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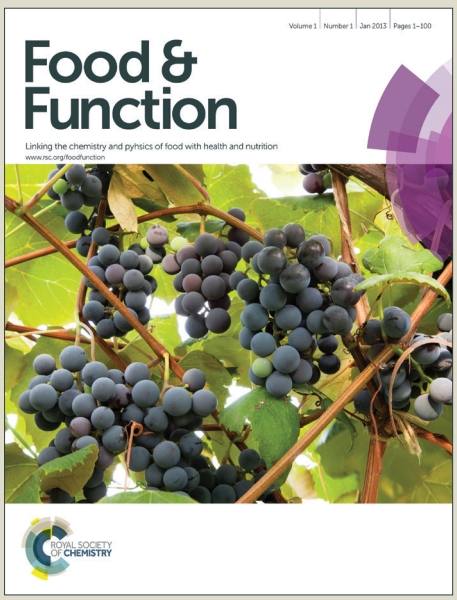

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Estrogen Modulation Properties of Mangiferin and Quercetin and the Mangiferin Metabolite Norathyriol

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Abstract

Mango fruit contain many bioactive compounds, some of which are transcription factor regulators. Estrogen receptor alpha (ERα) and beta (ERβ) are two regulators of gene transcription that are important in a variety of physiological processes and also in diseases including breast cancer. We examined the ability of the mango constituents quercetin, mangiferin, and the aglycone form of mangiferin, norathyriol, to activate both isoforms of the estrogen receptor. Quercetin and norathyriol decreased the viability of MCF-7 breast cancer cells whereas mangiferin had no effect on MCF-7 cells. We also determined that quercetin and mangiferin selectively activated ERα whereas norathyriol activated both ERα and ERβ. Despite quercetin, mangiferin and norathyriol having similar polyphenolic structural motifs, only norathyriol activated ERβ, showing that bioactive agents in mangoes have very specific biological effects. Such specificity may be important given the often-opposing roles of ERα and ERβ in breast cancer proliferation and other cellular processes.

Keywords: estrogen receptor, mangiferin, norathyriol, quercetin, phytoestrogen

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INTRODUCTION

Fruit and vegetables form part of a healthy diet with epidemiological and experimental evidence supporting their positive health benefits.[1] While traditionally the vitamin and mineral components of fruit and vegetables were considered paramount in their effects on human health, we are now aware that a range of other chemicals within fruit and vegetables, known as phytochemicals, are responsible for many of the positive health outcomes including the ability to help protect against and prevent certain diseases.[2]

Many individual phytochemicals have anti-cancer effects and may be protective for some cancers.[2-4] Mechanistically their bioactivity has been attributed to different actions including antioxidant effects, the genomic modulation of gene expression and epigenetic changes.[4] Some phytochemicals also act as phytoestrogens, which are plant-derived compounds that mimic mammalian estrogens and may be important in breast cancers.[5, 6]

Breast cancer is a major cause of death in women and its incidence is linked to exposure to estrogen. Paradoxically estrogen-active compounds can both protect against and contribute towards breast cancer.[7] For example, the synthetic nonsteroidal estrogen diethylstilbestrol is linked to an increased incidence of breast cancer in the daughters of women that used this chemical to reduce the risk of miscarriage during pregnancy[8], whereas women with a diet high in soy during adolescence, which contains the phytoestrogen genistein, have been reported to have a lower risk of postmenopausal breast cancer.[9] The likely physiological outcome appears to be based in part on both the timing and level of exposure to the phytoestrogen.[7]

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The genomic effects of estrogens are transduced via two estrogen receptors, ERα and ERβ[10], through a classic mechanism of the ligand binding to the receptor, followed by receptor conformational change, translocation to the nucleus and recognition and binding to estrogen response elements (ERE) in the DNA upstream of target genes. The downstream effect will ultimately depend on the receptor that has bound and whether the binding is via a homodimer or a heterodimer.[10, 11] The estrogen receptors have opposing effects on proliferation with ERα eliciting a proliferative response while ERβ is anti-proliferative. Evidence also suggests that ERβ can overcome the ERα proliferative response in cells where they are co-expressed, by forming an ERβ/ERα heterodimer that if activated, promotes an anti-proliferative response.[12]

Studies examining phytoestrogens and their ability to activate the ER suggest that depending on the precise molecule, they can activate either or both ERβ and ERα. For example, the activity of genistein on the expression of the anti-apoptotic protein Bax appears to be related to its ability to activate ERβ, although it can activate both ERα and ERβ.[13] While the majority of studies on phytoestrogens have been conducted on soy and botanical species from temperate crops[14], tropical fruit also contain many bioactive compounds with potential estrogenic effects and thus potential health benefits.[15]

The polyphenolic compounds quercetin and mangiferin are found in mangoes[16] and both compounds have a range of biological activities. Quercetin has been reported to attenuate a variety of pathways important in cancer growth and metastasis[17], whereas mangiferin shows both *in vitro* and *in vivo* antitumor, anti-inflammatory and antioxidant activity.[18] Our own studies have shown that these compounds are capable of modulating cellular migration and

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transcriptional activity.[19-21] Given the effects of other natural compounds on breast cancer we chose to study the effects of these two compounds and the aglycone form of mangiferin, norathyriol, on proliferation in breast cancer cells and to understand their effects by examining their ability to modulate the transcriptional pathways responsible for the induction of phase II detoxification enzymes and their ability to activate ERα and ERβ.

