Research Article

Novel Methodology and Process Optimization for the Synthesis of Flavones

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ABSTRACT

Flavones are medicinal natural products. Prevention and cure of disease using flavonoids as a phytochemicals are known. Due to its wide spectrum activities it attracts researchers of the world. Hence cheap easy lab feasible synthetic methodology is demand of pharmaceutical industries. In that point of view we optimized Claisen-Schmidt method for the synthesis of flavones. Different molar ratios, solvent, temperature and catalysts are examined during experiments. Also effect of structure and substituent on the product formation is notified. Effect of groups like hydroxyl, methoxy and other on starting material (i. e. 2-hydroxy acetophenone and aromatic aldehyde) was studied. Attention is given for the synthesis of basic flavone molecules which are pharmaceutically active and whose skeletal modification gives new lead molecules. Hydroxyl group is important on flavone molecule for its versatile biological activities. So alternate method for the synthesis of flavone is methylation of starting material or methylated starting material must be used. After comparison of hydroxylated starting material and menthoxylated starting material we found that later is easy to synthesize flavones.

Keywords: Chalcone, flavone, hydroxyl aromatic aldehyde and 2-hydroxy aromatic ketones

Received 07 Jan 2014 Received in revised form 25 Jan 2014 Accepted 27 Jan 2014

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INTRODUCTION

Flavonoids consist of polyphenol compounds of benzopyrane class constituting an important group of oxygen heterocyclic compounds distributed in plants as a secondary metabolite [1]. Flavones are natural products synthesized in plants via shikimate pathway. Flavones are secondary metabolite and plays various role in plants. As flavones are directly associated with human dietary ingredients and health, there is need to check structure and function relationsheep. The bioavaibility, metabolism, and bio-activity of flavones depend upon stereo chemical configuration, number of hydroxyl groups, substitution of functional groups in the structure of flavones. Diversity of structure gives them wide spectrum activities. They protect plant from UV rays, interact with soil microbes, attract insect for pollination [2-4] Tea, wine, fruits, herbs and vegetables are the main source of flavonoids for human being [5]. Recently scientists have focused on the health benefits of flavonoids for human health. Many reports were showed that flavonoids have various pharmacological activities [6, 7].

Flavonoids have both metabolic and infective disease activity. Most of flavonoids are shown to have antioxidant [8,9], anticancer [10-12,23],free radical scavenging capacity [13,14], hepato-protective [15-17], anti-inflammatory [18,19], antibacterial [20,21], anti-diabetic [22], antimicrobial [24], ant osteoporotic [25] activities, while some flavonoids shows potential antiviral activities [26,27]. Some of the drug molecules with particular activity is shown below (**Fig. 1**).Due to varied biological activity of flavonoid skeleton it is necessary to synthesize flavone in laboratory with simple and cheap method's we try to modify the solvent, molar ratio, yield, temperature and time span of the reaction. Basically flavones are synthesized by major five methods. The

most common methods are Claisen-Schmidt method, Baker-Venkatraman method and Ganguly's method [35]. In current work we have selected Claisen- Schmidt method for optimization. Claisen-Schmidt method involves two steps. In first step aldehyde and ketones were undergoes condensation to give chalcones. Second step involves oxidative coupling. In second step cyclization occurred and chalcone were converted to flavones (**Scheme 1**)

