ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2010; 1(4):338-344

ORIGINAL RESEARCH ARTICLE

Biological Activities of Some Derivatives of Pyrimidine, Oxadiazole and Indole in Combination

P.Muthumani[*1], R. Meera[1], Suraj Bansal Agarwal [1],P.Devi[2]

[1]*Department of Pharmaceutical Chemistry,* [2]*Department of Pharmacognosy, K.M. College of Pharmacy, Uthangudi, Madurai ,TamilNadu.*

Received 25 Aug 2010; Revised 15 Sep 2010; Accepted 01 Oct 2010

ABSTRACT

The derivatives of pyrimidine, oxadiazole and indole in combination compounds were synthesized by Conventional method and Micro wave method .The antimicrobial activity of the synthesized compounds were evaluated on *S.aureus* , *B.subtilis, E.coli* and *P.aeroginosa*. The anti oxidant activity was determined by DPPH assay method. The present investigation deals with the synthesized compounds possessing good antioxidant and antibacterial activity.

KEYWORDS:Pyrimidine, oxadiazole and indole in combination derivative compounds, Antimicrobial activity, Anti oxidant activity.

INTRODUCTION

Pyrimidine derivatives are highly effective in antitumour agent [1], antibacterial activity [2, 3], anti lukemic activity [4], HIV induced cytopathic effect [5]. Oxadiazole derivatives are useful in antibacterial activity[6,7,8] anti inflammatory activity[9,10], antitubercular activity [11], anticonvulsant activity [12], anti fungal activity [13,14], analgesic activity [15], insecticidal activity [16,17], anti cancer activity [18], anti HIV [19], plant growth regulating activity [20].Indole derivatives are effective in antibacterial activity [21], anti fungal activity[22,23,]Antitumour[24,25], antiviral [26,27], antioxidant [28], antimicrobial [29], progesterone antagonist [30], anti mitotic potency [31]. On the basis of our observation the present research work was carried out to evaluate the derivatives of pyrimidine, oxadiazole and indole in combination for antimicrobial and anti oxidant activity.

MATERIALS AND METHODS:

All the chemicals are analytical grade and were purified by the established methods. Melting points and were determined by open capillary tubes method purity and homogeneity of the compounds was routinely determined by thin layer chromatography on glass plates using silica gel G as absorbent and solvent system. Benzene: Ethylacetate: Methanol (8.5:1.4:0.1). Spots were visualized by iodine vapor by irradiation with UV light.[1]HNMRspectra was recorded on Bruker Ultra shield (300MHZ) spectrometer using DMSO (TMS as internal standard). The anti microbial activities of the synthesized compounds were evaluated on *S.aureus* , *B.subtilis, E.coli* and *P.aeroginosa*. The anti oxidant activity was determined by DPPH assay method.

SCREENING FOR ANTIBACTERIAL ACTIVITY [32, 33, 34]

Organisms used

Staphylococcus aureus, NCIM 2079, *Pseudomonas aeruginosa* NCIM 2036, *Escherichia coli* NCIM 2118 and *Bacillus subtilis* NCIM 2063 the strains were confirmed for their purity and identity by Gram's staining method and their characteristic biochemical reactions. The selected strains were preserved by sub culturing them periodically on nutrient agar slants and storing them under frozen condition. For the study, fresh 24 hour broth cultures were used after standardization of the culture.

Preparation of the inoculums

The inoculum for the experiment was prepared fresh in Mueller Hinton broth from preserved frozen slants. It was incubated at 37°C for 18-24 hours and used after standardization.

***Corresponding Author:** P.Muthumani, **Email:** meeraharsa@yahoo.com.

Drugs used 5-(4-aryl, 6-methyl –pyrimidin-2-one)- 2- imino indolino -1, 3, 4- oxadiazole.
Standard used: Ciprofloxacin (10 mcg/disc)
Vehicle used: DMSO (Di methyl sulfoxide

ANTIBACTERIAL SCREENING BY KIRBY –BAUER METHOD

Mueller Hinton agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 37°C before inoculation.

The organisms were inoculated in the plates prepared earlier, by dipping a sterile swab in the previously standardized inoculums, removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium three times, rotating the plates through an angle of 60° after each application. Finally the swab was pressed round the edge of the agar surface. It was allowed to dry at room temperature, with the lid closed. The sterile discs containing test drugs, standard and blank were placed on the previously inoculated surface of the Mueller Hinton agar plate and it was kept in the refrigerator for one hour to facilitate uniform diffusion of the drug.

