Evaluation of Antipyretic Activity and Structural Identification of Chemical Constituent from the Bark of*Plumeria acutifolia* Poir. (Tayoke Saga)

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Abstract

Investigations on acute toxicity, antipyretic activity and chemical constituents were done on the bark of *Plumeria acutifolia* Poir. (Tayoke Saga in Myanmar). The medium lethal doses (LD_{50}) of EtOH extract and water extract were found to be 4.9 (3.06 – 7.84) and 9.4 (6.02 – 14.66) g/kg body weight, respectively, in albino mice. Antipyretic activity studies in rat models revealed that water and ethanol extract of Tayoke Saga bark (1.5 and 0.75 g/kg body weight in dose, respectively) showed moderate reduction in yeast-induced pyrexia which was comparable to that of standard antipyretic drug paracetamol. Isolation of chemical constituents from active crude ethanol extract was done by solvent partition, successive column chromatographic separation and recrystallization. Plumieride, C, (3.5% yield, mp 154 ºC) was isolated from EtOH extract. The water and ethanol extract were able to reduce yeast-induced fever in albino rats indicating their efficacies as antipyretic drugs.

Keywords: antipyretic activity, *Plumeria acutifolia* Poir., LD_{50}

Introduction

Fever (also known as pyrexia, or a febrile response for the Latin word febris meaning fever) is a frequent medical symptom that describes and increases in internal body temperature to levels that are above normal (37 ^{o}C, 98.6 F). Fever is most accurately characterized as a temporary elevation in the body's thermoregulatory set-point, usually by about 1-2 ^{o}C. Fever differs from hyperthermia, which is an increase in body temperature over the body's thermoregulatory set-point (due to excessive heat production or insufficient thermoregulation, or both). Fever is not a disease but symptom of disease.

Pyrexia or fever is caused as a secondary impact of infection, tissue damage inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1β, α and TNF-α), which increase the synthesis of prostaglandin E2 (PGE 2) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature (Spacer *et al.,* 1994). A natural antipyretic agent with reduced or no toxicity is therefore, essential. It will be a cost effective alternative approach to study this plant for the development of an effective antipyretic agent.

Plumeria acutifolia Poir. (Tayoke Saga in Myanmar) is a small tree belongs to a family Apocynaceae. Because of their attractive flowers and values of traditional medicine, these plants are very famous in Myanmar (Hundley, 1987). Hot water extract of the bark of *Plumeria acutifolia* was practically found to be a potent remedy in the treatment of malaria which is one of the prevalent diseases in Myanmar (Maung Maung Htay *et al*., 2001).

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***Plumeria acutifolia* Poir. (Tayoke Saga)**

Family	:	Apocynaceae
Scientific name	:	*Plumeria acutifolia* Poiret
Synonym	:	*Plumeria accuminata* Aiton
	:	*Plumeria rubra* Linn
English name	:	Frangipani Tree or Jasmine Tree or Pagoda Tree
Myanmar name	:	Tayoke Saga

Plumeria, commonly known as frangipani, is a genus of shrubs and trees of the family Apocynaceae, native of tropical America; some as ornamental species are grown in the warmer regions of the world. About eight species are reported from India, but owing to the overlapping of characters in some species, it becomes difficult to fix their identity (The Wealth of India, 1969).

In Myanmar, four species of "Tayoke saga" plant, namely, *Plumeria acutifolia* Poir., *Plumeria rubra* L., *Plumeria alba* L. and *Plumeria obtusa* L. are recorded by Hundley in 1987. Because of their attractive flowers and values of traditional medicine, these plants are very famous in Myanmar (Hundley, 1987).

Materials and Methods

Sampling of Plant Materials

Pleumeria acutifolia Poir. (Tayoke Saga) used in this study was collected from Zalun Township, Ayeyarwaddy Region, in February, 2004 and identified at the Department of Botany, Dagon University. The stem bark was cut into small parts. After being air dried at room temperature for 2 weeks, these parts were made powder by using grinding machine and stored in air-tight container to prevent moisture changes and contamination.

Phytochemical Investigation of *Plumeria acutifolia* Poir.

