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Metal complexes of Flavonoids: Their synthesis, characterization, and enhanced anti-oxidant and anti-cancer activities

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Abstract

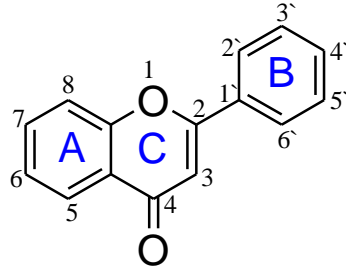
Flavonoids are polyphenolic compounds of natural origin. They are extensively studied within drug discovery programmes due to their wide ranging biological activities such as anti-microbial, anti-oxidant, anti-tumor, neuroprotective and cardioprotective properties. The ability of flavonoids to coordinate with metal atoms has provided new leads for drug discovery programmes, with better pharmacological activities and clinical profiles than the parent flavonoids. In this review, the enhanced anti-oxidant and anti-cancer activities of flavonoid metal complexes versus the parent flavonoids are discussed. Possible mechanisms of action for the metal complexes, such as DNA binding and apoptosis induction, are also presented alongside an overview of the synthesis of the metal complexes, and the different techniques used for their characterization.

Keywords

Flavonoid, metal, flavonoid metal complex, anti-oxidant, anti-cancer.

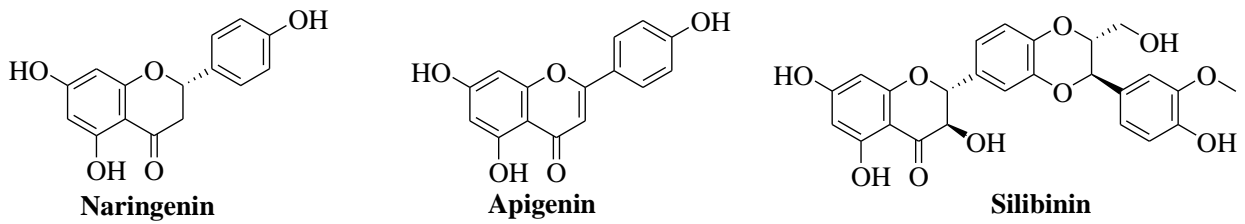
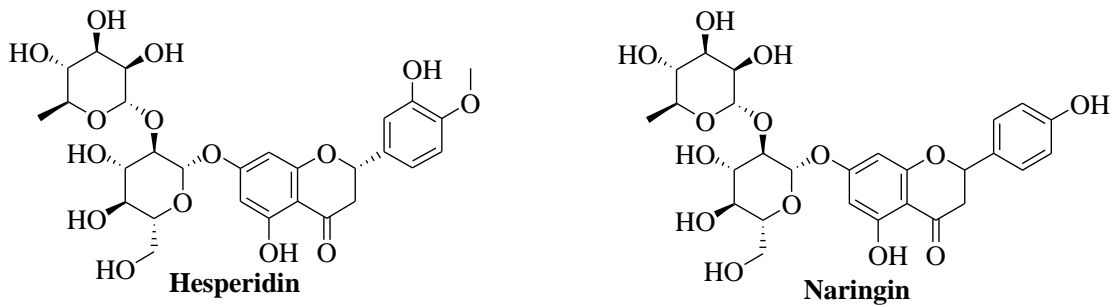
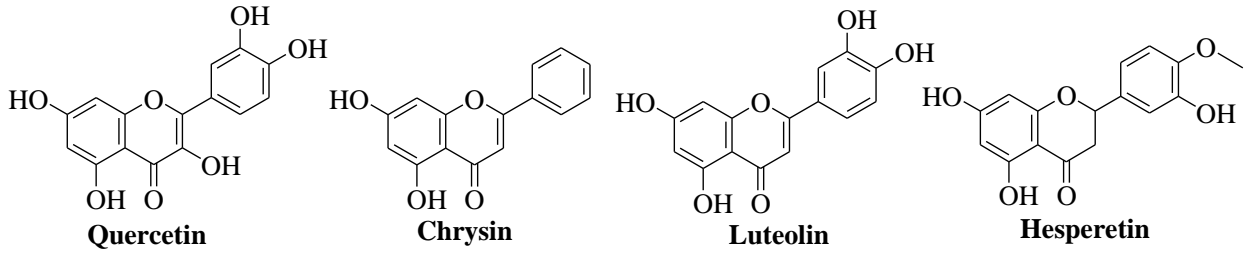
Introduction

Flavonoids are phytochemicals that are mainly found in tea, citrus fruit, berries, apples and legumes. Chemically, they are polyphenolic compounds with a C₆-C₃-C₆ ring system (**Figure 1**) and they are often found in esterified or glycosylated forms [1]. It was not until the 1990's that research on flavonoids witnessed significant progress, with the number of publications increasing by approximately 6-fold, from 524 in 1990 to 3147 in 2017. As a result it is now well established that flavonoids have a wide and diverse range of biological activities [2, 3] such as anti-viral [4-6], anti-bacterial [7-10], neuroprotective [11-13], cardioprotective [14, 15], anti-oxidant [16-18] and anti-cancer [3, 19, 20] properties.

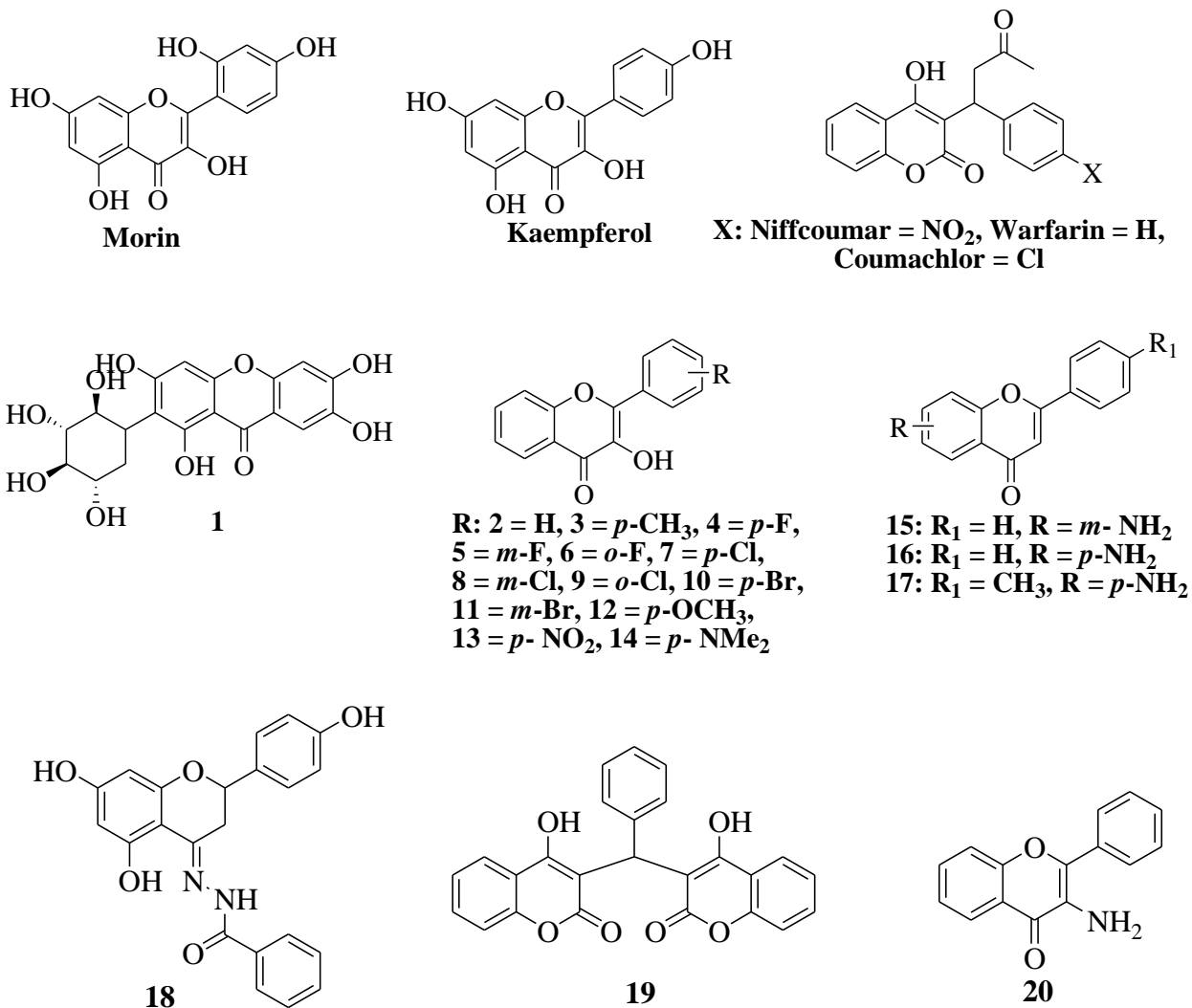


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Basic skeleton of flavonoids



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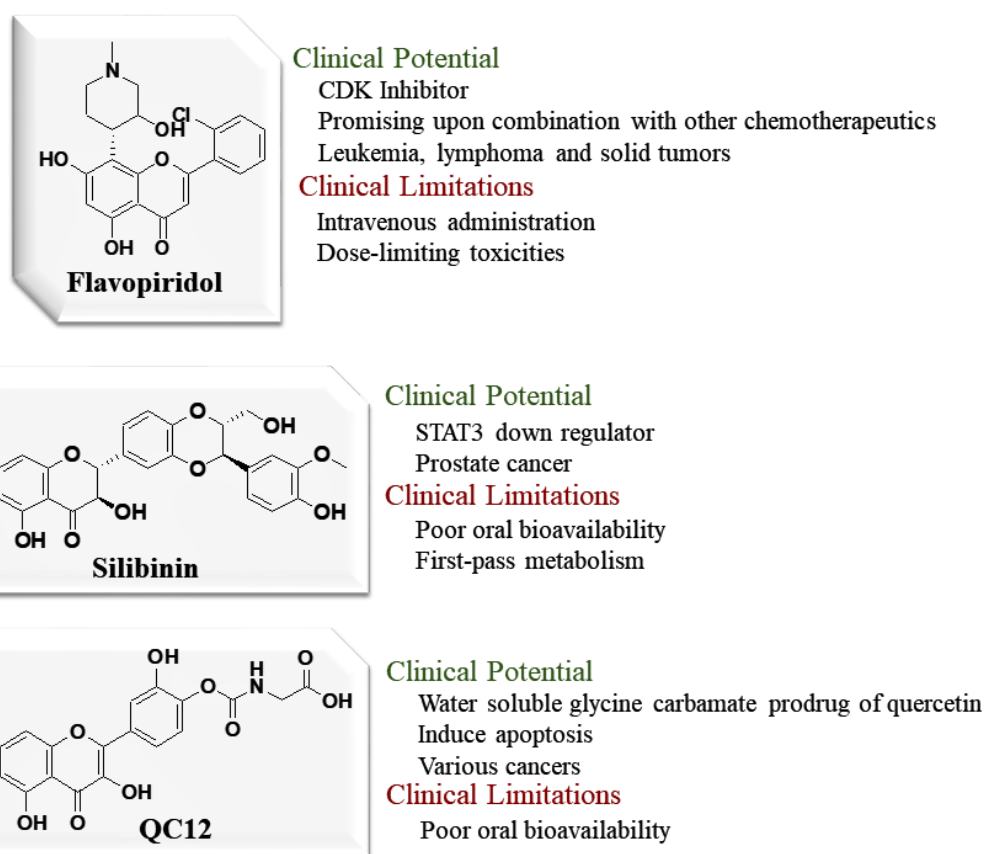
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34

