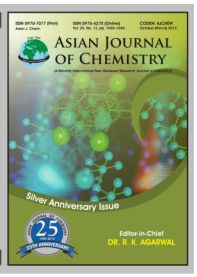
Asian Journal of Chemistry; Vol. 25, No. 17 (2013), 9565-9568

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15078A



Structural Elucidation of Bioactive Compound from the Fruit of *Parkia timoriana*

BIPLAB DE[1,*], SWARNALI NATH CHOUDHURY[2] and S.B. PAUL[3]

[1]Regional Institute of Pharmaceutical Science and Technology, Abhoynagar, Agartala-799 005, India
[2]Department of Science And Technology, ICFAI University Tripura, Kamalghat, Agartala-799 210, India
[3]Department of Chemistry, Assam University, Dargakona, Silchar-788 011, India

*Corresponding author: E-mail: biplab_32@yahoo.co.in

(*Received*: 1 January 2013; *Accepted*: 4 October 2013) AJC-14230

The analgesic activity of the methanolic extract of fruit of *Parkia timoriana* (DC.) Merr. and its separated fractions were studied in acetic acid induced model. The *in vivo* antiinflammatory was also studied using carageenan induced rat paw edema method. The result of both studies indicated that the extract and its components possessed significant analgesic activity as well as antiinflammatory activity at the dose 100 mg/Kg bw. Both the activities were compared with standard drug aspirin for analgesic and diclofenan sodium for antiinflammatory. The structure of the bioactive compound has been characterized on the basis of spectral data such as IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies.

Key Words: *Parkia timoriana*, Analgesic activity, Antiinflammatory activity.

INTRODUCTION

Medicines of plant origin have been used as a form of therapy for the relief of pain since ancient time[1]. In management of pain, the presently used drugs are well known for having side and toxic effects are either narcotics, if used, *e.g.*, opioids or non-narcotics *e.g.*, salicylates and corticosteroids *e.g.*, hydrocortisone. Introducing a new synthetic product as drug, approximately 3000-4000 compounds are to be synthesized, screened and tested and hence cost of development goes up from 0.5-5.0 million dollars. Thus these synthetic drugs are very expensive. Plants represent a large number of untapped source of structurally novel compounds that might serve as lead for the development of novel drugs[2].

To overcome these problems many workers have aimed at knowing the analgesic activity of different medicinal plants and using them for the treatment of pain management as possible alternatives to chemically synthetic drugs[3-7].

Parkia timoriana (DC.) Merr. is a deciduous, medium-sized tree up to 50 m tall. The leaves are arranged alternate. The primary rachis is a stalk of 18-42 cm long and the pinnate leaf comes with 14-31 pairs of leaflet. It is distributed in India, Bangladesh, Myanmar, Thailand and throughout Malaysia (except New Guinea), including the Philippines[8].

Parkia timoriana are considered beneficial in the treatment of hepatalgia, oedema, nephritis, diabetes and colic, probably as a result of their diuretic and relaxing activity. They are also used as an anthelmintic. The leaves are used against jaundice[9]. Pods are used in bleeding piles. Bark extract is given in diarrhoea and dysentery. Bark and leaves are employed for making lotion applied to sores and skin infections[10].

EXPERIMENTAL

Sample preparation: The fruit of *Parkia timoriana* (DC.) Merr. were collected from Agartala in the month of April 2011 and were shed dried for 7 days. Since certain compounds get denatured in sunlight, it is dried under shade to avoid decomposition. The dried fruits were then crushed to fine powder. The powdered plant materials (100 g) were defatted with petroleum ether. After washing with petroleum ether the residue were extracted exhaustively with 100 mL distilled methanol by using soxhlet apparatus. The extract was filtered through cotton followed by vacuum suction.

Separation: To separate the components present in the extract of fruit of *Parkia timoriana,* solvent extraction technique was adopted based on trial and error basis and resulted to give total four components (Y_1-Y_4).

