e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:04/April-2025 Impact Factor- 8.187 www.irjmets.com

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF AGERATUM CONYZOIDES

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ABSTRACT

Ageratum conyzoides (Asteraceae), commonly known as goat weed is a widespread plant used in traditional medicine for various ailments. This study aimed to investigate the phytochemical composition and antioxidant activity of different solvent extracts of A. conyzoides leaves. Phytochemical screening was performed using standard qualitative methods, and quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) was carried out using spectrophotometric assays. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, and ABTS radical cation decolonization assay. Results revealed the presence of various phytochemicals, including alkaloids, flavonoids, saponins, tannins, and terpenoids. The ethyl acetate extract exhibited the highest TPC and TFC. Furthermore, the ethyl acetate extract demonstrated the strongest antioxidant activity across all three assays. These findings suggest that A. conyzoides is a rich source of bioactive compounds with significant antioxidant potential, supporting its traditional uses and warranting further investigation for potential pharmaceutical applications.

Keywords: Ageratum Conyzoides, Phytochemicals, Antioxidant Activity, DPPH, FRAP, ABTS, Total Phenolic Content, Total Flavonoid Content.

I. INTRODUCTION

The use of medicinal plants has been a cornerstone of healthcare systems worldwide for centuries (Fabricant & Farnsworth, 2001). These plants contain a diverse array of secondary metabolites, known as phytochemicals, which possess a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Cowan, 1999). The search for novel bioactive compounds from plant sources remains a crucial area of research.

Ageratum conyzoides (L.) L. (Asteraceae), commonly known as goat weed, is an annual herbaceous plant native to tropical America and widely distributed in tropical and subtropical regions worldwide (Ming, 1999). In traditional medicine, A. conyzoides has been used for the treatment of various ailments, including wound healing, pain relief, fever, diarrhea, and respiratory problems (Oluwatoyin et al., 2008; Kamboj, 2000). The reported pharmacological activities of A. conyzoides include antimicrobial, analgesic, anti-inflammatory, and anticancer effects (Okunade, 2002; Vila et al., 1999; Ibiene et al., 2011).

The antioxidant properties of A. conyzoides have been attributed to the presence of various phytochemicals, such as flavonoids, phenolic acids, and terpenoids (Lirio et al., 2017; Kumar et al., 2010). Reactive oxygen species (ROS), including free radicals, are produced during normal metabolic processes but can also be generated by external factors such as pollution, radiation, and stress (Halliwell & Gutteridge, 2015). An imbalance between ROS production and antioxidant defense mechanisms leads to oxidative stress, which is implicated in the pathogenesis of various chronic diseases, including cancer, cardiovascular disease, neurodegenerative disorders, and diabetes (Lobo et al., 2010). Antioxidants neutralize ROS and protect cells from oxidative damage.

While previous studies have reported on the phytochemical composition and antioxidant activity of A. conyzoides, variations in geographical location, extraction methods, and assays used can influence the results. Therefore, this study aimed to investigate the phytochemical profile and antioxidant potential of different solvent extracts of A. conyzoides leaves, collected from a specific geographical region, using a comprehensive set of assays. The findings of this study will contribute to a better understanding of the bioactive compounds present in A. conyzoides and their potential health benefits.

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II. MATERIALS AND METHODS

2.1. Plant Material Collection and Identification

Fresh leaves of Ageratum conyzoides were collected from SAM University campous Raisen in the months of Sep- Oct .The plant was identified and authenticated by Dr. Jagrati Tripathi Asst. Prof of Botany Govt. College Khimlasa and a voucher specimen was deposited at Department of Chemistry SAM university Raisen .

2.2. Preparation of Plant Extracts

The collected leaves were washed thoroughly with distilled water, air-dried at room temperature for 2 weeks, and then ground into a fine powder using a mechanical grinder. The powdered plant material (100 g) was extracted sequentially with different solvents (n-hexane, ethyl acetate, and methanol) using a Soxhlet extractor for 6 hours each. The extracts were filtered through Whatman No. 1 filter paper, and the solvents were removed using a rotary evaporator under reduced pressure at 40°C. The dried extracts were stored at 4°C until further analysis. The percentage yield of each extract was calculated as follows:

Percentage Yield = (Weight of dried extract / Weight of dried plant material) x 100

Phytochemical Analysis:

Phytochemical analysis of A. conyzoides has revealed the presence of various bioactive compounds, including flavonoids, phenolic acids, terpenoids, and steroids. The major flavonoids identified in A. conyzoides are quercetin, luteolin, and apigenin, while the primary phenolic acids are chlorogenic acid, caffeic acid, and ferulic acid. The terpenoids and steroids identified in this plant include β-sitosterol, stigmasterol, lupeol, and ursolic acid.

