ISSN 2350-1030

International Journal of Recent Research in Physics and Chemical Sciences (IJRRPCS)
Vol. 12, Issue 2, pp: (1-6), Month: October 2025 – March 2026, Available at: www.paperpublications.org

ANTIOXIDANT ACTIVITIES AND NEUROPROTECTIVE EFFECTS OF *PSIDIUM GUAJAVA* (GUAVA) AQUEOUS LEAF EXTRACT ON THE NICOTINE-INDUCED PREFRONTAL CORTEX TOXICITY IN ADULT MALE WISTAR RATS

[1]Christian Chiemeka Ozor, [2]Christian Chizuakupue, [3]Anthony Ifeanyi Egwuatu

Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology (ESUT), Enugu State, Nigeria.

DOI: https://doi.org/10.5281/zenodo.18110223

Published Date: 31-December-2025

Abstract: **Background: Nicotine induces oxidative stress and alters brain functions, causing addiction and impeding brain development in children and young people. *Psidium guajava* (Guava) displays strong medicinal value and it is consumed for food and pharmacologic activities. Aim: This study aimed at investigating the antioxidant and neuroprotective effects of *Psidium guajava* (Guava) aqueous leaf extract on the nicotine-induced prefrontal cortex toxicity in adult male wistar rats. Methodology: Thirty (30) adult male wistar rats (150-200g) were divided into six (6) groups (n=5). Group A (control group) received only food and water *ad libitum*. Group B (Untreated) was injected subcutaneously with 0.1ml of 1% nicotine every 48 hours till the end of the experiment. Group C, D, E and F received same but were treated daily with 200, 400, 600mg/kg of PGALE and 100mg/kg of Vitamin E respectively. The experiment lasted 28 days. The animals were sacrificed 24 hours after last treatment under ketamine (100mg/ml) as anesthesia. Neatly harvested brains were used for the biochemical estimation of tissue oxidative stress markers via homogenation and also for histological studies stained with H&E. Results: Nicotine treatment led to a significant decrease in Superoxide Dismutase (SOD), an increase in Malondialdehyde (MDA) and degenerative injuries in the microstructure of the prefrontal cortex evident by well-developed vacuolated cytoplasm and shrinkage of neurons. High doses of PGALE and Vitamin E led to a significant improvement in the antioxidant activity and preserved the normal histoarchitecture of the brain prefrontal cortex. Conclusion: Treatment with *Psidium Guajava* demonstrated strong dose-dependent antioxidant and neuroprotective potentials. This study justifies its use in traditional medicine.**

***Keywords: Psidium Guajava*, Oxidative stress, Prefrontal cortex, Nicotine.**

1. INTRODUCTION

Cigarettes smoking via conventional cigy2garettes or electronic cigarettes are a very common practice in the modern society. However, these practices can alter brain functions and cause conditions such as cognitive decline, loss of memory, mood disorders and drug dependence on humans as well as experimental animals (Kuncorowati *et al*., 2020).

Electronic cigarettes can lead to muscle spasms, tremors and can cause direct injuries to neurons (Qasim et al., 2017). Nicotine is a very prominent content of cigarettes and is known to induce oxidative stress and alter brain functions, cause addiction and impede brain development in children and young people (Qasim *et al*., 2017).

The prefrontal cortex of the human brain plays a role in cognitive function, regulating behavior and memory (Kuncorowati *et al.*, 2020). Any neurotoxic alteration on the brain's prefrontal cortex will lead to disturbances in these important functions (Akkoc and Ogeturk, 2017)

Medicinal plants contain active ingredients which are useful in the synthesis of drugs. They are a rich source of bioactive phytochemicals or bio-nutrients (Mamta *et al.,* 2013). *Psidium guajava* (Guava) and its all parts have an old history of medicinal value (Nwinyi *et al.,* 2008). It is consumed both as food and as folk medicine due to its pharmacologic activities (Deguchi and Miyazaki, 2010). Ethanolic extracts of stem have a high anti-diabetic activity (Rai *et al.,* 2007; Mukhtar *et al.*, 2006).

