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Selection of Bioavailability Markers for Herbal Extracts Based on *In Silico* Descriptors and Their Correlation to *In Vitro* Permeability

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Abstract: Bioavailability data for herbal supplements in humans is not readily available or is difficult to obtain, because of the complexity of the composition and the diversity of the constituents. Potency of an herbal extract is due to the synergistic interactions between several constituents. Thus, the use of *in silico* methods is an attractive alternative to predict the qualitative intestinal permeability of the active constituents for the selection of appropriate bioavailability markers. Molecular descriptors such as CLogP, minimal cross-sectional area and polar surface area of 37 active components from selected herbal extracts such as milk thistle, kava, ginkgo, ginseng, valerian, black cohosh and garlic were estimated. *In vitro* permeability of the compounds was determined by SimBioDAS an *in vitro* epithelial cell permeability assay. Based on the *in silico* descriptors and their relationship with the *in vitro* permeability, the qualitative intestinal permeability of the active compounds was predicted. Bioavailability and bioequivalence markers were predicted for kava, *Ginkgo biloba* and milk thistle. Choosing a compound which has the least intestinal permeability as a marker is the most conservative approach toward ensuring the bioavailability of the entire extract.

Keywords: Kava; ginkgo; milk thistle; *in silico*; CLogP; minimal cross-sectional area; permeability; bioavailability

Introduction

The sale of herbal supplements has been increasing exponentially, with total sales from all channels estimated to approximately $4.4 billion.[1] Retail sales from the food, drug and mass market (FDM) only have been steadily decreasing from $731 million in 1998 to $250 million in 2005 attaining a plateau since 2003. The data reported for FDM sales does not include sales from large warehouse buying clubs like Wal-Mart and Sam's Club.[1] If this data were obtained, reported sales would be twice the totals reported in 2005. Though FDM sales have decreased in the past decade, total sales from all channels have shown a steady growth, which probably indicates that consumers are turning toward buying herbal supplements from sources other than large warehouse buyers and through the Internet. Table 1 describes some of the top ranked herbal supplements based on their retail sales in 2005 and their comparative rank in 2001 and 2002.[1−4] It is interesting to note that Kava, which was ranked 11 and 13 in 2001 and 2002 respectively, did not show up in sale rankings in 2005, due to reports of hepatotoxicity coming to light in 2003.[5,6]

While the sale of herbal supplements has been increasing steadily, the quality of these products has not been highly

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Table 1. Ranking of Herbal Supplements Based on Retail Sales in 2005, 2002 and 2001

herb	ranking based on retail sales		
	2005	2002	2001
garlic	1	1	3
echinacea	2	3	2
saw palmetto	3	5	6
Ginkgo biloba	4	2	1
cranberry	5	9	9
soy	6	4	5
ginseng	7	6	4
black cohosh	8	8	10
St. John's wort	9	7	7
milk thistle	10	11	12
evening primrose	12	12	13
valerian	13	10	8
grape seed	15	15	14
bilberry	16	14	15
kava		13	11

scrutinized. Finding data of questionable accuracy is not uncommon, which is often designed to sell products rather than provide unbiased information. The corresponding lack of bioavailability data in humans due to diversity in its active components, a synergistic pharmacologic effect or the mere lack of pure reference standards further complicates the issue. Solubility, partition coefficient and intestinal permeability are the fundamentals for oral bioavailability Thus, adopting methods to predict these parameters using molecular descriptors gives a certain directional focus in a scientific and economic way. By estimating molecular descriptors such as the predicted octanol−water partition coefficient (CLogP), polar surface area (PSA) and the minimal cross-sectional area (MCSA) for the active components of various herbal extracts, an attempt has been made to correlate these descriptors with the *in vitro* epithelial permeability, in order to help select bioavailability and bioequivalence markers for herbal extracts resulting in safe and efficacious products. Prediction of oral bioavailability has always been difficult due to the complex process of absorption.[7] Lipinski's rule of 5 is one of the most commonly used models for the prediction of passive intestinal permeability mainly due to its least complex nature and ease of use.[8,9] Though it has its advantages of being simple, easy to interpret and fast to compute, the main drawback of the rule of 5 is that it cannot be applicable to molecules derived from natural sources or natural products, like active components from herbal extracts.[10]

