Antioxidant effects of some selected flavonoids: A structure-activity relationship based study

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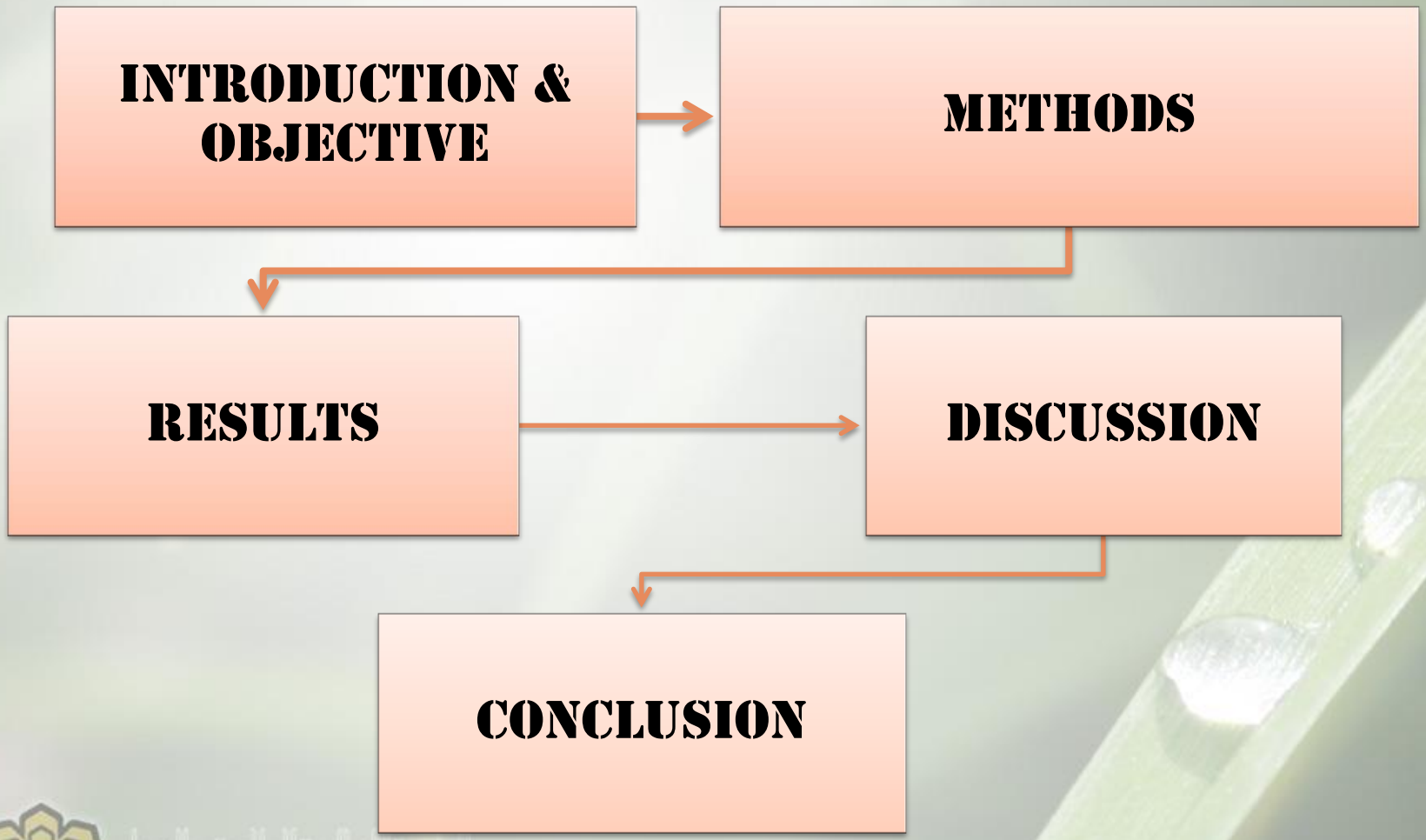
Garden of Knowledge and Virtue

