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***Research Article** (Open Access)*

IN VITRO ANTI-OXIDANT POTENTIAL AND CYTOTOXICITY STUDY ON SW982 RHEUMATOID ARTHRITIS CELL LINE OF *NYCTANTHES ARBORTRISTIS* FLOWER

Shammi Sharma[1] and *Jaya Prakash Yadav[1,2]
[1]Department of Genetics M.D., University, Rohtak- 124001, Haryana, India
[2]Indira Gandhi University, Meerpur, Rewari- 122502, Haryana, India
**Author for Correspondence: yadav1964@rediffmail.com*

ABSTRACT
It is widely recognized that enhanced oxidative stress, characterized by the overproduction and accumulation of free radicals, plays a significant role in the pathogenesis and progression of various human ailments. *Nyctanthes arbortristis*, commonly known as the Night-flowering Jasmine, has long been recognized for its therapeutic properties in traditional medicine. In this study, we investigated the in vitro antioxidant potential and cytotoxicity of *Nyctanthes arbortristis* flower extract using the SW982 rheumatoid arthritis cell line. The flower extract was prepared using standard protocols and subjected to various antioxidant assays, including DPPH, ABTS, H_2O_2, and NO assay. Our results revealed significant antioxidant activity of *Nyctanthes arbortristis* flower extract, evidenced by dose-dependent scavenging of DPPH, ABTS, H2O2, and NO. The flower aqueous and oil extract showed antioxidant potentials. The extracts phytochemical profiling through GCMS and HPLC identified various bioactive constituents like beta sitosterol, stigmasterol and quercetin. Furthermore, the extract demonstrated dose-dependent cytotoxic effects on SW982 cells, indicating that the oil extract was highly toxic then the aqueous extract. This study demonstrates that flower oil and aqueous extract is the best solvent for extracting phenolics and flavonoids from *N. arbortristis*. High phenolic and flavonoids content support the antioxidant capability of *N. arbortristis* as a possible natural antioxidant source. These findings underscore the therapeutic potential of *Nyctanthes arbortristis* flowers in combating oxidative stress and warrant further exploration of its pharmacological applications in rheumatoid arthritis.

***Keywords:** Antioxidant, phenolics, flavonoids, MTT, cytotoxicity, SW982, HPLC, GCMS*

INTRODUCTION
The *Nyctanthes arbortristis* tree, which belongs to the Oleaceae family, is a deciduous tree that is indigenous to Southeast Asia, including India and Myanmar (Parekh & Chanda, 2007). Different portions of this plant, which is commonly known as the Night-flowering Jasmine or Parijat, have been utilised in traditional medicine for millennia due to the unique pharmacological qualities that it possesses (Khatune *et al.,* 2001). The blooms of *Nyctanthes arbortristis*, in particular, have garnered interest due to the possible therapeutic advantages that they possess, which include anti-inflammatory, analgesic, antipyretic, and antioxidant qualities (Agrawal and Pal, 2013). Phytochemical studies on *Nyctanthes arbor-tristis* report the presence of essential phytoconstituents such as nyctanthic acid, β-sitosterol, oleanolic acid, lupeol, ascorbic acid and phenolic compounds like tannins, glycosides and flavonoids etc. (Kannadhasan *et al.,* 2012). Rheumatoid arthritis, often known as RA, is a chronic inflammatory condition that is characterised by inflammation of the synovial joints. This inflammation ultimately results in increasing joint destruction and disability (Baecklund *et al.*, 2006). Oxidative stress, which is caused by an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defence systems, is a significant factor in the pathophysiology of rheumatoid arthritis (RA) (Ahmed *et al.*, 2019). As a result, natural antioxidants that

are generated from medicinal plants have garnered a substantial amount of interest as prospective therapeutic agents for the management of rheumatoid arthritis and the difficulties that are associated with it (Kang & Ji. 2012). Over the past several years, there has been an increasing interest in investigating the antioxidant and cytotoxic characteristics of plant extracts in relation to a variety of illness situations, such as cancer and autoimmune diseases (Sharma *et al*., 2021). An important in vitro model for researching the pathogenesis of rheumatoid arthritis (RA) and assessing prospective therapeutic approaches is the SW982 cell line, which was generated from the synovial tissue of a patient who was diagnosed with RA. Utilising the SW982 RA cell line, the purpose of this work was to explore the antioxidant capacity and cytotoxic effects of floral extracts derived from *Nyctanthes arbortristis* in vitro. By gaining an understanding of the pharmacological qualities of *Nyctanthes arbortristis* flowers, one may get significant insights into the possible therapeutic uses of these flowers in the management of rheumatoid arthritis and other disorders associated to it.

MATERIALS AND METHODS

Collection of plant material and extracts preparation

Fresh flowers of *Nyctanthes arbortristis* were collected from Maharshi Dayanand University, Rohtak, in the morning between 6:00 and 8:00 AM. The collected flowers were then dried at room temperature for 3 to 5 days until completely dry. Subsequently, the dried flowers were powdered using a mechanical grinder. For the preparation of extracts, 50 grams of the powdered sample were taken for each extraction. The powdered sample was then subjected to extraction using a Soxhlet extractor with 300 ml of the desired solvent. The extraction process was carried out to ensure maximum extraction efficiency. After the extraction process, the solvent was evaporated to dryness using a rotary evaporator at 45°C. This resulted in the formation of dry extracts. The dry extracts were carefully collected and stored in a refrigerator at 4°C to maintain their stability and prevent degradation until further analysis or use in experiments (Parekh & Chanda, 2007).

Hydro distillation:

A conventional technique for extracting essential oils from different plant components is hydrodistillation. It is renowned for being easy to use and efficient in removing aromatic chemicals from plant sources. The plant material(flower) and water are normally contained in a round-bottom flask that is part of the hydro distillation equipment. The essential oil vapors from the plant material are carried by the steam produced when the flask is brought to a boil. Steam carrying the vapours of essential oils rises through the device and reaches a condenser. The steam condenses back into a liquid state as a result of the condenser cooling it. A Clevenger extractor or separator receives the condensed combination of water and essential oil that comes from the condenser. Due to variations in density, the oil and water in the Clevenger extractor split into two layers. The lighter essential oil separates and floats to the top of the water. To prevent deterioration from light and air, the obtained essential oil is kept in sealed, dark containers (Barros *et al.,* 2022).