Some of the molecular descriptors that come close to predicting drug permeability and eventually their bioavailability or fraction absorbed are the PSA, solubility, CLogP/distribution coefficient (LogD) and MCSA.[11,12] Though it is impossible to predict quantitatively the intestinal permeability of a drug from molecular descriptors alone, the PSA, CLogP and the MCSA are some of the important molecular descriptors that can be used in correlation with the *in vitro* cell permeability to get a certain qualitative idea of the intestinal permeability of the drug. The PSA of a molecule can be defined as the surface area associated with the hydrogen bonding acceptor atoms nitrogen and oxygen, and the hydrogen atoms bound to these heteroatoms.[13] An exhaustive account of the role of PSA in intestinal permeability is described by Artursson and Bergstrom.[14]

The MCSA, also termed as the collisional cross-sectional area, can be defined as that cross-sectional area of the solute molecule when it is partitioned into the lipid bilayer interior and is preferentially oriented with its longest axis along the bilayer normal.[15] The calculation of the MCSA using the general solute−solvent interaction (GSSI) model is based on the principle that all solution phase processes can be modeled in terms of one or more gas-to-solution transfer processes.[12] Formation of a cavity is a thermodynamically unfavorable process, and hence theoretically the alignment of the solute molecule along its longest axis minimizes the work required to create a cavity big enough so that the solute molecule can be accommodated into the lipid bilayer (solvent molecule). The extent of permeation of the solute through the lipid bilayer mainly depends on its cross-sectional area along the longest axis of the solute. Therefore, transcellular diffusion occurs when a cavity or opening of free volume with cross-sectional area equal to or greater than the minimum cross-sectional area of the solute is created.[15]

Drug lipophilicity is widely used as a predictor of membrane permeability since it is assumed that drug partitioning in the lipophilic cell membrane is a rate determining process for passive membrane permeation. Transcellular diffusion requires dehydration of the compound and entry into the lipid bilayer of the cell membrane. The compound travels through the cytoplasmic aqueous phase or along the lipid membranes of the cell and crosses the cell

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Table 2. Selected Herbal Extracts with Their Active Components and Rankings Based on Retail Sales

herbal extract	active compounds[5,18]	ranking		
		2001	2002	2005
kava	kawain (K), dihydrokawain (DK), methysticin (M), dihydromethysticin (DM), yangonin (Y), desmethoxy-yangonin (DY)	11	13	
milk thistle	silybin A (SbA), silybin B (SbB), isosilybin A (ISbA), isosilybin B (ISbB), silycristin (Sc), silydianin (Sd), taxifolin (Tx)	12	11	10
Ginkgo biloba	ginkgolide A (GA), ginkgolide B (GB), ginkgolide C (GC), ginkgolide J (GJ), isorhamnetin (Ih), kaempferol (Kf), quercetin (Q)	1	2	4
ginseng	ginsenoside Rb1(GRb1), ginsenoside Rb2 (GRb2), ginsenoside Rc (GRc), ginsenoside Rd (GRd), ginsenoside Re (GRe), ginsenoside Rf (GRf), ginsenoside Rg1 (GRg1), ginsenoside Rg2 (GRg2)	4	6	7
black cohosh	26-deoxyactein (dAcn), 26-deoxyacteol (dAcl), actein (Acn)	10	8	8
echinaceae	caftaric acid (CfA), chicoric acid (CcA)	2	3	2
garlic	alliin (Aln), deoxy-alliin (dAln)	3	1	1
valerian	hydroxy-valerenic acid (HVA), valerenic acid (VA)	8	10	13

membrane again to exit.[16] Hence transcellular diffusion must depend on the lipophilicity of the compound.

Herbal supplements are crude extracts or semipurified extracts manufactured to contain a definite amount of a particular constituent or a group of constituents, which are called marker compound(s). Since potency requires biological assessment of an extract, the presence of marker compounds does not guarantee the potency of an extract. Even if the marker compound demonstrates bioactivity, the biological activity depends on the composition of the rest of the extract. Other components, even those showing no direct physiological effect, can influence the absorption, distribution, metabolism and excretion of the active compounds. Such a background matrix can also lead to a difference in solubility, oil−water partitioning, permeability and bioavailability of any single compound in the given extract. Thus, it becomes necessary to know the physicochemical and biopharmaceutic properties of each of the compounds present.