Guava contains a large number of antioxidants and phytochemicals including essential oils, polysaccharides, minerals, vitamins, enzymes, and triterpenoid acid alkaloids, steroids, glycosides, tannins, saponins with high contents of flavonoids, carotenoids and fructose sugar (Smith and Siwatibau, 1975; Khan and Ahmad, 1985). Guava has a higher content of vitamin C, vitamin A and is also a good source of important dietary fibers (Das, 2011).

2. MATERIALS AND METHODS

Plant Material and Extraction

Fresh matured leaves of *Psidium guajava* were plucked from the stems of a Guava tree in Independence layout of the Enugu metropolis of Enugu State. Identification and authenticated was carried out at the Faculty of Agriculture, Enugu State University of Science and Technology, Agbani, Enugu.

The leaves were washed to remove dirt and air-dried at room temperature for 7 days. The dried leaves were grinded using an electronic grinder and the aqueous extraction was done according to the methods of Kanu *et al*., (2022). The resultant crude extract was stored at −4°C until required for use.

Experimental Animals

Thirty adult male wistar rats weighing 150-200g were procured for this study. This study was carried out in the Animal facility of the Enugu State University of Science and Technology College of Medicine, Parklane, Enugu. All animals were provided easy access to water and standard poultry pellets as food. The animals were kept and maintained under standard laboratory conditions and handling was done following international guidelines on the use of experimental animals. Ethical approval was gotten from the university's ethical clearance committee with the ethical right permission number: ESUCOM/FBMS/ETR/23/007.

Experimental Design

The rats were randomly divided into six (6) groups (n=5). Group A (control group) received only food and water till the end of the experiment. Group B (Nicotine-only group) was injected subcutaneously with 0.1ml of 1% nicotine every 48 hours till the end of the experiment. This dosage was adopted from Mamdouh et al., (2003). Group C, D, E and F were injected subcutaneously with 0.1ml of 1% nicotine every two days but however, treated daily with 200, 400, 600mg/kg of *Psidium Guajava* Aqueous Leaf Extract (PGALE) and 100mg/kg of Vitamin E respectively. Extract doses were adopted from Kanu *et al.*, (2022). The experiment lasted 28 days.

Animal Sacrifice and Histological Study

The animals were sacrificed 24 hours after their last administration under ketamine (100mg/ml) as anesthesia. The cranial cavities were accessed and the brains neatly harvested. Each harvested brain was divided into two equal halves. Half was

ISSN 2350-1030

International Journal of Recent Research in Physics and Chemical Sciences (IJRRPCS)
Vol. 12, Issue 2, pp: (1-6), Month: October 2025 – March 2026, Available at: www.paperpublications.org

immediately homogenized for the biochemical estimation of oxidative stress markers in the brain tissue while the remnants were immediately fixed with 10% formaldehyde solution for 72 hours. The prefrontal cortices were isolated from the fixed tissues and were processed using the standard protocols for histological tissue processing and stained with hematoxylin and eosin for histological studies. Photomicrographs were taken at x100 magnification.

Biochemical Estimation of Oxidative Stress Markers

The harvested brain tissues were homogenized in phosphate buffer (pH 7.6), and then centrifuged at 16,000 rpm for 30 minutes at 4°C. The resultant supernatant was used to determine the antioxidant activity by the estimation of the antioxidant enzyme (Superoxide Dismutase) and also lipid peroxidation in the tissues (Malondialdehyde). Superoxide Dismutase (SOD) activity was determined according to the methods of Sun et al., (1988) and expressed as units per milligram of protein. The level of Malondialdehyde (MDA) was estimated using Draper and Hadley's double heating method (Draper and Hadley, 1990) and was expressed as nanomoles/gram of protein.

