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Health-Benign Preservative Potency of Synthesized Flavonoids through Antimicrobial, Antioxidant and Cytotoxic Activity

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Abstract

South Asian countries are mostly agrobased and advancing food and beverage industries would largely depend on proper preservation of agroproducts. Chemical additives like preservative, color, dye, fragrance etc. have become a serious concern as these are taken by human body or used externally everyday. Very often these create some health problems as these are toxic, allergic and sometimes carcinogenic in nature. So there is a continuous effort to reduce the health risks through the use of natural, health beneficiary, cheap and easily available additives in this recently growing potential field. Preservatives usually inhibit the spoiling of food constituents or other things by inhibiting the growth of microorganism or air oxidation. Naturally occurring color pigments flavonoids specially chalcones and related compounds have withdrawn intensive interest as these are free from harmful nitrogenous chromophore (-CO-CH=CH-) with numerous biodynamic properties. Some analogues of natural chalcones being synthesized and characterized by spectral techniques (UV, IR, 1H and 13C NMR). Finally screened through antimicrobial, antioxidant and cytotoxic activity. Most of the compounds show very high antimicrobial activity against G+ and G□ bacteria as compared to standard. Specially 2′, 5′-dihydroxy-2, 4, 6- trimethoxychalcone, an orange-red dye showed very high antioxidant activity, IC50 0.92 at DPPH modeling and very less cytotoxicity LC50 71.75. Moreover, some chalcone have very good potency as colorant as produces transparent and appealing color while added to carbomer gel, major ingredients of cosmetics, ultrasonic, ECG gel etc. Gel forms remained undisturbed with constant pH for 6 months. So the observation is very much supportive and indicative of high prospects of chalcone as s food, beverage, cosmetics additives for a healthy and green society.

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I. INTRODUCTION

Safe and hygienic food, beverage, cosmetics etc. are a requirement for a healthy and green society. The use of synthetic additives in the food or cosmetics industry have been declining in recent years; this is mainly because man seeks to reduce risk in suffering from diseases [1].The principal classes of chemical additives like preservative, color, dye, fragrance etc. have become a serious concern as these are taken by human body or used externally everyday. Preservative is a natural or chemical additives to products such as food, beverages, pharmaceutical drugs, paints, biological samples, cosmetics, wood, etc. to protect decomposition trough microbial growth or some other undesirable chemical changes [2]. Expiry date is also importantly determined by this factor. So Antimicrobial and Antioxidant activity should be vital for preservative action.

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Some commercial preservatives such as Nitrites and nitrates are applied in raw meat to prevent its spoilage from microorganisms or oxidation process and also to retain the fresh red color. However, excessive amount of nitrites in blood during pregnancy may case the risk of blue baby syndrome. [3]. Preservative like sodium benzoate, potassium sorbate are popularly used in fruit juice industry which have high risk to be converted into carcinogenic benzene [3]. Besides some synthetic colors originated from azo (-N=N-) and other nitrogenous chromophore like Tetrazine (lemon color) are in wide industrial application in soft drinks, ice-cream etc. which are toxic and allergic [4] specially very harmful for children. Due to growing health threats of chemical preservatives, there is continuous effort to introduce natural, health beneficiary, cheap and easily available additives in the recently growing industries.

Flavonoids (scheme-1) can be synthesized in whole parts of the plant. They 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. [5]. So its distribution in plants is very vital and influenced by different factors, including variation and exposure degree of light. The production of high oxidized flavonoids is accelerated by light [6].Flavonoids cannot be synthesized in human body but its dietary intake to improve health beneficial effects and increase their amount in humans [1].

Chalcone (scheme-1), a distinct subclass of flavonoid, natural color pigments are safe for food and dyes were reported to exert antimicrobial [7], antioxidant [7] and radical-scavenging activities because of polyphenolic groups and have been recently recommended for use as food colorants and preservative. These are mostly yellow colored compounds for which the chromophore -CO-CH=CH- conjugated to aromatic ring is responsible and lightening and deepening of color take place for the presence of other auxochromes [8].Chemically chalcones are 1, 3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system (-CO-CH=CH-) and a nonnitrogenous class of chromophore.

