Pharmacometrics of Stilbenes: Seguing Towards the Clinic

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Abstract: Stilbenes are small molecular weight (~200-300 g/mol), naturally occurring compounds and are found in a wide range of plant sources, aromatherapy products, and dietary supplements. These molecules are synthesized *via* the phenylpropanoid pathway and share some structural similarities to estrogen. Upon environmental threat, the plant host activates the phenylpropanoid pathway and stilbene structures are produced and subsequently secreted. Stilbenes act as natural protective agents to defend the plant against viral and microbial attack, excessive ultraviolet exposure, and disease. One stilbene, resveratrol, has been extensively studied and has been shown to possess potent anti-cancer, anti-inflammatory and anti-oxidant activities. Found primarily in the skins of grapes, resveratrol is synthesized by *Vitis vinifera* grapevines in response to fungal infection or other environmental stressors. Considerable research showing resveratrol to be an attractive candidate in combating a wide variety of cancers and diseases has fueled interest in determining the disease-fighting capabilities of other structurally similar stilbene compounds. The purpose of this review is to describe four such structurally similar stilbene compounds, piceatannol, pinosylvin, rhapontigenin, and pterostilbene and detail some current pharmaceutical research and highlight their potential clinical applications.

Keywords: Piceatannol, Pinosylvin, Rhapontigenin, Resveratrol, Pterostilbene, Stilbene.

1. INTRODUCTION

Currently, there is a plethora of interest in elucidating the health benefits associated with the consumption of fruits and vegetables rich in phytochemicals. Recently, an epidemiological study has shown an inverse relationship between consumption of fruits and vegetables with incidence of cardiovascular disease, stroke, and mortality [1]. Research has demonstrated that diets rich in fruits and vegetables substantially lower the risk of cancer development and cancer-related mortality [2,3,4,5]. Several diverse compounds exist in fruits and vegetables that may be responsible for their associated health-benefits. The general classes of the natural compounds that have been isolated from various foodstuffs are flavonoids, stilbenes, isoflavonoids, and lignans. All of these compounds are structurally similar and are derived *via* the phenylpropanoid pathway (Figs. **1, 2**).

1.1. Stilbene Structure

Stilbenes are small (MW 210-270 g/mol), naturally occurring compounds found in a wide range of plant sources, aromatherapy products, and dietary supplements.

Stilbenes exist as stereoisomers in *E* and *Z* forms, depending on where functional groups are attached in relation to one another on either side of the double bond. Naturally occurring stilbenes overwhelmingly exist in the *Z* (*trans*) form. It has been postulated and scientifically verified that the *E* and *Z* forms of stilbenes elicit different pharmacological activities. Research has revealed the *Z* form to exhibit more potent activity compared to the *E* form across various anti-cancer and anti-oxidant assays. One such study demonstrated *trans*-resveratrol to be ten times more potent in its ability to induce apoptosis in the HL60 leukemia cell line compared to *cis*-resveratrol [6]. Additional research has shown *trans*-stilbene compounds to be significantly more potent in their ability to inhibit cyclooxygenase I (COX-I) activity compared to *cis*-stilbene compounds [7]. These compounds, namely flavonoids, isoflavonoids, and lignans, have also generated much scientific research in their potential clinical applications in the treatment of diseases. Comparatively, little research has been conducted in determining the potential cardioprotective, anti-inflammatory and chemoprotective activities of most stilbenoid compounds. There are several stilbenes that have been recognized and classified (Table **1**). Based on our current phytochemical knowledge, it is postulated that many more stilbene compounds have yet to be identified. Stilbenoid compounds can be either constitutive and confined to the wood pulp of the host, or induced in response to environmental stressors. Induction of stilbene synthesis and secretion occurs in the fruit and/or leaves of its host. Stilbenes that have been induced are often referred to as phytoalexins, due to their protective actions upon secretion [8]. These secondary metabolites act as protective agents to defend the plant against viral and microbial attack, excessive ultraviolet exposure, and disease [9]. Upon environmental threat, the plant host activates the phenylpropanoid pathway and stilbene structures are produced and secreted as a consequence. Which specific stilbene that is produced depends largely on its host, the region of origin, and the environmental stimuli. The most well-known and well-characterized stilbene compound is resveratrol. Primarily found in peanuts, red wine, and grapes [10-12], resveratrol has been shown to be a potent anti-inflammatory, anti-cancer and chemoprotective agent [13-15] (Tables **2-4**). Based on encouraging therapeutic evidence, resveratrol research has fueled a great deal of interest in characterizing structurally similar stilbene com-

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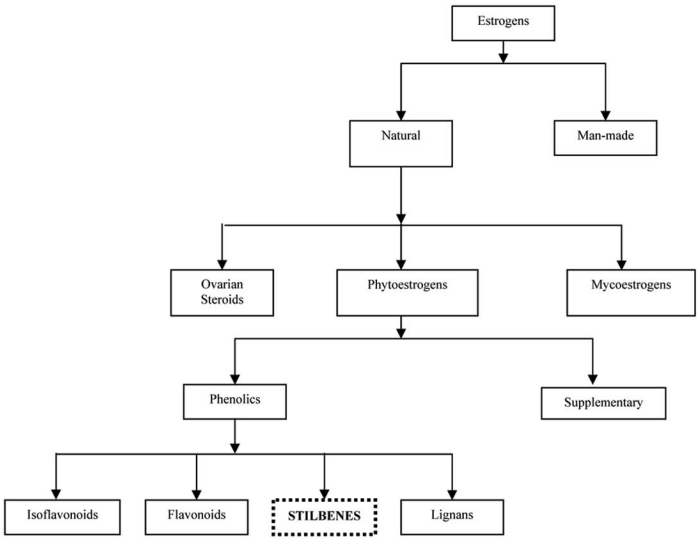


Fig. (1). Relationship between stilbene, flavonoid, lignan, and isoflavonoid formation.

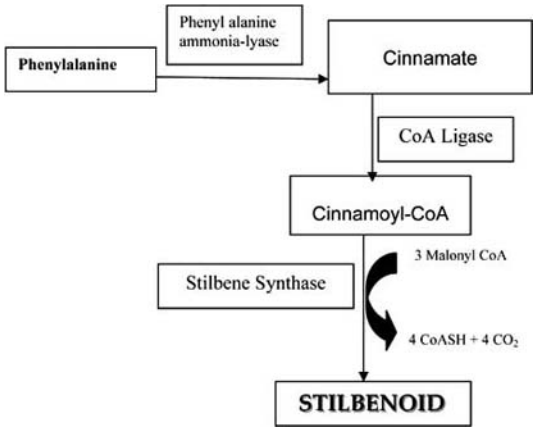


Fig. (2). Phenylpropanoid synthesis pathway of stilbenoids.

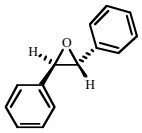
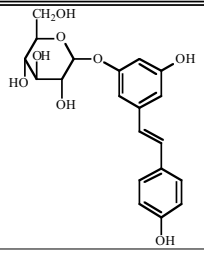
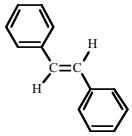
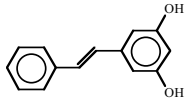
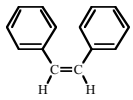
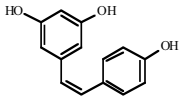
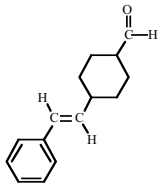
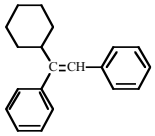
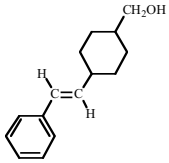
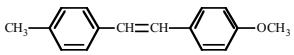
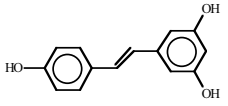
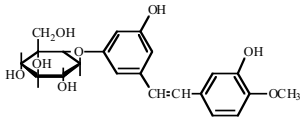
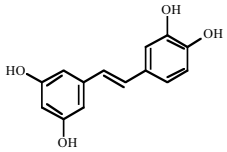
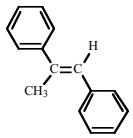
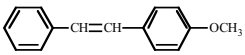
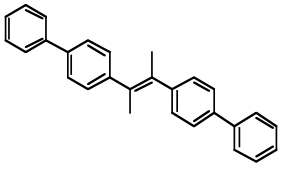
pounds and in synthesizing modified stilbenes substituted with various functional groups. The purpose of this review is to describe four such structurally similar stilbene compounds: piceatannol, pinosylvin, rhapontigenin, and pterostilbene and review their applications in current pharmaceutical and agricultural research (Table **5**).

2. PICEATANNOL

Piceatannol (trans-3, 4, 3', 5'-tetrahydroxystilbene) $C_{14}H_{12}O_4$, MW 244.2 g/mol, log P 2.442, is a naturally occurring stilbene present in sugar cane, berries, peanuts, red wines, and the skin of grapes [16,17,18]. First isolated and characterized from *Euphorbia lagascae* in 1984, piceatannol is synthesized in response to fungal attack, ultraviolet exposure, and microbial infection [19]. Induction of piceatannol synthesis is also evident during the ripening of grapes and increases during the fermentation process of wine production due to β-glucosidase activity of bacteria [20]. Constitutive piceatannol has also been identified in the heartwood of *Cassia garrettiana*, a common plant family in Asian countries [21].

Recent research has shown piceatannol to be a metabolite of resveratrol *via* the cytochrome P450 1A2 and 1B1 enzymes. One study employing human liver microsomes found that *trans*-resveratrol metabolism produces two main metabolites, with one being piceatannol. This biotransformation was dependent on the cytochrome P450 1A2 enzyme as evidenced through the investigator's use of enzyme specific inhibitors and protein antibodies. The rate of resveratrol hydroxylation forming piceatannol was rapid, reporting a K_m of 21μM with a V_{max} of 86pmol^{-1}mg^{-1} microsomal protein. Further investigation using several CYP enzymes (1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) has detected that CYP1A1 is the major enzyme involved in the biotransformation of resveratrol into piceatannol [22]. Research using human lymphoblast expressed cytochrome P450 1B1 microsomes has also shown *trans*-resveratrol to be biotransformed into piceatannol along with two other as of yet unidentified tetrahydroxylated stilbene compounds [23]. These data have led investigators to postulate that resveratrol may act as a pro-drug for production of piceatannol and other stilbenes.

