



RESEARCH ARTICLE

Analysis of *Trigonella foenum-graecum* Seeds, *Musa paradisiaca*, and *Citrus sinensis* Peels as a Poultry Feed Supplement

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ABSTRACT

Alternate feed resources could play an important role in meeting the challenges to the poultry in Pakistan. In the present study, physical and chemical characterization of medicinally important fenugreek seeds and agro-waste of orange and banana peels were investigated. It was found that banana peels have 10% moisture contents followed by fenugreek seeds i.e., 8%. The nutritional characteristics showed that fenugreek seeds and orange peels contain 39% crude fiber followed by 25% in banana peels. The protein value in fenugreek seeds and orange peels was found to be 24%. The fat content was estimated as 2, 3, and 2% in fenugreek seeds, banana peels, and orange peels, respectively. The antibacterial activity of aqueous, methanolic, and ether extracts of orange peels presented 19±0.3 mm, 18±0.6 mm, and 14±0.8 mm zone of inhibition against *Bacillus (B) atrophaeus* while 12±0.4 mm, 13±0.1 mm and 14±0.8 mm against *Salmonella (S) typhimurium*, respectively. Moreover, ether extract of banana peels showed remarkable antibacterial activity against *B. atrophaeus* with an 18±0.6 mm zone of inhibition followed by aqueous extracts with a 14±0.8 mm zone of inhibition against *S. typhimurium*. Furthermore, methanolic extracts of fenugreek seeds exhibited potent antibacterial activity against both bacterial strains with a 17±0.9 mm zone of inhibition. Such resources from agro wastes can provide birds with nutrients, natural antioxidants, and antibiotics properties.

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INTRODUCTION

Pakistan is the 11th largest poultry producer in the world. Poultry is a vital segment of agriculture in Pakistan that contributes 1.3% to the national GDP (Hussain et al., 2015). Although poultry production is significant, however, it is not meeting the supply and demand challenges due to the rapid increase in the population of the country. Other factors such as the sudden increase in the cost of feed grains and supplements also affected its production (Devendra and Leng, 2011; Memon et al., 2020). Poultry farming is mainly reliant on its feed. Up to 70% of the cost of production is spent on feed. Efficiency and availability of feed resources are the main factors influencing the protein production (Buza et al., 2014; Makkar and Beever, 2013). To increase the production of white meat commercially high protein

feed is used. It is a need of time to find alternate nonconventional, cheaper feed sources not being used in the human food chain. Globally 45% of fruits and vegetables are wasted (Faostat, 2015). Most of this is either being dumped or causing environmental pollution. Some of it is also recycled as fertilizer but its contribution is very less (Jahid et al., 2018). Fruits and vegetable waste provides birds with nutrients and it can fulfill the demand and supply. Fruits and vegetable waste would be a cheaper source of high nutrients for birds so it would be economically attractive for the farmers (Chatterjee, 2014). In the present study, physical and chemical characterization of fenugreek seeds and peels of oranges and bananas was studied. The antibacterial potential of fenugreek seeds and banana and orange peels extracted in different solvents was also observed. This combination of seeds and fruit peels is

very much important related to nutraceuticals or dietary supplements and can be used in the preparation of animal feed at a low cost with other benefits of natural antibiotics and antioxidants.

MATERIALS AND METHODS

Raw material

Fenugreek seeds were purchased from the local market of Faisalabad. Seeds were washed, dried at 40°C, and ground into a fine powder. Freshly harvested bananas and oranges were taken from the local market of Lahore. The uppermost colored covering of the fruits was removed and heated in an oven at 100°C for 24 hours. After heating, peels of fruits and fenugreek seeds were ground into a fine powder. The samples were subjected to proximate analysis for estimation of moisture content, ash percentage, crude fiber, crude protein, and crude fat by AOAC 2000 methods (Maisarah et al., 2014).

Crude protein analysis

The Kjeldahl method was used for crude protein analysis. The raw material was hydrolyzed to convert bound nitrogen into ammonium. The reaction was performed in a digester for 2 h in concentrated sulphuric acid (raising the temperature to boiling point) containing copper tablets as a catalyst. Water is added to hydrolysate after cooling. The amount of total nitrogen was estimated by titration. The nitrogen content is converted into protein content by multiplying it with the “Jones factor” i.e., species-specific ranging from 5.7 – 6.25 (Maisarah et al., 2014).

