

ABSTRACT

(In Malaysia, fatal food poisonings associated with the consumption of puffer fish have occurred for decades, but the causative species or toxins have never been documented. To date, there has been little study on morphological and verification of toxin profiles on puffer fish especially on costal Sabah and Sarawak waters. Attempts was made to identify the morphology and verify the toxin profile in Tetraodontidae species of puffer fish commonly found in Sabah and Sarawak coastal waters.) Seven species puffer fish namely *Lagocephalus lunaris*, *Lagocephalus spadiceus*, *Lagocephalus sceleratus*, *Xenopterus naritus*, *Takifugu oblongus*, *Tetraodon nigroviridis* and *Arothron stellatus* were collected and morphological identified as well as toxin profile verification using biological mouse bioassay analysis and analytical Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Most significant characteristics that drawn attention for morphology identification of Tetraodontidae species is having beak like teeth structure consisting of two teeth at upper jaw and lower jaw. It is interesting to note that *Lagocephalus lunaris* and *Lagocephalus spadiceus* could be distinguish to different species by identified the distribution patterns of small spines on the dorsal body. Based on morphological identification, *Xenopterus naritus* and *Takifugu oblongus* were having high abundant of small prickle on lower part bodies compared to others collected species. With respect to *Arothron stellatus*, the distributions of small prickles were numerous on dorsal body with small black spot until to the tail. To isolate unknown toxin from puffer fish, five grams of different tissues such as muscle, skin, liver, gonad and eggs was minced and extracted with 0.1% acetic acid (AcOH). The crude toxin was assessed and preliminary screening for tetrodotoxin (TTX) detection by using biological mouse bioassay and analytical TLC analysis. Mouse bioassay

analysis revealed that all edible tissues of all collected species were non toxic and safe for human consumption except reproductive organ for *L. lunaris*, *X. naritus*, *T. oblongus* and *T. nigroviridis* where gave clinical neurotoxin symptom to the mice and lead to the death. TLC analysis gave positive detection of TTX by producing the fluorescent yellow spots for extracts of *L. lunaris*, *X. naritus*, *T. oblongus* and *T. nigroviridis* where R_f values were 0.78 and 0.22 with, pyridine: ethyl acetate: acetic acid: water (15:5:3:6) and 1-butanol: acetic acid: water (2:1:1), respectively. Meanwhile, for extracts of *Lagocephalus spadiceus*, *Lagocephalus sceleratus* and *Arothron stellatus* did not produce any single one fluorescent yellow spot in TLC analysis. Verification of toxin profile and quantitative toxin in extracted of puffer fish was carried out using HPLC, GC-MS and LC-MS. Quantitative results from these analyses indicate that *L. lunaris*, *X. naritus*, *T. oblongus* and *T. nigroviridis* collected from coastal Sabah and Sarawak waters confirmed exhibit tetrodotoxin (TTX) in extracted tissues based on verification using HPLC, LC-MS and GC-MS analysis by comparing the retention time of sample extracts to the retention time of authentic TTX. Meanwhile, there was no detection of TTX in *L. spadiceus*, *L. sceleratus* and *A. stellatus*. These finding is in agreement with the results from mouse bioassay and TLC analysis. From this study, it was clearly demonstrated that local puffer fish from coastal East Malaysian waters were exhibit TTX as toxin principles and the toxin profiles could be drawn with the aim of provide the informative data as a safety guidelines for local people in order to safeguard public health from puffer fish poisoning.

