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New Insights on Health Benefit of Some Synthesized Biomimic Flavonoids in Cosmetics Products

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Abstract

Variety chemicals are commonly used in cosmetics as dye, colorant, thickener, fragrance, whitening agent, preservative, binding agent etc. Some of these poses little or no risk but in reality some such as Benzophenone, Formaldehyde, Coal tar etc. have been linked to serious health threats, including cancer, reproductive and neurological harm even developmental delays. Flavonoids specially chalcones and related compounds are mostly polyphenolic phytochemicals which have withdrawn intensive interest as these are natural color pigments, UV protector of plants with non-nitrogenous chromophore -CO-CH=CH- in conjugation with aromaticity and numerous biodynamic properties. So some new and structurally modified chalcones were synthesized by Claisen-Schmidt Condensation, characterized by spectral techniques (UV, IR, ¹H NMR) and screened through antioxidant activity, namely Gallic acid equivalent antioxidant capacity (GEAC) assays, total antioxidant method. Among the selected chalcones 2 – chloro – 4' – methyl chalcone resulted in the greatest GEAC, 45.61 µg/mL as well as total antioxidant in BHT equivalence 260.11 µg/mL The potency of cosmetics additives of the compounds were examined as applied on carbomer gel (polyacryl amide), major cosmetic thickening ingredients and the stability of color and gel state being confirmed through unchanged pH (about 6.54 - 6.98) for three months. Compounds form very attractive transparent colors with gel and the binding mechanism can be explained by IR and UV spectroscopic technique.

Keywords: Cosmetics chemicals, Biomimic Flavonoids, Antioxidant Activity, GEAC, Total Antioxidant Activity, Carbomer gel Binding Efficacy.

1. Introduction

Variety chemicals are commonly used in cosmetics pose little or no risk, some have been linked to serious health problems, including cancer, reproductive and neurological harm, and developmental delays. Cosmetic chemicals enter the body through the skin, inhalation, ingestion and internal use and pose the same risks as food chemicals.¹ In addition to the risks posed by intentionally added ingredients, cosmetics can be contaminated with heavy metals, including arsenic, cadmium, lead, mercury and nickel. Over the last few decades, the number of chemicals added to cosmetics and other products has increased a lot in these industries which have become a serious concern as these are taken by human body or used externally everyday.²

Benzophenone commonly found in lip balm, nail polish, foundations, sunscreens, fragrance, shampoo, conditioner, hair spray, moisturizers can cause severe skin and eye irritation, create allergies, an endocrine (hormone) disruptor, and can lead to developmental and reproductive toxicity, possibly carcinogenic (cancer causing), Carcinogenic Coal tar can be found in eye makeup, hair dyes and anti-dandruff shampoos. Formaldehyde commonly used preservative in nail polish, soaps, shampoos, body wash, bubble bath, lotions, and deodorants, is a very known carcinogenic and has been linked to neurotoxicity, developmental toxicity, even asthma.³ So in modern life there is again a great demand to revive the age-old art of use of natural agents as beautification of skin and care purpose.

Green tea, soybeans, red wine and aromatic plants are popularly taken as anti-aging dietary worldwide. The main reason behind is all these contain flavonoids, a class of over 4000 plant-derived chemicals (or 'phyto - chemicals') which share a similar molecular structure.

Structurally, flavonoids are a type of “polyphenol” – a group of molecules that, in plants, play various roles including: aiding reproduction, fighting against infection and protecting against damage from UV (ultraviolet) radiation in sunlight. This latter function is of particular interest – if flavonoids can protect plants from the harmful effects of UV rays, can they do the same to human skin?⁴

Flavonoids are a diverse group of plant polyphenols with antioxidant properties. Chalcones, flavones, isoflavone, aurones etc. belong to this class of compound. They are often responsible for the colors in many fruits, vegetables, and flowers. In plants, these pigments protect plants from environmental stress as they are potent antioxidants,⁵ and radical-scavengers.^{6,7} Flavonoids are important in cosmetics as they provide strong antioxidant protection and protection from UV radiation of our skin.⁸ Some flavonoids are also antiallergic, anti-inflammatory, antimicrobial.⁵ Flavonoids being synthesized in whole parts of the plant and provide color, fragrance and taste to the fruits, flowers and seeds which make them attractants for insects, birds and mammals for pollination and seed dispersal.⁹

This research proposes to synthesize new and structurally modified flavonoids like chalcones utilizing Claisen-Schmidt condensation Method.¹⁰⁻¹⁷ In the present study four substituted chalcones were synthesized; **3'**, **4'** – methylene dioxy – 4 – nitro chalcone, **3a**, **2** – chloro – 4' – methyl chalcone, **3b**, **2** – chloro – 2' – hydroxy chalcone, **3c**, 2' – hydroxyl – 2, 4, 6 – trimethoxy chalcone, **3d**, from their corresponding acetophenones (1a-1d) as well as aldehydes (2a-2c) and using NaOH/EtOH. They have been characterized by spectral data. (UV, IR, ¹H

NMR) and screened through antioxidant assay, namely Gallic acid equivalent antioxidant capacity (GEAC) assays, total antioxidant method. The potency of cosmetics additives of the compounds were examined as applied on carbomer gel (polyacryl amide), major cosmetic thickening ingredients and the stability of gel being studied by appearance and pH change of mixture. Compounds (3a-3d) form very attractive transparent colors with gel and the binding mechanism being observed by IR and UV spectroscopic technique.