Crude fat analysis

Gravimetric methods were used for crude fat analysis. It includes all kinds of fatty acids esters such as wax esters, and other non-fatty materials (Redondo et al., 2008).

Crude fiber analysis

2 g of finely powdered fenugreek seed, orange peel, and banana peel were boiled separately with 10% nitric acid and filtered. Residues were washed and boiled with 2.5% sodium hydroxide solution for 30 seconds and filtered. The residue was washed with hot water dried and transferred to a pre-weighed crucible to measure crude fiber content (Thiex, 2019).

Moisture and ash content analysis

Fine powder of calcinated fenugreek seed, orange, and banana peel was reduced to ash in a muffle furnace for 6 h at 525°C. The moisture content of ash was removed in a desiccator and weighed. The ash content was recorded as gram per 100-gram fresh weight. Moisture content was recorded by the weight loss on drying and heating of raw material. Ash content was also determined using a thermogravimetric approach (Thiex, 2019).

Heavy metal analysis

Heavy metals were analyzed on atomic absorption. Wet digestion of calcinated fenugreek seeds, and orange and banana peel were required for the preparation of the solution. A total of 5 mL of concentrated nitric acid was treated with 0.5 g of sample and stirred. The 4 mL of 33% hydrogen peroxide were added vigilantly in a fuming hood and slightly stirred. The solution was then heated on a hot plate at 95°C and boiling has refrained. Strong effervescence was produced. After 7-8 minutes the solution was removed from the hot plate when brown fumes become less dense. The solution was allowed to cool. The solution was filtered, washed with 5 mL of (1:1) HCl, and diluted up to 25 mL with distilled water. Metals (Pb, Cu, Zn, Cd, Co, Hg, As, and Ni) in the digested samples were analyzed in triplicate. A standard solution, and five dilutions were prepared for plotting the calibration curve (Robinson, 1960).

Antibacterial activity

Antimicrobial activity was studied on two microbes, *Bacillus (B) astrophaeus* (Gram-Positive) and *Salmonella (S) typhimurium* (Gram Negative). The bacterial strains were taken from the Microbiology laboratory, Food, and Biotechnology Research Center, PCSIR Laboratories Complex, Lahore, Pakistan. The bacterial cultures were maintained at 4°C. Mueller-Hinton agar plates were used for culturing. The ampicillin (500 µg/mL), and tetracycline (500 µg/mL) solution were incorporated before the liquid medium. Each sample was taken in dried powdered form (10 g) of each and was dissolved in three different solvents (distilled water, methanol, and ethanol), 100 mL each, kept soaked for 48 hours, and filtered. The microbes were grown on Mueller-Hinton agar. Sample solutions were impregnated on filter paper disks. The presence or absence of growth around the disks was a measure to check the antibacterial activity of the sample solutions (Murray and Zeiting, 1983).

RESULTS

The research was conducted to investigate the potential of waste products i.e. banana and orange peels and fenugreek seeds. The results reveal that the peels and the seed have strong pharmaceutical potential.

Physicochemical characterization

The results of physical and chemical characterization of dried and ground orange peels were given in Table 1. The moisture content represents the measure of water content and storage stability of the seeds and peels. The experimental result showed the percentage of moisture at 8%, 10%, and 5% in fenugreek seeds, banana peels, and orange peels, respectively.

