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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

Deepali Agarwal[1*], Jyotsna Dhanik[1], Ankita Verma[1] and Virendra Kumar Kasana[1]

[1]Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar- 263145, Uttarakhand, India.

Authors' contributions

This research work was carried out in collaboration between all authors. Author DA designed the study, performed the synthesis and wrote the first draft of the manuscript under the supervision of author VKK. Authors DA, AV and JD managed the spectral and statistical analysis of the study. Authors AV and JD managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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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).

**Corresponding author: E-mail: agarwal.deepali.15@gmail.com;*

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 a higher catalytic activity for 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 to the existing methods as catalysts provide less reaction time, easier workup and afford 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*] xan then-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 $NaHSO_4.SiO_2$ [8], strontium triflate [9], 12-tungstophosphoric acid [10], p-TSA [11,12], $Ce(SO_4)_2.4H_2O$ [13], p -TsOH [14], sodium hydrogen sulfite [15], zinc acetate [16], dibutylamine [17], trifluroacetic acid [18], $ZrOCl_2.8H_2O$ [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 liquid extract) [20]; the liquid extract of the lemon contains about 5% to 6% citric acid. Other main ingredients of lemon liquid extract 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 and 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 main constituents of liquid extract of *Citrus limonium* for the synthesis of above pharmacologically bioactive compounds.

2. EXPERIMENTAL

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

2.1 General Procedure for Liquid Extraction from *Citrus limonium*

Fresh lemon (*Citrus limonium*) of variety Pant Lemon-1 was procured from Horticulture

Research Center, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The lemon was sliced using a knife and then liquid was extracted immediately using domestic presser by pressing the pieces manually. Liquid extract was filtered through muslin cloth and then through an ordinary filter paper to remove solid material and to get liquid lemon extract. For each reaction, fresh liquid was extracted.

2.2 General Procedure for Linking Liquid Extract of *Citrus limonium* with β-cyclodextrin (*Citrus limonium*-Cyclodextrin Composite)

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

2.3 General Procedure for Synthesis of α-Aminophosphonate Derivatives

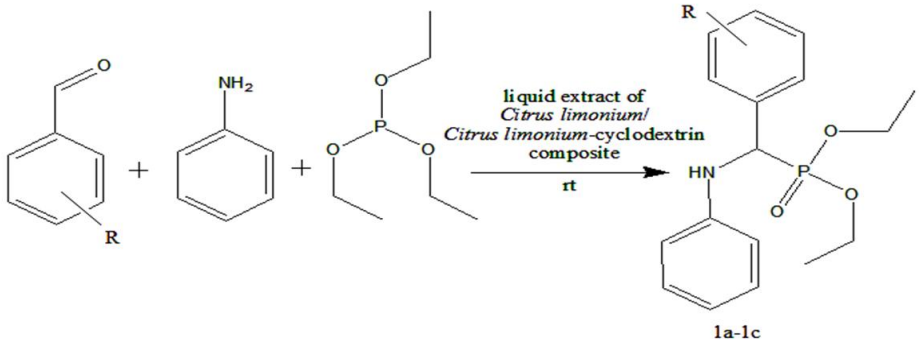
A mixture of substituted benzaldehyde (2 mmol), aniline (2 mmol), triethyl phosphite (3 mmol) and one drop of fresh liquid extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin composite (0.3 mmol) were taken in 100 mL round bottom flask and was stirred at room temperature (Scheme 1). 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.

2.4 General Procedure for Synthesis of 1*H*-benzo[*d*]imidazole Derivatives

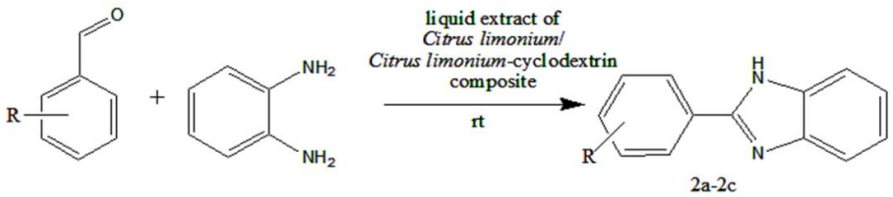
4 mmol substituted benzaldehyde, 4 mmol benzene-1,2-diamine and one drop of fresh liquid extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin composite (0.3 mmol) were taken in 100 mL round bottom flask and stirred on magnetic stirrer at room temperature (Scheme 2). After completion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

