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Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF DIFFERENT SUBSTITUTED BENZILIC ACID USING 2,2-DIPHENYL-1-PICRYL HYDRAZYL RADICAL, ABTS ASSAY METHOD

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ABSTRACT

Objectives: The aim of this study is to investigate the antioxidant activity of different substituted benzilic acid using DPPH assay and ABTS methods.

Methods: Compounds were synthesized based on benzil-benzilic acid rearrangement. The synthesized compound was confirmed by 1HNMR, Infrared, Mass, TGA, and UV analysis. Antioxidant activity of the compound was measured by radical scavenging assay method (2,2-diphenyl-1-picrylhydrazyl) and ABTS method.

Results: All synthesized compounds show moderate to extent antioxidant activity. The results for the antioxidant activity showed that the highest percentage of scavenger radicals was present in the 2'-chloro-4-methoxy-3-nitro benzilic acid.

Conclusion: The result showed that better activity in 2'-chloro-4-methoxy-3-nitro benzilic acid due to the presence of electro withdrawing and electro donating group than others. Thus, it could be served as potent antioxidant. All compounds were tested for their effect on the viability of cells and results demonstrated that they are not toxic towards the cell lines used.

Keywords: Synthesis, Antioxidant activity, 2, 2-diphenyl-1-picryl hydrazyl assay, ABTS assay.

INTRODUCTION

Antioxidants are compounds capable of preventing and even counteracting the damage caused in human tissue by the normal effects of physiological oxidation. Antioxidants have received increased attention in the last years from nutritionists and medical researchers for their potential activities in the prevention of several degenerative diseases such as cancer and cardiovascular disorder, as well as aging [1-4]. Recent evidence [5] suggests that free radicals which are generated in any bioorganic redox processes may induce oxidative damage in various components of the body (e.g., lipids, proteins, and nucleic acids) and may also be involved in processes leading to the formation of mutations. Furthermore, radical reactions play a significant role in the development of life-limiting chronic diseases such as cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, cataracts, and others [6]. The antimicrobial activity of substituted benzilic acid [7] and antitumor activity, antioxidant, antimicrobial activity for the compound benzyl [8] and has been reported. Antioxidants may be classified according to their mode of action as being free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation or oxygen scavengers that react with oxygen in closed systems [9]. A number of methods are available for the determination of free radical scavenging activity, but the assay employing the stable 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and its convenience [10]. This assay is the most widely used *in vitro* test to assess free radical scavenging capacities [11]. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. The ABTS radical is stable over a wide pH range and can be used to study pH effects on antioxidant mechanisms [12]. The ABTS radical is soluble in both aqueous and organic solvents and is not affected by ionic strength and can be used to measure the antioxidant capacity of hydrophilic and lipophilic compounds in test samples [13]. Antioxidants are added as Redox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical-induced decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule [14]. On the other hand, effects on the antioxidant activity of synthetic compounds were reported [15-17]. Consequently, the synthesis of new active derivatives with potential applications in this area and prepared by simple chemical procedures should be of increasing interest. In view of the considerable importance of the benzilic acid and its derivatives, the present work is aimed for testing of target compounds for their free radical scavenging activity using DPPH and ABTS radical scavenging methods. The investigation of antioxidant screening revealed that all the newly synthesized compounds showed potent to moderate radical scavenging activity when compared to the standard ascorbic acid.

METHODS

All chemicals and solvents were obtained from Sigma-Aldrich and S.D. Fine, India, AR. Different substituted benzilic acid derivatives were synthesized according to the synthetic scheme as shown below. Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and were uncorrected. Infrared (IR) and proton nuclear magnetic resonance (1H NMR) spectra and 13CNMR were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Advance (500 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane as an internal standard. The mass spectra were recorded on a gas chromatography-mass spectrometry spectrometer - JOEL GC Mate. The homogeneity of the compounds was monitored by ascending thin-layer chromatography on silica gel G (Merck) coated aluminum plates, visualized by iodine vapor (Figs. 1-4).

2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay

Procedure

The ability of the extracts to annihilate the DPPH radical was investigated by the method described by (Blois 1958). Stock solution

