Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 8 Issue 4, April - 2021

Dye and Preservative Efficacy Screening of Some Synthesized Substituted Chalcones through their Bioactivity

Mymuna Sabrina[1], Most. Sayima Siddika[2], M. Azizul Islam[4], Bilkis Jahan Lumbiny[5]*
Departmant of Chemistry
University of Rajshahi
Rajshahi-6205, Bangladesh
*blksjahan@yahoo.com

Abu Taher [3]
Department of Chemistry
Inha University
Incheon 402-751, South Korea

***Abstract*—Health benign chemical additives like preservative, color, fragrance, dye etc. are in continuous search to reduce the health risk specially child's as well as that of adults. Processed food marketed for children with appealing color, attractive fragrance and fresh look might cause their attention deficit hyperactivity disorder, disability, obesity, cardiovascular disease even cancer. Chalcone; an important class of colored biocompound having non-nitrogeneous chromophore were synthesized by Claisen-Schmidt condensation reaction. Compounds 2′- hydroxy-3,4-methylene dioxy chalcone, 3a, 3′, 4′- methylenedioxy-2, 4, 5-trimethoxy chalcone, 3b, 4′- benyloxy-2′, 4,- dihydroxy - 3-methyl chalcone 3c, 2´, 4- dihydroxy chalcone, 3d were characterized by spectral analysis and screened in vitro for their antibacterial activity against four pathogenic bacteria by disc diffusion. Most of the compounds show moderately good antibacterial activity against both G^+ and G^- bacteria. Interestingly Staphylococcus aureus showed resistance to the standard Azithromycin antibiotics whereas compounds 3b (10 mm) and 3c (8 mm) showed better activity than drug (0 mm). Moreover, in vitro antioxidant activity using diphenylpicrylhydrazyl (DPPH) model was carried out and very good radical scavenging efficacy being found. Synthesized compounds easily penetrate through carbomer gel while added and produces appealing colors without any change in pH for six months supports dye efficacy of these compounds.**

Keywords— Health benign, Preservatives, Dye, Chalcones, Antimicrobial, Antioxidant activity

I. INTRODUCTION

The growing economy of Bangladesh is mostly rely on agriculture and rapid advancement of industries like textile, leather, food, cosmetics etc. Tropical climate of Bangladesh allows quick spoilage of food and other products due to microbial growth and air oxidation. So health benign, safe chemical additives are in continuous search to protect decomposition trough microbial growth or some other undesirable chemical changes and to achieve sustainable economic growth. The principal classes of widely used chemical additives like preservative, color, dye, fragrance etc. in these industries have become a serious concern as these are taken by human body or used externally everyday [1]. Over the last few decades, the number of chemicals added to foods and other products has skyrocketed [2]. Preservatives are added to foods to keep them fresh. Some synthetic dyes are added to foods to make them look more appealing or in lotions and beauty products to make them feel, look, and smell nice [2]. In a policy statement entitled "Food Additives and Child Health" [3] the American Academy of Pediatrics warns about these harms — and points out that they often are worse for children. Children are smaller, so their "dose" of any given chemical ends up being higher [3] and might increase their attention deficit hyperactivity disorder, disability, obesity, cardiovascular disease even cancer [2]. Flavonoids (Fig.1.), 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 [4]. Chalcone (Fig. 1.), a distinct subclass of flavonoid, natural color pigments are safe for food and dyes were reported to exert antimicrobial [5], antioxidant [5] and radical-scavenging [6] activities because of polyphenolic groups and have been recently recommended for use as food colorants and preservative. Chemically chalcones are 1, 3-diphenyl-2-propene-1-one, a non-nitrogenous class of chromophore in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system (-CO-CH=CH-).These are mostly yellow colored compounds and lightening and deepening of color take place for the presence of other auxochromes [7].

So some structurally modified chalcones; 2′-hydroxy-3, 4 - methylenedioxy chalcone, 3a, 3′, 4′-methylenedioxy - 2, 4, 5-trimethoxy chalcone, 3b, 4′-benzyloxy-2′, 4,- dihydroxy - 3-methyl chalcone, 3c, 2´, 4 - dihydroxy chalcone, 3d were synthesized by Claisen-Schmidt reaction and characterized by

spectral techniques (UV, IR, ^{1}H NMR,^{13}C NMR). Preservative efficacy were screened through antimicrobial and antioxidant activity .

