INVITRO ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF MUNTINGIA CALABURA

G.C. Gilbis Tamil Priya[1*], Dr. N. Yasmin[2]

Abstract

The present research revealed the invitro antioxidant and cytotoxic properties of ethanolic leaf extract of Muntingia calabura. Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific assemblage. since epidermiological studies has pointed that predominant consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer. In the present study, the antioxidant property of ethanolic leaf extract of Muntingia calabura was studied by DPPH Assay and ABTS radical scavenging assays. The cytotoxic activity was assessed using the 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. The results of antioxidant assay in ethanolic leaf extract of M. calabura reported that DPPH assay depicted the IC_{50} Value of 82.57 µg/ml followed by ABTS assay showed the IC_{50} Value of 175.97 µg/ml respectively. The cytotoxic activity of ethanolic leaf extract of M. calabura was carried out against SK-MEL cell line and here the percentage of viability decreased in the increase in the concentration of M. calabura extract and reported the IC_{50} value of 82.57 µg/ml. The ethanolic leaf extract of M. calabura inhibited the viability of SK-MEL cancer cell lines and these reported the anticancer property of ethanolic leaf extract of M. calabura respectively. From the experimental results it was clear that Muntingia calabura leaf extract was affluent in antioxidant polyphenol and possess evidential antioxidant activity. This work further exploits the possibility of phytomedicine over chemotherapy in disease prevention and control. Thus, the consolidation of edible M. calabura leaves into regular diet could forestall the risk of cardiovascular diseases, ageing, inflammations and cancers due to antioxidant compounds present in the leaf extracts.

Key words: Muntingia calabura leaves, Antioxidant, Cytotoxicity, Anticancer property, Phytochemicals.

Research Scholar, (Register No- 20123092272009)
[1*] PG and Research Department of Zoology, Muslim Arts College, Thiruvithancode,
[1*]Affiliated to Manonmaniam Sundaranar University, Abishekapetti, Tirunelveli, Tamil Nadu, India.
[2]Assistant Professor,PG and Research Department of Zoology, Muslim Arts College, Thiruvithancode,
[2]Affiliated to Manonmaniam Sundaranar University, Abishekapetti, Tirunelveli, Tamil Nadu, India.

***Corresponding Author:** - G.C. Gilbis Tamil Priya
[*] PG and Research Department of Zoology, Muslim Arts College, Thiruvithancode,
[*]Affiliated to Manonmaniam Sundaranar University, Abishekapetti, Tirunelveli, Tamil Nadu, India.

DOI: 10.53555/ecb/2021.10.4.11

Introduction

Medicinal plants are now preferred above pharmaceutical medications by many people (Dehpour et al., 2009). Antioxidants are important because they may reduce oxidative stress, which is one of the factors that can harm biological molecules (Farhat et al., 2013). Cancer is the major cause of death worldwide, claiming over 6 million lives every year. In the recent years alternative therapies have gained importance over conventional cancer therapies for the treatment of cancer (Amin et al., 2009). Exogenous antioxidants can be found as natural antioxidants which are the way out for oxidative stress management because they can affect endogenous antioxidant systems and balance oxidative homeostasis (Jadoon et al. 2015). Oxidative stress is naturally inhibited by enzymatic antioxidant compounds such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non enzymatic antioxidant compounds such as glutathione, alpha-tocopherol, ascorbic acid and ubiquinone (Bhattacharryya et al., 2014). Chemotherapy is restricted by both intrinsic and acquired cell resistance to drugs. Muntingia calabura Linn is a plant that belongs to the Elaeocarpaceae family, commonly known as cherry tree. The tree bears small red fruits with enormous tiny yellow seeds. The fruits are so sweety and juicy, which attracts the people to eat. This plant has been used as the Peru traditional medicine in reducing the swell of the prostate gland and alleviating headaches and cold as well as pains associated with gastric ulcers. The fruits and leaves possess the antioxidant activity (Shih et al., 2006). It was used by Vietnam and the Philippines to reduce ulcers, fever, headaches, to be utilized as tranquilizers. M. calabura fruit is often consumed directly and its leaves can be utilized as a substitute for tea (Mahmood et al. 2014).

