020

Accepted 15 April 2020

Published 22 April 2020

Original Research Article

ABSTRACT

Thiobarbituric acid reactive species (TBARS) assay was used to study the antioxidant potential of *Bridelia ferruginea*, a widely used medicinal plant in Nigeria, sub-tropical Africa and parts of Asia. The aqueous extract of *Bridelia ferruginea* stem bark showed inhibition against the formation of TBARS induced by the pro-oxidant, sodium nitroprusside, in the liver and brain tissue homogenates of the locally bred male and female albino-Wistar rats. The inhibition of TBARS is an indication of the antioxidant potential of the plant extract. The extent of antioxidant potential depends on concentrations, showing varying degrees of inhibition with different concentrations. It showed a 54.16% inhibition in the liver and 60.65% in the brain, both at a concentration of 0.33 mg/ml, with IC₅₀ values of 3.00 ± 1.58 mg/ml and 2.99 ± 1.59 mg/ml for the liver and brain homogenates respectively. The results suggest the effectiveness of the aqueous extract of *Bridelia ferruginea* stem bark in reversing the effect of lipid peroxidation that may result from sodium nitroprusside overload.

*Corresponding author: E-mail: adeseun4u2c@gmail.com;

Keywords: *Bridelia ferruginea*; antioxidant; oxidative stress; pro-oxidants; sodium nitroprusside; TBARS.

1. INTRODUCTION

All over the world, several hundreds of plants are good sources of medicinal agents and are used in traditional medicine for a large number of different purposes [1-3]. As a result, plants are one of the bedrocks for modern medicines [4].

Bridelia ferruginea (Family: Euphorbiaceae) is an indigenous medicinal plant in Nigeria. Its common names in Nigeria include Kirni, Kizni (Hausa), Maren (Fulani), Iralodan (Yoruba), Ola (Igbo) [5]. Its habitat is the savannah, especially in the moister regions from Guinea to Zaire and Angola. The bark is dark grey, rough and often marked scaly [6]. *Bridelia ferruginea* has been extensively used in human history. It is proven to possess potent antimicrobial effects against some micro-organisms known to cause enteric and secondary upper respiratory tract infections [7]. The bark extract has also been used for coagulation of milk [8]. The rich phytochemical constituents of this plant (Table 1) is thought to be the active ingredients, responsible for its diverse pharmacological functions.

Pro-oxidants are chemicals that induce oxidative stress, usually through either creating reactive oxygen species (ROS) or inhibiting antioxidant systems [10]. Sodium nitroprusside is an inorganic compound with the formula $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$ [11]. This red-coloured salt, which is often abbreviated 'SNP', is a potent vasodilator [12]. It is administered intravenously in cases of acute hypertensive emergency.

Sodium nitroprusside breaks down in circulation to release nitric oxide (NO), which is a reactive oxygen species with short half-life (< 30 s) [10]. Although nitric oxide acts independently, it may also cause neuronal damage in cooperation with other ROS [12]. This results in oxidative stress, which is an imbalance between the systemic manifestation of reactive oxygen species and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [13]. Oxidative stress can damage cells and tissues (e.g. liver and brain). In humans, oxidative stress is thought to be involved in the development of many diseases or may make worse their symptoms. These include cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure and chronic fatigue syndrome [1]. An overdose of the analgesic paracetamol (acetaminophen) can cause fatal damage to the liver, partly through its production of reactive oxygen species [14,15]. This informed the choice of using the liver and brain tissues for this study.