Over the last six decades synthetic as well as isolation methods to obtain chalcones and its derivatives are being researched throughout the world [9]. Chalcones and its derivatives are still an object of sustained interest thus the present study has been extended by synthesizing eight substituted chalcones; 2′ - hydroxychalcone**(3a)**, 2′ - hydroxy - 4 - methoxychalcone**(3b)**, 2′ - hydroxy - 2, 4, 5 - trimethoxychalcone**(3c)**, 2′, 5′ - dihydroxy - 2, 4, 6 - trimethoxychalcone**(3d)**, 4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone**(3e)**, 2′ - hydroxy - 3′ - C - prenylchalcone**(4a)**, 2′- hydroxy - 5′ - C - prenylchalcone**(4b)**, 2′, 5′ - dihydroxy - 2, 4, 6 - trimethoxy - 3′ - C - prenylchalcone**(4c)** based on Claisen-Schimdt condensation method [9] from substituted benzaldehyde and acetophenone in good yield (scheme-1). They have been characterized by spectral data. Antimicrobial preservatives prevent degradation by bacteria. So the potency of the development of new preservative is highly based on *in vitro* antimicrobial screening. The synthesized chalcones**(3a-3e, 4a-4c)** were screened *in vitro* for their antibacterial activity against two pathogenic bacteria. In addition the synthesized chalcones**(3a-3e, 4a-4c)** were evaluated for *in vitro* antioxidant activity using diphenylpicrylhydrazyl (DPPH) model. Observation for antioxidant activity is expressed in terms of percent scavenging of DPPH radical and the inhibitory concentration 50% (IC_{50}). Some chalcone have very good potency as colorant as produces transparent and appealing color while added to carbomer gel, major ingredients of cosmetics, ultrasonic, ECG gel etc. Gel forms remained undisturbed with constant pH for 6 months.Cytotoxic activities of the same compounds were undertaken *in vivo* by brine shrimp lethality test (BST) and expressed by lethal concentration 50%, (LC_{50}) which indicates these are less toxic and

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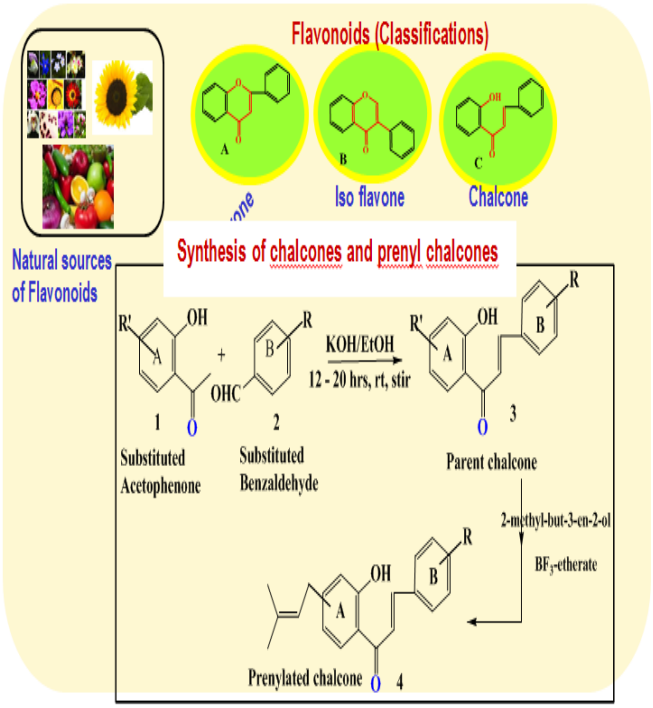
could be a better sorce as health-benign preservative.



Scheme-1: A fig. for Natural sources of Flavonoids, Classificaton of Flavonoids as flavone, isoflavone and chalcone, general synthetic scheme for chalcones and prenylchalcones where substituents of ring A is represented as R′ and that of Ring B as R.

II. EXPERIMENTAL

A. Materials

GR grade starting materials; substituted acetophenone, substituted benzaldehyde, resorsinol, BF_3-etharate, 2-methyl-but-3-en-2- ol 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.

B. Synthesis (scheme-1) and characterization of substituted chalcones (3a-3e) by Claisen-Schimdt condensation [9]

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-3e**). 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′ - hydroxychalcone(3a): $C_{15}H_{12}O_2$;Solid and lemon yellow ; m. p., **UV:** λ_{max} (CH_3OH): 196 and 233 nm, IR (KBr, cm^{-1}); 3414.06 (-OH), 3046. 91 (C=C-H, olifinic str.), 1640.01 (C=O), ^{1}H NMR, (400 MHz, $CDCl_3$); δ_H 12.803 (s, 1H, C_2-OH), 7.923 (d, *J* = 8.4 Hz, 1H), 7.921 (d, *J* = 14.8 Hz, 1H, C_β-H), 7.668 -7.661 (m, 2H), 7.657 (d, *J* = 14.8 Hz, 1H, C_α-H), 7.498 (t, 1H), 7.441-7.436 (bs, 3H) 7.029 (d, 1H, *J* = 8.4 Hz), 6.944 (t, 1H), ^{13}C-NMR (100 MHz, $CDCl_3$); δ_c193.77 (1C, C=O), 163.62, 145.49 (1C, C_β), 136.42, 134.63, 130.94, 129.67, 129.06 (2C), 128.68 (3C), 120.17 (1C, C_α), 120.4, 118.87.

