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The *In-vitro* Antimicrobial and Antioxidant Activities of some Diels-Alder Diaryl Methanone Adducts

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Abstract: The *in-vitro* antimicrobial and antioxidant activities of some 2-(9*H*-fluorene-2-yl)-3-(substituted phenyl)bicyclo [2.2.1]hept-5-ene-2-yl methanones by disc-diffusion as well as serial dilution against their microbial strains and DPPH radical scavenging activities. The titled substrates were synthesized by environmentally benign water mediated Diels-Alder reaction of 9*H*-2-fluorenyl chalcones and cyclopentadiene in good yields. **Conclusion**: The present results confirm the positive correlation between the acute phase response of inflammation and oxidative stress in non-diabetic and diabetic obese subjects.

Keywords: *In-vitro* antimicrobial activity, Antioxidant activity, Diels-Alder adduct, Environmentally benign reaction.

1 Introduction

Methanones possess very important and valuable biological activities such as antibacterial[1], antifungal[2], antioxidant[3], insect antifeedants[4], physotoxic[5], anti-inflammatory[6], tyrosine kinase inhibition[7], CEPT inhibitors[8], anti-tuberclousis[9], urase inhibition[10], molecular docking[11], anti-tumour[12] and cytotoxic[13] due to presence of carbonyl and association of other groups like vinyl[14], allyl[15], acyl[16], imine[17] and electron-donating as well as electron-withdrawing substituents[18]. These ketones are structurally important and versatile intermediates for carbon-carbon block buildings such as synthesis of heterocyclics[19], epoxides[20], falvonones[21], chromones[22] and bicyclo methanones[23] by Diels-Alder adduct formations.

Mumtaz et al., have synthesized and evaluated the antimicrobial and phytotoxic effects of some diaryl pyrazoline methanone derivatives[5]. The *in-vitro* antimicrobial and anti-inflammatory activities of some benzofuro carboxamides were studied by Lavanya et al.,[6]. Wang research group have evaluated and reported the herbicidal and insecticidal activities of some aryl sulphonyl pyrolyl methanones[24]. Christ and his co-workers have studied the LEDGF/p-75-integrase interaction potential of quinolone ketones [25]. The *in-vitro* protein tyrosine kinase inhibitory activities of phenyl furyl methanones were reported by Zheng et. al.,[7]. The Diels-Alder adduct methanones also possess antimicrobial, antioxidant and insect antifeedants[26]. Senbagam et al., have studied the antimicrobial activities of some unsaturated methanones[27]. Recently, Thirunarayanan have synthesized and evaluated the biological activities of some bicyclo methanones[28,29]. Literature survey reveals that there is no report available regarding the synthesis and evaluation of in-vitro biological and antioxidant activities of some Diels-Alder adducts methanones derived from 9*H*-2-fluorenyl chalcones and cyclopentadiene by environmentally benign water mediated Diels-Alder reaction. Therefore the author wishes to report in continuation of earlier work[30], to synthesized and evaluation of biological activities of some 2-(9*H*-fluorene-2-yl)-3-(substituted phenyl)bicyclo [2.2.1]hept-5-ene-2-yl methanones by disc-diffusion as well as serial dilution against their microbial strains and DPPH radical scavenging activity methods.

2 Materials and Methods

2.1 Chemistry of Compounds

2.1.1 General

Chemical used in this present investigation were procured from Sigma-Aldrich and Merck companies. Mettler FP51 melting point apparatus was utilized for finding melting points of all bicyclo[2.2.1]hepene-2-yl methanones with open capillaries and are uncorrected. Infrared spectra (KBr, 4000-400 cm-1) were recorded on Thermo scientific Nicolet iS5, US made Fourier transform spectrophotometer.

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Table 1. The physical constants and analytical data of 2-(2-fluorenyl)-3-(substituted phenyl) bicyclo[2.2.1]hept-5-ene-2-yl-methanones.