2.5 General Procedure for Synthesis of 6-amino-2,4-dihydro-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile Derivatives

To a 100 mL round bottom flask, 5 mmol substituted benzaldehyde, 5 mmol methyl 3-oxobutanoate, 5 mmol hydrazine hydrate,



Scheme 1. Synthesis of α-aminophosphonate derivatives



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

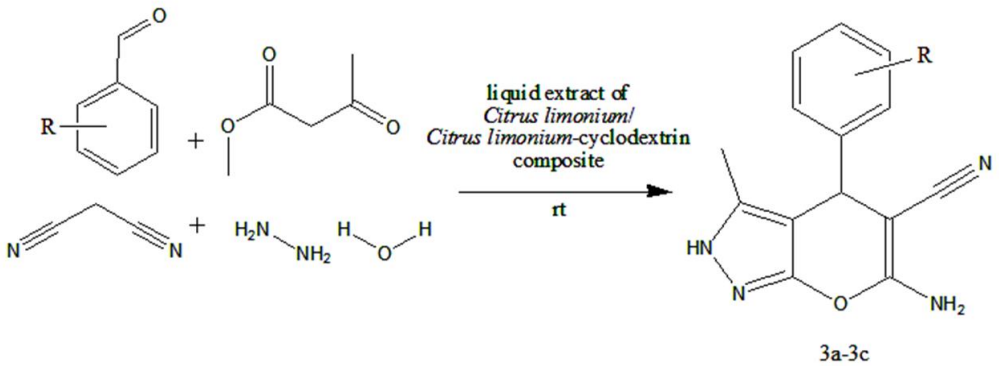
5 mmol malononitrile and one drop of fresh liquid extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin composite (0.3 mmol) were taken and stirred at room temperature (Scheme 3). Reaction progress was monitored over silica gel TLC plates using hexane and ethyl acetate (80:20) solvent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

2.6 General Procedure for Synthesis of 9,10-dihydrobenzo[*a*]xanthen-11(12*H*)-one Derivatives

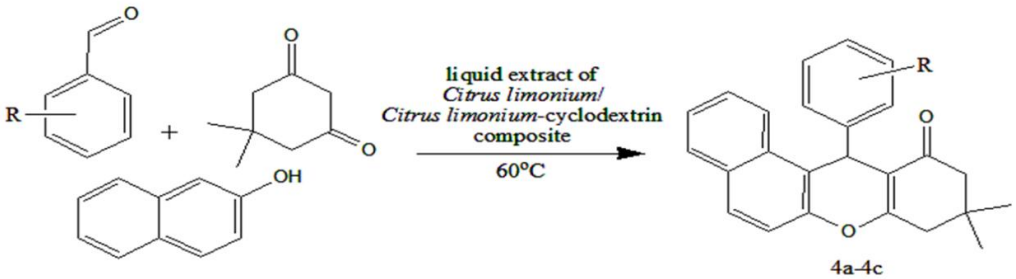
A mixture of 5 mmol substituted benzaldehyde, 5 mmol β-naphthol and 5 mmol 5,5-dimethylcyclohexane-1,3-dione in presence of one drop of fresh liquid extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin composite (0.3 mmol) was taken in 100 mL round bottom flask and stirred vigorously 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 the product. The precipitate obtained was then washed successively with cold aqueous ethanol. The crude product was recrystallized with dichloromethane to afford pure product.

2.7 General Procedure for Synthesis of 3,4-dihydro-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-trione Derivatives