Herbal extracts are complex mixtures, and hence isolation of each active compound from the extract is difficult. Performing bioavailability studies would be very difficult for each of the active compound in the selected herbal extracts. Further due to lack of reliable bioavailability data in humans and the difficulty to obtain pure and isolated compounds from the extracts, the use of *in silico* methods provides an attractive alternative to predict the biopharmaceutical properties of these compounds. Therefore the use of *in silico* methods to estimate the molecular descriptors of each of the compounds would give a certain directional focus on which compound can be selected as a probable bioavailability marker.

Bioavailability studies would be carried out by identifying the compound that shows least intestinal permeability as predicted by the *in silico* descriptors hence explaining that if the least permeable compound has a certain bioavailability, the rest of the active compounds in the extract would have higher bioavailability than the marker compound. Hence identifying markers which are least permeable in a group of compounds for a particular herbal extract is probably the most conservative approach toward demonstrating the bioavailability of the entire extract.

In the following work 37 active compounds from 8 top selling herbal supplements were chosen such that they covered a wide molecular weight range from 200 to 1000 accompanied with structural diversity. Effective permeability for 31 of these compounds was determined by the SimBioDAS system, an *in vitro* epithelial cell permeability assay.[17]

Materials and Methods

Selection of herbal supplements was primarily based on their highest consumption and ranking according to retail sales.[2,3,5,6] As mentioned earlier, studying all the known active compounds in a herbal extract would be a tedious task. Table 2 gives the list of herbal extracts and their corresponding active compounds under investigation. The second criterion for selection was structural diversity which included kavalactones from kava, terpene trilactones and flavonol aglycons from ginkgo, flavonolignan isomers from milk thistle and the ginsenosides from ginseng to name a few. All together they spanned a wide range of structural diversity and molecular weights.

Calculation of Predicted Octanol−Water Partition Coefficient (CLogP). In the following work SYBYL6.8, which incorporates the program CLogP, was used to predict the octanol−water partition coefficient of the molecules.[19] The program divides the molecule into basic fragments and calculates the log $P_{o/w}$ by the summation of hydrophobic contributions of these fragments.[20] CLogP has been tested

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on an elaborate database which includes nearly 8000 compounds and yields good results ($r = 0.970$).[21]

Estimation of Molecular Descriptors. Since the mdl.mol format is a 2-D representation of the compounds, the program CONCORD was used to create 3D coordinates of each atom in the molecules. In order to estimate molecular descriptors such as polar surface area and minimal cross-sectional area, it is necessary that the compounds are represented as geometrically optimized 3-D structures. We used CONCORD for the conversion of 2-D or crude 3-D input structures to accurate and geometrically optimized 3-D structures.[22]

CONFORT, a powerful conformational analysis tool, based on a novel algorithm for conformational searching, performs exhaustive and rapid analysis of 3-D drug sized molecules.[23] We used CONFORT to identify various global energy and maximally diverse conformers, and only those with energy <2.5 kcal/mol were selected. The conformer with the maximum nonpolar surface area was selected for the calculation of PSA, and the one with minimal cross-sectional area was identified for the calculation of the same.

The accessible surface area required for the calculation of the PSA and MCSA was determined using SAVOL3, a widely distributed program for calculating the mentioned molecular descriptors.[24] The algorithm computes the molecular (or solvent accessible) surface area by summing the nonoccluded surface area of each atom in the molecule. This can be done by imagining a slicing plane (passing through the two poles of the sphere) being rotated incrementally about its axis, thereby cutting the sphere into many double lunar segments (imagine, for example, two slicing planes intersecting at the center of the sphere at an angle of 1° from each other; the resulting pairwise spherical segments are what is referred to as double lunar segments).[25] The nonoccluded surface area of each double lunar segment is calculated by analytically summing up portions which are not contained in the van der Waals sphere of a neighboring atom. The precision of the surface area depends on the angle of increment used for rotating the slicing plane.

SAVOL3 was incorporated along with the GSSI[12,25] (general solute−solvent interaction) model for the calculation of the minimum cross-sectional area of the solute. The GSSI model is based on a semiempirical approach to enable the prediction of solution phase properties (free energies of desolvation, partition coefficients, and membrane permeabilities).

