Table 1 N-terminal sequence of the main peaks eluted from the chromatographic steps. Assignment of the peaks to protein families by BLAST against a *Tityus* venom database

Peak	N-terminal sequence	Protein family (Uniprot ID)
6	KDKMKAGWERLTSQSEYACP…	Ts19 Frag-II (P86822)
	KIKEKIIEAKDKMKAGWERL…	Ts19 Frag-I (P86822)
7	KKDGYPVEYDNCAYICWNYDNAY…	Ts3 (P01496)
	KDKMKAGWERLTSQSEYACPAID…	Ts19 Frag-II (P86822)
8	KIKEKIIEAKDKMKAGWERLTSQSEYACPAIDKFCEDHCAAKKAVGKCDDFKCNCIK	Ts19 Frag-I (P86822)
	KEGYAMDHEGCKFSCFIRPAGFCDGYCKTHLKASSGYCAWPACYCYGVPD…	Ts2 (P68410)
9	KIKEKIIEAKDKMKAGWERLTSQSEYA…	Ts19 Frag-I (P86822)
	KKDGYPVEYDNCAYICWNYDNAYCDKL…	Ts3 (P01496)
	KEGYAMDHEGCKFSCFIRPAGFCDGYC…	Ts2 (P68410)
10	GREGYPADSKGCKITCFLTAAGYCNTECTL…	Ts4 (P45669)
11	GREGYPADSKGCKITCFLTA…	Ts4 (P45669)
12	GREGY…	Ts4 (P45669)
13	KLVALIPNDQLRSILKAVVHKVAKTQFGCPAYEGYCNDHCNDIERKDG…	Ts8 (P69940)
	GLREKHVQKLVALIPNDQLRSILKAVVHKVAKTQFGCPAYEGYCNDHC…	Ts8 propeptide (P69940)
	GREGYPADSKG…	Ts4 (P45669)
9.2	KIKEKIIEAK…	Ts19 Frag-I (P86822)
	KEGYAMDHEG…	Ts2 (P68410)
9.3	KIKEKIIEAKDKMKA…	Ts19 Frag-I (P86822)
	KEGYAMDHEGCKFSC…	Ts2 (P68410)

…, the primary sequence was not completely determined

whose precursor, known as Ts19, was determined through a transcriptomic study of the Ts venom gland [33, 34]. Posteriorly, two mature fragments of Ts19, named Ts19 Frag-I and Ts19 Frag-II, were deposited in the UniProt databank [28; Swiss-Prot: P86822]. The post-translational engineering of Ts19 toxin and its fragments, named post-splitting, has been recently suggested. Moreover, Ts19 Frag-II presents a specific and significant blocking effect on Kv1.2 [35].

The corresponding molecular mass of the 57 amino acid residues of oxidized monoisotopic toxin (S-S) Ts19 Frag-I (peak 9.3) sequenced through Edman degradation was calculated as 6,458 Da. The average molecular mass of the same peak was determined as 6,575 Da through MALDI-TOF mass spectrometry, linear mode. The difference between these masses corresponds to the amino acid residue (Leu or Ile) of the C-terminal region. Since the Ts19 Frag-I shares high identity with the β-KTx-like toxins TstKMK from *T. stigmurus* and TtrKIK from *T. trivittatus* and with Ts19, which presents a Leu in the C-terminal, we deduced that the amino acid residue to complete the entire sequence from Ts19 Frag-I is Leu. These 58 amino acid residues were submitted to ProtParam, a tool that predicted the pI 8.57. The composition of Ts19 Frag-I contains a high content of Lys residues, which explains the predicted basic isoeletric point. A similar result was observed experimentally with Ts15 [36]. The theoretical mass of oxidized monoisotopic (S-S) Ts19 Frag-I (peak 9.3) calculated by the Sequence Editor was 6,571 Da, indicating the six cysteine residues that form three disulfide bonds, as observed in the β-KTx family [6]. Ts19 Frag-I was classified into the β-KTx class (subfamily) 2, since it shares high similarity with other β-KTxs belonging to this class (Fig. 5).

The Ts19 Frag-I presents nine additional amino acid residues in the N-terminal region when compared with Ts19 Frag-II. Interestingly, the N-terminal region of Ts19 Frag-I starts with the amino acid residues KIK. Other toxins that have KIK in their N-terminal region showed cytolytic, antimicrobial and hemolytic activities [7, 8]. The Ts19 Frag-II identified in the fractions VIIIA and VIIIB from Ts (the present work) was previously identified in the fractionation of Tsv on a C18 column and corresponds to 0.8 to 1.8 % of the total venom protein [37].

The peak 9.3 is constituted mainly (63.7 %) by Ts19 Frag-I (6,570.0 Da) and by peptides of 6,985.2 Da and 7,441.5 Da, whose N-terminal sequences corresponded to Ts2 and Ts3-KS, respectively. The respective theoretical molecular masses of oxidized monoisotopic (S-S) Ts2 and Ts3-KS calculated by the Sequence Editor are 6,985 Da and 7,442 Da [1], confirming that the proteins identified by Edman degradation are correct.