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Synthesis, Antimicrobial and Antioxidant Activities of Novel series of Cinnamamide Derivatives having Morpholine Moiety

Gangadhara Seelolla, Prasad Cheera and Venkateswarlu Ponneri*

Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, AP, India

Abstract

Structurally morpholine containing Cinnamamide derivatives have been synthesized in quantitative yields by the reaction of different substituted cinnamicacids with morpholine. The structures of the newly synthesized compounds were confirmed by their IR, LC-MS, ^{1}H & ^{13}C-NMR spectral data. The synthesized compounds were evaluated for their antimicrobial activity against bacterial species *Bacillus subtilis* and *Escherichia coli* etc and in vitro antioxidant activity by employing DPPH, hydrogen peroxide, and nitric oxide radical scavenging assays. Among these prepared compounds 4i and 4j showed significant antioxidant, antibacterial as well as antifungal activity and these were found to be the most potent activity molecules when compared with that of standard drugs.

Keywords: Morpholine; Cinnamic acid; Amide; Antioxidant property; Antimicrobial activity

Introduction

Cinnamic acid, which is ubiquitous in cinnamon oil and many other balsams, is a naturally occurring aromatic fatty acid of low toxicity with a long history of human exposure [1]. Commercially, it is widely applied in the perfume industry because of its floral odor. In recent years, cinnamic acid derivatives have attracted much attention due to their antioxidative [2], antitumor [3] and antimicrobial properties [4]. Though some of the research papers on cinnamic acid derivatives were reported as antitubercular agents [5-8]. Among these cinnamic acid derivatives such as cinnamamides were reported to have many different biological activities such as anticancer, antimitotic, antioxidant and seed-germination inhibitory effects [9-11] and some of the cinnamamide derivatives exhibit a variety of biological activities, for example as shown in Figure 1, the genus piperaceae molecules (A) have been widely studied, due to the biological properties of secondary metabolites from these plants [12-14]. The natural piperidine derivative (B) showed good insecticidal activity against the fall armyworm, *Spodoptera frugiperda* [15]. Compounds (C) represent a novel series of potent small molecule inhibitors that not only have excellent in vitro profiles but also have activity *in vivo* [16]. N-(2-hydroxy ethyl) cinnamamide (D) was found to posses good anticonvulsant activity and also low toxicity [17]. . In addition, in the course of screening compounds for pharmacological action, it was observed that α-phenyl-N,N-diethyl cinnamamide potentiated Nembutal hypnosis in rats [18].

On the other hand, among the family of heterocyclic compounds, the heterocycles with N, S and O atoms have attracted the attention of chemical research due to their wide spectrum of biological activities. N and O atoms containing Morpholine has its unique position in heterocyclic chemistry, and its derivatives are gaining considerable importance due to diverse biological activities such as antimalarial [19], antibacterial [20], antimicrobial [21,22], antidepressant [23], antiproliferative [24], hypocholesterolemic [25], antibiotic, antileukemic [26,27]etc.

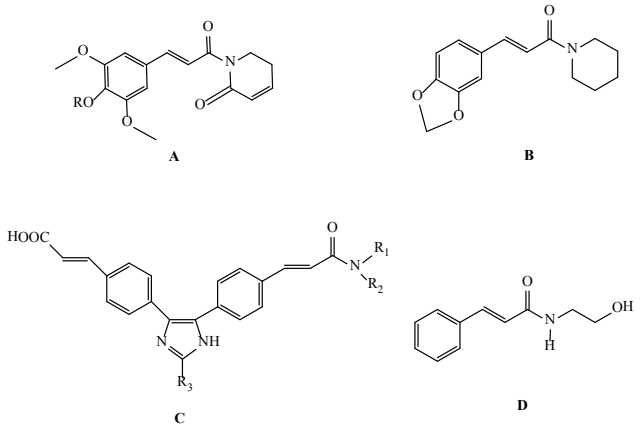

Figure 1. Examples of molecule containing cinnamamide derivatives.

