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Phenethylamide derivatives: Synthesis and evaluation of antimicrobial and antioxidant activity

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ABSTRACT

Phenethylamidediesters ***(2-6)*** *were synthesized by reacting [3-ethoxy-4-(ethoxycarbonyl)phenyl]acetic acid with a series of C-protected aminoacids viz., Alanine, Proline, Phenylalanine, Tyrosine and 2-amino-5-(benzyloxy)-5-oxopentanoic aciddiesters and these compounds were hydrolyzed using sodium hydroxide to their respective diacids* ***(7-11)****. The newly synthesized compounds were tested for antimicrobial activity against Escherichia coli, Staphylococcus Aureus, Pseudomonas Aeruginos,Aspergillus Flavus, ChrysosporiumKeratinophilum, Candida Albicansand antioxidant activity by DPPH method. Compounds* ***6****,* ***7****,* ***8,*** *and* ***11*** *were most active as antimicrobials and* ***6, 10*** *and* ***11****have shown promising antioxidant activity.*

Key words: antimicrobial; antioxidant; amides; Phenethylamide.

INTRODUCTION

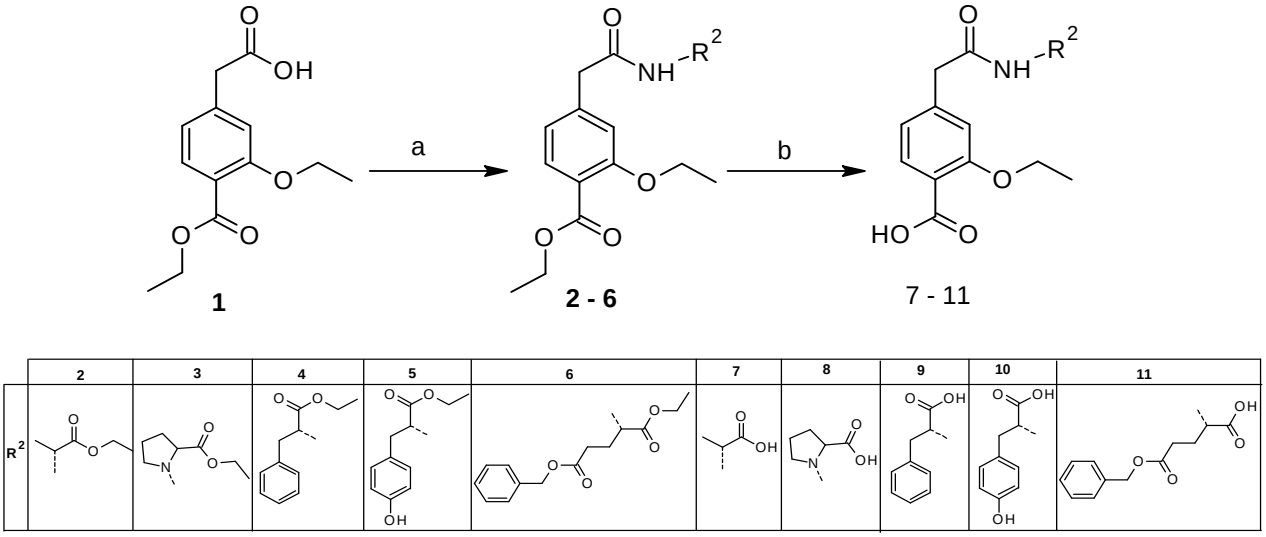
In nature, protein synthesis involving a sequence of peptide coupling reactions (amide bond formation between two α-aminoacids or peptides) is very complex, probably to safe guard the unique and precisely defined aminoacids sequence of every protein. This barrio is overcome in vivo by a selective activation process catalyzed by enzymes, where the required aminoacid is transferred into an intermediate amino ester. This intermediate is then involved in a process mediated by the coordinated interplay of more than a hundred macro molecules, including m-RNA's, t-RNA's, activating enzymes and protein factors, in addition to ribosomes [1].The amide functionality is a common feature in small or complex synthetic or natural molecules. For example, it is ubiquitous in life, as proteins play important role in virtually all biological process such as enzymatic catalysis (nearly all known enzymes are proteins), transport/storage (hemoglobin), immune protection (antibodies) and mechanical support (collagen). Amides also play a key role for medicinal chemists. And in-depth analysis of the comprehensive medical

chemistry data base revealed that the carboxamide group appears in more than 25% of known drugs [2]. This can be expected, since carboxamides are neutral, are stable and have both hydrogen-bond accepting and donating properties.

