OPEN ACCESS

Phytopharmacological Communications

DOI: doi.org/ 10.55627/ppc.004.001.0548

Research Article

FT-IR Analysis, Phytochemical Content and Antioxidant Activity of *Murraya koenigii* Leaf Extracts

Javeria Anwar, Sana Khan, Muhammad Asad Anwar*

Department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology Islamabad

***Correspondence:** asad.anwar@cust.edu.pk

Abstract

Murraya koenigii (M. koenigii) which belongs to the family *Rutaceae* is a medicinal plant that is rich with phytochemicals. Phytochemicals are bioactive compounds that can be used as sources for prevention and treatment of many diseases. This study aims to determine the total phenolic content (TPC), total flavonoid content (TFC), total antioxidant activity (TAA), and characterisation of phytochemicals of the *M. koenigii* leaves; after applying different drying methods and a variety of solvents for extraction of phytochemicals. Sun-dried, shade-dried, and fresh *M. koenigii* leaves are employed in the study along with the use of n-hexane, methanol, and distilled water as a solvent for extraction of phytochemicals based on polarity. The maceration method is used for extraction, the Folin-Ciocalteu method for evaluation of total phenolic content, the colorimetric test for total flavonoid content, phosphomolybdate assay for antioxidant activity analysis, and Fourier transform infrared spectroscopy (FT-IR) for determining various functional groups present in these phytochemicals. Phytochemical contents, antioxidant activities and functional groups are compared in different types of leaves as well as different solvents. The results of the study revealed that shade-dried leaves extracted using distilled water and fresh leaves extracted using methanol have high TAA which is 0.371 ± 0.02 µg/mL and 0.375 ± 0.01 µg/mL, respectively. This high antioxidant activity is due to high phenolic and flavonoid content in these samples, 67.03±3.10, 0.75±0.13 (mg GAE/g dry weight) for *M. koenigii* Shade Dried Water extract (MKShDW) and 112.94±1.03, 0.52±0.08 (mg QE/g Fresh Weight) for *M. koenigii* Fresh Methanolic extract (MKFrMe), respectively. Thus, these leaves can be used as a good source of natural antioxidants. Various functional groups are observed in the FT-IR analysis of this study such as alkanes, alcohols/phenols, aldehydes, esters, ethers, amines, amides, etc.

Keywords: *Murraya koenigii*, Phytochemicals, Solvents, Drying Methods, Antioxidant activity, FT-IR analysis.

1. Introduction

Murraya koenigii (M. koenigii), also known as "curry leaf" or "Kari patta" (Rana and Yamini 2022, 1-6) in Pakistan, is a commonly used aromatic plant in South Asian countries, particularly India and Pakistan. It has been used for centuries for its medicinal properties and as a spice in culinary. The plant belongs to the family *Rutaceae* (Abuga et al. 2020) and is a deciduous shrub of about 16–15 inches or a small tree of about 3-5 meters with woody stems and smooth leaves (2-4 cm long) that are strongly aromatic. It also has small white, 5-petaled fragrant flowers (Sharma et al. 2020). Its fruit is an edible small, glandular berry, clustered closely, enclosing two green seeds and blackens when ripe (Abeysinghe et al. 2021). According to the "Flora of Pakistan", the curry leaf plant is commonly found along the foothills of the Himalayas and has spread to other regions due to migration, from the River Ravi towards Tamil Nadu and Assam and to Chittagong in Pakistan, India, and Bangladesh respectively (Datta et al. 2023, 271-287). *M. koenigii* has been utilized as a medicinal plant, flavorant, and aromatic with widespread ethnobotanical use for centuries. The

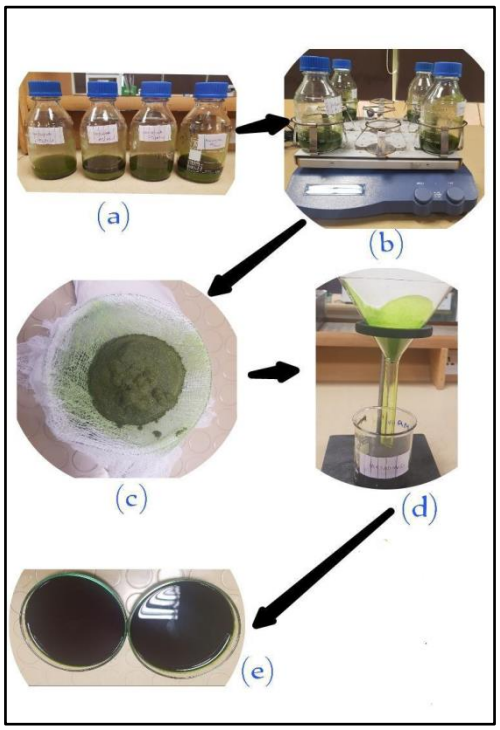

Figure 1: (a) Dried sample mixed with solvent (b) the mixture was placed on the shaker in bottles (c) the extract was strained using a muslin cloth (d) the resulting extract was passed through a filter paper (e) the filtrate was transferred into petri plates for drying in fumehood

dried leaves of this plant are often utilized as a seasoning in Pakistani cuisine. The various parts of the curry leaf plant, including stems, bark, seeds, fruits, roots, and leaves, play a significant role in fighting infection and boosting immunity. Consuming the leaves raw cures dysentery and the extract of its root is administered to control kidney pains and reduce fever (Azmin and Chng 2020, 26-28). In traditional Ayurveda, curry leaves were used to treat hypertension, hysteria, cough, rheumatism, and hepatitis (Raghu 2020, 1-8). *M. koenigii* has a variety of chemical components that interact to produce their pharmacodynamic response. The presence of phytochemicals such as alkaloids, essential oils, phenolics, terpenoids, tocopherol, -carotene, and lutein as well as minerals, protein, and fat has been suggested as the cause of the bioactivity of curry leaf (Balakrishnan et al. 2020, 3-7). Although the curry leaf plant produces a relatively small number of phytochemicals, it possesses hepatoprotective, anti-hypercholesterolemic, anti-diabetic, diabetic, antibacterial, anti-inflammatory, and antioxidant activity (Balakrishnan et al. 2020, 13-17).