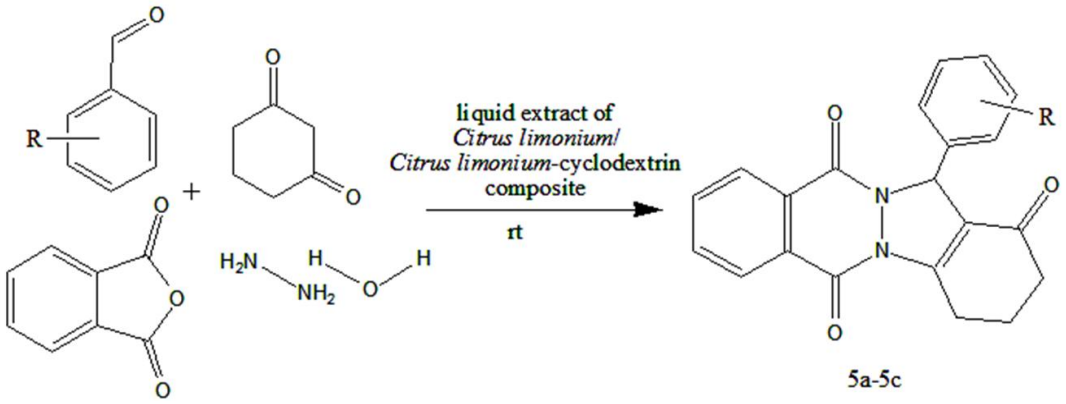
4 mmol phthalic anhydride, 6 mmol hydrazine hydrate and 2 mL distilled water were mixed in 100 mL round bottom flask and stirred on magnetic stirrer. Then, 4 mmol substituted benzaldehyde, 4 mmol cyclohexane-1,3-dione and one drop of fresh liquid extract of *Citrus limonium* (0.07 g)/ *Citrus limonium*-cyclodextrin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



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



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



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

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

All values represented in Table 1 are mean of three replicates (n=3) with standard deviation ranging from ±0.7 to ±1.8. Statistical analysis was performed using software The Unscrambler X 10.5. Entire data were 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 reaction 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).

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

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 *Citrus limonium*		*Citrus limonium*-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)-1H-benzo[d]imidazole
2b:2-(3-nitrophenyl)-1H-benzo[d]imidazole; 2c:2-(4-chlorophenyl)-1H-benzo[d]imidazole
3a:6-amino-4-(4-methoxyphenyl)-2,4-dihydro-3-methylpyrano[2,3-c]pyrazole-5-carbonitrile; 3b:6-amino-4-(3-chlorophenyl)-2,4-dihydro-3-methylpyrano[2,3-c]pyrazole-5-carbonitrile
3c:6-amino-4-(4-fluororophenyl)-2,4-dihydro-3-methylpyrano[2,3-c]pysrazole-5-carbonitrile ; 4a:12-(4-methoxyphenyl)-9,10-dihydro-9,9 dimethyl-8H-benzo[a]xanthen-11(12H)-one
4b:12-(4-chlorophenyl)-9,10-dihydro-9,9-dimethyl-8H-benzo[a]xanthen-11(12H)-one;4c:12-(3-nitrophenyl)-9,10-dihydro-9,9-dimethyl-8H-benzo[a]xanthen-11(12H)-one
5a:13-(3-methoxyphenyl)-3,4-dihydro-2H-indazolo[2,1-b]phthalaine-1,6,11(13H)-trione; 5b:13-(4-chlorophenyl)-3,4-dihydro-2H-indazolo[2,1-b]phthalazine-1,6,11(13H)-trione
5c:13-(4-nitrophenyl)-3,4-dihydro-2H-indazolo[2,1-b]phthalazine-1,6,11(13H)-trione

Table 2. Synthesis of Compound 1a, 2a and 4a using main constituents of liquid extract of *Citrus 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 *Citrus limonium* (R15)	0.07 g (1 drop)	3 min	94%	15 min	87%	10 min	85%

3.1 Characterization of *Citrus limonium*-β-cyclodextrin Composite

3.1.1 SEM analysis

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

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 Fig. 2b exhibited numerous peaks with high intensity as compared to XRD pattern of β-cyclodextrin, Fig. 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 (Fig. 2b) [26-27].

3.2 Characterization of Synthesized Compounds

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

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 1.2 (3H, t, OCH_2-CH_3), 1.4 (3H, t, OCH_2-CH_3), 3.3-3.5 (1H, m, OCH_2-CH_3), 3.7-3.85 (1H, m, OCH_2-CH_3), 4.2 (3H, s, OCH_3), 4.35-4.5 (2H, m, OCH_2-CH_3), 4.7 (1H, br s, N-H), 5.4 (1H, dd, NH-CH-), 6.7-7.6 (9H, m, Ar-H).

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 1.25 (3H, t, OCH_2-CH_3), 1.35 (3H, t, OCH_2-CH_3), 3.7-3.85 (1H, m, OCH_2-CH_3), 4.05-4.15 (1H, m, OCH_2-CH_3), 4.2-4.35 (2H, m, OCH_2-CH_3), 5.2 (1H, br s, N-H), 4.9 (1H, dd, NH-CH-), 6.7-7.4 (9H, m, Ar-H).