Amides are known to play a pivotal role in molecular recognition, being important components in supramolecular chemical anion sensorstechnology [3, 4]. Amide derivatives were associated with broad spectrum of biological activities including antituberculosis [5], anticonvulsant [6], analgesic, anti-inflammatory [7], insectidal [8], antimicrobial [9], and antitumor [10] properties. Morpholine derivatives find their wide spectrum of antimicrobial activity and exhibit anthelmintic, bactericidal, and insecticidal activity [11]. Amide derivatives also show anti-platelet activity [12].Compounds having amino acid moieties are known to possess a wide range of biological and pharmacological activity [13] and organic acid esters known to be used for reducing virus and bacterial populations [14].Currently there is a tendency to use aminoacid/peptidyl residues during the prodrug design process. The literature reports that bioactive compounds show enhanced activity when linked to aminoacids [15-18]. The presence of unusual aminoacid and heterocyclic building blocks has stimulated interest in new synthetic methodologies and strategies to obtain a target structure. In this connection, we have synthesizedPhenethylamidediestersand their corresponding diacidsby coupling C-protected aminoacids Alanine, Proline, Phenylalanine, Tyrosine and 2-amino-5-(benzyloxy)-5-oxopentanoic aciddiesterswith [3-ethoxy-4-(ethoxycarbonyl)phenyl]acetic acid followed by hydrolysis. The present study was undertaken with a view to find the efficacy of these compounds as antimicrobial and antioxidant agents.

MATERIALS AND METHODS

Scheme 1: Synthesis of phenethyl amides



Reagents and conditions: (a) DCM, ethyl ester, EDCI.HCl (3.5 eq.), HOBt (1.0 eq), DIPEA(3 eq.), RT 10 h; (b) THF, NaOH 3 eq.), 85° C for 12 h.

Chemistry

[3-ethoxy-4-(ethoxycarbonyl)phenyl]acetic acid**1** was prepared as per the know method [19]. Compounds **2-11**(**Scheme 1**) were obtained by coupling of **1** with ethyl esters of Alanine, Proline, Phenylalanine, Tyrosine and 2-amino-5-(benzyloxy)-5-oxopentanoic acid followed by hydrolysis using aqueous sodium hydroxide. All the compounds were characterized by using spectroscopic methods. ^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX400 spectrometer operating at 400 MHz, with Me_4Si as internal standard. The chemical shifts are expressed as δ values in parts per million (ppm) and the coupling constants (J) are given in hertz

(Hz). Mass spectra were determined by the EPSRC Mass Spectrometry Centre (Swansea, UK). Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck) and TLC was carried out on precoated silica plates (kiesel gel 60 F_{254}, BDH). Melting points were determined on an electrothermal instrument and are uncorrected. All reagents involved in the experiments were commercially available and used without further purification. The yields were of purified compounds and were not optimized.

Synthesis of phenethyl amides (2-11)

General method for synthesis of ethyl esters: To a stirred solution of acid in ethanol, was added thionyl chloride (4 equivalents) at 0° C. The contents were stirred at 80° C for 4 h under nitrogen atmosphere. The reaction completion was monitored by TLC. The solvent was removed under vacuum, added ice cold water and basified with 10% $NaHCO_3$ solution. Then the product was extracted to ethyl acetate and the organic layer was washed with saturated NaCl solution, dried over anhyd. Na_2SO_4 and concentrated to residue to obtain the ethyl ester.

General method for amide coupling:To a stirred solution of equimolar acid and amine in anhydrous dichloromethane, was added at 0° C EDCI.HCl (3.5 eq.), HOBt (1.0 eq) and Diisopropyl ethylamine (DIPEA) (3 eq.). The reaction mass was stirred at room temperature under nitrogen atmosphere for 10 h. The reaction completion was monitored by TLC. The reaction mass was washed with sodium bicarbonate (10% solution) followed by water and saturated sodium chloride solution. The organic layer was dried over sodium suphate and concentrated under vacuum to residue. The residue was purified by column chromatography using 60-120 mesh silica gel and pet-ether and ethyl acetate as eluent.

