IJPSR (2021), Volume 12, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 November 2020; received in revised form, 04 March 2021; accepted, 23 June 2021; published 01 October 2021

EVALUATION OF ANTIOXIDANT ACTIVITY OF LEAVES EXTRACT OF *MORINGA CONCANENSIS* NIMMO ON ISOLATED FROG'S HEART

Sridhar Vanga [*1] and Shravan Kumar Dholi [2]

Department of Pharmaceutical Chemistry [1], Department of Pharmacology [2], Vaageswari Institute of Pharmaceutical Sciences, Beside LMD Police Station, Ramakrishna Colony, Karimnagar - 505481, Telangana, India.

Keywords:

Moringa cocanensis Nimmo, Antioxidant activity, Ringer solution, Frog's heart

Correspondence to Author:
Vanga Sridhar

Assistant Professor,
Department of Pharmaceutical Chemistry, Vaageswari Institute of Pharmaceutical Sciences,
Ramakrishna Colony, Karimnagar - 505481, Telangana, India.

E-mail: sreedher.vanga@gmail.com

ABSTRACT: *Moringa Cocanensis Nimmo* is one of the important medicinal plants belonging to the family moringaceae. *Moringa Cocanensis Nimmo* has antipyretic, antihelmintic, antimicrobial, hepatoprotective, and anticancer activities. It is one of the most noted systems of medicine in the world. Ayurveda is based on the hypothesis that everything in the universe is composed of five basic elements *viz*. space, air, energy, liquid, and soil. The traditional system was proved to be more effective when compared to synthetic drugs due to reduction in side effects, adverse effects, and even a little increase in dose was not that dangerous when compared to the synthetic drugs. It was even found to be more economical and safer in all means. Hence, we have choosed the plant *Moringa Concanensis Nimmo*, evaluated its medicinal property and proved its antioxidant nature for replacing synthetic drug formulations. When ringer solution containing H_2O_2 perfused to heart preparation, the muscaranic actions of acetylcholine were not observed much, indicating the oxidative stress on frog heart induced by H_2O_2; this might be due to the desensitization of receptors. The cardiac arrest was proposed at 19.25^{th} min. This result supports the frog heart model for induction of oxidative stress by H_2O_2. In the presence of the extract of the plant, cardiac arrest was observed at 27, 30 min respectively, *i.e.*, heart was protected longer period with plant juice, against H_2O_2 induced oxidative stress when compared with the control.

INTRODUCTION: Herbal medicine is also called as botanical medicine or phytomedicine. It refers to using a plant's seeds, berries, stem, roots, leaves, bark or flowers for medicinal purposes. Herbal medicine is the practice of health care using herbs instead of drugs. Herbs are extremely useful for the treatment of disease and sickness because they are natural and often have fewer adverse side effects than pharmaceutical drugs. Though drugs generally take less time to work. They often treat symptoms: herbs usually treat the disease itself, though they take longer to start working. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(10).5469-75

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(10). 5469-75

Moringa concanensis Nimmo belongs to the family Moringaceae. The plant is distributed in the Ooty, Yercaud area of the Salem district in Tamilnadu, and it is the second species occurring in India. *Moringa concanensis* is restricted in its distribution. *Moringa concanensis Nimmo* is used for various human ailments on its own.

The preparation of the drug from this plant is very easy and simple. The plant Kattumurungai is entirely different from the Murungai *(Moringa oleifera*). Leaves and flowers are larger in size than *Moringa oleifera*. The bark is very smooth and very hard in both the plant, respectively. These plant leaves are used to reduce the cholesterol and body weight and diabetis, constipation. The juice of the leaves is used in cases of headache, and it is also applied in the eyes in fainting fits due to nervous debility. The leaves are nutritive and cooling and are useful in removing all kinds of excessive pain. The aqueous extract of the leaves has been found to possess anti-fertility activity [3-11].

MATERIALS AND METHODS:

Collection of Leaves: The young fresh leaves of *Moringa concanensis* were collected during December and January from Karimnagar, Telangana.

FIG. 1: *MORINGA CONCANENSIS NIMMO* PLANT

Preparation of Plant Extract: The dried powdered plant material of *Moringa concanensis* was extracted with water using the maceration extraction method. After exhaustive extraction, the collected aqueous extract was subjected to evaporation to obtain the pure drug of extract.

