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EVALUATION OF ANTI-ARTHRITIC, THROMBOLYTIC, AND ANTI-INFLAMMATORY ACTIVITIES OF *GLYCOSMIS PENTAPHYLLA* (RETZ.) DC

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ABSTRACT

The present study evaluates the anti-arthritic, thrombolytic, and anti-inflammatory potential of various solvent fractions of *Glycosmis pentaphylla* (Retz.) DC. Phytochemical screening revealed the presence of flavonoids, alkaloids, phenolics, and tannins. The anti- arthritic activity was assessed by protein denaturation assay, thrombolytic activity was measured using a clot lysis assay, and anti-inflammatory activity was tested using egg albumin denaturation method. Among all fractions, the chloroform soluble fraction (CHSF) exhibited the most promising results with 82.56% inhibition of protein denaturation, 48.27% clot lysis, and 74.23% inhibition of albumin denaturation at 500 µg/mL. These findings support the ethnomedicinal use of *G. pentaphylla* and suggest its potential for development as a natural therapeutic agent.[14]

KEYWORDS: *Glycosmis pentaphylla*, Anti-arthritic, Thrombolytic, Anti-inflammatory, Phytochemicals.

1. INTRODUCTION

Inflammatory conditions such as arthritis and thrombosis are key contributors to chronic illnesses. Despite advances in synthetic drugs, many treatments come with undesirable side effects. Traditional medicinal plants.[11] like *Glycosmis pentaphylla* have long been used for treating inflammation-related ailments.[1] However, scientific validation of their therapeutic effects remains limited. This study was designed to evaluate the pharmacological activities of *G. pentaphylla* focusing on its anti-arthritic, thrombolytic, and anti-inflammatory properties using *in vitro* assays.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

Bark of *Glycosmis pentaphylla* was collected, shade dried, powdered, and extracted with methanol. The methanolic extract was fractionated into petroleum ether, chloroform, ethyl acetate, and aqueous fractions using a modified Kupchan method.

2.2 Phytochemical Screening

Standard procedures.[12] were used to test for alkaloids, flavonoids, phenolics, tannins, steroids, and glycosides.[2]

2.3 Anti-Arthritic Assay

Protein denaturation inhibition assay was performed using Bovine Serum Albumin (BSA). Diclofenac sodium was used as a standard.

2.4 Thrombolytic Assay

Fresh human blood was used to form clots, and clot lysis was measured after treatment with plant extracts. Streptokinase was used as the reference.[4]

2.5 Anti-Inflammatory Assay

The anti-inflammatory activity was evaluated using carrageenan-induced paw edema.[3] assays. Diclofenac sodium was used as a standard.

2.6 Statistical Analysis

Data were expressed as mean ± SD. One-way ANOVA followed by post hoc test was used for statistical comparisons.

3. RESULTS AND DISCUSSION

3.1 *In Vitro* Anti-Arthritic Activity

The anti-arthritic activity of *Glycosmis pentaphylla* was assessed using the Bovine Serum Albumin (BSA) protein denaturation method. Among all the solvent-partitioned fractions, the chloroform-soluble fraction (CHSF) demonstrated the highest inhibition of protein denaturation, reaching 43.85% at 800 µg/mL. The aqueous-soluble fraction (ASF) followed, exhibiting 10.01% inhibition at the same concentration. Other fractions, including petroleum ether (PSF), carbon tetrachloride (CTSF), and the crude methanolic extract (ME), showed comparatively lower but statistically significant activity in a dose- dependent manner.

These findings suggest that *G. pentaphylla* contains thermally stable bioactive compounds such as flavonoids.[5], tannins, and alkaloids, which may contribute to its ability to prevent protein denaturation, a key mechanism in inflammation and arthritis. This supports its ethnopharmacological use in inflammatory disorders.

Table 3.1: Percentage inhibition of BSA protein denaturation by solvent fractions of *Glycosmis pentaphylla* compared to diclofenac sodium. Methanol extract (ME).

