WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 5, 977-991. Research Article ISSN 2277– 7105

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT PROPERTY, DNA DAMAGE INHIBITION ACTIVITY AND CYTOTOXICITY OF ASTER (*CALLISTEPHUS CHINENSIS*) FLOWER WASTE

Shruti A. Kakodkar[1]*, Sneha N. Kshirsagar[1], Aparna S. Kelkar[1], Akhila M. Nair[1], Pranjali P. Dhawal[2], Kshitij V. Satardekar[2], Siddhivinayak S. Barve[2]

[1]Department of Biotechnology, KET's V. G. Vaze College of Arts, Science & Commerce, Mulund, Mumbai-400081, Maharashtra, India.

[2]KET's Scientific Research Centre, V.G Vaze College Campus, Mulund, Mumbai- 400081, Maharashtra, India.

Article Received on
05 Feb. 2019,

Revised on 26 Feb. 2019,
Accepted on 19 March 2019

DOI: 10.20959/wjpr20195-14575

***Corresponding Author**
Shruti A. Kakodkar
Department of Biotechnology, KET's V. G. Vaze College of Arts, Science & Commerce, Mulund, Mumbai-400081, Maharashtra, India.

ABSTRACT

Temple floral waste has become a major concern that is affecting the environment and living habitat due to its improper disposal and subsequent garnering of microorganisms. Considering this, the current study focused on evaluating potential therapeutic properties of aster (*Callistephus chinensis*) flower waste (AFW) that are often used as offerings in Indian temples and festivities. Ethanolic extract of AFW was investigated for its phytochemical constituents, total phenolic and flavonoid contents, antioxidant property, DNA damage inhibition activity and cytotoxicity. Total phenolic and flavonoid contents were determined colorimetrically using Folin-Ciocalteu reagent and aluminum chloride respectively. Antioxidant activity was studied using DPPH assay and FRAP assay. AFW extract showed the presence of phytochemicals like flavonoids, phenols, cardiac glycosides and saponins. Total phenolic and flavonoid contents were found to be 251.71±0.0029 mg gallic acid equivalent/g and 32.05mg quercetin equivalent/g respectively. The extract displayed IC_{50} value at 374.869µg/mL indicating strong antioxidant property. Also, reducing antioxidant potential of the extract increased with increase in extract concentration. DNA damage inhibition study showed that the extract could prevent hydroxyl radicals from causing DNA damage. Cytotoxicity study by MTT assay revealed no deleterious effect of the extract on mouse fibroblast cell line at low

concentrations. In conclusion, AFW extract showed presence of important metabolites such as phenols and flavonoids, strong antioxidant property and ability to protect DNA from hydroxyl radical induced damage. These observations highlight promising therapeutic potential of AFW extract against disorders resulting due to free radical oxidative stress. Further examinations are warranted to explore and isolate active phytotherapeutic constituents.

KEYWORDS: Aster flowers, phenols, flavonoids, DNA damage, Cytotoxicity.

INTRODUCTION

Free radicals and reactive oxygen species (ROS) produced in biological systems can damage cell structures, DNA, lipids and proteins leading to several health problems such as cardiovascular diseases, cancer, aging, inflammations and skin disorders.[1-3] Antioxidants are molecules capable of scavenging such free radicals and ROS and, thus are imperative in preventing the onset of diseases induced due to oxidative stress. Cellular endogenous antioxidants such as glutathione reductive enzymes and superoxide dismutase, under elevated levels of ROS, fail to protect cells from damage.[4] Supplementation of diet or medicine with exogenous synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate have been reported to alleviate oxidative stress.[5] However, the toxic effects of these synthetic antioxidants are a major setback, restricting their application on a comprehensive scale.[5] Owing to this, numerous research studies are now focusing on plants for obtaining natural antioxidants due to their ability to synthesize antioxidants and abundant availability.[6] Research studies have shown that various phytochemicals like phenols and flavonoids that are abundantly present in plants possess impressive antioxidant activity.[7-9]

India is a country endowed with diverse cultural and religious traditions. Fresh flowers of aster and marigold form an integral part of these traditions as decorations or offerings in temples. These floral decors, offerings and unsold flowers from flower markets are often disposed improperly as waste, leading to their decay by disease causing microorganisms.[10-12] Thus proper management of floral waste is important owing to the deleterious effects they pose on the environment and human health. Due to the increasing awareness of environmental sustainability, utilization of floral waste for producing valuable products has become the focus point of several research studies.[11] Studies on extracts of waste marigold, rose and saffron flowers were shown to possess the ability to act as effective natural dyes for

dyeing textile fabrics and leather.[10, 13-15] Additionally, rose flower waste obtained from Indian temples of Chennai have been successfully used for extracting essential oils.[16] Our previous research study on wasted aster (*Callistephus chinensis)* flowers showed that its natural dye could potentially replace synthetic indicators such as phenolphthalein and methyl red in titration analysis.[17] Previous phytochemical studies conducted on fresh flowers and leaves of aster cultivars in India have revealed their potential role as an antioxidant, antimicrobial and anticarcinogenic agent.[18, 19] However, to the best of our knowledge, a detailed phytotherapeutic analysis of aster flower waste (AFW) at biochemical and molecular level has not been explored in India.

