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Evaluation of Antioxidant Potential through DPPH and ABTS Assays: A Study on Polyherbal Formulation

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Abstract

The study investigates the antioxidant potential of a polyherbal formulation (PHF) using DPPH radical scavenging, ABTS radical scavenging assays, and total phenolic content (TPC) estimation. The DPPH assay revealed that the hot water extract of PHF showed a maximum scavenging activity of 83.6%, comparable to the standard Gallic acid (86.6%), while the ethanolic extract exhibited 54.5% activity. ABTS assay demonstrated higher antioxidant activity in the ethanolic extract (86.5%) compared to the hot water extract (82.3%). The IC50 values confirmed the superior radical scavenging capacity of the ethanolic extract for ABTS and hot water extract for DPPH. TPC analysis revealed 72.6 mg/g of phenolic content in the hot water extract, significantly higher than the ethanolic extract (31.22 mg/g). The findings highlight the potential use of PHF hot water extract as an effective natural antioxidant alternative.

Keywords: Antioxidant activity, DPPH assay, ABTS assay, polyherbal formulation, total phenolic content, Gallic acid.

Introduction

Antioxidants play a vital role in neutralizing free radicals and reducing oxidative stress, thereby preventing cellular damage and mitigating the risk of diseases such as cancer, cardiovascular disorders, and aging-related complications [1, 2]. Among various methods used to evaluate antioxidant potential, DPPH and ABTS assays are widely employed for their simplicity and reliability [3, 4].

The DPPH assay is based on the ability of antioxidants to donate hydrogen atoms, reducing the stable purple-colored DPPH radical to a yellow non-radical form [5]. The degree of decolorization correlates with the scavenging potential of the antioxidant [6]. The ABTS assay, on the other hand, utilizes a green-colored radical cation, which undergoes decolorization upon reaction with hydrogen donors [7, 8].

Plant-based polyherbal formulations are gaining popularity for their potent antioxidant properties attributed to their rich phenolic content [9]. Total phenolic content (TPC) estimation provides a quantitative measure of phenolics, strongly correlated with antioxidant capacity [10-16].

This study evaluates the antioxidant potential of a polyherbal formulation using DPPH and ABTS assays and determines its phenolic content to establish its efficacy as a natural alternative to synthetic antioxidants.

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Material and Method

Collection of Plant Material:

The plant materials were collected from western ghat and Bangalore rural zone. In the polyherbal preparation, leaves of *Tinnospora cordifolia, ocimum sanctum, Gymnema sylvestre* were selected, where as in the plants like *Withania somnifera* and *Coleus forskohlii*, the root part of the plant were selected, and the plant like *Phyllanthus emblica*, the fruit portion of the plant was selected to prepare the polyherbal formulation and to screen the antidiabetic and antioxidant therapeutic value through in vitro and in vivo studies.

Table 1: Authenticated plant species

S.N	Botanical Name	Family	Part Traded
1.	*Gymnema sylvestre* (Retz.). ex Sm.	Apocynaceae	Leaves
2.	*Phyllanthus emblica* L.	Phyllanthaceae	Fruits
3.	*Tinospora cordifolia* (Wild.) Miers	Menispermaceae	Leaves
4.	*Ocimum tenuiflorum* L.	Lamiaceae	Leaves
5.	*Withania somnifera* (L.) Dunal	Solanaceae	Roots
6	*Coleus forskohlii* (willd.) Briq.	Lamiaceae	Roots

DPPH radical scavenging activity

The free radical DPPH with an odd electron gives a maximum absorption at 517 nm which develops purple color. When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor who is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form, results in reaction in yellow color with respect to the number of electrons captured. It is evident that more the decolorization more is the reducing ability. It is most accepted model for evaluating the free radical scavenging activity of any new drug. In this when a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, resulting it gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of violet coloration.

Principle

The antioxidant which reacts with stable free radical, hence DPPH and converts it to 1,1-diphenyl–2-picryl hydrazine. The efficacy to scavenge the stable free radical DPPH is measured by decrease in the absorbance at 517 nm. When the degree of discoloration occurs it is indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability.

Preparation of working solutions:

DPPH (0.659mM): 2.6mg of DPPH dissolved and made up to 10ml in HPLC grade methanol.

Positive control (1000µg/ml): 1mg of Gallic acid is dissolved and made up to 1 ml HPLC grade methanol.

Sample preparation: 5mg of the sample was dissolved in 5mL of water followed by dilutions made in water and the final concentrations to the sample were tested were 200, 400, 600, 800 and 1000µg/mL concentration.

