GREEN EXTRACTION AND COMPUTATIONAL EVALUATION OF CAFFEIC ACID AND THIADIAZOLE HYBRIDS FROM SPENT COFFEE GROUNDS AND PEELS: A DRUG–HERB SYNERGISTIC APPROACH

Project Reference No.: *49S_MSC_0327*

College : *St. Philomena's College (Autonomous), Mysuru*
Branch : *Department of Chemistry*
Guide(s) : *Dr. Zabiulla*
Dr. Saleem Javid
Student(S) : *Mr. Hemanth Gowda K J*
Mr. Yadunandan S
Ms. Sufiya Nikhat

Keywords:

Green extraction; Caffeic acid; Thiadiazole; Molecular docking; Antioxidant activity; Waste valorization.

Introduction:

Oxidative stress caused by free radicals is a major contributing factor to several chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. The development of effective antioxidant molecules capable of neutralizing reactive oxygen species (ROS) is therefore an important area of research in medicinal chemistry [1].

Caffeic acid, a naturally occurring phenolic compound, has attracted significant attention due to its strong antioxidant activity arising from its catechol moiety, which facilitates hydrogen donation and radical stabilization. Structural modification of caffeic acid through derivatization, particularly via amide linkage, has been reported to enhance its stability, lipophilicity, and biological activity [2]. Incorporation of phenoxy groups further improves electron delocalization, thereby increasing free radical scavenging efficiency.

Similarly, thiadiazole derivatives represent an important class of heterocyclic compounds known for their diverse pharmacological properties, including notable antioxidant activity. [3, 4].

Recent studies indicate that combining aromatic systems such as phenoxy groups with biologically active scaffolds enhances conjugation and facilitates electron transfer mechanisms, which are crucial for antioxidant activity. Such structural modifications are also known to influence structure–activity relationships (SAR), leading to improved pharmacological profiles [5].

In addition, agro-waste materials such as spent coffee grounds and passion fruit peels are rich and sustainable sources of phenolic compounds, including caffeic acid derivatives. The application of green extraction techniques using eco-friendly solvents and energy-efficient methods has gained significant importance for the recovery of such bioactive compounds. These approaches not only reduce environmental impact but also enhance the efficiency and selectivity of extraction processes, contributing to sustainable waste valorization [6, 7].

Therefore, the design and evaluation of caffeic acid and thiadiazole derivatives, along with the utilization of naturally derived caffeic acid from agro-waste sources through green extraction methods, represent a promising strategy for developing potent antioxidant agents with potential therapeutic applications.