Cpd	X	M.W.	M.p. (°C)	Yield (%)	Micro analysis (%)		
					C	H	N
1	H	362	109-110	65	89.45 (89.47)	6.08 (6.12)	---
2	3-NH_2	377	92-93	61	85.95 (85.91)	6.10 (6.14)	3.69 (3.71)
3	3-Cl	397	99-100	62	81.75 (81.70)	5.28 (5.33)	---
4	4-Cl	397	111-112	63	81.73 (81.70)	5.26 (5.33)	---
5	4-$N(CH_3)_2$	405	88-89	62	85.91(85.89)	6.68 (6.71)	3.39 (3.45)
6	4-OH	378	79-80	62	85.73 (85.69)	5.84 (5.86)	---
7	4-OCH_3	392	116-117	65	85.74 (85.68)	6.12 (6.16)	---
8	3-NO_2	402	122-123	61	79.64 (79.59)	5.14 (5.19)	3.49 (3.44)
9	4-NO_2	402	112-113	61	79.62 (79.59)	5.11 (5.19)	3.46 (3.44)

Values in parentheses are calculated

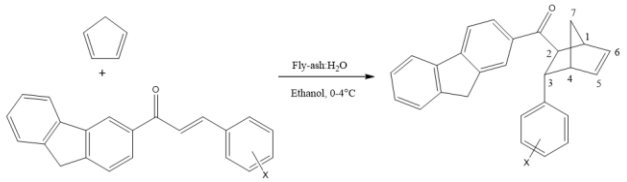
The NMR spectra of selective compounds were recorded in Bruker AV 400 spectrometer operating at 400 MHz for ^{1}H NMR spectra and 100 MHz for 13C NMR spectra in $CDCl_3$ solvent using TMS as internal standard. Electron impact and chemical ionization mode FAB+ mass spectra were recorded with a SHIMADZU spectrometer.

2.1.2 Synthesis of 9H-fluorenyl chalcones

The substituted styryl 9*H*-fluorenyl ketones were synthesized by literature method[31]

2.1.3 Synthesis of 2-(9H-fluorene-2-yl)-3-(substituted phenyl)bicyclo [2.2.1]hept-5-ene-2-yl methanones

An equal molar quantities of substituted styryl 9*H*-fluorenyl ketones (2 mmol) in 15 mL of ethanol, cyclopentadiene (2 mmol) and 0.4g of fly-ash in 20 mL of water were stirred on magnetic stirrer for 6 h in 0-4°C **(Scheme 1).** The completion of the reaction was monitored by a thin layer chromatogram. Kept the reaction mixture in an overnight. Extraction of organic layer with dichloromethane (10 mL), washed with water and brine (10mL), dried over on anhydrous Na_2SO_4 and concentration of the extract afforded the solid product. Further this solid was recrystallized with ethanol.



Entry	1	2	3	4	5	6	7	8	9
X	H	3-NH_2	3-Cl	4-Cl	4-$N(CH_3)_2$	4-OH	4-OCH_3	3-NO_2	4-NO_2

Scheme 1: Synthesis of (9*H*-fluoren-2-yl)-3-(substituted phenyl)(bicyclo[2.2.1]hept-5-en-2-yl)ketones by water mediated fly-ash catalyzed Diels-Alder reaction of 9*H*-2-fluorenyl chalcones and cyclopentadiene.

The physical constants and analytical data of synthesized (9*H*-fluoren-2-yl)-3-(substituted phenyl)(bicyclo[2.2.1]hept -5-en-2-yl)ketones were presented in Table 1.

The spectroscopic data of synthesized (9*H*-fluoren-2-yl)-3-(substituted phenyl) (bicyclo [2.2.1] hept-5-en-2-yl)ketones were summarized as follows.

(Fluorene-2-yl)(3-phenylbicyclo[2.2.1]hept-5-en-2-yl)methanone (**1**): IR (KBr, cm^{-1}): ν = 2998, 2925, 1599, 1448, 1417, 1032, 745; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.699 (dd, 1H, H_1, J = 6 and 6 Hz), 3.487 (t, 1H, H_2, J = 16 Hz), 3.352 (t, 1H, H_3, J = 15.5 Hz), 2.655 (dd, 1H, H_4, J = 4 and 6 Hz), 6.445 (d, 1H, H_5, J = 15Hz), 1.822 (dd, 1H, H_7, J = 8 and 6 Hz), 1.622 (dd, 1H, $H_{7'}$, J = 6 and 8 Hz), 4.226(s, 2H, CH_2 fluorene ring), 6.245-8.218(m, 12H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 198.21(CO), 45.62(C_1), 53.25(C_2), 44.29(C_3), 45.98(C_4), 137.25(C_5, C_6), 45.65(C_7), 36.98(CH_2 fluorene ring), 120.89-147.25 (Ar-C); Mass (m/z)=362[M^+], 286, 285, 197, 193, 169, 165, 120, 89, 92, 91, 77,76, 28.