Plates were prepared in triplicate and they were then incubated for 18- 24 hours. Observations were made for zone of inhibition around the discs containing the drugs and compared with that of the standard. All the compounds synthesized were tested for antibacterial activity against gram positive and gram negative bacteria. Saturated solutions of the compounds were first studied for activity and the compounds with zones greater than 15 mm were to be taken up for quantitative studies.

SCREENING FOR ANTIFUNGAL ACTIVITY

Organisms used

Candida albicans NCIM 3102 and *Aspergillus niger* NCIM 596

Drugs used : 5-(4-aryl, 6-methyl –pyrimidin-2-one)-2-imino indolino -1,3,4-oxadiazole.
Standard used : Fluconazole (10 mcg /disc)
Vehicle used : Dimethyl sulphoxide

ANTIFUNGAL SCREENING

Sabouraud dextrose agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to prevent the condensate falling on the agar surface. The plates were dried at 25°C before inoculation.

The organisms (*Candida albicans* NCIM 3102 and *Aspergillus niger* NCIM 596) were inoculated in the plates prepared earlier, by dipping a sterile swab in the previously standardized inoculums, removing the excess of inoculums by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium three times, rotating the plates through an angle of 60° after each application. Finally the swab was pressed round the edge of the agar surface. It was allowed to dry at room temperature, with the lid closed. The sterile discs containing test drugs, standard and blank were placed on the previously inoculated surface of the Sabouraud dextrose agar plate and it was kept in the refrigerator for one hour to facilitate uniform diffusion of the drug.

Plates were prepared in triplicate and they were then incubated for 24-48 hours, after placing them in refrigerator for one hour to facilitate uniform diffusion. Observations were made for zone of inhibition around the discs containing the drugs and compared with that of the standard, Fluconazole. All the compounds synthesized were tested for antifungal activity. Saturated solutions of the compounds were first studied for activity and the compounds with zones greater than 15 mm were to be taken up for quantitative studies.

ANTIOXIDANT STUDIES [35, 36]

FREE RADICAL SCAVENGING ACTIVITY BY DPPH ASSAY METHOD

Chemicals used

- 2,2-Diphenyl -1-Picrylhydrazyl (DPPH)
- Methanol, distilled
- Ascorbic acid

Procedure:

Free radical scavenging activity of the test compounds were determined by DPPH assay method and compared with (EC_{50}) of Ascorbic acid as standard.

Drug stock solutions (1µg/ml) were diluted to final concentrations of 2,4,6,8 and 10µg/ml in methanol. Minimum amount of dimethyl sulphoxide was used to solubilise the samples. One ml of 0.3 mM(12 gm in 100 ml) DPPH

methanol solution was added to 2.5 ml of drug solution of different concentrations and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm and converted to percentage antioxidant activity (AA %). It was calculated by the following formulae:

%Reduction in Absorbance =100-[{(Abs_{sample}-Abs_{blank})/$Abs_{control}$}×100]

Methanol (1ml) and drug solution (2.5 ml) was used as a blank. DPPH solution (1ml, 0.3 mM) and methanol (2.5 ml) was used as a control. Ascorbic acid was the standard solution.

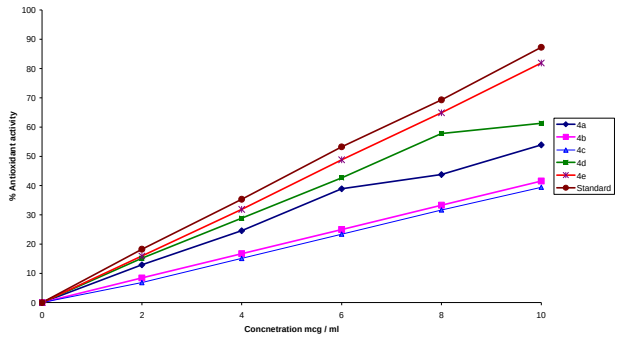
The EC_{50} values were calculated by linear regression of plots where the abscissa represented the concentration of the compounds (µg /ml) and the ordinate, the average percentage of antioxidant activity.

