Research Article

Exploring the phytochemical properties and Anti-inflammatory studies on the aerial parts of *Rosmarinus officinalis.L*

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Abstract

The inflammatory response is the body's reaction to a harmful stimulus. The inflammatory response of the host is essential for blocking and treating the infectious process, but it is also frequently responsible for illness signs and symptoms. The current study investigates the anti-inflammatory potential of the aerial portions of *Rosmarinus officinalis*. The crude extracts were also subjected to a fluorescence analysis. A plethysmograph was used to test the anti-inflammatory activity of carrageenan induced rat hind paw in vivo. Wistar strain rats weighing 150-200gm were placed into four groups of four animals each. The first group acted as the control and got distilled water, while the second group of animals received the conventional medicine Diclofenac sodium at a dose of 20mg/kg body weight (orally). The third and fourth animal groups were given Ethanolic extract of Rosmarinus officinalis l orally at doses of 200 and 400 mg/kg body weight, respectively. The volume of paw oedema was assessed in the control, standard, and treatment groups one, two, three, and four hours after Carrageenan injection. The percentage of oedema inhibition was determined. The current study covers preliminary phytochemical and anti-inflammatory investigations on Rosmarinus officinalis L aerial parts.

Keywords: Oedema, Carrageenan, Anti-inflammatory, Inflammation

1. Introduction

Rosmarinus officinalis is an aromatic, perennial evergreen shrub in the mint family(Labiatae) native to the hills of the Mediterranean, Portugal, and northwestern Spain. It grows to be a tall shrub that really can reach heights of 4 to 6 feet. *Rosmarinus officinalis L* and its taxonomicparts have multivarious medicinal uses. The leaves and flowers are acrid, thermogenic

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and digestive, anthelmintic, and anti-inflammatory, emmenagogue, sudorific, antipyretic, Expectorant, anti-bacterial and depurative, they are useful in colic, dyspepsia, Verminbsis, arthralgia, chronic skin eruptions, psoriasis, cough and catarrh in children, amenorrhea, dysmenorrhea, intermittent fevers and ulcers. In view of its pharmacological and biological properties we decided to study the preliminary phytochemical screening of aerial parts of *Rosmarinus officinalis L* which is widely used in indigenous medicine

2. Material and methods

2.1 Phytochemical investigation of the Aerial part of *Rosmarinus officinalis*

The phytochemical investigation on the aerial parts of plant involves the following extraction of plant material, Preliminary phytochemical studies, Identification of constituents by TLC fluorescence analysis of powder and behavior of powdered drug with chemical reagents

2.2 Extraction of plant material

The plant material was collected in the month of September. The leaves stem, flowers of the plant were dried in the shade . Then the shade-dried material was subjected to successive solvent extraction process by hot continuous percolation method using Soxhlet apparatus. The solvent were used in increasing polarity, such as Hexane, chloroform, andEthanol.

2.3 Preparation of Extracts

The coarse powder was extracted with Hexane by hot continuous percolation method using soxhlet apparatus. After completion of extraction, it was filtered, and solvent wasremoved by distillation under reduced pressure. The extract was stored in a desiccator. A yellowish brown colour residue was obtained.

The marc left after Hexane was dried and extract with chloroform by hot continuous percolation method using soxhlet apparatus. After completion of extraction, it was filtered, and solvent was removed by distillation under reduced pressure. The extract was stored in a dessicator. A thick greenish coloured residue was obtained.

The marc left after chloroform extraction was dried and extracted with Ethanol by hot continuous percolation method using soxhlet apparatus. After completion of extraction, it was filtered, and solvent was removed by distillation under reduced pressure. The extract was stored in a dessicator. A brown-coloured residue was obtained.

2.4 Identification of plant constituents by phytochemical test

The various extract of the plant of *Rosmarinus officinalis* was subjected to chemical testsfor identification of its active constituents.

