

In vitro Neuroprotective and Antioxidant Studies on Extract of *Woodfordia fruticosa* Flowers and its Phytochemical Analysis

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ABSTRACT

Aim: Medical illnesses known as Neurodegenerative Diseases (NDDs) have generated considerable attention in the science-based community. The *in vitro* approach for determining the antioxidant potentials of *W. fruticosa* flowers and their neuroprotective effects. **Materials and Methods:** *Woodfordia fruticosa* flower extract samples that underwent GC-MS analysis. DPPH and ABTS Radical Scavenging assays were performed. The neuroprotective potential of the selected extract was assessed using IMR-32 by MTT assay. **Results:** Twenty-one compounds were found in the methanolic leaf extract of *Woodfordia fruticosa*, according to the GC-MS analysis. Using the DPPH and ABTS assays, *in vitro* antioxidant activity was assessed. The standard and test samples produced various concentrations (12.5, 25, 50, 100, and 200 g/mL). Percentage suppression of the anti-oxidant action is shown. Ten distinct acetone extract compounds and nine different ethanol extraction components were found. Both preparations are more effective in scavenging free radicals as their concentration increases. **Conclusion:** The current research demonstrates *W. fruticosa*'s ability to protect neurons from Lipopolysaccharide-induced neurotoxicity in neuronal cells. Maximum cytotoxic effect was found in the MTT solubilization found better at high-end concentrations than at Lower concentrations among both extracts.

Keywords: Antioxidant, Anti proliferative, Hexadecanoic acid, Furfural, Ayurvedic, Cytotoxic.

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INTRODUCTION

The Lythraceae family includes the sprawling, leafy shrub *Woodfordia fruticosa* (L.). Many experts in different South-East Asian regions have suggested the plant as a conventional remedy. The blossoms are widely utilised in the Indian subcontinent as well as other South Asian nations to make the fermenting Ayurveda known as Aristhas and Asavas.^[1] The plant's flowers are

traditionally used to treat wounds, ulcers, and to reduce blood in an emergency. The use of the entire plant in ayurvedic medicines has historically given it importance.^[2] Additionally, it has been claimed that the dried blossoms of this plant can cure wounds and peptic ulcers by enhancing granulation and reducing discharge. Bright crimson, pungent, and bitter flowers that are used specifically to make herbal remedies have uterine sedative and anthelmintic properties.. additionally this plant used to for the treatment of dysentery, sprue, intestinal complaints, rheumatism, dysuria, hematuria, wounds, bleeding injuries, otorrhoea, leucorrhoea, and dysmenorrhoea were reported among this plant. In contrast to certain other minor non-phenolic components like steroids and triterpenoids, it includes a significant amount of phenolics, including hydrolyzable tannins and flavonoids.^[3] This plant's anti-inflammatory,

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anticancer, hepatoprotective, immunomodulatory, antibacterial, and free radical scavenging activities are present in all parts, making it a significant therapeutic resource.^[4] Recently, this genus has yielded a variety of bioactive chemicals, including flavonoids, anthraquinone glycosides, polyphenols, and tannins (particularly those of the macrocyclic hydrolysable class). This chemical's metabolites and compounds, especially those from the flowers and leaves, have some significant therapeutic effects.^[5]

There is a high demand for the blooms in both local and foreign markets that are focused on the production of herbal remedies, despite the fact that diverse components of the plant have long been recognised in medicinal purposes.^[6] The flowers of this plant serve as an anthelmintic and uterine sedative in Indian systems of medicine because they are caustic, cooling, poisonous, and aromatic. These flowers are also helpful for toothaches, leucorrhoea, menorrhagia, dysentery, leprosy, and blood disorders. A class of substances known as antioxidants prevents the production of free radicals or prevents the spread of free radicals through a variety of processes. They support the prevention or mitigation of oxidative damage to cell macromolecules brought on by a potential xenobiotic or a developing pathogenic situation (diabetes).^[7,8] It is anticipated that addressing OS or increasing innate antioxidant potential through reactive supplementation will have a preventive role on the treatment of various illnesses, particularly diabetic and neurological diseases.^[9]

MATERIALS AND METHODS

Plant Material

Woodfordia fruticosa flowers were collected from Kozhikode, Kerala. The plant was identified and authenticated by the Botanical Survey of India, Southern regional centre, Coimbatore.

Preparation of ethanol and acetone extract

Flowers from *Woodfordia fruticosa* were thoroughly cleaned by washing them multiple times in pure water. The material is then completely air-dried under shadow, ground into a coarse powder, and extracted with ethanol and acetone for 50 hr. Under reduced pressure, all traces of alcohol and acetone are eliminated, yielding semi-solid extracts.

