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Metabolomic and elemental analysis of camel and bovine urine by GC–MS and ICP–MS



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Abstract Recent studies from the author's laboratory indicated that camel urine possesses antiplatelet activity and anti-cancer activity which is not present in bovine urine. The objective of this study is to compare the volatile and elemental components of bovine and camel urine using GC–MS and ICP–MS analysis. We are interested to know the component that performs these biological activities. The freeze dried urine was dissolved in dichloromethane and then derivatization process followed by using BSTFA for GC–MS analysis. Thirty different compounds were analyzed by the derivatization process in full scan mode. For ICP–MS analysis twenty eight important elements were analyzed in both bovine and camel urine. The results of GC–MS and ICP–MS analysis showed marked difference in the urinary metabolites. GC–MS evaluation of camel urine finds a lot of products of metabolism like benzene propanoic acid derivatives, fatty acid derivatives, amino acid derivatives, sugars, prostaglandins and canavanine. Several research reports reveal the metabolomics studies on camel urine but none of them completely reported the pharmacology related metabolomics. The present data of GC–MS suggest and support the previous studies and activities related to camel urine.

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1. Introduction

Urine is produced in mammals by the kidneys. It is transparent, sterile and slight yellowish in nature. The urine contains urea, amino acids, creatinine, organic acids, ammonia, toxins and inorganic salts. All these components are water soluble so they are easily excreted. The medical use of human urine and its extracts has been known for centuries ([Armstrong](#),

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1971; Burzynski et al., 1977). Urine analysis can be performed to study various types of renal disorders like bladder, ovary and kidney diseases. To achieve and identify the polar metabolites of the camel and bovine urine, we widely used the GC–MS technique.

The milk and urine of camel are reported to be useful in treating several diseases (Al-Haider et al., 2011). Camel urine has a lot of chemical constituents which can act as antibacterial, antifungal, antiviral and anticancer agents (Al-Yousef et al., 2012). In the Arabian region, people usually wash their hair with urine. Camel urine is used in Asian countries to treat diabetic neuropathy (Agarwal et al., 2009). Camel milk has been studied for its medical value in relation to chronic diseases like hepatitis (Sharmanov et al., 1982), peptic ulcer (Sharmanov et al., 1981) and food allergies (Shabo et al., 2005). Camel milk also shows antimicrobial and antiviral activities which are mainly due to the presence of lysozyme and lactoferrin, respectively (Ikeda et al., 2000; Benkerroum et al., 2004; Redwan et al., 2014). The published data for camel urine is less and some reports support that it has anticancer and antiplatelet activities.

The therapeutic efficacies from clinical studies of camel urine were investigated by some of researchers (Ohaj, 1993; Ohag, 1998). Profiling from NMR and GC–MS of urinary acids and metabolites is very useful tool in these days. These days, there is an urgent need to explore, identify and characterize the components present in camel urine.

Camel urine showed potent platelet inhibition activity (Al-Haider et al., 2011) and anticancer (Al-Harbi et al., 1996; Al-Kabarity et al., 1987; Al-Haider et al., 2014) activity which is not detectable in bovine and human urine. The aim of the current study was to identify and characterize the camel urine components that contribute to the anticancer and antiplatelet effects. The results and data of this work could be useful in linking the composition of bovine and camel urine with various biological activities.

2. Materials and methods

2.1. GC–MS

2.1.1. Sample collection

Camel urine was obtained from healthy, virgin and lactating domesticated camels (camel dromedaries). All animals were female and aged between 2 and 10 yr. All the animals were raised on a private farm, were disease free and were provided free access to water and feed. Camel urine was obtained during feeding with the help of experienced camel attendants. Approximately 250–300 ml of urine sample from each of the animals was collected. Urine was first collected directly into stainless steel containers and then transferred to suitable glass vials. Urine samples were then carried out to the laboratory and stored at -80°C till further use. Bovine urine was also collected by the same process.

2.1.2. Sample preparation

Various solvents like ethyl acetate, methanol, chloroform, petroleum ether and dichloromethane (DCM) were selected and tried for extraction of the urinary components. DCM was

selected on the observation that it has a low boiling point and most of the urinary components were easily dissolved in DCM and the derivatization reaction was completed within 20 min.