Many efforts to find and develop novel and more potent antioxidants without side effects, and effective antimicrobial derivatives become increasingly important in the food and the cosmetic industries, and in medicine. Because of the constrained availability of natural cinnamic acid analogues, the establishment of their biological activities is still limited. Therefore, we synthesized a series of morpholine containing cinnamamide derivatives and evaluated their antimicrobial effects and antioxidant properties.

Experimental Section

Chemistry

Melting points were determined in open capillaries on a Mel–Temp apparatus and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized with UV light. Merck silica gel (60–120 mesh) was used for column chromatography. The IR spectra were recorded on a Perkin-Elmer BX1 FTIR Spectrophotometer using KBr pellets and the wave numbers were given in cm^{-1}. ^{1}H NMR (400 MHz), and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer in $CDCl_3$/DMSO-d_6 solution using TMS as an internal standard. All chemical shifts were reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent 1100 LC/MSD instrument with method API–ES at 70 eV.

***Corresponding author:** Venkateswarlu Ponneri, Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, AP, India, Tel: 91-9393600444, E-mail: chem.ponneri@gmail.com

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The microanalyses were performed on a Perkin Elmer 240C elemental analyzer. The antioxidant property was carried out by using Shimadzu UV–2450s spectrophotometer.

General Procedure for the Synthesis of trans cinnamamides (4a-j)

In this reaction, a mixture of phenyl substituted cinnamic acid (4a-j) and $SOCl_2$ (which acted as both reactant and solvent) was stirred and refluxed at 80–90°C for 4 hrs. Remaining $SOCl_2$was removed by distillation under reduced pressure. Thus, the intermediate acid chloride (2a-j) was added drop wise to 0°C cooled solution of morpholine (3) in dry pyridine to give product cinnamamide (4a-j) with good yield as described in scheme 1.

(E)-1-morpholino-3-phenylprop-2-en-1-one (4a)

Color less solid; Yield 72 %; mp: 158-160°C; IR (KBr, cm^{-1}): 1660 (C=O), 1580 (C=C), 1170 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.48 (d, 2H, -ArH), 7.22 (t, 1H,-ArH), 6.83 (dd, 2H, -ArH), 6.72 (d, 1H, =CH), 7.11 (d, 1H, =CH), 2.50 (t, 2H, $-CH_2$), 2.14 (t, 2H, $-CH_2$); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 158.4, 140.2, 135.3, 127.7, 127.3, 126.4, 115.8, 59.6, 44.8; MS: m/z 218 $(M+H)^+$ for $C_{13}H_{15}NO_2$; Anal. calcd.for $C_{13}H_{15}NO_2$: C 71.87, H 6.96, N 6.45; Found: C 71.80, H 6.94, N 6.40%.

(E)-3-(2-chlorophenyl)-1-morpholinoprop-2-en-1-one (4b)

Pale yellowish brown solid in 70 %; mp: 188-190°C; IR(KBr, cm^{-1}): 1675 (C=O), 1550 (C=C), 1140 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.80 (d, 1H, -ArH), 7.57 (d, 1H, -ArH), 7.26 (t, 2H, -ArH), 7.39 (d, 1H, =CH), 7.99 (d, 1H, =CH), 3.50 (t, 2H, $-CH_2$), 3.72 (t, 2H, $-CH_2$); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 165.2, 138.9, 134.6, 133.6, 130.3, 130.1, 127.6, 126.9, 119.9, 66.8, 47.4; MS: m/z 252 $(M+H)^+$ for $C_{13}H_{14}ClNO_2$; Anal. calcd.for $C_{13}H_{14}ClNO_2$: C 62.03, H 5.61, N 5.56; Found: C 61.94, H 5.59, N 5.51%.