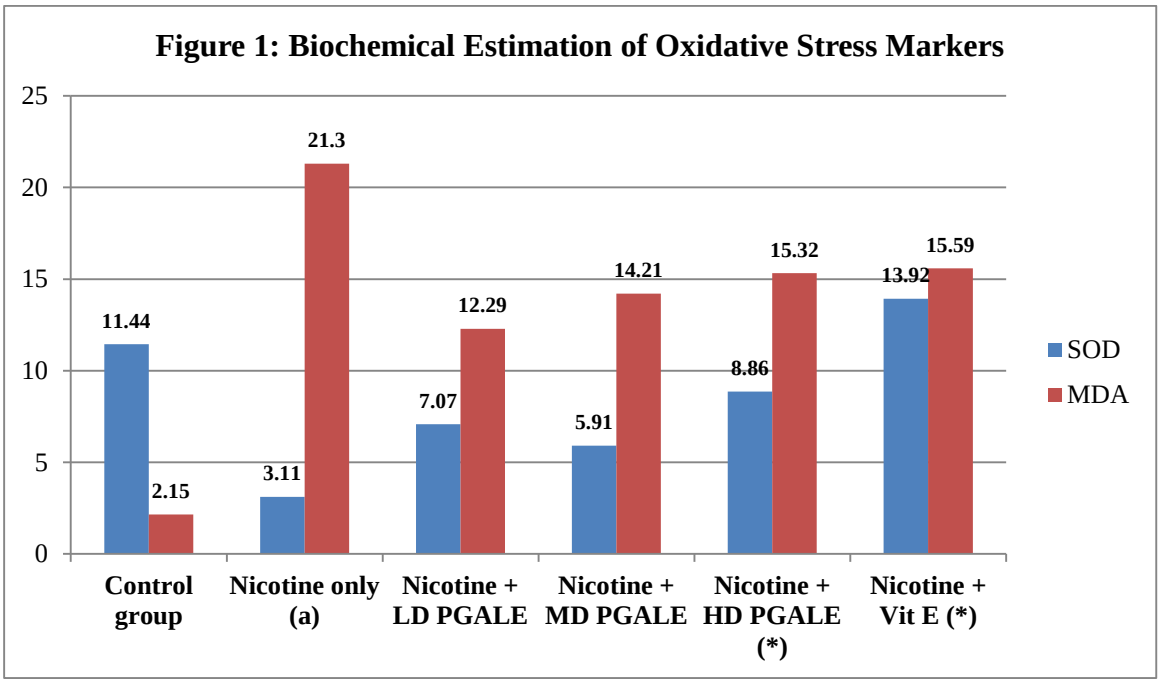
Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS version 21) for Windows (SPSS Inc., Chicago, USA). Numerical results from the estimation of the oxidative stress markers were presented in charts. Data were compared by one way analysis of variance (ANOVA). Statistical significance were be taken at $p<0.05$.

3. RESULTS

Biochemical Analysis

Figure 1: Biochemical Estimation of Oxidative Stress Markers



Relative to the control group, there was a significant decrease ($p<0.05$) in Superoxide Dismutase (SOD) activity and a significant increase ($p<0.05$) in Malondialdehyde (MDA) activity seen in the animal group treated with Nicotine only **(a)**. PGALE and Vitamin E treated groups displayed a significant increase ($p<0.05$) in Superoxide Dismutase (SOD) activity and a significant decrease ($p<0.05$) Malondialdehyde (MDA) activity relative to the animal group treated with Nicotine only **(*)**.

ISSN 2350-1030

International Journal of Recent Research in Physics and Chemical Sciences (IJRRPCS)
Vol. 12, Issue 2, pp: (1-6), Month: October 2025 – March 2026, Available at: www.paperpublications.org