2.1.3. Derivatization

Freeze dried samples were dissolved in a suitable amount of DCM. The samples were transferred to GC vials in an appropriate aprotic solvent such as DCM. Traces of methanol were removed because it could react with the reagent. About 80 μL of BSTFA and 50 μL of pyridine were added to the sample. This amount is enough for a sample containing 100 μg of total derivatizable material and dissolved in 100 μL of solvent.

The vials were capped tightly which were heated at 65°C for 20 min. The heating step was performed to ensure the completion of reaction. After heating, the samples were allowed to cool down at room temperature and injected on the GC/MS. Derivatized samples were stored in the freezer in order to extend their lifespan. BSTFA is reported to be very corrosive for metal syringe needles and plungers. Therefore, the rinsing solvents were properly labeled and all syringes that come in contact with BSTFA were cleaned thoroughly, first using methanol and then DCM. This also includes the autosampler syringe on the GC/MS. Finally the vials were then tightly screwed and stored in the refrigerator until GC–MS analysis.

The GC–MS analysis was performed in a Perkin Elmer Clarus 600 gas chromatograph linked to a mass spectrometer (Turbomass) available at Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. An aliquot of 2 mL of extract was injected into the Elite-5MS column of 30 m, 0.25 μm film thickness, and 0.25 mm internal diameter.

2.1.4. Capillary column using the following temperature program

The GC–MS system starts with the initial oven temperature of 60°C for 5 min, increasing to 240°C at a rate of 15°C for 5 min, and then to 300°C at a rate of 15°C for 5 min. The injector temperature was maintained at 200°C . The interface temperature was 250°C . Helium was used as a mobile phase at a flow rate of 1.0 mL/min. Mass spectral detection was carried out in electron ionization mode by scanning at 40–600 (m/z). Finally, unknown compounds were identified by comparing the spectra with that of the National Institute of Standard and Technology library. The total time required for analyzing a single sample was 31 min.

2.1.5. Metabolites detected by GC–MS

The metabolomics study of urine by GC–MS gives a lot of information regarding the volatile products. In this experiment the comparison between the cows (Table 1) and camel urine (Table 2) showed entirely different components depending on the climate, food and categories. A total of 33 detectable peaks were selected for metabolite identification in the National Institute of Standard and Technology (NIST) 2005 Library and Wiley Access Pak v7 May, 2003 library. Approximately 14 components are identified in bovine and 20 components are identified in camel urine on the basis of Match factor. Most of the volatile components are identified are alcohols, alkanes, acids, amines, sugars and ketones.

Table 1 Urinary components present in bovine urine.

S. No	Name	RT	Area	Height	N area %	Area %
1.	Propanedioic acid	11.2	60,824	1,187,105	1.3	0.6
2.	Glycine	12.1	40,126	443,992	0.9	0.4
3.	L-Alanine	11.8	74,424	826,929	1.6	0.8
4.	Dihydroxyacetone	12.7	22,139	427,901	0.5	0.2
5.	Butanedioic acid	15.0	288,059	1,761,715	6.2	2.8
6.	Hippuric acid	15.4	4,638,166	105,749,376	100.0	45.7
7.	Ribitol	16.4	39,684	461,918	0.9	0.4
8.	D-Glucuronic acid	16.5	55,984	508,308	1.2	0.6
9.	Hexadecanoic acid	16.6	90,162	2,308,675	1.9	0.9
10.	Myo-inositol	16.8	249,413	5,282,377	5.4	2.5
11.	Trans-9-octadecanoic acid	17.7	612,651	9,735,627	13.2	6.0
12.	Octadecanoic acid	17.9	83,853	2,069,838	1.8	0.8
13.	Arabinofuranose	19.3	37,164	574,082	0.8	0.4
14.	Prostaglandin F1A	24.0	17,981	173,956	0.4	0.2

Table 2 Urinary components present in camel urine.

