Comparative Analysis of Phenolic Content and Antioxidant Activities in Herbal Plants

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Abstract:
This study evaluates the phenolic content and antioxidant activities of four selected herbal plants: Curcuma amada (rhizome), Coleus forskohlii (root), Cissus quadrangularis (whole plant), and Acorus calamus (rhizome). After processing the plant samples, total phenolics, flavonoid content, and antioxidant properties were measured. Notable findings include the highest total phenolic content in Cissus quadrangularis (37.52 mg gallic acid/g), while Curcuma amada exhibited the highest levels of flavonoids (20.03 mg/g quercetin equivalent), DPPH radical scavenging activity (65.96%), and superoxide anion scavenging activity (64.49%). Correlations were found between phenolic content and antioxidant activities across the plants. Overall, the herbal plants studied demonstrated significant antioxidant capacities, highlighting their potential health benefits.

Keywords: Antioxidant activities, phenolic content, herbal plants, flavonoids, free radical scavenging

Introduction:

Herbal plants have long served as a crucial source of active natural substances, boasting an impressive variety of chemical compositions, biological effects, and modes of action. These plants are abundant in phytochemical constituents, notably polyphenols such as flavonoids, phenylpropanoids, phenolic acids, and tannins. These compounds are instrumental in neutralizing free radicals and exhibiting potent antioxidant properties (Nickavar et al., 2007). Herbal medicine has been a cornerstone of traditional healthcare practices for centuries and remains relevant today. Its enduring popularity stems from its diverse biomedical advantages and its deep-rooted cultural importance across numerous regions worldwide, which plays a vital role in supporting human health (Megraj et al., 2011).

Literature Review:

Polyphenols, a major class of phytochemical compounds, exhibit significant biological effects primarily due to their antioxidant capabilities. These include free radical scavenging, inhibition of peroxidation, and chelating transition metals (Melo et al., 2005). Polyphenols share a common chemical pattern with one or more phenolic groups, allowing them to function as hydrogen donors and neutralize free radicals. The continuous utilization of oxygen by human cells produces free radicals, generating reactive oxygen species (ROS) such as superoxide anion (O2−), hydroxyl radicals (HO·), non-radical species like H_2O_2, single oxygen, and nitric oxide (NO). Phytocompounds such as flavonoids and phenolic acids, which are commonly present in plants, possess multiple biological and pharmacological activities, including antioxidative, cytotoxic, anticancer, antimicrobial, antiviral, and anti-inflammatory properties, offering a broad range of potential therapeutic benefits (Middleton et al., 2000).

Recent studies continue to explore the potential health benefits of polyphenols and their derivatives. For instance, research by Zhang et al. (2021) has highlighted the potential role of polyphenols in neuroprotection and the prevention of neurodegenerative diseases. Additionally, a review by Gupta and Dhiman (2020) emphasized the antimicrobial and antiviral activities of various polyphenolic compounds, noting their potential applications in combating infectious diseases. Furthermore, studies by Liska et al. (2019) suggest that polyphenolic compounds may play a role in the regulation of inflammation and metabolic health. These recent findings underscore the importance of polyphenols in modern medical research and their potential applications in health and disease management.

Materials and Methods:

In this study, four edible herbal plants – Curcuma amada, Coleus forskohlii, Cissus quadrangularis, and Acorus calamus – were selected for investigation. The rhizomes of Curcuma amada and Acorus calamus, the root of Coleus forskohlii, and the entire Cissus quadrangularis plant were utilized for extraction. To prepare the extracts, 5 grams of each dried plant sample were processed with a solvent mixture comprising acetone, methanol, and water in a total volume of 250 milliliters. The extraction process involved centrifugation to separate solid particles from the liquid phase, followed by filtration through filter paper to obtain a clear extract. The resulting filtered extracts were then stored in screw cap bottles to preserve their integrity until the analysis could be performed. The collected extracts were subsequently utilized in assessing the antioxidant activities of the selected plants.

Results and Discussion:

The study evaluated the antioxidant activities of four selected herbal crops, presenting the findings in Table - 1 and Figure - 1.

Total Phenolic Content

Phenolic compounds are a crucial group of phytochemicals that act as primary antioxidants and free radical scavengers. These compounds play a significant role in human health due to their capacity to neutralize free radicals and provide biological protection (Sulaiman et al., 2011). The total phenolic content of the samples varied from 19.94 to 37.52 mg gallic acid/g, with the highest content observed in Cissus quadrangularis (37.52 ± 0.66 mg/g), aligning with previous findings by Nagani et al. (2012), and followed by Coleus forskohlii (31.03 ± 0.29 mg/g). A significant difference ($P < 0.05$) was noted among all samples in terms of total phenolic content. Phenolic compounds can donate hydrogen atoms to free radicals, thus functioning as antioxidants (Sulaiman et al., 2011). Their potential as natural preservatives in the food industry is garnering interest due to their ability to retard lipid oxidation, enhancing the quality and nutritional value of foods (Wojdyło et al., 2007).