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Table 3. *In silico* and *In Vitro* Permeability Data for Kava Constituents

compound	mol wt	CLogP	PSA (Å^2)	MCSA (Å^2)	$P_{eff} \times 10^{-6}$ (cm/s)	SD $\times 10^{-6}$ (cm/s)
DY	228.24	2.79	89.232	92.250	21.667	1.033
DK	232.28	2.15	83.790	107.726	19.333	0.816
DM	276.28	1.71	136.049	113.722	19.333	0.816
K	230.26	2.04	92.568	106.461	20.000	1.095
M	274.27	1.61	145.773	105.66	20.333	0.816
Y	258.27	2.71	108.388	97.242	65.667	16.848

Measurement of *In Vitro* Permeability. The Caco-2 cell line is one of the most commonly used *in vitro* models for studying intestinal absorption. Previous studies in our laboratory have shown that permeability coefficients can be used to classify drugs into various high and low permeability solubility classes and such data can also be used in combination with various *in silico* descriptors to determine lead compounds for drug development.[26] We adopt a similar approach when studying herbal extracts, by using the *in vitro* permeability data for each of the active compounds along with the PSA and the MCSA to point toward a suitable and meaningful bioavailability marker for each herbal extract. *In vitro* permeability (P_{eff}) for 31 of the 37 compounds was determined by using the SimBioDAS system developed by Kinetana Inc.[17,27,28] Effective permeability experiments were performed at Kinetana Inc., Alberta, Canada, considering the greater benefit to cost ratio in determining the effective permeability of each of the active compounds. The SimBioDAS is an *in vitro* epithelial cell based permeability assay, the monolayers of which achieve differentiation and confluence after three days. The formation of the monolayer and the integrity of the cells is confirmed by the TEER (transepithelial electrical resistance) and monitoring the permeability of Lucifer Yellow as a pass/fail marker. Previous studies[29] have shown permeability data obtained using the SimBioDAS system for 35 validation compounds is in correlation with the respective human *in vivo* data after certain area correction. The intralaboratory results obtained have been found to be more consistent and less scattered as compared to the Caco-2 cell model. Detailed information about the experimental process can be obtained by contacting the inventors of SimBioDAS.

Results

Tables 3, 4, 5, 6 and 7 show the calculated molecular

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Table 4. *In Silico* and *In Vitro* Permeability Data for Milk Thistle Constituents

compound	mol wt	CLogP	PSA (Å^2)	MCSA (Å^2)	$P_{eff} \times 10^{-6}$ (cm/s)	SD $\times 10^{-6}$ (cm/s)
ISbA	482.44	1.94	350.239	123.707	N/A[a]	N/A
ISbB	482.44	1.94	350.593	133.146	N/A	N/A
SbA	482.44	1.95	349.809	146.32	1.888	1.020
SbB	482.44	1.94	348.589	125.89	1.863	0.989
Sc	482.44	1.38	410.998	131.682	1.312	0.804
Sd	482.44	−0.39	368.061	157.108	N/A	N/A
Tx	304.25	1.02	347.423	101.285	2.200	0.636

[a] N/A = data not available.

Table 5. *In silico* and *In Vitro* Permeability Data for *Ginkgo biloba* Constituents

compound	mol wt	CLogP	PSA (Å^2)	MCSA (Å^2)	$P_{eff} \times 10^{-6}$ (cm/s)	SD $\times 10^{-6}$ (cm/s)
B	326.30	−2.64	271.405	139.435	3.891	0.130
GA	408.40	−1.55	283.058	128.28	6.203	0.261
GB	424.40	−1.45	313.477	132.547	3.921	0.169
GC	440.40	−2.48	309.311	134.402	4.252	0.245
GJ	424.40	−2.58	326.152	134.414	3.195	0.513
Ih	316.26	1.75	282.536	109.498	N/A[a]	N/A
Kf	286.24	1.90	307.353	96.256	0.833	0.079
Q	302.24	1.30	353.742	111.565	0.763	0.101

[a] N/A = data not available.