EXPERIMENTAL

Chemicals

Quercetin and mangiferin were purchased from Sigma Aldrich (Sydney, Australia). Norathyriol was isolated and purified as previously described.[22] DMEM and fetal bovine serum (FBS) were obtained from JRH (Sydney, Australia). DMSO was purchased from Sigma Aldrich. GW9662 was purchased from Calbiochem (EMD Biosciences, Inc., San Diego, CA). ICI 182,780 was purchased from Astra Zeneca (North Ryde, Australia), 17β-estradiol (E2) from Sigma Aldrich (Sydney, Australia).

Plasmids

The human ERα and ERβ plasmids pRST7-ERα and pRST7-ERβ, respectively, and the estrogen response element (ERE) plasmid pGL2-TATA-Inr-Luc-3XERETATALuc were obtained from Prof Donald McDonnell (Duke University Medical Center, Durham, USA) and have been described previously.[23] The pGL2-TATA-Inr-Luc-3XERETATALuc reporter contains three copies of the vitellogenin ERE.[24] The human ARE plasmid GCShARE4-tk-Luc was obtained

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from Dr Marcus Calkins (School of Pharmacy, University of Wisconsin, Madison, USA) and has been previously validated.[25]

Cell Culture

MCF-7 and Cos-7 cells were maintained and plated in high glucose DMEM supplemented with 10% FBS, L-glutamine (4 mM), penicillin G (100 U/mL) and streptomycin sulfate (100 µg/mL). Cells were maintained at 37 °C in a humidified 5% CO_2/95% air incubator. Prior to reaching approximately 90% confluence, cells were trypsinized with a 0.05% trypsin/0.53 mM EDTA solution and resuspended in fresh growth medium before plating onto a new growth surface.

Cell viability - MTS Assay

As described previously[26], MCF-7 cells were plated at 5×10^3 cells/well into a 96-well plate and were allowed to adhere for 24 h. The medium was replaced with phenol red- and serum-free complete media supplemented with 1.5% fatty acid-free BSA and the appropriate compound every two days. GW9662 was used as a positive control for inhibition of proliferation as it inhibits the growth of MCF-7 breast tumor cells.[27] All chemical stock solutions were made in DMSO. Control wells containing 10% FBS in phenol red-free growth media were also included in each experiment. DMSO was added to all control wells and 96 h after the addition of the compounds a cell viability assay was performed to quantitate viable cell numbers. Viable cell number was approximated using an MTS assay.[28] CellTiter 96®Aqueous One Solution Reagent (Promega, Australia; 20 µL) was added to each well containing 100 µL of culture medium. The plates were incubated for 120 min at 37^{o}C and absorbance values were obtained at 490 nm using a model 550 microplate reader (Bio-Rad laboratories, Australia).

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Transient transfection and transactivation assays

For gene reporter assays examining transactivation of the ARE and ERE, MCF-7 cells and Cos-7 cells were used, respectively. Cells were transfected at approximately 70% confluency. Prior to reaching confluence, cells were trypsinized with a 0.05% trypsin/0.53 mM EDTA solution and resuspended in fresh growth medium before plating onto a new growth surface. MCF-7 cells were plated at 5×10^4 cells/well and Cos-7 cells were plated at 1.2×10^4 cells/well into 96-well plates and allowed to adhere for 24 h. All transient transfections used LipofectAMINE 2000 reagent (0.8 μL/well) (Promega) and were performed in serum- and antibiotic-free media as described previously[29] and according to the manufacturer's directions. For gene reporter assays examining transactivation of the ARE each transfection consisted of the transfection control plasmid pSV-b-Gal (250 ng) and the gene reporter human ARE plasmid GCShARE4-tk-Luc (300 ng). For gene reporter assays examining transactivation of the ERE, each transfection consisted of the transfection control plasmid pSV-b-Gal (250 ng) or the gene reporter ERE plasmid pGL2-TATA-Inr-Luc-3XERETATALuc (250 ng) and 100 ng of ST7-ERα or pRST7-ERβ plasmid (for ERα or ERβ activity, respectively). Five hours after transfection, the medium was replaced with serum- and phenol red-free complete medium supplemented with 1.5% BSA and the appropriate chemical compound. All chemical stock solutions were in DMSO, and DMSO was added to all control wells. The highest concentration of DMSO added to the cell cultures was 0.65% v/v, which was used for all experimental and control wells. After a further 19 h, the cells were either lysed with 1 x luciferase lysis buffer (Promega) for ARE or ERE activity or else lysed with 1 x reporter lysis buffer (Promega) for β-galactosidase (β-gal) assay. ERE and ARE-driven reporter luciferase activity was measured using the Bright-Glo Luciferase

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Assay System (Promega) in a NOVOstar or FLUORstar fluorescence microplate reader (BMG Labtechnologies, Offenburg, Germany).

β-Galactosidase activity

MCF-7 and Cos-7 cells transiently transfected as above were assayed for β-gal activity as previously described.[30] Briefly, following lysis in 1 x reporter lysis buffer, 2 × β-gal assay buffer (50 µL; 200 mM sodium phosphate buffer pH 7.3, 2 mM MgCl2, 100 mM β-mercaptoethanol, 1.33 mg/mL o-nitrophenyl β-galactopyranoside) was added and the plates were incubated for 120 min at 37 °C prior to the reading of absorbance (415 nm) using a Bio-Rad model 550 microplate reader.