The pathogenesis of various conditions involves the participation of reactive oxygen and reactive nitrogen species, which include free radicals. The living organism must maintain an equilibrium between the formation of these reactive species and their accumulation in cells and tissues, as well as the biological system's capacity to detoxify them. The absence of this balance results in a condition known as oxidative stress, which occurs when the concentration of reactive species is too high, leading to harmful effects on humans, plants, and animals. Antioxidants utilized in medicine and industry can be classified into; natural and synthetic. Synthetic antioxidants, which are commonly used, are believed to have detrimental effects on the human body, prompting a shift towards natural antioxidants derived from plants (Sen and Chakraborty 2011). One of the most prominent natural antioxidant groups is polyphenolic compounds that possess one or more hydroxyl groups

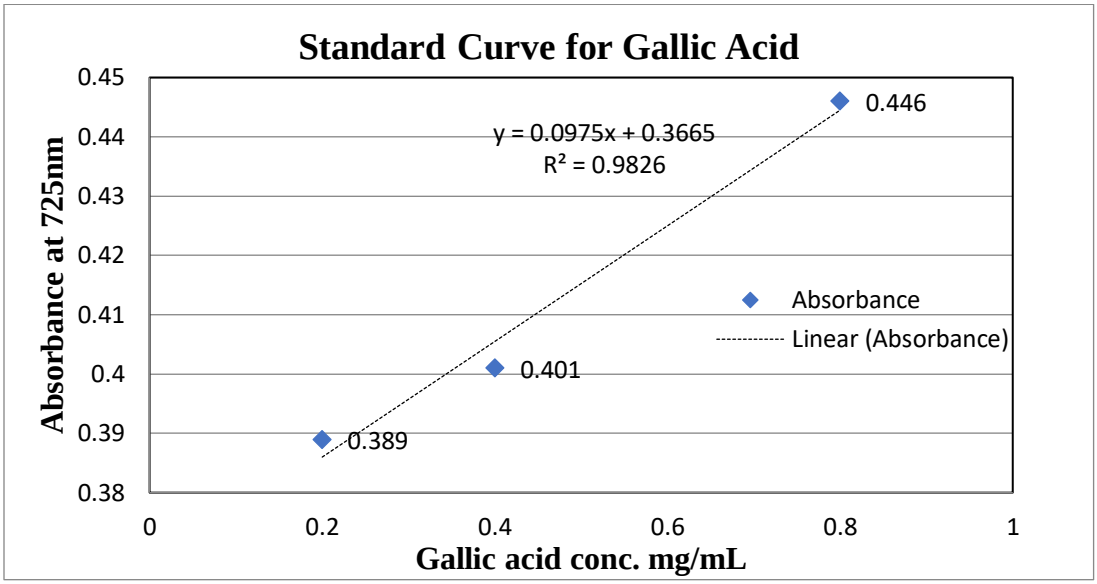

Figure 2: Scatter plot diagram for concentrations of Gallic Acid.

Table 1: Total Phenolic Contents and Total Flavonoid Contents in *M. koenigii* leaf extracts.

Leaf Extract Samples	Total Phenolic Content (mg GAE/g Dry Weight)	Total Flavonoid Content (mg QE/g Dry Weight)
MKSuDMe	6.67±0.40	0.55±0.14
MKSuDnH	21.05±1.74	0.46±0.06
MKSuDW	30.16±1.39	0.41±0.30
MKShDMe	2.05±1.94	0.58±0.03
MKShDnH	1.31±0.84	0.43±0.12
MKShDW	67.03±3.10	0.75±0.13
MKFrMe	112.94±1.03	0.52±0.08
MKFrnH	1.66±0.29	0.50±0.04
MKFrW	41.50±0.66	0.37±0.14

within their chemical structure. These compounds can be grouped into two main classes: phenolic acids and flavonoids (Olszowy 2019). Different organic bioactive compounds like crystalline glycosides, carbazole alkaloids, koenigin, girinimbin, iso-mahanimbin, koenine, koenidine, and koenimbine show exceptional antioxidant properties (Asema et al. 2021, 1798-1801). The mechanism of action of the antioxidant activity of curry leaves involves the ability of these phytochemicals to scavenge free radicals and reactive oxygen species (ROS) in the body by neutralizing free radicals after donating an electron or hydrogen atom to stabilize them and prevent them from causing further damage. Curry leaves' antioxidant qualities are attributed to their ability to activate innate antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (Gill and Sharma 2014, 126–29; Rehana et al. 2017). It was discovered that the antioxidant effect may be caused by the plant's high total phenolic content and total flavonoid content as well as its capacity to scavenge free radicals (Aryal et al. 2019, 96). The purpose of our

study was to determine the effect of different drying methods on the phytochemical content and antioxidant activity of *M. koenigii* leaves.

2. Materials and Methods

2.1. Apparatus and Equipment

The utilized instruments and equipment include a UV-visible spectrophotometer (UV-1602 spectrophotometer - BMS - MA State, USA), a water bath, dry bath, a rotary flask shaker (BIORAYS), Nicolet iS10 FT-IR Spectrometer (Thermo Scientific), glass pipette, micropipettes, electronic analytical balance, test tubes, flasks, petri plates, Eppendorf/ culture tubes, Eppendorf tubes rack.

2.2. Chemicals

n-Hexane (Sigma), distilled water, Methanol (Sigma), Folin-Ciocalteu reagent (FC reagent) (Sigma), Sodium carbonate (Sigma), Gallic acid (Sigma), Quercetin (Sigma), Aluminum chloride (Sigma), Potassium acetate (Sigma), Ascorbic acid (Sigma), Sulphuric Acid (Sigma), Sodium Phosphate (Sigma), and Ammonium Molybdate (Sigma).

2.3. Sample Collection and Authentication

The fresh leaf samples of *M. koenigii* were collected from the Islamabad region. The authenticity of the samples was verified by the Pakistan Museum of Natural History, Islamabad. Voucher specimens, accession number 043157, were deposited at the Pakistan Museum of Natural History, Islamabad.

2.4. Preparation of Samples

The collected *M. koenigii* leaf samples were partitioned and cleaned to eliminate any attached foreign matter. For drying, leaves were allowed to sun-dry for a period of 5 days, while some were shade-dried at room temperature for 15 days and subsequently pulverized into a fine powder by using a mechanical blender. To achieve a particle size smaller than 1.40 mm, sun-dried and shade-dried ground leaf samples were also sieved. To ensure their preservation, all the powdered materials were stored in sterile bags in a dry location. The maceration method of extraction was used to obtain curry leaf extracts. n-hexane, methanol, and water were employed as the extracting solvents in concentrations of 25g/100ml for each sun-dried, shade-dried, and fresh sample (Figure 1). The fresh leaf sample was extracted with methanol, n-hexane, and distilled water separately, using a blender. To determine solvent efficiency, the percentage yield was calculated from the extracts by using the formula; % yield = weight of dry extract/ weight of dry sample X 100.