Presentation outline



General Overview of Flavonoids

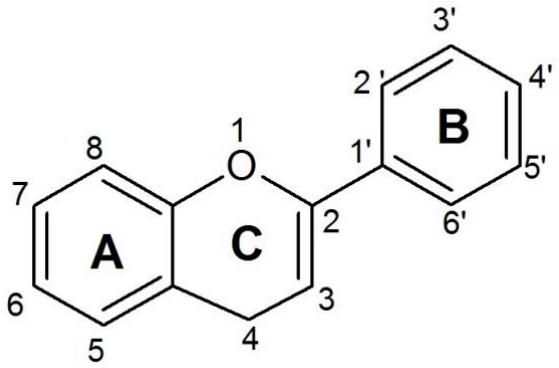
- Hydroxylated phenolic substances- potent free radical scavengers and considered therapeutics against free radical mediated diseases.

- Classified according to their side group positions and substitutions (e.g. flavones, flavonols, flavanones, flavanonols, flavanols, anthocyanins & chalcones)

- Protective effects : ascribed to their capacity to transfer hydrogen or electrons free radical [1], activate antioxidant enzymes [2], chelate metal catalyst [3], reduce α-tocopherol radicals [4] and inhibit oxidases [5].

Objective

To investigate the antioxidant and radical scavenging activities of some selected flavonoids with respect to identify key positions responsible for antioxidant effects as well as the effect of derivatisation on the antioxidative effects



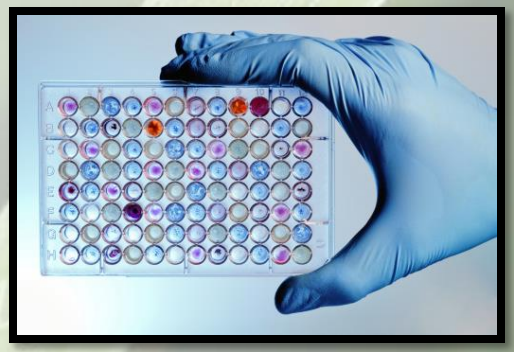
METHODOLOGY

Isolation of:

- 3 compounds from *Tetracera indica* (wogonin, norwogonin, tectochrysin),
- 4 compounds from *Tetracera scanden* (hypolaetin, isoscutellarein, kaempferol, quercetin).
- Chrysin, 8-hydroxy-7-methoxyflavone, (+)catechin and (-)epicatechin were bought from Sigma, Germany)

Semi-synthetic analogs:
Methyl ether and Acetates of wogonin and norwogonin

Antioxidant studies : 1-diphenyl-2-picryl hydrazyl (DPPH), Dot blot, ABTS+ radical scavenging Xanthine Oxidase inhibitory and ferric reducing antioxidant powder (FRAP) assays.



RESULTS

Spectral data (H-NMR) of tested flavonoids

Wogonin (5,7-dihydroxy-8-methoxyflavone**;** Norwogonin 8-methyl ether): **^{1}H-NMR** [600 MHz, Acetone-d_6, δ (ppm)]: 6.67 (s, 1H, H-3), 6.20 (s, 1H, H-6), 7.97 (m, 2H, H-2′/H-6′), 7.50 (m, 3H, H-3′/H4′/H5′), 3.84 (s, -OCH_3, 3H, H-8a), 12.43 (s. 1H, OH-5) [6].

Methyl ether of wogonin (5,7,8-trimethoxyflavone**): ^{1}H-NMR** [600 MHz, Acetone-d_6, δ (ppm)]: 6.77 (s, 1H, H-3), 6.73 (s, 1H, H-6), 8.11 (m, 2H, H 2′/H-6′), 7.63 (m, 3H, H-3′/H4′/H5′), 3.95 (s, 2 X -OCH_3, 6H, H-7a, H-8a), 4.07 (s, -OCH_3, 3H, H-5a) [7].