Total Flavonoid Content

Flavonoids are naturally occurring polyphenolic compounds found in herbal plants, as well as in various vegetables, nuts, fruits, and beverages like coffee, tea, and red wine (Hertog et al., 1993). These compounds exhibit a broad spectrum of chemical and biological activities, including free radical scavenging through their hydroxyl groups (Miliauskas et al., 2004). In the current study, the flavonoid content ranged from 4.27 to 20.03 mg/g quercetin equivalent, with Curcuma amada having the highest content (20.03 ± 0.81 mg/g), followed by Acorus calamus (12.01 ± 0.61 mg/g), Cissus quadrangularis (6.50 ± 0.77 mg/g), and Coleus forskohlii (4.27 ± 0.33 mg/g). Significant differences ($P < 0.05$) were observed in flavonoid content among all samples.

DPPH Radical Scavenging Activity

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) is a stable, nitrogen-centered free radical effectively neutralized by antioxidants, measured spectrophotometrically at 517 nm. The reduction of DPPH by proton or electron donation changes its color from violet to yellow, indicating antioxidant activity. This method is commonly used for quick evaluation of plant extracts' antioxidant properties (Srividya et al., 2014). The DPPH radical scavenging activity ranged from 19.21% to 65.96%, with Curcuma amada exhibiting the highest activity (65.96 ± 1.19%) and Coleus forskohlii following close behind (61.81 ± 1.37%). Significant differences ($P < 0.05$) were observed among the samples. Nenadis et al. (2003) noted that the mango ginger extracts' ability to scavenge DPPH radicals may be due to the presence of hydrogen-donating groups in antioxidant compounds.

Superoxide Anion Scavenging Activity

Although superoxide anions are weaker oxidants, they generate highly reactive hydroxyl radicals and singlet oxygen, causing oxidative stress (Srividya et al., 2014). Superoxide anions play a critical role in the formation of reactive oxygen species (ROS), including hydrogen peroxide, hydroxyl radicals, and singlet oxygen, which induce oxidative damage to lipids, proteins, and DNA (Wickens, 2001). In this study, superoxide anion radical scavenging activity ranged from 29.48% (Coleus forskohlii) to 64.49% (Curcuma amada). While significant differences ($P < 0.05$) were observed between Curcuma amada and Cissus quadrangularis, there was no significant difference between Acorus calamus and Coleus forskohlii.

Reducing Power

Previous studies have established a strong correlation between the reducing power of plant extracts and their antioxidant activities (Chanda and Kaneria, 2012; Mbaebie et al., 2012). The Fe3+ reduction is often used as an indicator of electron-donating activity, a key mechanism in phenolic antioxidant action and its correlation with other antioxidant properties. The reducing power of the samples varied from 0.39 to 0.57, with Coleus forskohlii showing the highest reducing power (0.57 ± 0.09) followed by Acorus calamus (0.49 ± 0.07). No significant difference ($P < 0.05$) was noted in reducing power among the samples.

TBARS % (Thiobarbituric Acid Reducing Substance)

The percentage of TBARS in the samples ranged from 137.86% to 319.86% (Cissus quadrangularis). TBARS and malondialdehyde (MDA) percentages are inversely proportional to antioxidant activity; as TBARS increases, antioxidant activity decreases. Curcuma amada demonstrated the highest antioxidant activity based on TBARS percentage (137.86 ± 2.29%), followed by Acorus calamus (185.71 ± 5.41%). A significant difference ($P < 0.05$) was noted in TBARS percentages across the samples.

Correlation Analysis

The Pearson's correlation coefficient (r) was used to evaluate relationships between various antioxidant parameters across the herbal crops studied. High positive correlation was observed between total phenolic content and DPPH radical scavenging activity (r = 0.789), consistent with Wojdyło et al.'s (2007) findings. This suggests phenolic compounds' significant contribution to the antioxidant properties of herbal crops. Total phenolic content also showed positive linear correlations with reducing power (r = 0.791) and TBARS% (r = 0.740) in Acorus calamus extracts, but these were not statistically significant ($P < 0.05$). DPPH radical scavenging activity demonstrated positive correlations with total flavonoid content (r = 0.954, $P < 0.05$) and TBARS% (r = 0.997, $P < 0.01$), both statistically significant.

Conclusion:

Herbal plants have been integral to traditional medicine systems worldwide for centuries, providing remedies for chronic conditions such as cardiovascular diseases, skin disorders, and various infections (Megraj et al., 2011). The study confirms that herbal plants are rich in diverse phytochemicals with potent antioxidant properties, particularly phenolic compounds. Their therapeutic potential, along with their limited side effects compared to allopathic medicines, makes them appealing sources of natural antioxidants and possible preventive agents against common health issues. Further exploration of these plants as potential natural antioxidants and therapeutic agents is warranted to uncover their full benefits and contributions to human health.

