Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF CINNAMALDEHYDE'S MANNICH BASES

G.VISHNUVARDHANARAJ[a], D.TAMILVENDAN[b], M.AMALADASAN[c]

[a]Department of Chemistry A.V.V.M Sri Pushpam College (Autonomous) Poondi, Thanjavur, [b]Department of Chemistry, National Institute of Technology, Tiruchirappalli, [c]Department of Chemistry St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India.
Email: Vishnuchem28@gmail.com

Received: 03 May 2013, Revised and Accepted: 10 Jun 2013

ABSTRACT

Objectives: The Mannich bases of Cinnamaldehyde derivatives were synthesized from o-toluidine,urea and thiourea by Mannich condensation reaction.

Methods: The synthesized compounds TOCT and TOCU were characterized by UV-Vis, FT-IR, FT-Raman, ^{1}H NMR, ^{13}C NMR, 2d NMR, FAB-MASS, and elemental analysis and were evaluated for their antibacterial activity against a panel of five pathogenic bacterial strains namely, *(Staphylococcus aureus*, (Gram +ve) *Klebsiella Pneumonia,(Gram –ve), Salmonella typhi, (*Gram –ve*), Escherichia coli*,(Gram –ve), and *Pseudomonas aeraginosa*,(Gram –ve),by two fold serial dilution method and antioxidant activity

Results: The synthesized compounds showed significant inhibitory activity against the microbes with concentration ranged from 50µg - 1.562 µg,using straptomycine as a standard.

Conclusion: The synthesized compound TOCT shows excellent antibacterial activity against *Staphylococcus aureus,*TOCU showed good antibacterial activity against only *Escherichia coli* and *Pseudomonas aeraginosa*.the synthesized compounds exhibit significant antioxidant activity using ascorbic acid as a standard.

Keywords: Cinnamaldehyde's derivatives, Antibacterial, Antioxidant activity.

INTRODUCTION

Microorganisms are closely associated with the health and welfare of human beings. Some microorganisms are beneficial, while others are detrimental.[1], They also help in the production of important products like penicillin, interferon, and alcohol. On the other hand, certain microorganisms can cause disease. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Hence, the constant need for new, safe and effective new molecules. The organic heterocyclic compounds containing hetero atoms are well known to possess significant biological activities. Such as bactericidal, fungicidal, herbicidal and insecticidal activities.[2],Shortly developments in biomedical point to the involvement of free radicals in many diseases.[3],The oxidative stress (OS), includes by reactive oxygen species (ROS), can be described as a dynamic imbalance between the amounts of free radicals generated in the body and levels of antioxidants to quench and or/scavenge them and protect the body against their deleterious effects.[4], Excessive amounts of ROS may be harmful because they can initiated biomolecular oxidations which lead to cell injury, aging, cancer, atherosclerosis, cirrhosis, cataracts and death, [5]. For these reasons, antioxidants are of interest for the treatment of many kinds of cellular degeneration.[6], So that it received great attention to synthesis new organic molecules with enhanced biological activity

The present study was undertaken in an attempt to synthesize some new Mannich bases of Cinnamaldehyde, o-toluidine, urea and thiourea and carry out their antibacterial and antioxidant studies.[7], The Mannich reaction is a three-component condensation in which a compound containing an active hydrogen atom is allowed to react with an aldehyde or ketone, and a primary or secondary amine with concomitant release of water to produce a base known as Mannich base. The formation of C-N-C bond in this amino methylation process makes the Mannich reaction an extremely useful synthetic transformation. Mannich bases are broad range of application in the area of biomedical, biomimetic, antioxidants,and antimicrobial properties.[8,-11], Amide derivatives of Mannich bases have been reported to have antiepileptic, anticonvulsive.[12] Cinnamaldehyde and its derivatives has been reported to inhibit the growth of *Staphylococcus aureus* , *Escherichia coli*, *Clostridium botulinum*, *Salmonella enteric, Serovar typhimurium.*[13-15]