Considering the hazardous impact of floral waste and limited research conducted on AFW in India, the aim of the present study was to obtain a suitable extract from wasted aster flowers and, further determine its therapeutic value by analyzing its phytochemical constituents, antioxidant property, DNA damage inhibition activity and cytotoxicity on normal mammalian cells.

MATERIALS AND METHODS

Collection and Identification: Wasted pink hued aster flowers collected from local markets of Mumbai were used for the analyses. The taxonomic authentication performed by Blatter Herbarium, St. Xavier's College, Mumbai confirmed that the flowers belonged to the plant *Callistephus chinensis* (L.) also known as *Aster chinensis* (L.).

Extraction of Phytochemicals: Ethanolic extract was obtained from AFW by performing a previously described Soxhlet extraction procedure.[17] The extract obtained was dried and stored at 4°C until further use. Further analyses were performed using this dried extract.

Preliminary Phytochemical analysis: The ethanolic extract obtained from AFW was subjected to qualitative phytochemical analysis for determining the presence of flavonoids, saponins, tannins, terpenoids, phenolic compounds and cardiac glycosides.[20]

Total phenolic content: The total phenolic content in AFW extract was determined using a modified procedure mentioned by Alhakmani *et al.* [21] 500 µL of AFW extract (1000 µg/mL) was mixed with 750 µL of Folin-Ciocalteu reagent and incubated for 10 mins. The solution was then neutralized with 750 µL of 2% Na_2CO_3 solution and incubated in dark for 30 min for color development. The absorbance was measured at 765nm wavelength on UV-Visible

Spectrophotometer (Cary 50, Varian). Similar reaction set was prepared using varied concentrations of standard gallic acid (100-1000 µg/mL). The content of phenolic compounds in AFW extract was determined with the help of standard calibration curve obtained using gallic acid. The total phenolic content was expressed as gallic acid equivalent (GAE) in mg/g of dry extract.

Total Flavonoid Content: A modified aluminium chloride method was used for quantifying total flavonoid content of AFW extract.[22] The reaction mixture was prepared by adding 30µL of 5% $NaNO_2$ to 500 µL of 100 µg/ml extract. The tube was then incubated at room temperature for 5 min. After incubation, 30µL of 10% $AlCl_3$ was added and, the reaction mixture was further incubated at room temperature for 5 min. Next, 200 µL of 1M NaOH was then added and finally the volume was made up to 1mL using distilled water. The mixture was allowed to stand for 30 min and absorbance was measured spectrophotometrically at 510 nm. Similar experiment was performed for varied concentrations of standard Quercetin (20-100 µg/mL). Quercetin standard curve and its linear equation were used to determine total flavonoid content in the extract. Flavonoid content was expressed as quercetin equivalent (QE) in mg/g dry weight of extract.

Antioxidant Activity

DPPH (2, 2-diphenyl-1- picrylhydrazyl) Free Radical Scavenging Assay: DPPH is a free radical, which on accepting a hydrogen atom from an antioxidant gets reduced and changes its color from deep purple to light yellow. This color change can be analyzed using spectrophotometer at 517 nm wavelength. Free radical scavenging activity of our extract was measured using DPPH assay with minor modification.[20] Different concentrations of AFW extract ranging between 10-100 µg/ml were prepared in methanol. DPPH reagent solution was freshly prepared in methanol. Test solution was prepared by adding 500µL of each concentration and 500µL DPPH solution. Colour blank for each respective extract concentration was prepared by adding 500µL of extract and 500µL of methanol in order to negate the absorbance imparted by the extract's characteristic colour. Negative control tubes were prepared by adding 500µL of DPPH and 500µL of methanol. All tubes were incubated in dark at room temperature for 30 min and absorbance was measured at 517 nm. Similar procedure was performed using standard ascorbic acid. Calibration curves for extract and standard ascorbic acid were plotted as percent scavenging activity *versus* concentration. The absorbance of sample (Absorbance of test - Absorbance of color blank) was expressed as *As*

and, absorbance of negative control was expressed as *Ac*. Percentage radical scavenging activity was calculated by the formula.

