

SYNTHESIS AND CYTOTOXIC, ANTI OXIDANT ACTIVITIES OF NEW CHALCONE DERIVATIVES

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ABSTRACT

Chalcones belong to an important class of flavonoids, which may be prepared by Claisen reaction. They possess a wide range of biological activities and industrial applications. Kostanecki was the first to give the term chalcone and who did pioneering work in the synthesis of naturally coloring compounds. Cytotoxicity against tumour cell lines may be the result of disruption of the cell cycle, inhibition of angiogenesis, interference with p53-MDM2 interaction, mitochondrial uncoupling or induction of apoptosis. Structural requirements for cytotoxic activity vary according to the mechanisms of action. Chemoprotection by chalcones may be a consequence of their antioxidant properties, mediated via inhibition or induction of metabolic enzymes, by an anti-invasive effect or a reduction in nitric oxide production. Chalcones are synthesized by conventional and microwave assisted synthesis methods. By microwave assisted synthesis, a considerable increase in the reaction rate has been observed and that too, with better yields. The compounds have been screened for cytotoxic activity and antioxidant activity.

Key words: Claisen-Schmidt condensation, Microwave irradiation, Cytotoxic activity, Antioxidant activity.

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INTRODUCTION

Chalcones are abundantly present in nature from ferns to higher plants¹. They are aromatic compounds with an unsaturated side chain and are often cytotoxic *in vitro*². Chalcones have also been reported to be anti-inflammatory, analgesic and antipyretic³. Some chalcones possess bactericidal, antifungal and insecticidal activity and some of their derivatives are reported to be antimutagenic⁴. Chalcones are 1,3-diphenyl-2-propene-1-one^{5,6}, in which two aromatic rings are linked by a three carbon α , β -unsaturated carbonyl system. These are abundant in edible plants and are considered to be the precursors of flavonoids and isoflavonoids. Chalcones are synthesized by Claisen-Schmidt condensation, which involves cross aldol condensation of appropriate aldehydes and ketones by base catalyzed or acid catalyzed followed by dehydration. Chalcone is a common natural pigment and one of the important intermediate in the biosynthesis of flavonoids⁷. Synthetic and naturally occurring chalcones have been extensively studied and developed as one of the pharmaceutically important molecules. Chalcone derivatives are screened for their anti-inflammatory activity⁸, chemopreventive activity⁹, cardiovascular disease¹⁰, anticancer activity¹¹, cytotoxic activity¹², antiproliferative activity¹³, antimalarial activity¹⁴, antiviral activity¹⁵, anti-HIV activity¹⁶. Therefore, in the present investigation it has been considered worthwhile to synthesize some new chalcone derivatives by conventional and microwave irradiation methods and comparison between two methods.

Microwave-induced organic reaction enhancement (MORE) chemistry¹⁰ is gaining popularity as a non-conventional technique for rapid organic synthesis. Important features of this technique are easy access to very high temperature, good control over energy input in a reaction, higher yields and rapid synthesis of organic compounds.

The synthesized compounds were purified by recrystallization and chromatography. The compounds were characterized by ¹H NMR and IR analysis. The compounds were tested for their cytotoxic activity and antioxidant activities by standard methods.

EXPERIMENTAL

General procedure for the synthesis of chalcones by Claisen-Schmidt condensation ¹⁷⁻²¹

Synthesis of chalcones (1-5)

(a) **(Conventional)**. Equimolar quantities (0.001mol) of 2-acetyl-5-methyl-furan and respective aldehydes (0.001mol) were mixed and dissolved in minimum amount (3ml) of alcohol. To this, aqueous potassium hydroxide solution (0.003mol) was added slowly and mixed occasionally for 24 hrs, at room temperature. Completion of the reaction was identified by observing on precoated TLC plates of Merck. After completion of the reaction, the reaction mixture was poured into crushed ice, if necessary acidified with dil HCl. The solid separated was filtered and dried. It was purified by recrystallization or by column chromatography performed on silica gel (100-200 mesh, Merck) using ethylacetate and hexane mixture as mobile phase.

(b) **(MWI)**. Equimolar quantities (0.001mol) of 2-acetyl-5-methyl-furan and respective aldehydes (0.001mol) were mixed and dissolved in minimum amount (3ml) of alcohol. To this, aqueous potassium hydroxide solution (0.003mol) was added slowly and mixed. The entire reaction mixture was microwave irradiated for about 2-6 minutes at 180 watts .