(3-(3-Aminophenyl)bicyclo[2.2.1]hept-5-en-2-yl)(fluorene-2-yl)methanone(**2**): IR (KBr, cm^{-1}): ν = 3458, 3025, 2847, 1601, 1428, , 1448, 1325, 1105, 759, 625; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.785 (dd, 1H, H_1, J = 8 and6 Hz), 3.574 (t, 1H, H_2, J = 16.5 Hz), 3.415(t, 1H, H_3, J = 16 Hz), 2.865 (dd, 1H, H_4, J = 6.4 and 8 Hz), 6.294 (d, 2H, $H_{5,6}$, J = 16Hz), 1.893(dd, 1H, H_7, J = 8.5 and 6.5 Hz), 1.708(dd, 1H, $H_{7'}$, J = 6.5 and 8 Hz), 4.51(s, 2H, CH_2 fluorene ring), 6.321(s, 2H, NH_2), 6.416-8.238m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 198.54(CO), 45.25(C_1), 53.20(C_2), 44.22(C_3), 45.82(C_4), 138.24(C_5, C_6), 45.62(C_7), 36.28(CH_2 fluorene ring), 119.25-147.24 (Ar-C); Mass (m/z)=377[M^+], 361, 285, 193, 165, 141, 103, 77, 62, 61, 52, 28, 16.

(3-(3-Chlorophenyl)bicyclo[2.2.1]hept-5-en-2-yl)(fluorene-2-yl)methanone (**3**): IR (KBr, cm^{-1}): ν = 2958, 1854, 1598, 1425, 1438, 1258, 1104, 968, 728, 635, 428; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.704(dd, 1H, H_1, J = 8.8 and 9.6 Hz), 3.413 (t, 1H, H_2, J = 18 Hz), 3.382 (t, 1H, H_3, J =16 Hz), 2.771(dd, 1H, H_4, J =

10 and 10.2 Hz), 6.412 (d, 2H, $H_{5,6}$, *J* = 18 Hz), 1.891(dd, 1H, H_7, *J* = 9.6 and 6.4 Hz), 1.542 (dd, 1H, $H_{7'}$, *J* = 8 and 12 Hz), 4.51(s, 2H, CH_2 fluorene ring), 6.615-8.524(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 199.25 (CO), 46.85 (C_1), 53.68 (C_2), 45.68 (C_3), 47.65 (C_4), 136.17 (C_5, C_6), 46.35 (C_7), 36.89(CH_2 fluorene ring), 119.23-147.87(Ar-C); Mass (m/z)=397[M^+], 399[M^{2+}], 361, 344, 309, 285, 233, 231, 203, 195, 193, 168, 165, 111, 90, 52, 47, 35, 28,

(3-(4-Chlorophenyl)bicyclo[2.2.1]hept-5-en-2-yl)(fluorene-2-yl)methanone (**4**): IR (KBr, cm^{-1}): ν = 2992, 2952,1854, 1525, 1436, 1225, 1112, 925, 725, 615, 464; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.808(dd, 1H, H_1, *J* = 9.6 and 6.4 Hz), 3.442(t, 1H, H_2, *J* = 17 Hz), 3.365 (t, 1H, H_3, *J* =16 Hz), 2.416(dd, 1H, H_4, *J* = 6.4 and 7.6 Hz), 6.365 (d, 2H, $H_{5,6}$, *J* = 15 Hz), 1.859 (dd, 1H, H_7, *J* = 13.6 and 9.6 Hz), 1.646(dd, 1H, $H_{7'}$, *J* = 9.6 and 9 Hz), 4.24(s, 2H, CH_2 fluorene ring), 6.621-8.358(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 198.35 (CO), 46.78 (C_1), 53.69 (C_2), 44.95 (C_3), 46.38 (C_4), 137.28 (C_5, C_6), 46.29 (C_7), 36.22(CH_2 fluorene ring), 121.23-148.87(Ar-C); Mass (m/z)=397[M^+], 399[M^{2+}], 361, 344, 309, 285, 233, 203, 195, 168, 165, 111, 91, 52, 47, 36, 28.