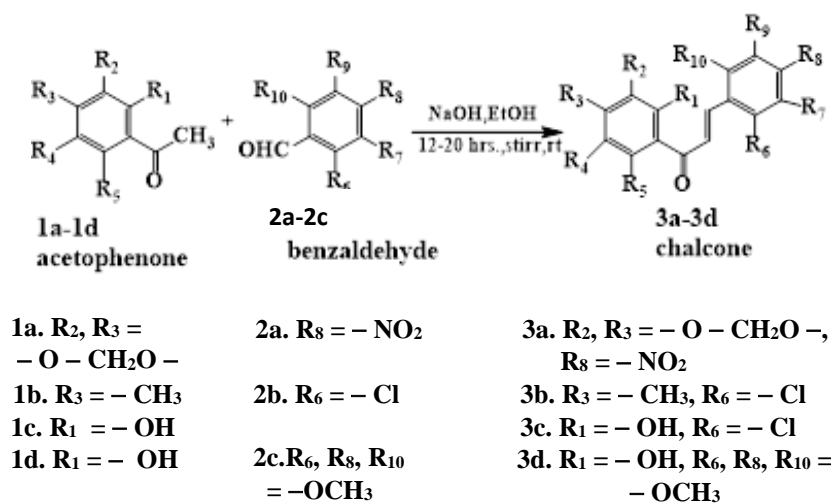
2. Materials and methods

2.1 Chemicals and apparatus

Commercially available substituted acetophenones (1a-1d) and substituted benzaldehydes (2a-2c) were purchased and utilized together for the synthesis of substituted chalcones (3a-3d). Other necessary chemicals and reagent grade solvent were purchased from E-Merck or Sigma-Aldrich company and purified if required.

2.2 Synthesis of Substituted Chalcones (3a-3d)

Four substituted chalcones were synthesized: 3', 4' – methylene dioxy – 4 – nitro chalcone, **3a**, 2 – chloro – 4' – methyl chalcone, **3b**, 2 – chloro – 2' – hydroxy chalcone, **3c**, 2' – hydroxyl – 2, 4, 6 – trimethoxy chalcone, **3d** using conventional Claisen-Schmidt condensation method. The chalcones (**3a-3d**) were obtained from equimolar quantities of the corresponding acetophenones (**1a-1d**) as well as aldehydes (**2a-2c**) in presence of NaOH/EtOH as shown in (Scheme 1). The structures of the above compounds were assigned on the basis of spectral data; UV, IR and ¹H NMR.



Scheme 1: General synthetic route of chalcone (3a-3d).

2.3 Characterization of Synthesized Chalcones (3a-3d)

(i) 3', 4' – methylene dioxy – 4 – nitro chalcone, 3a

C₁₆H₁₁O₅N, Solid and yellowish brown, yield 63%; m. p. 119-120 °C, λ_{max} (CH₃OH; nm) ; 280, 205, IR (KBr, cm⁻¹): 3124.23 (C=C–H, olefinic, str), 2917.12 (aliphatic –C–H, asym.-str.), 2800.00 (aliphatic –C–H, sym.-str.), **1663.11 (C=O, conjugated keto group)**, 1606.25, 1595.26 (C=C, aromatic), ¹H NMR, δ_H (400 MHz, DMSO-d₆, 11H); 8.30 (s, 2H, C₃, C₅–H), 8.280 (s, 2H, C₂, C₆–H), 8.154 (d, J = 16.0 Hz, 1H, C_β–H), 7.892-7.891(m, 1H, C_{6'}–H), 7.773 (d, J = 15.6 Hz, 1H, C_α–H), 7.684-7.679 (m, 1H, C_{2'}–H), 7.118 (s, 1H, C_{5'}–H), 6.176 (bs, 2H, –OCH₂O–).

(ii) 2 – chloro – 4' – methyl chalcone, 3b

C₁₆H₁₃OCl, light brown solid, yield 60%; m. p. 115-116 °C, λ_{max} (CH₃OH; nm); 255, 205 IR (KBr, cm⁻¹); 3100.00 (C=C–H, olefinic, str), 3000 (aliphatic –C–H, asym.-str.), 2930.40 (aliphatic –C–H, sym.-str.), **1625.82 (C=O, conjugated keto group)**, 1510.49 (C=C, aromatic), 1474.26, 1440.94, 1408.43, 1384.50 (–CH₃ bend), 1226.77, 1182.28, 1119.84 (C–O–C, str), 1035.65 1066.80, 886.28 (C–O str), 824.98 (C–H, bending aromatic), 752.87 (C–Cl).