Histological Analysis

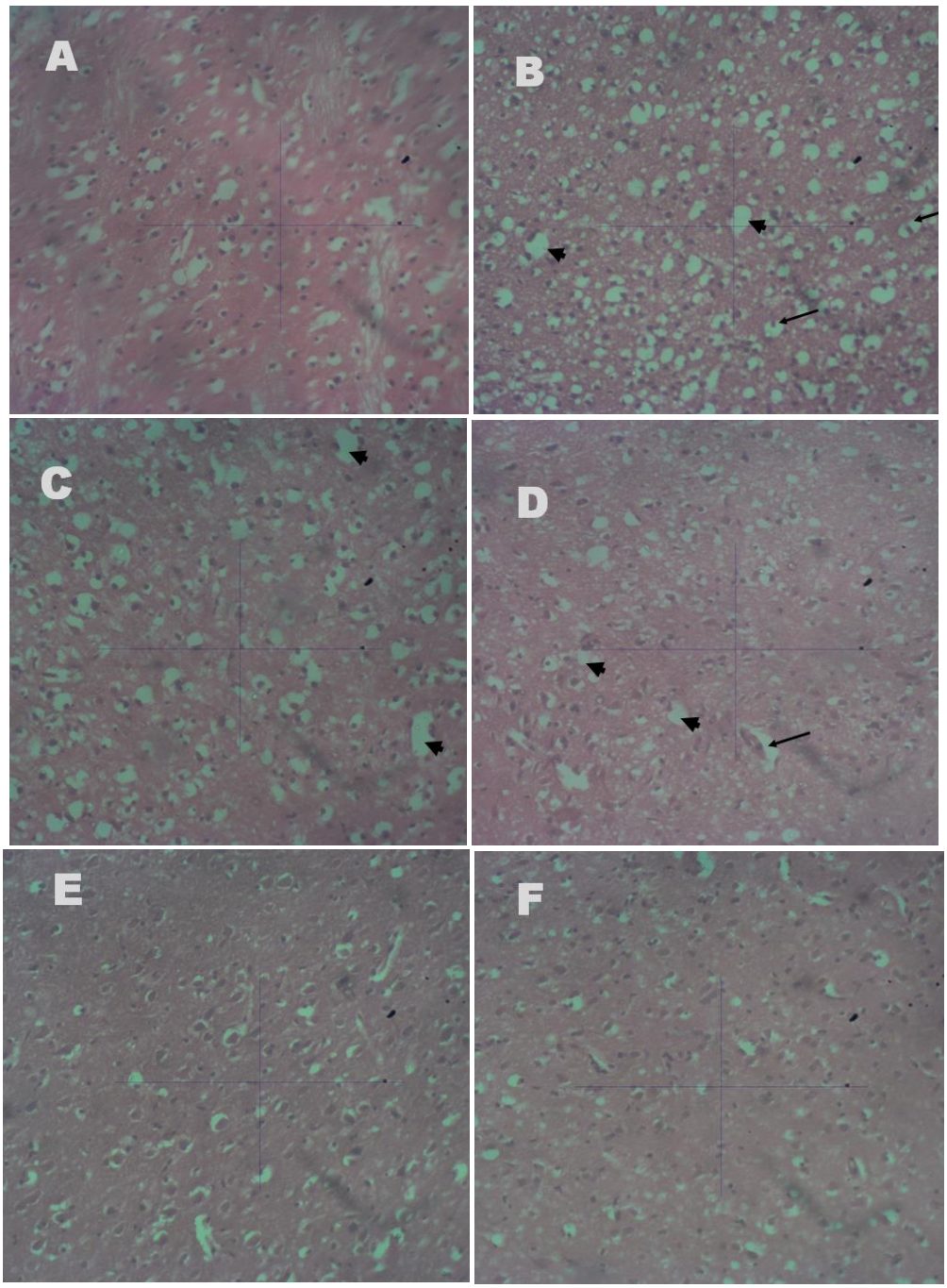


Figure A: Photomicrograph of a section of the prefrontal cortex of the control group with normal neuronal cells of different categories, numerous glia cells and a normal condensed neurofibrilary network. H&E.x100. **Figure B:** Untreated group showing numerous neurons with well developed vacuolated cytoplasm (Arrow heads) and shrinkage of neurons (Arrows). H&E.x100. **Figure C:** Nicotine + LD PGALE showing numerous neurons with vacuolated cytoplasm (Arrow heads). Mild depletion of the neurofibrilary network. H&E.x100. **Figure D:** Nicotine + MD PGALE showing neurons with mild vacuolated cytoplasm (Arrow heads). Blood vessels (Arrow). Normal neurofibrilary network. H&E.x100. **Figure E:** Nicotine + HD PGALE showing normal histological appearance. H&E.x100. **Figure F:** Nicotine + Vitamin E. Histology appears normal. H&E.x100.