MATERIALS AND METHODS

All reagents were commercially available and used without further purification. Solvents were distilled from appropriate drying agents subsequently prior to use. The C, H and N were analyzed on a Carlo – Erba 1106 elemental analyzer. IR spectra were recorded on a shimadzus FTIR affinity 1 spectrophotometer in KBr medium, all melting points were taken in open capillary tubes in ^{o}C by using richerkjung Heizbank melting point apparatus, Ultraviolet – visible (UV – Vis) absorption spectra were recorded on a Perkin-Elmer Lambda 35 spectrophotometer at the wavelength of maximum absorption (λ_{max}) in DMSO at room temperature. Raman spectra were Recorded on Bruker RFS 27, ^{1}H NMR, ^{13}C NMR and 2d NMR spectra were recorded on a Bruker Advance DPX 400 MHz ultra-shield FT –NMR spectrophotometer in DMSO-d_6 with TMS as internal standard, chemical shifts are expressed in (6 units ppm). SAIF IIT Madras-36. The mass spectral studies were recorded on JEOL D - 300 (EI) mass spectrometer. Antimicrobial screening of the Mannich bases were carried out by using agar- well diffusion and two fold serial dilution method. The five human pathogenic bacteria were purchased from MTCC Chandigarh, India. (*Staphylococcus aureus* (Gram +ve) MTCC No 96121, *Klebsiellapnemonia* (Gram –ve) MTCC No 3384, *Salmonella typhi* (Gram –ve) MTCC No 1771, *Escherichia coli* (Gram –ve) MTCC No1302, *Psudomonas aeruginosa* (Gram –ve) MTCC No 4727) and were used for the antibacterial studies. Antioxidant screening of the Mannich bases were recorded on Perkin Elmer Lambda 35, UV-Visible spectrophotometer.

Synthesis of Mannich Bases

1-{(2E) -1-[(2-methylphenyl) amino]-3-phenylprop-2-en-1-yl) thiourea (TOCT)

Thiourea (0.76g, 0.01M), o- toluidine (1.06mL, 0.01M), and cinnamaldehyde(1.32 mL, 0.01M,)were taken in equimolar ratio. A concentrated ethanolic solution of thiourea and o- toluidine was prepared. Cinnamaldehyde was added in drops with constant

stirring of the solution. The mixture first becomes oily yellow and then slowly turned into a pale yellow solid mass which was separated by suction filtration and washed several time with distilled water. The product (Fig.1) was dried at 45°c and recrystallized from methanol by slow evaporation method. [16]

1-{(2E)-1-[(2-methylphenyl) amino]-3-phenylprop-2-en-1-yl}urea (TOCU)

urea (0.60g, 0.01M), and o-toluidine 1.06 mL, 0.01M) were dissolved in minimum amount of ethanol and the contents were mixed well at room temperature until a homogeneous solution was obtained. Cinnamaldehyde (1.32 mL, 0.01M) was added to this solution slowly with constant stirring for an hour cooling gave off orange yellow solid product (Fig.2) and it was washed with distilled water several times, dried in an air oven at 50°C and recrystallized with methanol by slow evaporation method.[17]