The Hexane, chloroform, and Ethanol extracts were subjected to thin layer chromatography in different solvent system. The TLC plates were made with silica gel "G" and activated. The extracts were spotted by means of the micropipette and dried, developed in solvent system I & II separately

Table 1: Data Showing the Extractive Values of Aerial Parts of *Rosmarinus Officinalis*

Plant Name	Part used	Method of Extraction	Hexane	Chloroform	Ethanol
Rosmarinus officinalis	Aerialparts	Hot continuous Percolation	7. 25%	6.56%	12.36%

Table 2: Data showing the preliminary phytochemical screening of various extracts of *rosmarinus officinalis*

Extracts	Alkaloids	Carbohydrates	Phytosterols	Phenols	Protein	Saponins	Gums	Fats	Flavonoids
Hexane	-	-	+	-	-	-	-	-	+
Chloroform	+	+	-	+	+	-	-	-	+
Ethanol	+	+	-	+	+	-	-	-	+

2.5 DETECTION OF COMPOUNDS

The compounds were detected by placing in Iodine chamber and the colour intensity of the compounds observed under UV light or sprayed with Vanillin in sulphuric acid. The Rf values were calculated

2.5.1 IDENTIFICATION OF PLANT CONSTITUTENTS BY CHROMATOGRAPHIC METHOD (TLC)[23−24]

Of the various methods of separating and isolating plant constituents the chromatographic procedure originated by Tswett is one of the most useful technique for general application. All finely divided solids have the power to adsorb other substances on their surface to a greater or lesser extent; similarly, all substances are capable of being adsorbed. Some much more readily than others this phenomenon of selective adsorption is the fundamental principle of chromatography. In this work the chromatographic methods used are Thin Layer Chromatography (TLC).

The extract was taken in a capillary tube and it was spotted on a TLC plate, 2 cm above its bottom. Most solution for application were between 0.1 to 1% strength. The starting points equally sized as far as possible and had a diameter ranging from 2 to 5cm.

2.5.2 FLUORESCENE ANALYSIS[25−26]

Fluorescence analysis was carried out for the crude powder and extract separately using acid and alkali medium. The colour intensity of the powder and extract were observed in day light and UV light in UV chamber. The powdered drug also treated with various chemical reagents in separate glass dish and the change of colour was noted and tabulated

2.6 Invivo anti-inflammatory activity of ethanolic extract of *rosmarinus officinalis* in wistar rats

Anti-inflammatory activity was studied by Carrageenan induced rat hind paw oedema, measured by Plethysmograph (Mercury displacement method) Wistar strain rats of either sex between 150-200gm were divided into 4 groups of 4 animals each. The first group served as the control and received the distilled water, second group of animals were administered with standard drug Diclofenac sodium, 20mg/kg body weight(orally). The third and fourth groups of animals were treated with Ethanolic extract of *Rosmarinus officinalis l* at a dose of 200 and 400 mg/kg body weight orally.

The volume paw oedema was measured in control, standard and treated groups accordingly 1, 2, 3 and 4 hours after Carrageenan injection. The percentage inhibition of oedema was calculated. The data was analyzed by one way ANOVA followed by Dunnett's t- test.

According to this test there was a significant difference between the drug treated groups and control at the level of $P < 0.001$ and $P < 0.01$.

3. Results and discussion

The chloroform and methanolic extracts were subjected to TLC and the solvent ratio is mentioned in (Table: 3)

Table 3: Solvent system used for TLC and their ratio

SOLVENT COMPOSITION	RATIO	CODE
TOLUENE:ETHYL ACETATE:DIETHYLAMINE	7:2:1	A
TOLUENE:ETHYLACETATE	93:7	B

Thin layer chromatography (TLC) was performed on Chloroform, and Ethanol extracts in various solvent systems. The TLC plates were produced and activated with silica gel G. The extracts were spotted using a micropipette and dried before being developed in solvent systems I and II separately. Three spots were identified Yellow, yellowish green and brown in UV light and iodine chamber detection in solvent A with chloroform extract. A total of two spots were identified in purple and brown in solvent system B with vanillin in sulphuric acid and their respective Rf values are tabulated in Table 4

Table 4: Thin layer chromatography of *Rosmarinus officinalis extracts*

Extract	Solvent system	Detection	Spots	Colour	R_f Value
Chloroform	A	Iodine Chamber	I	Yellow	**0.82**
			II	Yellowish	**0.63**
		U-V light		Green	
			III	Brown	**0.26**
	B	Vanillin in Sulphuric Acid	I	Purple	**0.73**
			II	Brown	**0.46**
Ethanol	A	Iodine chamber	I	Yellow	**0.92**
			II	Orange-Brown	**0.67**
		U-V light	III	Brown	**0.32**
	B	**Vanillin in Sulphuric Acid**	**I**	**Purple**	**0.78**
			II	**Brown**	**0.52**

3.1 Fluorescence analysis

Separate fluorescence analyses were performed on the crude powder and extract using acid and alkali media. The colour intensity of the powder and extract was measured in both daylight and UV light in a UV chamber. In a separate glass dish, the powdered drug was likewise treated with several chemical reagents, and the change in colour was documented and tabulated.(Table 5 & 6)

