

Electronic supplementary information (ESI)

2. Materials and Methods

2.1. Phytochemical Tests

2.1.1. Alkaloid Procedures

Wagner Reagent

The Wagner reagent was prepared by adding 1g of iodine and 1g of potassium iodide to 50 mL of distilled water. Subsequently, 10 mL of 5% hydrochloric acid (HCl) was added. The test was then conducted by adding the reagent to 1 mL of both groups of seed extracts (SEs). A prominent brown or yellow precipitate indicated the test as positive (Dahanayake et al., 2019).

2.1.2. Tannin Procedures

2.1.2.1. Ferric Chloride Test

A 2% ferric chloride solution was prepared. The test for 1mL SEs was conducted using this reagent (Dahanayake et al., 2019).

2.1.2.2. Lead Acetate Test

A solution was prepared by measuring 4 g of lead acetate and 0.5 mL of acetic acid, followed by adding 100 mL of distilled water. The test was then performed by adding 10 drops of the reagent to 1mL SEs sample (Dahanayake et al., 2019).

2.1.2.3. Phlobatannins test (HCl test)

The Phlobatannins test (HCl test) was conducted by preparing a 20 mL solution of 37% HCl. Subsequently, 1 mL SEs were added to 5 mL of HCl (Priya and Ravindhran, 2015).

2.1.3. Free Anthraquinone test

1 mL of SEs was introduced into 1.5 mL of 10% HCl, followed by the addition of 1.5 mL of chloroform along with one drop of 10% ammonia (Ryavalad and Melappa, 2014).

2.1.4. Flavonoid – Shinota test

1 mL of SEs were diluted by 5 mL of distilled water, then 1mL of sodium hydroxide (NaOH) was added to each sample (Ahmed and Saddam, 2024).

2.1.5. Carbohydrate - Molisch test

Molisch reagent was prepared by mixing 3.75 g of α -naphthol and 25 mL of ethanol. Then 1mL of the SEs were added to 1 mL of distilled water to dilute extracts. Subsequently, 2-3 drops of the reagent were added to the samples, followed by the addition of 2 drops of H_2SO_4 to conduct the test (Usman, Abdulrahman and Usman, 2009).

2.1.6. Saponin – Frothing test

1 mL of SEs were added to 10 mL distilled water for dilution, followed by shaking for 20 minutes and allowing it to stand for 20 minutes (Priya and Ravindhran, 2015).

2.1.7. Cardiac Glycoside test

2 mL of SEs were added to 1mL of concentrated acetic acid, followed by the addition of 2 drops of 2% ferric chloride and 0.5 mL of concentrated H_2SO_4 (Nalado and Abduljabbar, 2023).

2.1.8. Phenolic test

1 mL of SEs were added to 1mL of distilled water for dilution, followed by the addition of 4 drops of 5% ferric chloride (Ahmed and Saddam, 2024).

2.1.9. Coumarin's test

The Coumarin's test involved adding 1 mL of SEs to 1 mL of 10% NaOH (Shaikh and Patil, 2020).

2.1.10. Amino acids

2 mL of SEs were added to 5mL of 10% NaOH, followed by the addition of ninhydrin reagent (Jaganathan, Shanmugavadivu and Ganesh, 2018).

2.1.11 Lieberman test

The Lieberman test began with the addition of 1.5 mL of lead acetate to 1 mL of SEs, followed by filtration of the extracts. The filtrate was then shaken with 2.5 mL of chloroform in a separatory funnel and left until evaporated. The remaining part was melted on a watch glass in 2.5 mL acetic anhydride, followed by the addition of 1 mL of concentrated H₂SO₄, the extract was precipitated, and no filtrate could be obtained to perform the test (Priya and Ravindhran, 2015).

2.1.12. Salkowski Test

The Salkowski test is used to detect the presence of steroids or specifically cholesterol in a sample. The Salkowski test was conducted by adding 1 mL of SEs to 0.5 mL of H₂SO₄ and then adding 2 mL of chloroform (Dahanayake et al., 2019).