Evaluation of antioxidant capacity:

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay:

An evaluation of the antioxidant activity of the test samples was carried out by determining their capacity to scavenge free radicals. This capability was assessed by monitoring the change in optical density of DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. There were several different quantities of flower oil and aqueous extracts that were made. These concentrations were 20, 40, 60, 80, and 100 µg/ml. DPPH, which is a stable free radical with a nitrogen centre, is responsible for the violet-blue hue that floral oil solutions obtain. When a substrate that is capable of contributing hydrogen atoms is introduced into the DPPH solution, the reaction results in the formation of a yellow product known as diphenyl picryl hydrazine. To make a DPPH solution with a concentration of 0.5 mmol/L, 95% methanol was utilised. The test tube was filled with 1 ml

of extracts of varying strengths and 2 mL of DPPH solution at a concentration of 0.5 mnol/L. The mixture was allowed to sit at room temperature and exposed to darkness for a period of half an hour (Manzocco *et al.*, 1998).

Inhibition of DPPH radical (%) = [(A0 - At)/ (A0)] ×100

Where A_0 = control absorbance

At = test samples absorbance

ABTS (2, 2'- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) assay:

The ABTS antioxidant assay involves determining the antioxidant capacity of plant extracts using the ABTS radical cation decolorization method. First, a 7 mM ABTS stock solution is prepared and mixed with 2.45 mM potassium persulfate in a 1:1 ratio to generate ABTS+ radicals. This mixture is incubated in the dark at room temperature for 12-16 hours. After incubation, the ABTS+ solution is diluted with PBS to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Plant extracts are then prepared at various concentrations in methanol, and 20 µL of each extract is added to 980 µL of the ABTS+ working solution. The mixture is incubated for 6-10 minutes at room temperature, and absorbance is measured at 734 nm. A control using methanol instead of the extract and a blank without ABTS+ are also prepared.

%inhibition = [(Abs control – Abs sample)]/ (Abs control)] × 100

Where A_0 = control absorbance

At = test samples absorbance

Hydrogen Peroxide (H_2O_2) assay:

Ruch *et al.*, (1989) used this approach to test the capacity of a number of plant extracts to scavenge hydrogen peroxide throughout their research. Phosphate buffer with a concentration of 50 mmol/L and a pH of 7.4 was used to create a hydrogen peroxide solution with a concentration of 40 mmol/L. For the purpose of this experiment, hydrogen peroxide was combined with plant extracts at different quantities, namely 20, 40, 60, 80 and 100 µg/ml. After ten minutes, the absorbance at 230 nm was measured using a blank solution that contained phosphate buffer but did not contain any hydrogen peroxide.

Scavenged H_2O_2 (%) = [(A_0 -At)/A_0] × 100

Where A_0 = control absorbance

At = test samples absorbance

Nitric oxide assay:

A typical way to test nitric oxide scavenging activity is the Griess-Ilosvoy reaction. Sodium nitroprusside, a nitric oxide donor, decomposes in an aqueous solution at physiological pH (7.2) to release NO, which interacts with oxygen to generate NO_2- in the presence of Griess reagent. In PBS, flower oil and aqueous plant flower extracts (20-100 µg/ml) were mixed with 10 mM sodium nitroprusside. The combination sat at 30°C for two hours. A control reaction combination with ethanol instead of extract was also made. Following incubation, 0.5 ml of Griess reagent was introduced into the reaction mixture. The Griess reagent typically consists of 1% sulfanilamide, 2% phosphoric acid (H_3PO_4), and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride. The absorbance of the solution at 550 nm was then measured using a spectrophotometer. The Griess-Ilosvoy reaction produces nitrite ions, which may be quantified to assess the test sample's nitric oxide scavenging activity (Ebrahimzadeh *et al.,* 2008).

Radical scavenging activity (%) = [(A_0 -At)/A_0] × 100

Where,

A_0 = control absorbance

At = test samples absorbance

Determination of total phenolic content

A typical assay measures a sample's total phenolic content, which indicates its antioxidant and health-promoting qualities. This is often done with the Folin-Ciocalteu test. Different quantities of flower oil, and aqueous extracts (20-100 µg/ml) were produced Mix the sample with Folin-Ciocalteu. Place the mixture at room temperature for a certain time to allow the phenolic compounds and Folin-Ciocalteu reagent to react. Stop the process using sodium carbonate (Na_2CO_3) after incubation. Use a spectrophotometer to measure the blue solution's absorbance at 725 nm. Compare the sample's absorbance to a standard curve made with known amounts of a phenolic chemical (e.g., gallic acid) under the same circumstances to determine its total phenolic content. Report findings in milligrammes of gallic acid equivalents (GAE) per g or ml of sample (Wolfe *et al.,* 2023).

Determination of total flavonoid content

Another popular assay measures a sample's total flavonoid content, which indicates its antioxidant potential and health benefits. Extracts of flower oil and aqueous extract prepared at 20-100 µg/ml concentrations. A standard solution is made by dissolving a known amount of quercetin in the sample extraction solvent. Measure a volume of sample extract and add a colorimetric reagent like aluminium chloride ($AlCl_3$) that interacts with flavonoids to generate coloured complexes. To generate coloured complexes, incubate the mixture at room temperature or under controlled conditions. Use a spectrophotometer to measure the absorbance of the coloured solution at 415 nm for aluminium chloride. The sample's total flavonoid content may be calculated by comparing its absorbance to a standard curve with known concentrations of the standard under the same circumstances. Results in mg of flavonoid standard equivalents per g or ml of sample (Bahorun *et al.,* 1996).

Determination of the effect of plant extracts on cell proliferation by MTT assay:

Cell culture:

With the help of NCCS Pune, we were able to acquire the human synovial cell line SW982. In T-50 flasks, the SW982 cells were grown with 10% Dulbecco's Modified Eagle Medium (DMEM), 10% Fatal Bovine Serum (FBS), and 5% carbon dioxide (CO2). In addition, the cells were utilised for the purpose of conducting a cytotoxicity investigation on various plant extracts, which was then followed by an MTT assay (Morgan 1998).

