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# Biopotential Extraction of Methanol Extract from the Leaves of Persicaria Hydropiper with an Emphasis on Cytotoxicity and Lipid Peroxide Inhibition

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**Abstract** This comprehensive study examined the properties of microwave-assisted extract of *Persicaria hydropiper* leaves (MAEPH), a traditionally used medicinal plant, with a focus on its total flavonoid content, antioxidant activity, lipid peroxide inhibition, and cytotoxic effects on MCF7 cells. The results shed light on the possible medicinal applications and advantages of MAEPH. A reliable regression equation was used to precisely measure the crucial parameter known as total flavonoid content, proving that MAEPH is a rich source of these strong antioxidants. The fact that MAEPH displayed concentration-dependent nitric oxide scavenging action suggests that it has the potential to reduce inflammation and oxidative stress, both of which are related to a number of health issues. Through $IC_{50}$ values and comparisons with ascorbic acid, its effectiveness was further demonstrated. By significantly inhibiting the production of lipid peroxide in both goat liver and egg yolk models, MAEPH's strong antioxidant activities were validated, showing its ability to protect cellular membranes and lipids from oxidative damage. Notably, the study investigated the cytotoxicity of MAEPH on MCF7 cells, revealing dose-dependent effects with a calculated $IC_{50}$ value, suggesting its potential to inhibit breast cancer cell proliferation. Although MAEPH's cytotoxicity may not be as high as that of the traditional cancer and cytotoxic drug Cyclophosphamide, its lower toxicity highlights its potential as a more bearable option. In conclusion, this study highlights MAEPH's potential as a natural source of antioxidants and a possible candidate for cancer treatment. These results lay a solid groundwork for future research, including *in vivo* studies, into the health advantages and possible uses of MAEPH. This study offers fresh opportunities for the creation of medicines and substances that promote health, making contributions to the fields of natural medicine and pharmaceutical sciences.

**Keywords:** *Persicaria hydropiper*, Flavonoid content, Antioxidant activity, Cytotoxic effects, Nitric oxide scavenging, Lipid peroxide inhibition.

## 1. Introduction

The beginning and progression of numerous diseases, including cancer, are significantly influenced by oxidative stress. It is a condition that arises from an imbalance between the body's ability to combat the production of reactive oxygen species (ROS) with antioxidants and the capability of the body to do so. This imbalance can cause DNA mutations, cellular damage, and the start of intricate processes that aid in the aetiology of many diseases. One of these, cancer, is one of the most prevalent and well-researched diseases with a direct connection to oxidative stress [1]. Uncontrolled cell proliferation and the ability of malignant cells to avoid apoptosis, the normal process of programmed cell death, are characteristics of cancer. By causing damage to cellular components, including DNA, oxidative stress is a key factor in the development of cancer. ROS can produce mutations and chromosomal abnormalities when they interact with DNA, which are characteristics of cancer. These genetic changes may activate oncogenes, inactivate tumour suppressor genes, and affect cell cycle control, which will encourage the development of cancerous cells [1, 2].

Additionally, oxidative stress not only contributes to cancer but also causes it. Numerous cancer risk factors, including exposure to ionising radiation, environmental toxins, and cigarette smoke, produce ROS and cause oxidative stress. For instance, tobacco smoke contains a number of compounds that stimulate the generation of ROS and have the potential to harm DNA. This harm may compound over time and result in the progress of cancer. Furthermore, oxidative stress and chronic inflammation, another well-known risk factor for cancer, are strongly linked. When immune cells combat an infection, inflammation encourages the creation of ROS, which causes cellular damage and may start malignant changes [3-5].