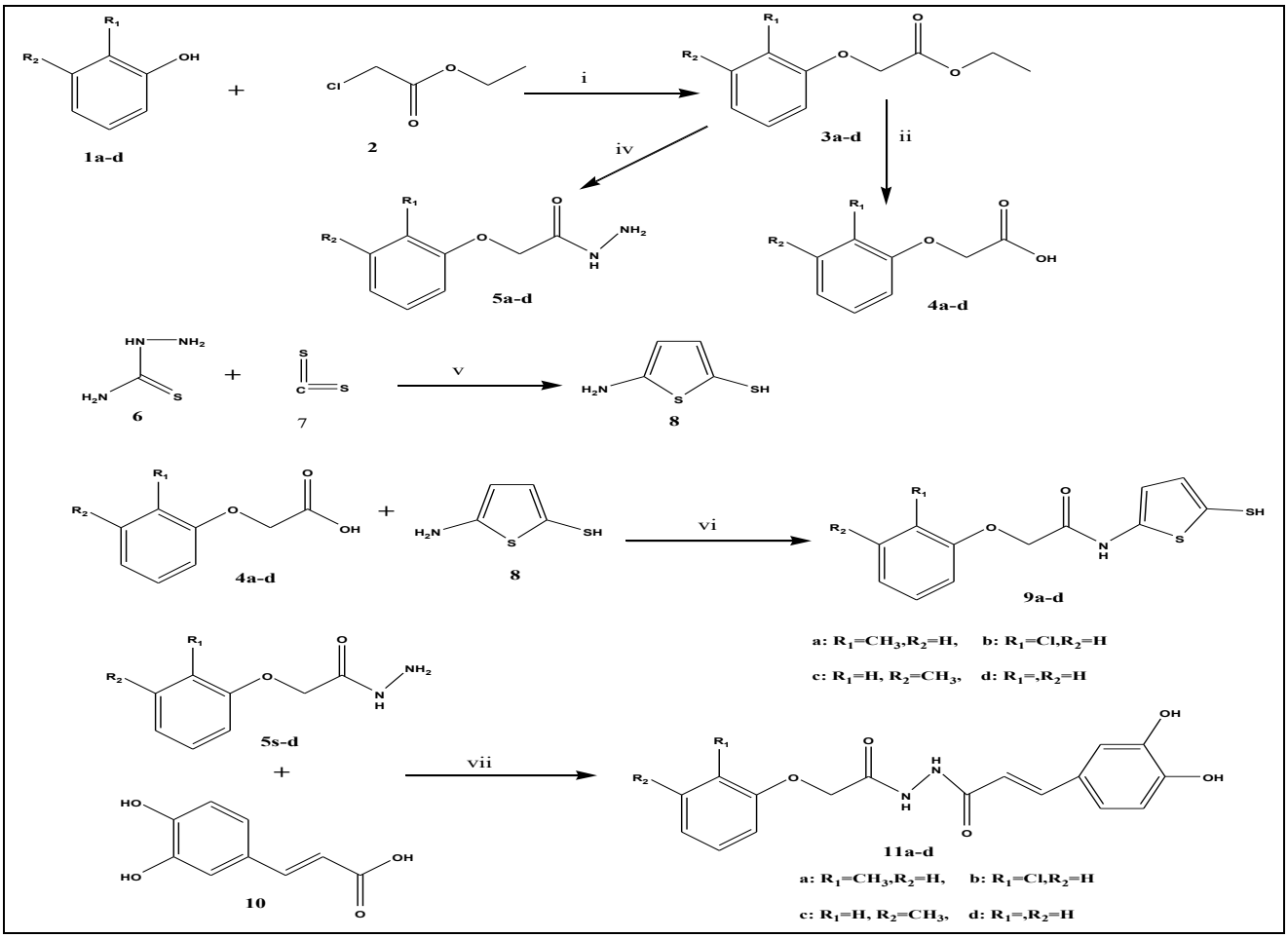
Objectives:

1. To extract caffeic acid and its derivatives from spent coffee grounds and passion fruit peels using green extraction techniques.
2. To synthesize caffeic acid derivatives appended with phenoxy groups via amide linkage.
3. To synthesize thiadiazole derivatives appended with phenoxy groups via amide linkage.
4. To evaluate antioxidant activity of extracted and synthesized compounds and perform computational studies

Methodology:

Synthesis:

The target compounds, thiadiazole derivatives appended with phenoxy groups via amide linkage (**9a–d**) and caffeic acid derivatives appended with phenoxy groups via amide linkage (**11a–d**), were successfully synthesized through a multistep synthetic procedure as illustrated in the scheme. All the synthesized compounds were characterized using spectroscopic techniques including ^{1}H NMR, ^{13}C NMR, and mass spectrometry.



Initially, substituted ethyl aryloxy acetates (**3a–d**) were synthesized by etherification of substituted hydroxy aryl compounds (**1a–d**) with ethyl chloroacetate (**2**) in the presence of anhydrous potassium carbonate using dry acetone as solvent. The obtained esters (**3a–d**) were subjected to alkaline hydrolysis using aqueous alcoholic sodium hydroxide under reflux conditions to yield the corresponding substituted aryloxy acetic acids (**4a–d**).
Simultaneously, a portion of the esters (**3a–d**) was treated with hydrazine hydrate in ethanol to obtain substituted aryloxy acetohydrazides (**5a–d**).

For the preparation of the thiadiazole intermediate, thiosemicarbazide (**6**) was reacted with carbon disulfide (**7**) in the presence of anhydrous sodium carbonate in absolute ethanol under reflux conditions. The reaction mixture was further heated at 75–80 °C, and upon completion, the product was acidified with concentrated hydrochloric acid to yield **5-amino-1,3,4-thiadiazole-2-thiol (8)**.

Finally, the target compounds were synthesized via coupling reactions. The substituted aryloxy acetic acids (4a–d) were coupled with 5-amino-1,3,4-thiadiazole-2-thiol (8) to obtain the **thiadiazole derivatives (9a–d)**. Similarly, substituted aryloxy acetohydrazides (5a–d) were coupled with caffeic acid (**10**) to yield the **caffeic acid derivatives (11a–d)**. These coupling reactions were carried out using lutidine as base and TBTU as coupling agent in dimethyl sulfoxide (DMSO), affording the desired products in good yields.