Table 1. Names, Molecular Formula, and Structures of Identified Stilbenes

Name	Formula	Structure	Name	Formula	Structure
trans -stilbene oxide	$C_{14}H_{12}O$		*trans*-Piceid	$C_{20}H_{22}O_8$	
trans-stilbene	$C_{14}H_{12}$		*trans*-Pinosylvin	$C_{14}H_{12}O_2$	
cis-stilbene	$C_{14}H_{12}$		*cis*-Resveratrol	$C_{14}H_{12}O_3$	
trans-4-Stilbene-carbox-aldehyde	$C_{15}H_{12}O$		alpha-cyclohexyl-stilbene	$C_{20}H_{22}$	
trans-4-Stilbene-methanol	$C_{15}H_{14}O$		4,4'-dimethylstilbene	$C_{16}H_{16}$	
trans-Resveratrol	$C_{14}H_{12}O_3$		Rhaponticin	$C_{21}H_{24}O_9$	
trans-Piceatannol	$C_{14}H_{12}O_4$		*trans-a*-methylstilbene	$C_{15}H_{14}$	
trans-4-methoxy-stilbene	$C_{15}H_{14}O$		*trans* -4,4' Diphenylstibene	$C_{26}H_{20}$	

(Table 1. Contd….)

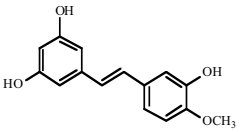
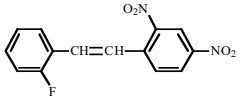
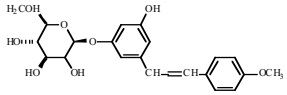
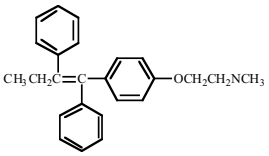
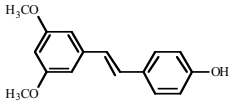
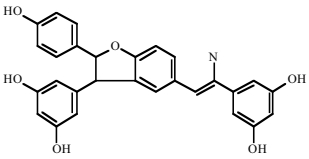
Name	Formula	Structure	Name	Formula	Structure
Rhapontigenin	$C_{15}H_{16}O_4$,		2,4-dinitro-2'-fluorostilbene	$C_{14}H_9FN_2O_4$	
De-oxyrhaponticin	$C_{21}H_{24}O_8$		Tamoxifen	$C_{26}H_{29}NO$	
Pterostilbene	$C_{16}H_{16}O_3$		Resveratrol-trans-dihydrodimer	$C_{28}H_{30}O_6$	

Table 2. Summary of Resveratrol Cancer Studies

Cell Type	Conclusions	Reference
Leukemia (B lymphocyte)	Inhibition of proliferation, induction of apoptosis	Billard *et al.* 2002
Leukemia (HL60)	Induction of apoptosis, inhibition of cell growth	Dorrie *et al.* 2001 Kang *et al*. 2003
Leukemia (Adult T-cell Leukemia)	Apoptosis induction	Hayashibara *et al.* 2002
Leukemia (THP-1)	Apoptosis induction, inhibition of cell growth	Tsan *et al*. 2000, Pemdurthi *et al*. 2002
Breast (MDA-MB-231)	Apoptosis induction, inhibition of cell proliferation and growth	Mgbonyebi *et al*. 1998, Scarlatti *et al*. 2003
Breast (MCF-7)	Apoptosis induction, growth inhibition	Mgbonyebi *et al*. 1998, Serrero *et al*. 2001, Lu *et al.*1999
Colon (HCT-116)	Apoptosis induction	Mahyar-Roemer *et al*. 2001, Wolter *et al.* 2002
Colon (Caco-2)	Apoptosis induction	Wolter *et al.* 2002
Colon (F344 rat model)	Inhibits colon carcinogenesis	Tessitore *et al*. 2000
Prostate (LnCap)	Growth inhibition	Hsieh *et al.* 2000, Stewart *et al.* 2004
Prostate (DU-145)	Apoptosis induction, growth inhibition	Lin *et al*. 2002, Kampa *et al.* 2000
Prostate (PC-3)	Apoptosis induction, growth inhibition	Stewart *et al.* 2004
Liver (HepG2)	Growth inhibition, decrease in hepatocyte growth factor-induced HepG2 cell invasion	De Ledinghen *et al.* 2001
Liver (Fao rat model)	Cell cycle arrest, proliferation inhibition	Delmas *et al.* 2000
Melanoma (A431)	Apoptosis induction	Ahmad *et al.* 2001, Adhami *et al*. 2001
Melanoma (A375)	Apoptosis induction	Niles *et al.* 2003
Melanoma (SK-Mel-28)	Apoptosis induction	Niles *et al.* 2003, Larrosa *et al*. 2003
Ovarian (PA-1)	Apoptosis induction	Yang *et al.* 2003
Endrometria	Proliferation inhibition	Bhat *et al.* 2001

Table 3. Pharmacological and Pharmacokinetic Studies of Resveratrol

Animal Model	Route of Administration	Conclusions	References
Rat	Oral	No estrogen agonism on estrogen targets in reproductive and nonreproductive tissues, resveratrol may be an estrogen antagonist	Turner *et al.* 1999
Rat	IV	Significant cardiac bioavailability, affinity for kidney tissue	Bertelli *et al.* 1996
Rat/mouse	IP	All resveratrol present in urine and serum in conjugated form	Yu *et al.* 2002
Rat	Oral	^{14}C-*trans*-resveratrol prefers stomach, liver, kidney, intestine tissues and is eliminated in bile and urine	Vitrac *et al.* 2004
Rat	IV and Oral	Extensive enterohepatic recirculation is evident, intestine is important in its presystemic glucuronidation, undergoes extensive first pass glucuronidation, bioavailability approximately 38%, biliary excretion predominates	Marier *et al*. 2002
Mouse	Oral	Reduction in tumor weight and volume, reduction in tumor metastasis to the lung	Kimura *et al*. 2000
Mouse	IG	Increase in lymphocyte proliferation, IL-2 production	Feng *et al.* 2002
Mouse	Topical	Inhibition of UVB-mediated skin edema	Afaq *et al.* 2003
Gerbil	IP	Crosses blood-brain barrier, protects against cerebral ischemic injury	Wang *et al.* 2002
Rabbit	IG	Reduction in endothelial function	Zou *et al*. 2003
Human	Oral	Adequate absorption of pharmacologically active concentrations of resveratrol from grape juice causes reduction in risk of artherosclerosis	Pace-Asciak *et al*. 1996
Human	Oral	Resveratrol exists in urine and serum predominately in conjugated form	Yu *et al.* 2002
Human	Oral	Metabolites are inactive in antiviral HIV assay compared to parent resveratrol	Wang *et al.* 2004
Human microsomes	-	Irreversible inhibitor of CYP 3A4, reversible inhibitor of CYP 2E1	Piver *et al.* 2001
Human	Oral	Due to poor bioavailablitiy, resveratrol alone is unlikely to be responsible for beneficial health effects, antioxidant activity.	Vitaglione *et al.* 2005

2.1. Receptor Interaction

Piceatannol is an antagonist of the aryl-hydrocarbon (Ah) receptor. The Ah receptor is an orphan, cytosolic receptor that has been implicated in dioxin toxicity. Agonists of this receptor include halogenated aromatic compounds such as 2,3,7,8-tetrachlorodibenzo-*n*-dioxin (TCDD) and polycyclic aromatic carbohydrates such as benzo(a)pyrene. Depending on the extent of exposure, AhR agonists have the potential to involve extensive tissue damage and can induce tumor growth and progression in a number of organs through the activation of cytochrome P450 1A1 enzyme. Piceatannol and resveratrol compete inhibiting AhR binding sites, effectively displacing TCDD [24].

In addition to acting as an antagonist to the Ah receptor, piceatannol also acts as a potent tyrosine kinase inhibitor [25,26,27]. Tyrosine kinases act as important intracellular mediators in several signaling pathways involved in the activation of mitogen-activated protein kinase (MAPK), pro-inflammatory mediators, and transcription factors [28]. Piceatannol has been characterized as a spleen tyrosine kinase (Syk) inhibitor. Several studies have employed piceatannol as a Syk inhibitor and it has quickly become the standard in signal transduction assays. Syk is a small cytoplasmic non-receptor protein tyrosine kinase (PTK) that is widely expressed in haematopoetic cells, including B and T lymphocytes, and has been detected in epithelial cells [29]. Many studies have utilized piceatannol as a Syk inhibitor in attempt to attenuate asthmatic responses and inflammation. Research has demonstrated that piceatannol is a potent and selective inhibitor of mast cell degranulation, bronchial constriction, bronchial edema, and anaphylaxis [30-32]. One study determined that Syk activation was one of the earliest signaling responses in antigen-induced inflammation associated with asthmatic response. This activation was ablated by treatment with piceatannol, thus effectively inhibiting the inflammatory response and subsequent cytokine activation. These data suggest piceatannol to be a possible strategy in treating and preventing antigen-induced asthmatic responses [33].

Due to its estrogen structural similarity it isn't surprising that investigators have determined that piceatannol acts as a selective estrogen receptor modulator (SERM) in human breast cancer cell lines. Piceatannol is an agonist or partial agonist to the estrogen receptor across breast cancer cells expressing either wild-type or mutant estrogen receptors. In

Table 4. Molecular Targets of Resveratrol

Activity	Target
Transcription Regulation	↓AR ↓AP-1 ↓NF-κB ↓β-catenin
Cell Cycle	↓Rb ↓Cyclin A,B1,D ↑Cdk2 ↑p21 ↑p27
Apoptosis and Growth	↑p53 ↓TNF ↑FasL ↓IL-1β ↑Bax ↑Adenyl-cyclase ↓Bcl-2 ↓EGF ↓IL-6 ↓Survivin ↑Caspases ↑Ceramide
Invasion	↓Cox-2 ↓iNOS ↓VCAM-1 ↓ICAM-1 ↓IGF-1R ↓VEGF ↓Tissue factor
Cytokines and Kinases	↑NAG-1 ↑TGF- β ↓PC-GF ↓PKC ↓Syk ↓PKD ↓Erk1/2 ↓CKII
Miscellaneous	↓DNA polymerase (I, III) ↓CYP1A1 ↓Ribonucleotide reductase ↓Tubulin polymerization

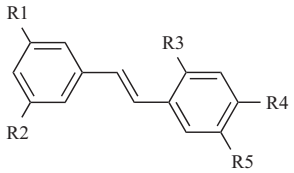
comparison, resveratrol was reported to have a "super-agonistic" effect in one particular transfected breast cancer cell line, MCF-7. This cell line was transfected with estrogen-responsive reporter constructs, thus showing resveratrol to be a potent activator of estrogen receptors and subsequent estrogen regulated activity in tested cells. In cell lines with mutated estrogen receptors, resveratrol and piceatannol-induced estrogen receptor activation was significantly attenuated lending further evidence that these two stilbenes in particular are potent estrogen receptor modulators. Interestingly, investigators have also reported resveratrol to have a weak stimulatory effect in the estrogen-negative breast cancer cell line, MDA-MB-231, suggesting that resveratrol and possibly other stilbene compounds may elicit their effects through a parallel, estrogen-independent pathway [34].