(3-(4-Dimethylaminophenyl)bicyclo[2.2.1]hept-5-en-2-yl)(fluorene-2-yl)methanone (**15**). IR (KBr, cm^{-1}): ν = 3268, 28897, 1569, 1439, 1025, 865, 629; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.668 (dd, 1H, H_1, *J* =11.2 and 8.8 Hz), 3.915 (t, 1H, H_2, *J* = 29 Hz), 3.843 (t, 1H, H_3, *J* = 19 Hz), 2.887 (dd, 1H, H_4, *J* = 17.6 and 10.8Hz), 6.582 (d, 2H, $H_{5,6}$, *J* = 17 Hz), 1.845 (dd, 1H, H_7, *J* = 9.6 and 12 Hz), 1.623 (dd, 1H, $H_{7'}$, *J* = 9.6 and 11.2 Hz), 6.713 (s, 6H, $2CH_3$); 4.86(s, 2H, CH_2 fluorene ring), 6.236-8.158(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 190.58(CO), 46.89 (C_1), 52.68 (C_2), 44.75 (C_3), 46.42 (C_4), 136.82 (C_5, C_6), 46.12(C_7), 36.52(CH_2 fluorene ring), 41.25(CH_3), 118.36-147.25(Ar-C); Mass (m/z)=405[M^+], 390, 375, 361, 353, 285, 212, 193, 165, 120, 92, 91, 76, 44, 30, 28, 15.

(3-(4-Hydroxyphenyl)bicyclo[2.2.1]hept-5-en-2-yl)(fluorene-2-yl)methanone (**6**). IR (KBr, cm^{-1}): ν = 3448, 2998, 1602, 1428, 1038, 687, 524; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.852(dd, 1H, H_1, *J* =8.8 and 6 Hz), 3.81 (t, 1H, H_2, *J* = 29 Hz), 3.741 (t, 1H, H_3, *J* = 16 Hz), 2.736 (dd, 1H, H_4, *J* = 6.6 and 7.2 Hz), 6.258(d, 2H, $H_{5,6}$, *J* = 16 Hz), 1.800 (dd, 1H, H_7, *J* = 4.8 and 4 Hz), 1.526 (dd, 1H, $H_{7'}$, *J* = 7.2 and 8 Hz), 5.24 (s, 1H, OH), 4.28(s, 2H, CH_2 fluorene ring), 6.212-8.147(m, 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 197.29(CO), 46.72 (C_1), 53.28 (C_2), 45.28 (C_3), 46.52 (C_4), 138.29 (C_5, C_6), 46.44(C_7), 36.18(CH_2 fluorene ring), 119.35-145.38(Ar-C); Mass (m/z)=378[M^+], 361, 326, 290, 288, 285, 213, 193, 185, 165, 93, 91, 52, 28, 17.

(3-(4-Methoxyphenyl)bicyclo[2.2.1]hept-5-en-2-yl)(fluorene-2-yl)methanone (**7**). IR (KBr, cm^{-1}): ν = 2994, 2804, 1609, 1537, 1498, 1045, 638, 524; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.765 (dd, 1H, H_1, *J* = 6 and 8 Hz), 3.847 (t, 1H, H_2, *J* = 18 Hz), 3.362 (t, 1H, H_3, *J* = 17 Hz), 2.676 (dd, 1H, H_4, *J* = 4.8 and 10.4 Hz), 6.365 (d, 1H, $H_{5,6}$, *J* =18 Hz), 1.800 (dd, 1H, H_7, *J* = 6.4 and 8 Hz), 1.682 (dd, 1H, $H_{7'}$, *J* = 8 and 10 Hz), 3.671(s, 3H, OCH_3), 4.17(s, 2H, CH_2 fluorene ring), 6.118-7.658(m. 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 196.89(CO), 46.68 (C_1), 53.68 (C_2), 45.39 (C_3), 46.89 (C_4), 134.68 (C_5, C_6), 45.92 (C_7), 36.85(CH_2 fluorene ring), 59.38 (OCH_3), 121.37-147.98(Ar-C); Mass: 392[M^+], 377, 361, 340, 302, 285, 199, 193, 165, 107, 91, 52, 31, 28, 15.

(3-(3-Nitrophenyl)bicyclo[2.2.1]hept-5-en-2-yl)(fluorene-2-yl)methanone (**8**)**:** IR (KBr, cm^{-1}): ν = 2984, 2824, 1598, 1425, 1265, 1082, 722, 528; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.895(dd, 1H, H_1, *J* = 8 and 10 Hz), 3.97 (t, 1H, H_2, *J* = 18 Hz), 3.892(t, 1H, H_3, *J* = 17 Hz), 2.947(dd, 1H, H_4, *J* = 10 and 6 Hz), 6.724 (d, 1H, $H_{5,6}$, *J* =16 Hz), 1.997 (dd, 1H, H_7, *J* = 7.2 and 6 Hz), 1.550(dd, 1H, $H_{7'}$, *J* = 7.6 and 9.2 Hz), 4.83(s, 2H, CH_2 fluorene ring), 6.215-7.851(m. 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 199.28(CO), 47.06 (C_1), 53.29 (C_2), 45.85(C_3), 46.65 (C_4), 138.25 (C_5, C_6), 46.24 (C_7), 36.95(CH_2 fluorene ring), 123.68-147.89(Ar-C); Mass: 407[M^+], 381, 361, 285, 242, 214, 193, 165, 122, 90, 52, 46, 26.