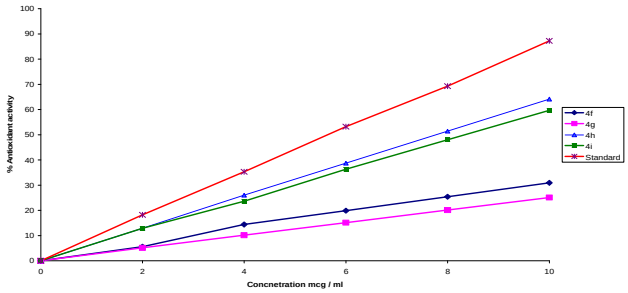
RESULTS

The free radical scavenging activity was carried out for the synthesized compounds 5-(4-aryl, 6-methyl-pyrimidin-2-one) -2-imino indolino -1,3,4-oxadiazole (4a-4i). The tests were carried out both for the compounds synthesized by conventional method (4a-4i) and Micro Wave (Ma_4-Mi_4) method. EC_{50} were found out by plotting graph of % inhibition Vs concentration in mcg/ml. The values were compared with that of the standard.

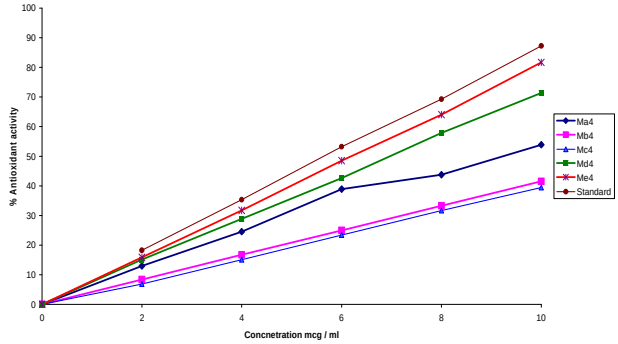
In the series of synthesized compounds 4-isopropyl phenyl substituted compound (4e) showed good free radical scavenging activity (EC_{50} 6.2) .Moderate activity was shown by 3,4,5-trimethoxy phenyl substituted compound (4d), 4-N,N dimethyl phenyl substituted compound (4h) (EC_{50} 6.9 and 7.5 respectively). Phenyl substituted compound (4a) and 4-isopropyl methyl substituted compound (4i) showed minimum antioxidant activity (EC_{50} 8.9 and 8.1 respectively). All other products (4b, 4c, 4f, and 4g) were found to be devoid of any antioxidant activity against the standard drug ascorbic acid (EC_{50} 5.8). Compounds synthesized by Microwave method (Ma_4-Mi_4) also showed good correlation with the conventional products in the anti oxidant study. (**Table 3,4) Fig 1,2,3,4.**