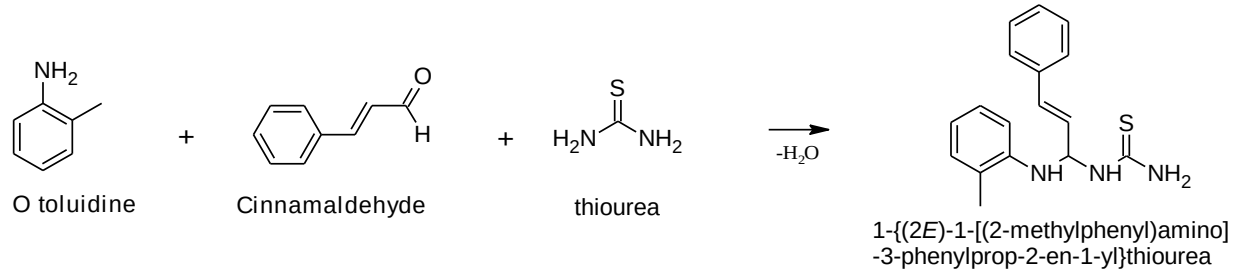

Fig. 1: 1-{ (2E) -1-[(2-methylphenyl) amino]-3-phenylprop-2-en-1-yl)thiourea

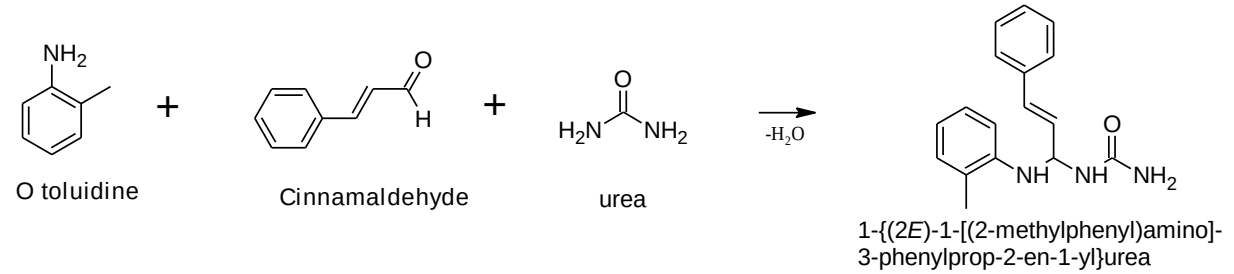

Fig. 2: 1-{(2E)-1-[(2-methylphenyl) amino]-3-phenylprop-2-en-1-yl} urea

Spectral data

1-{(2E) -1-[(2-methylphenyl) amino]-3-phenylprop-2-en-1-yl) thiourea (TOCT)

M.F: C_{17} H_{19} N_3 S , yield: 94%, m.p 68 – 70°C, Mol.wt: 297. FT IR KBr ν in cm^{-1}: 3058,3000 (-NH), 3042,2942 (CH aromatic and aliphatic), 1603 (C=C),(ν C=N) , (б NH), 1574 (б NH ipb) , 1375,1293,1255 (ν C=S), (ν C-N stretching),(б C-N) 1108, 1147, 1255 (C-N-C) 1448,1376 (CH symmetrical and Asymmetrical stretching), 688, 719 (Mono substituted benzene ring), 747, 783 (disubstituted toluidine aromatic ring), 1603 (skeletal vibration of benzene ring), 1187 (ν C=S) 612 (б S-C-N), (π C-S) 551 (б C-S) 980 (CH opb of Vinyl group) FT Raman polycrystalline powder ν in cm^{-1}: 3058 (- NH) 3018 (CH aromatic), 2985 (CH aliphatic) 1575 (б NH), 1359 1282 (C-N stretching), (б CH) 1150, (C-N-C), 618 (б S- C-N) 451(opb of ring C-C+ π C=S) 980 (CH opb of vinyl group), 1600 (skeletal vibration of benzene ring), 820 (C=C opb of ring), 130 (skeletal bending vibration). ^{1}H NMR (400MH_z DMSO d_6) б 8.27, (S,NH), 8.24 (S,NH_2), 6.94,6.96 (d,2H), 7.08 – 7.22 (m, 4H toluidine aromatic ring), 7.34- 7.69 (m, 5H benzene ring), 2.50 (S, 3H CH_3), ^{13}C NMR (400 MH_z, DMSO d_6) б 162.01 (S, 1C, C=S), 151.16 (S,1C,CH),18.05 (S,1C,CH_3) 117.98 (S,1C), 151.16 (S,1C),135.83(S,1C),125.94 – 131.59(m,4C,toluidine aromatic ring), 129.34 – 131.59(m,5C, benzyl ring) 144.42 (S,1C), 2d NMR(400 MH_z, DMSO d_6) The ^{1}H -^{1}H and ^{1}H – ^{13}C correlation (TOCT)which shows the better result corresponding to the those of ^{1}H NMR and ^{13}C NMR spectral data of the 2d NMR spectral study of the (TOCT) substantiates to the ^{1}H NMR and ^{13}C NMR spectral assignments. Mass (positive mode) *m/z*: 297 (C_{17} H_{19} N_3S), base peak *m/z*: 221 (C_{16} H_{16} N^+), 116 (C_9 H_8 $^+$), 106 (C_7 H_8 N^+), 77 (C_6 H_5^+). 76 (C H_3 N_2 S^+) Calculated: C 42. 50%, H 47.50%, N 7.50%, S 2.50%. Found: C 42.47%, H 47.48%, N 7.49%, S 2.47%.