$$\frac{Ac - As}{Ac} \times 100$$

Ferric Reducing Antioxidant Potential (FRAP) Assay: A modified FRAP assay was performed as per the method described by Sharma *et al.*[23] Series of extract concentrations ranging between 10-100 µg/ml were prepared using 0.2M sodium phosphate buffer (pH 6). Test solutions were prepared by adding 500 µL of 1% $K_3Fe(CN)_6$ to 500 µL extract of respective concentration. Color blank for each extract concentration was set up in order to negate the effect of extract colour on absorbance. Similar set was prepared using standard ascorbic acid which served as positive control. All the tubes were incubated at 50°C in water bath for 30 min. After 30 min, 500 µL of 10% trichloracetic acid was added to each tube to terminate the reaction, followed by centrifugation at 3000 rpm for 10 min. After centrifugation, 500 µL distilled water was added to 500 µL supernatant. Lastly, 100 µL of 0.1% $FeCl_3$ was added to test and standard solutions while, 100 µL of distilled water was added to color blank. This was followed by measurement of absorbance at 700 nm.

H_2O_2-UV Induced DNA damage protection assay: Modified Comet assay was performed for assessing the property of AFW extract to protect DNA from damage caused by exposure to ultraviolet radiation and hydrogen peroxide.[24] UV-A tube (Philips, India) with radiation emission range of 340-400 nm and peaked at 365 nm was used. UV–B Bulb source (Philips, India) peaked at 312 nm was used with emission of radiation from 280-320 nm.

pBR322 plasmid DNA was used as a DNA source for the assay. For the assay, test mixture was prepared by adding 5µL of 1mg/mL AFW extract to 1 µL of 10 ng of plasmid DNA. This was followed by addition of 1µL of 0.1M H_2O_2. The volume was made up to 10µL with Tris EDTA (TE) buffer (pH 8). The reaction mixture was irradiated with UV-A and UV-B light having intensities of 1.8 mW/cm^2 and 5 mW/cm^2 respectively for 15 min. Complete plasmid DNA damage which served as positive control was obtained by adding 1µL of H_2O_2 and 8 µL of TE buffer to 1µL plasmid DNA followed by UV irradiation. Additionally, two separate aliquots of plasmid DNA of volume 1 µL were separately exposed to UV light and 0.1M H_2O_2 respectively to assess their individual effect on the DNA. Negative control consisted of untreated DNA sample suspended in TE buffer. An aliquot of plasmid DNA was also treated with only AFW extract in order to assess whether the extract had any damaging

effect on the DNA. For this, 1 µL of plasmid DNA was treated with 5µL of AFW extract and volume was made up to 10µL with TE buffer. The treated DNA samples were visualized and analyzed using 1% agarose gel electrophoresis.

MTT Assay: A modified MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay method was performed to screen the cytotoxic effect of AFW extract on normal mouse fibroblast cell line (NIH/3T3, ATCC: CRL-1658). Cell lines were cultured in DMEM (HiMedia) supplemented with 10% inactivated FBS (Gibco, Thermo Scientific) and 1% Pen-Strep (HiMedia), and further grown at 37°C in a humidified atmosphere of 5% CO_2 in air. The assay was completed in five passages using trypsin (Gibco, Thermo Scientific).

The cells were seeded in 96-well plates at a density of 2-4 X 10^4 cells/well. The plate was incubated in 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 24 hours. After the monolayer of cells was achieved the cells were treated with different concentrations of AFW extract (0.2-2 mg/mL diluted in serum-free media) for 24 hrs. MTT solution 100 µL (0.5 mg/mL) was added to the wells and incubated at 37°C in a humidified atmosphere of 5% CO_2 for 4 hrs. Upon the formation of formazan crystals the cells were lysed in 100% dimethyl sulphoxide (DMSO). The absorbance was measured after 45 min using a microplate reader at a wavelength of 560 nm. Phosphate buffer saline (PBS) served as negative control and 25% DMSO served as positive control for cytotoxicity. Cell viability was calculated using the following formula,

Cell Viability= [Absorbance of plant extract–(Absorbance of PBS- Absorbance of DMSO)] X 100 / [Absorbance of PBS-Absorbance of DMSO]

The IC_{50} values were calculated to determine the concentration of extract capable of showing 50% cell viability.

Statistical Analysis: All experiments were performed in triplicates. The results were expressed as mean ± standard deviation (SD). Results were interpreted as statistically significant when *p*-value was less than 0.05.

RESULTS AND DISCUSSION

The current scenario of increasing mismanagement of waste flowers in India has left a tremendous impact on the environment. Thus, there is a need to develop a useful remedial

measure to reduce their harmful impact on the ecosystem. Waste flowers are often managed by using solid state fermentation to produce useful products such as compost, biogas, bioethanol pigments etc.[25] However, as highlighted earlier, research studies are now focusing on exploring floral waste to obtain active phytochemical constituents that may be useful for medicinal purposes.[16] Phytochemical studies have revealed that vegetative parts of the plants such as flowers, stem, roots and leaves are rich sources of natural antioxidants.[26] Taking this into consideration and the impending impact of waste flowers on the environment, our present study was focussed on extracting useful phytochemicals from AFW and further exploring their therapeutic value. The current study aimed to provide an experimental information base highlighting various crucial phytotherapeutic properties of AFW such as total phenolic and flavonoid contents, antioxidant activity, ability to exert protection against DNA damage and cytotoxicity on normal cells.

