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WOUND HEALING ACTIVITY of-7-O-glucoside from the flowers of *CLERODENDRUM PHILOMIDES*

KEYWORDS: Clerodentrum philomides, Apigenin-7-O-glucoside, Wound healing,.

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ABSTRACT *The fresh flowers of Clerodendrum philomides is found to contain apigenin and apigenin 7-O-glucoside. The isolated compound is characterized by choromatography and UV spectral techniques. . The ethyl acetate extract of Clerodendrum phlomidis is investigated for its wound healing property.The flower extract has immense potential for the management and treatment of wounds.*

INTRODUCTION

Clerodendrum philomides is a wild shrubby plant belonging to verbenaceae family. It is used in ayurveda and yuinani. The white flowers from Clerodendrum philomides were chosen for phyto-chemical investigation and the results are given below.

Experimental

Extraction and fractionation

The fresh flowers of Clerodendrum philomides of 2Kg were collected from the river bank of Cauvery in Thanjavur District, and were refluxed with MeOH (4x500 ml). The alcoholic extract was concentrated in vacuo and the aqueous concentrate was successively fractionated with Benzene, free ether (3x250 ml) and ethyl acetate (4x250 ml). Ethyl acetate fraction only taken for further studies.

Ether Fraction (Apigenin)

The residue from the ether fraction was taken up in Me_2O and left in ice-chest for few days, when a yellow needle of MeOH m.p.278-280C (Yield 60g) was separated. It is soluble in organic solvents but insoluble in water. It developed a reddish orange colour with Mg-HCl and yellow colour with NaOH. It appeared pale yellow under UV as well as on exposure to NH_3.

It responded to Wilsons's boric acid, Horhammer-Hansel and Gibb's test but did not answer for Molisch's test.

Ethyl Acetate Fraction (Apigenin-7-O-glucoside)

The residue from ethyl acetate fraction was taken up in Me_2CO and left in ice-chest for two days when a pale yellow solid was separated. It came out as pale yellow plates when recrystallised from hot MeOH. It developed a greenish brown colour with alcoholic Fe^{3+}, forming yellow precipitate with basic lead acetate solution and reduced ammoniacal $AgNO_3$ but not Fehling's solution./ It gave yellow colour with aqueous NaOH, intense yellow with Conc H_2SO_4 and magenta colour with Mg-HCl. It appeared deep purple colour under UV which turned yellow on exposure to NH_3. It responded to Wilson's Boric Acid, Gibbs and Molisch's test but not answered Horhammer-Hansel test./

Hydrolysis of the glycoside

To a solution of the glycoside(100mg) in hot aqueous MeOH(10 ml, 50%), an equal volume of dil. H_2SO_4(7%) was added and the mixture gently refluxed at 100^0C for hours. The excess of alcohol was distilled of in vacuo and the resulting aqueous solution was extracted with glycoside was subjected to partial hydrolysis by treatment with 10% formic acid in cyclohexane and the resulting solution extracted with ethyl acetate and subjected to Paper Chromatography. The R_f values are indicated in Table (Table 1).

Identification of aglycone (Apigenin)

The residue from the ether fraction of the hydroxylate was taken up with Me_2O and left under chilled condition for a few days, when a yellow solid was obtained. The colour reactions, chromatographic behavior and UV spectral data were all similar to those for the free aglycone described earlier. The R_f values are indicated in the table.

Table-1 .R_f(X100) values of constituents of the flowers of Clerodendrum philomides
(Whatmann No.1, Ascending, 30±2^{0}C)

Compound	*Developing Solvents				
	a	b	c	d	e
Aglycone(1)	35	65	91	95	78
Aglycone(authentic)	35	65	91	95	78
Glycoside(2)	57	75	55	70	80
Glycoside(authentic)	57	75	55	70	80

(1) Aglycone-Apigenin
(2) Glycoside-Apigenin-7-O-glucoside
***Solvent key**
a. 30% aq.AcOH
b. 60% aq.AcOH
c. n-BuOH:AcOH:H_2O=4:1:5 BAW (Upper Phase)
d. Phenol saturated water
e. Forestal (AcOH:Conc.HCl:H_2O)=30:3:10

Identification of sugar (Glucose)

The filtrate after the removal of all aglycone was neutralized with $BaCO_3$. The concentrated filtrate was examined by paper chromatography and gave R_f values (Table-2) corresponding to those of glucose.