(iii) 2 – hydroxy – 2' – chloro chalcone

C₁₅H₁₁O₂Cl; light yellow solid, yield 62%; m.p: 82 - 83 °C, λ_{max} (CH₃OH, nm) ; 310, 200, IR (KBr, cm⁻¹); 3435.96 (–OH, broad), 2927.35 (aliphatic –C–H, asym. -str.),

1691.61 (C=C, olefinic str.), 1640.69 (C=O, conjugated keto group), 1582.78 (C=C, aromatic), 1485.10, 1440.65, 1365.99, 1339.09, 1289.79, 1260.30, 1204.99 (C–O–C, str) 1154.58, 1050.62, 1019.43, 973.45 (C–O str), 863.70, 852.38, 825.16, 787.61 (C–H, bending aromatic), 755.12 (C–Cl), ¹H NMR, δ_{H} (500 MHz, CDCl₃, 11H); 12.786 (s, 1H, –O–H), 8.325 (d, 1H, $J=15$ Hz C _{β} –H), 7.930 (d, 1H, $J=10$ Hz, C _{β'} –H), 7.788 (d, 1H, $J=7.5$ Hz, C₃–H), 7.655 (d, 1H, $J=15$ Hz, C _{α} –H), 7.530 (t, 1H, C₅–H), 7.483 (dd, 1H, $J=7.5$ Hz C _{β'} –H), 7.390–7.340 (m, 2H, C₄–H, C₆–H), 7.058 (d, 1H, $J=7.5$ Hz, C _{β'} –H), 6.970 (d, 1H, $J=7.5$ Hz, C₄–H).

(iv) 2' – hydroxyl – 2, 4, 6 – trimethoxy chalcone, 3d

C₁₈H₁₈O₅, orange-yellow solid, yield 61%; mp 126–128 °C, λ_{max} (CH₃OH, nm); 360, 265, IR (KBr, cm⁻¹); 3413.38 (–OH); 2931.33 (–CH₃, asym.-str.) 2839.28 (–CH₃, sym-str.), 1964.00 (C=C str.), 1624.25 (s, C=O, conjugated keto group), 1548.28 (C=C, aromatic), ¹H NMR, δ_{H} (400 MHz, CDCl₃, 18H); 8.356 (d, $J=15.6$ Hz, 1H, C _{β} –H), 7.898 (d, $J=15.6$ Hz, 1H, C _{α} –H), 7.362 (m, 1H, C _{β'} –H), 7.011–6.993 (m, 1H, C₃–H), 6.885–6.863 (m, 2H, C_{4'}, C_{5'}–H), 6.124 (bs, 2H, C₃, C₅–H) 3.890 (s, 9H, –OCH₃).

2.4 Antioxidant Activity

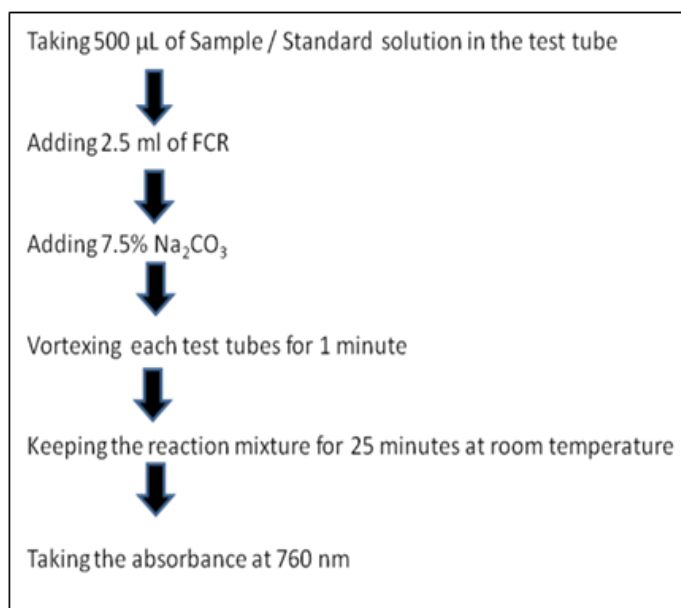
2.4.1 Antioxidant Activity by Gallic acid equivalent antioxidant capacity (GEAC) assays

Phenolic compounds are important plant constituents with

redox properties responsible for antioxidant activity.¹⁸ The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. As a basis, phenolic content was measured using the Folin–Ciocalteu Reagent (FCR).¹⁹ It is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric *in vitro* assay of phenolic and polyphenolic antioxidants, also called the Gallic Acid Equivalence method (GAE/GAEC).²⁰ The reagent does not measure only phenols, but will react with any reducing substance. It therefore measures the total reducing capacity of a sample.

2.4.1.1 Procedure

Firstly, the stock solution of samples and Gallic acid were prepared by dissolving 1mg of sample/standard compound in 1mL 70% methanol. Then solution of different concentrations (2–16 $\mu\text{g/mL}$) of Gallic acid were made. The concentration of sample solution was made 50 $\mu\text{g/mL}$. From these solutions 0.5mL sample/standard solution was taken in a test tube, then 2.5mL of FCR was added. finally 2 mL of sodium carbonate solution was added. After preparing reaction mixture, the mixture was vortexed for 1minute. The reaction mixture's absorbance was measured at 760 nm after being kept at room temperature for 25 minutes. The results were derived from a calibration curve and expressed in Gallic Acid equivalents in $\mu\text{g/mL}$. Schematically-



