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Evaluation of microbial contamination in rose water samples extracted from *Rosa damascena* petals

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Abstract
Plants extract have extensively used in various countries as alternative medicines. However, contaminations of microbes in these medicines possibly affect human health. The current study was performed toward an evaluation of the pathogenic abundance in freshly extracted and marketed rose water samples. Twelve samples of rose water were utilized in that study, making serial dilution (1:10, 1:100, 1:1000) of all samples indivisually.1ml of each dilution sample was dropped on muller-Hinton agar sterile plates and incubated for 24 hrs at 37 ºC for bacterial growth. All the commercial samples show contamination of both gram-positive and gram-negative bacilli. However, the sample which is freshly prepared in the lab showed no contamination. The existence of bacteria in samples recommended the fact that aseptic management throughout the processing of plant extracts and appropriate storage and packaging is necessary.

Keywords: bacterial contamination, muller –Hinton agar, bacilli, rose water

Introduction
Herbal medicine consists of herbs, herbal distillates, herbal resources, preparations, and refined yield of herbs. Such therapeutic preparations were used from the prehistoric period to care for a broad variety of diseases [1-3]. However, the remedial use of herb goes keen on swift turn down in Western countries when an additional conventional man-made drug was generally accessible. In distinguish, numerous rising countries sustained to obtain advantage from medicinal herbalist prosperous knowledge. For instance, Ayurvedic medicine (India), Kampo medicine (Japan), traditional Chinese medicine (TCM), and Unani medicine (the Middle East and South Asia) are still used by a huge population [4]. According to the review of the World Health Organization (WHO), about 70-80% of the world population particularly in the developing countries relies on non-conventional medicines mainly of herbal origins for their chief healthiness concern. As medicines derived from plants source are comparatively reachable and low cost than synthetic drugs [5].
In the traditional system of medicine, numerous plants related medicine are used for human health-care [6] as well as they mortal promote as safe and secure exclusive of any hazardous effects. As the utilization of herbal products by patients is escalating gradually, there is a call for pharmacists and physicians to have awareness about the safety of these preparations [3].
According to technical guidelines of WHO for the evaluation of microbial features of plant extracts, the purpose of micro-biological contaminants and limit tests for entire possible aerobic bacteria and fungi point out the superiority of herbal preparations. According to the British Pharmacopoeia standards (2004), species of Salmonella and Shigella bacteria should not there in plant-based medicines planned for *in-vivo* utilization at all stage [7]. Further micro-organisms experienced and must obey with limits put in local, nationwide or global pharmacopeias [8]. Given the entire contamination possibility and based on the recommended strategy or standards, current studies were planned to evaluate the microbiological contamination in commercial and freshly prepared rose water samples. Determine the nature of microbes and determine the extent of such contamination.
Rosa damascena which is also documented as gul-e-surkh is solitary of the principally immediate sweet-smelling and therapeutic plants as a matter of course used for a variety of strength needs. It is an erect shrub up to 2 meters in height. Rose plant is cultured all over the world for a motive that of its pleasant appearance and fragrance. It is the most famous than any other flower all over the world. It has referred to as a king of flowers. There are over 200 rose species, and more than 180000 cultivars variety of the plant has been known, among them the

Rosa damascena is one of the most important generals of the Rosaceae family. Apart from its uses as an ornamental plant in frivolous greenhouse and gardens. They are primarily cultivated for use in the perfume, medicine, and food industry. The plant has exposed various biological and pharmacological actions. It has been used in Unani medicine (Tibb-e-Unani)since the ancient era [9]. Rose plant used for the managing of numerous complain and described in the ethnobotanical texts, and a variety of uses have been reported such as in aching throat, puffy tonsillitis, slimming to women and old people, uterine hemorrhages, and urticaria. Locally they are useful to heal aphthae [10]. The most advantageous effect of *Rosa damascena* in ancient medicine is joint with the curative of abdominal chest pain, intensification the heart, heal of menstrual bleeding and digestive disorders, and reduce inflammation mainly of the neck. This plant is also used as a mild laxative [11]. flora of the plant is excellent for the eyes. ease a pain, toothache, stomatitis, settlement the lungs, kidney, and liver. It is also used in the warmth of the body, chronic fever, inflammation, and intestinal affection and to decrease too much perspiration [12]. Rosewater forms an agreeable medium for the grounding of lotions and collyrium [13].

Because of the entire contamination possibility and based on the recommended strategy or standards, current studies were planned to evaluate the microbiological contamination in commercial and freshly prepared rose water samples. Determine the nature of microbes and determine the extent of such contamination.

Materials and Methods

Sample collection / preparation of rose water

Total twelve samples of rose water were used in the current study, six samples were collected from the local market, four samples were provided by Mohammad Hashim Tajir Surma laboratories and the final sample were ready in the lab using hydro distillation method as the report by (Verma *et al*., 2011) [14]. All samples were filtered through Whatman filter paper and stored at 6 ºC in a refrigerator for further analysis and study. Sample and the voucher specimen number (RD-01-12) is available in the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi herbarium.

Chemicals

Chemicals were utilized in the study are of Analytical grade, Muller –Hinton agar (Oxide), Methanol (Merck), Distilled water (Local Preparation). Experiments were done carefully with appropriate controls.