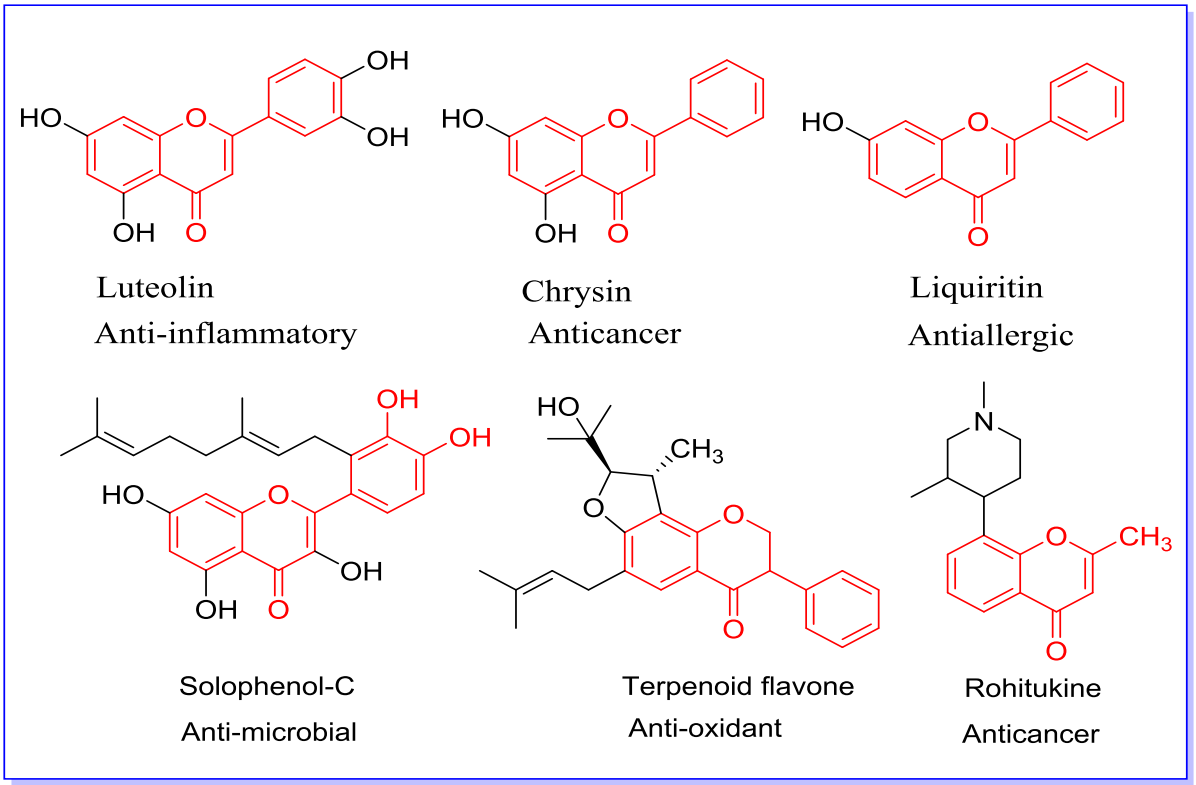


Figure 1: Flavones with Biological Activity (28- 32)

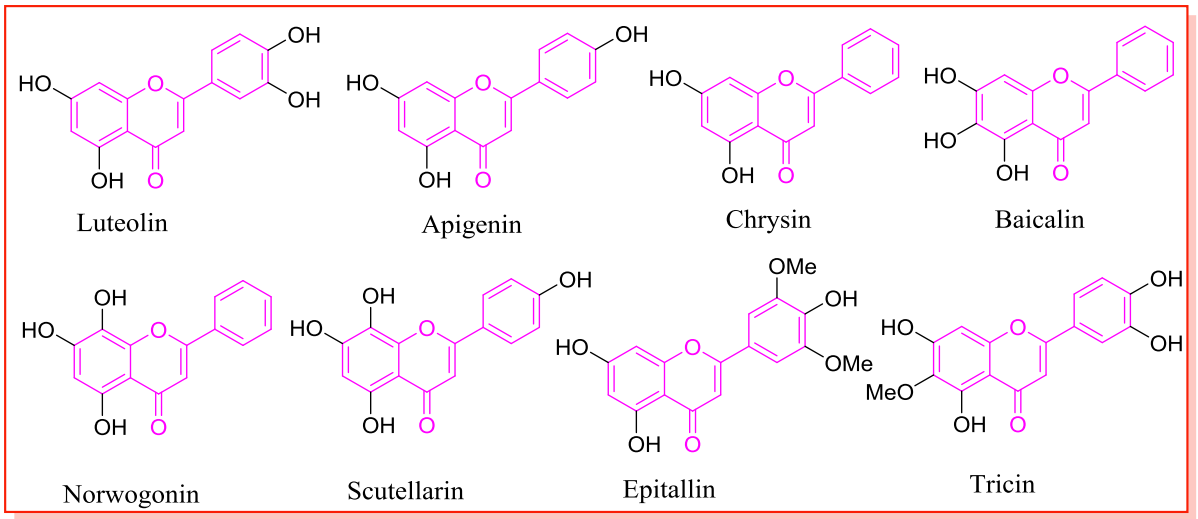


Figure 2: Examples of Basic Flavone Molecules

Flavonoids with large number of hydroxyl group in ring A, B, C have potent biological activity. Various synthetic report in past literature indicated that very few people synthesized flavones with more hydroxyl groups as a substituent. Most of people synthesized flavones and checked their biological activities [35]. Some people modified methodology, invented novel methods also, but no one reported that

synthesis of flavone with highly substituted OH group is difficult. This remained problem. Initially we thought that it is problem of work up that we were not able to isolate the product.