(E)-3-(2-methoxyphenyl)-1-morpholinoprop-2-en-1-one (4c)

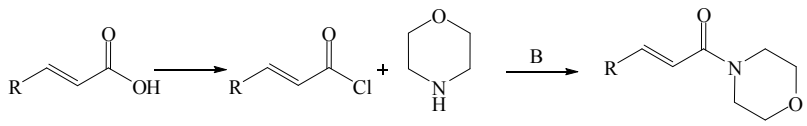
Color less solid; Yield 68 %; mp: 168-170°C; IR (KBr, cm^{-1}): 1680 (C=O), 1545 (C=C), 1130 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.33 (dd, 1H, -ArH), 6.97 (d, 1H, -ArH), 6.93 (dd, 1H, -ArH), 6.89 (d, 1H, -ArH), 7.91 (d, 1H, =CH), 7.46 (d, 1H, =CH), 3.87 (s, 3H, $-OCH_3$),3.70 (m, 8H, -CH); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 166.2, 158.2, 138.6, 130.7, 129.0, 124.2, 120.6, 111.6, 111.2, 66.8, 55.4, 53.3; MS: m/z 248 $(M+H)^+$ for $C_{14}H_{17}NO_3$; Anal. calcd. for $C_{14}H_{17}NO_3$: C 68.00, H 6.93, N 5.66; Found: C 67.91, H 6.90, N 5.60%.

(E)-3-(3-methoxyphenyl)-1-morpholinoprop-2-en-1-one (4d)

Pale yellow solid in 70 %; mp: 169-171°C; IR(KBr, cm^{-1}): 1665 (C=O), 1530 (C=C), 1070 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.34 (d, 1H, -ArH), 7.32 (d, 1H, -ArH), 6.95 (dd, 1H, -ArH), 7.28 (d, 1H,-ArH), 7.49 (d, 1H, =CH), 7.24 (d, 1H, =CH), 3.81 (s, 3H, $-OCH_3$), 3.62 (m, 8H, -CH); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 160.9, 158.4, 140.1, 136.7, 130.3, 127.7, 122.1, 115.8, 111.7, 107.6, 62.2, 55.5, 46.2; MS: m/z 248 $(M+H)^+$ for $C_{14}H_{17}NO_3$; Anal. calcd.for $C_{14}H_{17}NO_3$: C 68.00, H 6.93, N 5.66; Found: C 67.91, H 6.90, N 5.60%.

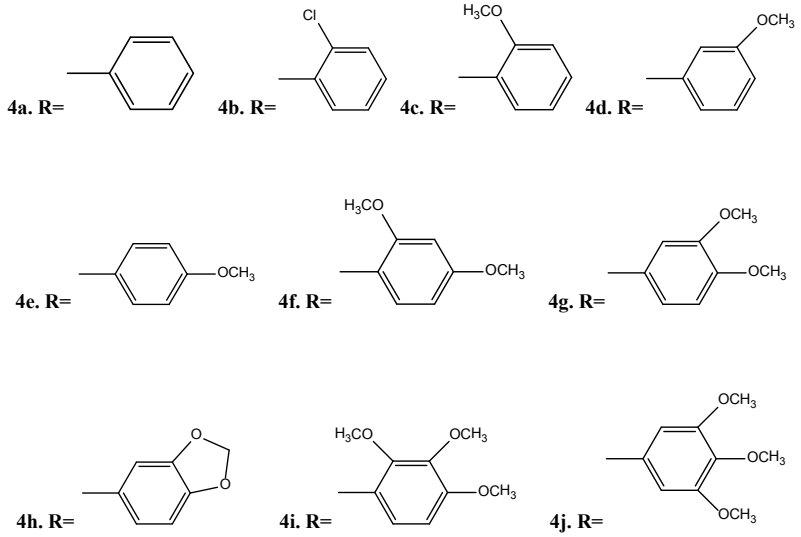
(E)-3-(4-methoxyphenyl)-1-morpholinoprop-2-en-1-one (4e)

Color less solid in 71 %; mp: 166-169°C; IR(KBr, cm^{-1}): 1640 (C=O),



1 a-j **2 a-j** **3** **4 a-j**

A: $SOCl_2$, reflux, 80–90 °C, 4 hours B: Pyridine, reflux, 80–90 °C, 4 hours



Scheme 1:

1540 (C=C), 1090 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.58 (d, 2H, -ArH), 6.95 (d, 2H, -ArH), 7.29 (d, 1H, =CH), 7.04 (d, 1H, =CH), 3.62 (s, 3H, -OCH_3), 3.43 (m, 8H, -CH); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 162.9, 159.2, 140.1, 132.7, 128.3, 115.8, 111.7, 66.2, 55.8, 43.0; MS: m/z 248 $(M+H)^+$ for $C_{14}H_{17}NO_3$; Anal. calcd. for $C_{14}H_{17}NO_3$: C 68.00, H 6.93, N 5.66; Found: C 67.91, H 6.90, N 5.60%.