2′ - hydroxy - 4 – methoxychalcone (3b): $C_{16}H_{14}O_3$; Solid and pineapple yellow; m. p. 87-88 ºC **UV:** λ_{max} (CH_3OH): 197 nm, IR (KBr, cm^{-1}); 3414.07 (-OH), 3024.51 (C=C-H olifinic str.), 2969.85 (C-H, asym.-str.), 2839.64 (C-H, sym-str.), 1922.67 (C=C olifinic str.), 1640.50 (C=O), ^{1}H NMR, δ_H(500 MHz, $CDCl_3$); 12.803 (s, 1H, $C_{2'}$-OH), 7.925 (d, *J* = 8Hz, 1H), 7.910 (d, *J* = 16 Hz, 1H, C_β-H), 7.640 (dd, *J* = 8 Hz), 7.545 (d, *J* =16 Hz, 1H, C_α-H), 7.490 (t, 1H), 7.030 (d, 1H, *J* = 8 Hz) , 6.960-6.940 (m, 1H), 6.950 (d, 2H), 3.870 (s, 3H), ^{13}C-NMR (125 MHz, $CDCl_3$); δ_c192.80 (1C, C=O), 163.60, 159.80, 145.10 (1C, C_β), 135.90, 130.20, 118.70 (1C, C_α), 118.20, 114.20 (2C), 55.80 (OCH_3).

2′ - hydroxy - 2, 4, 5 – trimethoxychalcone (3c): $C_{18}H_{18}O_5$, Solid and dark yellow, m. p. 120-122 ºC, **UV:** λ_{max} (CH_3OH): 196, 259 and 307 nm, IR (KBr, cm^{-1}); 3416.30 (-OH), 3009.49 (C=C-H olifinic str.),

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2964.99 (C-H, asym.-str.), 2864.28 (C-H, sym-str.), 1660.01 (C=O, conjugated keto group), ^{1}H NMR, δ_H(400 MHz, $CDCl_3$); 13.080 (s, 1H,-OH), 8.170 (d, *J* = 16 Hz, 1H, C_β-H), 7.640 (d, *J* = 8.6 Hz, 1H), 7.390 (d, *J* = 16 Hz, 1H, C_α-H), 7.370 (m, 1H), 7.010 (m, 1H), 6.920 (d, 1H, *J* = 2.6 Hz), 6.590 (s, 1H), 6.100 (s, 1H), 3.730 (s, 9H, $OC\underline{H}_3$), ^{13}C-NMR (100 MHz, $CDCl_3$); δ_c187.01 (1C, C=O), 158.50, 153.00, 147.80, 142.80 (1C, C_β), 139.81, 135.71, 131.11, 123.90, 123.30, 121.61 (1C, Cα), 116.20, 113.81, 108.20, 100.61, 56.40 ($O\underline{C}H_3$), 56.30 (2C, $O\underline{C}H_3$).

2′, 5′ – dihydroxy - 2, 4, 6 - trimethoxychalcone (3d): $C_{18}H_{18}O_6$; Orange solid; m. p. 175-176 ºC, **UV:** λ $_{max}$ (CH_3OH): 196, 242 and 384 nm, IR (KBr, cm^{-1}): 3415.26 (-OH), 3000.50(-C=C-H, olifinic, str.), 2940.52 (C-H, asym.-str.), 2840.49 (C-H, sym-str.), 1605.56 (C=O, conjugated keto group), ^{1}H NMR, (400 MHz, $CDCl_3$);δ_H 12.769 (s, 2H, $C_{2'}$ and $C_{5'}$-OH), 8.363 (d, *J*=15.6 Hz, 1H, C_β-H), 7.906 (d, *J* = 16 Hz, 1H, C_α- H), 7.340 (dd, *J* = 4.0 & 2.4 Hz, 1H), 7.012-6.982 (m, 1H), 6.894 (t, 1H), 6.877 (bs, 2H), 3.916 (s, 6H,-OCH_3), 3.861 (s, 3H, -OCH_3), ^{13}C-NMR (100 MHz, $CDCl_3$); δ_c194.89 (1C, C=O), 163.75, 162.09 (2C), 159.00, 157.69, 147.18 (1C, C_β) ,137.05, 123.93 (1C, C_α), 120.44, 119.48, 119.05, 114.81, 106.55, 90.61 (2C), 55.85 (2C, -$O\underline{C}H_3$), 55.40 (1C, -$O\underline{C}H_3$).