An antioxidant is a molecule that inhibits the oxidation of other molecules. As oxidative stress appears to be an important part of many human diseases, the use of antioxidants in pharmacology is being intensively studied, particularly as treatments for stroke and neurodegenerative diseases [1]. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer and coronary heart disease. The effectiveness of the aqueous

Table 1. Phytochemical Analysis of *Bridelia ferruginea* Leaf and Stem Bark [9]

Test	<i>Bridelia</i> leaf	<i>Bridelia</i> bark
Alkaloids	Present	Present
Flavonoids	Flavonoid present Catechol tannin suspected	Flavonoid present Catechol tannin suspected
Tannin	Tannin confirmed Taniferous confirmed	Tannin confirmed Taniferous confirmed
Cardiac glycosides	Deoxysugar present Steroidal ring present Steroidal nucleus present Cardenoloids present	Deoxysugar present Steroidal ring present Steroidal nucleus present Cardenoloids present
Anthraquinone	Free anthraquinone present Bound anthraquinone present	Free anthraquinone present Bound anthraquinone present
Phlobatinnins	Present	Present
Saponin	Saponin confirmed	Saponin confirmed
Cyanogenic glycosides	Anthocyanin absent/negative	Anthocyanin absent/negative

extract of *Bridelia ferruginea* stem bark, as an antioxidant, in inhibiting iron (II) sulphate induced oxidative stress has been studied [1]. Therefore, the objective of this research was to investigate the antioxidant potential of the stem bark of *Bridelia ferruginea* plant on sodium nitroprusside induced lipid peroxidation.

2. MATERIALS AND METHODS

2.1 Acquisition and Authentication of the Plant Material

Dried bark of *Bridelia ferruginea* was collected from the Okesa market, Ado-Ekiti, Ekiti State, Nigeria and authenticated in the Department of Plant Science, Ekiti State University, Ado-Ekiti, by a botanist with herbarium code number UHAE 46.

2.2 Sample Preparation of Plant Sample

1 g of the bark was pounded using mortar and pestle. It was then soaked in 100 ml of distilled water for 24 h. The crude extract was thereafter filtered using a Whatman filter paper and kept in a refrigerator until further analysis.

2.3 Animal Preparation

All animal procedures were in strict accordance with the NIH guidelines for the care and use of laboratory animals. 21 locally bred male and female albino-Wistar rats with an average weight of 200 ± 20 g, fed on standard diet and allowed water *ad libitum* were used for *in vitro* studies. The animals were grouped according to their gender and housed (two rats per cage) in a well ventilated room.

2.4 Production of Thiobarbituric Acid Reactive Species (TBARS) from Animal Tissues

The rats were sacrificed with cervical dislocation. The liver and brain tissues were quickly removed and placed on ice. 1 g of the tissues from each animal were homogenized in cold 0.1 M Tris-HCl buffer pH 7.4 (1: 10 w/v) with about ten up and down strokes at approximately 1200 rpm in a Teflon glass homogenizer. The homogenates were centrifuged for 10 min at 3000 rpm to yield a pellet that was discarded and the supernatants were used for the assay. Production of thiobarbituric acid reactive species (TBARS) was determined using standard methods of [16] as described by [17].

100 μ l of the supernatants with or without 50 μ l of the freshly prepared pro-oxidant (sodium nitroprusside), 30 μ l 0.1M Tris-HCl buffer (pH 7.4), different concentrations of the plant extracts (0.33 - 3.33 mg/ml) and an appropriate volume of distilled water to make up the total volume of 300 μ l, were incubated at 37°C for 1 hour. The colour reaction was carried out by adding 200, 500 and 500 μ l each of 8.1% sodium dodecyl sulphate (SDS), 1.3M acetic acid buffer (pH 3.4) and 0.6% Thiobarbituric acid (TBA) (pH 6.0) respectively. The reaction mixture was further incubated at 97°C for 1 hour and the absorbance of the reaction mixture was read, after cooling the tubes, at a wavelength of 532 nm, using a spectrophotometer. All experiments were carried out in triplicates.

2.5 Statistical Analysis

Data were analyzed statistically by one-way Analysis of Variance (ANOVA), followed by Duncan's multiple range test (DMRT) where appropriate. Statistical comparisons were performed with student's t-test. Differences were considered significant at $P < .05$. The correlation coefficient (R^2) between the parameters tested was established by regression analysis.

