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Comparative Analysis of Phytochemicals and Antioxidant Activities of Genus Cocculus in Gujarat State, India

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Abstract:

Menispermaceae is an important angiosperm family that contains several medicinally important plants including the genus Cocculus. In Gujarat state of the Indian subcontinent, two species; Cocculus hirsutus (L.) Diels. and Cocculus pendulus (J. R. Forst. & G. Forst.) Diels are reported. This study presents a comparative report of these two species. While both species are immensely used by the tribal folk in various forms, they are also abundantly utilised in western medicine. Phytochemical analysis of the leaf extracts of both species report the presence of several important phytochemicals like alkaloids, flavonoids, saponins, tannins, carbohydrates, etc. On testing the Total Flavonoids Contents (TFC) of both the species, it was concluded that TFC was more in C. hirsutus (L.) leaf extracts as compared to C. pendulus (Forst) leaf extracts. The antioxidant activity in the leaf extracts of C. hirsutus (L.) and C. pendulus (Forst) were studied through the DPPH radical scavenging activity which was significantly more in C. hirsutus (L.) than C. pendulus (Forst). Though much analysis and research work has been reported for Cocculus hirsutus (L.), there are yet many studies to be done on the aspects of Cocculus pendulus (Forst).

Keywords: Cocculus hirsutus (L.), Cocculus pendulus (Forst), phytochemical analysis, total flavonoids content (TFC), antioxidant activity, DPPH

1. INTRODUCTION

Menispermaceae is an important angiosperm family containing several medicinally important plants. The genus Cocculus of the family Menispermaceae comprises about 35 species, out of which two species of Cocculus are found in Gujarat; Cocculus pendulus (Forst) Diels (synonym: Cocculus leaeba) and Cocculus hirsutus (L.) (synonym: Cocculus villosus). Cocculus is a perennial climber spread throughout the tropical and subtropical areas. In Asia, it has been reported from India, Myanmar, Nepal and Pakistan and southern China. It is also distributed in Central Arabia and in Africa. In India, it is found in Andhra Pradesh, Gujarat, Haryana, Jammu & Kashmir, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Tamil Nadu, Uttar Pradesh, and Rajasthan.

Both species of Cocculus are scandent twiners and similar in morphology except a few minor differences. Leaves are ovate to oblong, obtuse or subacute with a sharp short point, and subcordate at a base. C.

hirsutus (L.) has soft hairs on both sides of its leaves which are lacking in C. pendulus (Forst). Flowers are unisexual. Inflorescences cymose or thyrsoid, terminal, or axillary. Male flowers have 6-9 sepals, in 2-3 series, imbricate aestivation, the outer sepals are smaller and petals are 6 in number, normally bifid or emarginated apex, auricled below, stamens are usually 6-9, with free anthers, sub-globose and, cells bursting transversely. Female flowers are similar to male flowers in sepals and petals, carpels are 3-6 in number, and style is subulate, cylindrical and reflexed. Fruits are drupes, curved and obovoid in shape, slightly compressed from the lateral sides, and style scar is near the base, with a bony endocarp, and perforated on both sides. Seeds are curved, horse-shoe shaped, endocarp faintly tubercled, endospermic, and liguliform cotyledons.

2. MATERIALS AND METHODOLOGY

A. Collection

The sample plant of Cocculus hirsutus (L.) was collected from the Gujarat University campus, Navrangpura, Ahmedabad district of Gujarat State. The plant sample of Cocculus pendulus (Forst) had been collected from near Parpada Road, Palanpur, Banaskantha district of Gujarat State.

B Extraction

The extraction process of the plant samples was done through the Cold Extraction method. The dried crude extract was utilized to prepare the extract series of concentration 1mg plant extract/1 mL solvent for the experiment.

C Phytochemical Screening – Qualitative Analysis

The select plant samples were phytochemically screened to evaluate the presence of various plant metabolites like alkaloids, flavonoids, phenols, carbohydrates, reducing sugars, saponins, tannins, glycosides, and proteins. Solvents like aqueous, methanol and chloroform are tested by the standard protocols of Harborne, A. J. (1998).

D Quantitative Analysis

- Determining the Total Flavonoids Content

The Total Flavonoids Content (TFC) in the methanolic leaf extracts of C. hirsutus (L.) and C. pendulus (Forst) was determined by the aluminium chloride method using quercetin as standard by taking 30 mg of Quercetin in 30mL of methanol making it the concentration of 1mg/1mL of standard solution. The Total Flavonoids Content were evaluated by preparing triplicate series to minimise error. 100 µL 10% aluminium chloride was added to all the series followed by addition of 100 µL 1M potassium acetate. The absorbance is measured at a wavelength (λ) of 415 nm. The results were expressed as means (±SD) mg of Quercetin equivalents per gram (mg QE/g).