Preliminary phytochemical analyses were performed in order to know the different types of chemical constituents present in the bark of *Plumeria acutifolia* Poir. Phytochemical investigation on plant sample was done according to standard procedures.

Study on Acute Toxicity of Bark of *Plumeria acutifolia* Poir

The acute toxicity test for ethanol and water extracts of bark of *P. acutifolia* Poir. was performed in mice by determining the LD_{50} according to the method of Litchfield and Wilcoxon (1949).

Preparation of Text Extracts

(i)Ethanol Extract

Dried powdered sample (100 g) was refluxed with 95% ethanol (300 mL) for three hours. Evaporation to dryness provided ethanol extract of Tayoke Saga bark.

(ii) Water Extract

Dried powered sample (100 g) was refluxed with distilled water (300 mL) for 3 hours. Evaporation to dryness provided water extract of Tayoke Saga bark

Animals and Apparatus

90 albino mice of both sexes (weighing 28-38 g), mouse cage and '18' gauge intragastric needles

Procedure

90 albino mice of both sexes (weighing 28-38 g) were used in this study. Food was withheld for the period 12 hours. Mice were separated into 9 groups and each group contains 10 mice. Each group was placed separately in the 9 mouse cages.

The given doses of ethanol extract were 1, 0 ,2.0 ,4.0, and 8.0 g/kg and that of water extract were 1.5, 3.0, 6.0, 12.0 and 16.0 g/kg. After giving the extract orally each group of mice was allowed to free access to water. Then, the animals were observed for 14 days and the medium lethal dose (LD_{50}) of each extract was investigated (Loomis,1968).

Antipyretic Activity of Bark of *Plumeria acutifolia* Poir.

Preparation of Test Samples

Both extracts of Tayoke Saga bark (3 g each) were dissolved in propylene glycol (1 mL) and diluted with distilled water (9 mL) 30 minutes before experiment.

Animals and Apparatus

30 Adults albino rats of either sexes (body weight 180-250 g), mouse cage and '18' gauge intragastric needles

Procedure

Yeast induced by pyrexia was applied to evaluate the antipyretic activity of the extracts. The rats were divided into four groups (six animals in each) and the body temperature of each rat was recorded by measuring rectal temperature at predetermined time intervals. Fever was induced by injecting 15 % suspension of Brewer's yeast (*Saccharomyces cerevisiae*), following a standard method (Murugesan *et al.,* 2000). In brief the rats were allowed to remain quite in the cage for sometimes. A clinical thermometer was inserted 3-4 cm deep into the rectum, after fastened the tail, to record the basal rectal temperature. The animals were then given a subcutaneous injection of 10 mL/kg of 15 % W/V Brewer's yeast suspended in 0.5 % W/V methylcellulose solution and the animals were returned to their housing cages. 19 hours after yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately the water extract was administered orally at dose of 1.50 g / kg to the first group of animals; ethanol extract was administered orally at dose of 0.75 g / kg to the second group; the third group received 5 mL / kg of propylene glycol as vehicle control and the last group was administered with 250 mg / kg of paracetamol (45 g paracetamol was dissolved in 0.5 mL propylene glycol and diluted with 4.5 mL distilled water) as drug control. Rectal temperature of all the rats was recorded at 19h, immediately before extract or vehicle or paracetamol administration and again at 1 h interval up to 24 h, after yeast injection (Chattopadhyay *et al.,* 2002)

Statistical Analysis

The statistical analysis was carried out with Microsoft (Excel) software. Difference of the parametric data of body temperature was examined by student "t" test.

Preparation of Active Ethanol Extract of Tayoke Saga Bark

Air dried bark powder sample (400 g) was percolated with 95 % EtOH (1200 mL) for one week at room temperature. Ethanol crude extract (32.16 g, 8.02 % yield) was obtained after removal of the solvent. The crude extract was then dissolved in distilled water and partitioned with chloroform (5 x 100 mL). Removal of chloroform extract (6.0 g, 1.5 % yield). The defatted layer was concentrated under reduced pressure to provide the defatted ethanol extract (25.0 g, 6.25 % yield). The defatted ethanol extract was used to screen the active chemical constitution.

