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The Anti-oxidants and Phytoconstituents of Tiliacora racemosa, Capsicum annum, Trigonella foenum-graecum leaves

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ABSTRACT

Herbs with medicinal properties play an increasingly important role in research to know their functions on disease prevention and treatment property. As the ecosystem is full of potential herbs it becomes very important to study about their beneficial pharmacological responses. This study characterizes the phytochemical composition and antioxidant activity of three medicinal herbs including the leaves of Tiliacora racemosa, Capsicum annum, Trigonella foenum-graecum leaves. These three herbs are well known and familiar for its broad pharmacological responses. It has beneficial responses on all the physiological body systems. By following various chemical methods for phytoconstituents and UV spectrometer for antioxidant studies were carried out using ascorbic acid as standard. The antioxidant studies carried out to determine the potentiality of the herbs selected- Reducing Power Assay and H2O2 radical scavenging assay. Results revealed that the presence of flavonoids, phenols, tannin's, alkaloids, glycosides, phenols, terpenoids and glycosides despite the well-known antioxidant properties of these plant species, our study suggests all the three herbs can stand out as a relatively more valuable plant source of natural bioactive molecules for developing novel functional property with potential for not only promoting human health against the diseases but also improving bio-valorisation and environment. This study compared and demonstrated these plant extracts as valuable sources of bioactive compounds, likely for preparing novel functional products in various industries for folkloric advantages. These three herbs possess antioxidant properties as well as which can act as scavengers against free radicals, which is one of the causes for various types of diseases.

Methanolic extract of all three herbs is a potential source of natural antioxidants and serves as an effective free radical scavenger and/or inhibitor. Hence, these herbs might be a good plant-based pharmaceutical product for several diseases caused by free radicals, supported by phytoconstituents with presence of abundance amount is surely to be considered for its pharmacological responses.

Key words: *Tiliacora racemosa: Capsicum annum; Trigonella foenum-graecum; Antioxidants; Phytoconstituents; free radicals; Reducing power assay; H2O2 radical scavenging assay.*

Introduction

There is strong evidence that many dangerous pathophysiological processes, such as cancer, diabetes and cardiovascular and neurodegenerative diseases, are associated with the accumulation of free radicals. A free radical is an atom or molecule that has an unpaired electron and is therefore unstable. This unstable radical has the tendency to become stable through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells, thus causing protein and DNA damage [1]. Such radical-caused cell damage can become more widespread due to weakened cellular antioxidant defence systems. All biological systems have innate antioxidant defence mechanisms that remove damaged molecules, but these mechanisms can be inefficient. Therefore, dietary intake of antioxidants is imperative to protect cells from damage caused by free radicals [2,3].

Antioxidants are substances that prevent and stabilize the damage caused by free radicals by supplying electrons from antioxidants to these damage cells. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body. Consumption of antioxidant-enriched fruits and vegetables is known to lower the risk of several

diseases caused by free radicals [4-6]. Such health benefits are mainly due to the presence of phytochemicals such as polyphenols, carotenoids, and vitamin E and C. Although phenolic compounds are commonly found in both edible and in non-edible herbs, cereals, fruits, vegetables, oils, spices, and other plant materials, scientific information on the antioxidant properties of endemic plants is scare because the availability of endemic plants is limited to certain regions and only known by local populations [7,8]. Therefore, the assessment of such properties remains an interesting and useful task, particularly to find promising sources of natural antioxidants for functional foods and/or nutraceuticals. Figure 1 [9,10].

Methodology

Preparation of Plant Extract

The freshly collected leaves of the three herbs was collected from local market, cleared from dirt and then the leaves were dried under shade for about 15 days and they were pulverized in the laboratory [11]. Followed by soxhlation to obtain the methanolic extract as the solvent was better suited to obtain all the active constituents needed to study further activities. Authenticated by Dr.P Suresh Kumar, Botanist Govt degree college, Hyderabad T.S voucher number- BSI/SRC/10/23/2020/Tech/1521, 1023, 304 respectively.

