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Evaluation of anti-inflammatory activity of leaf extract fractions of *Alangium salvifolium*

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Abstract

The objective of this study was to evaluate the anti-inflammatory activity of active fractions of *Alangium salvifolium* leaf extract.From results, it has been inferred that the methanol and aqueous extracts of *Alangium salvifolium* contain the maximum number of phytoconstituents like alkaloids, glycosides, carbohydrates, flavonoids, tannins and polyphenol. While the alkaloids were absent in aqueous extracts of *Alangium salvifolium*. Based on the qualitative chemical test, it has been observed that chemically therapeutic compounds were present in sufficient amounts in methanolic and aqueous extracts of *Alangium salvifolium*. By the help of column chromatography the bioactive fractions were isolated which might be responsible for the therapeutic activity. The eight fractions (F1 to F8) were isolated from the methanol extract of *Alangium salvifolium*. The F6 and F7 indicate the presence of phenolic compounds and flavonoids. It is scientifically documented that flavonoid molecules possess anti-inflammatory activity on various animal models of inflammation. The isolated compound namely F6 and F7 exhibited anti-inflammatory effect on carrageenan-induced paw oedema. The F6 isolated from *Alangium salvifolium* produces maximum anti-inflammatory effect compared to F7. This study confirmed that flavonoid fraction obtained from *Alangium salvifolium* leaves extract are responsible for its anti inflammatory activity and the effects observed are attributable due to the presence of flavonoids in the plant.

Keywords: *Alangium salvifolium*, anti-inflammatory, extract, column chromatography, flavonoids

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Introduction

Plants are natural and traditional sources of medication in large parts of the world. A wide variety of herbs singly and in mixture have been extensively investigated in basic biological sciences to evaluate their chief as well as supplementary, complementary and synergistic action in health and diseases (Rajeshwari & Andallu, 2012).

Inflammation is a very complex response that occurs as a result of an injury, infection or another stimulus, in which several cell types and secreted factors elicits protective immunity, tissue repair and resolution of tissue damage (Howcroft et al., 2013).

Alangium salvifolium is a deciduous, rambling shrub or tree, belongs to Family Alangiaceae and has a documented medicinal activities (Chopra et al., 1956). The plant was also reported for its anti fungal activity, anti microbial activity, cardiac activity and anti fertility activity (Shetty, 2003; Xavier et al., 2005; Jain et al., 2010; Kumar et al., 2010).

The anti-inflammatory and anti- arthritis activity of *Alangium salvifolium* leaves extract were scientifically reported. However, no report has yet been published about the active component(s) responsible for anti-inflammatory activity of *Alangium salvifolium* leaves extract. The main aim of this study was to explore the anti-inflammatory activity of active fraction of *Alangium salvifolium* leaf extract.

Material and Methods

All chemicals used were of analytical grade. Chemicals and reagents used for the preparation of buffers, analytical solutions were used as obtained.

Collection and preparation of plant material

The leaves of *Alangium salvifolium* were collected for region of Gwalior, Madhya Pradesh. The leaves were shade dried and ground to coarse powder for extraction.

Preparation of extracts (Harborne, 2005; Kokate et al., 2000)

500 gram of powdered of leaves of *Alangium salvifolium* was packed in soxhlet apparatus and extracted with different polarity of solvent. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The petroleum extracts were stored in refrigerator for further experimental work.

The extracts were weighed and their percentage value was recorded and also the physical appearance and color was evaluated and recorded and thereafter, were stored in refrigerator for further experimental work.

Qualitative chemical tests (Kandelwal et al., 1996)

Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids.

Fractionation of active crude extract with different solvents

Packing of chromatographic column

Wet packing method was adopted for packing the column. Slurry of activated silicagel (neutral) was prepared in solvent system and was poured into the column with the help of a hollow glass cylinder. The column was previously filled with solvent system. While pouring the slurry the column was continuously tapped with a rubber cork so that a compact column was formed devoid of any air bubble. The solvent was eluted thereafter at a steady rate till solvent head remained about 2-3 cm above the column.

Preparation of sample

About 10 gm of extract was mixed with 50 gm of silica gel for CC (60-120 mesh) & few quantity of an appropriate solvent was mixed. This mixture was triturated in a mortar till a homogenous & dry free flowing mixture was obtained.