Statistical Analysis

Prism V4.03 software (GraphPad Software Inc., San Diego, CA) was used for statistical analysis. Significance was determined using one-way analysis of variance with the Tukey's test for all pairwise multiple comparisons for normally distributed data of equal variance.

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RESULTS

MCF-7 breast cancer cell viability

The effects of the mango constituents quercetin, mangiferin, and the mangiferin aglycone norathyriol (Fig. 1) were assessed on cell viability in MCF-7 breast cancer cells at concentrations of 100 μM. Quercetin at a concentration of 100 μM significantly inhibits cancer cell growth in three different bladder cancer cell lines.[31] As expected[27], the positive control GW9662 (100 μM), a PPARγ antagonist, significantly inhibited cell viability in the MCF-7 breast cancer cells compared to control (Fig. 2). A significant ($P < 0.05$) decrease in MCF-7 breast cancer cell viability was also observed with quercetin (100 μM) and norathyriol (100 μM), however, mangiferin had no significant effect on cell viability.

Activation of the ARE

To examine the abilities of quercetin, mangiferin and norathyriol to protect against oxidative stress we analyzed their capacities to activate the ARE. While the known ARE inducer tBHQ significantly ($P < 0.05$) activated the ARE at a concentration as low as 0.03 μM (Fig. 3), there was no activation of ARE by any of the compounds tested. However, quercetin (100 μM) did significantly ($P < 0.05$) inhibit activation of the ARE.

ERα and ERβ transactivation

To characterize the possible estrogen receptor modulation properties of quercetin, mangiferin and norathyriol we looked at their ability to activate ERα and ERβ in Cos-7 cells. Estradiol significantly ($P < 0.05$) activated ERα directed activation of the ERE at a concentration of 10 nM (Fig. 4A). Mangiferin significantly ($P < 0.05$) activated ERα at concentrations greater than 100

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μM (Fig. 4B), while quercetin only modestly activated ERα at a concentration of 30 μM but at no other concentration (Fig. 4C). Norathyriol activated ERα and displayed a biphasic activation effect, having significant activity at concentrations of 10, 30 and 100 μM but having no significant activity at higher concentrations of 300 and 1000 μM (Fig. 4D).

To confirm that activation of ERα by mangiferin and norathyriol was specific, Cos-7 cells were treated with the estrogen receptor antagonist ICI 182,780.[32] The activation of ERα by its endogenous agonist estradiol was abolished by ICI 182,780 (100 nM; Fig. 5), as was the activation of ERα by mangiferin (100 μM) and norathyriol (30 μM) (Fig. 5). Additionally in the presence of submaximal estradiol (5 nM) both mangiferin (100 μM) and norathyriol (30 μM) further activated ERα whereas no such effect was seen with quercetin (30 μM) (Fig. 6).

Similar to ERα, we examined the ability of mangiferin, quercetin and norathyriol to activate ERβ. Estradiol, the endogenous agonist, was able to transactivate ERβ (Fig. 7A), as was norathyriol in a biphasic manner with activation only at 10 and 30 μM (Fig. 7D). However, neither mangiferin nor quercetin had any ability to activate ERβ (Fig. 7B & 7C). The activation of ERβ by estradiol (20 nM) and norathyriol (30 μM) was abrogated by ICI 182,780 (100 nM; Fig. 8). Furthermore quercetin, mangiferin and norathyriol were unable to further significantly increase the transactivation of ERβ by estradiol (20 nM) (Fig. 9).

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DISCUSSION

Phytochemicals in tropical fruits, like those from more temperate fruits and vegetables, can have biological activity. Mangoes are recognized as having medicinal properties and mango fruit are an important economic crop.[15] We have previously shown that the frequently found fruit and vegetable polyphenolic quercetin and the aglycone derivative of the mango signature compound mangiferin, norathyriol, can inhibit the transactivation of peroxisome proliferator-activated receptor isoforms[21]. This work extended the assessment of mango bioactivity effects on MCF-7 cells, ARE activity and estrogen modulation properties of quercetin, mangiferin and of norathyriol, a metabolite of mangiferin.

Both quercetin and norathyriol at 100 μM significantly reduced viable MCF-7 cell numbers after 96 h in culture, whereas mangiferin did not show any inhibition of cell viability, highlighting the importance of conversion to the aglycone metabolite for some type of bioactivity, as we have seen previously for the modulation of transactivation of the peroxisome proliferator-activated receptors [21]. In agreement with this result, quercetin has previously been shown to inhibit the proliferation of a wide range of cancer cell lines[31, 33, 34] via modulation of several different pathways including inhibition of protein kinases.[33]

Induction of phase II detoxification enzymes via the ARE is thought to confer health protective benefits against carcinogens.[35] However, we observed a lack of potency of the phytochemicals quercetin, mangiferin and norathyriol to activate the ARE, whereas previous work has shown that quercetin can activate the ARE in a HepG2 cell model.[36] Our results reinforce the concept

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that ARE activity will depend on variables including the sequence context of the ARE, the cell type and the inducer.[35]