4 - hydroxy - 3′, 4′, 5′ - trimethoxychalcone (3e) : $C_{18}H_{18}O_5$;brownish yellow; m. p. 177-178 ºC, UV: λ_{max}(CH_3OH): 197 nm, IR (KBr, cm^{-1}): 3413.88 (-OH), 3100.00 (-C=C-H, olifinic, str.), 2944.52 (C-H, asym.-str.), 2834.07 (C-H, sym-str.), 1640.58 (C=O, conjugated keto group), ^{1}H NMR,(400 MHz, CDCl) ; δ_H 9.850 (s, 1H, –OH), 7.770 (d, *J* = 16 Hz, 1H, C_β-H), 7.550 (d, *J* = 8.4 Hz, 2H,), 7.340 (d, *J* =15.6 Hz, 1H, C_α- H), 7.220 (d, 2H), 6.890 (d, *J* = 8.4 Hz, 2H,), 3.900 (s, 9H,-$OC\underline{H}_3$), ^{13}C-NMR (100 MHz, $CDCl_3$); δ_c 189.67 (1C, C=O), 158.35, 153.15 (2C), 144.94 (1C, C_β), 142.41, 133.78, 130.52 (2C), 127.57, 119.34 (1C, C_α), 116.07 (2C), 105.95 (2C), 60.97 (1C,-$O\underline{C}H_3$), 56.38 (2C,-$O\underline{C}H_3$).

C. Synthesis and characterization of substituted prenylchalcones (4a-4c, scheme-1)

To a stirred solution of chalcone (**3a** or **3d**, 2.0 g) in dry dioxane (20.0 mL) was added gradually borontrifluoride-etherate (2.6 mL) at room temperature during the course of 30 minutes this was added a solution of 2-methyl-but-3-en-2- ol (2.5 mL) in dry dioxane (2.5 mL) and the solution stirred for 6 hrs., kept at room temperature overnight and diluted with moist ether (150 mL). The ethearal layer was washed with water and dried over anhydrous $NaSO_2$. It was evaporated to dryness and the residue on column chromatography over silica gel and elution successively with n-hexane or a mixture of n-hexane-acetone (3:1). The n- hexane fraction obtained from 3a produces **4a** and n-hexane-acetone (3:1) fraction yields **4b.** Prenylchalcone**4c** beingobtained in the above way starting with 3d.

2′ - hydroxy - 3′ - C – prenylchalcone (4a) : $C_{20}H_{20}O_2$, gummy substance light, yellow; UV: λ $_{max}$ (CH_3OH): 197 nm, ^{1}H NMR, δ_H(500 MHz, $CDCl_3$), 7.935 (dd, *J* = 8.0 Hz, 1H), 7.830 (d, *J* = 16.0 Hz, 1H, C_β-H), 7.680 (d, *J* =16.0 Hz, 1H, C_α-H), 7.670-7.650 (m, 1H), 7.460-7.420 (m, 2H), 7.200-7.160 (m, 3H), 7.030 (d, *J* = 8.0 Hz, 1H), 5.500 (s, 1H), 4.050-3.900 (m, 1H,), 3.40-3.20 (m, 2H), 1.80 (s, 6H, -CH_3), ^{13}C-NMR (125 MHz, $CDCl_3$); δ_c 192.80 (1C, C=O), 161.50, 145.10 (1C, C_β), 138.20, 135.20, 131.80, 129.90, 129.06 (2C), 128.68 (3C), 123.50, 123.10, 122.50, 121.70, 118.70 (1C, Cα), 24.60, 22.60, 18.60.