(3-(4-Nitrophenyl)bicyclo[2.2.1]hept-5-en-2-yl)(fluorene-2-yl)methanone (**9**)**:** IR (KBr, cm^{-1}): ν = 2998, 2895, 1614, 1528, 1255, 1032, 852, 624, 529; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.884(dd, 1H, H_1, *J* = 12 and 8 Hz), 3.441 (t, 1H, H_2, *J* = 18 Hz), 3.762(t, 1H, H_3, *J* = 17 Hz), 3.455(dd, 1H, H_4, *J* = 8.8 and 6.4 Hz), 6.724 (d, 1H, $H_{5,6}$, *J* =15 Hz), 1.920 (dd, 1H, H_7, *J* = 6.4 and 8Hz), 1.718(dd, 1H, $H_{7'}$, *J* = 6.7 and 8 Hz), 4.93(s, 2H, CH_2 fluorene ring), 6.228-7.882(m. 11H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ = 199.98(CO), 47.72 (C_1), 53.61 (C_2), 45.38(C_3), 46.59(C_4), 138.25 (C_5, C_6), 46.35 (C_7), 37.25(CH_2 , fluorene ring), 119.38-147.95(Ar-C); Mass: 407[M^+], 381, 361, 355, 317, 285, 280, 242, 214, 193, 168, 165, 127, 122, 90, 52, 46, 26.

2.2 Evaluation of antimicrobial activities

2.2.1 Disc-diffusion method

The antimicrobial activities of prepared 2-(2-fluorenyl)-3-(substituted phenyl) bicyclo[2.2.1]hept-5-ene-2-yl-methanones were evaluated using the antimicrobial activities by measuring the zone of inhibition against their bacterial and fungal strains using the procedure of Bauer-Kirby disc-diffusion technique reported [26-30, 32,]. There are two gram-positive pathogenic strains (*Staphylococcus*

aureus, *Enterococcus faecalis*) and four gram-negative strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) were applied for evaluation of antimicrobial activities. The Bauer-Kirby[32] disc diffusion technique was adopted at the concentration of 250 µg/mL of compounds with ampicillin and streptomycin as standards. The disc diffusion technique was used for the evaluation of antifungal activity of ketones against *Candida albicans* strain and the dilution method was adopted for *Penicillium sp*. and *Aspergillus niger* strains. The dilution concentrations of the compounds are 50 µg/mL and Griseofulvin was employed as standard drug.

2.2.2 Serial dilution method

Based on the Bauer-Kirby[32] disc diffusion experimental results of mm of maximum zone of inhibition values were compared with the potency of all ketones with standard drugs against bacterial and fungal strains by determination of minimum inhibitory concentration (MIC) of test compounds using two-fold serial dilution method.

This Test was done in the seeded broth (10^{-6} to 10^{-7} cfu/mL). The test compound were taken at different concentrations ranging from 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.13 µg/mL, 1.56 µg/mL, 0.78 µg/mL and 0.39 µg/mL for finding MIC by using seeded broth as diluent. Similarly, the standard solution of ciprofloxacin drug was prepared at the concentration of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25.5 µg/mL, 6.25 µg/mL, 3.13 µg/mL, 1.56 µg/mL, 0.78 µg/mL and 0.39 µg/mL of sterile distilled water and DMSO were maintained throughout the experiment simultaneously as control.

The study involves a series of 10 assay tubes for the test compounds against each strain. In the first assay tube, 1.6 mL of seeded broth was transferred and 0.4 mL of the test solution was added followed by mixing thoroughly to obtain a concentration of 200 µg/mL. To the remaining 12 assay tubes with 1 mL of seeded broth was transferred, then from the first assay tube / mL of the content was pipetted out and added into the second assay tube followed by mixing thoroughly. This type of dilution was repeated up to 10^{th} assay tube serially. The same procedure was followed for standard drugs. Duplicates were also maintained, these were done under aseptic conditions.

