



ISSN: 2395-7476
IJHS 2016; 2(21): 125-131
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www.homesciencejournal.com
Received: 24-03-2016
Accepted: 25-04-2016

Adeline Dorcas F
Department of Home Science,
Women's Christian College,
Chennai – 600006.

Sheila John
Department of Home Science,
Women's Christian College,
Chennai – 600006.

Estherlydia
Assistant Professor, Food
Chemistry and Food Processing,
Loyola College, Chennai –
600034.

Priya Iyer
Department of Biotechnology,
Women's Christian College,
Chennai – 600006.

Priyadarshini S
Department of Home Science,
Women's Christian College,
Chennai – 600006.

Correspondence
Adeline Dorcas F
Department of Home Science,
Women's Christian College,
Chennai – 600006.

Study on the antimicrobial property of bitter orange (*Citrus aurantium* L.) Peel powder and developing recipes using the powder

Adeline Dorcas F, Sheila John, Estherlydia, Priya Iyer, Priyadarshini S

Abstract

The presence of flavonoids, alkaloids and essential oil in citrus peels makes it a potent inhibitor for various microorganisms. The citrus peel possesses many beneficial constituents such as phytochemicals, essential oil, fibre and pectin which provide numerous health benefits to humans but these peels are often discarded or fed to animals. The main objective of the study was to assess the antimicrobial activity of Bitter orange (*Citrus aurantium* L.) peel powder and to develop nutritious recipes incorporating the peel powder. The antimicrobial activity of different extracts (aqueous, acetone and ethanol) of peel powder using agar well diffusion method indicated that acetone extract exhibited highest inhibition for *E.coli*, *Salmonella typhi*, *Enterobacter spp* and *Aspergillus Niger* while ethanol exhibited highest inhibition for *Enterobacter spp*. The recipes were classified into four different categories namely breakfast and lunch, gravies and side dishes, desserts and sweets and quick and easy to make recipes.

Keywords: phytonutrients, antioxidant, bitter orange peel powder

1. Introduction

The sour orange (*Citrus aurantium* L.) is believed to be native to South-east Asia, possibly India (Khan, 2007) [5]. Sour orange or Khatta is also known as Bitter orange, is indigenous to India (Manay, 2008) [6]. The plant bears large and round fruit having thick rind and acid pulp (Das, 2009) [2]. Today, the best-known use of bitter orange is for marmalade, the characteristic British jam made from these aromatic fruit. Bitter orange has been used traditionally as a sedative, an appetite stimulant, an insecticide for mosquitos and dyspepsia. Tropicallly, bitter orange is used for inflammation of eyelids, conjunctivae, muscle pain, rheumatic pain and phlebitis (Roth, 2010) [9]. Preparation from peel, flowers and leaves of bitter orange (*Citrus aurantium* L.) are popularly used in order to minimize central nervous system disorders (Pultrini *et al.*, 2006) [7]. The dried outer peel of the fruit of bitter orange, with the white pulp layer removed, is used medicinally. The leaves are also commonly used in many folk traditions.

Bitter orange peel contains a volatile oil with limonene (about 90%), flavonoids, coumarins, triterpenes, vitamin C, carotene, and pectin. The flavonoids have several useful properties, being anti-inflammatory, antibacterial and antifungal (Suryawanshi, 2011) [10]. Citrus essential oil is also the most popular of natural essential oil, which is intensively accumulated in oil glands of the peel. The Essential oil is recognized not only for its aromatic functions but also for its physiological properties, such as chemoprevention against cancer and aromatherapy effects. Bitter orange was found therapeutically effective in application of various conditions such as digestion and flatulence, cardiovascular health, Cancer, diabetes and bacterial infection. Naturally occurring antimicrobial compounds could be used as food preservatives to protect food quality and extend the shelf life of foods and beverages. Plants constitutes of antimicrobial compounds in various parts such as bark, stalk, leaves, roots, flowers, pods, seeds, stems, hull, latex and fruit rind (Kaneria *et al.*, 2009; Aref *et al.*, 2010; Rajaei *et al.*, 2010) [4, 1, 8]. The antimicrobial activity of the essential oils present in citrus peels is well recognized.

