https://doi.org/ 10.33472/AFJBS.6.6.2024.1213-1226


# Metabolite Profiling and GC-MS Analysis of *Allium fistulosum* extract for Antioxidant and Cancer Therapeutics

**Vetrikodi, N.* and Jeyanthi K. A.**

Department of Biotechnology, Thanthai Hans Roever College, Affiliated to Bharathidasan University, Perambalur – 621212, Tamil Nadu, India

*Corresponding Author

**N. Vetrikodi**

Department of Biotechnology, Thanthai Hans Roever College, Affiliated to Bharathidasan University, Perambalur – 621212, Tamil Nadu, India

Article Info

Volume 6, Issue 6, 2024

Received: 03 March 2024

Accepted: 30 April 2024

doi: 10.33472/AFJBS.6.6.2024.1213-1226

**Abstract**

*Allium fistulosum*, a widely cultivated perennial plant in Asia, has a rich history of traditional medicinal use. This study aims to comprehensively characterize the metabolite profile of *A. fistulosum* ethanol extract using GC-MS analysis, evaluate its antioxidant potential, and perform molecular docking studies with identified compounds. *A. fistulosum* ethanol extract exhibited significant antioxidant activity with IC50 values of 63.12 µg/ml (NORSA), 72.79 µg/ml (ABTS), 78.35 µg/ml (DPPH), and 47.45 µg/ml (HRSA). Phytochemical analysis revealed phenolic compounds (1.2 mg/g), flavonoids (0.86 mg/g), phytosterols (3.3 mg/g), tannins (5.12 mg/g), total steroids (11.2 mg/g), protein (13.6 mg/g), sugar (0.86 mg/g), and lipids (21.8 mg/g). The compound 3 (1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester) identified through GC-MS analysis were utilized for molecular docking studies, revealing a substantial binding affinity of -7.4 kcal/mol. *A. fistulosum* ethanol extract exhibited significant antioxidant activity with $IC_{50}$ values. This research sets the stage for further investigations into the development of targeted therapies for cancer treatment.

**Keywords**: *Allium fistulosum*, phytochemicals, ethanol extract, antioxidant activity, GC-MS

## INTRODUCTION

Phytochemicals, bioactive compounds found in plants, have garnered substantial attention due to their potential health-promoting properties (Yan et al., 2023; Banwo et al., 2021; Kothari et al., 2020). Within the *Allium* genus, encompassing garlic, onions, leeks, and related species, extensive research has unveiled their phytochemical composition and associated health benefits (Ahmed et al., 2021; Chakraborty et al., 2022;

Guillamón et al., 2021; Rocchetti et al., 2022). *Allium* species produce a rich array of secondary metabolites, including polyphenols, organosulfur compounds, saponins, polysaccharides, and tannins (Bhat et al., 2020; Kim et al., 2023; Khan et al., 2017). Their bioactive constituents has antimicrobial (Packia Lekshmi et al., 2015; Lanzotti et al., 2014), antioxidant (Štajner et al., 2008; Kurnia et al., 2021; Stajner and Varga 2003), antitumor (Asemani et al., 2019; Nouroz et al., 2015; Rauf et al., 2022), immunoregulatory, antidiabetic (Sabiu et al., 2019; Vu et al., 2020), antioxidant and anti-inflammatory properties (Lee et al., 2021; Momoh et al., 2022; Alam et al., 2023) and various other activities (Zeng et al., 2017; Fernández-Bedmar et al., 2019). These studies underscore the pivotal role of *Allium* species as sources of health-promoting compounds.

Notably, they contain an abundance of organosulfur compounds, such as allicin and its derivatives, which have been associated with various health benefits. Extensive research on these well-known species has firmly established them as valuable sources of phytochemicals with a wide array of health benefits (Ozma et al., 2023; Bede and Zaixiang 2020; Okoro et al., 2023; Sagar et al., 2022; Elegbeleye et al., 2022). Nevertheless, there is a need to broaden our understanding by investigating lesser-known species like *A. fistulosum*. Such research may unveil unique bioactive compounds, enriching our knowledge of natural antioxidants and their potential applications in functional foods and nutraceuticals. *A. fistulosum* (Wang et al., 2023; Vlase et al., 2013) which was commonly called as welsh onion.

Welsh onion rich with dietary fibers including vitamins A, C, E, and K, as well as carbohydrates, proteins, lipids, magnesium, calcium, iron, and potassium, which comprehensively plays a crucial role in promoting digestive health by facilitating digestion, preventing constipation, and mitigating the risk of colon diseases (Gao et al., 2021; Sung et al., 2018; Balkrishna et al., 2023). Recent research endeavors have shed light on the diverse positive effects of *A. fistulosum* on human health. Studies have suggested its potential in preventing cardiovascular diseases and inhibiting the onset of cancer (Țigu et al., 2021; Dubey et al., 2023; Zolfaghari et al., 2021; Ravindranath et al., 2022). The objective of this study was to comprehensively investigate the metabolic profile and *in vitro* biological potential of *A. fistulosum*. To achieve this, a multifaceted approach was employed, including the estimation of polyphenolic contents and thorough analysis using gas chromatography-mass spectrometry (GC-MS) on the ethanol extract of *A. fistulosum*.