The phytoconstituents of M. calabura stem bark consist of amino acids, flavonoids, saponins, proteins, triterpenoids, sterols, steroids, alkaloids, phenolic compounds, tannins, glycosides, and carbohydrates (Safrida and Sabri, 2009). The nutritional value of M. calabura L. per gram of the leaves consists of 204.0±3.46mg carbohydrates, 002.04±0.15mg protein, and 001.41±0.07mg amino acids, whereas, that per gram of the fruit consists of 75.33±4.61mg carbohydrate, 06.44±0.15mg protein, and 00.88±0.07mg amino acids (Krishnaveni and Dhanalakshmi, 2014). In addition, the pericarp of M. calabura also has many important health benefits. The high phytochemical content of M. calabura leaf extract, especially flavonoids and polyphenols causes the leaf extract of M. calabura to have antioxidant activity (Zakaria et al. 2011, 2014a; Balan et al. 2015), antiproliferative (Zakaria et al. 2011), antinociceptive (Zakaria et al. 2014a), anti-inflammatory (Balan et al. 2015), and anticarcinogenic (Nasir et al. 2017). This study aimed to determine the antioxidant and cytotoxic activity of M. calabura leaves ethanolic extract.

Materials and Methods

Plant material:

The fresh leaves of Muntingia calabura was collected as sample, It was collected from the Mulagumoodu Junction near Azhagiamandapam, Kanyakumari District. The leaf sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The bark was air dried under room temperature. The dried plant samples were cut and grinded to make it in powder form and pass through 30 mesh SS screen fitted. The powdered samples were stored in clean, dry and sterile container for further use. Chemical reagents All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. Mumbai, India), SD Fine- Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade. Quercetin and gallic acid were kindly provided by Scan Research Laboratories, Bhopal (India).

Extraction of plant material

For the extraction of extract from shade dried and powdered bark, the following process was used. Extraction by soxhletion method The soxhletion technique was used to extract 52.8 gram of powdered M. calabura bark using ethanol as solvent. The extracts were evaporated at temperatures higher than their boiling points. Finally, the dried ethanolic leaf extract % of M. calabura yields were determined.

Antioxidant activity

Antioxidant activity DPPH free radical scavenging assay

The activity of DPPH scavenging was determined using a modified technique (Parkhe and Jain, 2018) The spectrophotometer was used to test the DPPH scavenging activity. The stock solution (6 mg in 100 mL methanol) was produced to give an initial absorbance of 1.5 mL in 1.5 mL methanol. After 15 minutes, there was a decrease in absorbance in the presence of sample extract at various concentrations (10-100 g/ml). 1.5 ml of DPPH and 1.5 ml of varying concentrations of the test sample were placed in a succession of volumetric flasks, and the final volume was adjusted to 3 ml using methanol. Three test samples were collected and

processed in the same way. Finally, the average was calculated. For each concentration, the absorbance at zero time was measured. After 15 minutes at 517 nm, the absorbance of DPPH with varied concentrations showed a final reduction. The following equation was used to compute the % inhibition of the free radical DPPH: % inhibition = [(absorbance of control - absorbance of sample) / absorbance of control] × 100%. IC50 was computed based on the proportion of DPPH radicals scavenged, even though the activity is represented as a 50 percent inhibitory concentration (IC50). The stronger the antioxidant activity, the lower the IC50 value.

