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PHYTOCHEMICAL PROFILING AND ANTIOXIDANT EVALUATION OF ROOT ETHANOL EXTRACT OF MAESA INDICA (ROXB.) SWEET

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ABSTRACT
Background: Healing herbs have long been used in traditional medicine due to their therapeutic properties and rich content of bioactive molecules. Despite its traditional applications, research on the root part of *Maesa indica* is scarce. This study focuses on exploring the phytochemical composition and antioxidant potential of the ethanol extract of *M. indica* roots. **Methodology:** Secondary metabolites were identified using Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (LC-Q-TOF-MS). Antioxidant activities were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the metal chelating activity assay. Quantification of total phenolic content (TPC) and flavonoids was also conducted. **Results and Discussion:** Preliminary phytochemical analysis revealed the presence of flavonoids, phenols, steroids, and saponins. LC-Q-TOF-MS profiling identified seven primary secondary metabolites. The root extract exhibited high phenolic content (380.91 ± 23.52 µg/mg) and moderate flavonoid concentration (114.21 ± 6.25 µg/mg). Antioxidant activity of root extract was demonstrated by DPPH radical scavenging showed strong activity (IC_{50}: 88.78 µg/mL) and moderate ferrous ion chelating activity (IC_{50}: 172.31 µg/mL), suggesting effective free radical neutralization. **Conclusion:** The findings highlight the root extract of *M. indica* as a promising source of natural antioxidants. Compared to previous studies on aerial parts of the plant, the root extract offers comparable or enhanced antioxidant capacity, suggesting its value in future pharmaceutical and nutraceutical formulations.

INTRODUCTION

The reactive chemicals associated with the evolution of various diseases, including tumors, heart conditions, hepatitis, asthma, cataracts, and immunodeficiency, are known as reactive oxygen species [1]. Small quantities of chemical substances are specified as antioxidants, which are used to stop or reduce oxidation caused by oxidants. Phenolic chemicals (carotenoids, vitamins A, C, and E, flavonoids, phenolic compounds, tannins, anthocyanins, lignins, and other natural substances with strong antioxidant potential) can be found in plants. An extraction procedure is necessary to obtain these antioxidant molecules [2]. Plants, vegetables, fruits, nuts, and spices have been reported to be a significant source of dietary active antioxidants that protect the body against various oxidative stress-induced conditions [3].

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Antioxidant compounds, including phenolics and flavonoids, work to eliminate free radicals, such as hydrogen peroxide and lipid peroxides, thereby preventing the formation of oxidative stress, which can cause degenerative diseases [4]. Phytochemistry contains several semi-polar elements, including various types of secondary metabolites, and is a somewhat complex field. These components can be successfully sorted and identified using LC-MS methods [5].

The nitrogen compounds (alkaloids and amines), phenolic compounds (phenolic substances, flavonoids, coumarins, quinines, and other polyphenols), vitamins, terpenoids, and other secondary metabolites of herbal origin are among the many different types of bioactive constituents that show antioxidant properties. In many regions of the world, particularly India, shrubs are utilized in traditional medicine to address a range of illnesses due to their antioxidant properties [6].

Maesa indica (Roxb.) Sweet, often called wild tea or wild berry, is a tall, smooth, evergreen plant that grows up to 4 m tall and belongs to the Primulaceae family. It is indigenous to China and Southern India and can be found in moist deciduous forests and evergreen to semi-evergreen forests at elevations of up to 1800 meters. Additionally, it is situated in a area with high humidity and a variety of evergreen and semi-evergreen species. This plant contains several phytochemicals, including saponins, tannins, flavonoids, fixed oil, phenolics, polysaccharides, and glycosides [7]. *M. indica* has anticancer, antimutagenic, and spermicidal capabilities. Leaf extracts from *M. indica* are used as an anthelmintic and blood purifier, and they can possess larvicidal properties. In traditional Chinese medicine, the plant is utilized to treat cancer.

