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Chemical Constituents of Donkey Dung (Anbarnasara): Questioning the Recent Claims Concerning Therapeutic Effects

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Abstract

Background: Various animal excrements have been used as a medicine for the treatment of different diseases in the past. Today, people still use some of these excrements, especially female donkey dung (Anbarnasara,) by smelling the smoke of burnt dung for some ailments like sinusitis and bronchitis. Recently, some studies have focused on the therapeutic activities of Anbarnasara but no one has studied the active ingredients of it.

Objective: The aim of this study was to identify the chemical constituents and antibacterial activities of Anbarnasara and its smoke.

Materials and Methods: Female donkey dungs were collected from Shahreza in May 2017. The substance was burnt and the smoke was led to a beaker of methanol by a distillation apparatus. Then, the methanolic extract was dried via a rotary evaporator. Antibacterial activity of the smoke and also the total methanolic extract of Anbarnasara were tested on *Staphylococcus aureus* and *Escherichia coli* via well diffusion method. Chemical constituents were analyzed through gas chromatography-mass spectrometry (GC–MS) .

Results: The results showed that Anbarnasara and its smoke have a very weak antibacterial activity. Regarding chemical constituents, both total methanolic extract and smoke extract predominantly contained toluene, xylene, and dibutyl phthalate.

Conclusion: Previous studies showed xylene as one of the major components of the smoke of Anbarnasara. These articles also reported the presence of some plant-based compounds such as glyoxal, syringol, and limonene in the smoke. The presence of these compounds is probably due to the donkeys' diet. In our study, the presence of dibutyl phthalate, an industrial plasticizer, among the components is probably due to environmental factors. These evidences suggested that Anbarnasara is under the influence of environmental factors like vegetation and pollutions and therefore, should be used as a medicine with caution. Recent claims regarding the antibacterial, cytotoxic, and wound healing activities of Anbarnasara should be considered independent of these environmental factors.

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Background

Traditional medicine (TM) and complementary and alternative medicine (CAM), according to the World Health Organization (WHO), are attracting more and more attention within the context of health care provision and health sector reform. Many factors are contributing to the widespread use of TM/CAM.[1] TM underlines the use of traditional therapies for the maintenance of health and the prevention, diagnosis, improvement, or treatment of physical and mental illnesses.[2] Excrements as therapeutic agents have a long history of use in TM. When the spirits were considered as the cause of diseases, disgusting excrements were prescribed to drive out the spirits from ill bodies.[3] Although the explanation of the pathology of diseases was changed during the thousand years of human life, the therapeutic role of excrements still remained in traditional systems of medicine.

In Persian traditional medicine manuscripts, the dung of various animals (e.g. horse, dog, fox, donkey, crocodile, etc) has been mentioned for treatment of various

ailments. Among the aforementioned items, donkey dung is still present in apothecary shops of Iran with the name of Anbarnasara (Table 1).[4] Although apothecaries insist on the nature of Anbarnasara as female donkey dung, sex of the donkey has not been discussed in Persian medical manuscripts.[5]

In today's folk medicine of Iran, the smoke produced by burning Anbarnasara is supposed to have amazing therapeutic effects including strong antimicrobial and anti-allergic activities. Based on these suppositions, which have not been mentioned in Persian traditional medicine, some investigations have been recently published showing antibacterial, wound healing, and cytotoxic effects of the smoke.[6-8] All of these studies, except one, have been conducted on the therapeutic effects, not the active components which are responsible for these activities. The aim of this study was to investigate the major chemical components of Anbarnasara.

Materials and Methods

Extraction Procedure

Female donkey dungs were collected from Shahreza, Isfahan, Iran in May 2017. To prepare the total methanolic extract, 200 g of dried Anbarnasara was powdered and soaked in methanol and shaken overnight. Then, the extract was filtered and dried via rotary evaporator. For smoke extract, 200 g of dried Anbarnasara was powdered and a Round-bottom flask was half filled with it. This flask was heated by a heating mantle to burn the powdered Anbarnasara. The raised smoke was collected using a distillation apparatus to another flask filled up with methanol. The extraction yield (w/w) was calculated as the weight of dry extract/weight of dry starting material ×100.