ABTS radical reduction Assay

ABTS radicals were prepared by reacting the ABTS solution (28 mg ABTS in 10 mL aqueous) with potassium persulfate (15 mg in 10 mL distilled water) in a dark room for 12 h. Then the volume of the ABTS radical solution made up to 50 mL with ethanol pro analysis. The sample lyophilisate stock solution was made to various concentrations (1-1000 µg/mL) by taking a particular sample volume and adding one mL of ABTS radical solution. The sample mixture was sufficient to 5 mL with ethanol pro analysis. Then mixtures were homogenized and incubated in a dark room for 30 mins, then measured the absorption was at 752 nm. The ABTS solution was blank (1 mL ABTS solution to 5 mL distilled water), and quercetin was used as a positive control. The inhibitory effect was calculated by the formula [(Ablank – Asample)/Ablank X 100%]

Cytotoxic activity

Cytotoxicity testing was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) according to the method Ayob et al., 2013 and Zhang et al., 2008).In this assay, SKMEL cells were harvested after reaching 80% confluence. Before starting the MTT assay, SKMEL cells were optimized at different seeding densities ranging from 2.0 × 103 cell/mL to 1.0 × 106 cell/mL in light to determine appropriate seeding number for the experiment. The final absorbance was measured using a BioRad microplate reader (Shinagawa-ku, Tokyo, Japan) at a wavelength of 575 nm. Cytotoxic activity was recorded as IC50, which is the concentration necessary to reduce the absorbance of treated cells by 50% compared to the control (untreated cells)

Result and Discussion

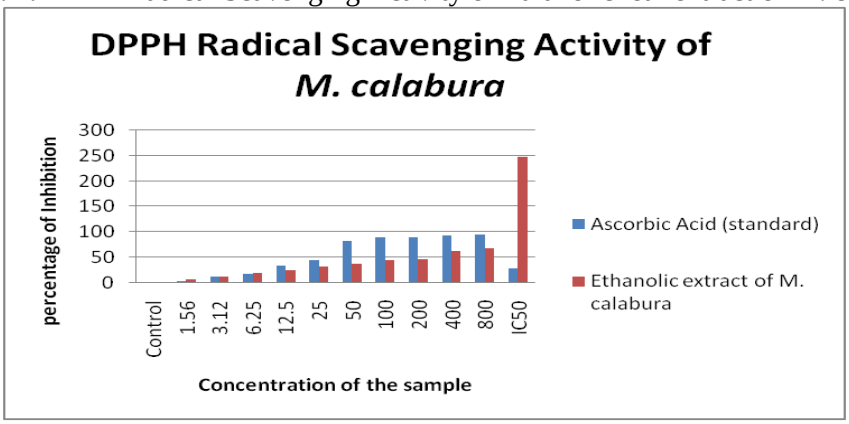
DPPH Radical scavenging activity

In the DPPH Radical scavenging analysis, the standard ascorbic acid reported highest percentage of inhibition of 94.95% at 800 µg/ml concentration of the standard, followed by 3.36 % to 94.95% of inhibition range was observed at the concentration range of 1.56 to 800 µg/ml concentration of standard at 515 nm. Here the least percentage of DPPH activity of 3.36% was represented in the standard concentration of 1.56 µg / ml. The IC_{50} value of the standard ascorbic acid was 28.19 µg/ml. Comparatively, In the DPPH radical scavenging activity of M. calabura, the highest percentage of inhibition of 67.93% was observed at 800µg/ml concentration of the ethanolic leaf extract of M. calabura sample and the least percentage of inhibition of 3.36% was represented at 1.56 µg / ml of the sample. The ethanolic leaf extract of M. calabura showed the inhibition range of 5.37 to 67.93% at the concentration level of 1.56 µg/ml to 800µg/ml of the sample. Relatively, the IC50 value of ethanolic leaf extract of M. calabura was 247.12 µg/ml. The results of DPPH assay of standard ascorbic acid and ethanolic leaf extract of M. calabura was illustrated in Table. 1 and fig. 1.

