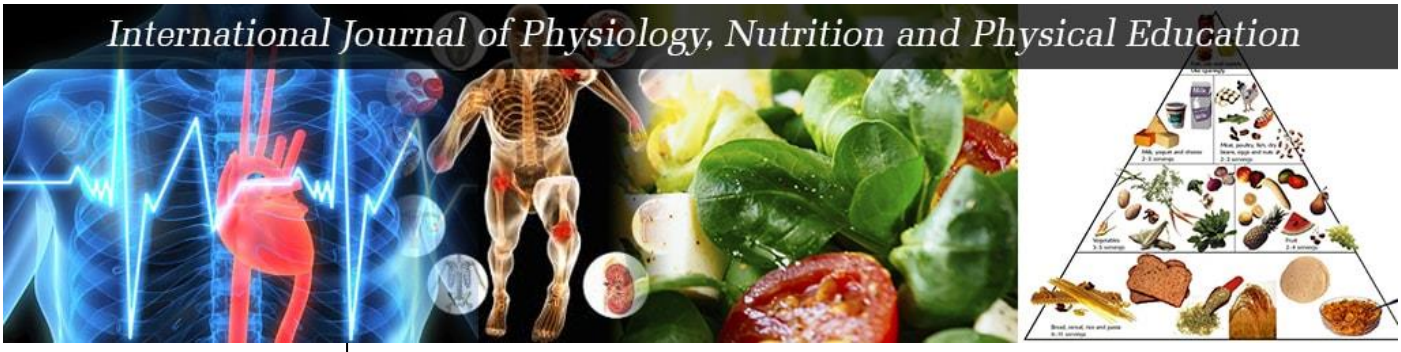

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Safety study of Arand leaves (*Ricinus communis* Linn.)

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Abstract
India is a mother of herbs for the development of Ayurveda, Unani, Siddha, Homeopathy and other natural herbs based health science. Herbal medicine plays an important role in the development of potent therapeutic agents. There is a belief of common man that herbal drugs are safe and free from side effects is false. Herbs contain many constituents and some of them are toxic, however the adverse effect of phyto therapeutic agents is less frequently compared with synthetic drug. The use of herbal medicinal products and supplements has increased tremendously over the past three decades with almost 80% of people worldwide relying on them. Since safety continues to be a major issue with the use of herbal remedies, safety is a fundamental principle in the provision of herbal medicines and herbal products for health care, and a critical component of quality control, so it is necessary to improve safety of herbal drugs by developing certain quality control parameters and by following WHO guidelines. Safety study of herbal drugs and food items are now mandatory as per WHO guidelines to prevent their toxicity likely to arise due to the toxic material they may absorb from the soil or from air. Plants are vulnerable to be contaminated with harmful ingredients, therefore the current study includes the determination of Aflatoxins, Heavy metals, Pesticidal residue, and Microbial load of Arand leaves (*Ricinus communis* Linn.) used as herbal drug. The findings showed that all the safety parameters were below the permissible limit as per WHO guidelines. Hence we can say that the drug Arand leaves is free from toxicity.

Keywords: unani medicine, arand, safety study, WHO, permissible limit

1. Introduction
Unani medicine is an oldest system of traditional medicines, where Arand (*Ricinus communis* Linn.) is a very important drug. It has been used in Unani medicine since centuries for its great medicinal values and belongs to the family Euphorbiaceae (Wallis, 1985) [20]. The castor is one of the major oil seed crops of India and India is the second largest producer of castor seed in the world. The castor crop is valuable since a substantial portion of the oil produced is exported (Anonymous, 1999) [2]. Oil of *Ricinus communis* Linn. (Castor) was mentioned in the ancient Egyptian *Ebers Papyrus* (around 1550 BC) which contains more than 700 natural products (Saad and Said, 2011) [5]. In Unani litrature two types of this plant are described white and red (Ghani, 2010; Hakeem, 2002) [11, 12]. One type having big seeds and its oil is used for burning and of other type are small and used for medicinal purpose, red variety is more potent than white, the red variety is known as *Jogaya Arand* or *Lal Arand* (Ghani, 1911; Ghani, 2010) [10, 11].