The percentage of ash content in fenugreek seeds, orange, and banana peels were 2.0%, 4.0%, and 7.0%, respectively. The experimental value of fiber percentage

Table 1: Physical and chemical properties of samples

Physical and chemical parameters	Orange peel	Banana peel	Fenugreek seeds
Moisture content	5 ±0.15	10±0.15	8±0.07
Ash content	7±0.1	4±0.10	2±0.10
Fiber content	4±0.05	25±0.20	39±0.05
Protein content	5±0.07	1±0.15	24±0.10
Fat content	5±0.05	3±0.07	2±0.05

Table 2: Analysis of heavy metals in samples

Metals	Sample		
	Fenugreek seeds	Orange Peel	Banana Peel
Pb	Not detected	Not detected	Not detected
Cd	Not detected	Not detected	Not detected
Cu	Not detected	Not detected	Not detected
Ni	Not detected	Not detected	Not detected
Co	Not detected	Not detected	Not detected
Hg	Not detected	Not detected	Not detected
Zn	Not detected	0.01± 0.03	Not detected
As	0.02± 0.04	Not detected	Not detected

Table 3: Antibacterial activity of banana and orange peel and Fenugreek seeds

Bacterial Species	Solvents	Zone of Inhibition (mm)				
		Control	Antibiotic cefotaxime	Orange peel	Banana peel	Fenugreek seed
<i>Bacillus atrophaeus</i> (Gram-Positive)	Ether	11±0.70	17±0.9	14±0.8	18±0.6	13±0.1
	Water			19±0.3	12±0.4	16±0.2
	Methanol			18±0.6	11±0.7	17±0.9
<i>Salmonella typhimurium</i> (Gram Negative)	Ether	11±0.70	12±0.4	14±0.8	11±0.7	12±0.4
	Water			12±0.4	14±0.8	15±0.5
	Methanol			13±0.1	11±0.7	17±0.9

was 39% in fenugreek seed, 4%, and 25% in peels of orange and banana, respectively. Ground powder of fenugreek seeds was processed for the estimation of nutritional value. The crude fiber, protein, and fat content of fenugreek seeds were found as 39%, 24%, and 2%, respectively.

The results of the chemical characterization of banana peels exhibited 25% of crude fiber. Peels of bananas have a low amount of protein and fat contents as 1% and 3%, respectively.

Heavy metals in fenugreek seeds (FS), orange peel (OP), and banana peel (BP)

Fruit peels make an important part of total fruit weight and if they are not managed further, they can cause serious environmental pollution in form of smell, soil and water pollution, and harborage for insects, hence it is necessary to examine the presence of heavy metals. It was found that none of the potentially toxic metals were present (Table 2) in any of the tested samples of fenugreek seeds and orange, and banana peels except zinc, which was found in orange peel (0.01 ppb), and fenugreek seeds exhibited astatine (0.02 ppb). However, both were in lower concentration

Antibacterial activities of fenugreek seeds, orange and banana peels

Evaluation of the antibacterial activity of water, ether, and methanolic extracts of fenugreek seeds and banana and orange peels was determined initially by the disc

diffusion method (Table 3) against *Bacillus atrophaeus* (gram-positive) and *Salmonella typhimurium* (gram-negative). Cefotaxime antibiotic was used as a standard (Gast and Porter Jr, 2020; Stapels et al., 2018). The water and metabolic extracts of orange peels presented a strong activity against *B. atrophaeus* with a diameter of inhibition zone of 19.0 mm, and 18.0 mm, respectively. The extract of banana peels showed remarkable antibacterial activity against *B. atrophaeus* with a diameter of inhibition zone of 17.0 mm and did not show any remarkable activity against *S. typhimurium*. The water and methanolic extracts of fenugreek seeds also showed significant antibacterial activities against both the tested bacterial species with an inhibition zone of 16.0 mm against *B. atrophaeus*, and 15.0 mm against *S. typhimurium* (water extracts), and 17.0 mm against both the species (methanolic extracts). On contrary, very low antibacterial activity was exhibited by water and methanolic extracts of banana peels (12.0 mm and 11.0 mm, respectively) against *B. atrophaeus*. Moreover, ether extracts of banana peels showed valuable antibacterial activity with an 18.0 mm zone of inhibition against *S. typhimurium*.

A comparison of antibacterial activities of positive control showed that *S. typhimurium* exhibited resistance against cefotaxime antibiotics. On the other hand, water and methanolic extracts of fenugreek seeds displayed significant antibacterial activities.