Melting point of all compounds were determined in open capillaries by Melting Point Apparatus (Melting Point Apparatus, Indo, M-AB-92) and are uncorrected. The I.R. spectra were recorded (in Affinity-1 FTIR Spectrophotometer IR solution Version 1.50SU Shimadzu Corporation) in KBr pellets. ^{1}H and ^{13}C NMR were run on Mercury-400BB in DMSO, while mass spectra was performed in Agilent 7890 GC coupled with 5975 MS.

Screening of analgesic activity: Acetic acid induced writhing in mice[11].

Albino mice of either sex (weighing 25-30 g) were used as per experimental protocols approved by Institute of Bioresources and sustainable development (IBSD), Department of Biotechnology, Govt. of India, Imphal, Manipur, India. The animals were housed under standard environmental condition (25 ± 2 ºC) and relative humidity (50 ± 5 %) and fed with standard diet and water *ad libitum*. The animals were acclimatized to laboratory environment for a period of 14 days before performing the experiments.

The mice were divided into five groups of five animals each. The first group comprised the control; the remaining four groups were administered test dose. The test doses were prepared in distilled water to get the desired concentration of the extract and the separated compounds.

Acetic acid (1 % v/v, 10 mL/kg) was injected into the peritoneal cavities of mice, which were placed in a large plastic tray and the intensity of nociceptive behaviour was quantified by the number of writhes was counted for 10 min beginning from 5 min after the acetic acid injection. Test drugs and control vehicle were administered 0.5 h before the acetic acid injection. The writhing response consists of a contraction of the abdominal muscle turning of trunk (twist) together with a stretching of the hind limbs. The antinoceptive activity was expressed as the writhing scores over 20 min. Per cent inhibition of writhing was calculated using the relation:

Inhibition of writhing (%)

$$= 100\left[1 - \frac{\text{Mean writhing number of treated mice}}{\text{Mean writhing number of control mice}}\right]$$

Results are given in Table-1.

Antiinflammatory activity[12]: Antiinflammatory activity was evaluated using 0.1 mL of carrageenan (1 % w/v) induced hind paw edema method. Albino rats of either sex between 150-200 g were selected for the studies. The animals were kept on diet and allowed food and water ad- libitum. They were housed in polypropylene cages maintained under condition (12 h light/12 h dark cycle ± 3 ºC, 35-60 % humidity). The rats were divided into four groups of five rats each. The test groups-3 and 4 received 200 mg/Kg of compound Y_4 and extract Y_E by oral route. The positive control received (2nd group) standard diclofenac sodium (8 mg/Kg P.O.) by oral route. All the suspensions were administered 1 h before the injection of Carrageenan (0.1 mL of 1 % w/v). The hind paw volume was measured plethysmometrically before and after the carrageenam injection at hourly intervals for 5 h and percentage inhibition of inflammation was calculated using the relation:

Inhibition of oedema (%)

$$= 100\left[1 - \frac{\text{Mean paw volume of treated rats}}{\text{Mean paw volume of control rats}}\right]$$

Results are given in Tables 2 and 3.

RESULTS AND DISCUSSION

Highest mean writhing response of 12.2 ± 1.855 was shown by the animals after the intake of extract, Y_E given at a dose of 100 mg/Kg bw. and also almost the same number of writhing response, 12 ± 0.837 was shown by the animals after the intake of Y_3 given at a dose of 50 mg/Kg bw. Mean writhing response shown by the animals after the intake of Y_2 was 9 ± 2.025, while component Y_4 showed least writhing response of

TABLE-1
ANALGESIC ACTIVITY (ACETIC ACID-INDUCED WRITHING) OF *Parkia timoriana* (DC.) Merr.