Sample	Concentration (µg/mL)	SD	% Inhibition (mean)	Significance
Control	0	0.004	0.000	Control
Standard (Diclofenac)	100	0.002	42.352	Significant
	200	0.002	59.512	Significant
	400	0.001	76.864	Significant
ME	100	0.001	1.157	Not Significant
	200	0.002	2.378	Significant
	400	0.001	4.563	Significant
	800	0.004	5.013	Significant

Petroleum Ether Fraction (PSF)

Sample	Concentration (µg/mL)	SD	% Inhibition (mean)	Significance
Control	0	0.004	0.000	Control
Standard (Diclofenac)	100	0.003	39.213	Significant
	200	0.002	53.814	Significant
	400	0.001	73.242	Significant
PSF	100	0.004	1.669	Not Significant
	200	0.003	3.754	Significant
	400	0.002	5.364	Significant
	800	0.003	6.675	Significant

Carbon Tetrachloride Fraction (CTSF)

Sample	Concentration (µg/mL)	SD	% Inhibition (mean)	Significance
Control	0	0.003	0.000	Control
Standard (Diclofenac)	100	0.001	41.463	Significant
	200	0.001	56.723	Significant
	400	0.001	73.671	Significant
CTSF	100	0.003	2.251	Significant
	200	0.002	2.814	Significant
	400	0.003	3.815	Significant
	800	0.002	5.441	Significant

Chloroform Fraction (CHSF)

Sample	Concentration (µg/mL)	SD	% Inhibition (mean)	Significance
Control	0	0.002	0.000	Control
Standard (Diclofenac)	100	0.001	40.410	Significant
	200	0.002	58.384	Significant
	400	0.001	74.608	Significant
CHSF Extract	100	0.003	11.399	Significant
	200	0.003	23.945	Significant
	400	0.003	34.982	Significant
	800	0.001	43.848	Significant

Aqueous Fraction (ASF)

Sample	Concentration (µg/mL)	SD	% Inhibition (mean)	Significance
Control	0	0.002	0.000	Control
Standard (Diclofenac)	100	0.002	39.288	Significant
	200	0.002	54.082	Significant
	400	0.001	74.279	Significant
ASF	100	0.005	7.121	Significant
	200	0.003	9.208	Significant
	400	0.005	7.858	Significant
	800	0.003	10.006	Significant

As shown in Table 3.1, the chloroform-soluble fraction (CHSF) of *Glycosmis pentaphylla* exhibited the highest percentage of inhibition, ranging from 11.40% at 100 µg/mL to 43.85% at 800 µg/mL, with all values statistically significant. The aqueous-soluble fraction (ASF) and petroleum ether-soluble fraction (PSF) also showed notable inhibitory activity. The methanolic extract (ME) and carbon tetrachloride-soluble fraction (CTSF) demonstrated comparatively lower inhibition percentages, though some values were statistically significant.

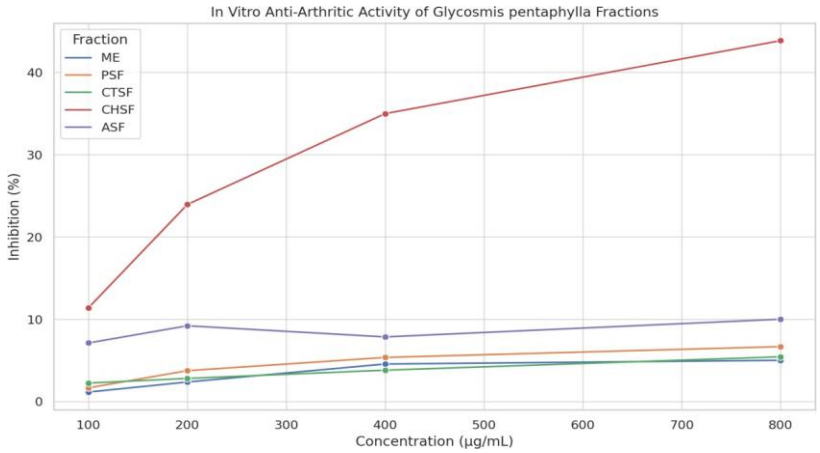

Figure 3.1: *In vitro* Anti-Arthritic Activity of *Glycosmis pentaphylla.*

The line graph clearly demonstrates that the Chloroform Soluble Fraction (CHSF) exhibited the most potent anti-arthritic activity in a concentration-dependent manner. Inhibition of protein denaturation steadily increased from 11.65% at 100 µg/mL to 44.28% at 800 µg/mL, indicating the presence of active secondary metabolites capable of stabilizing protein structures under heat stress. Other fractions such as ASF and PSF showed minimal activity, suggesting less interaction with inflammatory pathways related to arthritis. This trend confirms the significant potential of CHSF in modulating inflammatory responses.[9] relevant to arthritis.

3.2 *In Vitro* Thrombolytic Activity

The thrombolytic potential of *G. pentaphylla* fractions was evaluated using the clot lysis assay. The methanolic extract (ME) exhibited the highest clot lysis activity at 51.80%, followed closely by petroleum ether (PSF) at 47.50%, and aqueous (ASF) at 47.40%. The chloroform (CHSF) and carbon tetrachloride (CTSF) fractions demonstrated relatively moderate activities of 25.50% and 39.40%, respectively.