Preliminary phytochemical analysis of the extract -The extract was subjected to preliminary phytochemical investigations to identify various phytoconstituents present in methanolic extract of herbs according to the method prescribed in Khandelwal book [12-14]. These are the following test performed:

a) Tests for Alkaloids:

Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute HCl. Shake well and filter. With filtrate, perform the following tests:

•**Mayer's test**: 2-3ml, filtrate with few drops Mayer's reagent gives cream coloured precipitate.

b) Test for Flavonoids

• **Shinoda test**: To dry powder or extract, add 5ml of 95% Methanol, few drops of concentrated HCl and 0.5g Mg turnings, pink colour observed [15].

• To small quantity of residue, add lead acetate solution. Yellow colour ppt a formed [16,17]. Addition of increasing amount of NaOH to residue, shows yellow coloration, which decolorizes after addition of acid.

c) Test for Phenols

• **Ferric chloride test**: A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour [18].

d) Test for Saponins

• **Foam test**: To 2 ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins [19].

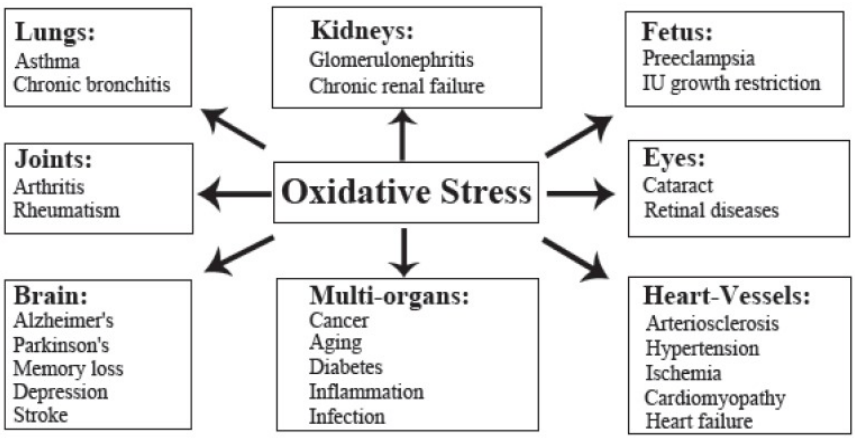

Figure 1: Oxidative stress-induced diseases in humans.

e) Test for Steroids

•**Liebermann-Burchard test**: 2 ml of extract was treated with drops of chloroform, acetic anhydride and conc. Sulphuric acid and observed for the formation of dark pink or red colour [20].

f) Test for Tannins

• **Braymer's test**: 2 ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution [21].

Tests for Cardiac Glycosides

g) Test for Terpenoids

• **Liebermann's Burchard's Test**: To 1ml of methanolic extract, add 1ml of chloroform, 2-3ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid. Pink to red coloration indicates the presence of terpenoids [22].

• In vitro antioxidant assays

Reducing Power Assay

Principle:

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm [23,24].

Potassium ferricyanide $\xrightarrow{\text{Antioxidant}}$ Potassium ferrocyanide

$$\xrightarrow{\text{Ferric chloride}}$$

Ferric ferrous complex (700 nm)

Preparation of reagents:

1. **Phosphate buffer pH 6.6**: Potassium dihydrogen phosphate (62.5 ml 0.2 M) was added to 250 mL volumetric flask and also 20.5 ml of 0.2 M NaOH and made up to volume 250 ml with distilled water.

2. **Potassium dihydrogen phosphate (0.2 M) solution**: Potassium dihydrogen phosphate (2.72 g) was dissolved in distilled water and volume made up to 100 ml [25].

3. **Sodium hydroxide solution (0.2 M) solution**: 0.8 g of sodium hydroxide was dissolved in distilled water and volume made up to 100 ml [26].