General method for ester hydrolysis: To a stirred solution of ethyl ester in THF, was added aqueous NaOH solution (30%, 3 eq.) and the contents were stirred at 85° C for 12 h. the reaction completion was monitored by TLC. The solvent was removed under vacuum; the aqueous layer was washed with MTBE to remove the non-polar impurities. The aqueous layer was acidified with citric acid (pH 2-3) and product was extracted to ethyl acetate. The organic layer was washed with saturated NaCl solution, dried over anhyd. Na_2SO_4 and concentrated to residue to obtain the acid.

*Synthesis of ethyl 2-ethoxy-4-(2-(1-ethoxy-1-oxopropan-2-ylamino)-2-oxoethyl)benzoate(**2**)*

Yellow solid (47%), ^{1}HNMR(DMSO-d_6, δ ppm); 400MHz;1.07(t, J = 3.08Hz, 3H), 1.24 (t, J = 3.01Hz, 3H), 1.34(t, J = 3.44 Hz, 3H), 2.75-2.81(m, 1H), 2.89 (d, J = 7.2Hz, 3H), 3.74(s, 2H), 3.99-4.09(m, 4H), 4.24 (q, J = 7.1Hz,2H), 6.85(d, J = 7.7Hz, 1H), 6.95(d, J = 3.9Hz, 1H), 7.6 (t, J = 8Hz,1H), 8.27(d, J = 8.3 Hz, 1H); MF = $C_{18}H_{25}NO_6$, MW=351.39;$[m/z]^+$= 352.2.

*Synthesis of ethyl 1-(2-(3-ethoxy-4-(ethoxycarbonyl)phenyl)acetyl)pyrrolidine-2-carboxylate(**3**)*

Yellow solid (57%), ^{1}HNMR(DMSO-d_6, δ ppm); 400MHz; 1.08 (t, J = 3.08Hz, 3H), 1.24 (t, J = 3.09Hz, 3H), 1.39 (s,3H), 1.82-1.90 (m,2H), 2.10-2.14 (m, 1H), 3.47-3.69 (m, 4H), 3.74 (s,2H), 4.04 (q, J = 6.0Hz,2H), 4.12 (q, J = 5.8Hz,2H), 6.85 (d, J = 8.0Hz,1H), 6.98 (d, J = 4Hz, 1H), 7.55 (t, J = 8.0Hz,1H); MF=$C_{19}H_{25}NO_6$; MW= 363.40; $[m/z]^+$= 364.6.

*Synthesis of ethyl 2-ethoxy-4-(2-(1-ethoxy-1-oxo-3-phenylpropan-2-ylamino)-2-oxoethyl)benzoate(**4**)*

White solid (71%), ^{1}HNMR(DMSO-d_6, δ ppm); 400MHz; 1.09 (t, J = 7.08 Hz, 3H), 1.24 (t, J = 3.12Hz, 3H), 1.29 (t, J = 7.12Hz, 3H), 2.88-2.93(m,1H), 3.03(dd, J_1= 5.6Hz, J_2 = 13.68Hz, 1H), 3.46(s, 1H), 3.99-4.06(m, 4H), 4.23 (q, J = 7.0-8Hz, 2H), 4.44-4.48(m,1H), 6.76 (dd, J_1 = 1.2,Hz, J_2 = 7.88,Hz, 1H), 6.95 (s, 1H), 7.17-7.27 (m, 5H), 7.51 (d, J = 7.88Hz, 1H), 8.59(d, J = 7.76Hz, 1H); MF = $C_{24}H_{29}$ NO_6; MW = 427.49; $[m/z]^+$= 428.5.

*Synthesis of ethyl 2-ethoxy-4-(2-(1-ethoxy-3-(4-hydroxyphenyl)-1-oxopropan-2-ylamino)-2-oxoethyl) benzoate(**5**)*

White solid (64%), ^{1}HNMR(DMSO-d_6, δ ppm); 400MHz; 1.08 (t, J = 3.08Hz, 3H), 1.24(t, J = 3.08Hz, 3H), 1.34(t, J = 3.32Hz, 3H), 2.76-2.81(m, 1H), 2.89(dd, J_1 = 5.72Hz, J_2 = 13.82Hz, 1H), 3.46 (s, 1H), 3.99-4.06 (m, 4H), 4.23 (q, J = 7.08Hz, 2H), 4.33-4.39(m, 1H), 6.62(d,J = 2.72Hz, 2H), 6.76(dd, J_1 =

1.24Hz, J_2 = 12.01Hz, 1H), 6.97(d, J = 8.44Hz, 3H), 7.52 (d, J = 7.88Hz, 1H), 8.53(d, J = 7.72Hz, 1H), 9.24(s, 1H); MF = $C_{24}H_{29}NO_7$; MW = 443.48; $[m/z]^+$=444.5.

