

Original Research Article

Evaluation of synergistic effect of different compositions of liquid extract of *Citrus limonium* and its linkage with β -cyclodextrin for the one-pot multicomponent synthesis of pharmacological bioactive compounds with application of exploratory analysis

ABSTRACT

The ability of liquid extract of *Citrus limonium* and *Citrus limonium*- β -cyclodextrin composite to promote multicomponent reactions of pharmacologically bioactive compounds from a diversity of aromatic aldehydes has been described. Development of *Citrus limonium*- β -cyclodextrin composite was characterized using scanning electron microscopy (SEM) and X-ray diffraction pattern (XRD). Liquid extract of *Citrus limonium* efficiently catalyzed one pot two and three component reaction of α -aminophosphonates, 1*H*-benzo[*d*]imidazoles and 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones, whereas, *Citrus limonium*- β -cyclodextrin composite showed higher catalytic activity of four component reaction of 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitriles and 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-triones. The method is superior over the existing methods as catalyst provides less reaction time, easier workup and affords good to excellent yields under solvent free condition. Application of principal component analysis and variable correlation analysis extract the information and further evaluate the catalytic efficiency of liquid extract of *Citrus limonium* and *Citrus limonium*- β -cyclodextrin composite. Also, PCA applied on catalytic activity of different constituents of liquid extract of *Citrus limonium* and their different ratios gave the idea of their synergistic effect for the synthesis of above pharmacological compounds.

Keywords: *Citrus limonium*, *Citrus limonium*- β -cyclodextrin composite, pharmacologically bioactive compounds, principal component analysis, variable correlation analysis.

1. INTRODUCTION

Synthetic drugs have successfully been used to protect human beings from diseases. α -aminophosphonates, 1*H*-benzo[*d*]imidazoles, 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-

carbonitriles, 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones, 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-triones are known for their broad spectrum pharmacological activities such as, antineoplastic activity [1], anti-inflammatory and analgesic activity [2], antiulcer, antipsychotic, antiprotozoal, antifungal [3-5], antimicrobial, anticancer, anticonvulsant, vasodilator and molluscicidal activity [6], enzyme inhibitors [7], etc.

In recent years, numerous methods have been reported for the synthesis of these biologically active compounds. Generally, catalysts such as NaHSO₄.SiO₂ [8], strontium triflate [9], 12-tungstophosphoric acid [10], p-TSA [11, 12], Ce(SO₄)₂.4H₂O [13], p-TsOH [14], sodium hydrogen sulfite [15], zinc acetate [16], dibutylamine [17], trifluoroacetic acid [18], ZrOCl₂.8H₂O [19] have been reported for their synthesis. However, these methods have drawbacks such as application of expensive, thermally unstable and toxic catalyst, long reaction time, harsh reaction conditions, tedious workup and purification of the products. Thus, a mild, convenient and high yielding procedure using inexpensive and non toxic catalyst would be desirable.

Lemons are the important commercial citrus fruit indigenous to north- west regions of India. They have been widely cultivated in tropical and sub-tropical parts of the world. *Citrus aurantium*, *Citrus indica*, *Citrus limonium* are some important species of lemon. Lemons have particularly high concentrations of citric acid, which can constitute as much as 8% of the dry weight of these fruits (about 47 g/L in the juices) [20]; the juice of the lemon contains about 5% to 6% citric acid. Other main ingredients of lemon juices are moisture (85%), carbohydrates (11.2%), protein (1%), vitamin-C (0.5%), fat (0.9%), minerals (0.3%), fibers (1.6%) and some other organic acids [21]. Herein, keeping in view, the broad spectrum pharmacological activities of α -aminophosphonates, 1*H*-benzo[*d*]imidazoles, 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitriles, 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones, 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-triones and in continuation of our programme [22-24] i.e. development of convenient and environmentally benign procedures using green catalysts, we report liquid extract of *Citrus limonium* and *Citrus limonium*- β -cyclodextrin composite as a catalyst. In addition, principal component analysis and variable correlation analysis were applied to evaluate the catalytic efficiency of catalysts and

54 main constituents of liquid extract of *Citrus limonium* for the synthesis of above pharmacologically
55 bioactive compounds.

56 **2. EXPERIMENTAL**

57 All the reagents and chemicals were obtained from Hi-media and used without further
58 purification. The purity of new synthesized compounds and development of reactions was monitored
59 by thin layer chromatography (TLC) on Merck pre-coated silica gel G and visualized by UV light.

60 **2.1. General procedure for liquid extraction from *Citrus limonium***

61 Fresh lemon (*Citrus limonium*) of variety Pant Lemon-1 was procured from Horticulture
62 Research Center, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.
63 The lemon was sliced using knife and then juice was extracted immediately using domestic presser by
64 pressing the pieces manually. Juice was filtered through muslin cloth and then through ordinary filter
65 paper to remove solid material to get liquid lemon juice. For each reaction, fresh juice was extracted.

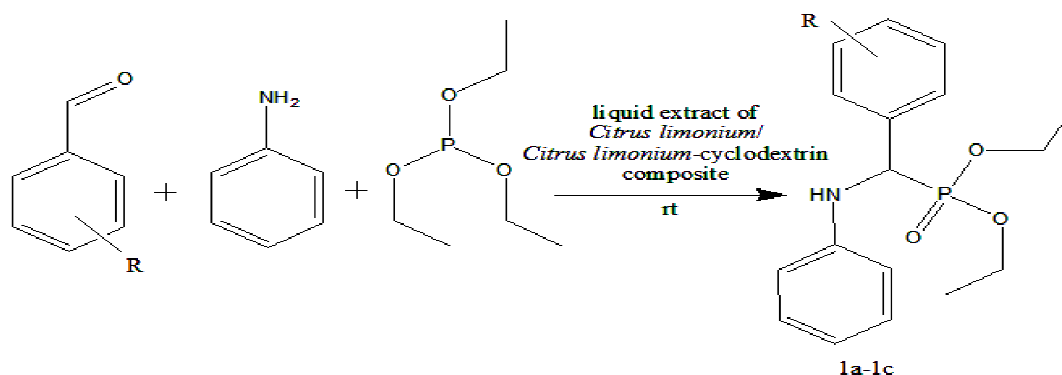
66 **2.2. General procedure for linking liquid extract of *Citrus limonium* with β -cyclodextrin (*Citrus* 67 *limonium*-cyclodextrin composite)**

68 2 g/5 mL aqueous β -cyclodextrin was taken in 50 mL beaker on hot plate with magnetic stirrer at
69 300 rpm. To this, 10 mL of fresh liquid extract of *Citrus limonium* was added drop by drop and slowly
70 temperature was raised to 55°C with continuous stirring for 6 hr. The composite formed was then
71 sieved and oven dried at 55°C for 24 hr.