Table 5: Fluorescence Analysis of Extract

SL. NO.	EXTRACTS	DAYLIGHT	UVLIGHT
1.	HEXANE	Yellowish brown	Green
2.	CHLOROFORM	Dark green	Bluish green
3.	ETHANOL	Green	Greenish brown

Table 6: Fluorescence Analysis of Powder

SL.	REAGENT	DAYLIGHT	UVLIGHT
1	NaOH in Methanol	Greyish green	Light green
2	Powder + HCl	Grey	Dark green
3	Powder + NaOH in Water	Green	Green
4	Powder + Dil.H_2SO_4	Dark brown	Purple
5	Powder + Dil.HNO_3	Yellowish brown	Bluish green

3.2 *Invivo* anti-inflammatory activity of ethanolic extract of *rosmarinus officinalis* in wistar rats

At first hour, Diclofenac sodium, Ethanolic extract of *Rosmarinus officinalis L* 200mg/kg and 400mg/kg exhibited good anti-inflammatory activity compared to control group ($P < 0.001$). At second hour also diclofenac sodium, ethanolic extract of *Rosmarinus officinalis* L 200mg/ kg and 400mg/kg exhibited good anti-inflammatory($P<0.001$). At third hour diclofenac sodium ($P<0.001$) exhibited good anti-inflammatory activity compared to ethanolic extract of *Rosmarinus officinalis L* 200mg/kg ($P<0.001$) and 400mg/kg ($P<0.001$). At fourth hour also diclofenac sodium and ethanolic extract of *Rosmarinus officinalis L* 400mg/kg exhibited good anti-inflammatory activity compared to ethanolic extract of *Rosmarinus officinalis L* 200mg/kg ($P<0.001$). It means diclofenac sodium showed highest antiinflammatory activity followed by ethanolic extract of Rosmarinus officinalis L 200mg/kg

Hence the results of the present investigation conclude that the ethanolic extract of *Rosmarinus officinalis L* 200mg/kg and400mg /kg. showed more anti-inflammatory activity than200mg/kg dose. Further work is progress to isolate the constituents responsible for the anti- inflammatory activity in our laboratory

Table 7: **Invivo Anti-inflammatory activity of Ethanolic Extract of *Rosmarinus officinalis* in albino rats**

DRUG	ROUTE	DOSE mg /kg	Paw Volume			
			1 Hour	2 Hour	3 Hour	4 Hour
Control	P.O	1 ml/kg	0.225 ± 0.025	0.275 ± 0.025	0.325 ± 0.00216	**0.04 ±0.005**
Diclofenac sodium	P.O	20 mg /kg	0.075** ± 0.025 (66.66%)	0.125** ± 0.025 (54.54%)	0.15** ±0.00288 (53.84%)	**0.2**± 0.0408 (50%)**
ETHANOL 200 mg /kg	P.O	200 mg /kg	0.125** ± 0.025 (44.44%)	0.175** ± 0.04787 (36.36%)	0.275* ± 0.0570 (15.38%)	**0.325** ± 0.025 (18.75%)**
ETHANOL 400 mg /kg	P.O	400 mg /kg	0.1** ± 0.003 (55.55%)	0.15** ±0.028 72(45. 45%)	0.225** ±0.0350 (30.76%)	**0.275** ± 0.0478 (31.25%)**
One- way ANOVA		**F** **P**	**9.223 <0.001**	**3.9522 <0.01**	**1.094 <0.5**	**6.1855 <0.001**