In classical Unani literature Arand leaves have been found to possess as emetic (*Muqi*), (Dey, 1980; Kabiruddin, 2007) [7, 14], purgative (*Mushil*) (Dey, 1973; Dey, 1980 Kabiruddin, 2007) [6, 7, 14], analgesic (*Musakkin*) (Ghani, 1911) [10], resolvent (Ahmad, 1930), antidote (*Tiryaq*) (Ghani, 1911; Ghani, 2010) [10, 11], anti-inflammatory (*Mohallil-e-Waram*) (Ghani, 2010) [11], galactopoietics (*Muwallid-e-Laban*) (Dey, 1973; Dey, 1980; Ross, 2001) [6, 7, 18], anti-colic (*Daaf-e-Qulanj*) (Ghani, 2010) [11] etc.Pulp and oil used asstrong purgative (*Qawi mushil*) (Dey, 1973; Hasan, 1894) [6, 13], laxative (*Mullaiyan)* (Safiuddin, 2013) [19], purgative of cold humor (*Mushil-e-Akhlat-e-Baridah*), emmenagogue (*Mudir-e-Haiz*) (Dey, 1973; Dey, 1980; Kabiruddin, 2007; Ross, 2001) [6, 7, 14, 18], anti-helmintic (*Qatil-e-Kiram-e-Shikam*) (Dey, 1980; Kabiruddin, 2007) [7, 14]

Arand leaves indicated inbruises and fractures (*Zarba wa Saqta*), pain (*Dard*), alopecia areata (*Da-us-Salab*) (Ghani, 1911) [10], pain due to flutelence (*Dard-e-Reehi*) (Ghani, 1911) [10], cough (*Sua'al*) (Ghani, 1911; Kabiruddin, 2007; Safiuddin, 2013) [10, 14, 19],

burn (*Ahraaq-e-Aaza*) (Dey, 1980; Ghani, 1911) [7, 10], dama (*Asthma*) (Dey, 1980; Ghani, 1911; Safiuddin, 2013) [7, 10, 19] etc. Pulp and oil indicated in qabz (*Constipation*) (Safiuddin, 2013) [19], rheumatism (*Hudar*) (Dey, 1980; Ross, 2001) [7, 18], gout (*Niqris*) (Kabiruddin, 2007) [14], facial paralysis (*Laqwah*) (Kabiruddin, 2007; Safiuddin, 2013) [14, 19], paralysis (*Falij*) (Dey, 1973; Dey, 1980; Kabiruddin, YNM; Kabiruddin, 2007; Safiuddin, 2013) [6, 7, 14, 19] etc.

Herbs normally carry a large number of bacteria and moulds, often originating in soil or derived from manure. Current practices of harvesting, production, transportation, and storage may cause additional contamination and microbial growth proliferation of microorganism may result from failure to control the moisture level of herbal medicines during transportation and storage (Anonymous, 2007) [4]. Aflatoxins are naturally occurring mycotoxins that are produced principally by some starins of *Aspergillus flavus* and most strains of *Aspergillus parasiticus*, and also produced by some other species like *A. nomius*, *A. ochraceoroseus*, *A. bombycis*, *A. pseudotamari*. The four major aflatoxins B_1, G_1, B_2, and G2 are fungal secondary toxic metabolites. Aflatoxins are the strongest natural carcinogens and their main target organ is liver. Aflatoxin B_1 (AFB1) is the most potent natural carcinogen known. The International Agency for Research on Cancer (IARC) has classified aflatoxin B_1 as group I carcinogen. Ingestion of contaminated herbal plants and herbal medicines is regarded as potential source of heavy metal toxicity. Heavy metals are released into the environment by both natural and variety of anthropogenic sources. The presence of heavy metals in plant tissues is primarily dependent upon their availability and concentration in the soil. They can also be directly deposited on the plant surface from the atmosphere. Heavy metals are persistent in nature due to their long biological half-life. The major heavy metals of health concern are arsenic, cadmium, lead and mercury. Cultivation and collection of medicinal plants in the immediate vicinity of industrial area which utilizes these metals and area where these metals have been improperly disposed is highly discouraged because plants from these areas are prone to high concentration of heavy metals, hence increase the risk of contamination when taken (Ezeabara *et al.*, 2014) [18].