S. No	Name	RT	Area	Height	N area %	Area %
1.	Pyrotartaric acid	11.2	35,371	903,663	0.2	0.1
2.	Propane dioic acid	12.3	175,789	881,756	0.9	0.6
3.	Aminomalonic acid	12.5	796,841	16,794,296	4.2	2.6
4.	Erythritol	12.8	162,312	3,961,203	0.8	0.5
5.	Canavanine	13.0	574,171	6,362,680	3.0	1.9
6.	Creatinine	13.2	269,862	5,460,907	1.4	0.9
7.	Galactose	14.0	60,467	349,291	0.3	0.2
8.	Ribitol	14.8	27,077	584,557	0.1	0.1
9.	Azelaic acid	15.0	316,206	2,567,412	1.6	1.0
10.	Hippuric acid	15.4	19,138,354	386,707,360	100.0	63.0
11.	N-Phenyl acetyl glycine	15.6	2,228,299	44,340,772	11.6	7.3
12.	D-Glucuronic acid	16.4	122,990	1,337,466	0.6	0.4
13.	Hexadecanoic acid	16.6	101,347	2,436,548	0.5	0.4
14.	Benzene propanoic acid	16.8	69,294	1,200,123	0.4	0.2
15.	Trans-9-decanoic acid	17.7	583,098	7,878,695	3.0	1.9
16.	Prostaglandin F1A	18.0	8840	156,630	0.1	0.08
17.	Psuedo uridine	18.6	143,953	2,744,591	0.8	0.5
18.	Melibiose	22.6	154,818	2,361,507	0.8	0.5
19.	D-Galactose	23.3	40,949	781,510	0.2	0.1
20.	2-Deoxy-galctopyranose	23.5	213,684	3,725,774	1.1	0.7

2.2. ICP-MS

2.2.1. Sample preparation for ICP-MS analysis

One gram of freeze dried sample and 50 ml of 20% nitric acid was added to an Erlenmeyer flask. The mixture was heated to 70–85 °C for 48 h. During the heating period the volume of the flask was maintained at the same level intermittently adding 20% nitric acid. After the completion of digestion the contents of the Erlenmeyer flask were filtered using a Nalgene filter (Thermo scientific) unit. The filtrate was collected in a 100 ml volumetric flask and allowed to cool. After cooling the volume was made up to 100 mL using Milli Q water and analyzed with ICP-MS.

2.2.2. ICP-MS analysis

For the elemental analysis of bovine and camel urine the instrument ELAN-DRC-II, Perkin Elmer, USA was utilized. The instrumental setting details for the analysis done are given in the Table 3. Other details are given below.

2.2.3. Calibration of ICP/MS

In the present study, all instrument optimizations were performed by using about 1 ppb tuning solution containing various elements such as Mg, Co, Pb, Na, Fe, Cu, Be, In, Se, U, Rh, Pb and Ba in 1% HNO₃. This includes various aspects of instruments such as mass calibration, resolution, nebulizer gas flow, AutoLens calibration and daily performance checks.

Table 3 Instrumental setting (ELAN-DRC II, Perkin Elmer) for the ICP/MS based elemental analysis of Bovine and Camel freeze dried urine.

ELAN-DRC II, Perkin Elmer Parameters for the analysis of camel and bovine freeze dried urine	
RF Power: 1100 watts	
Plasma gas flow: 18 L/min	
Auxiliary gas flow: 1 L/min	
Nebulizer gas flow: 0.76 L/min	
Peristaltic pump speed: 1.0 mL/min	
Nebulizer/spray chamber PFA-ST/Peltier-cooled cyclonic	
Spray chamber temp: 2 °C	
Detector mode dual lens/AutoLens enabled	
Sampler/skimmer cones nickel	
Scanning mode: peak hopping	
Number of points/peak: 1	
Number of sweeps/reading: 20	
Number of readings/replicate: 1	
Number of replicates: 3	

The tuning solution was prepared by diluting about 50 µL of a 10 ppm stock solution in order to get 500 mL of 1% HNO₃. The stock solution containing multi-elements was prepared from 1000 ppm single element stock solution of the elements listed above by diluting about 500 µL of each element into 50 mL of 1% HNO₃.

2.2.4. Internal standards for ICP/MS

For all analysis, a multi-element (BDH Chemicals) internal standard solution containing 20 ppb was used. Li and Sc were selected as internal standards for the analysis of Be and Al, respectively. Li was often naturally present in real samples which could result in poor recoveries during analysis. In these situations, Sc was used instead of Li. The solution of internal standard was prepared by diluting 1 mL of the stock solution (10 ppm) into 500 mL of 1% nitric acid. Because internal

Table 4 Metabolites detected in bovine urine by GC–MS.