Beyond the first stages of the disease, oxidative stress and cancer are linked. Oxidative stress also affects how quickly and aggressively cancer develops. Higher levels of oxidative stress are present in invasive cancer cells, which may enhance their capacity for immune system evasion and metastasis. These cells frequently have higher apoptosis resistance, which enables them to endure and flourish in a ROS-rich environment. Oxidative stress can also influence how well a cancer therapy works. ROS production is frequently used in radiation therapy and chemotherapy to cause cancer cells to die. However, as cancer cells have evolved to exist in a pro-oxidant environment, high levels of oxidative stress in the tumour microenvironment can increase the resistance of cancer cells to these treatments. This opposition may reduce the efficacy of therapeutic measures [1, 6]. Cancer is among the conditions that has been studied the most in relation to oxidative stress, but it is not the only condition that is impacted by this correlation. Numerous additional illnesses, such as autoimmune diseases, cardiovascular diseases, diabetes, and neurodegenerative conditions like Alzheimer's and Parkinson's disease, clearly link oxidative stress to their development. In these circumstances, oxidative stress results in protein, lipid, and DNA damage, which feeds into the pathophysiology at play and advances the disease. Because it consumes so much oxygen, the brain is especially susceptible to oxidative stress, and oxidative damage is a major factor in the emergence of neurodegenerative disorders [6].

The body uses a complex system of antioxidant defence to lessen the harmful consequences of oxidative stress. Exogenous (obtained from food) and endogenous (made by the body) antioxidants both neutralise ROS and support cellular equilibrium. In order to prevent and treat diseases brought on by oxidative stress, it is crucial to provide an appropriate intake of antioxidants through a balanced diet and lifestyle choices. Inference: Redox imbalance has a crucial role in the development of many diseases, including cancer, and is supported by the relationship between oxidative stress and these ailments [2, 7, 8]. While oxidative stress is frequently a side effect of the illness, it can also play a part in the onset, development, and resistance to treatment of the illness. Understanding this complex link is crucial for the creation of tailored treatments and preventative measures intended to lessen the negative effects of oxidative stress on human health [6, 9].

Cytotoxicity, or the capacity of particular substances or therapies to cause cell death, is of utmost significance in the context of diseases like cancer. It acts as a key mechanism for getting rid of or stopping the development of cancerous cells. Cytotoxicity is a technique used in a number of cancer therapies, such as chemotherapy and targeted therapies, to selectively kill malignant cells while causing the least amount of harm to healthy cells. Inducing cytotoxicity is crucial for preventing cancer spread and recurrence in addition to helping to reduce

tumour burden. Furthermore, because it offers crucial insights into how chemicals, medications, and environmental variables affect living things, the study of cytotoxicity is crucial for assessing the efficacy and safety of these factors. Understanding and utilising cytotoxicity are essential components of medical research and clinical practise, and they have a significant impact on the creation of innovative therapies and diagnostic tools to treat a variety of disorders [10-12].

*Persicaria hydropiper* (L.) Delarbre is a member of the Polygonaceae family. Synonyms for this species include *Persicaria hydropiper* var. projectum Stanford, *Polygonum hydropiper* L., *Persicaria hydropiper* (L.) H. Gross, and *Persicaria hydropiper* (L.) Opiz. The plant is also known as pakarmul or in bishkatali Bangladesh, daun senahun in Malaysia, and la liao in China. It is frequently known as marsh-pepper smartweed, marsh-pepper knotweed, smartweed, or water pepper [13]. *P. hydropiper* is widespread throughout the planet and is native to tropical and temperate parts of Asia regions, together with the Caucasus, Western Asia, Middle Asia, Siberia, Russian Far East, Eastern Asia, China, Indo-China, the Indian Subcontinent, and Malesia; Australia and Northern Africa. The plant typically thrives in wet locations near water's edge and in marshes, and it predominates in fields of crops [13]. The goal of the current study is to investigate potential pharmacological evaluation of microwave assisted extract of leaves of *Persicaria hydropiper*, a historically used medicinal plant, in several models of oxidative stress and cytotoxicity [14].

## 2. Material and Methods

### 2.1 Chemicals and Drugs

Signova pharmaceuticals Pvt. Ltd. provided free samples of quercetin and cyclophosphamide as gift. Himedia Biosciences Company in Maharashtra, India provided the Sodium Nitroprusside Griess Reagent, Thiobarbituric Acid, Sodium Dodecyl Sulfate, and Tris-KCl Buffer. From Loba Chemie Pvt Ltd, Maharashtra, India, we purchased trichloroacetic acid and MTT. All other chemicals and reagents were acquired only from reliable, verified vendors and were of high quality.

