



Antibacterial and Reduced Cancer Cell Growth by Fuzhuan Tea, Chinese Fermented *Camellia sinensis*

Amy C. Keller¹, Corey D. Broeckling², Tiffany L. Weir³, Elizabeth P. Ryan^{1,3}

¹Animal Cancer Center, Departments of Clinical Sciences ²Proteomics and Metabolomics Facility ³Food Science and Human Nutrition
Colorado State University, Fort Collins, CO 80526

INTRODUCTION

Dark teas, such as Pu'er and Fuzhuan, are *Camellia sinensis* L. (Theaceae) that have undergone a microbial fermentation process (1). Fuzhuan tea, whose leaves are characterized by the production of yellow cleistothecia from the fungus *Eurotium cristatum*, is produced in Hunan Province, China, and is used medicinally as a preventative and remedy for food-borne illnesses, dysentery, and to aid general nutrition and digestion (1-4).

Several reports suggest that the fermentation process required for production of Fuzhuan tea alters its phytochemical profile. Catechins, commonly associated with non-fermented tea's bioactive properties, are reduced in Fuzhuan tea compared to non-fermented green teas (3). However, it has been reported that *E. cristatum* and other dominant fungi may impart unique chemical components to the tea; several novel triterpenoids have already been identified from leaf extracts (2).

These findings, alongside Fuzhuan tea's inhibitory effects on gastrointestinal tumor growth (5), provided a strong rationale for the comparison of metabolomic profiles of water-soluble extracts of Fuzhuan tea with several non-fermented green teas for the purpose of identifying novel bioactive components. In addition, the teas were tested for antibacterial and cancer antiproliferative activity.



Fuzhuan tea (*Camellia sinensis*).

Eurotium cristatum

METHODS

Metabolomic analysis was performed by direct injection of water-soluble tea extracts redissolved in MeOH onto an ultra performance liquid chromatography instrument (UPLC) coupled to a Q-ToF Micro mass spectrometer. Data was analyzed and candidate compounds identified by comparison with KEGG and Metlin databases. The identification requirements for level 3 in metabolomic analysis were applied as previously described by the Chemical Analysis Working Group Metabolomics Standards Initiative (6).

Antibacterial activity of non-fermented green and Fuzhuan tea extracts were measured in vitro against *Staphylococcus aureus* (NCTC 8325) and *Shigella sonnei*. Extracts were also tested to determine effects on growth of the colon cancer cell line, Caco-2.