Isolation of Chemical Constituent from Defatted Ethanol Extract of Tayoke Saga Bark

Defatted ethanol extract (5.0 g) was dissolved in ethanol and thoroughly adsorbed on silica gel (3 g). The adsorbed material after being dried was transferred to a silica gel column (50 g; 2.0 cm in diameter packed in ethyl acetate : ethanol (49:1). The column was eluted consecutively with ethyl acetate : ethanol (19:1), ethyl acetate: ethanol (9:1), ethyl acetate : ethanol (4:1), ethyl acetate : ethanol (3:1), ethyl acetate : ethanol (2:1) and finally with ethyl acetate : ethanol (1:1).

A quantity of 10 mL was collected for each fraction and the column chromatography was monitored by TLC using the solvent system of ethyl acetate and ethanol mixture. The fraction which gave similar TLC pattern were combined together and concentrated. In this way, three major fractions, F_4-F_6, were obtained.

The fraction F_5 after crystallization in ethyl acetate – methanol provided compound C as colourless needles (2.80 g, 3.5 % in yield, m.p. 154 °C, R_f = 0.25, EtOAc : EtOH, 9:1). The isolated compound was physicochemically characterized and then structurally identified by modern spectroscopic technique such as UV, FT IR, ^{1}H NMR, ^{13}C NMR and EI MS.

Results and Discussion

Phytochemical Investigation of *Plumeria acutifolia* Poir.

Preliminary phytochemical tests were carried out on stem bark of plant samples. It was found that alkaloids, carbohydrates, glycoside, saponins, flavonoids, α-amino acid, phenolic compounds, steroids, terpenoids and tannins were found to be present in the stem bark of *P. acutifolia* Poir.

Acute Toxicity of Bark of *Plumeria acutifolia* Poir.

The acute toxicity test was done to determine the symptomatology consequent, degree of toxicity to administration of the drug and to find out the medium lethal dose (LD_{50}) of the drug. Usually the acute lethality of a compound is determined on the basis of deaths occurring in 24 hours but the survivors should be observed for at least 14 days in order to detect delayed effects (Looms, 1986). Since the route of administration selected should be the intended route for administration of the tested drug given to the human during therapy. The oral route was chosen for this test.

Table 1 Table Calculated for LD_{50} of LD_{50} of Ethanol Extract of *P. acutifolia acutifolia* Poir.(Bark)

No.	Dose (g/kg)	Dead/ Tested	Observed % Death		Expected Death (%)	Observed Minus Expected Death	$(Chi)^2$	LD_{50} (g/kg)
			Actual value(%)	Corrective Value (%)				
1.	8	7/10	70	-	75	-5	0.015	
2.	4	5/10	50	-	40	10	0.05	
3.	2	2/10	20	-	11	9	0.075	4.9
4.	1	0/10	0	0.54	1.6	-1.06	0.0066	
							(c)0.1466	

Table 2 Table Calculated for Watery Extract of *P.* Poir.(Bark)

No.	Dose (g/kg)	Dead/ Tested	Observed % Death		Expected Death (%)	Observed Minus Expected Death	$(Chi)^2$	LD_{50} (g/kg)
			Actual value(%)	Corrective Value (%)				
1.	16	6/10	60	-	74	-14	0.090	
2.	12	4/10	40	-	62	-22	0.140	
3.	6	3/10	30	-	30	0	-	9.4
4.	3	3/10	30	-	10	20	0.450	
5.	1.5	0/10	0	0.7	2	-1.3	0.007	
							(c)0.687	

In this experiment, lethal activity and determination of medium lethal dose (LD_{50}) of ethanol and water extracts of *P. acutifolia* Poir. was done according to the method of Litchfield and Wilcoxon. Oral administration of EtOH extract did not produce any mortality in mice up to a dose level of 8 g/kg. The sign of toxicity included diarrhea, muscle weakness, lethargy and death. LD_{50} of EtOH extract was found to be 4.9 (3.06-7.84) g/kg.

Water extract showed that no lethality of the mice upto14 days, with a maximum dose level of 1.5 g/kg. However, 60 % of the mice were dead at the dose 16 g/kg. Signs of toxicity were similar to those of ethanol extract including diarrhea, muscle weakness, lethargy and death. LD_{50} of water extract was found to be 9.4 (6.02-14.66) g/kg.