### 2.2 Collection of the plant

Between September and May 2021, we gathered *Persicaria hydropiper* leaves in their natural environment. Mr. P. Agarwal, Botanist, c/o Herbal Waves, Himachal Pradesh, India, recognized, identified and authenticated the plants by comparing them to the proper voucher specimens at the Department of Pharmacognosy, Herbarium.

### 2.3 Preparation of the extract: Microwave assisted technique

The leaves of the plant were cleaned and shade dried followed by pulverization using a motorized crusher. The plant was extracted using a microwave-assisted procedure [15]. A quantity of dry powder (5 gm) was dissolved in 100 ml of methanol (20:1) at a 70:1 ratio before being microwaved at a setting of 160 W. After five minutes, the process was completed and the temperature was lowered to normal temperature. After that, the microwave started a new cycle for 1 minute. To obtain the final extract, the extract had been filtered and then concentrated on an aqua bath. The final extract had been codenamed as MAEPH (Microwave Assisted Extract of *Persicaria hydropiper* leaves).

### 2.4 Determination of total flavanoid content

Using a technique previously reported, the concentration of flavonoids in the MAEPH was measured spectrophotometrically [16]. The amount of flavonoids in an extract were expressed as the corresponding mg of quercetin per gm of extract. One millilitre of MAEPH (1 mg/mL) and one millilitre of a 2 percent methanolic AlCl3 solution were combined to create the sample. The samples were kept at room temperature for an hour during incubation. The absorbance at 415 nm was measured using spectrophotometry (Shimadzu 1800, UV spectrophotometer). For statistical calculations that resulted in the computation of the mean absorbance value, the samples were produced and analysed in triplicate. Similarly, the standard quercetin curve was plotted using the similar method. The amount of flavonoids in an extract were expressed as the corresponding amount of quercetin per gram of plant extract).

### 2.5 Nitric oxide radical scavenging activity

According to a previously reported technique, the scavenging effect of the extract on nitric oxide radical was measured [17]. In phosphate buffered saline, Na- nitroprusside (1 mL, 6 millimol/L) was combined with various extract concentrations (10–1,000 µg/mL), distilled water, and phosphate buffered saline. After 150 minutes of room temperature incubation, added Griess reagent at 0.6 mL. At 546 nm, the pink coloured chromophore's absorption was measured. Quercetin was the preferred standard flavanoid substance. Three replicates of each experiment were performed. The % inhibition was figured out as follows:

$$\%\ \textbf{inhibition} = \left(\frac{100 - A_{SAMPLE}}{A_{CONTROL}}\right) \times 100$$

### 2.6 Inhibition of lipid peroxidation using egg yolk

Utilizing egg yolk as a lipid-rich media, a modified thiobarbituric acid reactive species (TBARS) experiment was used to measure the lipid peroxide product generated [18]. In a nutshell, 0.4 mL of the extract (10-100 µg/mL) was combined with 0.8 mL of egg yolk (15 percent v/v). After that, 1.5 mL of distilled water was added to the capacity. The mixture was then mixed with 0.07 mL of $FeSO_4$, and it was incubated for 30 minutes at 37 $^{O}$C. Then, 1.7 ml of TBA in SDS (Sodium dodecyl sulphate) and 1.7 ml of acetic acid were added. After thoroughly mixing the mixture, it was heated for an hour at 96 OC. Butanol (5 ml) was added after cooling, and the combination was centrifuged for 11 minutes at 5000 rpm. The organic top layer's absorbance at 532 nm was measured, and the % inhibition was determined using the formula below:

$$\%\ \textbf{inhibition of lipid peroxidation} = \left(\frac{100 - A_{SAMPLE}}{A_{CONTROL}}\right) \times 100$$