GC-MS analysis

The sample extract underwent GC-MS profiling using a Shimadzu GC-MS. Chromatographic circumstances It was a 30 mm long, 0.25 mm internal diameter, and

0.25 mm thick Elite-5 MS columns. The sample was injected into the carrier gas, helium, at a flow rate of 1 microliters per minute. The oven is kept at a constant 280°C. The entire time required for the GC to run was 50 min. It was possible to calculate the relative percentage amount of each component by measuring the mean peak area to the overall area.

In vitro antioxidant Assay

DPPH Radical Scavenging assays and ABTS assays were used to assess the antioxidant potential of the extracts.

DPPH Radical Scavenging assay

The DPPH assay was used to measure the oxidative activity of ethanol and acetone preparations. A reference without the test substance is also made. Samples of concentrations ranging from 12.5 g/mL to 200 g/mL were combined with a 2 mL reaction mixture with 0.2 mL DPPH in ethanol, and this reaction mixture was incubated at room temperature in the dark for 20 min. Ascorbic acid was employed as the positive control when measuring absorption read at 517 nm.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) assay

By using the ABTS test, the antioxidant activity of ethanol and acetone were evaluated. ABTS reagent with different concentration of extract. Antioxidant properties found in the extract lowered the amount of produced radical monocation. At 734 nm, absorbance was observed. Ascorbic acid served as the benchmark.

In vitro neuroprotective effect

cultured in Dulbecco's modified eagles' medium, the neuroprotective potential of the chosen extract was evaluated. After the cell line has grown sufficiently, Lipopolysaccharide (1 g/mL) is used to cause neuro inflammatory. Following an hour of incubation, prepared extracts were added to the appropriate wells. After a 24-hr incubation period, the sample content in the wells was withdrawn, and MTT solution was applied to all test and cell control wells. The wells were then incubated again for 4 hr. The MTT solubilization solution (DMSO) was added after the supernatant was removed, and the absorbance was assessed using a microplate reader at a wavelength of 540 nm.

RESULTS

The plant is identified *Woodfordia fruticosa* and accessed as BSI/ SRC/ 5/ 23/ 2020/ Tech/63 by Dr. M.U. Sharief, The Scientist 'E' and Head of the office, Botanical Survey of India (Southern Region)

Coimbatore. The GC-MS spectrum of ethanolic extract (Figure 1) and its corresponding Phytochemicals were detected from the chromatogram is shown in Table 1. Figure 2 shows GC-MS spectrum of acetone extract, and the compounds identified using NIST are listed in Table 2. Octacosane is the major compound found in ethanol extract and 1,2,3 benzenetril was the major compound detected in acetone extract. In Figure 3, the antioxidant strength of *Woodfordia fruticosa* Ethanolic Extract (WFFE) and *Woodfordia fruticosa* Acetone Extract (WFFA) is expressed in percentage terms about their capacity to scavenge DPPH free radicals between 12.5 to 200 µg and found to be increased by increasing concentration. Acetone extract demonstrated 89% (IC₅₀ = 24.83 g/mL), which is equivalent to the positive control ascorbic acid, while ethanol extract had the greatest radical scavenging activity (90%) 200 micrograms (IC₅₀ = 15.8 g/mL). The graph in Figure 3 showing the percentage of inhibition and concentration demonstrates a steady increase in the scavenging ability of plant extract. Using the ABTS scavenging assay, the antioxidant of the *Woodfordia fruticosa* flower and leaf extracts in ethanol and acetone was examined. Figure 4 depicts the profound and considerable percentage scavenging action of *Woodfordia fruticosa* flower extracts. Ethanol extract shows a minimum of 18% at 12.5 µg and a maximum of 79% at 200 µg with IC₅₀ 775µg. Extract of acetone shows a minimum of 15% at 12.5 µg ABTS scavenging and a maximum of 78% at 200µg with IC₅₀ 703 µg.

The bar graph displays the percentage of cell viability in the presence of neuroblastoma cell visibility stated over amounts of plant extract. Cell viability that matched control cells was taken to be 100%. The extract acted on cells in a dose-dependent manner, as observed through MTT assays between 6.25 to 100µg. The WFF ethanolic extract shows proliferative inhibition 56≥59≥65≥70≥93% and 57≥62≥68≥69≥76% by acetone extract (Figure 5). Cell viability was assessed following a 24-hr incubation with fractions of WFFE and WFFA in culture media at escalating concentrations (6.25, 12.5, 25, 50, and 100 g/mL).