3. RESULTS

Table 2 shows the inhibitory effect of aqueous extract of *Bridelia ferruginea* stem bark on sodium nitroprusside - induced lipid peroxidation in rat liver homogenate. As detailed in the table, there is an increase in the formation of TBARS in the control, when its absorbance value of 0.760 ± 0.041 is compared with the normal (0.434 ± 0.019). This indicates a high level of lipid peroxidation in the control, resulting from the accumulation of nitric oxide, a metabolic intermediate of sodium nitroprusside, in the liver tissue [12]. Aqueous extract of *Bridelia ferruginea* significantly reduced lipid peroxidation in the liver tissue, in a concentration dependent manner, showing varying degrees of inhibition with different concentrations. The extract showed the strongest inhibition in the liver of the rat, at a concentration of 0.33 mg/ml with $54.16 \pm 3.46\%$ inhibition when compared to other concentrations, while the lowest inhibition ($3.86 \pm 2.61\%$) was observed at a concentration of 3.33 mg/ml. The IC_{50} value, which is a measure of the effectiveness of the extract in inhibiting lipid peroxidation in the liver tissue is 3.00 ± 1.58 mg/ml.

Table 2. The inhibitory effect of aqueous extract of *Bridelia ferruginea* stem bark on sodium nitroprusside induced lipid peroxidation in a rat liver homogenate

Concentration (mg/ml)	Absorbance (532nm)	% Inhibition	Linear Equation (R ²)	IC ₅₀ (mg/ml)
Normal	0.434 ± 0.019 ^{de}	-	y = -12.55x +	3.00 ± 1.58
Control	0.760 ± 0.041 ^a	-	65.16	
0.33	0.349 ± 0.045 ^e	54.16 ± 3.46	R ² = 0.995	
0.67	0.467 ± 0.076 ^{cd}	38.83 ± 6.65		
1.33	0.558 ± 0.075 ^{bc}	26.80 ± 5.99		
2.67	0.655 ± 0.076 ^{ab}	13.90 ± 6.58		
3.33	0.731 ± 0.054 ^a	3.86 ± 2.61		

Results are expressed as mean of three experiments ± Standard Deviation. Values with different letters are significantly different (P < .05)

Table 3. The inhibitory effect of aqueous extract of *Bridelia ferruginea* stem bark on sodium nitroprusside induced lipid peroxidation in a rat brain homogenate

Concentration (mg/ml)	Absorbance (532nm)	% Inhibition	Linear Equation (R ²)	IC ₅₀ (mg/ml)
Normal	0.310 ± 0.045 ^d	-	y = -14.63x	2.99 ± 1.59
Control	0.602 ± 0.026 ^a	-	+76.19	
0.33	0.235 ± 0.030 ^e	60.65 ± 4.01	R ² = 0.986	
0.67	0.304 ± 0.039 ^d	40.80 ± 3.81		
1.33	0.407 ± 0.047 ^c	35.16 ± 12.14		
2.67	0.520 ± 0.022 ^b	21.46 ± 12.62		
3.33	0.568 ± 0.026 ^a	6.00 ± 4.77		

Results are expressed as mean of three experiments ± Standard Deviation. Values with different letters are significantly different (P < .05)

The inhibitory effect of *Bridelia ferruginea* stem bark extract on sodium nitroprusside-induced lipid peroxidation in a rat brain homogenate is shown in Table 3. The TBARS formation and the inhibitory potential of the extract on the brain homogenate is also concentration dependent as those of the liver homogenate. The plant extract had the highest percentage inhibition of 60.65 ± 4.01% in the brain homogenate at a concentration of 0.33 mg/ml and the lowest percentage inhibition of 6.00 ± 4.77% at a concentration of 3.33 mg/ml. There is also an increase in the formation of TBARS in the control, with an absorbance value of 0.602 ± 0.026 when compared with the normal value of 0.310 ± 0.045. The IC₅₀ value is 2.99 ± 1.59 mg/ml.