## MATERIALS AND METHODS

### Collection and Preparation of Plant extract

Wild *A. fistulosum* specimens were collected from Tamil Nadu Agricultural University (TNAU), Coimbatore. Fresh plant material, including leaves and bulbs, was collected and thoroughly cleaned to remove any contaminants and then air-dried. The dried plant material was coarsely ground, and 50 grams of the ground material was extracted with 500 mL of 70% ethanol in a Soxhlet extractor for 8 hours. The

extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator at a controlled temperature (<40°C) to obtain the ethanol extract.

**Phytochemical Screening**

**Total phenolics**: It was determined using the Folin Ciocalteu's method (Patel and Kajal 2010; Bhalodia et al., 2011). Absorbance was measured at 750 nm using a UV-visible spectrophotometer. Gallic acid as the standard, and a calibration curve was generated using standard gallic acid. The total phenolic content of the sample was expressed as milligrams of gallic acid equivalent weight (GAE) per 100 grams of dry mass.

**Total flavonoid**: The determination of total flavonoid content was carried out using the aluminum chloride colorimetric assay (Patel and Kajal 2010; Kumar et al., 2008). The absorbance was measured at 510 nm using a UV-visible spectrophotometer. The total flavonoid content in the polyherbal formulation was expressed as milligrams of quercetin equivalents per 100 grams of dry mass.

**Total phytosterols**: The quantitative analysis of total phytosterols content was determined described by Araújo et al. (2013) by Liebermann-Buchard (LB) reagent. The absorbance of the mixture was measured at 626 nm.

**Tannin**: It is determined using the Folin-Ciocalteu method (Tamilselvi et al., 2012). The absorbance was measured at 700 nm using a UV spectrophotometer. Results were expressed in terms of milligrams of tannic acid equivalents per gram of dried sample.

**Total sugar**: It was quantified using the phenol-sulfuric acid method (Albalasmeh et al., 2013) by Benedict's reagent whereas the measurement of absorbance at 595 nm using an Elisa reader.

**Lipid:** It was quantified using the vanillin method (Markou et al., 2021). Total protein content in the extract was determined using the Bradford assay (Marks et al., 1985). Absorbance was measured at 595 nm using a microplate reader.

**Antioxidant Activities**

***DPPH Assay***

The DPPH method described by Jeong et al., (2010) albeit with certain modifications Firstly, a 0.1 mM DPPH solution was prepared in methanol, and 100 µl of this solution was added to 300 µl of the ethanol extract at various concentrations (500, 250, 100, 50, and 10 µg/mL). These mixtures were vigorously shaken and allowed to stand at room temperature for 30 minutes. Subsequently, their absorbance was measured at 517 nm using a UV-VIS spectrophotometer, with ascorbic acid as the reference.

The capability to scavenge the DPPH radical was calculated by

DPPH scavenging effect (% inhibition) = [(absorbance of control - absorbance of the reaction mixture) / absorbance of control] × 100

***ABTS Radical Scavenging Assay***

The method described by Arnao et al., (1990) albeit with certain modifications. ABTS solution and various concentrations (500, 250, 100, 50, and 10 µg/ml) were incubated in equal quantities at room temperature in darkness (14hrs). Subsequently, the absorbance was recorded at 734 nm after 7 minutes using a spectrophotometer and the percentage inhibition was calculated as follows:

ABTS Radical Scavenging Activity (%) = ($Abs_{control}$ - $Abs_{sample}$) / $Abs_{control}$ x 100

***Nitric Oxide Radical Scavenging Assay (NORSA)***

The sample extracts were prepared from a 50 mg/mL crude extract and subsequently serially diluted with DMSO to create concentrations ranging from 500 to 10 µg/mL. 150 µl aliquot of the extract was then mixed Griess reagent (150 µl). Following a 30-minute incubation period, 100 µL of the reaction mixture was transferred to a 96-well plate, and the absorbance was measured at 540 nm using a UV-Vis microplate reader (Molecular Devices, GA, USA) (Marcocci et al., 1994; Ebrahimzadeh et al., 2010)

$$NORSA\ (\%) = [(A_{control} - A_{test}) / A_{control}] \times 100$$

***Hydrogen peroxide scavenging assay***

The assay was conducted in accordance with the method originally detailed by Ruch et al., (1989) with minor adaptations. Various concentrations of the ethanol extract (500, 250, 100, 50, and 10 µg/ml) were introduced into a 43 mM hydrogen peroxide solution (0.6 ml). After incubation of 10 minutes, the absorbance of hydrogen peroxide at 230 nm and calculating the percentage of inhibition as

$$\%\ inhibition = [(Control - Test) / Control] \times 100$$

**GC-MS Analysis**

GC-MS analysis was done to obtain the chemical profile of *A. fistulosum* using the SHIMADZU QP2010S GC-MS system. The operating conditions were as follows: the oven temperature was programmed at 70°C, the ion source temperature was set at 200°C, and the sample injection mode was splitless with a sampling time of 2 min, the carrier gas employed was helium (99.99%) at a flow rate of 1.00 mL/min. The scan range covered 50-500 m/z. The identification of chemical compounds was based on the peaks observed at various mass-to-charge ratios. Further verification was performed by comparing the obtained data with the standard spectra available in the mass spectral libraries of the National Institute of Standards and Technology (NIST-11) and WILEY 8 library.