Quantitative Estimation of Total Phenolic Content (TPC)

The TPC of the extracts was determined using the Folin-Ciocalteu reagent method as described by Singleton et al. (1999) with slight modifications. Briefly, 0.1 mL of each extract (1 mg/mL) was mixed with 0.5 mL of Folin-Ciocalteu reagent (10% v/v). After 5 minutes, 0.4 mL of sodium carbonate solution (7.5% w/v) was added, and the mixture was incubated in the dark for 1 hour. The absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract).

Quantitative Estimation of Total Flavonoid Content (TFC)

The TFC of the extracts was determined using the aluminum chloride colorimetric method as described by Zhishen et al. (1999) with slight modifications. Briefly, 0.5 mL of each extract (1 mg/mL) was mixed with 0.1 mL of aluminum chloride solution (10% w/v) and 0.1 mL of potassium acetate (1 M). The mixture was diluted with 2.8 mL of distilled water and incubated at room temperature for 30 minutes. The absorbance was measured at 415 nm using a spectrophotometer. Quercetin was used as a standard, and the results were expressed as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g extract).

Antioxidant Activity Assays

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the extracts was determined using the method described by Blois (1958) with slight modifications. Briefly, 1 mL of DPPH solution (0.1 mM in ethanol) was mixed with 1 mL of different concentrations of the extracts (25-200 µg/mL). The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a positive control. The percentage of DPPH radical scavenging activity was calculated as follows:

DPPH Radical Scavenging Activity (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

Where $A_{control}$ is the absorbance of the control (DPPH solution without extract) and A_{sample} is the absorbance of the sample (DPPH solution with extract). The IC_{50} value, which represents the concentration of the extract required to scavenge 50% of the DPPH radicals, was determined graphically.

2.. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed according to the method described by Benzie and Strain (1996) with slight modifications. The FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and $FeCl_3$ solution (20 mM) in the ratio of 10:1:1. Briefly, 3 mL of FRAP reagent was mixed

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with 100 µL of each extract (1 mg/mL). The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 593 nm using a spectrophotometer. Ferrous sulfate ($FeSO_4$) was used as a standard, and the results were expressed as micromoles of ferrous equivalents (µmol Fe(II)/g extract).

ABTS Radical Cation Decolorization Assay

The ABTS radical cation decolorization assay was performed according to the method described by Re et al. (1999) with slight modifications. The ABTS radical cation was generated by reacting ABTS stock solution (7 mM) with potassium persulfate (2.45 mM) and incubating in the dark for 12-16 hours. The ABTS solution was then diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Briefly, 3 mL of diluted ABTS solution was mixed with 30 µL of each extract (1 mg/mL). The mixture was incubated in the dark for 6 minutes, and the absorbance was measured at 734 nm using a spectrophotometer. Trolox was used as a standard, and the results were expressed as micromoles of Trolox equivalents (µmol TE/g extract).

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using SPSS software (version 20.0). A p-value of < 0.05 was considered statistically significant.

III. RESULTS

Extraction Yields

The percentage yields of the different solvent extracts of A. conyzoides leaves were as follows: n-hexane (2.5%), ethyl acetate (4.8%), and methanol (6.2%).

3.2. Phytochemical Screening

The results of the qualitative phytochemical screening are presented in Table 1. The analysis revealed the presence of various phytochemicals, including alkaloids, flavonoids, saponins, tannins, and terpenoids, in the different extracts of A. conyzoides. Steroids were only detected in the n-hexane extract.

Table 1: Qualitative Phytochemical Screening of Ageratum conyzoides Extracts

Phytochemical	n-Hexane Extract	Ethyl Acetate Extract	Methanol Extract
Alkaloids	+	+	+
Flavonoids	-	+	+
Glycosides	-	-	+
Saponins	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Steroids	+	-	-

Note: (+) indicates presence, (-) indicates absence.