Acetate of wogonin (5,7-diacetoxy-8-methoxyflavone): **^{1}H-NMR** [600 MHz, Acetone-d_6, δ (ppm)]: 6.97 (s, 1H, H-3), 6.77 (s, 1H, H-6), 8.10 (m, 2H, H-2′/H-6′), 7.64 (m, 3H, H-3′/H4′/H5′), 4.09 (s, -OCH_3, 3H, H-8a), 2.41 (s, -$OCOCH_3$, 3H), 2.34 (s, -$OCOCH_3$, 3H) [8].

Techtochrysin (5-hydroxy-7-methoxyflavone): **^{1}H-NMR** [600 MHz, MeOD-d_4, δ (ppm)]: 6.43 (s, 1H, H-3), 6.34 (d, J = 2.4 Hz, 1H, H-6), 6.50 (d, J = 2.4, 1H, H-8), 7.87 (dd, J=1.8,4.2Hz, 2H, H-2′/H-6′), 7.44 (m, 3H, H-3′/H-4′/H5′), 3.74 (s, 3H, 7-OCH_3) [9].

Norwogonin (5,7,8-trihydroxyflavone): **^{1}H-NMR** [600 MHz, Acetone-d_6, δ (ppm)]: 6.77 (s, 1H, H-3), 6.36 (s, 1H, H-6), 7.37 (m, 3H, H-3′/H4′/H5′), 8.13 (m, 2H, H-2′/H-6′), 12.34 (s, 1H, OH-5) [10].

Acetate of norwogonin (5,7,8-triacetoxyflavone): **^{1}H-NMR** [600 MHz, Acetone-d_6, δ (ppm)]: 6.98 (s, 1H, H-3), 6.85 (s, 1H, H-6), 8.00 (m, 2H, H-2′/H-6′), 7.67 (m, 3H, H-3′/H4′/H5′), 2.48 (s, -$OCOCH_3$, 3H), 2.37 (s, 2 X -$OCOCH_3$, 6H) [8].