Table. 1. DPPH Radical Scavenging Activity of Ethanolic leaf extract of M. calabura

S.No	Concentration (µg / ml)	Ascorbic Acid (standard)	Ethanolic extract of M. calabura
1.	Control	0.8229	0.8577
2.	1.56	3.36	5.37
3.	3.12	11.61	10.86
4.	6.25	17.22	18.78
5.	12.5	32.98	25.15
6.	25	44.26	31.25
7.	50	82.19	36.02
8.	100	88.75	43.80
9.	200	89.75	45.23
10.	400	92.11	61.29
11.	800	94.95	67.93
12	**IC50**	**28.19**	**247.12**

Figure. 1. DPPH Radical Scavenging Activity of Ethanolic leaf extract of M. calabura



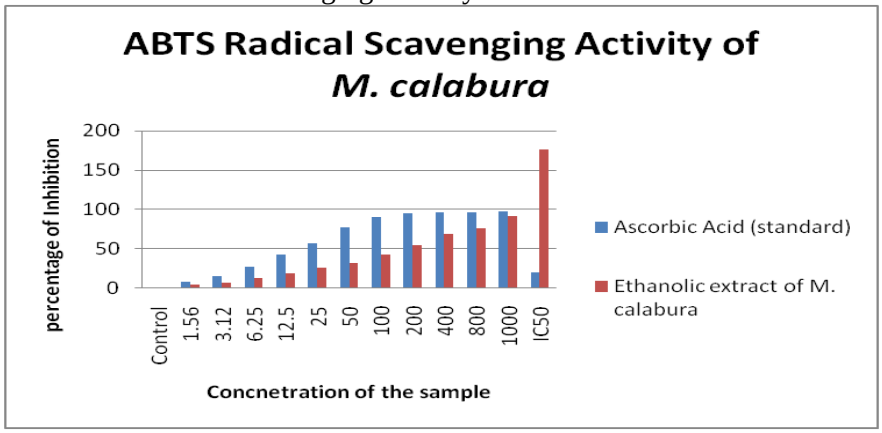
ABTS radical reduction Analysis

In the ABTS radical reduction analysis, the standard ascorbic acid reported highest percentage of inhibition of 97.24 % at 1000 µg/ml concentration of the standard, followed by 8.20 % to 97.24% of inhibition range was observed at the concentration range of 1.56 to 1000 µg/ml concentration of standard at 515 nm. Here the least percentage of DPPH activity of 8.20% was represented in the standard concentration of 1.56 µg / ml. The IC_{50} value of the standard ascorbic acid was 19.69µg/ml. Comparatively, In the ABTS radical reduction analysis of M. calabura, the highest percentage of inhibition of 90.86% was observed at 1000µg/ml concentration of the ethanolic leaf extract of M. calabura sample and the least percentage of inhibition of 4.54% was represented at 1.56 µg / ml of the sample. The ethanolic leaf extract of M. calabura showed the inhibition range of 4.54 to 90.86% at the concentration level of 1.56 µg/ml to 1000µg/ml of the sample. Relatively, the IC50 value of ethanolic leaf extract of M. calabura was 175.97 µg/ml. The results of ABTS radical reduction assay of standard ascorbic acid and ethanolic leaf extract of M. calabura was illustrated in Table. 2 and fig. 2.

Table.2. ABTS Radical Scavenging Activity of Ethanolic leaf extract of M. calabura

S.No	Concentration (µg / ml)	Ascorbic Acid (standard)	Ethanolic extract of M. calabura
1.	Control	0.7837	0.7568
2.	1.56	8.20	4.54
3.	3.12	14.80	6.59
4.	6.25	27.58	12.73
5.	12.5	43.04	18.87
6.	25	56.42	25.89
7.	50	76.73	31.32
8.	100	90.48	42.60
9.	200	94.72	54.33
10.	400	96.10	69.31
11.	800	96.40	76.11
12.	1000	97.24	90.86
13	**IC50**	**19.69**	**175.97**

Figure. 2. ABTS Radical Scavenging Activity of Ethanolic leaf extract of M. calabura



