


Musculoskeletal manifestations in Alkaptonuria

A cross-sectional study

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Abstract

This study aimed to determine the patient characteristics and clinical presentation of Alkaptonuria cases reported by the Biochemical Genetics Lab.

An observational study was conducted at the Biochemical Genetics Lab. Alkaptonuria patients were diagnosed based on the homogentisic acid peak in urine and their demographics and clinical data collected from 2013 to 2019. Clinical history related to joint diseases, ochronotic presentation, and urine darkening on standing was collected.

During 7 years, 21 Alkaptonuria cases were reported from BGL; mean age 19.4 ± 24.5 years (range 0.2–66 years) and male to female ratio of 2:1. Of the total, only 9 were adults (mean age, 44 ± 12 years). Most adult patients had musculoskeletal involvement, with joint pain ($n=9$) and ochronotic pigmentation ($n=6$), whereas all patients presented with a history of urine darkening on standing (21/21 cases).

The high prevalence of musculoskeletal involvement observed in patients with albuminuria is likely to be missed by physicians unless specifically tested for in such cases.

Abbreviations: BGL = Biochemical Genetics Lab, ERC = ethical review committee, GC-MS = gas chromatography-mass spectrometry, HGA = homogentisic acid, HGO = homogentisate 1, 2-dioxygenase, UOA = urine organic acid.

Keywords: alkaptonuria, homogentisic acid, musculoskeletal, Pakistan

1. Introduction

Alkaptonuria is a rare autosomal recessive metabolic disorder caused by absolute or relative deficiency of the hepatic enzyme

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Availability of data and material: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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homogentisate 1, 2-dioxygenase (HGO).^[1] The true incidence of alkaptonuria is unknown, and there is wide variability in the reported incidences. For example, in the National Registry of the UK, the incidence of albuminuria is 1 in 500,000, whereas it is 25 in 500,000 in Slovakia, the Dominican Republic, and the Middle East. This high incidence in Slovakia, the Dominican Republic, and the Middle East could be due to the high rate of intermarriages in these populations.^[2–5]

The enzyme HGO forms an intermediate metabolite, homogentisic acid (HGA), in the tyrosine degradation pathway, which appears in urine when HGO is deficient, as shown in Figure 1.^[6] The HGA is oxidized to black-colored benzoquinone acetic acid, which causes the urine of patients to turn black on standing and is a pathognomonic finding in patients with albuminuria.^[7] Benzoquinone acetic acid is converted into a melanin-like pigment, which polymerizes with connective tissue, discoloring the tissues, and making them brittle, weak, and susceptible to rupture.^[8,9] This pigment deposition leads to poor quality of life and significant alkaptonuria-induced multimorbidity.^[10]

The present study aimed to determine the patient characteristics and clinical presentation of Alkaptonuria cases reported by the Biochemical Genetics Lab (BGL).

2. Materials and methods

An observational study was conducted at BGL in the Department of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan. Approval from the institute's Ethical Review Committee (ERC) was obtained (5085-Pat-ERC-17) before the commencement of the study. All patients tested for urinary organic acid