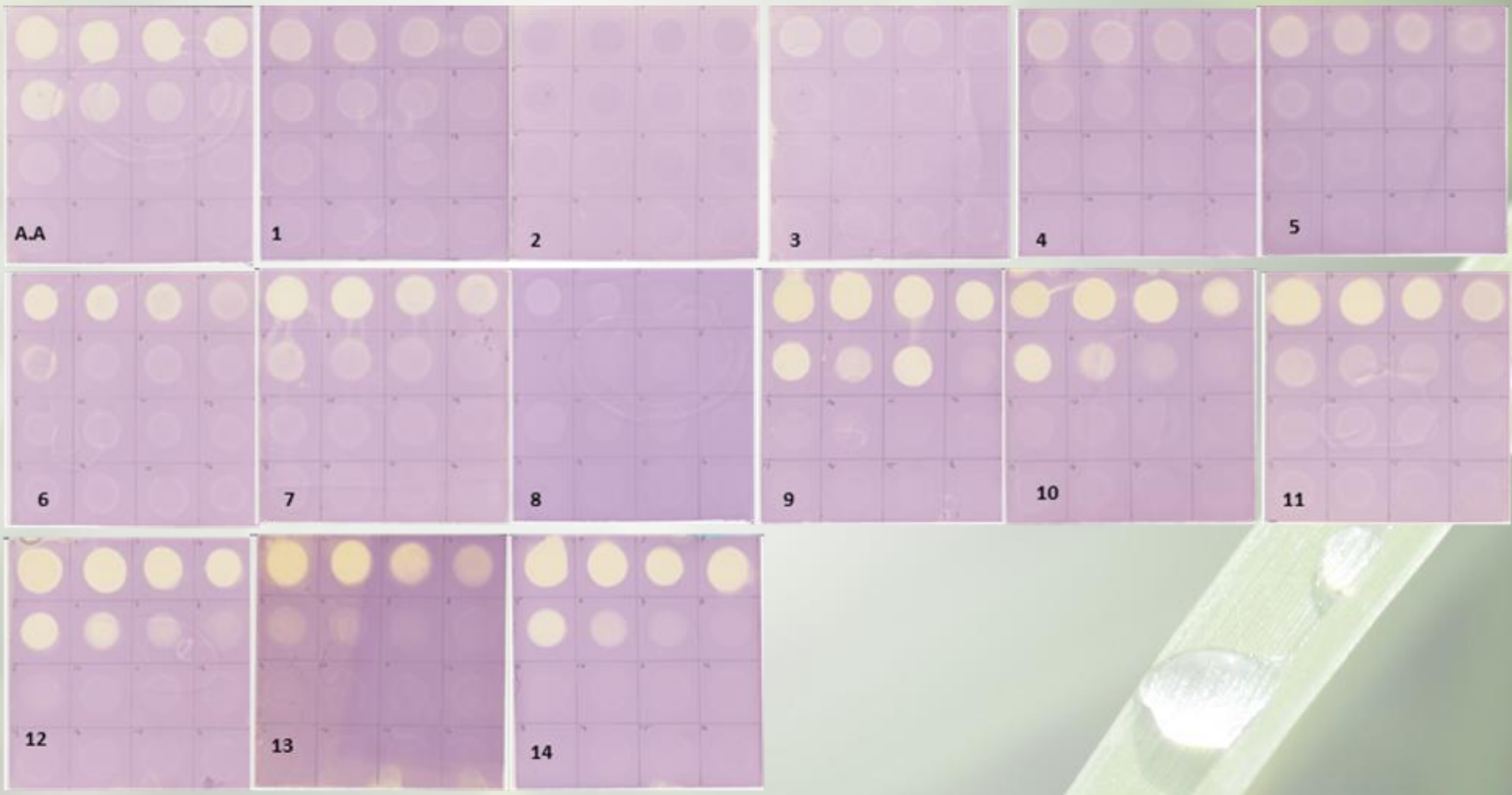
Isoscutellarein (4′,5,7,8-tetrahydroxyflavone): **^{1}H-NMR** (600 MHz, Acetone-d_6, δ (ppm)): 6.26 (1H, s, H-6) 6.72 (1H, s, H-3), 6.93 (2H, d, J = 8.4 Hz,3′, 5′) 8.00 (2H, d, J=8.4 Hz, H-2′, 6′), 8.81 (1H, s, OH-4′), 10.44 (1H, s, OH-8), 10.59 (1H, s, OH-7), 12.36 (1H, s, OH-5) [11].

Hypolaetin (3′,4′,5,7,8-pentahydroxyflavone (8-Hydroxyluteolin)): **^{1}H-NMR** (600 MHz, Acetone-d_6, δ (ppm)): 6.27 (1H, s, H-6), 6.59 (1H, s, H-3), 6.90 (1H, d, J = 2.4 Hz, H-2′), 7.43 (1H, d, J = 2.4 Hz, H-5′), 7.46 (1H, dd, J = 2.4, 2.4 Hz, H-6′), 8.77 (1H, s, OH-4′), 9.52 (1H, s, OH-3′), 10.64 (1H, s, OH-7), 9.98 (1H, s, OH-8), 12.37 (1H, s, OH-5) [11].

Kaempferol (4′, 3, 5, 7-tetrahydroxyflavone): **^{1}H-NMR** (600 MHz, Acetone-d_6, δ (ppm)): 6.27 (1H, d, J = 1.8 Hz, H6), 6.54 (1H, d, J = 1.8 Hz, H-8), 7.02 (2H, dd, J = 2.4, 9 Hz, H-3′, 5′), 8.16 (2H, dd, J = 1.8, 8.4 Hz, H-2′, 6′), 12.17 (1H, s, OH-5) [11].