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 1.25 (3H, t, OCH_2-CH_3), 1.35 (3H, t, OCH_2-CH_3), 3.65 (2H, q, OCH_2-CH_3), 4.25 (2H, q, OCH_2-CH_3), 2.5 (3H, s, CH_3), 4.25 (1H, br s, N-H), 4.65 (1H, dd, NH-CH), 6.55-7.35 (9H, m, Ar-H).

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 3.55 (3H, s, -OCH_3), 6.55-7.4 (4H, m, Ar-H), 7.7-8.2 (4H, m, Ar-H).

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

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

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 6.7-7.5 (4H, m, Ar-H), 7.65-8.25 (4H, m, Ar-H).

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

^{1}H NMR (DMSO, TMS, 400 MHz): δ (ppm) 1.55 (3H, s, -OCH_3), 2.5 (3H, s, -CH_3), 5.15 (1H, s, -CH), 6.5 (2H, br s, -NH_2), 7.2-7.55 (4H, m, Ar-H), 12.05 (1H, s, -NH).

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

^{1}H NMR (DMSO, TMS, 400 MHz): δ (ppm) 2.7 (3H, s, -CH_3), 5.3 (1H, s, -CH), 6.4 (2H, br s, -NH_2), 7.25-7.6 (4H, m, Ar-H), 12.1 (1H, s, -NH).

Table 1, Entry 3c: 6-amino-4-(4-fluororophenyl)-2,4-dihydro-3-methylpyrano[2,3-*c*]pysrazole-5-carbonitrile

^{1}H NMR (DMSO, TMS, 400 MHz): δ (ppm) 2.6 (3H, s, -CH_3), 5.25 (1H, s, -CH), 6.45 (2H, br s, -NH_2), 7.35-7.7 (4H, m, Ar-H), 12.2 (1H, s, -NH).

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 0.85 (3H, s, $-CH_3$), 1.05 (3H, s, $-CH_3$), 1.4 (3H, s, $-OCH_3$), 2.45 (2H, s, $-CH_2$), 2.6 (2H, s, $-CH_2$), 5.7 (1H, s, -CH), 6.6-7.95 (10H, m, Ar-H).

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 1.1 (3H, s, $-CH_3$), 1.25 (3H, s, $-CH_3$), 2.5 (2H, s, $-CH_2$), 2.7 (2H, s, $-CH_2$), 5.55 (1H, s, -CH), 6.7-8.1 (10H, m, Ar-H).

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 1.2 (3H, s, $-CH_3$), 1.4 (3H, s, $-CH_3$), 2.35 (2H, s, $-CH_2$), 2.65 (2H, s, $-CH_2$), 5.6 (1H, s, -CH), 6.55-8.2 (10H, m, Ar-H).

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

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 1.3 (2H, m, $-CH_2$), 1.55 (2H, t, $-CH_2$), 3.05 (2H, t, $-CH_2$), 3.8 (3H, s, $-OCH_3$), 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-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-trione

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 1.15 (2H, m, $-CH_2$), 1.4 (2H, t, $-CH_2$), 3.2 (2H, t, $-CH_2$), 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-2*H*-indazolo[2,1-*b*]phthalazine-1,6,11(13*H*)-trione

^{1}H NMR ($CDCl_3$, TMS, 400 MHz): δ (ppm) 1.3 (2H, m, $-CH_2$), 1.55 (2H, t, $-CH_2$), 3.45 (2H, t, $-CH_2$), 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 Tables 1 and 2 were analyzed separately using principal component analysis. PCA on Table 1 generated scores and loadings plots of two principal components (Fig. 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 the synthesis of 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 the synthesis of 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).

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. Fig. 4 and Fig. 5 represents 2D (PC1-2) 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*]xanthen-11(12*H*)-ones.

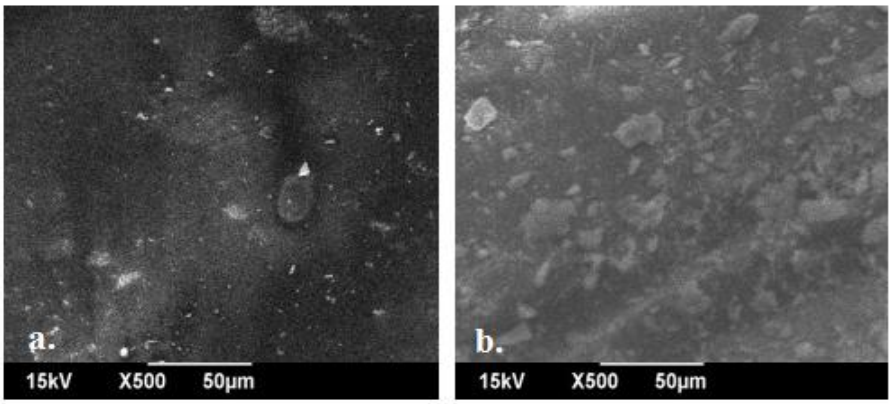


Fig. 1. a). SEM micrograph of β-cyclodextrin, b). SEM micrograph of *Citrus limonium*-β-cyclodextrin composite