Table 6. *In Silico* and *In Vitro* Permeability Data for Data for Ginsenosides (Ginseng)

compound	mol wt	CLogP	PSA (Å^2)	MCSA (Å^2)	$P_{eff} \times 10^{-6}$ (cm/s)	SD $\times 10^{-6}$ (cm/s)
GRb1	1109.29	4.544	647.745	224.914	0.160	0.128
GRb2	1079.27	5.232	603.788	232.126	N/A[a]	N/A
GRc	1079.27	4.299	622.799	282.806	0.283	0.258
GRd	947.15	5.726	556.208	273.938	0.197	0.062
GRe	947.15	3.869	623.308	295.768	0.987	0.372
GRf	801.01	4.726	417.294	209.152	N/A	N/A
GRg1	801.01	4.932	438.223	245.672	2.033	0.922
GRg2	785.01	4.758	350.105	209.695	N/A	N/A

[a] N/A = data not available.

descriptors (PSA, MCSA and CLogP) and the effective permeability (P_{eff}) for the 37 compounds.

The CLogP for the actives ranged between −2.70 for (Aln) to +6.7 for the highly lipophilic acteols from black cohosh. The ginsenosides are found to be the largest molecules in terms of size and have the highest MCSA values and corresponding low permeability. The kava lactones from kava are found to be lipophilic followed by the flavonolignans from milk thistle, and the flavonol aglycons from *Ginkgo biloba*. Though flavones are present as glycosides in *Ginkgo biloba*, they are known to be hydrolyzed to aglycons (Q, Kf and Ih) after oral administration of *Ginkgo biloba*. Hence the aglycons are taken into consideration when studying permeability. The terpene trilactones from ginkgo are found to be least lipophilic.

Discussion

In the following work, an attempt has been made to study the interrelationships between the *in silico* descriptors and *in vitro* permeability to see whether these correlations help us select the least permeable component in select herbal extracts (kava, *Ginkgo biloba* and milk thistle). Selecting the component which has the least intestinal permeability is the most conservative approach to ensure the bioavailability of a multicomponent herbal supplement.

Hence, while selecting a bioavailability marker for an herbal extract three criteria need to be fulfilled:

1. The selected marker should have one of the least permeable among its active components in the extract.

2. The proportion of the selected marker in the extract should be sufficient for its precise quantitative determination in biological fluids after oral administration.(e.g., Sc in milk thistle, K in kava).

3. The selected marker should be easily available as a reference standard, at a reasonable cost and acceptable purity for routine analysis.

Since the identification of the least permeable component is primarily based on prediction by *in silico* descriptors, it does not account for the interactions between the components in an extract and their presystemic metabolism. Thus, prediction of intestinal permeability is done on a merely qualitative basis.

A plot of the predicted octanol−water partition coefficient versus the polar surface area is shown in Figure 1. The plot depicts an overall trend that the polar surface area of all the components (except the outliers) increases as the components move from the lipophilic scale to the hydrophilic scale. This holds true for the kava components, all the ginkgo terpenes and 3 isomers of silymarin (Tx, Sd, Sc). The ginsenosides and the acteols from black cohosh can be considered as outliers due to their unrealistic high values of CLogP. These high CLogP values can be attributed to their large molecular weight (between 600−1100 g/mol) and structure. It has been reported that the efficiency of partition coefficient predicting programs such as CLogP decreases as the molecular weight or the size of the compound increases.[30] Thus, the ginsenosides and the acteols can be considered as outliers. High polar surface area indicates the greater hydrophilic nature of the compound, and thus a lower polar surface area implies greater lipophilicity and hence higher permeability, which is again true in the case of kava compounds, which are lipophilic, have a low polar surface area and are the smallest and simplest of the molecules.

Figure 2 is a plot of the effective permeability determined using SimBioDAS (P_{eff}) versus the polar surface area for the active herbal components. The plot depicts a clear trend that as the PSA increases, the P_{eff} of the components decreases. If metoprolol ($P_{eff} = 10 \times 10^{-6}$ cm/s) is