Furthermore, puerperalism, boils, gallbladder stones, paraplegia, and lactopenia are treated with it as it contains numerous phytoconstituents belongs to different groups like fatty acids and its derivatives such as 2,3-dihydroxypropyl octadeca-9,12-dienoate, hexadeconoic acid, *2,3-Dihydroxypropanoic hexadecanoic anhydride*, steroidal compounds like β-sitosterol, stigmasterol, hydrocarbon like dodecane, quinones like maesaquinone, kiritiquinone, anthroquinone like chrysophanol, flavonoids like quercetin 3-rhaminoside, rutin, catechin, quercetin, phenolic acids like chlorogenic acid, synthetic compound like nitrendipine and monoterpenes such a β-thujone are just several of its many medicinally active ingredients [8].

The plant exhibits a range of biological properties, including antibacterial, antileishmanial, anti-angiogenic, antiviral [7], and antiviral [9] effects. This plant has been shown to have a wide variety of phytochemical groups, including carbohydrates, fixed oil, tannins, flavonoids, phenolics, saponins, & glycosides [10].

Traditional remedies are just one of the many medical benefits of this plant that have been recognized for a long time. There is also evidence that the Chinese utilized it to treat cancer. Nevertheless, there are no previous reports on the free radical scavenging activity of root ethanol extract in the literature reviews. While several studies have investigated the leaf and aerial parts of *M. indica* for their bioactive compounds and pharmacological properties [10], no published research has reported the antioxidant potential of its root part specifically. The first study on the antioxidant properties of the root extracts is presented in this article. Compared with other plant parts, the root of *M. indica* has not been the subject of as much research. Therefore, this study fills a crucial gap in the literature by presenting the first comparative account of the antioxidant potential and phytochemical composition of root ethanol extract, thereby establishing their biomedical relevance.

MATERIALS AND METHODS

Collection of plant materials

Fresh root samples of *M. indica* were collected from Agumbe, Western Ghats, Karnataka, India. The plant material was identified and confirmed by a taxonomist, and a voucher specimen (number HJCB2092) was submitted to the Department of Applied Botany at Kuvempu University in Shankaraghatta, Shivamogga.

Preparation of plant extract

The collected root material is washed thoroughly with tap water and left to dry in the shade for 25 to 30 days. The samples were then ground in a mixer grinder into coarse powder after drying. Petroleum ether, ethyl acetate, chloroform, ethanol, and distilled water were the solvents of increasing polarity used to extract the powdered samples using a soxhlet extractor at elevated temperatures. The solvent in the siphon tube was extracted until it became colorless. The residue from each extraction was then shade-dried. After being concentrated using a rotary evaporator at a regulated temperature and lower pressure, the crude extract was weighed, labeled, and kept in sealed glass vials at 4°C for further investigation.

Preliminary qualitative phytochemical analysis

The preliminary phytoconstituents, including alkaloids, tannins, glycosides, phenols, flavonoids, sterols, saponins, terpenoids, carbohydrates, and proteins, were determined in the ethanol extract of *M. indica* using standard protocols [11].

Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (LC-Q-TOF-MS) profiling and analysis

LC-Q-TOF-MS is a beneficial approach in determining a metabolite profile in a sample. Using a separation system with higher resolution is key to this method for identifying metabolite compounds in the extract. Several previous studies used the LC-MS/MS method to identify phytochemical constituents of plants [12]. The Xevo G2-XS QTof model was used to perform metabolite profiling of the root ethanol extract. 20 µL of the ethanol sample was injected with needle wash onto an Acquity UPLC system (Waters, USA) equipped with a BEH C18 column (50 x 4.6 mm, 2.6 µm) to perform chromatographic separations. Throughout the run, solvents A (0.1% formic acid in water) and B (acetonitrile) were used, with a source temperature of 150°C and a capillary voltage of 3.0 kV. The obtained data were matched with a mass library for metabolite analysis.

Total phenol content estimation:

Gallic acid was included as a standard antioxidant, which was used to determine the total phenolic content of the extract. 0.5 mL of Folin-Ciocalteu reagent was added to 100 µg of suitably diluted extract, and a 7% sodium carbonate solution (2 mL) was added. The mixture was then incubated at room temperature for 10 minutes. After one minute of boiling, the mixture's color absorbance was measured at 750 nm using a spectrophotometer with slight modification [13].