Phytochemical Analysis of AFW extract: Table 1 enlists the results of phytochemical analysis of ethanolic AFW extract. Ethanolic extract of AFW showed presence of vital secondary metabolites like flavonoids and phenols that are reported to possess strong antioxidant properties due to their high redox potential.[7-9, 26] The extract also showed the presence of saponins which are known antimicrobial, antiviral and anticancer agents.[27] The extract also contained cardiac glycosides which are known to be useful for the treatment of congestive heart failure. [27]

Table. 1: Phytochemical analysis of AFW extract (Key: + Present, - Absent).

Phytochemicals	AFW extract
Flavonoids	+
Phenols	+
Tannins	-
Terpenoids	-
Cardiac Glycosides	+
Saponins	+

Total phenolic and flavonoid contents: Due to the detection of phenols and flavonoids in preliminary phytochemical analysis, the extract was further analyzed to determine its total phenolic and flavonoid contents. The total phenolic content was analyzed using Folin Ciocalteu assay. The linear equation was obtained from the calibration curve of standard gallic acid; $A= 0.0028X - 0.294$, $R^2= 0.9787$, where A is the absorbance and X is amount of gallic acid in µg (Fig. 1). Based on this equation, the total phenolic content in the extract was found to be 251.71 ± 0.0029 mg of GAE/g thereby, indicating the presence of high phenolic

content. The total flavonoid content, on the other hand, was determined using aluminium chloride colorimetric assay. The linear equation based on the calibration curve of standard Quercetin was A= 0.0035X - 0.022, R^2= 0.9887, where A is the absorbance and X is amount of quercetin in µg (Fig. 2). Using this equation, the total flavonoid content was found to be 32.05mg QE/g. Phenols display strong antioxidant property due to the reduction of ROS by the hydroxyl group attached to their aromatic benzene ring.[28] On the other hand, several properties of flavonoids attribute to their antioxidant property, for e.g. their ability to quench free radicals, metal ion chelation and suppression of enzymes involved in free radical formation.[8] The presence of both phenols and flavonoid in the extract strongly suggests that these constituents could be major contributing factors to the antioxidant property of AFW extract.

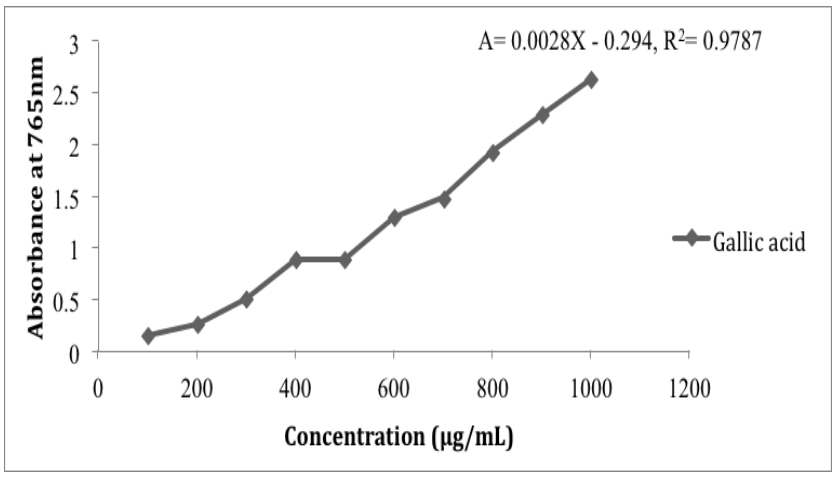

Figure. 1: Standard Calibration curve of Gallic acid for the determination of total phenolic content of AFW extract.

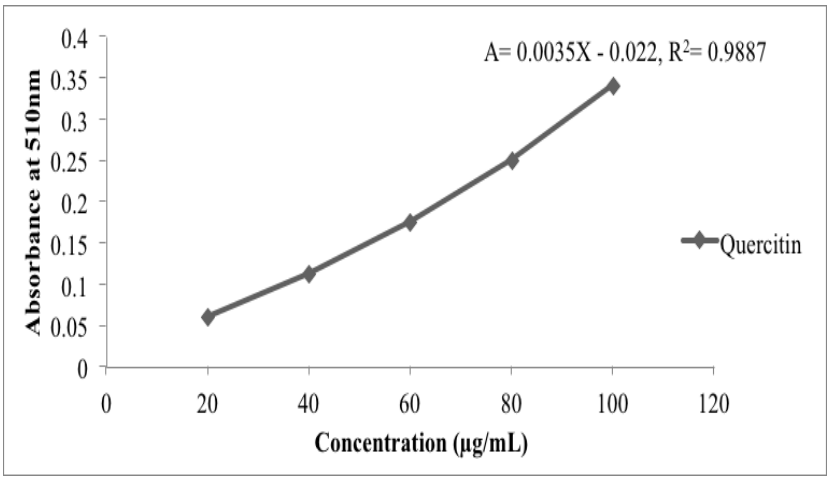

Figure. 2: Standard Calibration curve of Quercetin for the determination of total flavonoid content of AFW extract.