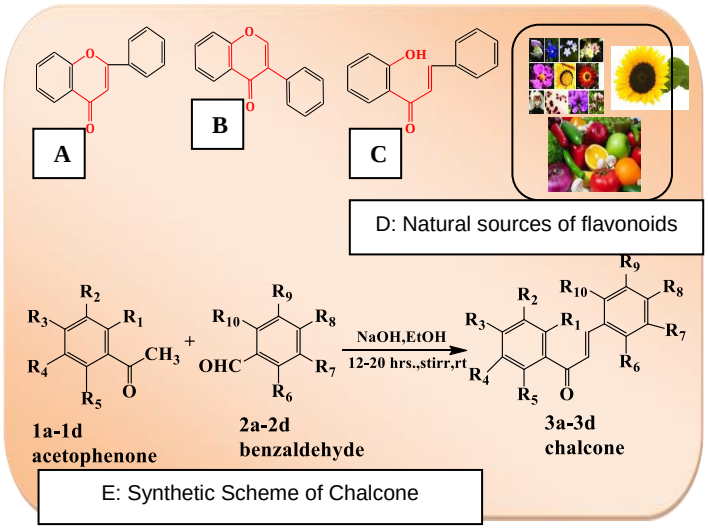


Fig. 1. Classification of flavonoids , flavones (A) , isoflavones (B) , Chalcone (C), Natural sources of Flavonoids (D) and general synthetic scheme for chalcones (E)

In addition dyeing efficacy was tested by their penetrating capacity onto carbomer gel and color stability as well.

II. RESEARCH METHOD

2.1 Materials

GR grade starting materials; substituted acetophenone (1a-1d), substituted benzaldehyde (2a-2d), were purchased from Sigma Aldrich and used without further purification. Column chromatography was performed on silica gel (Merck, 60-120 mesh). Other chemicals were reagent grade EtOH, MeOH, NaOH, diphenylpicrylhydrazyl (DPPH) and purchased from Sigma-Aldrich company.

2.2 Procedures of Synthesis and characterization

Synthesis (Fig.1.) of substituted chalcones (**3a-3d**) were carried out by conventional method [1, 8, 9, 10, 11, 12, 13 and 14]. Substituted acetophenones (0.01 mol) and substituted benzaldehydes (0.01 mol) in ethanolic (30 mL) solution were mixed in presence of NaOH (20%, 15.0 mL). The reaction mixture was stirred for 12 hrs and kept overnight at room temperature. Then it was diluted with ice cold water and acidified with ice cold dil. HCl. until a solid being precipitated (**3a-3d**). The precipitate was filtered, washed and dried. For some cases it was further purified by column chromatography using pet ether-ethyl acetate solvent system.

2'- hydroxy-3,4-methylenedioxy chalcone, 3a

$C_{16}H_{12}O_4$, Solid and yellow; m. p.155-156 ºC, λ_{max} (CH_3OH, nm) ; 369.5, 423.5, IR (KBr, cm^{-1}): 3435.83 (-OH), **1641.01 (C=O, conjugated keto group)**, ^{1}H NMR, δ_H (400 MHz, $CDCl_3$, 12H); 12.881 (s. 1H), 7.884 (d, 1H, *J*=8.0 Hz), 7.826 (d, 1H, *J* =15.2 Hz, **C_β-H)**, 7.486-7.448 (m, 1H), 7.467 (d, 1H, *J* =15.2 Hz, **C_α-H**), 7.163-7.126 (m, 2H), 7.007 (d, 1H, *J*=8.4 Hz), 6.941-6.903(m, 1H), 6.841(d, 1H, *J=7.6 Hz*), 6.022 (s,2H, -OC$\underline{H_2}$O-), ^{13}C-NMR (100 MHz, $CDCl_3$, 16C); δ_c 193.54, 163.54, 150.30, 148.50, 145.33, 136.22, 129.51, 129.07, 125.75, 120.07, 118.79, 118.60, 117.99, 108.75 , 106.74, 101.75

3', 4'- methylenedioxy-2, 4, 5 - trimethoxy chalcone (3b)

$C_{19}H_{18}O_6$, dark yellow solid; m. p. 140-141 ºC, λ_{max} (CH_3OH, nm ; 378.5, 411.5, 533.5, IR (KBr, cm^{-1}); **1635.74 (s, C=O, conjugated keto group)**, ^{1}H NMR, δ_H (400 MHz, $CDCl_3$, 18H); 8.211 (d, *J* =15.6 Hz, 1H, **C_β-H**), 7.773 (d, *J* =7.6 Hz, 1H), 7.655 (s, 1H), 7.566 (d, *J* =15.2 Hz, 1H, **C_α-H**), 7.010 (d, *J*= 8.0 Hz, 1H), 6.652 (s, 2H), 6.180 (s, 2H, -OC$\underline{H_2}$-O), 4.056 (s, 9H, -OC$\underline{H_3}$), ^{13}C-NMR (100 MHz, $CDCl_3$, 19C); δ_c 188.68, 154.49,152.29,151.20,148.01, 143.01, 139.48, 133.39, 124.39,119.69,115.43, 111.34, 108.33, 107.71, 101.67 , 96.75 , 56.26 (3C)

