

Assessing Anti-inflammatory Activities and Compounds in Switchgrass (*Panicum virgatum*)

Khanh-Van Ho^{1,2,3,4}, Novianus Efrat¹, Kathy L. Schreiber⁵, Phuc H. Vo¹, Marco N. De Canha⁶, Analike Blom van Staden⁶, Bianca D. Payne⁶, Carel B. Oosthuizen⁶, Danielle Twilley⁶, Zhentian Lei^{7,8}, Lloyd W. Sumner^{7,8}, Charles R. Brown⁹, Namrita Lall^{1,6,10} and Chung-Ho Lin^{1*}

¹ Center for Agroforestry, School of Natural Resources, University of Missouri, Columbia, Missouri, United States

² Department of Chemistry, University of Missouri, Columbia, Missouri, United States

³ Molecular Imaging and Theranostics Center, University of Missouri, Columbia, Missouri, United States

⁴ Department of Food Technology, Can Tho University, Can Tho, Vietnam

⁵ Cell and Immunobiology Core, University of Missouri, Columbia, Missouri, United States

⁶ Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa

⁷ Metabolomics Center, University of Missouri, Columbia, Missouri, United States

⁸ Department of Biochemistry, Bond Life Sciences Center, University of Missouri, Columbia, Missouri, United States

⁹ Department of Veterinary Pathobiology, University of Missouri, Columbia, Missouri, United States

¹⁰ College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, India

* Corresponding Author: Chung-Ho Lin, PhD

Research Associate Professor

University of Missouri

Center for Agroforestry

203 ABNR Natural Resources Building

University of Missouri

Columbia, Missouri 65211, United States

Phone: 573-882-6283

Email: LinChu@missouri.edu

SUPPLEMENTARY INFORMATION

Supplementary Table S1. Putative identification of the secondary metabolites with known anti-inflammatory activities in switchgrass through untargeted metabolomics analyses.

Compound	Reference
4-Hexylresorcinol	Ahn, <i>et al.</i> [1], Frankos, <i>et al.</i> [2]
Aescin	Sirtori [3]
Altholactone	Al Momani, <i>et al.</i> [4], Jiang, <i>et al.</i> [5],
Auranofin	Thangamani, <i>et al.</i> [6]
Baicalin	He, <i>et al.</i> [7]
Bergenin	Srivastava [8]
Bruceine B	Bawm, <i>et al.</i> [9]
Coumarin	Venugopala, <i>et al.</i> [10]
Dioscin	Aumsuwan, <i>et al.</i> [11]
Formononetin 7-O-rutinoside	Singh, <i>et al.</i> [12]
Gambogic acid	Kashyap, <i>et al.</i> [13]
Kaempferol-7-rhamnoside	Sim, <i>et al.</i> [14]
Nevadensin 5-gentibioside	Alhusainy, <i>et al.</i> [15]
Okanin 3',4'-diglucoside	Kil, <i>et al.</i> [16]
Osthenol-7-O-beta-D-gentiobioside	Kuo, <i>et al.</i> [17]
Petunidin 3-glucoside	Huang, <i>et al.</i> [18]
Quercetin	Li, <i>et al.</i> [19]
Quercetin-3-glucoside	Walton, <i>et al.</i> [20]
Quercitrin	David, <i>et al.</i> [21]
Rhoifolin	Qin, <i>et al.</i> [22]
Rutin trihydrate	Ganeshpurkar and Saluja [23]
Tenylidone	Panic, <i>et al.</i> [24]

Supplementary Table S2. *In vitro* biological activities of the extracts derived from the four switchgrass cultivars. Antibacterial and antimycobacterial activities were evaluated against 2 bacterial strains *Cutibacterium acnes* and *Mycobacterium smegmatis*, respectively. Anticancer activity was investigated using human colorectal adenocarcinoma (HT-29) and human malignant melanoma (UCT-MEL-1) cell lines.

Extract	Antibacterial*	Antimycobacterial*	Anticancer ⁺		Porcine elastase ⁺	Mushroom tyrosinase ⁺
			HT-29	UCT-MEL-1		
<u>Cultivar</u>						
Alamo	>500	>1000	>400	>400	>500	>1000
Kanlow	>500	>1000	>400	>400	>500	>1000
Liberty	>500	>1000	>400	>400	>500	>1000
Show Me	>500	>1000	>400	>400	>500	>1000
<u>Control</u>						
Tetracycline	1.56	-	-	-	-	-
Ciprofloxacin	-	0.31	-	-	-	-
Actinomycin D	-	-	0.040	0.011	-	-
Ursolic acid	-	-	-	-	20.80	-
Kojic acid	-	-	-	-	-	0.45

* Values are expressed as minimum inhibitory concentration (MIC in $\mu\text{g/mL}$)

⁺ Values are shown as fifty percent inhibitory concentration (IC_{50} , $\mu\text{g/mL}$)