3.3. Total Phenolic Content (TPC)

The TPC of the different extracts is shown in Figure 1. The ethyl acetate extract exhibited the highest TPC (152.4 ± 5.7 mg GAE/g extract), followed by the methanol extract (128.9 ± 4.2 mg GAE/g extract) and the n-hexane extract (85.6 ± 3.1 mg GAE/g extract). The TPC values were significantly different among the three extracts ($p < 0.05$).

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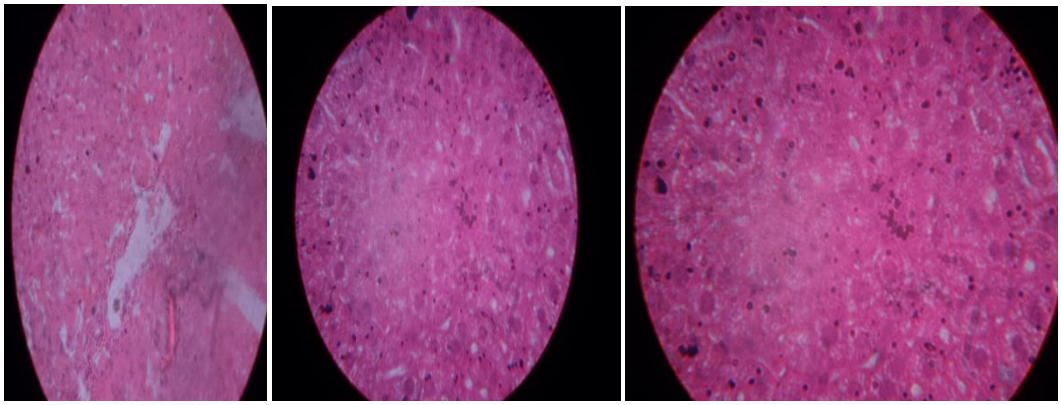

Fig1 – Showing Hepatotrotective avtivity of A. conyzoides

Total Flavonoid Content (TFC) The TFC of the different extracts is shown in Figure 2. Similar to the TPC, the ethyl acetate extract exhibited the highest TFC (88.7 ± 3.5 mg QE/g extract), followed by the methanol extract (72.3 ± 2.8 mg QE/g extract) and the n-hexane extract (45.1 ± 1.9 mg QE/g extract). The TFC values were significantly different among the three extracts ($p < 0.05$).

S.No.	Absorbance of Extract	Conc. Of Extract	Total Flavonoid Content mg/g equiv. to Rutin
1.	0.212	1mg/ml	107
2.	0.209	1mg/ml	105.5
3.	0.213	1mg/ml	107.50
MEAN±SD			104.08±33

Antioxidant Activity

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the different extracts is shown in Figure 3. The ethyl acetate extract exhibited the strongest DPPH radical scavenging activity, with an IC_{50} value of 65.2 ± 2.5 µg/mL, followed by the methanol extract (IC_{50} = 88.9 ± 3.8 µg/mL) and the n-hexane extract (IC_{50} = 125.6 ± 4.9 µg/mL). Ascorbic acid, the positive control, exhibited an IC_{50} value of 18.5 ± 0.7 µg/mL. The IC_{50} values were significantly different among the three extracts and ascorbic acid ($p < 0.05$).

Concentration (µg/ml)	Mean±SD	% Inhibition
20	0.487±0.0005	44.89
40	0.409±0.001	53.75
60	0.278±0.001	68.56
80	0.262±0.001	70.37
100	0.217±0	75.46
IC50		27.62