RESULTS

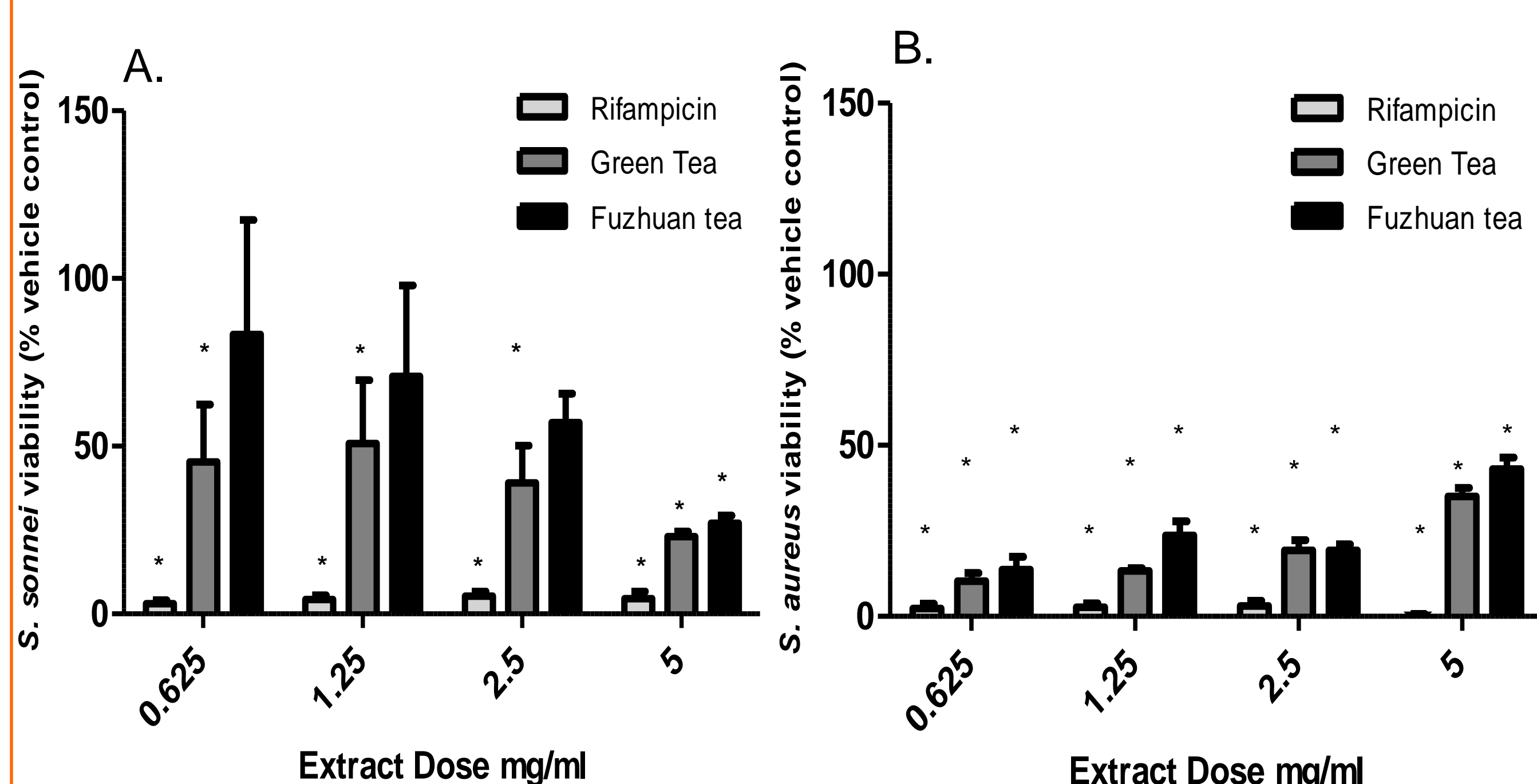


Figure 1. Growth inhibitory effects of Fuzhuan tea and non-fermented green tea extracts on viability of pathogenic bacteria were determined in vitro using direct inhibition assays in 96-well plates (A) *Shigella sonnei* (MIC 5 mg/ml, $p < 0.01$) and (B) *Staphylococcus aureus* following a 24 hour incubation (MIC 0.625 mg/ml, $p < 0.01$)