35 **Figure 1.** Chemical structures of flavonoids from which organometallic derivatives have been
 36 prepared

37 With respect to the beneficial health effects of flavonoids, the greatest impact has been seen
 38 within the anti-cancer field [2]. Flavonoids are known to interfere with an array of targets
 39 affecting cancer growth and progression. For example, they have been shown to induce cell cycle
 40 arrest and apoptosis [21] in addition to inhibiting mitotic spindle formation [22] and
 41 angiogenesis [23, 24]. Despite the advantages of having a compound that can interact with
 42 different targets, this can be a limitation due to limited selectivity. Indeed, this has been one of
 43 the biggest obstacles in the use of flavonoids as potential drugs due to its direct relation with
 44 adverse effects and poor *in vivo* toxicity profiles. However, despite the extensive research and
 45 number of structures and the activity reported for them, no flavonoid has to date reached the

46 market and only a limited number are in clinical investigation as anti-cancer agents, eg
 47 flavopiridol (phase-II) [25, 26], silibinin (phase-II) [27, 28], quercetin (phase-II) [29, 30] and the
 48 quercetin derivative QC12 (phase-I) [31] (**Figure 2**). Arguably, the main drug development
 49 challenge is the poor bioavailability of flavonoids, resulting from water insolubility and
 50 susceptibility to glucourinidation and/or methylation by intestinal and liver metabolism (first
 51 pass effect). There are several studies on the pharmacokinetics of flavonoids indicating their poor
 52 bioavailabilities. Wu et al., for instance, reported the absolute oral bioavailability of silibinin in
 53 rats to be approximately 0.95% [32]. In another study, flavopiridol's mean oral bioavailability
 54 following bolus intra-gavage was shown to be 20% [33]. Moreover, a study by Gugler et al.
 55 failed to detect any plasma quercetin concentrations in subjects receiving 4 g orally [34].



56

57

Figure 2. Flavonoids in clinical trials as anti-cancer agents

58 Due to the presence of hydroxyl and oxo groups within flavonoids, they possess metal chelating
 59 abilities that can have profound effects on their pharmacokinetic and pharmacological properties
 60 [35, 36]. The flavonoid structure, type of chelating metal and the pH of the surrounding medium

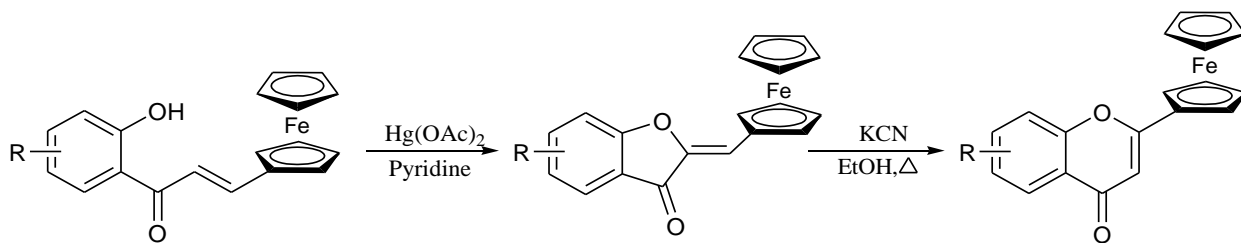
61 are determining factors of the preferred coordination site [37]. Metal atoms have beneficial
62 biological properties such as chelating free radicals and binding to DNA [38]. These properties
63 can cause synergistic effects when combined with other active chemical entities such as the
64 flavonoids. Accordingly, metal complexation is reported to enhance the anti-oxidant, anti-
65 proliferative and chemical properties of parent flavonoids. This review will therefore highlight
66 important advances within the field of metal-flavonoid complexes with particular emphasis on
67 their synthesis, methods of characterization, anti-oxidant and anti-proliferative activities.

68

69

70 **Synthesis and characterization of flavonoid metal complexes**

71 Flavonoid metal complexes are generally synthesized by dissolving a flavonoid salt in an
72 alcoholic or aqueous solution, followed by the addition of the metal salt also in alcoholic or
73 aqueous solution. The reaction can be carried out under different conditions of stirring and/or
74 heating, normally a base is used to deprotonate the hydroxyl groups and facilitate metal
75 coordination. The complex usually precipitates from solution and is then filtered and air dried
76 (**Table 1**). Preparations of ferrocenyl flavonoid complexes, where the ferrocenyl moiety acts as a
77 replacement for ring B, follow a different synthetic approach. Ferrocenyl aurones are obtained
78 via a classic Claisen-Schmidt condensation of ferrocene carboxaldehyde and various 2-
79 hydroxyacetophenones to obtain the corresponding ferrocene chalcones [39, 40]. The
80 synthesized chalcones are then treated with mercury acetate in pyridine to form the ferrocenyl
81 aurones that can be isomerized into their flavone counterparts by heating in ethanol at reflux with
82 potassium cyanide (**Figure 3**) [39, 40].



85 Ferrocenyl Chalcones

Ferrocenyl Aurones

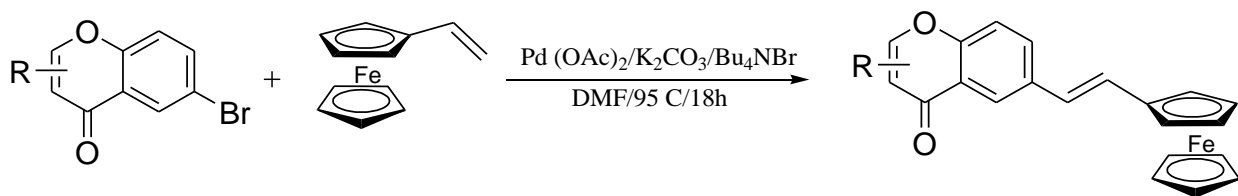
Ferrocenyl Flavones

86

Figure 3. Synthesis of ferrocenyl flavonoids

87 Kowalski et al. reported the synthesis of ferrocenyl flavonoids using the palladium-catalyzed

88 Heck cross-coupling reaction of vinyl ferrocene with 6-bromochromones (**Figure 4**) [41].

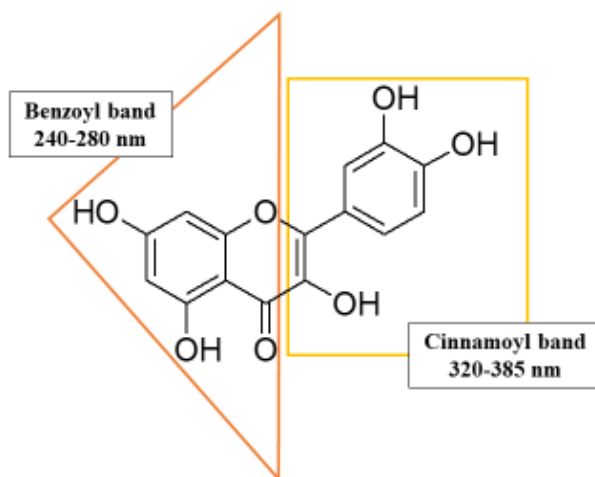


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Figure 4. Synthesis of ferrocenyl flavonoids using Pd

91 Structural characterization of metal complexes can be achieved using a number of methods
92 (**Table 1**). Fourier transform infra-red (FT-IR) spectroscopy can indicate the coordination site of
93 the metal. Complexation of the metal atom with the C=O group, for instance, causes an increase
94 in the bond length which is manifested as a decrease in the frequency of the C=O peak.
95 Coordination with OH groups leads to disappearance of their characteristic broad peaks ν OH at
96 3600-3200 cm^{-1} . However, this disappearance can be masked by the presence of other OH
97 groups in the flavonoid structure and in the H₂O molecules incorporated in the metal sphere
98 during complexation. Therefore, IR spectroscopy is not a helpful tool in determining which OH
99 groups are specifically coordinated to the metal atom. ¹H NMR spectroscopic analysis can
100 provide a better alternative where the disappearance of an OH peak from the ¹H NMR spectrum
101 corresponds to coordination at this particular OH group. For example, the disappearance of the 5-
102 OH peak at δ 10.52 ppm of the **lanthanum-18** complex proved coordination at this OH in ring
103 A [42]. Heteroatom NMR spectroscopic analysis is also extremely useful. For example,
104 Tabassum et al. used ¹¹⁹Sn NMR spectroscopic analysis to prove the presence of stannous atoms
105 in the synthesized bi-metallic complexes **31** and **32** (**Figure 6**) [43]. ¹⁹⁵Pt NMR spectroscopic
106 analysis was also used to prove the structure of complex **68** [44]. Electron Paramagnetic
107 Resonance (EPR) spectroscopy is also a helpful technique which is similar to NMR spectroscopy
108 but more sensitive as it explores the interaction between an external magnetic field and unpaired
109 electrons rather than nuclei. This is attributed to the fact that the amount of energy absorbed by a
110 spinning electron is higher than that absorbed by a nucleus. This technique is more difficult to
111 use as it requires background knowledge of subjects like quantum mechanics and mathematical
112 techniques [45]. However it was used by Tabassum and Naso et al. groups to elucidate structures

113 of complexes (**31** and **32**) and (**33**, **37**, **VOhespiridin**, **VOsilibinin** and **VOmorin**), respectively
114 [43, 46-49].



115

116 **Figure 5.** UV absorption bands using quercetin as an illustrative example

117 Flavonoids display absorption bands in the UV-Vis region due to electronic $\pi - \pi^*$ transitions
118 [50, 51]. These transitions are responsible for the two characteristic benzoyl and cinnamoyl
119 bands of flavonoids at 240-280 nm and 320-385 nm, respectively (**Figure 5**) which are
120 bathochromically shifted after chelating with metals [52]. The observation of a red shift in one
121 band rather than the other verifies chelation with that particular ring [53-55]. Although the shift
122 is small in many cases, it gives a good insight when overlapped with the parent's spectrum. UV-
123 Vis absorption bands are also characteristic to binding in other sites. Analysis of the spectra of
124 **VOhespiridin** showed complexation with the rutinose sugar part [46]. Its electronic spectra
125 showed bands at 358, 520 and 702 nm in agreement with the three band pattern at ca. 420, 500
126 and 700 nm observed for similar sugar vanadyl(IV) complexes coordinated through deprotonated
127 cis-hydroxyl groups [56]. UV-Vis spectra can also help in detecting the stoichiometry of the
128 complex. This is achieved by monitoring the absorption intensity of the ligand through a range of
129 concentrations until the reaction is completed [46, 47, 57, 58]. Different methods can then be
130 used to represent the data, e.g. Job's method, from which ligand-metal ratio can be interpreted.
131 Similarly, fluorescence of free parents can be compared to their complexes at a specific
132 wavelength. Peaks from complexes are higher in intensity and are shifted to lower wavelengths
133 ca. 15-20 nm [55]. Elemental analysis and mass spectrometry provide complementary

134 information about the molecular formulae of the resulting complexes. In a recent study by Wang
135 et al. the elemental analysis of a lead-luteolin complex was determined by scanning electron
136 microscope (SEM) equipped with energy dispersive spectroscopy (EDS) [59]. The experimental
137 values of weight and atomic number percentages of carbon, oxygen and lead atoms favored a 2:1
138 rather than 1:1 complex [59]. Analysis of the fragmentation pattern in mass spectrometry
139 specifies how many metal atoms are coordinated in the complex [43, 60-64]. Roy et al. and Naso
140 et al. reported the synthesis of two different complexes (**36** and **37** respectively) using the same
141 ligand (**Luteolin**) and metal (**Vanadium**) [49, 65]. The two complexes are reported to differ in
142 their stoichiometries and metal coordination sites which may have resulted from different
143 ligand/metal ratios added during the synthesis process. Thus for complex **36**, two luteolin
144 molecules are reported to chelate to the oxidovanadium(VI) group via the 4-C=O and 5-OH
145 functionalities, whilst **37** is reported to contain only one luteolin chelated to the 3' and 4' cis-OH
146 groups in ring B. Among the characterization techniques provided by Roy and his group, mass
147 spectrometry data supported their structural hypothesis for complex **36**. Thus peaks at m/z
148 623.11, 639.89 and 658.21 represented two luteolin + one vanadium, two luteolin + vanadium
149 oxide and two luteolin + vanadium oxide with one molecule of water, respectively. When
150 deducing the structure of complex **37**, Nato et al. demonstrated that the FT-IR spectrum showed
151 no change in the C=O and the C2=C3 bands, in addition to an increase of 44 cm⁻¹ of the 4'-OH
152 band, indicating chelation with ring B. EPR and UV-Vis spectra of complex **37** also supported
153 the same findings. Thermal gravimetric studies provide information on the heat-induced
154 decomposition of the complex as a function of temperature or time. Dehydration happens during
155 the early stages at temperatures ranging from 100-250 °C. This is followed by decomposition of
156 the ligand at higher temperatures (400-600 °C). At temperatures as high as 900 °C, the complex
157 decomposes completely leaving the metal oxide [42, 43, 55, 60, 62, 66].