MTT assay protocol:

The MTT Assay was used to determine the cytotoxicity of the provided substances using the SW982 cell line (bought from NCCS Pune). A popular technique for determining cell viability and proliferation is the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. The cells of interest should be cultured until they attain the required confluence or passage number in a suitable growth medium under conventional conditions (e.g., 37°C, 5% CO2). Cells should be plated in 96-well plates at a density appropriate for the test. 5,000–10,000 cells are usually planted per well. Once the cells have attached themselves to the plate, which normally takes an overnight period, apply different quantities of flower oil and aqueous extract (0, 1, 10, 50, 100, 250, 500, and 1000 µg/ml). Make a stock solution of MTT in growth medium or phosphate-buffered saline (PBS) after the intended treatment duration. MTT is usually applied at a final 0.5 mg/ml concentration. Once the MTT solution (50 µl) has been added to each well containing cells, incubate the plate at 37°C for 2-4 hours to allow metabolically active cells to generate formazan crystals. After the incubation time, take the medium out of the wells with caution so as not to disrupt the formazan crystals. Dissolve the formazan crystals generated by live cells by adding 100 µl of dimethyl sulfoxide (DMSO) solubilizing agent; this releases the purple formazan solution. Measure the formazan solution's absorbance in each well using a microplate reader at a wavelength that is usually between 550 and 600 nm. By comparing the absorbance values of treated wells to control wells (i.e., cells that have not been treated), determine the proportion of viable cells in each treatment group. To find out how the

treatments affect cell viability, graph and evaluate the data using the relevant statistical techniques (Morgan 1998).

GC-MS analysis of flower extracts:

GC-MS was used to identify the primary phenolic acids and flavonoids in the flower oil and aqueous extract. Using a capillary column and helium as the carrier gas, the SCIO-SQ436-GC was used to investigate the GC/MS analysis of floral extract. A 1 mg/ml concentration of floral extract was made into a solution. After that, this solution was injected into the column at a temperature of 400°C and a flow rate of 1 ml/min. The oven temperature was increased by 8°C every minute for two minutes at 80°C, until it reached 250°C. After keeping the temperature constant for 25 minutes, running for a total of 49 minutes at 280°C at an increase of 8°C per minute (Kumari *et al.,* 2020).

HPLC analysis of flower extracts:

An autosampler (YL 9150) equipped with 100 UV-visible photodiode detectors was used in the Agilent 1200 infinite series HPLC system to analyse extracts that were flower oil and aqueous, 5 µl (100–200 micrograms per millilitre) of the standard and plant extract stock solution (1 mg/ml) were introduced by autosampler. On a C-18 column, the separation was carried out at room temperature. Methanol to acetonitrile (60:40 v/v) makes up the mobile phase. The isocratic mode is used to achieve separation, and a flow rate of 1 ml/min is used. After the samples were processed for 12 minutes, a UV photodiode detector picked them up at 254 nm (Javed *et al.,* 2024).

Statistical analysis:

Every experiment was carried out in triplicate, and the mean ± standard deviation was used to represent the findings. Graph Pad Prism-6 software was used to determine the IC50.

RESULTS

Antioxidant potential of Nyctanthes arbortristis flower extracts:

Analyses using DPPH were carried out to determine the radical scavenging activity (RSA) of various floral extracts. The floral oil extract of the plant had a high inhibitory percentage (68.23%), as shown in Figure 1(a), followed by the aqueous extract (54.72), and then compared with the ascorbic acid as the standard (91%). As part of the radical scavenging test, the 50% inhibitory concentrations (IC50 values) of both the standards and the samples are presented in Table 1. Additionally, the IC50 values for the floral oil and aqueous extracts were found to be 69.16 and 87.96 µg/ml, respectively. In comparison, the standard value for ascorbic acid was found to be 30.31 µg/ml. The methanolic extract exhibited an RSA inhibition of 65.12% in the ABTS scavenging experiment, whereas the aqueous extract exhibited a 57.33% inhibition at a concentration of 100 µg/ml. This information is illustrated in Figure 1(b). In terms of the IC50 values, the floral oil and aqueous extracts were found to be 66.97 and 87.48 µg/ml, respectively. While this was going on, the IC50 value of ascorbic acid was 48.83 µg/ml, as seen in Table 1. It was determined whether or not the floral extract of *Nyctanthes arbortristis* possessed a percentage of RSA activity against nitric oxide (NO) radicals. As shown in Figure 1(c), the findings demonstrated that the scavenging activity of the methanolic extract was 68.90%, followed by the aqueous extract, which had a scavenging activity of 61.67% at a concentration of 100 µg/ml. The average inhibitory concentration (IC50) values for the floral oil and aqueous extracts were 69.92 and 87.38 µg/ml, respectively. During this time, the value for ascorbic acid was recorded as 53.77 µg/ml, as shown in table 1. *Nyctanthes arbortristis* flower extracts have a powerful potential to scavenge OH radicals due to their antioxidant properties. The flower oil of the plant exhibits a scavenging activity of 70.27 percent, followed by aqueous (55.27 percent) and ethyl acetate (54.15 percent) at a concentration of 100 µg/ml respectively, as seen in Figure 1(d). Specifically, the IC50 values for the floral oil and aqueous extracts were 56.81 µg/ml and 71.62 µg/ml, respectively. Ascorbic acid, on the other hand, demonstrated an IC50 value of about 46.09 µg/ml, as seen in Table 1.