- Determining the Antioxidant Activity

The Antioxidant activity of C. hirsutus (L.) and C. pendulus (Forst) was tested by the DPPH radical scavenging method in methanolic and aqueous leaf extracts of both plants. Firstly, a 4 mg of DPPH (2,2

diphenyl-1-picrylhydrazyl) was measured and added to 100 mL methanol to make it 0.04mg/ 1 mL of DPPH solution. It was stored in a dark area as DPPH is light sensitive. Ascorbic acid had been taken as standard. A stock solution series was prepared with 0.5mg/1mL Ascorbic acid solutions (in distilled water and methanol) and the respective solvents (distilled water and methanol) with concentrations ranges from 25 µL to 200 µL. The experiment was carried out in triplicate series in order to minimise error. All the results are symbolised as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

By the testing of certain phytochemicals and antioxidant assay reveals a direct comparison between Cocculus hirsutus (L.) and Cocculus pendulus (Forst). The qualitative screening of both species reported significant results indicating presence of various phytochemicals.

The results of the phytochemical analysis of C. hirsutus (L.) resemble to that stated by Patil, S. A. (2014). On preliminary phytochemical analysis of the leaves of C. hirsutus (L.), no phenolic contents as well as proteins were detected whereas glycosides, carbohydrates, alkaloids, and flavonoids proved to be present. These results are similar to Madhavan, V., et al., (2010).

The results of the phytochemical analysis of C. pendulus (Forst) indicated presence of alkaloids, saponins, tannins, glycosides, flavonoids, carbohydrates, and reducing sugars. Rabari, H, et al., (2010) have mentioned the presence of alkaloids in the chloroform leaf extracts of C. pendulus (Forst).

The Total Flavonoids Content in C. hirsutus (L.) was 262.8936 ±16.160 QE/g of sample. The Total Flavonoids Content in C. pendulus (Forst) was 199.2727 ± 9.172 QE/g of sample.

In the DPPH Radical Scavenging Assay (RSA) for determining the Antioxidant Activity, the IC_{50} value of the standard, Ascorbic acid was 108.61. The IC_{50} value of the methanolic extract of C. hirsutus (L.) by DPPH Radical Scavenging Assay was 67.23. The IC_{50} value of the aqueous extract of C. hirsutus (L.) by DPPH Radical Scavenging Assay was 12.10. The IC_{50} value of the methanolic extract C. pendulus (Forst) by DPPH Radical Scavenging Assay was 376.39. The IC_{50} value of the aqueous extract of C. hirsutus (L.) by DPPH Radical Scavenging Assay was 342.54.

Table 1: Results of Qualitative Phytochemical Screening in C. hirsutus (L.) and C. pendulus (Forst)

Sr No	**Name of the Test**	**Cocculus hirsutus (L.)**			**Cocculus pendulus (Forst)**		
		Distilled Water	**Methanol**	**Chloroform**	**Distilled Water**	**Methanol**	**Chloroform**
Test for Alkaloids							
1.	Dragendroff's Test	+	+	+	+	+	+
2.	Hager's Test	-	-	-	-	-	-

3.	Wagner's Test	-	-	+	-	-	+
4.	Mayer's Test	-	+	+	-	+	+
Test for Flavonoids							
1.	Alkaline Reagent Test	+	-	-	+	-	-
2.	Ferric Chloride Test	-	+	+	-	+	+
Test for Proteins							
1.	Millon's Test	-	-	-	-	-	-
Test for Saponins							
1.	Foam/Frothing Test	-	-	+	-	-	+
Test for Carbohydrates							
1.	Barfoed's Test	-	-	-	-	-	-
2.	Molisch's Test	+	+	+	-	+	+
Test for Tannins							
1.	Sodium Hydroxide Test	-	+	+	-	+	+
Test for Phenols							
1.	Ferric Chloride Test	-	-	-	-	-	-
Test for Reducing Sugars							
1.	Benedict's Test	+	+	+	+	+	+
Test for Glycosides							
1.	Aqueous NaOH Test	-	+	-	-	+	-

(+) = presence of phytochemical (-) = absence of phytochemical

Table 2: Results for Total Flavonoids Content (TFC) for standard (Quercetin)