158 Nevertheless, the best proven technique to confirm a theoretical structure is X-ray
159 crystallography. Unfortunately, it is not feasible to apply this to all synthesized molecules due to
160 the specific crystal requirements needed to run an X-ray. Only structures of complexes **49**, **51**, **53**
161 and **55** [67, 68] were proven by X-ray crystallography.

162
163 In general, all the used characterization methods can provide helpful information of varying
164 importance. NMR and UV-Vis spectroscopy are the most insightful if X-ray is not feasible.

165 Other methods like IR spectroscopy, elemental analysis and mass spectrometry can complement
166 the results obtained and offer the advantages of being easy to interpret and available in most
167 research facilities.

168 **Table 1.** Summary of synthetic conditions and characterization methods for flavonoid metal complexes

<i>Flavonoid Solution</i>	<i>Metal</i>	<i>Base used and pH</i>	<i>Final product Formula</i>	<i>Characterization</i>	<i>Ref</i>
<i>Quercetin.2H₂O in EtOH</i>	NiCl ₂ ·6H ₂ O	NaOEt 6-7	Ni(Que) ₂ (H ₂ O) ₂	UV IR Elemental Analysis	[54]
	CuCl ₂ ·2H ₂ O	NaOEt 6-7	Cu(Que) ₂ (H ₂ O) ₂	UV IR Elemental Analysis	[53]
	La acetate Nd acetate Eu acetate Gd acetate Tb acetate Dy acetate Tm acetate Y acetate	NaOEt <i>N.A.</i>	La(Que) ₃ (H ₂ O) ₆ Nd(Que) ₃ (H ₂ O) ₆ Eu(Que) ₃ (H ₂ O) ₆ Gd(Que) ₃ (H ₂ O) ₆ Tb(Que) ₃ (H ₂ O) ₆ Dy(Que) ₃ (H ₂ O) ₆ Tm(Que) ₃ (H ₂ O) ₆ Y(Que) ₃ (H ₂ O) ₆	UV IR ¹ H NMR TG-DTA Fluorescence analysis Electrochemistry Elemental analysis	[55]

<i>[Cu(Que)₂(H₂O)₂]</i> <i>in MeOH</i>	SnCl ₄	N.A.	[Cu(Que) ₂ (H ₂ O) ₆ -Sn ₂ Cl ₄]	IR ¹ H, ¹³ C & ¹¹⁹ Sn NMR EPR ESI-MS TG-DTG	[43]
	SnCl ₄	N.A.	[Zn(Que) ₂ (H ₂ O) ₆ -Sn ₂ Cl ₄]	IR ¹ H, ¹³ C & ¹¹⁹ Sn NMR EPR ESI-MS TG-DTG	[43]
<i>Chrysin in EtOH</i>	Vanadyl acetylacetonate	N.A. 5	VO(Chry) ₂ EtOH	UV-Vis IR EPR Spectrophotometric titrations	[58]
	Ph ₃ GeBr	Na ₂ CO ₃ N.A.	Chry-Ge. C ₂ H ₆ O	IR ¹ H & ¹³ C NMR Elemental analysis	[69]

	La acetate	NaOH N.A.	La(Chry) ₂ .OAc(H ₂ O) ₇	IR ¹ H NMR Elemental analysis TG-DTG Spectrophotometric titrations	[57]
<i>Luteolin in H₂O</i>	VOSO ₄ .H ₂ O	NaOH 6	VO(Lut) ₂	UV-Vis IR ¹ H NMR ESI-MS	[65]
<i>Luteolin</i>	50% aqueous solution of VOCl ₂	NaOH 5	[VO(Lut)(H ₂ O) ₂]Na·3H ₂ O	UV-Vis IR EPR	[49]
<i>Luteolin in EtOH</i>	Mn(CH ₃ COO) ₂	N.A. 4	MnO-Lut	Elemental analysis UV-Vis IR Elemental analysis TG-DTG	[70]
<i>Hesperidin in H₂O</i>	50% aqueous solution of VOCl ₂	NaOH 12	[VO(Hesp)(OH) ₃]. Na ₄ (H ₂ O) ₃	UV-Vis IR EPR Spectrophotometric	[46]

<i>Hesperetin in EtOH</i>	CuCl ₂ ·2H ₂ O	NH ₃ solution 7-8	[Cu(Hespt) ₂ (H ₂ O) ₂] · H ₂ O	titrations	[66]
				UV-Vis IR ESI-MS TG-DTG	
<i>Naringin in MeOH</i>	Cu acetate in distilled H ₂ O	N.A.	[Cu (Nar)] ⁺ [CH ₃ COO] ⁻ · (H ₂ O) ₅	UV-Vis	[71]
				IR ¹ H NMR ESI-MS Elemental analysis	
<i>Naringenin in EtOH</i>	CuCl ₂ ·2H ₂ O	NH ₃ solution 7-8	[Cu(Narg) ₂ (H ₂ O) ₂] · H ₂ O	UV-Vis	[66]
				IR ESI-MS TG-DTG	
<i>Apigenin in EtOH</i>	CuCl ₂ ·2H ₂ O	NH ₃ solution 7-8	[Cu(Apg) ₂ (H ₂ O) ₂] · H ₂ O	UV-Vis	[66]
				IR ESI-MS TG-DTG	
<i>Silibinin in EtOH</i>	50% aqueous solution of VOCl ₂	NaOCH ₃ 9	Na ₂ [VO(Sil) ₂] · (H ₂ O) ₆	IR	[47]
				EPR Spectrophotometric titrations	

<i>Morin in MeOH</i>	50% aqueous solution of VOCl ₂	NaOCH ₃ 5	[VO(Mor) ₂ H ₂ O] · (H ₂ O) ₅	UV-Vis IR EPR Spectrophotometric titrations	[48]
<i>Kaempferol in EtOH</i>	ZnCl ₂ ·2H ₂ O	NaCl 8-10	[Zn(Kaem) ₂ (H ₂ O) ₂] · H ₂ O	UV-Vis IR ¹ H NMR ESI-MS Elemental analysis	[64]
<i>Nifflcoumar sodium salt in H₂O</i>	Ce(NO ₃) ₃ ·6H ₂ O La(NO ₃) ₃ ·6H ₂ O Nd(NO ₃) ₃ ·6H ₂ O	N.A. 4-5	Ce(NS) ₃ · (H ₂ O) ₄ La(NS) ₃ · (H ₂ O) ₄ Nd(NS) ₃ · (H ₂ O) ₆	IR ¹ H NMR Elemental analysis	[72]
<i>Nifflcoumar in H₂O</i>	Aqueous solution of ZrCl ₄	NaOH 5	Zr(Niff) ₂ (OH) ₄ (H ₂ O) ₅	IR ¹ H NMR Elemental analysis TG-DTG	[73]
<i>Warfarin in H₂O</i>	Aqueous solution of ZrCl ₄	NaOH 5	Zr(War) ₂ (OH) ₄ (H ₂ O) ₂	IR ¹ H NMR Elemental analysis TG-DTG	[73]

<i>Coumachlor in H₂O</i>	Aqueous solution of ZrCl ₄	NaOH 5	Zr(Coum) ₂ (OH) ₄ (H ₂ O) ₆	IR ¹ H NMR Elemental analysis TG-DTG	[73]
<i>1 in 50% EtOH</i>	GeO ₂ in deionized H ₂ O	NaOH 7	<i>N.A.</i>	UV-Vis IR ¹ H NMR MS Elemental analysis TG-DTG	[62]
<i>2-11 in MeOH</i>	[Ru(η ⁶ -p- cymene)Cl ₂] ₂ in CH ₂ Cl ₂	NaOMe <i>N.A.</i>	<i>N.A.</i>	¹ H & ¹³ C NMR Elemental analysis X-ray for (49, 51, 53, 55)	[67, 68]
<i>15-18 in CH₂Cl₂</i>	[Ru(η ⁶ -p- cymene)Cl ₂] ₂ in CH ₂ Cl ₂	<i>N.A.</i>	<i>N.A.</i>	UV-Vis spectra IR ¹ H NMR FAB/EI-MS	[61, 68, 74]
<i>7, 12-14 in EtOH</i>	[Ru(DMSO) ₄ Cl ₂] in EtOH	TEA <i>N.A.</i>	[Ru(DMSO) ₂ (7) ₂] ₂ NaNO ₃ (H ₂ O) ₂ [Ru(DMSO) ₂ (12) ₂] ₂ NaNO ₃ . H ₂ O [Ru(DMSO) ₂ (13) ₂]. (NO ₃) ₂ (H ₂ O) ₂ [Ru(DMSO) ₂ (14) ₂] ₂ NaNO ₃ (H ₂ O) ₅	IR ¹ H NMR ESI-MS Elemental analysis	[63]

<i>18 in EtOH</i>	La(NO ₃) ₃ ·6H ₂ O	TEA N.A.	N.A.	UV-Vis spectra IR ¹ H NMR Elemental analysis TG-DAT	[42]
<i>19 in H₂O</i>	Aqueous solution of Ce La Nd	NaOH 5	Ce(19)(OH)(H ₂ O) ₂ La(19)(OH)·H ₂ O Nd(19)(OH)·H ₂ O	IR ¹ H & ¹³ C NMR Elemental analysis	[75]
<i>20 in EtOH</i>	Aqueous solution of K ₂ PtCl ₄	N.A.	cis - [Pt(20) ₂ Cl ₂]	IR ¹ H & ¹⁹⁵ Pt NMR	[44]