(E)-3-(2,4-dimethoxyphenyl)-1-morpholinoprop-2-en-1-one (4f)

Color less solid in 70 %; mp: 174-176°C; IR(KBr, cm^{-1}): 1666 (C=O), 1536 (C=C), 1140 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.78 (d, 1H, -ArH), 6.60 (d, 1H, -ArH), 6.55 (s, 1H, -ArH), 7.69 (d, 1H, =CH), 7.02 (d, 1H, =CH), 3.85 (s, 3H, -OCH_3), 3.59 (s, 3H, -OCH_3), 3.38 (m, 8H, -CH); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 165.22, 161.98, 158.93, 136.3, 129.1, 116.4, 114.8, 105.8, 98.2, 66.2, 55.6, 55.5, 44.3; MS: m/z 278 $(M+H)^+$ for $C_{15}H_{19}NO_4$; Anal. calcd.for $C_{15}H_{19}NO_4$: C 64.97, H 6.91, N 5.05; Found: C 64.90, H 6.88, N 5.01%.

(E)-3-(3,4-dimethoxyphenyl)-1-morpholinoprop-2-en-1-one (4g)

Color less solid in 68 %; mp: 176-179°C; IR(KBr, cm^{-1}): 1683 (C=O), 1526 (C=C), 1133 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.35 (s, 1H, -ArH), 7.19 (dd, 1H, -ArH), 7.08 (d, 1H, -ArH), 7.44 (d, 1H, =CH), 6.95 (d, 1H, =CH), 3.82 (s, 3H, -OCH_3), 3.71 (s, 3H, -OCH_3), 3.60 (m, 8H, -CH); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 160.2, 150.5, 149.5, 138.7, 128.3, 122.1, 115.8, 111.7, 107.6, 68.6, 55.6, 48.4; MS: m/z 278 $(M+H)^+$ for $C_{15}H_{19}NO_4$; Anal. calcd.for $C_{15}H_{19}NO_4$: C 64.97, H 6.91, N 5.05; Found: C 64.90, H 6.88, N 5.01%.

E)-3-(benzo[d][1,3]dioxol-5-yl)-1-morpholinoprop-2-en-1-one (4h)

Pale yellow solid in 71 %; mp: 179-181°C; IR(KBr, cm^{-1}): 1675 (C=O), 1580 (C=C), 1149 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.41 (s, 1H, -ArH), 7.15 (dd, 1H, -ArH), 6.92 (d, 1H, -ArH), 7.45 (d, 1H, =CH), 7.12 (d, 1H, =CH), 6.02 (s, 2H, -OCH_2), 3.70 (m, 8H, -CH); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 164.2, 150.5, 148.4, 139.5, 128.3, 127.3, 122.1, 118.8, 108.4, 104.6, 66.2, 49.2; MS: m/z 262 $(M+H)^+$ for $C_{14}H_{15}NO_4$; Anal. calcd.for $C_{14}H_{15}NO_4$: C 64.36, H 5.79, N 5.36; Found: C 64.26, H 5.77, N 5.31%.

(E)-1-morpholino-3-(2,3,4-trimethoxyphenyl)prop-2-en-1-one (4i)

Color less solid in 68 %; mp: 196-199°C; IR(KBr, cm^{-1}): 1645 (C=O), 1560 (C=C), 1123 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.20 (d, 1H, -ArH), 6.66 (d, 1H, -ArH), 7.79 (d, 1H, =CH), 6.87 (d, 1H, =CH), 3.87 (s, 9H, -OCH_3), 3.71 (m, 8H, -CH); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 165.8, 154.6, 152.7, 142.1, 138.0, 123.0, 121.8, 115.5, 107.1, 66.4, 60.7, 60.4, 55.6, 48.2; MS: m/z 308 $(M+H)^+$ for $C_{16}H_{21}NO_5$; Anal. calcd. for $C_{16}H_{21}NO_5$: C 62.53, H 6.89, N 4.56; Found: C 62.44, H 6.86, N 4.50%.