DISCUSSION

The model designed for the management of food waste highly prefers “prevention and reduction”, followed by “repurposing and re-use” to the least preferred “disposal” (Tedesco et al., 2021). This study is based on the repurposing and recycling of selected waste products i.e. banana and orange peels and fenugreek seeds. The results reveal that the peels and the seed have strong pharmaceutical potential. The demand for food supply for increasing population offer recycling of biodegradable fruits and vegetable wastes in the development of nutraceuticals, other valuable products, and feed for livestock (Socas-Rodríguez et al., 2021).

Our study shows that banana peels and fenugreek seeds have a high amount of dietary fiber (25 and 39%, respectively). The amount of fiber in banana peels is lower than reported by Emaga et al., 2007 (45-50%) but comparable with the results of Tedesco et al., 2021 (25%). The percentage of crude fiber found in banana peels is high and makes it a good source of dietary fiber (Eshak, 2016; Siyal et al., 2016). Crude fiber mainly contains constituents of cell walls including cellulose, hemicellulose, and lignin. Endogenous enzymes cannot digest these compounds. On the other hand, intestinal microorganisms degrade these compounds to produce energy. However, it reduces nutrients and digestibility, and the fiber content promotes the growth of friendly gut bacteria, promoting animal health (Jha and Berrocoso 2015; Jha et al., 2019). Supplements of banana peels can be used to increase the growth of chicks in poultry (Siyal et al., 2016). This significant amount of crude fiber refers to the utilization of banana peels in the manufacturing of livestock feed, nutraceuticals, and pharmaceuticals (Fidrianny et al., 2014).

Proteins are a source of amino acids and are essential for the growth and development of animals and human beings (Coultrate, 2009). The protein content found in orange peels (5%) is in harmony with the results of a study conducted on orange peels (3.94%) and seeds (6.1%) by Oikeh et al., (2013). On contrary, findings by Gotmare and Gade (2018) reported a very high amount of protein content (12.43%). On the other hand, protein content found in banana peels is very low (1%) as compared to the work of Emaga et al., (2007) and Pyar and Peh (2018), which is reported as 5%. The difference might be due to regional differences in climate, soil, temperature, and cultivation practices of the plant (Buba and Ngura, 2015). It is also important to note that generally fruits are not taken as a source of protein so a low value of protein content is found (Marles 2017). Fenugreek seeds were found to contain a high amount of protein (24%), which is similar to the results of Mullaicharam et al., (2013) and Işıklı and Karababa (2005). In contrast to our results, Buba and

Ngura (2015) reported a very low amount of protein content (2.74%) in fenugreek seeds. Fenugreek seeds contain a good amount of protein with a very balanced composition of amino acids (Singh et al., 2013).

The moisture content represents the measure of water content and storage stability of the seeds and peels. The experimental result showed the percentage of moisture at 8%, 10%, and 5% in fenugreek seeds, banana peels, and orange peels, respectively. Our findings on orange peels and fenugreek seeds are comparable with previous studies reported by Gotmare and Gade (2018), and Buba et al. (2015). On the other hand, Hassan Pyar reported 50% moisture content in banana peels which is very higher than our results (Pyar and Peh 2018). The difference may be due to the genetic makeup of varieties and climatic conditions. (Butt and Anjum, 1997). Water content determines the shelf life of fruits and vegetables, higher the water content, the more prone it to decay, spoilage, and degradation (Hausmann et al., 2016). The ash content of orange peels (7%) and fenugreek seeds (2%) are similar to the results of Gotmare and Gade (2018) (7.8%) and Buba et al. (2015) (2.9%), respectively. In contrast to ash contents observed in our study (4%), several studies reported a high ash content, which is 8.8% (Moniruzzaman et al., 2014; Pyar and Peh, 2018). Estimation of ash content has no nutritional importance as such, but its value demonstrates the presence of minerals in the sample (Moniruzzaman et al., 2014).

The aim of this study is to use orange and banana peels in the manufacturing of livestock or poultry feed. Therefore, it is important to test the toxicity of these wastes. None of the potentially toxic metals was present in any of the tested samples of fenugreek seeds and orange, and banana peels. The absence of heavy metals suggests that there are no serious safety issues and fruits and vegetable wastes can be used in the livestock or poultry feed if followed an adequate hygienic-sanitary protocols (Tedesco et al., 2021).

