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Separation And Characterization of Active Phytoconstituents (Phenols, Carbonyls, Alkenes, And Carboxylic Acid) Of Extracts of Ziziphus Nummularia Leaves and Fruits by Maceration Process and Hot Continuous Extraction Method by Soxhlet Apparatus with Proton NMR (Nuclear Magnetic Resonance) Spectroscopy

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ABSTRACT

Diabetes mellitus is a complex set of metabolic disorders characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism 1 resulting from defects in insulin secretion, insulin action or both 2. Recent evidence suggests that oxidative stress may contribute to the pathogenesis of Type-2 Diabetes mellitus by increasing insulin resistance or impairing insulin secretion 3.Elevated extra and intra cellular glucose concentrations results in oxidative stress 4 which was reported both in animals in experiment and in diabetic patients 5. The source of oxidative stress is a cascade of ROS leaking from the mitochondria. This process has been associated with the onset of type 1 diabetes (Type-1DM) via the apoptosis of pancreatic beta-cells, and the onset of type 2 diabetes (Type-2DM) via insulin resistance 6.A possible benefit of vegetables and fruits is from their antioxidant components and thus a contribution to reduction of systemic oxidative stress 7. Vegetables and fruits have been shown to contain high concentrations of antioxidants, which might reduce the risk of diabetes especially Type-2 DM. In NMR spectrum signals are proportional to their molar concentration making the direct comparison of concentration of all compounds possible without the need of calibration curve of each individual compound. It reflects the real molecular levels of metabolites present in the plant.

Keywords: Ziziphus Nummularia, Antioxidants, Oxidative stress, Nuclear Magnetic Resonance..

INTRODUCTION

Diabetes mellitus is a complex set of metabolic disorders characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both 8. Recent evidence suggests that oxidative stress may contribute to the pathogenesis of Type-2 Diabetes mellitus by increasing insulin resistance or impairing insulin secretion 9. Globally, the number of people suffering from diabetes is increasing at an alarming rate 10.

The long-term, relatively specific complications of diabetes mellitus are predominantly vascular and include the development of retinopathy, nephropathy and neuropathy. People with diabetes also have a significantly increased risk of cardiac, peripheral arterial and cerebro-vascular disease 8. Herbal medicines have long been used for the treatment of diabetes mellitus. This is because such herbal plants have hypoglycaemic properties and other beneficial effects. Herbal medicines have the advantage of usually having no or less side-effects 11. Most of these plants have antioxidant activities 12 and hence, prevent or treat hard curable diseases, other than having the property of combating the toxicity of toxic 13 or other drugs 14. Several studies examining dietary patterns and incidence of Type-2 diabetes have been also shown that vegetables and fruits are important components of the dietary patterns

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

associated with a decreased risk of Type-2 diabetes 12, 16.

Characterization of Ziziphus nummularia

Ziziphus nummularia, also called Jharberi, is species of *Ziziphus* native to the western India and south-eastern Pakistan and south Iran 10. *Ziziphus nummularia* leaves and fruits are used for mental retardation, preventing frequent attacks of colds and influenza, treating diarrhoea, dysentery and colic, indigestion, inflammation of gums and tonic 1. The unripe fruits of the plant are prescribed in the management of vomiting, burning sensations and as tonic, while dried fruits are useful as an anticancer, sedative, stomach ache and in treatment of anaemia, bronchitis, burns, chronic fatigue, diarrhoea, hysteria, loss of appetite and pharyngitis 15. Chemical constituents are present in leaves and fruits of *Ziziphus nummularia* are Glutamine synthetase, nitrate reductase and glutamine dehydrogenase, Ascorbic acid (vitamine C), nummularine-T, nummularine-M and N, Nummularine E, nummularine S, frangufoline, nummularine R, sterols and/or triterpenes, alkaloids, flavonoids, tannins and saponins 12, nummularine-P (I), mauritine-D, jubanine B, nummularine O (I), Jubanine-A, -B, and mauritine-C, N-desmethyl-jubanine-B (I) (Miana and Shah 1985), nummularogenin, (25S)-3 β-hydroxy-5 β-spirostane-2,12-dione (I), nummularine B, nummularine M (I), nummularine N (II), Zizynummin, sitosterol, stigmasterol, betulinic acid, oleanolic acid, ceanothic acid,β-D-glucosides of sitosterol and stigmasterol, n-octacosanol and quercetin-3-O-galactoside 17.