**RESULT AND DISCUSSION**

***Phytochemical Content Analysis***

The results of the phytochemical analysis are presented in Fig. 1. *A. fistulosum* ethanol extract was found to contain 1.2 mg/g of phenolic compounds, which are renowned for their antioxidant properties and various health benefits, including anti-inflammatory and anticancer effects. The presence of phenolic compounds in *A. fistulosum* suggests their potential as a natural source of antioxidants. Additionally, flavonoids, another

class of bioactive compounds, were detected with a content of 0.86 mg/g. Flavonoids are recognized for their ability to scavenge free radicals and possess various pharmacological activities, such as anti-inflammatory, anti-allergic, and cardioprotective effects (Kothari et al., 2020; Rocchetti et al., 2022).

The extract was also found to contain 3.3 mg/g of phytosterols, which are structurally similar to cholesterol and have been shown to lower blood cholesterol levels, making them beneficial for cardiovascular health. The presence of phytosterols suggests its potential use in functional foods designed to promote heart health. A notable content of tannins, 5.12 mg/g, was observed in the extract. Tannins are known for their astringent properties and are often used in traditional medicine for their antidiarrheal and wound-healing effects. The presence of tannins in *A. fistulosum* suggests its possible use in herbal remedies and pharmaceutical preparations. The total steroid content in the *A. fistulosum* ethanol extract was determined to be 11.2 mg/g. Steroids are a diverse group of compounds with various biological activities. The presence of steroids in *A. fistulosum* suggests the potential for diverse pharmacological effects, including anti-inflammatory and immune-modulating properties (Tamilselvi et al., 2012; Albalasmeh et al., 2013; Bhat 2020).

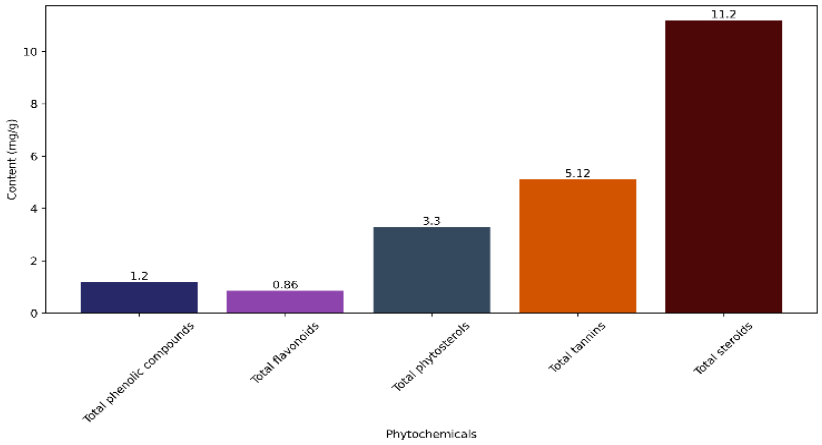


**Figure 1. Phytochemical Content Analysis of *A. fistulosum* Ethanol Extract**

The macronutrient composition of the extracted sample was analyzed shown in Fig. 2. revealing the following findings: Protein content is an essential nutritional parameter, as proteins play a crucial role in various physiological functions and are vital for overall health (Araújo et al., 2013). The total protein content was determined to be 13.6 mg/g. The total sugar content in the extracted sample was measured to be 0.86 mg/g. The observed sugar content falls within the range, indicating that the extracted sample contains a moderate number of sugars.

The total lipid content in the extracted sample was quantified at 21.8 mg/g. This suggests that the extracted sample contains a notable amount of lipids, which could have implications for its dietary and nutritional properties. The quantified macronutrient data for the extracted sample provide valuable insights into its nutritional profile (Markou et al., 2021; Arnao et al., 1990; Jeong et al., 2010).

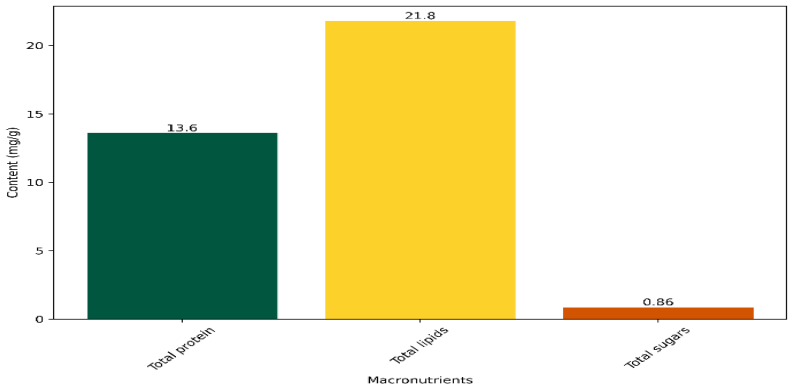


**Figure 2. Quantification of Macronutrient Concentrations (mg/g)**

***Evaluation of Antioxidant Activities***

Figure 3 presents detailed information regarding the percentage of inhibition observed at varying concentrations of the tested samples by DPPH radical scavenging assay. Additionally, the mean percentage of inhibition for each tested sample concentration is provided, highlighting the concentration-dependent inhibitory responses exhibited by the samples. Furthermore, the IC50 value for the tested sample, indicating the concentration at which a 50% inhibition rate is achieved, has been determined to be 78.35 µg/ml. The results validate its potential utility in diverse health-promoting applications, underlining its role as a potent source of natural antioxidants (Kurnia et al., 2021; Štajner et al., 2008).