Since ERα and ERβ are both activated by the agonist estradiol, our studies used Cos-7 cells to enable the overexpression of individual estrogen receptor isoforms for assessment. Norathyriol and mangiferin dose-dependently transactivated the ERα receptor and this transactivation was increased in the presence of submaximal estradiol. This activity was abrogated in the presence of the receptor antagonist ICI 182,780 indicating that transactivation occurs through selective activation of the ERα receptor. However, only norathyriol activated ERβ and again this activity could be decreased in the presence of ICI 182,780 indicating an ERβ receptor-mediated mechanism. Given that norathyriol but not mangiferin activated ERβ, the presence of the glycone in the mangiferin structure may cause steric hindrance that prevents ERβ activation. Previous work shows that the removal of a glycone group increases the estrogen bioactivity of soy demonstrating the potential negative modulating effect of glycones on receptor activity[37]. Norathyriol's activation of both ERα and ERβ was biphasic, as is the action of the flavanol kaempferol on ER activation in human breast cancer cells[38] and of genistein, a soy component, on proliferation in breast cancer cells[39]. Hence, the ultimate functional response of the cell to estrogen receptor isoform activation will be influenced by the concentration of the individual diet-derived components at the site of action.

The concentration of dietary compounds, such as flavonoids, used in *in vitro* studies to effect gene expression changes, is higher than the steady state concentrations reached *in vivo* [40]. However, concentrations of serum quercetin of 100 μM have been attained when feeding rats

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0.5% quercetin in their diet over 10 days[41] and in humans a normal Western diet translates to a plasma level <100 nM, which can be raised to micromolar concentrations with dietary supplementation.[17] Compounds may accumulate with long-term ingestion allowing for an increased *in vivo* concentration[40], indeed quercetin is absorbed in humans but is believed to be only slowly eliminated[42]. Hence, the concentrations we used in our *in vitro* studies appear appropriate to help define possible pathways sensitive to quercetin. Although mangiferin is well tolerated in vivo in oral doses[43], its bioavailability and serum levels reached with chronic ingestion are unclear. Likewise levels of norathyriol reached with chronic mangiferin ingestion have not been fully evaluated.

Our studies have helped demonstrate that the effects of these mango components vary according to the nature of the transcription factor. This is exemplified by the ability of mangiferin to activate ERα but not ERβ. The lack of the ability of mangiferin to reduce MCF-7 cell proliferation may be due to differential activation or heterodimer formation of ERα and ERβ.[12] Further research is required to identify other transcription factors and receptors that show similar differential sensitivity to the effects of mango components such as quercetin and mangiferin and the putative metabolite norathyriol. Collectively, the results from this study provide further evidence that constituents of mango and/or their metabolites may contribute to health outcomes through the modulation of specific transcription factors.

More research is being directed towards the identification of the bioactive molecules in specific fruit and vegetables. The mechanisms of bioactive action extend beyond antioxidant activity to the modulation of complex cellular signaling pathways including regulation of receptors and

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transcription factors. The soy isoflavone genistein modulates a number of transcriptional pathways including estrogen signaling pathways and pathways that involve alteration of cell survival, cell cycle and apoptosis[44] and resveratrol, a polyphenol found in red wine, is a modulator of NF-κB and AP-1 mediated pathways.[45]

CONCLUSION

In summary the mango signature molecules mangiferin and norathyriol dose-dependently transactivated the ERα receptor by a mechanism inhibited by the estrogen receptor antagonist ICI 182,780. Norathyriol (but not mangiferin) transactivated the ERβ receptor and ICI 182,780 also inhibited this activation. These results are consistent with the hypothesis that dietary phytochemicals from tropical fruit, particularly mango components and metabolites may modulate receptor activation and gene transcription.

Acknowledgments

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Abbreviations: β-gal, β-galactosidase; ANOVA, analysis of variance; ARE, antioxidant responsive element; E2, 17β-estradiol; ER, estrogen receptor; ERE, estrogen response element; FBS, foetal bovine serum; ICI, ICI 182,780; MTS, (3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt; tBHQ, *tert*-butylhydroquinone

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Figure Legends

Figure 1: Chemical structures of quercetin, norathyriol and mangiferin.

Figure 2: Effect of quercetin, mangiferin, norathyriol on MCF-7 breast cancer cell viability

Cell viability was measured using an MTS assay with GW9662 as a positive control. Data are presented as mean ± SEM (n = 4). Results are representative of three independent experiments. An asterisk (*) indicates a significance of $P < 0.05$ when compared to the control, using one-way analysis of variance with the Tukey's post test.

Figure 3: The effects of quercetin, mangiferin and norathyriol on ARE transactivation

Gene reporter gene assays were performed using MCF-7 cells transiently transfected with a luciferase reporter construct containing the human ARE. β-Gal was co-transfected and the luciferase activities were normalized with respect to β-gal transfection. Fold change is given relative to untreated controls and is presented as mean ± SEM (n = 3). Results are representative of two independent experiments. An asterisk (*) indicates a significance of $P < 0.05$ when compared to control (0 μM tBHQ), using one-way analysis of variance with the Tukey's post test.

Figure 4: The effects of quercetin, mangiferin and norathyriol on ERα transactivation

Gene reporter gene assays were performed using Cos-7 cells transiently transfected with a human ERE luciferase reporter construct and the human ERα. β-Gal was co-transfected and the luciferase activities were normalized with respect to β-gal transfection. Fold change is given

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relative to untreated controls and is presented as mean ± SEM (n = 3). Results are representative of three independent experiments. An asterisk (*) indicates a significance of $P < 0.05$ when compared to control (0 μM estradiol), using one-way analysis of variance with the Tukey's post test.