Plant Material and Authentication

The leaves and fruits of *Ziziphus nummularia* used in this study were obtained from cultivated plants grown in Mohanlal Sukhadia University Udaipur Rajasthan.

As the plant material was not collected from the wild, no special permissions or licences were required. All procedures adhered to institutional and national ethical guidelines for the use of plant materials in research. Fresh leaves and fruits of *Ziziphus nummularia* (Burm. f.) Wight & Arn. were collected from the botanical garden of Government Science College, Udaipur, Rajasthan, India (approximate coordinates: 24.5854° N, 73.7125° E).

Proton NMR Analysis:

The NMR analysis preformed for identification of phytoconstituents present in leaf and fruit extracts of *Z. nummularia*. The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed to yield an NMR spectrum for the nucleus concerned 18, 19. The purified sample was placed in an inert solvent [deuterochloroform (CDCl3), deuterium oxide (D2O), carbon tetrachloride (CCl4) or deuterated dimethyl sulphoxide (DMSO)] and the solution was placed between the poles of a powerful magnet. The different chemical shifts of the proton according to their molecular environments within the molecule were measured in the NMR apparatus relative to a standard, usually tetramethyl silane (TMS). Chemical shifts () are measured in ppm units, where op

$$6 \times \Delta = /10\nu\nu\delta \,.$$

Δν being the difference in absorption frequency of the sample and the reference compound (TMS) in Hertz units 20. NMR performed at Punjab University, Chandigarh

RESULT AND DISCUSSION

NMR Analysis of Extract:

The results of NMR spectra of leaves and fruits extracts of *Ziziphus nummularia* prepared by maceration and soxhlation are given in the Table 4.18 and 4.19. The peak range, percentage area and their functional groups are shown in the tables. The range of 3.310 – 3.127 ppm signifies the presence of phenols in the leaves extract by maceration and the range of 0.994 – 0.901 ppm signifies the presence of phenols in leaves extract through soxhlation. Phenols were

also reported in the range of 3.725 - 3.556 in fruits extract prepared by soxhlet method.

Based on the proton NMR data the leaves of Ziziphus nummularia contains phenols, carbonyls, alkenes and carboxylic acid.

Table: 1.1 NMR analysis of leaves extract by maceration:

SNO.	Peak range	Functional groups	% area
1	3.310 – 3.271	alcohols,phenols ethers, or esters	0.89
2	3.261 – 3.2440	alcohols,phenols ethers, or esters	1.65
3	3.169 – 3.127	alcohols,phenols ethers, or esters	3.64
4	2.8852 – 1.234	Aromatics , carbonyls, (ketones, esters, aldehydes, acids, amides) • alkenes	0.58
Fruits extract spectra maceration			
5	4.90 – 4.89	Alkene sp2 hybridized C-H's	1.65

Table: 1.2 NMR analysis of leaves and fruits extract by soxhlation:

s.no	Peak range	Functional groups	% area
1	1.760 – 1.721	carbonyls, (ketones, esters, aldehydes, acids, amides) • alkenes, or • aromatics	0.63 – 1
2	1.410 – 1.299	carbonyls, (ketones, esters, aldehydes, acids, amides) • alkenes, or • aromatics	2.82 – 2.92
3	1.169 – 1.113	carbonyls, (ketones, esters, aldehydes, acids, amides) • alkenes, or • aromatic	0.85 – 1.84
4	0.994 – 0.901	alcohols,phenols • carboxylic acids	3.08
Fruits extract by soxhlet method			
1	3.725 – 3.670	alcohols, phenols • ethers, or • esters	3.58
2	3.602 – 3.5561	alcohols, phenols • ethers, or • esters	3.82
3	3.196 – 3.066	alcohols, • ethers, or • esters	1.99
4	2.95 – 2.67	alcohols, • ethers, or • esters	0.49 – 0.83