2′ - hydroxy - 5′ - C - prenylchalcone (4b) : $C_{20}H_{20}O_2$, Gummy and yellow, UV: λ $_{max}$ (CH_3OH): 196 and 241 nm ^{1}H NMR, (500 MHz, $CDCl_3$), δ_H7.930 (d, *J* = 15 Hz, 1H, C_β-H), 7.720-7.700 (m, 1H), 7.640 (d, *J* =10 Hz, 2H), 7.550 (d, *J* =15 Hz, 1H, Cα-H), 7.450 (bs, 3H), 7.190-7.130 (m, 1H), 6.980-6.960 (m, 1H), 5.500 (s, 1H), 5.200 (s, 1H), 3.700-3.600 (m, 2H), 1.820 (s, 3H), 1.740 (s, 3H), ^{13}C-NMR (125 MHz, $CDCl_3$); δ_c 192.80 (1C, C=O), 160.60, 145.10 (1C, C_β), 136.20, 135.20, 131.80,

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131.50, 130.50, 128.60 (2C), 128.50 (2C), 127.90, 123.10, 120.90 , 117.40, 118.70 (1C, C_α), 34.00, 24.60, 18.60.

2′, 5′ - dihydroxy -2, 4, 6 - trimethoxy - 3′- C - prenylchalcone (4c) : $C_{23}H_{26}O_6$, wine red semi solid, UV: λ_{max} (CH_3OH): 196 nm, ^1H NMR,(400 MHz, $CDCl_3$), δ_H 8.330 (d, *J* = 16.0 Hz, 1H, C_β-H), 7.420 (d, *J* =16.0 Hz, 1H, C_α-H), 7.080 (*J* = 8.0 Hz, 1H), 7.040 (dd, *J* = 8.0 Hz, 1H), 7.670-7.650 (m, 1H), 6.090 (s, 2H), 5.750 (bs, 1H), 5.350 (s, 2H), 3.830 (s, 9H), 3.210 (s, 2H), 1.820 (s, 3H),1.700 (s, 3H),^{13}C-NMR (100 MHz, $CDCl_3$); δ_c 192.80 (1C, C=O), 160.60, 159.60 (2C), 154.10, 149.60, 134.70, 131.80, 123.90, 123.10, 122.50 (1C, C_β), 121.30 (1C, C_α), 115.40, 107.00, 104.20, 90.90 (2C), 56.20, 55.8, 28.10, 24.60 , 18.60.

D.Preservative Potency through Antimicrobial activity determination

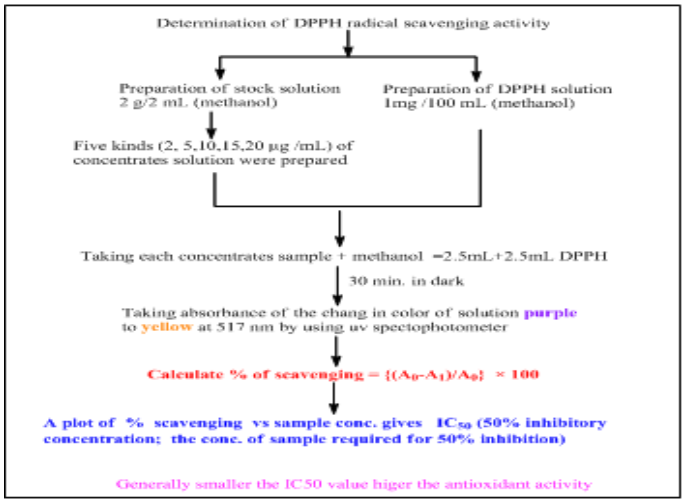
As South Asian countries lie in tropical zone so food preservation from the growth of microorganism is dominant features in all food sectors as it increases the health-related issues in consumers [10]. Antimicrobial preservatives prevent degradation of substances by bacteria. *In vitro* antibacterial activities of the test chemicals **(3a-3e, 4a-4c)** were studied using Two pathogenic bacteria viz. *Bacillus caerius* (G^+, B_1) and *Eschericia coli* (*E. coli*, G^-, B_3) at 250 (µg $disc^{-1}$). Disc diffusion, a primary assay technique, was followed [9]. The bioactivity is expressed by the diameter of zone of inhibition in mm comparing to those of the standard drug (ciprofloxacin, C-50). Higher the zone of inhibition higher the activity.