Gas Chromatography-Mass Spectrometry

Volatile components of the smoke condensate of Anbarnasara were identified using gas chromatography-mass spectrometry (GC-MS). The chemical composition of the smoke of Anbarnasara was analyzed using gas chromatograph (Agilent 7000, Triple Quad, GC 7890A) with a HP-5MS 5% phenyl methyl siloxane capillary column (30.00 m × 0.25 mm, 0.25 µm film thickness). Oven temperature was kept at 50˚C for 2 minutes initially, and then raised at 10˚C/min to 290˚C and kept at this temperature for 10 minutes. The whole run time was 36 minutes. Injector and detector temperatures were set at 290˚C and 300˚C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min. The syringe size was 10 µL and the injection volume was 1 µL. Peak areas were used for obtaining quantitative data. The GC was coupled to an Agilent 5975 C [Agilent Technologies] mass selective detector. Retention indices were calculated for all components using a homologous series of n-alkanes (C5-C24) injected in conditions equal to samples. Identification of smoke components was accomplished based on the comparison of their retention times with those of authentic standards and their mass spectral fragmentation patterns (Wiley/ChemStation data system) and the calculated retention indices with the retention

Table 1. Traditional Indications of Anbarnasara in Medical Manuscripts of Persian Traditional Medicine

Indication	Preparation	Route of administration	References
Nosebleed	Extract of fresh one with camphor	Nasal drop	Akhawayni
	Extract of fresh one	Nasal drop	Avicenna, Jorjani, Ansari Shirazi
		Putting saturated lint with its extract on nostril	Jorjani
	Dried one while spraying vinegar on it	Smelling	Ansari Shirazi
	Extract of fresh one	Nasal snuffing drop	Mo'men
	Burned one	Insufflation	Mo'men
	With melting snow and leek extract	Nasal snuffing drop	
	With vinegar	Topically applied to forehead	Aqili
Epilepsy	Water extract	Nasal drop	Aqili
	Dried one while spraying vinegar on it	Smelling	Aqili
	Extract of fresh one	Nasal drop	Aqili
Kidney stones	Extract of fresh one	Oral	Ansari Shirazi, Mo'men
Bleeding (as clotting agent)	Dried unburned one	Externally applied as a powder on lesions	Rhazes
	Burned one	Externally applied as a powder on lesions	Rhazes, Avicenna, Ansari Shirazi
	Fresh one	Applied on lesions	Ansari Shirazi
Scorpion bite	Wine Extract of dried one (from a donkey fed on herbage)	Oral	Rhazes, Ansari Shirazi, Aqili
Abortion (as abortifacient)	Fumigating the dried one	Smelling	Jorjani
	Extract of fresh one	Oral	Mo'men

indices of the NIST Mass Spectrometry Data Center.[9]

Preliminary Determination of Antibacterial Activities

Staphylococcus aureus 6539-P and *Escherichia coli* ATCC 8739 were transferred onto the surface of Mueller-Hinton agar plates using sterile cotton swabs. Wells with 8 mm diameters were cut in the plates using a sterile crock borer. Aliquots of 100 µL of 500 mg/mL of the methanolic extracts of Anbarnasara and its smoke were individually delivered into the wells. After overnight incubation at 37°C, the inhibition zones around wells were measured in mm using a caliper.

Results

Gas Chromatography-Mass Spectrometry Analysis

As it is shown in Table 2, the major peak of Anbarnasara methanolic extract (RT=5.728 minutes) belongs to Toluene [mass 92] in a mixture with tropylium ion [mass 91]. The second major peak shows dimethyl benzene [xylene] pattern in GC-MS in which mass 106 shows the whole structure and mass 91 shows the molecule after losing a methyl group. As GC-MS just shows the mass of molecules, it is not possible to confirm the 3D structure of the molecule as ortho-, meta- or para- xylene. Based on GC-MS library, the third major peak with the mass of 278 can show the presence of dibuthyl phthalate in the methanolic extract.

Regarding the smoke, the major peak area shows mass of 92 which (in the same way) belongs to Toluene. Tropylium ion with mass 91 is a significant peak in the chromatogram. The second major peak with RT=7.28 belongs to dimethyl benzene (xylene). Based on the breaking pattern, the third major peak area in the chromatogram [mass 278] belongs to dibuthyl phthalate.

Antibacterial Activities

The result showed that methanolic extract of Anbarnasara and its smoke has no considerable antibacterial activity (Table 3).

Table 3. Antibacterial Activity of Methanolic and Smoke Extracts of Anbarnasara via Well Diffusion Method.