In NORSA, the ethanolic extract of *A. fistulosum* exhibited significant antioxidant activity, as indicated by its capacity to scavenge nitric oxide (NO) radicals effectively. The results for the tested sample concentrations (in µg/ml) in triplicates. Figure 4 illustrates a dose-dependent relationship between sample concentrations (µg/ml) and inhibition percentages. As concentrations decrease, inhibition decreases as well. At 500 µg/ml, mean inhibition is about 68.82%, while at 10 µg/ml, it's approximately 37.73%. These results reveal the concentration-dependent inhibitory effects. The IC50 value is 63.12 µg/ml, indicating the concentration for 50% inhibition. Marcocci et al. (1994) reported the nitric acid scavenging activities of the *Ginkgo* plants.

The ABTS assay revealed notable antioxidant prowess of the extract. Figure 5 provides the percentage of inhibition at various concentrations of tested samples, measured in triplicates, along with their mean values. Ascorbic acid exhibited the highest mean inhibition at 65.88%, followed by decreasing inhibition as the sample concentrations decreased. The IC50 value, representing the concentration at which a 50%-inhibition rate is achieved, was determined to be 72.79 µg/ml. Similar results were reported by Vu et al. (2020) and Lee et al. (2021).

In the HRSA, the ethanolic extract of *A. fistulosum* displayed substantial hydroxyl radical (OH•) scavenging activity. The figure presents OD values at 230 nm for various concentrations of tested samples. The mean OD value for the control (ascorbic acid) was 0.813. The tested sample concentrations (ranging from 500 µg/ml to 10 µg/ml) and ascorbic acid exhibited varying OD values at 230 nm. The figure provides data on the percentage of inhibition for various concentrations of tested samples, with triplicate

measurements for each concentration. Ascorbic acid is used as the control, and it exhibits the highest mean inhibition at approximately 88.72%. The IC50 value for the tested sample, representing the concentration at which a 50% inhibition rate is achieved, was determined to be 47.45 µg/ml. Similar results were reported by Patel and Kajal (2010), Bhalodia et al. (2011), Kumar et al. (2008), Wilson and Demmig-Adams (2007).

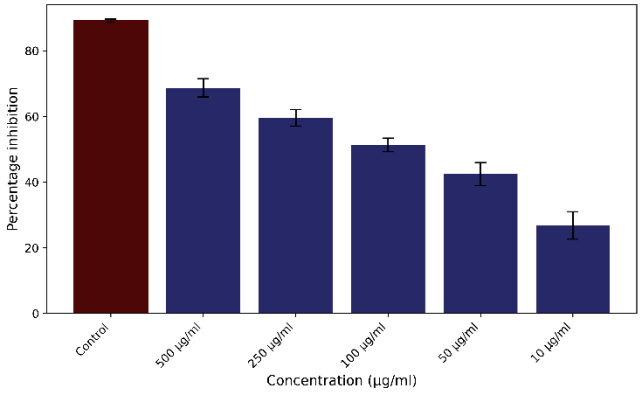


**Figure 3. Mean ± S.D Quantitative Analysis of DPPH Radical Scavenging by *A. fistulosum* Ethanol Extracts (n = 3)**

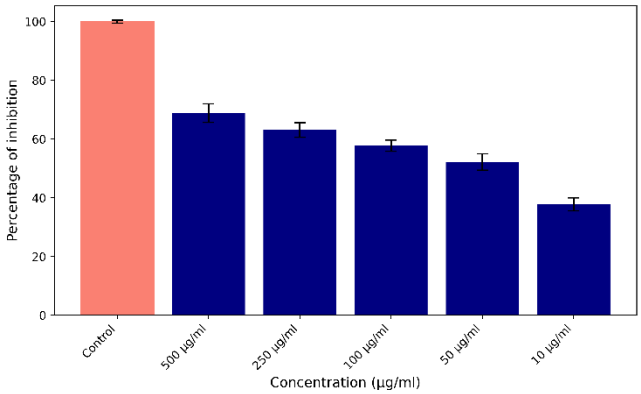


**Figure 4. Mean ± S.D Quantitative Analysis of Nitric Oxide Radical Scavenging by *A. fistulosum* Ethanol Extracts (n = 3)**