4. DISCUSSION

Many constituents of cigarette have been known to be toxic to the brain, cardiovascular, and pulmonary systems and is a significant cause of oxidative stress. (Breese *et al.,* 1997; Ciobica *et al.,* 2012). Nicotine from a smoked cigarette has been demonstrated to be circulated to the brain in as little as 7 seconds after inhalation (Maisto *et al.,* 2004). Nicotine binds to brain tissues with high affinity, and the receptor binding capacity is increased in smokers compared with nonsmokers (Perry *et al.,* 1999). *Psidium guajava* (Guava) have displayed a long history of medicinal value and is consumed both as food and for its pharmacologic activities (Nwinyi *et al.,* 2008; Deguchi and Miyazaki, 2010).

This study indicated that Nicotine led to oxidative stress within the brain tissue. This was evident by the significant decrease in the antioxidant enzyme (Superoxide Dismutase) and an increase in the lipid peroxidation within the tissues (figure 1). The level of Malondialdehyde (MDA) present in a tissue acts as a marker of the free radical production that increases at the end of lipid peroxidation in the tissue (Kadir *et al.,* 2015). This study corresponds with previous studies and Nicotine has been shown to induce oxidative stress within tissues (Swami *et al.*, 2006; Chowdury and Walker, 2008)

Treatment with high doses of *Psidium Guajava* Aqueous Leaf Extract (PGALE) and Vitamin E led to a significant increase in the antioxidant enzyme activity (Superoxide Dismutase) and a significant decrease in the lipid peroxidation of the brain tissues. According to previous studies, Vitamin E is a strong antioxidant and has been seen to reverse nicotine-induced oxidative stress within tissues (Mohamed *et al.*, 2010). *Psidium guajava* (Guava) have also been documented to possess antioxidant properties and contains high levels of vitamin A and C (Khan and Ahmad, 1985; Das, 2011).

Nicotine treatment also led to degenerative injuries in the microstructure of the prefrontal cortex evident by well developed vacuolated cytoplasm and shrinkage of neurons (Figure B). This can be said to be the effect of the oxidative stress induced by Nicotine treatment on the brain tissue.

High doses of PGALE and Vitamin E restored the normal histoarchitecture of the brain prefrontal cortex (Figure E and F) relative to the groups treated with low and medium doses (Figure C and D) which still displayed mild signs of tissue degeneration evident by vacuolated cytoplasm and mild depletion of the brain neurofibrilary network. The Neuroprotective property of the high doses of PGALE can be suggested to be caused by its effect in improving the antioxidant enzyme activity and reduction of the rate of lipid peroxidation in the brain tissue.

Previous studies by Jimenez-Escrig *et al.,* (2001) and Yamashiro *et al.,* (2003) demonstrated that the aqueous extract from guava leaves have antioxidant and radical-scavenging activity linked to its polyphenol content and thus may prevent tissues from injurious harm caused by oxidative stress.

5. CONCLUSION

Nicotine administration increased the oxidative stress status of the brain and resulted to degenerative injuries in the microstructure of the prefrontal cortex. Treatment with *Psidium Guajava* demonstrated a strong dose-dependent antioxidant and neuroprotective potential via improving the antioxidant enzyme activity, reduction of the rate of lipid peroxidation in the brain tissue and preservation of the normal histoarchitecture of the brain prefrontal cortex. This study justifies its use in traditional medicine.

REFERENCES

[1] Akkoc RF, Ogeturk M. The Prefrontal Cortex: A Basic Embryological, Histological, Anatomical, and Functional Guideline. Journal Of Human Anatomy and Physiology 2017; 1(1): 1-4

[2] Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, Leonard S (1997). Effect of smoking history on nicotine binding in human postmortem brain. *J Pharmacol Exp Ther*; 282(1):7–13

[3] Chowdury P, Walker A. (2008). A cell-based approach to study changes in the pancreas following nicotine exposure in an animal model of injury. Langenbecks Arch Surg; 393: 547-550

[4] Ciobica A., Padurariu M., Hritcu5 L. (2012). The Effects of Short-Term Nicotine Administration on Behavioral and Oxidative Stress Deficiencies Induced in a Rat Model of Parkinson's disease. Psychiatria Danubina; Vol. 24, No. 2, pp 194-205

[5] Das AJ (2011). Review on nutritional, medicinal and pharmacological properties of Centella asiatica (Indian pennywort). *J Biol Act Prod from Nat.;*1(4): 216–28.