The analysis of fenugreek seeds and peel extracts against microorganisms reveals the possibility of using the peel wastes and fenugreek seeds as a novel sources and also low-cost natural antibiotics (Kumar et al., 2021). The results also provide a clear understanding that the best selection of solvent makes the sample more effective against microorganisms. Hence, choosing a suitable solvent is very important for selective extraction from natural sources.

Antimicrobial activity of peel extracts and fenugreek seeds was tested against gram-positive and gram-negative microorganisms and the results are shown in Table 3. Results were fairly comparable with the tested standard antibiotic (Cefotaxime) and show the very dynamic antimicrobial activity of aqueous extract of the orange peel and fenugreek seeds against both gram-negative (15 mm zone of inhibition (ZI)) and gram-

positive bacteria (19 mm ZI by orange peels and 16 mm ZI by fenugreek seeds). In comparison to our results, Egbuonu and Osuji (2016) reported less growth inhibition of *E. coli* (11.33 mm ZI) and *S. aureus* (14 mm ZI) by aqueous extracts of orange peels. The author also mentioned high inhibitory activity by orange peels as compared to orange seeds (Egbuonu and Osuji 2016). Remarkable antibacterial activities were also exhibited by methanolic extracts of orange peels against *Bacillus atrophaeus* (18 mm ZI). Methanolic extracts of fenugreek seeds revealed significant antibacterial activity against both gram positive and gram negative strains (17 mm ZI). In agreement with the present study, Upadhyay et al. (2008) presented the antibacterial activity of fenugreek seed extracts against *E. coli*, *B. cereus*, *L. acidophilus*, and *Pneumococcus* (Upadhyay et al., 2008). Another study has confirmed that Fenugreek seed extracts exhibited antimicrobial activity against *E. coli*, *S. typhi*, *V. cholerae*, *S. sonnei*, *S. aureus*, *M. hitea*, *B. subtilis* and *L. bacillus* (Ritu et al., 2010). In case of banana peels, only ether extracts exhibited inhibitory activity against *Bacillus atrophaeus* (18 mm ZI). These results are higher than the work of Rita et al., (2020), which reported that methanolic extracts of banana peels showed inhibition against *E. coli* (gram +ive, 13.37 mm ZI) and against *S. aureus* (gram negative, 15.17 mm ZI). By closing nut in a shell, the current study cleared that gram positive bacteria are more susceptible to different solvent extracts of samples. This may be due to the different constituents of the cell walls of both bacteria (Okigbo and Mmeka 2008; Rita et al., 2020).

The burden of agro-waste of fruits and vegetables thrown in the environment can be decreased by utilizing the wastes as natural feed for poultry (Tedesco et al., 2021). Among the non-processed feed, maize and other cereals are considered the best organic feed but these crops would be expensive because humans consume these cereals crops as a cheap protein sources (Kasapidou et al., 2015). For these reasons, agri-waste proved to be the best alternative source. In the present study, it has been found that high fiber contents are present in banana and orange peels. Many studies recommended the use of banana and orange peels in the poultry feed (reviewed by Sugiharto et al., 2018). The application of fruit peels and fenugreek seed will help in improving the quality of poultry meat. However, the nutritional values of orange and banana peels and fenugreek seeds are principally important to design and formulate a balanced diet to promote the growth of poultry meat without any health hazard and to minimize feed cost by alternating maize which is a costly crop (Siyal et al., 2016).

Conclusion

A combination of fenugreek seeds and peels of banana and oranges in animal diet supplements can provide a

cheap source of dietary fiber, and proteins with additional benefits of antioxidants found in orange peels particularly. The antimicrobial properties may boost the immune system against microbial attacks and in this way; the use of synthetic antibiotics can be reduced significantly. In short, the use of fruits and vegetable waste in animal diets can handle a) the high cost of animal feed, b) the recycling of domestic waste and c) an effective alternative against synthetic biotics.

Author's contributions

KY conceived the idea, and designed the project. IG participated in the design of the study. NN performed crude analysis, and antibacterial study and helped in writing. HZ also helped in writing the manuscript. NK wrote the manuscript and formatting. All authors read and approved the final manuscript.

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