QUANTITATIVE SCREENING OF ANTIOXIDANT ACTIVITY DPPH ASSAY METHOD FIG1,2





QUANTITATIVE SCREENING OF ANTIOXIDANT ACTIVITY FOR COMPOUNDS SYNTHESIZED BY MW METHOD DPPH ASSAY METHOD FIG 3, 4



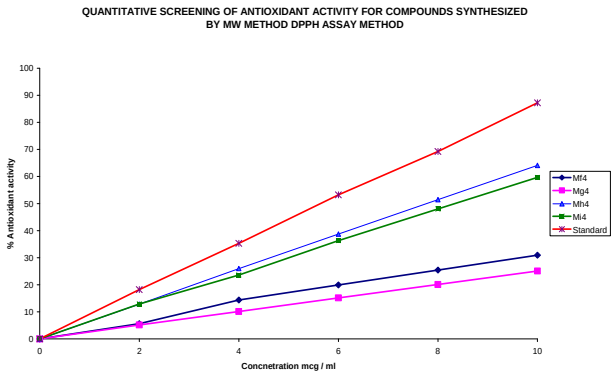


TABLE: 3 QUANTITATIVE SCREENING OF ANTIOXIDANT ACTIVITY DPPH ASSAY METHOD

Sr. no	Compound Code	Absorbance	Absorbance at 516 nm					IC_{50} µg/ml
			2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml	
1	Control	($Abs_{control}$)	.9426	.9426	.9426	.9426	.9426	
2	4a	$Abs_{sample} - Abs_{blank}$	.8223	.7127	.6127	.5316	.4353	8.9
		% Reduction in Absorbance (AA%)	12.93	24.56	38.89	43.78	53.91	
3	4b	$Abs_{sample} - Abs_{blank}$	.8646	.7866	.7086	.6306	.5526	-
		% Reduction in Absorbance (AA%)	8.45	16.72	25	33.27	41.54	
4	4c	$Abs_{sample} - Abs_{blank}$	.8798	.8018	.7238	.6458	.5678	-
		% Reduction in Absorbance (AA%)	6.84	15.11	23.39	31.66	39.44	
5	4d	$Abs_{sample} - Abs_{blank}$	.8012	.6719	.5423	.3991	.2718	6.9
		% Reduction in Absorbance (AA%)	15.18	28.89	42.64	57.83	71.33	
6	4e	$Abs_{sample} - Abs_{blank}$	.7924	.6420	.4825	.3310	.1706	6.2
		% Reduction in Absorbance (AA%)	15.93	331.89	48.81	64.88	81.90	
7	4f	$Abs_{sample} - Abs_{blank}$	.8906	.8086	.7566	.7046	.6526	-
		% Reduction in Absorbance (AA%)	5.61	14.39	19.91	25.42	30.94	
8	4g	$Abs_{sample} - Abs_{blank}$	.8956	.8486	.8016	.7546	.7076	-
8	4g	$Abs_{sample} - Abs_{blank}$	.8956	.8486	.8016	.7546	.7076	-
		% Reduction in Absorbance (AA%)	5.16	10.15	15.13	20.12	25.1	
9	4h	$Abs_{sample} - Abs_{blank}$	.8228	.6991	.5791	.4593	.3397	7.5
		% Reduction in Absorbance (AA%)	12.88	26.01	38.73	51.44	64.14	
10	4i	$Abs_{sample} - Abs_{blank}$	.8226	.7213	.6016	.4914	.3817	8.1
		% Reduction in Absorbance (AA%)	12.90	23.65	36.35	48.04	59.68	
11	Ascorbic acid	$Abs_{sample} - Abs_{blank}$	.7723	.6113	.4424	.2912	.1217	5.8
		% Reduction in Absorbance (AA%)	18.24	35.32	53.24	69.28	87.26	

TABLE: 4 QUANTITATIVE SCREENING OF ANTIOXIDANTACTIVITY FOR COMPOUNDS SYNTHESIZED BY MW METHOD DPPH ASSAY METHOD

Sl .No	Compoud Code	Absorbance	Absorbance at 516 nm					IC_{50} µg/ml
			2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml	
1	Control	($Abs_{control}$)	.9426	.9426	.9426	.9426	.9426	
		$Abs_{sample} - Abs_{blank}$	.8221	.7125	.6126	.5316	.4351	
2	Ma_4	% Reduction in Absorbance (AA%)	12.97	24.58	38.91	43.78	53.89	9.0
		$Abs_{sample} - Abs_{blank}$	.8651	.7866	.7089	.6306	.5528	
3	Mb_4	% Reduction in Absorbance (AA%)	8.42	16.72	24.98	33.27	41.53	-
		$Abs_{sample} - Abs_{blank}$	.8798	.8018	.7238	.6458	.5678	
4	Mc_4	% Reduction in Absorbance (AA%)	6.84	15.11	23.39	31.66	39.44	-
		$Abs_{sample} - Abs_{blank}$	.8010	.6714	.5421	.3988	.2717	
5	Md_4	% Reduction in Absorbance (AA%)	15.16	28.85	42.62	57.85	71.35	6.8
		$Abs_{sample} - Abs_{blank}$	.7928	.6435	.4851	.3390	.1725	
6	Me_4	% Reduction in Absorbance (AA%)	15.89	31.73	48.52	64.03	81.69	6.1
		$Abs_{sample} - Abs_{blank}$	.8908	.8086	.7569	.7049	.6528	
7	Mf_4	% Reduction in Absorbance (AA%)	5.60	14.39	19.90	25.40	30.92	-
		$Abs_{sample} - Abs_{blank}$	.8959	.8486	.8019	.7548	.7079	
8	Mg_4	% Reduction in Absorbance (AA%)	5.14	10.15	15.16	20.11	25	-
		$Abs_{sample} - Abs_{blank}$	.8228	.6991	.5794	.4596	.3399	
9	Mh_4	% Reduction in Absorbance (AA%)	12.88	25.98	38.71	51.41	64.12	7.9
		$Abs_{sample} - Abs_{blank}$	.8226	.7213	.6016	.4914	.3817	
10	Mi_4	% Reduction in Absorbance (AA%)	12.90	23.65	36.35	48.04	59.68	8.1
	Ascorbic	$Abs_{sample} - Abs_{blank}$	.7723	.6113	.4424	.2912	.1217	
11	acid	% Reduction in Absorbance (AA%)	18.24	35.32	53.24	69.28	87.26	5.8

IJPBA, Sep.-Oct. 2010, Vol. 1, Issue, 4

DISCUSSION

Antibacterial activity

All the derivatives of newly synthesized compound, 5-(4-aryl, 6-methyl–pyrimidin-2-one)-2-imino-indolino-1,3,4-oxadiazole (4a-4i) molecules were screened for antibacterial activity against both gram positive and gram negative organism by agar diffusion method (KBmethod).