3.Results and Discussion

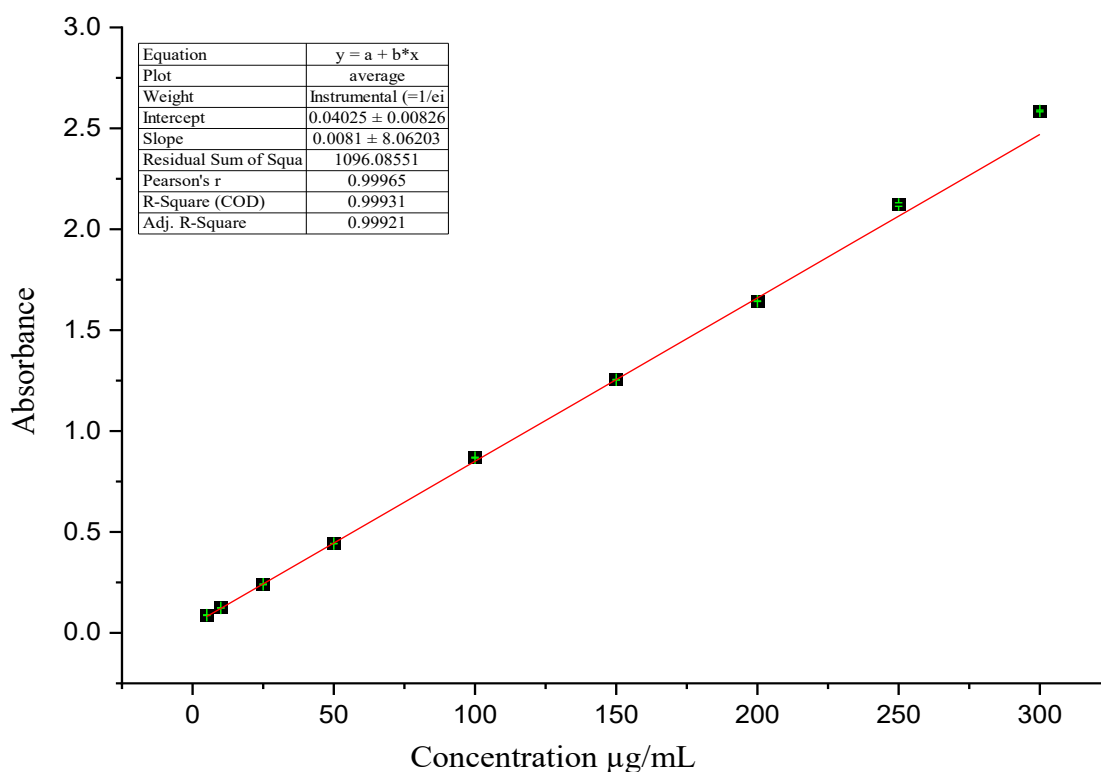
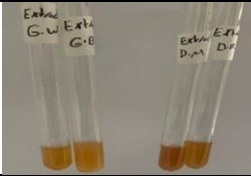
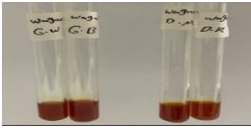
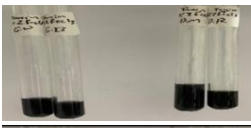
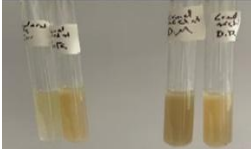

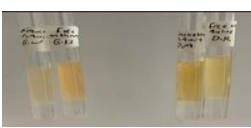
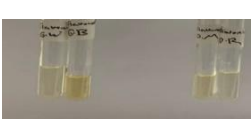
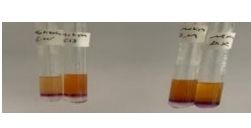
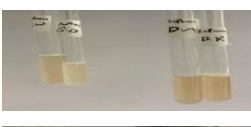

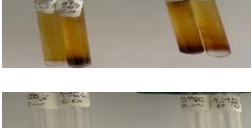


Figure S 3.2. Standard gallic acid calibration curve for TPC.

Table S 3.1. Qualitative analysis of phytochemicals of the extracts (WGSE, BGSE, ADSE, BDSE).

Phytochemical test		Result				Pictures
		WGS E	BGSE	ADS E	BDS E	
Alkaloid	Wagner's test	+	+	+	+	
	Ferric Chloride test	+	+	+	+	
Tannins	Lead Acetate	+	+	+	+	
	HCl test	+	+	+	+	
Free Anthraquinone		+	+	+	+	
Flavonoids	Shinota test	+	+	+	+	
Carbohydrates	Molisch test	+	+	+	+	
Saponin	Forthing test	+	+	+	+	
Phenol	Phenolic test	+	+	+	+	
Gardiac Glycoside		+	+	+	+	
Terpenoids	Salkowski test	+	+	+	+	