2. Material and Methods

2.1 Sample Preparation

Arand (*Ricinus communis* Linn.) leaves was used as drug, and were collected from Ajmal khan Tibbiya College, A.M.U, Aligarh and were properly identified according to the botanical and Unani literature, and then confirmed in Pharmacognosy section of Department of Ilmul Advia, Ajmal khan Tibbiya College, and in Botany of Department, A.M.U. Aligarh. A herbarium sample of the test drug was prepared and submitted to *Mawalid-e-salasa* museum of the Department of Ilmul Advia after identification for further reference- Voucher no.SC-0212/17. Arand leaves dried in shade and were powdered in electrical grinder and there after the drug was passed through the sieve no. 80 to confirm its fineness and uniformity of particle size. Finally the powder was stored in air tight container for further studies related to the microbiological determination test, Pesticides residue, Aflatoxins and heavy metals.

3. Microbiological Determination Tests

3.1 Total viable aerobic count (TVC)

The total viable aerobic count of the material being examined is determined as specified in the test procedure, using Plate count Method.

3.2 Pre-treatment of the test drug

Depending on the nature of the herbal drug sample used, it was dissolved using a suitable method and any antimicrobial property present in the sample was eliminated by dilution or neutralization. Buffered Sodium Chloride-Peptone Solution, pH 7.0 (MM1275-500G, Himedia Labs, Mumbai, India) was used for diluting the test sample.

4. Test procedures

Plate count for bacteria and fungi

For bacteria

Petridishes 9 to 10 cm in diameter were used then add to each petridish a mixture of1 ml of the pretreated test sample and about 15 ml of the liquefied casein-soybean digest agar at a temperature not exceeding 45°C. Alternatively, the pretreated test sample was spread on the surface of the solidified medium in a petridish of the same diameter. Two dishes were prepared with the same dilution. They were inverted and incubated at 30-35 °C for 5 days, unless a more reliable count was obtained in a shorter time. The number of colonies so formed was counted and the results were calculated using the plates with the greatest number of colonies, up to a maximum of 300 (Lohar, 2007) [15].

For fungi

This method proceeded as described in the test for bacteria butSabouraud glucose agar with antibiotics in a petridish of 90 mm diameter was used in place of Casein soyabean digest agar and the plate was incubated at 20 to 25 °C for 5 days, unless a more reliable count was obtained in a short period of time. The number of colonies so formed was counted and the results were calculated using the plates with not more than 100 colonies (Lohar, 2007) [15].

Pesticidal residue

Organochloride, *Organophosphorous* and *Pyrethroids* compound c are specific pesticide residues and the test for the assessment of specific pesticide residues were conducted using GC/MS-MS (Ramkrishnan *et al.*, 2015) [17].

Aflatoxins

LCMS-MS was used to detect the possible presence of aflatoxins B_1, G_1, B_2 and G_2 which are highly dangerous contaminants in any material of plant origin (Maritxell ventura *et al.*, 2004) [16].

Heavy metals

Atomic absorption spectrophotometer (AAS) was used in the determination of heavy metals and some nonmetal elements in the atomic state. Here heavy metals including Arsenic, Mercury, Cadmium and lead were determined in the test sample using Atomic Absorption spectrophotometry (Lohar, 2007) [15].

5. Result and Discussion

Table 1: Heavy metal analysis of Arand (*Ricinus communis* Linn.)

S. No	Test Parameters	Test Result (mg/kg)	LOQ (mg/kg)	Permissible limits (mg/kg)
1.	Lead as Pb	Not detected	2.50	Not more than 10
2.	Mercury as Hg	Not detected	0.5	Not more than 1
3.	Arsenic as As	Not detected	1.25	Not more than 3
4.	Cadmium as Cd	Not detected	0.25	Not more than 0.3

LOQ= Limit of Quantification

Table 2: Microbial Load in Arand

S. No	Parameters	Test Results (cfu/gm)	Permissible Limit
1.	Total Bacterial Count	7000	Not more than 1x 10^5cfu/g
2.	Total Yeast and Mould	780	Not more than 1x10^3cfu/g