72 **2.3. General procedure for synthesis of α -aminophosphonate derivatives**

73 A mixture of substituted benzaldehyde (2 mmol), aniline (2 mmol), triethyl phosphite (3 mmol)
74 and one drop of fresh liquid extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin
75 composite (0.3 mmol) were taken in 100 mL round bottom flask and was stirred at room temperature
76 (**Scheme 1**). After completion of the reaction as indicated by TLC using hexane and ethyl acetate

(80:20) solvent system, the reaction mixture was extracted with water and dichloromethane to give pure α -aminophosphonate.

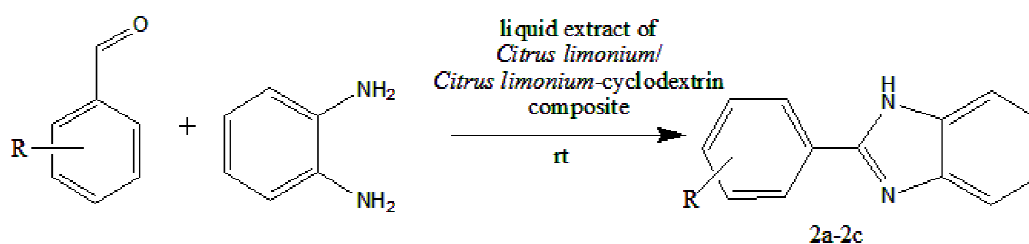


79

80 **Scheme 1. Synthesis of α -aminophosphonate derivatives**

81 2.4. General procedure for synthesis of 1H-benzo[d]imidazole derivatives

82 4 mmol substituted benzaldehyde, 4 mmol benzene-1,2-diamine and one drop of fresh liquid
 83 extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin composite (0.3 mmol) were taken
 84 in 100 mL round bottom flask and stirred on magnetic stirrer at room temperature (**Scheme 2**). After
 85 completion of reaction, as indicated by silica gel TLC plates using hexane and ethyl acetate (70:30)
 86 solvent system, solid product formed was washed with water and dried. Recrystallization was done
 87 using ethanol.

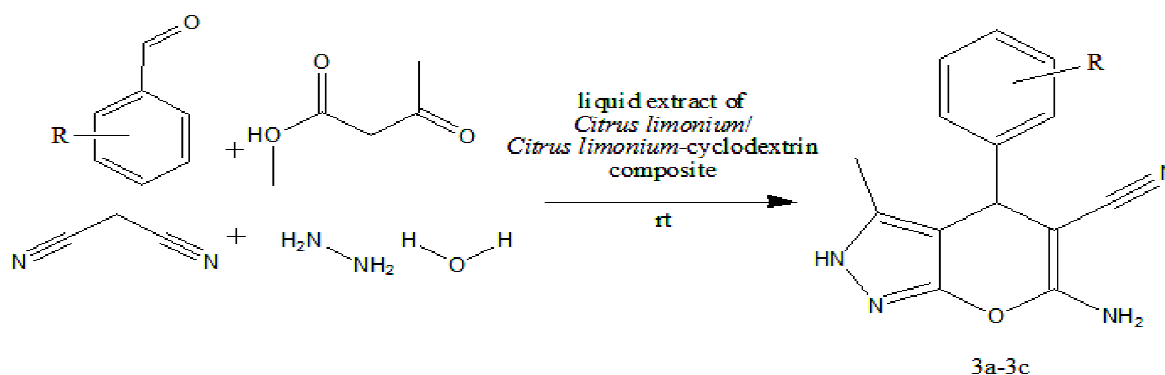


88

89 **Scheme 2. Synthesis of 1H-benzo[d]imidazole derivatives**

90 2.5. General procedure for synthesis of 6-amino-2,4-dihydro-3-methylpyrano[2,3-c]pyrazole-5- 91 carbonitrile derivatives

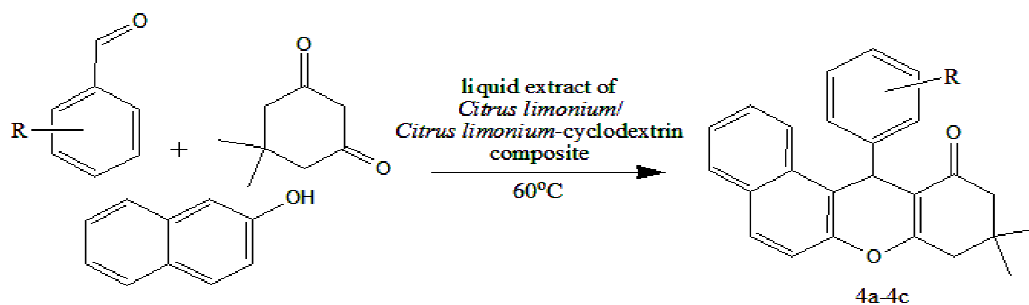
To a 100 mL round bottom flask, 5 mmol substituted benzaldehyde, 5 mmol methyl 3-oxobutanoate, 5 mmol hydrazinehydrate, 5 mmol malononitrile and one drop of fresh liquid extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin composite (0.3 mmol) were taken and stirred at room temperature (**Scheme 3**). Reaction progress was monitored over silica gel TLC plates using hexane and ethyl acetate (80:20) solvent system. After completion of reaction, the crude solid product of reaction mixture was washed with deionized water and crystallized with ethanol to get pure product.



Scheme 3. Synthesis of 6-amino-2,4-dihydro-3-methylpyrano[2,3-c]pyrazole-5-carbonitrile derivatives

2.6. General procedure for synthesis of 9,10-dihydrobenzo[a]xanthen-11(12H)-one derivatives

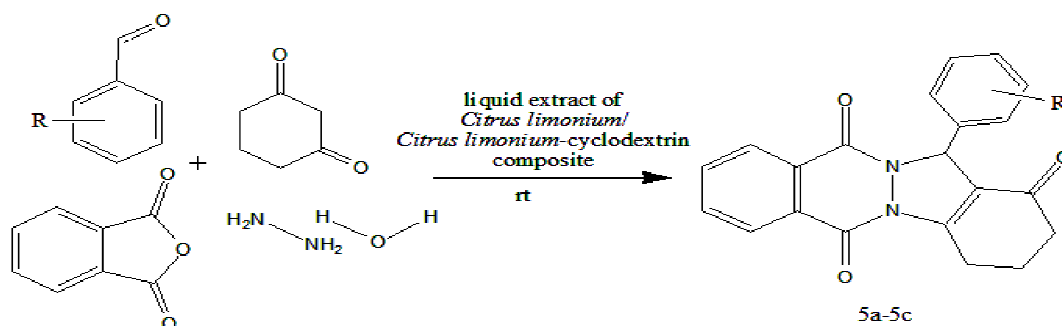
A mixture of 5 mmol substituted benzaldehyde, 5 mmol β -naphthol and 5 mmol 5,5-dimethylcyclohexane-1,3-dione in presence of one drop of fresh liquid extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin composite (0.3 mmol) was taken in 100 mL round bottom flask and stirred vigorously at 60° C (**Scheme 4**). After completion of the reaction, as observed by TLC plates using hexane and ethyl acetate (80:20) solvent system, ice cold water was added to facilitate the precipitation of product. The precipitate obtained was then washed successively with cold aqueous ethanol. The crude product was recrystallized with dichloromethane to afford pure product.