Ethanol extract showed lower LD_{50} than that of water extract, however, LD_{50} of both extracts have more than 0.5 g/kg. According to the toxicity scale of Hodge and Sterner, any compound with an oral LD_{50} of between 500-5000 mg/kg should be considered practically nontoxic (CCHOS,1999). Therefore, both extracts were hoped to be safety as oral drugs.

Antipyretic Activity of Bark of *Plumeria acutifolia* Poir.

The results of effect of ethanol and water extracts of *P. acutifolia* bark on yeast induce pyrexia in rats are depicted in Table 3 and Figure 1. The experimental rats showed a mean increase of about 1.01 ^{o}C in rectal temperature,19 hours after Brewer's yeast injection. Hyperthermia was continued throughout the test (over 5 h) in vehicle group. The mean rectal temperature of control rats at -19, 0 and 5 h were 37.68 ± 0.19, 38.78 ± 0.05 and 38.81 ± 0.03 ^{o}C respectively.

The mean temperature of paracetamol receiving group (0.25 g/kg dose) at -19, 0, 1, 2, 3, 4 and 5 h were 38.05 ± 0.1 ,38.95 ± 0.03, 37.97 ± 0.11, 37.37 ± 0.13, 37.18 ± 0.26, 37.22 ± 0.31 and 37.68 ± 0.22 ^{o}C, respectively. Paracetamol reduced rectal temperature and antipyretic effects continued for 5 h with the maximum reduction (196.6 %) at 3 h ($p<0005$) after administration.

Mean rectal temperature of aqueous extract receiving group (1.50 g/kg dose) at -19, 0, 1, 2, 3, 4 and 5 h were 37.85 ± 0.45, 39.03 ± 0.07, 38.6 ± 0.14, 38.4 ± 0.11, 38.23 ± 0.02, 37.98 ± 0.08 and 37.98 ± 0.08 ^{o}C, respectively. Aqueous extract significantly reduced rectal temperature and antipyretic effects continued for 5 h with maximum reduction (88.98 %) at 4 h ($p<0.00005$) after receiving the treatment.

Table 3 Effect of Test Drugs (Aqueous and Ethanol Extracts of *P. acutifolia* and Paracetamol) on Yeast Induced Pyrexia in Albino Rats

Treatment	Dose (g/kg)	Rectal temperature (°C)		Rectal temperature after administration of drug (°C)				
		Normal (A)	19 h after yeast administraton (B)	1h (C_1)	2h (C_2)	3h (C_3)	4h (C_4)	5h (C_5)
Control	5 ml	37.68±0.19	38.78±0.05	38.73±0.05 (4.54±0.52)	38.83±0.03 (4.54±0.31)	38.92±0.04 (12.72±1.35)	38.85±0.05 (6.36±0.23)	38.81±0.03 (2.72±0.1)
Parace-tamol	0.25	38.05±0.1	38.95±0.03*	37.97±0.11**** (108.88±10.84)	37.37±0.13***** (175.55±17.83)	37.18±0.26**** (196.66±18.55)	37.22±0.31*** (192.22±17.47)	37.68±0.22*** (141.11±13.01)
Water extract	1.5	37.85±0.12*	39.03±0.07*	38.6±0.14 (36.44±3.19)	38.4±0.11* (53.38±5.22)	38.23±0.02** (67.79±7.52)	37.98±0.08***** (88.98±9.20)	37.98±0.08***** (88.98±9.20)
Ethanol extract	0.75	37.85±0.12	38.85±0.05	38.43±0.03 (42±4.73)	38.1±0.08** (75±7.74)	38.13±0.1***** (72±6.67)	37.96±0.05***** (89±9.57)	37.96±0.07***** (89±9.35)

$$\% \text{ reduction} = \frac{B - C_n}{B - A} \times 100; \text{ where } n = 1, 2, 3, 4 \text{ or } 5$$

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$; **** $p < 0.0005$; ***** $p < 0.00005$.