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Table 7. *In Silico* and *In Vitro* Permeability Data for Black Cohosh, Echinaceae, Garlic and Valerian

herb	compound	mol wt	CLogP	PSA (Å^2)	MCSA Å^2)	$P_{eff} \times 10^{-6}$ (cm/s)	SD $\times 10^{-6}$ (cm/s)
black cohosh	dAcn	660.83	6.124	186.216	152.839	11.067	2.551
	dAcl	600.78	6.781	184.475	164.142	5.267	1.457
	Acn	676.83	5.419	269.289	182.592	2.883	0.366
echinaceae	CfA	312.23	−1.540	420.790	104.414	9.750	0.683
	CcA	474.37	0.139	516.139	167.085	11.500	0.837
garlic	Aln	177.22	−2.708	215.180	83.821	1.333	0.308
	dAln	161.22	−1.198	202.245	83.847	2.400	0.456
valerian	HVA	250.33	2.650	172.723	116.400	5.800	1.006
	VA	234.33	4.737	117.788	124.200	39.667	10.328

considered as a cutoff limit to distinguish between high permeability and low permeability compounds, we see that most of the herbal components fall into the low permeability class with the exception of the kava components. The plot depicts a linear decrease in permeability with the increasing PSA ($R^2 = 0.72$) indicating that the decrease in permeability is probably due to the solvation of the compound and the increasing molecular size. It can be inferred from this plot that, from a group of components in each extract, the one which has a low permeability and a high PSA would indicate lower intestinal permeability and can be selected as a probable marker. For the kava compounds, Y is the only component which has a very high permeability ($P_{eff} = 65.66 \times 10^{-6}$ cm/s) whereas the other 5 components have permeabilities in a close range with each other ($19.3-21.6 \times 10^{-6}$ cm/s). Between these low permeability components, M and DM have the highest PSA (~140 Å^2) followed by K (92.56 Å^2). Y cannot be considered as a marker, mainly due to its high permeability. K is one of the major components of the kava extract, in proportion and in terms of pharmacologic effect, and thus K is considered as a marker, for kava.

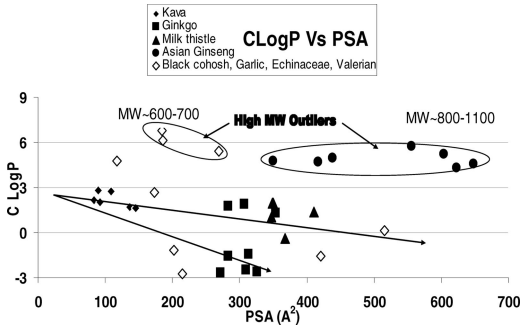

Figure 1. Plot of CLogP versus polar surface area.

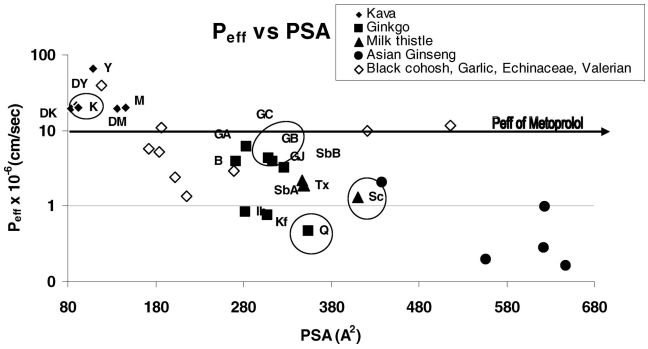

Figure 2. Plot of permeability (P_{eff}) vs polar surface area (PSA).

For the ginkgolides in *Ginkgo biloba*, GJ has the highest polar surface area accompanied by the lowest permeability, and can be considered as a marker. The proportion of GJ in the extract is very minimum as compared to the other ginkgo terpenes like GA, GB or GC, and the next least permeable component among the terpenes is GB. GB is also easily available commercially as a reference standard, and hence GB can be considered as a marker for the ginkgo terpenes. Similarly Q can be considered as a marker for the flavonol glycosides in *Ginkgo biloba*.

Among the silymarin isomers, Sd has a high polar surface area and is expected to be one of the least permeable compounds in the extract, due to its very low lipophilicity (−0.39). Sd is one of the minor components of silymarin and also suspected to be pharmacologically inactive as compared to the silybins,[31] whereas the next least permeable compound Sc has a very high proportion in the extract and is easily quantifiable. Thus, Sc can be selected as a marker for the silymarin isomers.

Based on the plot of effective permeability versus PSA we have we have K as a marker for kava, GB as a marker for the ginkgo terpenes, Q as a marker for the flavonol glycosides and Sc as a marker for milk thistle.