4. **Potassium ferricyanide (1% w/v) solution**: Potassium ferricyanide (1 g) was dissolved in water and volume made up to 100 mL in volumetric flask.

5. **Ferric chloride solution** (0.1% w/v): Ferric chloride (25 mg) was dissolved in distilled water and volume made up to 25 mL in volumetric flask.

Method:

1. To 1 mL of test and standard compounds added 2.5 mL of potassium ferricyanide (1 % w/v), 2.5 mL of phosphate buffer pH 6.6 and incubated at 50^0C for 30 min.
2. To 2.5 mL of above supernatant liquid added 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ solution (0.1% w/v).
3. The absorbance of ferric ferrous complex was measured using phosphate buffer pH 6.6 as control at 700 nm using UV-Visible spectrophotometer and estimated the increase in absorbance.
4. The percent increase in reducing power was calculated using the following equation.

$$\textbf{Percentage increase in reducing power } (\%) = \frac{\textbf{Abs}_{\textbf{test}} - \textbf{Abs}_{\textbf{blank}}}{\textbf{Abs}_{\textbf{blank}}} X\ \textbf{100}$$

Where 'Abs_{test}' is absorbance of test solution

'Abs_{blank}' is absorbance of blank.

H_2O_2 Radical Scavenging Assay

Principle- Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

Preparation of reagents:

1. **Phosphate buffer solution pH 7.4:** Add 250.0 mL of 0.2 M potassium dihydrogen phosphate to 393.4 mL of 0.1 M sodium hydroxide and make up the volume to 1000 mL with the distilled wáter [27,28].
2. **Potassium dihydrogen phosphate (0.2 M) solution:** Potassium dihydrogen phosphate (2.72 g) was dissolved in distilled water and volume made up to 100 ml.
3. **Sodium hydroxide solution (0.1 M) solution:** 0.4 g of sodium hydroxide was dissolved in distilled water and volume made up to 100 ml.

Method

1. A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (Ph 7.4).

2. Test compounds (10–50 µg/M) were added to hydrogen peroxide solution (0.6 mL).

3. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

$$\% \, H_2O_2 \text{ activity} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} X\ 100$$

Where, Abs (control): Absorbance of the control

Abs (sample): Absorbance of the extract/standard.

In vitro Antioxidant Assays

The methanolic extract of all the three herbs was subjected to *in vitro* antioxidant activity. *In vitro* anti-oxidant activity was performed using reducing power assay & hydrogenperoxide scavenging assay.

Result

Following is the result of phytoconstituents present in three different herbs using methanol extract by following chemical methods to determine it Figure 2,3,4,5 Table 1.

Anti-oxidant studies- The methanolic extract of all the three has shown antioxidant activity in reducing power assay. The reducing ability of a compound generally depends on the presence of reductants which have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom.

The reducing capacity of a compound can be known by measuring Fe3+- Fe2+ complex. Fe3+ reduction is due to electron donating activity of herbs, which is an important mechanism of antioxidant action. Increase in absorbance indicates an

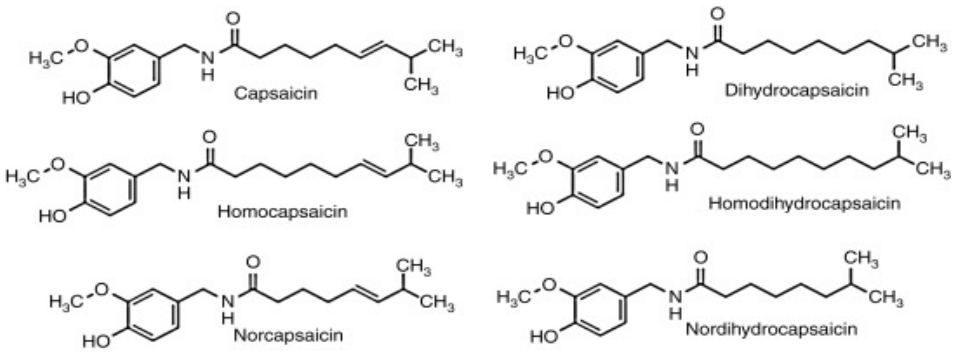

Figure 2: The above chemical structure shows the presence of various active constituents present in *Capsicum annum*.