Values are expressed in mean SEM, n=4 (4 animals in each group) df = 3/12

One- way ANOVA followed by Dunnett's t –test

**P<0.001, *P<0.01 was considered to be statistically significant.

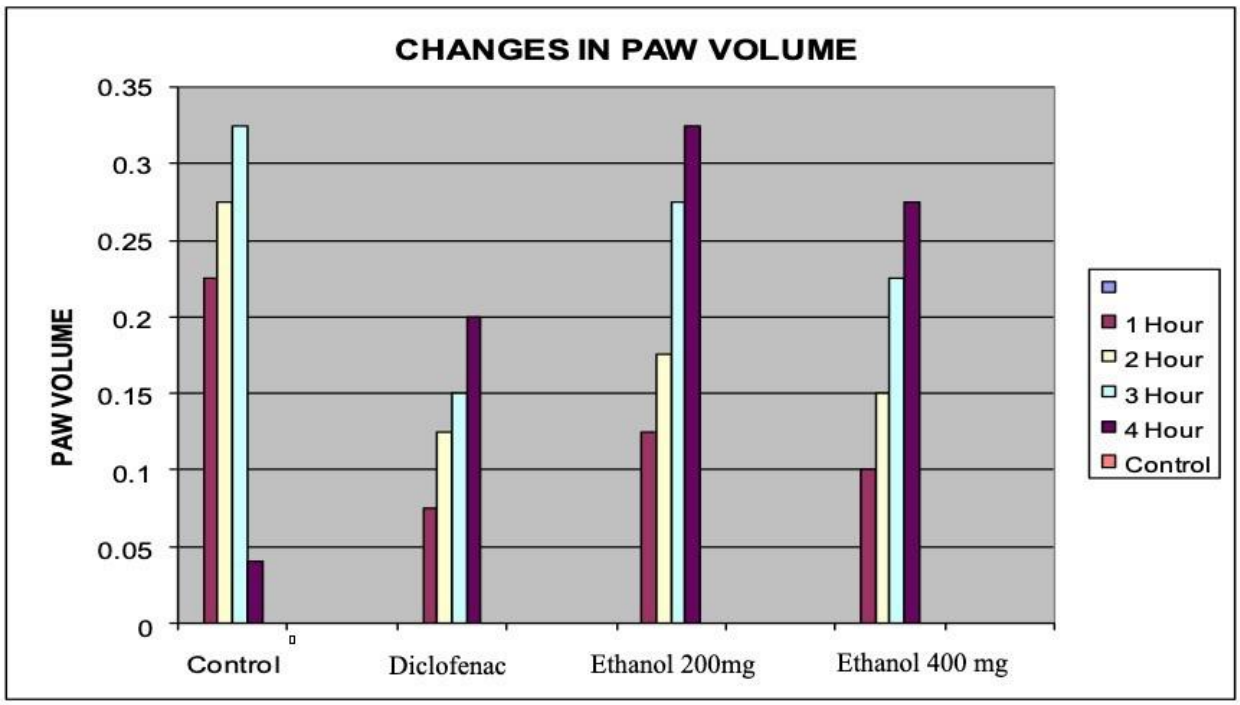

Figure 1: Changes in Paw volume with Control, Standard and Ethanolic Extract (200,400 mg)

4. Discussion

The present studies involved the findings of Phytochemical and Anti-inflammatory activity on aerial parts of Rosmarinus officinalis. It opens up vital for further studies of this plant.

The dried powder of the aerial parts of plant was extracted with different solvents of increasing polarity and the percentage of extract where calculated (Table:1). The ethanolic extract possessed maximum extractive value.

Various extracts were subjected to Phytochemical studies. The extracts answered positively for Carbohydrates, alkaloids, Proteins, Terpenoids, Steroids and Flavonoids and answered negatively for Glycosides, saponins and Fixed oil & Fat. (Table:2)

Chloroform and ethanolic extracts were subjected to TLC using two different solvent system Toluene: Ethyl acetate: Dimethylamine (7:2:1), Toluene: Ethyl acetate (93:1). The Rf value of differentextracts measured, and number of spots noted (Table:4). The chloroform and ethanolic extract showed maximum spots with solvent system A. The presence of alkaloids were identified in solvent system A and Terpenoids were identified in solvent system B Fluorescence analysis was performed for the extracts and crude powders using various reagents and the fluorescence nature was observed by compared with day light and UV light and reported in (Table:5&6)

The anti-inflammatory activity was studied by Carrageenan induced rat hind paw oedema measured by Plethysmograph(Mercury Displacement Method)Wistar strain rats of either sex between 150-200gm were divided into four groups of four animal each. The first group served as a control and received the distilled water, and second group of animals were administered with standard drug. Diclofenac Sodium 200mg/Kg body weight orally. The third and fourth groups of animals were treated with EERO (Ethanolic Extract of *Rosmarinus officinalis*) at a dose of 200 and 400 mg/Kg body weight. **(**Table:7)

The volume paw oedema was measured in control, standard and treated groups accordingly 1,2,3 and 4 hours after carrageenan injection. The percentage inhibition of oedema was calculated.

The data was analyzed by one way ANOVA followed by Dunnett's t-Test . According to this test there was a significant difference between the drug treated groups and control at the level of $P<0.001$and $P<0.01$

5. Conclusion

The present study provided the Botanical information, phytoconstituents and Anti-inflammatory activity present in this *Rosmarinus officinalis*. It will support for the structural elucidation of active constituents.

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