DPPH radical scavenging activity of extract: Based on the above analysis it is observed that the extract contains high percentage content of phenolics and flavonoids which are known to be strong antioxidants. In view of this, the AFW extract was further analyzed for its potential antioxidant property. Two simple and inexpensive antioxidant assays were performed i.e. DPPH and FRAP assays. The graph of percentage scavenging activity of AFW extract *vs.* concentration of AFW extract and percentage scavenging activity of ascorbic acid *vs.* its concentration is displayed in Fig. 3. It was observed that DPPH free radical scavenging activity increased with increase in concentration of AFW extract (R^2= 0.9358, $p < 0.001$) (Table 2). The IC_{50} value which reflects concentration of extract required to inhibit 50% of DPPH radical activity was estimated from the linear regression equation y=0.0881x + 16.974. The analysis showed IC_{50} value to be at 374.869 µg/mL. The lower IC_{50} value of the extract highlights its strong antioxidant activity. Interestingly, the strong antioxidant property of AFW extract observed in the current study is in agreement with that observed in an earlier study conducted on fresh aster flowers.[18]

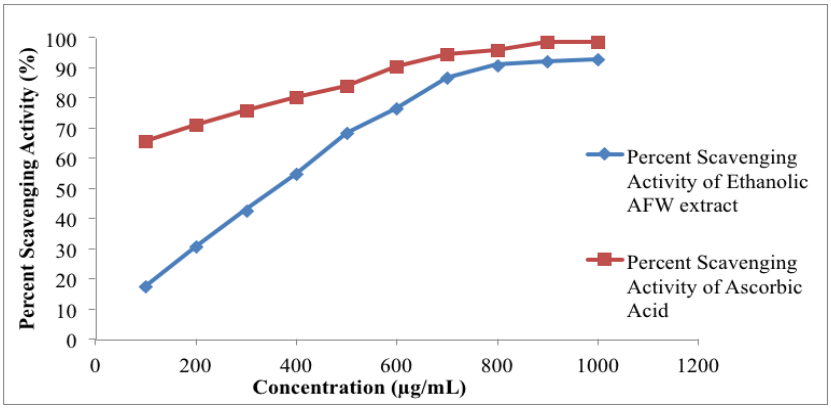

Figure. 3: DPPH free radical scavenging activity of AFW extract and standard ascorbic acid.

Table. 2: Percentage Scavenging Activity of AFW extract at various concentrations.

Concentration of AFW extract (µg/mL)	% scavenging activity
100	17.4654
200	30.8582
300	42.8029
400	54.9148
500	68.6085
600	76.6930
700	86.8118
800	91.0970
900	92.2815
1000	92.8934

FRAP analysis of extract: FRAP analysis is considered to be a simple method for determining antioxidant property.[29] It is based on the principle of antioxidant mediated reduction of ferric-tripyridyltriazine complex to a coloured, reduced ferrous form which can be evaluated spectrophotometrically at 700 nm. A sample is said to possess high reducing potential when the absorbance increases with increase in sample extract concentrations. FRAP analysis revealed a strong linear correlation between various concentrations of AFW extract and absorbance at 700 nm (Fig. 4, R^2= 0.9945, $p < 0.001$) indicating that the redox potential of the AFW extract increased with increase in its concentration (Table 3). These results further support the idea that the observed antioxidant activity of our extract may be majorly due to phenols and flavonoids present in it.

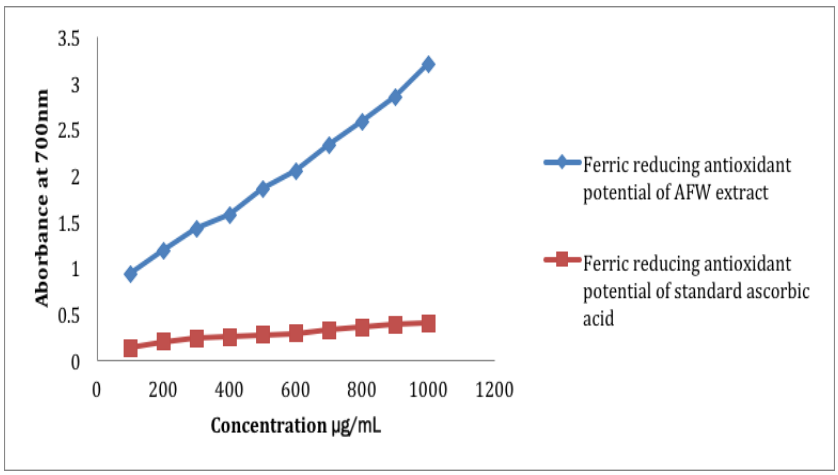

Figure. 4: Ferric reducing antioxidant potential of AFW extract and ascorbic acid.