Scheme 2: Schematic representation of procedure of GEAC assay

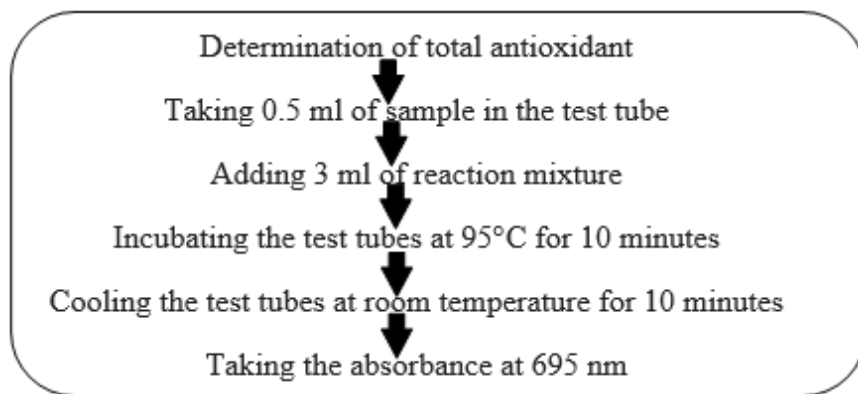
2.4.2 Total Antioxidants Determination by UV Spectroscopy

Despite the physiological role of oxidant molecules, oxidative stress (OS) could underlie several human diseases. When the levels of antioxidants are too low or too high, OS occurs, leading to damage at the molecular, tissue and cellular levels. Therefore, antioxidant compounds could represent a way to modulate OS and/or to maintain proper redox balance.²¹

2.4.2.1 Procedure

Firstly, the stock solution of samples/standard Butylated hydroxyl toluene (BHT) were prepared by dissolving 1mg

of sample/standard compound in 1mL 70% methanol. Then the stock solution of BHT was diluted to make different concentration (2–16 $\mu\text{g/mL}$). The sample concentration of 3a–3d were 71.42 $\mu\text{g/mL}$. From stock solution, 0.5 mL sample/standard solution was taken in a test tube, then 3mL of reagent mixture (1mL H₂SO₄ + 1mL [NH₄]Mo₇O₂₄.4H₂O, Ammonium molybdate + 1mL Na₃PO₄) was added. After preparing reaction mixture, test tubes were incubated at 95°C for 10 minutes. The reaction mixture's absorbance was measured at 695 nm after being cooled at room temperature for 10 minutes. The results were derived from a calibration curve and expressed in BHT equivalents in $\mu\text{g/mL}$.



Scheme 3: Schematic representation of procedure of Total antioxidant assay

2.5 Application on Carbomer Gel

2.5.1 Coloration on commercial carbomer gel

Commercial carbomer gel (polyacryl amide) is major ingredients of cosmetics, especially thickening agent of shampoo, lotion, cream etc. or used as ultrasonic or ECG gel. 2 mL sample 5% ethanolic solutions of 3a-3d (cosmetics grade) were prepared for investigation. Each of these ethanolic solutions were added to 5 g of commercial carbomer gel (ECG) (polyacryl amide) kept in four

different containers. The solutions were properly mixed with the gel. Nice color penetration being observed. The change in color, physical state, odor and pH were observed for three months. Sample-gel mixture was studied by spectrophotometer and λ_{\max} of the mixtures were determined and compared with that of sample and carbomer gel. Besides dried Sample-gel mixture was investigated by IR spectrum thus compared with sample.

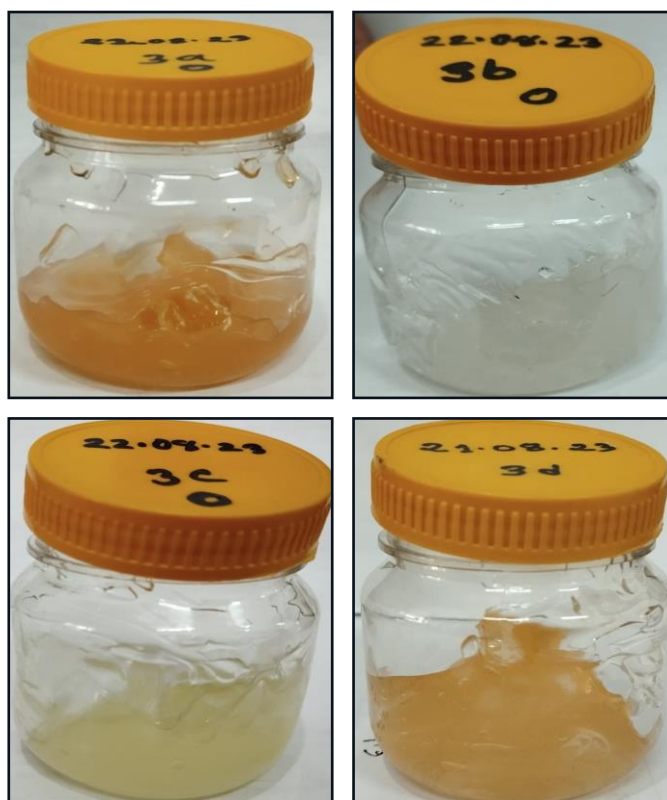


Fig. 1 Application of (3a-3d) on Carbomer gel (ECG gel (KONIX) to observe coloration

3. Results and Discussion

3.1 Results for Antioxidant Activity by GEAC Method

Chalcones basic structure includes two aromatic rings bound by α , β -unsaturated carbonyl group, a unique template associated with diverse application. Due to the presence of the reactive keto-vinyl group, chalcones and their analogues have been reported to be antioxidant. Hydroxyl, methoxy and benzyloxy substituents are associated with antioxidant properties.