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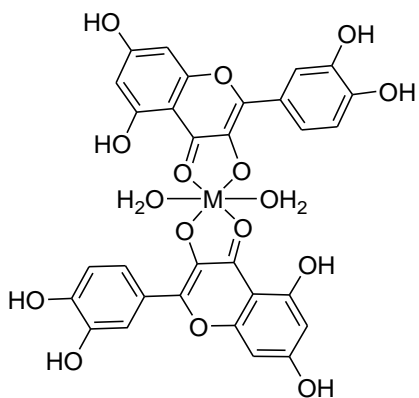
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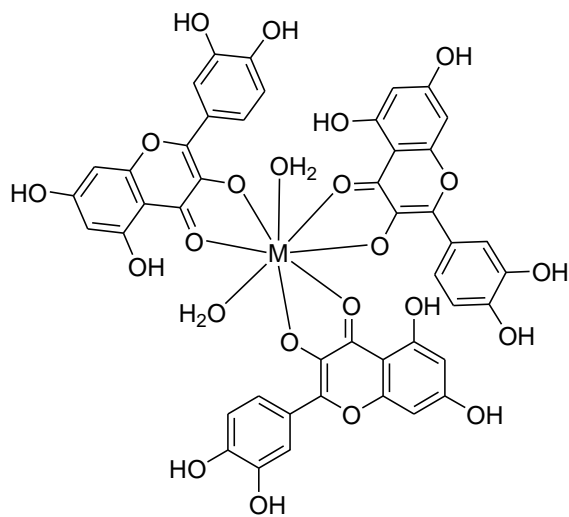
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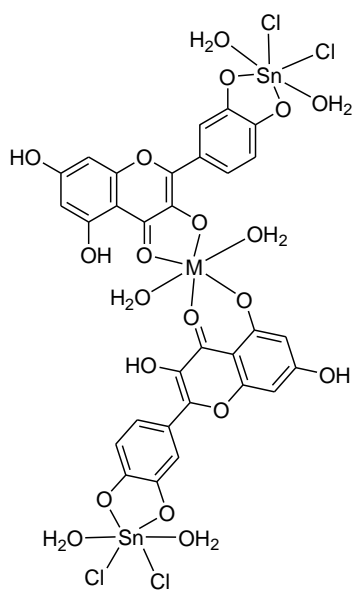
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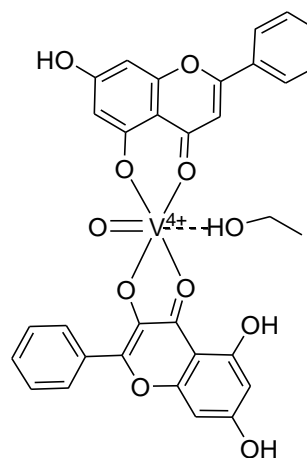
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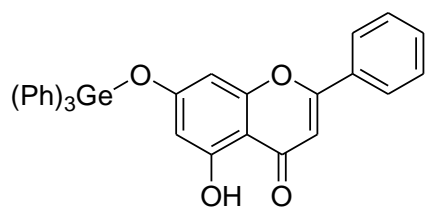
**23, M = La; 24, M = Nd; 25, M = Eu;
26, M = Gd; 27, M = Tb; 28, M = Dy;
29, M = Tm; 30, M = Y**



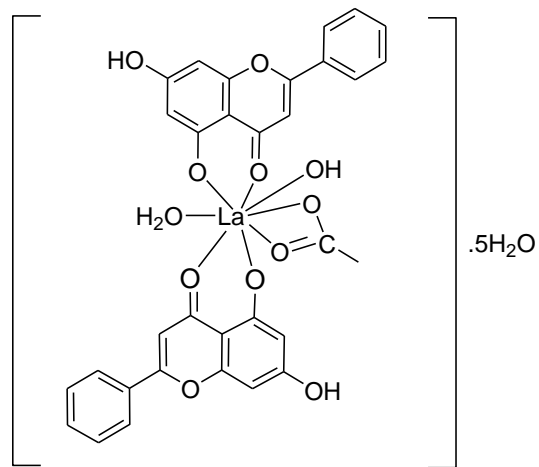
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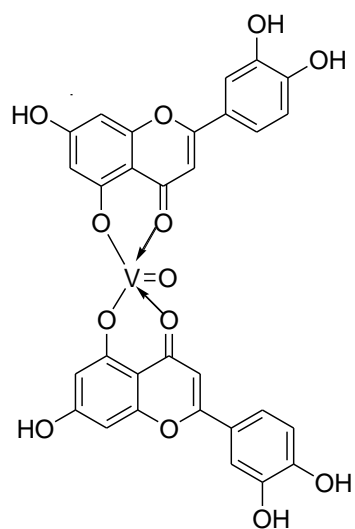
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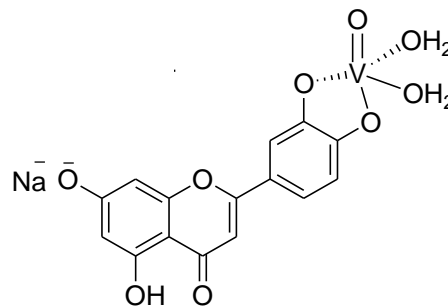
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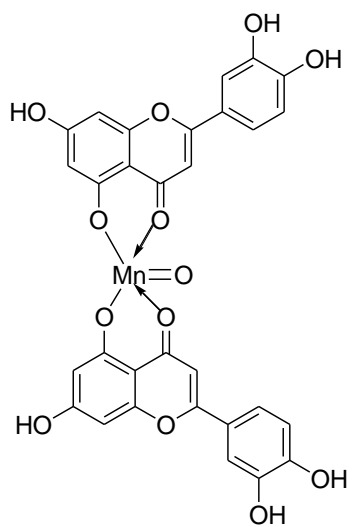
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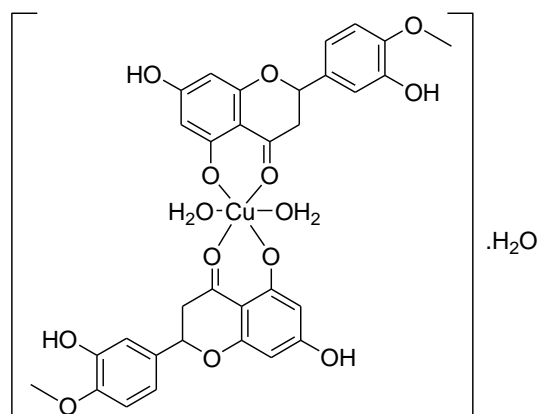
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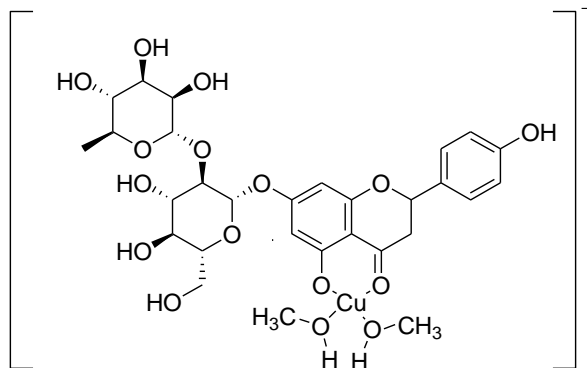
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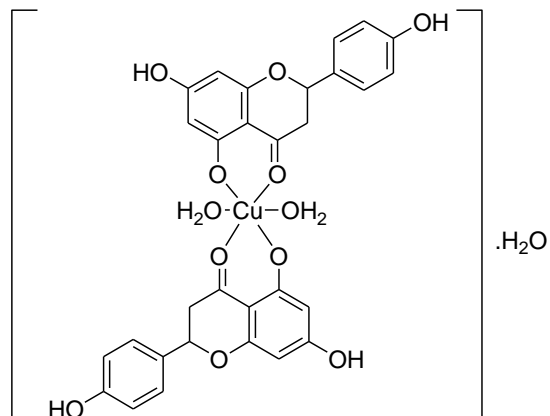
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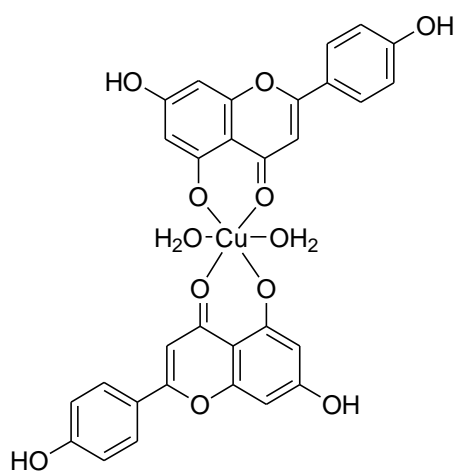
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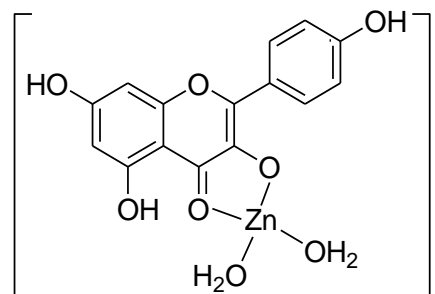
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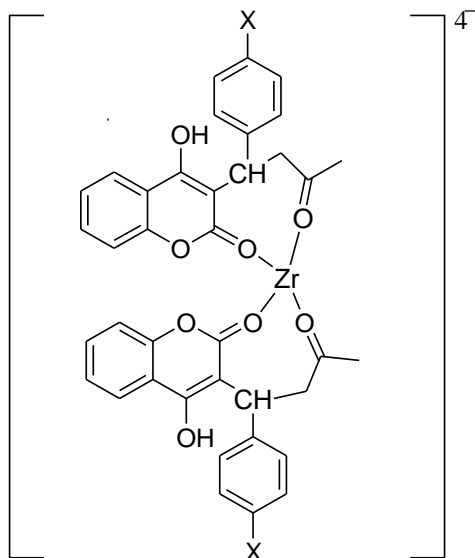
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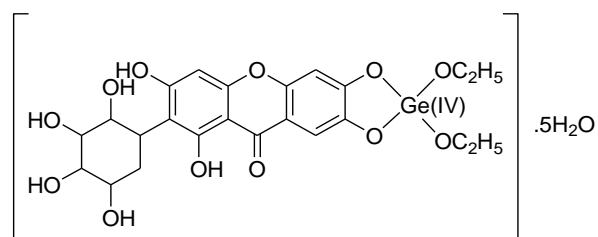
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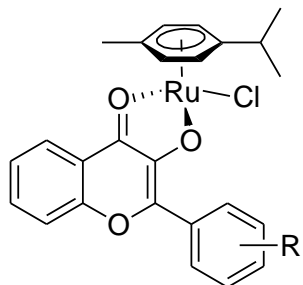
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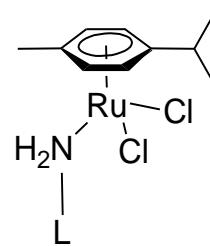
44, X = NO₂; 45, X = H; 46, X = Cl



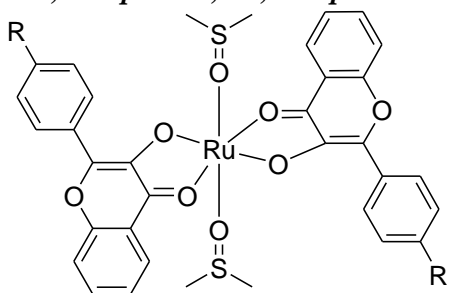
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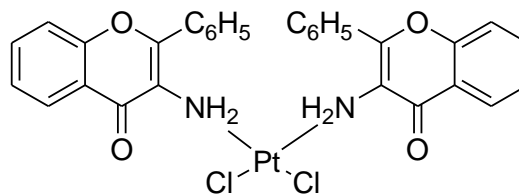
48, R = H; 49, R = *p*-CH₃; 50, R = *p*-F;
 51, R = *m*-F; 52, R = *o*-F; 53, R = *p*-Cl;
 54, R = *m*-Cl; 55, R = *o*-Cl; 56, R = *p*-Br;
 57, R = *m*-Br; 58, R = *p*-OCH₃;
 59, R = *p*-NO₂; 60, R = *p*-NMe₂



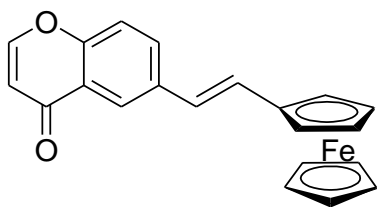
61, L = Cpd 15; 62, L = Cpd 16;
 63, L = Cpd 17



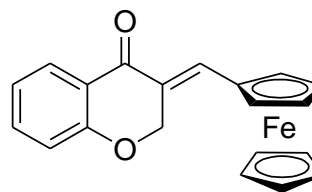
64, R = Cl; 65, R = OCH₃; 66, R = NO₂;
 67, R = NMe₂



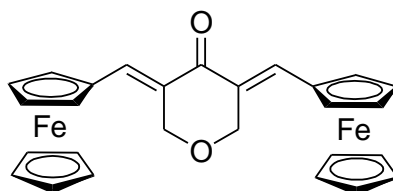
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Figure 6. Chemical structures of flavonoid metal complexes

177 **Anti-oxidant activities of flavonoid metal complexes**

178 Complexation of flavonoids with metals often increases the anti-oxidant activity of the parent
 179 flavonoids as shown in table 2. Metal complexes demonstrate enhanced activities in free radical
 180 scavenging assays such as DPPH· (1,1-diphenyl-2-picrylhydrazyl radical), ABTS⁺ [2,20-azino-
 181 bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt)], H₂O₂, OH· and O₂^{·-}. Superoxide
 182 dismutase (SOD) like activity is often used to measure the anti-oxidant activities of the
 183 complexes as it is considered to be one of the body's first line free radical defense enzymes.