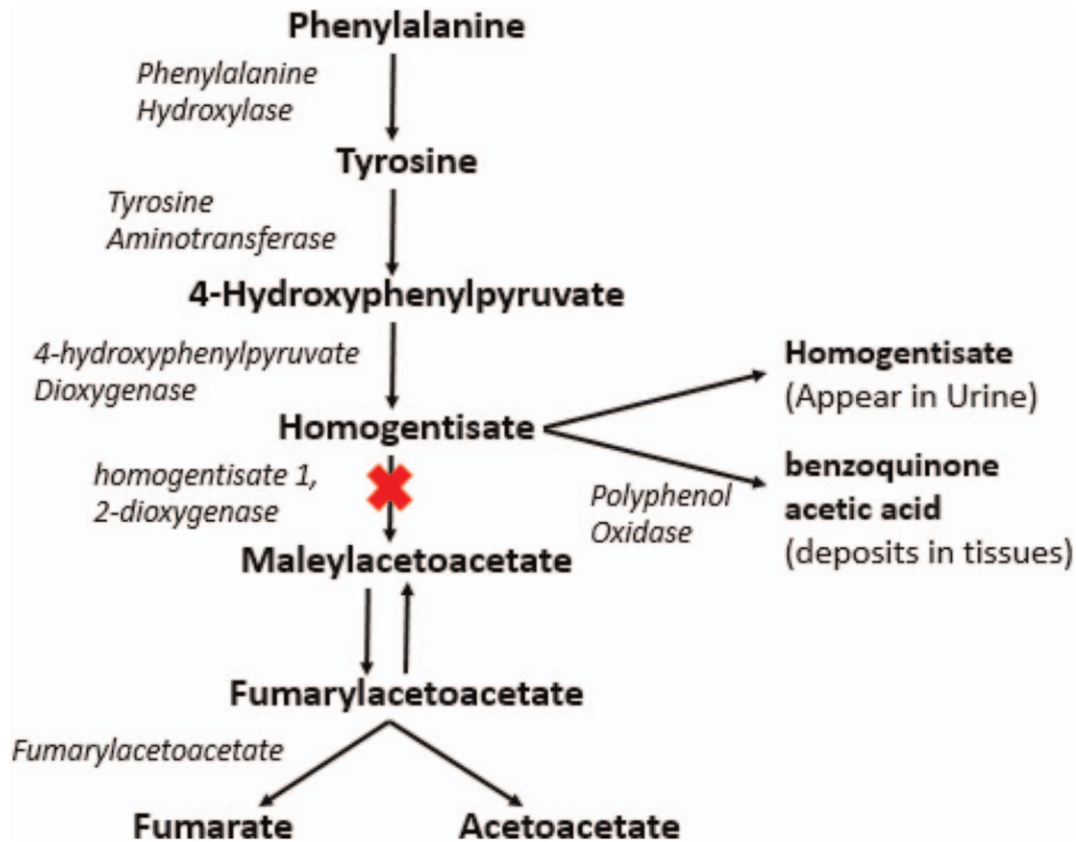


Figure 1. The metabolic pathway of tyrosine metabolism, showing block in the pathway at the level of homogentisate 1, 2-dioxygenase leading to Alkaptonuria.

(UOA) analysis from January 2013 to December 2019 were reviewed, and only those who presented with alkaptonria, including those diagnosed with other inherited metabolic disorders, were excluded. Clinical details were collected by telephone interviews with patients or guardians after verbal informed consent. Details including demographics, birth, drug and family history, symptomatology, and clinical manifestations related to joint involvement were captured on a structured clinical history form.

Urine organic acid was analyzed by gas chromatography-mass spectrometry (GCMS) on an Agilent GC-MS system (Agilent Technologies, US) using ethyl acetate and derivatization by bis (trimethylsilyl) acetamide compound. The 3, 3 Dimethyl glutaric acid is used as an internal standard. Patients were diagnosed with Alkaptonuria based on the presence of an HGA peak on the UOA chromatogram, appearing at a retention time of 16 minutes (Fig. 2A). Ion spectrum matching of HGA was performed with the qualifying ion reference library (Fig. 2B).

3. Results

Twenty-one cases of alkaptonuria were reported over a 7-year period. The mean and standard deviation of the patients was mean age 19.4 ± 24.5 years (range 0.2–66 years) and male to female ratio of 2:1 (Table 1). Nine patients presented after the second decade of life, whereas 8 cases presented within the infantile period. Parental consanguinity was present in 15 of 21 patients with alkaptonuria. The pathognomonic feature of urine darkening on standing or dark-colored staining of underclothing

was observed in all patients (21/21). Most patients had symptoms for 12.2 ± 6.6 years (range 3–25 years).