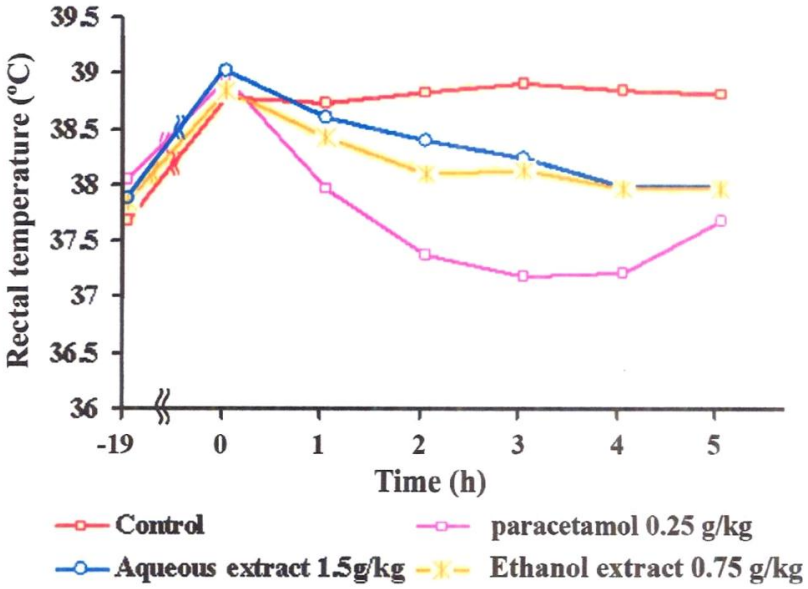


Figure 1 Antipyretic effect of test drugs (aqueous and ethanol extracts of *P. acutifolia* and Paracetamol) on yeast induced pyrexia in albino rats

The temperature of ethanol extract receiving group (0.75 g/kg dose) at -19, 0, 1, 2, 3, 4, and 5 h were 37.85 ± 0.12, 38.85 ± 0.05, 38.43 ± 0.03, 38.1 ± 0.08, 38.13 ± 0.1, 37.96 ± 0.05 and 37.96 ± 0.07 °C, respectively. Ethanol extract also showed antipyretic effect and continued for 5 h. The % reduction at 5 h was 89 % ($P<0.00005$).

These observations clearly revealed that water and ethanol extracts have marked antipyretic activity in yeast-induced fever in albino rats. Percentage

reduction in rectal temperature was calculated by considering the total fall un temperature to normal levels as 100%.

Further investigation on chemical constituents of ethanol extracts reveled non-steroidal compounds constituents the extract. In general, non-steroidal drugs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus (Clark and Cumby, 1975; Zeil and Krupp, 1975). Therefore, the antipyretic activity of ethanol extract of *P. acutifolia* may be attributed to the inhibition of prostaglandin synthesis in Hypothalamus.

Studies on Spectroscopic Data and Identification of Isolated Compound

Compound (C): colourless needles crystal; m.pt. 154 ºC; UV λ_{max} (EtOH): 225 nm; FT IR ν_{max} (KBr): 3379, 2909, 2851, 1755, 1697, 1633, 1436, 1288, 1266,1099, 1076, 1039, 865, 786 cm^{-1}; ^1H NMR (CD_3OD): δ 1.27 (d, J = 6.42Hz, 3H, H-14), 2.50 (t, J = 1.7 Hz, 1H, H-9),
2.8 (dd, 1H, H-3′), 2.9 *(t, J* = 8 Hz, 1H, H-5), 3.02 (m, 2H, H-6′), 3.4 (dd, 1H, H-4′), 3.69 (s,3H, H-16), 3.8 (dd, 1H, H-2′), 4.35 (m, 1H, H-5′), 4.39 (m, 1H, H-13), 4.5 (d, 1H, H-1′), 5.1 (*d, J* = 4.6 Hz, 1H, H-1), 5.5 (d, J = 5.6 Hz, 1H, H-7), 6.3 (dd, 1H, H-6), 7.2 (s, 1H, H-10), 7.49 (*s*,1H, H-3);^{13}C NMR (CD_3OD): δ 22.3 (C-14), 48.6 (C-16), 51.2 (C-6′), 60.9 (C-5), 69.8 (C-9), 72.9 (C-8), 76.4 (C-2′), 77.2 (C-3′), 78.5 (C-4′), 79.3 (C-5′), 91.9 (C-13), 95.78 (C-1), 98.2 (C-1′), 109.4 (C-7), 129.3(C-6), 137.4 (C-10), 139.5(C-3), 148.3(C-11), 150.9 (C-4), 166.2(C-12), 170.5(C-15) ; EI MS m/z: 470 $[M]^+$, Molecular formula $C_{21}H_{26}O_{12}$.