Sr. No.	Sample Conc. (µg / mL)	Solvent - methanol	Absorbance
1.	0	1000	0.1016
2.	200	800	0.3938
3.	400	600	0.7733
4.	600	400	0.991
5.	800	200	1.2211
6.	1000	0	1.7834

Table 3: Results for TFC for C. hirsutus (L.) and C. pendulus (Forst)

	C. hirsutus (L.) Extract (1 mL)			C. pendulus (Forst) Extract (1 mL)		
Sr no	Absorbance	QE/g of sample	Standard Deviation	Absorbance	QE/g of sample	Standard Deviation
1.	0.512	274.32	16.16	0.401	0205.75	9.17
2.	0.475	251.46		0.38	192.78	
Average	0.4935	262.89		0.3905	199.27	

Graph 1: Total Flavonoids Content: Standard Calibration Curve

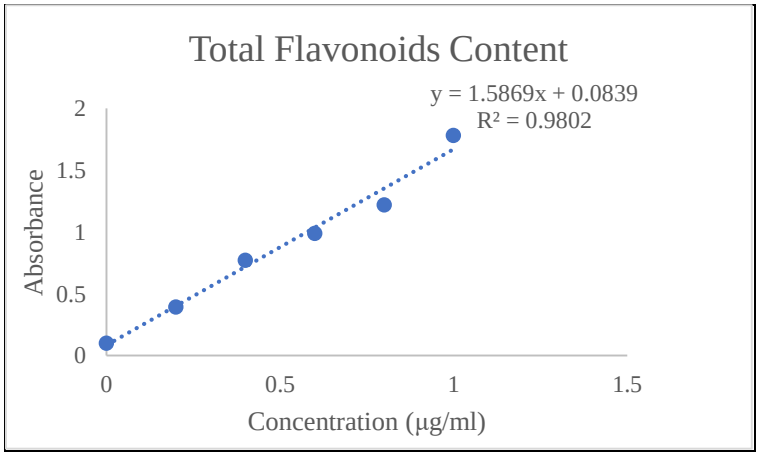


Table 4: Results for % inhibition by Standard (Ascorbic Acid) for Antioxidant Activity

Sr. No.	Concentration (µg/ml)	% RSA	IC50 value
1	25	28.055 ± 1.208	108.61
2	50	49.529 ± 0.577	

3	75	53.563 ± 8.276	
4	100	85.774 ± 0.210	
5	125	87.594 ± 0.105	

Table 5: Results for % inhibition by methanolic extracts of C. hirsutus (L.) and C. pendulus (Forst)

	Antioxidant Activity in C. hirsutus (L.) Methanolic Extract			**Antioxidant Activity in C. pendulus (Forst) Methanolic Extract**		
Sr. No.	**Concentration (µg/ml)**	**% RSA**	**IC50 value**	**Concentration (µg/ml)**	**% RSA**	**IC50 value**
1	25	73.94 ± 1.998	67.23	25	47.649 ± 1.377	376.39
2	50	76.25 ± 1.260		50	50.985 ± 0.820	
3	75	79.43 ± 3.580		75	53.260 ± 0.277	
4	100	81.64 ± 1.952		100	54.413 ± 0.090	
5	125	82.37 ± 0.998		125	59.265 ± 2.055	

Graph 2: Antioxidant Activity in Methanolic Extracts of C. hirsutus (L.) and C. pendulus (Forst)

