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Spectrophotometric Assays For Flavonoids Diosmin, Quercetin, Rutin And Morin With Copper, Molybdenum, Lead And Tungsten

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Abstract: Metallic salts viz. Cu, Mo, Pb and W have been observed to react with flavonoids rather selectively forming chromophores enabling their use for colorimetric determination of the flavonoids. Optimized methods have been provided for assaying diosmin (5 to 100 µg with Cu), morin (2 to 25 µg with Pb, 5 to 40 µg with W), quercetin (1 to 40 µg with Mo or W, and 20 to 200 µg with Pb) and rutin (10 to 30 µg with Mo, 10 to 400 µg with W and 30 to 150 µg with Pb). The metallic salts have been employed as copper sulfate or acetate 0.5 µmole, ammonium molybedate 5 µmoles, lead acetate 5 and 25 µmoles, and sodium tungstate 100 µmoles per sample.
Key-words: Metallic salts, Diosmin, Morin, Quercetin, Rutin, Flavonoid.

Introduction

Flavonoids are known to interact with a number of metallic elements including notably Al, Tb, Cu and Fe. Trivalent aluminium interacts with flavonoids like fisetin (tetrahydroxylated flavone)[1], 3-hydroxyflavone[2], quercetin[3], 3'4'-dihydroxy-flavone[4], phyto-flavonoids[5,6], morin and rutin[7], and a large number of others for which it has been routinely employed for colorimetric measurements of flavonoids in general[11-14]. Iron and Copper have shown interaction with phyto-flavonoids[5], and a number of standard flavonoids[15]. Additionally, copper has shown affinity with quercetin[16], and antimony has been found to interact with flavonols to allow their colorimetric determination[17]. Terbium has proved as a useful for fluorimetric determination of flavonoids in pharmaceuticals[18]. In view of these observations, it was desired to look for interaction of other metallic salts with some commonly known flavonoids such as diosmin, morin, quercetin and its glycoside rutin, and to explore the possibility of employing these for their colorimetric assays. A preliminary screening revealed that the salts containing Cu, Mo, W and Pb were worth investigation while those containing Bi, Ag, Fe^{++} and Zn failed to elicit any characteristic color reaction with the test flavonoid. Consequently, systematic studies were planned to exploit potential elements for their use in assaying test flavonoids. Since the test flavonoids viz., diosmin, quercetin, rutin and morin structurally contain phenolic functions such as resorcinol in all, catechol (in quercetin and rutin) and guaiacol (in diosmin), these phenolic substances were also studied separately for their possible color reactions with the test metallic salts to appreciate whether or not these are likely to interfere or participate in the color reaction.

Materials And Methods

The experiments were carried out at an ambient temperature of 23.1 ± 0.4^0C. The drugs and chemicals used were of standard purity and quality obtained from reputed sources in India. Spectrophtometric measurements were made with UV-Visible Spectrophotometer, Model UVmini-1240 (Shimadzu Corporation, Japan).

Reagents

Copper acetate/sulfate solutions: Stock solutions prepared as 2 % (w/v) copper acetate monohydrate or 2.5 % copper sulfate pentahydrate (w/v) in water, equivalent each to 100 µmoles per mL solution. Working solutions included either 0.02 % copper acetate or 0.025 % copper sulfate solution equivalent to 0.5 µmoles per mL solution.

Lead acetate solution: 0.95% (w/v) lead acetate trihydrate in water, equivalent to 25 µmoles per mL solution.

Ammonium molybedate solution: 200 mg salt dissolved in water to make 113.2 mL solution, equivalent to 10 µmoles per mL solution.

Sodium tungstate solution: 3.3 % (w/v) solution of sodium tungstate in water, equivalent to 100 µmoles per mL solution (pH approximately 11.5). Neutralized sodium tungstate solution was prepared by adding 1 mL 10 % HCl per 10 mL stock sodium tungstate and 9 mL water equivalent to 50 µmoles per mL solution. (pH ca.6.7).