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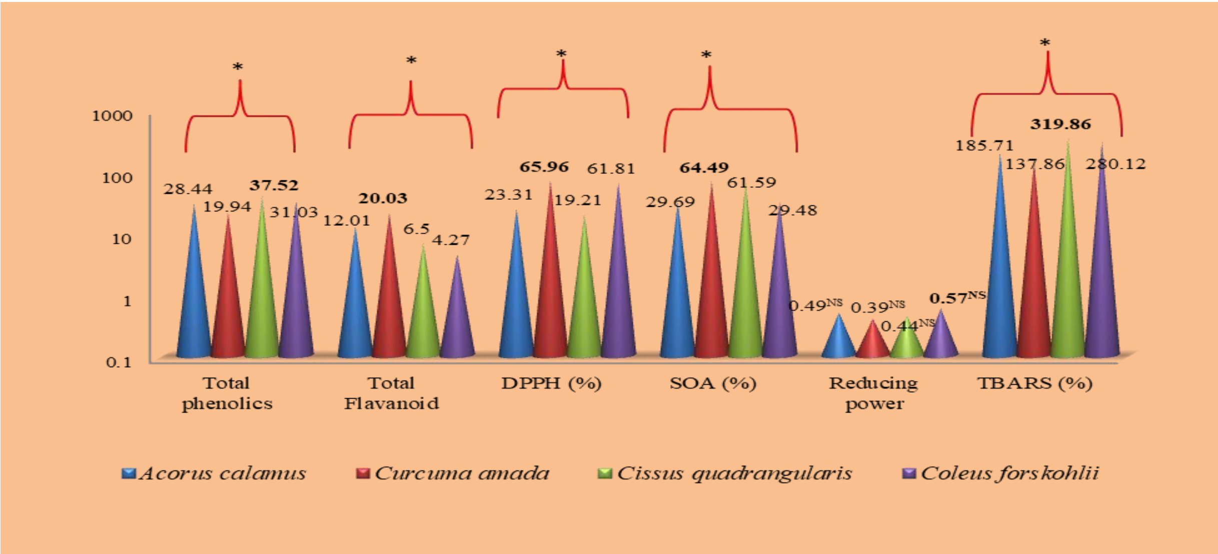
Table - 1: Evaluation of Antioxidant Properties in Chosen Medicinal Herbs

Plants	Total Phenolics	Total Flavanoid	DPPH (%)	SOA (%)	Reducing Power	TBARS (%)
Acorus calamus	28.44±0.60*	12.01±0.61*	23.31±0.55*	29.69±0.77	0.49±0.07	185.71±5.41*
Curcuma amada	19.94±0.34*	20.03±0.81*	65.96±1.19*	64.49±0.50*	0.39±0.01	137.86±2.29*
Cissus quadrangularis	37.52±0.66*	6.50±0.77*	19.21±0.27*	61.59±0.83*	0.44±0.00	319.86±4.50*
Coleus forskohlii	31.03±0.29*	4.27±0.33*	61.81±1.37*	29.48±0.68	0.57±0.09	280.12±0.90*
CD	0.96	1.26	1.83	1.35	0.11	7.131
SE(m)	0.29	0.38	0.55	0.41	0.03	2.153

Values are expressed as Mean±SD; *Significant at 0.05 level (P<0.05)

Note: TP - Total phenolics expressed as mg/g gallic acid equivalent; **TF-** Total flavanoid expressed as mg/g Quercetin equivalent; **DPPH** – 1, 1-diphenyl-2-picryl-hydrazil, activity is expressed in % inhibition; **RP** – Reducing power expressed in absorbance; **SOA** – Super oxide anion scavenging activity is expressed in %; **TBARS-** Thio Barbituric acid Reducing substance expressed in %.



*P<0.05, **P<0.01

Table - 3: Correlation coefficient of antioxidant activity of Curcuma amada

Properties	TP	DPPH	SOA	RP	TF	TBARS
TP	1.000					
DPPH	0.079	1.000				
SOA	0.763	0.704	1.000			
RP	0.265	-0.940*	-0.420	1.000		
TF	-0.796	-0.666	-0.999**	0.372	1.000	
TBARS	-0.951*	-0.383	-0.925*	0.045	0.944*	1.000

*P<0.05, **P<0.01

Table - 4: Correlation coefficient of antioxidant activities of Cissus quadrangularis

Properties	TP	DPPH	SOA	RP	TF	TBARS
TP	1.000					
DPPH	0.401	1.000				
SOA	-0.485	0.607	1.000			
RP	-0.949*	-0.669	0.184	1.000		
TF	0.077	0.944*	0.834	-0.388	1.000	
TBARS	-0.336	-0.998**	-0.660	0.616	-0.965**	1.000

*P<0.05, **P<0.01

Table - 5: Correlation coefficient of antioxidant activities of Coleus forskohlii

Properties	TP	DPPH	SOA	RP	TF	TBARS
TP	1.000					
DPPH	0.997**	1.000				
SOA	-0.459	-0.394	1.000			
RP	0.627	0.681	0.405	1.000		
TF	-0.999**	-0.999**	0.431	-0.651	1.000	
TBARS	-0.688	-0.634	0.960**	0.134	0.665	1.000

*P<0.05, **P<0.01