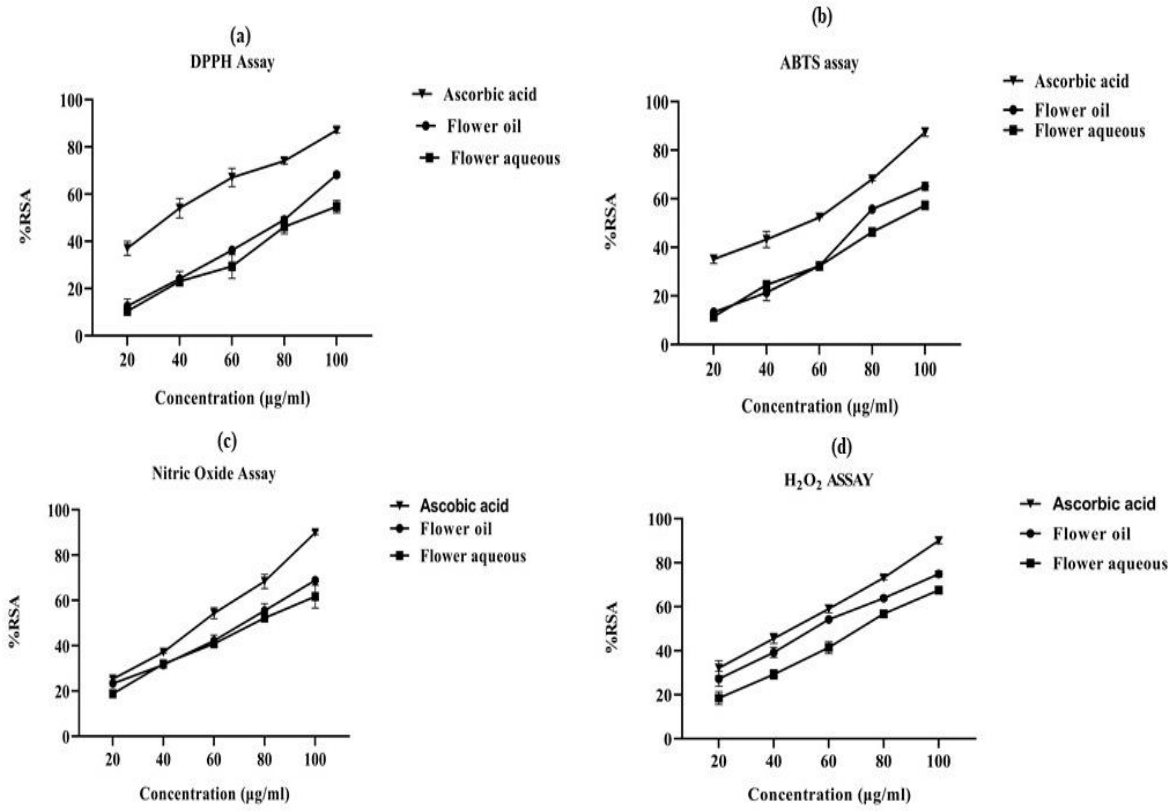

Figure. 1. Radical scavenging assays of *Nyctanthes arbortristis* flower oil and aqueous extracts compared with standard compound ascorbic acid (a) DPPH (b) ABTS (c) Nitric oxide (d) H_2O_2 assay.

Table 1: IC50 value of methanol, aqueous and ethyl acetate extracts of *Nyctanthes arbortristis* flower various biochemical assays

Sr. no.	Plant extracts	DPPH IC50 (µg/ml)	ABTS IC50 (µg/ml)	Nitric oxide (NO) (µg/ml)	Hydrogen Peroxidase (H_2O_2) (µg/ml)
1	Ascorbic acid	29.31±0.08	45.83±0.04	52.68±0.14	44.32±0.158
2	Flower Oil	69.16±0.001	66.97±0.72	69.92±0.005	56.81±0.01
3	Flower aqueous	87.96±0.06	87.48±0.56	87.34±0.321	71.62±0.35

Total Phenolics Content (TPC):

Through the use of the total phenolics content assay, the concentration of phenolic compounds that were present in the floral extracts of *Nyctanthes arbortristis* was ascertained. A representation of the TPC of the plant in each of the several types of solvent. In accordance with the information shown in table 2, the

polyphenol content of the floral extracts was found to be 52.14 mg GAE/g in flower oil and 156.22 mg GAE/g in aqueous extract.

Table 2: Total Phenolic and flavonoids contents of *Nyctanthes arbortristis* flower extract.

Plant Flower Extracts	Total Phenolic Content (mg GAE /g sample)	Total Flavonoid Content (mg QE/g sample)
Flower oil	52.14±0.12	84.91± 0.045
Aqueous	156.22±0.36	164.32±0.025

Total Flavonoid Content (TFC):

A quantitative phytochemical screening analysis is used to determine the total flavonoid content of the *Nyctanthes arbortristis* flower extract. This analysis is used to determine the amount or quantity of flavonoid phytochemical compounds present in there. The findings showed that the floral oil extract has flavonoid components with a concentration of 84.91 mg GAE/g, while the aqueous extract has a concentration of 164.32 mg GAE/g, as shown in table 2.

Cytotoxicity study of flower extract by MTT assay:

The evaluation of the toxicity of plant materials is an essential step that must be taken prior to the production or use of pharmaceuticals. The MTT test was utilised in this investigation to determine whether or not crude flower oil and aqueous plant flower extracts presented any cytotoxic effects on SW982 cells. The findings of the study indicated that the aqueous extracts, with an IC50 value of 231.1 ± 0.07 µg/ml, exhibited a lower level of toxicity in comparison to the floral oil extracts, which had an IC50 value of 28.09 ± 0.3208 µg/ml, when compared to the untreated control cells, as indicated in table 4. After being subjected to aqueous and floral oil extracts, the viability of SW982 cells was significantly reduced. Based on the results of the MTT experiment, the range of viability for floral oil and aqueous samples was 65% to 31.11% and 85.55 to 34.32%, respectively, as depicted in Figure 2. According to these findings, the cytotoxic profile of these extracts is one of the most important factors to consider when considering their prospective uses in the creation of drugs or in therapeutic usage.