Table. 3: FRAP analysis of AFW extract at various concentrations.

Concentration of AFW extract (µg/mL)	Absorbance of AFW extract ± SD
100	0.9257± 0.0169
200	1.1858 ± 0.0359
300	1.4167 ± 0.0089
400	1.5708 ± 0.09100
500	1.8515 ± 0.01789
600	2.0381 ± 0.06243
700	2.3279 ± 0.05968
800	2.5743 ±0.002404
900	2.8362 ±0.039103
1000	3.1995± 0.127633

H_2O_2-UV induced DNA damage inhibition activity of AFW extract: AFW extract was further tested to determine its potential application as an inhibitor of H_2O_2-UV induced DNA damage. The DNA damage results were visualized and analyzed using agarose gel

electrophoresis (Fig. 5). Hydroxyl radicals were generated as a result of the reaction between hydrogen peroxide and UV radiation. These hydroxyl radicals have a tendency to react with the nitrogenous bases of DNA thereby, producing base radicals and sugar radicals. This in turn breaks the phosphate backbone, consequently leading to DNA strand break.[24] Plasmid DNA exposed to hydrogen peroxide in the presence of UV resolves more into single stranded nicked DNA and double stranded nicked and linear DNA. On the other hand, the covalently closed circular form (ccc) gets depleted (well 1). Addition of AFW extract to plasmid DNA and subsequent H_2O_2-UV treatment resulted in the conservation of native super-coiled ccc form (well 2). This indicates that the extract conferred a protective effect on the DNA against hydroxyl radicals, thereby preventing it from undergoing strand break. This DNA conformation profile was the same as that observed in untreated intact plasmid DNA (well 3). It was further observed that ccc conformation of the DNA was not affected when DNA was separately treated with either UV radiation or H_2O_2 (well 4 and well 5). It was also seen that DNA when treated with only AFW extract did not undergo any damage (well 6). This observation is of significance as it shows that AFW extract protects DNA against oxidative damage without itself causing any DNA damage.

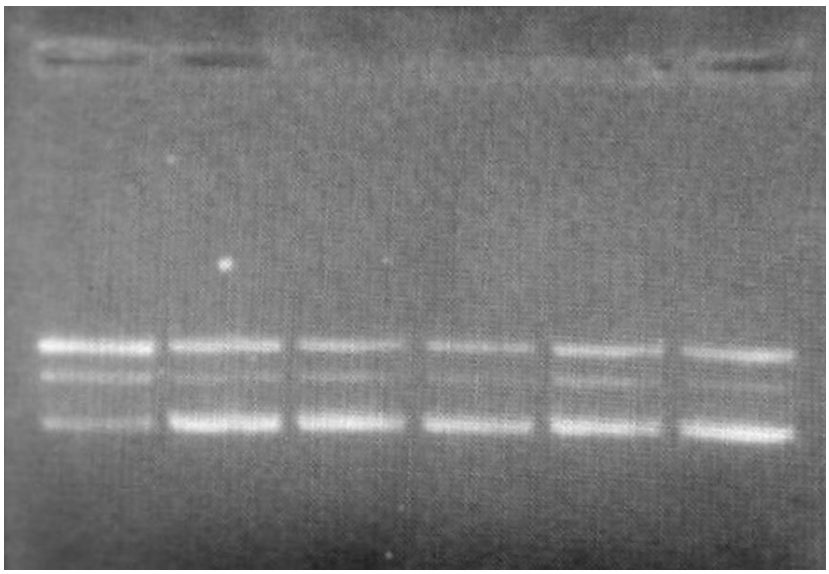

Figure. 5: Analysis of H_2O_2-UV induced plasmid DNA damage and its prevention by AFW extract. Left to right, well 1: plasmid DNA treated with H_2O_2 in presence of UV radiation, well 2: plasmid DNA treated with H_2O_2, UV radiation and AFW extract, well 3: Untreated Plasmid DNA, well 4: plasmid DNA exposed to UV, well 5: plasmid DNA treated with H_2O_2, well 6: plasmid DNA treated with only AFW extract.