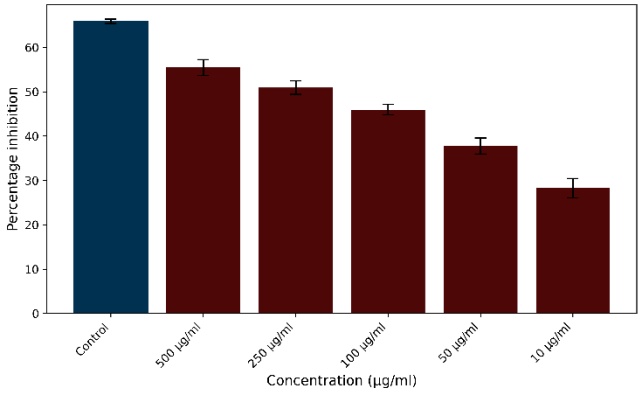


**Figure 5. Mean ± S.D Quantitative Analysis of ABTS by *A. fistulosum* Ethanol Extracts (n = 3)**

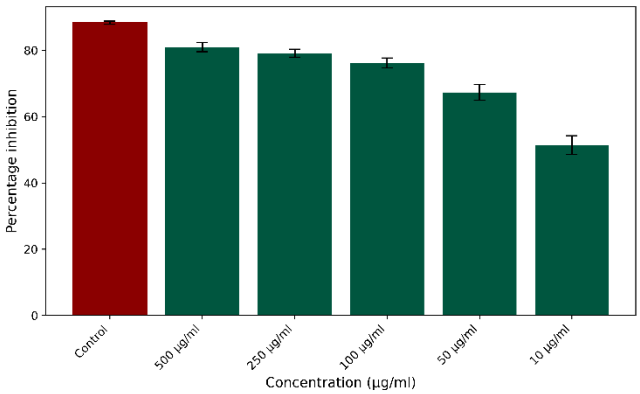


**Figure 6. Mean ± S.D Quantitative Analysis of Hydrogen peroxide by *A. fistulosum* Ethanol Extracts (n = 3)**

**GC-MS analysis**

The full scan GC-MS chromatogram is presented in Figure 7. Table 1 presents a comprehensive list of ten different chemical compounds, each identified by its name, molecular weight, molecular formula, retention time, and area percentage in the *A. fistulosum*. Compound 4, identified as "1,3-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester," is the most abundant compound, constituting a significant portion of the *A. fistulosum* (41.87% area). This indicates its importance in the chemical composition of the *A. fistulosum* whereas similar bioactive compounds were reported by Marrelli et al. (2018) and Zeng et al. (2017).

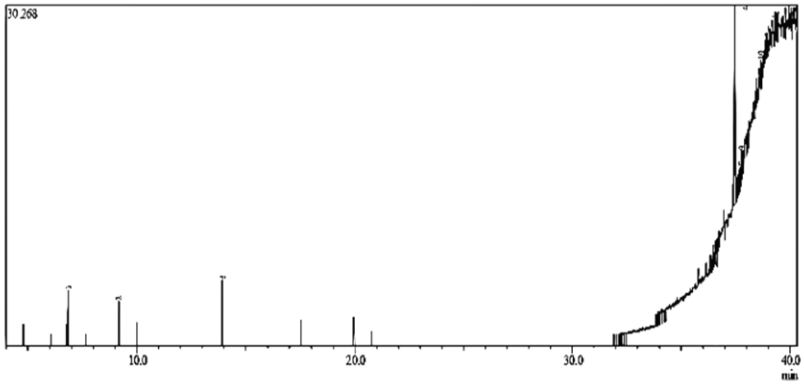


**Figure 7. GC-MS of ethanol extract of *A. fistulosum***

**Table. 1. Compound Analysis Data with Molecular Properties and Retention Times**

| S.No | Name | Mol. Weight | Molecular Formula | Retention Time | Area % |
|---|---|---|---|---|---|
| 1 | 1-Methylbutyl Nitrite | 117 | $C_5H_{11}NO_2$ | 6.85 | 8.89 |
| 2 | 1-Methylbutyl Nitrite | 117 | $C_5H_{11}NO_2$ | 9.18 | 6.43 |
| 3 | 3-Alpha`-Methylcholest-5-en-3-Beta-ol Nitrite | 429 | $C_{28}H_{47}NO_2$ | 13.91 | 10.49 |
| 4 | 1,3-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester | 390 | $C_{24}H_{38}O_4$ | 37.46 | 41.87 |

| | | | | | |
|---|---|---|---|---|---|
| 5 | 1H-Furo[3,4-C] pyrrole-4-Carboxylic Acid 6-(2-Furanyl) hexahydro-1,3-dioxo-4-phenyl-, Methyl Ester, (3A. Alpha.,4. Beta.,6. Beta.,6 | 341 | $C_{18}H_{15}NO_6$ | 37.50 | 2.18 |
| 6 | 1,2-Di(hydroxydiphenylmethyl)indane | 482 | $C_{35}H_{30}O_2$ | 37.58 | 4.64 |
| 7 | Isoquinoline, 1,2,3,4-Tetrahydro-7-Methoxy-2-Methyl-1-[(3-Nitrophenyl)methyl]-8-(Phenylmethoxy)-,(.+-.)- | 418 | $C_{25}H_{26}N_2O_4$ | 37.63 | 7.82 |
| 8 | Methyl 3-Methyl-5-Oxy-2-Phenoxyhexanedithioate | 282 | $C_{14}H_{18}O_2S_2$ | 37.73 | 4.63 |
| 9 | 14,19-Dioxoundecacyclo [9.9.0.0(1,5).0(2,12).0(2,18).0.(3,7).0(6,10).0(8,12).0(11,15).0(13,17).0(16,20)] icosane-4-syn,9-syn-dica | 338 | $C_{22}H_{14}N_2O_2$ | 37.76 | 10.67 |
| 10 | tert-Butyldimethylsilyl 3-Methyl-4-((2,2,3,3,3- | 412 | $C_{17}H_{21}F_5O_4Si$ | 38.67 | 2.39 |

Several compounds, such as compound 5, "1H-Furo[3,4-C] pyrrole-4-Carboxylic Acid 6-(2-Furanyl) hexahydro-1,3-dioxo-4-phenyl-, Methyl Ester," and compound 10, "tert-Butyldimethylsilyl 3-Methyl-4-((2,2,3,3,3-," are present in relatively small amounts (2.18% and 2.39% area, respectively). The compounds were identified using GC-MS analysis, and further confirmation was done by comparing their mass spectra with the reference spectra in the NIST-11 and WILEY 8 libraries.

The identified compounds from the GC-MS analysis of ethanol extract of *A. fistulosum* exhibit diverse functional groups, suggesting a range of potential biological activities. Compound 4, characterized by ester groups and aromatic rings, may possess anti-inflammatory and antioxidant properties (Elegbeleye et al., 2022; Sagar et al., 2022). Compound 5, featuring furoyl, carboxyl, and ester groups, could potentially be employed for its anti-inflammatory and analgesic effects (Ravindranath et al., 2022). Compound 7, containing isoquinoline and nitrophenyl groups, aligns with the characteristics of isoquinoline alkaloids known for their antimicrobial and anti-inflammatory activities (Chang et al., 2016; Zolfaghari et al., 2021). Compound 9's complex polycyclic structure hints at versatile biological activities, possibly encompassing antiviral, anticancer, or antimicrobial properties (Corzo-Martínez et al., 2007; Ozma et al., 2023; Upadhyay 2017).