Figure 5: The effect of mangiferin and norathyriol on the transactivation of ERα in the presence and absence of ERα antagonist ICI 182,780

Gene reporter assays were performed using Cos-7 cells transiently transfected with a human ERE luciferase reporter construct and the human ERα. β-Gal was co-transfected and the luciferase values for ERα transactivation were normalized with respect to β-gal transfection. Fold change is given relative to untreated controls and is presented as mean ± SEM from 3 separate experiments performed in triplicate (n = 3). An asterisk (*) denotes significance at $P < 0.05$ compared to the respective compound in the absence of ICI 182,780, using one-way analysis of variance with the Tukey's post test.

Figure 6: Effects of quercetin, mangiferin and norathyriol on ERα transactivation in the presence and absence of estradiol

Gene reporter gene assays were performed using Cos-7 cells transiently transfected with a human ERE luciferase reporter construct and the human ERα. β-Galactosidase was co-transfected and the luciferase values for ERα transactivation were normalized with respect to β-galactosidase transfection. Fold change is given relative to untreated controls and is presented as mean ± SEM from 3 separate experiments performed in triplicate (n = 3). An asterisk (*) denotes significance at $P < 0.05$ compared to the respective compound in the absence of E2, using one-

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way analysis of variance with the Tukey's multiple comparison post test. A hash (#) denotes significance at $P < 0.05$ compared to the estradiol (5 nM) alone using one-way analysis of variance with the Tukey's multiple comparison post test.

Figure 7: The effects of quercetin, mangiferin and norathyriol on ERβ transactivation

Gene reporter gene assays were performed using Cos-7 cells transiently transfected with a human ERE luciferase reporter construct and the human ERβ. β-Galactosidase was co-transfected and the luciferase activities were normalized with respect to β-galactosidase transfection. Fold change is given relative to untreated controls and is presented as mean ± SEM (n = 3). Results are representative of three independent experiments. An asterisk (*) indicates a significance of $P < 0.05$ when compared to control, (0 μM estradiol) using one-way analysis of variance with the Tukey's post test.

Figure 8: The effect of norathyriol and estradiol on the transactivation of ERβ in the presence and absence of ERβ antagonist ICI 182,780.

Gene reporter gene assays were performed using Cos-7 cells transiently transfected with a human ERE luciferase reporter construct and the human ERβ. β-Galactosidase was co-transfected and the luciferase values for ERα transactivation were normalized with respect to β-galactosidase transfection. Fold change is given relative to untreated controls and is presented as mean ± SEM from 3 separate experiments performed in triplicate (n = 3). An asterisk (*) denotes significance at $P < 0.05$ compared to the respective compound in the absence of ICI 182,780, using one-way analysis of variance with the Tukey's post test.

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Figure 9: Effect of quercetin, mangiferin and norathyriol on the transactivation of ERβ in the presence and absence of estradiol

Gene reporter gene assays were performed using Cos-7 cells transiently transfected with a human ERE luciferase reporter construct and the human ERβ. β-Galactosidase was co-transfected and the luciferase values for ERα transactivation were normalized with respect to β-galactosidase transfection. Fold change is given relative to untreated controls and is presented as mean ± SEM from 3 separate experiments performed in triplicate (n = 3). An asterisk (*) denotes significance at $P < 0.05$ compared to the respective compound in the absence of E2, using one-way analysis of variance with the Tukey's post test for all pair wise multiple comparisons.

References

1. W. C. Willett and M. J. Stampfer, *Annu Rev Public Health*, 2013, 34, 77-95.
2. J. L. Slavin and B. Lloyd, *Adv Nutr*, 2012, 3, 506-516.
3. C. C. Neto, J. W. Amoroso and A. M. Liberty, *Mol Nutr Food Res*, 2008, 52 Suppl 1, S18-27.
4. D. Vauzour, A. Rodriguez-Mateos, G. Corona, M. J. Oruna-Concha and J. P. Spencer, *Nutrients*, 2010, 2, 1106-1131.
5. M. M. Liu, Y. Huang and J. Wang, *Anticancer Agents Med Chem*, 2012, 12, 1306-1313.
6. S. Jenkins, A. M. Betancourt, J. Wang and C. A. Lamartiniere, *J Steroid Biochem Mol Biol*, 2012, 129, 191-200.
7. L. Hilakivi-Clarke, S. de Assis and A. Warri, *J Mammary Gland Biol Neoplasia*, 2013, 18, 25-42.

Food & Function Accepted Manuscript

8. L. Hilakivi-Clarke, *Breast Cancer Res*, 2014, 16, 208.

9. L. N. Anderson, M. Cotterchio, B. A. Boucher and N. Kreiger, *Int J Cancer*, 2013, 132, 1683-1692.

10. S. Nilsson, S. Makela, E. Treuter, M. Tujague, J. Thomsen, G. Andersson, E. Enmark, K. Pettersson, M. Warner and J.-A. Gustafsson, *Physiol Rev*, 2001, 81, 1535-1565.