2.5. Total Phenolic Content (TPC) Determination

The TPC of fresh and dried leaf extracts from *M. koenigii* was analyzed using the Folin-Ciocalteu reagent (FC reagent) by using the method described by (Tabassum et al. 2019) with slight optimization. In this analysis, the DMSO-dissolved plant extract (1.0 mg/mL) was mixed with the FC reagent. Each plant extract sample (1.0 mg/mL) was dispensed into a test tube in a volume of 300µL. To this, 1.5 mL of FC reagent was added, and the mixture was allowed to incubate at room temperature for 5 minutes. Subsequently, 1.5 mL of a 7% Na_2CO_3 solution was added to the test tube, and the solution was left at room temperature for 90 minutes. The absorbance was measured at 725 nm of the resulting solution. All the tests were performed in triplicates. Gallic acid equivalents (GAE) of the fraction in mg/g were used to determine the TPC. A stock solution of 10 mg/mL of gallic acid was prepared, afterwards dilutions with a concentration range of 200-800 µg/mL were used to obtain the standard curve.

2.6. Total Flavonoid Content (TFC) Determination

The total flavonoid levels were determined using a calorimetric test as described by (Tabassum et al. 2019) with modifications. In this method, 500 µL of the DMSO dissolved extracts (1 mg/mL) were combined with 1.5 mL of methanol in a test tube. To this premixed solution, 100 µL of 10% Aluminum chloride, 100 µL of 1M Potassium Acetate, and 2.8 mL of distilled water were added. The resulting mixture was incubated for thirty minutes at room temperature, and the absorbance was measured at 415 nm. All the tests were performed in triplicates. The TFC was calculated as mg Quercetin equivalents (QE) per gram of dried extract. A

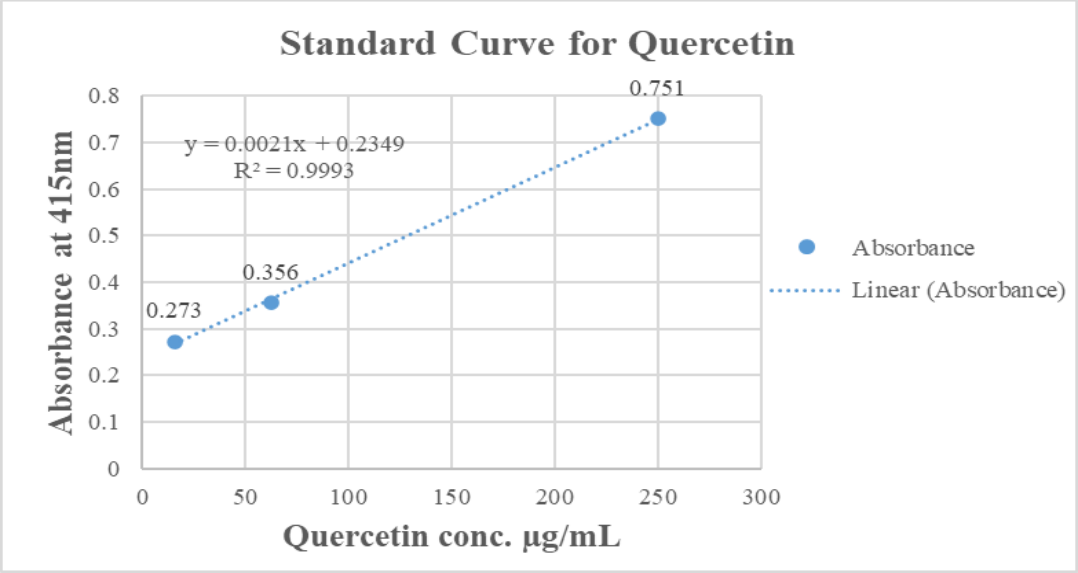

Figure 3: Scatter plot diagram for Quercetin concentrations.

Table 2: Total antioxidant activity of *M. koenigii* leaf extracts.

Leaf Extract Samples	Total Antioxidant Activity (Absorbance at 695 nm of 50 µg/mL of Sample)
MKSuDMe	0.356±0.01
MKSuDnH	0.395±0.02
MKSuDW	0.331±0.33
MKShDMe	0.353±0.02
MKShDnH	0.340±0.01
MKShDW	0.371±0.02
MKFrMe	0.375±0.01
MKFrnH	0.362±0.00
MKFrW	0.305±0.07
Ascorbic Acid	0.867±0.01
Quercetin	0.422±0.00

stock solution of 10 mg/mL of quercetin was prepared, afterwards dilutions with a concentration range of 15.6-250 µg/mL were used to obtain the standard curve.

2.7. Antioxidant Activity Determination

The TAA of the fractions was determined by the phosphomolybdate method as described by (Saeed et al. 2012). Dilutions of 50 µg/mL, 500 µg/mL, and 1000 µg/mL of plant extract (1mg/mL) were prepared. Briefly, 100µL extract dilution was mixed with 1 ml of reagent solution (0.6M Sulphuric Acid, 28 mM Sodium Phosphate, and 4 mM Ammonium Molybdate) in test tubes. The tubes were closed and incubated in a dry bath at 95°C for 90 minutes. Afterwards, the samples were allowed to cool down to room temperature and their absorbance was measured at 695 nm against a blank. For blank, 100µL of DMSO was mixed with the reagent solution and incubated under the same

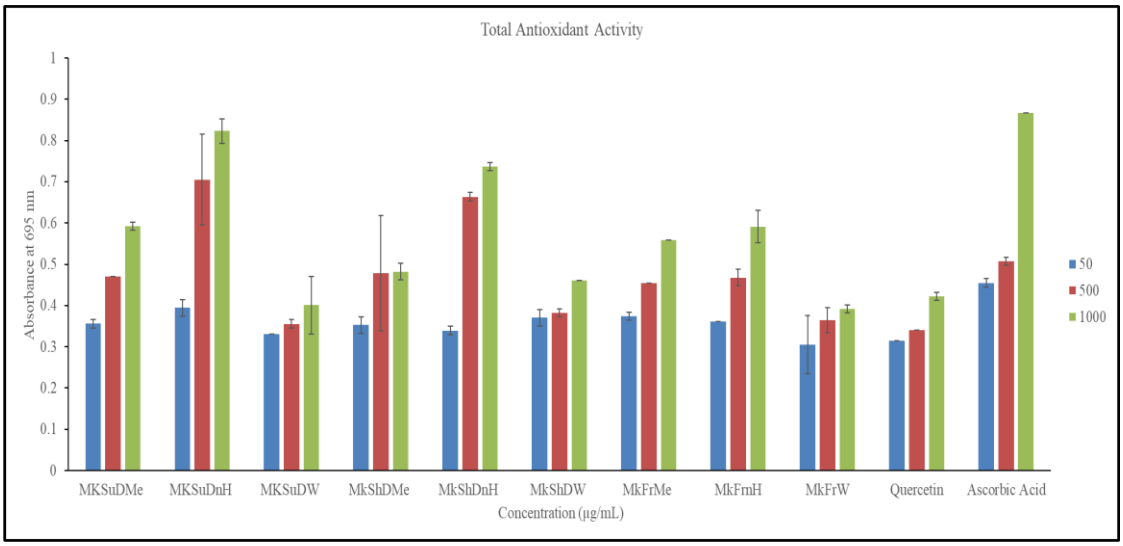

Figure 4: Total antioxidant activity of *M. koenigii* leaf extracts.

conditions (90 min at 95°C). All tests were performed in duplicates. Ascorbic acid and quercetin were used as the reference standards.