### 2.7 Determination of Fe2+/ascorbate-induced lipid peroxidation using liver homogenate

The method reported earlier by Ohkawa et al., 1979 was used to perform lipid peroxidation [19]. The reaction mixtures were incubated for 65 minutes at 37 °C with varying extract concentrations (10-100 µg/ml). Additionally, they included 0.39 mL of 0.17 mmol/L ferrous ammonium sulphate, 0.08 mL ascorbic acid, and various doses of the extracts along with 0.3 mL of liver homogenate in 35mmol/L tris buffer. The substance that reacted with thiobarbituric acid as a result of this process was measured as follows: To stop the reaction, an aliquot of the reaction mixture (0.5 mL) was combined with 1.7 mL of 0.16 mol/L Tris-KCl buffer and 0.6 mL of 35 percent TCA. This mixture was then placed in a water bath for 47 minutes at 75 °C. It was then refrigerated in ice before being centrifuged to remove precipitates for 11 minutes at room temperature and 4000 rpm. The absorbance of the pink, clear supernatant was assessed at 532 nm in contrast to a control sample. The experiment was done three times with catechin as the standard.

$$\%\ \textbf{inhibition of lipid peroxidation} = \left(\frac{100 - A_{SAMPLE}}{A_{CONTROL}}\right) \times 100$$

### 2.8 Cell viability

The MTT assay was used to examine how MAEPH affected the growth and proliferation of cancer cells. In this approach, 104 cells were grown in 104-well plates at a density of 100 µL per well, and they were then incubated for a whole night. Following that, the cells were treated to various concentrations of the reference drug (10-1000 nM) and MAEPH (100-1000 nM). After the initial one day of incubation, 12 mL of MTT solution was added to each well, and the plate was subsequently incubated at 37 °C with 6 percent carbon dioxide for an additional 5 hours. The MTT-containing medium was carefully removed after the requisite amount of time, 110 mL of DMSO was added to each well, and then ELISA readers were used to measure each well's absorbance at 570 nm (Bio-Tek Instrument, USA)[20].

$$\% \textbf{ Viability} = \left(\frac{100 - A_{SAMPLE}}{A_{CONTROL}}\right) \times 100$$

### 2.9 Statistical analysis and data interpretations

One-way ANOVA was employed to statistically analyse the outcomes of various experiments. Dunnett's test was run as *post hoc* in order to analyse the ANOVA test. Significant results were defined as a *P* value 0.05 or less. The mean ± SD (standard deviation) of all data are presented. GraphPad Prism Software (Version 8) was utilised for all the graphing, plotting and statical analysis.

## 3. Results

### 3.1 Determination of total flavanoid content

Because these polyphenolic chemicals have so many health advantages, measuring the total flavonoid concentration is important in the domains of pharmacology and plant science. Fruits, vegetables, beverages, and other plant-based foods include a variety of phytochemicals called flavonoids. They have a significant impact on human health, and it is vital for a number of reasons to comprehend and quantify their composition. Flavonoids are famous for their strong antioxidant qualities first and foremost. They are capable of successfully scavenging dangerous free radicals, which are connected to oxidative stress and the emergence of a variety of chronic illnesses, including as cancer, heart problems, and neurological issues. Researchers and medical professionals can find sources of powerful antioxidants that improve general health and wellbeing by calculating the total flavonoid content of foods and dietary supplements. Flavonoids have also been allied to anti-microbial, anti-inflammatory, and anti-allergic activities. Determining the total flavonoid content is crucial for pharmaceutical quality assurance and standardisation of natural and herbal products. It makes sure that herbal treatments, which are frequently used for their therapeutic capabilities, are reliable and effective. The concentration of total flavanoid contents in this investigation was represented as milligram per gm of plant extract. By means of an equation derived from the equation of the regression analysis of the standard graph of Quercetin, the amount of total flavonoid components in the extract was articulated as grams of Quercetin:

$y = 0.0191x + 0.0832$, $R^2 = 0.9598$ (MEAPH)

Where, x = concentration. and y = absorbance.