1-{(2E)-1-[(2-methylphenyl) amino]-3-phenylprop-2-en-1-yl} urea (TOCU)

M.F: $C_{17}H_{19}N_3O$, Yield: 90% m.p 62-65 °C, Mol.wt: 281. FTIR KBr ν in cm^{-1}: 3444 (-NH), 3028, 2924 (CH aromatic and aliphatic), 1674 (C=O), 1589 (б NH)1072 ,1107 (C-N-C), 1448,1375 (CH symmetrical and Asymmetrical stretching),748 (CH opb of disubstituted toluidine aromatic ring),550 (б O-C-N) 445 (Opb ring C=C) 1627 (ν ring) 690 (CH opb of Monosubstituted benzene ring), 979 (CH Opb of Vinyl group) FT Raman polycrystalline powder ν in cm^{-1} : 3058 (-NH), 3010,2986 (CH aromatic and aliphatic), 1629 (C=O), 1576 (б NH)1150,1111 (C-N-C), 1483, 1359 (CH Symmetrical and Asymmetrical stretching), 982 (CH opb of vinyl group), 1589,(Skeletal vibration of benzene ring), 451 (C=O bending),314 (C=C opb of aromatic ring), 187, 129 (skeletal bending vibration), ^{1}H NMR (400 MH_z, DMSO d_6), б 8.27 (S, NH), б 8.24(S,NH_2), б5.44 (S,CH),7.09 – 7.23 (m, 4H toluidine aromatic ring), 7.34 – 7.70 (m, 5H phenyl ring), 6.94, 6.96 (d, 2H), 2.51 (S, 3H), ^{13}C NMR (400 MH_z, DMSO d_6) б 162.09 (S,2C, C=O), 135.88 (S,1C, CH), 118.04 (S,C), 151.21(S,C) 126.03-131.68 (m, 4C, toluidine aromatic ring),129.40-135.92 (m, 5C, benzyl ring), 144.50 (S,2C), (18.13, 1C, CH_3), 2d NMR(400 MH_z, DMSO d_6) The ^{1}H -^{1}H and ^{1}H – ^{13}C correlation (TOCU) which shows the better result corresponding to the those of ^{1}H NMR and ^{13}C NMR spectral data of the 2d NMR spectral study of the (TOCU) substantiates the ^{1}H - NMR and ^{13}C- NMR spectral assignments.FAB Mass (Positive mode) *m/z*: 281 ($C_{17}H_{19}N_3O$), base peak *m/z*:221 (C_{16} $H_{16}N^+$), 116 (C_9 H_8^+), 106 (C_7 H_8 N^+) 77 (C_6 H_5^+), 59 (C H_3 N_2 O^+) . Calculated: C 42.50%, H 47.50%, N 7.50%, O 2.50%. Found: C 42.48%, H 47.49%, N 7.48%, O 2.48%.

RESULT AND DISCUSSION

The meting points of the synthesized compounds were found in open capillary tubes and readings were uncorrected. The results of IR spectra were given in spectral details heading which showed absorption bands for aromatic C-H,N-H,C-N,C-N-C,C=N,C=O,C=S,NH_2 and CH_3 groups. The results of the ^{1}HNMR

spectra given under spectral details heading showed that the number of hydrogen atoms present in all the synthesized compounds were exact when compared to the number of hydrogen atoms in the expected compounds, the molecular mass of the synthesized compounds were nearer to the molecular mass of the expected compounds.