11. F. Delaunay, K. Pettersson, M. Tujague and J. A. Gustafsson, *Mol Pharmacol*, 2000, 58, 584-590.

12. E. Powell, E. Shanle, A. Brinkman, J. Li, S. Keles, K. B. Wisinski, W. Huang and W. Xu, *PloS one*, 2012, 7, e30993.

13. S. M. Adams, M. V. Aksenova, M. Y. Aksenov, C. F. Mactutus and R. M. Booze, *PloS one*, 2012, 7, e37540.

14. Ososki AL and Kennelly EJ, *Phytother Res*, 2003, 17, 845-869.

15. J. T. Pierson, R. G. Dietzgen, P. N. Shaw, S. J. Roberts-Thomson, G. R. Monteith and M. J. Gidley, *Mol Nutr Food Res*, 2012, 56, 357-387.

16. Barua AB and Olson JA, *J Chromatogr B Biomed Sci Appl*, 1998, 707, 69-79.

17. M. Russo, C. Spagnuolo, I. Tedesco, S. Bilotto and G. L. Russo, *Biochem Pharmacol*, 2012, 83, 6-15.

18. M. Masibo and Q. He, *Compr Rev Food Sci F*, 2008, 7, 309-319.

19. N. H. Daud, C. S. Aung, A. K. Hewavitharana, A. S. Wilkinson, J. T. Pierson, S. J. Roberts-Thomson, P. N. Shaw, G. R. Monteith, M. J. Gidley and M. O. Parat, *J Agric Food Chem*, 2010, 58, 5181-5186.

Food & Function Accepted Manuscript

20. A. S. Wilkinson, B. M. Flanagan, J. T. Pierson, A. K. Hewavitharana, R. G. Dietzgen, P. N. Shaw, S. J. Roberts-Thomson, G. R. Monteith and M. J. Gidley, *J Food Sci*, 2011, 76, H11-18.

21. A. S. Wilkinson, G. R. Monteith, P. N. Shaw, C. N. Lin, M. J. Gidley and S. J. Roberts-Thomson, *J Agric Food Chem*, 2008, 56, 3037-3042.

22. L. Chun-Nan, C. Cheng-Hsiung, M. Arisawa, M. Shimizu and N. Morita, *Phytochemistry*, 1982, 21, 205-208.

23. J. M. Hall, D. P. McDonnell and K. S. Korach, *Mol Endocrinol*, 2002, 16, 469-486.

24. J. D. Norris, D. Fan, M. R. Stallcup and D. P. McDonnell, *J Biol Chem*, 1998, 273, 6679-6688.

25. R. T. Mulcahy, M. A. Wartman, H. H. Bailey and J. J. Gipp, *J Biol Chem*, 1997, 272, 7445-7454.

26. K. M. Suchanek, F. J. May, J. A. Robinson, W. J. Lee, N. A. Holman, G. R. Monteith and S. J. Roberts-Thomson, *Mol Carcinog*, 2002, 34, 165-171.

27. M. S. Jill, A. Y. Elisabeth and H. G. Jason, *Br J Pharmacol*, 2004, 143, 933-937.

28. C. J. Goodwin, S. J. Holt, S. Downes and N. J. Marshall, *J Immunol Meth*, 1995, 179, 95-103.

29. H. M. Faddy, J. A. Robinson, W. J. Lee, N. A. Holman, G. R. Monteith and S. J. Roberts-Thomson, *Int J Biochem Cell Biol*, 2006, 38, 255-266.

30. N. Gopisetty Venkata, J. A. Robinson, P. J. Cabot, B. Davis, G. R. Monteith and S. J. Roberts-Thomson, *Toxicol Lett*, 2006, 163, 224-234.

31. L. Ma, J. M. Feugang, P. Konarski, J. Wang, J. Lu, S. Fu, B. Ma, B. Tian, C. Zou and Z. Wang, *Front Biosci*, 2006, 11, 2275-2285.

Food & Function Accepted Manuscript

32. S. H. Chung and P. F. Lambert, *Proc Natl Acad Sci U S A*, 2009, 106, 19467-19472.

33. G. Russo, M. Russo, C. Spagnuolo, I. Tedesco, S. Bilotto, R. Iannitti and R. Palumbo, in *Advances in Nutrition and Cancer*, eds. V. Zappia, S. Panico, G. L. Russo, A. Budillon and F. Della Ragione, Springer Berlin Heidelberg, 2014, vol. 159, ch. 11, pp. 185-205.

34. D. W. Lamson and M. S. Brignall, *Altern Med Rev*, 2000, 5, 196-208.

35. T. Nguyen, P. J. Sherratt and C. B. Pickett, *Annu Rev Pharmacol Toxicol*, 2003, 43, 233-260.

36. S. Tanigawa, M. Fujii and D. X. Hou, *Free Radic Biol Med*, 2007, 42, 1690-1703.

37. K. Oerter Klein, M. Janfaza, J. A. Wong and R. J. Chang, *J Clin Endocrinol Metab*, 2003, 88, 4077-4079.

38. S. M. Oh, Y. P. Kim and K. H. Chung, *Arch Pharm Res*, 2006, 29, 354-362.

39. T. T. Rajah, N. Du, N. Drews and R. Cohn, *Pharmacology*, 2009, 84, 68-73.

40. S. M. Kuo, *Adv Exp Med Biol*, 2002, 505, 191-200.

41. C. Manach, C. Morand, O. Texier, M. L. Favier, G. Agullo, C. Demigne, F. Regerat and C. Remesy, *J Nutr*, 1995, 125, 1911-1922.

42. P. C. Hollman, M. vd Gaag, M. J. Mengelers, J. M. van Trijp, J. H. de Vries and M. B. Katan, *Free Radic Biol Med*, 1996, 21, 703-707.