Figure 2.1 Chromatogram of NMR spectra of leaves extract using maceration method

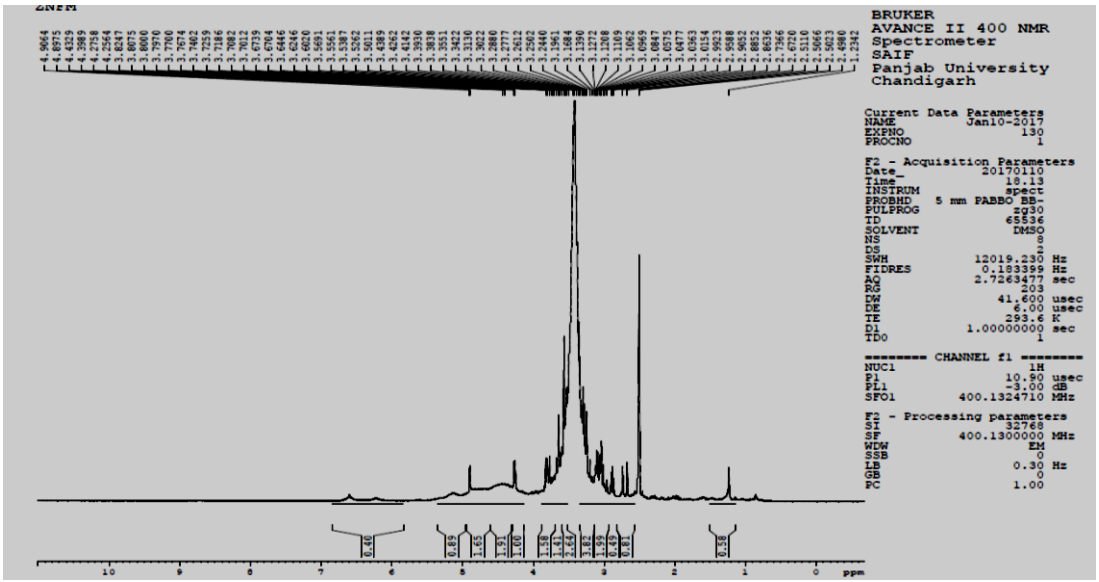


Figure 2.2 Chromatogram of NMR spectra of fruits extract using maceration method