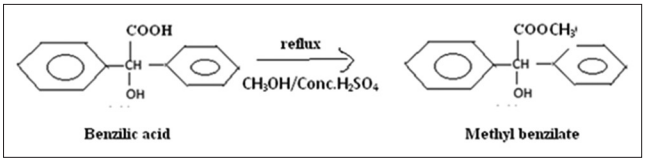


Fig. 1: Schematic representation of methyl benzilate

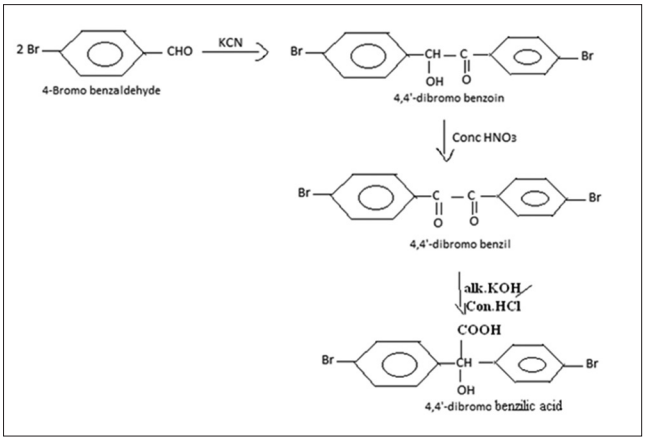


Fig. 2: Schematic representation of 4,4'-dibromo benzilic acid

of leaf extracts was prepared to the concentration of 1 mg/ml. 100 µg of each extract were added, at an equal volume, to methanolic solution of DPPH (0.1 mM). The reaction mixture is incubated for 30 minutes at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard controls [18]. The annihilation activity of free radicals was calculated in % inhibition according to the following formula:

% of inhibition=[(A of control−A of Test)/A of control]*100

ABTS radical scavenging assay

Procedure

ABTS assay was performed according to the protocol [19]. The stock solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution followed by incubation for 12 hrs at room temperature in the dark to yield a dark-colored solution containing ABTS•+ radicals. Working solution was prepared freshly before each assay by diluting the stock solution by mixing of stock solution to 50% methanol for an initial absorbance of about 0.700 (±0.02) at 745 nm, with temperature control set at 30°C. The free radical scavenging activity was assessed by mixing 300 µl of different fractions (25-250 µg/ml in respective solvents) with 3.0 ml of ABTS working standard. The decrease in absorbance was measured exactly 1 minute after mixing the solution; the final absorbance was noted up to 6 minutes. Data for each assay were recorded in triplicate. Ascorbic acid was used as positive controls. The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula:

% scavenging = [(A0−As)/A0]×100

Where, A0 is absorption of control and As is absorption of tested extract solution.

RESULT AND DISCUSSION

Various researchers have used the scavenging effect of a chemical on DPPH radical and ABTS assay as a quick and reliable parameter to assess the *in vitro* antioxidant activity. In this study, we determined the free radical scavenging capacities of the substituted benzilic acid using DPPH and ABTS assays. DPPH and ABTS assays have been widely used to determine the antioxidant capacities of plant extracts and synthesized