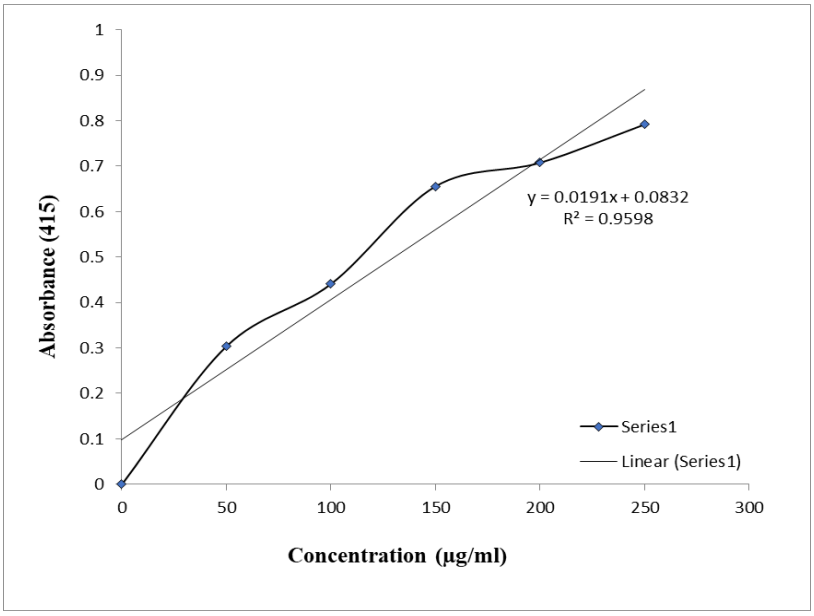


**Figure 1.** Standard curve of Quercetin for estimation of total flavanoid content in MAEPH.

### 3.2 Nitric oxide radical scavenging activity

Reactive nitrogen species (RNS), also known as nitric oxide, have the ability to pathologically alter the structural and functional behaviour of many cellular components [21]. In the current experimental setup, the sodium nitroprusside-PBS system's ability to reduce the production of nitrite in a linear, time-dependent manner served as an evaluation of the test sample's scavenging of nitric oxide. Figure 6 depicts the test sample's and the standard compound's concentration-dependent scavenging of nitric oxide (Ascorbic acid). It has been found that the test sample's concentrations (50–250 µg/ml) are all expected to display scavenging activity of nitric oxide. The test sample's ability to scavenge nitric oxide was found to be less effective than ascorbic acid. At 250 µg/ml concentration, the test sample and standard were determined to block nitric oxide by a percentage of 69.87 + 0.26 and 78.38 + 0.93, respectively (Figure 2). The data's equations from linear regression analysis were used to generate the IC50 values, which were 172.35 (test sample) and 123.32 µg/ml (ascorbic acid), respectively.

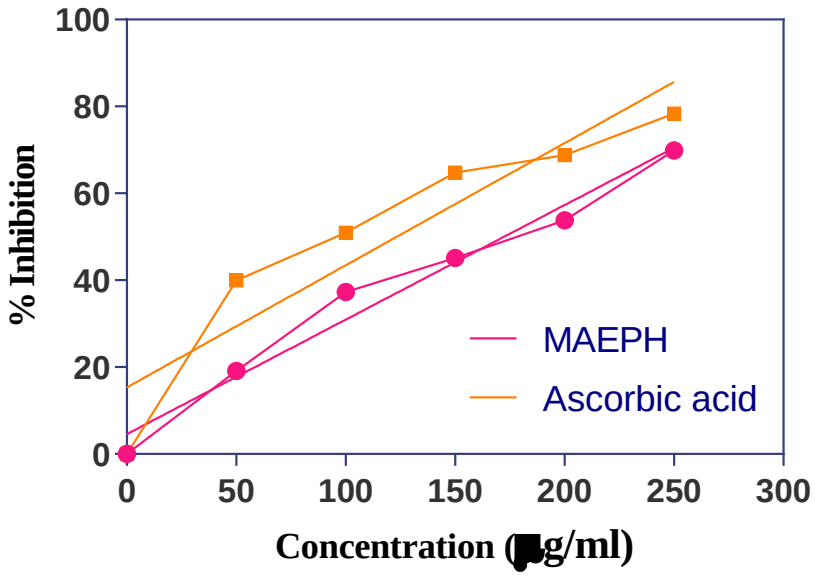


**Figure 2.** Depicting the Nitric oxide scavenging effect of MAEPH.

### 3.3 Lipid peroxidation inhibition employing egg yolk model

Using egg yolk as a lipid-enriched medium, a modified thiobarbituric acid reactive species (TBARS) experiment was employed to assess the amount of generated peroxide of lipid. In compared to conventional ascorbic acid, MAEPH was found to significantly suppress LPO at various dosages (table 1). But when compared to ascorbic acid and shown in an egg yolk model, MAEPH significantly inhibited LPO.