Procedure

24mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved using 100 ml of methanol; which protected from light by covering the test tubes through aluminium foil. 2ml of DPPH solution was added to 1ml of methanol and absorbance was taken immediately at 517nm for control reading. Various concentrations of hot water and ethanol extracts of PHF (200-1000µg/ml) as well as standard drug, Gallic acid was taken and diluted using 1ml of methanol and to which 2ml of DPPH was added. The absorbance was recorded after 48 hrs at 517nm using methanol as blank on UV-visible spectrophotometer [338]. The IC50values for each drug compounds as well as standard preparation were calculated. The DPPH free radical scavenging activity was derived using the following formula:

% of scavenging activity = [1-Absorbance of sample/Absorbance of control] × 100

The effective concentration of sample required to scavenge DPPH radical by 50% (IC50value) was obtained by plotting graph of % scavenging activity Vs concentrations

ABTS radical scavenging assay

It is evident that Free radicals are generally very reactive molecules possessing an unpaired electron which are produced continuously in cells either as by-products of metabolism or through leakage from mitochondrial respiration. The free radicals produced *in-vivo* include the active oxygen species like super-oxide radical O2-, hydrogen peroxide (H2O2) and hypochlorous acid (HOCl), oxygen free radicals responsible for many pathological conditions. These Free radicals and Reactive with Oxygen Species (ROS) cause DNA damage, lipid peroxidation, protein damage. They are known to be involved wide variety of disorders like cancer, cardiovascular diseases, inflammatory diseases, asthma and aging. Free radicals like the hydroxyl radical, hydrogen peroxide, super-oxide anion etc. Which mediate with inflammatory response, for the production of migratory factors, by cyclic nucleotides and eicosanoids. Super- oxide radicals amplify the inflammation process, increasing vascular permeability, adhesion of polymorph nuclear leucocytes to the endothelium and results in platelet aggregation.

Principle:

In this type of antioxidant with an ability to

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

donate a hydrogen atom will quench the stable free radical, a special process which is associated with the result in change in absorption which can be followed spectroscopically. In this experiment stable ABTS radical has a green color which is measured at 734nm.

Preparation of working solutions:

Phosphate buffer saline 10mM [PBS] (pH-7.4 at 25°C): A sachet of PBS was dissolved and made to using 1000ml of de-ionized water.

Ammonium persulfate [APS] (2.45mM): APS weighing around 5.59mg was dissolved & made up to 10ml of 10mM PBS and pH is adjusted to 7.4.

Positive control (1000µg/ml):

Stock 1: 1mg of Gallic acid is dissolved & made up to 1 ml 10mM PBS pH

7.4 (1000µg/ml). Followed by further dilutions are made as required**Sample preparation:** Around12.5 mg of sample was dissolved in 5 ml PBS. It is further extended as for preparing dilutions using vehicle buffer. The final concentration of sample tested was 10, 25, 50,100 and 200µg/ml for confirmation.

ABTS (7mM):38.41mg of ABTS is dissolved in 10ml of 10mM PBS pH 7.4.

ABTS radical solution:

Stock 1(3.5mM ABTS): 7mM of ABTS measuring around 10 ml was mixed with 10ml of 2.45mM APS and incubated in dark (stored in an amber colored bottle) for about 16hrs at 25°C in to evolve the ABTS radicals.

Working solution (0.238mM ABTS): Stock 1 measuring 680µl is made to 10ml with 10mM PBS with pH 7.4. Care should be taken that the working solution should have an absorbance of ≥1.0 at 734nm.

Procedure:

The assay was performed as per Auddy*et al* (2003). In this method, 250µl total reaction volume containing 20µl of 10mM PBS pH 7.4/ vehicle buffer/ positive control/ test solutions with various concentrations than 230µl of ABTS radical solution (0.238mM) was added and mixed and read immediately at 734nm with microplate reader.

The results obtained will be calculated using the formula:

Estimation of total phenolic content:

Phenolic content of the biological material reflects its antioxidant property. Colorimetric reactions are widely used in the UV/VIS spectrophotometric method, which is widely used to perform rapid and applicable in routine laboratory use then this method measures the total concentration of phenolic hydroxyl groups in the plant extract. Polyphenols in plant extracts which is reacts with specific redox reagents (Folin- Ciocalteau reagent) to form a blue complex which is measured by visible-light spectrophotometry. This reaction forms a blue chromophores complex

Preparation of working solutions:

Folin-Ciocalteau reagent: It was prepared by dissolve 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water was added. To this 5 ml 85% phosphoric acid and 10 ml concentrated hydrochloric acid was added. Reflux for 10 hr and add 15 g lithium sulfate, 5 ml water and 1 drop

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bromine once again. Reflux for 15 min. Cool to room temperature and bring to 100 ml with water.