E. Additive Potency through pH Test and Color Effect

This is one of the ancient methods such as pickling and adding honey restrict microorganism growth by modifying the pH level. 2 mL 0.1% ethanolic sample solution was added on commercial carbomer gel (polyacryl amide), major ingredients of cosmetics, ultrasonic or ECG gel. Color stability, pH and odor being observed for six months

F. Preservative Potency through Antioxidantl activity determination

In addition the synthesized chalcones**(3a-3e, 4a-4c)** were screened for *in vitro* free radical scavenging activity using diphenylpicrylhydrazyl (DPPH) model [9]. Observation for antioxidant activity is expressed in terms of percent scavenging of DPPH radical and the inhibitory concentration 50% (IC_{50}, scheme-2)as compared to standard ascorbic acid. Lower the IC_{50} value higher the activity



Scheme-2: A flowchart to determine Antioxidant activity through DPPH modeling

G. Cytotoxicity Bioassay

Lethality assay (*in vivo)* being investigated in a simple zoological organism, such as Brine shrimp lethality test (BST) in support of preliminary screening [11, 12] of toxicity of physiologically active plant extract or synthesized compounds. Cytotoxic activities of the same compounds were undertaken *in vivo* by brine shrimp lethality test (BST) and expressed by lethal concentration 50%, (LC_{50}) (scheme-3).

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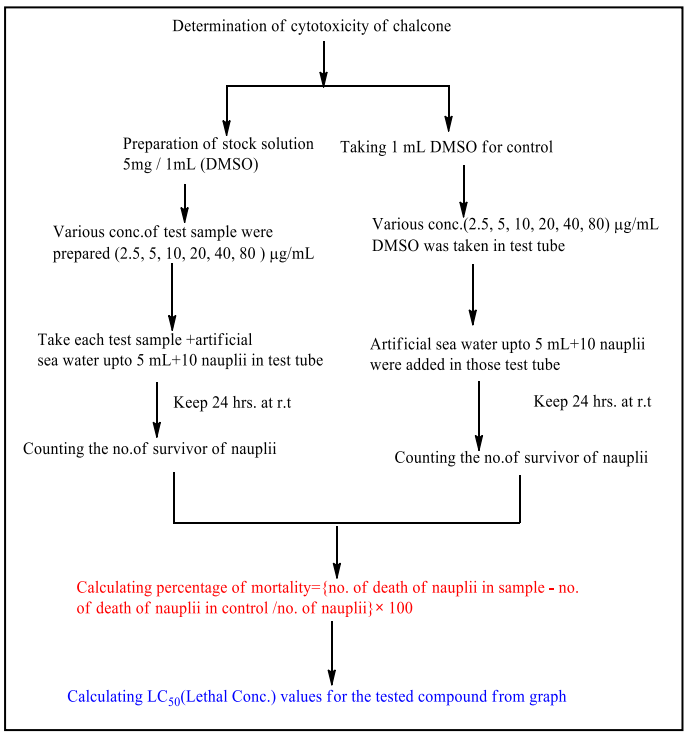

Scheme-3: A flowchart to determine Cytotoxic activity through BST modelling

III. RESULTS AND DISCUSSIONS

A. Structure Elucidation

Some unique spectroscopic structural features for chalcones are known which contains two aromatic rings connected by a keto-vinyl chain, -CO-CH=CH- [9]. 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 IR spectra of chalcones show usually a shift of band for carbonyl group near 1625-1650 cm^{-1}, characteristic shift to lower wavenumber for an α, β- unsaturated carbonyl group. In the ^{1}H NMR spectra the α-H and β-H of chalcones resonate at δ 6.7 -7.4 and δ 7.3 -7.7 as two doublets with large copling constants (J = 16-17 Hz) as an evidence of the olefin bond has *trans* geometry. In the ^{13}C NMR spectra of chalcones, the carbonyl carbon appears between δ 188.6 and 194.6. The α and β carbon atoms give rise to signals in between δ 116.1-128.1 and δ 136.9-145.4 respectively. Similar values are obtained for the synthesized compounds (**3a-3e, 4a-4c**)

B. Antibacterial activity

Bacillus cereus (G^+) bacteria is known to spread food born illness whereas *E. coli* (G^-)causes urine infection, diarrhoea diseases etc. So activity against these organisms will enhance preservative property along with antibiotic potency. The microbiological data shows that all compounds (**3a-3e, 4a-4c**) were found to be high to moderately active against both bacterial strains and presented in Tabular form (Table-I) and in Fig. 1 and Fig. 2.