2. Materials and Methods

2.1 Antimicrobial activity of Bitter orange peel powder

The antimicrobial activity of different extracts of peel powder was studied by agar well diffusion method on four bacteria and one fungi. The organisms studied for antimicrobial

activity includes *E.coli*, *Salmonella typhi*, *Shigella spp.*, *Enterobacter spp.* and *Aspergillus niger* The Mueller-Hinton agar medium was inoculated with freshly prepared cells of each bacteria and fungi to yield a lawn of growth. After solidification of the agar, well was made using well cutter and extract solutions (100 µg/ml) were placed on the plates. After incubation at 37 °C for 24 hours for bacteria and 25 °C for 72 hours for fungi, the antimicrobial activity was measured as diameter of the zone of inhibition formed around the well. The diameter of zone of inhibition around each well was measured and recorded at the end of the incubation period. Interpretation of zones of inhibition of test cultures was adopted from Johnson and Case, (1995) [3]. Diameter zone of inhibition of 10 or less indicates test product is resistant to test organism, diameter zone of inhibition of 11 to 15 indicates test product has intermediate resistance to test organism, diameter zone of inhibition of 16 or more indicates that the test product has susceptible resistance to test organism.

2.2 Sensory evaluation of products with Bitter orange peel powder

Twenty five recipes were formulated incorporating 10-20 g of Bitter orange peel powder. Twenty post graduate students were chosen as panel members to score the sensory attributes using a five point hedonic score card. The options on the score card for the evaluation were excellent, very good, good, satisfactory and poor for each sensory attributes such as appearance, colour, texture, flavour and taste.

Criteria used for selection of panel members were

Inclusion criteria

- Basic knowledge and idea of food ingredients, its importance in cooking and techniques of sensory evaluation
- willingness to participate in the study
- Subjects should not be allergic to any specific food

Exclusion criteria

- If infected with any common ailments (cold, blocked nose) the subject will not be permitted to participate in the study
- If undergoing any treatment or medication
- Subjects allergic to any specific foods

The judges were instructed to taste the foods displayed and to fill the five point hedonic score card for the recipes. The options on the score card for the evaluation were excellent, very good, good, satisfactory and poor for each sensory attributes such as appearance, colour, texture, flavour and taste. A glass tumbler filled with drinking water was provided to rinse the mouth. Individual scores were given for appearance, colour, texture, flavor and taste.

Description	Score
Excellent	5
Very good	4
Good	3
Satisfactory	2
Poor	1

The recipes formulated and standardized were classified into include four categories which include

Breakfast and lunch dishes

1. Bitter orange podi fried idli
2. Vegetable kichili powder dosa

3. Bitter orange podi millagu rice
4. Bitter orange podi ragi adai
5. Bitter orange powder aloo paratha
6. Bitter orange podi aval upma
7. Bitter orange powder bread upma

Gravies and side dishes

8. Bitter orange powder paneer capsicum masala
9. Bitter orange powder mango pachidi
10. Bitter orange powder double beans masala
11. Bitter orange powder pakoda gravy
12. Bitter orange powder jack fruit seed masala
13. Bitter orange podi appalla kootu

Desserts and sweets

14. Bitter orange podi gulab jamun
15. Bitter orange powder bread sweet
16. Bitter orange powder rava laddu
17. Bitter orange powder coconut burfi
18. Bitter orange powder fruit custard
19. Bitter orange powder sweet somas
20. Bitter orange powder diamond cuts

Quick and easy to make recipes

21. Tangy, sour, spicy sprout salad
22. Bitter orange powder sweet green gram sundal
23. Bitter orange powder masala sweet corn
24. Bitter orange powder masala pori
25. Bitter orange podi medhu bonda.

2.2 Statistical Analysis

The data obtained were subjected to the following statistical analysis:

1. Arithmetic mean
2. Standard deviation

3. Result and Discussion

3.1 Antimicrobial Activity of *Citrus aurantium* L. peel powder

In the present study, the antimicrobial activity of different extracts of *Citrus aurantium* L. peel powder were studied by agar well diffusion on four bacterial strains namely, *E.coli*, *Salmonella typhi*, *Shigella spp.* and *Enterobacter spp.* and one fungal strain *Aspergillus niger*.

The zone of inhibition of the three extracts aqueous, acetone and ethanol on the gut microorganisms are presented in Table 1.