Phenolic solutions: Catechol and resorcinol were each made 0.2 % (w/v) in water. Guaiacol was prepared as 1% (w/v) in 10% ethanol. A cocktail of phenolic substances was also prepared in 0.05 N acetic acid containing per mL 2 mg each of phenol, resorcinol, catechol, guaiacol, hydroquinone and tannic acid.

Acetic acid solution: A 10% (v/v) solution of glacial acetic acid in water. Approximately 0.5 N acetic acid prepared by adding 13 mL water to each 5 mL of 10% acetic acid solution.

EDTA solution: 0.1 M EDTA disodium in water.

Diosmin: Synthetic diosmin purified from Venex-500 tablets (Elder Pharmaceuticals Ltd., Mumbai) served as a Laboratory standard. The tablets with gross weight 1032 ± 5 mg $table^{-1}$ (n=9) were finely pulverized with pestle and mortar. Each tablet was labeled to contain 500 mg synthetic diosmin (B.P.). Each 207 mg gross tablet powder, equivalent to 100 mg synthetic diosmin, was added 20 ml distilled water, extracted at room temperature for one hour, and filtered over Whatman Filter No. 1. The residue was washed with about 50 mL water to remove water soluble impurities and coloring excipients, and then reconstituted with 10 ml water, and added 4 ml 2.5 N NaOH to solubilize diosmin, and volume was made 50 ml with water. The sample was filtered, and the filter paper washed with water to get 100 ml volume to get 0.1 % (w/v) labeled synthetic diosmin in 0.1 M NaOH. Further dilutions made in 0.1 N NaOH as per need. The stock solution was stored well stoppered in refrigerator. Diosmin was quite stable in alkaline medium, and when kept stoppered when not in use. Samples of diosmin on exposure to air or on rendering acidic developed opalescence.

Morin: Stock solution was prepared as 0.1% in methanol. Working solutions were made by dilution in methanol.

Rutin: Stock solution was prepared in acid-ethanol as 0.2 % (w/v) rutin in 55% (v/v) ethanol and 5 % (v/v) acetic acid in water. A separate solution was prepared in methanol as 0.2 % (w/v), and diluted in methanol as and when required.

Quercetin: Stock solution was prepared in acid-ethanol as 0.2 % (w/v) quercetin in 55% (v/v) ethanol and 5 % (v/v) acetic acid in water.

Analytical techniques for flavonoids with metallic salts

Assay of diosmin with copper salts

Each milliliter of 0.1N NaOH solution containing 50 µg of diosmin was added 2 mL of aqueous solution containing copper acetate 0, and 0.5 through 50 µmoles. The color was monitored at 380 and 400 nm to find the concentration of copper appropriate to use for the assay Based on these results, each milliliter of 0.1 N NaOH solution containing diosmin 0 and 5 through 100 µg was added 0.5 mL of 0.02% copper acetate solution or

0.025 % copper sulfate solution and 1.5 mL water, allowed to stand at room temperature for 20-30 minutes and read at 380 and 400 nm.

Assay of morin with lead acetate

Each milliliter of methanol containing 2 through 25 µg of morin was added 0.1 mL of 0.5 N acetic acid solution followed by 0.2 mL of 0.95% lead acetate solution and 1.7 mL water. The color was monitored at 405 nm following standing at room temperature for 20-30 minutes.

Assay of morin with sodium tungstate

Each milliliter of methanol containing 5 through 40 µg of morin were added 2 mL of pH adjusted sodium tungstate solution, and the color monitored at 405 nm following standing at room temperature for 20-30 minutes.

A comparative study over 16 through 30 µg of morin in methanol was also conducted

while using either pH adjusted tungstate or alkaline tungstate as 100 µmoles per sample, and the color monitored at 405 nm.

Assay of quercetin and rutin with sodium tungstate

Each milliliter of acid-ethanol containing either quercetin (1 through 50 µg) or rutin (10

through 400 µg) was added 1 mL water and 1 mL 3.3 % sodium tungstate solution. The color was monitored at 405 nm at 20-30 minutes.