Scheme 1: **Claisen-Schmidt Synthesis of Flavone [33, 34]**

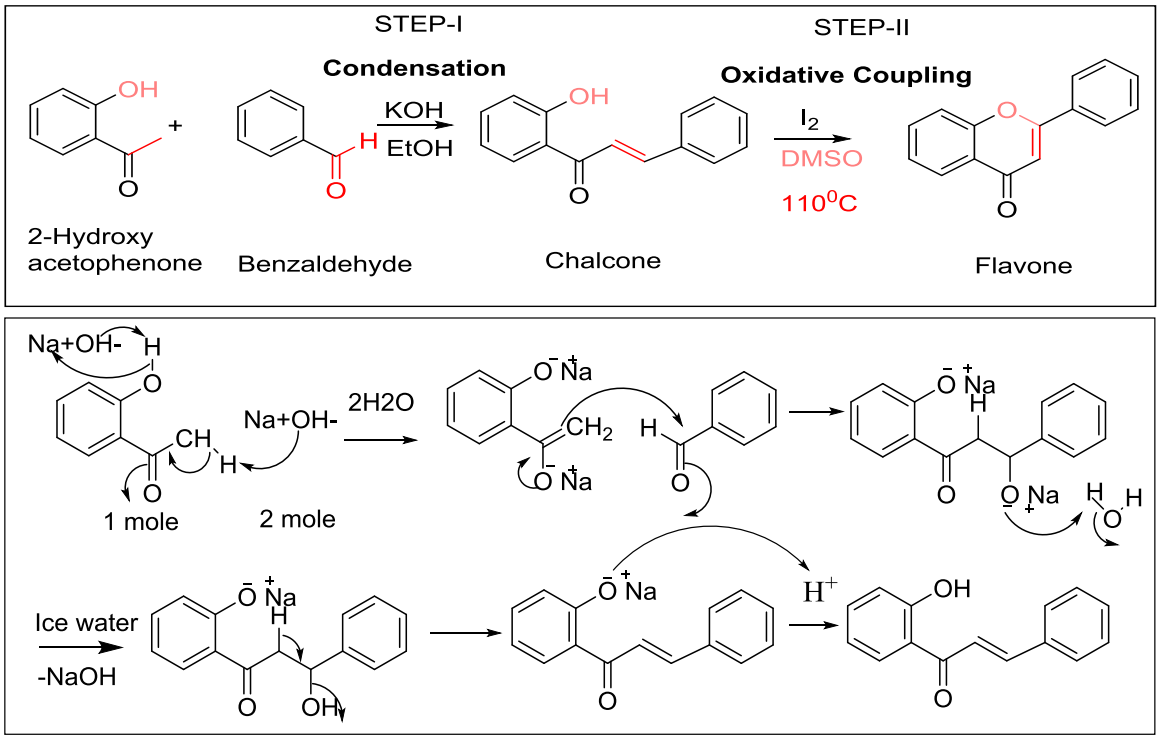


Figure 2: **Mechanism of Formation of Chalcones**

But after careful observation, past literature study and understanding of reaction mechanism of chalcone (**Fig. 3**) we concluded that OH groups undergoes H-bonding. As per our other observation polymerisation also occurred (**Fig.4**).

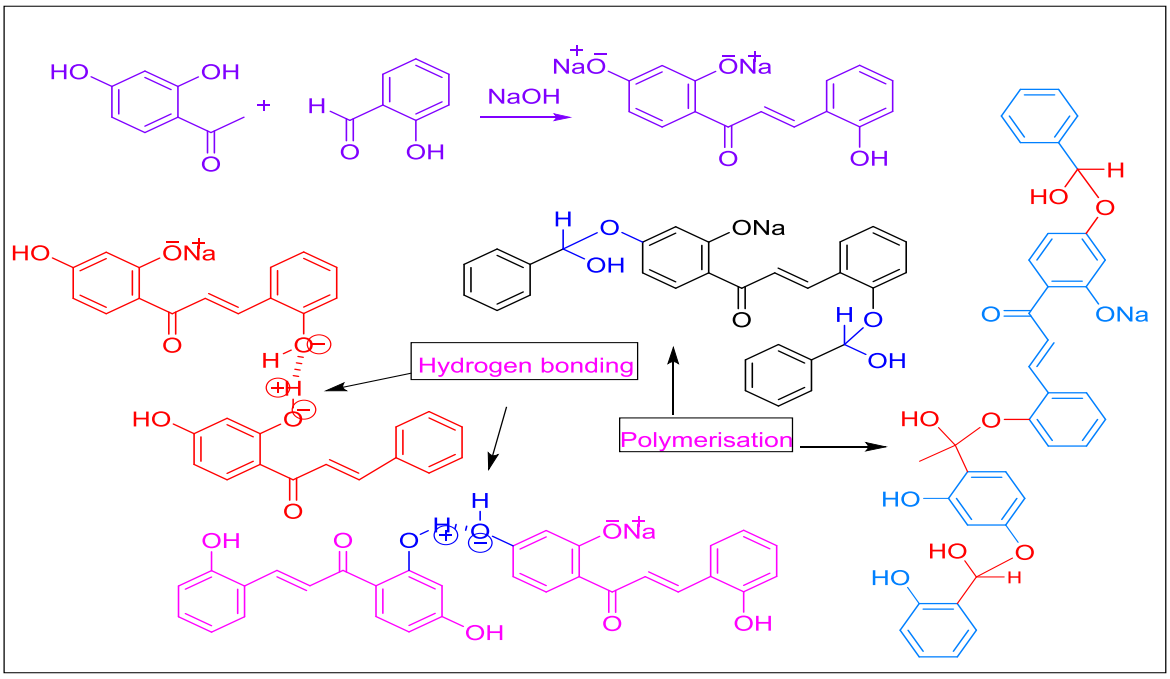


Figure 3: H-bonding and Polymerization in Chalcones

MATERIALS AND METHODS

All material purchased from Sigma-Aldrich and Merck chemicals (Gujarat).

Experimental

All materials purchased from Sigma, Aldrich and solvents from Merck Chemicals.