(E)-1-morpholino-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (4j)

Color less solid in 68 %; mp: 176-179°C; IR(KBr, cm^{-1}): 1666 (C=O), 1525 (C=C), 1136 (C-O); ^{1}H-NMR (DMSO-*d6*) *δ*: 7.04 (s, 2H, -ArH), 7.45 (d, 1H, =CH), 7.15 (d, 1H, =CH), 3.62 (s, 9H, -OCH_3); ^{13}C NMR (DMSO-*d6*) δ (ppm) : 162.0, 150.5, 138.7, 135.3, 130.7, 115.8, 107.6, 66.2, 60.6, 56.5, 44.2; MS: m/z 330 $(M+Na)^+$ for $C_{16}H_{21}NO_5$; Anal. calcd. for $C_{16}H_{21}NO_5$: C 62.53, H 6.89, N 4.56; Found: C 62.44, H 6.86, N 4.50%.

Pharmacological Screening

Antioxidant screening (*in vitro*)

The compounds 4a-j were tested for antioxidant property by DPPH, NO and H_2O_2 methods.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the test compounds (25, 50, 75 and 100 µg/mL) in methanol were added to 4 ml of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I %) of free radical production from DPPH was calculated by the following equation

% of scavenging = [(A control - A sample) / A blank] x 100 → *Eq*. (1)

Where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried at in triplicate.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods of Green et al. and Marcocci et al. Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 mL of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 µg/mL) of the test compounds and incubated for 150 min at 25°C and 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Nitric oxide scavenging activity was calculated by using Equation (1).

Hydrogen peroxide (H_2O_2) scavenging activity

The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch *et al*. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 25, 50, 75 and 100 g/ml concentrations of the test compounds in 3.4 ml phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percent of scavenging of H_2O_2 was calculated by using *Eq*. (1)

Results and Discussion

Chemistry

Target compounds (4a-j) were prepared as outlined in Scheme 1. In order to investigate how substituents on the phenyl ring in cinnamamides influence the activities, a mixture of substituted cinnamic acid (1a-j) and $SOCl_2$ (which acted as both reactant and solvent) was stirred and refluxed at 80-90°C for 4 hrs. Remaining $SOCl_2$was removed by distillation under reduced pressure. Thus, the intermediate acid chloride (2a-j) was added drop wise to 0°C cooled solution of morpholine (3) in dry pyridine to give product cinnamamide (4a-j) with good yield.

The structure of all newly synthesized morpholine containing cinnamamide derivatives were determined by IR, NMR and Mass spectroscopic analysis and the results were in agreement with the proposed structures. In ^{1}H NMR spectra, the aromatic protons resonated at δ 6.55–7.80 ppm. The morpholine ring proton resonated with two triplets around δ 2.50 and 3.72 The conjugated double bond of cinnamicacid showed two doublet of which C–H proton near to aromatic ring (Ph–C<u>H</u>=HC–) resonated at δ 7.11–7.99 while C–H proton towards the carbonyl end (Ph–CH=C<u>H</u>–CO) resonated at δ 6.72–7.46. The synthesized compounds exhibited characteristic peak of

cinnamicacid double bond appeared at 1525–1580 cm^{-1} and carbonyl peak appeared at 1640–1683 cm^{-1}. ^{13}C NMR further confirmed the structures of the desired products and the $(M+H)^+$ molecular ion peak of all the compounds were confirmed by their molecular weights.

Antimicrobial activity

All the synthesized compounds (4a-j) were screened for their antimicrobial activity determined by well plate method [28,29]. The antimicrobial values showed that most of the synthesized derivatives exhibited excellent activities against different strains of bacteria and moderate activity against fungi. Ciprofloxacin and Fluconazole were used as the standard drugs for antimicrobial and antifungal testing, respectively.