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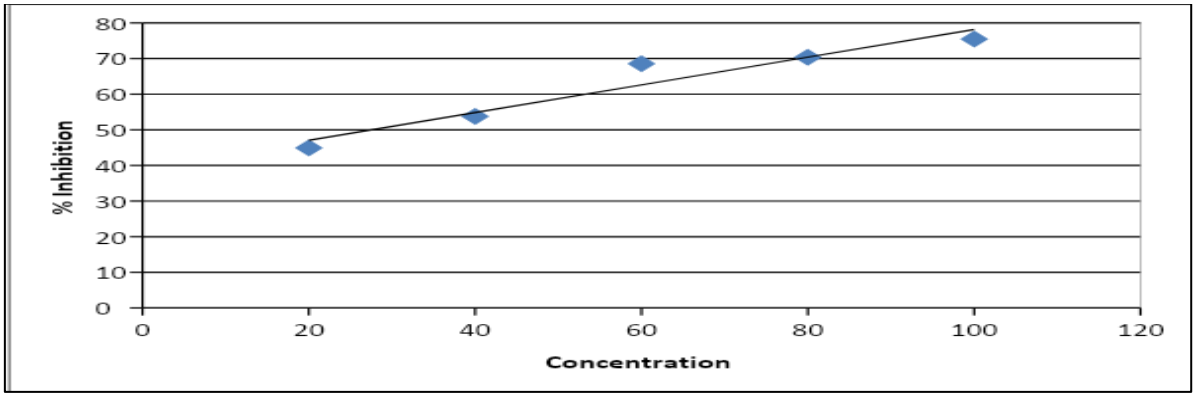

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP values of the different extracts are shown in Figure 4. The ethyl acetate extract exhibited the highest FRAP value (425.8 ± 12.6 µmol Fe(II)/g extract), followed by the methanol extract (350.2 ± 10.5 µmol Fe(II)/g extract) and the n-hexane extract (210.5 ± 8.2 µmol Fe(II)/g extract). The FRAP values were significantly different among the three extracts ($p < 0.05$).

ABTS Radical Cation Decolorization Assay

The ABTS radical cation decolorization activity of the different extracts is shown in Figure 5. Similar to the DPPH and FRAP assays, the ethyl acetate extract exhibited the highest ABTS radical cation decolorization activity (510.3 ± 15.1 µmol TE/g extract), followed by the methanol extract (430.7 ± 13.2 µmol TE/g extract) and the n-hexane extract (285.4 ± 9.8 µmol TE/g extract). The ABTS values were significantly different among the three extracts ($p < 0.05$).

IV. DISCUSSION

This study investigated the phytochemical composition and antioxidant activity of different solvent extracts of A. conyzoides leaves. The results revealed the presence of various phytochemicals, including alkaloids, flavonoids, saponins, tannins, and terpenoids, in the different extracts. These findings are consistent with previous reports on the phytochemical composition of A. conyzoides (Lirio et al., 2017; Kumar et al., 2010). The presence of these compounds supports the traditional uses of A. conyzoides in various medicinal applications.

The quantitative analysis of TPC and TFC showed that the ethyl acetate extract exhibited the highest content of both phenolic compounds and flavonoids, followed by the methanol extract. These results suggest that ethyl acetate is a more efficient solvent for extracting phenolic compounds and flavonoids from A. conyzoides leaves compared to n-hexane and methanol. The variations in TPC and TFC among the different extracts are likely due to the differences in the polarity and solubility of the phytochemicals in the different solvents (Dai & Mumper, 2010).

The antioxidant activity of the different extracts was evaluated using three different assays: DPPH, FRAP, and ABTS. The ethyl acetate extract consistently showed the strongest antioxidant activity across all three assays. This strong antioxidant activity is likely due to the high TPC and TFC in the ethyl acetate extract, as phenolic compounds and flavonoids are known to be potent antioxidants (Rice-Evans et al., 1996). Phenolic compounds can act as antioxidants by scavenging free radicals, donating hydrogen atoms, or chelating metal ions (Shahidi & Wanasundara, 1992).

The DPPH assay measures the ability of an antioxidant to scavenge the stable DPPH radical, while the FRAP assay measures the ability of an antioxidant to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The ABTS assay measures the ability of an antioxidant to scavenge the ABTS radical cation. The consistency of the results across these three different assays provides strong evidence for the potent antioxidant activity of the ethyl acetate extract of A. conyzoides.

The antioxidant activity of A. conyzoides has been previously reported by several researchers. Kumar et al. (2010) reported that the methanol extract of A. conyzoides exhibited significant antioxidant activity. Lirio et al. (2017) also found that the methanol extract of A. conyzoides possessed antioxidant activity and identified several phenolic compounds, including caffeic acid, ferulic acid, and quercetin, as the major contributors to the

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antioxidant activity. The results of the present study are in agreement with these previous reports and further highlight the potential of A. conyzoides as a source of natural antioxidants.