2.8. FT-IR Analysis

An FTIR spectroscopic analysis was performed to identify the functional groups present in *M. koenigii* leaves. The FT-IR spectra of 9 samples, *M. koenigii* Sun-Dried Methanolic extract (MKSuDMe), *M. koenigii* Sun-Dried n-Hexane extract (MKSuDnH), *M. koenigii* Sun-Dried Water extract (MKSuDW), *M. koenigii* Shade Dried Methanolic extract (MKShDMe), *M. koenigii* Shade Dried n-Hexane extract (MKShDnH), MKShDW, MKFrMe, *M. koenigii* Fresh n-Hexane extract (MKFrnH) and *M. koenigii* Fresh Water extract (MKFrW), were recorded on a Nicolet iS10 FT-IR instrument within the wavelength range of 400-4000 cm-1.

2.9. Statistical Analysis

Microsoft Excel was used to perform the statistical analysis as well as for regression analysis. Mean values and standard deviations from three replicates of each experiment were calculated and reported as the mean value ± the standard deviation.

3. Results

3.1. Total Phenolic Content (TPC)

The TPC of the different *M. koenigii* extracts varied significantly as shown in Table 1. To determine the number of phenols in each fraction, TPC methodology was employed. Different solvents were used to extract phenols with varying polarity. The TPC trend determined shows the highest phenolic content in MKFrMe, whereas the lowest is MKShDnH. The overall trend seen was, MKFrMe > MKShDW > MKFrW > MKSuDW > MKSuDnH > MKSuDMe > MKShDMe > MKFrnH > MKShDnH. Gallic acid was used as the standard for TPC. The corresponding absorbance values recorded for different concentrations of gallic acid were in the range of 0.389-0.446. As the concentration of gallic acid increased, there was a discernible increase in absorbance, suggesting a proportional relationship between the concentration of gallic acid and the absorbance measured as shown in the scatter plot (Figure 2).

3.2. Total Flavonoid Content

Spectrophotometric measurement of flavonoids in different samples was done using the TFC determination method (Table 1). The majority of oxidizing chemicals, including singlet oxygen, and other free radicals are effectively scavenged by flavonoids. Within the various fractions, the TFC varied between the lowest 0.37 mg QE/g of MKFrW extract and the highest, 0.75 mg QE/g of MKShDW extract. The trend formed is as; MKShDW > MKShDMe > MKSuDMe > MKFrMe > MKFrnH > MKSuDnH > MKShDnH > MKSuDW > MKFrW.

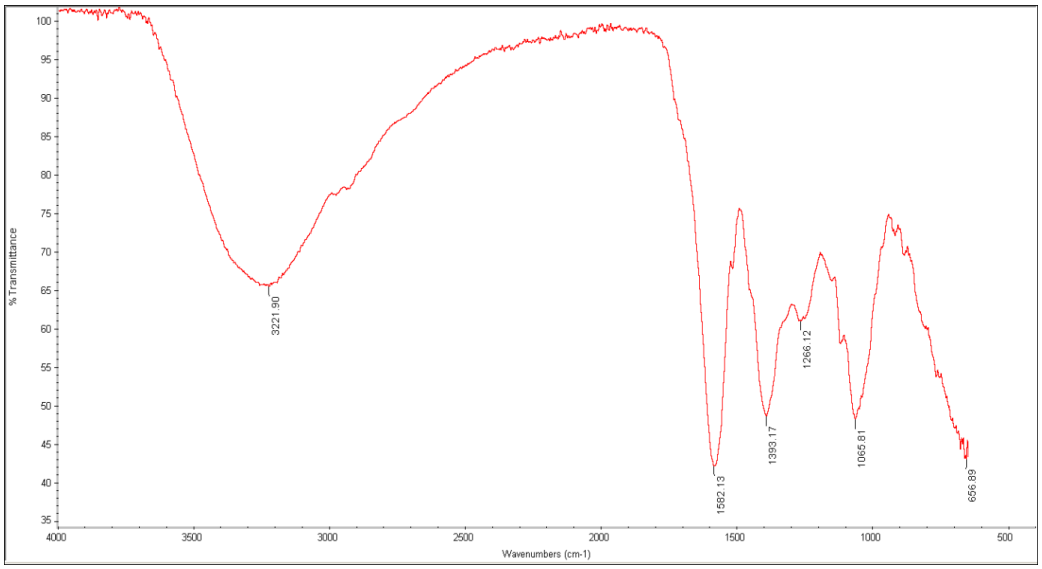

Figure 5: FT-IR spectrum representing potential bands in MKShDW (*M. koenigii* Shade Dried Water Extract)

Table 3: FT-IR Spectral peak values of MKShDW with functional groups from literature (Cseke et al., 2006).

Peak Value	Functional Group Assignment	Possible Compound
656.89	C-I	Halo compound
1065.81-1266.12	C-O, C-O(Aryl), C-N	Alcohol/Phenols, Ether, Ester, Amide, Amine
1393.17	-CH_3, O-H	Alkanes, Alcohol/Phenols, Acid
1582.13	C= C, N-H	Aromatics, Amine
3221.90	O-H, N-H	Alcohol/Phenols, Amine, Amide

Quercetin was used as a standard for TFC. The absorbance values of different concentrations of quercetin were recorded in the range of 0.273-0.751. As the concentration of quercetin increased, there was a noticeable rise in absorbance, indicating a proportional relationship between the concentration of quercetin and the absorbance measured as shown in the scatter plot (Figure 3).