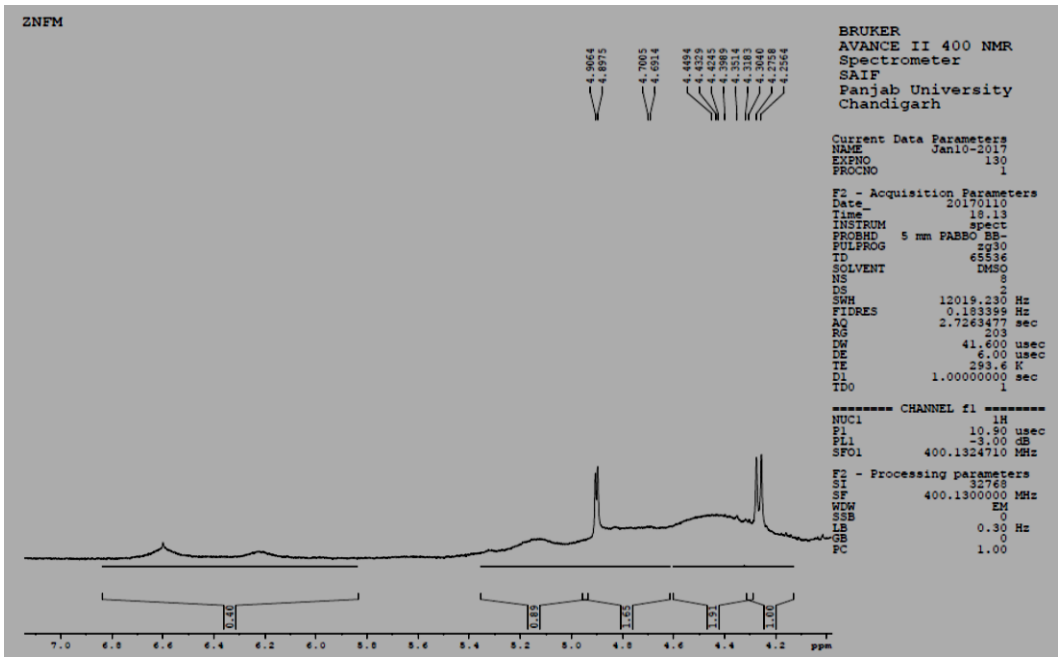


Figure 2.3 Chromatogram of NMR spectra of leaves extract using soxhlet method

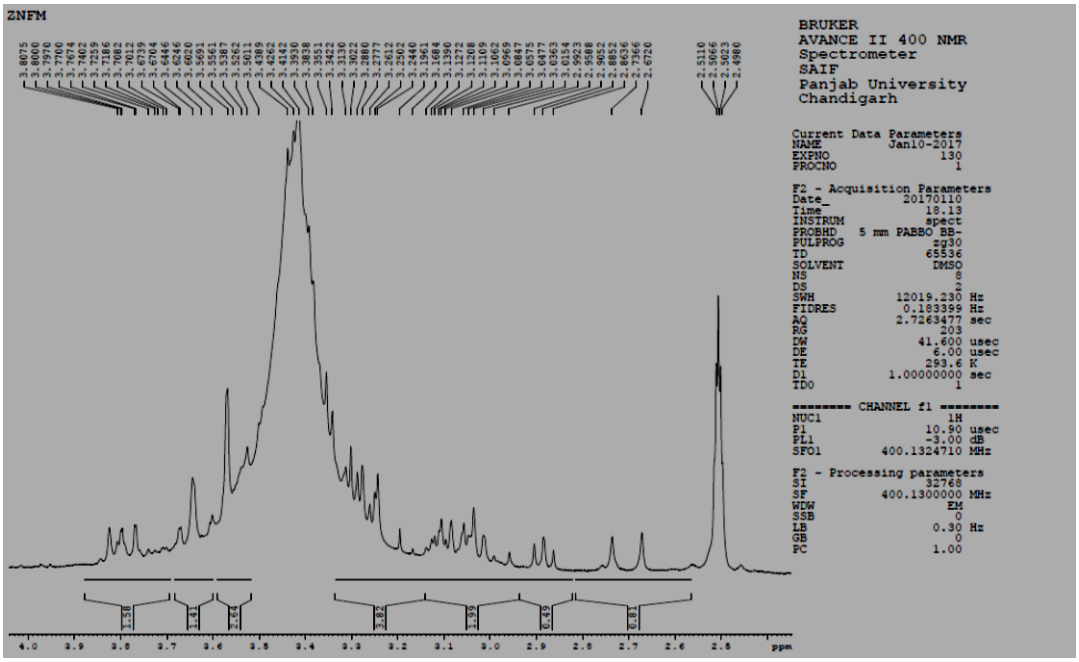
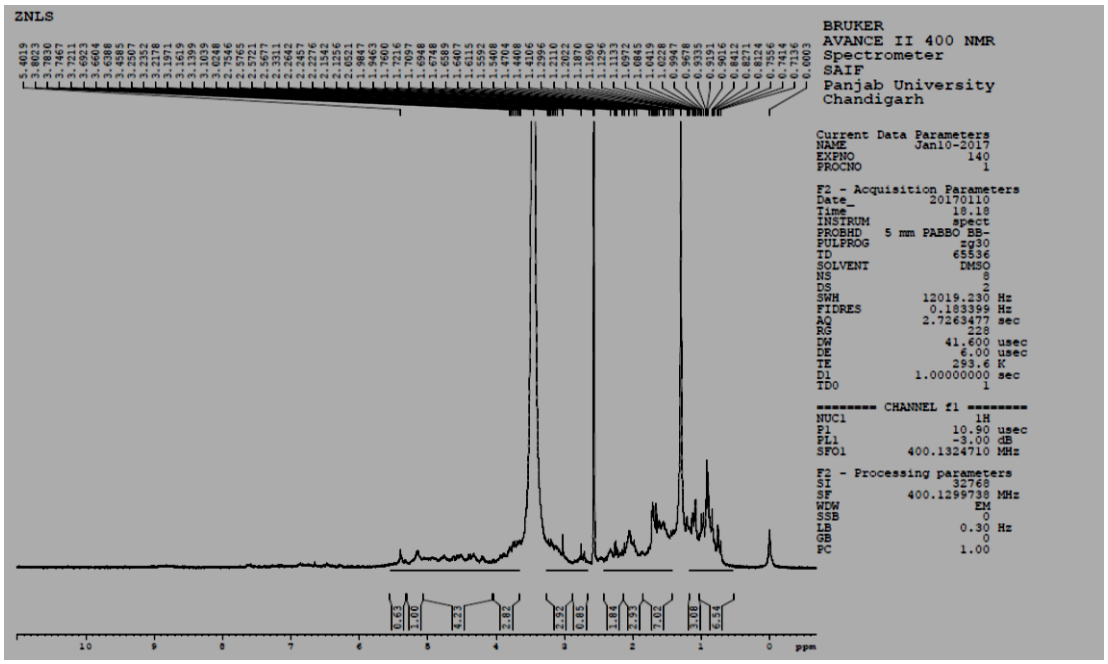