*Synthesis of 1-ethyl 5-phenyl 2-(2-(3-ethoxy-4-(ethoxycarbonyl)phenyl)acetamido)pentanedioate(**6**)*
Pale brown solid (56%), ^{1}HNMR(DMSO-d_6,δ ppm); 400MHz; 1.06 (t, J = 3.1Hz, 3H), 1.22 (t, J = 3.2Hz, 3H), 1.32 (t, J = 3.4Hz, 3H), 1.89-1.93 (m, 2H), 2.25 (t, J = 7.6Hz, 2H), 2.55 (t, J = 13.2Hz, 2H), 3.13 (s, 1H), 4.03 (q, J = 6.7Hz, 2H), 4.16-4.19 (m, 4H), 4.24(q, J = 7.6Hz, 2H), 6.84 (d, J = 7.6Hz, 1H), 7.01 (s, 1H), 7.52 (d, J = 8Hz, 1H), 7.52-7.62 (m, 5H), 8.45 (d, J = 7.2Hz, 1H); MF = $C_{27}H_{33}NO_8$; MW = 499.55; $[m/z]^+$=500.4.

*Synthesis of 4-(2-(1-carboxyethylamino)-2-oxoethyl)-2-ethoxybenzoic acid(**7**)*
Pale yellow solid (66%), ^{1}HNMR(DMSO-d_6,δ ppm); 400MHz; 1.06 (t, J = 3.10Hz, 3H), 2.74-2.80 (m, 1H), 2.90 (d, J = 7Hz, 3H), 3.72 (s, 2H), 4.19 (q, J = 6.7Hz, 2H), 6.90 (d, J = 7.1Hz, 1H), 6.93 (d, J = 4Hz, 1H), 7.7 (t, J = 8Hz, 1H), 8.24 (d, 8.2Hz, 1H), 12.24 (s, 2H); MF = $C_{14}H_{17}NO_6$; MW = 295.28; $[m/z]^+$=296.5.

*Synthesis of 1-(2-(4-carboxy-3-ethoxyphenyl)acetyl)pyrrolidine-2-carboxylic acid(**8**)*
White solid (56%), ^{1}HNMR(DMSO-d_6,δ ppm); 400MHz; 1.08 (t, J = 3.10 Hz, 3H), 1.80-1.89 (m, 2H), 2.10-2.13 (m,1H), 3.47-3.70 (m, 4H), 3.75 (s,2H), 4.02 (q, J = 5.8Hz, 2H), 6.85 (d, J = 7.78Hz, 1H), 6.95 (d, J = 4.01 Hz, 1H), 7.59 (t, J = 7.9Hz, 1H) 12.12 (s, 2H); MF = $C_{16}H_{19}NO_6$; MW = 321.32; $[m/z]^+$=322.3.

*Synthesis of 4-(2-(1-carboxy-2-phenylethylamino)-2-oxoethyl)-2-ethoxybenzoic acid(**9**)*
White solid (56%), ^{1}HNMR(DMSO-d_6, δ ppm); 400MHz; 1.27 (t, J = 6.8Hz, 3H), 2.82-2.88 (m, 1H), 3.06 (dd, J_1 = 4.8Hz, J_2 = 13.2 Hz, 1H), 3.69 (t, J = 6.0Hz, 1H), 3.74 (s, 2H), 4.12 (q, J = 6.8Hz, 2H), 4.14 (d, J = 5.6Hz, 1H), 6.69 (d, J = 7.6Hz, 1H), 6.88 (s, 1H), 7.07-7.15 (m, 5H), 7.41 (d, J = 6.8Hz, 1H), 7.7 (s, 1H), 12.14 (s, 2H); MF = $C_{20}H_{21}NO_6$; MW = 371.38; $[m/z]^+$=372.4.