In vitro Antibacterial activity [18]

The synthesized compounds TOCT and TOCU were screened for antibacterial activity against the five pathogenic bacteria such as *Staphylococcus aureus,* ,(Gram +ve) *Klebsiella Pneumonia*, ,(Gram -ve) *Salmonella typhi,* ,(Gram -ve) *Escherichia.coli,* ,(Gram -ve) *Pseudomonas aeruginosa*,(Gram -ve) Purchased from MTCC, Chandigarh, and maintained by periodical sub culturing on nutrient agar medium. Determination of MIC and MBC values of the synthesized compounds were evaluated by using the two fold serial micro dilution method. 20 mg of synthesized compounds dissolved in 2 ml of DMSO was used as a stock solution. The concentration ranged from 50µg - 1.562 µg the results are tabulated in TOCT shows minimum inhibitory concentration range between 6.25-25µg/ml against- *Staphylococcus aureus* (6.25 µg/ml) *Klebsiella pneumonia* (25 µg/ml), *Salmonella typhi* (12.5 µg/ml), *Escherichia.coli* (25 µg/ml) and *Pseudomonas aeruginosa* (25 µg/ml). TOCU exhibited minimum inhibition concentration range between 12.5-25 µg/ml against *Staphylococcus aureus* (25 µg/ml) *Klebsiella pneumonia* (25 µg/ml), *Salmonella typhi* (25 µg/ml), *Escherichia.coli* (12.5µg/ml) and *Pseudomonas aeruginosa* (12.5 µg/ml). For MBC, TOCT shows Minimum bactericidal concentration against only *Staphylococcus aureus* (12.5µg/ml), TOCU shows Minimum bactericidal concentration against *Escherichia.coli (*25 µg/ml*)* and *Pseudomonas aeruginosa* (25 µg/ml).This result indicate that TOCT has significant activity than TOCU.

Table1: in vitro anti bacterialactivity Mannich bases of cinnamaldehyde derivatives

Concentration of Mannich bases (50-1.562µg)	SA		KB		SAL		*E.Coli*		PA	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
TOCT	6.25	12.5	25	-	12.5	-	25	-	25	-
TOCU	25	-	25	-	25	-	12.5	25	12.5	25

SA- *Staphylococcus aureus,* KB- *Klebsiella pneumonia*, SAL- *Salmonella typhi,* E.coli *Escherichia.coli* PA - *Pseudomonas aeruginosa*

In vitro Antioxidant activity [19-21]

DPPH radical-scavenging activity was determined by (shimada *et al.*,1992). Hydrogen peroxide scavenging activity was estimated by replacement titration method (Zhang, 2000). The Fe^{3+} reducing power Assay was determined by the method. (Oyaizu, 1986).

Free radical scavenging activity using DPPH radical method

This model was very easy and worldwide accepted model for estimation of free radical scavenging activity. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The TOCT and TOCU possess the significant free radical scavenging activity at various concentrations. The free radical scavenging activities increased against the concentration of synthesized compounds increased from 20 – 80µg/mL. The maximum free radical scavenging activity has been observed in TOCT followed by TOCU at concentration of 80µg/mL. This result was compared with L ascorbic acid as positive control. From the experimental data TOCT showed maximum percentage scavenging activities than TOCU (Fig.3).