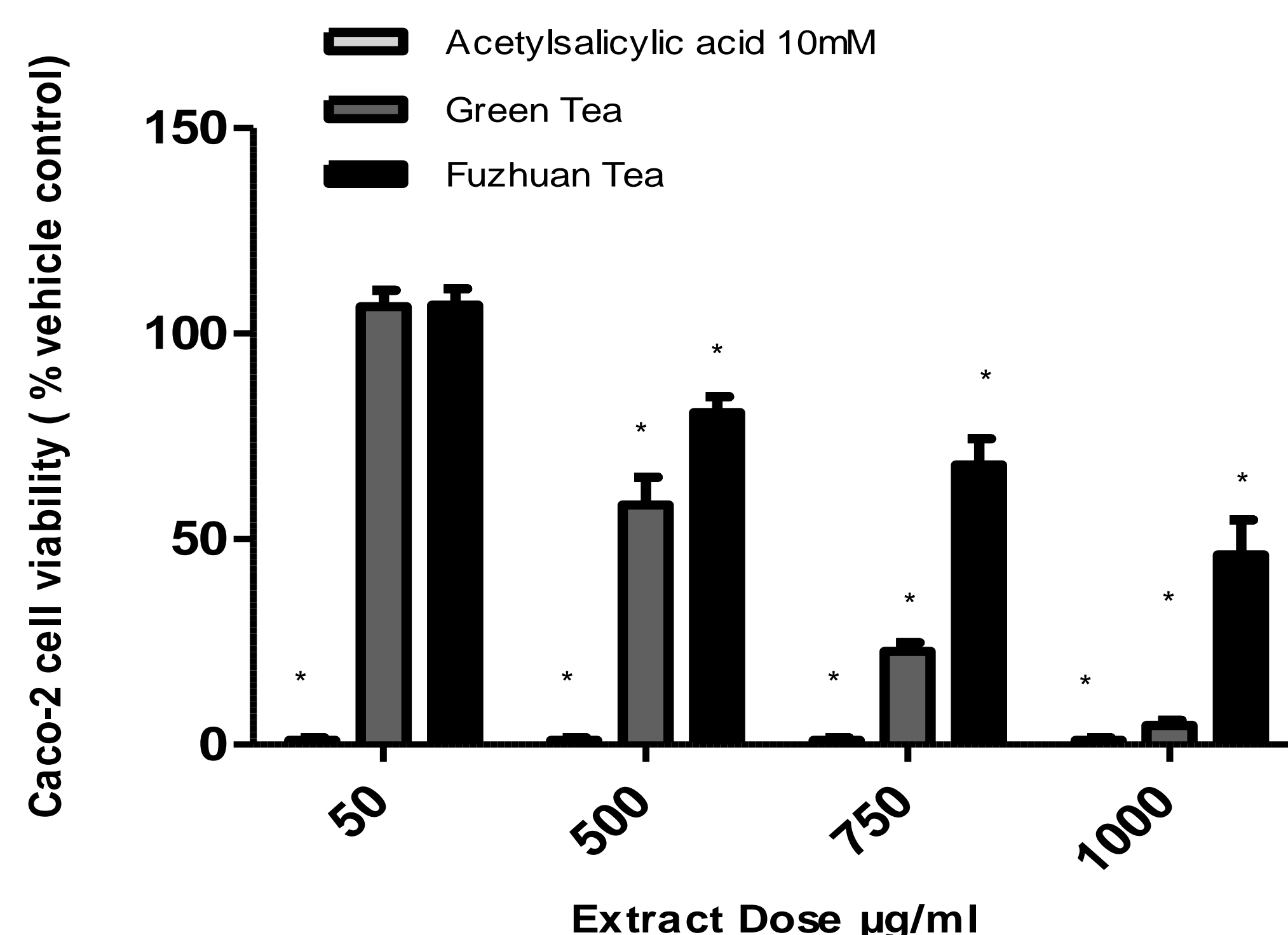


Figure 2. Fermented Fuzhuan tea and non-fermented green tea significantly decreased Caco-2 viability compared with the vehicle control after 72 hours.