A recent study found piceatannol to be a potent and selective inhibitor of the COX-2 pathway. Investigators demonstrated piceatannol to inhibit COX-1 at an IC_{50} of 4.713 μM (1.15 μg/mL) and to inhibit COX-2 at an IC_{50} of 0.0113 μM (0.003 μg/mL), yielding a selectivity index of 417.08. Resveratrol inhibited COX-1 at an IC_{50} of 0.535 μM (0.12 μg/mL) and COX-2 at an IC_{50} of 0.996 (0.23 μg/mL), yielding a selectivity index of 0.54. Comparatively, celecoxib, a selective COX-2 inhibitor currently utilized and available with a prescription, has a selectivity index of 546.41. Further investigations have demonstrated that piceatannol was able to bind directly to the COX-1 and COX-2 enzyme isoforms, inhibiting their activity. The authors concluded that piceatannol is a potential therapeutic compound that inhibits COX-2 activity, which may have utility in various disease states associated with COX-2 activation [35].

2.2. Anti-Oxidant Activity

Considerable research has been generated demonstrating that resveratrol is a potent anti-oxidant across a variety of assays. Due to the structural similarities between piceatannol and resveratrol, it has been hypothesized that piceatannol also possesses potent anti-oxidant activity. One study determined piceatannol to be significantly more potent in inhibiting Cu^{2+} induced lipid peroxidation in low-density proteins compared to resveratrol [36]. Moreover, these investigators found piceatannol to be equally active in scavenging 1,1 diphenyl-2-picryl hydrazyl (DPPH), a stable free radical often employed in anti-oxidant assays compared to resveratrol. Further investigations revealed piceatannol to be a potent superoxide scavenger, suggesting a possible role in cardioprotection following ischemia [37]. Other studies demonstrated the ability of piceatannol to inhibit carcinogen-induced preneoplastic lesion formation in a mouse mammary gland model. Piceatannol and resveratrol both significantly inhibited lesion formation, inhibiting 89-90% of lesions formed compared to a control group [38]. Recent research has shown piceatannol to be a more effective scavenger of nitric oxide and hydrogen peroxide compared to resveratrol. Piceatannol also exhibited highly selective cytotoxicity towards activated microglial cells that were secreting high concentrations of nitric oxide. The study also examined the ability of piceatannol and resveratrol to inhibit inducible nitric oxide synthase (iNOS) enzyme activity and determined that both stilbenes had little inhibitory effect. Piceatannol appears to be as effective as ascorbic acid or Trolox® (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), two well-characterized and potent anti-oxidants, in its free radical scavenging capacity [39]. Current research has also shown piceatannol to be equal to and in many cases, surpasses the anti-oxidant capacity of resveratrol. Many investigators have hypothesized that the additional hydroxyl group of piceatannol makes it more reactive and is therefore a more potent free radical scavenger compared to resveratrol [40].

Table 5. Structures of Stilbenes



Stilbene	R1	R2	R3	R4	R5
Rhapontigenin	OH	OH	H	OCH_3	OH
Resveratrol	OH	OH	H	OH	H
Rhaponticin	*O*-Glucose	OH	H	OCH_3	OH
Piceatannol	OH	OH	H	OH	OH
Pinosylvin	OH	OH	H	H	H
Pterostilbene	OCH_3	OCH_3	H	OH	H

2.3. Anti-Cancer Activity

Several studies have been conducted over the last decade to investigate the possible anti-cancer activity of piceatannol. Investigators have defended piceatannol to be effective in inhibiting the development of preneoplastic lesions in a mouse mammary gland model with 77% inhibition at a concentration of 10 μg/mL (40.9 μM) [7]. Other investigators have reported that piceatannol induces apoptosis in a human prostate cancer cell line (NRP-154) [41]. Piceatannol has also demonstrated potent anti-cancer activity in Lewis lung carcinoma (LLC) bearing mice and increased survival time and rate in carcinectomized mice. In this study, piceatannol significantly inhibited both tumor growth and metastasis in a concentration-dependent manner. Investigators also suggested that the anti-cancer and anti-metastatic activities of piceatannol appear to be attributed to its capacity to inhibit the formation of human umbilical vein endothelial cells (HUVECs), thus effectively cutting off nutrition for the tumor to grow and inhibiting the tumor from spreading. Inhibition of HUVEC formation occurred at concentrations of 10 μM (2.4 μg/mL) [42].

It has been recently reported that piceatannol attenuates the proliferation rate in the human colon adenocarcinoma cell (Caco-2) model. Investigators found this effect to be concentration-dependent at 0-200 μM (0-50 μg/mL). In addition, investigators measured the cell cycle distribution in treated cells and reported a significant accumulation of cells in the S phase of the cell cycle. Further experimentation showed the presence of specific S phase associated cyclins and cyclin-dependent kinases. The authors also examined piceatannol in HCT-116 (human colorectal adenoma) cells and found identical results. The investigators concluded that piceatannol is an effective anti-cancer agent that inhibits proliferation and growth in colon cancer cell models *via* arresting the cell cycle at the S phase [43].

Recent investigations of resveratrol and piceatannol have been conducted in BJAB Burkitt-like lymphoma cells and in an *ex vivo* model with leukemic lymphoblasts of 21 patients diagnosed with childhood lymphoblastic leukemia. Results suggest that both piceatannol and resveratrol are potent inducers of apoptosis in BJAB cells at an IC_{50} of 25 μM (6.1 μg/mL). Surprisingly, piceatannol, and not resveratrol was determined to effectively induce apoptosis in the *ex vivo* model [44]. In the laboratory it has been determined that piceatannol is most active in HL60 leukemia and HCT-116 colon cancer cells with an IC_{50} of ~2-3 μg/ml [45].

2.4. Cardioprotective Activity and the "French Paradox"

Resveratrol and piceatannol have been detected in red wine and have long been associated with cardioprotection. The "French paradox" described in 1992 fueled interest in the cardioprotective effects of these hydroxystilbenes and since then have generated a great deal of research focused on elucidating their mechanism of action of stilbenes upon cardiac tissue and function [46]. The French paradox is described as an anomaly in which southern French citizens, who smoke regularly and enjoy a high-fat diet, boast a very low mortality rate of coronary heart disease. Scientists have attributed this unlikely relationship to moderate consumption of the anti-inflammatory and anti-oxidant polyphenolic compounds, such as piceatannol and resveratrol, in red wine [47-49]. Piceatannol and resveratrol have been shown to elicit a number of cardioprotective activities including inhibition of low-density lipoprotein (LDL) oxidation, mediation of cardiac cell function, suppression of platelet aggregation, and attenuation of myocardial tissue damage during ischemic events. One study in particular demonstrated the cardioprotective activity of piceatannol [50]. This research attempted to elucidate the mechanism of action through which piceatannol protects myocardial tissue during myocardial ischemic events associated with cardiac arrhythmia. The investigators proposed that since oxidative stress is a major contributor to myocardial ischemia, treatment with the anti-oxidant stilbene piceatannol might protect cardiac function. Piceatannol was administered to rats that had undergone left main coronary artery occlusion by three different procedures (30-minute occlusion, 5 minute occlusion followed by 30 minute reperfusion, 4 hour occlusion) *via* infusion. Investigators reported that piceatannol infusion

significantly reduced instances of ventricular tachycardia and ventricular fibrillation across all experimental groups. Moreover, piceatannol infusion completely prevented mortality in the 30-minute occlusion and the 5 minute-occlusion followed by reperfusion groups. Investigators also reported a significant decrease in lactate dehydrogenase levels and an increase in nitric oxide levels in the blood. Cardiac infarct size was nearly halved in the 4-hour occlusion group treated with piceatannol. The authors concluded that piceatannol is a potent chemoprotective agent and elicits its action *via* its anti-oxidant properties [50].

3. PINOSYLVIN

Pinosylvin (trans-3', 5' dihydroxystilbene) $C_{14}H_{12}O_2$, MW 212.26 g/mol, log P 3.69, is a naturally occurring stilbene found in several species of pine tree wood extracts and in eucalyptus [51-54]. A high content of constitutive pinosylvin is found in the heartwood of pine tree species. Induced pinosylvin is found in the pine needles of trees challenged by infection or environmental stress [55].

3.1. Anti-Fungal Activity

Several studies have been conducted in the arena of pinosylvin's capacity to protect various pine species against disease and decay [56-58]. A recent study evaluated and compared pinosylvin and resveratrol in *in vitro* fungal and wood decay assays. Despite structural similarities, the anti-fungal capacities of resveratrol and pinosylvin were substantially different. Pinosylvin effectively inhibited growth of fungal-induced wood decay in birch and aspen tree samples. At the same concentrations (50 μg/mL or ~236 μM), resveratrol demonstrated a growth enhancing effect [59]. The majority of the data available investigating the stilbene pinosylvin characterizes pinosylvin based on its potent anti-fungal and anti-bacterial activities in pine tree species. Only a handful of studies have been conducted employing pinosylvin as a therapeutic agent and little is known of its possible anti-cancer, anti-oxidant and cardioprotective effects.

3.2. Anti-Cancer Activity

Early work has shown pinosylvin to be a potent inhibitor of human lymphoblastoid cells. A 30 μg/mL (~142 μM) concentration of pinosylvin significantly inhibited lymphoblastoid cell growth and was found to be the most potent of the compounds tested [51].

Investigators tested pinosylvin and other wood-derived compounds in both *in vivo* breast cancer models in trout and in *in vitro* breast cancer cell lines. It was reported that pinosylvin had estrogenic effects, lending evidence that, like piceatannol and resveratrol, pinosylvin is an estrogen receptor modulator. Pinosylvin showed potent anti-proliferation activity in both the estrogen dependent breast cancer cell line MCF-7 and the T-47D cell line, reporting LEC (lowest effective concentration) of 1 μM (0.2 μg/mL), which inhibited proliferation by 27%. Pinosylvin was not found to be estrogenic in the *in vivo* model, although only one concentration, 1 μM was tested. The purpose of this set of experiments was to determine whether estrogenic wood extracts released from paper mills were adversely effecting trout reproduction. Based on these data, it appears that pinosylvin may possess potent anti-cancer activity in addition to anti-fungal and anti-bacterial activities [60]. In the laboratory it has been determined that pinosylvin is most active in HepG2 liver cancer cells and MDA-MB-231 estrogen negative breast cancer cells with an IC_{50} of ~10-12 μg/ml [61].