Total flavonoid estimation

5 mL of chromogen reagent (0.1% cinnamaldehyde solution in a cooled mixture of 75 mL methanol and 25 mL concentrated HCl) was added to 200 µg of the sample. The absorbance at 640 nm was measured following a 10-minute incubation period. In µg catechin equivalents (CE)/mg of extracts, the total flavonoid concentration was reported with slight modification [14].

Determination of antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: Samples at varying concentrations (0–100 µg/mL) were combined with 2 mL of 2, 2-Diphenyl-1-picrylhydrazyl (100 µM) to make a total of 3 mL. The resultant solution was subsequently allowed to rest at room temperature for 45 minutes in the dark. Absorbance was determined at 517 nm. The sample's ability to scavenge free radicals was measured and reported in IC_{50} values in relation to vitamin C, a common antioxidant with slight modification [15].

Ferrous-ion chelating assay

The ferrous-ion chelating effect of samples was determined [16]. 0.05 mL of 2 mM ferric chloride was added to 0.05 mL of correctly diluted samples at varying concentrations (0 to 250 µg/mL). The reaction was initiated by adding 0.1 mL of 5 mM Ferrozin, and the mixture was allowed to stand for 10 minutes at room temperature. Absorbance was measured at 562 nm by comparing the colors with the blank, and the results were reported as IC_{50} values relative to the standard antioxidant ethylene diamine tetraacetic acid (EDTA).

Statistical analysis

All tests were performed in triplicates, and the results are expressed as Mean ± standard error of mean. The data were analyzed using one-way ANOVA. Significance was set at 0.05, and comparisons were made against the reference controls.

RESULTS

Qualitative assessment of phytochemicals:

Phytochemical screening of root ethanol extract showed the existence of glycosides, alkaloids, phenols, flavonoids, and saponins (Table 1). A wide range of phytochemicals was found in the root ethanol extract of *M. indica* in five different solvents. Numerous phytochemicals, including flavonoids, phenols, steroids, saponins, and tannins, were visible in the ethanol extract. Only saponins were present in the petroleum ether extract; however, a greater amount of phytochemicals were found in the ethanol extract. Root ethanol extract has thus been utilized in an array of biological processes. Ethanol was chosen as the solvent of focus because it demonstrated superior efficiency in extracting a broader spectrum of bioactive compounds compared to the other solvents tested, making it more suitable for subsequent biological evaluations.

Liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) profiling

M. indica root ethanol extract showed the existence of seven major peaks (Figure 1). Among the identified compounds,

flavonoids, polyphenols, and anthraquinones are present. The mass spectra of each constituent were determined using the MassBank library.

The compound names, along with their retention times (RT), mass-to-charge ratios (m/z), and molecular formulas, are listed in Table 2. The results revealed the presence of 1-hydroxyanthraquinone, diphyllin, 4-hydroxypropranolol, apigenin 7-O-neohesperidoside, rutin, di(2-ethylhexyl)amine, and feruloyl dehydrotyramine.

Determination of total phenol and flavonoid content

Total phenolic content in root ethanol extract was expressed as equivalent to gallic acid (EGA) and was estimated to be 380.91 ± 23.52 µg/mg of dry material. It was found that the total flavonoid concentration in the samples was 114.21 ± 6.25 µg/mg of dry extract, equivalent to catechin.

DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated in terms of IC_{50} values. The ethanol root extract exhibited significant free radical scavenging activity with IC_{50} values of 88.78±1.64 µg/mL for scavenging DPPH radicals in comparison to standard ascorbic acid (IC_{50}: 24.43±0.03 µg/mL).

Ferrous-ion chelating assay

The outcomes of the ferrous ion chelating assay of root ethanol extract demonstrated that the fraction of Ferrozine-Fe^{2+} complex formation inhibition is 172.31±1.40 µg/mL, and the standard EDTA showed an IC_{50} value of 45.74±0.8 µg/mL.