Sample Preparation for Bacterial Contamination Assay

Each rose water sample (1ml) was taken in a test tube individually and adds 9ml of sterile distilled water was added and shake well. Serial dilution was prepared by transferring 1ml of first dilution (10^{-1}) from each serial test tube into another 9ml of distilled water to make (1:100) procedure was repeated for making (1:1000) dilution. The same procedure was repeated on all twelve samples of rose water.

Bacterial Contamination Assay

The Muller –Hinton agar was taken in Petri dishes and marked as original, (1:10), (1:100), (1:1000) dilution, with the assist of the sterilized pipette, serially diluted each sample was taken and dropped. 1ml of each sample on the surface of Muller-Hinton agar plates and the drops spread. The plates were left upright for dry then incubated at 37 ^{O}C for 24 hours. After 24 hours bacterial growth on plates was observed, results were noted; the following equation calculated the colony count.

CFU= per ml=A average number of colonies for a dilution *50*dilution factor (Thoha *et al*., 2012) [15].

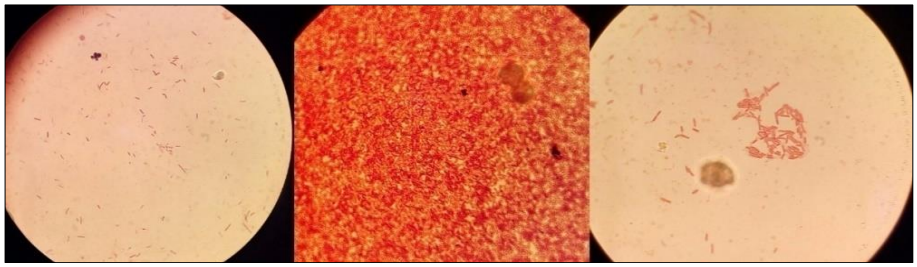
Result and Discussion

Table-1 indicates the outcome of bacterial contamination as recognized later than the microbiological analysis of all the rose water samples. Fig-1 shows the bacterial contamination. The bacterial contamination was prominent in all commercial samples experienced in the present study, sample#06 shows 130 CFU/ml. whereas sample#12 which was freshly extracted in the lab, showed no bacterial contamination. The bacterial species which were identified counting both gram-positive and gram-negative bacilli.

Table 1: Bacterial contamination in rose water samples

Sample	Original	1:10 (10^{-1})	1:100 (10^{-2})	1:1000(10^{-3})
01	TNTC	TNTC	TNTC	TNTC
02	TNTC	TNTC	TNTC	TNTC
03	TNTC	TNTC	TNTC	TNTC
04	TNTC	TNTC	230	200
05	TNTC	150	100	67
06	TNTC	260	250	230
07	TNTC	120	105	100
08	TNTC	180	150	130
09	TNTC	200	185	120
10	TNTC	TNTC	TNTC	TNTC
11	TNTC	TNTC	TNTC	TNTC
12	-	-	-	-

●: source & manufacturer's name are available upon request
Note: TNTC = Too numerous to count (-) = no colony found
Formula: CFU/ml = average number of colonies for a dilution × 50 ×dilution factor
Sample#05:150 × 50× 10^{-1} = 75 CFU/ml
Sample#06: 260 × 50× 10^{-1}= 130 CFU/ml
Sample#07: ×120× 50× 10^{-1}= 60 CFU/ml
Sample#08: ×180× 50× 10^{-1}= 90 CFU/ml
Sample#09:200 × 50× 10^{-1}= 100 CFU/ml



Gram-positive Bacilli Gram-negative Bacilli Gram-negative Bacilli

Fig 1: Bacterial Contamination Presence of Gram (+) and Gram (-) Bacteria Rose Water Samples

In the point of view of Pakistan, where several populations are based on medicine derived from a plant source, the evaluation of pathogens in these medicines is instantly essential. Based on this recommendation, attempted to recognize and enumerate the pathogenic micro-organisms in samples randomly collected from local markets and prepared in the lab. The determination of the colony-forming unit of any solution and suspension was known as the Miles and Misra Method. In 1938 Miles and Misra initially invented this method. This procedure is effortless, speedy, and unforced to process, but it necessitates a highly capable microbiologist and also restricted environmental conditions. Any bacteria when growing on the agar forming visible colonies. On the first step, the proper incorporation of the solution on agar surface and place the plates in an incubator in an inverted position by providing the positive condition that is temperature and humidity. The bacteria were full-fledged within 18-24 hours. Since 1938 this method of calculating CFU is most frequently used. The result of bacterial contamination was mention in table 1. Eleven out of twelve extracts were found to be microbiologically contaminated while only 1 extract showed no growth on Muller –Hinton agar. Growth on all the plates showed three distinct types of colonies. Their microscopic examination revealed Gram-positive Bacilli and Gram-negative Bacilli mention in Fig.1.

Though, this study gives a highlight actuality with the aim that manufacturer is supposed to create the confident, lowest possible intensity of micro-organisms in the unprocessed supplies, refined dosage form, and the packaging components to sustain suitable superiority, safety, and strength of the medicines. Quality has to be built throughout the process start from an assortment of propagating materials to the final products reaching the consumers. Finally, based on the suggestive data previously reported and considering the contamination status as revealed from our study, we recommend that there is an urgent need for constant monitoring and control of the microbiological standards of herbal medicines available in the local markets.

Conclusion

Then, based on the obtained results, it is proposed that producers of herbal distillates consider the hygiene conditions, using correct and suitable pasteurization, considering the structural condition of the workshop, and also packaging appropriately to reduce the secondary contamination and increase the quality of the finished product.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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