3.3. Anti-Oxidant and Anti-Inflammatory Activity

A study comparing the anti-oxidant capacities of resveratrol and pinosylvin was conducted using lipid peroxyl radicals ($LOO^{\cdot}$). Interestingly, investigators determined that pinosylvin is less efficient in scavenging free radicals compared to resveratrol. Previous data have shown piceatannol to be a more potent anti-oxidant compared to resveratrol in its capacity to scavenge free radicals. Based on this evidence, it appears that the addition of hydroxyl moieties to the stilbene structure increases anti-oxidant capacity of stilbenes. These findings are consistent with the findings that trans-stilbene (with no hydroxyl moieties attached) is relatively ineffective in scavenging free radicals [62].

4. RHAPONTIGENIN

Rhapontigenin, (3,3', 5 –trihydroxy-4'-methoxystilbene) $C_{15}H_{16}O_4$, MW 238 g/mol, is a stilbene found in Korean rhubarb rhizomes, most abundantly in the *Rhei undulatum* species [63]. Rhaponticin, the glycosylated parent compound of rhapontigenin, has long been employed in Korea, Japan, and China as an oral hemostatic agent in treating Oketsu, a disease characterized by poor circulation, pain, and chronic inflammation [64]. Rhaponticin has also been recommended by health professionals in Asian countries to treat and prevent allergies [65]. Rhapontigenin, the dominant metabolite of rhaponticin, is determined to be the active molecule [66-68].

4.1. Anti-Allergic Activity

Rhapontigenin was found to inhibit histamine release from activated mast cells. Of the metabolites from the rhizome of *rheum undulatum* tested, rhapontigenin showed the most potent inhibition of histamine release from mast cells and inhibition of a passive cutaneous anaphylaxis reaction (PCA). Moreover, investigators tested disodium cromoglycate, a commercially available anti-allergy drug, and found rhapontigenin to elicit a more potent inhibitory effect in the histamine release and PCA assays [66].

Rhapontigenin and piceatannol were comparatively studied in a passive cutaneous anaphylaxis reaction (PCA) and antigen-induced histamine release assays. Results showed that rhapontigenin effectively inhibited histamine release by 90% at a concentration of 20 μg/mL (~78 μM). Piceatannol elicited nearly identical effects, inhibiting 86% histamine release at a concentration of 20 μg/mL (~83 μM). Additionally, rhapontigenin and piceatannol inhibited PCA in rats at concentrations of 20 μg/ml (~78 μM and ~83 μM) and 100 μg/mL (~388 μM and ~410 μM), respectively. In a delayed hypersensitivity test using sheep blood cell-induced delayed-type hypersensitivity (SRBC-DTH) in mice, rhapontigenin and piceatannol both exhibited inhibitory

activity in similar concentrations of 100 μg/mL. Based on results of this set of experiments, investigators concluded that rhubarb extracts possess anti-allergic activity and this activity is likely due to stilbene content [63].

Recent research comparing rhaponticin to rhapontigenin in a passive cutaneous anaphylaxis reaction (PCA) assay showed rhapontigenin to be a far more potent inhibitor of anaphylaxis compared to rhaponticin. Mice were administered intraperitoneal doses of either stilbenes glycosylated or aglycone compound. Rhapontigenin inhibited anaphylaxis by 85% at a dose of 50mg/kg. However, when mice were administered oral doses of either stilbene, rhaponticin was reported to exhibit a more potent inhibition. This work supports evidence that rhaponticin is a pro drug of rhapontigenin and metabolism of rhaponticin into rhapontigenin is necessary to produce anti-allergic activity [65].

4.2. Anti-Oxidant and Anti-Inflammatory Activity

Rhapontigenin, piceatannol, and resveratrol were employed to assess their capacities to inhibit nitric oxide (NO) production in lipopolysaccharide-activated macrophages. All three stilbenes effectively inhibited NO production. Of the stilbenes examined, piceatannol was the most potent, with an IC_{50} of 23 μM (5.6 μg/mL), followed by rhapontigenin (IC_{50}≈48 μM or 12.4 μg/mL) and lastly resveratrol (IC_{50}≈68 μM or 15.5 μg/mL). It has been suggested that the oxygen groups attached to the stilbene structure are crucial for the observed activity, as evidenced by the observation that addition of the glucoside moiety reduced pharmacological activity [65]. Further research found piceatannol, resveratrol, and rhapontigenin to inhibit nuclear factor kappa B (NF-κB) activation. Although none of the stilbenes tested inhibited inducible nitric oxide synthase (iNOS) enzymatic activity, they effectively inhibited iNOS induction and the activation of the downstream mediator NF-κB, thus inhibiting the overproduction of NO, which is implicated in pathological pro-inflammatory processes [67].

4.3. Anti-Cancer Activity

Recent investigations have determined that rhapontigenin is a potent inhibitor of human cytochrome P450 1A1 enzyme. This enzyme is implicated in the biotransformation of a number of carcinogenic and immunotoxic compounds. Seven compounds were tested and rhapontigenin was found to elicit the most potent competitive inhibitory effect. Moreover, rhapontigenin was highly selective in inhibiting CYP 1A1 enzyme activity, reporting an IC_{50} value of 0.4 μM (0.1 μg/mL) inhibition of CYP 1A1 compared to an IC_{50} value of 400 μM (103.2 μg/mL) inhibition of CYP1A2. Investigators concluded that rhapontigenin is a potent inhibitor of CYP 1A1 enzyme and warrants further study as a possible therapeutic and anti-cancer agent [68].

In addition to potently inhibiting P450 1A1, rhapontigenin is an active inhibitor of CYP 1B1. This enzyme is expressed and detected in a number of cancers such as prostate and breast cancers. Rhapontigenin potently inhibits CYP 1B1 activity with an IC_{50} of 9 μM (2.3 μg/mL). Several other CYP enzymes were tested (1A2, 2E1, 3A4, 2D6, and 2C8), and the inhibitory activity was limited to 1A1 and 1B1 enzymes [68, 69]. The laboratory has demonstrated that rhaponticin has little activity. On the other hand, rhapontigenin has been shown to have greatest anti-cancer activity in HepG2 liver cancer cells with an IC50 of 80-120 μg/ml [153].

4.4. Cardioprotective and Platelet Activity

Rhapontigenin has long been employed as an anti-coagulant drug in Asian countries. Research has shown rhapontigenin to inhibit platelet aggregation induced by arachidonic acid and collagen [70]. One study compared rhaponticin and resveratrol in its capacity to inhibit platelet aggregation *in vitro*. Resveratrol was a more potent inhibitor of both collagen and ADP-induced platelet aggregation compared to rhaponticin. Resveratrol inhibited collagen-induced aggregation at an IC_{50} of 11.60±2.1 μg/mL (~5 μM), and inhibited ADP-induced aggregation at an IC_{50} of 17.75±3.3 μM (~77.8 μg/mL). Rhaponticin inhibited collagen-induced aggregation at an IC_{50} of 52.34±4.1 μg/mL (~124.6 μM), and inhibited ADP-induced aggregation at an IC_{50} of 112.07±16.93 μM (~267.5 μg/mL). Interestingly, the investigators did not examine rhapontigenin, the active metabolite of rhaponticin. Based on available data, it can be suggested that rhapontigenin would elicit a more potent inhibition of platelet aggregation compared to rhaponticin, an outcome that would have been likely in an *in vivo* model. The authors concluded that the presence of stilbenes in rhubarb likely accounts for anti-platelet aggregation [71].

5. PTEROSTILBENE

Pterostilbene (trans-3,5-dimethoxy-4'-hydroxystilbene) $C_{16}H_{16}O_3$, MW 256.3 g/mol, log P 3.99, is a stilbene found in deerberry and rabbiteye blueberries, unripe Pinot noir and *Botrytis vinifera* infected Chardonnay grapes, and immature berries of Pinot and Gamay varieties [16,72,73]. Pterostilbene production was discovered at a high concentration in Xarello grape variety and at low levels in the leaf extracts of other grape varieties [74]. Due to variability in concentration, it has been suggested that pterostilbene synthesis in *Vitis* species is specific for each species [74]. It should be noted that pterostilbene has not yet been identified in any red wines [75]. Pterostilbene has, however, been identified as the main phenolic compound in darakchasava, a traditional Ayurvedic medicinal drink from India used to treat cardiovascular and other ailments [76]. Additionally, pterostilbene has been found in *Pterocarpus marsupium,* a tree whose heartwood is used in another Ayurvedic remedy as a nutraceutical therapy for diabetes [77]. Moreover, pterostilbene is known to be found in the stem bark of *Guibourtia tessmanii*, a tree found in central Africa that is commonly used in folk medicine [78]. Finally, it has been discovered that pterostilbene and a glycosylated pterostilbene are present in conventionally and organically grown commercially available blueberry products [Remsberg *et al.* unpublished].

5.1. Anti-Oxidant Activity

Pterostilbene has been shown to elicit significant anti-oxidant activity *in vitro* that is comparable to the activity of resveratrol. Research has demonstrated that pterostilbene inhibits citronellal thermo-oxidation by an EQ value of 355μM (~90.9μg/ml), and that pterostilbene scavenges for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals with an EC_{50}

value of approximately 30μM (~7.68μg/ml) [77]. Additionally, pterostilbene inhibits 2,2`-azo-bis(2-amidinopropane) (ABAP) derived peroxyl radicals with a total reactive anti-oxidant potential of 237 ± 58μM (~60.7μg/ml) as compared to resveratrol at 253 ± 53μM (~57.7μg/ml) [80]. Further investigations have demonstrated that pterostilbene protects against lipid peroxidation by reducing thiobarbituric acid reactive substance (TBARS) production by 61% in normal human fibroblasts [79]. Additional research employing a plant model quantified electrolyte leakage after inducing oxidative damage *via* the herbicide, acifluorfen. It has been demonstrated that pterostilbene was effective in reducing oxidative damage in comparable concentrations to resveratrol in this model [80]. Interestingly, other investigations measured the rate constants of pterostilbene with peroxyl radicals and demonstrated that pterostilbene acted only as a mild anti-oxidant in a homogeneous solution [81]. However, this same research also showed evidence that cis-hydroxy-stilbenes have less anti-oxidant activity than the trans isomer [81].