Representative procedure for the synthesis of chalcone and flavones

Step I

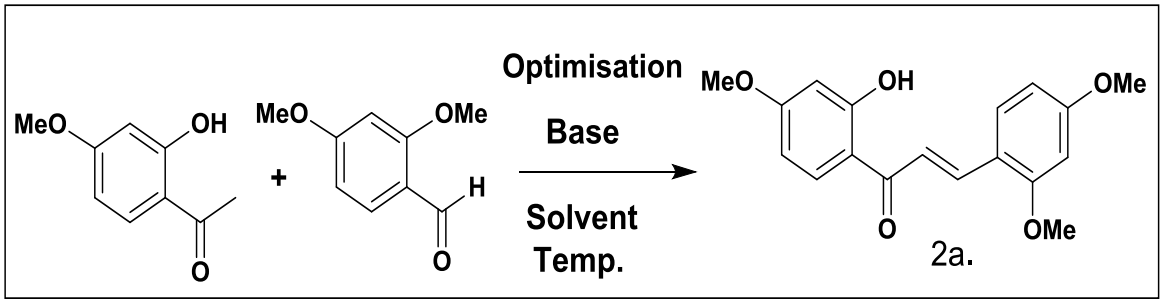
One mole each substituted 2-hydroxy acetophenone and substituted aromatic aldehyde was added in a RBF. Apparatus with stirrer and water condenser was assembled. Use hot plate with stirrer. Solvent was added to dissolve the reactant. Then 2 mole of NaOH was dissolved in distilled water (small quantity). NaOH solution was added with dropping funnel under stirring. Colour of reaction mixture was changed. Mostly yellow colour formed which indicates that reaction was initiated. Reaction mixture was stirred further till single spot appears on TLC. The reaction mass was cooled if reaction was carried out at heating condition. Reaction mass was poured on crushed ice. The reaction mass was neutralized with suitable dilute acid.Recrystalised from cold ethanol. Yield & M. P. was measured. Pure sample was given for analysis mentioning percent solubility in suitable solvent.

Step II

In a two neck RBF one mole of chalcone was added and reflux condenser was attached. Through the neck of condenser 10 mole % of iodine was added. The assembly was placed in oil bath and 110^0C temperature was maintained carefully. After 1.5 hours TLC was cheked.When difference of spot occurs on TLC work up was carried out. Saturated sodium thiosulphate solution was used to remove excess iodine. Sometimes brine may be used to remove inorganic impurities. For extraction of flavone ethyl acetate or chloroform was used.

Step I Process optimization for the synthesis of chalcone

Scheme 2: Ref. chalcone for optimization



By using Claisen-Schmidt method we synthesized above chalcone 2a-2j (**Table 7**). We modified and optimized the process for the synthesis of chalcone. Inorganic bases, iodine, solvents, molar ratio, temperature and time span of reaction was corrected (**Table 1-6**). Ethanol as a good solvent and NaOH/KOH are good bases for the synthesis of chalcone (**Table 3 & 4**). We also invented that 1:1:2 is very good molar ratio (**Table 3**) for the synthesis of chalcones. We also carry out reaction from RT to reflux condition and find out that 70^0C is good temperature for the synthesis (depending upon melting point of chalcone). We also concluded that hydroxyl group interfered in the formation of chalcone (**Fig.3**).

Table1-6: process optimization for synthesis of chalcone

Table1. Catalyst Optimization

Entry	Catalyst	M.ratio	Solvent	Yeild
2a.	NaOH	1:1:2	EtOH	85
2a.	KOH	1:1:2	EtOH	82
2a.	Na2CO3	1:1:2	EtOH	75
2a.	K2CO3	1:1:2	EtOH	70
2a.	Iodine	1:1:10%	EtOH	80

Table2. Mole ratio of Iodine Optimization

Entry	Catalyst	M.ratio	Solvent	Yeild
2a.	Iodine	1:1:5 mole%	Dioxane	50
2a.	Iodine	1:1:10 mole%	Dioxane	60
2a.	Iodine	1:1:15 mole%	Dioxane	75
2a.	Iodine	1:1:20 mole%	Dioxane	80
2a.	Iodine	1:1:25 mole%	Dioxane	80

Table3.Mole ratio optimization

Entry	Catalyst	M.ratio	Solvent	Yeild
2a.	NaOH / KOH/ Na2CO3	1:1:1	EtOH	60
2a.	NaOH / KOH/ Na2CO3	1:1:1.5	EtOH	65
2a.	NaOH / KOH/ Na2CO3	1:1:2	EtOH	85
2a.	NaOH / KOH/ Na2CO3	1:1:2.5	EtOH	85
2a.	NaOH / KOH/ Na2CO3	1:1:3	EtOH	85

Table 4. Solvent Optimization

Entry	Catalyst	M.ratio	Solvent	Yeild
2a.	NaOH/KOH/K2CO3	1:1:2	EtOH	85
2a.	NaOH/KOH/K2CO3	1:1:2	MeOH	82
2a.	NaOH/KOH/K2CO3	1:1:2	Acetone	75
2a.	NaOH/KOH/K2CO3	1:1:2	Dioxane	70
2a.	Iodine	1:1:2	THF	80