43. Y. Prado, N. Merino, J. Acosta, J. A. Herrera, Y. Luque, I. Hernández, E. Prado, G. Garrido, R. Delgado and I. Rodeiro, *J Pharm Pharmacogn Res*, 2015, 3, 13-23.

44. A. M. Mahmoud, W. Yang and M. C. Bosland, *J Steroid Biochem Mol Biol*, 2014, 140, 116-132.

45. T. E. Kraft, D. Parisotto, C. Schempp and T. Efferth, *Crit Rev Food Sci Nutr*, 2009, 49, 782-799.

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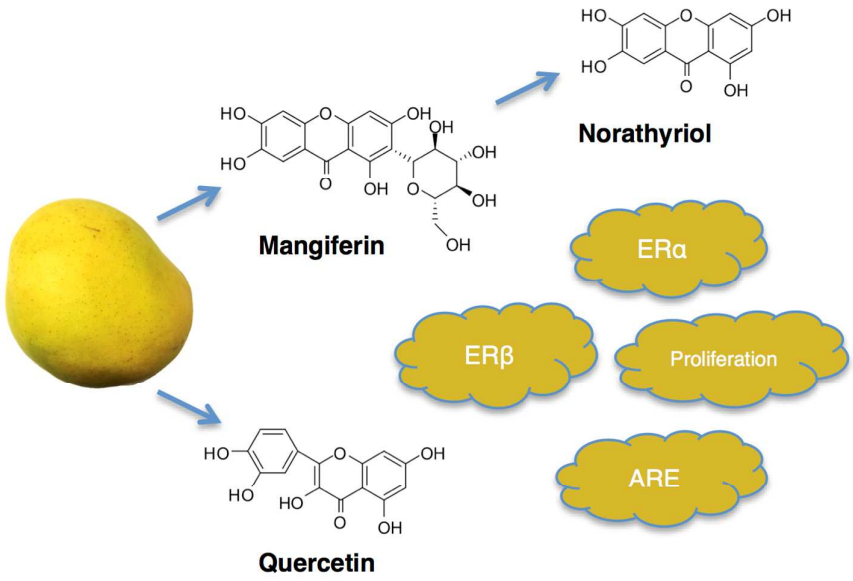
Mango fruit contain many bioactive compounds, some of which are transcription factor regulators.



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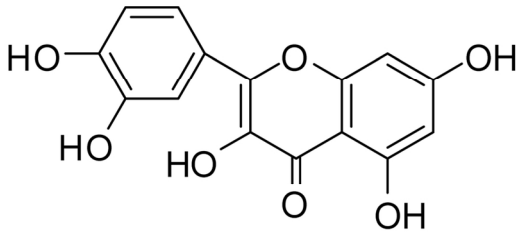

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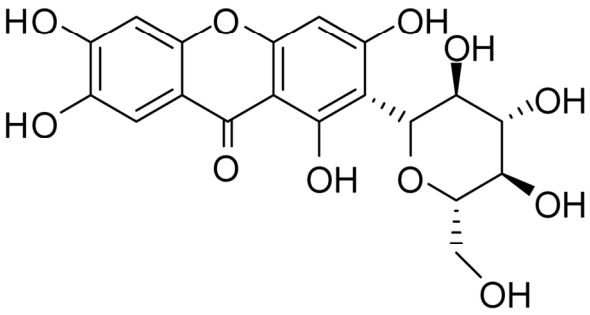
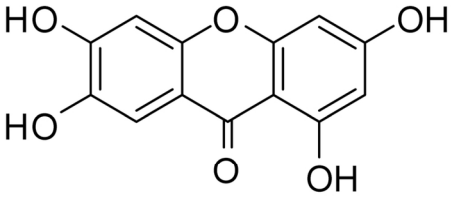


Mangiferin



Norathyriol

Figure 1
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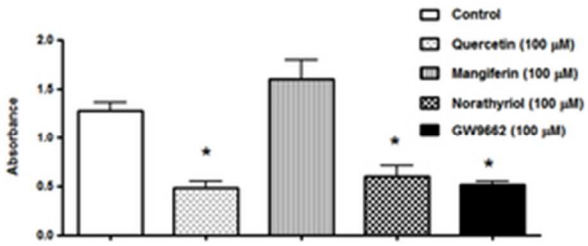

Figure 2
25x12mm (300 x 300 DPI)

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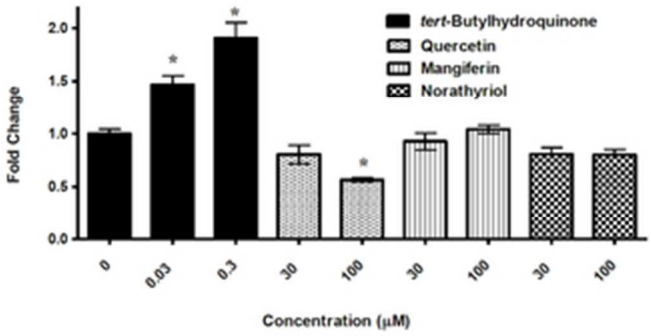