Phosphate buffer saline 10mM [PBS] (pH-7.4 at 25°C): One sachet of PBS is dissolved in 1000ml of de-ionized water.

Positive control (1000µg/ml):

Stock 1: 1mg of Gallic acid is dissolved and made up to 1 ml 10mM PBS pH 7.4 (1000 µg/ml). Further dilutions are made if it is required.

Sample preparation: 12.5 mg of sample was dissolved in 5 ml PBS, further dilutions, were prepared with vehicle buffer. The final concentration is
1.0 ml.

Procedure:

The total phenolic content in hot water and ethanol extracts of polyherbal formulation was estimated by Folin-Ciocalteau method with some modifications, 1.5ml of Folin-Ciocalteau reagent was added to 1 ml of each polyherbal formulation extracts and kept for 5 minutes. Then 4ml of 7.5% Sodium bicarbonate was added to total volume was made up to 10ml by distilled water. The above solution was kept for incubation at room temperature for 30 minutes duration. Absorbance was measured at 765 nm using 1 cm cuvette in a UV-VIS lambda 25 spectrophotometer. Gallic acid solution (1.0- 100µg/ml) was used to produce standard curve. The total phenolic content was expressed in mg of Gallic acid Equivalents (GAE) mg / g of extract.

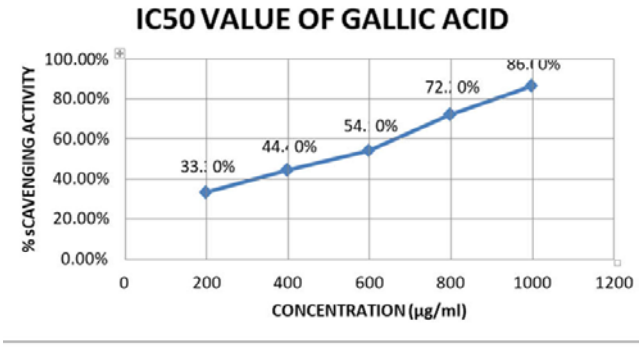
Results and Discussion

DPPH scavenging activity

The DPPH radical scavenging activity was recorded in terms of % Inhibition as shown in Table-2. It is evident from the table that Ethanolic extract of PHF showed minimum DPPH scavenging activity (54.5%) and Hot water PHF showed has maximum DPPH scavenging activity (83.60%). The Results obtained were compared with standard used (Gallic acid) which exhibited 86.60% scavenging activity. Higher % Inhibition indicates better scavenging activity or antioxidant potential. The amount of extract required for 50% inhibition of DPPH activity (IC50) is also shown in table-3. It is evident from the results hot water extract showed percentage of inhibition, which is similar to selected standard Gallic acid. Hence the polyherbal formulation using hot water extract can be used as safe alternative for the synthetic standard gallic acid.

Table 2: DPPH radical scavenging activity of Gallic acid at 1mg/ml concentration

Gallic acid	Concentration (µg/ml)	% Inhibition	IC50 Value
	200	33%	481µg/ml
	400	44.4%	
	600	54.1%	
	800	72.2%	
	1000	86.6%	



Graph 1: IC50 Value of Gallic acid at 1mg/ml

Table 3: DPPH radical scavenging activity of PHF

	Extract	Concentration(µg/ml)	% Inhibition	IC50 Value
Polyherbal formulation	Hot water	200	13.2%	760µg/ml
		400	15.8%	
		600	43.7%	
		800	53.60%	
		1000	83.60%	
		200	15.5%	

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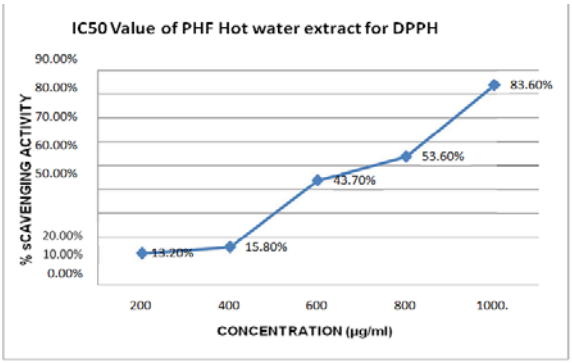
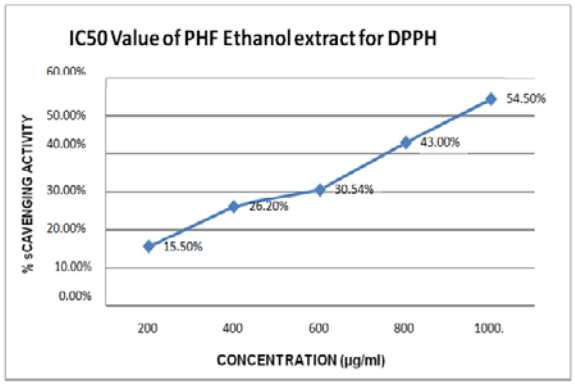
	Ethanol	400	26.2%	939µg/ml
		600	30.54%	
		800	43.00%	
		1000	54.5%	