**Table 1.** Percentage lipid peroxidation inhibition of MAEPH in the egg yolk homogenates model

| Media | Concentration (µg/ml) | MAEPH (%) | Ascorbic acid (%) |
|---|---|---|---|
| Egg yolk | 100 µg/ml | 72.84 ± 1.06* | 84.13 ± 1.24* |
| | 80 µg/ml | 63.81 ± 1.12* | 75.42 ± 1.82* |
| | 40 µg/ml | 55.73 ± 1.21* | 69.62 ± 1.18* |
| | 20 µg/ml | 44.57 ± 1.04* | 51.18 ± 1.06* |
| | 10 µg/ml | 32.61 ± 1.01* | 45.07 ± 1.09 |

The mean of three replicate measurements plus the standard deviation (SD) is used to express values. Significant differences from ascorbic acid at *p < 0.05 and **p < 0.01.

### 3.4 Inhibition of Fe2+/ascorbate-induced lipid peroxidation using liver homogenate

As previously mentioned, goat liver homogenates were employed as a lipid-rich media to assess the lipid peroxide produced using a modified thiobarbituric acid reactive species (TBARS) assay [18]. Various concentration of MAEPH solution (10-100 µg/ml) was subjected to lipid peroxidation inhibition assay in this goat liver homogenates model (Table 2).

**Table 2.** Inhibition of Lipid peroxidation (LPO) appraisal *in vitro* using liver homogenate ***p < 0.01*

| Media | Concentration (µg/ml) | MAEPH (%) | Catechin (%) |
|---|---|---|---|
| **Liver homogenate** | 100 µg/ml | 80.45 ± 1.13** | 88.39 ± 1.41** |
| | 80 µg/ml | 71.14 ± 1.02** | 84.24 ± 1.29* |
| | 40 µg/ml | 62.36 ± 1.22* | 73.27 ± 1.80* |
| | 20 µg/ml | 51.76 ± 1.14* | 59.82 ± 1.56* |
| | 10 µg/ml | 40.13 ± 1.00* | 53.77 ± 1.02* |

The mean of three replicate measurements plus the standard deviation (SD) is used to express values. Significant differences from Catechin at *p < 0.05 and **p < 0.01.

### 3.5 Evaluation of cytotoxicity using MTT assay

The $IC_{50}$ (50 percent growth inhibition) of MAEPH against MCF7 cells at various doses was calculated using an MTT test. Figure 3 displays the outcomes of the tests, which used various amounts of MAEPH. When compared to control and typical drug concentrations, it was discovered that MAEPH doses between 100 nM and 1000 nM significantly affected MCF7 cells on MTT assays. The MAEPH concentration with the highest level of cytotoxicity against the MCF7 cell was determined to be 1000 nM, with a viability of 36.24±1.21 percent. Growth inhibition percentage rose as MAEPH concentration grew, and the assay's IC 50 value was 111 µg/ml. As a result of the investigation into the concentrations of Standard drug (Cyclophosphamide) that showed the most cytotoxicity against the MCF7 cell, it was found that the highest concentration of 1000 nM was found to have a viability rate of 8.08 ±0.97% of the cells (Table 3). It was found that the growth inhibition percentage increased with increasing MAEPH concentration.