4'- benzyloxy- 2', 4 - dihydroxy, 3-methyl chalcone, 3c

$C_{23}H_{20}O_4$; light brown; m.p. 275-276 ºC , λ_{max} (CH_3OH, nm ; 394.5, 485.0, IR (KBr, cm^{-1}): 3435.81 (-OH), **1626.29 (s, C=O, conjugated keto group),** ^{1}H NMR, δ_H (400 MHz, $CDCl_3$, 20H) ; 12.718 (s, 2H), 7.631 (d. 1H, *J* = 8.4 Hz), 7.403-7.336 (m,7H), 7.386 (d, *J* = 13.6 Hz, 1H, **C_β-H**), 7.369 (d. 1H *J* =14.8 Hz, **C_α- H**), 6.523-6.496 (m, 3H), 5.086 (s, 2H), 2.548 (s, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$, 23C); δ_c 202.61, 165.19 (3C), 135.78, 132.36(2C),128.81 (3C), 128.72 (2C), 128.33, 127.54 (3C), 114.10, 108.16, 101.89 (2C), 70.23, 22.99

2', 4- dihydroxy chalcone, 3d

$C_{15}H_{12}O_3$; deep brown solid; m. p. 284-285 ºC λ_{max} (CH_3OH, nm ; 340.5, 533.0, IR (KBr, cm^{-1}): 3401.19 (-OH) **1605.03 (C=O, conjugated keto group),** ^{1}H NMR, δ_H (400 MHz, $CDCl_3$, 12H); 12.830 (s, 1H), 11.750 (s, 1H), 7.931-7.758 (m, 2H), 7.880 (d, *J* =14.0 Hz, 1H**,** **C_β-H**), 7.655-7.379 (s, 2H), 7.530 (d, *J* =17.6 Hz, 1H, **C_α-H**), 7.393 (d, 1H, *J* = 8.4 Hz), 7.104 – 6.897 (m, 2H). 6.887 (d, 1H, *J* = 8.4 Hz)

2.3 Preservative Efficacy

As Bangladesh lies in tropical zone so preservation of agroproducts from the growth of microorganism as well as air oxidation being very urgent issue. So *in vitro* Antibacterial and antioxidant activity were examined to explain their preservative potency.

2.3.1 Antibacterial Activity

In vitro antibacterial activities of the test chemicals **(3a-3d)** were studied using four pathogenic bacteria *Bacillus cereus (*B_1 **G^+,** food borne illness germs**),** *Staphyllococcus aureus (*B_2, **G^+,** skin infection germs) *Escherichia coli (*B_3, **G^-,** urine infection, diarrhea germs) *Pseudomonas,* B_4, **G^-**, respiratory

infection germs) were collected from the Pharmaceutical Microbiology laboratory, Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh and studied through Disc diffusion, a primary assay technique [1, 8,11,14]. The bioactivity is expressed by the diameter of zone of inhibition in mm comparing to those of the standard drug (Azithromycin, Az-30). Higher the zone of inhibition higher the activity.

2.3.2 Antioxidant Activity

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 1, 1-diphenyl-2 picrylhydrazyl (DPPH) radical [1,8,11,14] by UV Spectrophotometer. The electron in the DPPH free radical gives a strong absorption maximum at 517 nm and purple in color in methanolic solution. The color turns purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to no. of electrons captured. The activity is measured in terms of % inhibition and minimum (50%) inhibitory concentration IC_{50} from graph.

Inhibition (%) = $[(A_{517}$ of control $- A_{517}$ of sample$)/A_{517}$ 0f control$] \times 100$ [A = Absorbance]

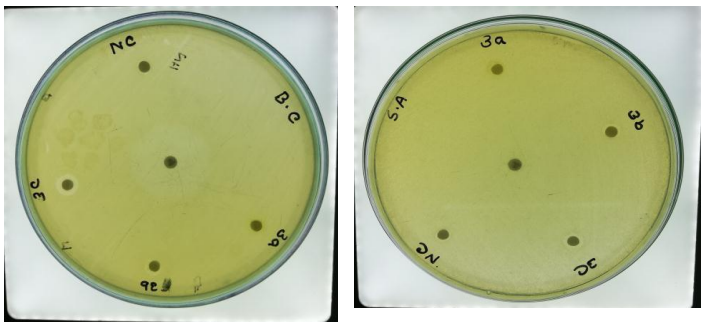
2.4 Dye efficacy Test

Dye effect was tested while 2 mL 0.1% ethanolic sample solution (tolerable range for food and cosmetics) was added on 5 g of commercial carbomer gel (polyacryl amide), major ingredients of cosmetics, ultrasonic or ECG gel. Color stability, pH and odor being observed for six months