Observation: Weight of the fresh leaves collected: 2kg**.** Weight of the dried leaves: 500 g. Weight of the powdered drug: 400. Weight of the powder subjected to extraction: 100g**.** Weight of the extract obtained: 14g. % yield of extract obtained: 14%

Animals: Frogs of Rana Tagrina species from the animal house of Vaageswari Institutes Of pharmaceutical Sciences, Karimnagar, was used for the studies.

Evaluation of Anti Oxidant Activity

Isolated frog Heart Preparation Using *Symes Cannula*: An Indian frog (*Rana tigrina*) was stunned by head-blow using a steel rod and pithed. The skin and abdomen were cut and opened.

The pectoral girdle was cut using a bone cutter and removed the pericardium carefully. Syme's cannula was connected to the reservoir containing the frog Ringers solution and introduced immediately into the heart's sinus venosus.

The connecting blood vessels were cut, and the heart was isolated from the animal and mounted on a stand. The heart was then covered with a thin layer of cotton wool and poured some frog Ringer solution periodically to prevent drying. The heart was connected to the Starlings heart lever and adjusted for recording the responses of the heart. The level of frog Ringer solution in the *Syme's cannula* was maintained by fixing a glass tube into the cork fixed to the reservoir (Marriott's bottle) tightly. The heart was allowed to stabilize, and when the heart rate and cardiac output were taken, the recordings were made on a slow rotating drum, to which a soothed kymograph paper was affixed. The study protocol was approved by the Institutional Animal Ethical Committee, Vaageswari Institute of Pharmaceutical Sciences, JNTU, Hyderabad.

FIG. 2: DISSECTION OF FROG

H_2O_2 Induced Oxidative Stress on Isolated Frog Heart: 230 µl of H_2O_2 in 400 ml of frog Ringer solution was used to induce oxidative stress on isolated frog hearts. The parameters studied include cardiac output, force of contraction, heart rate and cardiac arrest.

Acetylcholine at 10 ng, 20 ng dose levels elicited its muscarinic action like negative ionotropic, negative chronotropic and decreased cardiac output.

The same dose levels were repeated in continuous perfusion of frog Ringer solution containing H_2O_2 to the heart preparation and observed the parameters. The time taken to induce cardiac arrest was compared with those of treated heart.

Effect of *Moringa concanensis* Aqueous Leaf Extract on Oxidative Stress: Influence of *Moringa concanensis* extract on oxidative stress was studied by perfusing frog Ringer solution containing extract of the plant and H_2O_2 solution on the isolated frog heart preparation.

The parameters studied include the force of contraction, heart rate, and cardiac output (n=4). The time taken for the cardiac arrest was noted by continuously perfusing the frog Ringer solution containing juices of medicinal plant and H_2O_2 solution.

When isolated frog heart was perfused with normal frog Ringer solution, acetylcholine elicited muscarinic action *i.e.*, negative ionotropic, negative chronotropic and decreased cardiac output at 10 ng, 20 ng dose levels. But, in continuous perfusion of frog Ringer solution containing H_2O_2 to the heart preparation, the muscarinic actions were not observed much (less effect), indicating that the damage of muscarinic receptors by H_2O_2 continuous exposure and this might be no specific damage to the receptor due to oxidative stress induced by H_2O_2.

Finally, it produced cardiac arrest at 19.25th min (average) and was taken as a control (n=4).

RESULTS AND DISCUSSION:

TABLE 1: H_2O_2 INDUCED OXIDATIVE STRESS ON ISOLATED FROG HEART PREPARATION

	Heart Rate (Beats/min)	Cardiac Output (ml)	Cardiac Arrest (min)
Study I	30	6	20
Study II	44	9	22
Study III	36	7	18
Study IV	32	6.2	17
Average	35.5	7.05	19.25

TABLE 2: THE ACTIVITY OF ASCORBIC ACID ON ISOLATED FROG HEART PREPARATION

	Heart Rate (Beats/min)	Cardiac Output (ml)	Cardiac Arrest min
Study I	32	6	30

TABLE 3: THE ANTIOXIDANT ACTIVITY OF PLANT EXTRACT ON ISOLATED FROG HEART PREPARATION

	Heart rate (Beats/min)	Cardiac Output(ml)	Cardiac Arrest(min)
Study I	36	7	26
Study II	38	8	30
Average	37	7.5	28