*Synthesis of 4-(2-(1-carboxy-2-(4-hydroxyphenyl)ethylamino)-2-oxoethyl)-2-ethoxybenzoic acid(**10**)*
White solid (66%), ^{1}HNMR(DMSO-d_6, δ ppm); 400MHz; 1.29 (t, J = 6.8Hz, 3H), 2.69-2.75 (m, 1H), 2.90 (dd, J_1 = 4.4 Hz, J_2 = 13.8Hz, 1H), 3.3 (s,1H), 3.98 (q, J = 6.8Hz, 2H), 4.28-4.34 (m, 1H), 6.60 (d, J = 8.4Hz, 2H), 6.68 (d, J = 0.8Hz, 1H), 6.93 (t, J = 7.2Hz, 3H), 7.48 (d, J = 8Hz, 1H), 8.35 (d, J = 8.4Hz, 1H), 9.19 (s, 1H), 12.5 (s, 2H); MF = $C_{20}H_{21}NO_7$; MW = 387.38; $[m/z]^+$ = 388.5.

*Synthesis of 4-(2-(1-carboxy-4-oxo-4-phenoxybutylamino)-2-oxoethyl)-2-ethoxybenzoic acid(**11**)*
White solid (56%), ^{1}HNMR(DMSO-d_6,δ ppm); 400MHz; 1.28 (t, J = 6.7Hz, 3H), 1.89-1.94 (m, 2H), 2.25 (t, J = 7.6Hz, 2H), 2.55 (t, J = 14Hz, 2H), 3.13 (s, 1H), 4.03 (q,J = 6.8Hz, 2H), 4.16 (q, J = 8.0Hz, 2H), 6.85 (d, J = 7.6Hz, 1H), 7.00 (s,1H), 7.52 (d, J = 8.0 Hz, 1H), 7.54-7.63 (m, 5H), 8.45 (d, J =7.6Hz, 1H), 12.34 (s, 2H); MF = $C_{23}H_{25}NO_8$; MW = 443.44; $[m/z]^+$ = 444.3.

Pharmacology

Bacterial and Fungal strains
The following bacteria and fungi were used for the experiment. Bacteria: S*taphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. All bacterial strains were maintained on nutrient agar medium at ±37°C. Fungi: *Aspergillus flavus*, *Chrysosporiumkeratinophilum* and *Candida albicans* MTCC 227 are used in this study. All fungi strains were maintained on potato dextrose agar (PDA) at ±25 °C. These cultures are obtained from the Department of Microbiology, Kuvempu University.

Antimicrobial activity
The newly synthesized compounds were tested against a panel of pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus* and P*seudomonas aeruginosa*. Microorganism strains were maintained on nutrient agar medium at 37°C. The cultures were inoculated in fresh 10 ml Nutirent Broth to yield an initial suspension of approximately 10–100 cfu/ml. All broths were then incubated statically at the aforementioned temperatures for microorganisms, for 18–24 h so that all cells were in the stationary

phase. Susceptibility of the test organism to the extract was determined by employing in the well plate technique. The bacterial suspensions were diluted tenfold in distilled water, and 0.1 ml from the appropriate dilution was spread plated on nutrient agar in order to give a population of approximately 106 cfu/plate. The wells were dug in each Petri plate by sterilized cork borer. The compounds were dissolved in DMSO and appropriate dilutions were made (1mg/ml and 0.5mg/ml). The same procedure was repeated for different micro-organisms. Each experiment was carried out in triplicate. After the inoculation and addition of organism and compound, the Petri plates were incubated in inverted position for 18 h at 37°C. After the incubation, the zone of inhibition was measured and the values for Dimethylsulphoxide (DMSO) were subtracted to get the actual values.

Antifungal Activity

The fungal strains used in this study were *Candida albicans*, *Aspergillus Flavus* and *Chrysosporium Keratinophilum*. The required amounts of each fungal strain were removed from the stock and suspended in 5ml of distilled water with 2 drops of Tween 80. This suspension was uniformly spread on Petri plates containing Potato dextrose agar media using sterile swabs. After applying the samples into the wells formed by using the same technique for tests on bacteria, the plates were incubated at 25 °C for 3 days. The plates were then examined for the presence of zones of inhibition and the results were recorded. Flucanazole was used as a positive control at a concentration of 1 mg/mL.