DISCUSSION

Analysis of the phytochemical properties of the medicinal plants used to show and isolate the drug, lead compounds, and components from the parts of the plant. Their phytochemical properties can identify the unique biological activity of the plants [17]. The present qualitative phytochemical investigation of *M. indica* revealed that the plant extracts contains several different chemical components (Table 1). Numerous biological properties have been identified in these phytochemicals. The antibacterial, antioxidative, antiulcer, anticarcinogenic, antitumor, and anti-inflammatory properties of terpenoids have been well-documented in recent studies [18-20]. Similarly, flavonoids exhibit antibacterial, anticancer, anti-diabetic, anti-aging, and anti-inflammatory properties, as demonstrated in several studies [21, 22]. Steroids have also been reported to exhibit antimicrobial and cardiotonic-like effects in modern pharmacological research [23].

Table 1: Group test for the detection of secondary metabolites root extracts of *Maesa indica*

Phytochemical tests	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Distilled water
Alkaloids	-	-	-	-	-
Flavonoids	-	-	-	+	-
Phenols	-	-	+	+	-
Glycosides	-	-	-	-	+
Steroids	-	-	-	+	-
Saponins	+	-	-	+	+
Tannins	-	-	-	+	-

Note: The symbols + for presence and - for absence indicate the results

Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (LC-Q-TOF-MS) profiling

LC-Q-TOF-MS profiling of the root ethanol extract of *M. indica* showed the presence of 10 major peaks, as shown in Figure 1. 1-Hydroxyanthraquinone, Diphyllin, 4-Hydroxypropranolol, Apigenin 7-O-neohesperidoside, Rutin, Di(2-ethylhexyl)amine, and Feruloyl dehydrotyramine.

Among these, 1-hydroxyanthraquinone is an anthraquinone that can be utilized as an anticancer agent [24], and diphyllin is a polyphenol used as an antitumor, antiviral, and antibacterial agent [25]. 4-Hydroxypropranolol is a potent antioxidant [26], and rutin, a flavonoid compound, has been proven to exhibit antimicrobial, anti-inflammatory, anticancer, and antidiabetic properties [27]. In addition, apigenin 7-O-neohesperidoside, a glycosylated form of apigenin, is recognized for its potent antioxidant and anti-inflammatory properties, with studies supporting its neuroprotective and hepatoprotective effects [28]. Compound identification was confirmed by comparing the obtained mass spectral data (m/z values of precursor and product

ions), retention times, and characteristic fragmentation patterns with those available in published literature and databases such as the MassBank library. Each compound was matched based on both the accurate mass and MS/MS fragmentation behavior, and peaks were annotated only when confidence in spectral matching was high [29, 30]. The FCR reagent was used to evaluate the total phenolic content of plant ethanol extracts, which was then expressed in terms of mg gallic acid (GAL) equivalent/g dry weight. Gallic acid equivalent (EGA), which measures the total phenolic content of the *M. indica* root ethanol extract, was found to be 380.91 ± 23.52 µg/mg of dry material. This number indicates that the plant roots have a comparatively high phenolic content, which may indicate strong antioxidant activity. Antioxidants play a crucial role in maintaining the body's homeostatic balance and in preventing and treating various illnesses. However, synthetic antioxidants are somewhat hazardous. Therefore, consuming natural antioxidants from food is the best option because they are crucial for both illness prevention and treatment [31].