5.2. Anti-Cancer Activity

Limited research has demonstrated pterostilbene to have cancer chemopreventive properties in both *in vitro* and *in vivo* experiments. *In vitro* research using highly malignant B16 melanoma F10 cells has shown inhibition of growth by ~40% with 60 minute per day exposure to pterostilbene at a bioavailable concentration of 40μM (10.24μg/ml) [82]. When the B16 cells were exposed to both pterostilbene (40μM or 10.24μg/ml) and quercetin (20μM or 6.04μg/ml), cancer growth reduction was raised to ~56% [82]. Pterostilbene and quercetin may interfere with the molecular signaling during cell division [82].

In an *in vitro* examination of tumor cell adhesion to the endothelium using hepatic sinusoidal endothelium (HSE) cells, pterostilbene demonstrated ~60% inhibition of adhesion as compared to trans-resveratrol with ~47% [82]. A pterostilbene and quercetin combined test demonstrated a ~68% adhesion inhibition [82]. Additionally, inhibition of metastatic growth of the melanoma cells in the liver was examined *via* intravenous administration of pterostilbene (20 mg/kg per day) to mice. Administration of pterostilbene inhibited growth by ~34%, while pterostilbene and quercetin together inhibited growth by ~73% and increased survival by two-fold [82].

Pterostilbene was evaluated *in vitro* to determine its ability to induce apoptosis in leukemia cells of different sensitivity and drug resistances and in lymphoma cell lines. In HL60 promyelocytic leukemia cells, it was found that trans-pterostilbene (AC_{50} ~70μM or ~17.9μg/ml) was less active than resveratrol (AC_{50} ~50μM or ~11.4μg/ml) at inducing apoptosis. Similarly, pterostilbene inhibited cell growth (IC_{50} ~35μM or ~8.96μg/ml) less potently than resveratrol (IC_{50} ~5μM or ~1.14μg/ml) [6]. However, the cis-pterostilbene was a potent apoptosis-inducing agent with an AC_{50} equal to 5μM (~1.28μg/ml) compared to resveratrol [6]. In a subsequent study, pterostilbene was found to be an active apoptotic agent on leukemia cells that express the anti-apoptotic oncogene Bcr-Abl and on cells that express the multidrug resistant (MDR) phenotype [83]. Pterostilbene also demonstrated activity in lymphoma cell lines with a mutation of the Fas gene (HUT78B1 and HUT78B3) that were surprisingly resistant to apoptosis by trans-resveratrol and piceatannol [83]. Moreover, pterostilbene was found to be nontoxic to normal hemopoietic stem cells when concentrations that elicited apoptosis in leukemia cell lines were employed [83]. A pan-caspase-inhibitor Z-VAD-fmk did not inhibit apoptosis induced by pterostilbene; and therefore, it has been suggested that apoptosis is activated by pterostilbene through a caspase-independent mechanism [83].

Preliminary research has also been undertaken on pterostilbene's ability to inhibit cyclooxygenase (COX) enzymes. In comparison to resveratrol, pterostilbene demonstrated only moderate inhibition of COX-1 with an IC_{50} equal to ~19.8μM (~5.07μg/ml) and slight inhibition of COX-2, with an IC_{50} approximately ~83.9μM (~21.5μg/ml) [80]. Interestingly, resveratrol showed both COX-1 and -2 inhibition with an IC_{50} equal to ~1.1μM (~0.251μg/ml) and ~1.3 μM (~0.296μg/ml), respectively [80].

5.3. Anti-Diabetic Activity

Pterostilbene and *Pterocarpus marsupium* extracts that have been show to contain pterostilbene have reported anti-diabetic properties. Research demonstrated that pterostilbene can lower the blood glucose levels in streptozotocin-induced hyperglycemic rats by 42% [77]. *Pterocarpus marsupium,* which contains pterostilbene in its heartwood, has also been shown to have anti-hyperglycemic properties [84]. A study on the prevention of hyperglycemia and insulin resistance employed rats that were given a high-fructose diet supplemented with *P. marsupium* extracts [84]. This treatment for 30 days significantly lowered serum glucose levels in the rats [84]. A *P. marsupium* extract also showed significant protection against hypertriglyceridemia and hyperinsulinemia [84].

5.4. Anti-Fungal Activity

Pterostilbene is highly fungitoxic. It inhibits germination of conidia of *Botrytis cinerea in vitro* at concentrations between 10 and 50mM (2.56mg/mL and 12.8mg/mL) [85]. Investigation into its effects on the conidia showed that pterostilbene rapidly destroys ribosomes, the endoplasmic reticulum, and the membranes of the nucleus and mitochondria, and disorganizes the plasma membrane [85]. Pterostilbene has demonstrated enzyme-mediated degradation that is initiated by laccase produced by *B. cinerea* [86]. The metabolite produced by this enzymatic reaction has a dehydrodimeric structure which is insoluble in water [86]. Research suggests that the fungus creates an insoluble dimer with a higher molecular weight in an attempt to escape the action of phytoalexins like pterostilbene [86].

6. STILBENES CONCENTRATIONS IN FOODSTUFFS

6.1. Sources

Stilbenes are naturally occurring phytochemicals that can be found on a broad spectrum of foods and food products (Table **6**). Resveratrol is the most widely studied stilbene, and it has been identified in grapes [10, 87-92], and wine [11, 87-89, 93-94]. It has been also detected on different

Table 6. Stilbene Sources from Foodstuff

Stilbene	Foodstuff	Reference
Resveratrol	Grape	Jeandet *et al*. 1995a, Wang *et al*. 2002, Vian *et al*. 2005, Liew *et al*. 2005, Gonzalez-Barrio *et al*. 2005, Yilmaz *et al*. 2004, Roldan *et al*. 2003
	Wine	Cantos *et al*. 2003, Wang *et al*. 2002, Vian *et al*. 2005, Liew *et al*. 2005, Shu *et al*., 2005, Gambuti *et al*., 2004
	Beer Hop	Tedesco *et al*. 2005, Jerkovic *et al*. 2005, Callemien *et al*. 2005
	Bilberry	Ehala *et al*. 2005, Lyons *et al*. 2003, Rimando *et al*. 2004
	High-bush, Low-bush, Rabbit eye, Elliot's Blueberry	Lyons *et al*. 2003., Rimando *et al*. 2004
	Cowberry	Ehala *et al*. 2005
	Cranberry	Ehala *et al*. 2005, Wang *et al*. 2002, Rimando *et al*. 2004
	Deerberry	Rimando *et al*. 2004
	Ligonberry	Rimando *et al*. 2004
	Sparkleberry	Rimando *et al*. 2004
	Partridgeberry	Rimando *et al*. 2004
	Strawberry	Ehala *et al*. 2005
	Red Currant	Ehala *et al*. 2005
	Rhubarb	Kageura *et al*. 2001
	Pistachio	Tokusoglu *et al*. 2005
	Peanut	Liu *et al*. 2003, Tokusoglu *et al*. 2005, Wang *et al*. 2005, Frank *et al*. 2003, Chen *et al*. 2002, Chung *et al*. 2000, Sanders *et al*. 2000, Sobolev *et al*. 1999, Schoppner *et al*. 1984
	Peanut Butter	Sobolev *et al*. 1999, Ibern-Gomez *et al*. 2000
Piceatannol	Grape	Cantos *et al*. 2003
	Wine	Cantos *et al*. 2003
	Sugar Cane	Brinker *et al*. 199.
	Rhubarb	Kageura *et al*. 2001
	Deerberry	Rimando *et al*. 2004
	High Bush Blueberry	Rimando *et al*. 2004
Pinosylvin	Pine nuts	Skinnider *et al*. 1986, Kodan *et al*. 2002, Chiron *et al*. 2000, Wollenweber *et al*. 2003, Lee *et al*. 2005, Schanz *et al*. 1992
Rhapontigenin	Rhubarb	Matsuda *et al*. 2001, Matsuda *et al*. 2000, Ko *et al*. 1999, Suresh *et al*. 2004
Pterostilbene	Grape	Adrian *et al*. 2000, Pezet *et al*. 1998, Douillet-Breuil *et al*. 1999
	Rabbit Eye Blueberry	Rimando *et al*. 2004
	Deerberry	Rimando *et al*. 2004
	Organic and Conventional Commercial Blueberries	Remsberg *et al*. unpublished

(Table 6. Contd…)

Stilbene	Foodstuff	Reference
Astringin	Grape	Waffo-Teguo *et al*. 2001, Landrault *et al*. 2002
	Wine	Carando *et al*. 1999, Ribeiro de Lima *et al*. 1999, Vitrac *et al*. 2005
Piceid	Grape	Bavaresco *et al*. 2003
	Wine	Vian *et al*. 2005, Ribeiro de Lima *et al*. 1999, Vitrac *et al*. 2005, Moreno-Labanda *et al*. 2004
	Beer Hop	Jerkovic *et al*. 2005, Callemien *et al*. 2005
Viniferin	Grape	Huang *et al*. 2005, Zhang *et al*. 2004, Pezet *et al*. 2003
	Wine	Vitrac *et al*. 2005

berries: bilberry [16, 98, 99], high-bush, low-bush, rabbit eye, and Elliot's blueberry [16, 99], cowberry [98], cranberry [16, 87, 98], deerberry [16], ligonberry [16], sparkleberry [16], partridgeberry [16], strawberry [98], and red currant [98]. Furthermore, it can be found in rhubarb [67], pistachios [100], peanuts [12, 100-107], peanut butter [106, 108], and more recently, it has been found to be present in significant amounts in beer hops [95-97].

Piceatannol has been suggested to be involved in the French paradox because it is also present in grapes and wine [18], but it has been reported to be found also on sugar cane [17], rhubarb [67], and some varieties of berries: deerberry [16], and high-bush blueberry [16]. Pinosylvin has been found in pine nuts [52-55, 109, 110], while rhapontigenin has been reported to be present in rhubarb species [63, 64, 111, 112]. Pterostilbene has been detected in different varieties of berries: rabbit eye blueberry [16], deerberry [16], and organic and conventional commercial blueberries [Remsberg *et al.*, unpublished]. Even though it is present in grapes [72-74], it has not yet been identified in any red wines [75].

There are some other stilbenes present in grapes and wines that have been implicated in the French paradox and that are under current pharmaceutical investigation. Some of these stilbenes include, astringin, that can be found in grapes [7, 113], and wines [114, 115, 138], piceid found also in grapes [116], wine [88, 115, 117, 138], and recently detected in beer hops [96, 97], and viniferin also detected in grapes [118-120] and wine [115].

The broad spectrum of foods in which these stilbenes have been identified to be present also leads to the possibility of detection in many other foods in future studies. There is a current need for more sensitive and specific methods for detection of stilbenes in more foods and food products to better quantify the intake in our diet and to better predict their pharmacological and physiological effects in our bodies.