Quercetin (3,3′,4′,5,7-pentahydroxyflavone): **^{1}H-NMR** [600 MHz, Acetone-d_6, δ (ppm)]: 6.28 (d, J = 2.4 Hz, 1H, H-6), 6.53 (d, J = 1.8, 1H, H-8), 7.01 (d, J = 8.6 Hz, 1H, H-5′), 7.71 (dd, J = 2.0, 8.4 Hz, 1H, H-6′), 7.84 (d, J = 2.2 Hz, 1H, H-2′), 12.16 (1H, s, OH-5) [11].

Dot Blot Assay



Rapid antioxidant screening by dot blot assay on a silica sheet stained with a DPPH solution in MeOH at 16 different concentrations viz., 1000 - 0.03 µg/mL applied from top to down. A.A is ascorbic acid (positive control), 1- wogonin, 2- methylether (wogonin), 3- acetate (wogonin), 4- tectochrysin, 5- 8-hydroxy-7-methoxyflavone, 6-chrysin, 7-norwogonin, 8-acetate (norwogonin), 9-isoscutellarein, 10-hypolaetin, 11-kaempferol, 12-quercetin, 13- (+)catechin, 14-(-)epicatechin.

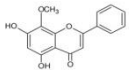
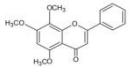
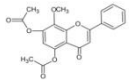
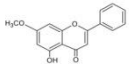
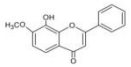
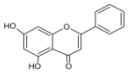
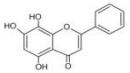
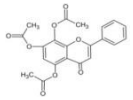
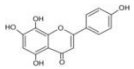
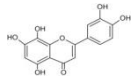
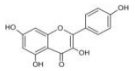
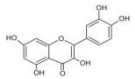
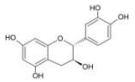
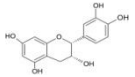
IC_{50} values of flavonoids for DPPH, $ABTS^{+}$ & Xanthine Oxidase inhibition assays

Samples (µg/ml)	DPPH	$ABTS^{+}$	Xanthine Oxidase
Ascorbic acid	4.75 ± 0.91^{GHa}	-	-
Trolox	-	1.76 ± 0.15^{FG}	-
Allupurinol	-	-	0.16 ± 0.30^{D}
Wogonin	$>100^{B}$	52.63 ± 2.99^{D}	NA
Methyl-ether (wogonin)	$>200^{A}$	$>200^{A}$	NA
Acetate (wogonin)	$>200^{A}$	$>200^{A}$	NA
Tectochysin	$>100^{B}$	45.59 ± 4.75^{E}	$>100^{A}$
8-hydroxy-7-methoxyflavone	68.24 ± 3.70^{C}	3.19 ± 0.15^{F}	NA
Chrysin	$>100^{B}$	$>100^{B}$	$>100^{A}$
Norwogonin	35.61 ± 1.68^{D}	1.24 ± 0.19^{FG}	NA
Acetate (norwogonin)	$>100^{B}$	78.99 ± 66.5^{C}	NA
Isoscutellarien	5.23 ± 0.53^{GHa}	1.73 ± 0.06^{FG}	$>100^{A}$
Hypolaetin	3.69 ± 0.11^{Ha}	0.80 ± 0.03^{FG}	$>100^{A}$
Kaempferol	10.89 ± 0.86^{EF}	1.36 ± 0.22^{FG}	16.36 ± 0.93^{B}
Quercetin	7.76 ± 0.99^{FG}	0.83 ± 0.01^{FG}	8.58 ± 0.72^{C}
(+)catechin	14.34 ± 1.55^{EF}	0.62 ± 0.05^{G}	NA
(-)epicatechin	9.92 ± 0.33^{F}	0.70 ± 0.08^{G}	NA