A calibration curve of Gallic acid was made by taking absorbance at 760 nm preparing four different concentrated solution as well as blank solution (Table-1) and plotting absorbance (deducting value of the Blank) vs concentration graph (Fig.2). The results for compounds 3a-3d were derived from a calibration curve ($y = 0.1118x + 0.0288$, $R^2 = 0.9945$) of Gallic acid (2–16 $\mu\text{g/mL}$) and expressed in Gallic acid equivalents (GAE) in $\mu\text{g/mL}$ presented in

Table-2 and graphically by Fig. 3.

Table-1: Absorbance of Gallic Acid solution at 760 nm.

Concentration of Gallic acid ($\mu\text{g/mL}$)	Absorbance
2	0.331*
4	0.461
8	0.865
16	1.840
Blank	0.120

$$*\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{blank}}$$

Table-2: Determination of Gallic Acid equivalent of sample.

Sample	Concentration of sample ($\mu\text{g/mL}$)	Absorbance	Gallic acid equivalent ($\mu\text{g/mL}$)
3a	50	0.156	27.91
3b	50	0.255	45.61
3c	50	0.13	23.26
3d	50	0.194	34.70

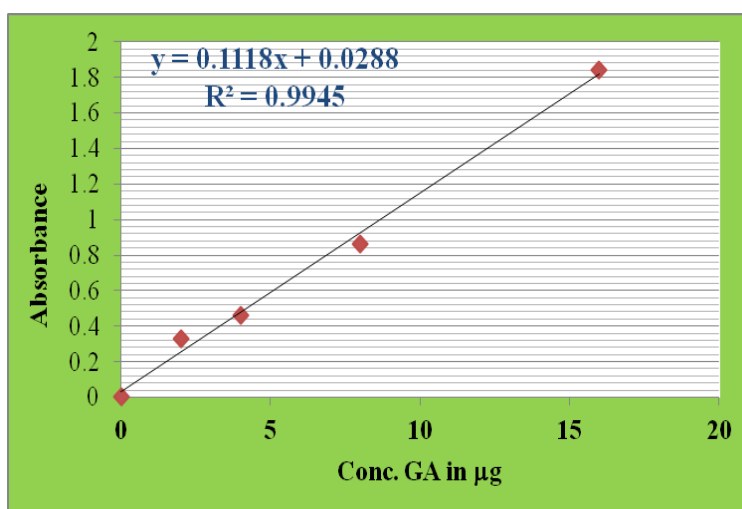


Fig. 2 Calibration curve for Gallic acid.

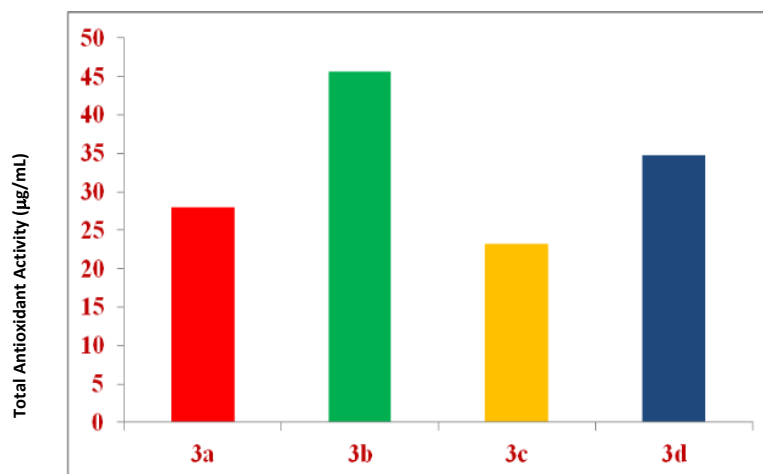


Fig. 3 Bar diagram for the GEAC of samples 3a-3d in $\mu\text{g/mL}$

Compound 3c and 3d contain phenolic group and compound 3d showed very good GEA capacity, 34.70 $\mu\text{g/mL}$. Although compound 3b don't have any phenolic group but presence of chlorine enhanced GEAC value. Compound 3b has the highest value 45.71 $\mu\text{g/mL}$. Due to the lack of phenolic $-\text{OH}$ group compound 3a shows moderately good value, 27.91 $\mu\text{g/mL}$. Although compound 3c contains both $-\text{OH}$ and $-\text{Cl}$ group it shows least value 23.26 $\mu\text{g/mL}$. Since $-\text{OH}$ group is in 2 positions of left ring and $-\text{Cl}$ occupy 2 positions in the right ring so some steric

congestion may arise. Moreover, due to nearness of these two groups a dipole-dipole interaction may exist and reduce the free radical generation process.

3.2.2 Results for Total Antioxidant Activity by Total Antioxidant Method

A calibration curve of BHT was made by taking absorbance at 695 nm preparing three different concentrated solution as well as blank solution (Table-4) and plotting Absorbance (deducting value of the Blank) vs

concentration graph (Fig.4).

Table-3: Absorbance of BHT solution at 695nm.