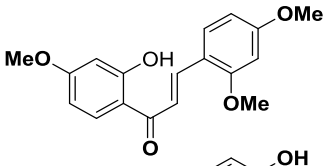
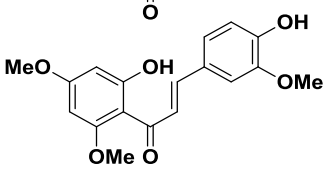
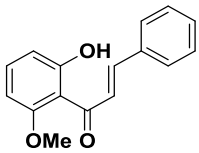
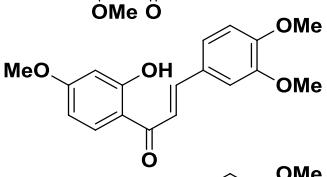
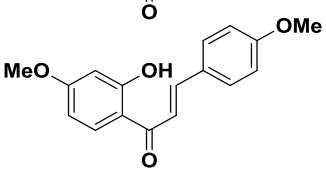
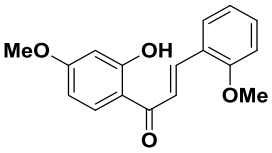
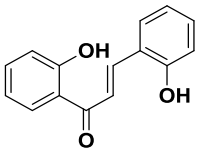
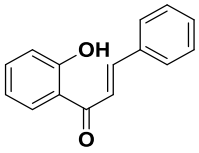
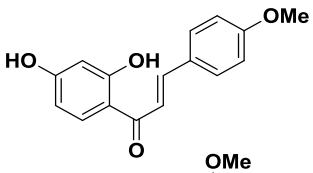
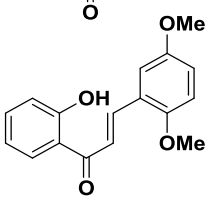
Table5. Temperature Optimization

Entry	Catalyst	M.ratio	T(0C)	Time(Hrs)
2a.	NaOH	1:1:2	40	3.0
2a.	NaOH	1:1:2	50	2.2
2a.	NaOH	1:1:2	60	2.0
2a.	NaOH	1:1:2	70	1.5
2a.	NaOH	1:1:2	80	1.0
2a.	NaOH	1:1:2	90	0.5

Table6.Optimized conditions for chalcones

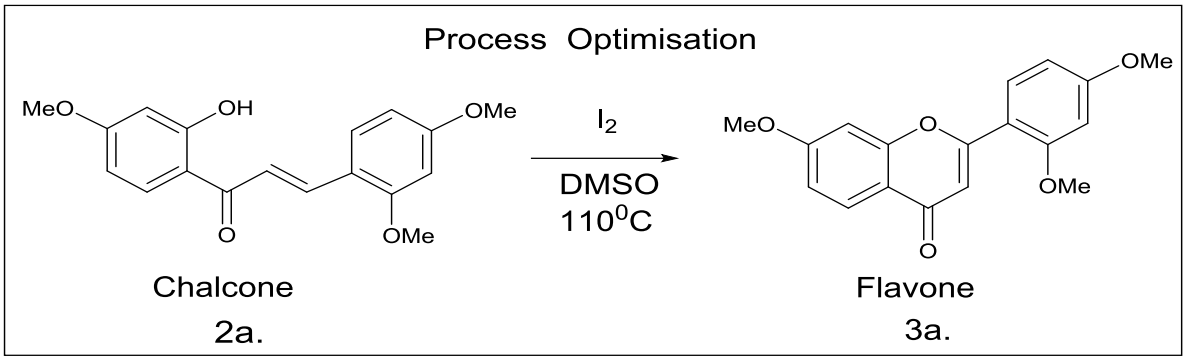
Catalyst	M.ratio	Solvent	T(^{0}C)	t (Hrs.)	Yeild
NaOH	1:1:2	EtOH	70	1.5	85
KOH	1:1:2	EtOH	70	2.0	85
Na2CO3	1:1:2	EtOH	70	3.0	85
K2CO3	1:1:2	EtOH	70	4.0	85
Iodine	1:1:20%	EtOH	70	3.0	85

Table 7: Molecules Synthesized by Optimized Process

Entry	Chalcone Molecule	Catalyst	Mole ratio	Solvent	Temp.	Melting Point
2a.		NaOH	1:1:2	Ethanol	60^0C	120^0C
2b.		NaOH	1:1:2	Ethanol	60^0C	170^0C
2c.		NaOH	1:1:2	Ethanol	60^0C	63^0C
2d.		NaOH	1:1:2	Ethanol	60^0C	208^0C
2e.		NaOH	1:1:2	Ethanol	60^0C	115^0C
2f.		NaOH	1:1:2	Ethanol	60^0C	92^0C
2g.		NaOH	1:1:2	Ethanol	60^0C	158^0C
2h.		NaOH	1:1:2	Ethanol	60^0C	156^0C
2i.		NaOH	1:1:2	Ethanol	60^0C	178^0C
2j.		NaOH	1:1:2	Ethanol	60^0C	96^0C

Step II

Process optimization for synthesis of flavone from chalcone (Oxidative coupling)



Scheme 3: Reference compound for optimization

Above scheme is used for oxidative coupling of chalcone to flavones. Various solvents, catalysts, mole percept are analysed (**Table 8-11**). We concluded that DMF is good solvent and iodine is good catalyst. Various lewis acids were analysed for the oxidative coupling of chalcone to flavones. But iodine was the best catalyst. Iodine forms cyclic intermediate with chalcone which helps to convert chalcone to flavones.10 mole per cent or 1:0.1 mole of chalcone and iodine gives best yield of product. We also noticed that solvent plays important role in the synthesis of flavones.DMSO was good solvent for the synthesis of flavone.