112 **Scheme 4. Synthesis of 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-one derivatives**

113 **2.7. General procedure for synthesis of 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-**
 114 **trione derivatives**

115 4 mmol phthalic anhydride, 6 mmol hydrazine hydrate and 2 mL distilled water were mixed in
 116 100 mL round bottom flask and stirred on magnetic stirrer. Then, 4 mmol substituted benzaldehyde, 4
 117 mmol cyclohexane-1,3-dione and one drop of fresh liquid extract of *Citrus limonium* (0.07 g) / *Citrus*
 118 *limonium*-cyclodextrin composite (0.3 mmol) were added to this mixture (**Scheme 5**). The completion
 119 of reaction, as monitored on TLC using hexane and ethyl acetate (80:20) solvent system, the solid
 120 residue was collected and was dissolved in warm ethanol. The solid crude product was purified by
 121 recrystallization in aqueous ethanol.



123 **Scheme 5. Synthesis of 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-trione**
 124 **derivatives**

125 **2.8. Scanning electron microscopy (SEM) and X-ray Diffraction (XRD)**

The morphology of samples was determined by scanning electron microscopy using BRUKER-JEOL JSM-6610LV. XRD patterns were obtained using powder X-ray diffractometer- BRUKER-D8 ADVANCE.

2.9. Statistical Analysis

Statistical analysis was performed using software The Unscrambler X 10.5. Entire data was analyzed using principal component analysis to evaluate the catalytic efficiency of liquid extract of *Citrus limonium* and main components present in it, and *Citrus limonium*-cyclodextrin composite for the synthesis of α -aminophosphonates, 1*H*-benzo[*d*]imidazoles, 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitriles, 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones, 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-triones.

3. RESULTS AND DISCUSSION

Table 1 represents the percent (%) yield and reaction time for α -aminophosphonates (1a-1c), 1*H*-benzo[*d*]imidazoles (2a-2c), 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitriles (3a-3c), 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones (4a-4c) and 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-triones (5a-5c) obtained by multicomponent reactions using liquid extract of *Citrus limonium* and *Citrus limonium*-cyclodextrin composite. Liquid extract of *Citrus limonium* effectively catalyze two and three component reactions, and gave α -aminophosphonates (1a-1c), 1*H*-benzo[*d*]imidazoles (2a-2c) and 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones (4a-4c) in excellent yield, whereas, *Citrus limonium*-cyclodextrin composite exhibit higher catalytic efficiency for four component reactions, i.e. 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitriles (3a-3c) and 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-triones (5a-5c).

Table 1. % Yield and reaction time of synthesized bioactive compounds using liquid extract of *Citrus limonium* and *Citrus limonium*-cyclodextrin composite

Entry	Liquid extract of <i>Citrus limonium</i>		<i>Citrus limonium</i> - β -cyclodextrin composite	
	Time (min)	% Yield	Time (min)	% Yield
1a	3	94	35	76
1b	5	91	30	71
1c	10	88	40	79
2a	15	87	30	69

2b	20	90	35	66
2c	20	92	20	74
3a	40	77	15	92
3b	45	75	25	91
3c	30	78	35	88
4a	10	85	20	71
4b	15	84	25	75
4c	25	90	30	68
5a	35	74	20	85
5b	20	69	30	88
5c	40	72	35	90

1a: Diethyl [1-(2-methoxyphenyl)-1phenylamino] methylphosphonate	1b: Diethyl [1-(3-nitrophenyl)-1phenylamino] methylphosphonate
1c: Diethyl [1-(4-methylphenyl)-1phenylamino] methylphosphonate	2a: 2-(3-methoxyphenyl)-1 <i>H</i> -benzo[<i>d</i>]imidazole
2b: 2-(3-nitrophenyl)-1 <i>H</i> -benzo[<i>d</i>]imidazole	2c: 2-(4-chlorophenyl)-1 <i>H</i> -benzo[<i>d</i>]imidazole
3a: 6-amino-4-(4-methoxyphenyl)-2,4-dihydro-3-methylpyrano[2,3- <i>c</i>]pyrazole-5-carbonitrile	3b: 6-amino-4-(3-chlorophenyl)-2,4-dihydro-3-methylpyrano[2,3- <i>c</i>]pyrazole-5-carbonitrile
3c: 6-amino-4-(4-fluorophenyl)-2,4-dihydro-3-methylpyrano[2,3- <i>c</i>]pyrazole-5-carbonitrile	4a: 12-(4-methoxyphenyl)-9,10-dihydro-9,9-dimethyl-8 <i>H</i> -benzo[<i>a</i>]xanthen-11(12 <i>H</i>)-one
4b: 12-(4-chlorophenyl)-9,10-dihydro-9,9-dimethyl-8 <i>H</i> -benzo[<i>a</i>]xanthen-11(12 <i>H</i>)-one	4c: 12-(3-nitrophenyl)-9,10-dihydro-9,9-dimethyl-8 <i>H</i> -benzo[<i>a</i>]xanthen-11(12 <i>H</i>)-one
5a: 13-(3-methoxyphenyl)-3,4-dihydro-2 <i>H</i> -indazolo[2,1- <i>b</i>]phthalazine-1,6,11(13 <i>H</i>)-trione	5b: 13-(4-chlorophenyl)-3,4-dihydro-2 <i>H</i> -indazolo[2,1- <i>b</i>]phthalazine-1,6,11(13 <i>H</i>)-trione
5c: 13-(4-nitrophenyl)-3,4-dihydro-2 <i>H</i> -indazolo[2,1- <i>b</i>]phthalazine-1,6,11(13 <i>H</i>)-trione	

149

150 Further, to explore the possibility of catalytic activity of the main constituents of liquid
151 extract of *Citrus limonium*, the reaction was carried out for the synthesis of diethyl [1-(2-
152 methoxyphenyl)-1phenylamino] methylphosphonate (1a), 2-(3-methoxyphenyl)-1*H*-
153 benzo[*d*]imidazole (2a) and 12-(4-methoxyphenyl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-
154 11(12*H*)-one (4a), using some of its major constituents as catalyst (**Table 2**). The perusal of table 2
155 reveals that no major constituent was able to catalyze the reaction individually. Using different
156 combinations of constituents catalyze selective reactions with low yield and long reaction time.
157 Combination of ascorbic acid, citric acid, glucose, fructose, citrulline and water in the ratio
158 1:1:0.5:0.5:1:2 gave 1a, 2a and 4a in 64%, 59% and 55% yield in 9.5 hr, 11 hr and 10 hr respectively
159 (**Table 2**). It may be concluded that some synergistic effect is responsible for unprecedented catalytic
160 activity of *Citrus limonium* for one pot two and three component synthesis of α -aminophosphonates,
161 1*H*-benzo[*d*]imidazoles and 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones.