Figure 3
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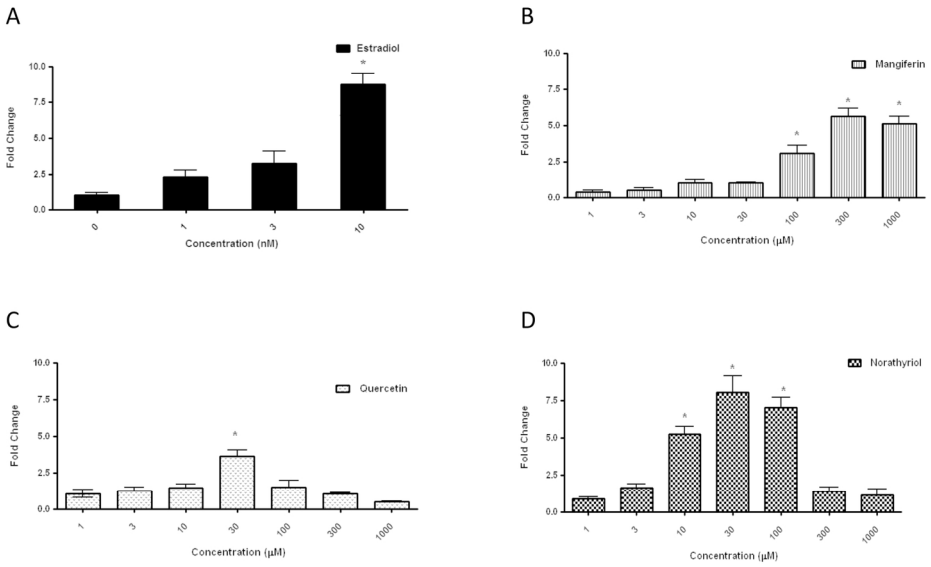

Figure 4
155x97mm (300 x 300 DPI)

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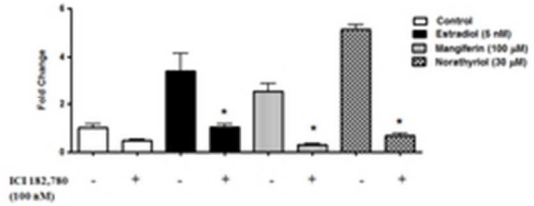

Figure 5
23x9mm (300 x 300 DPI)

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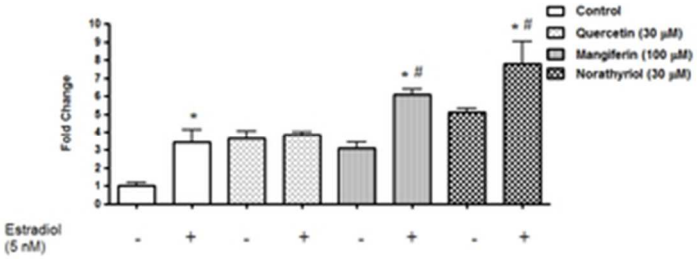

Figure 6
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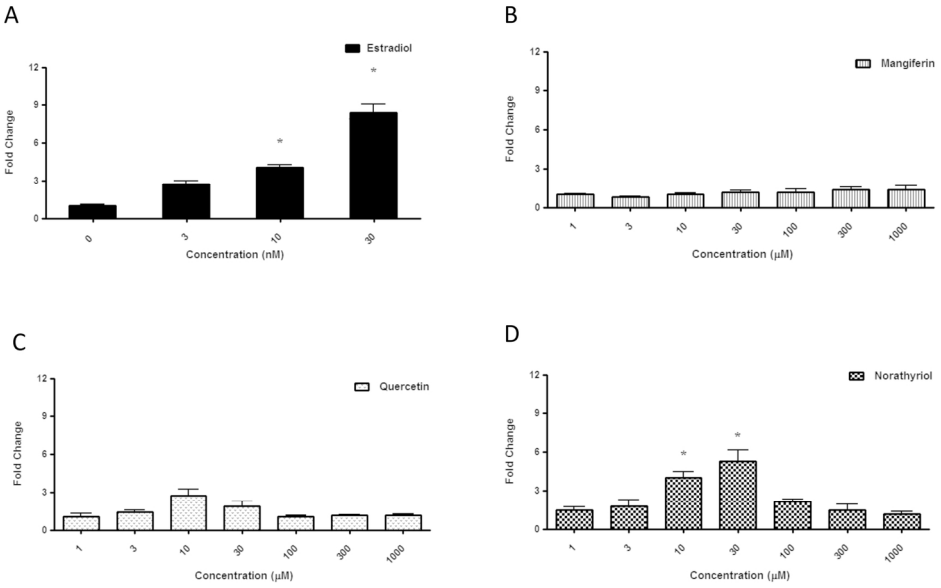

Figure 7
154x97mm (300 x 300 DPI)

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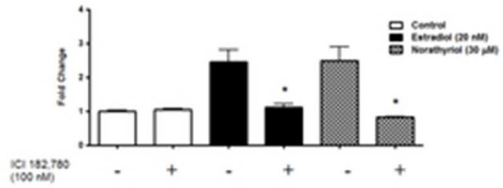

Figure 8
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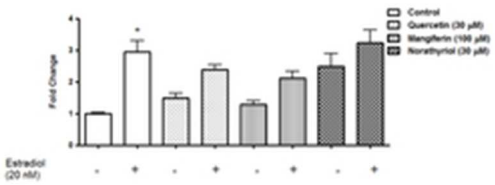

Figure 9
21x7mm (300 x 300 DPI)

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