The racks of assay tubes were placed inside the incubator at 37 ± 1°C for 24 h. After the incubation period, the assay tube concentrations were again streaked into nutrient agar plate due to the turbidity of drug microorganism mixture. The lowest concentration of the test compounds, which caused apparently a complete inhibition of growth of organisms, was taken as minimum inhibitory concentration. The solvent control tube was also observed to find whether there was any inhibitory action. The sterile distilled water and DMSO did not show any inhibition. For the antifungal activity, the similar procedure was adopted with fungal strains.

In order to understand the results of serial dilution method, the potency of synthesized compounds against tested bacterial & fungal strains are calculated with respect to the reference (standards) using the following equation

$$\text{Potency (\%)} = \frac{\text{MIC } (\mu\text{g/mL}) \text{ of reference compound}}{\text{MIC } (\mu\text{g/mL}) \text{ of tested compound}} \times 100 \quad \ldots\ldots \quad (1)$$

2.3 Measurement of antioxidant activity

The antioxidant activity of all prepared methanones were measure by DPPH radical scavenging activity technique [28-30] reported in literature. The antioxidant activity was expressed in terms of IC50 (µg/mL, concentration required to inhibit DPPH radical formation by 50%). α-Tocopherol was taken as a positive control. The radical scavenging activity was calculated as:

$$\text{DPPH radical scavenging activity (\% of inhibition)} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

3 Results and Discussion

3.1 Antimicrobial activities

3.1.1 Antibacterial assay by disc-diffusion method

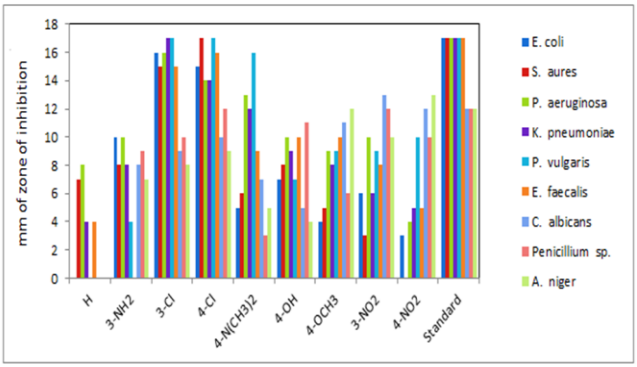


Figure 1: The antibacterial and antifungal activities – correlation chart of zone of inhibition of (2-fluorenyl)-3-(substituted phenyl bicyclo [2.2.1]hept-5-en-2-yl) ketones.

The observed antibacterial activities of prepared methanones by means of measurement of zone of mm of inhibition of disc-diffusion method were presented in Table 2. The correlation diagram was illustrated in Figure 1.

Table 2: Antibacterial[a], antifungal[b] and antioxidant activities[c] by means of mm of zone of inhibition (2-fluorenyl)-3-(substituted phenylbicyclo[2.2.1]hept-5-en-2-yl)methanones.

Cpd.	X	Antibacterial activity						Antifungal activity			Antioxidant activity (DPPH radical scavenging)
		E. coli	*S. aures*	*P. aeruginosa*	*K. pneumoniae*	*P. vulgaris*	*E. faecalis*	*C. albicans*	*Penicillium sp.*	*A. niger*	
1	H	---	7	8	4	---	4	---	---	---	26.63±1.02
2	3-NH_2	10	8	10	8	4	---	8	9	7	23.81±1.94
3	3-Cl	16	15	16	17	17	15	9	10	8	19.58±1.09
4	4-Cl	15	17	14	14	17	16	10	12	9	22.95±1.54
5	4-$N(CH_3)_2$	5	6	13	12	16	9	7	3	5	21.45±1.64
6	4-OH	7	8	10	9	7	10	5	11	4	37.95±1.24
7	4-OCH_3	4	5	9	8	9	10	11	6	12	35.54±1.11
8	3-NO_2	6	3	10	6	9	8	13	12	10	19.54±1.54
9	4-NO_2	3	---	4	5	10	5	12	10	13	11.04±1.82
Standard		17	17	17	17	17	17	12	12	12	39.14±1.57

a: standard = Ampicillin; b: standard= Griseofulvin; c:Standard= α-Tocopherol.