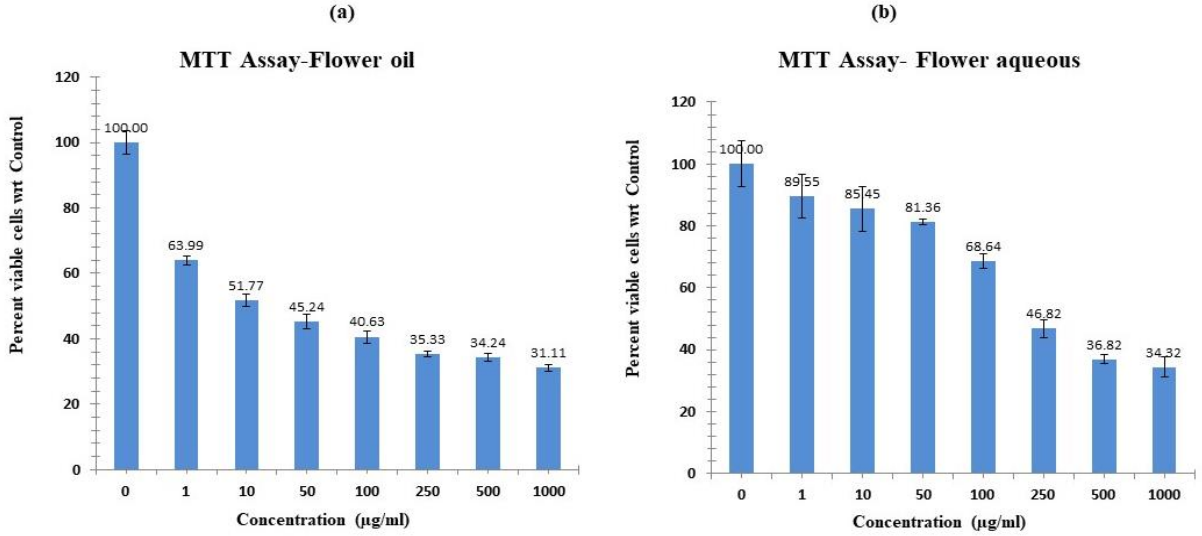

Figure 2: MTT assay of flower extracts showing different cell viability at different concentrations (a) flower oil (b) aqueous

Table 4: IC50 value of different flower extracts of cytotoxicity by MTT assay.

Sr. no.	Plant extract	MTT IC50 (µg/ml)
1	Methanolic	28.09 ± 0.3208
2	Aqueous	231.1 ± 0.071

Phytochemical profiling of flower extracts through GCMS:

In the flower oil, 16 compounds have been identified according to the percent area and retention time (RT) through the GCMS analysis (Figure 3 and Table 5). Major phytochemical constituents are Benzyl alcohol (3.76%) (RT 4.765), 2-Pentadecanone,6,10,14-trimethyl (4.70%) (RT 17.112), Benzyl benzoate (2.4%) (RT 16.35), Phytol (5.542%) (RT 20.513), Hexadecanoic acid, methyl ester (7.83%) (RT 18.291), 1H-Imidazole,1-methyl-5-nitro (6.74%) (RT 23.28), 1-Heptacosanol (9.93%) (RT 24.264), Tetrapentacontane (7.25%) (RT 15.564), etc.

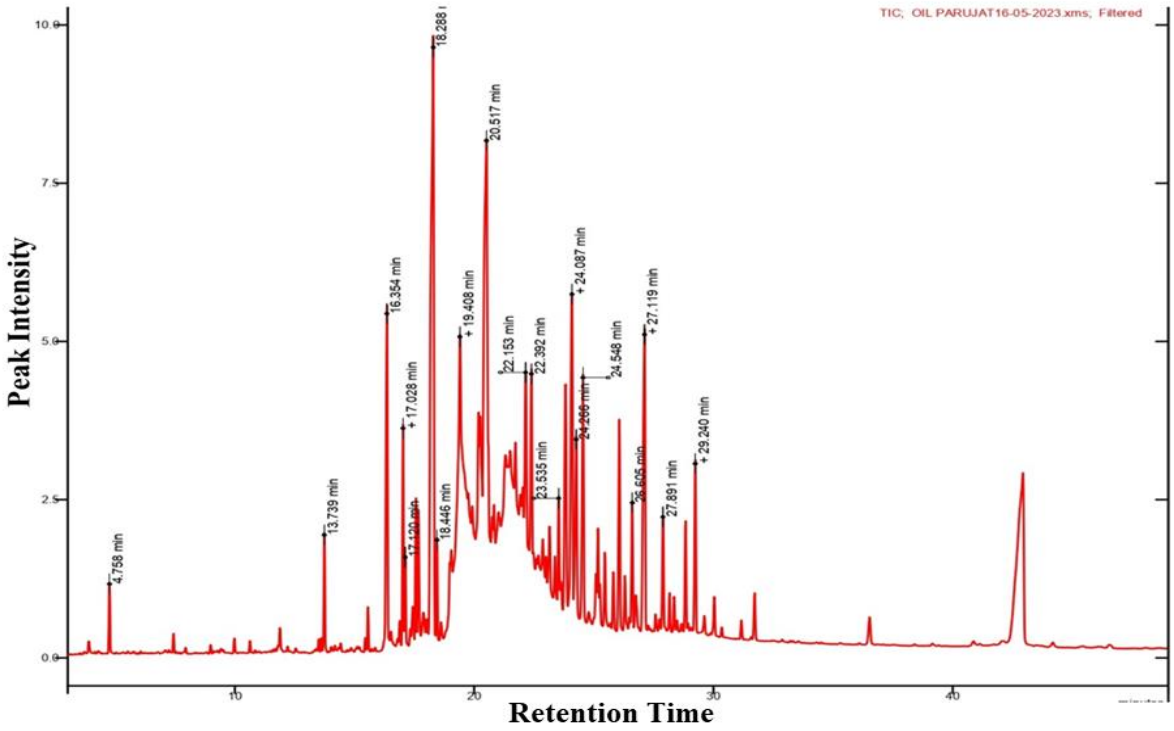

Figure 3: GC-MS chromatogram of flower oil extract.

In the flower aqueous extract, 5 bioactive compounds were identified with their per cent area and retention time depicted in Figure 4 and Table 6. The main bioactive constituents were Dodecene (5.8%) (RT 7.384), Nonadecane (9.07%) (RT 18.231), Isophorene (6.949%) (RT 11.049), 2- Propenoic acid, 3- phenyl methyl ester (5.24%) (RT 10.763), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (1.66%) (RT 17.406), Dibutyl phthalate (5.50%) (RT 18.584).

Table 5: Phytochemical compounds present in flower oil.