## CONCLUSION

In this study, we conducted a comprehensive evaluation of the phytochemical composition and antioxidant capacity of the ethanol extract from *A. fistulosum*. Our findings revealed substantial levels of phenolic compounds, flavonoids, phytosterols, and tannins, underscoring *A. fistulosum*'s potential as a valuable

source of these bioactive constituents. Furthermore, we determined significant macronutrient content, including protein, sugar, and lipids, enhancing the extract's nutritional value. GC-MS analysis provided detailed insights into the extract's chemical complexity. Antioxidant assessments highlighted the robust antioxidant potential, making it a compelling candidate for mitigating various radical species. These findings collectively emphasize the potential of *A. fistulosum* as an exceptional natural source of antioxidants and bioactive compounds. This study provides significant promise for the development of functional foods and nutraceuticals, contributing to the advancement of natural product-based therapeutics. This research not only deepens our scientific understanding of *A. fistulosum* but also underscores its practical applications in health, nutrition, and pharmaceuticals.

## CONFLICT OF INTEREST

The authors confirm that this manuscript content has no conflict of interest.

## ACKNOWLEDGEMENT

Authors are thank to the Principal, Management and Department of Biotechnology, Thanthai Hans Roever College, Perambalur for their institutional support.

## REFERENCES

Ahmed, S. S., Fahim, J. R., Youssif, K. A., Amin, M. N., Abdel-Aziz, H. M., Brachmann, A. O., ... & Hamed, A. N. E. Cytotoxic potential of *Allium* sativum L. roots and their green synthesized nanoparticles supported with metabolomics and molecular docking analyses. *South African Journal of Botany*, 2021, *142*, 131-139.

Alam, A., Al Arif Jahan, A., Bari, M. S., Khandokar, L., Mahmud, M. H., Junaid, M., & Haque, M. A. *Allium* vegetables: Traditional uses, phytoconstituents, and beneficial effects in inflammation and cancer. *Critical Reviews in Food Science and Nutrition*, 2023, *63*(23), 6580-6614.

Albalasmeh, A.A., Berhe, A.A. and Ghezzehei, T.A. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate polymers*, 2013, *97*(2), 253-261.

Araújo, L.B., Silva, S.L., Galvão, M.A., Ferreira, M.R., Araújo, E.L., Randau, K.P. and Soares, L.A. Total phytosterol content in drug materials and extracts from roots of Acanthospermum hispidum by UV-VIS spectrophotometry. *Revista Brasileira de Farmacognosia*, 2013, *23*(5), 736-742.

Arnao, M.B., Acosta, M., Del Rio, J.A. and Garcia-Canovas, F. Inactivation of peroxidase by hydrogen peroxide and its protection by a reductant agent. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 1990, *1038*(1), 85-89.

Asemani, Y., Zamani, N., Bayat, M., & Amirghofran, Z. *Allium* vegetables for possible future of cancer treatment. *Phytotherapy Research*, 2019, *33*(12), 3019-3039.

Balkrishna, A., Chaudhary, M., Sharma, H., Srivastava, D., Kukreti, A., Kumar, A., & Arya, V. (2023). Phytochemistry, pharmacology, and medicinal aspects of *Allium fistulosum* L.: A narrative review. *Journal of Applied Pharmaceutical Science*, 2023, *13*(10), 107-118.

Banwo, K., Olojede, A. O., Adesulu-Dahunsi, A. T., Verma, D. K., Thakur, M., Tripathy, S.,& Utama, G. L. Functional importance of bioactive compounds of foods with Potential Health Benefits: A review on recent trends. *Food Bioscience*, 2021, *43*, 101320.

Bede, D., & Zaixiang, L. (2020). Dietary Polysaccharides from *Allium* species: A critical review in dietary polysaccharides from *Allium* Species: Extraction, characterization, bioactivity, and potential utilization. *Acta Sci. Agric*, 4, 1-15.

Bhalodia, N.R., Acharya, R.N. and Shukla, V.J. Evaluation of *in-vitro* Antioxidant Activity of hydroalcoholic seed extratcs of *Cassia fistula* linn. *Free Radicals and Antioxidants*, 2011, *1*(1), 68-76.

Bhat, R. Bioactive compounds of *Allium* species. Bioactive Compounds in Underutilized Vegetables and Legumes, 2020, 1-20.

Chakraborty, A. J., Uddin, T. M., Zidan, B. R. M., Mitra, S., Das, R., Nainu, F., ... & Emran, T. B. *Allium* cepa: A treasure of bioactive phytochemicals with prospective health benefits. Evidence-Based Complementary and Alternative Medicine: eCAM, 2022.