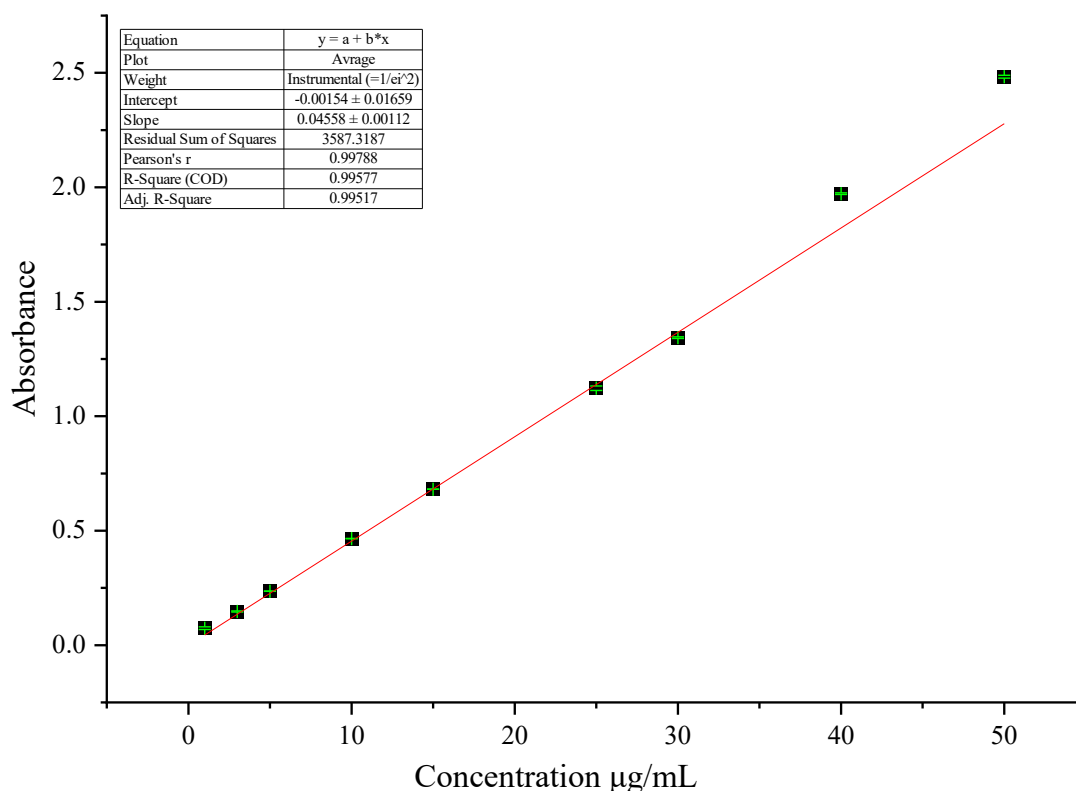


Figure S 3.3. Standard quercetin calibration curve for TFC.

References

- Ahmed, H. and Saddam, A. (2024) 'IMPACT OF GRAPE SEEDS EXTRACT AGAINST ALLOXAN INDUCED DIABETES IN MICE', *Iraqi Journal of Agricultural Sciences*, 55 (6), pp. 2085-2093. Available at.
- Dahanayake, J.M. et al. (2019) 'Comparative Phytochemical Analysis and Antioxidant Activities of Tamalakyadi Decoction with Its Modified Dosage Forms', *Evid Based Complement Alternat Med*, 2019 pp. 6037137. Available at: <http://dx.doi.org/10.1155/2019/6037137>.
- Jaganathan, V., Shanmugavadivu, M. and Ganesh, S. (2018) 'Preliminary phytochemical screening and anti-bacterial activity of date seed methanolic extract', *Intl J of Adv Res in Biol Sci*, 5 (2), pp. 209-215. Available at.
- Nalado, Y. and Abduljabbar, T. (2023) 'Qualitative and Quantitative Phytochemical Analysis of Aloe barbadensis Miller Leaf Extracts', *UMYU Scientifica*, 2 pp. 24-30. Available at: <http://dx.doi.org/10.56919/usci.2123.004>.
- Priya, S.E. and Ravindhran, R. (2015) 'Phytochemical analysis and antimicrobial properties of extracts from aerial parts of Phyla nodiflora (L) Greene', pp. Available at.
- Ryavalad, C. and Melappa, G. (2014) 'First report of anticancer agent, lapachol producing endophyte, Aspergillus niger of Tabebuia argentea and its in vitro cytotoxicity assays', *Bangladesh Journal of Pharmacology*, 9 pp. 129-139. Available at: <http://dx.doi.org/10.3329/bjp.v9i1.15622>.
- Shaikh, J.R. and Patil, M. (2020) 'Qualitative tests for preliminary phytochemical screening: An overview', *International journal of chemical studies*, 8 (2), pp. 603-608. Available at.
- Usman, H., Abdulrahman, F. and Usman, A. (2009) 'Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of Ficus thonningii (Moraceae)', *Afr J Tradit Complement Altern Med*, 6 (3), pp. 289-295. Available at: <http://dx.doi.org/10.4314/ajtcam.v6i3.57178>.