In a separate experiment, catechol 50 through 2000 µg in 1 mL water was added 1 mL acid-ethanol and 1 mL sodium tungstate solution and monitored at 405 nm.

Assay of quercetin and rutin with ammonium molybdate

Each milliliter of acid-ethanol containing either quercetin (1 through 40 µg) or rutin (10 through 30 µg) was added 1 mL water and 1 mL ammonium molybdate solution, 5 µmoles. The color was monitored at 405 nm at 20-30 minutes.

In separately conducted assays, catechol 10 through 1000 µg in 1 mL water was added 1 mL each of acid-ethanol and ammonium molybdate, 5 µmoles, and monitored at 405 nm. Catechol over 50 through 500 µg in 2 mL water, in absence of acid-ethanol, was also monitored at 430 nm using similar mass of Mo to check influence of acid-ethanol on color reaction.

Assay of quercetin and rutin with lead acetate

Each milliliter of acid-ethanol containing quercetin (20 through 200 µg) or methanol containing rutin (30 through 150 µg) was added 1 mL water and 1 mL 0.95% lead acetate solution, 25 µmoles or 1 mL 0.2 % solution, 5 µmoles respectively. The color was monitored respectively at 405 and 430 nm at 20-30 minutes.

In a separate experiment, catechol 100 through 4000 µg in 1 mL water and 1 mL acid-ethanol was treated with 1 mL 0.95% lead acetate solution, 25 µmoles. The color was monitored at 405 nm at 20-30 minutes.

Results And Discussions

In a general screening test using 75 µg rutin in methanol, 10 µmoles each of ferrous sulfate, zinc sulfate, silver nitrate or bismuth sub nitrate failed to elicit any color reaction while ammonium molybedate (2 to 5 µmoles), copper sulfate (0.5 to 2 µmoles), sodium tungstate (30 to 100 µmoles) and lead acetate (5 to 25 µmoles) elicited color reaction. Rutin (180 to 900 µg) in acid-ethanol did not react to lead acetate or copper salts while in methanol or pH methanolic and pH adjusted rutin reacted effectively.

Quercetin (50µg), rutin (50 µg) and morin (20 µg) when added each 2 mL of pH adjusted sodium tungstate (100 µmoles) developed color with maximum absorbance at 405 nm while 100 µg diosmin failed to react. The color intensity, with mean absorbance values within parenthesis, was in the order:

Quercetin (0.85) > Morin (0.43) > Rutin (0.31)

Absorbance at 405 nm was more than at 430 nm.with responsive flavonoids following the order: rutin (4.5 folds) > morin (1.8 folds) > quercetin (1.1 folds). Morin in methanol reacted to all test salts. For instance, morin

40 µg reacted positively (absorbance values within parenthesis) to Mo 5 µmoles (0.76), tungstate 100 µmoles (0.68), copper 10 µmoles (0.90) and lead 10 µmoles (1.1).

Diosmin and metallic salts

Diosmin (up to 500 µg) failed to develop color with ammonium molybedate (5 µmoles), lead acetate (25 µmoles) or sodium tungstate (100 µmoles) whereas it reacted effectively with copper salts as little as 0.5 µmoles. This provided rationale for using copper sulfate or copper acetate to assay diosmin.

Diosmin with copper salts (sulfate or acetate) developed color with maximum absorbance at 380 and 400 nm. An evaluation over 0.5 through 50 µmoles of copper with 50 µg diosmin revealed mean absorbance at 0, 0.5, 1 and 3 µmoles of copper respectively as 0.180±0.003, 0.262±0.002, 0.242 ±0.003 and 0.107 ± 0.004 (n=3 each) reflecting per cent increase in absorbance at 0.5 and 1.0 µmoles, respectively as, 46±1 and 34 ±2 compared to standard, and higher copper concentration, 3 µmoles, caused decrease in color intensity ($P<0.01$, n=3). This allowed use of 0.5 µmole of copper salt for the assay. At this concentration, the assay was linear over 5 through 100 µg diosmin (Table 1) with either copper salt and at either 380 or 400 nm. At 380 nm the absorbance values over 5 through 50 µg are significantly more than at 400 nm ($P<0.01$, n= 5 each). In general absorbance values tend to be more with copper acetate than with copper sulfate, and more at 380 nm than at 400 nm. Thus, copper salts can be employed for assaying diosmin over 5 through 100 µg at 0.5 µmoles.