S. No.	Animal No.	Treatment	Dose	Number of writhing (in 10 min duration)	Responders (n/n)	Mean of writhing ± standard error mean (SEM)	Inhibition (%)
1	1	Control water + (acetic acid)	10 mL/kg + 0.1 mL/kg	12	5/5	16.6 ± 1.166	0
	2			18			
	3			18			
	4			17			
	5			18			
2	1	Sample Y_2 + (acetic acid)	100 mg/kg + 10 mL/kg	7	5/5	9 ± 2.025	45.784
	2			6			
	3			7			
	4			8			
	5			17			
3	1	Sample Y_E + (acetic acid)	100 mg/kg + 10 mL/kg	11	5/5	12.2 ± 1.855	26.506
	2			6			
	3			16			
	4			12			
	5			16			
4	1	Sample Y_4 + (acetic acid)	100 mg/kg + 10 mL/kg	3	5/5	6.4 ± 1.568	60.446
	2			6			
	3			12			
	4			7			
	5			4			
5	1	Sample Y_3 + (acetic acid)	50 mg/kg + 10mL/kg	14	5/5	12 ± 0.837	27.711
	2			11			
	3			14			
	4			10			
	5			11			

TABLE-2
EFFECT OF *Parkia timoriana* (DC.) MERR. ON CARRAGEENIN INDUCED PAW EDEMA IN RATS

Treatment	Paw volume (mL)					
	0 h	1 h	2 h	3 h	4 h	5 h
Normal control (1 mL dist. Water P.O.)	0.2	0.4	0.5	0.6	0.5	0.5
	0.2	0.5	0.6	0.6	0.6	0.5
	03	0.4	0.5	0.6	0.5	0.5
	0.2	0.4	0.5	0.6	0.6	0.6
	0.3	0.5	0.6	0.7	0.7	0.7
Standard diclofenac sodium (8 mg/kg P.O.)	0.2	0.35	0.4	0.4	0.3	0.25
	0.2	0.3	0.45	0.5	0.4	0.3
	0.2	0.4	0.4	0.35	0.3	0.3
	0.3	0.45	0.5	0.5	0.35	0.35
	0.2	0.35	0.35	0.3	0.2	0.2
Sample Y_4	0.3	0.5	0.6	0.6	0.5	0.5
	0.2	0.3	0.5	0.6	0.4	0.4
	0.2	0.3	0.5	0.5	0.5	0.4
	0.3	0.4	0.5	0.5	0.4	0.4
	0.2	0.3	0.4	0.4	0.3	0.3
Sample Y_E	0.3	0.4	0.6	0.7	0.5	0.4
	0.3	0.4	0.5	0.5	0.4	0.4
	0.2	0.5	0.7	0.5	0.5	0.5
	0.3	0.5	0.6	0.6	0.5	0.4
	0.2	0.4	0.5	0.5	0.5	0.4

TABLE 3
ANTIINFLAMMATORY ACTIVITY: % PROTECTION BY *Parkia timoriana* (DC.) MERR.

Treatment	Mean ± SEM of paw volume (mL)						
	0 h	1 h	2 h	3 h	4 h	5 h	Protection (%)
Normal control (1 mL dist. water P.O.)	0.24±0.024	0.44±0.024	0.54±0.024	0.62±0.02	0.58±0.037	0.59±0.04	0
Standard diclofenac sodium (8 mg/kg P.O.)	0.22±0.02	0.37±0.025	0.42±0.025	0.41±0.04	0.31±0.033	0.28±0.025	50
Sample Y_4	0.24±0.024	0.36±0.04	0.5±0.031	0.52±0.037	0.42±0.037	0.4±0.031	28.58
Sample Y_E	0.26±0.024	0.44±0.024	0.58±0.037	0.56±0.040	0.48±0.02	0.42±0.02	25

6.4 ± 1.568. On calculating the percentage of inhibition of the separated components, Y_4 showed a percentage of writhing inhibition of 60.446 %, Y_2 showed a percentage of inhibition of 45.784 % while the extract, Y_E and the component Y_3 showed a percentage of inhibition of 26.506 and 27.7 %, respectively. Hence it was found that component Y_4 is the most active in analgesic property if compared with the standard Aspirin which has a percentage of inhibition of 80 %. The values were considered significant when $p < 0.001$, as compared with test groups vs control group.