Metabolites	Pathway
Propane dioic acid	Citric acid cycle
Glycine	Amino acid
L-Alanine	Amino acid glycolysis
Dihydroxyacetone	Glycolysis and intermediate of fructose metabolism
Butanedioic acid	Citric acid cycle
Hippuric acid	Carboxylic acid found in herbivores
Ribitol	Riboflavin and flavin mononucleotide
D-Glucuronic acid	Androgens, minerocorticoids and fatty acid metabolism
Hexadecanoic acid	Fatty acid
Myo-inositol	Glucose 6 phosphate
Trans-9-octadecanoic acid	Fatty acid
Octadecanoic acid	Fatty acid
Arabinofuranose	Carbohydrate
Prostaglandin F1A	Arachdionic acid

Table 5 Metabolites detected in camel urine by GC–MS.

Metabolites	Pathway
Pyrotartaric acid	Amino acid lysine and tryptophan
Propane dioic acid	Amino acid
Aminomalonic acid	Calcium binding properties to proteins
Erythritol	Insecticide
Canavanine	Amino acid
Creatinine	Creatinine phosphate
Galactose	De Ley Doudoroff pathway
Ribitol	Riboflavin and Flavin mononucleotide
Azelaic acid	Tyrosine inhibitor
Hippuric acid	Carboxylic acid found in herbivores
N-phenylacetyl glycine	Amino acid
D-Glucuronic acid	Androgens, minerocorticoids and fatty acid metabolism
Hexadecanoic acid	Fatty acid
Benzene propanoic acid	Fatty acid
Trans-9-octadecanoic acid	Fatty acid
Prostaglandin F1A	Arachdionic acid
Pseudo-uridine	Nucleoside
Melibiose	Glycolysis
D-Galactose	Glycolysis
2-Deoxy-galactopyranose	Glycolysis

Table 6 Inorganic constituents in freeze dried camel urine estimated by ICP–MS.

S. No.	Element name (Symbol)	Level of element (ppm of camel urine)
1.	Lithium (Li)	0.006807
2.	Boron (B)	0.031970
3.	Sodium (Na)	647.344755
4.	Magnesium (Mg)	15.105697
5.	Aluminum (Al)	0.159909
6.	Phosphorus (P)	0.471004
7.	Chlorine (Cl)	0.0002358
8.	Potassium (K)	280.7982
9.	Calcium (Ca)	0.023656
10.	Silver (Ag)	0.000300
11.	Chromium (Cr)	1.963761
12.	Manganese (Mn)	0.017308
13.	Iron (Fe)	1.705473
14.	Cobalt (Co)	0.001637
15.	Nickel (Ni)	0.086647
16.	Copper (Cu)	0.038132
17.	Zinc (Zn)	0.05477
18.	Arsenic (As)	0.000359
19.	Selenium (Se)	0.002612
20.	Strontium (Sr)	0.103021
21.	Platinum (Pt)	0.000239
22.	Gold (Au)	0.001787
23.	Mercury (Hg)	0.000258
24.	Tin (Sn)	0.000461
25.	Antimony (Sb)	0.001004
26.	Iodine (I)	0.000256
27.	Barium (Ba)	0.013403
28.	Cadmium (Cd)	0.000086

Table 7 Inorganic constituents in freeze dried bovine urine estimated by ICP-MS.

S. No.	Element name (Symbol)	Level of element (ppm of bovine urine)
1.	Lithium (Li)	0.008723
2.	Boron (B)	0.010121
3.	Sodium (Na)	12.615121
4.	Magnesium (Mg)	1.456404
5.	Aluminum (Al)	0.294056
6.	Phosphorus (P)	5.967582
7.	Chlorine (Cl)	0.0001456
8.	Potassium (K)	33.654251
9.	Calcium (Ca)	0.002356
10.	Silver (Ag)	0.000446
11.	Chromium (Cr)	2.663761
12.	Manganese (Mn)	0.016310
13.	Iron (Fe)	1.14256
14.	Cobalt (Co)	0.001631
15.	Nickel (Ni)	0.087564
16.	Copper (Cu)	0.025632
17.	Zinc (Zn)	0.08650
18.	Arsenic (As)	0.000376
19.	Selenium (Se)	0.000796
20.	Strontium (Sr)	0.005005
21.	Platinum (Pt)	0.000579
22.	Gold (Au)	0.005673
23.	Mercury (Hg)	0.000236
24.	Tin (Sn)	0.000489
25.	Antimony (Sb)	0.000224
26.	Iodine (I)	0.000213
27.	Barium (Ba)	0.032678
28.	Cadmium (Cd)	0.000128

standard was added online, no internal standard spikes were added to individual blanks, standards and samples.