Dubey, N., Dubey, N., Bhadoria, U., Shah, K., & Chauhan, N. S. Targeting colorectal cancer using dietary flavonols. Cancer Innovation, 2023

Ebrahimzadeh, M.A., Nabavi, S.M., Nabavi, S.F., Bahramian, F. and Bekhradnia, A.R., Antioxidant and free radical scavenging activity of *H. officinalis* L. var. angustifolius, *V. odorata*, *B. hyrcana* and *C. speciosum*. *Pak J Pharm Sci*, 2010, *23*(1), 29-34

Elegbeleye, J. A., Krishnamoorthy, S., Bamidele, O. P., Adeyanju, A. A., Adebowale, O. J., & Agbemavor, W. S. K. Health-promoting foods and food crops of West-Africa origin: The bioactive compounds and immunomodulating potential. *Journal of Food Biochemistry*, 2022, *46*(11), e14331.

Fernández-Bedmar, Z., Demyda-Peyrás, S., Merinas-Amo, T., & del Río-Celestino, M. Nutraceutic potential of two *Allium* species and their distinctive organosulfur compounds: A multi-assay evaluation. *Foods*, 2019, *8*(6), 222.

Gao, S., Liu, X., Liu, Y., Cao, B., Chen, Z., & Xu, K. (2021). The spectral irradiance, growth, photosynthetic characteristics, antioxidant system, and nutritional status of green onion (*Allium fistulosum* L.) grown under different photo-selective nets. *Frontiers in plant science*, 2021, *12*, 650471.

Guillamón, E., Andreo-Martínez, P., Mut-Salud, N., Fonollá, J., & Baños, A. Beneficial effects of organosulfur compounds from *Allium* cepa on gut health: A systematic review. *Foods*, 2021, *10*(8), 1680.

Jeong, J.H., Jung, H., Lee, S.R., Lee, H.J., Hwang, K.T. and Kim, T.Y. Anti-oxidant, anti-proliferative and anti-inflammatory activities of the extracts from black raspberry fruits and wine. *Food Chemistry*, 2010, *123*(2), 338-344.

Khan, S. A., Jameel, M., Kanwal, S., & Shahid, S. Medicinal importance of *Allium* species: a current review. *Int J Pharm Sci Res*, 2017, *2*(3), 29-39.

Kim, S. H., Yoon, J. B., Han, J., Seo, Y. A., Kang, B. H., Lee, J., & Ochar, K. Green Onion (*Allium fistulosum*): An Aromatic Vegetable Crop Esteemed for Food, Nutritional and Therapeutic Significance. *Foods*, 2023, *12*(24), 4503.

Kothari, D., Lee, W. D., & Kim, S. K. *Allium* flavonols: Health benefits, molecular targets, and bioavailability. *Antioxidants*, 2020, *9*(9), 888.

Kumar, T.S., Shanmugam, S., Palvannan, T. and Kumar, M.B. Evaluation of antioxidant properties of Elaeocarpus ganitrus Roxb. Leaves, 2008

Kurnia, D., Ajiati, D., Heliawati, L., & Sumiarsa, D. Antioxidant properties and structure-antioxidant activity relationship of *Allium* species leaves. *Molecules*, 2021, *26*(23), 7175.

Lanzotti, V., Scala, F., & Bonanomi, G. Compounds from *Allium* species with cytotoxic and antimicrobial activity. *Phytochemistry* Reviews, 2014, *13*, 769-791.

Lee, J. Y., Seo, K. H., Lee, E. Y., Ji, Y. J., Lee, Y. J., Kang, M. H., ... & Kim, H. D. Antioxidant and Anti-Obesity Potentials of Korean-Native Wild Vegetables (*Allium* species). *Horticulturae*, 2021, *7*(12), 541.

Marcocci, L., Maguire, J.J., Droylefaix, M.T. and Packer, L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochemical and biophysical research communications*, 1994, *201*(2), 748-755.

Markou, G., Eliopoulos, C., Argyri, A. and Arapoglou, D.Production of arthrospira (Spirulina) platensis enriched in β-glucans through phosphorus limitation. *Applied Sciences*, 2021, *11*(17), 8121.

Marks, D.L., Buchsbaum, R. and Swain, T. Measurement of total protein in plant samples in the presence of tannins. *Analytical Biochemistry*, 1985, *147*(1), 136-143.

Momoh, B. J., Okere, S. O., & Anyanwu, G. O. The anti-obesity effect of *Allium* cepa L. leaves on high fat diet induced obesity in male wistar rats. *Clinical Complementary Medicine and Pharmacology*, 2022, *2*(3), 100035.

Nouroz, F., Mehboob, M., Noreen, S., Zaidi, F., & Mobin, T. A review on anticancer activities of garlic (*Allium sativum* L.). *Middle East J Sci Res*, 2015, *23*(6), 1145-1151.

Okoro, B. C., Dokunmu, T. M., Okafor, E., Sokoya, I. A., Israel, E. N., Olusegun, D. O., & Ugbogu, E. E. A. (2023). The Ethnobotanical, Bioactive Compounds, Pharmacological Activities and Toxicological Evaluation of Garlic (*Allium* sativum): A Review. *Pharmacological Research-Modern Chinese Medicine*, 100273.

Ozma, M. A., Abbasi, A., Ahangarzadeh Rezaee, M., Hosseini, H., Hosseinzadeh, N., Sabahi, S., & Kafil, H. S. A critical review on the nutritional and medicinal profiles of garlic's (*Allium sativum* L.) bioactive compounds. *Food Reviews International*, 2023, *39*(9), 6324-6361.

Packia Lekshmi, N. C. J., Viveka, S., Jeeva, S., & Raja Brindha, J. Antimicrobial spectrum of *Allium* species-a review. *Indian J Sci*, 2015, *15*(44), 1-5.