Table 1: Results for antimicrobial activity of *Citrus aurantium* L. peel powder against gut microorganisms

Microorganisms	Zone of inhibition (mm)		
	Aqueous	Acetone	Ethanol
<i>E.coli</i>	-	15	11
<i>Salmonella typhi</i>	-	17	-
<i>Shigella spp.</i>	-	13	-
<i>Enterobacter spp.</i>	-	15	23
<i>Aspergillus niger</i>	-	20	10

The *Citrus aurantium* L. peel powder extract against four bacteria and one fungal strain were examined using three extracts ethanol, acetone and aqueous. The acetone extract of *Citrus aurantium* L. peel powder showed positive results against all the microorganisms. The maximum inhibition was observed for *Aspergillus Niger* which had 20 mm zone of inhibition. The ethanol extract of *Citrus aurantium* L. peel powder showed positive results against *E.coli*, *Enterobacter*

spp. and *Aspergillus Niger*. While the microorganisms exhibited no antimicrobial activity in aqueous extract of *Citrus aurantium* L. peel powder. Among the three extract of *Citrus*

aurantium L. peel powder, acetone was the best, since it had zone of inhibition for all the studied gut organisms.

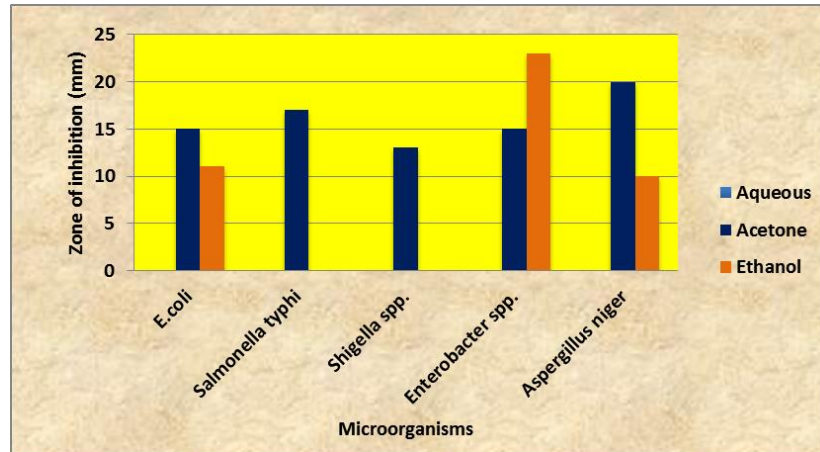


Fig 1: Comparison of zone of inhibition exhibited by *Citrus aurantium* L. peel powder using ethanol, acetone and aqueous extract against the gut microorganisms

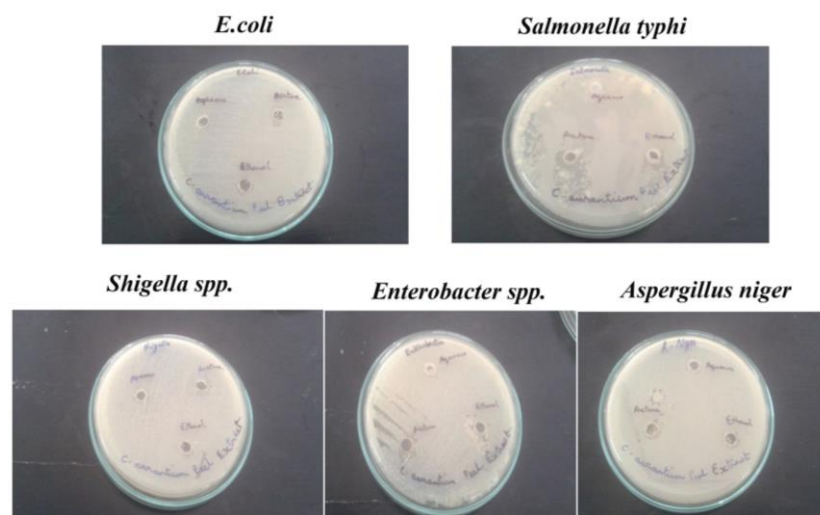


Fig 2: Zone of Inhibition exhibited by *Citrus aurantium* L. peel powder using ethanol, acetone and aqueous extract against the gut microorganisms