Because of its high boiling point expected temperature maintained. As per our opinion radical reaction occur in the formation of flavones. This conclusion is supported by past synthesis. Since it is possible to prepare flavone without solvent and study of mechanism indicates flavones may be formed via radical reaction. Temperature was important factor to initiates the reaction. 110⁰C is good temperature for the synthesis of flavones. If temperature increased above 120⁰C a specific type of smell is there indicating decomposition of reaction. So under optimized condition we synthesized different flavones (**Table 12**).

Table 8-11: Process optimization for highly hydroxylated flavones

Table8.Different catalyst

Entry	Catalyst	Time(Hrs)	Yield
3a.	AlCl3	3.5	25
3a.	ZnCl2	3.5	05
3a.	SnCl2.H2O	3.5	50
3a.	BF3.OEt2	3.5	00
3a.	FeCl3	3.5	05
3a.	HgCl2	3.5	05
3a.	SnCl4	3.5	00
3a.	Iodine	3.5	80

Table9.Different solvents

Entry	Catalyst	Solvents	Yield(%)
3a.	Iodine	Dioxane	56
3a.	Iodine	Acetonitrile	00
3a.	Iodine	Methanol	12
3a.	Iodine	Ethanol	33
3a.	Iodine	THF	15
3a.	Iodine	DMSO	60
3a.	Iodine	Free	80
3a.	Iodine	Acetone	20

Table 10.Different mole ratios

Entry	Catalyst	Solvents	Yield(%)
3a.	I_2 (05%)	DMSO	45
3a.	I_2 (7.5%)	DMSO	70
3a.	I_2 (10%)	DMSO	80
3a.	I_2 (10.5%)	DMSO	80
3a.	I_2 (12.5%)	DMSO	80
3a.	I_2 (15%)	DMSO	80
3a.	I_2 (20%)	DMSO	80
3a.	I_2 (25%)	DMSO	80

Table 11.Temp.optimization

Entry	Catalyst (10 mole%)	Temp. (⁰C)	Time (HrS.)	Yield (%)
3a.	DMSO/I2	100	1.0	50
3a.	DMSO/I2	105	2.5	55
3a.	DMSO/I2	110	1.5	80
3a.	DMSO/I2	115	1.0	85
3a.	DMSO/I2	120	1.0	80
3a.	DMSO/I2	125	0.45	80
3a.	DMSO/I2	130	0.45	Decomp.
3a.	DMSO/I2	135	0.45	Decomp.

Spectral Data of some Flavones

2-(2, 4-dimethoxyphenyl)-7-methoxy-4H-chromen-4-one (3a)
IR (cm^{1}) 3068,2849, 1641,1459,1365,1315, 1083961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'),7.015(C-4'), 6.97(C-5'), 9.89 (C-OH).

2-(2-hydroxyphenyl)-4H-chromen-4-one (3b)
IR (cm^{-1})- 3068,2849,1641,1459,1365,1315, 1083,961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'), 7.015(C-4'), 6.97(C-5'), 9.89 (C-OH).

7-hydroxy-2-(3-hydroxyphenyl)-4H-chromen-4-one (3c)
IR (cm^{-1}) -
3068,2849,1641,1459,1365,1315,1083, 961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'), 7.015(C-4'),6.97(C-5'),9.89 (C-OH).

5-hydroxy-2-(3-hydroxyphenyl)-4H-chromen-4-one (3d)
IR (cm^{-1}) -
3068,2849,1641,1459,1365,1315, 1083,961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'), 7.015(C-4'), 6.97(C-5'), 9.89 (C-OH).

6-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (3e)
IR (cm^{-1}) -
3068,2849,1641,1459,1365,1315,1083, 961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'),7.015(C-4'),
6.97(C-5'), 9.89 (C-OH).

7-hydroxy-2-(3, 4, 5-trihydroxyphenyl)-4H-chromen-4-one (3f)
IR (cm^{-1}) -
3068,2849,1641,1459,1365,1315, 1083,961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'),7.015(C-4'),
6.97(C-5'), 9.89 (C-OH).

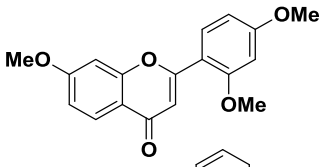
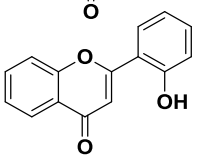
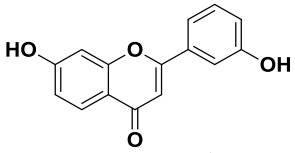
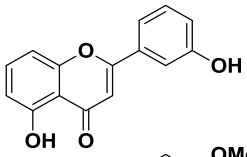
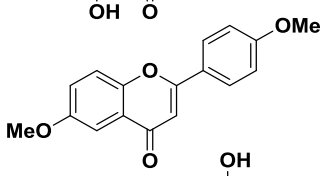
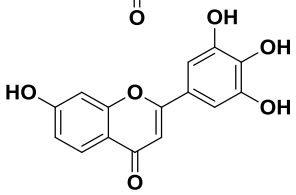
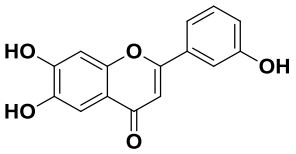
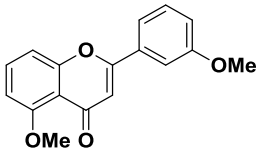
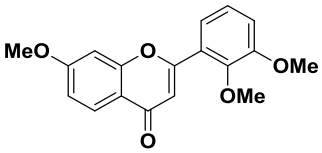
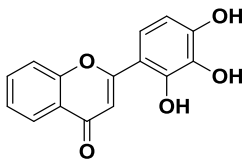
6, 7-dihydroxy-2-(3-hydroxyphenyl)-4H-chromen-4-one (3g)
IR (cm^{-1})- 3068,2849,1641,1459,1365,1315, 1083,961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'),7.015(C-4'),
6.97(C-5'), 9.89 (C-OH).