Keywords: Puffer fish, Morphology, TTX, HPLC, LC-MS, GC-MS

**KAJIAN MORFOLOGI DAN TOKSIN BAGI SPESIS IKAN BUNTAL DARIPADA
PERAIRAN SABAH DAN SARAWAK**

ABSTRAK

Di Malaysia, keracunan makanan yang dikaitkan oleh ikan buntal telah lama berlaku tetapi spesies atau toksin penyebab tidak direkodkan. Sehingga hari ini, hanya terdapat sedikit kajian terhadap morfologi dan profil toksin telah dijalankan terhadap ikan buntal terutama di perairan timur Malaysia iaitu Sabah dan Sarawak. Dalam kajian ini, pelaksanaan telah dilakukan untuk mengenalpasti morfologi dan toksin terlibat di dalam tisu ikan buntal yang telah ditangkap di perairan pantai Sabah dan Sarawak. Sebanyak tujuh spesies ikan buntal terdiri daripada spesies Tetraodontidae iaitu Lagocephalus lunaris, Lagocephalus spadiceus, Lagocephalus sceleratus, Xenopterus naritus, Takifugu oblongus, Tetraodon nigroviridis dan Arothron stellatus telah dikenalpasti ciri-ciri morfologi dan toksin profil disahkan menggunakan bioesei tikus, TLC, HPLC, GC-MS dan LC-MS. Hasil daripada kajian ini, menunjukkan bahawa ikan buntal mempunyai ciri-ciri morfologi yang unik kerana mempunyai struktur gigi yang seakan paruh dimana dua gigi berada di rahang atas dan dua gigi lagi berada di rahang bawah. Daripada hasil kenalpasti morfologi, Lagocephalus lunaris dan Lagocephalus spadiceus boleh dibezakan dengan mengenalpasti corak taburan duri kecil pada bahagian badan dorsal ikan tersebut. Manakala Xenopterus naritus dan Takifugu oblongus pula mempunyai taburan duri kecil yang banyak di bahagian bawah badan ikan tersebut. Hal ini berlainan dengan Arothron stellatus di mana ikan ini mempunyai taburan duri kecil dan bintik hitam yang banyak di bahagian dorsal badan ikan sehingga ke bahagian ekor. Beberapa analisis telah dijalankan untuk merekodkan toksikologi ikan buntal yang ditangkap di perairan pantai Sabah dan Sarawak. Lima gram tisu ikan yang berbeza seperti

isi, kulit, hati, gonad dan telur telah dicampurkan dengan 0.1% asid asetik dan telah dianalisis dengan menggunakan bioesei tikus, TLC, HPLC, GC-MS dan LC-MS. Hasil daripada analisis bioesei tikus menunjukkan bahawa tisu pembiakan daripada Lagocephalus lunaris, Xenopterus naritus, Takifugu oblongus dan Tetraodon nigroviridis adalah bertoksik dan memberikan simptom neurotoksin yang menyebabkan kematian kepada tikus tersebut. TLC analisis juga memberikan hasil positif untuk kehadiran tetrodotoksin di dalam sampel analisis dengan menghasilkan bintik kuning dengan nilai R_f masing-masing. Manakala untuk ekstrak tisu daripada Lagocephalus spadiceus, Lagocephalus sceleratus dan Arothron stellatus tidak menghasilkan bintik kuning dalam analisis TLC. Pengesahan profil toksin di dalam ekstrak ikan buntal telah dijalankan lebih lanjut dengan menggunakan HPLC, GC-MS dan LC-MS. Hasil kuantitatif daripada tiga analisis ini menunjukkan bahawa ekstrak daripada Lagocephalus lunaris, Xenopterus naritus, Takifugu oblongus dan Tetraodon nigroviridis yang telah ditangkap di perairan pantai Sabah dan Sarawak mengandungi TTX. Keputusan analisis ini telah disahkan dengan membandingkan R_t sampel yang diuji dengan R_t TTX tulen. Sementara itu, tiada pengesanan kehadiran TTX di dalam ekstrak tisu Lagocephalus spadiceus, Lagocephalus sceleratus dan Arothron stellatus. Hasil daripada analisis ini mengukuhkan lagi hasil kajian daripada analisis bioesei tikus dan TLC. Kajian ini menunjukkan bahawa ikan buntal yang ditangkap daripada perairan Sabah dan Sarawak mengandungi TTX sebagai sifat toksin dan profil toksin dapat dilakarkan untuk tujuan menyediakan data sebagai garis panduan bagi mengelakkan keracunan ikan buntal di kalangan orang tempatan.

Kata kunci: Ikan buntal, Morfologi, TTX, HPLC, LC-MS, GC-MS