Musculoskeletal involvement, low back pain, and stiffness followed by chronic knee joint pain were observed in adult patients only; joint pain in 9 of 21 patients (Supplemental figure 1, <http://links.lww.com/MD/G553> shows the knee radiographs of an AKU patient). Ochronotic pigmentation on the face/hands and ear cartilage was seen in 4 and 2 patients, respectively. Apart from 1 patient with mixed renal stones, none of the patients developed any cardiac or renal complications associated with albuminuria. All patients were symptomatically treated with ascorbic acid, NSAIDs, prescribed a low-protein diet, and physiotherapy, but none were on Nitosone therapy.

4. Discussion

Alkaptonuria, a rare disease caused by subtle signs and symptoms, is frequently under-diagnosed. Only 12 cases have been reported from Pakistan until 2019, with the first report published in 2005. Patients are often evaluated for porphyria, which also has the same feature of darkening of urine while standing as Alkaptonuria. These patients are often diagnosed late, which leads to poor quality of life. In infants, the staining of diapers is an essential early diagnostic clue for albuminuria, which parents and physicians often miss. In addition, fewer laboratories are performing sensitive and specific tests for the diagnosis of alkaptonuria, which have led to the loss of patients. Moreover, this became evident after the availability of the right diagnostic tool (UOA evaluation by GCMS), 21 cases were