Table-I: Results of the antibacterial activity of the compounds (3a-3e, 4a-4c) against *Bacillus cereus* (G^+)and *E.coli* (G^-)

Compound No.	Molecular formula	Diameter of the zone of inhibition (mm)			
		Bacillus cereus (G+)		*E. coli* (G^-)	
		250 µg $disc^{-1}$	*C-50	100µg $disc^{-1}$	*C-50
3a	$C_{15}H_{12}O_2$	24	40	18	40
3b	$C_{16}H_{14}O_3$	28		**30**	
3c	$C_{18}H_{18}O_5$	30		**30**	
3d	$C_{18}H_{18}O_6$	**38**		20	
3e	$C_{18}H_{18}O_5$	12		06	
4a	$C_{20}H_{20}O_2$	08		06	
4b	$C_{20}H_{20}O_2$	12		06	
4c	$C_{23}H_{26}O_6$	20		26	

* Ciprofloxacin-50

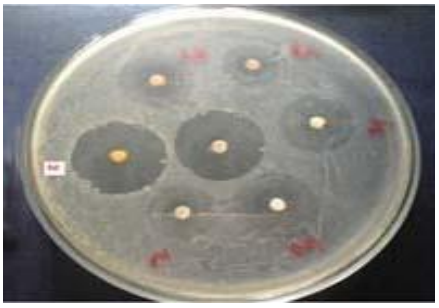

Fig. 1: Photographic plate of the zone of inhibition at the concentration of 250 µg $disc^{-1}$ against *Bacillus caerius*(G^+)

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Fig. 2: Photographic plate showing the zone of inhibition at the concentration of 250 μg disc^{-1} againstEscherichia coli (G$^-$).

Compounds **3b, 3c** and **3d** exhibited fairly good potentialities against both G$^+$ and G$^-$bacteria and in some cases very high activity as same as the standard drugs. Prenyl derivative of compound **3d**, compound **4c** shows very high activity against (G$^-$) pathogen than other prenyl derivatives.This indicates that the presence of electron releasing hydroxyl (-OH) and methoxy groups (-OCH_3) cause better antimicrobial effects. **4c**contains lipophilic prenyl group and enhance cell membrane permeability thus more susceptible to (G$^-$)**.** In **3e** three methoxy groups are occupied in ring A and in three adjacent carbons. So steric effect may be a cause of poor activity.

C. pH and color effect

Compounds 3b and 3d penetrates uniformly throughout the gel to form transparent color gel and color stabilizes specially no change in the gel state. pH of the gel was observed for six months and vales remained nchanged indicating no bacterial growth. No unwanted smell being found indicating no side reactions or atoxidation. Transparent color gel is a very special quality and can have a high potency to be used as ultrasonic or ECG gel .

D. Antioxidant activity

DPPH radical scavenging (Fig. 3) data corresponding IC_{50} values (Fig. 4) of synthesized chalcones**(3a-3e, 4a, 4c)** are represented in Tabular form (Table -II) respectively.

Table-II: DPPH radical scavenging data of synthesized chalcones(3a-3e, 4a,4c) and their corresponding IC_{50} values

Compound No	Conc. μg /mL	Absorbance at 517 nm	% Inhibition	*IC_{50}
3a	2	0.210	23.36	54.94
	5	0.203	25.91	
	10	0.191	30.29	
	15	0.189	31.02	
	20	0.186	32.12	
3b	2	0.184	32.85	36.89
	5	0.180	34.31	
	10	0.178	35.04	
	15	0.165	39.78	
	20	0.160	41.61	
3c	2	0.109	10.60	74.73
	5	0.233	14.96	
	10	0.223	18.61	
	15	0.216	21.17	
	20	0.220	19.71	
3d	2	0.120	56.20	0.92
	5	0.110	59.65	
	10	0.093	66.06	
	15	0.082	70.07	
	20	0.076	72.26	
3e	2	0.222	18.98	93.01
	5	0.219	20.07	
	10	0.216	21.17	
	15	0.210	23.36	
	20	0.205	25.18	
4a	2	0.221	19.34	65.67
	5	0.213	22.26	
	10	0.208	24.08	
	15	0.201	26.64	
	20	0.197	28.10	
4c	2	0.252	8.03	103.31
	5	0.240	12.41	
	10	0.235	14.23	
	15	0.232	15.33	
	20	0.230	16.06	

***Ascorbic acid is the standard and IC_{50} 0.08 (μg /mL)**

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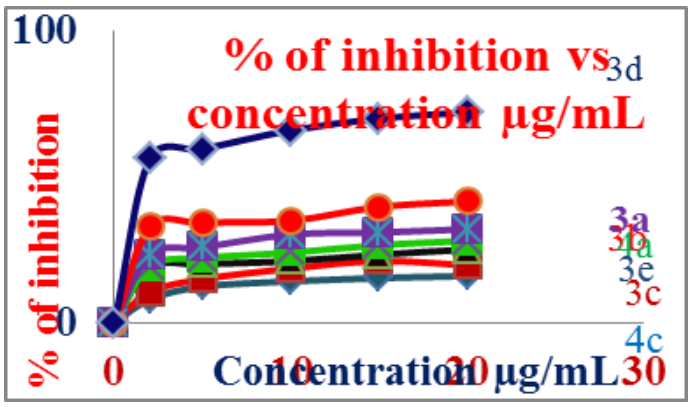

Fig. 3: DPPH radical scavenging activity of synthesized chalcones (3a-3e, 4a, 4c) without standard Ascorbic acid and 4b.