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Table-2 R_f (x100) Values of the sugar from the Glycoside from Clerodendrum philomides (Whatmann No.1, Ascending, 30±2°C)

Compound	*Developing solvents			
	a	b	c	d
Sugar from Glycoside	25	17	38	37
Glucose (authentic)	25	17	38	37

***Solvent**
a. n-BuOH:27% aq.AcOH=1:1
b. n-BuOH :AcOH:H_2O=4:1:5 BAW (upper Phase)
c. Phenol saturated with water
d. Forestal (AcOH:Conc.HCl:H_2O)=30:3:10

Results and Discussion
Structural and spectral interpretation
The flowers of Clerodendrum philomides have been found to contain Apigenin. UV spectral data with methanol indicated that it was a flavone, while on addition of NaOAc indicating free OH at 7th position . Addition of H_3BO_3 indicated the presence of catecholic hydroxyl groups addition of $AlCl_3$ indicating the presence of free OH and at C-5 (Harborne and Mabry, 1982). UV spectral data of the compound is λ_{max}^{MeOH} nm 267, 296 (sh), 336; +NaOMe 275, 324, 392; +$AlCl_3$, 276, 301, 348, 384; +($AlCl_3$-HCl) 276, 336; +NaOMe 275, 324, 392; +$AlCl_3$ 276, 301, 348, 384; +($AlCl_3$-HCl) 276, 299, 340, 381; NaOAc 274,301(sh),376;and+(NaOAc-H_3BO_3)268, 302(sh),338 nm. From the above data the compound has been identified as Apigenin.

UV spectral data with methanol indicated that it was a flavone glycoside, while on addition of NaOAc indicating the occupation of 7th-position. Addition of H_3BO_3 indicated the presence of catecholic hydroxyl groups. Addition of $AlCl_3$ indicating the presence of free OH at C-5. UV spectral data of compound is λ_{max}^{MeOH} nm 226, 266, 333:+NaOMe 240, 272, 305 sh, 390; +$AlCl_3$,274,300sh, 345, 385 sh; +($AlCl_3$-HCl)275 294sh, 340, 380; +NaOAc 252sh, 263, 350,385sh ;and +(NaOAc – H_3BO_3) 264, 300sh, 398 nm. From the above data compound was identified as Apigenin – 7-O glucoside.

Wound Healing Activity
Wounds are any damage to break off the skin tissues. The damage may be caused by accidents, incisions from surgery or other traumas. Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. A therapeutic agent selected for the treatment of wounds should ideally improve one or more phases of healing without producing deleterious side effects. Several natural products, which are composed of active principles, like tri terpenes, alkaloids, flavonoids, and biomolecules have been reported to promote the process of wound healing.

Wound healing herbals encourage blood clotting, fight infection and accelerate the healing of wounds. Plants or chemicals entities derived from plants need to be identified and formulated for treatment management of wounds. Clerodendrum philomides is a variable manual and is distributed throughout India. The flowers are said to be useful in chronic rheumatism. The juice is applied in psoriasis and other chronic skin eruption. The whole plant is used for analgesic,antipyretic, anti-rheumatic, anti-inflammatory, antibacterial treatment and antioxidant activity etc. The plant Clerodendrum philomides is valued in the Indian traditional system of medicines, ayurveda for their multiple health benefits. However, the medicinal values of plant pertaining to wound has yet to be reported, Therefore the aim of treating wound is to either shorten the time required for healing or to minimize the undesired consequences.

The process of wound healing involves a variety of process such as inflammation, cell proliferation and contraction of the collagen lattice formed. The present investigation revealed that the ethyl acetate extract of Clerdendrum philomides were proved to wound healing activity by comparing it with the standard soframycin ointment. Petroleum ether extracts exhibit very poor wound healing activity. Rabbits were used as animal models where a control is used for the purpose of deciding the healing property. The clinical observations on wound healing were as follows.

The studies on excision wound healing model reveals that all the three groups showed decreased wound area from day to day. The wound control showed a time dependent increase in percent from 59.26% to 77.77% from 8th day to 12th day and 77.77% to 94.44 from 12th day to 16th day while complete wound closure and epithelisation was observed on 16th day of wound induction compared with day zero which was taken as 0%. Scab over the wound started appearing from 8th and 12th day in both standard and Apigenin treated groups respectively./

All readings are found to be statistically significant and comparable with control. In soframycin treated group significant increased in the epithelisaion period and decreased percent wound contraction were observed when compared to control. On 12th day standard and Apigenin drug treated animals were showed significantly greater wound closure as compared to control animals. A few macrophages and lymphocytes were seen beneath the newly formed epidermis and also seen fibrous tissue which filled and defect at the dermis. However, on 6th post wounding day, Group- I animals showed 77.77% of healing (which may be due to self-immunity) whereas soframycin treated animals showed 98.15% healing on the other hand the Apigenin treated group showed 94.44% of wound healing. It was evident that number of neutrophils, lymphocytes and macrophages were moderate at the level of studies.