Application of sample

The mixture as prepared above is fed very slowly into the column with the help of a hollow glass cylinder without disturbing the silica bed. Thereafter appropriate solvent system was poured into the column for elucidation of components.

Collection of eluting sample

Elute was collected at the rate of 20 drops per minute & each fraction was of about 100 ml. Each fraction was subjected to TLC on silica gel G. The fractions with same R_f were pooled together & concentrated to obtain pure compounds or a mixture of 2-3 compounds (Skoog et al., 2004).

Isolation of Compounds from Alangium salvifolium leaves extract

The *Alangium salvifolium* extract was subjected to column chromatography using silica gel (60-120 mesh size), and eluted with the following solvent ratios of Hexane: dichloromethane (DCM), 100:0, 75:25, 50:50, 25:75, 0:100, then with 75:25, 50:50, 25:75, 0:100, DCM:Ethanol (EtOH). Finally eluted with 75:25, 50:50, 25:75, 0:100, EtOH:Methanol (MeOH). The fractions (25 ml) were collected from the column. The elute collected were monitored by thin layer chromatography (eluent: DCM-MeOH, 9:1 and 3:2) for homogeneity and the similar fraction were pooled together. The eight different fractions were collected and dried. The fraction F1, F2 and F3 were containing waxy material; the fractions F4 and F8 were powder but quantity was very little. The yield of fraction F5, F6 and F7 were 315 mg, 440 mg and 365 mg, respectively. The three

fractions were further analyzed for phytochemical screening to determine the nature of isolated compound.

Pharmacological activity

Selection of animals

Male Wistar rats (150-200 gm), were used, and kept in qurantine for 10 days under standard husbandry conditions (27.3 ^{o}C, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and were given standard food and water *ad libitum*. All experiments were approved by the institutional ethical committee and were carried out according to the animal ethics committee guidelines.

Effect of isolated fraction of Alangium salvifolium on Carrageenan- induced oedema

Albino Wistar rats of either sex weighing between (150-200 gm) were divided into various groups and six animals in each group. The groups were as follows:

Group I (control group) – Treated with distilled water

Group II – Treated with standard drug Aceclofenac at 10 mg/kg body weight

Group III – Treated with F6 at 50 mg/kg body weight

Group IV – Treated with F7 at 50 mg/kg body weight

Acute inflammation was produced by injecting 0.1ml of 1% carrageenan suspension in normal saline into the subplantar region of right hind paw after 60 minutes of drug administration. The control group was administered only distilled water. The isolated compound and standard drugs administered intraperitoneally 1 h before carrageenan suspension administration.

A mark was made on the leg at the malleous to facilitate uniform dipping at subsequent readings. The volume of paw oedema volume was measured with the help of plethysmograph by mercury displacement method immediately before and five hours after the drug administration (Oyanagui, 1984). The inhibition of oedema in various treated groups was then calculated by using statistical analysis.

Results and Discussion

Extraction of plant material

The yield of petroleum extracts of leaves of *Alangium salvifolium* was 1.7%. The yield of methanol and aqueous extracts of leaves of *Alangium salvifolium* were 19.6% and 24.1%, respectively.

Phytochemical screening

Preliminary phytochemical investigations of the extracts of leaves of *Alangium salvifolium* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates (Table 1).

Table 1 Phytochemicals present in leaves of *Alangium salvifolium* extracts

Phytoconstituent	Pet Ether	Methanol	Aqueous
Alkaloids	-	+	-
Glycosides	-	+	+
Carbohydrates	-	+	+
Tannins and Phenolic compound	-	+	+
Flavonoids	-	+	+
Steroid test	-	-	-
Protein	-	+	-
Fat and oil test	+	-	-

+ = Present, - = Absent

Isolation of compound from methanol extracts

The methanol extract of *Alangium salvifolium* leaf was subjected to column chromatography using silica gel (60-120 mesh size), and eluted with the following solvent ratios of Hexane: dichloromethane (DCM), 100:0, 75:25, 50:50, 25:75, 0:100, then with 75:25, 50:50, 25:75, 0:100, DCM:Ethanol and finally eluted with 75:25, 50:50, 25:75, 0:100, Ethanol:Methanol. The fractions (25 ml) were collected from the column. The elute collected were monitored by thin layer chromatography (eluent: DCM-MeOH, 9:1 and 3:2) for homogeneity and the similar fraction were pooled together. The eight different fractions were collected and dried. The fraction F1, F2 and F3 were containing waxy material; the fractions F4 and F8 were powder but quantity was very little. The yield of fraction F5, F6 and F7 were 315 mg, 440 mg and 365 mg, respectively. The three fractions were further analyzed for phytochemical screening to determine the nature of isolated compound. The three fractions were further analyzed for phytochemical screening to determine the nature of isolated compound.