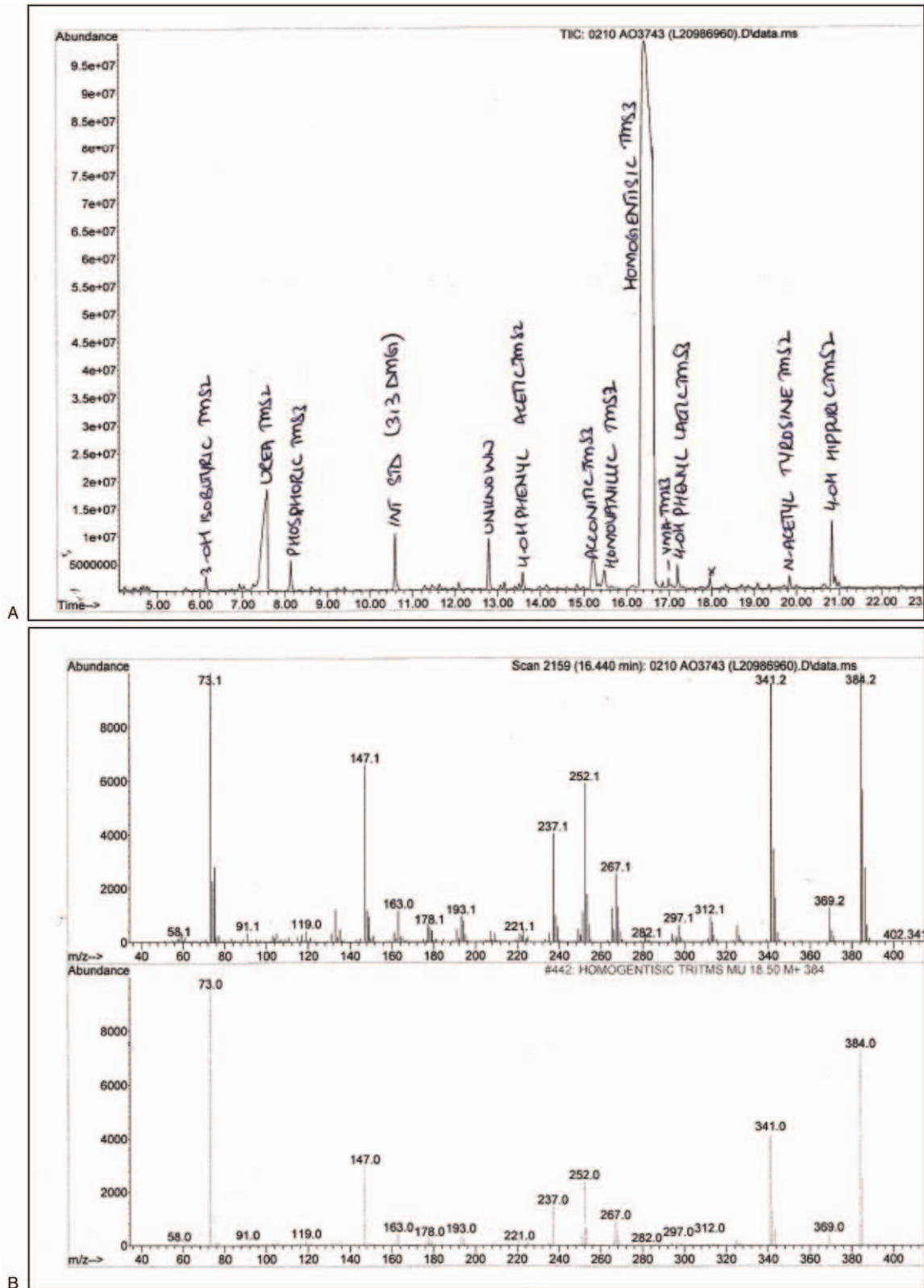


Figure 2. (A) Urine organic acid chromatogram performed by gas chromatography-mass spectrometry, showing a peak of homogentisic acid (HGA). The x-axis represents the retention/elution time, while the y-axis represents the abundance of molecules. HGA appears as a trimethylsilyl derivative at a retention time of 16 minutes, measured against an internal standard (3,3 dimethylglutarate). HGA is reported as a large, moderate, or small peak relative to the peak of the internal standard. (B) Qualifying ion spectrum of homogentisic acid. The x-axis shows the mass to charge ratio, while on the y-axis is the abundance of molecules, the above picture shows the qualifying ion spectrum of an Alkaptonuria patient compared to the mass to charge ratio of homogentisic acid from qualifying ions reference library shown in the lower figure. The qualifying ions of homogentisic acid were 341, 284, and 252.

Table 1
Clinical presentation of patients with alkaptonuria reported by BGL.

No.	Year of UOA testing/publication	City of residence	Sex	Presenting age in years	Duration of symptoms in years	Common clinical features		
						Ochronotic pigmentations	Joint pain	Urine darkening on standing
Biochemical genetics laboratory data, n=12 (2013–2019)								
1.	2019	Nawab Shah	M	66	25	Yes	Yes	Yes
2.	2019	Rawalkot	F	47	15	Yes	Yes	Yes
3.	2019	Karachi	F	0.8	–	No	No	Yes
4.	2019	Peshawar	M	1	–	No	No	Yes
5.	2019	Rawalkot	M	36	7	No	Yes	Yes
6.	2018	Lahore	M	0.5	–	No	No	Yes
7.	2018	Hyderabad	M	2.2	–	No	No	Yes
8.	2018	Karachi	M	0.2	–	No	No	Yes
9.	2018	Karachi	F	1.5	–	No	No	Yes
10.	2018	Karachi	M	1.7	–	No	No	Yes
11.	2017	Lahore	M	28	10	No	Yes	Yes
12.	2017	Rawalpindi	F	35	8	Yes	Yes	Yes
13.	2017	Karachi	M	1.8	–	No	No	Yes
14.	2017	Karachi	F	0.75	–	No	No	Yes
15.	2016	Karachi	F	0.9	–	No	No	Yes
16.	2016	Lahore	M	55	20	Yes	Yes	Yes
17.	2016	Hyderabad	M	0.1	–	No	No	Yes
18.	2016	Lahore	M	57	15	Yes	Yes	Yes
19.	2015	Lahore	M	0.3	–	No	No	Yes
20.	2015	Lahore	M	40	7	No	Yes	Yes
21.	2014	Rawalpindi	F	33	3	Yes	Yes	Yes

F=female, M=male, UOA=urine organic acid.

diagnosed in only 7 years, whereas literature reports only 12 cases from Pakistan in >70 years.

