

# ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF BITTER ORANGE (*Citrus aurantium* L.) AND SWEET ORANGE (*Citrus sinensis* L. Osbeck) PEEL EXTRACTS

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## Introduction

Citrus processing generates a substantial amount of peel waste, which is a valuable source of bioactive compounds with potential antimicrobial properties. This study investigated the antioxidant and antibacterial activities of bitter orange (BO) and sweet orange (SO) peels.

## Materials and Methods

The fruits were washed and air-dried at room temperature. The flavedo and albedo were removed with a fruit peeler and crushed.

□ total phenolics (TP) - Folin-Ciocalteu colorimetric method with gallic acid as standard  
 □ antioxidant activity (AA) – ABTS and DPPH assay - methods with Trolox as standard; measurements were performed in three parallels

Extracts were prepared by extraction in an ultrasound bath (25 °C, 15 min.) using ethanol (1:10), vacuum concentrated, and tested by agar disc-diffusion (5 mm discs) at 25 mg mL<sup>-1</sup> in DMSO.

Four bacterial strains (*E. coli*, *S. enteritidis*, *L. monocytogenes*, *S. aureus*) were tested using the disc diffusion method. Bacterial suspensions (0.5 McFarland) were swabbed onto Mueller-Hinton agar. Sterile discs (5 mm) were loaded with 10 µL of orange extracts, alongside control antibiotics (tetracycline, penicillin G, cefotaxime, erythromycin, ceftazidime, piperacillin). After 1-hour pre-diffusion at 4°C, plates were incubated at 37°C, and inhibition zones were quantified with a digital caliper. All assays were conducted in duplicate.

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**Table 1 Total phenolics and antioxidant activity of BO and SO**

Sample	TP (g kg <sup>-1</sup> )	AA (µmol 100 mL <sup>-1</sup> )	
		ABTS	DPPH
Flavedo BO	16.388 ± 0.562	95.004 ± 1.151	19.666 ± 1.962
Albedo BO	49.394 ± 0.308	201.937 ± 5.105	27.395 ± 0.259
Flavedo SO	22.579 ± 1.274	122.652 ± 3.271	23.675 ± 0.733
Albedo SO	17.770 ± 1.932	106.996 ± 3.335	5.752 ± 0.543

**Table 2 Bitter orange and sweet orange antibacterial activity**

Tested bacteria		Bitter orange		Sweet orange	
		flavedo	albedo	flavedo	albedo
G (-)	<i>E. coli</i>	7.98 <sup>a*</sup>	6.53 <sup>ab</sup>	7.74 <sup>b</sup>	7.94 <sup>a</sup>
	<i>S. enteritidis</i>	8.61 <sup>b</sup>	7.19 <sup>b</sup>	9.10 <sup>c</sup>	8.13 <sup>a</sup>
G (+)	<i>L. monocytogenes</i>	0	0	0	0
	<i>S. aureus</i>	0	0	0	0

\* inhibition zones in mm; a, b, c - Rows with different letters are statistically significantly different ANOVA, Bonferroni post-hock test (p < 0.05)

## Conclusions

- BO contains a significantly higher proportion of TP and greater antioxidant activity than SO.
- In BO's, the albedo was a richer source of phenolic compounds than the flavedo, while in SO, the flavedo had higher TP content and greater AA than the albedo, regardless of the assay used.
- Antibacterial activity screening revealed species-specific susceptibility among G(-) bacteria.
- *S. enteritidis* showed significantly greater susceptibility to SO extracts (both flavedo and albedo), whereas
- *E. coli* exhibited consistent inhibition patterns across all citrus extract types, with no significant differences between BO and SO varieties or tissue types.

✓ Higher concentrations of citrus peel extracts may provide a naturally derived, consumer-acceptable alternative for pathogen control in specific applications.  
 ✓ Additionally, valorisation of orange peel waste can significantly reduce agro-industrial waste by transforming it into a source of value-added antibacterial and antioxidant extracts, thereby supporting a circular economy.