162 **Table 2. Synthesis of Compound 1a, 2a and 4a using main constituents of liquid extract of *Citrus***
 163 ***limonium***

S.No.	Constituent	Weight	1a		2a		4a	
			Time	Yield	Time	Yield	Time	Yield
1	Ascorbic acid (R1)	0.176 g	15 hr	-	12 hr	-	10 hr	-
2	Citric acid (R2)	0.192 g	15 hr	-	12 hr	-	10 hr	-
3	Sucrose (R3)	0.342 g	15 hr	-	12 hr	-	10 hr	-
4	Glucose (R4)	0.180 g	15 hr	-	12 hr	-	10 hr	-
5	Fructose (R5)	0.180 g	15 hr	-	12 hr	-	10 hr	-
6	Citrulline (R6)	0.175 g	15 hr	-	12 hr	-	10 hr	-
7	Water (R7)	1 mL	15 hr	-	12 hr	-	10 hr	-
8	Citrulline: Citric acid: Water (R8)	1:1:2	10 hr	52%	12 hr	-	10 hr	-
9	Ascorbic acid: Glucose: Citrulline: Water (R9)	1:0.5:1:2	9.5 hr	50%	12 hr	-	10 hr	-
10	Citric acid: Glucose: Citrulline: Water (R10)	1:0.5:1:2	10 hr	53%	12 hr	54%	10 hr	48%
11	Ascorbic acid: Citric acid: Citrulline: Water (R11)	1:1:1:2	12 hr	49%	12 hr	51%	9.5	54%
12	Ascorbic acid: Citric acid: Fructose: Water (R12)	1:1:0.5:2	12 hr	-	12 hr	-	10 hr	-
13	Ascorbic acid: Citric acid: Glucose: Water (R13)	1:1:0.5:2	10 hr	-	12 hr	-	10 hr	-
14	Ascorbic acid: Citric acid: Glucose: Fructose: Citrulline: Water (R14)	1:1:0.5:0.5:1:2	9.5 hr	64%	11 hr	59%	10 hr	55%
15	Liquid extract of <i>Citrus limonium</i> (R15)	0.07 g (1 drop)	3 min	94%	15 min	87%	10 min	85%

164

165 3.1. Characterization of *Citrus limonium*- β -cyclodextrin composite

166 3.1.1. SEM Analysis

167 The SEM images of β -cyclodextrin and *Citrus limonium*- β -cyclodextrin composite are shown
 168 in figure 1a and 1b respectively. SEM micrograph of β -cyclodextrin has smooth homogeneous surface
 169 (**Figure 1a**), whereas, *Citrus limonium*- β -cyclodextrin composite has rough surface with cracks and
 170 heterogeneous crystalline particles of different sizes (**Figure 1b**) [25]. The morphological
 171 modification observed for *Citrus limonium*- β -cyclodextrin composite indicated the linking of liquid
 172 extract of *Citrus limonium* with β -cyclodextrin.

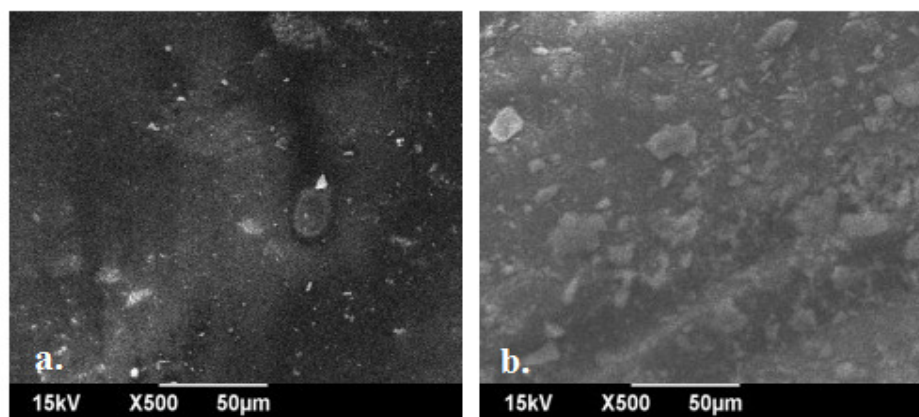
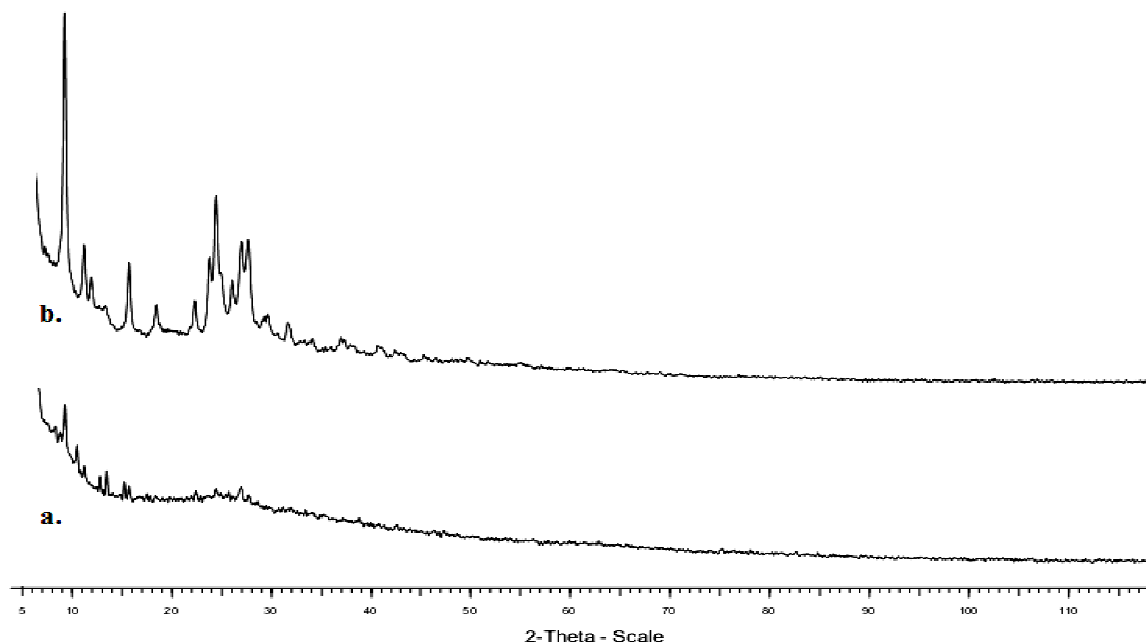


Figure 1. a). SEM micrograph of β -cyclodextrin, b). SEM micrograph of *Citrus limonium*- β -cyclodextrin composite

3.1.2. X-ray Diffraction (XRD) Analysis

The formation of *Citrus limonium*- β -cyclodextrin composite was further confirmed by XRD pattern on the basis of degree of crystallinity. The XRD pattern of *Citrus limonium*- β -cyclodextrin composite shown in figure 2b exhibited numerous peaks with high intensity as compared to XRD pattern of β -cyclodextrin, figure 2a [25]. The change in XRD pattern confirmed the linking of *Citrus limonium* with β -cyclodextrin, since crystallinity shifted to more crystalline structure which thereby resulted in increase in number and intensity of peaks (Figure 2b) [26-27].