Concentration ($\mu\text{g/mL}$)	Absorbance
16	0.406*
8	0.111
2	0.041
blank	0.048

*Absorbance_{sample} – Absorbance_{blank}

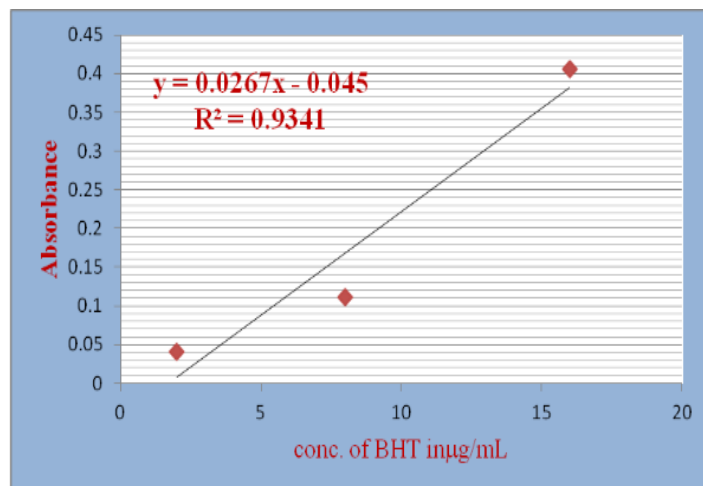


Fig. 4 Calibration curve for BHT

The results for compounds 3a-3d were derived from a calibration curve ($y = 0.0267x - 0.045$, $R^2 = 0.9945$) of BHT (2–16 $\mu\text{g/mL}$) and expressed in BHT equivalents in $\mu\text{g/mL}$ presented in Table-4 and graphically by Fig.5.

Table-4: Absorbance of sample solution at 695nm.

Sample	Absorbance	Total antioxidant equivalent to BHT ($\mu\text{g/mL}$)
3a	0.346	181.44
3b	0.494	260.11
3c	0.331	173.58
3d	0.353	185.12

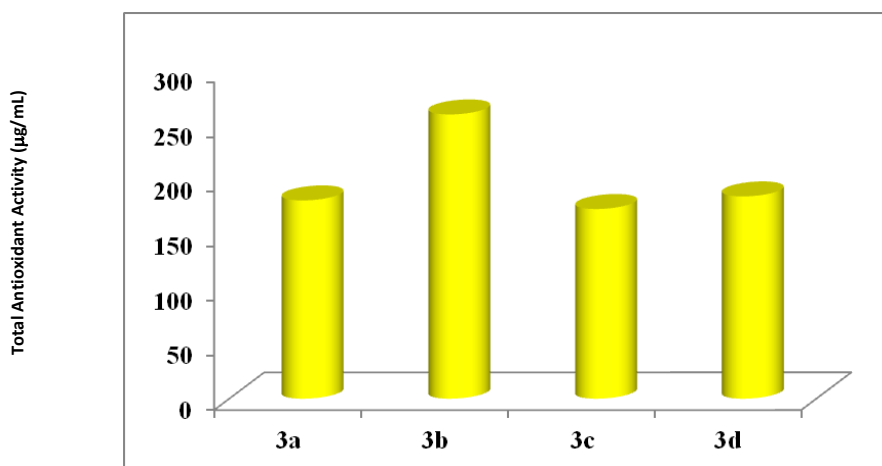


Fig. 5 Bar diagram for the Total Antioxidant Content of samples 3a-3d in $\mu\text{g/mL}$

The trend is similar as GEAC value. Compound 3b has the highest value 260.11 $\mu\text{g/mL}$ BHT equivalent. Compound 3c and 3d contain phenolic group and compound 3d showed very good Total Antioxidant, 185.12 $\mu\text{g/mL}$. Due to the lack of phenolic $-\text{OH}$ group compound 3a shows moderately good value, 181.44 $\mu\text{g/mL}$. Although compound 3c contains both $-\text{OH}$ and $-\text{Cl}$ group it shows least value 173.58 $\mu\text{g/mL}$. The reason might be similar as

being found in GEAC assay.

3.2.3 Results for Application on Carbomer Gel

3.2.3.1. pH Test and Color Effect

Color stabilizes and gel state remained undisturbed and no layer separation being observed. pH of the gel remained unchanged about a month and no unwanted smell being found.

Table-5: pH value of sample with carbomer gel in day of mixing and in different time interval upto three months.

Sample	pH	pH (After 24 hrs.)	pH (After 15 days)	pH (After 3 months)
3a	6.84	6.72	6.60	No significant change
3b	6.98	6.96	6.85	
3c	6.97	6.54	6.70	
3d	6.79	6.66	6.59	
blank	6.76			

3.2.3.2 Binding Efficacy study by UV Spectroscopy

Chalcones, a group of compounds with two aromatic rings connected by a keto-vinyl chain, -CO-CH=CH- so these have some unique structural features in spectroscopy. The oxygenated chalcones usually possess UV absorption maxima in the range of 340-390 nm and chalcones lacking B-ring oxygenation may have their absorption at considerably shorter wavelengths and a minor peak usually appears in the range of 220-270 nm. The functional moiety

in colorless polyacryl amide is $[-CH_2-CH(CONH_2)-]$ and usually possess UV absorption maxima in the range below 300 nm. UV absorption maxima being studied for sample-gel mixture and shift of wavelength and intensity (Fig. 6, Fig.7) are presented in Table-6. Wavelength and intensity of sample - gel mixture were compared to that of sample and gel for the prediction of binding interaction of sample with gel.