3.3. Total Antioxidant Activity

The assay for phosphomolybdate reduction has been widely utilized to ascertain the overall antioxidant potential of samples. The extract's antioxidant activity was as follows: MKSuDnH > MKFrMe > MKShDW > MKFrnH > MKSuDMe > MKShDMe > MKShDnH > MKSuDW > MKFrW. The leaf extract samples' absorbance values were between 0.305 ± 0.07 µg/ml and 0.395 ± 0.02 µg/ml, at 695 nm (Table 2). As shown in Figure 4, all the leaf extracts prepared in different solvents and standard compounds showed an increase in antioxidant activity in a dose-dependent manner. From Figure 4, it was observed that the TAA of curry leaves was highest for Ascorbic acid followed by Quercetin as they were standards. It was followed by MKSuDnH extract, followed by TAA of MKShDnH extract. Next in descending order, was the TAA of MKFrMe extract, followed by that of MKShDW, then the TAA of MKFrnH. The TAA of MKShDMe extract using methanol is lower than the TAA of MKShDW. The second lowest is the TAA of the MKSuDW extract. While the lowest TAA is of MKFrW extract. On the other hand, the highest TAA was shown by a sun-dried n-hexane extract.

3.4. FT-IR Analysis of Leaf Extracts

The peak values found in the FT-IR spectrum were used to determine the functional groups of the active components contained in the three extracts

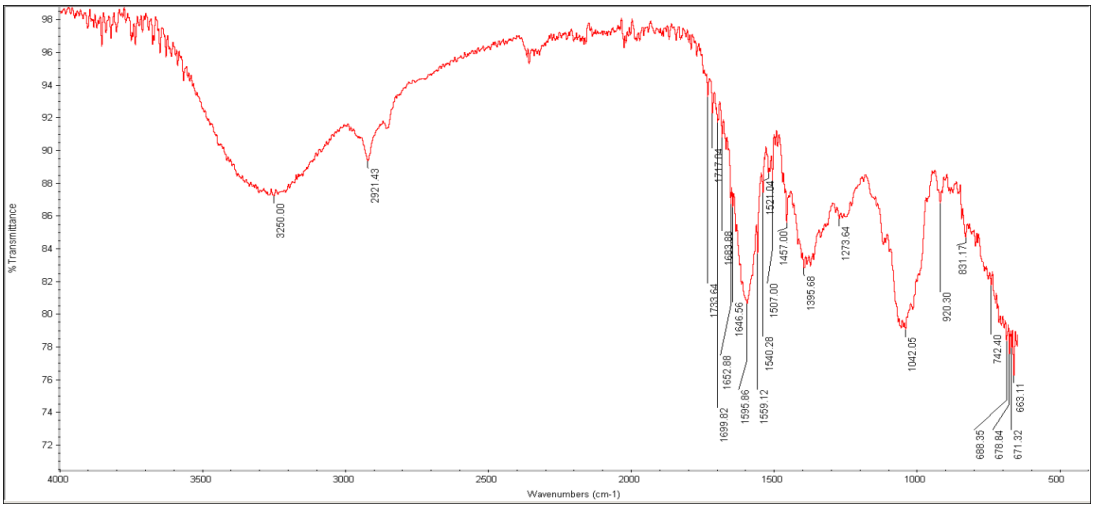

Figure 6: FT-IR spectrum representing potential bands in MKFrMe (*M. koenigii* Fresh Methanol Extract)

Table 4: FT-IR Spectral peak values of MKFrMe with functional groups from literature (Cseke et al., 2006).

Peak Value	Functional Group Assignment	Possible Compound
3250.00	C=C-H	Alkenes, Aromatics
2921.43	-C-H, -O-H (Dimer)	Alkanes, Acids
1717.04-1733.64	C=O	Acids, Esters
1646.56-1699.82	C=C, C=O (unsat.), C=O, N-H	Alkenes, Aldehydes, Acids, Amines
1595.86	C=C, N-H (pri), N-H (sec)	Aromatics, Amines
1521.04-1559.12	N-H (sec)	Amines
1395.68-1507.00	C=C	Alkanes, aromatics, alcohols/phenols, acids
1042.05-1273.64	Alkyl, C-O, Aryl, C-N	Alcohols/phenols, Ethers, Esters, Acids, Amines
920.30	O-H	Acids
742.40-831.17	(CH_2), N-H	Alkanes, Amines
663.11-688.35	C-Br, C-Cl	Halo compounds

having the best antioxidant activity. Each potential peak was given a numerical label (Figure 5 to Figure 7), and (Table 3 to Table 5) provided the potential functional groups of the peak values.

4. Discussion

The percentage yield of fresh and shade-dried samples is highest for water extracts and least for n-hexane extracts, depicting the solubility of phenolic compounds in polar solvents. The sun-dried sample has the highest percentage yield for methanolic extract followed by water and n-hexane extracts; these results support previous studies such as (Ali et al. 2021, 985-992). Phenolics are a more potent antioxidant than carotenoids, vitamins C and E, as demonstrated through in vitro studies. It is believed that phenolics possess strong antioxidant properties. Additionally, phenolics have been partially responsible for the inverse relationship observed between the consumption of fruits and vegetables and the occurrence of oxidative stress-related disorders, including cancer, osteoporosis, and cardiovascular diseases (Scalbert et al. 2005, 287-306). The high TPC observed in MKFrMe suggests that freshly harvested leaves possess a rich pool of phenolic compounds, likely due to reduced enzymatic degradation and minimal loss of bioactive constituents during processing (Salve et al. 2020, 584-89). Our study showed that sun-dried samples had more phenolic

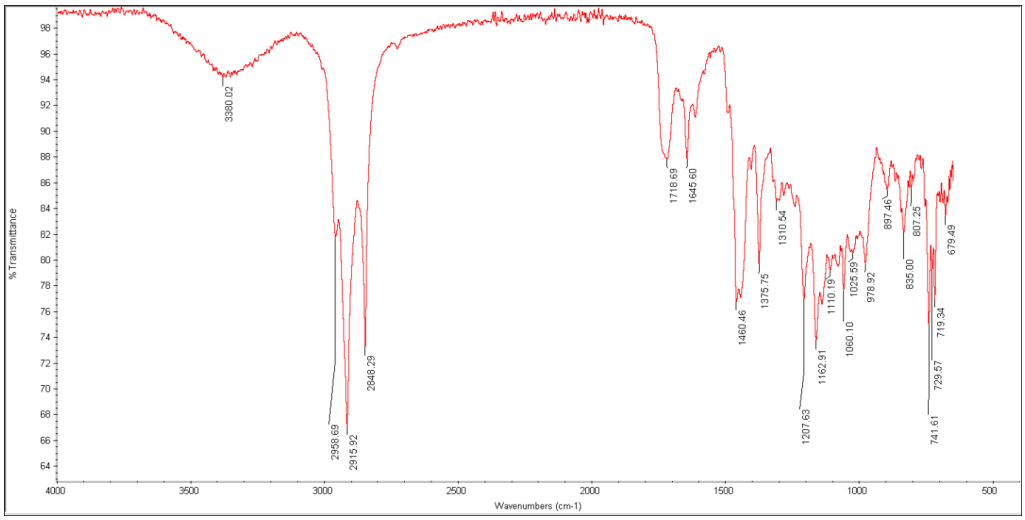

Figure 7: FT-IR spectrum representing potential bands in MKSuDnH (*M. koenigii* Sun-dried n-Hexane Extract)

content than shade-dried samples, although fresh samples showed higher phenolic content than sun-dried ones. This result is consistent with the study by (Roshanak et al. 2015) which shows that high temperatures are responsible for phenol preservation. His study also shows the higher TAA of sun-dried samples contributed by TPC compared to shade-dried samples.