Sr. no.	Retention time	Compound name	Area	Molecular formula	Molecular weight
1	4.765	Benzyl alcohol	3.761e+9	C7H8O	108
2	13.732	1,3-Benzenediol, monobenzoate	6.331e+9	CC13H10O3	214
3	16.357	Benzyl benzoate	2.402e+10	C14H12O2	212
4	17.029	3,7,11,15-Tetramethyl-2-hexadecane-1-ol	1.158e+10	C22H42O2	338
5	17.113	2-Pentadecanone,6,10,14-trimethyl	4.706e+9	C18H36O	268
6	18.443	Isophytol	4.999e+10	C20H40O	70.37
7	18.291	Hexadecanoic acid, methyl ester	7.835e+10	C17H34O2	270
8	19.402	[1-(3,3-Dimethyloxiron-2-ylmethyl0-3,7-dimethylocta-2,6-dimethyl]	1.440e+10	C18H34OSi	294
9	20.513	Phytol	5.542e+10	C20H40O	296
10	22.161	1-Heptacosanol	1.082e+10	C27H56O	396
11	22.396	Triacontane	1.122e+10	C30H62	422
12	23.288	1H-Imidazole,1-methyl-5-nitro….	7.576e+9	C4H5N3O2	127
13	24.096	Oxirane, hexadecyl	2.671e+10	C18H36O	268
14	24.264	1-Heptacosanol	9.937e+9	C27H56O	396
15	24.549	Hentnacontane	1.832e+10	C31H64	436
16	26.603	Tetrapentacontane	7.254e+9	C54H110	758

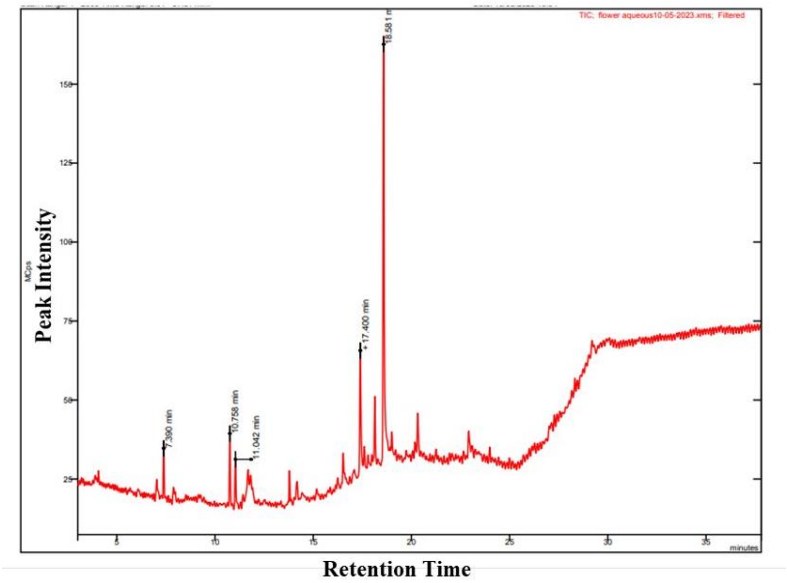

Figure 4: GC-MS chromatogram of flower oil extract

Table 6: Phytochemical compounds present in flower aqueous extract

Sr. no	Retention time	Compound name	Area	Molecular formula	Molecular weight
1	7.384	Dodecene	5.891e+7	C12H26	170
2	10.763	Nonadecane	9.007e+7	C19H40	268
3	11.049	Isophorene	6.949e+7	C9H14O	138
4	17.406	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.664e+8	C16H22O4	278
5	18.584	Dibutyl phthalate	5.501e+8	C16H22O4	278

HPLC analysis of flower extracts:

The identification of bioactive components was accomplished by the use of HPLC fingerprinting analysis on floral oil and aqueous extracts. The high-performance liquid chromatography (HPLC) technology was used to ascertain the levels of the beneficial substances stigmasterol, beta-sitosterol, and quercetin, as illustrated in Figure 5(a-c). Flower oil extract displays 10 peaks at retention time, as shown in Figure 5 (d). These peaks are as follows: 2.476, 2.632, 3.057, 3.326, 3.587, 3.961, 4.192, 4.439, 5.071, and 7.391. The floral oil extract identified these compounds stigmasterol (RT 2.476), beta-sitosterol (RT 2.632), and quercetin (RT 4.556) at their specific retention time.

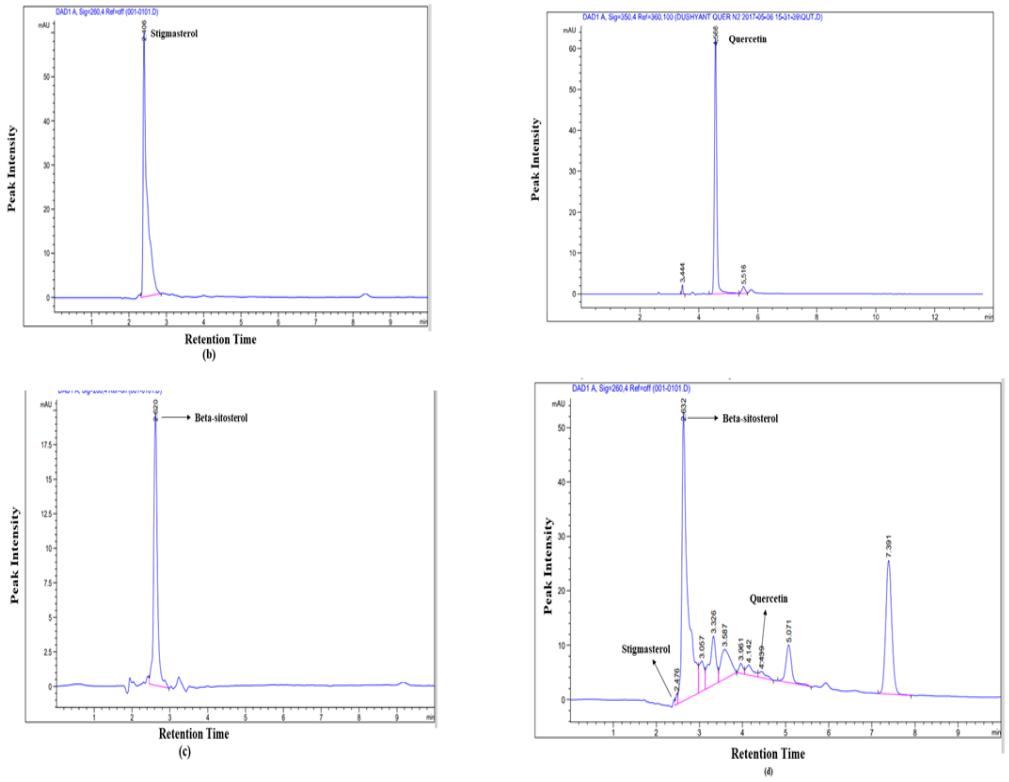

Figure 5: HPLC chromatogram of the standard (a) Quercetin (b) Beta-sitosterol (c) Stigmasterol & flower extracts (d) Flower aqueous.

