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RESEARCH ARTICLE

PROFILING OF BIOACTIVE COMPOUNDS OF *AMARANTHUS DUBIUS* BY LC–MS/MS ANALYSIS

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ABSTRACT

Reliable and reproducible techniques for identification and quantification of bioactive compound usually require derivatization. However, techniques such as LC–MS/MS may perhaps side line the derivatization with significant accuracy. Several physiological processes apparently requires that the pool be studied in the seed itself. The present analysis revealed a higher concentration of bioactive compound especially Auranofin, Beauvericin, β-Carotein, conessine, Hyperforin, Hypericin, Morphine, Panaxydol, Primaquine, ropivacaine, Siphonaxanthin, Sphingosine, Strophanthidin, Sulfamerazine, syringin, Trimethoprim,Tuftsin, vincristine bioactive compound. The study constitutes first report of use of LC–MS/MS method for analysing the bioactive compound of amaranth grain**.**

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INTRODUCTION

Amaranth, also widely recognized as rajgira (king seed), ramdana (God's seed), seems to be a very nutritive pseudocereal with such an amazingly protein content when tried to compare instead to true cereal crops. Unlike most standard grains, including such wheat, rice, and corn, which have been low in lysine, amaranth is a great source of protein even though, when combined with many other cereals, it would provide a ‘‘balanced" protein content. Amaranth species are therefore receiving a lot of attention in trying to develop companies as a result to overcome protein malnutrition. (Escudero *et al*., 2006). However, these studies focused on investigation of bioactive compound and medicinal properties of these compound through LC-MS/MS analysis.

MATERIALS AND METHODS

A commercial seed sample of *Amaranthusdubius* was collected and used for the studies. A working standard mixture of 1 mg/L was prepared by diluting the intermediate stock standard solution, from which the calibration standards within the range 1–50 ng/mL were prepared by serial dilution with methanol.

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LC–MS/MS analysis: The Methanolic extract of *Amaranthusdubius* leaves studied with the help of 6200 series TOF/6500 series Q-TOF B.05.01 (B5125.3) LC-MS instrument. Precursor ions were selected in Q1 with an isolation width of 2 D and fragmented in the collision cell, applying a slope of collision energies in the range 5–45 eV. nitrogen is used as Collision gas and product ions were identified with a collision RF of 150/400 Vpp, transfer time of 70 ms, pre-pulse storage of 5 ms, pulse frequency of 10 kHz, and spectra rate of 1.5 Hz for collision-induced dissociation (CID) of in-source fragment ions, with the in-source CID energy increased from 0 to 100 V. Accurate mass spectra were acquired in the m/z range of 50–1000 at an acquisition rate of 2 spectra per seconds. Internal calibration was carried using signals at m/z 121.0509 (protonated purine) and 922.0098 (protonated hexakis (1H,1H, 3Htetrauoropropoxy) phosphazine) in positive mode. Both raw HPLC–QTOFMS (Agilent 6540 UHD QTOF LC-MS) full single MS and MS/MS data, and for data mining based on molecular formulae estimations and fragment patterns was processed with Mass Hunter Workstation software (Qualitative Analysis). Using the algorithm employed for full single MS data, ions with identical elution profiles and related m/z values (representing different isotopes of the same compound) were extracted by molecular features extraction (MFEs).

RESULT AND DISCUSSION

The content of 18 Bioactive compound studied using LC–MS/MS from seed sample of leaves amaranth has been summarised in Table 1.

Table No. 1. Profiling of bioactive compounds of *Amaranthusdubius*byLC–MS/MS analysis

Name	Formula	Score	Mass	RT	Properties	Reference
Auranofin	C20 H34 Au O9 P S	61.06	678.1335	5.5008	Colorectal Cancer Cells	Marzo*et al*., 2017
Beauvericin	C45 H57 N3 O9	87.78	783.9602	7.5621	Antioxidant capacity, Antifungal Miconazole Activity	Mallebrera*et al*., 2017,
β-Carotein	C40 H56	66.55	536.4365	5.6594	Antioxidant Activity	Mueller and Boehm, 2011
Conessine	C24 H40 N2	82.94	356.3195	1.1669	Anti-malarial property, Antibacterial Activities	Dua*et al*., 2013. Patrice *et al*., 2007
Hyperforin	C35 H52 O4	75.38	536.3872	20.5162	HT-1080 (Epithelial), C-26 (colon), Human Myeloid Tumor Cells	Dona *et al*., 2004. Merhi*et al*., 2011
Hypericin	C30 H16 O8	64.52	504.0853	19.2992	Antifungal properties	Sytar*et al*. 2016
Morphine	C17 H19 N O3	72.82	285.1366	8.4056	Antioxidant properties	Gulcin*et al*., 2004
Panaxydol	C17 H24 O2	79.99	260.1786	8.5745	HepG2 cell line (human Hepatocarcinoma)	Guo*et al*., 2009
Primaquine	C15 H21 N3 O	81.81	259.1696	6.4646	HL60 Cells (Leukemia), Antiplasmodial Agents	Yan *et al*., 2011.
Ropivacaine	C17 H26 N2 O	65.78	274.2031	6.8633	Antifungal Activity	Oki *et al*., 1989.
Siphonaxanthin	C40 H56 O4	70.55	600.4186	18.0252	HL60 Cells (Leukemia). Anti-Obesity effect	Ganesan*et al*., 2011. Li and Zhuosi, 2015
Sphingosine	C18 H37 N O2	74.11	299.2841	11.692	Lung cancer. Bone repair	French *et al*., 2010. Sartawi*et al*., 2017.
Strophanthidin	C23 H32 O6	81.71	404.2199	8.6531	Lung cancer	Reddy *et al*., 2019
Sulfamerazine	C11 H12 N4 O2 S	77.32	264.0697	4.9936	Antimicrobial Activity	Othman *et al*., 2020
Syringin	C17 H24 O9	56.29	372.1406	15.8456	Antiulcer activity	Sundaram and Rao, 2016
Trimethoprim	C14 H18 N4 O3	73.9	290.1382	6.4883	Antimicrobial Activity	Araujo *et al*., 2017
Tuftsin	C21 H40 N8 O6	78.83	500.3073	20.7226	L 210 leukaemia cells	Nishioka, 1979
vincristine	C46 H56 N4 O10	82.86	824.3956	10.7573	Gastric Cancer Cell Line	Xue*et al*., 2012

The sample was analysed by LC–MS/MS and the peaks were compared with those of reference compounds analysed under the same conditions. Above compundssome bioactive properties like anti oxidants, anti malarial, anti bacterial etc.Compounds like sphingosinestrophanthidin show activity against lung cancer. Auranofin shows activity against Colorectal Cancer, Vincristine shows activity against gastric cancer. This investigation revealed that *A. dubius*consist medicinally and nutritionally important compounds *A. dubius* can used in pharmaceutical industries to produce essential drugs for disease like malaria or cancer etc.

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