184 However, it is important to note that even where the complexes showed better SOD like activity
 185 than their free parents they are still far above the limit for good SOD like activity which is IC₅₀ ≤
 186 20 μM.

187 **Table 2.** Summary of anti-oxidant activities of reviewed flavonoids and their metal complexes

Complex	Assay	Results	Ref
		Enhanced activity at 120 μM,	
23		90%	
24		85%	
25	O ₂ ^{·-}	84%	
26		97%	[55]
27		77%	
28		92%	
29		87%	
30		88%	
		<i>quercetin = 42%</i>	
	DPPH·	Enhanced activity at 100 μM, 45%	
33		<i>chrysin = 18%</i>	[58, 76]
	ABTS ⁺	Enhanced activity 3.96 mM	
		<i>chrysin = 0.9 mM</i>	

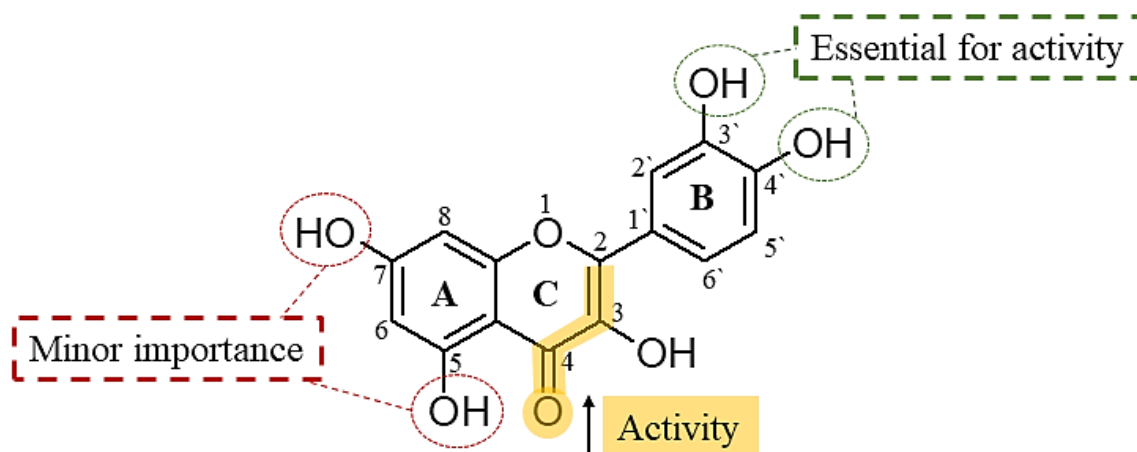
	SOD	IC ₅₀ = 157 μ M <i>chrysin = no activity</i>	
	OH \cdot	Enhanced activity at 100 μ M, 75% <i>chrysin = 45%</i>	
36	DPPH \cdot	Enhanced activity at 100 μ M, 40% <i>luteolin = 25%</i>	[65]
	ABTS ⁺	Enhanced activity at 10 μ M, 64% <i>luteolin = 40%</i>	
37	DPPH \cdot	Lower activity IC ₅₀ = >100 μ M <i>luteolin = 66.7 μM</i>	[49]
	OH \cdot	Enhanced activity IC ₅₀ = 17 μ M <i>luteolin = 50.7 μM</i>	
	O ₂ \cdot^-	Lower activity IC ₅₀ = 417 μ M <i>luteolin = 384 μM</i>	
38	DPPH \cdot	Enhanced activity at 1 mM, 90% <i>luteolin = 75%</i>	[70]
	OH \cdot	Enhanced activity at 1 mM, 80% <i>luteolin = 74%</i>	
VOhesperidin	DPPH \cdot ABTS ⁺	No enhancement in activity	[46]
40	DPPH \cdot	Enhanced activity at 10 μ M, 35.5% <i>naringin = 2%</i>	[71]

VMorin	SOD	Slightly enhanced activity IC ₅₀ = 45 μM <i>morin</i> = 66 μM	[48]
	DPPH·	Similar activity at 10 μM, 22% <i>morin</i> = 15%	
	ABTS ⁺	Similar activity at 10 μM, 81% <i>morin</i> = 76%	
	OH·	Enhanced activity at 10 μM, 26% <i>morin</i> = 2%	
	ROO·	No effect on radical	
47	OH·	Enhanced activity at 100 μM, 65% <i>2</i> = 43%	[62]
	DPPH·	Lower activity at 80 μM, 60% <i>2</i> = 95%	

188

189 As shown in table 2, rare earth metal complexes (**23-30**) increased O₂⁻ scavenging activity of
190 quercetin by, on average, 2-fold [55]. The difference in activity between the eight metal
191 complexes is not significant, however, the gadolinium complex (**26**) showed the best activity.
192 Whether this increase in activity is attributed to incorporation of the metal atom or to the higher
193 molar ratio of quercetin present in the complex (as opposed to 1 quercetin molecule) needs
194 further investigation. **Figure 7** illustrates the main structural features responsible for the anti-
195 oxidant activity of flavonoids. The 3', 4' *ortho*-dihydroxyl group is the most significant
196 contributor to flavonoids' anti-oxidant activity [3]. These two catechol moieties form *ortho*-
197 semiquinone radicals that are highly stabilized by the electron delocalization and intra-molecular
198 hydrogen bonding. The combination of C2=C3 and 4-C=O group in ring C also assists in the

199 delocalization of the π -electrons in ring B. This in turn influences the dissociation of phenolic
 200 hydroxyl groups as well as the stability of the formed phenoxy radicals in ring B [3]. Ring A
 201 *meta*-hydroxyl groups are less important than ring B dihydroxyl groups which are oxidized more
 202 readily [77]. The highest increase in activity can be seen upon complexation of metals with
 203 flavonoids lacking the essential structural features of anti-oxidant activity (**Figure 7**), such as
 204 chrysin and naringin [78]. For instance, complexation of vanadium metal with chrysin (**33**)
 205 increased ABTS⁺ scavenging activity from 0.9 mM for chrysin to 3.96 mM [58]. This value is
 206 close to that of quercetin (4.7 mM) [79] that fulfills anti-oxidant structural activity requirements
 207 (**Figure 7**) and is therefore one of the best flavonoid anti-oxidants.

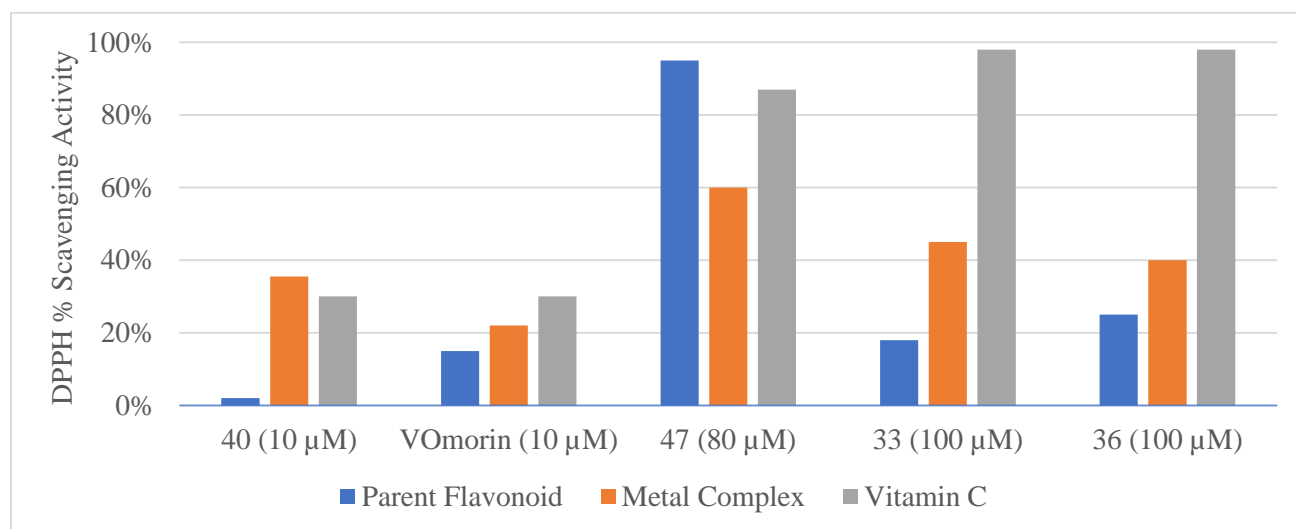


208

209 **Figure 7.** Summary of structural features responsible for anti-oxidant activity of flavonoids

210 As illustrated in **Figure 8**, metal complexation demonstrated a positive impact on the DPPH[•]
 211 scavenging activity of ligands which was higher than that of vitamin C in only one case, the Cu-
 212 Naringin complex **40**. Despite the witnessed increase in DPPH[•] scavenging activity of chrysin
 213 (18%) upon complexation with vanadium (**33**) (45%) at 100 μ M, it is still far less than that of
 214 ascorbic acid (98%) at the same concentration [58]. However, metal complexation did not
 215 always result in enhanced DPPH[•] scavenging activity. The vanadium-luteolin complex **37**
 216 showed a loss in the DPPH[•] scavenging activity of luteolin due to chelation of the vanadium
 217 metal with the 3' and 4'-OH groups that are essential for anti-oxidant activity of flavonoids [49].
 218 Pi et al. attributed the loss of the polyphenol ligand's (2) high DPPH[•] scavenging activity (95%)

219 after coordination with germanium (**47**) to steric hindrance factors [62]. This might be caused by
220 the presence of the two ethyl ether groups rather than the germanium atom.



221
222 **Figure 8.** DPPH scavenging activity of metal complexes compared to their parent flavonoids
223 and vitamin C at different concentrations

224 Cytotoxic activities of flavonoid metal complexes

225 One of the milestones in the history of chemotherapy was the discovery of cisplatin in 1969 [80].
226 Cisplatin is effective and still widely used against various types of cancers, such as testicular,
227 ovarian, breast, bladder, lung cancer and brain tumors. It demonstrates remarkable curing rates
228 for testicular cancer (over 90% and near 100% with early discovery) [81]. Inside the body,
229 cisplatin crosslinks DNA and initiates apoptosis. Despite this promising profile, cisplatin suffers
230 from limitations such as nephrotoxicity, neurotoxicity and drug resistance [82, 83]. This ongoing
231 interest in the design and development of metal-based anti-cancer drugs has also influenced the
232 flavonoid field, where a number of flavonoid-metal complexes have been synthesized and
233 evaluated as anti-cancer agents.