3.2 Sensory evaluation of 25 recipes using Bitter orange peel powder

The 25 recipes formulated were divided into four different categories which includes:

1. Breakfast and Lunch dishes
2. Gravies and side dishes
3. Desserts and sweets
4. Quick and easy to make recipes

Acceptability and palatability of recipes for breakfast and lunch dishes

The recipes made under the category of breakfast and lunch were bitter orange podi fried idli, vegetable kichili powder dosa, bitter orange podi millagu rice, bitter orange podi ragi adai, bitter orange powder aloo paratha, bitter orange podi aval upma and bitter orange powder bread upma. The overall acceptability and palatability of recipes for breakfast and lunch are presented in Table 2.

Table 2: Overall acceptability and palatability of recipes for breakfast and lunch dishes

Recipes	Overall acceptability (Mean±S.D)
Bitter Orange Podi Fried Idli	4.52 ± 0.67
Vegetable Kichili Powder Dosa	4.49 ± 0.58
Bitter Orange Podi Millagu Rice	4.47 ± 0.67
Bitter Orange Podi RagiAdai	4.02 ± 0.94
Bitter Orange Powder Aloo Paratha	4.39 ± 0.67
Bitter Orange Podi Aval Upma	4.15 ± 0.69
Bitter Orange Powder Bread Upma	4.54 ± 0.64

From Table 2, it can be inferred that bitter orange podi fried idli and bitter orange powder bread upma were rated as excellent by the panelist whereas the other dishes were rated very good. The addition of bitter orange peel powder gave a

distinct taste to the dishes. These breakfast and lunch dishes can be given as packed lunches to children as they are nutritious and tasty.



Fig 3: Overall acceptability and palatability of recipes for breakfast and lunch dishes Acceptability and palatability of recipes for gravies and side dishes

The recipes made under the category of gravies and side dishes were bitter orange powder paneer capsicum masala, bitter orange powder mango pachadi, bitter orange powder double beans masala, bitter orange powder pakoda gravy, bitter orange powder jack fruit seed masala and bitter orange podi appala kootu.

The overall acceptability and palatability of recipes for gravies and side dishes are presented in Table 3.

Table 3: Overall acceptability and palatability of recipes for gravies and side dishes

Recipes	Overall acceptability (Mean±S.D)
Bitter Orange Powder Paneer Capsicum Masala	4.68±0.54
Bitter Orange Powder Mango Pachadi	4.52±0.60
Bitter Orange Powder Double Beans Masala	4.71±0.56
Bitter Orange Powder Pakoda Gravy	4.48±0.58
Bitter Orange Powder Jack fruit seed Masala	4.59±0.62
Bitter Orange Podi Appala Kootu	4.5±0.58

From Table 3, it was found that all the dishes were rated as excellent among the panelists except bitter orange powder pakoda gravy was rated as very good. These dishes go well with rice and all bread varieties. The Addition of bitter orange peel powder gave a bitter-tangy twist to the gravies.

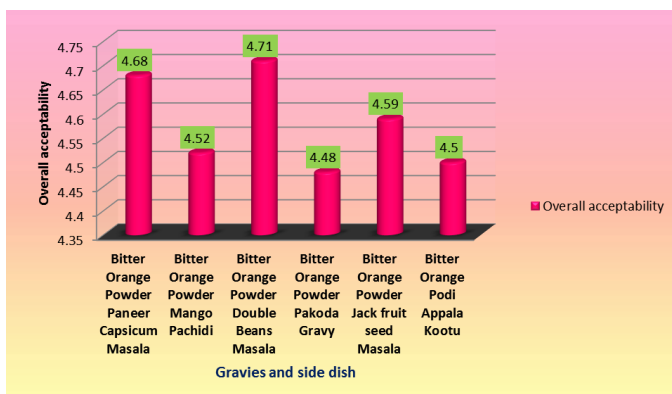


Fig 4: Overall acceptability and palatability of recipes for gravies and side dishes Acceptability and palatability of recipes for desserts and sweets

The recipes made under the category of desserts and sweets were bitter orange podi gulab jamun, bitter orange powder

bread sweet, bitter orange powder rava laddu, bitter orange powder coconut burfi, bitter orange powder fruit custard, bitter orange powder sweet somas and bitter orange powder diamond cuts.