Table-6: λ_{max} (nm) value of sample, Carbomer gel and Sample - carbomer gel along with Absorbance value determined by UV Spectroscopy

Sample	λ_{max} , nm (Absorbance)	Sample – Carbomer gel mixture	λ_{max} , nm (Absorbance)
3a	280 (0.291), 205 (0.277)	3a + Gel	270 (1.722), 205(0.911)
3b	255 (6.016), 205 (5.958)	3b + Gel	270 (2.755), 250 (3.188)
3c	310 (3.198), 200 (1.484)	3c + Gel	325 (1.237), 250 (1.988)
3d	360 (0.505), 265 (0.58)	3d + Gel	265 (0.256), 245 (0.089)
Carbomer Gel	260, 240 Low intensity		

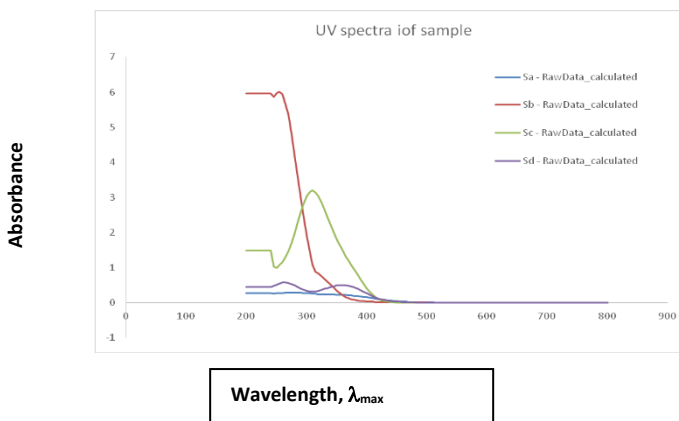


Fig. 6: Compiled UV Spectra of Samples (3a-3d)

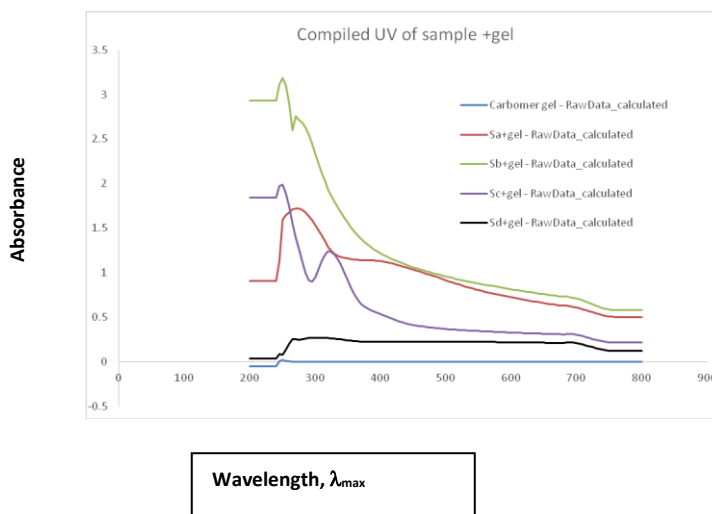


Fig. 7: Compiled UV Spectra of Samples (3a-3d) with Gel

In UV spectroscopy λ_{max} $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ are shifted to shorter wavelength when the sample mixed with gel

specially for compound 3a and 3d. Deep coloration of compound 3a and 3d in Fig. 1 also support this observation. Such blue shift indicates more energy is required for $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transition which is a clear indication of binding interaction²² and high potency of the compounds as cosmetics ingredients.

Compounds form Specially very attractive transparent colors with gel and the binding mechanism being observed by IR spectroscopic technique as -CO-CH=CH- group in chalcones show a characteristic sharp peak around 1600-1650 cm^{-1} whereas amide group show peak near at 1725 cm^{-1} for C=O group. Wave number for sample mixed gel, carbomer gel and sample are shown in Table- 7 and Fig. 8 and Fig. 9.

3.2.3.3 Binding Efficacy study by IR Spectroscopy

Table-6: IR wavenumber (cm^{-1}) value of sample 3a-3d, Carbomer gel, and Sample with carbomer gel determined by IR Spectroscopy.

Sample	Wavenumber (cm^{-1})	Sample Carbomer – gel mixture	λ_{max} , nm (Absorbance)
3a	1663.11	3a + Gel	1634,1737
3b	1625.88	3b + Gel	1631,1733
3c	1640.69 , 3435.96	3c + Gel	1631, 1739, 3428
3d	1634.25, 3413.28	3d + Gel	1631, 1731, 3420
Carbomer Gel	260, 240 Low intensity		