234

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238 **Cytotoxic activity of quercetin metal complexes**

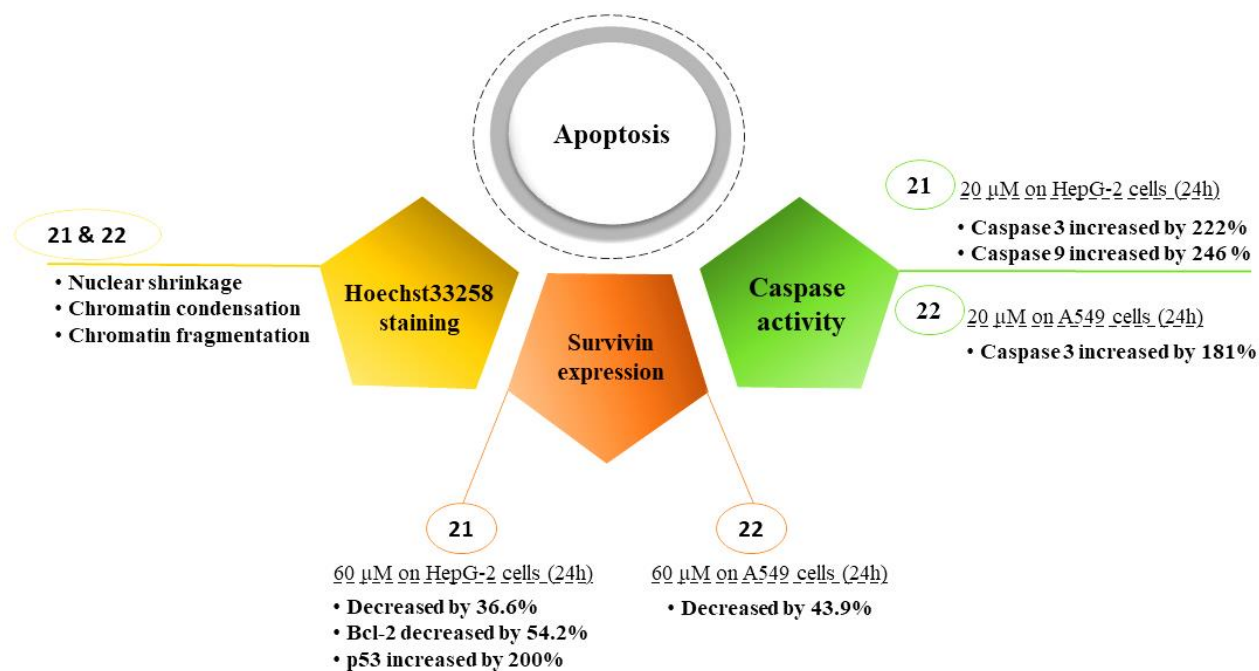
239 **Table 3.** Summary of *in vitro* anti-tumor activity of quercetin metal complexes

Complex	Cell line	Activity (μM) (IC_{50} or suppression % or GI_{50})	Quercetin activity (μM)	Ref.
21	HepG2	14.4 ± 0.9 *	33.7 ± 1.0 *	[84]
	SMMC7721	19.6 ± 0.6 *	42.1 ± 1.0 *	
	A549	35.6 ± 0.8 *	72.1 ± 1.2 *	
22	A549	21.5 ± 0.5 *	34.9 ± 1.0 *	[53]
23	Bel-7402	46.03% ♦	20% ♦	[55]
25	Bel-7402	62.32% ♦	20% ♦	
26	Bel-7402	45.30% ♦	20% ♦	
31	U373MG, PC3, Hop62, HL60, HCT15 and HeLa	< 8.7 •	N.A.	[43]
32	HeLa	7.7 •	N.A.	

240 * = IC_{50} , ♦ = Suppression % at 10 μM , • = GI_{50}

241 Hepatocellular carcinoma (HepG2), hepatoma (SMMC7721), lung carcinoma (A549), liver cancer (Bel-
242 7402), central nervous system (U373MG), prostate cancer (PC3), lung cancer (Hop62), leukemia
243 (HL60), colon carcinoma (HCT15) and cervical cancer (HeLa).

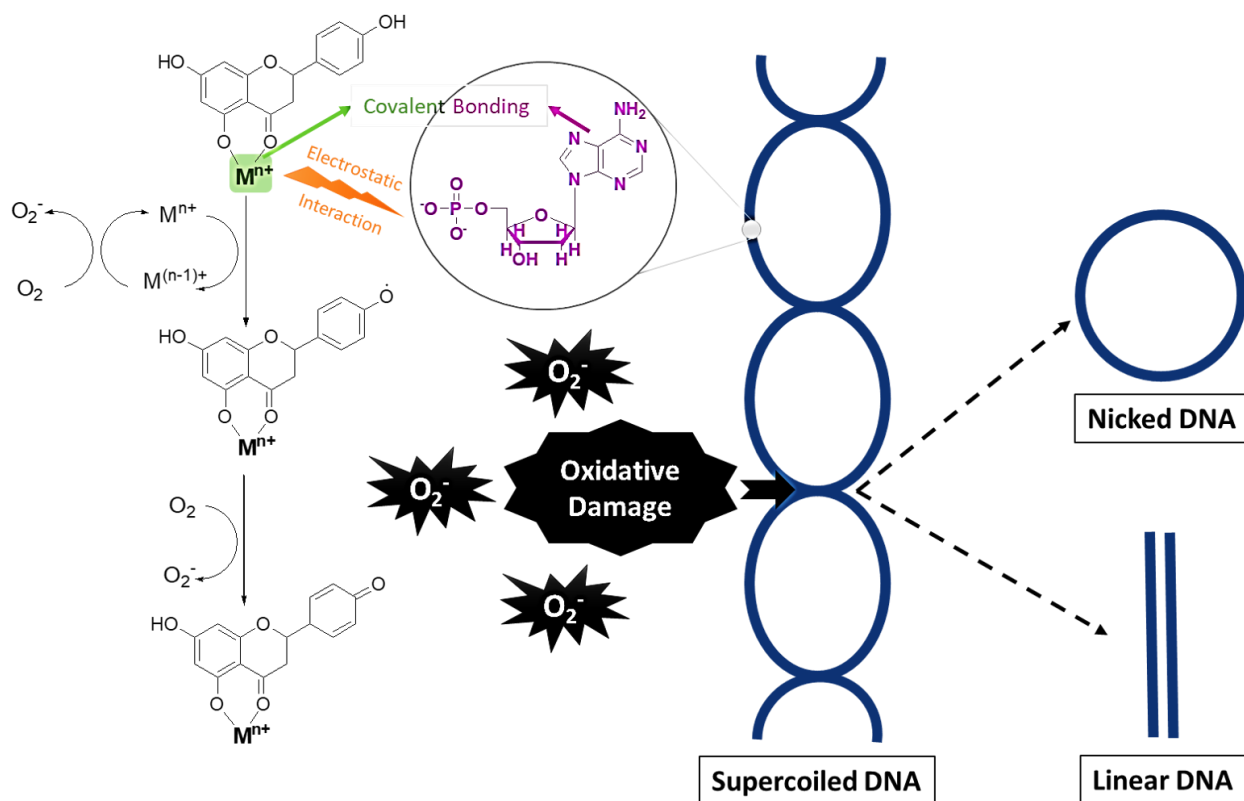
244
245 It is evident that complexation with metals enhances the anti-proliferative activity of quercetin.
246 Compound **21** (Ni-quercetin) had better activity on the hepatic cancer cell lines (HepG2 and
247 SMMC7721) than on the lung carcinoma cell line (A549). Pro-apoptotic ability has been
248 investigated for complexes **21** and **22** (Cu-quercetin) as a possible cytotoxic mechanism (**Figure**
249 **9**) [53, 84]. This is indicated by changes in the levels of key apoptotic proteins like survivin and
250 caspases along with morphological changes observed using the DNA-sensitive Hoechst33258
251 stain. Possible mechanisms of action of **21** on the A549 cell line were not evaluated. This might
252 have given a better idea of the behavior of complexes in different cell lines and the reasons
253 behind the witnessed difference in activities.



254

255 **Figure 9.** Summary of pro-apoptotic studies done on complexes 21 and 22. Survivin expression
 256 was measured by immunocytochemistry and caspase activity by absorption spectra.
 257

258 Metal atoms in flavonoid complexes can interact with DNA nucleotides by either electrostatic
 259 interactions with the phosphate groups, or covalent binding to nucleobases (**Figure 10**). The
 260 ROS and free radicals resulting from flavonoids' auto-oxidation are thus generated in proximity
 261 to the DNA [37]. This leads to oxidative damage of DNA and induces apoptosis (**Figure 10**) [85,
 262 86]. Flavonoid metal complexes are also more planar than the free ligands which facilitates their
 263 binding to DNA via intercalation [37]. Tan et al. reported the selective binding of the nickel
 264 quercetin complex (**21**) to GC-rich DNA sequences using fluorescence emission and molecular
 265 modelling. This, in addition to decreased survivin activity, led them to conclude that down
 266 regulation of survivin via interference with the GC-rich survivin protein promoter gene might be
 267 a plausible mechanism by which the complex **21** exerts its anti-tumor activity [84].



268

269 **Figure 10.** Plausible mechanism of oxidative DNA damage by flavonoid metal complexes

270 The quercetin rare earth metal complexes (**23**, **25** and **26**) showed increased activities compared
 271 with free quercetin on the liver cancer cell line (Bel-7402) (**Table 3**) [55]. Complex **23** (La-
 272 quercetin) can intercalate with DNA as evidenced by DNA fluorescence studies. Tabassum and
 273 her colleagues were able to synthesize two unique hetero-bimetallic complexes quercetin- Cu^{II} -
 274 Sn_2^{IV} (**31**) and quercetin- Zn^{II} - Sn_2^{IV} (**32**) [43]. The Cu complex (**31**) showed significant activity
 275 on various human cancer cell lines (**Table 3**) except for ovarian cancer (A2780). The Zn
 276 complex (**32**), however, did not demonstrate good activity except on the HeLa cell line (**Table**
 277 **3**). Complexes **22**, **31** and **32** were able to promote the conversion of substrate DNA to cleaved
 278 forms (nicked and linear) as studied by gel electrophoresis [43, 53, 54]. To better understand
 279 whether this DNA cleavage is promoted through an oxidative pathway, the effect of different
 280 radical scavengers was evaluated where only H_2O_2 markedly promoted plasmid DNA breakage
 281 caused by the complexes. Increased level of thiobarbituric acid reactive species (TBARS) with
 282 increasing concentrations of complex **22** backs up the role of oxidative damage as a mechanism
 283 of CT-DNA cleavage [53, 54]. At 30 μM of complex **31**, DNA relaxation activity of

284 Topoisomerase-I was significantly inhibited. This does not necessarily correlate with direct
 285 inhibition of the enzyme and may instead result from strong binding with DNA which prevents
 286 the enzyme from exercising its function [43]. To exploit binding of complexes **31** and **32** with
 287 the minor groove of DNA, the complexes were docked into the DNA duplex of sequence
 288 d(CGCGAATTCGCG)₂ dodecamer (PDB ID: 1BNA). Both complexes bound to the narrow
 289 minor groove region of DNA within the GC rich region and were stabilized by hydrogen
 290 bonding (2.8–3.0 Å) between the –OH groups of quercetin with O4/N2 atoms of C8/G8 bases in
 291 DNA.

292 Complex **31** also showed good binding modes with the human-DNA–Topo-I complex (PDB ID:
 293 1SC7) and bovine erythrocyte Cu, Zn superoxide dismutase (PDB ID: 2SOD) [43].