To comprehend plant extracts' possible health advantages and antioxidant qualities, it is essential to look at their TFC. Among the many biological roles that flavonoids exhibit are anti-inflammatory, antioxidant, and anti-carcinogenic properties (Cushnie and Lamb 2005). Shade-dried leaves had higher TFC across all solvents than fresh or sun-dried leaves. Sun-dried leaves had slightly higher TFC content than fresh extracts and higher TFC than MKShDnH. This is similar to the trend of TPC and coincides with (Roshanak et al. 2015) study that showed high TAA of sun-dried extracts. This might also be because the drying temperature was not high enough to degrade the flavonoids. The fresh extracts show overall reduced TFC compared to sun-dried and shade-dried leaves. This might be due to incomplete extraction or enzymatic degradation. Fresh leaves might have intact cell walls that hinder the complete release of flavonoids during extraction compared to dried samples where cell walls might be more disrupted as well as, the presence of active enzymes in fresh samples that can degrade flavonoids soon after harvest.

Adding more antioxidants to one's diet may be a long-term approach to avoid lifestyle-related non-communicable diseases like cancer, cardiovascular disease, and hypertension. This is because free radicals harm the human body (Tomar, Banerjee, and Kaushik 2017). The TAA of the polyphenol extracts obtained from plants was estimated using the phospho-molybdenum test to examine both the absolute and comparative effects. The phospho-molybdenum assay's total antioxidant activity is dependent on the conversion of Mo VI to Mo V and the creation of a green Mo V complex, which has a maximum absorbance at 695 nm (Behera, Senapati, and Parida 2022).

Because volatile chemicals and enzymatic activity are preserved during the extraction process, fresh leaf extracts often display stronger antioxidant activity than shade-dried extracts. Volatile substances found in fresh leaf extracts can greatly enhance their antioxidant capacity. Certain substances, such as flavonoids and phenols, are frequently heat-sensitive and may be lost or deteriorated during the drying process as indicated in (Liyanage et al. 2017). Studies indicate that fresh leaf extracts have a greater concentration of volatile chemicals, which enhances their antioxidant

Table 5: FT-IR Spectral peak values of MKSuDnH with functional groups from literature (Cseke et al., 2006).

Peak Value	Functional Group Assignment	Possible Compound
679.49	C-Br, C-Cl	Halo compound
719.34-978.92	$(CH_2)_n$>4, N-H	Alkanes, Amine
1025.59-1207.63	O-H, C-O, Aryl, C-N	Alcohol/Phenols, Ether, Ester, Amine, Acids
1310.54-460.46	$-CH_3$, O-H, C-O, Aryl, $-CH_2$, C= C	Alkanes, Alcohol/Phenols, Amine, Aromatics, Amine
1645.60	C= C, N-H	Alkenes, Amide
1718.69	C= O	Aldehydes, Ketone, Esters, Acids
2848.29-2958.69	-C-H, O= C-H, O-H	Alkanes, Aldehydes, Acids
3380.02	O-H, N-H	Alcohol/Phenol, Amine, Amide

capacity. Enzymes found in fresh methanolic leaf extracts and extracts from shade water can also support antioxidant activity. Peroxidases and polyphenol oxidase are two examples of enzymes that are involved in the production and metabolism of phenolic compounds, which are important sources of antioxidant activity. Sun-dried methanolic and aqueous extracts have less antioxidant capacity as a result of these enzymes being denatured or rendered inactive during the drying process as in a previous study (Liyanage et al. 2017, 101). The temperature of sun-dried n-hexane extract may not be too high to break down phenolic and flavonoid compounds, or it may contain extracted lipophilic bioactive chemicals that could lead to a high potential for antioxidants. Studies highlight how crucial enzymatic activity is to preserving the antioxidant potential of fresh plant extracts. According to the results of this study, for fresh samples as well as sun-dried samples the TAA of samples extracted using methanol is higher than the one using distilled water which is following previous study (Ali et al. 2021). While in shade-dried samples TAA is higher for the distilled water extracted sample than the methanol extracted sample, which might be an effect of the dryness conditions applied. The drying process exposes sensitive substances to heat and air, which can cause oxidative deterioration, especially when it is carried out in the shade. The TAA of shade-dried extracts may be decreased by this degradation due to the long duration of drying following a previous study (Saifullah et al. 2019). It has been proved that all the extracts have the capability of quenching free radicals and converting them to a more stable state.

Plant extracts FT-IR spectra may alter as a result of drying for several reasons, including the loss of volatile chemicals. This may have an impact on the peak intensities linked to those particular chemicals. Some functional groups can undergo oxidation, which can create new peaks or change the intensity of already existing ones. In a similar way peak intensity and resolution may be affected by changes to the sample's physical structure brought about by grinding and drying. It has been revealed that the type of plant, the drying process, and the storage environment all affect the degree of spectral variances. According to some studies, it may be better to use fresh samples to get a more accurate representation of the chemical makeup (Ray et al. 2022). The number of peaks and number of functional groups found in shade-dried and sun-dried samples are more in samples extracted using n-hexane followed by methanol and least are in water. The differences in the number of peaks in fresh, sun-dried, and shade-dried leaves might be due to different temperatures and drying conditions applied as indicated in a previous study (Iwansyah et al. 2020, 8586). While fresh leaves sample the peak numbers and functional groups are more in water followed by methanol and least are in n-hexane extracts which might be due to polarity differences among these solvents. Different

types of compounds were characterized in the leaf extracts such as Alkanes, Alkenes, Alcohols/ Phenols, Esters, Ether, Acids, Aldehydes, Amines, Amides etc. Most of the extracts contain hydroxyl groups which depict the antioxidant activity of these extracts thus supporting previous studies (Bhardwaj et al. 2016).