Extraction:

Caffeic acid–rich fractions were successfully extracted from spent coffee grounds and passion fruit peels using multiple green extraction techniques. All extracts were systematically labeled for clear identification and comparative analysis.

1. **Direct Solvent Extraction**

Direct solvent extraction was carried out using a 70:30 ethanol–water mixture. The powdered coffee grounds and passion fruit peels were heated at 60–70 °C for several hours. The extracts obtained after filtration and concentration were labeled as:

- **C-cd1** (coffee – direct extract)
- **C-pd1** (passion fruit – direct extract)

2. **Sequential Solvent Extraction**

Sequential solvent extraction was performed using hexane, ethyl acetate, and ethanol.

From **spent coffee grounds**, the extracts were labeled as:

- **C-cs1** (hexane fraction)
- **C-cs2** (ethyl acetate fraction)
- **C-cs3** (ethanol fraction)

From **passion fruit peels**, the extracts were labeled as:

- **C-ps1** (hexane fraction)
- **C-ps2** (ethyl acetate fraction)
- **C-ps3** (ethanol fraction)

3. **Ultrasound-Assisted Extraction (UAE)**

Ultrasound-assisted extraction was carried out using ethanol–water solvent under ultrasonic irradiation. The extracts obtained were labeled as:

- **C-cu1** (coffee – UAE extract)
- **C-pu1** (passion fruit – UAE extract)

4. Aqueous Extraction

Aqueous extraction was performed using distilled water as a solvent. The extracts obtained were labeled as:

C-ca1 (coffee – aqueous extract)
C-pa1 (passion fruit – aqueous extract)

Code	Meaning	Code	Meaning
C-cd1	Coffee – Direct extract	**C-pd1**	Passion fruit – Direct extract
C-cs1	Coffee – Sequential (hexane)	**C-ps1**	Passion fruit – Sequential (hexane)
C-cs2	Coffee – Sequential (ethyl acetate)	**C-ps2**	Passion fruit – Sequential (ethyl acetate)
C-cs3	Coffee – Sequential (ethanol)	**C-ps3**	Passion fruit – Sequential (ethanol)
C-cu1	Coffee – Ultrasound extract	**C-pu1**	Passion fruit – Ultrasound extract
C-ca1	Coffee – Aqueous extract	**C-pa1**	Passion fruit – Aqueous extract

Table 1: The *in-vitro* antioxidant activity in DPPH method

Compounds	Conc. (µg/ml)				
	25	**50**	**75**	**100**	**IC_{50}**
9a	66.74 ± 0.61	70.52 ±	73.86 ± 0.81	77.42 ±	17.35 ± 0.58
9b	56.42 ± 1.54	60.28 ±	64.15 ± 1.72	68.36 ±	23.68 ± 1.48
9c	68.36 ± 0.52	72.15 ±	75.68 ± 0.72	79.24 ±	16.82 ± 0.49
9d	62.88 ± 1.12	66.74 ±	70.18 ± 1.32	73.65 ±	19.12 ± 1.05
11a	73.65 ± 0.41	77.28 ±	80.74 ± 0.63	84.36 ±	15.36 ± 0.35
11b	66.45 ± 1.12	70.18 ±	74.36 ± 1.22	78.54 ±	18.94 ± 1.10
11c	**75.82 ± 0.32**	**79.14 ±**	**83.26 ± 0.52**	**86.91 ±**	**14.72 ± 0.28**
11d	71.84 ± 0.38	75.96 ±	79.42 ± 0.58	83.05 ±	15.88 ± 0.42
C-cd1	61.48 ± 1.28	65.12 ±	69.05 ± 1.44	72.86 ±	19.86 ± 1.18
C-pd1	57.36 ± 1.42	61.08 ±	64.92 ± 1.60	68.74 ±	22.36 ± 1.34
C-cs1	60.25 ± 1.31	64.18 ±	68.02 ± 1.51	71.84 ±	20.54 ± 1.22
C-cs2	74.92 ± 0.26	78.36 ±	82.45 ± 0.48	85.78 ±	14.98 ± 0.22
C-cs3	72.15 ± 0.34	76.42 ±	79.88 ± 0.55	83.14 ±	15.42 ± 0.31
C-ps1	58.86 ± 1.45	62.74 ±	66.38 ± 1.65	70.12 ±	21.68 ± 1.36
C-ps2	71.88 ± 0.37	75.94 ±	79.36 ± 0.60	82.68 ±	15.96 ± 0.38
C-ps3	69.75 ± 0.42	73.86 ±	77.24 ± 0.66	80.59 ±	16.48 ± 0.44
C-cu1	64.25 ± 1.22	68.36 ±	72.18 ± 1.28	76.04 ±	17.92 ± 1.12
C-pu1	59.82 ± 1.34	63.45 ±	67.36 ± 1.51	71.28 ±	21.54 ± 1.26
C-ca1	52.84 ± 1.65	56.72 ±	60.38 ± 1.84	64.15 ±	25.92 ± 1.52
C-pa1	49.75 ± 1.78	53.62 ±	57.28 ± 1.95	61.04 ±	27.48 ± 1.64
Ascorbic acid	78.45 ± 0.28	82.36 ±	86.92 ± 0.42	90.14 ±	12.84 ± 0.18
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ± SD.