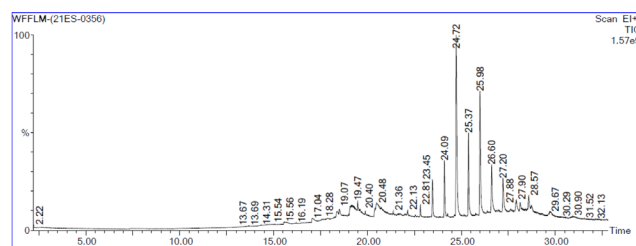


Figure 1: Chromatogram of ethanolic extract of *Woodfordia fruticosa*.

Table 1: Compounds of ethanol extract of *Woodfordia fruticosa*.

Peak	Retention Time	Area%	Name	Base m/z
1	19.150	3.62 6.474	N-Hexadecanoic acid	43.1946
2	19.470	1.289	1-Hexyl-1-nitrocyclohexane	43.2645
3	23.452	4.420	Hexatriacontane	57.3163
4	24.087	5.658	tetratetracontane	57.3163
5	24.718	27.223	octacosane	57.3163
6	27.904	2.527	Octadecane, 1-chloro	43.2645
7	28.114	1.244	URS-12-EN-28-OL	43.1946
8	28.569	2.158	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.BETA.,4.ALPHA.,5.ALPHA.)-	41.2362

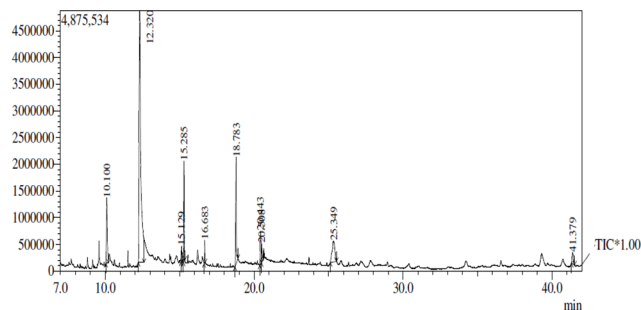


Figure 2: Chromatogram of acetone extract of *Woodfordia fruticosa*.

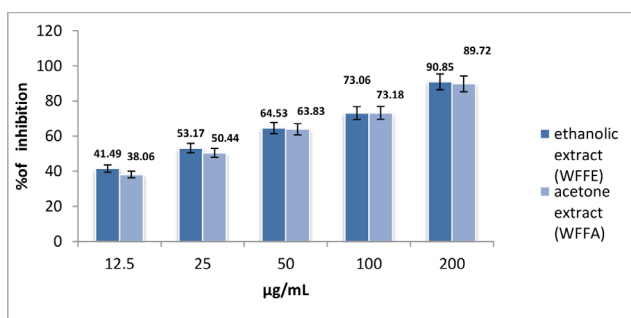
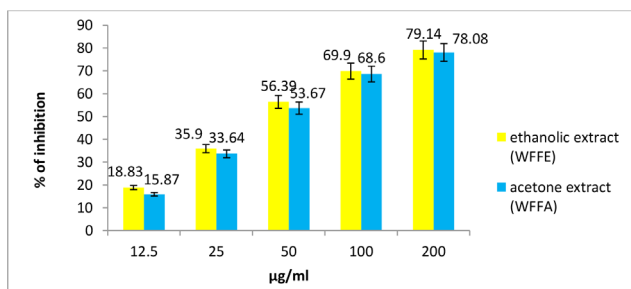
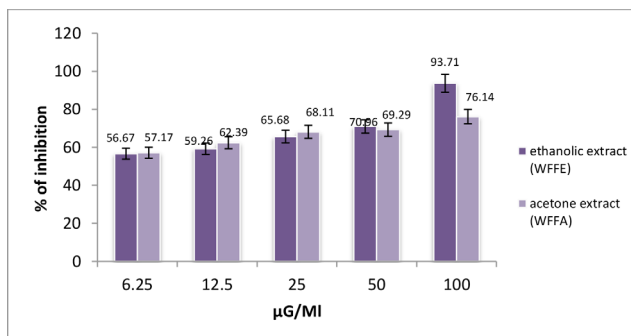
Of the studied fractions, fraction WFFE substantially and concentration-dependently reduced the cell toxicity. Very intriguingly, both fractions demonstrated neuroprotectivity equivalent to and equal to control at greater concentrations when the neuroprotective effect caused by fractions was compared with control.