**Table 3.** Cytotoxicity of the MAEPH as compared to standard

| Concentration (nM) | MAEPH | Standard (Cyclophosphamide) |
|---|---|---|
| 0 | 99.99±1.69 | 99.99±1.85 |
| 100 | 89.82±1.44 | 61.51±1.61 |
| 200 | 86.75±1.28 | 41.22±1.80 |
| 300 | 77.92±1.47 | 22.47±1.21 |
| 400 | 68.61±1.27 | 26.99±1.23 |
| 500 | 60.88±1.23 | 16.78±1.44 |
| 750 | 44.87±1.16 | 12.92±0.98 |
| 1000 | 36.24±1.21 | 8.08 ±0.97 |

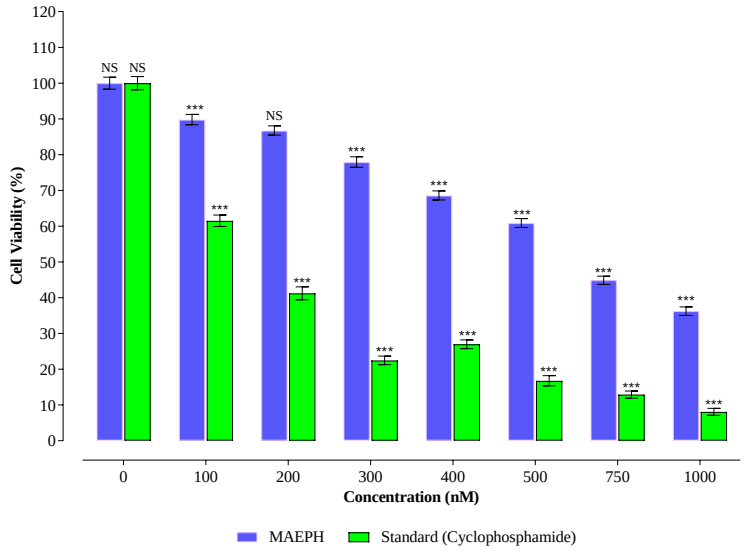


**Figure 3.** Cytotoxicity in terms of cell viability (%) of the MAEPH and Standard (Cyclophosphamide)

## 4. Discussion

With a focus on its total flavonoid content, antioxidant activity, lipid peroxide inhibition, and cytotoxic effects on MCF7 cells, the current study examines the characteristics of microwave assisted extract of leaves of *Persicaria hydropiper* (MAEPH), a traditional medicinal plant. These findings shed important light on the possible medical uses and advantages of MAEPH. The measurement of the total flavonoid concentration in MAEPH is one of the study's key components. Flavonoids are well-known for having strong antioxidant capabilities, which makes them important for maintaining health and well-being. The amount of total flavonoid content in this study is represented in milligrams per gramme of extract. This quantification technique used a regression equation built from a graph of the standard quercetin concentration. This quantification technique's accuracy and precision are demonstrated by the high R-squared value of 0.9598. The results indicate that flavonoids, which are crucial antioxidants capable of scavenging damaging free radicals, are present in significant amounts in MAEPH. This finding affirms the possibility of using MAEPH as a natural source of antioxidants, which may help shield cells and tissues from oxidative damage, hence enhancing general health.

An essential component of MAEPH's possible health advantages is its ability to scavenge nitric oxide, which is explored in this study as well. Nitric oxide is an essential component of many physiological processes, and maintaining its balance is essential for good health [22, 23]. The reduction of linear time-dependent nitrite production in the sodium nitroprusside-PBS system was employed in this study to evaluate the scavenging of nitric oxide by MAEPH. The outcomes show that between 50 and 250 µg/ml, MAEPH demonstrates concentration-dependent nitric oxide scavenging activity. This shows that MAEPH may have an impact on how the body regulates the levels of nitric oxide, which is crucial because an excess of nitric oxide is linked to a number of pathological disorders, such as inflammation and oxidative stress. The measured percentage inhibition values (69.87±0.26 percent for MAEPH and 78.38±0.93 percent for ascorbic acid at a concentration of 250 µg/ml) show that MAEPH can effectively scavenge nitric oxide, despite the fact that ascorbic acid was shown to have a somewhat higher reference compound's nitric oxide-scavenging activity. The derived and then calculated $IC_{50}$ values for MAEPH and ascorbic acid were found to be 172.35 µg/ml and 123.32 µg/ml, correspondingly and the calculated $IC_{50}$ values further highlight the concentration at which MAEPH and ascorbic acid demonstrate their inhibitory effects on nitric oxide. These numbers quantify each compound's efficiency in preventing the generation of nitric oxide and support MAEPH's possible health advantages.