III RESULTS AND DISCUSSIONS

3.1 Results for Antimicrobial Screening

Antibacterial activities of all the compounds **(3a-3d)** were employed as test against four bacteria and The result of antibacterial screening is presented as tabular form in Table-1 and Fig. 2. (for G^+ bacteria) and Table-2 and Fig. 3. (for G^- bacteria)



(I) (II)

Fig. 2. Photographic representation of the zone of inhibition at the concentration of 250 µg / disc against (I) *Bacillus cereus* (G^+, B_1), (II) *Staphyllococcus aureus* (G^+, B_2)

TABLE 1. Results of the antibacterial activity of the compounds (**3a-3d)** against *Bacillus cereus.*(G^+, B_1) and *Staphyllococcus aureus* (G^+, B_2) at 250 (µg/disc)

Molecular formula	Diameter of the zone of inhibition (mm)			
	Bacillus cereus (G^+)		*Staphyllococcus aureus* (G^+)	
	250 µg/ disc	*Az-30	250µg $disc^{-1}$	*Az-30
3a	**0**	**34**	**0**	**0**
3b	**8**		**8**	
3c	**11**		**10**	
3d	**0**		**0**	

*Az-30 means Azithromycin 30µg/ disc

The trend for antibacterial (*Bacillus cereus,* G^+, B_1) activity at conc. 250µg/ disc

_Az_-30 (34mm) > 3c (11 mm) > 3b (8 mm) > 3a, 3d (0 mm)

It was found that the inhibition zones of compound **3b** and **3c** were more effective but smaller than standard.

Antibiotic resistance is one of the biggest threats to global health, food security and development today [15]. Antibiotic resistance occurs when bacteria changes in response to the use of this medicines and able to cause serious diseases and this could be a major public health problem. For example *Staphylococcus aureus* are now almost always resistant to benzyl penicillin which worked in the past [15].

The trend for antibacterial activity (*Staphylococcus aureus,* G^+, B_2*)* at conc. 250 µg/ disc

3c (10 mm) > 3b (8 mm) > 3a, 3d (0 mm) = *Az–30 (0 mm)

Very interestingly the standard Azithromycin does not inhibit the microbial growth of B_2 but compounds 3b (10 mm) and 3c (8 mm) showed better activity than drug (0 mm). So some intensive focus should be given to these compounds.

The trend for antibacterial (*E. Coli* , G^-, B3) *activity at conc.* 250 µg /disc (Table -2)

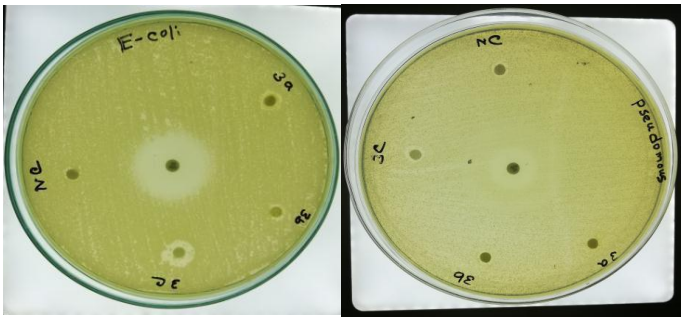
***Az*-30 (32mm) > 3c (15 mm) > 3b (10 mm) > 3a (11 mm)**

The trend for antibacterial *Pseudomonus* , G^-, B4) *activity at conc.* 250 µg/ disc (Table -2)

***Az*-30 (15 mm) > 3c (11 mm) > 3b (8 mm) > 3a, 3d (0 mm)**

TABLE 2.: Results of the antibacterial activity of the compounds (3a-3d) against *E.Coli* (G^-, B_3) at 250 (µg /disc) and *Pseudomonus* (G^-, B_4) at 250 (µg /disc)

Molecular formula	Diameter of the zone of inhibition (mm)			
	E.Coli (G^-, B_3)		*Pseudomonus* (G^-, B_4)	
	250 µg/ disc	*Az-30	250µg/ disc	*Az-30
3a	**11**	**32**	**0**	**15**
3b	**10**		**8**	
3c	**15**		**11**	
3d	**0**		**0**	