6.2. Factors Affecting Stilbene Concentrations

Stilbenes concentrations are influenced by a variety of different factors that can be subdivided into two subclasses: pre-harvest and post-harvest. During pre-harvest, the important factors are: plant genetics, farming practices (organic versus conventional), weather, light exposure, pests and pest management, fertility of the soil, harvest time, and ripeness of the plant. During post-harvest, time since the food leaves the farm and makes its way to the consumer, the important factors are: storage, and processing (cooking or industrial processing) [121].

6.2.1. Pre-Harvest

It has been suggested that plants contain five classes of phytochemicals that affect human health: minerals, vitamins, proteins, carbohydrates, and secondary plant metabolites [121, 122]. Organic farming has been suggested to improve the overall health benefits of foods because of the possibility of an increase in secondary metabolites, vitamins, and minerals compared to conventionally farmed plants. The importance of organic versus commercial farming practices has been observed in marionberries [124], corn [124], peaches [125], pears [125], grapes and wines [126]. These studies reported higher levels of phytochemicals in the organic produce compared to the conventional produce. Conversely, there are some reports where the levels of polyphenolics detected have been inconsistent [122, 127]. This variability is expected because the quality of the produce from farming is directly related to the weather, soil and water quality. There is only one report where a stilbene (resveratrol) was measured in conventional and organic wines [126]. The investigators found that organic wines have on average and 32% higher concentration levels of resveratrol than conventional wines [126].

Organic farming is usually performed in fields that contain lower levels of available nitrogen compared to conventional farming, which leads to lower levels of nitrate and protein levels in organic foods (with the exception of grains) [128]. There is a report where three different soil treatments (tilled soil conventional method, trifolium cover crop sowed soil, and soil covered with natural, mixed meadow) are used for organic crops and their polyphenols content is compared to the levels of conventional crops grown using the tilled soil conventional method for conventional crops [123]. These investigators found that the trifolium cover crop organic crops contained higher levels of polyphenols compared to conventional crops [123]. The specific farming practice may also lead to differences in polyphenol content; this is reflected in a study reporting that the raised bed system leads to higher concentrations of ascorbic acid and polyphenols in some plants [128].

Furthermore, it has been observed that organic crops contain higher secondary metabolite concentrations than conventional crops, because they require an intrinsic method to be able to cope as well or better than conventional crops against natural pests [129].

Some studies have reported an increase on polyphenol content after genetic modification [130, 131]. For instance, the amounts of quercetin, kaempferol, and naringenin (flavonoids with similar chemical structure to stilbenes) are higher in genetically modified tomatoes compared to the parent genotype [131]. Also it has been reported that genetically modified tomatoes presented new polyphenols that were not identified previously [130]. Thus, if the genetic engineered (GE) cultivar would be available on the market, it would need an extensive safety testing before it can be approved for marketing [121].

It is generally considered that the polyphenols content increases with maturity. A study showed that the total levels of phenolics increase during maturity on grapes during ripening [132], however, another study showed the opposite pattern in grapes [133]. These discrepancies can be explained to the differences in the variety of the grape studied as well as farming methods. For instance, blackberries and strawberries have been shown to have higher anti-oxidant and phenolic content during the green stage, whereas red raspberries have higher levels during the red stage [134]. In addition, a recent study has shown that the amounts on naringenin (a flavonoid with similar structure to stilbenes) increase with maturity having the highest levels at the breaker stage, declining in red-ripe fruit [131]. Further studies examining pre-harvest factors on stilbenes concentrations in fruits and other foodstuffs are warranted.

6.2.2. Post-Harvest

The processing of foods has long been thought to actually reduce the nutritional value of foods [135]. However, it has been demonstrated in tomatoes that thermal processing during the manufacture of ketchup leads to an increase on lycopene and total anti-oxidant capacity but to a decrease in vitamin C. It has also been shown that commercial squeezing of oranges reduces the phenolics content by 22% compared to hand squeezing [136]. Freezing and pasteurization may also reduce the level of phenolic compounds, whereas vitamin C is increased during orange juice processing [136]. It has also been shown that thermal processing increases the total anti-oxidant capacity despite lowering the vitamin C content in tomatoes and sweet corn [137].

Therefore, it can be observed that different plant growing conditions and processing techniques may lead to differences in phytochemical content. More research needs to be conducted in order to understand the different variables during pre- and post-harvest, and to be able to identify the different phytochemicals present among different foods and between different species and cultivars. Currently, there is only one report that compares the amount of any stilbenes; in this case, resveratrol in organic and conventional wines [124]. Given its pharmacological importance and with this paucity of data on stilbenes in the literature, there is a definite need to examine and compare the concentration of stilbenes in organic and conventional produce and processed products.

7. PHARMACOKINETICS OF RESVERATROL

Based on structural similarities, it may be inferred that pinosylvin, piceatannol, rhapontigenin, and pterostilbene could display similarities in pharmacokinetic disposition to that of resveratrol. Several studies have determined the pharmacokinetic parameters of resveratrol *in vitro* using microsomes, and *in vivo* employing animal and human models.

7.1. Resveratrol Concentration in Wine

It has been estimated that the concentration of resveratrol in wine ranges from as little as 0.2mg/L to up to 10.6mg/L, depending largely on grape type and environment [138]. One study measuring the resveratrol content across 120 different types of wine from Portugal and France found the highest resveratrol content in French red wines at an average concentration of 5.6 mg/L. Interestingly, the investigators reported the average concentrations of piceatannol (13.1 mg/L) to be nearly 3 times higher compared to average resveratrol concentrations (5.6mg/L) in French red wines [139]. Another study found Chilean and Canadian red wines to have an average resveratrol concentration of 2.5mg/L compared to an average resveratrol concentration of 1.25mg/L in red wines produced in the United States [140]. Two studies found the average concentration of resveratrol to be ~7.15mg/L in a wide range of Pinot Noir types tested [141] and 7.74mg/L in a wide variety of Merlots examined [142]. It is evident based on these data and investigation that environment is critical in determining resveratrol and piceatannol concentration in wine and that validated assays for these compounds are imperative. Resveratrol has also been produced in the form of dietary supplements ranging in concentration of 10-20mg [143]. One important question to consider when determining concentrations of resveratrol in wines or supplements is whether the concentrations of resveratrol following oral administration and absorption are sufficient to produce the pharmacological effects associated with resveratrol exposure *in vitro*. One study addressed this inquiry by quantifying resveratrol concentrations in rat tissues following red wine administration. The researchers administered a 4mL dose of red wine with a known content of resveratrol (6.5mg/L) to a group of 42 rats *via* intragastric intubation. The dose administered corresponded to a concentration of approximately 86 μg/kg, representing an average amount of resveratrol that a human subject would ingest from one glass of red wine. At various time points following administration, groups of six rats were sacrificed and blood, heart, liver and kidney samples were taken. Resveratrol concentrations were determined. The maximum resveratrol concentration in the blood was 20.2ng/mL and was reached 1 hour after administration. The extracted livers demonstrated an average concentration of 20.7ng/g, the hearts contained 2.2ng/g, and the kidneys showed an average 20 ng/g resveratrol concentration. A second group of rats were administered a 43 μg/kg dose of wine (2mL red wine) daily over the course of 15 days. This experimental design was implemented to examine resveratrol concentrations achieved over time, resembling a situation in which a human

subject might ingest a glass of wine daily. Results demonstrated the resveratrol concentration in the liver to be 53.5ng/g, the heart to be 3.1ng/g, and the kidney to be 44.1ng/g. Comparing these results with previous research delineating the *in vitro* concentrations yielding pharmacological activity, the researchers concluded that over time, it is possible to achieve concentrations of resveratrol in the blood, heart, liver and kidneys *via* daily moderate consumption of red wine that is sufficient to elicit pharmacological activity shown in *in vitro* studies [144].

Toxicity studies have been conducted to determine whether high doses of resveratrol can be tolerated in the rat model. In one study, researchers administered a 20mg/kg dose to male rats daily for 28 days. Food utility indexes measuring feeding efficiency, hematological variables such as red blood cells, white blood cells, and platelet counts, glucose, cholesterol, triglycerides, high density lipoproteins (HDL)/ low density lipoproteins (LDL), liver enzymes, and clinical biochemical variables were monitored and compared to control rats. Across all but one variable, resveratrol treated rats were identical to control rats. The only quantifiable variable that differed significantly was aspartate aminotransferase levels, which were 30% higher in resveratrol treated rats compared to controls. Alanine aminotransferase levels in both groups were identical. The researchers also extracted the vital organs of the resveratrol-treated rats and found no evident pathology. It appears that the administration of a high dose of resveratrol yielded no determinable toxicological effects and that resveratrol appears to have a large safety margin [143].

7.2. Resveratrol Pharmacokinetic Parameters

Several studies employing both human and rat liver microsomes as well as in intact animal models have revealed that resveratrol is metabolized extensively *via* glucuronidation [145-147]. Sulfation of resveratrol, yielding a sulfated metabolite, is also apparent, although it appears that glucuronidation is the predominant metabolic pathway. One study incubating rat liver microsomes with resveratrol in a uridine 5'-diphosphate-glucuronic acid (UDPGA) system determined that resveratrol was extensively metabolized as evidenced by high performance liquid chromatography (HPLC) analysis. This metabolite was later confirmed *via* mass spectrometry to be a glucuronide [144]. Human subjects have been utilized experimentally to elucidate the metabolic pathway of resveratrol in man. Subjects received a dose of either 0.03mg/kg or 1mg/kg *via* oral administration. Urine samples were taken at various time points over the course of 24 hours. Researchers plotted cumulative resveratrol excretion of both dose groups and determined that all resveratrol was excreted 2-3 hours following administration in the 0.03mg/kg group and all resveratrol to be excreted 7-10 hours following administration in the 1mg/kg dose group. Resveratrol was shown to exist predominately in its glucuronidated form. Plasma samples taken from rats administered resveratrol showed that at all time points analyzed, 90% of the total resveratrol detected in the plasma existed as a glucuronidated metabolite [145].