Gram-positive organisms screened:

Staphylococcus aureus, Bacillus subtilis

Gram-negative organisms screened:

Escherichia coli, Pseudomonas aeruginosa

Concentration at saturated solution /disc was used for all the test compounds and results were compared with the standard drug, Ciprofloxacin at 10 µg/disc concentrations and DMSO as the vehicle. The results were interpreted as per KB method (Kirby- Bauer method).

All the test compounds synthesized were found to be resistant towards both the gram positive and gram negative organisms tested and did not show any zone of inhibition**.(Table 1**)

Antifungal activity

All the newly synthesized derivatives were screened for antifungal activity against *Aspergillus niger* and *Candida albicans* by agar diffusion method (KB method) using Fluconazole (10 µg/disc) as the standard and DMSO as the vehicle by Kirby Bauer method.

Screening was done for the newly synthesized compounds at concentration of saturated solution /disc. The test micro organism *Candida albicans* as well as *Aspergillus niger* were found to be resistant to all the compounds synthesized and did not show any zone of inhibition. (**Table 2**)

TABLE 1: QUANTITATIVE SCREENING OF ANTIBACTERIAL ACTIVITY

Sl. No	Compound code	Zone of inhibition in mm diameter			
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	4a	-	-	-	-
2	4b	-	-	-	-
3	4c	-	-	-	-
4	4d	-	-	-	-
5	4e	-	-	-	-
6	4f	-	-	-	-
7	4g	-	-	-	-
8	4h	-	-	-	-
9	4i	-	-	-	-
10	Stand	20	28	21	33

Resistant Moderately sensitive sensitive

(<12 mm) (12-17 mm) (≥18 mm)

TABLE NO2:QUANTITATIVE SCREENING OF ANTIFUNGAL ACTIVITY

Sl.No	Compound Code	Zone of inhibition in mm diameter	
Sl.No	Compound Code	Zone of inhibition in mm diameter	
		Aspergillus niger	*Candida albicans*
1	4aQ	-	-
2	4b	-	-
3	4c	-	-
4	4d	-	-
5	4e	-	-
6	4f	-	-
7	4g	-	-
8	4h	-	-
9	4i	-	-
10	Standard	24	24

Resistant Moderately sensitive Sensitive

(<12 mm) (12-17 mm) (≥18 mm)

Antioxidant activity

All the derivatives of newly synthesized 5-(4-aryl, 6-methyl –pyrimidin-2-one)- 2-imino indolino-1,3,4-oxadiazole (4a-4i), molecules were screened for antioxidant activity by DPPH assay method, and compared with standard drug (Ascorbic acid). In the series of synthesized compounds, 4e showed good free radical scavenging activity.4d and 4h showed moderate activity, whereas compound 4a and 4i showed minimum antioxidant activity. The compounds synthesized by micro wave method (Ma_4-Mi_4) were found to be in correlation with the products synthesized by conventional method.

CONCLUSION

BIOLOGICAL STUDIES

Antimicrobial studies

All the compounds synthesized were resistant towards the test gram positive and gram-negative organisms. **(***Staphylococcus aureus,. Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa).*

The synthesized compounds were also found to be resistant towards the fungi tested (*Aspergillus niger* and *Candida albicans)* and did not show any zone of inhibition.

Antioxidant Studies

DPPH assay method was followed to evaluate the free radical scavenging activity of the synthesized compounds. Among them 4e from both microwave and conventional method showed good antioxidant activity. Where as 4d and 4h from conventional and MW method showed moderate activity. Compound 4a and 4i showed little antioxidant activity. Good correlation was obtained with the results of the products of Micro Wave and conventional method.

References

1. Gangjee A, Jianminglu Y, Roy L, Kisliuk William HH. Guilia S.McGuire JJ: *Journal of medicinal chemistry*. 2003 ; 40(4): 591.
2. Pshy K, Bardhan MM, Panda CS: *Indian journal of chemistry*. 2003; 42B:910.