DPPH scavenging activity

The target compounds were tested for 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity with Butylated hydroxytoluene (BHT) as standard according to the previously reported procedure [20]. This method is based on the reduction of free radical DPPH by free radical scavengers. The procedure involves the measurement of decrease in absorbance of DPPH at 517 nm, which is proportional to the activity of free radical scavenger added to DPPH reagent solution. A stock solution of test compounds (1 mg/mL) and DPPH (0.004%) was prepared in 95:5 methanol: water. To 3 mL of freshly prepared DPPH solution in test tube, was added stock solution of test compound (100 µg) and reacted for 15 min and the absorbance was measured at 517 nm using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). BHT was used as a reference standard and dissolve in distilled water to make the stock solution with the same concentration (1 mg/mL). Ascorbic acid was used as control sample and 95% methanol served as blank. % scavenging of the DPPH free radical was measured by using the following equation:

% scavenging activity = (Absorbance of the control - Absorbance of the test sample) / Absorbance of the control × 100.

Table 1.Antibacterial activity of phenethyl amides

	Escherichia coli		***Staphylococcus Aureus***		***Pseudomonas Aeruginosa***	
Concentration in (mg/mL)	1	0.5	1	0.5	1	0.5
DMSO	0	0	0	0	0	0
Streptomycin	18	14	16	12	16	13
2	07	05	08	06	04	03
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	11	09	10	08	12	09
7	12	09	09	07	10	07
8	10	08	08	06	09	07
9	03	01	05	03	04	02
10	05	03	04	02	03	01
11	09	07	08	06	09	07

RESULTS AND DISCUSSION

Antibacterial activity

The in vitro antibacterial activities of the target compounds are as shown in the Table 1. In addition, the inhibition zones formed by standard antibiotic Streptomycin, and DMSO (negative controls), are listed. As can be seen from **Table 1**, the compounds **6**, **7**, **8**, and **11** showed good antibacterial activity towards all bacteria when compared with the other compounds.

Antifungal activity

The antifungal activity of different test compounds is shown in the **Table 2**. The compounds **6**, **7**, **8**, and **11**showed good activity when compare to the other compounds.

Table 2.Antifungal activity of Phenethyl amides

	Aspergillus Flavus		***Chrysosporium Keratinophilum***		***Candida Albicans***	
Concentration (mg/mL)	1	0.5	1	0.5	1	0.5
DMSO	0	0	0	0	0	0
Flucanazole	14	10	16	14	23	20
2	03	0	05	03	04	01
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	09	06	10	07	11	08
7	07	05	09	07	10	08
8	07	04	10	08	09	07
9	05	03	03	01	04	02
10	03	01	04	02	03	0
11	08	05	10	07	11	08

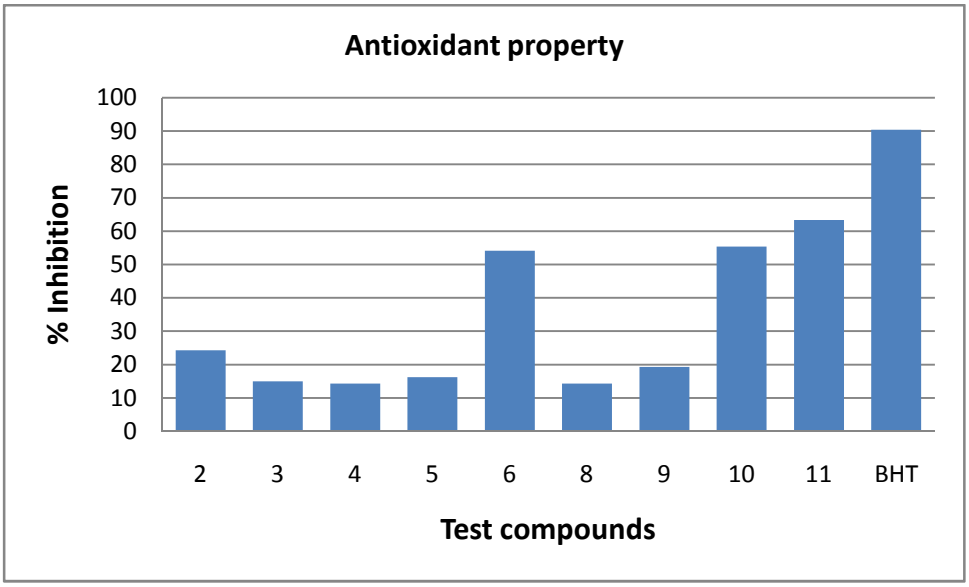


Fig. 1.% Inhibition of phenethyl amides

DPPH Scavenging activity

Antioxidant properties Organic compoundswere evaluated to find a new source of antioxidant. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds. The compounds were screed for DPPH radical scavenging activity according to the method described and the results of the screening are shown in table as comparable with BHT, known antioxidant. The

compounds **6, 10** and **11** show comparable activity with BHT, but other compounds were not shows good activity when compare with the standard (**Table 3**). The graphical representation of these results is shown in **Fig. 1**.