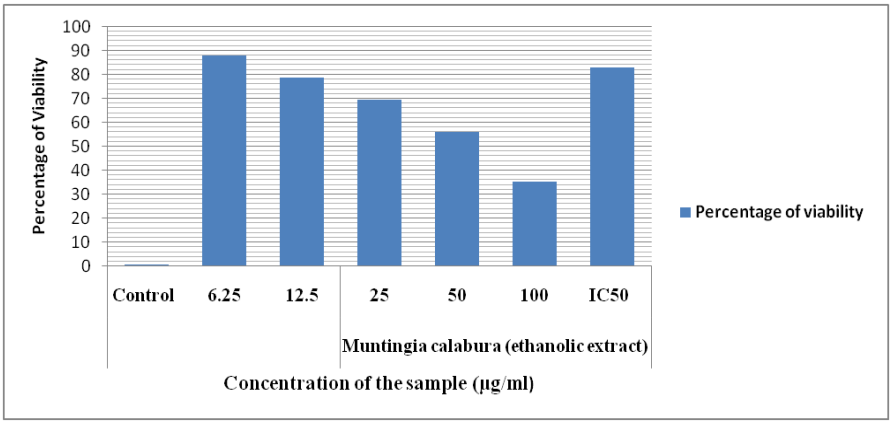
Cytotoxicity activity of ethanolic leaf extract of calabura

The SKMEL cell lines (human skin melanoma) were treated with ethanolic leaf extract of M. calabura. The percentage of cell viability of SKMEL cell lines treated with different concentrations of M. calabura. extracts (6.25µg/ml to100 µg/ml) and their cytotoxic activity against SKMEL cell lines was observed under an inverted phase contrast microscope. The cytotoxic activity of ethanolic leaf extract of M. calabura showed higher percentage of viability of 87.56 % at 6.25 µg/ml of ethanolic leaf extract of M. calabura 78.39 , 69.20 55.75, 35.39 percentage of viability in 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml concentrations of ethanolic leaf extract of M. calabura. The results showed that higher the concentration of ethanolic leaf extract of M. calabura lower the viability of cell lines followed by higher the cytotoxic activity on cell lines. Dose dependant reduction in cell viability was observed in SK-MEL cells administered with different concentrations of the sample. The IC_{50} value of ethanolic leaf extract of M. calabura in SKMEL cell line was 82.57 µg/mL of the sample. The results of cytotoxicity activity of ethanolic leaf extract of M. calabura was illustrated in Figure.4.6.2. (a) and Table: 4.6.2.(a).

Table. 3. Percentage of viability for varying concentration of ethanolic leaf extract of M. calabura on SKMEL cell line

Plant name	Concentration (µg/ml)	Percentage of viability
Muntingia calabura (ethanolic extract)	Control	0.653
	6.25	87.56
	12.5	78.39
	25	69.20
	50	55.75
	100	35.39
	IC50	**82.57**

Figure. 3. Percentage of viability for varying concentration of ethanolic leaf extract of M. calabura on SKMEL cell line



Discussion

Antioxidant property plays an important role in reducing chronic diseases like cancer and cardiovascular (CAD) diseases. Crude plant extracts are screened on cell culture, to determine their efficiency as a potential alternate drug and also to check their efficiency in clinical application as suggested by Balamurugan et al. (2014).M. calabura L. or locally known as „Kerukup Siam“, belongs to the family Elaeocarpacea (Morton, 1987). This plant is native to American continent and is widely cultivated in warm areas of Asian region, including Malaysia (Chin, 1989). The leaf is used to provide relief from gastric ulcers and to reduce swelling of the prostate gland as reported in Peru folklore medicinal uses. Ninan Jisha et al., (2019) reported the antioxidant property of aqueous, petroleum ether, chloroform, methanol and ethyl acetate extract of M. calabura using DPPH, FRAP and ABTS assays. Among all the extracts methanolic extract of M. calabura recognized the least IC 50 value representing the highest antioxidant property among all the four extracts viz., ethyl acetate extract, aqueous extract, chloroform extract and petroleum ether extract. Mohamed Azmathulla Khan et al., (2015), depicted the in vitro antioxidant properties of different extracts of Muntingia Calabura roots