(III) (IV)

Fig. 3. Photographic representation of the zone of inhibition at the concentration of 250 µg/ disc against (III) ***E.Coli*** and (IV) *Pseudomonus*

Compounds **3c** and **3b** exhibited fairly good potentialities against both bacteria and in some cases about near to that of the standard drugs. Very interesting results are found with this drug resistant bacteria *Staphylococcus aureus*. The standard Azithromycin does not inhibit the microbial growth but compounds 3b and 3c showed better activity than drug. This led us to conclude that the presence of electron releasing hydroxyl (-OH), benzyloxy and methyl groups in 3c and methoxy group ($-OCH_3$) in 3b are responsible for the antimicrobial effects. So some intensive focus should be given to these compounds as a better option for Antibiotic resistant micro-organism.

3.2 Results for Antioxidant Screening

The antioxidant activity is measured in terms of % inhibition and minimum (50%) inhibitory concentration IC_{50} . Generally smaller the IC_{50} value higher the percent inhibition higher the antioxidant activity.

TABLE 3. DPPH radical scavenging data of synthesized chalcones **(3a-3d)**

Compound No	Conc. µg /mL	% Inhibition	*IC_{50}
3a	**15**	**76.85**	<15 µg /mL
	25	**77.08**	
	50	**78.47**	
3b	**5**	**72.20**	<5 µg /mL
	15	**74.53**	
	25	**76.85**	
	50	**79.17**	
3c	**2**	**74.30**	<5µg /mL
	15	**75.92**	
	25	**76.16**	
	50	**75.92**	
	100	**79.86**	
3d	**5**	**83.79**	< 5µg/mL
	15	**86.17**	
	50	**94.21**	
	100	**94.91**	

*Ascorbic acid is the standard and IC_{50} 0.08 (µg /mL). The Table 3. excludes some concentration which are insignificant. Blank Absorbance of 0.032% DPPH solution (A_0) = 0.432.

At 5 µg /mL concentration % inhibition shows the following trend (Table 3.).

3d (83.79%) > 3c (74.30%) > 3b (72.20%)

Thus the compound **3d** showed highest activity. As it contains two Phenolic -OH group and suppose to produce phenoxide free radical easily. Compond 3c has two phenolic grop –OH radical can be stabilized by benzyloxy group.

3.3 Results for Dye Screening

2 mL sample solutions (0.1% ethanolic solution) were added to 5 g of commercial carbomer gel (polyacryl amide), major ingredients of cosmetics, ultrasonic or ECG gel. Color stabilizes and gel state remained undisturbed. pH of the gel remained unchanged about six months (around 6.50) and no unwanted smell being found. Compounds 3a and 3b penetrates uniformly throughout the gel to produce very attractive transparent and appealing orange (3b) and lemon (3c) color (Fig. 4.) . Transparent color gel is a special type of gel and can be applicable for ultrasonic test, ECG etc.

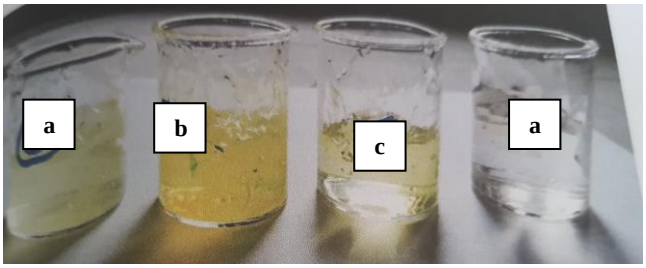


Fig. 4. Application of sample on carbomer gel (a) for 3a (b) for 3b (c) for 3c (d) carbomer gel

(I) (II)

Fig . 5. (I) ECG gel (SONEX), made up of carbomer gel (II) application of 3b compound on ECG gel (SONEX) produce transparent lemon color

IV CONCLUSION

Compounds 3′, 4′- methylenedioxy-2, 4, 5 - trimethoxy chalcone (3b) and 4′- benzyloxy- 2′, 4 - dihydroxy, 3-methyl chalcone, 3c showed high potency as preservative and dye. These two compounds showed good activity against selected four pathogenic bacteria. Even against *Staphyllococcus aures* (G^+, B_2) it has activity whearas this organism showed resistance to standard drug, Azithromycin. Numerous polar functional groups in 3b and 3c provide more binding sites to act as good dye. So chalcones have a high potential to be applied as safe dye in place of toxic azo dyes and others as well as a health friendly preservative.

V ACKNOWLEDGEMENT

We are thankful to the Faculty of Science, Rajshahi University to provide research grants to partially fulfill this project.

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