4. DISCUSSION

Sodium nitroprusside is an anti-hypertensive drug that acts by relaxing the vascular smooth muscle and consequently dilates peripheral arteries and veins. However, sodium nitroprusside has been reported to cause cytotoxicity through the release of cyanide and/or nitric oxide (NO) [12]. Nitric oxide is a kind of the

reactive oxygen species. The over production of reactive oxygen species can directly attack the polyunsaturated fatty acids of the cell membrane and induce lipid peroxidation, consequently resulting in cell and tissue damage [12]. To measure the extent of lipid peroxidation induced by sodium nitroprusside in this study, the thiobarbituric acid reactive species (TBARS) assay was used. This is because there exists a strong correlation between TBARS as a marker of lipid peroxidation in tissues [18]. This is evident in the fact that most of the aldehydes that react with thiobarbituric acid (TBA) are derived from peroxides and unsaturated fatty acids during the test procedure [19]. According to the results of this study, the aqueous extract of *Bridelia ferruginea* stem bark showed ability to inhibit TBARS formation in the liver and brain homogenates used. This agrees with the results of [1], when the aqueous extract of *Bridelia ferruginea* stem bark was used to inhibit TBARS formation induced by iron (II) sulphate. It also agrees with the work of [18], where the ethanolic extract of *Bridelia ferruginea* stem bark is discovered to have an effective TBARS inhibiting ability in the liver and brain of albino rats. The strongest TBARS inhibition ability of the plant

extract was observed at the lowest concentration (0.33 mg/ml). With increasing concentrations, the inhibitory effects diminished significantly, which suggests the dose – dependent manner in which the extract works. Comparatively, the highest TBARS inhibition was recorded in the brain homogenate ($60.65 \pm 4.01\%$), as against the liver homogenate ($54.16 \pm 3.46\%$).

The inhibition of TBARS by the aqueous extract of *Bridelia ferruginea* stem bark is an indication of the antioxidant potential of the plant extract [1]. The protection offered by the aqueous extract of *Bridelia ferruginea* on tissues (liver and brain) induced with lipid peroxidation in this study is most likely due to its rich phytochemical constituents, such as flavonoids and alkaloids [9]. Phytochemicals, which are chemicals derived from plants, are known to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers. In laboratory studies, many phytochemicals act as antioxidants, neutralizing free radicals and removing their power to create damage [20].

5. CONCLUSION

The results from this study further confirms the potency of the antioxidant activity of the aqueous extract of *Bridelia ferruginea* against TBARS induced lipid peroxidation. It also indicates the possible effectiveness of its use in reversing the effect of lipid peroxidation that may result from sodium nitroprusside overload. Human trial is however needed to validate this claim.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Adeleye OA, Oloyede OI, Bolarinwa TO and Adebayo OA. Inhibitory Effects of Aqueous Extract of *Bridelia ferruginea* Stem Bark on Iron (II) Sulphate – Induced Oxidative Stress in Brain and Liver of Albino Wistar Rats. Journal of Applied Life Sciences International. 2017;4(2):1-7. [Article no. JALSI.36752] DOI: 10.9734/JALSI/2017/36752
2. Christian AG, Ahunna AG, Nwakaego EM, Chimsorom CK, Chile AE. Antimalarial potential of the ethanolic leaf extract of *Pseudocedra kotschyi*. Journal of Acute Disease. 2015;4(1):23-27.
3. Baldé AM, Traoré MS, Baldé MA, Barry MS, Diallo A, Camara M, *et al.* Ethnomedical and ethnobotanical investigations on the response capacities of Guinean traditional health practitioners in the management of outbreaks of infectious diseases: The case of the Ebola virus epidemic. Journal of Ethnopharmacology. 2016;182:137-149.
4. Awodele O, Amagon KI, Agbo J, Prasad, MNV. Toxicological evaluation of the aqueous stem bark extract of *Bridelia ferruginea* (Euphorbiaceae) in rodents. Interdisciplinary Toxicology. 2015;8(2):89-98.
5. Alowanou GG, Olounlade AP, Azando EVB, Dedehou VFGN, Daga FD, Hounzangbe-adote M. A review of *Bridelia ferruginea*, *Combretum glutinosum* and *Mitragina inermis* plants used in zootherapeutic remedies in West Africa: Historical origins, current uses and implications for conservation. Journal of Applied Biosciences. 2015;87(1):8003-14.
6. Rashid MA, Gustafson KR, Cardellina JH, Boyd MR. A new podophyllojoxin Derivative from *Bridelia ferruginea*. Nat. Prod. Lett. 2000;14:285-292.
7. Adeoye AO, Abaeli AM, Owowumi CJ, Olukoya DK. Antimicrobial activity of *Bridelia ferruginea*: Book of abstract of the Symposium on drug production from natural product. Drug Research and Production Unit, Obafemi Awolowo University, Ile-Ife. 1998;24.
8. Orafidiya LO, Lamikanra A and Adedeji JA. Coagulation of milk as an index of astringency of the bark extract of *Bridelia ferruginea* Benth. and lime juice for the formulation of a traditional gargle Ogun Efu. Phytotherapy Res. 1990;4:189-194.
9. Owoseni AA, Ayanbamiji TA, Ajayi YO and Ewegbenro IB. Antimicrobial and phytochemical analysis of leaves and bark extracts from *Bridelia ferruginea*. African Journal of Biotechnology. 2010;9(7):1031-1036.