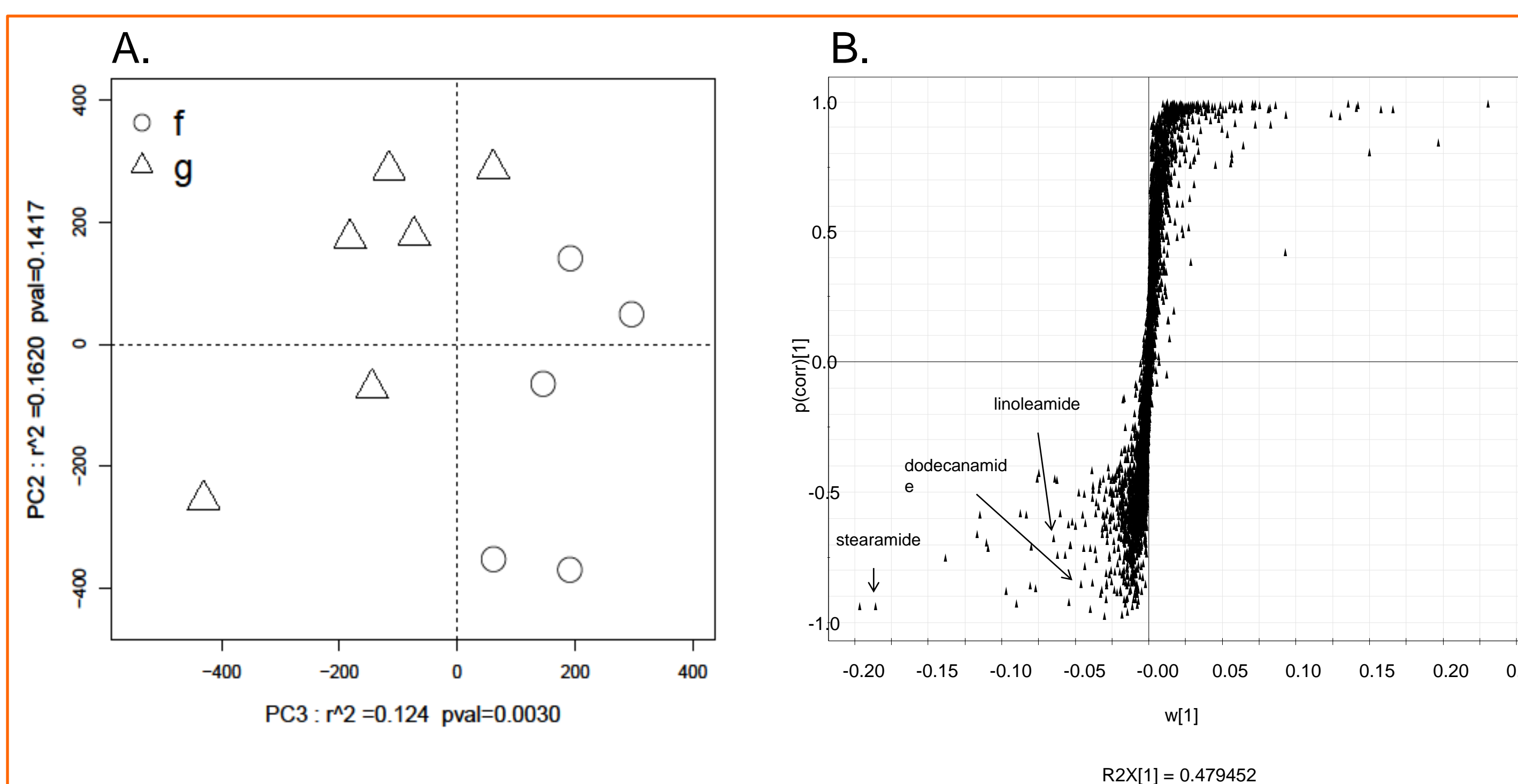


Figure 3. A. Principal component analysis of aqueous extracts of Fuzhuan tea versus non-fermented green tea. Component 2 (16.2%) and component 3 (12.4%, $p = 0.003$) are shown.

B. Partial least squares discriminant analysis (PLS-DA) S-plot shows a binary comparison of average values of Fuzhuan tea ($n = 5$) and non-fermented green tea ($n = 1$).

Table 1. Candidate compounds found in Fuzhuan tea and non-fermented green tea.

Candidate	Class	m/z	RT (minutes)	Fold-change	p value
dodecanamide ^a	fatty acid amide	200.207	4.89	1.59	<0.05
glycosylated flavonoid ^a	flavonoid	786.227	5.40	2.78	<0.01
linoleamide ^a	fatty acid amide	280.270	5.51	7.20	<0.05
stearamide ^a	fatty acid amide	284.288	7.74	1.49	<0.05
glycosylated flavonoid ^a	flavonoid	743.195	2.57	51.77	< 0.001
glycosylated flavonoid ^a	flavonoid	889.248	2.58	35.54	< 0.001
epigallocatechin ^{a,b}	flavonol	307.085	1.63	1.52 (higher in green tea)	NS
epicatechin gallate ^{a,b}	flavonol	459.091	1.73	8.14 (higher in green tea)	< 0.001
Caffeine ^{a,b}	alkaloid	195.088	1.85	1.07	NS

^aMetabolite identifications made according to Level 3 (6).

^bAlso previously reported in Fuzhuan tea (7).

CONCLUSIONS

- ❖ Fuzhuan tea dose dependently and significantly inhibited viability of *S. sonnei* with a minimum inhibitory concentration (MIC) of 5 mg/ml as compared with the untreated vehicle control ($p < 0.01$).
- ❖ When tested with *S. aureus*, both the fermented Fuzhuan tea and non-fermented green tea had a MIC of 0.625 mg/ml as compared with the vehicle control ($p < 0.01$).
- ❖ Fuzhuan tea significantly decreased Caco-2 viability after 72 hours and had a MIC of 500 µg/ml and an LD₅₀ of 1 mg/ml as compared with vehicle control.
- ❖ Principal component analysis (PCA) revealed that Fuzhuan tea and non-fermented green tea phytochemical profiles are maximally separated by PC3, which accounts for 12.4% of the total variation in the dataset.
- ❖ Fuzhuan tea has unique chemical components compared to non-fermented green tea as well as antibacterial activity.
- ❖ Future directions: confirm the identity of unique Fuzhuan tea compounds and test bioactivity of Fuzhuan tea extract in vivo.

REFERENCES

- Xu A., et al. *International Journal of Food Microbiology* 2011; 146: p. 14-22.
- Ling T.-J., et al. *Journal of Agricultural and Food Chemistry* 2010; 58: p. 4945-4950.
- Weir, T. et al. In press: *HerbalGram* 2011.
- Fu D., et al. *Food Res Int* 2011; doi:10.1016/j.foodres.2011.07.008.
- Song L.B., et al. *Journal of Tea Science* 2009; 29: p. 191-195.
- Sumner L.W., et al. *Metabolomics* 2007; 3: p. 211-221.
- Wu, Y.Y., et al. *African Journal of Biotechnology*; 9: p. 6748-6754.

ACKNOWLEDGMENTS

Fuzhuan tea (PHatea) and travel support was provided by a gift from the Golden Flower Trading Company (Lone Tree, Colorado), and research was supported by the Animal Cancer Center, Colorado State University.