Antibacterial activity: All the synthesized compounds were screened against Gram +ve bacterial strains such as *Staphylococcus aureus* and *Bacillus subtilis* and Gram –ve bacterial strains such as *Klebsiella pneumoniae* and *Escherichia coli*. The results obtained as MIC is presented in Table 1. The Compounds 4f and 4i were showed good activity 12.5 and 6.12 µg/mL respectively against the Gram positive bacterial strain *Staphylococcus aureus*, when compared with standard ciprofloxacin (12.5 µg/mL). When *Bacillus subtilis* was introduced as another Gram positive bacterial strain for conducting the antibacterial test, it was found that 4b and 4i both compounds were also exhibited good activity 12.5 µg/mL equivalent to the standard ciprofloxacin. Some of the compounds 4b, 4c, 4e and 4j were screened moderate activity and rest of the compounds 4d and 4g was does not show any activity against Gram +ve bacterial strains. The compounds 4b and 4i were showed MIC value 25 µg/mL equivalent to that reported by the standard ciprofloxacin (25 µg/mL) against Gram negative bacterial strain *Klebsiella pneumonia*. When *E.coli* was introduced as another gram-negative strain for conducting the antibacterial test, it was found that Compounds 4f and 4i were exhibited MIC value 12.5 µg/mL equivalent to that reported by the standard ciprofloxacin (12.5 µg/mL) against Gram negative bacterial strain *Escherichia coli*. Some of the compounds 4c, 4e and 4j showed less MIC values and 4g compound does not show any activity against Gram negative bacterial strains. By comparing all these values, the compound which is having highest electron releasing substituent on benzene ring in cinnamamide derivative was found potent activity against all the tested organisms (Table 1).

Antifungal activity: All the synthesized compounds 4a-j was also screened for their antifungal activity against two fungal strains such as *Candida albicans* and *Aspergillus flavus*. Fluconazole was taken as a standard drug throughout the experiment. Compound 4b was found to be the excellent and potent antifungal activity with MIC 3.12 µg/mL and 6.25 µg/mL against *C. albicans* and *A. flavus* respectively, while standard drug Fluconazole with 6.25 µg/mL. Only two compounds 4i and 4j were also showed good activity with 3.12 µg/mL and 6.25 µg/mL against *Aspergillus flavus* and *Candida albicans* respectively and rest of the compounds 4c, 4a, 4d, 4f exhibit very poor activity compared with standard Fluconazole (Table 1).

Antioxidant screening (*in vitro*)

In the present study, the antioxidant activity of the synthesized compounds was assessed in vitro by the 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide (NO) and hydrogen peroxide (H2O2) [30-34]. These methods were based on measuring the continual absorbance decrease of the methanolic solution of the DPPH at 517 nm, in the presence of antioxidant compound. The DPPH has an odd electron so it can accept an electron or hydrogen free radical. In the presence of antioxidant, this odd electron becomes paired due to H transfer from antioxidant and hence DPPH absorbance decreases. The ability of newly synthesized compounds (4a-j) act as hydrogen donors or free radical scavengers was tested by in vitro antioxidant assays involving DPPH radical, NO radical, H_2O_2 and the results were compared with that of standard antioxidant Ascorbic acid. In DPPH method, the newly synthesized compounds showed moderate to good antioxidant activity compared to the standard (Table 2). Among the newly synthesized compounds 4f, 4i and 4j were found to be good antioxidant activity compared with that of the standard Ascorbic acid (18.33 ± 0.04) with the IC_{50} values 19.44 ± 0.14, 19.43 ± 0.08 and 19.09 ± 0.09 µg/mL respectively. Some of the compounds 4c, 4e and 4g were exhibited moderate activity with the IC_{50} values 21.06 ± 1.23, 21.44 ± 0.33 and 20.04 ± 1.18 µg/mL respectively and the remaining compounds 4a and 4b were exhibited less activity and compounds 4d and 4h were does not showed any activity. In DPPH scavenging method only two compounds 4i and 4j showed good antioxidant activity because of these two compounds having more electron releasing methoxy groups on phenyl ring in cinnamamide.