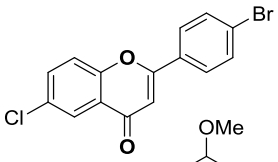
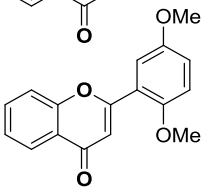
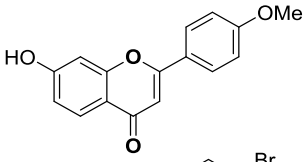
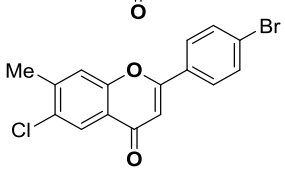
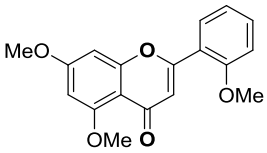
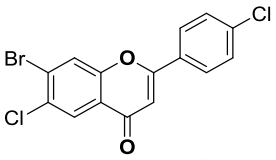
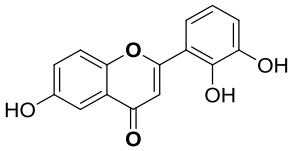
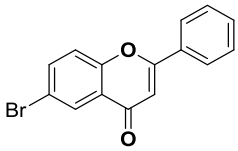
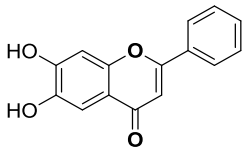
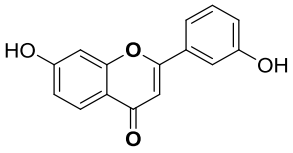
5-methoxy-2-(3-methoxyphenyl)-4H-chromen-4-one (3h)
IR (cm^{-1}) -
3068,2849,1641,1459,1365,1315, 1083,961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'), 7.015(C-4'), 6.97(C-5'), 9.89 (C-OH).

2-(2, 3-dimethoxyphenyl)-7-methoxy-4H-chromen-4-one (3i)
IR (cm^{-1}) -
3068,2849,1641,1459,1365,1315, 1083,961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'),7.015(C-4'),
6.97(C-5'), 9.89 (C-OH).

2-(2, 3, 4-trihydroxyphenyl)-4H-chromen-4-one (3j)
IR (cm^{-1})-
3068,2849,1641,1459,1365,1315, 1083,961,736.
^{1}HNMR (300MHz**)** 6.70(C-3), 7.85(C-5), 7.82(C-7), 6.89(C-8), 7.04(C-2'), 6.99(C-3'),7.015(C-4'),
6.97(C-5'), 9.89 (C-OH).

Table12: Synthesis of flavone under optimized condition

Entry	Chalcone Molecule	Catalyst	Mole ratio	Solvent	Temp.	Melting Point
3a.		Iodine	1:10 mole %	DMSO	110^0C	124^0C
3b.		Iodine	1:10 mole %	DMSO	110^0C	247^0C
3c.		Iodine	1:10 mole %	DMSO	110^0C	270^0C
3d.		Iodine	1:10 mole %	DMSO	110^0C	280^0C
3e.		Iodine	1:10 mole %	DMSO	110^0C	194^0C
3f.		Iodine	1:10 mole %	DMSO	110^0C	290^0C
3g.		Iodine	1:10 mole %	DMSO	110^0C	280^0C
3h.		Iodine	1:10 mole %	DMSO	110^0C	138^0C
3i.		Iodine	1:10 mole %	DMSO	110^0C	178^0C
3j.		Iodine	1:10 mole %	DMSO	110^0C	252^0C

Entry	Flavone Molecule	Catalyst	Mole ratio	Solvent	Temp.	Melting Point
3k.		Iodine	1:10 mole %	DMSO	110^{0}C	245^{0}C
3l.		Iodine	1:10 mole %	DMSO	110^{0}C	108^{0}C
3m.		Iodine	1:10 mole %	DMSO	110^{0}C	257^{0}C
3n.		Iodine	1:10 mole %	DMSO	110^{0}C	239^{0}C
3o.		Iodine	1:10 mole %	DMSO	110^{0}C	178^{0}C
3p.		Iodine	1:10 mole %	DMSO	110^{0}C	222^{0}C
3q.		Iodine	1:10 mole %	DMSO	110^{0}C	280^{0}C
3r.		Iodine	1:10 mole %	DMSO	110^{0}C	192^{0}C
3s.		Iodine	1:10 mole %	DMSO	110^{0}C	252^{0}C
3t.		Iodine	1:10 mole %	DMSO	110^{0}C	270^{0}C

All melting point of compounds matches with the reported m. p. (source – chemspider)

CONCLUSION

We first time predicted that hydroxyl group interfere in the formation of chalcone. As hydroxyl group protected by suitable reagent we will easily synthesize chalcone and then flavone via Claisen-Schmidt method. Oxidative coupling of chalcone to flavone is second important step and suitable per cent of iodine is necessary.