DISCUSSION

The chromatograms exhibited the presence of numerous biologically active compounds, especially fatty acid derivatives. Applying the WILEY 8 database and the National Institute of Standards and Technology (NISTII), phytoconstituents were detected. The comparison of retention duration and peak, computer matching, and the distinctive fragmentation patterns of the mass spectra were utilised as criteria for defining phytochemical constituents. Ethanol extract showed 8 different peaks and acetone showed 10 different peaks with two sterols. Both solvent extracts demonstrated positive results for their presence in their respective solvents in the current study of phytochemical analysis

Table 2: Compounds of acetone extract of *Woodfordia fruticosa*.

Peak	Retention Time	Area	Area%	Name	Base m/z
1	10.100	4876531	7.73	5-Hydroxymethyl furfural	97.05
2	12.320	37980864	60.22	1,2,3Benzenetriol	126.05
3	15.129	699380	1.11	Galactitol, 1,2:4,5-di-o-isopropylidene-	59.05
4	15.285	3554876	5.64	Diisopropylidene mannitol	101.10
5	16.683	748544	1.19	Tetradecanoic acid	73.05
6	18.783	5562231	8.82	Hexadecanoic acid	73.05
7	20.443	2252457	3.57	Linoleic acid	67.05
8	20.508	1040231	1.65	Alpha.-linolenic acid	79.10
9	25.349	4980632	7.90	Chondrillasterol	271.20
10	41.379	1372863	2.18	Gamma.-sitosterol	55.05

**Figure 3: DPPH assay of *Woodfordia fruticosa* flowers (acetone and ethanol extract).****Figure 4: ABTS assay of *Woodfordia fruticosa* flowers (acetone and ethanol extract).****Figure 5: In vitro neuroprotective effect determination by MTT *Woodfordia fruticosa* flower cell viability.**

for such significant metabolites, and the findings also suggested that *Woodfordia fruticosa* leaves are excellent sources of these bioactive molecules. In accordance with

results, ethanol extract showed that plant consists of a high alkane and benzenetriol group in acetone which help in antioxidant activity as well as the neuroprotective activity.^[10,11] The outcome revealed here suggests that an ethanol extract of *W. fruticosa* flowers has antioxidant and neuroprotective properties. Further evaluation of WFFE showed high antioxidant ability as observed by DPPH followed by ABTS assay. On the basis of the Literature survey, different solvent extracts of *Woodfordia fruticosa* exhibited maximum antioxidant in the polar than non-polar solvent extraction method.^[12,13] Studies have examined the biological functions of the extract with fatty acids and sterol compounds, which have been suggested to be possible antioxidants and free-radical scavengers. A similar antioxidant report was given by Chaturvedi *et al.*^[14] These results support earlier research that indicated *Woodfordia fruticosa* floral acetone fraction has the strongest ABTS radical scavenging property than ethanol extract.^[15] This finding implies that phytochemical substances found in fractions were probably to postpone the degeneration of neurons in culture media.^[16] The hepatoprotective and anti-proliferative activity of *Woodfordia fruticosa* was well documented.^[17,18] alcoholic extract from WFFE flower showed their promising protective effects against IMR-32 -induced cell death after the treatment of these extracts. Results from the cell viability assay confirmed the ability of ethanol extract to enable cell proliferation by protecting the cells from oxidative damage. the effect of *Woodfordia fruticosa* flower of suspension cell culture bioactive substances have neuropharmacological and neuroprotective effects were reported for the first time in this study. Preventing the oxidative damage of cells by phytochemical has the ability to control neurological disorders. The variety of neuroprotective mechanisms of natural plant extracts may allow researchers to screen drugs for neurological disorders.^[19,20] Recent studies suggest that oxidative stress plays a significant role in the pathogenesis of numerous neurodegenerative

cascades. The present data on the antioxidant activity of the flowers would help to evaluate the traditional knowledge from a scientific perspective. Further study is required to be carried out to analyze the phytochemicals of these medicinal flowers in detail and to explore the efficacy and dose *in vivo* on neuroprotective.

CONCLUSION

Phytochemical analysis of the *Woodfordia fruticosa* flower extract reveals many substances with strong antioxidant and neuroprotective properties. In conclusion, the current investigation demonstrated that the levels of ABTS and DPPH activity in plant extracts greatly varied. The extracts' active components are primarily responsible for their antioxidant and neuroprotective properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Flowers of *Woodfordia fruticosa* extracted with acetone and ethanol and its phytochemical reveals the presence of fatty acid esters along with some alkane, sterols URS-12-EN-28-OL and furfural. Both extracts showed significant antioxidant in DPPH and ABTS assay method. *In vitro* neuroprotective effect determination by MTT *Woodfordia fruticosa* of both extract exhibited more than 50% inhibition at 6.5µg/mL but maximum inhibition 93% recorded in ethanol extract. These findings suggest the potential use of *W. fruticosa* flowers in the treatment of neurological disorders.