The phytochemical investigation of F5 of *Alangium salvifolium* leaves revealed the presence of alkaloids, glycosides and carbohydrates. The F6 and F7 indicate the presence of glycoside, flavonoids, tannins & phenolic compound.

Anti-inflammatory studies

The effect of the F6 and F7 isolated from *Alangium salvifolium* on carrageenan- induced paw oedema is presented in (Table 2 & Fig 1).

The animals administered only distilled water, the subplantar injection of carrageenan produced a local oedema that increased progressively from 0.31 ml after the first hour to reach a maximum within 4 h. The administration of fraction F6 and F7 (50 mg/kg) revealed significant ($P<0.05$) reduction in oedema in the rats compared with the same time of the distilled water treated group. Aceclofenac (10 mg/kg) produced a significant ($P<0.05$) decrease in oedema at the 2 hour compared with the same time of the distilled water treated group.

The effect of the isolated compound in this model may be attributed to the inhibition of the release of pro-inflammatory mediators of acute inflammation, especially prostaglandins.

Table 2 Effect of isolated fraction from *Alangium salvifolium* leaves extract on carrageenan induced paw oedema

Group	Paw volume after induction				
	1 hr	2 hr	3 hr	4 hr	5 hr
Control	0.32 ± 0.008	0.73 ± 0.018	0.94 ± 0.010	1.22 ± 0.012	1.06 ± 0.017
Aceclofenac (10 mg/kg)	0.26 ± 0.024	0.43 ± 0.023*	0.35 ± 0.014*	0.27 ± 0.016*	0.23 ± 0.008*
F6 (50 mg/kg)	0.29 ± 0.010	0.57 ± 0.010	0.64 ± 0.008*	0.52 ± 0.012*	0.43 ± 0.008*
F7 (50 mg/kg)	0.28 ± 0.008	0.64 ± 0.010	0.71 ± 0.025*	0.54 ± 0.020*	0.47 ± 0.018*

Values are expressed as mean ± SD, n = 6 in each group. *P<0.05 compared to control group

Conclusion

Alangium salvifolium are rich in secondary metabolite such as alkaloid, glycoside, flavonoids, polyphenol etc. In the present study an attempt was made to isolate the various active constituent and evaluate their anti-inflammatory and anti-arthritis activity. It was concluded that the F6 and F7 isolated from *Alangium salvifolium* leaves extract exhibited moderate to highly anti-inflammatory activity. It suggest that the anti-inflammatory activity of F6 and F7 due to presence of polyphenol and flavonoids. This scientific study revealed the efficacy of the isolated compound and it would definitely have wide scope in future.

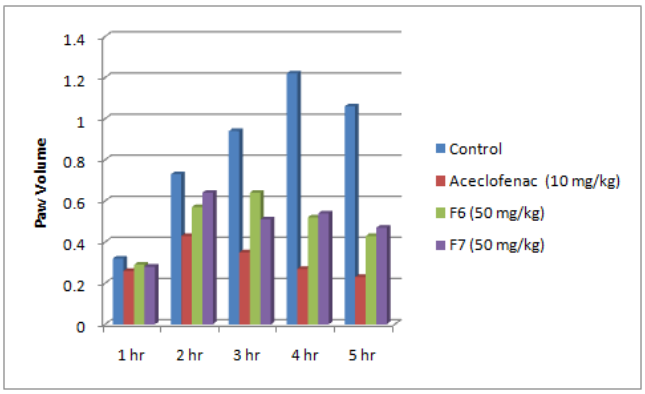

Figure 1 Effect of isolated fraction from *Alangium salvifolium* leaves extract on carrageenan induced paw oedema

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