Table 1:Assay of diosmin with copper salts[a]

Diosmin, µg	Copper sulfate		Copper acetate	
	380 nm	400 nm	380 nm	400 nm
5	0.031±0.001	0.024 ±0.001	0.041 ± 0.001	0.034 ± 0.001
15	0.107 ± 0.001	0.082 ±0.002	0.115 ± 0.001	0.093 ± 0.001
25	0.181 ± 0.001	0.150 ±0.001	0.195 ± 0.001	0.163 ± 0.001
50	0.363 ± 0.001	0.331 ±0.001	0.372 ±0.002	0.343 ± 0.003
100	0.708 ± 0.004	0.702 ±0.004	0.715 ± 0.003	0.711 ± 0.004
Statistical analysis				
r ± s.e.	0.9998 ±0.0002	0.9996 ±0.0004	0.9997 ± 0.0003	0.9997 ± 0.0003
b ± s.e.	0.00711 ± 0.00006	0.00721 ±0.00009	0.00708 ± 0.00008	0.00719 ±0.00008

The values are mean ± s.e of five observations each.
[a] 0.5 µmole per sample

Morin and metallic salts

Morin has shown response with all test metallic salts. In 1 mL methanol 40 µg morin when treated with Mo (5 µmoles), Cu (10 µmoles), and tungstate (100 µmoles) in 2 mL water exhibited mean absorbance at 405 nm respectively as 0.762± 0.009, 0.899±0.006, 0.676 ±0.011 (n= 5 each). Lead acetate (5 or 10 µmoles) under similar conditions developed opalescence. However addition of 0.2 mL 10 % acetic acid rendered transparency in color with peak absorbance value of 1.072 ± 0.023 (n=5). Blank included 40 µg morin (0.2 mL of 0.2 % (w/v) in methanol) in 3 mL water. However, the present study investigated only use of tungstate and lead acetate for assaying morin. Morin developed excessive color with alkaline pH. This necessitated adjusting pH of sodium tungstate solution (ca. pH 11.5) with addition of 10% HCl (pH made 6.4 to 6.7). Morin 5 through 40 µg in methanol showed linear response with 100 µmoles of pH adjusted sodium tungstate at 405 nm (Table 2). Morin in methanol developed opalescence with 5 µmoles lead acetate in absence of acetic acid while acetic acid 0.1 through 0.4 mL 0.5N rendered samples transparent. Using 5 µmoles lead and 0.1 mL 0.5 N acetic acid, 2 through 25 µg morin in 1 mL methanol provided linear response at 405 nm (Table 3). A comparison of tungstate and lead based regression estimates over linear ranges of morin suggest that lead (b=0.0327) provided 1.4 times better chromagenic sensitiivity than tungstate (b=0.0234), and over comparable masses of 5, 10 and 20 µg morin, the mean absorbance values with lead were 2.8 ± 0.5 folds more than with tungstate and ratio of their regression estimates was about 1.7 ($P<0.01$).

Table 2: Assay of morin using sodium tungstate

Morin, µg	Sodium tungstate[a]
5	0.041 ±0.001
10	0.114 ± 0.002
20	0.336 ± 0.002
30	0.606 ± 0.002
40	0.840 ± 0.001
Statistical analysis	
r± s.e.	0.9978 ± 0.0020
b± s.e.	0.0234± 0.0007

The values are mean ± s.e. of five observations each
[a] 100 µmole sodium tungstate (pH ca. 6.7)