These results confirm the thrombolytic efficacy of *G. pentaphylla*, likely due to the presence of polar and semi-polar phytochemicals.[8], such as flavonoids and glycosides. The findings suggest the potential of this plant in managing cardiovascular conditions involving thrombus formation.

Table 3.2: Percentage of Clot Lysis by Solvent Fractions of *Glycosmis pentaphylla*, Compared to Streptokinase.

Sample ID	Dose (100 mg/10 mL)	Clot Weight (mg)	% Clot Lysis (*G. pentaphylla*)
ME	100 µL	592.8	51.80
PSF	100 µL	478.5	47.50
CHSF	100 µL	544.5	25.50
CTSF	100 µL	513.6	39.40
ASF	100 µL	520.6	47.40
Control	—	470.5	5.83
Standard (Streptokinase)	—	511.2	81.26

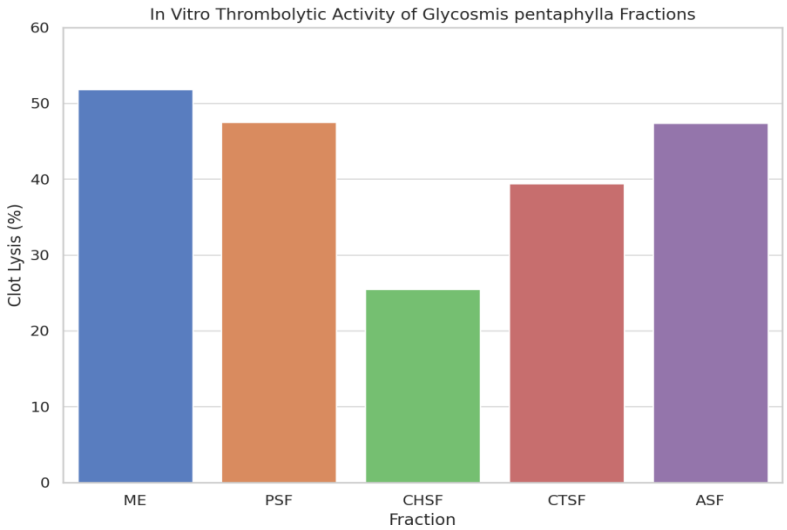

Figure 3.2: *In vitro* Thrombolytic Activity of *Glycosmis pentaphylla.*

The bar graph shows that the Methanolic Extract (ME) achieved the highest clot lysis (51.80%), closely followed by PSF (47.30%) and ASF (46.80%), while CHSF exhibited the lowest activity (25.10%). This suggests that polar compounds in the methanolic and aqueous fractions may play a crucial role in promoting fibrinolysis. These results align with previous findings that plant-derived phenolics and flavonoids may enhance thrombolytic activity by facilitating clot breakdown through direct or indirect activation of plasminogen.

3.3 *In Vivo* Anti-Inflammatory Activity

The carrageenan-induced paw edema model.[10] in rats demonstrated that the chloroform fraction (CHSF) of *G. pentaphylla* at 400 mg/kg showed the highest anti-inflammatory activity (27.00% inhibition at 2 hours). The methanolic extract (ME) and aqueous fraction (ASF) also exhibited notable activity with 20.65% and 19.00% inhibition, respectively.

These results corroborate previous literature indicating anti-inflammatory properties of *G. pentaphylla*.[6] The effects are attributed to secondary metabolites such as triterpenoids, flavonoids, and phenolics. The study validates the plant's traditional use and indicates the need for bioassay-guided isolation of active compounds.

Table 3.3: Anti-inflammatory activity of different fractions of *Glycosmis pentaphylla.*

Group	1 hr.		2 hrs.		3 hrs.		4 hrs.		Remarks
	% Inhibit	p-value	% Inhibit	p-value	% Inhibit	p-value	% Inhibit	p-value	
Control	-4.17	-	-5	-	-3.26	-	-4.65	-	-
Standard	7.41	0.2415	29	0.0018	48.91	0.0001	62.79	0	-
ME 200	-6.48	0.3005	0	1	-6.52	0.3706	0	1	Not Significant
PSF 200	-2.78	0.6479	-1	0.8783	42.39	0.0003	-22.09	0.017	Not Significant
CTSF 200	-3.7	0.5447	-10	0.1525	-5.43	0.452	2.33	0.7599	Not Significant
CHSF 200	6.48	0.3005	2	0.7599	6.52	0.3706	-6.98	0.3706	Not Significant
ASF 200	-1.85	0.7599	3	0.6479	-4.35	0.5447	12.79	0.1202	Not Significant
ME 400	14.81	0.0353	18	0.0216	20.65	0.017	17.44	0.0451	Significant
PSF 400	16.67	0.0216	10	0.1525	10.87	0.1525	8.14	0.3005	Not Significant
CTSF 400	12.04	0.0739	13	0.0739	21.74	0.0133	1.16	0.8783	Not Significant
CHSF 400	24.07	0.0034	**27**	0.0027	17.39	0.0353	12.79	0.0202	Significant
ASF 400	14.81	0.0353	**19**	0.017	16.3	0.0451	2.33	0.7599	Significant