Graph 2: IC50 Value of PHF Hot water extract for DPPH



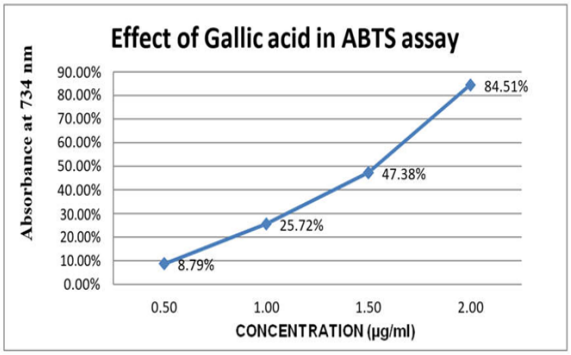
Graph 3: IC50 Value of PHF Ethanol extract for DPPH

ABTS radical scavenging assay

The ABTS radical scavenging activity was recorded in terms of % Inhibition as shown in table-5 and graph-5, graph-6. It was observed from the table that Ethanolic extract of PHF showed maximum ABTS scavenging activity (86.5%) and Hot water PHF has minimum DPPH scavenging activity (82.30%). The Results obtained were compared with standard Gallic acid which exhibited 84.51% scavenging activity. Higher % Inhibition indicates better scavenging activity or antioxidant potential. The amount of extract required for 50% inhibition of ABTS activity (IC50) is also shown in table-4 and graph-5, graph-6. The amounts of ABTS activity (IC50) were recorded as 38.6 µg/ml and 72.76 µg/ml in ethanolic extract and hot water extract. It is evident that IC50 value of the extract shows a safe administration when compared to the IC50 value of the standard drug (Gallic acid) 1.34 µg/ml (Table-4, Graph-5).

Table 4: ABTS radical scavenging activity of Gallic acid

	Concentration (µg/ml)	% Inhibition	IC50 (µg/ml) (95% C.I.)
Standard (Gallic acid)	0.5	8.79%	1.34 (1.23-1.46)
	1.0	25.72%	
	1.5	47.38%	
	2.0	84.51%	



Graph 4: Standard Gallic Acid

Table 5: ABTS radical scavenging activity of PHF

	Extract	Concentration(µg/ml)	% Inhibition	IC50 Value
Polyhedral formulation	Hot water	10	13.33%	72.76µg/ml
		25	20.77%	
		50	33.10%	
		100	55.11%	
		200	82.30%	
	Ethanol	10	10.0%	38.6µg/ml
		25	24.75%	
		50	67.25%	
		100	70.63.%	
		200	86.5%%	

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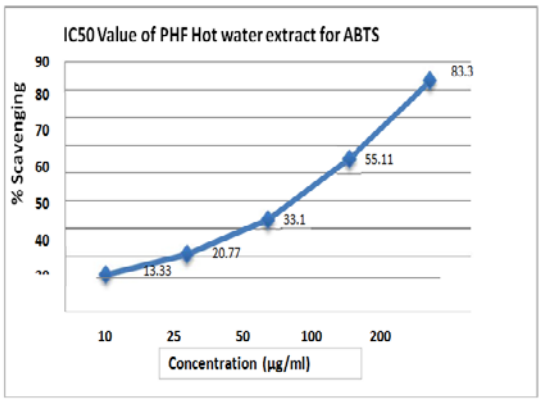
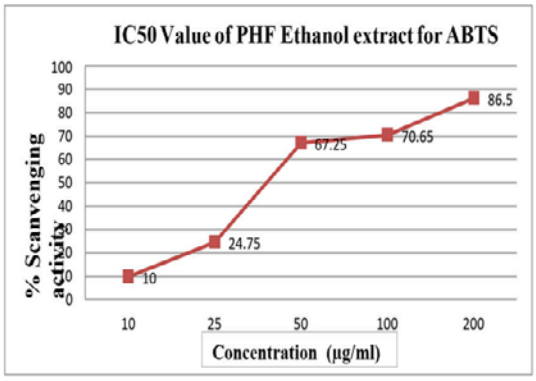