using various assays. The antioxidant and free radical scavenging activity was 4.31 to 9.13mg. Zakaria et al., (2011) possessed the antioxidant assays in aqueous, chloroform and methanol extract of M. calabura, in different concentrations (20, 100 and 500 µg/ml) using the DPPH radical scavenging and superoxide scavenging assays among these methanol extract, followed by the aqueous and chloroform extract of M. calabura exhibited the highest antioxidant activity in both assays.Chen et al. (2004) continued the cytotoxicity studies of M. calabura, where 15 bioactive compounds isolated from the MEMCSB were tested against P-388, A549, and HT-29 cells using the MTT colorimetric method. In the present study, the antioxidant and cytoxicity property of ethanolic leaf extract of M. calabura leaves were demonstrated in varied concentration range of 1.56 to 1000 µg / ml of sample extracts using DPPH and ABTS assays representing different Inhibition activity (IC 50 value). DPPH antioxidant assay showed ethanolic extract of M. calabura noted higher percentage of inhibition representing IC50 value of 247.12 µg / ml. ABTS antioxidant assay demonstrated higher percentage of inhibition with the IC 50 value of 175.97 µg / ml. The cytotoxic activity of ethanolic leaf extract of M. calabura represented the IC50 value of 82.57µg / ml.

Conclusion of the study

This study demonstrated that ethanolic leaf extract of M. calabura Linn. are rich in phytochemicals with potent antioxidant activity and has the ability to suppress the activity of cancer causing cell lines.The demand for plants used as traditional medicine formulations by the community is also increasing because plant derived medicines have proven to be healthier and do not cause as many side effects as those derived from chemicals. Crude protein of Muntingia calabura leaves extract showed a significant antioxidant activity and might be an alternative to synthetic antioxidants available in the market. Hence the advance acquisition are needed to purify the protein and to assess the in vivo antioxidant activity on animal models.

Conflict of Interest

There is no conflict of interest

References

1. Amin A, Gali-Muhtasib H, Ocker M, Schneider-Stock R. 2009. 'Overview of major classes of plant derived anticancer drugs'. Int J Biomed Sci, 5(1): 1-11.
2. Ayob Z., Abd Samad A., Mohd Bohari S. P. Cytotoxicity activities in local Justicia gendarussa crude extracts against human cancer cell lines. Jurnal Teknologi (Sciences and Engineering) 2013;64(2):45–52. doi: 10.11113/jt.v64.2043. [CrossRef] [Google Scholar]
3. Balamurugan V, Balakrishnan V, Robinson JP, Ramakrishnan M. Anticancer and apoptosis-inducing effects of Moringa concanensis using hepG2 cell lines. Bangladesh J Pharmacol. 2014; 9(4):604-09.
4. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. 2014. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev 94: 329-354.
5. Chen JJ, Lin RW, Duh CY, et al. (2004). Flavones and cytotoxic constituents from the stem bark of Muntingia calabura. J Chin Chem Soc 51:665–70 [Crossref], [Web of Science ®],
6. Chin WY (1989) A guide to the wayside trees of Singapore. BP Singapore Science Centre, pp: 145.
7. Dehpour AA, Ebrahimzadeh MA, Fazel NS, et al, 2009. Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition, Grasas y aceites, 60(4), 405-412. 2.
8. Farhat MB, Landoulsi A, Chaouch-Hamada R, et al, 2013. Characterization and quantification of phenolic compounds and antioxidant properties of Salvia species growing in different habitats, Industrial Crops and Products, 49, 904-914.
9. Jadoon S, Karim S, Asad MHHB, Akram MR, Khan AK, Malik A, Chen, C, Murtaza G. 2015. Anti-aging potential of phytoextract loaded pharmaceutical cream for human skin cell longevity. Oxid Med Cell Longev 2015. DOI: 10.1155/2015/709628.
10. Krishnaveni M and Dhanalakshmi R. Qualitative and quantitative study of phytochemicals in Muntingia calabura L. leaf and fruit. World Journal of Pharmaceutical Research. 2014; 3(6): 1687–1696.
11. Mahmood ND, Nasir NLM, Rofiee MS, Tohid SFM, Ching SM, Teh LK, Salleh MZ, Zakaria ZA. 2014. Muntingia calabura: A review of its traditional uses, chemical properties, and pharmacological observations. Pharm Biol 52 (12): 1598-1623.
12. Mohamed Azmathulla Khan Y, Dinesha R, Subhas Chandrappa M, Santhosh Kumar N, Harsha R K, Chikkanna D., 2015, “In vitro anti-inflammatory activity of proteins isolated from muntingia calabura plant root”, WJPR, Vol 4, (10)., pp. 2766-73.