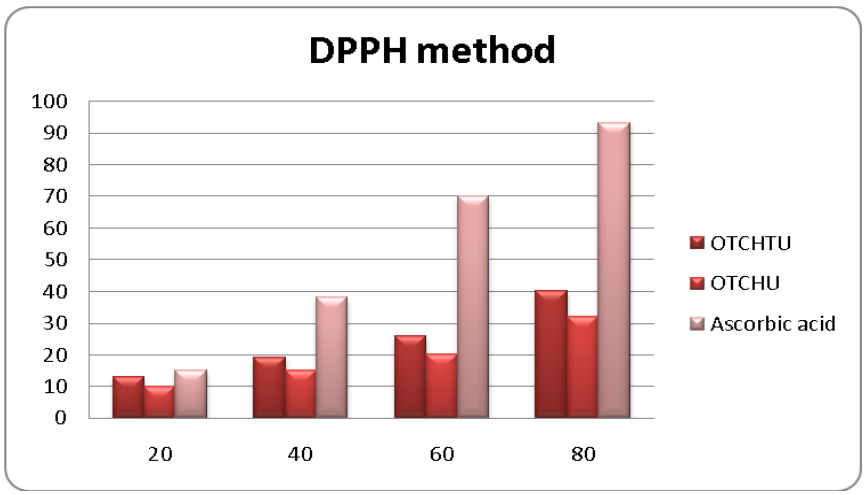

Fig. 3: Free radical scavenging activity of Mannich bases by using DPPH method

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross the cell membranes rapidly, once diffuse inside the cell, Hydrogen peroxide can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. The present study was observed that the Hydrogen peroxide scavenging effect of TOCT, TOCU and standard L ascorbic acid on the Hydrogen peroxide scavenging activity decreases in the following order: L ascorbic acid > TOCT > TOCU at concentration of 80µg/mL, respectively. The potential of L-ascorbic acid to scavenge Hydrogen peroxide is directly proportional to the concentration (Fig.4). TOCT exhibited maximum percentage of inhibition effect at 80µg /mL of concentratin.

Reducing power activity

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued

hydrogen abstraction, reductive capacity and radical scavenging. The present study showed reducing power of TOCT, TOCU and standard L ascorbic acid. Decreased the reducing power in the following order: L ascorbic acid > TOCT > TOCU at concentration of 80µg/mL, respectively (Fig 5). TOCT showed maximum absorbance at 80µg /mL of concentration.

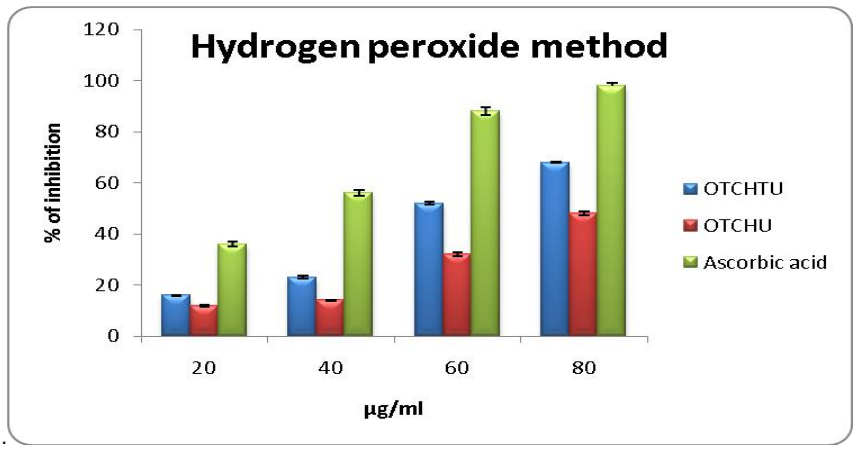

Fig. 4: Free radical scavenging activity by using H_2O_2 method

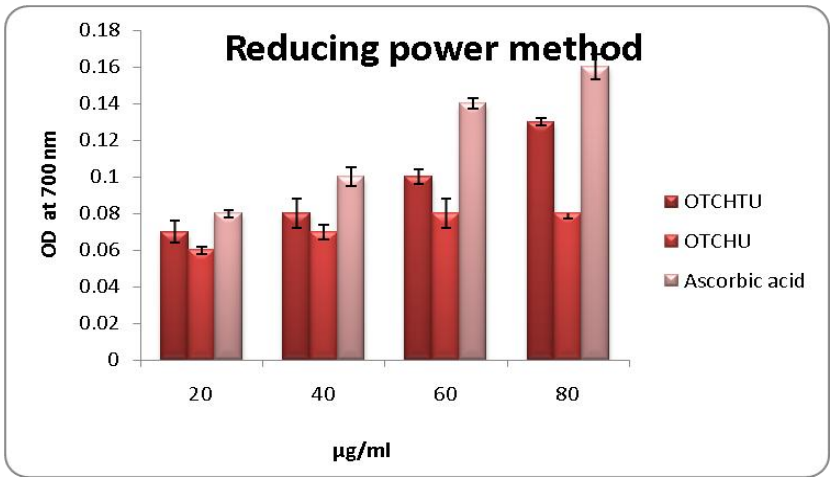

Fig. 5: Free radical scavenging activity by using reducing power method

CONCLUSION

The newly synthesized organic compounds have been characterized on the basis of above spectroscopic method and these compounds have been assigned for various biological evaluation, antibacterial and antioxidants, studies. In all the case TOCT has significant activities than TOCU may be owing to the presence of thioamide moiety, heteroatom's, chiral nature of the organic compound.