184 **Figure 2. a). XRD of β -cyclodextrin, b). XRD of *Citrus limonium*- β -cyclodextrin composite**

185 **3.2. Characterization of synthesized compounds**

186 ^1H NMR spectra were recorded at ambient temperature on a Bruker Avance II 400MHz NMR
187 spectrophotometer using CDCl_3 or DMSO as a solvent and TMS as an internal standard.

188 **Table 1, Entry 1a: Diethyl [1-(2-methoxyphenyl)-1phenylamino] methylphosphonate**

189 ^1H NMR (CDCl_3 , TMS, 400 MHz): δ (ppm) 1.2 (3H, t, $\text{OCH}_2\text{-CH}_3$), 1.4 (3H, t, $\text{OCH}_2\text{-CH}_3$), 3.3-3.5
190 (1H, m, $\text{OCH}_2\text{-CH}_3$), 3.7-3.85 (1H, m, $\text{OCH}_2\text{-CH}_3$), 4.2 (3H, s, OCH_3), 4.35-4.5 (2H, m, $\text{OCH}_2\text{-CH}_3$),
191 4.7 (1H, br s, N-H), 5.4 (1H, dd, NH-CH-), 6.7-7.6 (9H, m, Ar-H).

192 **Table 1, Entry 1b: Diethyl [1-(3-nitrophenyl)-1phenylamino] methylphosphonate**

193 ^1H NMR (CDCl_3 , TMS, 400 MHz): δ (ppm) 1.25 (3H, t, $\text{OCH}_2\text{-CH}_3$), 1.35 (3H, t, $\text{OCH}_2\text{-CH}_3$), 3.7-
194 3.85 (1H, m, $\text{OCH}_2\text{-CH}_3$), 4.05-4.15 (1H, m, $\text{OCH}_2\text{-CH}_3$), 4.2-4.35 (2H, m, $\text{OCH}_2\text{-CH}_3$), 5.2 (1H, br s,
195 N-H), 4.9 (1H, dd, NH-CH-), 6.7-7.4 (9H, m, Ar-H).

196 **Table 1, Entry 1c: Diethyl [1-(4-methylphenyl)-1phenylamino] methylphosphonate**

197 ^1H NMR (CDCl_3 , TMS, 400 MHz): δ (ppm) 1.25 (3H, t, $\text{OCH}_2\text{-CH}_3$), 1.35 (3H, t, $\text{OCH}_2\text{-CH}_3$), 3.65
198 (2H, q, $\text{OCH}_2\text{-CH}_3$), 4.25 (2H, q, $\text{OCH}_2\text{-CH}_3$), 2.5 (3H, s, CH_3), 4.25 (1H, br s, N-H), 4.65 (1H, dd,
199 NH-CH), 6.55-7.35 (9H, m, Ar-H).

200 **Table 1, Entry 2a: 2-(3-methoxyphenyl)-1H-benzo[d]imidazole**

201 ^1H NMR (CDCl_3 , TMS, 400 MHz): δ (ppm) 3.55 (3H, s, $-\text{OCH}_3$), 6.55-7.4 (4H, m, Ar-H), 7.7-8.2
202 (4H, m, Ar-H).

203 **Table 1, Entry 2b: 2-(3-nitrophenyl)-1H-benzo[d]imidazole**

204 ^1H NMR (CDCl_3 , TMS, 400 MHz): δ (ppm) 6.6-7.25 (4H, m, Ar-H), 7.6-8.15 (4H, m, Ar-H).

205 **Table 1, Entry 2c: 2-(4-chlorophenyl)-1H-benzo[d]imidazole**

206 ¹H NMR (CDCl₃, TMS, 400 MHz): δ (ppm) 6.7-7.5 (4H, m, Ar-H), 7.65-8.25 (4H, m, Ar-H).

207 **Table 1, Entry 3a: 6-amino-4-(4-methoxyphenyl)-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-**
208 **carbonitrile**

209 ¹H NMR (DMSO, TMS, 400 MHz): δ (ppm) 1.55 (3H, s, -OCH₃), 2.5 (3H, s, -CH₃), 5.15 (1H, s, -
210 CH), 6.5 (2H, br s, -NH₂), 7.2-7.55 (4H, m, Ar-H), 12.05 (1H, s, -NH).

211 **Table 1, Entry 3b: 6-amino-4-(3-chlorophenyl)-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-**
212 **carbonitrile**

213 ¹H NMR (DMSO, TMS, 400 MHz): δ (ppm) 2.7 (3H, s, -CH₃), 5.3 (1H, s, -CH), 6.4 (2H, br s, -NH₂),
214 7.25-7.6 (4H, m, Ar-H), 12.1 (1H, s, -NH).

215 **Table 1, Entry 3c: 6-amino-4-(4-fluorophenyl)-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-**
216 **carbonitrile**

217 ¹H NMR (DMSO, TMS, 400 MHz): δ (ppm) 2.6 (3H, s, -CH₃), 5.25 (1H, s, -CH), 6.45 (2H, br s, -
218 NH₂), 7.35-7.7 (4H, m, Ar-H), 12.2 (1H, s, -NH).

219 **Table 1, Entry 4a: 12-(4-methoxyphenyl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-**
220 **11(12*H*)-one**

221 ¹H NMR (CDCl₃, TMS, 400 MHz): δ (ppm) 0.85 (3H, s, -CH₃), 1.05 (3H, s, -CH₃), 1.4 (3H, s, -
222 OCH₃), 2.45 (2H, s, -CH₂), 2.6 (2H, s, -CH₂), 5.7 (1H, s, -CH), 6.6-7.95 (10H, m, Ar-H).

223 **Table 1, Entry 4b: 12-(4-chlorophenyl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-**
224 **11(12*H*)-one**

225 ¹H NMR (CDCl₃, TMS, 400 MHz): δ (ppm) 1.1 (3H, s, -CH₃), 1.25 (3H, s, -CH₃), 2.5 (2H, s, -CH₂),
226 2.7 (2H, s, -CH₂), 5.55 (1H, s, -CH), 6.7-8.1 (10H, m, Ar-H).

227 **Table 1, Entry 4c: 12-(3-nitrophenyl)-9,10-dihydro-9,9-dimethyl-8*H*-benzo[*a*]xanthen-11(12*H*)-**
228 **one**

229 ¹H NMR (CDCl₃, TMS, 400 MHz): δ (ppm) 1.2 (3H, s, -CH₃), 1.4 (3H, s, -CH₃), 2.35 (2H, s, -CH₂),
230 2.65 (2H, s, -CH₂), 5.6 (1H, s, -CH), 6.55-8.2 (10H, m, Ar-H).