Screening of other biological activities of switchgrass. The four cultivars of switchgrass were tested for potential biological activities against several targets based on the stipulated health benefits. Antibacterial activity against *Cutibacterium acnes* (ATCC 6919) and the anti-proliferative activity against human colorectal adenocarcinoma (HT-29) and pigmented human malignant melanoma (UCT-MEL1) were determined using the method described by Lall, *et al.* [25]. Antimycobacterial activity against *Mycobacterium smegmatis* (MC² 155) was determined using the method described by Reid, *et al.* [26]. Anti-elastase activity against porcine pancreatic elastase was performed according to the methods described by Lall, *et al.* [27]. The tyrosinase inhibitory potential was determined using a colorimetric assay as described in Lall, *et al.* [28]. We found that the cultivars at the highest concentration tested (>0.4 mg/mL) did not exhibit activity against the selected microbes (*C. acnes* and *M. smegmatis*), antiproliferative activity against the selected cancerous cell lines (HT-29 and UCT-MEL-1) or enzyme inhibition (against elastase and tyrosinase) (Supplementary Table 2).

REFERENCES

1. Ahn, J.; Kim, S.-G.; Kim, M.-K.; Kim, D.-W.; Lee, J.-H.; Seok, H.; Choi, J.-Y. Topical delivery of 4-hexylresorcinol promotes wound healing via tumor necrosis factor- α suppression. *Burns* **2016**, *42*, 1534-1541.
2. Frankos, V.H.; Schmitt, D.F.; Haws, L.C.; McEvily, A.J.; Iyengar, R.; Miller, S.A.; Munro, I.C.; Clydesdale, F.M.; Forbes, A.L.; Sauer, R.M. Generally recognized as safe (GRAS) evaluation of 4-hexylresorcinol for use as a processing aid for prevention of melanosis in shrimp. *Regul. Toxicol. Pharmacol.* **1991**, *14*, 202-212.
3. Sirtori, C.R. Aescin: pharmacology, pharmacokinetics and therapeutic profile. *Pharmacol. Res.* **2001**, *44*, 183-193.
4. Al Momani, F.; Alkofahi, A.S.; Mhaidat, N.M. Altholactone displays promising antimicrobial activity. *Molecules* **2011**, *16*, 4560-4566.
5. Jiang, C.; Masood, M.; Rasul, A.; Wei, W.; Wang, Y.; Ali, M.; Mustaqeem, M.; Li, J.; Li, X. Altholactone inhibits NF- κ B and STAT3 activation and induces reactive oxygen species-mediated apoptosis in prostate cancer DU145 cells. *Molecules* **2017**, *22*, 240.
6. Thangamani, S.; Mohammad, H.; Abushahba, M.F.; Sobreira, T.J.; Seleem, M.N. Repurposing auranofin for the treatment of cutaneous staphylococcal infections. *Int. J. Antimicrob. Agents* **2016**, *47*, 195-201.
7. He, P.; Wu, Y.; Shun, J.; Liang, Y.; Cheng, M.; Wang, Y. Baicalin ameliorates liver injury induced by chronic plus binge ethanol feeding by modulating oxidative stress and inflammation via CYP2E1 and NRF2 in mice. *Oxid. Med. Cell. Longev.* **2017**, 2017.
8. Srivastava, N. Evaluation of quantitative variation of secondary metabolites in *Bergenia ciliata* (Haw.) using high performance thin layer chromatography. *J. Biomed. Res.* **2014**, *28*, 328.
9. Bawm, S.; Matsuura, H.; Elkhateeb, A.; Nabeta, K.; Nonaka, N.; Oku, Y.; Katakura, K. In vitro antitrypanosomal activities of quassinoid compounds from the fruits of a medicinal plant, *Brucea javanica*. *Vet. Parasitol.* **2008**, *158*, 288-294.
10. Venugopala, K.N.; Rashmi, V.; Odhav, B. Review on natural coumarin lead compounds for their pharmacological activity. *BioMed Res. Int.* **2013**, 2013.
11. Aumsuwan, P.; Khan, S.I.; Khan, I.A.; Ali, Z.; Avula, B.; Walker, L.A.; Shariat-Madar, Z.; Helferich, W.G.; Katzenellenbogen, B.S.; Dasmahapatra, A.K. The anticancer potential of steroidal saponin, dioscin, isolated from wild yam (*Dioscorea villosa*) root extract in invasive human breast cancer cell line MDA-MB-231 in vitro. *Arch. Biochem. Biophys.* **2016**, *591*, 98-110.
12. Singh, K.B.; Dixit, M.; Dev, K.; Maurya, R.; Singh, D. Formononetin, a methoxy isoflavone, enhances bone regeneration in a mouse model of cortical bone defect. *Br. J. Nutr.* **2017**, *117*, 1511-1522.
13. Kashyap, D.; Mondal, R.; Tuli, H.S.; Kumar, G.; Sharma, A.K. Molecular targets of gambogic acid in cancer: recent trends and advancements. *Tumor Biol.* **2016**, *37*, 12915-12925.
14. Sim, M.-O.; Jang, J.-H.; Lee, H.-E.; Jung, H.-K.; Cho, H.-W. Antioxidant effects of *Geranium nepalense* ethanol extract on H₂O₂-induced cytotoxicity in H9c2, SH-SY5Y, BEAS-2B, and HEK293. *Food Sci. Biotechnol.* **2017**, *26*, 1045-1053.
15. Alhusainy, W.; Williams, G.M.; Jeffrey, A.M.; Iatropoulos, M.J.; Taylor, S.; Adams, T.B.; Rietjens, I.M. The natural basil flavonoid nevadensin protects against a methyleugenol-