Table 3: Assay of morin using lead acetate

Morin, µg	Lead acetate[a]
2	0.065 ±0.001
5	0.152 ± 0.002
10	0.311 ± 0.002
15	0.507 ± 0.002
20	0.662 ± 0.001
25	0.799 ± 0.003
Statistical analysis	
r± s.e.	0.9988 ± 0.0010
b± s.e.	0.0327 ± 0.0036

The values are mean± s.e of five observations each.
[a] 5 µmole lead acetate with 0.1 mL 0.5N acetic acid

Quercetin and metallic salts

Quercetin is chemically an aglycone of rutin. Copper 0.5 µmole failed to react with 20 µg quercetin in acid-ethanol while 100 µg responded lightly. Compared to copper other salts interacted with quercetin effectively facilitating their use in optimizing assays. For instance, ammonium molybdate at 5 µmoles exhibited linear response over 1 through 40 µg in acid-ethanol (Table 4). Lead acetate at 5 and 12.5 µmole failed to elicit an appreciable response with 20 to 100 µg quercetin in acid-ethanol while 25 µmoles induced linear response over 20 through 200 µg (Table 5). Quercetin 100 µg in acid-ethanol added tungstate 2 through 100 µmoles showed concentration related change in absorbance at 405 nm with peak absorbance, 0.258, at 100 µmoles compared to 0.08 and 0.20, respectively, at 2 and 50 µmoles Mo. Using 100 µmoles Mo per sample provided linear response over 1 through 50 µg quercetin in acid-ethanol (Table 6). Comparing regression coefficients with test metallic salts over linear ranges of quercetin has revealed sensitivity order as Mo (b= 0.0176) > W (b= 0.0147) >> Pb (b= 0.0017) suggesting that Mo and W formed a more chromogenic complex with quercetin than that formed by Pb.

Table 4: Assay of quercetin using ammonium molybdate

Quercetin, µg	Molybedate[a]
1	0.038 ±0.001
3	0.061± 0.001
10	0.175 ± 0.001
20	0.361 ± 0.002
30	0.522 ± 0.005
40	0.727 ± 0.004
Statistical analysis	
r± s.e.	0.9991 ± 0.0007
b± s.e.	0.0176± 0.0003

The values are mean± s.e of four observations each.
[a] 5 µmoles Mo added per sample

Table 5: Assay of quercetin using lead acetate

Quercetin, µg	Lead acetate[a]
20	0.028 ±0.001
50	0.066± 0.001
100	0.182 ± 0.001
200	0.324± 0.001
Statistical analysis	
r± s.e.	0.9946 ± 0.0054
b± s.e..	0.00168± 0.00009

The values are mean± s.e of four observations each.
[a] 25 µmole lead acetate added per sample

Table 6: Assay of quercetin using tungstate

Quercetin, µg	Tungstate[a]
1	0.141 ±0.002
3	0.170± 0.004
10	0.266 ± 0.002
20	0.404± 0.003
30	0.537±0.002
40	0.730 ± 0.014
50	0.856 ± 0.003
Statistical analysis	
r± s.e.	0.9987 ± 0.0009
b± s.e.	0.0147± 0.0003

The values are mean± s.e of four observations each.
[a] 100 µmole sodium tungstate per sample

Table 7: Assay of rutin using lead acetate

Rutin, µg	Lead acetate[a]
30	0.060 ±0.001
45	0.090± 0.001
75	0.170 ± 0.001
150	0.398± 0.002
Statistical analysis	
r± s.e.	0.9985 ± 0.0015
b± s.e.	0.0029± 0.0001

The values are mean± s.e of four observations each.
[a] 5 µmole lead acetate added per sample