Figure 2.4 Chromatogram of NMR spectra of fruits extract using Soxhlet method



CONCLUSION

In the field of plant senses, a natural product chemistry metabolomics has been developed to measure all metabolites both qualitatively and quantitatively, which can provide a clear picture of living organism under certain conditions. The plant metabolon is very complex as the metabolites present in a plant are all different regarding their polarity chemical behaviour, stability, and concentration. This makes the analysis of metabolites in one single experiment extremely difficult. In view of this, instead of aiming to analyse or all individual metabolites qualitatively and quantitatively a more realistic approach is to have a general view of all metabolites present in an organism under certain conditions. In this context NMR is very suitable method to carry out such analysis because it allows the simultaneous detection of diverse groups of secondary metabolites (flavonoids, alkaloids, phenolic compounds and so on). Besides abandoned primary metabolites (sugar, amino acid). In NMR spectrum signals are proportional to their molar concentration making the direct comparison of concentration of all compounds possible without the need of calibration curve of each individual compound. It reflects the real molecular levels of metabolites present in the plant. The NMR results of the present study also confirmed the presence of phenolic compounds, carbonyls, alkenes and carboxylic acid in the leaves and fruit of Ziziphus nummularia.

NMR spectra confirming the presence of flavonoids and phenolic compounds in the leaves and fruits extract of *Z.* nummularia.

Declarations

Ethical Approval:

Not applicable, as no studies involving human participants, no animals were performed in this research.

Consent to Participate:

Not applicable. The plant material of Ziziphus nummularia used in this study was cultivated in a private garden located in Mohanlal Sukhadia University. As the material was not collected from the wild, no special permissions or licenses were required. The study followed all institutional and national guidelines for the ethical use of plant materials in research. The leaves and fruits of *Ziziphus nummularia* used in this study were obtained from cultivated plants grown in Mohanlal Sukhadia University Udaipur Rajasthan.

As the plant material was not collected from the wild, no special permissions or licences were required. All procedures adhered to institutional and national ethical guidelines for the use of plant materials in research. No voucher number was assigned. The plant material was identified and authenticated by Dr. Geeta Swami, Professor, Department of Botany, Meera Girls College, Udaipur, Rajasthan, India. The collected plant materials were cleaned, shade-dried, and powdered for further use. No voucher number was assigned. The collection and use of *Ziziphus nummularia* plant materials complied with institutional, national, and international guidelines. The plant material was collected from a non-protected area with due permission from the authorities of Government Science College, Udaipur. The species is not listed as endangered or protected under the Wildlife Protection Act (1972). The plant was identified by Dr. Geeta Swami, Professor, Department of Botany, Meera Girls College, Udaipur, Rajasthan. No herbarium and voucher number was prepared at the time of identification.