294 Cytotoxic activity of chrysin, luteolin and silibinin metal complexes

295 **Table 4.** Summary of *in vitro* anti-tumor activity of metal complexes of chrysin, luteolin and
 296 silibinin

Complex	Cell line	Activity (µM) (IC ₅₀ or suppression %)	Flavonoid activity (µM)	Ref.
33	MG-63	16 *	>100 *	[76]
	HT-29	45% ◊	No effect ◊	[87]
VOsilibinin	HT-29	45% ◊	No effect ◊	
34	HepG2	30% ♦	20% ♦	[69]
	Colo205	25% ♦	10% ♦	
	MCF-7	45% ♦	20% ♦	
	MCF-7 10A	15% ♦	10% ♦	
35	A549	73.5 % ♦	4.9 % ♦	[57]
37	A549	60.5 *	66.3 *	[49]
	MDAMB-231	17 *	88.3 *	

297 * = IC₅₀, ♦ = Suppression % at 10 µM, ◊ = Suppression % at 100 µM
 298 Osteosarcoma (MG-63), colon adenocarcinoma (HT-29), hepatocellular carcinoma (HepG2), caucasian
 299 colon adenocarcinoma (Colo205), breast cancer (MCF-7), breast cell line (MCF-7 10A), lung carcinoma
 300 (A549) and triple negative breast cancer (MDAMB-231).
 301

302 The vanadium chrysin complex (**33**) showed significant cytotoxic activity on a human
303 osteosarcoma cell line (MG-63) with an IC_{50} value of 16 μ M (**Table 4**) [76]. This activity is
304 higher than the reported activity of cisplatin on the same cell line ($IC_{50} = 28.5 \mu$ M), yet lower
305 than that of doxorubicin ($IC_{50} = 3.35 \mu$ M) [88]. According to Leon et al. complex **33** showed a
306 multi-factorial anti-tumor mechanism of action. For example, it demonstrated cytotoxicity to
307 lysosomes and mitochondria of MG-63 cell line (at concentrations from 2.5 to 25 μ M) as
308 evidenced using neutral red (NR) uptake and the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-
309 diphenyltetrazolium bromide (MTT) assays, respectively. Genotoxicity was also evident through
310 induced micronuclei formation and DNA breaks using the Comet assay, in the concentration
311 range 2.5-5 μ M. In addition to that, compound **33** lowered GSH/GSSG ratio in MG-63 cell line
312 especially at higher doses (40%, 50 and 100 μ M). In a protein array platform test, **33** upregulated
313 82 proteins and downregulated 9 such as PKB/AKT, Cdk 4, 6 and 7, NAK, and JNK [89]. It
314 induced apoptosis via:

- 315 • Externalization of the phosphatidyl serine (PS), at the outer plasma membrane leaflet
316 measured by Annexin V-FITC fluorescent probe.
317
- 318 • Alteration of the mitochondria membrane potential (MMP) measured by Rhodamine 123 that
319 leads to the release of cytochrome c and finally to DNA fragmentation.
320
- 321 • Activation of caspase pathway measured by caspase 3 activation (350% basal at 100 μ M).
322

323 In another report the anti-tumor activities of vanadium complexes of chrysin (**33**) and silibinin
324 (**VOsil**) were tested on the HT-29 cell line (**Table 4**) [87]. **33** and **VOsil** showed better anti-
325 proliferative activities than cisplatin at 100 μ M (inhibition rate = 12 %) on the same cell line.
326 However, **33** did not show pro-apoptotic effects in the MG-63 cell line despite it causing cell
327 cycle arrest in the G2/M phase after 24 and 48 hours incubation. **VOsil**, on the other hand,
328 increased the percentages of apoptotic/necrotic cells, induced caspase 3 activation and reduced
329 nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) that controls DNA
330 transcription and is aberrantly activated in many cancers. Moreover, **VOsil** fully inhibited
331 topoisomerase-IB using plasmid relaxation assay at 100 μ M. The germanium complex of
332 chrysin, (**34**) also had activity on several targets. Its cytotoxic effect on HepG2, Colo250 and
333 MCF-7 cell lines was not significantly high nor much better than chrysin [69, 90]. The mean
334 apoptotic population detected on MCF-7 and Colo205 cell lines by Annexin-V were 19.5% and

335 12.5% for 20 µg/ml, respectively. Additionally, cellular ROS production increased in MCF-7
 336 cell line upon addition of high doses of complex **34**. This could be a possible etiology for
 337 apoptosis alongside with caspase-3, 8 and 9 activation whose levels increased from 100% to
 338 214.5%, 181.1% and 286.0%, respectively at 20 µg/ml. Some caspase activation was also
 339 observed on the Colo205 cell line with an increase from 100% to 129.3%, 146.5 and 119.3%, for
 340 caspase-3, 8 and 9, respectively. Cell cycle arrest at the G2/M phase was detected on MCF-7 and
 341 Colo205 (high doses) cell lines and at the S phase with low doses of the complex on the Colo205
 342 cell line. Complex **35** (La-chrysin) showed a significant increase (73.5%) in the anti-proliferative
 343 activity of chrysin (4.9%) on A549 cell lines. The complex intercalated with DNA base pairs as
 344 illustrated by an increase in viscosity and fluorescence of DNA upon addition of complex **35**
 345 [57]. The vanadium luteolin complex (**37**) significantly improved the cytotoxic activity of its
 346 parent on the MDAMB-231 cell line (IC₅₀ = 17 µM) as shown in table 4 [49]. However, it
 347 showed a trivial improvement on the A549 cell line (IC₅₀ = 60.5 µM, luteolin IC₅₀ = 66.3 µM).
 348 Naso et al. carried out high content cytotoxicity assays on complex **37** to investigate its
 349 mechanism of action on the MDAMB-231 cell line. The tests included ROS production, MMP,
 350 plasmatic membrane damage, nuclear membrane damage and mitotic arrest. Complex **37** showed
 351 significant increase in the levels of ROS production (650%) and percentage of depolarized cells
 352 (20%). Lactate dehydrogenase (LDH) levels in the culture media were measured as an indication
 353 for plasmatic membrane damage. Complex **37** demonstrated 212% increase compared with the
 354 basal levels as opposed to 98% for luteolin. The vanadium complex (**37**) also showed DNA
 355 damage and mitotic arrest activities using H2AX and PHH3 assays, respectively.

356 **Cytotoxic activity of hesperidin, naringin, hesperitin, naringenin, apigenin, morin,**
 357 **kaempferol, warfarin and mendiaxon metal complexes**
 358

359 **Table 5.** Summary of *in vitro* anti-tumor activity of hesperidin, naringin, hesperitin, naringenin,
 360 apigenin, morin, kaempferol, warfarin and mendiaxon metal complexes.

Complex	Cell line	Activity (suppression %)	Flavonoid Activity	Ref.
VOhesperidin	Caco-2	60% (at 100 µM)	No effect	[46]
40	K562	38.4% (at 100 µM)	13.2% (at 100 µM)	[71]
La-18	HL60	50% (at 0.01 µM)	N.A.	[42]
	A549	100% (at 0.003 µM)	N.A.	

39	HeLa	22.5%	15%	
	SGC-7901	45%	30%	
	HepG2	45%	30%	
41	HeLa	24%	27%	[66]
	SGC-7901	25%	33%	
	HepG2	35%	15%	
42	HeLa	25%	10%	
	SGC-7901	45%	5%	
	HepG2	35%	5%	
VOmorin	T47D	43% (at 10 μ M)	No effect	[48]
	SKBR3	38% (at 10 μ M)	14% (at 10 μ M)	
43	EC9706	72% (at 30 μ g/ml)	45 % (at 30 μ g/ml)	[64]
45	HL60	30% (at 100 μ M)	<i>N.A.</i>	[73]

361 Colon adenocarcinoma (Caco-2), chronic myeloid leukemia (K562), leukemia (HL60), lung carcinoma
362 (A549), cervical carcinoma (HeLa), gastric carcinomas (SGC-7901) and hepatocellular carcinoma
363 (HepG2), breast cancer (T47D), breast cancer overexpressing Herceptin-2 (SKBR3) and oesophageal
364 cancer cell line (EC9706).

365
366 As shown in table 5, **VOhesperidin** showed 60% inhibition in cellular proliferation of the colon
367 adenocarcinoma cell line (Caco-2) while its parent hesperidin showed no effect at all on the same
368 cell line at 100 μ M [46]. The Cu naringin complex (**40**) exhibited better inhibition of the K562
369 cell lines (**Table 5**). Cell cycle analysis showed a 50% decrease in viable cells at the S/G2/M
370 phases after 24 h. The number of hypodiploid cells tremendously increased from 5.5% (control)
371 to 38.4% indicating the complex led to cell death in the first 24 h [71]. The lanthanum complex
372 with ligand **18** exhibited a stronger suppression rate (100% inhibition at 0.003 μ M) than cisplatin
373 (100 % inhibition at 0.004 μ M) on the A549 cell line [42]. Lanthanum complexes like **35** and
374 **La-18** have significantly high suppression rates on A549 cell line.

375 As shown in table 5, complexation of copper with the flavonoids hesperetin (**39**) and apigenin
376 (**42**) did enhance the anti-proliferative activity of their free flavonoids whilst the naringenin
377 complex (**41**) did not show any improvement except on the HepG2 cell line [66]. **41** and **42** had
378 the same suppression rate (35%) on the HepG2 cell line. This indicates that unsaturation of ring
379 C has no effect on the activity whilst the addition of an OH group on ring B, as in complex **39**,
380 results in 30% increase in the activity. This may be due to the enhanced anti-oxidant activity of

381 compounds featuring two *m*-hydroxyl groups on ring B (**Figure 7**). Several DNA binding studies
 382 (UV-Vis spectral, fluorescence and CD measurements) were performed with the complex **39** that
 383 indicated DNA binding via intercalation with higher affinity than the free ligand.

384 The vanadium morin complex (**VOmor**) showed promising cytotoxic activity on human breast
 385 cancer cell lines (**Table 5**). It did not have deleterious effects on the non-tumorigenic breast
 386 epithelial mammal cells indicating its selectivity towards cancer cells [48]. Naso et al. suggest
 387 that the **VOmor** complex causes cell death by induction of apoptosis. This can be correlated to
 388 perturbation of the mitochondrial membrane potential which results in release of cytochrome c
 389 from the mitochondria to cytosol. This process leads to the activation of caspases-9, 3 and 7.
 390 However, mitochondrial membrane potential was only observed in the SKBR3 cell line which
 391 suggests the apoptotic potential on the T47D is achieved via a different mechanism that needs
 392 further investigation. The MTT assay was used to determine the anti-proliferative activity of the
 393 Kaempferol Zn complex (**43**) on EC9706 cells (**Table 5**) [64]. Cell viability decreased nearly by
 394 half in the EC9706 cell line while no significant effect was observed on the normal kidney cells
 395 (HK-2 cells). Atomic force microscopy (AFM) morphological data showed that complex **43**
 396 could deform and shrink EC9706 cells at the nanoscale. To measure apoptosis induction ability,
 397 Annexin V-FITC/PI was used. The apoptosis ratio of EC9706 cells increased from $3.4 \pm 0.9\%$
 398 for control cells to $33 \pm 7.6\%$ upon raise of **43** concentrations from 0 to 30 $\mu\text{g/mL}$, respectively.
 399 Complex **43** was able to increase the intracellular calcium ion level in cancer cells that can
 400 mediate cell death.