DISCUSSION

Both researchers and the general public are paying special attention to the function that antioxidants play in sustaining human health (Halliwell & Gutteridge 2015; Papas 2019). This is due to the amount of new data that suggests oxidative stress plays a critical role in the development of a variety of illnesses and diseases. It has been suggested by Parekh and Chanda (2008) that the antioxidant properties of the plant are due to the high phenolic content of the plant, more especially flavonoids and phenolic acids. Chemicals that include phenolic compounds have the ability to boost antioxidant activities by functioning as effective metal ion chelators, hydrogen donors, and free radical scavengers. Multiple studies have demonstrated that *Nyctanthes arbortristis* contains antioxidant activity. These studies were conducted using in vitro technology. According to Pyrzynska and Pekal (2013), the floral extracts of *Nyctanthes arbortristis* contain phenolic compounds capable of performing the function of antioxidant molecules. The initial concentration (IC50) values for the floral oil and the aqueous extracts were 69.16 and 87.96, respectively as given in table 1. Due to the fact that the IC50 value of the floral oil extract was lower than that of the aqueous extract, it can be concluded that the flower oil extract performed better in terms of antioxidant inhibition than the aqueous extract. When it comes to reactive oxygen species (ROS), the hydroxyl radical (OH) is the most reactive radical. It is the radical that interacts with nucleotides, amino acids, polysaccharides, and lipids in the body. All of the other biochemical assays, including ABTS, NO, and H2O2, produced the same results. These results were likewise consistent. The TPC and TFC tests were carried out in order to ascertain the amount of phenolic and flavonoid phytochemicals that were present in the floral extracts of *Nyctanthes arbortristis*. This was done since phenolic compounds and flavonoid compounds are both extremely powerful antioxidants. According to the findings of the study, the aqueous extract contained a significantly higher quantity of phenolics and flavonoids than the floral oil did. The evaluation of the toxicity of plant materials is an essential step that must be taken prior to the production or use of pharmaceuticals. In order to determine the cytotoxicity of plant flower extracts, an MTT test was carried out on the SW982 synovial fluid arthritic cell line. In comparison to the cells that were not subjected to any treatment, the viability of the cells in the experiment reduced as the amount of exposure to aqueous and floral oil extract increased. Following the completion of our research, we found that the aqueous extracts of *Nyctanthes arbortristis* were significantly less hazardous to SW982 cells than the floral oil. It appears from these data that the cytotoxic profile of these extracts is extremely important for the possible uses of these extracts in the creation of drugs or in therapeutic usage. As part of the phytochemical profiling process, each and every extract was put through GCMS and HPLC analysis. Both GCMS and HPLC procedures are utilised in order to determine which substances are volatile and which are non-volatile. Through the use of high-performance liquid chromatography (HPLC), each of the extracts was evaluated in relation to the reference substances quercetin, stigmasterol, and beta-sitosterol. These chemicals are renowned for their antioxidant action, according to the findings of other researchers who have investigated them. Stigmasterol is an unsaturated phytosterol that may be found in a broad variety of natural sources. It is also one of the most frequent plant sterols, according to Bakrim *et al.,* 2022. Stigmasterol is a member of the tetracyclic triterpene class. According to the findings of a study that was conducted by Shanthakumar *et al.,* (2013), stigmasterol that was extracted from the ethyl acetate extract of *Spondias mombin* bark works in conjunction with a phytosterol to reduce the generation of reactive oxygen species (ROS). Additionally, it has been found that the extract of Cucumis sativus has a considerable amount of antioxidant activity when subjected to the FRAP and DPPH assays. In a study conducted by Anjani *et al.,* 2023, the ethanolic extract of Cucumis sativus peels was shown to contain the flavonoid quercetin. This flavonoid was identified and defined by spectroscopic and chromatographic analysis. Using an extract of ethyl acetate, the flavonoid quercetin was successfully extracted. According to Wang *et al.,* (2013), the antioxidant activity of β-sitosterol was demonstrated in vitro by the use of the DPPH test, which is often employed to evaluate the antioxidants' capacity to scavenge free radicals of the environment. It has been determined via this inquiry that β-

sitosterol had an antioxidant effect. This is due to the fact that its free radical scavenging rate at a concentration of 100 µg/mL was greater than forty percent (Zhang *et al.,* 2023).
For the purpose of this investigation, we intend to make use of gas chromatography and mass spectrometry techniques in order to determine the bioactive chemicals that are present in the floral and aqueous extracts of Nyctanthes arbortristis. Phytochemical profiling of flower oil identified following bioactive constituents benzyl alcohol (RT 4.765), 2-pentadecanone,6,10,14-trimethyl (RT 17.112), benzyl benzoate (RT 16.35), phytol (RT 20.513), hexadecanoic acid,methyl ester (RT 18.291), 1h-Imidazole,1-methyl-5-nitro (RT 23.28), 1-heptacosanol (RT 24.264), and Tetrapentacontane (RT 15.564), Dodecene (5.8%) (RT 7.384), Nonadecane (RT 18.231), Isophorene (RT 11.049), 2-propenoic acid, 3-phenyl methyl ester (RT 10.763), and Dibutyl phthalate (RT 18.584) were among the primary bioactive ingredients that were found by the phytochemical profiling of the aqueous extract. This in-depth characterization offers the framework for understanding the chemical makeup of the extract, which in turn provides vital insights into the possible bioactivity and therapeutic uses of the extract. It is possible that these chemicals are the area of the floral oil and aqueous extract that have the ability to serve as antioxidants. Additionally, additional research might be conducted to investigate the precise characteristics and interactions of the compounds that have been found, which would contribute to a more comprehensive knowledge of *Nyctanthes arbortristis* as a valuable source of bioactive phytochemicals.