5. Conclusion

Based on the results received for this study, it can be assumed that fresh methanolic curry leaves and shade-dried leaves extracted by using distilled water have high antioxidant activity; the reason for this can be the high TPC and TFC found in these. As both methanol and distilled water are polar solvents, these can extract most of the bioactive compounds present in curry leaves. Thus, these can be used as natural sources of phenolics, flavonoids, and antioxidants for treating different diseases. FT-IR data also supports phytochemical content and antioxidant activity in *M. koenigii* leaves.

Data Availability

All the relevant data of this manuscript is available with the authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Funding

N/A

Author Contributions

JA and SK performed experimental work, data collection and evaluation, literature search, and manuscript preparation. MAA supervised the research work, compiled results, and refined the manuscript for publication. The authors read and approved the final manuscript for publication.

Acknowledgments

The authors are thankful to the Department of Bioinformatics and Biosciences, Capital University of Science & Technology, Islamabad, Pakistan.

References

Abeysinghe, D. Thelma, K. A. H. Kumara Hasanka Kumara. K. A. D. Kaushalya, Chandrika Udumalagala Gamage, and D. D. D. H. Alwis. 2021. "Phytochemical Screening, Total Polyphenol, Flavonoid Content, In Vitro Antioxidant and Antibacterial Activities of Sri Lankan Varieties of *Murraya Koenigii* and *Micromelum Minutum* Leaves." *Heliyon* 7 (7): e07449. https://doi.org/10.1016/j.heliyon.2021.e07449

Abuga, Ibrahim, Shaida Fariza Sulaiman, Ridhwan Abdul Wahab, Kheng Leong Ooi, and Mohammad Syaiful Bahari Abdull Rasad. 2020. "In vitro antibacterial effect of the leaf extract of Murraya koenigii on cell membrane destruction against pathogenic bacteria and phenolic compounds identification." European Journal of Integrative Medicine 33: 101010.

Ali, Ayesha Idris, Virginia Paul, Amit Chattree, Ranu Prasad, Ajit Paul, and Daniel Amiteye. 2021. "Evaluation of the use of different solvents for phytochemical constituents and antioxidants activity of the leaves of *Murraya koenigii* (Linn.) Spreng. (Rutaceae)." *Plant Archives* 21 (1): 985-992. DOI: https://doi.org/10.51470/PLANT-ARCHIVES.2021.v21.no1.137

Aryal, Sushant, Manoj Kumar Baniya, Krisha Danekhu, Puspa Kunwar, Roshani Gurung, and Niranjan Koirala. 2019. "Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables From Western Nepal." *Plants* 8 (4): 96. https://doi.org/10.3390/plants8040096.

Asema, Syed Ummul Khair, Anis Ahmed Shaikh, Mazahar Farooqui, and Samreen Fatema. 2021. "Phytochemical analysis of *Murraya*

koenigii leaves extract and its biological activity." *The International Journal of Analytical and Experimental Modal Analysis* 3 (1): 1798-1801.

Azmin, Siti Nuurul Huda Mohammad and Huck Ywih Chng. 2020. "Physio-chemical properties of curry leaf." In *Curry Leaf (Murraya koenigii): The Story of a Potential Miracle Plant*, 26-28. LAMBERT Academic Publishing. DOI: https://www.researchgate.net/publication/340234052

Balakrishnan, Rengasamy, Dhanraj Vijayraja, Song-Hee Jo, Palanivel Ganesan, In Su-Kim, and Dong-Kug Choi. 2020. "Medicinal Profile, Phytochemistry, and Pharmacological Activities of *Murraya koenigii* and its Primary Bioactive Compounds." Antioxidants 9 (2): 101. https://doi.org/10.3390/antiox9020101.

Behera, P. C., M. R. Senapati, and D. R. Parida. 2022. "Anti-oxidative Effect of Polyphenols of *Litsea Glutinosa* Leaf: An In-Vitro Assay." *E-Planet* 20 (1): 40-46.

Bhardwaj, Khushboo, Sunil Kumar, and Sudarshan Ojha. 2016. "Antioxidant Activity and FT-IR Analysis of *Datura innoxia* and *Datura metel* Leaf and Seed Methanolic Extracts." *African Journal of Traditional, Complementary and Alternative Medicines* 13 (5): 7-16. DOI:https://doi.org/10.21010/ajtcam.v13i5.2

Chan, Kelvin, and T.X. Lin. 2009. "Treatments used in complementary and alternative medicine." Dans *Side effects of drugs annual*. , 745–56. https://doi.org/10.1016/s0378-6080(09)03148-1.

Cseke, L. J., A. Kirakosyan, P. B. Kaufman, S. L. Warber, J. A. Duke, and H. L. Brielmann. 2006. "Characterization of Natural Products." In *Natural Products from Plants*, edited by Taylor and Francis, 319-388. CRC, Florida, USA.

Cushnie, T.P. Tim, and Andrew J. Lamb. 2005. "Antimicrobial activity of flavonoids." *International Journal of Antimicrobial Agents* 26 (5): 343–56. https://doi.org/10.1016/j.ijantimicag.2005.09.002.

Datta, H. S., D. Bora, M. D. Purkayastha, M. Choudhury, and M. Neog. 2023. "*Murraya koenigii* (L.) Spreng." In *Himalayan Fruits and Berries*, edited by T. Belwal, I. Bhatt, and H. Devkota, 271–287. Academic Press, UK. DOI: https://doi.org/10.1016/b978-0-323-85591-4.00044-1

Gill, Naresh Singh, and Bhartendu Sharma. 2014. "Study on Antioxidant Potential of *Murraya Koenigii* Leaves in Wistar Rats." *Pakistan Journal of Biological Sciences* 17 (1): 126–29. https://doi.org/10.3923/pjbs.2014.126.129.