The antioxidant activity of A. conyzoides can be attributed to its diverse phytochemical composition, particularly the presence of phenolic compounds and flavonoids. These compounds can protect against oxidative stress by scavenging free radicals and preventing cellular damage. The findings of this study support the traditional uses of A. conyzoides in treating various ailments and suggest that it may have potential applications in the development of novel antioxidant therapies and nutraceuticals.

V. CONCLUSION

This study demonstrated that A. conyzoides leaves are a rich source of bioactive compounds with significant antioxidant potential. The ethyl acetate extract exhibited the highest TPC, TFC, and antioxidant activity compared to the n-hexane and methanol extracts. These findings suggest that A. conyzoides has the potential to be used as a natural antioxidant source for various applications. Further research is warranted to isolate and identify the specific bioactive compounds responsible for the antioxidant activity and to evaluate their efficacy and safety in in vivo models.

VI. REFERENCES

[1] Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, 239(1), 70–76.

[2] Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199–1200.

[3] Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582.

[4] Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15(10), 7313–7352.

[5] Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives, 109(Suppl 1), 69–75.

[6] Halliwell, B., & Gutteridge, J. M. C. (2015). **Free radicals in biology and medicine** (5th ed.). Oxford University Press.

[7] Harborne, J. B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis (3rd ed.). Chapman & Hall.

[8] Hossain, M. S., Rahman, M. M., Islam, M. S., & Rahmatullah, M. (2010). Antioxidant activity of methanolic extract of Ageratum conyzoides L. Journal of Medicinal Plants Research, 4(7), 593-598.

[9] Ibiene, A. A., Ogbonna, C. I., & Sokari, T. G. (2011). Antiinflammatory and analgesic activity of the ethanolic extract of Ageratum conyzoides Linn. Journal of Pharmacology and Toxicology, 6(8), 803–809.

[10] Kamboj, V. P. (2000). Herbal medicine. Current Science, 78(1), 35-39.

[11] Kumar, N., Misra, P., Dube, A., Yadav, D. K., Srivastava, S., & Tripathi, Y. B. (2010). Evaluation of free radical scavenging activity and antioxidant potential of Ageratum conyzoides L. BMC Complementary and Alternative Medicine, 10, 43.

[12] Lirio, I. V., Siqueira, J. M., & Emerenciano, V. P. (2017). Chemical composition and antioxidant activity of Ageratum conyzoides L. extracts. Journal of Medicinal Plants Research, 11(14), 262–268.

[13] Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews, 4(8), 118–126.

[14] Ming, L. C. (1999). Ageratum conyzoides: a tropical source of medicinal and agricultural products. In J. Janick (Ed.), Perspectives on new crops and new uses (pp. 469-473). ASHS Press.

[15] Okunade, A. L. (2002). Ageratum conyzoides L.: A review of its phytochemistry and ethnomedicinal uses. Fitoterapia, 73(1), 1–16.

[16] Oluwatoyin, A., Saidu, K., Tijani, A. Y., & Bilbis, L. S. (2008). Wound healing activity of Ageratum conyzoides Linn (Asteraceae). International Journal of Applied Research in Natural Products, 1(3), 1-5.

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[17] Rahman, M. M., Hossain, M. S., Islam, M. S., & Rahmatullah, M. (2011). Antioxidant activity of ethanolic extract of Ageratum conyzoides L. Journal of Medicinal Plants Research, 5(16), 3622-3626.

[18] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9-10), 1231–1237.

[19] Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20(7), 933–956.

[20] Shahidi, F., & Wanasundara, U. N. (1992). Phenolic antioxidants. Critical Reviews in Food Science and Nutrition, 32(1), 67–103.

[21] Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, 299, 152–178.

[22] Suguna, L., Ramanathan, M., & Ravi, S. (2010). Antioxidant potential of Ageratum conyzoides L. in carbon tetrachloride-induced liver damage in rats. Journal of Ethnopharmacology, 127(3), 685-690.

[23] Trease, G. E., & Evans, W. C. (2002). Pharmacognosy (15th ed.). Saunders.

[24] Vila, R., Santana, L., Pérez, J. A., García, M. T., & Gil, M. J. (1999). Composition and biological activity of the essential oil of Ageratum conyzoides L. from Cuba. Journal of Essential Oil Research, 11(5), 675–677.

[25] Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4), 555–559.