The spectral data of all synthesised compounds is mentioned below

1-(5-methylfuran-2-yl)-3-phenylprop-2-en-1-one (1) : Mol. Formula: C₁₄H₁₂O₂ , Conventional method Yield 61, Microwave Irradiation 74%, m.p. 124 ± 2°C. IR (cm⁻¹) : 3020 (C-H aromatic stretching), 2924 (C-H methyl stretching), 1660 (C=O), 1604 (HC=CH), 1214 (C-O-C). ¹H NMR (δ ppm) : 2.45 (3H, s, C-5'-CH₃), 6.22 (1H, d, J=4.2 Hz, C-4'-H), 7.25 (1H, d, J=15.6 Hz, CO-CH=), 7.37-7.43(5H, m, C-2", 3", 4"and 5", 6"-H), 7.64 (1H, d, J=4 Hz, C-3'-H),

3-(4-fluorophenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one (2) : Mol. Formula: C₁₄H₁₁FO₂ , Conventional method Yield 65, Microwave Irradiation 74%, m.p. 92 ± 2°C. IR (cm⁻¹) : 1644 (C=O), 1591 (HC=CH), 1215 (C-O-C), 798 (C-F). ¹H NMR (δ ppm) : 2.13 (3H, s, C-5'-CH₃), 7.45 (1H, d, J=4 Hz, C-4'-H), 7.64 (1H, d, J=16 Hz, CO-CH=), 7.70 (2H, d, J=8.4 Hz, C- 3" and 5"-H), 7.75 (1H, d, J=4.2 Hz, C-3'-H), 7.84 (2H, d, J=8.4 Hz, C- 2" and 6"-H), 8.00 (1H, d, J=16 Hz, Ar-C-H=).

3-(4-chlorophenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one (3) : Mol. Formula: C₁₄H₁₁Cl O₂ , Conventional method Yield 78, Microwave Irradiation 86%, m.p. 126 ± 2°C. IR (cm⁻¹) : 3016 (C-H), 2924 (C-H), 1651 (C=O), 1602 (HC=CH), 1212 (C-O-C), 800 (C-Cl). ¹H NMR (δ ppm) : 2.41 (3H, s, C-5'-CH₃), 6.25 (2H, d, J=4 Hz, C- 3' and 4'-H), 7.26 (2H, d, J=8.2 Hz, C-3" and 5"-H), 7.40 (1H, d, J=16 Hz, CO-CH=), 7.58 (2H, d, J=9.4 Hz, C-2" and 6"-H), 8.1 (1H, d, J=16.4 Hz, Ar-C-H=).

3-(2,4-dichlorophenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one (4) : Mol. Formula: C₁₄H₁₀Cl₂ O₂ , Conventional method Yield 64, Microwave Irradiation 73 %, m.p. 120 ± 2°C. IR (cm⁻¹) : 3020 (C-H), 2924 (C-H), 1657 (C=O), 1601 (HC=CH), 1215 (C-O-C), 798 (C-Cl). ¹H NMR (δ ppm) : 2.44 (3H, s, C-5'-CH₃), 6.23 (1H, d, J=4 Hz, C- 4'-H), 7.26-7.30 (2H, m, C-5" and 6"-H), 7.35 (1H, d, J=15.6 Hz, CO-CH=), 7.45 (1H, s, C-3"-H), 7.69 (1H, d, J=8 Hz, C-3' -H), 8.16 (1H, d, J=16 Hz, Ar-C-H=).

1-(5-methylfuran-2-yl)-3-(4-nitrophenyl) prop-2-en-1-one (5) : Mol. Formula: C₁₄H₁₁NO₄ , Conventional method Yield 53, Microwave Irradiation 61 %, m.p. 174 ± 2°C. IR (cm⁻¹) : 1657 (C=O), 1603 (HC=CH), 1511 (Ar-NO₂), 1213 (C-O-C). ¹H NMR (δ ppm) : 2.46 (3H, s, C-5'-CH₃), 6.25 (1H, d, J=4 Hz, C- 4'-H), 7.30 (1H, d, J=4.2 Hz, C-3'-H), 7.44 (1H, d, J=16 Hz, CO-CH=), 7.75 (2H, d, J=9.2 Hz, C-2" and 6" -H), 7.81 (2H, d, J=9.4 Hz, C-3" and 5"-H), 8.24 (1H, d, J=16.4 Hz, Ar-C-H=).