10. Puglia CD, Powell SR. Inhibition of cellular antioxidants: A possible mechanism of toxic cell injury. *Environ. Health Perspect.* 1984;57:307–311.
11. Butler AR, Megson IL. Non-Heme Iron Nitrosyls in Biology. *Chemical Reviews.* 2002;102(4):1155–1165.
12. Bates JN, Baker MT, Guerra R, Harrison DG. Nitric oxide generation from sodium nitroprusside by vascular tissue. *Biochem. Pharmacol.* 1991;42:S157-S165.
13. Petrov A, Perekhvatova N, Skulachev M, Stein L, Ousler G. SkQ1 ophthalmic solution for dry eye treatment: Results of a phase 2 safety and efficacy clinical study in the environment and during challenge in the controlled adverse environment model. *Advances in Therapy.* 2016;33(1):96-115.
14. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. *Toxicol. Sci.* 2002;65 (2):166–76.
15. James LP, Mayeux PR, Hinson JA. Acetaminophen-induced hepatotoxicity. *Drug Metab. Dispos.* 2003;31(12):1499–506.
16. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry.* 1979;95(2):351-58.
17. Puntel RL, Nogueira CW, Rocha JBT. Krebs cycle intermediates modulate thiobarbituric reactive species (TBARS) production in Rat Brain *in vitro*. *Neurochemical Research.* 2005;30:225-35.
18. Oloyede OI, Babalola SO. *In vitro* antioxidant activity of ethanolic extract of *Bridelia ferruginea* (stem bark). *Academic Research International.* 2012;2(3):1-6. [ISSN: 2223-9944]
19. Gutteridge JMC. Lipid Peroxidation and Antioxidants as Biomarkers of Tissue Damage. *Clinical Chemistry* 1995;41(12): 1819-1828.
20. American Institute for Cancer Research (AICR). Health talk: What's the difference between an antioxidant and a phytochemical? Published: November 16, 2015 [Accessed: April 2, 2020] Available:<https://www.aicr.org/resources/blog/healthtalk-whats-the-difference-between-an-antioxidant-and-a-phytochemical/>