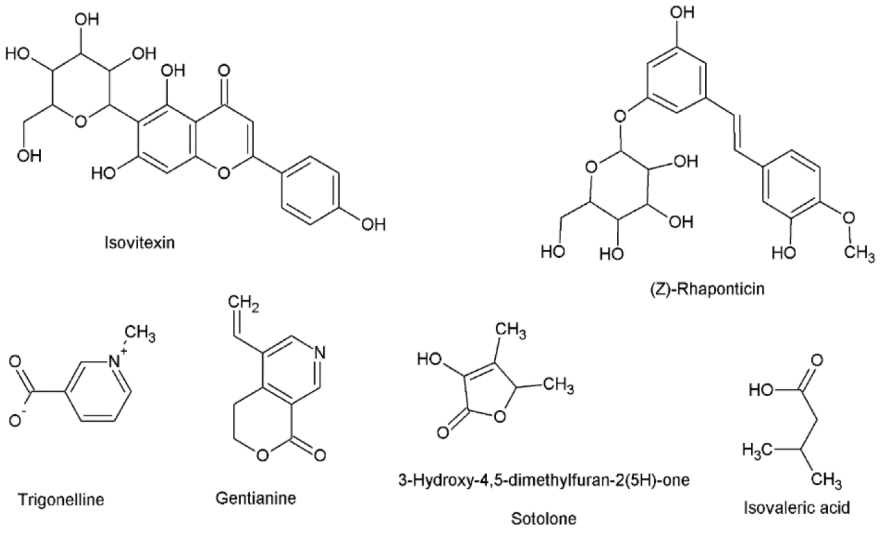

Figure 3: The above chemical structure shows the active constituents present in *Trigonella foenum-graecum* leaves.

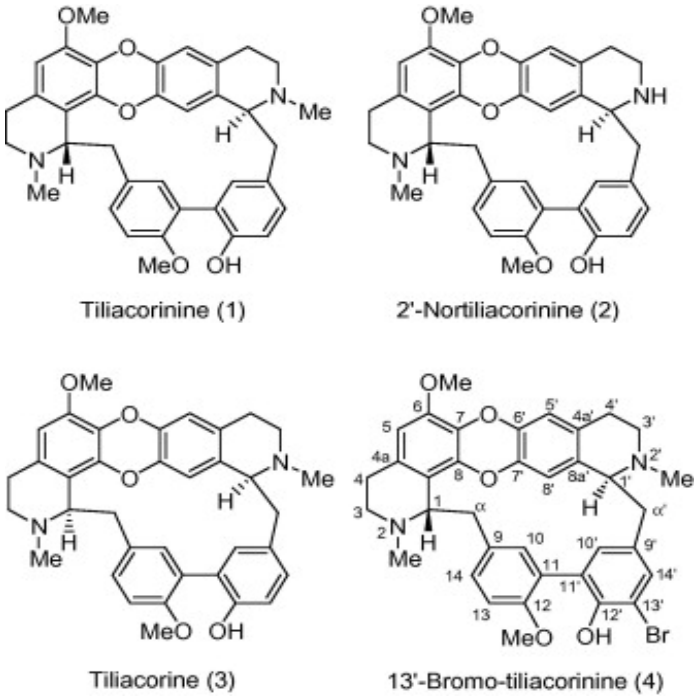

Figure 4: The above chemical structure shows the active constituents present in *Tiliacora racemosa.*