Cytotoxicity test

Brine shrimp lethality bioassay (BSLT)

Brine shrimp lethality test have been used as bioassay for a variety of toxic substances. This method has also been applied to plant extracts in order to facilitate the isolation of biologically active compounds^{19, 20, 21}. A general bioassay that appears capable of detecting a broad spectrum of bioactivity, present in

crude extracts and in synthetic compounds is the brine shrimp lethality bioassay, rather than more tedious and expensive *in vitro* and *in vivo* antitumor assays. Furthermore, it does not require animal serum as is needed for cytotoxicities.

Procedure

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of medicinal plants. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial sea water under constant aeration for 38 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 5 ml of brine solution. In each experiment, test substances whose activities are to be checked were added to the vial according to their concentrations and maintained at room temperature for 24 h under the light and surviving larvae were counted.

Experiments were conducted along with control (vehicle treated), different concentrations (1-5000 µg / ml) of the test substances in a set of three tubes per dose. Replicas should be maintained to get accurate results.

Statistical analysis

The percentage lethality was calculated from the mean survival larvae of compounds treated tubes and control. ED₅₀ values were obtained by (best-fit line method) plotting a graph, taking concentration on X-axis and percentage inhibition on Y-axis, at 50% of the percentage inhibition the line was drawn from Y-axis and aligned with the concentration on X-axis then got the ED₅₀ values.

Antioxidant activity

Free radicals are formed constantly in human system either as accidental products during metabolism or deliberately during the process of phagocytosis; or due to environmental pollutants, ionizing radiations, ozone, heavy metal poisoning, cigarette smoking and chronic alcohol intake. Free radicals being highly reactive can oxidize biomolecules leading to tissue injury and cell death.

In the present study, two *in vitro* antioxidant models 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH[•]) scavenging activity (as it is a model for lipophilic radicals which initiate lipid peroxidation) . The IC₅₀ values of chalcones tested for their antioxidant activity. Solvent used in both the tests for compounds was DMSO (Dimethylsulphoxide).

DPPH free-radical scavenging activity

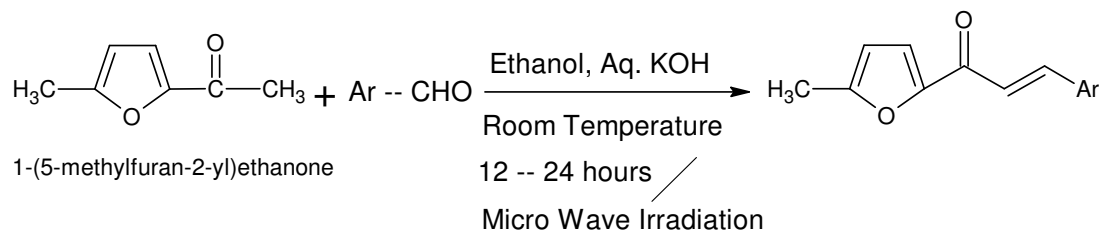
DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured by the method of Lamaison *et al.* The reaction mixture contained 1.5X10⁻⁷ M methanolic solution of DPPH and various concentrations of the test substances and were kept in dark for 50 minutes. Optical density (OD) of samples was measured at 517 nm against a blank, and IC₅₀ values were calculated (using linear regression analysis) by plotting a graph, taking concentration on X-axis and percentage inhibition on Y-axis, at 50% of the percentage inhibition the line was drawn from Y-axis and aligned with the concentration on X-axis then got the IC₅₀ values.

RESULTS AND DISCUSSION

In the present study, we have performed the synthesis of chalcone derivatives by conventional and microwave irradiation method in Scheme -1 but to reduce the reaction time, it was decided to synthesize the compounds with microwave irradiation, which can be more effective, faster, and energy efficient in addition; we have compared those with others that were obtained via conventional heating methods and results were mentioned in Table-1 and Table-2.

Brine shrimp lethality test have been used as bioassay for variety of toxic substances, all the chalcones (**1-5**) were tested for cytotoxic activity by the BSLT bioassay method. All the compounds were found to possess cytotoxic activity. Among them, compounds **1**, **3** showed dose dependent cytotoxic activity at concentrations of (**1**) 24.27 µg/ml, (**3**) 37.05 µg/ml, respectively. The remaining compounds exhibited less activity when compared to the above compounds at various concentration levels. Podophyllotoxin is used as a standard drug for BSLT assay method. By comparing the results compound **1**, **3** found to be the best among all the tested compounds. The results and complete data of test presented in Table-3. The potency of the chalcone derivatives was estimated by ED₅₀ values. Few of the chalcone

derivatives showed good percentage inhibition but their ED₅₀ values were more. Hence they were less potent among the tested compounds with respect to ED₅₀ values.