MTT Assay

Evaluation of both therapeutic and toxicological effects of a potential pharmacological agent is of great significance as it gives a better insight regarding its usefulness as a medicine. *In-vitro* cytotoxic assays such as MTT assay provide a sensitive strategic approach for selecting natural products from plants that possess therapeutic value.[30] The assay principally measures the amount of cell death occurring due to the compound under study. As indicated by the aforementioned results, AFW extract was found to act as a strong natural antioxidant. Keeping this in view, we further subjected the extract to cytotoxic analysis for determining whether the extract has any toxic effect on normal mammalian cell line. As shown in Fig. 6, the regression line obtained from performing MTT assay reflected a linear relationship between concentration of AFW extract and percentage cell viability (R^2= 0.8614, p= 0.0003). The AFW extract exhibited cytotoxicity on normal mouse fibroblast cell line at concentrations above 1.27mg/mL. This result is of great significance as it shows that the extract does not cause toxicity on the normal cell line at lower concentrations. Previous Indian study conducted on aster flowers showed that ethanolic extracts of fresh aster flowers displayed promising anti-cancerous activity against human cancer cell line SKMEL-2 at 40 µg/mL and 80 µg/mL concentrations.[19] These results along with our current findings provide evidences showing that the ethanolic extract obtained from aster flowers can serve as a potential anticancer agent without causing any cytotoxicity on normal mammalian cells. However, additional *in-vivo* studies on aster flower extract are warranted to confirm these findings. Also, further investigations are necessary to determine which active components in the extract are responsible for its anticancer activity.

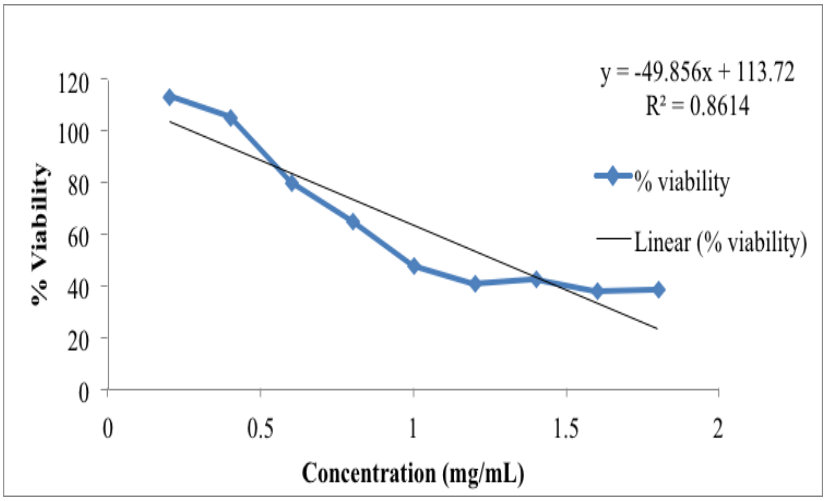

Figure. 6: MTT assay graph representing the relation between concentration of AFW extract and percentage cell viability.

CONCLUSION

In conclusion, the present study demonstrated that AFW could be utilized to extract valuable phytochemicals. The AFW extract showed high content of phenols and flavonoids along with strong antioxidant property. Also, DNA damage inhibition study showed that the extract has the capability of protecting DNA from genotoxic agents such as hydroxyl radicals. This could be attributed to the vast array of phytochemicals detected in the studied extract. The extract also displayed low cytotoxicity on normal mouse fibroblast cell line. This finding is of significant importance as it shows that ethanolic AFW extract is not deleterious to normal cells. However, further phytochemical studies are required to identify the active compounds present in the extract in order to understand the mechanism of action and applications of these constituents.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Biotechnology (DBT), Government of India for providing financial support under the STAR College Scheme. We would also like to extend our gratitude to KET's Scientific Research Centre for providing necessary research facilities for conducting the study.

REFERENCES

1. Partap S and Pandey S. A Review on Herbal Antioxidants. J Pharmacogn Phytochem, 2012; 1(4): 26-37.
2. Lien Ai Pham-Huy, Hua He, Chuong Pham-Huy. Free Radicals, Antioxidants in Disease and Health. Int J Biomed Sci., 2008; 4(2): 89-96.
3. Pai VV, Shukla P, Kikkeri NN. Antioxidants in dermatology. Indian Dermatol Online J. 2014; 5(2): 210-14.
4. Dong-Ping Xu, Ya Li, Xiao Meng, Tong Zhou, Yue Zhou, Jie Zheng, Jiao-Jiao Zhang, Hua-Bin Li. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int J Mol Sci., 2017; 18: 96.
5. Jiang Y, Han W, Shen T, Wang MH. Antioxidant activity and protection from DNA damage by water extract from Pine (Pinus densiflora) bark. Prev Nutr Food Sci., 2012; 17(2): 116-21.
6. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci., 2015; 11(8): 982-91.