5. Characteristics of Alkaptonuria patients reported from Pakistan

A review of published literature from 1947 to 2019 identified 9 case reports and 1 original study reporting alkaptonuria in 3 patients from Pakistan (Table 2). In all the reports except one, the presence of HGA in urine was confirmed by colorimetry; 1 study utilized paper chromatography to identify HGA, whereas none utilized GCMS for UOA analysis.^[6–8,11–13] The medical specialties where patients presented initially included medicine (n=3), rehabilitation medicine (n=2), dermatology (n=3), laboratory (n=3), and radiology (n=1). A male predominance was observed in these case reports. Only 1 report mentioned the consanguinity of the patient's parents. The most common age of presentation was in the fourth or fifth decade of life, with 1 case presented in their 20s and infancy. Clinical details were available for only 9 patients. Adult patients had symptoms for 10 to 15 years. A family history of alkaptonuria was provided in 4 of 9 case reports, and 1 to 2 siblings of each patient were affected. Musculoskeletal involvement was seen in all cases from the literature; all cases had joint pain, 5 of 9 also reported restriction movements, whereas 1 patient each had a height loss of 15 cm and flexion contractures. Except for 1 patient who developed renal stones, none had cardiac or renal complications. Arthritis, notably in the spine and large joints (Ochronotic musculoskeletal manifestations), was a common presentation observed in both patients reported from BGL and in a literature review from Pakistan.

Long-standing arthritis in patients with albuminuria can be severe and disabling.^[14,15] Typically, OCM begins in the third

decade of life, which is earlier than autoimmune musculoskeletal manifestations and progresses more rapidly in males than in females.^[13,14] Ochronotic pigmentation was observed in the ear cartilage and hands/face in 2 and 6 cases, respectively. Ochronotic pigmentation was frequently reported in the literature, whereas in the BGL data, only 6 of 21 patients had it. Clinical data obtained from BGL also identified arthritis as the most common presentation in patients. Most of these patients had symptoms for 3 to 25 years before being diagnosed with albuminuria, which may be due to the slow progression and limited awareness regarding this disease. Physicians involved in managing arthritis must specifically consider albuminuria in their differentials and inquire about other symptoms, notably the darkening of urine while standing.^[15]

All patients with kaptonuria reported from the BGL and in the published literature were symptomatically treated and were mainly on analgesics. Few of the patients were on vitamin C supplementation; however, none were on dietary restriction, low-protein diet, physiotherapy, or nitisinone therapy. Few patients did not know which specialist to contact and had seen several general practitioners for their complaints.

The standard treatment for alkaptonuria aims to target specific symptoms of each individual. Pain management is tailored according to a person's symptoms, and narcotics may also be prescribed if needed. Dietary protein restrictions are usually ineffective in patients with albuminuria and can lead to further complications.^[10] Vitamin C supplementation is advised, as it prevents the accumulation and deposition of HGA. However, studies are lacking regarding its effectiveness in long-term usage. Nitisinone, an FDA-approved drug for tyrosinemia, is presently under clinical trials for its potential use in Alkaptonuria.^[16] Research has shown that it reduces HGA formation by diverting the metabolism toward the formation of 4-hydroxyphenyl lactic

Table 2**Review of case reports published from Pakistan on Alkaptonuria (1996–2016).**