Consent to Publish:

Not applicable.

Data Availability Statement:

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests:

The author declares that there are no competing interests**.**

REFERENCES

1. Taverna, M. J. (2018). Epidemiology of Diabetes. In Dermatology and Diabetes (pp. 1-6). Springer, Cham.
2. World Health Organization (2006). The world health report: working together for health. World Health Organization.
3. Ceriello, A. and Motz, E. (2004). Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arteriosclerosis, thrombosis, and vascular biology, 24(5), 816-823.
4. Giugliano, D., Ceriello, A. and Paolisso, G. (1995). Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? Metabolism-Clinical and Experimental, 44(3), 363-368.
5. Ferrannini, E., Muscelli, E., Frascerra, S., Baldi, S., Mari, A., Heise, T., Broedl, U. C. and Woerle, H. J. (2014). Metabolic response to sodium-glucose co transporter 2 inhibition in type 2 diabetic patients. The Journal of Clinical Investigation, 124(2), 499-508.
6. West, I. C. (2000). Radicals and oxidative stress in diabetes. Diabetic Medicine, 17(3), 171-180.
7. Mirhoseini, M., Baradaran, A. and Rafieian-Kopaei, M. (2013). Medicinal plants, diabetes mellitus and urgent needs. Journal of Herbal Medicine Pharmacology, 2(2), 11- 17.
8. World Health Organization. (1999). Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus.
9. Ceriello, A. and Motz, E. (2004). Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arteriosclerosis, thrombosis, and vascular biology, 24(5), 816-823.
10. Montonen, J., Knekt, P., Järvinen, R. and Reunanen, A. (2004). Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care, 27(2), 362-366.
11. Nasri, H. and Shirzad, H. (2013). Toxicity and safety of medicinal plants. Journal of Herbal Medicine Pharmacology, 2 – 11.
12. Rafieian-Kopaei, M., Baradaran, A. and Rafieian, M. (2013). Plants antioxidants: From laboratory to clinic. Journal of Nephropathology, 2(2), 152-153.
13. Mardani, S., Nasri, H., Hajian, S., Ahmadi, A., Kazemi, R. and Rafieian-Kopaei, M. (2014). Impact of Momordica charantia extract on kidney function and structure in mice. Journal of Nephropathology, 3(1), 35 – 44.
14. Ardalan, M. R. and Rafieian-Kopaei, M. (2013). Is the safety of herbal medicines for kidneys

under question? Journal of Nephropharmacology, 2(2), 11-14.
15. Roshan, B. and Stanton, R. C. (2013). A story of micro albuminuria and diabetic nephropathy. Journal of Nephropathology, 2(4), 234 – 251.
16. Hajivandi, A. and Amiri, M. (2014). World kidney day 2014: Kidney disease and elderly. Journal of Parathyroid Disease, 2(1), 1- 4.
17. Sharma S, Nasir A, Prabhu KM, Murthy PS, Dev G (2003): Hypoglycemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxan induced diabetic rabbits. J. Ethnopharmacol 85: 201-206.
18. Kokate, C. K., Purohit, A. P., and Gokhale, S. B. (1995). A Text Book of Pharmocognocy. Nirali Prakashan Pune; 4th edition 40, 1-20.
19. Guevarra, B. Q. (2005). A Guidebook to Plant Screening: Phytochemical and Biological. University of Santo Tomas Publishing House. Manila, Edition revised. 22- 27.
20. Rastogi, R. P. and Mehrotra, B. N., (1998). Compendium of Indian Medicinal Plants.. Publication and Information Directorate of the Council of Scientific and Industrial Research, New Delhi, p. 408.

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