TABLE 4: PROTECTIVE EFFECT OF SAMPLE JUICE ON CARDIAC ARREST

Sample	Cardiac Arrest (min)
H_2O_2	19.25
Ascorbic acid	30
Moringa concanensis extract	28

FIG. 3: EFFECT OF H_2O_2 CONTINUOUS SUPPLY FROM THE RESERVOIR TO INDUCE OXIDATIVE STRESS ON ISOLATED FROG HEART

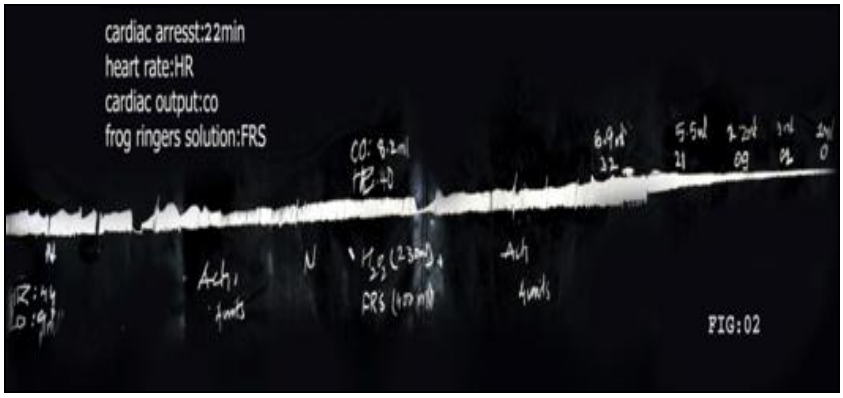

FIG. 4: EFFECT OF H_2O_2 CONTINUOUS SUPPLY FROM THE RESERVOIR TO INDUCE OXIDATIVE STRESS ON ISOLATED FROG HEART

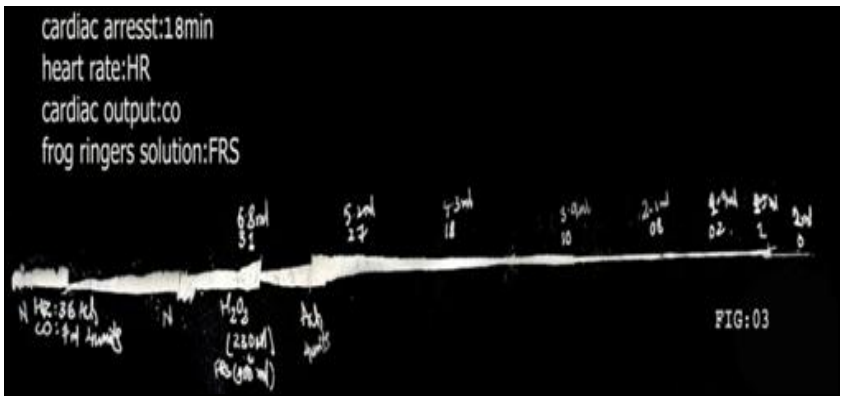

FIG. 5: EFFECT OF H_2O_2 CONTINUOUS SUPPLY FROM THE RESERVOIR TO INDUCE OXIDATIVE STRESS ON ISOLATED FROG HEART

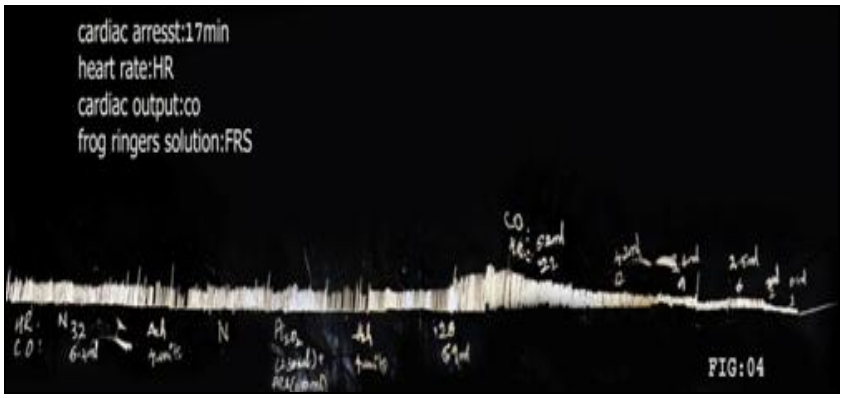

FIG. 6: EFFECT OF H2O2 CONTINUOUS SUPPLY FROM THE RESERVOIR TO INDUCE OXIDATIVE STRESS ON ISOLATED FROG HEART

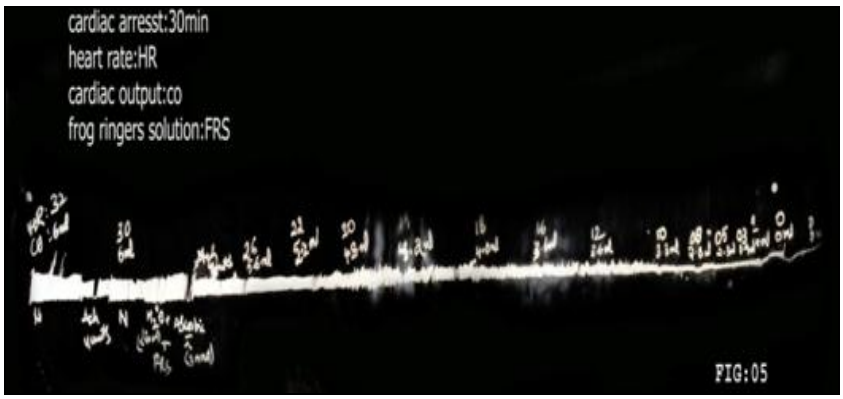