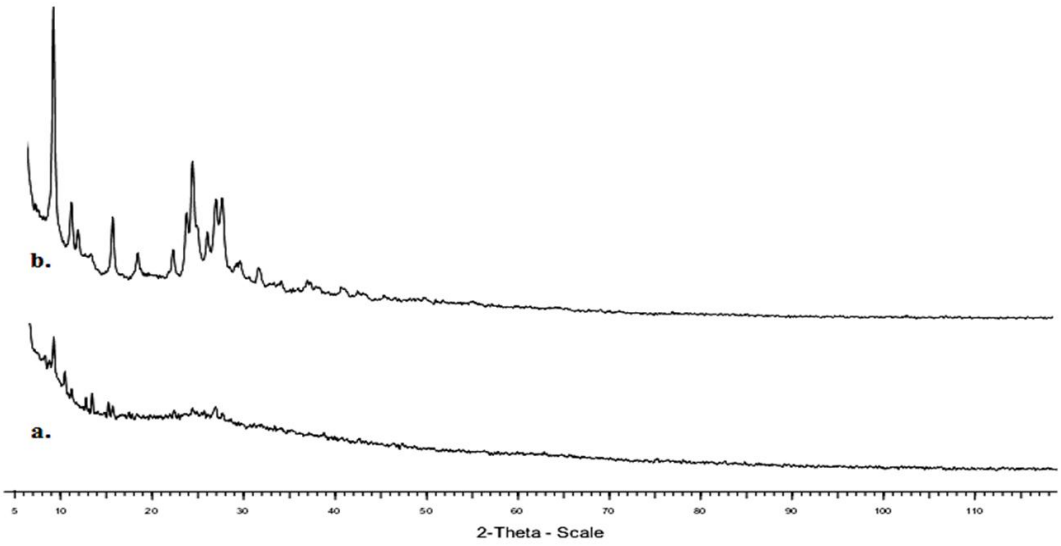
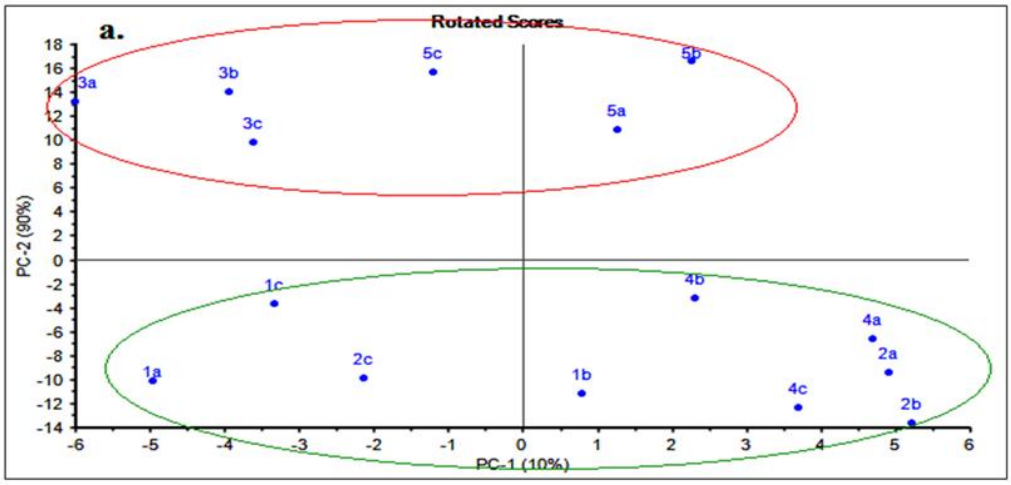


Fig. 2. a). XRD of β-cyclodextrin, b). XRD of *Citrus limonium*-β-cyclodextrin composite