Table 1, Entry 5a: 13-(3-methoxyphenyl)-3,4-dihydro-2H-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-trione

¹H NMR (CDCl₃, TMS, 400 MHz): δ (ppm) 1.3 (2H, m, -CH₂), 1.55 (2H, t, -CH₂), 3.05 (2H, t, -CH₂), 3.8 (3H, s, -OCH₃), 5.25 (1H, s, -CH), 6.3-6.8 (4H, m, Ar-H), 7.45-8.15 (4H, m, Ar-H).

Table 1, Entry 5b: 13-(4-chlorophenyl)-3,4-dihydro-2H-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-trione

¹H NMR (CDCl₃, TMS, 400 MHz): δ (ppm) 1.15 (2H, m, -CH₂), 1.4 (2H, t, -CH₂), 3.2 (2H, t, -CH₂), 5.4 (1H, s, -CH), 6.5-7.1 (4H, m, Ar-H), 7.5-8.3 (4H, m, Ar-H).

Table 1, Entry 5c: 13-(4-nitrophenyl)-3,4-dihydro-2H-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-trione

¹H NMR (CDCl₃, TMS, 400 MHz): δ (ppm) 1.3 (2H, m, -CH₂), 1.55 (2H, t, -CH₂), 3.45 (2H, t, -CH₂), 5.65 (1H, s, -CH), 6.65-7.35 (4H, m, Ar-H), 7.75-8.5 (4H, m, Ar-H).

3.3. Principal Component Analysis

Entire data of table 1 and 2 was analyzed separately using principal component analysis. PCA on table 1 generated scores and loadings plots of two principal components (**Figure 3**), which showed the distribution of synthesized pharmacologically active compounds using liquid extract of *Citrus limonium* (C1) and *Citrus limonium*-cyclodextrin composite (C2). Plots reveals that C1 grouped with 1a-1c, 2a-2c and 4a-4c, whereas, C2 grouped with 3a-3c and 5a-5c; showing high catalytic activity of *Citrus limonium* (C1) for α-aminophosphonates (1a-1c), 1*H*-benzo[*d*]imidazoles (2a-2c) and 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones (4a-4c); and of *Citrus limonium*-cyclodextrin composite (C2) for 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitriles (3a-3c) and 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-triones (5a-5c).

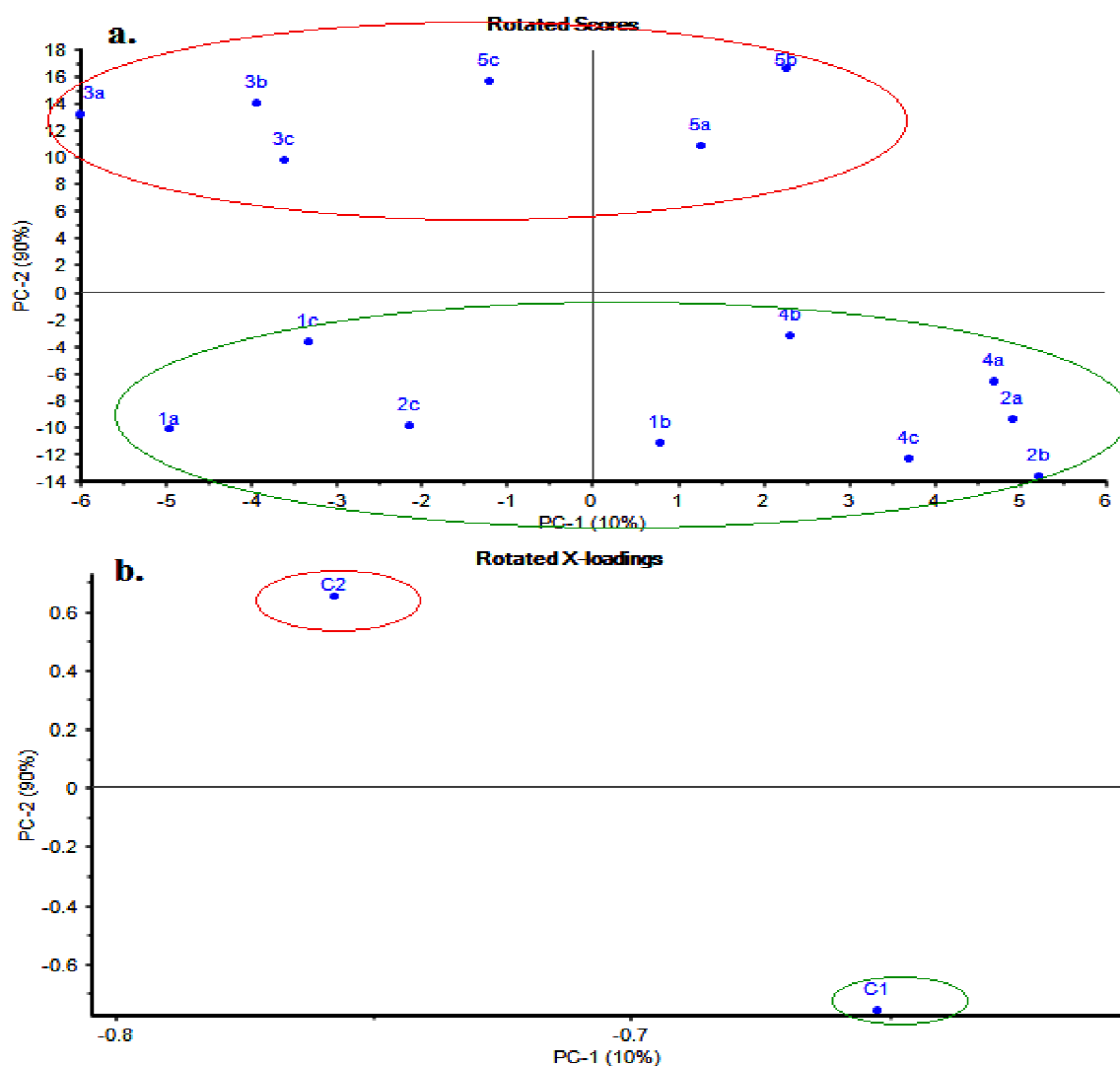


Figure 3. The scores plot (a) and loadings plot (b)

Correlation coefficient matrix of catalytic activity of the main constituents of liquid extract of *Citrus limonium* and its different combinations (**Table 3**) obtained via PCA technique on table 2 shows correlation in two groups as:

Group 1- R11, R8, R9, R10, R14

Group 2- R15, R8, R9, R11, R14

Perusal of table 3 evaluate that R11 has negative correlation with R8, R9 and R10; while R15 is negatively correlated with R11. Figure 4 and figure 5 represents 2D and 3D (PC1-2-3) scores

plots of three principal components respectively and distributed main constituents of liquid extract of *Citrus limonium* and its different combinations into four clusters as:

Cluster I- R1, R2, R3, R4, R5, R6, R7, R12, R13

Cluster II- R8, R9

Cluster III- R10, R11, R14

Cluster IV- R15

Constituents in cluster I was not able to catalyze the reactions. All the combinations of constituents in cluster II and III contain citrulline in common, which further describe its synergistic role for the synthesis of 1a, 2a and 4a. Citrulline in combination with citric and ascorbic acid increases its catalytic activity (R14). R15 (liquid extract of *Citrus limonium*) contributed to separate cluster (cluster IV) due to its unprecedented catalytic activity as a function of synergistic effects of its constituents for the synthesis of α -aminophosphonates, 1*H*-benzo[d]imidazoles and 9,10-dihydrobenzo[a]xanthene-11(12*H*)-ones.