FIG. 7: EFFECT OF $H_2O_2^+$ ASCORBIC ACID CONTINUOUS SUPPLY FROM THE RESERVOIR TO INDUCE OXIDATIVE STRESS ON ISOLATED FROG HEART

FIG. 8: EFFECT OF $H_2O_2^+$ EXTRACT CONTINUOUS SUPPLY FROM THE RESERVOIR TO INDUCE OXIDATIVE STRESS ON ISOLATED FROG HEART

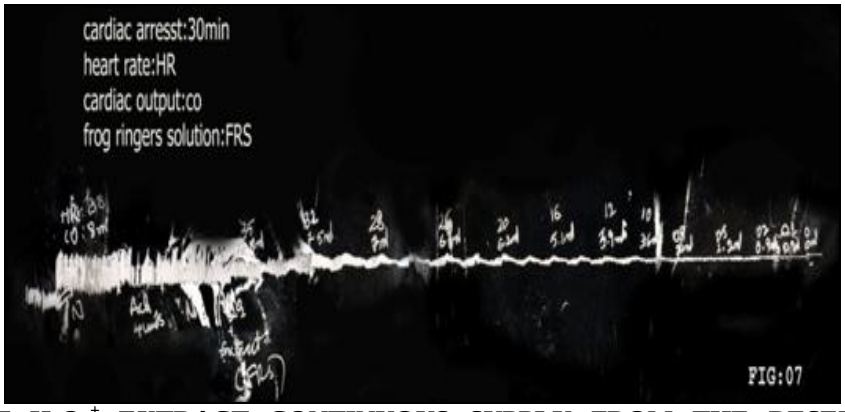

FIG. 9: EFFECT OF $H_2O_2^+$ EXTRACT CONTINUOUS SUPPLY FROM THE RESERVOIR TO INDUCE OXIDATIVE STRESS ON ISOLATED FROG HEART

The increased demand for medicinal plants has created an ecological crisis for medicinal herbs growing in the wild, raising the alarm about their rate of extinction. The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties due to the presence of flavonoids, a class of natural polyphenols found in green plant cells. In addition, leaf senescence is a crucial developmental stage in the life of plants.