REFERENCES

- Mishra S, Sonter S, Kumar Dwivedi M, Kumar Singh P. Anti sickling potential and chemical profiling of traditionally used *Woodfordia fruticosa* (L.) Kurz leaves. Arab J Chem. 2022;15(1):103539. doi: 10.1016/j.arabjc.2021.103539.
- Khare CP. Indian herbal remedies: rational western therapy, ayurvedic and other traditional usage Botany. Berlin, Heidelberg, New York: Springer-Verlag; 2004:483.
- Kaur R, Kaur H. The antimicrobial activity of essential oil and plant extracts of *Woodfordia fruticosa*. Arch Appl Sci Res. 2010;2:302-9.
- Shah AS, Juvekar AR. *In vitro* and *in vivo* immune stimulatory activity of *Woodfordia fruticosa* flowers on non-specific immunity. Pharm Biol. 2010;48(9):1066-72. doi: 10.3109/13880200903490497, PMID 20731559.
- Pratap KD, Suchandra AC, Nilendu P, Sukdeb B, Niranjan PS, Basudeb A. *Woodfordia fruticosa*: traditional uses and recent findings. J Ethnogr Pharmacol. 2007;110:189-99.
- Hemraj Upmanyu M, Gupta A, Jindal A, Jalhan S. Pharmacological activities of *Stephania glabra*, *Woodfordia fruticosa* and *Cissempele spareira*- a review. Int J Pharm Pharm Sci. 2012;4(3):16-23.
- Brewer MS. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Compr Rev. 2011;10(4):221-47. doi: 10.1111/j.1541-4337.2011.00156.x.
- Shankar K, Mehendale HM. Oxidative stress. 3rd ed. Amsterdam, The Netherlands: Elsevier; 2014;3:735-7.
- Sen S, Chakraborty R. Oxidative stress: diagnostics, prevention, and therapy. Washington, DC: ACS Publications; The role of antioxidants in human health; 2011;1-37.
- Bhatt LR, Seung HB. Radical scavenging activity of extracts and various fractions of *Woodfordia fruticosa* J Cosmet Pub Lic Health. 2005;1(2):68-70.
- Syed Y, Khan M. Chromatographic Profiling of ellagic acid in *Woodfordia fruticosa* Flowers and Their gastroprotective Potential in Ethanol-Induced Ulcers in Rats. Pharmacogn Res. 2016;8;Suppl 1:S1-11.
- Grover N, Patni V. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa* (L.) Kurz. Leaves. Int J Pharm Pharm Sci. 2013;5(13):291-5.
- Abegunde SM, Ayodele-Oduola RO. Comparison of efficiency of different solvents used for the extraction of phytochemicals from the leaf, seed and stem bark of *Calotropis procera* Int J Sci Res. 2013;4(7):835-8.
- Grover N, Patni V. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa* (L.) Kurz Leaves. Int J Pharm Pharm Sci. 2013;5(4):291-5.
- Chaturvedi PA, Ghatak AA, Desai NS. Evaluation of radical scavenging potential and total phenol content in *Woodfordia fruticosa* from different altitudes. J Plant Biochem Biotechnol. 2012;21(1):17-22. doi: 10.1007/s13562-011-0066-1.
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84. doi: 10.1016/j.biocel.2006.07.001, PMID 16978905.
- Chandan BK, Saxena AK, Shukla S, Sharma N, Gupta DK, Singh K, et al. Hepatoprotective activity of *Woodfordia fruticosa* Kurz flowers against carbon tetrachloride induced hepatotoxicity. J Ethnopharmacol. 2008;119(2):218-24. doi: 10.1016/j.jep.2008.06.020, PMID 18639619.
- Khan IA, Singh A, Mindala DP, Meena S, Vij B, Yadav AK, et al. Preclinical development of gastro-protective botanical candidate from *Woodfordia fruticosa* (Linn.) Kurz: chemical standardization, efficacy, pharmacokinetics and safety pharmacology. J Ethnopharmacol. 2019;241:112023. doi: 10.1016/j.jep.2019.112023, PMID 31195031.
- Law BNT, Ling APK, Koh RY, Chye SM, Wong YP. Neuroprotective effects of orientin on hydrogen peroxide-induced apoptosis in SH-SY5Y cells. Mol Med Rep. 2014;9(3):947-54. doi: 10.3892/mmr.2013.1878, PMID 24366367.
- Iriti M, Vitalini S, Fico G, Faoro F. Neuroprotective Herbs and Foods from Different Traditional Medicines and Diets. Molecules. 2010;15(5):3517-55. doi: 10.3390/molecules15053517, PMID 20657497.

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