Rutin and metallic salts

Rutin over 180 through 900 µg failed to interact with copper salt up to 2 µmoles but reacted with lead acetate, ammonium molybdate or sodium tungstate. Lead acetate up to 50 µmoles failed to interact with 150 µg of rutin in acid-ethanol due to acidic pH while reacted linearly over 30 through 120 µg rutin in methanol or pH adjusted acid-ethanol (r = 0.99) at 5 µmoles lead. with regression estimate b ± s.e. respectively as 0.00264±0.0002 and 0.00164 ± 0.00002 and pH correspondingly as 6.51 ± 0.02 and 6.69 ± 0.06 (n= 7 each) with no difference between mean values of absorbance (P>0.1). Absorbance values were better with methanolic rutin than with pH adjusted with ratio of regression coefficients found to be 1.6. Higher concentrations of lead salt, 25 µmoles, induced opalescence. For rutin assay lead acetate was employed at 5 µmoles over 30 to 150 µg of rutin in methanol providing linear response (Table 7). In presence of 1 through 2.5 µmoles of EDTA, there was concentration related decrease in color intensity by lead with 150 µg rutin (r=0.999) with mean per cent decrease in absorbance at 1, 1.5 and 2.5 µmoles EDTA, respectively as, 43, 56 and 85 compared to untreated sample. This reflected that the interaction between flavonoid and the lead was specific and prevented by lead chelator, EDTA. Rutin 10 through 30 µg in acid-ethanol showed linear response with 5 µmoles of Mo (Table 8). In a separate experiment the response at 1µmole Mo was linear but poor at 30 through 100 µg with regression coefficient at 5 µmoles of Mo (b=0.005) 1.3 times the value obtained at 1 µmoles of Mo (b=0.0038). Thus, 5 µmoles of Mo is optimal for use in the assay. Rutin 30 µg in acid-ethanol showed concentration related increase in absorbance at 405 nm over 30 through 300 µmoles of sodium tungstate with peak absorbance, 0.276, at 100 µmoles compared to those at 30 µmoles, 0.184, or 300 µmoles, 0.247. Thus, 100 µmoles sodium tungstate was employed for assaying rutin in acid-ethanol. This provided linear response over 10 through 400 µg (Table 9).

A comparison of regression estimates over linear ranges of rutin with test metallic salts has revealed: Mo (b = 0.0050) ≥ W (b= 0.0046) > Pb (b = 0.0029). The order is comparable to that obtained with quercetin. Thus, quercetin and rutin behaved in a similar pattern with overall absrbance values with quercetin more than those with rutin. Rutin is a glycoside of quercetin containing disaccharide rutinose. It behaved less intensely compared to quercetin suggesting possible interference in chromogenicity due to rutinose component.

Table 8: Assay of rutin using molybedate

Rutin, µg	Molybedate
10	0.037 ± 0.001
20	0.097 ± 0.001
30	0.136 ± 0.002
Statistical analysis	
r± s.e.	0.9926 ± 0.0085
b± s.e.	0.0050 ± 0.0003

The values are mean± s.e of four observations each.
[a] 5 µmoles Mo added per sample

Table 9: Assay of rutin using tungstate

Rutin, µg	Tungstate[a]
10	0.174±0.001
30	0.270± 0.002
50	0.377 ± 0.002
100	0.596± 0.005
400	1.978 ± 0.002
Statistical analysis	
r± s.e.	0.9999 ± 0.0001
b± s.e.	0.00461± 0.00003

The values are mean± s.e. of four observations each.
[a] 100 µmole sodium tungstate per sample

Effect of selected metallic salts on phenolics

Initial studies revealed that copper and lead salts failed to interact with phenolic cocktail containing equivalent of 1 mg each of phenol, catechol, guaiacol, hydroquinone, resorcinol and tannic acid while sodium tungstate and ammonium molybedate showed respectively light and intense colorations. This implied that these phenolics were unlikely to interfere with the assays using copper and lead salts. Resorcinol is common to diosmin, morin, quercetin and rutin. Additionally, the listed flavonoids respectively contain one molecule of guaiacol, resorcinol, catechol and catechol. This provided rationale to test the response of these phenolics individually with the test metallic salts to assess their possible interference or contribution to the color reaction by the interaction of metallic salts with the flavonoids. The observations revealed that resorcinol or guaiacol up to 2 mg each failed to react with 100 µmoles tungstate, 10 µmoles molybedate, 25 µmoles lead acetate or 0.5 µmole copper sulfate solutions. These observations implied that the reaction of metal with the flavonoids does not involve their resorcinol or guaiacol function. Catechol up to 2 mg too failed to react with copper or leads while both tungstate and molybdenum salts reacted linearly. Therefore, some degree of participation and interference is possible while assaying quercetin or rutin with tungstate or molybedate. To assess extent of possible interference or contribution by catechol, systematic evaluation of catechol was undertaken with these metallic salts.