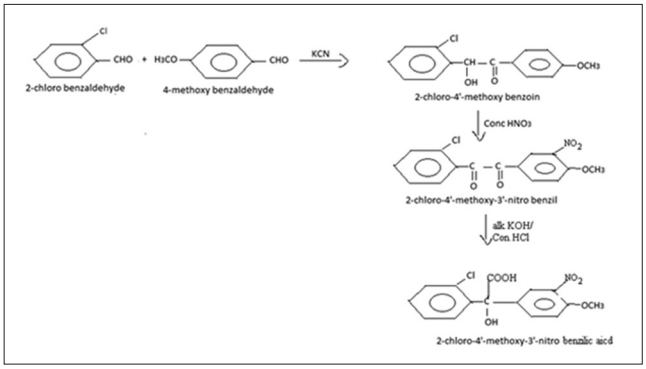


Fig. 3: Schematic representation of 2'-chloro-4-methoxy-3-nitro benzilic acid

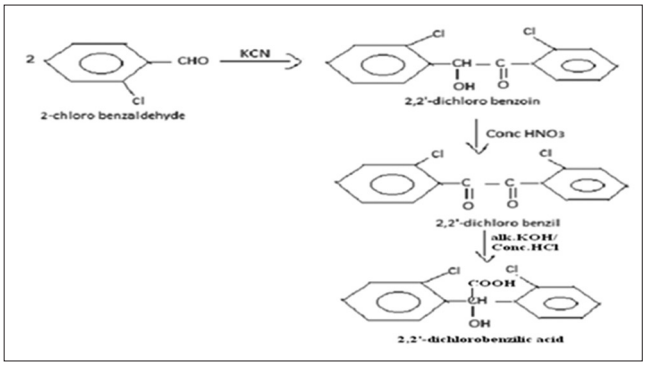


Fig. 4: Schematic representation of 2,2'-dichloro benzilic acid

compounds as they require relatively standard equipment and deliver fast and reproducible results. Indeed, an interlaboratory comparison of six methods for measuring antioxidant potential published recently showed that DPPH and ABTS assays are the easiest to implement and yield the most reproducible results [20]. As per chemical structural features, there were four different compounds synthesized under the study area. It is obvious that structural variation brings about the bioactivity and, of course, structural modification of molecules alters the biological activity in a regular trend. The results of free radical scavenging activity of benzilic acids at different concentrations are shown in Tables 1 and 2. The comparative antioxidant activity of compounds against ascorbic acid and substituted benzilic acid as a standard is shown by graphs. It is evident from results that free radical scavenging activity of these compounds was concentration dependent. Furthermore, substitution in the aromatic ring system with halogens such as chlorine or bromine sharply enhanced the antioxidant potency. This is why compounds 2, 3, and 4 were found to be more active than the compound 1. However, compound 3 was found to be the most efficacious antioxidant among all the listed compounds. Actually, the antioxidant efficacy of any compound depends strongly on its reducing property and compound 3 might have the higher reducing potential. It is thought that the chlorine atom because of its lone pair electron as well as its electronegativity power enhanced the formation and subsequent stabilization of the nitro group intervening aromatic system in case of compound 3. Due to extra stabilization, radical obtained from compound 3 would have the higher aptitude to trap free radical in a faster rate than the other similar type of molecules. In general, the presence of electron donor substituent shows moderate antioxidant property while electron withdrawing group enhances the DPPH and ABTS scavenging ability.

CONCLUSION

The reducing ability increases with increasing concentration. All substituted benzilic acid exhibited better reducing ability when compared with the standard ascorbic acid. Among the different benzilic

Table 1: Antioxidant values by ABTS assay

Test	MB (µg)			4,4'DBA (µg)			2'C3NBA (µg)			2,2'DCBA (µg)		
	20	60	100	20	60	100	20	60	100	20	60	100
Sample	4.5	27.6	48.9	9.2	37.5	56.0	11.3	20.5	82.3	12.05	39.8	56.8
Ascorbic acid	9.4	44	74	9.4	44	74	9.4	44	74	9.4	44	74

MB: Methyl benzilate, 4,4'DBA: 4,4'-dibromo benzilic acid, 2'C4MNBA: 2'-Chloro-4-methoxy-3-nitro benzilic acid, 2,2'DCBA: 2,2'dichloro benzilic acid

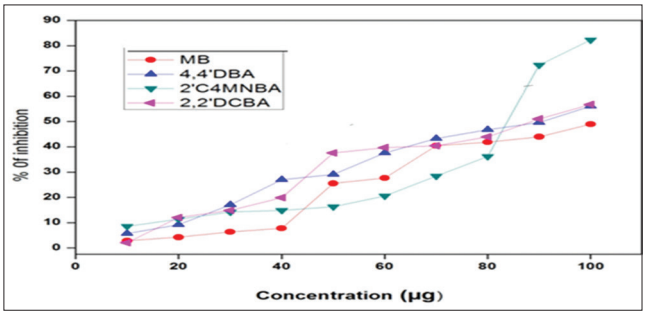
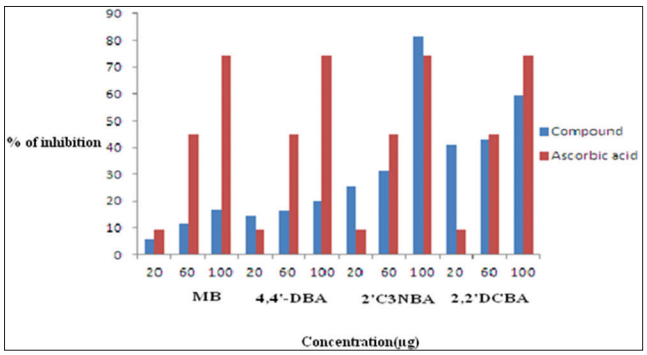


Table 2: Antioxidant values by DPPH assay

Concentration	MB (µg)			4,4'DBA (µg)			2'C3NBA (µg)			2,2'DCBA (µg)		
	20	60	100	20	60	100	20	60	100	20	60	100
Sample	6.0	11.3	16.8	14.6	16.4	20.2	25.3	31.3	81.2	41.3	43.1	59.5
Ascorbic acid	9.06	44.78	74.16	9.06	44.78	74.16	9.06	44.78	74.16	9.06	44.78	74.16

MB: Methyl benzilate, 4,4'DBA: 4,4'-dibromo benzilic acid, 2'C4MNBA: 2'-Chloro-4-methoxy-3-nitro benzilic acid, 2,2'DCBA: 2,2'dichloro benzilic acid



acid substituents, the scavenging ability is remarkably improved in the presence of the halogen-substituted benzilic acid and electron withdrawing group on the phenyl ring. Our studies, hence, reveal that different benzilic acid and its derivatives exhibited evident antioxidant properties *in vitro*. In summary, most of the synthesized compounds were a potential lead for antioxidant activity. On the basis of observed results, it may be concluded that the substitution favors the scavenging activity. The presence of chloro, methoxy, and nitro substitution in compound 3 increases the DPPH as well as ABTS free radical scavenging activity of the compounds.

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