13. Morton, J.F. (1987) Jamaica Cherry. In Fruits of Warm Climates. Miami: J.F. Morton; p.65Ninan Jisha a, A. Vysakh a, V. Vijeesha and MS Lathaa, Phytochemical and Antioxidant Studies on Leaf Extracts of Muntingia calabura L. International Journal of Pharmacy and Biological Sciences-IJPBSTM (2019) 9 (2): 1335-1340 Online ISSN: 2230-7605, Print ISSN: 2321-3272.
14. Parkhe G, Jain P, 2018. Study of antioxidant potential of hydroalcoholic extract of Anethum graveolens, Career Int J Sci Technol, 1(2), 39-45.
15. Safrida S, Sabri M. Effect of Muntingia calabura L. stem bark extracts on uric acid concentration and renal histopathology in diabetic rats. Medicina. 2019; 55(10): 695.
16. Shih C D, Chenn J J, Lee H H, 2006. 'Activation of nitric oxide signalling pathway Mediates Hypotensive effect of Muntingia calabura L.(Tiliaceae) leaf extract'. Am J Chin Med, 34.pp 857-72.
17. Zakaria ZA, Balan T, Suppalan V, Ahmad S, Jamaludin F. 2014b. Mechanism(s) of action involved in the gastroprotective activity of Muntingia calabura. J Ethnopharmacol 151 (2014): 1184-1193.
18. Zakaria ZA, Mohamed AM, Jamil NSM, Rofiee MS, Hussain MK, Sulaiman MR, Teh LK, Salleh MZ. 2011. In vitro antiproliferative and antioxidant activities of the extracts of Muntingia calabura leaves. Am J Chin Med 39 (1): 183-200.
19. Zakaria ZA, Sani MHM, Cheema MS, Kader AA, Teh LK, Salleh MZ. 2014a. Antinociceptive activity of methanolic extract of Muntingia calabura leaves: further elucidation of the possible mechanisms. BMC Compl Altern Med 14: 63. DOI: 10.1186/1472-6882-14-63.
20. Zakaria, Zainul Amiruddin & M.mohamed, A. & Mohd.jamil, N. & Rofiee, Mohd & Hussain, Mohd & Sulaiman, Mohd Roslan & Teh, Lay Kek & Salleh, M.Z.. (2012). In Vitro Antiproliferative and Antioxidant Activities of the Extracts of Muntingia calabura Leaves. The American Journal of Chinese Medicine. 39. 10.1142/S0192415X11008749.
21. Zhang Y., Chen A. Y., Li M., Chen C., Yao Q. Ginkgo biloba extract kaempferol inhibits cell proliferation and induces apoptosis in pancreatic cancer cells. Journal of Surgical Research. 2008;148(1):17–23. doi: 10.1016/j.jss.2008.02.036. [PMC free article] [PubMed] [CrossRef] [Google Scholar].