ACKNOWLEDGEMENT

We are thankful to CSIR – CECRI karaikudi, SAIF IIT –Madras for recording 1H NMR, 13C NMR, 2d NMR, FT-Raman, IR, and DRDO Lucknow for recording CHN values and mass spectrum.

REFERENCE

1. Dunn DB et al, Effects of 5-halogenated uracils on the growth of Escherichia coli and their incorporation into deoxyribonucleic acids. Biochemistry Journal 1957;67: 494-506.
2. Williams DR Metals, ligands and cancer. Chem. Rev 1972;72:203-213.
3. Ames BN et al, Shigenaga MK, Hagen TM Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993;90:7915-7922.
4. Shirwaikar A et al, in vitro antioxidant studies on the Benzyl tetra Isoquinoline alkaloid berberine. Biol . pharm.Bull. 2006;29:9,1906-1910.
5. Anwar F et al, antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare mill) seeds from Pakistan. Flav.Frag.J. 2009;24:170-176.
6. Tutour BL Antioxidant activities of algal extracts synergistic effect with vitamin E. phyto chem.1990;29:3759-3765
7. Elsayed et al, Mannich bases as synthetic Intermediates; Convenient synthesis of functionalized 1,2,4 – Trizepines, 1,4 – Diazepines and 1,5 Diazocines. Z. Naturforsch. 2011;66:577-584. C.Moura et al,Journal of organometalic chemistry 2009;694-950.
8. C, - Y. Chiang et al, Journal of Biological Inorganic chemistry. 2006;11,395.
9. Ames et al, Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993;90:7915-7922.
10. Pandeya et al,Synthesis, antibacterial, antifungal anti – HIV activities of norfloxacin Mannich bases. European Journal Medicinal chemistry. 2000;35:249-265.
11. Kwiatkowski et al, Structure of an anticonvulsant N–methyl – m – bromophenylsuccinimide. Acta Crystallography 1992; 32:206-208.
12. Bowels, et al., Inhibitory effects of flavor compounds on staphylococcus aureus WRRS B 124. Journal of food safety 1995;15:337-347.
13. Bowels et al, Antibotulinal properties of selected aromatic and aliphatic aldehydes. Journal of food production. 1993;56:788-794.

14. Helander et al, Characterization of the action of selected essential oil components on Gram – negative bacteria. Journal of Agricultural food chemistry.1998;46 3590- 3595.
15. Franklin et al, Crystal structure, conformation and vibrational analysis of a Mannich bases: 2[(Phenylamino) methyl] – isoindole -1,3-dione.Journalof chemistry Crystallography. 2011;41(8):1120-1123.
16. Tamilvendan et al, syntheses, spectral, crystallographic, antimicrobial, and antioxidant studies of few Mannich bases. Medicinal Chemical Research 2011;19:7,617-716.
17. Ilavenil S et al, Evaluation of Antibacterial Activity and Phytochemical Analysis of Crinum asiaticum. InternationalJournal of Current Research. 2010;3:83-88
18. Shimada et al, Antioxidative properties of xanthum on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry, 1992;40:945-948
19. Zhang X. Y. Principles of Chemical Analysis. Beijing: China Science Press. pp. 2000 275-276
20. Oyaizu M.Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. Japanese Journal of Nutrient 1986;44: 307-315
21. Ravichandran V et al, Synthesis and antimicrobial activity of Mannich bases of isatin and its derivatives with 2 – [(2,6 – dichlorophenylacetic acid. Arkivoc News Lett 2007;14: 51-57.
22. Tamil vendan D et al, syntheses, spectral, crystallographic, antimicrobial, and antioxidant studies of few Mannich bases. Medicinal Chemical Research 2011;19: 7, 617-716.