ACKNOWLEDGEMENT

Author is thankful to Dr. Rajeshwari Nair, Vice Principal, S.S.R., and College of ACS providing lab fascility to the research.

REFERENCES

1. Martens S, Mithofer A.Phytochemistry 2005;66:2399–407.
2. Mizutani M, Tanaka Y, Kusumi S. A kashi T. U. S. Patent 2008:7465569.
3. Pichersky E. Gang DR. Trends Plant Science 2000;5:439-45.
4. Dixon RA, Dye PM, Lamb CJ. Advances in Enzymology and Related Areas of Molecular Biology, 1983;55:1-136.
5. Shibata K, Nagai I, Kishida, M. J. Biol. Chem. 1916;28:93–108.
6. Mahmoodally AK, Subratty AH, Gurib-Fakim. Pharmaceutical Biology 2005;43:237-42.
7. Pandey, AK. National Acadamy Science Letters 2007;30:383-86.
8. Cook NC, Summan S. Journal of Nutritional Biochemistry 2006;54:6343-6351.
9. Havsteen B. Biochem. Pharmacol. 1983;32: 1141–1148.
10. Koen B, Ruth V, Guido V. Vjohannes VS. Journal of Biological chemistry 2005;280:5636-45.
11. Pines J. Semin. Cancer Biology, 1994;5:305–13.
12. Moon MJ, Lee SK, Song WK, Kim SW, Kim JI, Cho C. Bioorg. Med. Chem. 2006;14:237–246.
13. Kumar S, Pandey AK. The Scientific World Journal 2013.
14. Leopoldini M. , Russo N, Chiodo S, Toscano M, Journal of Agriculture and Food Chemistry 2006;54:6343-51.
15. Tapas AR, Sakarkar DM, Kakde RB, Tropical Journal of Pharmaceutical Research 2008;7:1089-99.
16. Zhu W, Jia Q, Wang Y, Zhang Y, Xia M. FreeRadical Biology and Medicines 2012;52:314-27.
17. Kim SM, Kang K, Jho EH. Phytotherapy Research 2011;25:1011-17.
18. Nishizuka Y. Nature 1988;334:661-65.
19. Hunter T. Cell1995;80:225-36.
20. Pandey AK, Kumar S, Bhargava A, Sharma B, Mishra A. Cellular andMolecular Biology 2011;57:16-25.
21. HarguchiH, Tanimoto K, TamuraY, Mizutani K, Kinoshta T. Phytochemistry 1998;48:125-129.
22. Kunimasa K, Kuranuki S, Matsuura N, Iwasaki N, Ikeda, M, Ito A, Sashida Y, Mimaki Y, Yano, M, Sato M, Igarashi Y, Okawa T. Bioorg. Med. Chem. Lett. 2009;19: 2062-64.
23. Liu, H. L. ; Jiang, W. B. ; Xie, M. X. Recent Pat. Anti-Cancer Drug Discovery 2010,5:152-164.
24. Tcushnie TP, Andrew JL International Journal of Antimicrobial Agents 2005;26:343–56.
25. Maurya R, Rawat P, Sharan K, Siddiqui JA, Swarnkar G, Mishra G, Manickavasagam K, Arya R, Chattopadhyay N World Pat. 2009: 110003.
26. Cushne TPT, LambAJ International Journal of Antimicrobial Agents 2005;26:343-56.
27. Critchfield JW, Butra ST, Folks TM AIDS Research andHuman Retroviruses 1996;12:39-46.
28. Saori I, TakahiroH, YukoS, Suichi M. Takesh O, Hirokazu TJ. . Agri. Food Chem. 2012;60:11765-70.
29. Ismail IS, Hiroshi M, Yasuke H, Nordin HL, Takahiro H, Motto S. Journal of Natural Products 2009;72:1879-83.
30. Cheng G, Jhingarui Z, Rui Z, Meng Z. Zhang J. Central Journal of Chemistry 2013;7:83.
31. Wenzang LU, Yang Y, Qingwang LI, FuzuLIU Latin American Journalof Pharmacy 2009, 28, 568-73.
32. Zheng W, Wang SY Journal of Agri. Food Chem. 2001;49: 5165-70.
33. Verma AK, Pratap R. Natural Product Report 2010;27:1571-93.
34. Kshatriya RB, Nazeruddin GM., Patil MS, Patel AC, More DB Int. Multi. e Journal, 2013;II(X):67-79.
35. Kshatriya RB, Shaikh YI, Nazeruddin GM. Oriental Journal of Chemistry 2013;29(4):1475-1487.