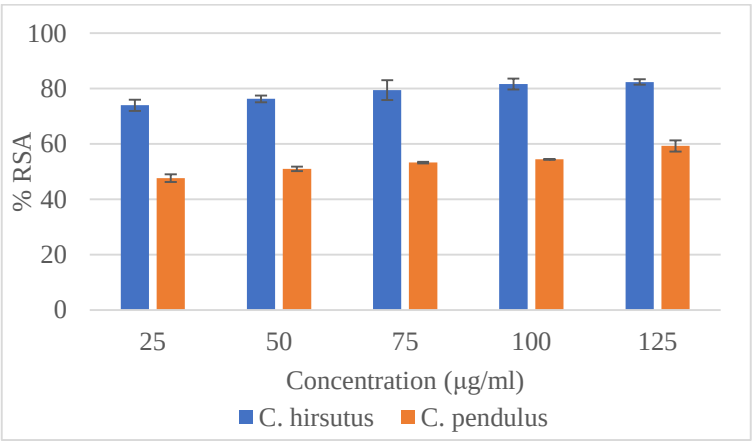
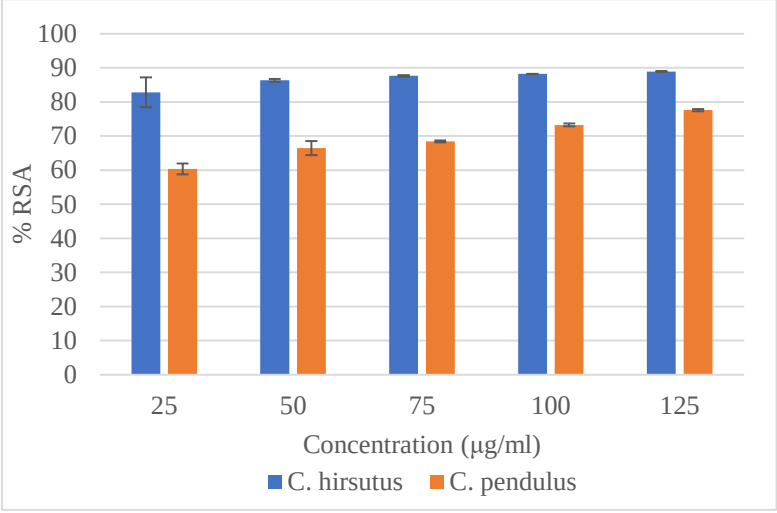


Table 6: Results for % inhibition by aqueous extracts of C. hirsutus (L.) and C. pendulus (Forst)

	Antioxidant Activity in C. hirsutus Aqueous Extract			Antioxidant Activity in C. pendulus Aqueous Extract		
Sr. No.	**Conc (µg/ml)**	**% RSA**	**IC50 value**	**Conc (µg/ml)**	**% RSA**	**IC50 value**
1	25	82.832 ± 4.323	12.10	25	60.357 ± 1.566	342.54
2	50	86.320 ± 0.410		50	66.424 ± 2.084	
3	75	87.655 ± 0.228		75	68.456 ± 0.277	
4	100	88.201 ± 0.052		100	73.218 ± 0.430	
5	125	88.929 ± 0.138		125	77.616 ± 0.328	

Graph 3: Antioxidant Activity in Aqueous Extracts of C. hirsutus (L.) and C. pendulus (Forst)



4. CONCLUSION

Cocculus hirsutus (L.) Diels and Cocculus pendulus (Forst) Diels are widely growing climbers. Both these species of Cocculus are much studied for their various phytochemicals and pharmacological activities. However, the field remains unexplored when it comes to comparative analysis of the two.

The species being rich in various phytochemicals, C. hirsutus (L.) and C. pendulus (Forst) are extensively used by the tribal folk. With a few minor changes morphologically and anatomically, it can subsequently

be concluded that both the species; Cocculus hirsutus (L.) and Cocculus pendulus (Forst) are ethnobotanically and medicinally important. Both species of Cocculus contain numerous phytochemicals and possess many medicinally important properties which can be efficiently utilised for the betterment of human health.

The comparative qualitative screening resulted the presence of alkaloids, flavonoids, saponins, carbohydrates, tannins, reducing sugars, and glycosides in C. hirsutus (L.) and C. pendulus (Forst). It can thus be concluded that the phytochemistry of both the species is the same.

When the Total Flavonoids Content was assessed, C. hirsutus (L.) (262.8936 ±16.160 QE/g of sample) reported to be higher in flavonoids contents as compared to C. pendulus (Forst) (199.2727 ± 9.172 QE/g of sample).

In the case of Antioxidant Activity by DPPH Radical Scavenging method, the aqueous extract of C. hirsutus (L.) had a lower IC50 value (12.10) proving to have a higher antioxidant activity than the methanolic extract of C. hirsutus (L.) (IC50 value = 67.23). Similarly, in the case of C. pendulus (Forst), the aqueous extract of C. pendulus (Forst) reported a lower IC50 value (342.54) proving to have a higher antioxidant activity than the methanolic extract of C. pendulus (Forst) (IC50 value = 376.39). The most antioxidant activity with a lower IC50 value was reported in C. hirsutus (L.) which was significantly more than that in C. pendulus (Forst).

This study concludes that although C. hirsutus (L.) and C. pendulus (Forst) both have similar set of phytochemicals, the quantitative analysis of both species gave significant results. C. hirsutus (L.) proved to have more Total Flavonoids Content as well as more Antioxidant Activity as compared to C. pendulus (Forst). Though much analysis and research work has been reported for Cocculus hirsutus (L.), there are yet many studies to be done on the aspects of Cocculus pendulus (Forst).

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