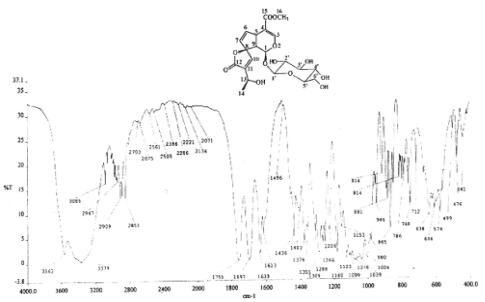


Figure 2 UV spectrum of isolated compound C

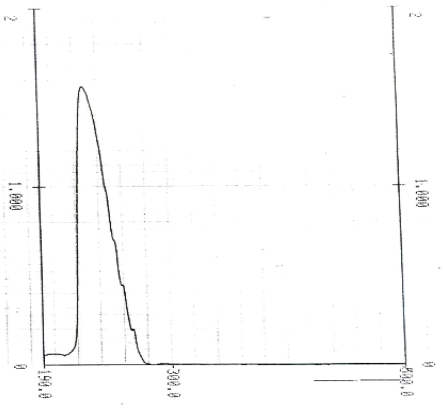
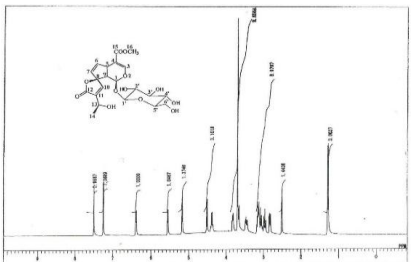


Figure 3 FT IR spectrum of isolated compound C



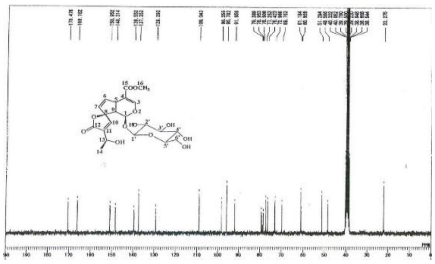


Figure 4 ^{1}H NMR spectrum of isolated compound C

Figure 5 ^{13}CNMR spectrum of isolated compound C

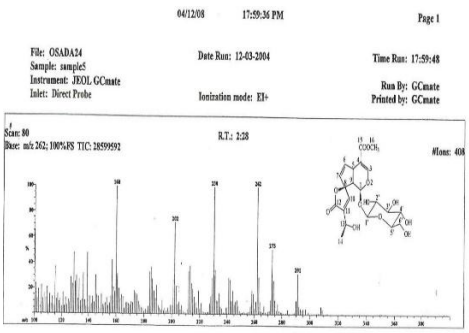


Figure 6Mass spectrum of isolated compound Cplumieride

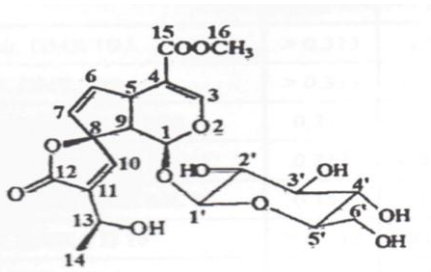


Figure 7 Chemical structure of

Conclusion

The LD_{50} values of ethanol extract and water extract in mice were 4.9 g/kg and 9.4 g/kg, respectively, indicating their safeties as oral drugs.

The water and ethanol extract of Tayoke Saga bark were able to reduce yeast-induced fever in albino rats indicating their efficacies as antipyretic drugs.

The compound plumieride (C) (3.5 % yield, m. p. 154 ^{o}C) was isolated from ethanolic extract of *P. acultifolia* Poir. And the structure was identified by UV, FT-IR, ^{1}H and ^{13}C NMR and MS spectroscopy.

Therefore, compound C may be main constituent of the plant responsible for antipyretic activity.

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