It was observed that the paw volume started decrease after 3 h in case of the standard diclofenac sodium and also for the component Y_4 and the extract Y_E. The paw volume reduction continued even after 4 h just as it was found for diclofenac sodium. The percentage of protection of the component Y_4 was little more than the extract Y_E. The percentage of protection found for component Y_4 was 28.58 % and that of the standard 50 %, but considering that Y_4, a natural product and the standard, diclofenac sodium, a synthetic compound, the percentage of protection of Y_4 can be considered to be appreciably good as compared to that of the standard diclofenac sodium. The results were found to be highly significant ($p < 0.001$) in comparison to the control.

Separated components could not show any activity was not reported here. The most active compound Y_4 was subjected for structure elucidation.

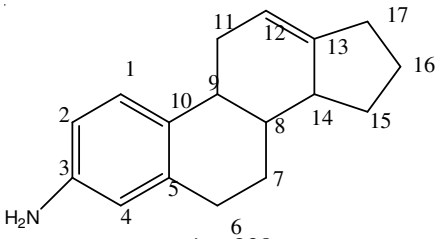
The melting point of the Y_4 compound was found to be 206-209 ºC. In the IR spectral studies in KBr pellets, the peak at 3342 cm^{-1} can be assigned to H-N-1º (aromatic) stretching. The peak at 2926 cm^{-1} can be correlated to -CH_3 stretching. The peak at 2853 cm^1 is due to >CH_2 sym stretching and that at 2721 cm^{-1} is due to >CH_2 asymmetry stretching. The peak at 1707 cm^{-1} is due to saturated acyclic C=O stretching. The peak at 1606 cm^{-1} is due to aromatic C-C stretching, at 1448 cm^1 for -O-CH_3, 1379 cm^{-1} for $(CH_3)_2$-C< symmetric stretching and that at 1151 cm^{-1} is for asymmetric stretching of $(CH_3)_2$-C<. The peak 1100 cm^{-1} is due to ⬭ and that at 1050 cm^{-1} is for ⬭. The peak at 972 cm^{-1} is due to CH=CH and that at 840 cm^{-1} is due to $C^{17}C^{14}C^{13}=C^{12}HC^{11}$ stretching.

In the ^{1}H NMR spectral analysis signal at 8.301(singlet) is for $^{22}CH_3$, 8.293 is for $^{20}CH_2$, 7.938 is for ^{4}C-H (Aro) and 6.901and 6.910 is for ^{1}C-H(Aro) and ^{2}C-H (Aro). The intense peak at 3.442 is for $^{23,24}CH_3$. Peak at 2.997(duplet) is due to 1º-ArNH_2, peak at 2.449 is for $^7CH_2$, 2.881, 2.888, 2.838 for ^{8}CH, ^{9}CH, ^{14}CH. The peaks at 2.770, 2.729, 2.721 is for $^{15}CH_2$, $^6CH_2$, $^{11}CH_2$, respectively. The peaks at 3.109, 3.029, 3.625 is for ^{17}CH, ^{12}CH and $^{16}CH_2$, respectively.

In the ^{13}C NMR spectra, the peak at 145.471 is due to ^{21}C, the peak at 83.688 is due to ^{18}C. The peak at 79.27 is due to ^{3}C and 79.143 is due to ^{2}C and ^{4}C, the peaks at78.944 and 78.609 are due to ^{1}C and ^{5}C, the peaks at 72.698 and 72.393 are due to ^{22}C and ^{10}C. The peaks at 71.943, 71.409 and 71.135 are for

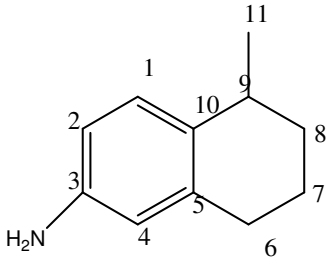
^{20}C, ^{19}C and ^{13}C, respectively. The peaks at 70.746 and 70.322 are for ^{12}C and ^{6}C. The peaks at 63.745 and 63.150 are for ^{17}C and ^{11}C, respectively. The peak at 59.741 is due to ^{14}C. The peak at 39.912 is due to ^{8}C and ^{9}C. The peak at 39.706 is due to ^{16}C. The peak at 39.508 is due to ^{23}C and ^{24}C. The peaks at 39.286 and 39.081 are due to ^{15}C and ^{7}C, respectively.