7. Verma K, Shrivastava D, Kumar G. Antioxidant activity and DNA damage inhibition in vitro by a methanolic extract of Carissa carandas (Apocynaceae) leaves. Journal of Taibah University for Science, 2015; 9(1): 34–40.
8. Banjarnahor SDS, Artanti N. Antioxidant properties of flavonoids. Med J Indones, 2014; 23(4): 239-44.
9. da Gama RM, Guimarães M, de Abreu LC, Armando-Junior J. Phytochemical screening and antioxidant activity of ethanol extract of Tithonia diversifolia (Hemsl) A. Gray dry flowers. Asian Pac J Trop Biomed, 2014; 4(9): 740-42.
10. Singh P, Borthakur A, Singh R, Awasthi S, Pal DB, Srivastava P, Tiwary D, Mishra PK. Utilization of temple floral waste for extraction of valuable products: A close loop approach towards environmental sustainability and waste management. Pollution, 2017; 3(1): 39-45.
11. Yadav I, Juneja SK, Chauhan S. Temple Waste Utilization and Management: A Review. International Journal of Engineering Technology Science and Research, 2015; 2(Special issue): 14-19.
12. Vankar PS, Shanker R, Wijayapala S. Utilization of Temple waste flower- Tagetus erecta for Dyeing of Cotton, Wool and Silk on Industrial scale. J Text Appar Technol Manag, 2009; 6(1): 1- 15.
13. Mahindrakar A. Floral Waste Utilization- A Review. Int J Pure App. Biosci, 2018; 6(2): 325-29.
14. Pervaiz S, Mughal TA, Najeebullah M, Khan FZ. Extraction of natural dye from Rosa damascena Miller.-a cost effective approach for leather industry. Int J Biosci, 2016; 8(6): 83-92.
15. Raja ASM, Pareek PK, Shakyawar DB, Wani SA, Nehvi FA, Sofi AH. Extraction of Natural Dye from Saffron Flower Waste and its Application on Pashmina fabric. Adv Appl Sci Res., 2012; 3(1): 156-61.
16. Perumal K, Moorthy TA, Savitha JS. Characterization of Essential Oil from Offered Temple Flowers Rosa damascena Mill. Asian J Exp Biol Sci., 2012; 3(2): 330-34.
17. Nair A, Kelkar A, Kshirsagar S, Harekar A, Satardekar K, Barve S, Kakodkar S. Extraction of natural dye from waste flowers of Aster (Aster chinensis) and studying its potential application as pH indicator. J Innovations Pharm Biol Sci., 2018; 5(4): 1-4.

18. Satyarum SK, Deshmukh PV. Antibacterial and antioxidant activity of flower extracts of aster and calendula sp. against skin pathogens. International Journal of Recent Trends in Science and Technology, 2016; 20(2): 197-200.
19. Satyarum SK, Deshmukh PV. In vitro antibacterial and anticancerous activity of flower and leaves of *Callistephus chinensis*. Life Sciences International Research Journal, 2016; 3(2): 62-5.
20. Dhawal P, Satardekar K, Hariharan S, Barve S. In-vitro analysis of Cinnamomum verum for formulation of bio-active cosmetic gel. Int J Pharm Sci Res., 2017; 8(7): 2988-95.
21. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in–vitro antioxidant and anti–inflammatory activity of flowers of Moringa oleifera. Asian Pac J Trop Biomed, 2013; 3(8): 623-27.
22. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. Journal of Taibah University for Science, 2015; 9(4): 449-54.
23. Sharma S, Vig AP. Preliminary phytochemical screening and in vitro antioxidant activities of Parkinsonia aculeata Linn. Biomed Res Int, 2014; Article ID 756184.
24. Fatima SS, Govekar SU, Satardekar KV, Barve SS, Dhawal PP. In vitro analysis of ethanolic extract of flowers of Calendula officinalis for antioxidant, antimicrobial and UV-H_2O_2 induced DNA damage protection activity. J Pharmacogn Phytochem, 2018; 7(5): 2378-83.
25. Waghmode MS, Gunjal AB, Nawani NN, Patil NN. Management of floral waste by conversion to value-added products and their other applications. Waste and Biomass Valor, 2018; 9(1): 33-43.
26. Padalia H, Chanda S. Evaluation of antioxidant efficacy of different fractions of Tagetes erecta L. flowers. IOSR J Pharm Biol Sci., 2014; 9(5): 28-37.
27. Njeru SN, Matasyoh J, Mwanikic CG, Mwendia CM, Kobiad GK. A Review of some Phytochemicals commonly found in Medicinal Plants. International Journal of Medicinal Plants. Photon, 2013; 105: 135-40.
28. Foti MC. Antioxidant properties of phenols. J Pharm Pharmacol, 2007; 59(12): 1673-85.
29. Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr Res., 2003; 23: 1719- 26.
30. Ogbole OO, Segun PA, Adeniji AJ. In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. BMC Complement Altern Med., 2017; 17(1): 494.