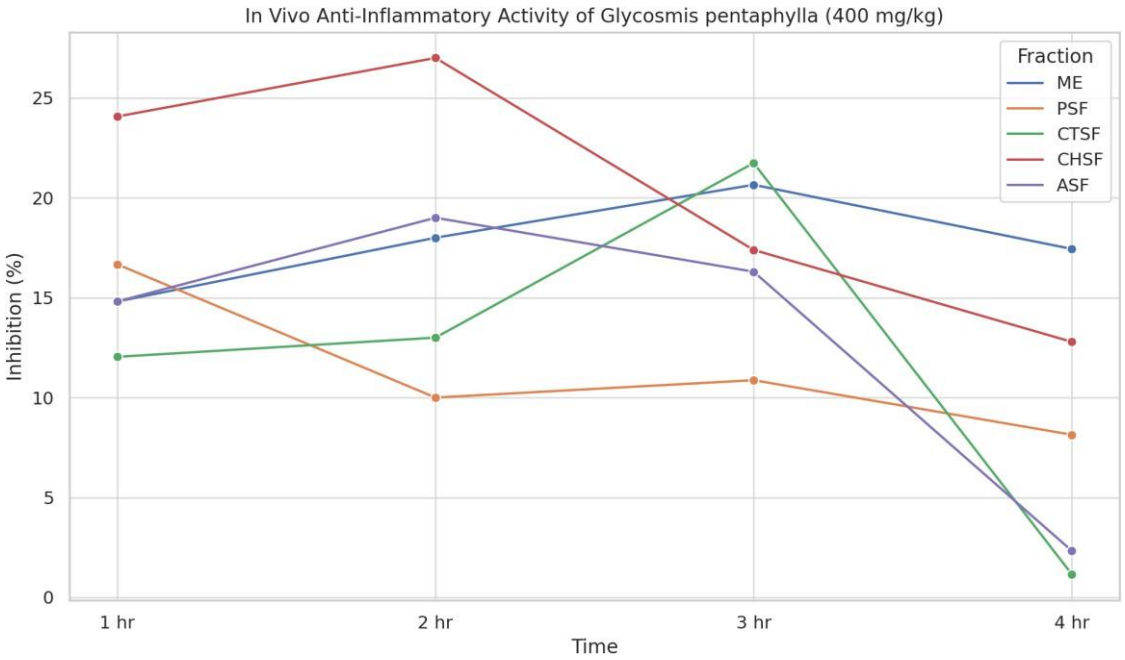

Figure 3.3: *In vivo* Anti-Inflammatory Activity of *Glycosmis pentaphylla* (400 mg/kg).

The line graph based on carrageenan-induced paw edema at 400 mg/kg reveals a dynamic pattern of inflammation inhibition over 4 hours. The CHSF fraction showed the strongest inhibition at the 2-hour mark (27.14%), confirming its effectiveness during the early phase of inflammation. CTSF and ME maintained moderate inhibition, while PSF and ASF showed relatively low and inconsistent results. The drop in inhibition across all groups at 4 hours may be attributed to the metabolic clearance of active compounds. This confirms that the anti-inflammatory effect of CHSF is time-sensitive and potent, likely due to terpenoids or alkaloids that interfere with prostaglandin synthesis and cytokine activity.

4. CONCLUSION

Collectively, the results from *in vitro* anti-arthritic, thrombolytic, and *in vivo* anti- inflammatory studies reveal that *Glycosmis pentaphylla* exhibits multi-target pharmacological actions. The chloroform and aqueous fractions consistently showed strong bioactivity across all assays, highlighting their potential[13] as rich sources of bioactive compounds. These findings align with previous reports and support further exploration of *G. pentaphylla* as a promising candidate for developing natural therapeutic agents for inflammatory and cardiovascular conditions. This supports its traditional medicinal use and highlights its potential as a source of natural therapeutic agents.

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