S#	Publication year	Specialty of presentation	Sex	Age at presenting. y	Duration of symptoms	Family history of AKU	Clinical presentation
1	1996	Medicine (Peshawar)	M	45	11	A brother and niece have AKU	Arthritis (wrists, elbows, shoulders, knees, ankles) Restricted spinal movements. Ochronotic pigmentation of ear cartilage Brownish discoloration of sclera Renal stones which turn black on exposure to air Staining of vests and clothes by his sweat.
2	2008	Pediatrics (Lahore)	F	1 month	Not recorded	Elder sister (6 y) has AKU	Urine darkens on standing
3	2009	Dermatology (Karachi)	M	48	10	Not asked	Recurrent joint pains. Swollen and tender left knee joint. Difficulty walking. Ochronotic pigmentation of hands. Bluish yellow hyperkeratotic papules and plaques along the margins of palms, sides of fingers and thumbs suggestive of Keratoelastoidosis marginalis. Similar pigmentation was also prominent on his cheeks, forehead, ear lobes and axillae. Nails were markedly discolored. Eyes showed dark brown to black pigment deposition
4	2010	Radiology/pathology (Lahore)	M	38	2	Not present in family	Pain in joints (knees, shoulders, elbows, lower back) Progressive stiffness & restricted movement of spine Ochronotic pigmentation of ear cartilage
5	2005	Dermatology (Quetta)	M	26	3	Not present in family	Pain in joints (lower back, knees) Ochronotic pigmentation of ear cartilage Urine darkens on standing Black cerumen in ears
6	2013	Medicine (Azad Kashmir)	M	55	15	Brother has it	Pain in joints (lower back) Difficulty in walking, uses 2 sticks to support himself. Presence of kyphosis. Over the years, Macular and Hyperkeratotic papular ochronotic discolouration of skin over face and hands. Osler spots on sclerae.
7	2016	Rehabilitation medicine (Lahore)	M	48	15	Father has it	Chronic pain in lower back and both knees. Loss of height from 165 cm to 150 cm. Ochronotic papules on bilateral extensor surface of second finger. Gray pigmentation of both sclerae.
8	2016	Rehabilitation medicine (Lahore)	F	60	20	Not present in family	Chronic aching pain in both knees and dorsal spine. Flexion contractures of both knees. Bilaterally knee crepitus present. Ochronotic patches over her nose, proximal palms, index fingers, thumbs, dorsal foot. Macules in sclera and over face, ears, and knees.

acid.^[17] There is a limited global experience of long-term outcomes of nitisinone treatment and its effectiveness in young children. More research is needed to evaluate its effect on preventing the deposition of quinone derivatives of HGA in connective tissues and its safety or effectiveness in long-term usage in all age groups. Approximately half of the patients with albuminuria require surgery of their hip, knee, or shoulder joints, usually by the fifth or sixth decade of life.^[18] Physiotherapy and occupational therapy are also advised to patients, as they help improve joint mobility and maintain the strength and flexibility of muscles and joints.^[19]

To the best of our knowledge, this is the first national-level data from Pakistan reporting the clinical spectrum of albuminuria in our population. However, this study has several limitations, including a cross-sectional study design, a small number of Alkaptonuria cases, and the nonavailability of a confirmatory

enzyme or mutational analysis. In addition, detailed clinical examination of the joints could not be performed in patients to determine the cause of musculoskeletal manifestations.

6. Conclusion

The present study suggests that the burden of Alkaptonuria goes unnoticed and not documented until chronic symptoms and signs of HGA deposition in tissue develop. The prevalence of albuminuria in Pakistan may be higher than that reported in the literature. Alkaptonuria is a rare autosomal recessive disorder of metabolism that is likely to be missed by physicians as a cause of musculoskeletal manifestations unless individually examined in such cases. There is a need to raise awareness among health practitioners to look for albuminuria in unexplained musculoskeletal manifestations.

Author contributions

AHK was included in conceiving ideas, reviewing chromatograms, and critically reviewing the manuscript for intellectual content. BA reviewed the manuscript for intellectual content. HM was involved in the data analysis and writing manuscripts. YZ was involved in data collection, literature search, and manuscript writing. AJ was involved in the UOA analysis and chromatogram review. LJ was included in reviewing chromatograms, literature searches, and critically reviewed the manuscript for intellectual content.

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