Iwansyah, Ade C., Tranh D. Manh, Yusuf Andriana, Muhammad A. Hessan, Faridah Kormin, Dang X. Cuong, Nguyen X. Hoan, Dang T. Yen, Pham V. Thinh, Lam T. Hai, and Truong N. Minh. 2020. "Effects of Various Drying Methods on Selected Physical and Antioxidant Properties of Extracts from *Moringa oleifera* Leaf Waste." *Sustainability* 12: 8586. DOI: 10.3390/su12208586

Joshi, Trapti, Tushar Jain, Rohit Mahar, Sumit K. Singh, Piush Srivastava, Sanjeev K. Shukla, Dipak K. Mishra, R. S. Bhatta, Dibyendu. Banerjee, and Sanjiv Kanojiya. 2018. "Pyranocarbazoles from *Murraya koenigii* (L.) Spreng. as Antimicrobial Agents." *Natural Product Research* 32 (4): 430–434. DOI: https://doi.org/10.1080/14786419.2017.1308363

Kaczorová, Dominika , Erna Karalija, Sabina Dahija, Renata Bešta-Gajević, Adisa Parić, and Sanja Ćavar Zeljković. 2021. "Influence of Extraction Solvent on the Phenolic Profile and Bioactivity of Two Achillea Species." *Molecules* 26 (6): 1601. DOI: https://doi.org/10.3390/molecules26061601

Liyanage, Ruvini, Chathuni Jayathilake, Rizliya Visvanathan, Swarna Wimalasiri, W. M. M. M. Dissanayaka, and Barana Chaminda. 2017. "Effect of Different Drying Methods on Antioxidant Activity of Star Fruits (Averrhoa carambola L.)." *Journal of Nutrition and Diet Supplements* 1 (1): 101. DOI: https://api.semanticscholar.org/CorpusID:52538645

Mandal, Suvra, Anupam Nayak, Manoj Kar, Samir K. Banerjee, Ashes Das, S. N. Upadhyay, R. K. Singh, Avijit Banerji, and Julie Banerji. 2010. "Antidiarrhoeal Activity of Carbazole Alkaloids from *Murraya koenigii* Spreng (Rutaceae) Seeds." *Fitoterapia* 81 (1): 72-74. DOI: 10.1016/j.fitote.2009.08.016

Olszowy, Małgorzata. 2019. "What Is Responsible for Antioxidant Properties of Polyphenolic Compounds from Plants?" *Plant Physiology and Biochemistry* 144: 135-143. DOI: https://doi.org/10.1016/j.plaphy.2019.09.039

Raghu, B. R. 2020. "Diversity and Distribution of Curry Leaf in India." *Journal of Horticultural Sciences* 15 (1): 1-8. DOI: https://doi.org/10.24154/JHS.2020.v15i01.001

Rana, Anita, and Yamini. 2022. "Antimicrobial properties and phytochemical analysis of different extracts of *Murraya koenigii*." Journal of Pharmacognosy and Phytochemistry 11, no. 6: 01-06.

Ray, Asit, Swagat Mohanty, Sudipta Jena, Ambika Sahoo, Laxmikanta Acharya, Pratap C. Panda, Parsuram Sial, Prasath Duraisamy, and Sanghamitra Nayak. 2022. "Drying Methods Affect Physicochemical Characteristics, Essential Oil Yield and Volatile Composition of Turmeric (Curcuma longa L.)." *Journal of Applied Research on Medicinal and Aromatic Plants* 26: 100357.

Rehana, Dilaveez, Dharmasivam Mahendiran, R. Senthil Kumar, and A. Kalilur Rahiman. 2017. "In Vitro Antioxidant and Antidiabetic Activities of Zinc Oxide Nanoparticles Synthesized Using Different Plant Extracts." *Bioprocess and Biosystems Engineering* 40 (6): 943–57. https://doi.org/10.1007/s00449-017-1758-2.

Roshanak, Sahar, Mehdi Rahimmalek, and Sayed A. H. Goli. 2015. "Evaluation of Seven Different Drying Treatments in Respect to Total Flavonoid, Phenolic, Vitamin C Content, Chlorophyll, Antioxidant Activity and Color of Green Tea (Camellia sinensis or C. assamica) Leaves." *Journal of Food Science and Technology* 53 (1): 721–729. DOI: https://doi.org/10.1007/s13197-015-2030-x

Salve, R. V., Syed H.M., More S.G., and Shinde E.M. 2020. "Effect of Different Drying Treatment on Composition, Nutritional and Phytochemical Content of Curry Leaves." *The Pharma Innovation Journal* 9 (7): 584–589. DOI: https://www.thepharmajournal.com/archives/2020/vol9issue7/PartI/9-7-93-429.pdf

Saeed, Naima, Muhammad R. Khan, and Maria Shabbir. 2012. "Antioxidant Activity, Total Phenolic and Total Flavonoid Contents of Whole Plant Extracts Torilis leptophylla L." *BMC Complementary and Alternative Medicine* 12: 221. DOI: https://doi.org/10.1186/1472-6882-12-221

Saifullah, M. D., Rebecca McCullum, Adam McCluskey, and Quan Vuong. 2019. "Effects of Different Drying Methods on Extractable Phenolic Compounds and Antioxidant Properties from Lemon Myrtle Dried Leaves." Heliyon 5 (12): 1-8. DOI: https://doi.org/10.1016/j.heliyon.2019.e03044

Scalbert, Augustin, Claudine Manach, Christine Morand, Christian Rémésy, and Liliana Jiménez. 2005. "Dietary Polyphenols and the Prevention of Diseases." *Critical Reviews in Food Science and Nutrition* 45 (4): 287–306. https://doi.org/10.1080/1040869059096.

Sen, Saikat, and Raja Chakraborty. 2011. "The Role of Antioxidants in Human Health." In Acs Symposium Series, 1–37. DOI: https://doi.org/10.1021/bk-2011-1083.ch001

Sharma, Nisha, Neha Minocha, and Nidhi Kushwaha. 2020. "A Review on the Activities of Aloe Vera and Curry Leaves." *International Journal of Recent Scientific Research* 11 (11): 39985-39991. DOI: http://dx.doi.org/10.24327/ijrsr.2020.1111.5602

Tabassum, Shahzadi, Muhammad Asad Anwar, Muhammad Gulfaraz, Muhammad Arshad, Uteuliyev Y. Sabitaliyevich, Sadykov B. Nurmurzayevich, and Muhammad S. Ahmad. 2019. "Bioactivity Evaluation and HPLC UV-VIS Based Quantification of Antioxidant Secondary Metabolites from Extract and Fractions of Bistorta amplexicaulis Rhizome." *Cellular*

and Molecular Biology 65 (1): 1-26. DOI: http://dx.doi.org/10.14715/cmb/2019.65.1.4

Tomar, Rajesh Singh, Sharmistha Banerjee, and Shuchi Kaushik. 2017. "Assessment of Antioxidant Activity of Leaves of *Murraya koenigii* Extracts and Its Comparative Efficacy Analysis in Different Solvents." *Journal of Pharmaceutical Sciences and Research* 9 (3): 288–291.

Tripathi, Yogesh C., Nishat Anjum, and Ashish Rana. 2018. "Chemical Composition and In vitro Antifungal and Antioxidant Activities of Essential Oil from *Murraya koenigii* (L.) Spreng. Leaves." *Asian Journal of Biomedical and Pharmaceutical Sciences* 8 (65): 6-13.