Absorption of a compound is of utmost importance in determining whether a xenobiotic can become a feasible pharmacotherapy for disease treatment. Upon ingestion or injection of a stilbene, the compound must first be adequately absorbed before it can elicit its desired pharmacological action upon its target. Resveratrol has been studied in a number of absorption assays to determine whether resveratrol is readily absorbed in animal and human models. One study employing the perfused rat intestine model reported that resveratrol is well absorbed and extensively metabolized *via* glucuronidation [147]. Another study using human colonic adenocarcinoma cells (Caco-2) found resveratrol to be easily absorbed across the colon cell monolayer. The Caco-2 model is widely employed in absorption and metabolism experiments in the pharmaceutical industry. This model addresses whether a compound can be transported across the apical side of the colon cell to the basolateral side. Samples can be taken from the basolateral side and assessed to determine the quantity of the compound that is successfully transported. Basolateral samples can also be used to identify and quantify the possible metabolites of the compound. Resveratrol is readily transported across a colon cell monolayer and is extensively metabolized *via* sulfation and glucuronidation [146]. Recent research employing ^{3}H-labeled resveratrol determined the liver to be the main organ involved in the biotransformation of resveratrol [148]. These studies support earlier work finding resveratrol to accumulate in the liver upon oral administration to mouse and rat models [144].

To determine the pharmacokinetic parameters of resveratrol, Bertelli *et al*. administered 4 mL of red wine containing a 28.24 μg/mL concentration of resveratrol to 36 rats *via* intragastric intubation. Groups of 6 rats were sacrificed at 30 minutes, 1, 2, 4, 6 and 12 hours post dose. Serum, heart tissue, liver tissue and the kidneys were removed and assessed for resveratrol content. Investigators reported that resveratrol is absorbed quickly and eliminated quickly with a plasma elimination half-life of 30 minutes. Moreover, concentrations of resveratrol were found to be higher in the liver and the kidneys compared to the plasma lending evidence that resveratrol distributes to secondary tissue compartments. The pharmacokinetic data was modeled to an open-two compartment model to determine the concentrations of resveratrol in plasma and tissue, namely heart, kidney and liver, over time. Results suggested that resveratrol enters the bloodstream after intestinal absorption and distributes predominately to liver and kidney tissues to a smaller degree, cardiac tissue. Investigators reported the area under the plasma concentration curve (AUC) values, a measure of total drug exposure over time, for plasma, heart, kidney and liver tissues. The AUC value for plasma was 26.31ng.h/mL compared to 6.50ng.h/ml for cardiac tissue (24% AUC plasma), 77.75ng.h/mL for kidney tissue (295% AUC plasma), and 57.35ng.h/mL for liver tissue (218% AUC plasma). Based on the interpretation of these data, investigators concluded that resveratrol is readily absorbed, distributed widely in heart, liver and kidney tissues, and is eliminated predominately *via* renal and biliary excretion [149].

Research characterizing the metabolism and pharmacokinetic parameters of resveratrol has shown that it is effectively absorbed, distributed, and metabolized upon

ingestion. Resveratrol is quickly biotransformed by the liver into a glucuronidated metabolite following intestinal absorption and is rapidly eliminated from the plasma and excreted in the urine and bile. It is also evident that humans can ingest and absorb pharmacologically active concentrations that have been shown to yield cardioprotective and chemoprotective activities. Based on these data, it is apparent that the development of reliable assays and validated methodology, the elucidation of the metabolic pathways and the characterization of the pharmacokinetic parameters of the structurally similar stilbene compounds piceatannol, pinosylvin, rhapontigenin, pterostilbene, etc. are warranted.

8. PHARMACOMETRICS OF STILBENES: ANTICANCER ACTIVITIES, ASSAY DEVELOPMENT, AND PHARMACOKINETICS OF PICEATANNOL, PINOSYLVIN, RHAPONTIGENIN, AND PTEROSTILBENE

8.1. Cell Culture Procedure

The HCT-116 (colon adenocarcinoma) HL60 (leukemia) cell lines were obtained from the American Type Culture Association (ATCC, Rockville, MD). The HL60 cells were maintained in RPMI 1640 medium and the HCT-116 cells were maintained in Mccoy's 5A medium. The cell lines were supplemented with 10% heat-inactivated fetal bovine serum (FBS) and penicillin-streptomycin (10mg/1L) and were incubated at 37°C in a 5% CO_2 atmosphere.

The optimal cell seeding numbers for each cell line were determined by preliminary cell seeding number experiments. Cells were seeded in numbers 1 x 10^4, 2 x 10^4, 3 x 10^4 and so on until the final cell seeding number 10 x 10^4 per well in a 96 well plate (Costar 3595). Cell plates were incubated at 37°C in a 5% CO_2 atmosphere for 72 hours. Following incubation, medium was aspirated and alamar blue (resazurin) fluorescent dye solution was diluted in fresh medium to make a 10% resazurin solution. The 10% solution was added directly to cells. The cell plates were incubated at 37°C in a 5% CO_2 atmosphere for 3 hours. The cell plates were subsequently removed from the incubator and placed at room temperature in a darkened drawer to protect from light for 30 minutes. Next, the cell plates were placed into the Cytoflour®4000 fluorescence multi-well plate reader (Applied Biosystems, USA). Fluorescence was read at an excitation of 485nm and an emission of 530nm. Standard curves of cell seeding number against fluorescence were generated. HL60 cells were seeded at a density of 5 x 10^4 cells/well. HCT-116 cells were seeded at a density of 3 x 10^4 cells/well.

8.2. Alamar Blue Assay

Alamar Blue (resazurin) fluorescent dye is an easy and accurate assay that has recently gained popularity in determining the cytotoxicity of many cell lines [150]. The resazurin non-fluorescent compound is metabolized into the fluorescent compound resorufin by intact and viable cells. This emission of fluorescence can be quantified using a cell plate reader and the number of viable cells following treatment can be determined. Cells were counted and seeded on 96 well plates. The seeded cells were incubated at 37°C in a 5% CO_2 atmosphere for 24 hours. Piceatannol, pinosylvin, resveratrol, rhaponticin, rhapontigenin, and pterostilbene were dissolved in methanol the day of the experiment and were diluted in medium to yield concentrations of 0.1, 1, 10, 50, and 100 μg/mL. Following aspiration of the medium, cells were treated with the stilbene solutions. Additional cells were treated with either methanol diluted in medium or medium only. Treated and control cells were incubated at 37°C in a 5% CO_2 atmosphere for 72 hours. After cell plates were removed from the incubator, medium was aspirated and replaced with 10% alamar blue (resazurin) fluorescent dye diluted in fresh medium. Cell plates were incubated at 37°C in a 5% CO_2 atmosphere for an additional 3 hours. Following incubation, cell plates were placed in a darkened environment for 30 minutes at room temperature. Next, the cell plates were placed into the Cytoflour®4000 fluorescence multi-well plate reader (Applied Biosystems, USA). Fluorescence was read at an excitation of 485nm and an emission of 530nm. The viable cell number (as a percent of control) in each cell line exposed to varying concentrations of stilbene was measured.

8.3. Results

Analysis of HL60 cell viability as a percent of the control following exposure showed greater activity in cells treated with piceatannol (IC_{50}~3 μg/mL or 12.3 μM), then resveratrol (IC_{50}~10 μg/mL or 43.9 μM), next pinosylvin (IC_{50}~38 μg/mL or 179.2 μM), and finally rhapontigenin (IC_{50}>100 μg/mL or >387.6 μM) (Fig. **3**). These data show piceatannol, pinosylvin, and rhapontigenin to possess anticancer activity, comparable to that of resveratrol.

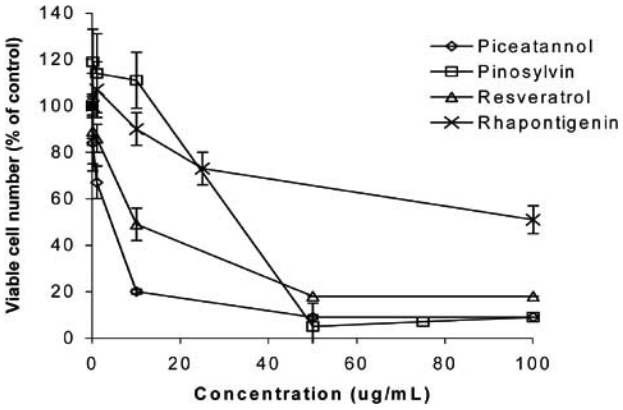


Fig. (3). Piceatannol, Pinosylvin, Resveratrol, and Rhapontigenin in HL60 leukemia cells.

Analysis of HCT-116 cell viability as a percent of the control following exposure showed greater activity in cells treated with pterostilbene ~7.4 μg/mL (~28.9 μM) compared to resveratrol (~21.1 μg/mL, ~92.5 μM) (Fig. **4**) [Remsberg *et al.* unpublished].

8.4. Methods of Analysis

Novel and simple high-performance liquid chromatographic methods have been developed for the determination of piceatannol, pinosylvin, rhapontigenin, and pterostilbene. Preliminary analysis showed piceatannol to elute at 19-20 minutes and the internal standard eluted at 28 minutes. An internal standard, 4-methylumbelliferone was chosen and

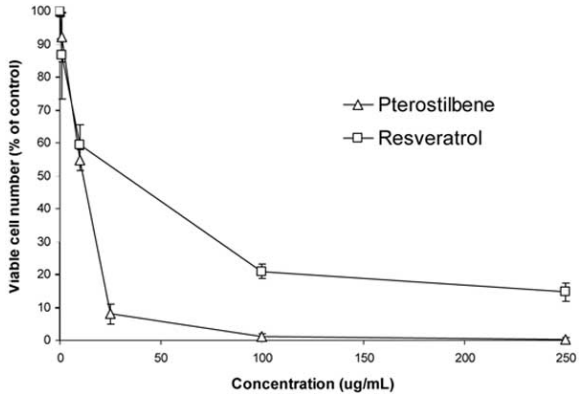


Fig. (4). Pterostilbene and Resveratrol in HCT-116 colon adenocarcinoma cells.

separation was achieved on a phenomenex C18 column (250 × 4.6 mm, ID, 5 µ) with fluorescence excitation at 320 nm and emission at 420 nm. The mobile phase used was methanol and 0.04% H_3PO_4 in HPLC water (34:66 v/v) and the flow rate was 1mL/min. An endogenous peak that was present in blank serum eluted at 12 minutes. The developed assay was validated in blank rat serum (Fig. **5**) [151].

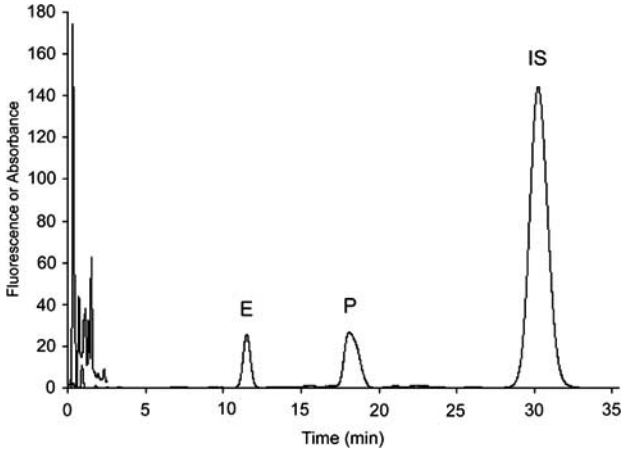


Fig. (5). HPLC chromatogram of piceatannol (P), internal standard (IS), and an endogenous peak present in blank serum (E).