Further all the newly synthesized compounds were subjected to the NO radical scavenging activity. The IC_{50} value of NO is the concentration of sample required to inhibit 50% of the NO radicals. Except 4b and 4d all the tested compounds exhibited strong NO radical scavenging with moderate to good IC_{50} values. The greater NO radical scavenging

Compound	Minimum inhibitory concentration (MIC) (µg mL^{-1})					
	Antibacterial activity				Antifungal activity	
	Gram +Ve bacteria		Gram –Ve bacteria			
	S. aureus	*B. subtilis*	*K. pneumonia*	*E.coli*	*C. albicans*	*A. flavus*
4a	100	50	>100	100	50	100
4b	25	12.5	25	50	3.12	6.25
4c	25	50	50	100	50	100
4d	–	–	100	50	25	50
4e	25	50	50	25	100	50
4f	12.5	25	100	12.5	50	>100
4g	–	–	>100	50	–	–
4h	50	100	–	–	100	>100
4i	6.12	12.5	25	12.5	12.5	3.12
4j	25	25	50	25	6.25	12.5
Ciprofloxacin	12.5	12.5	25	12.5	–	–
Fluconazole	–	–	–	–	6.25	6.25

(–) No activity. Lower MIC values indicate higher the antimicrobial activity.
S. aureus: Staphylococcus aureus, B. subtilis:Bacillus subtilis, K. pneumonia: Klebsiella pneumonia, E.coli: Escherichia coli, C. albicans: Candida albicans.

Table 1: Antimicrobial activity (MIC profiles) of the synthesized compounds (**4a–j**).

activity of the tested compounds was showed by compounds 4i and 4j showed 18.33 ± 0.09 and 18.86 ± 0.97 respectively (Table 3).

The prepared compounds (4a-j) having various concentrations (25, 50, 75 and 100 µg/mL) were subjected to H_2O_2 radical scavenging activity. The findings of present study (Table 4) indicated that the most of the prepared compounds exhibited moderate to good radical scavenging ability. The good H_2O_2 scavenging effect was detected in compounds 4i and 4j with the values of IC_{50} 18.02 ± 0.09 and 18.33 ± 0.10 respectively compared with the standard Ascorbic acid (17.80 ± 0.08). Rest of the compounds 4e, 4f, and 4g were showed moderate activity with IC_{50} values 20.76 ± 1.33, 20.01 ± 0.20 and 19.43 ± 0.16 respectively and the compounds 4a, 4b, 4c, and 4d exhibited less activity The reason would be the presence of electron releasing methoxy groups present on the phenyl ring.

Structure activity relationship (SAR) study was helped to reveal the effect of different substituent on the microbial strains, depending upon the different electronic environments developed on the aromatic ring by substituting the electron donating groups. It was observed that the compounds 4j (3,4,5–OCH_3), 4i (2,3,4–OCH_3), 4f (2,4–OCH_3) and 4b (2–Cl) with electronic donating groups exhibited good antimicrobial

Compound	Concentration (µg mL^{-1})				
	25	50	75	100	IC_{50}
4a	56.21 ± 0.76	57.15 ± 0.62	59.75 ± 0.65	60.20 ± 0.94	22.23 ± 0.30
4b	54.32 ± 0.27	55.35 ± 0.93	57.25 ± 0.07	61.16 ± 0.97	23.01 ± 1.41
4c	59.35 ± 0.25	60.34 ± 0.18	62.46 ± 0.16	64.16 ± 0.72	21.06 ± 1.23
4d	–	–	–	–	–
4e	58.29 ± 0.64	62.24 ± 0.96	64.54 ±1.08	68.72 ± 0.98	21.44 ± 0.33
4f	64.29 ± 0.27	65.26 ± 0.17	66.72 ± 0.23	67.14 ± 0.10	19.44 ± 0.14
4g	62.35 ± 0.10	62.66 ± 0.36	63.49 ± 0.89	69.14 ± 1.06	20.04 ± 1.18
4h	–	–	–	–	–
4i	64.32 ± 0.09	66.13 ± 0.20	67.14 ± 0.61	72.14 ± 0.02	19.43 ± 0.08
4j	65.45 ± 0.22	68.29 ± 0.92	70.39 ± 0.12	73.28 ± 0.90	19.09 ± 0.09
Ascorbic acid	68.04 ± 0.18	71.24 ± 0.15	72.43 ± 0.22	75.29 ± 0.03	18.37 ± 0.04
Blank	–	–	–	–	–