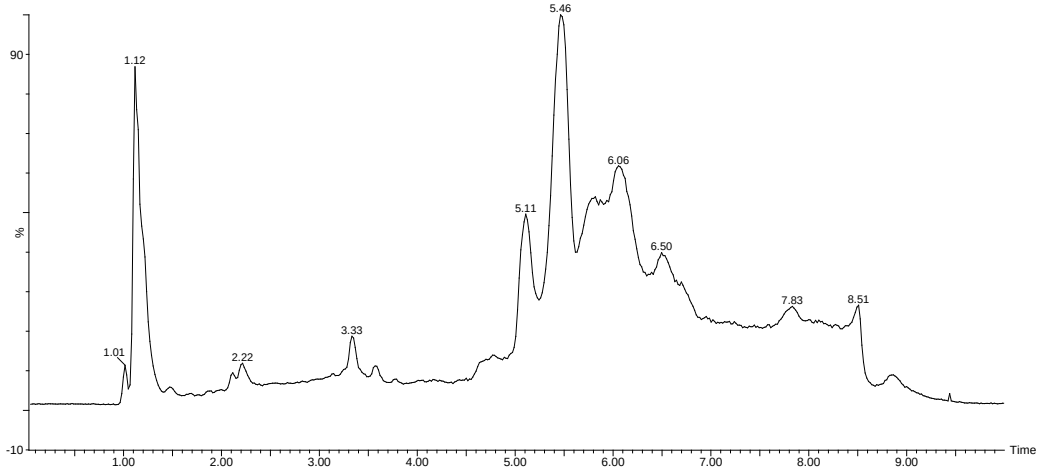

Figure 1: LC-Q-TOF-MS chromatogram of root ethanol extract of *Maesa indica*

Table 2: List of compounds detected in root ethanol extract of *Maesa indica*

SN	Compounds	RT (min)	Precursor m/z	Adduct	Formula	Ontology
1	1-Hydroxyanthraquinone	1.015	225.0717	M+H	$C_{14}H_8O_3$	Anthraquinones
2	Diphyllin	1.133	381.1658	M+H	$C_{21}H_{16}O_7$	N/A
3	4-Hydroxypropranolol	1.488	276.2207	M+H	$C_{16}H_{21}NO_3$	N/A
4	Apigenin 7-O-neohesperidoside	2.114	579.2676	M+H	$C_{27}H_{30}O_{14}$	Flavonoid
5	Rutin	2.119	611.2845	M+H	$C_{27}H_{30}O_{16}$	Flavonoid
6	Di(2-ethylhexyl)amine	3.332	242.3494	M+H	$C_{16}H_{35}N$	N/A
7	Feruloyl dehydrotyramine	5.092	312.4379	M+H	$C_{18}H_{21}NO4$	Ferulic acid & derivatives

The ethanol extract of *M. indica* root exhibits total flavonoid content (TFC) of 125.4 ± 5.3 µg QE/mg**,** comparable to catechins. Despite lower total phenolics, flavonoids remain a significant component of the extract's phytochemical profile, contributing to its moderate flavonoid content. Flavonoids are well-known for their potent antioxidant and anti-inflammatory properties, which likely enhance the bioactivity of the root extract. The extract demonstrated stong DPPH radical scavenging activity, with an IC_{50} value of 88.78 ± 1.64 µg/mL, which aligns with the antioxidant effects reported in other *maesa* species, particularly the roots [32]. The extract also demonstrated ferrous ion chelating activity, with an inhibition concentration (IC_{50}) of 172.31±1.40 µg/mL, indicating moderate ferrozine–Fe^{2+} complex inhibition (Figure 2). This chelating capacity mirrors findings in *M. indica* leaf extracts and other *Primulaceae* roots [33]. When compared to other well-established antioxidant plant sources, such as *Camellia sinensis* (green tea), which typically exhibits DPPH IC_{50} values between 20–40 µg/mL, and *Curcuma longa* (turmeric) with FRAP values around 300–500 µM, the antioxidant potential of *M. indica* root extract is relatively moderate to strong. This positions it as a promising, though slightly less potent, alternative to classical

antioxidant botanicals while still exceeding many lesser-known medicinal plants in terms of phenolic concentration and Ferrous ion chelation ability [34]. This study highlights, for the first time, the phytochemical richness and notable antioxidant activity of the root ethanol extract of *M. indica*. These findings suggest that the root extract may serve as a promising candidate for developing interventions targeting oxidative stress-related disorders, with relevance in future pharmaceutical research.