From the Table 2, the compounds **3** and **4** showed good antibacterial activities against *E.coli* strain. The methanone **2** showed enough activity. The remaining ketones **5-9** shows lesser than 50% activtity. The parent compound **1** was inactive against *E.coli* strain. Methanone **4** have beter antibacterial activity against *S. aures* strain. Compound **3** shows good antibacterial activity and the compound **2** have enough activity. The

Ketones **1, 6-8** were showed less than 50% antibacterial activity. The compound **9** was inactive against *S.aures* strain. Ketones **3-5** showed good antibacterial activity against *P. aeruginosa* strain. Methanones **2, 6, 7** and **8** showed enough antibacterial activity. Ketones **1, 7** and **9** have least antibacterial activity against *P. aeruginosa* strain. The methanone **3** had better antibacterial activity against *K. pneumoniae* strain. Ketones **4** and **5** shows good antibacterial activity. Compound **6** had enough activity against *K. pneumoniae* strain. Methanones **1, 2, 7, 8** and **9** had least antibacterial activity against *K. pneumoniae* strain. Compounds **3** and **4** shows better antibacterial activity against *P. vulgaris* strain. Methanones **5** and **9** shows good antibacterial activity. Ketones **7-9** shows enough activity and the ketones **2** and **6** have least activity. The parent compound **1** was inactive against *P. vulgaris* strain. The ketones **3** and **4** were showed good antibacterial activity against *E. faecalis* strain. Compounds **5-7** shows enough antibacterial activity. Ketones **2** and **9** have least antibacterial activity. The methanone **2** was inactive against *E. faecalis* strain.

3.1.1.2 Antifungal activity assay by disc-diffusion method

The observed antifungal activities of prepared methanones by means of measurement of zone of mm of inhibition of disc-diffusion method were presented in Table 2. The correlation diagram was illustrated in Figure 1. From the Table 2, the compounds **4, 7, 8** and **9**showed good antifungal activity against *C. albicans* fungal strain. The ketones **2, 3**, and **5** showed enough activity. Compound **6** had least activity. The parent compound **1** was inactive against *C. albicans* fungal strain. The methanone **4** and **8** shows better antifungal activity against *Penicillium sp*. strain. Compound 7 shows enough activity. Ketone **5** had least activity. The parent compound **1** was inactive against *Penicillium sp*. fungal strain. The methanone **9** had excellent and **7** had best antifungal activity against *A.niger* fungal strain. Ketones **2-4** and **8** showed good antifungal activity. The compounds **5** and **6** shows least activity. The parent ketone 1 was inactive against *A. niger* fungal strain.

3.1.1.3 Antibacterial activities by serial dilution method

The observed antibacterial activities of prepared methanones by means of measurement of minimum inhibitory concentration (MIC-potency) in serial dilution method were presented in Table 3. The potency correlation diagram was illustrated in Figure 2. From the Table 3, ketones **3** and **4** have shown maximum potency against *E.coli* strain. Here the +I effect of chlorine substituents enhanced the antibacterial activity. The compounds **1, 2** and **5-9** were shows less than 25% potency. Here the -I effect of nitro substituents, and electron donating nature of hydroxy and methoxy substituents reduces the antifungal activity against *E.coli*, *S.aures*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris* and *E. faecalis* strain. In addition the ketones **1, 8** and **9** also shows more than 40% antibacterial activities against *E. faecalis* strain. The +I effect of chlorine substituents enhanced the antibacterial activity. The –I effect of nitro substituents and the parent compounds also slightly enhance the activity. Meanwhile the +I effect of amino, hydroxyl, methoxy and methyl groups, electron donating nature substituents such as hydroxyl and methoxy groups reduces the bacterial activity against all strains.

Table 3: Antibacterial[a] and antifungal[b] activities[c] of (2-fluorenyl)-3-(substituted phenylbicyclo[2.2.1]hept-5-en-2-yl)methanones.

Cpd.	X	Antibacterial activity –MIC-poetency						Antifungal activity-MIC-potency		
		E. coli	*S. aures*	*P. aeruginosa*	*K. pneumoniae*	*P. vulgaris*	*E. faecalis*	*C. albicans*	*Penicillium sp.*	*A. niger*
1	H	16	3	25	10	19	32	21	18	7
2	3-NH_2	20	6	30	18	7	9	20	7	19
3	3-Cl	80	79	64	54	25	68	19	16	54
4	4-Cl	94	75	64	68	33	83	18	53	48
5	4-$N(CH_3)_2$	23	21	4	23	4	11	6	7	31
6	4-OH	18	11	7	21	17	17	33	48	32
7	4-OCH_3	12	7	6	18	3	10	17	33	17
8	3-NO_2	3	8	10	5	6	56	61	72	56
9	4-NO_2	6	10	4	3	8	43	58	68	65
Standard		9	9	9	9	9	9	7	7	7