A novel and simple high-performance liquid chromatographic method was also developed for determination of pinosylvin in rat serum. The internal standard used was 7-ethoxycoumarin. Separation was achieved on an amylose tris 3, 5 dimethylphenylcarbamate column (150 x 4.6mm, ID, 5µ) with UV detection at 308nm. The mobile phase used was acetonitrile and 0.1% H_3PO_4 in HPLC water (42:58 v/v) (Fig. **6**), and the developed assay was validated in blank rat serum [152].

A novel and simple high-performance liquid chromatographic method for the determination of rhapontigenin has also been recently validated. Separation was achieved on an

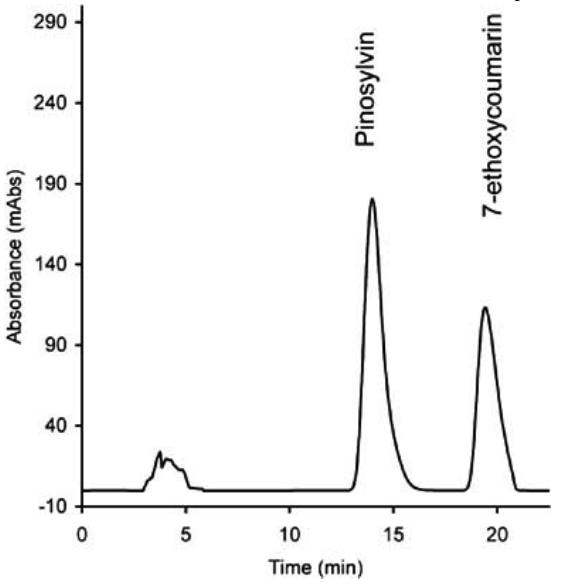


Fig. (6). Rat serum containing pinosylvin with a concentration of 10 µg/mL and 7-ethoxycoumarin (internal standard).

amylose tris 3, 5 dimethylphenylcarbamate column (150 x 4.6mm, ID, 5µ) with UV detection at 324nm. The internal standard used was daidzein and the mobile phase consisted of acetonitrile and 0.1% phosphoric acid (30:70, v/v) (Fig. **7**) [153].

There are a limited number of analytical approaches for the detection of pterostilbene in the literature [73, 74, 76, 78, 84]. Pterostilbene has been suggested to be assayed *via* high performance liquid chromatography using gradient elution, gas chromatography after methylation, and mass spectrometry; however, there remains no validated method of analysis in biological matrices. In the laboratory, a novel reverse phase HPLC method has recently been developed for the detection and quantification of pterostilbene using isocratic elution. A Phenomenex C18 column (250 x 4.60 mm) was employed with fluorescence excitation at 330 nm and emission at 374 nm (Fig. **8**). The mobile phase consisted of acetonitrile and HPLC water (50:50 v/v) with a flow rate

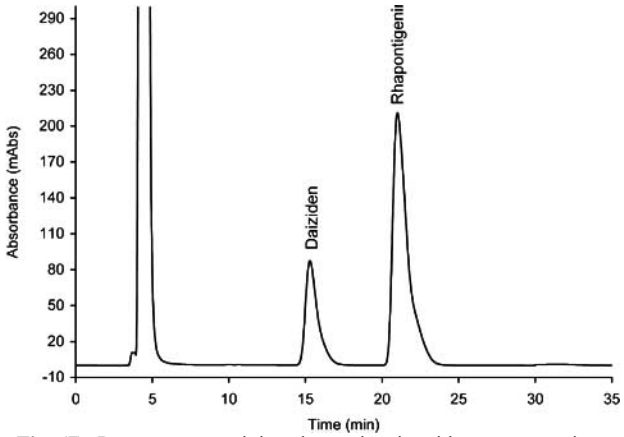


Fig. (7). Rat serum containing rhapontigenin with a concentration of 10 µg/mL and daidzein (internal standard).

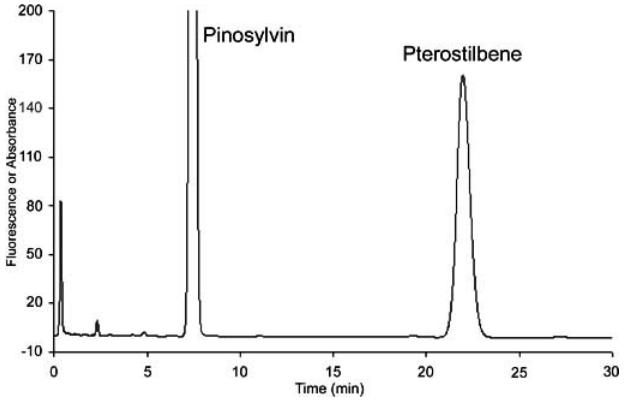


Fig. (8). Pterostilbene (100 µg/mL) and internal standard (pinosylvin) in methanol.

of 1.0 mL/min. Pinosylvin was used as an internal standard. A second but less sensitive HPLC method used a ChiralpakAd column with ultraviolet detection at 370 nm with a mobile phase of acetonitrile and 0.01% H_3PO_4 in HPLC water (42:58 v/v) was used with a flow rate of 0.4 mL/min [Remsberg *et al.* unpublished].

8.5. Preliminary Pharmacokinetics

8.5.1. Procedure

Male Sprague Dawley rats (n=4, average weight~325 g) were anesthetized using halothane and a silastic catheter was cannulated into the right jugular vein. The animals were placed in metabolic cages, allowed to recover overnight and fasted for 12 h before dosing. On the day of experiment, the animals were dosed intravenously with pinosylvin, piceatannol, rhapontogenin, or pterostilbene (10mg/kg). Serial blood samples (0.25 mL) were collected at 0, 1 min, 10 min, 0.25, 0.5, 1, 2, 4, 6, 12 and 24 h. After each sample collection, the cannulas were flushed with 0.25 mL of saline. Following centrifugation of the blood samples, serum was collected and stored at –70 °C until analyzed. The experimental animal protocols were approved by the Institutional Animal Care and Use Committee of Washington State University.

8.5.2. Results

The developed HPLC methods have been applied to the determination of pinosylvin, piceatannol, and rhapontigenin in pharmacokinetic studies in rats. There are no previously published studies or information of the pharmacokinetics of these four stilbenes in any species. The pharmacokinetics of pinosylvin, piceatannol, rhapontigenin, and pterostilbene appear to be qualitatively very similar to previous reports of resveratrol in the rat (Fig. **9**) [151, 152]. Pharmacokinetic analysis suggests significant biliary excretion and variable urinary excretion for each stilbene.

The laboratory has recently described a method of preparative enzymatic synthesis of the aglycone rhapontigenin from its glycosylated parent compound, rhaponticin (Fig. **10**). Many stilbenes are not commercially available and this method may have utility for the preparation, purification, and

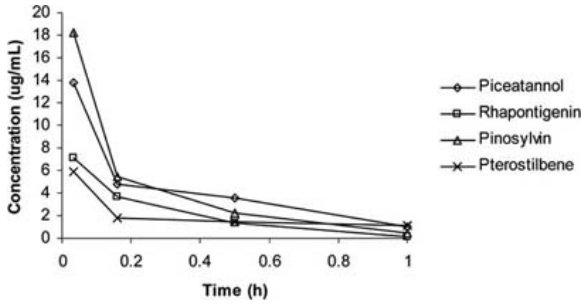


Fig. (9). Pharmacokinetic time course of rhapontigenin, pinosylvin, piceatannol, and pterostilbene in rat serum.

separation of other aglycone stilbenes and flavonoids from their glycosylated parent compounds.

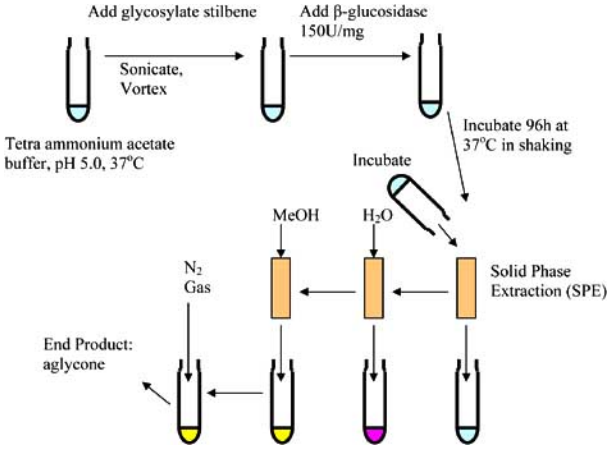


Fig. (10). Schematic of the developed method of preparative enzymatic synthesis of and aglycone stilbenes from the parent glycosylated stilbene.

CONCLUSION

Piceatannol, pinosylvin, rhapontigenin, and pterostilbene are stilbenes that are structurally similar and possess varying degrees of potency across many *in vitro* and *in vivo* assays. It is evident that hydroxyl and methoxy moieties and other substitutions attached to the general stilbene structure produce these varying degrees of pharmacological activity. Moreover, minute differences in structure have the capacity to significantly alter the pharmacokinetic disposition of these xenobiotics. These compounds are attractive candidates in therapeutic development due to apparent low toxicity and their anti-cancer, anti-hyperlipidemic, and anti-inflammatory activities. Preliminary data conducted in the lab has shown these stilbenes to possess anti-cancer activity in the HL60 leukemia cell line and the HCT-116 cell line. In addition, simple, sensitive, reproducible, and validated isocratic HPLC assays have been developed and validated for each of these stilbenes and have been used in the characterization of preliminary pharmacokinetic data. The relevance of pharmacokinetic data requires further elucidation, but experimental evidence suggests that these compounds may be attractive candidates for gastrointestinal (colorectal cancer, colitis,

gastrointestinal ulceration), and hepatic disorders (cirrhosis, hepatitis, hyperlipidemia). Further studies are ongoing in the laboratory to identify possible metabolic products of piceatannol, pinosylvin, rhapontigenin, and pterostilbene and to characterize their pharmacokinetic disposition and their disposition in a variety of organic and conventional foodstuffs, as well as their pharmacological activities in animal models seguing their development to the clinic.

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