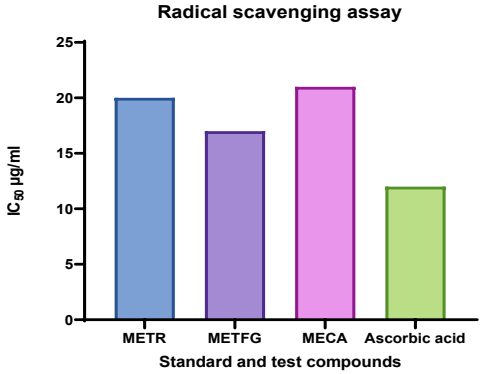

Figure 5: Above is graphical representation of herbs with their IC5O values

increase in reductive ability of hers. The reducing power activity of herbs might be due to presence of hydroxyl groups. It might be due to the presence of phenols and flavonoids the extract with adequate number of hydroxyl groups [29,30].

Tiliacora racemose, Trogenella feonum graceum and capsicum annum had shown dose dependent inhibition of free radicals and its IC50 value was found to be 32, 28, 34 µg/mL respectively.The potential of the extract was comparable to that of standard ascorbic acid (IC50 = 26 µg/mL). From the above result it is confirmed that *Trogenella feonum graceum* is more potent than other two herbs, because of its IC50 which is very close to ascorbic acid.

Hydrogen Peroxide Scavenging Assay

The in vitro antioxidant activity was performed using hydrogen peroxide scavenging assay. The IC50 value of the *Tiliacora racemose, Trogenella feonum graceum and capsicum annum* was 20, 17, 21 µg/mL & ascorbic acid was 12 µg/mL respectively. From the results it is clear that three herbs showed antioxidant activity Table 2,3,4,5,6,7.

Statistical analysis The percentage inhibition was calculated using: Percent Inhibition = Ac-As/ Ac x 100

Where, Ac is absorbance of control,

As is the absorbance of sample.

IC50 value (a concentration at 50% inhibition) was determined from the curve between percentage inhibition and concentration. All determinations were done in triplicate and the IC50 value was calculated by using the equation of line (Figure 6) [31-34].

Table 1: By the evidence of above constituents, it can be selected to perform various activities on physiological system disorders

S.no	Name of Phytochemical Constituents	Results Analysis of Methanolic Extract of Tiliacora Racemosa	Results Analysis of Methanolic Extract of Trigonella Foenum Graecum	Results Analysis of Methanolic Extract of Capsicum annum
1.	Alkaloids	+	+	+
2.	Phenols	+	+	+
3.	Saponins	+	+	+
4.	Tannins	+	+	+
5.	Flavonoids	+	+	+
6.	Anthraquinones	+	+	+
7.	Glycosides	+	+	+
8.	Terpenoids	+	+	+
9.	Steroids	-	-	-

Table 2: Reducing power assay of methanolic leaf extract of *Tiliacora racemosa*.

S.no	Compounds	Concentration	% Inhibition	IC_{50} Values
1.	Ascorbic acid	10	31.62 ± 0.40	26
		20	45.07 ± 0.25	
		30	56.67 ± 0.25	
		40	64.36 ± 0.10	
		50	70.08 ± 0.77	
2.	METR	10	22.08 ± 0.52	32
		20	35.57 ± 0.35	
		30	49.49 ± 0.33	
		40	61.26 ± 0.12	
		50	65.22 ± 0.15	

Table 3: Hydrogen peroxide scavenging assay of methanolic leaf extract of *Tiliacora racemosa.*

S.no	Compounds	Concentrations	% Inhibition	IC_{50} Values
1.	Ascorbic acid	10	48.62 + 0.41	12
		20	69.35 + 0.14	
		30	79.26 + 0.03	
		40	83.78 + 0.01	
		50	86.04 + 0.06	
2.	METR	10	43.27 + 0.48	20
		20	53.64 + 0.32	
		30	73.23 + 0.10	
		40	80.41 + 0.05	
		50	84.46 + 0.02	

Table 4: Reducing power assay of methanolic leaf extract of *Trigoella feonum graceum* [METFG].