Table 3: Test for Aflatoxins in Arand

S. No	Aflatoxins	Results	LOQ (mg/kg)	Permissible limit (mg/kg)
1.	Aflatoxin B_1	Not Detected	0.001	Not more than 0.5
2.	Aflatoxin G_1	Not Detected	0.001	Not more than 0.5
3.	Aflatoxin B_2	Not Detected	0.001	Not more than 0.1
4.	Aflatoxin G_2	Not Detected	0.001	Not more than 0.1

LOQ= Limit of Quantification

Table 4: Pesticidal residue in Arand

S. No	Pesticide Residue	Results	LOQ (mg/kg)	Permissible limit (mg/kg)
1.	Alachor	Not detected	0.002	0.002
2.	Aldrin and dieldrin	Not detected	0.04	0.05
3.	Azinophos-methyl	Not detected	0.04	1.0
4.	Bromopropylate	Not detected	0.08	3.0
5.	Chlordane	Not detected	0.04	0.05
6.	Chlorfenvinphos	Not detected	0.04	0.5
7.	Chlorpyrifos	Not detected	0.04	0.2
8.	Chlorpyrifos-methyl	Not detected	0.04	0.1
9.	DDT (Sum of p.p-DDT, p.p-DDE and p.p-TDE)	Not detected	0.04	1.0
10.	Deltamethrin	Not detected	0.10	0.5
11.	Diazinon	Not detected	0.04	0.5
12.	Dichlorvos	Not detected	0.04	1.0
13.	Diathiocarbamates (as CS_2)	Not detected	0.01	2.0
14	Endosulfan (Sum of Isomer and EndosulfanSulphate	Not detected	0.04	3.0
15.	Endrin	Not detected	0.04	3.0
16.	Ethion	Not detected	0.04	0.05
17.	Fenitrothion	Not detected	0.04	2.0
18.	Fenvalerate	Not detected	0.10	1.5
19.	Fonofos	Not detected	0.04	0.05
20.	Heptachlor	Not detected	0.04	0.05
21.	Hexachlorobenzene	Not detected	0.04	0.1
22.	Lindane (ϒ-Hexachlorocylohexane)	Not detected	0.04	0.6
23.	Malathion	Not detected	0.04	1.0
24.	Methidathion	Not detected	0.04	0.2
25.	Parathion	Not detected	0.04	0.5
26.	Parathion Methyl	Not detected	0.04	0.2
27.	Permethrin	Not detected	0.04	0.2
28.	Phosalone	Not detected	0.04	1.0
29.	Piperonyl butoxide	Not detected	0.04	3.0
30.	Primiphos Methyl	Not detected	0.04	4.0
31.	Pyrethrins (sum of isomer)	Not detected	0.10	3.0
32.	Quintozen (Sumof Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	Not detected	0.10	1.0

LOQ= Limit of Quantification

Table 5: Test for Specific Pathogens in Arand

S.No	Pathogens	Result (gm)	Permissible limit
1.	*E.coli* /gm	Absent	Absent
2.	*Salmonella* /gm	Absent	Absent
3.	*S.aureus* /gm	Absent	Absent
4.	*P.aeruginosa* / gm	Absent	Absent

6. Discussion and Conclusion

Unani medicine is recognized as one of the safest system of medicine because the drugs used in this system of medicine are prepared after applying several different procedures of purification and detoxification. However the possibility of contamination of a drug with toxicants mainly due to contaminated soil and atmosphere, in the crude drugs and

herbal products used in this system of medicine they may also contain the toxic substances which may cause serious side effects, therefore in order to ensure their quality and risk free therapeutic application safety studies of herbal drugs have become mandatory by WHO. In the present study all four parameters were undertaken to determine the safety/ toxicity of a drugs and it was found that in the test sample heavy metals (Arsenic, Mercury, Cadmium and Lead) were not detected, as aflatoxins cause serious adverse (hepatotoxity, carcinogenetic etc) effects on human and they were absent in the test drug sample. The microbial count (Bacterial, yeast and Mould) was found below permissible limit, this drugs is also free from Pesticide residue contamination. The findings indicated that the test drug Arand (*Ricinus communis* Linn.) is quite safe as per WHO requirements and can be used safely in the management of diseases, it is recommended for.

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