a: standard = Ampicillin; b: standard= Griseofulvin

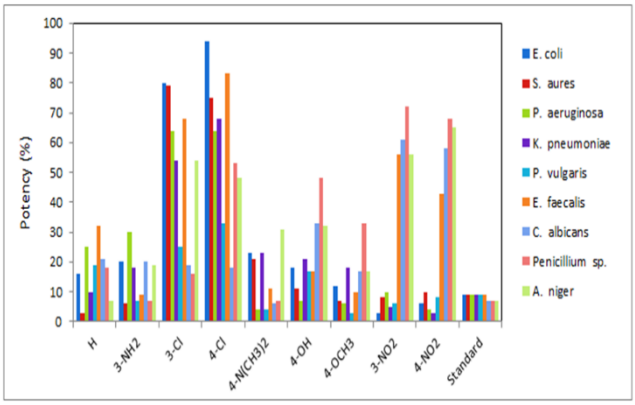


Figure 2: The correlation chart of antibacterial, antifungal-MIC-potencies of (2-fluorenyl)-3-(substituted phenyl bicyclo[2.2.1]hept-5-en-2-yl)methanones.

3.1.1.4 Antifungal activities by serial dilution method

The methanones **8, 9** and **6** shows significant antifungal activity against *C. albicans* fungal strains. The remaining ketones possess less than 25% of activity. Here the –I effect of nitro substituents and +I effect of hydroxyl groups enhances the fungal activity. The +I effect of halogens were absent for producing the fungal activity of ketones. The hyper conjugation of methyl group in dimethylamino also absent for developing the antifungal activity. The compounds **8** and **9** shows maximum antifungal potency against *Penicillium sp.* fungal strain. Ketone **4** had more than 50% of antifungal activity potency. The compounds **6** and **7** have more than 30% of antigunal potency. The ketones **1, 2, 3** and **5** possess less than 20% antifungal activity against *Penicillium sp*. fungal strain. Here the –I effect of nitro substituents enhances the fungal activity and the +I effect of 4-Cl, hydroxy and methoxy groups slightly enhances the antifungal activity. The +I effect of 3-Cl and dimethyl amino substituents were absent for producing the fungal activity of ketones. The prepared methanone 9 had maximum potency against *A.niger* fungal strain. The compound 3 and 8 shows more than 50% of antifungal activity. The ketones **4-6** shows more than 30 and less than 50% antifungal activity. The compounds **2, 7** have shown less than25% of antifungal potency. The parent compound had least antifungal activity against *A.niger* fungal strain. The –I effect of 4-nitro substituent enhances the antifungal activity. The +I effect of halogens, hydroxy and methoxy substituents shows 30-60% of antifungal activity. The +I effect of amino and methoxy substituents were absent for producing antifungal activity against *A.niger* fungal strain.

3.2 Antioxidant activity.

The measured antioxidant activities of synthesized 2-(9*H*-fluorene-2-yl)-3-(substituted phenyl)bicyclo [2.2.1]hept-5-ene-2-yl methanones were presented in Table 2. From the table, the compounds 6 and 7 shows enough antioxidant activity against α-Tocopherol. The +I effect and electron donating tendency of hydroxy and methoxy groups were responsible for the antioxidant activity.

4 Conclusions

More than 60% of 2-(9*H*-fluorene-2-yl)-3-(substituted phenyl)bicyclo [2.2.1]hept-5-ene-2-yl methanones were synthesized by environmentally benign water mediated Diels-Alder reaction of 9*H*-2-fluorenyl chalcones and cyclopentadiene. These ketones were characterized by their physical constants and spectroscopic data. The *in-vitro* antimicrobial and antioxidant activities of some 2-(9*H*-fluorene-2-yl)-3-(substituted phenyl)bicyclo [2.2.1]hept-5-ene-2-yl methanones by disc-diffusion as well as serial dilution against their microbial strains and DPPH radical scavenging activities. In disc-diffusion method, the halogens substituted compounds shows well and better

antibacterial activities against their strains. The halogen, nitro, amino, methoxy substituted compounds shows good, better and excellent antifungal activities. In serial dilution method, the chloro substituted ketones enhances the antibacterial activity against their bacterial strains. The nitro, chloro, dimethyl amino, hydroxy and methoxy substituents shows maximum, and more than 30-50% of antifungal activity against their fungal strains. The +I effect of halogen always enhances the antibacterial activities. The –I effect of nitro substituents enhances the fungal activities. The +I effect and electron donating tendencies of hydroxy and methoxy substituents slightly enhances the antifungal activity in some cases. The hydroxy and methoxy substituted methanones shows significant antioxidant activity against their standard.

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