Table 2: The *in-vitro* antioxidant activity of in lipid peroxidation method

Compounds	Conc. (µg/ml)				
	25	**50**	**75**	**100**	**IC_{50}**
9a	62.45 ± 0.72	66.18 ±	69.86 ± 0.93	73.24 ±	18.92 ± 0.64
9b	52.36 ± 1.62	56.74 ±	60.25 ± 1.86	64.12 ±	25.84 ± 1.52
9c	64.28 ± 0.63	68.52 ±	72.14 ± 0.85	75.68 ±	18.12 ± 0.55
9d	58.64 ± 1.24	62.48 ±	66.05 ± 1.48	69.72 ±	20.48 ± 1.12
11a	69.52 ± 0.48	73.86 ±	77.28 ± 0.65	80.92 ±	16.42 ± 0.38
11b	62.18 ± 1.18	66.45 ±	70.52 ± 1.34	74.36 ±	19.84 ± 1.08
11c	**72.36 ± 0.36**	**76.82 ±**	**80.94 ± 0.52**	**84.28 ±**	**15.68 ± 0.30**
11d	68.42 ± 0.41	72.75 ±	76.14 ± 0.58	79.86 ±	16.98 ± 0.44
C-cd1	57.84 ± 1.32	61.48 ±	65.72 ± 1.52	69.35 ±	21.12 ± 1.22
C-pd1	54.18 ± 1.45	58.26 ±	62.48 ± 1.64	66.12 ±	23.46 ± 1.36
C-cs1	56.12 ± 1.34	60.28 ±	63.95 ± 1.58	67.84 ±	22.36 ± 1.28
C-cs2	71.86 ± 0.28	75.94 ±	79.86 ± 0.45	83.14 ±	15.52 ± 0.24
C-cs3	69.42 ± 0.36	73.64 ±	77.12 ± 0.54	80.45 ±	16.08 ± 0.32
C-ps1	54.92 ± 1.48	58.86 ±	62.45 ± 1.72	66.18 ±	23.88 ± 1.42
C-ps2	69.84 ± 0.41	73.95 ±	77.36 ± 0.61	80.72 ±	16.62 ± 0.38
C-ps3	67.65 ± 0.46	71.78 ±	75.18 ± 0.67	78.54 ±	17.18 ± 0.46
C-cu1	60.25 ± 1.24	64.36 ±	68.18 ± 1.42	72.04 ±	19.36 ± 1.18
C-pu1	56.72 ± 1.38	60.45 ±	64.36 ± 1.58	68.28 ±	21.84 ± 1.32
C-ca1	50.84 ± 1.72	54.72 ±	58.38 ± 1.96	62.15 ±	27.42 ± 1.62
C-pa1	48.36 ± 1.86	52.18 ±	55.84 ± 2.05	59.62 ±	29.18 ± 1.74
Ascorbic acid	76.82 ± 0.26	80.94 ±	85.36 ± 0.42	88.72 ±	13.12 ± 0.20
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ± SD

Result and Conclusion:

1. The antioxidant activity of all tested samples was evaluated using DPPH and lipid peroxidation (LPO) assays, and the results showed a clear concentration-dependent increase in percentage inhibition. Overall, a wide variation in antioxidant potential was observed among the samples, allowing classification into high, moderate, and low activity groups.
2. Among all samples, **compound 11c** exhibited the **highest antioxidant activity**, showing maximum percentage inhibition and the lowest IC_{50} value, comparable to the standard ascorbic acid. Other samples such as **11a, 11d, and C-cs2 (ethyl acetate fraction of coffee)** also demonstrated **high antioxidant activity**, indicating strong free radical scavenging ability.
3. A group of samples including **C-cs3, C-ps2, C-ps3, 9c, and 9a** showed **moderate antioxidant activity**, with reasonably good inhibition values but slightly higher IC_{50}

values compared to the most active compounds. These results suggest the presence of active constituents, though with comparatively lower efficiency.

4. Samples such as **9d, 11b, C-cu1, C-pu1, C-cd1, and C-pd1** exhibited **lower to moderate activity**, indicating limited radical scavenging potential, possibly due to weaker electron-donating ability or lower phenolic content.
5. The **lowest antioxidant activity** was observed in **9b and aqueous extracts (C-ca1 and C-pa1)**, which showed minimum inhibition and higher IC_{50} values. This may be attributed to the presence of electron-withdrawing substituents or poor extraction efficiency of active phenolic compounds.
6. Overall, the variation in antioxidant activity can be correlated to structural features such as the presence of phenolic hydroxyl groups and electron-donating substituents, as well as the efficiency of extraction methods. The consistency in trends observed in both DPPH and LPO assays further supports the reliability of the results.

In conclusion,

7. The present study successfully demonstrated the development and evaluation of antioxidant agents through both synthetic and green extraction approaches. The results revealed that all tested samples exhibited concentration-dependent antioxidant activity, with significant variation based on structural features and extraction methods.
8. Among all, **compound 11c** showed the highest antioxidant activity, comparable to the standard ascorbic acid, indicating its potential as a promising antioxidant candidate. Samples containing electron-donating groups and phenolic moieties exhibited enhanced activity, whereas chloro-substituted compounds and aqueous extracts showed comparatively lower activity.
9. The findings also confirmed that **ethyl acetate and ethanol fractions obtained through sequential extraction** were more effective in isolating phenolic-rich compounds, leading to better antioxidant performance compared to other extraction methods.
10. Interestingly, the study highlights the importance of **structural modification and green extraction techniques** in improving antioxidant activity. The consistency observed in both DPPH and LPO assays further validates the reliability of the results. These outcomes suggest that the developed compounds and extracts have potential for further investigation in pharmaceutical and medicinal applications.

References:

1. M. A. Khan et al., “Recent advances in caffeic acid derivatives as antioxidant and therapeutic agents,” *European Journal of Medicinal Chemistry*, vol. 250, 2024.
2. Y. Li et al., “Design, synthesis and antioxidant evaluation of caffeic acid amide derivatives,” *Bioorganic Chemistry*, vol. 137, 2023.
3. S. Verma et al., “Synthesis and biological evaluation of 1,3,4-thiadiazole derivatives as antioxidant agents,” *Journal of Molecular Structure*, vol. 1285, 2024.
4. A. R. Patil et al., “Structure–activity relationship studies of thiadiazole derivatives,” *Bioorganic & Medicinal Chemistry Letters*, vol. 84, 2023.

5. P. Singh et al., “Phenoxy-based derivatives: synthesis and antioxidant potential,” *Synthetic Communications*, vol. 54, 2024.
6. R. Campos-Vega et al., “Spent coffee grounds as a source of phenolic compounds: green extraction approaches,” *Food Chemistry*, vol. 405, 2023.
7. L. Xu et al., “Green extraction of phenolic compounds from fruit peels and agro-waste: techniques and applications,” *Journal of Cleaner Production*, vol. 412, 2024.

Future Scope:

The future scope of this project includes:

1. **Completion of computational studies** such as molecular docking to understand the electronic properties and molecular interactions of the synthesized compounds.
2. **Detailed structure–activity relationship (SAR) studies** to further optimize substituents for improved antioxidant potential.
3. **Extension of biological evaluations** to include antimicrobial, antifungal, and anticancer activities.
4. **Isolation and purification of active constituents** from plant extracts for better activity correlation and standardization.
5. **In vivo studies and toxicity evaluation** to assess safety and therapeutic potential.