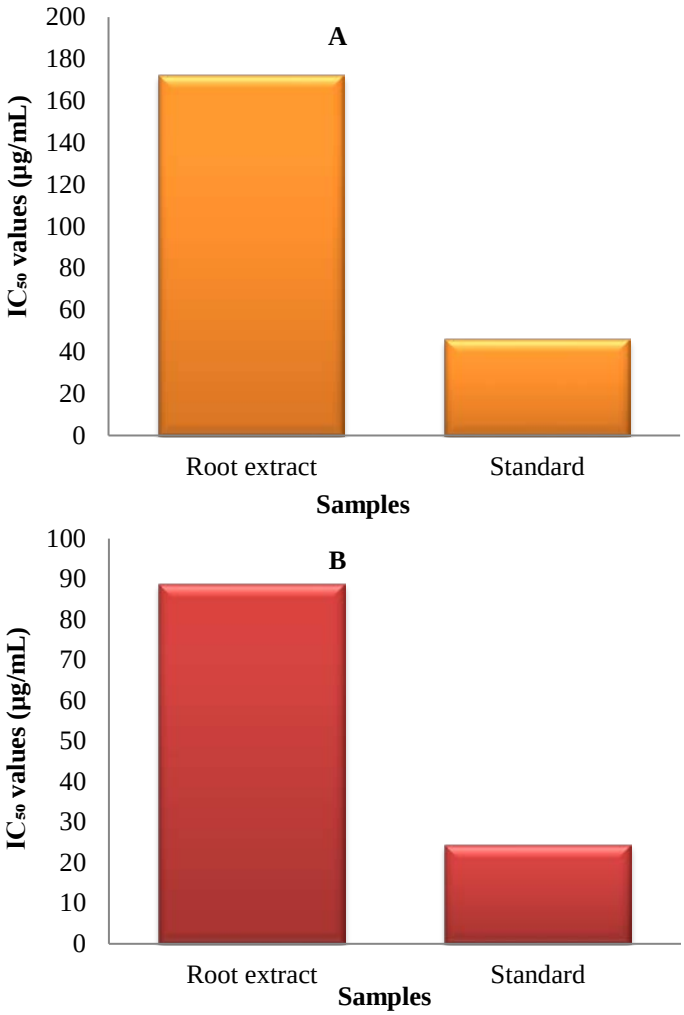

Figure 2: (A) IC_{50} values of ferrous ion chelating activity of *Maesa indica* root ethanol extract compared to the standard antioxidant EDTA. (B) IC_{50} values of DPPH free radical scavenging activity of *Maesa indica* root ethanol extract in comparison with ascorbic acid, shown in bar graph format.

CONCLUSION

The present study demonstrates that the ethanol extract of *Maesa indica* root is a promising source of bioactive constituents, supported by its rich phytochemical profile and significant antioxidant capacity. Preliminary screening confirmed the presence of key secondary metabolites, including phenolics, flavonoids, steroids, and saponins, which are known for their diverse biomedical activities. The high total phenolic content (380.91 ± 23.52 µg/mg) and moderate flavonoid levels (114.21 ± 6.25 µg/mg) correlate with strong antioxidant effects, as evidenced by DPPH radical scavenging (IC_{50}: 88.78±1.64 µg/mL), and ferrous ion chelating (172.31± 1.40 µg/mL). These results align with findings reported for other species within the Primulaceae family. However, this study is limited to in vitro evaluations and does not address the bioavailability, toxicity, or pharmacokinetics of the identified compounds. Further in vivo and mechanistic studies are essential to establish clinical relevance and therapeutic efficacy. This research underscores the therapeutic potential of *M. indica* root as a natural antioxidant source, advocating for its development as an alternative to synthetic antioxidants and its exploration in future pharmacological applications. Despite the study's encouraging results regarding the phytochemical diversity and antioxidant capacity of *M. indica* ethanol extract, the findings are solely based on in vitro tests, which may not accurately represent how the compounds behave biologically in living systems. The pharmacokinetics, metabolism, toxicity, and in vivo efficacy of the extract or their components are not examined in this study. These restrictions highlight the need for additional research to confirm the safety of the root extract, their mode of action, and their therapeutic potential under physiological conditions.

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FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Pooja K P conceptualized the study, designed the methodology, conducted the investigation, curated and analyzed the data, and contributed to drafting the original manuscript. Shrishail supervised the work, provided necessary resources, validated the findings, and contributed to the review and editing of the manuscript. Both the authors read and approved the final manuscript.

ABBREVIATIONS

TPC: Total phenolic content, FCR: Folin-Ciocalteu reagent, TPTZ: 2, 4, 6- Tris(2-pyridyl)-s-triazine

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