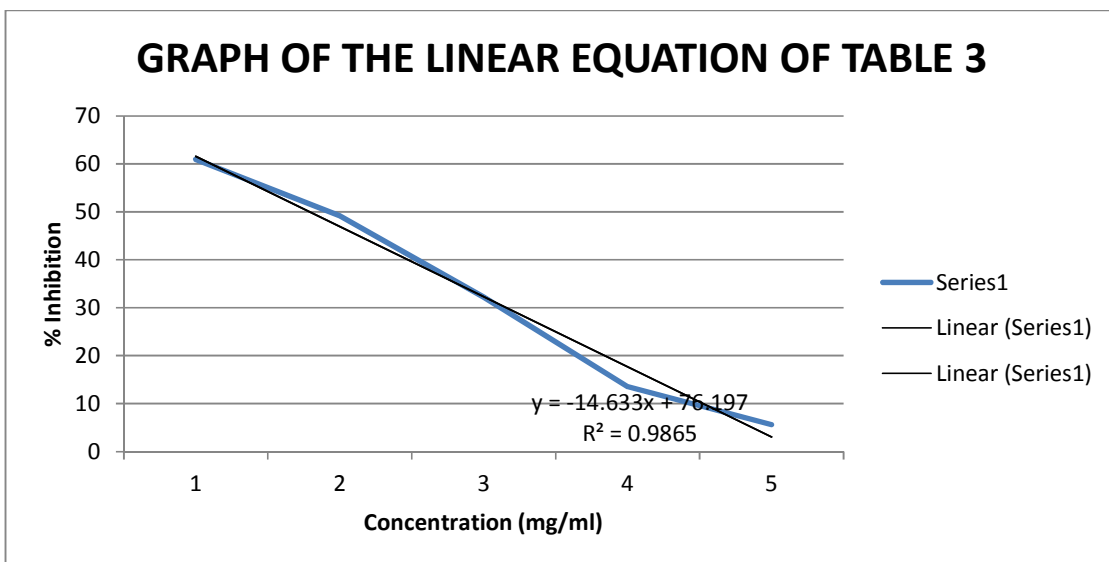
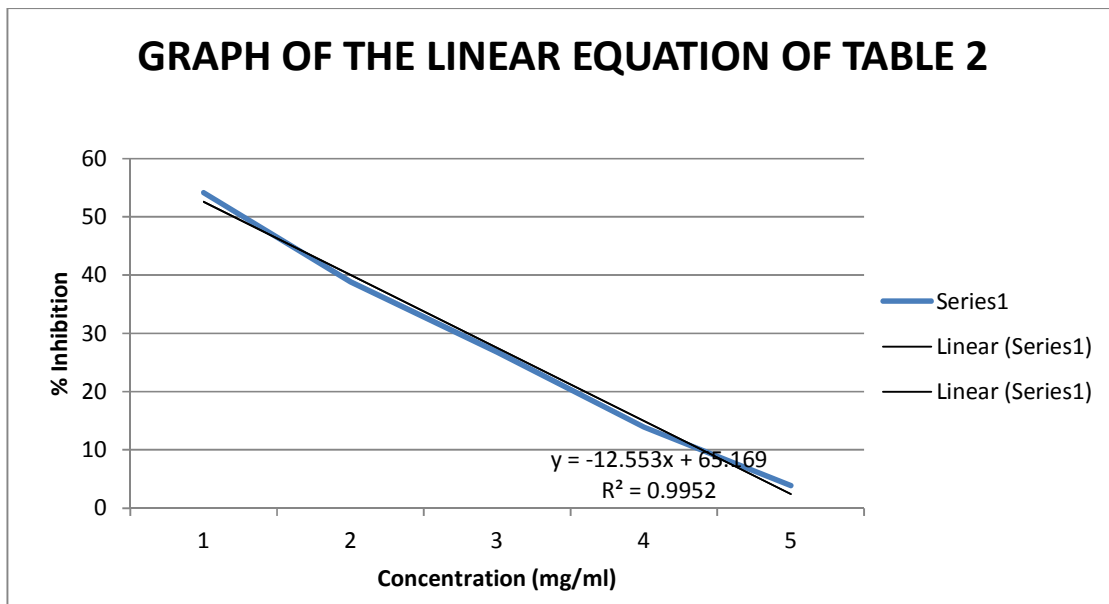
Appendix 1. Statistical Analysis

$$Mean = \frac{\sum x}{N}$$

$$Standard\ deviation = \frac{\sqrt{\sum(x - \bar{x})^2}}{N - 1}$$

$$Percent\ (\%) \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Test}}{\text{Absorbance of control}} \times 100$$

Appendix 2



© 2020 Adeleye; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/55967>