Table 3.% DPPH assay of Phenethyl amides

Compounds	DPPH Assay in %
2	24.3
3	15.0
4	14.3
5	16.2
6	54.1
8	14.3
9	19.3
10	55.4
11	63.3
BHT	**90.42**

CONCLUSION

In conclusion, the synthesized compounds **6**, **7**, **8**, and **11** showed antimicrobial activity and **6, 10** and **11**exhibited promising antioxidant activity. The most active compounds were 6 and 11 against antimicrobial activity and antioxidant activity respectively.

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REFERENCES

[1]. L.Stryer, In Biochemistry 4th ed.; W.H. Freeman; New York, **1995**; Chapter 2, pp 17-25.
[2]. A.K.Ghose, V.N.Viswanadhan,J.J.Wendolosk,*J.Comb. Chem.*,**1999**, 1, 55-68.
[3]. P.D.Beer, M.G.B.Drew, R.Jagasser, *J.Chem.Soc.*, Dalton Trans.,**1997**, 881-886.
[4]. A.Rojas, Hernandez, R.Pereda-Miranda, R.Mara, *J. Ethnopharmacol.*,**1992**, 35, 275-283.
[5]. Mohamed I. Hegab, Abdel-Samee M. Abdel-Fattah, Nabil M. Yousef, *Archiv der Pharmazie, Chemistry in Life Sciences*,**2007**, 340, 396-399.
[6]. NadeemSiddiqui, M. ShamsherAlam, WaquarAhsan, *ActaPharma.*,**2008**, 58, 445-454.
[7]. K.Gelewicz-Walesa,A.Pachuta-Stec, *Medical Academy in Lubin*, **2003**, 9,118-125.
[8]. T. L.Graybill, M. J.Ross, B.R.Gauvin, J. S.Gregory, A. L.Harris, M. A.Ator, J. M.Rinker, R. E.Dolle, *Bioorg. Med. Chem. Lett.*,**1992**, 2, 1375-1380.
[9]. NeelottamaKushwaha, Rakesh Kumar Saini, Swatantra K.S.Kushwaha,Intl.J.ChemTech Res., **2011**, 3, 1, 203-209.
[10]. Andre Warnecke, IndunaFichtner, Gretel Sab, Felix Kratz, *Archiv der Pharmazie, Chemistry in Life Sciences*,**2007**, 340, 8, 396-399.
[11]. T. A. Naik, K. H. Chikalia, *E-Journal of Chemistry*,**2007**, 4, 1, 60-66.
[12]. Klaus Rehse, JoschaKotthaus, LalehKhadembashi, *Archiv der Pharmazie, Chemistry in Life Sciences*,**2009**, 340, 8, 27-30.
[13]. Y. W.Chin, H. A. Jung, Y. Liu, N. Su, A. Castoro, W.J.Keller, A.D. Kinghorn, *J. Agric. Food Chem.*,**2007**, 55, 12, 4691-4697.
[14]. Richard F. Theiler, Earl P. Seitz, JR., US patent application US20100204323 (**2010**).
[15]. V.Suryanarayana, S. Sandeep, J.Meenakshi, S.Savitha, S.Prati Pal, K.ChamanLal, Rahul Jain, *Bioorg. Med. Chem.*,**2004**, 12, 239-247.
[16]. R. G.Sudhir, S. R. S.Rangan, C. A.Krishna, *J. Med. Chem.*,**1990**, 35, 5, 1505-1510.

[17]. C. L.Ana, P. S.Kezia, A. D. S.Ivone, M. D. A.Janete,J. B.Dalci, *Eur. J. Med. Chem.*,**2004**, 39, 1059-1065.
[18]. A.Christian, V.Emmanuelle, B.Helene, P.Claude, B.Jean, M.Bernard, *J. Med. Chem.*,**1984**, 27, 1161-1166.
[19]. Mohammad Salman, Purna C Ray, SujayBiswas, Naresh Kumar, U S Patent 6686497 (**2000**).
[20]. A. Braca, N.D. Tommasi, L.D. Bari, C. Pizza, M. Politi, I. Morelli, *J. Nat. Prod.*,**2001**, 64, 892–895.