The overall acceptability and palatability of recipes for desserts and sweets are presented in Table 4.

Table 4: Overall acceptability of recipes for desserts and sweets

Recipes	Overall acceptability (Mean±S.D)
Bitter Orange Podi Gulab jamun	4.67 ± 0.55
Bitter Orange Powder Diamond Cuts	4.33 ± 0.76
Bitter Orange Powder Rava Laddu	4.53 ± 0.63
Bitter Orange Powder Coconut Burfi	4.57 ± 0.63
Bitter Orange Powder Fruit Custard	4.51 ± 0.59
Bitter Orange Powder Sweet Somas	4.79 ± 0.39
Bitter Orange Powder Bread Sweet	4.32 ± 0.74

From Table 3, it can be inferred that all the dishes were graded as excellent among the panelists. But, bitter orange powder diamond cuts and bitter orange powder bread sweet were graded as very good. Including the bitter orange peel powder enhanced the flavor, nutrient content and aroma of these desserts, thus making it a healthier option.

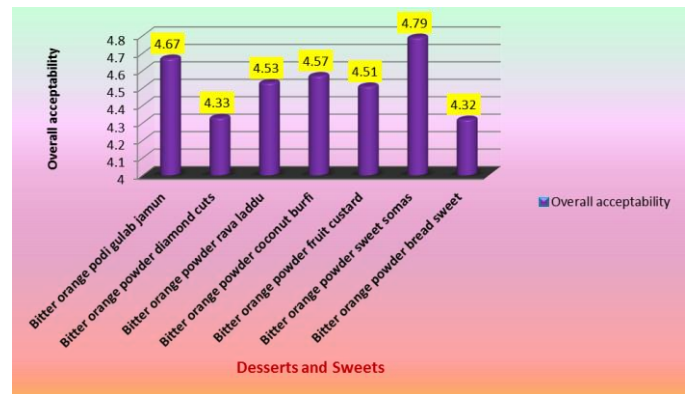


Fig 5: Overall acceptability and palatability of recipes for desserts and sweets Acceptability and palatability of quick and easy to make recipes

The recipes made under the category of quick and easy to make recipes were tangy, sour, spicy sprout salad, bitter orange powder sweet green gram sundal, bitter orange powder masala sweet corn, bitter orange powder masala pori, and bitter orange powder medhu bonda.

The overall acceptability and palatability of quick and easy to make recipes are presented in Table 5.

Table 5: Overall acceptability of quick and easy to make recipes

Recipes	Overall acceptability (Mean±S.D)
Tangy, Sour, Spicy Sprout Salad	4.41 ± 0.65
Bitter Orange Powder Sweet Green Gram Sundal	4.43 ± 0.63
Bitter Orange Powder Masala Sweet Corn	4.76 ± 0.43
Bitter Orange Powder Masala Pori	4.92 ± 0.27
Bitter Orange Podi Medhu Bonda	4.74 ± 0.50

From Table 5, it can be inferred that all the dishes were graded as excellent among the panelists. But, tangy, sour, spicy sprout salad and bitter orange powder sweet green gram sundal were graded as very good.