Table 3. Correlation matrix for catalytic activity of main constituents and their combinations of liquid extract of *Citrus limonium*

	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
R1	1.00														
R2	-	1.00													
R3	-	-	1.00												
R4	-	-	-	1.00											
R5	-	-	-	-	1.00										
R6	-	-	-	-	-	1.00									
R7	-	-	-	-	-	-	1.00								
R8	-	-	-	-	-	-	-	1.00							
R9	-	-	-	-	-	-	-	1.00	1.00						
R10	-	-	-	-	-	-	-	0.36	0.36	1.00					
R11	-	-	-	-	-	-	-	-0.80	-0.80	-0.84	1.00				
R12	-	-	-	-	-	-	-	-	-	-	-	1.00			
R13	-	-	-	-	-	-	-	-	-	-	-	-	1.00		
R14	-	-	-	-	-	-	-	0.90	0.90	0.74	-0.98	-	-	1.00	
R15	-	-	-	-	-	-	-	0.98	0.98	0.55	-0.91	-	-	0.97	1.00

Correlation analysis was done using coefficient values higher than 0.7.

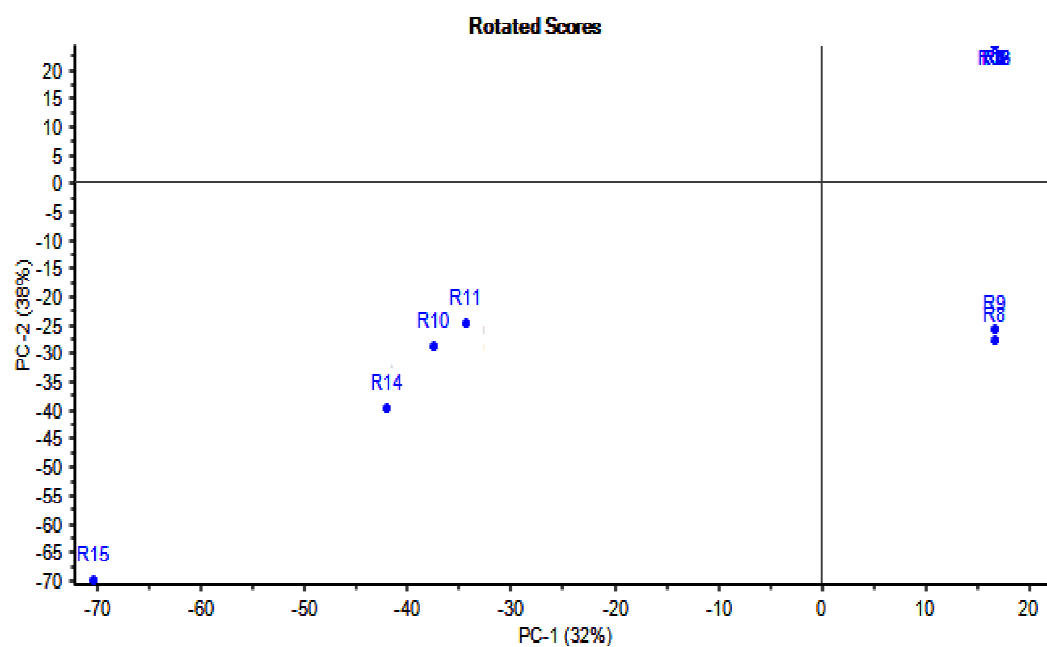


Figure 4. 2D PCA scores plot

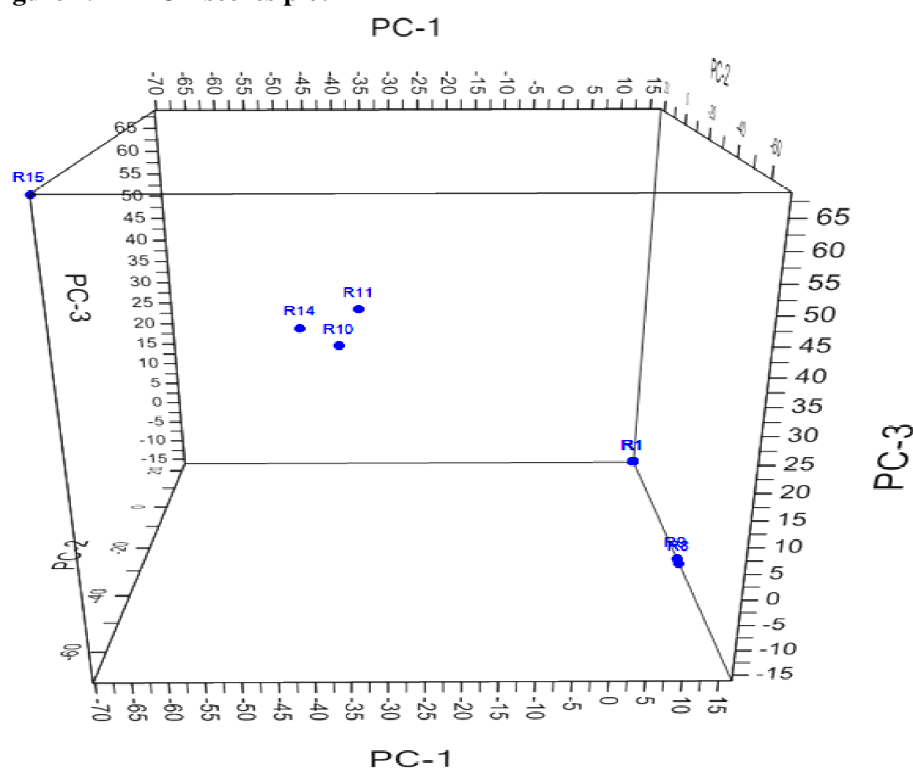


Figure 5. 3D PCA scores plot (PC1-2-3)

4. CONCLUSION

In conclusion, liquid extract of *Citrus limonium* and *Citrus limonium*- β -cyclodextrin composite permits the convenient, environmentally benign and cost effective one pot synthesis of α -aminophosphonates, 1*H*-benzo[*d*]imidazoles, 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-ones, 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitriles and 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-triones. The major advantages of this method are totally nonpolluting solvent free green approach for one pot synthesis of pharmacologically bioactive compounds within very short reaction time affording high yield. Principal component analysis and variable correlation analysis on data gave significant approach to evaluate catalytic efficiency of liquid extract of *Citrus limonium* and *Citrus limonium*- β -cyclodextrin composite, also, different constituents and their ratios of liquid extract of *Citrus limonium* were classified on the basis of its catalytic activity.