In addition to being tested for its antioxidant properties, MAEPH was also looked at for its potential to prevent the development of lipid peroxide. Lipid peroxides are reactive substances that can harm cells and are linked to

several ailments, for instance cardiovascular conditions and cancer. For this study's improved TBARS experiment, homogenates of egg yolk and goat liver were utilised as lipid-enriched media as the suitable media. The outcomes show that MAEPH efficiently prevents the development of lipid peroxide in both the goat liver homogenate model and the egg yolk model. This further supports MAEPH's role as an antioxidant by indicating that it may have the ability to shield cellular membranes and lipids from oxidative damage. Importantly, MAEPH's strong antioxidant effects are shown by the fact that it significantly inhibits lipid peroxide when compared to conventional ascorbic acid/catechin. This conclusion is particularly important to human health because oxidative lipid damage is a major contributor to the emergence of many chronic illnesses [9, 24, 25].

The cytotoxic effects of MAEPH on MCF7 cells are also investigated in the study, which is important in the context of prospective cancer treatments. The $IC_{50}$ (the concentration at which 50% growth inhibition occurs) of MAEPH on MCF7 cells was established using the MTT test, a commonly used technique for evaluating cell viability and proliferation. The findings show that MAEPH has cytotoxic effects on MCF7 cells that are dose-dependent and are present at doses ranging from 100 nM to 1000 nM. At 1000 nM, the maximum level of cytotoxicity was noted, and the cell viability was 36.24±1.21 percent. This shows that MAEPH may be able to stop MCF7 cells, a popular model for investigating breast cancer, from proliferating. The concentration at which MAEPH efficiently constrains the propagation of cancer cells is indicated by the computed $IC_{50}$ value of 111 µg/ml. With a cell viability of 8.08±0.97 percent at the highest concentration examined, the standard drug Cyclophosphamide demonstrated significantly higher cytotoxicity in comparison. Although MAEPH's cytotoxic effects might not be as strong as Cyclophosphamide's, it is important to take into account the different toxicity and side effects between natural substances like MAEPH and synthetic medications. MAEPH has less cytotoxicity than cyclophosphamide, which could point to a less toxic and more tolerant form of cancer treatment.

The results of your study offer significant new information on the possible medical uses and advantages of MAEPH. The accuracy of this method is demonstrated by the quantification of total flavonoid content using a trustworthy regression equation, which also demonstrates that MAEPH is a rich source of flavonoids, which are recognised for their antioxidant effects. The study on nitric oxide scavenging activity is equally encouraging because it shows how MAEPH may be able to control the free radical damage. The concentration-dependent nitric oxide scavenging shows that MAEPH can help reduce inflammation and oxidative stress, both of which are associated to a number of health concerns. Its effectiveness is reinforced by the comparison to ascorbic acid and the computation of IC50 values. Furthermore, MAEPH's powerful antioxidant properties are shown by the considerable suppression of lipid peroxide generation it exhibits in both egg yolk and goat liver homogenate models. This capability is crucial for preventing oxidative damage to cellular membranes and lipids, potentially lowering the risk of chronic illnesses. The study of MAEPH's cytotoxic effects on MCF7 cells offers a lot of promise for the treatment of cancer. While MAEPH might not have the same effectiveness as the common medicine Cyclophosphamide, its significantly reduced cytotoxicity makes it seem like a potentially safer and more palatable substitute. Additional investigation on the cytotoxic effects of MAEPH may shed light on its potential as an anticancer agent.

## 5. Conclusion

This work highlights the promising potential of microwave-aided extract of *Persicaria hydropiper leaves* (MAEPH) as a natural and organic source of antioxidants and as a cancer treatment aid and also an exciting possibility for cancer therapy is highlighted by this study. These results offer a strong basis for additional research into the health advantages and possible applications of MAEPH, including *in vivo* trials. The study also advances the realms of natural medicine and pharmaceutical sciences, opening up new opportunities for the creation of medicines and chemicals that promote health.

**Declaration of interest**

None declared

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Nil

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