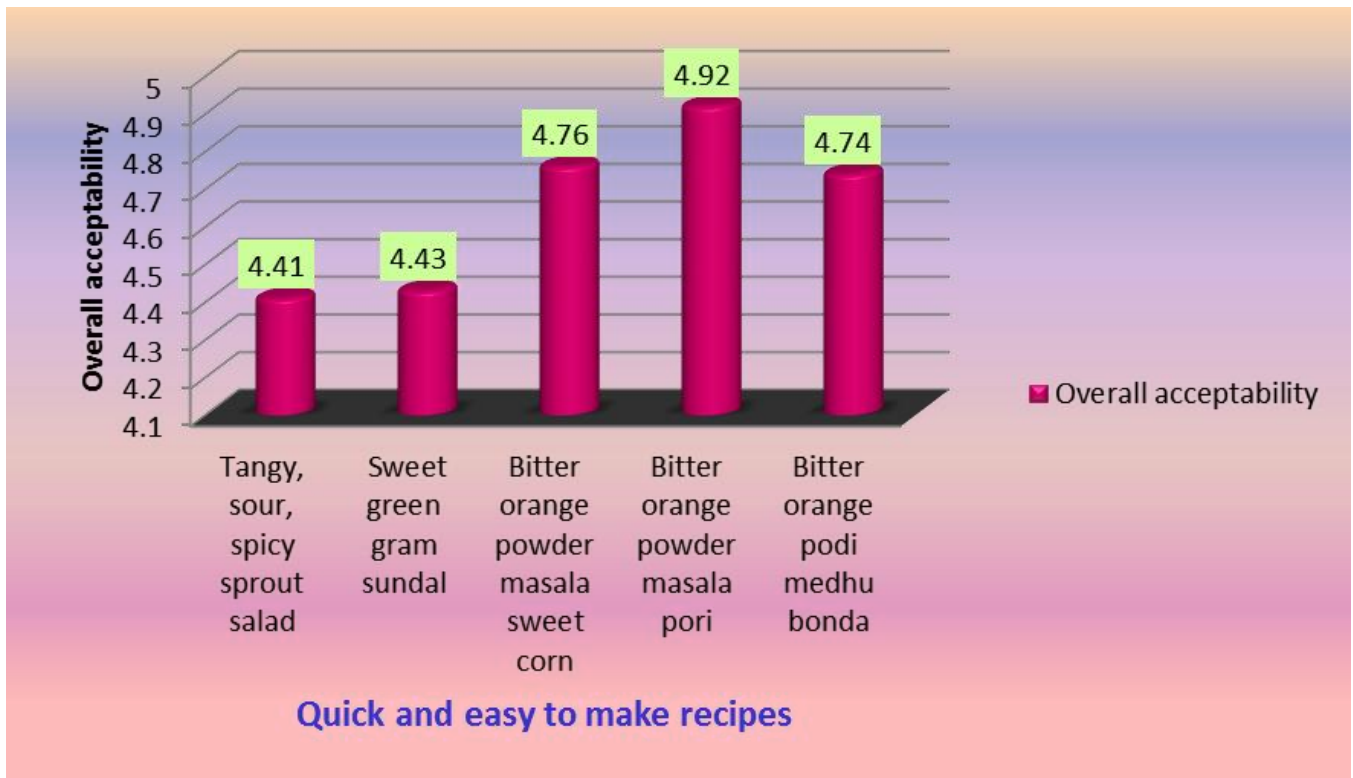


Fig 6: Overall acceptability and palatability for quick and easy to make recipes

4. Conclusion

The study was aimed to determine the antimicrobial potential of Bitter orange peels and an attempt to explore ways in which the Bitter orange peels could be included in our daily diet. In conclusion it can be stated that Bitter orange peel powder is a potential inhibitor of gut pathogens and is also a good value added addition to the diet as it rich in antioxidant,

phytonutrient and fibre. The addition of Bitter orange peel powder enhanced the nutrient content, taste and flavor of the recipes. Further research can be carried out by supplementing the peel as a food supplement for conditions like diabetes mellitus, cardio vascular diseases and obesity to test its effect as it is rich in antioxidants, phytonutrient and fibre.

Bitter orange podi fried idli



Vegetable kichili powder dosa



Bitter orange podi millagu rice



Bitter orange podi ragi adai



Bitter orange powder aloo paratha



Bitter orange podi aval upma



Bitter orange powder bread upma



Fig 7: Breakfast and lunch recipes

**Bitter orange powder
paneer capsicum masala**



**Bitter orange powder
mango pachidi**



**Bitter orange powder
double beans masala**



**Bitter orange powder
pakoda gravy**



**Bitter orange powder
jack fruit seed masala**



**Bitter orange podi
appalla kootu**



Fig 8: Gravies and side dishes

**Bitter oranger podi
gulab jamun**



**Bitter orange powder
bread sweet**



**Bitter orange powder
rava laddu**



**Bitter orange powder
coconut burfi**



**Bitter orange powder
fruit custard**



**Bitter orange powder
sweet somas**



**Bitter orange powder
diamond cuts**



Fig 9: Desserts and Sweets

**Tangy, sour, spicy
sprout salad**



**Bitter orange powder
sweet green gram sundal**



**Bitter orange powder
masala sweet corn**



**Bitter orange powder
masala pori**



**Bitter orange podi
medhu bonda.**



Fig 10: Quick and ready to make recipes

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