3. Results and discussion

The derivatized components of bovine and camel urine showed a remarkable difference which could be due to different anatomies of both the animals. Around 50 metabolites were analyzed in both camel and bovine urine but we selected some of the very important ones for their biological uses.

Bovine urine showed some metabolites from citric acid cycle, fatty acid synthesis, amino acid, glycolysis and the arachidonic acid pathway (Table 4). Malonic acid and succinic acid are important metabolites of the citric acid cycle and inhibits fatty acid oxidation resulting in hypoglycemia and can cause cardiomyopathy. Glycine and L-alanine metabolites in urine provide information regarding the amino acid pathway. Alanine is also a part of glycolysis. Fatty acids play a crucial role in energy metabolism in addition to the synthesis of the phospholipid bilayer and several hormones. Hexadecanoic acid, trans 9 octadecanoic acid and octadecanoic acid play a very crucial role as they are the biomarkers of fatty acid metabolism (Table 4). Ribitol is a biomarker of riboflavin and flavin mononucleotide. Arabinofuranose is a metabolite of carbohydrate metabolism.

Apart from the above metabolites which are present in bovine and camel urine different metabolites like canavanine, erythritol, benzenepropanoic acid and melibiose were also present (Table 5). Canavanine is excreted in camel urine in about 2% of the total urine content. According to the literature, canavanine is a toxic antimetabolite of L-arginine and showed potent antineoplastic activity (Rosenthal and Nkomo, 2000; Vynnytska-Myronovska et al., 2012; Vynnytska et al., 2011). These data support the study of Nujoud et al. and our previous work which showed the camel urine component depicts anticancer activity. Melibiose, D-galactose and 2-deoxy galactopyranose are the byproducts of glycolysis. Benzenepropanoic acid is also a fatty acid derivative and the study of this acid need to be explored further because this might responsible for the antiplatelet activity. The use of camel urine as antibacterial, antifungal, antiparasitic and ingredient of cosmetics was reported by researchers Christy (2000) and Natalie (2002). This study supports both the prior anticancer and antiplatelet activities and in the near future this area of biological activity of camel urine needs to be explored.

Inorganic elements play an essential role in the biological system. Several inorganic elements are needed by the organisms in addition to the four elements carbon, hydrogen, nitrogen and oxygen (constituting formation of organic molecules). There are various metals including Na, K, Mg, Ca, Fe, Zn, Cu, Mn, Cr, Mo and Se which are known to be essentially required for normal biological functions in human beings. The 28 important elements are given in the Tables 6 and 7. Na and K levels in camel urine are very high as compared to bovine

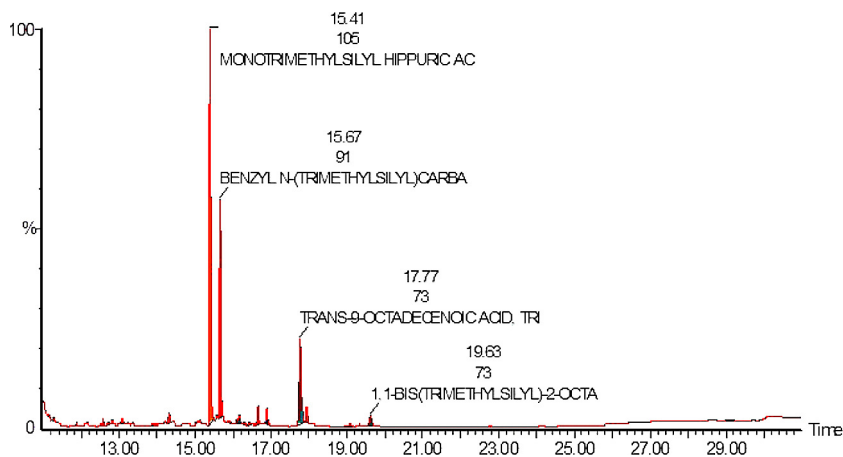


Figure 1 GC-MS spectra of camel urine.