REFERENCES

1. Schwaebe MK, Moran TJ, Whitten JP. Total synthesis of psorospermin. *Tetrahedron Lett.* 2005;46:827-829.
2. Hafez HN, Hegab MI, Ahmed-Farag IS, El-Gazzar ABA. 2008. A facile regioselective synthesis of novel spiro-thioxanthene and spiro-xanthene-9',2-[1,3,4]thiadiazole derivatives as potential analgesic and anti-inflammatory agents. *Bioorg. Med. Chem. Lett.* 2008;18:4538.
3. Kalyankar TM, Pekamwar SS, Wadher SJ, Tiprale PS, Shinde GH. Review on Benzimidazole Derivative. *Int. Journal of Chem. and Pharm. Sciences.* 2012;3(4):1-10.
4. Pandeya SN, Rai P. Review: Synthesis and biological activity of benzimidazole Derivatives. *Journal of Scientific Research in Pharmacy.* 2012;1(4):1-4.
5. Ryu CK, Park RE, Ma MY, Nho JH. Synthesis and antifungal activity of 6-arylamino-phthalazine-5,8-diones and 6,7-bis(arylthio)-phthalazine-5,8-diones. *Bioorg. Med. Chem. Lett.* 2007;17:2577-2580.
6. Das D, Banerjee R, Mitra A. Bioactive and pharmacologically important pyrano[2,3-*c*]pyrazoles. *J. Chem. Pharm. Res.* 2014;6(11):108-116.

- 312 7. Allen MC, Fuhrer W, Tuck B, Wade R, Wood JM. Renin inhibitors. Synthesis of transition-state
313 analog inhibitors containing phosphorus acid derivatives at the scissile bond. J. Med. Chem.
314 1989;32:1652-1661.
- 315 8. Das B, Laxminarayana K, Krishinaiah M, Srinivas Y. An Efficient and Convenient Protocol for
316 the Synthesis of Novel 12-Aryl- or 12-Alkyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one
317 Derivatives. Synthetic letters. 2007;20:3107-3112.
- 318 9. Li J, Tang W, Lu L, Su W. Strontium triflate catalyzed one-pot condensation of β -naphthol,
319 aldehydes and cyclic 1,3-dicarbonyl compounds. Tetrahedron Lett. 2008;49:7117-7120.
- 320 10. Wang HJ, Ren XQ, Zhang YY, Zhang ZH. Synthesis 12-Aryl or 12-Alkyl-8,9,10,12-
321 tetrahydrobenzo[a]xanthen-11-one Derivatives Catalyzed by Dodecatungstophosphoric acid. J.
322 Braz. Chem. Soc. 2009;20:1939-1943.
- 323 11. Bazgir A, Seyyedhamzeh M, Yasaei Z, Mirzaei P. A novel three-component method for the
324 synthesis of triazolo[1,2-*a*]indazole-triones. Tetrahedron Lett. 2007;48:8790–8794.
- 325 12. Jitender MK, Devanshi M. p-TSA-catalyzed one-pot synthesis of 12-aryl-8,9,10,12-
326 tetrahydrobenzo[a]xanthen-11-ones in ionic liquid and neat conditions Tetrahedron Lett. 2009;50:
327 4777-4780.
- 328 13. Mosaddegh E, Hassankhani A. A rapid, one-pot, four-component route to 2 H-indazolo[2,1-*b*]-
329 phthalazine-triones. Tetrahedron Lett. 2011;52: 488–490.
- 330 14. Xiangming H, Huiqiang M, Yulu W. p-TsOH Catalyzed synthesis of 2-arylsubstituted
331 benzimidazoles. ARKIVOC. 2007;Xiii:150-154.
- 332 15. Lopez SE, Restrepo J, Prerez B, Ortiz S, Salazar J. One Pot Microwave Promoted Synthesis of 2-
333 Aryl-1H-Benzimidazoles Using Sodium Hydrogen Sulfite. Bull. Korean Chem. Soc.
334 2009;30:1628-1630.
- 335 16. Patil VD, Medha G, Sharmesha M, Jaiswal A. A mild and efficient synthesis of benzimidazole by
336 using lead peroxide under solvent free condition. Dev Chemica Sinica. 2010;1(2):125-129.
- 337 17. Kalla RM, Kim RM, Kim I. Dibutylamine-catalysed efficient one-pot synthesis of biologically
338 potent pyrans. Tetrahedron Lett. 2015;56:717-720.

18. Borse AU, Patil NL, Patil MN, Mali RS. Synthetic utility of Kabachnik-Fields reaction: a convenient one-pot three-component synthesis of N -phenyl isoquinolone-1-phosphonates Tetrahedron Lett. 2012;53:6940-6942.
19. Bhanushali MJ, Nandurkar NS, Jagtap SR, Bhanage BM. $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$: An efficient catalyst for one-pot synthesis of α -amino phosphonates under solvent-free conditions. Synth. Commun. 2009;39:845-859.
20. Penniston KL, Nakada SY, Holmes RP, Assimos DG. Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. J. Endourol. 2008;22(3):567-570.
21. Patil S, Jadhav SD, Deshmukh MB. Natural Acid Catalyzed Multi-component Reactions as a Green Approach. Arch. Appl. Sci. Res., 2011, 3 (1):203-208.
22. Agarwal D, Agrwal A, Bairagi A, Kasana VK. Hydroxylamine Hydrochloride as an effective Catalyst for Formamide derivative Synthesis and their DPPH scavenging activity. Res. J. Chem. Sci. 2014;4(10):54-57.
23. Agarwal, D., Verma, A., Dhanik, J. and Kasana, V.K. Chemometric approach to evaluate catalytic activity of [CTAB/ 18-Crown-6]: A binary catalytic system for one pot green synthesis of 4-benzylidene-3-methylisoxazol-5(4H)-one derivatives at room temperature. Int. J. Chem. Stud. 2018;6(2):3003-3007.
24. Agrwal, A., Agarwal, D., Bairagi, A. and Kasana, V.K. A Mild and Environmentally benign Synthesis of Benzimidazoles: Relevance to the pectin hetero Polysaccharide as a Catalyst. Res. J. Recent. Sci. 2014;3:64-67.
25. Xi J, Qian D, Duan J, Liu P, Zhu Z, Guo J, Zhang Y, Pan Y. Preparation, Characterization and Pharmacokinetic Study of Xiangfu Siwu Decoction Essential Oil/ β -Cyclodextrin Inclusion Complex. Molecules. 2015;20:10705-10720.
26. Eid EM, Abdul AB, Suliman FEO, Sukari MA, Rasedeo A, Fatah SS. Characterization of the inclusion complex of zerumbone with hydroxypropyl- β -cyclodextrin. Carbohydr. Polym. 2011;83:1707–1714.

- 366 27. Periasamy R, Kothainayaki S, Rajamohan R, Sivakumar K. Spectral investigation and
367 characterization of host-guest inclusion complex of 4,4 '-methylene-bis (2-chloroaniline) with
368 beta- cyclodextrin. Carbohydr. Polym. 2014;114:558–566.