401 Cytotoxic activity of metal complexes 47-71

402 **Table 6.** Summary of *in vitro* anti-tumor activity of complexes 47-71

Complex	Cell line	Activity (μM) (IC ₅₀ or suppression%)	Flavonoid activity (μM)	Ref.
47	HepG2	65% \blacklozenge	35% \blacklozenge	
51		1.5 *	N.A.	
53	CH1	0.86 *	0.6 *	[67, 68]
54		1 *	N.A.	
55		1.2 *	N.A.	
61	DMBC12	0.96 *	N.A.	[61]

62		1.13 *	<i>N.A.</i>	
63		2.53 *	<i>N.A.</i>	
	DMBC11	4.14 *	<i>N.A.</i>	
64		16 *	17.2 *	
65	MCF-7	28 *	29.5 *	[63]
66		32.1 *	35.4 *	
67		36.2 *	38.4 *	
Ce-19	HL60	21.37 *	<i>N.A.</i>	[75]
69	CCRF-CEM	37.5 *	<i>N.A.</i>	[41]
70	Jurkat	2.97 *	<i>N.A.</i>	
		7.23 *	<i>N.A.</i>	[91]
71	HeLa	7.4 *	<i>N.A.</i>	

403 * = IC₅₀, ♦ = Suppression % at 160 μM

404 Hepatocellular carcinoma (HepG2), ovarian cancer (CH1), patient derived (not-established) melanoma
 405 (DMBC11 and DMBC12), breast cancer (MCF-7), lung carcinoma (A549), cervical carcinoma (HeLa),
 406 chronic myelogenous leukemia (K562), leukemia (HL60), T lymphoblast-like polymorph cancerous cell
 407 line (CCRF-CEM) and acute T-lymphoblastic leukemia (Jurkat)

408
 409 Pi et al. reported the DNA binding of the germanium complex of compound **1** (**47**) [62].
 410 Compound **47** showed an increase in fluorescence emission by 46% (at 550 nm) while that of the
 411 free parent **1** showed insignificant increase by 8% (at 536 nm). Intercalation with DNA was also
 412 confirmed by a UV absorption method through the hypochromism of complex **47** upon addition
 413 of CT-DNA. The anti-proliferative evaluation of **47** on the HepG2 cell line (**Table 6**) was
 414 followed by AFM that showed deformation of HepG2 cells and increase in the size of cell
 415 membrane particles. The effect on cell cycle, measured by flow cytometry, demonstrated that
 416 complex **47** causes cell cycle arrest at the G₀/G₁ phase.

417 The ruthenium complexes **48-57** were tested on a number of human cancer cell lines; CH1
 418 (ovarian carcinoma), SW480 (colon carcinoma), and A549 (non-small cell lung carcinoma),
 419 human urinary bladder (5637), human large cell lung (LCLC-103H), and human pancreatic
 420 carcinoma cell lines (DAN-G) [67, 68]. The most significant anti-proliferative activities were
 421 observed on the CH1 cell line with IC₅₀ values ranging from 0.89 to 7.9 μM (**Table 6**) [67]. The
 422 SW480 cell line also showed high sensitivity to these complexes (IC₅₀ from 3.4 - 26 μM) while
 423 A549 was the least affected with moderate activities (IC₅₀ from 8.6 - 30 μM). Kurzwehnart et al.

424 attempted to determine the mode of action of complexes **48-57** via CDK2 and topoisomerase II α
425 inhibition assays as well as flow cytometry cell cycle analysis. Despite showing inhibition of
426 CDK2 comparable with the standard reference roscovitine, CDK2 inhibition was excluded by the
427 authors as a mechanism of action. This is due to miscorrelation with the *in vitro* anti-tumor assay
428 activity pattern and poor influence on the G1/S transition of the cell cycle in which CDK2 is
429 involved. On the other hand, complexes **48-57** showed good inhibition of topoisomerase II α
430 catalytic activity at $\geq 10 \mu\text{M}$, which correlated well with the cytotoxic activity (compounds with
431 lowest IC₅₀ in MTT assay showed the highest topoisomerase II α inhibition).

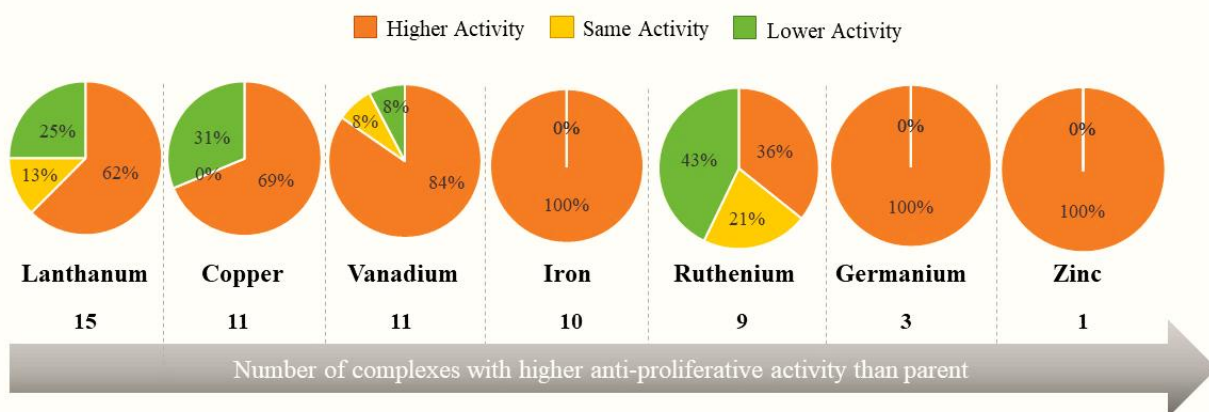
432 Compounds **61-63** showed promising IC₅₀ values on the patient derived (not-established)
433 melanoma cell lines (DMBC11 and DMBC12) as shown in table 6 [61]. Only complex **63**
434 exhibited potency on both DMABC11 and DMBC12 cell lines (IC₅₀ = 2.53 and 4.14 μM ,
435 respectively). Singh et al. developed four flavonoid ruthenium DMSO complexes (**64-67**) and
436 tested their anti-proliferative activities on the MCF-7 cell line (**Table 6**) in addition to their
437 action on different phases of the cell cycle [63]. Both the free ligands, and their ruthenium
438 complexes, showed similar activities with complex **64** being the most potent with IC₅₀ = 16 μM .
439 Analysis of the cell cycle showed that compound **64** dramatically increased the G1 phase and
440 decreased the S phase which might be explained by G1 arrest via inhibition of DNA synthesis in
441 the S phase.

442 Kostova et al. tested the cytotoxicity of three metal complexes (Cerium, lanthanum and
443 neodymium) with a bis-coumarin (**19**) [75]. Only the cerium complex demonstrated promising
444 anti-proliferative effect on the HL-60 cell line (**Table 6**).

445 Among four ferrocenylvinyl-flavone derivatives synthesized by Kowalski et al., compound **69**
446 was the most potent (**Table 6**) [41]. The mechanism of action was induction of apoptosis and
447 necrosis as shown by double staining of cells with Hoechst 33258 and propidium iodide.
448 Although anti-tumor activity was evaluated on four human cancer cell lines (HepG2, MCF-7,
449 MDA-MB-231 and CCRF-CEM), CCRF-CEM was the only affected cell line.

450 Janka and his colleagues also developed a number of ferrocenyl chalcones of which compound
451 **70** showed high activity on Jurkat cell line with IC₅₀ = 2.97 μM (**Table 6**) [91]. Complex **71** has
452 an additional ferrocenyl-vinyl group in its structure, however, it didn't show increase in the

453 activity on the HeLa cell line ($IC_{50} = 7.4 \mu M$) when compared with complex **70** ($IC_{50} = 7.23$
454 μM).



455

456 **Figure 11.** Summary of the effect of chelating different metals on the anti-proliferative activities
457 of parent flavonoids

458 It can be deduced from **Figure 11** that 64% of the complexes showed enhanced anti-proliferative
459 activities when compared to their parents while 11% maintained the same activity and 25% had
460 lower activity. It should be noted that not all of the complexes' activities were compared to their
461 respective parent flavonoid, depending on the availability of data. All of iron, zinc and
462 germanium complexes showed higher activity than the free flavonoids. However, the total
463 number of zinc complexes is too low to establish a clear view on the effect of complexation. On
464 the other hand, 84%, 69% and 62% of the vanadium, copper and lanthanum complexes,
465 respectively, exhibited better anti-proliferative activities than the free flavonoid. Ruthenium
466 metal complexes showed inconsistent results as 36% showed enhanced activity while 21% had
467 the same activity and 43% showed lower activities than their parent flavonoids.

468

469 **Future perspectives**

470

471 Flavonoids have gained significant interest from researches since the early times. This is due to
472 their therapeutic activities in a broad range of fields alongside their natural abundance, with the
473 anti-oxidant and anti-cancer effects of flavonoids being of the greatest impact. Consequently,
474 flavonoids like quercetin and flavopiridol have reached clinical trials as anti-cancer agents.
475 However, none of them are available on the market as anti-cancer drugs due to their poor

476 pharmacokinetic profiles. The structural ability of flavonoids to coordinate with metal atoms has
477 provided a new strategy for the development of flavonoid metal complexes with better
478 pharmacological profiles. Although chelation of flavonoids with metals enhanced the anti-
479 oxidant and anti-proliferative activities of their free parents in the majority of cases reported
480 herein, more research is needed to confirm if this is a predictable and general phenomenon.
481 Additionally, more research is required to probe whether this enhancement of activity is
482 sufficient to afford new drugs that offer enhanced clinical outcomes when compared to drugs
483 present in the market for the same indications. Larger scale studies probing the impact of
484 complexing different metal atoms with sets of structurally similar and/or different flavonoids on
485 anti-oxidant and anti-proliferative activities are therefore greatly needed. This would pave the
486 way for a better understanding of their structural activity relationships. Furthermore, the use of
487 computational studies like molecular modelling and quantitative SAR (QSAR) can provide
488 valuable information about possible targets and mechanisms of action of these complexes. This
489 will in turn lead to well informed and targeted structural designs.

490 **Executive summary**

491 **Introduction**

- 492 • Flavonoids are polyphenolic compounds with a C₆-C₃-C₆ ring system.
- 493 • Flavopiridol, silibinin and quercetin flavonoids have reached clinical trials as anti-cancer
494 agents for various tumors.
- 495 • Flavonoids have poor pharmacokinetic properties which limit their clinical applications.
- 496 • Flavonoids have the ability to form metal complexes that can change their pharmacological
497 and pharmacokinetic properties.

498 **Synthesis and characterization of flavonoid metal complexes**

- 500 • Flavonoid metal complexes are synthesized by reacting alcoholic or aqueous solutions of both
501 the flavonoid and the metal under stirring and/or heating conditions.
- 502 • Structural characterization techniques of flavonoid metal complexes are versatile and are often
503 complementary to one another.

504 **Anti-oxidant activities of flavonoid metal complexes**

- 506 • Flavonoid metal complexes demonstrate better radical scavenging activities than their free
507 parents.
- 508 • Anti-oxidant activity is measured by the ability to scavenge free radicals like DPPH[•], ABTS⁺,
509 H₂O₂, OH[•] and O₂^{•-} in addition to measuring SOD like activity.

- 510 • Some flavonoid metal complexes showed DPPH· scavenging activity higher or comparable to
511 vitamin C.

512

513 **Cytotoxic activities of flavonoid metal complexes**

- 514 • The discovery of cisplatin had an important role in directing researchers' focus on the
515 development of metal based drugs.
- 516 • Flavonoid metal complexes showed promising results on various human cancer cell lines.
- 517 • Cytotoxic activity of flavonoid metal complexes is caused by induction of apoptosis and/or
518 oxidative damage of DNA.
- 519 • 64% of the flavonoid metal complexes showed better anti-proliferative activities than their
520 parents, 11% maintained the same activity and 25% had lower activity.

521

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