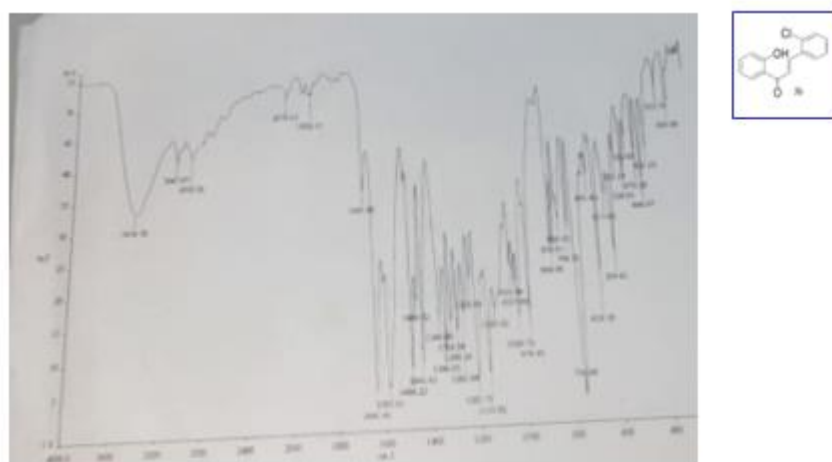


Fig. 8: IR spectrum of sample 3c.

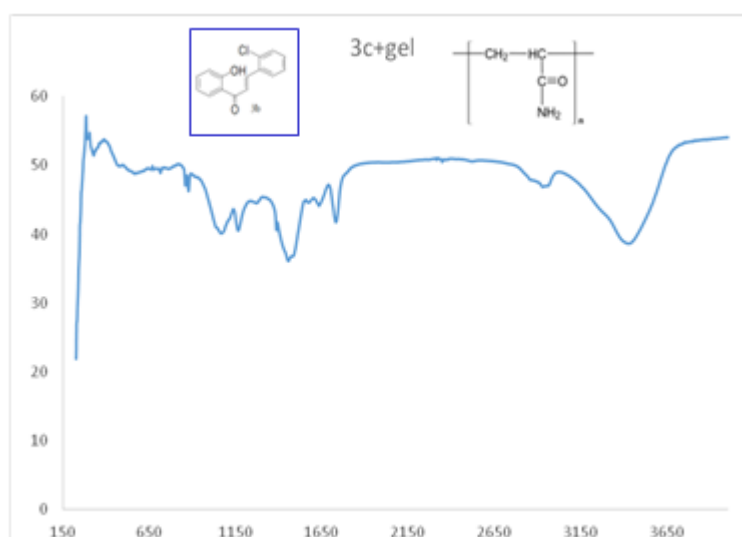


Fig. 9: IR spectrum of sample 3c and gel.

Sample mixed gel showed a broad peak for -OH group with low wave number value indicating Hydrogen bond formation. Besides in mixed sample a new broad peak around 1630-1635 cm^{-1} being found. The original sharp sample peak disappeared. Such shift to lower wave number

indicates more single bond character in C=O which can be obtained by binding of lone pair of electrons of Oxygen with some electrophile. Especially compound 3a and 3d showed better coloration as shown in Fig.1 as well as spectroscopic data also is in favor of binding interaction.

The peak around 1725 cm^{-1} might arise from excess polyacryl amide.

4 Conclusions

The trend for antioxidant activity by Galic Acid Equiuvalent Capacity (GEAC) at $50\text{ }\mu\text{g/mL}$ sample concentration

3b (45.61) > 3d (34.70) > 3a (27.91) > 3c (27.91)

The trend for Total antioxidant activity by Butylated hydroxyl toluene (BHT) Equivalent Capacity (GEAC) at $71.42\text{ }\mu\text{g/mL}$ sample concentration

3b (260.11) > 3d (185.12) > 3a (181.44) > 3c (173.58)

Highest activity in both case is observed for compound 2 – chloro – 4' – methyl chalcone, 3b.

Although compound 3b don't have any phenolic group but presence of chlorine enhanced antioxidant acitivity value. Compound 3c and 3d contain phenolic group and compound 3d showed very good activity. Due to the lack of phenolic –OH group compound 3a shows moderately good value. Although compound 3c contains both –OH and –Cl group it shows least value in each case. Since –OH group is in 2 positions of left ring and –Cl occupy 2 positions in the right ring so some steric crowd suppose to suppress the activity. Moreover, due to nearness of these two groups a dipole-dipole interaction may arise to decrease the free radical generation process.

The potency of cosmetics additives of the compounds were examined as applied on carbomer gel (polyacryl amide), major cosmetic thickening ingredients and the stability of gel being studied by appearance and pH change of mixture. Compounds (3a-3d) form very attractive transparent colors with gel and the binding mechanism being observed by IR and UV spectroscopic technique.

In UV spectroscopy λ_{max} $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ are shifted to shorter wavelength when the sample mixed with gel specially for compound 3a ($280\rightarrow 270\text{ nm}$) and 3d ($360\rightarrow 265\text{ nm}$). Deep coloration of compound 3a and 3d in Fig. 1 also supports this observation. Such blue shift indicates more energy is required for $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transition which is a clear indication of binding interaction and high potency of the compounds as cosmetics ingredients.

The binding mechanism also being observed by IR spectroscopic technique. In mixed sample a new broad peak around $1630\text{-}1635\text{ cm}^{-1}$ being found. The original sharp sample peak disappeared. Such shift to lower wave number indicates more single bond character in C=O which can be obtained by binding of lone pair of electrons of Oxygen with some electrophile. Especially compound 3a and 3d showed better coloration as shown in Fig.1 as well as spectroscopic data also is in favor of binding interaction.

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