[6] Deguchi Y, Miyazaki K (2010). Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf extract. *Nutr Metab (Lond)*.;7:9.

[7] Draper H., Hadley M. (1990). Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol; 186: 421–31.

[8] Jimenez-Escrig A, Rincon M, Pulido R, Sauracalixto F, (2001). Guava leaf extract as a new source of antioxidant dietary fiber. J Agric Food Chem. 49 (11): 5489-5493

[9] Kadir D., Ramazan Ö., H. Ramazan Y., Ayşe Y., Abdülhadi U., Kadir K., Arif D., Abdullah A. (2015) Paliperidone regulates intracellular redox system in rat brain: Role of purine mechanism, Redox Report, 20:4, 170-176,

[10] Kanu S., Ejeje J., Elom N., Eleazu C., Obeten U. (2022). Comparative Effect of Aqueous Extract of Psidium guajava Leaves and Arthemetar/Lumefantrine (coartem) on non-targeted parameters in Plasmodium berghei-Infected Mice. Research Square; 3(1): 1-15

[11] Khan MIH, Ahmad JA (1985). Pharmacognostic study of Psidiurn guajuva L. *J Med Plant Res.*; 23(2):95–103.

[12] Kuncorowati,C., Urfah, S., Maulana, D., Bahrudin, M. (2020). Effect of Electronic Cigarette on Brain Prefrontal Cortex of Male Wistar Rats. The Journal of Medical Research; 6(3): 98-102

[13] Maisto SA, Galizio M, Connors GJ (2004). Drug use and abuse. 4th ed. Belmont, CA. wadsworth/Thompson learning.;7: 321-329.

[14] Mamdouh A, Ghaly El, Sayed G, Khedr Abdel A. (2003). A comparative study of nicotine effect on the liver of albino Wistar rat. The Egyptian Journal of Medicine;10(8): 130-144.

[15] Mamta, S., Jyoti, S., Rajeev, N., Dharmendra, S. Abhishek, G. (2013). 6Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry; 1(6): 6168-182

[16] Mohamed N, Hapidin H, Othman F, Ahmad N, Muhammad N, Soelaiman I. (2010). Vitamin E reversed nicotine-induced toxic effects on bone biochemical markers in male rats. Arch Med Sci; 6, 4: 505-512

[17] Mukhtar HM, Ansari SH, Bhat ZA, Naved T, Singh P (2006). Antidiabetic activity of an ethanol extract obtained from the stem bark of Psidium guajava (Myrtaceae). *Die Pharmazie*.;61:725–7.

[18] Nwinyi O, Chinedu SN, Ajani OO (2008). Evaluation of antibacterial activity of Pisidium guajava and Gongronema latifolium. J Med Plants Res.;2(8): 189-92.

[19] Perry DC, Davila-Garcia MI, Stockmeier CA, Kellar KJ (1999). Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. *J Pharmacol Exp Ther*; 289(3): 1545–1552.

[20] Qasim H, Karim Z, Rivera J, Khasawneh F, Alshbool F. Impact of Electronic Cigarettes on the Cardiovascular System. Journal of The American Heart Association 2017; 6(9): 1-14

[21] Rai PK, Rai NK, Rai AK, Watal G (2007). Role of LIBS in elemental analysis of Psidium guajava responsible for glycemic potential. *Instrum Sci Technol.*;35:507–22.

[22] Smith RM, Siwatibau S (1975). Sesquiterpene hydrocarbons of fijian guavas. *Phytochemistry.*; 14(9):2013–5.

[23] Sun Y, Oberley LW, Li Y. (1988). A simple method for clinical assay of superoxide dismutase. Clin Chem; 34: 497–500

[24] Swami S, Suryakar AN, Katkam RV, Kumbar KM. (2006). Absorption of nicotine induces oxidative stress among bidi workers. Indian J Public Health; 50: 231-235

[25] Yamashiro S, Noguch K, Matsuzaki T (2003). Cardio protecting effect of extract from psidium guajava L and limonium wrighti, Okinawan medicinal plant against ischemiareperfusion injury in perfused rat hearts. Pharmacology 67 (3): 128-135.