Extracts[a]	Zone of Inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
Total methanolic	10[b]	9
Smoke	11	9
Gentamicin[c]	19	17

[a] 20 mg of the extracts was applied in well diffusion experiment.
[b] Diameter of the zone of inhibition (mm) including the diameter of Well (8 mm) for extracts and disk (6 mm) for the antibiotic.

Discussion

Based on Persian traditional medicine, Anbarnasara has been prescribed for the treatment of nosebleed, epilepsy, kidney stone, and scorpion bite (Table 1).[5,10-15] In nearly all of these indications, fresh Anbarnasara has been used. Surprisingly, using the smoke of Anbarnasara, which is widely suggested in Iran by traditional healers for treatment of various ailments, has been restricted to abortifacient.[13,15] This evidence points out that Persian traditional medicine attaches toxicity to the smoke of Anbarnasara.

Based on our results, toluene, xylene, and dibuthyl phthalate were the main constituents of Anbarnasara and its smoke. Previously, Safarpoor Dehkordi et al reported the presence of xylene in Anbarnasara smoke. They utilized Anbarnasara smoke in formulating a topical dosage form and measured wound healing effects of this ointment on rats.[6] Safarpour et al also reported the presence of guaiacol, syringol, and limonene in the smoke of Anbarnasara which are plant-based molecules. The existence of these herbal components in the smoke of Anbarnasara is related to rich vegetation of Shahrekord County where the donkeys ate grass.

Simultaneously, Joharchi et al reported the same results for wound healing effect and antimicrobial activity of Anbarnasara.[16] They also tried to analyze the content of the smoke of Anbarnasara via GC-MS which resulted in the identification of some herbal components including licochalcone A (probably from licorice root), limonene and carvacrol (from *Mentha* spp.), and terpenes (from different plants). They assumed that all these plant products may have a role in the efficacy of Anbarnasara.

In the present study, we observed the presence of dibutyl phthalate, a plasticizer, in both Anbarnasara and its smoke. We collected Anbarnasara from Shahreza, an industrial city in Isfahan province and it is probable that dibutyl phthalate as an environmental contaminant was entered donkey dung. These evidences suggested that Anbarnasara is under the influence of environmental factors like vegetation and pollutions and therefore, should be used as a medicine with caution.

Concerning antibacterial activities, neither methanolic extract of Anbarnasara nor its smoke extract has

Table 2. The Results of the Chromatography of Extract and Smoke

	Peak No.	Retention Time	Height	Area	Area %
Anbarnasara Smoke	1	5.76	6422551	14378916	91
	2	7.284	121639	323737	2.25
	3	20.751	137336	295914	2.06
Anbarnasara methanolic extract	1	5.728	28793197	111242026	90
	2	7.276	1181598	4623293	4.16
	3	20.773	2339201	4215885	3.79

considerable antibacterial effects. Previously, some investigations have reported antibacterial activities for the smoke of Anbarnasara. Shafiee et al showed antibacterial activities of its smoke against some gram-positive bacteria in human mouth.[7] Parvin et al also showed that the smoke of Anbarnasara has antibacterial activity against nosocomial infections.[8]

Some studies have been recently conducted on the cytotoxic activity of Anbarnasara. While Shafiee et al claimed that its smoke has no cytotoxic activity on L929 cell line,[17] Sadeghi-Aliabadi et al reported cytotoxic activity of this smoke not only on L929 (which is a normal cell) but also on immortal cancerous cells of HeLa and KB.[18] This contradiction could also be related to different sources of supplying or different time of collecting Anbarnasara, or even different grass eaten by donkeys.

Conclusion

As a conclusion and based on our results, Anbarnasara compositions did not show strong antibacterial effects on *Staphylococcus aureus* and *Escherichia coli*. It is probable that previous reports on antibacterial, anti-inflammatory, and wound healing activities of Anbarnasara depend on animal diet and environment. In Persian traditional medicine, the smoke of Anbarnasara does not have any significant therapeutic effect and is indicated that it is used as an abortifacient. These evidences suggested that Anbarnasara should be used as a medicine with caution.

Authors' Contributions

MMAA and AHB: Study design, supervision of the study and finalizing the manuscript; ZA and PSM: data collection; ZK and AB: Drafting of the manuscript.

Ethical Approval

The research followed the principles of Basel Declaration.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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