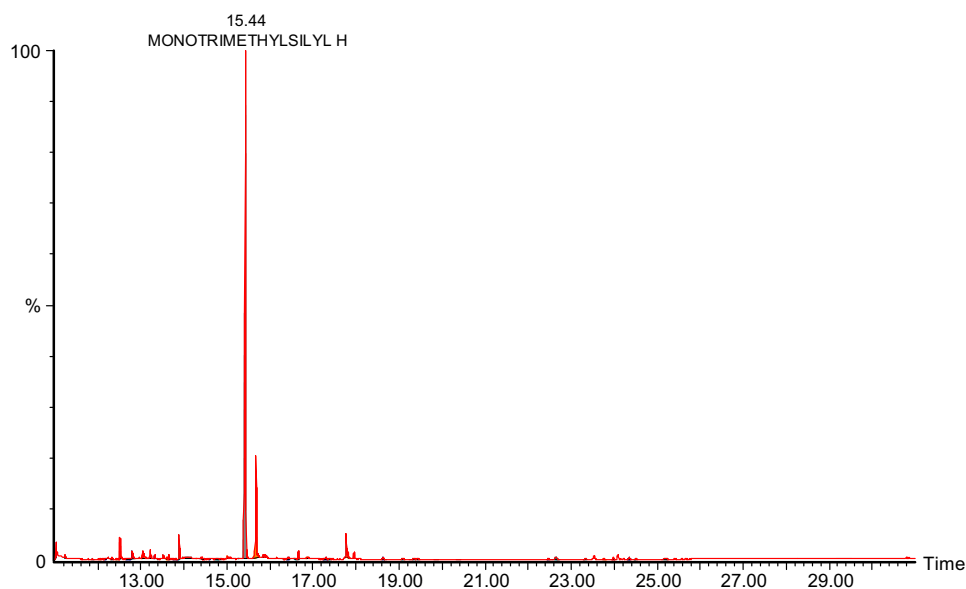


Figure 2 GC-MS spectra of bovine urine.

urine (Table 6). It suggests that the camel survives in dry xerophytic conditions to save the water and ionic balance in the body. The other interesting finding is the level of Mg in camel urine. The level of Mg in camel urine is high as compared to bovine urine. Mg is an important constituent of ATP and also plays an important role in the synthesis of DNA and RNA. It is also investigated as a cofactor in more than 300 enzymatic reactions which are required for the structural function of proteins, nucleic acids and mitochondria (Jahnen-Dechent and Ketteler, 2012). Fe is an essential element which is required for the activities of hemoglobin for its oxygen carrying capacity. Fe is also an integral part of important enzymes like cytochrome p450. Results show higher levels of Fe in camel urine in comparison with those of bovine.

Cr, which has been found in higher amounts in the bovine urine (Table 7), is reported to exhibit a significant role in the biological system. Studies have reported to find a positive correlation between Cr deficiency and the diabetes (Cefalu and Hu, 2004). The presence of Cr in the cow urine can be viewed as a source of Cr supplement and be regarded as a medicinal asset along with its other therapeutic uses.

Both camel and bovine urine typically contains more than 50% of N₂ and P and K content of whole sewage, and is widely considered better than commercially-available chemical fertilizers or stabilized sludge from sewage plants (see Figs. 1 and 2).

4. Conclusion

The results support the previous studies of anticancer and antiplatelet activity of camel urine and provide an important path of the exploration of the new field. The metabolites in camel urine like canavanine are also excreted in cattle, sheep and goats but the percentage is less compared to camel. Canavanine is a byproduct of amino acids and urea metabolism and it is an arginine analog and showed potent activity against tumor cells. Furthermore, benzenepropanoic acid may contribute to the antiplatelet activity of camel urine. The inorganic

elements in camel and bovine urine are almost same except sodium, potassium, iron, magnesium and chromium. The area of metabolomics needs to be explored more in the near future to know the other pharmacological effects of camel urine. The results of this study could be useful in linking the composition of bovine and camel urine with various biological activities in the near future.

5. Conflict of interest

The authors report no conflicts of interest associated with this manuscript.

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