Figure 3 shows the plot of effective permeability (P_{eff}) versus minimal cross-sectional area (MCSA). The plot depicts an approximate trend of decreasing permeability with the increase in the MCSA which is expected, as the MCSA is the area of the molecule when it is partitioned in the lipid

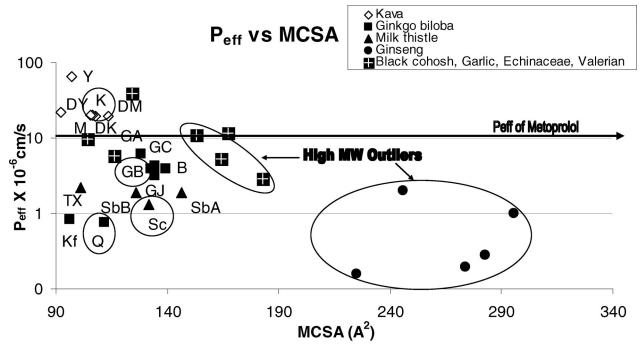

Figure 3. Plot of permeability (P_{eff}) vs minimal cross-sectional area (MCSA).

bilayer during the permeation process. Thus, larger the cross-sectional area, the more difficult it is for the molecule to permeate into the lipid bilayer, in this case the gastrointestinal membrane. Among the kava components, we have DM and DK with similar permeability values and increasing MCSA's. They are followed by kawain, which has the next highest MCSA and a similar permeability of 20×10^{-6} cm/s. As earlier, among the three, K is selected as a marker for kava based on its pharmacology and its major proportion in the extract. Also K is readily available as a reference standard.

Among the silymarin isomers, between Sc and SbA, Sc would be selected as a marker. Sd is not a choice due to its minor proportion in the extract. Even though SbA has a higher MCSA (146 $Å^2$) than Sc (131 $Å^2$), SbA is more lipophilic (1.95) as compared to Sc (1.38) and has a lower permeability value as compared to SbA. Hence, the low lipophilicity and the low permeability value of Sc makes it a potential marker candidate compared to SbA.

Among the ginkgo terpenes a choice needs to be made between B and GB as performance marker. The previous plot, based on P_{eff} versus PSA, predicted GB as a marker, due to its high polar surface area and proportion in the extract among the terpenes. Considering the permeability values, there is no significant difference between the permeability values of GB and B. Also GB has a moderately hydrophilic nature (CLogP $= -1.45$) as compared to B (CLogP $= -2.64$), which is highly hydrophilic. This increases the chance of GB being the least permeable compound, as compared to B, which would not be permeable at all due to high solvation. Thus, GB is predicted as a marker for the terpenes and Q which has a high MCSA (111 $Å^2$) and low permeability ($P_{eff} = 0.76 \times 10^{-6}$ cm/s) is predicted as a least permeable marker for the ginkgo flavonol glycosides.

Conclusion

From the interparameter relationships observed between the PSA, MCSA, CLogP and the effective permeability of the compounds, and maintaining the three criteria for the selection of an appropriate bioavailability/bioequivalence marker, the following active components are selected as markers for the respective herbal extracts:

I. K as a marker for the kava extract.

II. GB as a marker for the ginkgo terpenes and Q as a marker for the flavonol glycosides in *Ginkgo biloba*.

III. Sc is selected as a marker for the silymarin isomers in milk thistle.

Identification of the least permeable compound in an herbal extract for bioavailability testing is the most conservative approach toward ensuring the bioavailability of the entire extract.

Assigning a bioavailability and bioequivalence marker for a particular herbal extract would thus facilitate routine bioavailability and bioequivalence studies for herbal supplements being launched into the market thus regulating the inflow of therapeutically efficacious products in a cost-effective and economic manner.

Abbreviations Used

PSA, polar surface area; MCSA, minimal cross-sectional area. ◆, compounds of kava: K, kawain; DK, dihydrokawain; M, methysticin; DM, dihydromethysticin; Y, yangonin; DY, desmethoxyyangonin. ■, compounds of *Ginkgo biloba*: Q, quercetin; Kf, kaempferol; Ih, isorhamnetin; GA, ginkgolide A; GB, ginkgolide B; GC, ginkgolide C; GJ, ginkgolide J; B, bilobalide. ▲, compounds of milk thistle (*Silybum marianum*): SbA, silybin A; SbB, silybin B; ISbA, isosilybin A; ISbB, isosilybin B; Sc, silycristin; Sd, silydianin; Tx, taxifolin.

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