Therapeutic of Excision wound model -Experimental
Excision wound model was employed to study the rate of wound contraction and the time required for full epithelisation of the wounds. Healthy rabbits of either sex of approximately the same age, weighing about 150-250 gm were used for the study. They were fed with standard diet. They were individually housed, maintained in poly propylene cages under standard conditions. The animals were divided into three groups of six rabbits.

Group I : No treatment and served as control.
Group II: Test group with wound and treated with isolated Apigenin 7-O-glucoside
Group III: Test group with standard drug (Soframycin)

Animals were anaesthetized with slight vapour inhalation of Diethyl ether and predetermined area on the back of rat was shaved. The excision wound a circular piece of full thickness sized approximately 30mm^2 and 2 mm depth were made by cutting out the skin from the shaved

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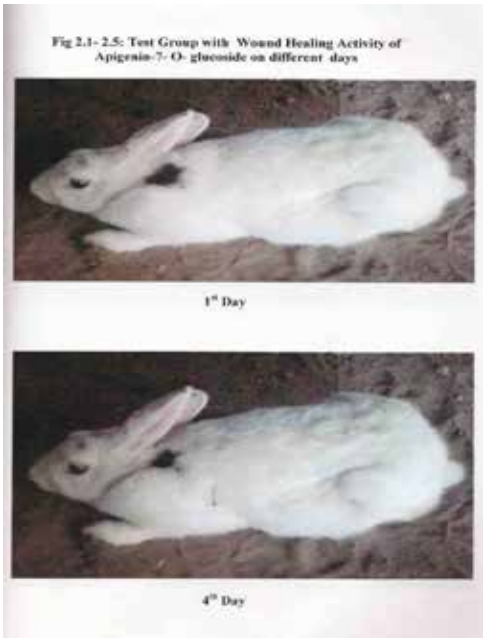
area. Wounds were traced on 1mm^2 graph paper on the day of wounding and on alternate days, until healing was complete. The isolated Apigenin is applied at a dose of 200mg per day for sixteen days. Wound areas were measured on days 0th,4th,8th,12th and 16th for all groups. Figs (fig-1 to fig-6)

Fig.1-6 Test group with wound healing activity of apigenin -7-O-glucoside on different days.



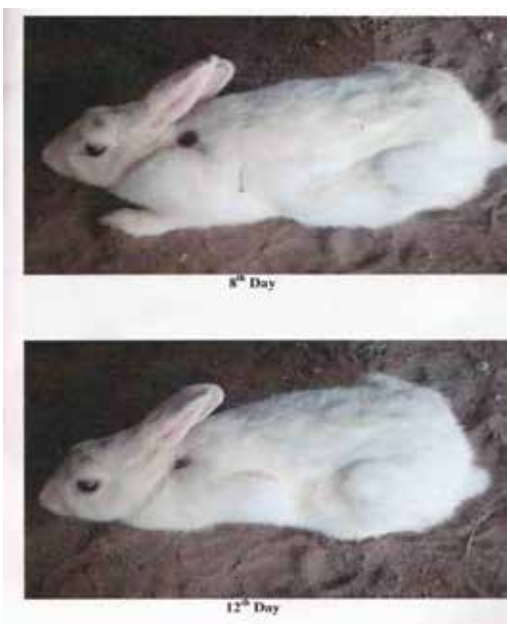


Table –3 Effects of Apigenin -7-O-glucoside from clerodendrum philomides On excision wound

Day	Group I	Group II	Group III
0	27.00 ± 1.340	27.00±1.032	27.00 ± 1.645
4	22.00 ± 11.628	21.39 ± 5.733	18.28 ± 9.987
8	20.91 ± 9.089	11.34 ± 59.84	09.31 ± 8.054
12	11.43 ± 3.189	06.34 ± 2.314	03.69 ± 1.892
16	06.39 ± 2.281	01.50 ± 1.913	0.578 ± 2.091

Group I – served as control.
Group II – Treatment with Apigenin -7-O-glucoside
Group III – Treatment with soframycin

Table – 4 effects of Apigenin -7-O-glucoside from Clerodendrum philomides On excision wound [% of Wound Closure]

Day	Group I	Group II	Group III
0	0%	0%	0%
4	18.52%	22.22%	33.33%
8	25.93%	59.26%	66.67%
12	59.26%	77.77%	88.89%
16	77.77%	94.44%	98.15%

SUMMARY AND CONCLUSION

The flowers of Clerodendrum philomides have been found to contain Apigenin-7-O-glucoside. The isolated compound have been duly characterized by chromatographic technique, study as well as by UV spectral studies.

The effects of the isolated flavonoid glycoside from the EtOAc fraction of Clerodendrum philomides against wound healing activity have been established.

On the basis of the results obtained in the present investigation, it is possible to conclude that the flowers extract of Clerodendrum philomides has significant wound healing activity.

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