S.NO	Compounds	Concentrations	% Inhibition	IC_{50} Values
1.	Ascorbic acid	10 20 30 40 50	31.62 ± 0.40 45.07 ± 0.25 56.67 ± 0.25 64.36 ± 0.10 70.08 ± 0.77	26
2.	METGF	10 20 30 40 50	25.68 ± 0.22 35.87 ± 0.45 51.59 ± 0.34 58.29 ± 0.19 62.27 ± 0.18	28

Table 5: Hydrogen peroxide scavenging assay of methanolic extract of trigonella feonum graceum [METGF]

S.NO	Compounds	Concentrations	% Inhibition	IC_{50} Values
1	Ascorbic acid	10 20 30 40 50	48.62 + 0.41 69.35 + 0.14 79.26 + 0.03 83.78 + 0.01 86.04 + 0.06	12
2	METGF	10 20 30 40 50	39.08 ± 0.43 46.57 ± 0.56 49.79 ± 0.83 59.16 ± 0.73 63.12 ± 0.65	17

Table 6: Reducing power assay of methanolic leaf extract of Capsicum annum/racemosa [MECA].

S.NO	Compounds	Concentrations	% Inhibition	IC_{50} Values
1	Ascorbic acid	10 20 30 40 50	31.62 ± 0.40 45.07 ± 0.25 56.67 ± 0.25 64.36 ± 0.10 70.08 ± 0.77	26
2	MECA	10 20 30 40 50	18.09 ± 0.51 27.51 ± 0.23 39.46 ± 0.54 49.24 ± 0.87 61.21 ± 0.71	34

Table 7: Hydrogen peroxide scavenging assay of methanolic leaf extract of *Capsicum annum/racemosa.*

S.NO	Compounds	Concentrations	% Inhibition	IC_{50} Values
1	Ascorbic acid	10 20 30 40 50	48.62 + 0.41 69.35 + 0.14 79.26 + 0.03 83.78 + 0.01 86.04 + 0.06	12
2	MECA	10 20 30 40 50	31.08 ± 0.23 42.57 ± 0.54 55.49 ± 0.56 67.26 ± 0.78 71.22 ± 0.49	21

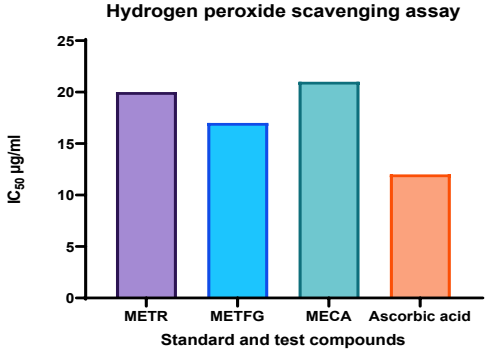

Figure 6: Above is graphical representation of all the three herbs with their IC5O values.

Discussion

The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention. The implication of oxidative stress in the etiology of several chronic and degenerative diseases suggests that antioxidant therapy represents a promising avenu treatment [35-37]. In the future, a therapeutic strategy to increase the antioxidant capacity of cells may be used to fortify the long-term effective treatment. However, many questions about antioxidant supplements in disease prevention remain unsolved. Further research is needed before this supplementation could be officially recommended as an adjuvant therapy. In the meantime, it is reminded that avoiding oxidant sources (cigarette, alcohol, bad food, stress, etc) must be considered as important as taking diet rich in antioxidants. Indeed, our health also depends on our lifestyle.

Conclusion

Due to the presence of various types of phytoconstituents all the three herbs possess to have properties of curing various kinds of diseases. The two methods used to study anti-oxidant properties provide the evidence of having rich inhibiting free radical properties. Further investigations and intensive research on these herbs will provide sufficient knowledge of their constituents and anti-oxidant properties which is going to be helpful in many diseases

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