In mass spectral analysis of the compound showed a molecular ion peak at *m/z* 383. The base peak $(M + 1)^+$ was recorded as *m/z* 239 for $C_{17}H_{20}N^+$-

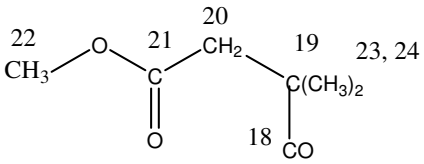


m/z = 239

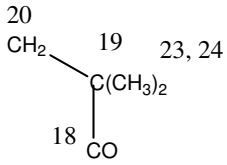
The other fragments $(m/z)^+$ obtained may be described as under-



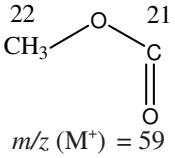
m/z(M^+) = 160



m/z (M^+) = 143

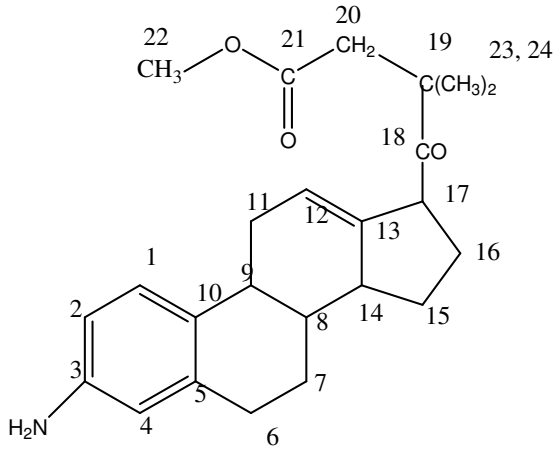


m/z (M^+) = 84



m/z (M^+) = 59

Based on the above spectral analysis the steroidal bioactive structure may be drawn as-



m/z = 383

Conclusion

The bioactive compound isolated from methanolic extract of *Parkia timoriana* (DC.) Merr. was found to be a steroidal component, which has analgesic and antiinflammatory activity.

REFERENCES

1. R.N. Almeida, D.S. Navarro and J.M. Barboso, *Phytomedicine*, **8**, 310 (2001).
2. K. Hostettmann, *Bull. Soc. Fib. Sci. Nat*., **76**, 51 (1987).
3. V.J. Galani and B.G. Patel, *Global J. Pharmacol*., **5**, 54 (2011).
4. A.B. Gokhale, A.S. Damre, K.R. Kulkarni and M.N. Saraf, *Phytomedicine*, **9**, 433 (2002).
5. A. Jain and E. Basal, *Phytomedicine*, **10**, 34 (2003).
6. D. Mantle, F. Eddeb and A.T. Pickering, *J. Ethnopharmacol*., **72**, 47 (2000).
7. B.K. Datta, S.K. Datta, M.M. Chowdhury, T.H. Khan, J.K. Kundu, M.A. Rashid, L. Nahar and S.D. Sarkar, *Pharmazie*, **59**, 222 (2004).
8. http://www.globinmed.com/index.
9. U.K. Yusuf and E.A.M. Zuhud, Plant Resources of South-East Asia No. 12(2): Medicinal and Poisonous Plants-II. Backhuys Publisher, Leiden, The Netherlands, pp. 404-408 (2001).
10. http://www.flowersofindia.net.
11. S.K. Kulkarni and M.K. Jain, in: A Handbook of Experimental Pharmacology, Vallabh Parkashan, Delhi, India, edn. 2, pp. 127 (1993).
12. C.A. Winter, E.A. Risley and G.W. Nuss, *J. Pharmacol. Exp. Ther*., **141**, 367 (1963).