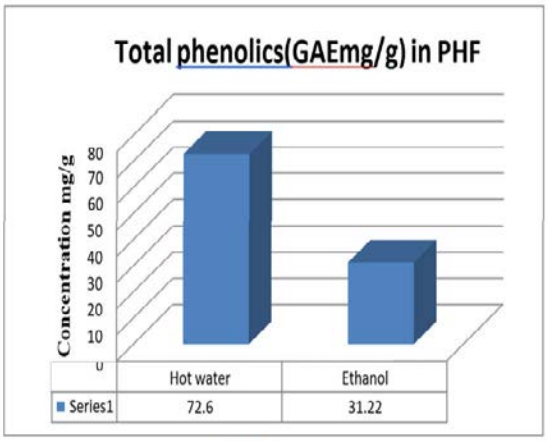
Graph 5: IC50 Value of PHF Hot water extract for ABTS



Graph 6: IC50 Value of PHF Ethanol extract for ABTS

Table 6: Total phenolic content of PHF in different extracts

Extracts	Concentration (mg/g)
Hot water	72.60
Ethanol	31.22



Graph-7

Estimation of total phenolic content

Since, there is a strong correlation between the antioxidant activity of plant extracts and their phenolic content; hence the total phenol was determined using Folin-Ciocalteau reagent. Gallic acid was used as standard compound. The total phenols content in two extracts of PHF was expressed as mg/g Gallic acid equivalent using the standard curve equation: **y=0.0116x+0.0478 (y = mx + c)**, where y is the absorbance at 765nm and x is the total phenolic content in different extracts of PHF expressed in mg/g. The phenol content in Ethanolic extract of Polyherbal formulation was found to be 31.22. The maximum phenolic content was found in PHF Hot water extract (72.60mg/g) shown in Table-6 and graph-7. Indicating its remarkable antioxidant activity.

Conclusion

This study highlights the strong antioxidant potential of the polyherbal formulation, particularly its hot water extract, as evidenced by DPPH and ABTS assays. The high total phenolic content further supports its efficacy. These findings suggest the potential of PHF as a natural and safe alternative to synthetic antioxidants, warranting further investigation for therapeutic applications.

References

1. International Diabetes Federation. *IDF Diabetes Atlas*. 10th ed. Brussels: International Diabetes Federation; 2021.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27(5):1047–53.

3. Ahmad A, Alghamdi SS, Mahmood K, Afzal M. Traditional medicinal plants for diabetes management: A review of phytochemical, pharmacological, and clinical evidence. *J Ethnopharmacol*. 2015;175:475–96.
4. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *J Adv Pharm Technol Res*. 2012;3(4):253–63.
5. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. *Diabetes Care*. 1989;12(8):553–64.
6. Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. *J Ethnopharmacol*. 2002;81(1):81–100.
7. Kwon YI, Apostolidis E, Shetty K. Inhibitory potential of wine and tea on alpha-amylase and alpha-glucosidase for management of hyperglycemia linked to type 2 diabetes. *BioFactors*. 2007;31(1):69–82.
8. Etxeberria U, de la Garza AL, Campión J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha-amylase. *Int J Mol Sci*. 2012;13(5):5951–73.
9. Bhaskaran K, Harish BN. Ayurvedic insights into diabetes management: A comprehensive review. *J Ayurveda Integr Med*. 2010;1(1):14–20.
10. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent alpha-amylase inhibitory activity of Indian ayurvedic medicinal plants. *BMC Complement Altern Med*. 2011;11:5.
11. Ali H, Houghton PJ, Soumyanath A. Alpha-amylase inhibitory activity of some Malaysian plants used to treat diabetes, with particular reference to *Phyllanthus amarus*. *J Clin Med Res*. 2006;36(6):643–8.
12. Khan A, Safdar M. Role of diet, nutrients, spices, and natural products in diabetes management. *J Clin Biochem Nutr*. 2003;31(1):16–20.
13. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr Sci*. 2002;83(1):30–8.
14. Pandey A, Tripathi P, Pandey RD, Srivastava R. Alternative therapies useful in the management of diabetes: A systematic review. *Diabetes Metab Syndr Clin Res Rev*. 2013;7(2):131–44.
15. Kumar S, Kumar D, Singh N, Bhatt V. Antidiabetic potential of *Tinospora cordifolia*. *Int J Pharm Sci Rev Res*. 2010;3(2):108–13.
16. Ragavan B, Krishnakumari S. Antidiabetic effect of *T. cordifolia* on normal and alloxan diabetic rats. *J Ethnopharmacol*. 2007;111(3):629–34.

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