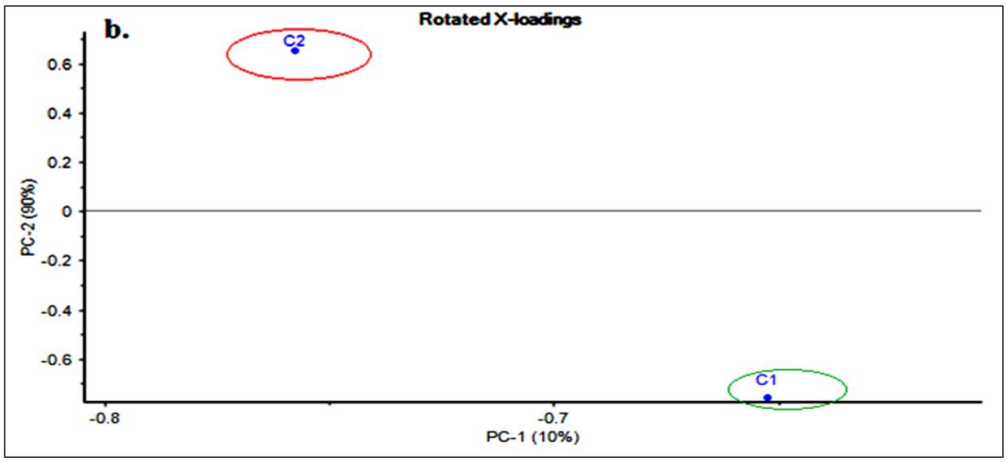


Fig. 3. The scores plot (a) and loadings plot (b)

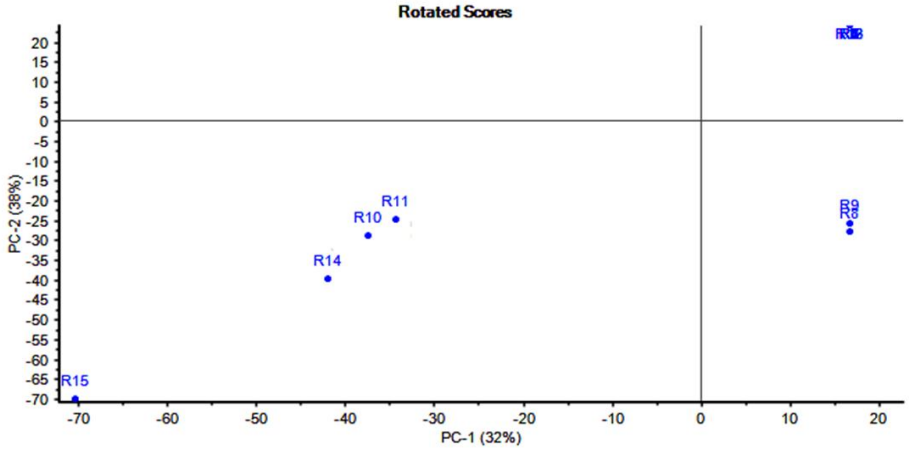


Fig. 4. 2D PCA scores plot

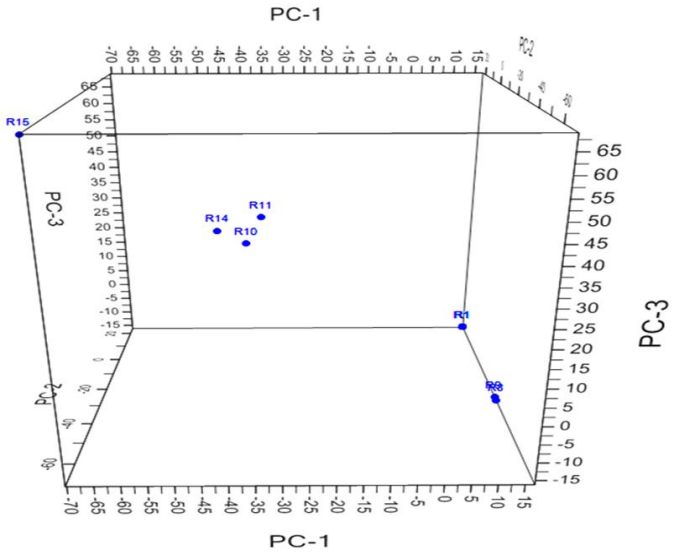


Fig. 5. 3D PCA scores plot (PC1-2-3)

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.

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 a significant approach to evaluate the 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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