Catechol 50, 100, 300 and 1000 µg in 1 mL acid-ethanol using 5 µmoles of Mo provided mean absorbance values at 405 nm respectively as 0.027 ± 0.001, 0.070 ± 0.001, 0.210 ± 0.003 and 0.785 ± 0.002 (r± s.e. = 0.9944 ± 0.0056, b± s.e. = 0.00076± 0.00004, n= 5 each). Under these conditions, 10 µg catechol remained undetectable. The reaction when monitored in absence of acid-ethanol and at 430 nm showed improved linearity and sensitivity over 50 through 300 µg (r ± s.e. = 0.9999 ± 0.0001; b ± s.e. = 0.00120 ± 0.00001; n= 5 each). Consequently, the color reaction of quercetin and rutin with Mo is unrelated to the presence of catechol because quercetin and rutin over the test range exhibited appreciable absorbance but contained estimated catechol equivalent from 1.66 µg (10 µg rutin) through 9.75 µg (30 µg quercetin) while 10 µg catechol failed to develop color with Mo. Besides, comparison of ratio of regression coefficients reveal that quercetin and rutin are, respectively, 23 and 6.6 folds more sensitive to color reaction with Mo than catechol implying that the quercetin is about 3.5 folds more sensitive than rutin. Mean absorbance with quercetin is generally 4-folds (4.0±0.3) more than rutin at comparable masses ($P<0.01$, n= 4 each).

Catechol 50, 100, 300,1000 and 2000 µg with 1 mL acid-ethanol while using 1 mL of 3.3 % sodium tungstate (100 µmoles) provided mean absorbance values respectively as 0.150 ± 0.001, 0.169 ± 0.001, 0.195 ± 0.003, 0.343± 0.004 and 0.576 ± 0.004 (r ± s.e. = 0.9988 ± 0.0011, b ± s.e. = 0.000220 ± 0.000005, n= 5 each). Quercetin and rutin are, respectively, 67 and 21 folds more sensitive to color reaction with tungstate than catechol implying that the quercetin is about 3.2 folds more sensitive than rutin. Mean absorbance with quercetin is generally 2-folds (2.1±0.2) more than rutin at comparable masses ($P<0.01$, n= 4 each). Thus participation of catechol moiety in color reaction of quercetin and rutin to either Mo or to Pb is negligible.

In general quercetin was providing more absorbance values than rutin with test metallic salts such that ratio of regression estimates: b (quercetin)/b (rutin) while employing Mo, and W were respectively found to be 3.5 and 3.2, while with lead rutin was somewhat more sensitive than quercetin with ratio of regression estimates, b (rutin)/b (quercetin), as 1.7. In a properly matched assay using 5 µmoles Mo quercetin was 4.1 ± 0.4 times more chromogenic than rutin with ratio of regression coefficients 3.52 over 10 through 30 µg masses. This implied that overall quercetin; aglycone of rutin was more chromogenic than its glycoside form rutin suggesting possible role of rutinose in suppressing color intensity in rutin with these metallic moieties.

Conclusions

Ability of metallic salt solutions to react with selected flavonoids extends opportunity to employ these for colorimetric determination of the flavonoids in addition to trivalent aluminium that is already in use. The study has provided alternative colorimetric methods for determination of flavonoids. Diosmin can be assayed with copper salts. Quercetin and rutin are assayable with Mo, W or Pb. Morin can be assayed by any of the test metallic salts. Further studies are required to optimize assays for morin using copper salts and molybedate, and for assaying rutin in methanol with copper salts.

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