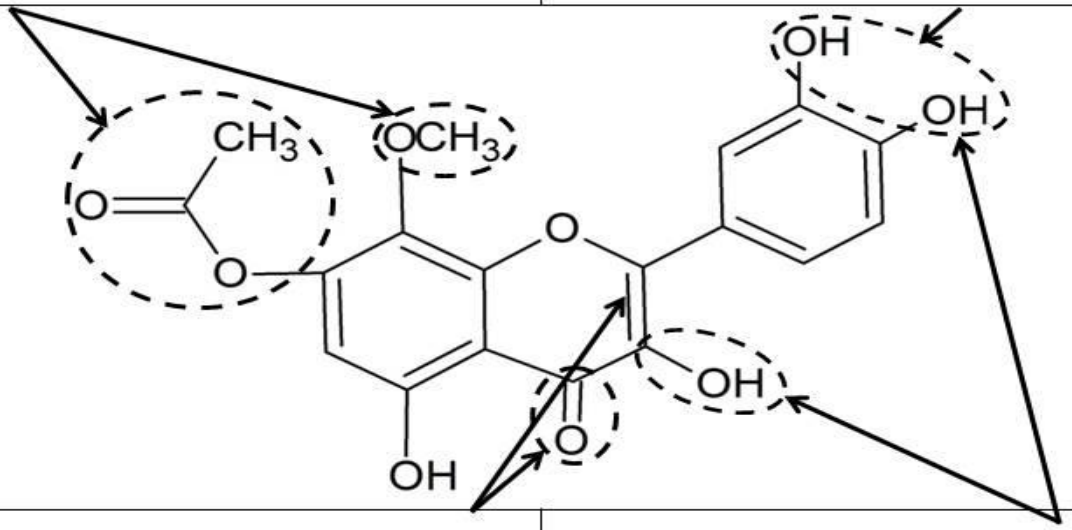
Result of FRAP in ascorbic acid equivalent (AAE)

Samples	FRAP (AAE µg)
Ascorbic acid (positive control)	114.58 ± 0.27^{Da}
Wogonin	39.15 ± 1.68^{E}
Methyl-ether (wogonin)	1.68 ± 0.18^{G}
Acetate (wogonin)	5.06 ± 3.24^{G}
Tectochysin	11.49 ± 0.32^{F}
8-hydroxy-7-methoxyflavone	104.92 ± 8.29^{D}
Chrysin	21.38 ± 1.86^{F}
Norwogonin	152.14 ± 7.30^{C}
Acetate (norwogonin)	39.63 ± 1.01^{E}
Isoscutellarien	262.91 ± 4.99^{A}
Hypolaetin	177.37 ± 1.82^{B}
Kaempferol	265.65 ± 5.46^{A}
Quercetin	138.93 ± 6.22^{C}
(+)catechin	148.12 ± 4.40^{C}
(-)epicatechin	152.07 ± 1.95^{C}

Discussion

Class	Compound	Chemical Structure
Flavone	Wogonin	
	Methyl-ether (wogonin)	
	Acetate (wogonin)	
	Tectochrysin	
	8-Hydroxy-7-methoxy flavone	
	Chrysin	
	Norwogonin	
	Acetate (norwogonin)	
	Isoscutellarein	
	Hypolaetin	
Flavonol	Kaempferol	
	Quercetin	
Flavanol	(+)catechin (2R,3S)	
	(-)epicatechin (2R,3R)	

Discussion

Methyl and acetate groups decreased antioxidant effects of flavonoids	A pair of hydroxyl group at position of C-3' and C-4' / C-4' and C-5' (catechol) enhanced the DPPH, FRAP and ABTS+ radical scavenging activities
	
The absence of C-2-C-3 double bond and ketonic group at C-4 reduced the xanthine oxidase inhibitory activity	Hydroxyl groups: the total number and the configuration of –hydroxyl group play an important role in regulating bioactivity of flavonoids

Conclusion

The results of this study will further help to understand the role of flavonoids as natural antioxidants which might facilitate in the development of nutritional products and semi synthetic analogs that retain substantial antioxidant capacity with minimal adverse effects.

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Thank you

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