Where, Ar = Phenyl (**1**), 4''- Fluoro phenyl(**2**), 4''- Chloro phenyl(**3**), 2,4''- Dichloro phenyl(**4**), 4''- Nitro phenyl(**5**)
Scheme-1

Table-1: Comparative reaction time and percentage yield of chalcone derivatives by conventional and microwave irradiation methods.

S.No.	Reaction time		Yield (%)	
	Conventional (hr)	MW (min)	Conventional	MW
1	24	3	61	74
2	24	4	65	74
3	24	3.5	78	86
4	24	3.5	64	73
5	24	4	53	61

The *in vitro* antioxidant activity and scavenging effects of the 5 chalcones were evaluated by using different reactive species assay containing DPPH radical scavenging activity. The potency of the chalcone derivatives was estimated by IC₅₀ values. The IC₅₀ values of chalcone derivatives used in the present study were given in Table-4.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured for all the chalcones (**1-5**). Among them, compounds **1, 2, 3, 4** and **5** showed a dose dependent inhibition of radicals at concentrations of **25, 50** and **100** µg/ml.

Ascorbic acid, the well known antioxidant was used in the test for comparing the results, compounds **5** appears to be the best among all the tested compounds. Few of the chalcone derivatives showed good percentage inhibition but their IC₅₀ values were more. Hence they were less potent among the tested compounds with respect to IC₅₀ values.

CONCLUSION

All the synthesized (**5**) compounds were purified by recrystallization or by column chromatography. The identification of compounds was established by single spot TLC, melting point and by spectral analysis involving IR, ¹H NMR, ¹³C NMR and elemental analysis. Since chalcones were widely reported to possess cytotoxic and antioxidant activities etc. All the chalcone derivatives were evaluated for the above mentioned activities and they have exhibited promising activity.

From the cytotoxic and antioxidant activities it was proven that most of the chalcone derivatives are potent and possessing cytotoxic and antioxidant activities.

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Table -2: Characterization of chalcone derivatives

Compound	Quantity (µg/ml) Percentage inhibition			
	25 µg/ml	50 µg/ml	100 µg/ml	IC ₅₀ µg/ml
1	7.24	12.31	16.03	76.12
2	4.05	7.12	11.04	65.04
3	9.11	10.03	12.04	81.15
4	8.35	9.24	10.47	75.20
5	10.14	11.85	13.69	49.18
Ascorbic acid	16.13 1 µg/ml	38.11 2.5 µg/ml	62.34 5 µg/ml	0.61

Table-3: Cytotoxic activity of chalcones by using Brine shrimp lethality test (Compounds 1-5)

S.NO	Compound	Solubility	ED ₅₀ µg/ml
1	Phenyl	DMSO	24.27
2	4''- Fluoro phenyl	-	39.26
3	4''- Chloro phenyl	-	37.05
4	2,4''- Dichloro phenyl	-	43.53
5	4''- Nitro phenyl	-	45.38
Standard	(Podophyllotoxin)	-	3.88

Table-4: Percentage inhibition of free radicals using DPPH method (Compounds 1-5)

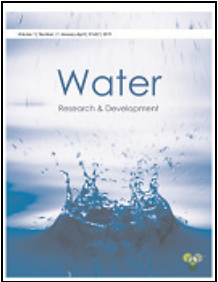
Compound	Rf value	M.P	Elemental analysis	
			Calculated(%)	Found(%)
1	0.62	124± 2°C	C: 79.17 H: 5.65 O: 15.08	C: 79.2 H: 5.68 O: 15.1
2	0.64	92± 2°C	C: 72.98 H: 4.77 O: 13.90	C: 72.95 H: 4.80 O: 13.87
3	0.66	126± 2°C	C: 68.12 H: 4.46 O: 12.97	C: 68.09 H: 4.49 O: 12.94
4	0.62	120± 2°C	C: 59.78 H: 3.55 O: 11.38	C: 59.81 H: 3.52 O: 11.35
5	0.56	174± 2°C	C: 54.18 H: 3.42 O: 24.88	C: 54.21 H: 3.45 O: 24.91

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