- induced marker of hepatocarcinogenicity in male F344 rat. *Food Chem. Toxicol.* **2014**, *74*, 28-34.
16. Kil, J.-S.; Son, Y.; Cheong, Y.-K.; Kim, N.-H.; Jeong, H.J.; Kwon, J.-W.; Lee, E.-J.; Kwon, T.-O.; Chung, H.-T.; Pae, H.-O. Okanin, a chalcone found in the genus *Bidens*, and 3-penten-2-one inhibit inducible nitric oxide synthase expression via heme oxygenase-1 induction in RAW264.7 macrophages activated with lipopolysaccharide. *J. Clin. Biochem. Nutr.* **2011**, 1112130134-1112130134.
 17. Kuo, P.-C.; Liao, Y.-R.; Hung, H.-Y.; Chuang, C.-W.; Hwang, T.-L.; Huang, S.-C.; Shiao, Y.-J.; Kuo, D.-H.; Wu, T.-S. Anti-inflammatory and neuroprotective constituents from the peels of *Citrus grandis*. *Molecules* **2017**, *22*, 967.
 18. Huang, W.; Yan, Z.; Li, D.; Ma, Y.; Zhou, J.; Sui, Z. Antioxidant and anti-inflammatory effects of blueberry anthocyanins on high glucose-induced human retinal capillary endothelial cells. *Oxid. Med. Cell. Longev.* **2018**, 2018.
 19. Li, X.; Jiang, Q.; Wang, T.; Liu, J.; Chen, D. Comparison of the antioxidant effects of quercitrin and isoquercitrin: Understanding the role of the 6"-OH group. *Molecules* **2016**, *21*, 1246.
 20. Walton, M.C.; McGhie, T.K.; Reynolds, G.W.; Hendriks, W.H. The flavonol quercetin-3-glucoside inhibits cyanidin-3-glucoside absorption in vitro. *Journal of agricultural and food chemistry* **2006**, *54*, 4913-4920.
 21. David, A.V.A.; Arulmoli, R.; Parasuraman, S. Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacogn. Rev.* **2016**, *10*, 84.
 22. Qin, X.-X.; Zhang, M.-Y.; Han, Y.-Y.; Hao, J.-H.; Liu, C.-J.; Fan, S.-X. Beneficial phytochemicals with anti-tumor potential revealed through metabolic profiling of new red pigmented lettuces (*Lactuca sativa* L.). *Int. J. Mol. Sci.* **2018**, *19*, 1165.
 23. Ganeshpurkar, A.; Saluja, A.K. The pharmacological potential of rutin. *Saudi pharmaceutical journal* **2017**, *25*, 149-164.
 24. Panic, G.; Vargas, M.; Scandale, I.; Keiser, J. Activity profile of an FDA-approved compound library against *Schistosoma mansoni*. *PLoS neglected tropical diseases* **2015**, *9*, e0003962.
 25. Lall, N.; Henley-Smith, C.J.; De Canha, M.N.; Oosthuizen, C.B.; Berrington, D. Viability reagent, PrestoBlue, in comparison with other available reagents, utilized in cytotoxicity and antimicrobial assays. *Int. J. Microbiol.* **2013**, 2013.
 26. Reid, A.; Oosthuizen, C.B.; Lall, N. In vitro antimycobacterial and adjuvant properties of two traditional South African teas, *Aspalathus linearis* (Burm. f.) R. Dahlgren and *Lippia scaberrima* Sond. *South African Journal of Botany* **2020**, *128*, 257-263.
 27. Lall, N.; Kishore, N.; Fibrich, B.; Lambrechts, I.A. In vitro and in vivo activity of *Myrsine africana* on elastase inhibition and anti-wrinkle activity. *Pharmacognosy magazine* **2017**, *13*, 583.
 28. Lall, N.; Van Staden, A.B.; Rademan, S.; Lambrechts, I.; De Canha, M.N.; Mahore, J.; Winterboer, S.; Twilley, D. Antityrosinase and anti-acne potential of plants traditionally used in the Jongilanga community in Mpumalanga. *South African Journal of Botany* **2019**, *126*, 241-249.