(−) showed no scavenging activity. Values were the means of three replicates ± SD

Table 2: The *in vitro* antioxidant activity of (**4a–j**) in DPPH method

Compound	Concentration (µg mL^{-1})				
	25	50	75	100	IC_{50}
4a	57.29 ± 0.84	60.16 ± 0.72	62.24 ± 0.63	63.42 ± 0.94	21.81 ± 1.30
4b	–	–	–	–	–
4c	63.40 ± 0.23	64.29 ± 0.19	65.19 ± 0.14	66.18 ± 0.78	19.71 ± 1.03
4d	–	–	–	–	–
4e	64.10 ± 0.64	65.20 ± 0.96	67.20 ±1.08	69.14 ± 0.98	19.50 ± 0.64
4f	62.32 ± 0.07	66.28 ± 0.07	69.25 ± 0.03	70.24± 0.10	20.05 ± 0.02
4g	63.19± 0.10	65.26 ± 0.06	68.76 ± 0.89	71.24 ± 1.06	19.78 ± 0.18
4h	62.28 ± 0.28	63.16 ± 0.17	64.26 ± 0.44	69.14 ± 1.26	20.07 ± 1.03
4i	68.19 ± 0.06	69.14 ± 0.03	72.34 ± 0.06	76.14 ± 0.22	18.33 ± 0.09
4j	66.27 ± 0.30	68.16 ± 0.62	71.32 ± 0.12	74.29 ± 0.40	18.86 ± 0.97
Ascorbic acid	69.24 ± 0.23	72.16 ± 0.02	76.29 ± 0.21	78.31 ± 0.15	18.05 ± 0.05
Blank	–	–	–	–	–

(−) showed no scavenging activity. Values were the means of three replicates ± SD

Table 3: The *in vitro* antioxidant activity (**4a–j**) in NO method.

Compound	Concentration (µg mL^{-1})				
	25	50	75	100	IC_{50}
4a	52.16 ± 0.14	54.29 ± 0.48	55.19 ± 0.63	57.29 ± 0.72	23.96 ± 0.82
4b	53.24 ± 0.49	54.16 ± 0.51	56.28 ± 0.14	59.14 ± 0.33	23.47 ± 1.44
4c	59.28 ± 0.42	60.24 ± 0.69	62.32 ± 0.14	64.18 ± 0.78	21.08 ± 0.23
4d	51.20 ± 0.64	52.60 ± 0.44	54.39 ± 0.36	56.27 ± 0.94	24.41 ± 1.25
4e	60.20 ± 0.45	61.14 ± 0.96	62.22 ±1.12	64.19 ± 0.38	20.76 ± 1.33
4f	62.44 ± 0.24	64.27 ± 0.15	65.29 ± 0.56	68.19 ± 0.19	20.01 ± 0.20
4g	64.33 ± 0.22	65.28 ± 0.15	67.19 ± 0.39	70.23 ± 1.14	19.43 ± 0.16
4h	–	–	–	–	–
4i	69.35 ± 0.06	72.39 ± 0.23	73.14 ± 0.16	75.22 ± 0.24	18.02 ± 0.09
4j	68.16 ± 0.20	70.19 ± 0.90	71.23 ± 0.22	74.28 ± 0.90	18.33 ± 0.10
Ascorbic acid	70.20 ± 0.12	72.14 ± 0.09	74.28 ± 0.14	76.14 ± 0.06	17.80 ± 0.08
Blank	–	–	–	–	–

(−) showed no scavenging activity. Values were the means of three replicates ± SD

Table 4: The *in vitro* antioxidant activity of (**4a–j**) in H_2O_2 method

results, displaying moderate to good MIC values and good antioxidant properties with good IC_{50} values.

Conclusion

In this work conclusion, a new series of trans cinnamamides were synthesized from starting materials namely morpholine and substituted cinnamic acids with good yield. All the title compounds were screened for their antimicrobial and antioxidant activity studies. The investigation of antimicrobial and antioxidant activity screening data reveals that, among the all the compounds only few of them are exhibiting moderate to good activity. Compounds 4i and 4j showed an excellent, almost equivalent to that of standard, rest of the compounds showed moderate to mild inhibition activity.

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