It is the time during which compounds synthesized by the plant during its growth phase are mobilized into younger tissues. Leaf senescence also indicates the beginning of the harvest period and has been shown to affect antioxidant activity. Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins, and DNA are all susceptible to attack by free radicals. Antioxidant may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation *etc*. Oxidative stress induced by hydrogen peroxide (H_2O_2) may contribute to the pathogenesis of ischemic-reperfusion injury in the heart. For the purpose of directly investigating the injury potential of H_2O_2 on the heart muscle, a cellular model of H_2O_2 induced myocardial oxidative stress was developed using monolayer rat cardiomyocyte cultures it was reported that an oxidant burden was established by hydrogen peroxide overload may elicit post-ischemic myocardial damage [9]. Earlier reports suggest that oxidative stress or cell damage was induced to the human colon carcinoma cells, Caco-2, cells by exposing hydrogen peroxide at concentrations varying from 0 to 250 μm *10+. It was reported that exposure of a high concentration of H_2O_2 increases apoptotic signals, eventually inducing apoptosis, which resulted in mitochondrial membrane potential disruption. This study induced oxidative stress on isolated frog hearts by perfusing frog Ringer solution containing H_2O_2. When Ringer solution containing H_2O_2 perfused to heart preparation, the muscarinic actions of acetylcholine were not observed much, indicating the oxidative stress on frog heart induced by H_2O_2; this might be due to the desensitization of receptors. The cardiac

arrest was produced at 19.25th min. This result supports the frog heart model for induction of oxidative stress by H_2O_2. In the presence of the extract of the plant, cardiac arrest was observed at were showed. Therefore the cardiac arrest time was prolonged by 27, 30 min respectively, *i.e.*, the heart was protected longer period with plant juice against H_2O_2 induced oxidative stress when compared with the control.

CONCLUSION: The traditional system was proved to be more effective when compared o synthetic drugs due to reduction in side effects, adverse effects, and even a little increase in dose was not that dangerous when compared to the synthetic drugs. It was even found to be more economical and safer in all means. Hence we Have choosen the plant *Moriga concanensis Nimmo* and evaluated its medicinal property, and proved its antioxidant nature for replacing the synthetic drug formulations. When ringer solution containing H_2O_2 perfused to heart preparation, the muscaranic actions of acetylcholine were not observed much, indicating the oxidative stress on frog heart induced by H_2O_2; this might be due to the desensitization of receptors. The cardiac arrest was proposed at 19.25th minute. This result supports the frog heart model for induction of oxidative stress by H_2O_2. In the presence of the extract of the plant, cardiac arrest was observed at 27, 30 min respectively, *i.e.*, the heart was protected longer period with [plant juice, against H_2O_2, induced oxidative stress when compared with the control. Plant authentication and IAEC approval number: BCP/2020/9/116

ACKNOWLEDGEMENT: We are grateful to the Management and Principal of Vaageswari Institute of Pharmaceutical Sciences, Karimnagar, Telangana, India, for their support and providing institutional facilities.

CONFLICTS OF INTEREST: There are no conflicts of interest.

REFERENCES:

1. Acharya, Deepak and Anshu S: Indegenious Herbal medicines: Tribal formulations and traditional herbal practices, Aavishkar Publishes Distributor, Jaipur India. ISBI. International journal of Green pharmacy 2008; 2: 126-28.
2. Sridhar V, Sunitha T, Prasad SH and Padmaja B: Ethnomedicinal practices in different communities of telangana for treatment of wounds. The American Journal of Science and Medical Research 2018; 4(1); 20-25.
3. Master Y and Somani V: Clonal micropropagation of threatened *Moringa concanensis Nimmo* (Moringaceae): sustainable alternative. Int J Pharm Sci & Res 2020; 11(6): 2807-13. doi: 10.13040/IJPSR.0975-8232.11(6).2807-13.
4. Sachan D, Jain SK and Singh N: *In-vitro* and *In-vivo* Efficacy of *Moringa oleifera* Plant Constituents in Urolithiasis as Antilithiatic Drug. International Journal of Science and Research 2011; 2(7); 1638-44.
5. Rani NZA, Husain K and Kumolosasi E: *Moringa genus*: A review of phytochemistry and pharmacology. Front Pharmacol 2018; 9: 1-26
6. Singh A, Mishra JN and Singh SK: Pharmacological importance of *Moringa concanensis Nimmo* leaf : An overview. Asian J Pharm Clin Res 2019; 12(2): 27-31.
7. Manokari M, Shekhawat MS and Ravindran CP: Effect of indole-3-butyric acid (IBA) on vegetative propagation of a rare medicinal plant *Moringa concanensis Nimmo* ex Dalz & Gibson using stem cuttings. World Sci News 2016; 58: 122-32.
8. Jun-jie Z, Yue-sheng Y, Meng-fei L, Shu-qi L, Yi T and Han-bin C: An efficient micropropagation protocol for direct organogenesis from leaf explants of an economically valuable plant, drumstick (*Moringa oleifera* Lam.). Ind Crops Prod 2017; 103: 59-63.
9. Shittu HO and Igiehon E: *In-vitro* morphogenetic response of Moringa oleifera Lam. leaf petiole explant to cytokinin and auxin concentrations. Int J Basic Sci Technol 2016; 2(1): 15-9.
10. Gayathri M, Kumar PS, Prabha AML and Muralitharan G: *In-vitro* regeneration of *Arachis hypogaea* and *Moringa oleifera* Lam. using extracellular phytohormones from Aphanothece sp. MBDU 515. Algal Res 2015; 7: 100-5.
11. Gardner PR, Nguyen DD and White CW: Aconitase is a sensitive and critical target of oxygen poisoning in cultured mammalian cells and in rat lungs. Proc Natl Acad Sci USA 1994; 91: 12248-52.
12. Khanam S, Shivprasad HN and Devi K: *In-vitro* antioxidant screening models: a review, Indian J Pharm Edu 2004; 38(4): 180-83.
13. Dhawal P, Satardekar K, Hariharan S and Barve S: *In-vitro* analysis of *Cinnamomum verum* for formulation of bio-active cosmetic gel. Int J Pharm Sci Res 2017; 8(7): 2988-95.doi: 10.13040/IJPSR.0975-8232.8(7): 2988-95
14. Pisoschi AM: The Role of Antioxidants in The Chemistry of Oxidative Stress: A review. European Journal of Medicinal Chemistry 2015; 97: 55-74.
15. Dholi S, Prasannalaxmi, Ashok, Vidhyasree and Naresh: Comparative Cardiotonic Activity of *Gymnema sylvesre* with digoxin on frog's heart. AJPRD 2013; 1(2): 143-47.
16. Dholi SK, Nag PA, Samatha P, Rajitha T, Susheel R, Rekha M, Rakesh R and Raparla R: Evaluation of skeletal muscle activity of *Aloe vera* extract on frog's Rectus abdominus muscle. Int J of Phytopharmacy Research 2015; 6(2): 61-64.
17. Dholi SK, Khanam R, Malathi S, Akhila P and Abhilash P: Evaluation of skeletal muscle activity of fenugreek seeds extract and fenugreek leaves extract on isolated frog's rectus abdominus muscle. IAJPS 2015; 2(4): 763-70.
18. Palle RK, Dholi SK and Thirupati N: Evaluation of skeletal muscle activity of fenugreek seeds extract and fenugreek leaves extract on isolated frog's rectus abdominus muscle. IAJPS 2017; 2(6): 431-38.
19. Koteshwarrao G and Dholi SK: evaluation of sketetal activity of tecomastans on isolated frog's rectus

abdominus muscle. World Journal of Pharmaceutical Research 2018; 3(5): 126-29.
20. Vanga S, Pingili M and Tharigoppula S: Phytochemical screening and evaluation of antifungal activity of gall extracts of Quercusinfectoria. Int J Pharm Sci Res 2017; 8(7): 1000-04.
21. Dholi SK, Donthula1 KK and Koteshwarrao G: comparative cardiotonic-activity of *Aloe-vera (Aloe barbadensis* miller) with digoxin on isolated frog's heart, IAJPS 2018; 05(07): 6853-56.
22. Rajashekhar CH and Dholi SK: evaluation of skeletal muscle activity of *Hibiscusrosa sinensis* leaves extract on isolated frog's rectus abdominus muscle. EJBPS 2019; 6(3): 473-78.
23. Sridhar V, Mamatha P and Prasad SH: Pharmacognostical, phytochemical screening and evaluation of antioxidant activity of ethanol and aqueous extracts of *Randia spinosa* leaves. Int J Pharm Sci & Res 2018; 9(11): 4854-58. doi: 10.13040/IJPSR.0975-8232.9(11).4854-58.

How to cite this article:
Vanga S and Dholi SK: Evaluation of anti-oxidant activity of leaves extract of *Moringa concanensis Nimmo* on isolated frog's heart. Int J Pharm Sci & Res 2021; 12(10): 5469-75. doi: 10.13040/IJPSR.0975-8232.12(10). 5469-75.