Patel, P.R. and Kajal, S.S. Antioxidant activity of some selected medicinal plants in western region of India. *Advances in Biological research*, 2010, *4*(1), 23-26.

Rauf, A., Abu-Izneid, T., Thiruvengadam, M., Imran, M., Olatunde, A., Shariati, M. A., Kazhybayeva, G. Garlic (*Allium* sativum L.): Its chemistry, nutritional composition, toxicity, and anticancer properties. *Current topics in medicinal chemistry*, 2022, *22*(11), 957-972.

Ravindranath, K. J., Mohaideen, N. S. M. H., & Srinivasan, H. (2022). Phytocompounds of onion target heat shock proteins (HSP70s) to control breast cancer malignancy. *Applied biochemistry and biotechnology*, 194(10), 4836-4851.

Rocchetti, G., Zhang, L., Bocchi, S., Giuberti, G., Ak, G., Elbasan, F., & Zengin, G. The functional potential of nine *Allium* species related to their untargeted phytochemical characterization, antioxidant capacity and enzyme inhibitory ability. *Food Chemistry*, 2022, *368*, 130782.

Ruch, R.J., Cheng, S.J. and Klaunig, J.E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 1989, *10*(6), 1003-1008.

Sabiu, S., Madende, M., Ajao, A. A. N., Aladodo, R. A., Nurain, I. O., & Ahmad, J. B. The Genus *Allium* (Amaryllidaceae: Alloideae): Features, phytoconstituents, and mechanisms of antidiabetic potential of *Allium* cepa and *Allium* sativum. *Bioactive food as dietary interventions for diabetes*, 2019, 137-154.

Sagar, N. A., Pareek, S., Benkeblia, N., & Xiao, J. Onion (*Allium* cepa L.) bioactives: Chemistry, pharmacotherapeutic functions, and industrial applications. *Food Frontiers*, 2022, *3*(3), 380-412.

Scherer, C., Jacob, C., Dicato, M., & Diederich, M. Potential role of organic sulfur compounds from *Allium* species in cancer prevention and therapy. *Phytochemistry Reviews*, 2009, *8*, 349-368.

Stajner, D., & Varga, I. S. I. An evaluation of the antioxidant abilities of *Allium* species. *Acta biologica szegediensis*, 2003, *47*(1-4), 103-106.

Štajner, D., Igić, R., Popović, B. M., & Malenčić, D. J. Comparative study of antioxidant properties of wild growing and cultivated *Allium* species. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 2008, *22*(1), 113-117.

Sung, Y. Y., Kim, D. S., Kim, S. H., & Kim, H. K. (2018). Aqueous and ethanolic extracts of welsh onion, *Allium fistulosum*, attenuate high-fat diet-induced obesity. *BMC complementary and alternative medicine*, 2018, *18*(1), 1-11.

Tamilselvi, N., Krishnamoorthy, P., Dhamotharan, R., Arumugam, P. and Sagadevan, E. Analysis of total phenols, total tannins and screening of phytocomponents in Indigofera aspalathoides (Shivanar Vembu) Vahl EX DC. *Journal of Chemical and Pharmaceutical Research*, 2012, *4*(6), 3259-3262.

Țigu, A. B., Moldovan, C. S., Toma, V. A., Farcaș, A. D., Moț, A. C., Jurj, A., ... & Pârvu, M. Phytochemical analysis and *in-vitro* effects of *Allium fistulosum* L. and *Allium sativum* L. extracts on human normal and tumor cell lines: A comparative study. *Molecules*, 2021, *26*(3), 574.

Vlase, L., Parvu, M., Parvu, E. A., & Toiu, A. (2013). Phytochemical analysis of *Allium fistulosum* L. and A. ursinum L. *Digest Journal of Nanomaterials & Biostructures*, 2013, *8*(1).

Vu, N. K., Kim, C. S., Ha, M. T., Ngo, Q. M. T., Park, S. E., Kwon, H., ... & Min, B. S. Antioxidant and Antidiabetic Activities of Flavonoid Derivatives from the Outer Skins of *Allium* cepa L. *Journal of agricultural and food chemistry*, 2020, *68*(33), 8797-8811.

Wang, J., Qiao, L., Liu, B., Wang, J., Wang, R., Zhang, N., & Yu, Y. Characteristic aroma-active components of fried green onion (*Allium fistulosum* L.) through flavoromics analysis. *Food Chemistry*, 2023, *429*, 136909.

Wilson, E. A., & Demmig-Adams, B. Antioxidant, anti-inflammatory, and antimicrobial properties of garlic and onions. *Nutrition & food science*, 2007, *37*(3), 178-183.

Yan, J. K., Zhu, J., Liu, Y., Chen, X., Wang, W., Zhang, H., & Li, L. Recent advances in research on *Allium* plants: Functional ingredients, physiological activities, and applications in agricultural and food sciences. *Critical Reviews in Food Science and Nutrition*, 2023, *63*(26), 8107-8135.

Zeng, Y., Li, Y., Yang, J., Pu, X., Du, J., Yang, X., & Yang, S. Therapeutic role of functional components in alliums for preventive chronic disease in human being. *Evidence-Based Complementary and Alternative Medicine*, 2017.

Zolfaghari, B., Yazdiniapour, Z., Sadeghi, M., Akbari, M., Troiano, R., & Lanzotti, V. Cinnamic acid derivatives from welsh onion (*Allium fistulosum*) and their antibacterial and cytotoxic activities. *Phytochemical analysis*, 2021, *32*(1), 84-90