CONCLUSION

The current study highlights the antioxidant potential of flower oil and aqueous extract derived from *Nyctanthes arbortristis*. Through various assays, it was demonstrated that both flower oil and aqueous extract exhibit significant antioxidant capacity. The plant aqueous extract has high amount of total phenolics and flavonoids content compared to the flower oil extracts. of flower o attributed to their high phenolic content. These findings align with traditional uses of *Nyctanthes arbortristis* as a natural antioxidant and support its ethnopharmacological applications. Furthermore, cytotoxicity studies conducted using the MTT assay indicated that all extracts, at different concentrations, were non-toxic. This suggests their safety for continued use in plant research and potential medication development. Analysis using GCMS and HPLC revealed the presence of various bioactive compounds in the flower extracts, including steroids, flavonoids, and phenolics. Notable antioxidants such as stigmasterol, quercetin, and beta-sitosterol were among the major constituents identified. These results underscore the potential health-promoting effects of phenolic and flavonoid constituents found in *Nyctanthes arbortristis* flowers. Additionally, they provide evidence supporting the plant's protective role against diseases associated with oxidative stress. Further exploration of these constituents could lead to the development of novel therapeutic interventions targeting oxidative stress-related conditions.

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ETHICAL STATEMENT

The animal-related research is not included in this article by any authors. None of the writers of this paper have conducted any studies on human subjects.

Conflict of interest

Shammi Sharma has no conflict of interest; Jaya Prakash Yadav has no conflict of interest.

REFERENCES

Agrawal J & Pal A (2013). *Nyctanthes arbor-tristis* Linn-A critical ethnopharmacological review. *Journal of Ethnopharmacology,* **146**(3), 645-658.

Ahmed KSZ, Ahmed SSZ, Thangakumar A & Krishnaveni R (2019). The therapeutic effect of *Parmotrema tinctorum* against complete Freund's adjuvant-induced arthritis in rats and identification of novel Isophthalic ester derivative. *Biomedicine* and *Pharmacotherapy*, **112**(108646), 1-11.

Baecklund E, Iliadou A, Askling J, Ekbom A, Backlin C, Granath F & Klareskog L (2006). Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis* and *Rheumatism*, ***54***(3), 692-70.

Bahorun T, Neergheen VS, Aruoma OI (1996). Phytochemical constituents of *Cassia fistula*. *African Journal of Biotechnology,* **5**(20), 1530-1540.

Bakrim S, Benkhaira N, Bourais I, Benali T, Lee LH, El Omari, N & Bouyahya A (2022). Health benefits and pharmacological properties of stigmasterol. *Antioxidants*, **11**(10), 1-32.

Barros M, Redondo L, Rego D, Serra C & Miloudi K (2022). Extraction of essential oils from plants by hydrodistillation with pulsed electric fields (PEF) pre-treatment. *Applied Sciences*, ***12***(16), 8107.

Ebrahimzadeh MA, Pourmorad F & Hafezi S (2008). Antioxidant activities of Iranian corn silk. *Turkish Journal of biology*, ***32***(1), 43-49.

Halliwell B & Gutteridge JM (2015). Free radicals in biology and medicine. Oxford University Press, USA.

Javed S, Anwar A, Javiad I, Shoaib A, Aljawdah HM & Kaushik P (2024). Anticancer activity of ethyl-acetate fraction of *Sorbaria tomentosa* and compounds identification through HPLC and LC-MS analysis. *Journal of King Saud University-Science*, **36**(2), 1-11.

Kang C & Ji LL (2012). Role of PGC-1α signaling in skeletal muscle health and disease. *Annals of the New York Academy of Sciences*, ***1271***(1), 110.

Kannadhasan R, Venkataraman S, SardharRajan S, Joseph FYiFanus (2012). Phytochemical constituents and antioxidant activities of *Nyctanthes arbor-tristis* Linn. Free Radicals and Antioxidants, **2**(3), 42-46.

Khatune NA, Mosaddik MA & Haque ME (2001). Antibacterial activity and cytotoxicity of *Nyctanthes arbor-tristis* flowers. *Fitoterapia*, **72**(4), 412-414.

Kumari R, Mishra RC, Sheoran R & Yadav JP (2020). Fractionation of antimicrobial compounds from *Acacia nilotica* twig extract against oral pathogens. *Biointerface Research in Applied Chemistry*, **10**, 7097-7105.

Manzocco L, Anese M & Nicoli MC (1998). Antioxidant properties of tea extracts as affected by processing. *LWT-Food Science and Technology*, **31**(7-8), 694-698.

Morgan DM (1998). Tetrazolium (MTT) assay for cellular viability and activity. *Polyamine protocols*, 179-184.

Papas AM (Ed.). (2019). Antioxidant status, diet, nutrition, and health. CRC press.

Parekh J, Chanda SV (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology*, **31**(1), 53-58.

Pyrzynska K, Pekal A (2013). Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Analytical Methods*, **5**(17), 4288-4295.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**(9-10), 1231-1237.

Shanthakumar B, Sathish M & Suresh AJ (2013). In vitro antioxidant activity of extracts and stigmasterol from flower of *Clerodendrum inerme* Linn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* **4**(4), 1411-1418.

Wang CZ, Yuan HH, Bao XL & Lan MB (2013). In vitro antioxidant and cytotoxic properties of ethanol extract of *Alpinia oxyphylla* fruits. *Pharmaceutical Biology*, **51**(11), 1419-1425.
Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, **51**(3), 609-614.
Zhang P, Liu N, Xue M, Zhang M, Liu W, Xu C & Zhou Y (2023). Anti-Inflammatory and Antioxidant Properties of β-Sitosterol in Copper Sulfate-Induced Inflammation in Zebrafish (*Danio rerio*). *Antioxidants*, **12**(2), 391.