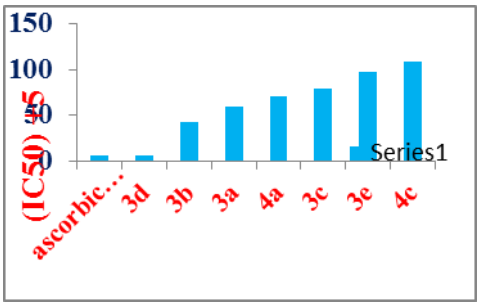

Fig. 4: Comparison of IC_{50}value of Ascorbic acid and synthesized chalcones (3a-3e, 4a,4c) withot4b.

Generally smaller the IC_{50} value higer the antioxidant activity thus the compound **3d** showed highest activity **(0.92)**. As it contains two Phenolic -OH group and suppose to produce phenoxide free radical easily and stabilized by electronic group methoxy occupied *ortho* and *para* position of Ring B. Presence of prenyl group reduce this activity.

E. Cytotoxicity

Brine Shrimp Lethality Assay of Chalcones**(3d, 3e, 4a-4c)** in DMSO is expressed in terms of percent mortality and Lethal concentration (LC_{50}) represented in Table-III.

For plant extracts LC_{50} value more than 1000 ppm indicating inactive nature, on the contrary LC_{50} values smaller than 30 ppm for pure compounds were considered toxic.[13] All compounds **(3d, 3e, 4a-4c)** showed a dose dependent cytotoxic activity at the tested concentrations. Compound **3d** was the most active than other compounds, presenting the highest LC_{50} of 71.75 ppm. Whereas from the result **3e, 4a, 4b, 4c** showed less activity. It is also known from the literature [6] chalcone containing *o*-methoxy substitution in ring-B reducing its ability to kill *Artemianauplii* due to steric hindrance to the receptor of specimen. In **3d** there are three methoxy functionality in ring B and in **3d** both the *ortho*position are occupied by methoxy group so **3d** is least toxic. Compounds **4a, 4b, 4c** these three compounds contain lipophillcprenyl functionality which enhance cell membrane permeability and increase toxicity. Among these three prenylatedchalcones**4c** contain *ortho, para*methoxy functionality and having the higher LC_{50} value 29.38 ppm. The present study also revealed that **3a, 3b, 3e, 4a, 4b** synthesized compounds are toxic against *Artemia sp.*

Table- III: Brine Shrimp Lethality Assay of Chalcones(3d, 3e, 4a-4c) in DMSO

Compound No	Conc. µg /mL	% of mortality	LC_{50}
3a	2.5	11.11	**71.75**
	5	12.5	
	10	14.28	
	20	14.28	
	40	28.57	
	80	57.14	
3e	2.5	33.33	**10.10**
	5	37.50	

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	10	62.50	
	20	71.43	
	40	71.43	
4a	2.5	55.55	**0.27**
	5	62.50	
	10	75.00	
4b	2.5	44.44	1.61
	5	50.50	
	10	57.14	
	20	57.14	
	40	85.71	
	80	85.71	
4c	2.5	33.33	29.38
	5	37.50	
	10	37.50	
	20	42.86	
	40	57.14	

IV. CONCLUSION

The strategy of synthesis of natural analogous color pigments flavonoids utilizes a simple Claisen-Schimdt condensation where waste minimization and environment friendly protocol being maintained. Preservative efficacy being examined through antimicrobial (pH resistance and antibacterial effect), antioxidant effect. Toxicity measurement indicates its impact on health. Compound 2′, 5′-dihydroxy-2, 4, 6- trimethoxychalcone, 3d, an orange-red dye showed high preservative potency as it has high antibacterial effect due to the presence of electron releasing groups such as methoxy, hydroxyl etc . It has very high antioxidant activity, IC_{50} 0.92 at DPPH modeling due to the presence of more phenolic –OH gops and very less cytotoxicity LC_{50} 71.75 due to the steric hinderance from trimethoxy functionality.

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