***Corresponding Author:** P.Muthumani, **Email:** meeraharsa@yahoo.com.

3. Sayeed HH ,Ahmed HS, Rashad AE: *Actapharm.* 2006;56:231.
4. Edward EK, Ebrahim N, Zhou A, Khalili P, Leonard W I., Balzarini J, Erik DC: *Journal of medicinal chemistry* 2003;46:995.
5. Sheriff A. Rostom H, Hesham T Fahmy Y. Manal N Saudi S: *Scientia pharmaceutica* ,2003;71:57.
6. Moglilaiah K. and Vidya K: *Indian journal of chemistry*, 2006; 45B: 1905-1906.
7. Moglilaiah K. and Reddy S Ch: *Indian journal of chemistry*. 2006; 44B: 768-772.
8. Sidhar D , Arjun M ,Jyothi M., Raviprasad T.,Sarangapani M: *Indian journal of heteropcyclic chemistry* .2006;16:61-62.
9. Ravindra K C, Vagdevi H M., VaidyaV P and Padmashal B: *Indian journal of chemistry* .2006;45:2506-2511.
10. Khan MSY. and Chawla G: *Indian journal of chemistry*, 2004;43(B):1302-1305**.**
11. Vasoya S L., Patel M R. Dobaria S V and Joshi H S: *Indian journal of chemistry* .2005;44B:405-409.
12. Khan MSY and Khan MR: *Indian journal of chemistry*.2001;11:119-122.
13. Frank PV and Kalluraya B*: Indian journal of chemistry*.2005;44B:1456-1459.
14. Agarwal T and Tiwari N: *Indian journal of chemistry*.1994; 42:603-606.
15. Khan MSY and Akhtar M :*Indian journal of chemistry*.2003;42(B): 900-904.
16. Mohan TP, Vishalakshi B, Bhar KS, Rao KS and Kendappa GN : *Indian journal of chemistry.*2004; 43 :1798-1801.
17. Holla BS, and Prasanna CS: *Indian journal of chemistry*.2004; 43B:864-869.
18. Bhat KS, Karthikeyan MS, Holla BS and Shetty NS: *Indian journal of chemistry* .2004;43B:1765-1769.
19. Shah HP and Shah BR: *Indian journal of of chemistry* .1998;37B: 180-182.
20. Lizheng and wang X*: Indian journal of chemistry* .2003;42B: 941-944.
21. Varma RS, Garg K P: *Acta pharmaceutica Jugoslavica,* 1980;30:4.
22. Singh SP, Singh AV, Gupta KC: *Acta pharmaceutica Jugoslavica*,. 1986;36(1):19.
23. Bhovi MN , Gadaginamath GS: *Indian journal of heterocyclic chemistry.,* 2004;4:15.
24. Labobuta I., Salama., H. Eshba M. Nabil H., Chirbini E., Eglal: *Acta pharmaceutica* Jugoslavica., 1988; 38(3):189.
25. Heckel, Armin., Roth., Gerald., J., Walter Rainer, Meel.V. Jacobus, Frank, Boehringer., German KG., PCT Int.Appl/. 2001:282.
26. Singh S P ,Jha R K , Zentralblat Fuer :Microbiolgie. 1989;144(2):105.
27. Sridhar SK, Pandeya SN. Bajpal. SK, Manjula H: *Indian drugs.*1999;36(6):412.
28. Tan,reiter,Manchester,Yan.,Yan,Sawi,Sain z,Mayo,Kohen,Allegra:Hardehan,Current topics in medicinal chemistry ,Bentham Science publisher, 2002:181-187.
29. El-Gendy, Ahmedy A.A , Aly M: Archives of pharmacol research., 2000;23(4):11.2.
30. Fensome, Andrew, Miller, LoriC, John,W, Zhang, JonesKB, Christopher: MPCT *Int Appl*.,2000:127.
31. Combeau Cecile ,Mailliet ,Patrick ,Chrion.,PCT Int.Appl. 2002:18.
32. Atlas BM : Laboratory Maual in Microbiology 4th ed,281.
33. Gnanasekhran P: Laboratory Manual in Microbiology 4th ed,1.
34. Boyd R F: Text book of General; Microbiology, 76.
35. Canpaigne E. Thomson R L, Westh V: *Journal of pharmaceutical chemistry.*1959;1:97.
36. Mensor L L, Menzes F S , Lelto G G, Reis A S. Dosantos T L. *Phtotheropy research.*2001;15:127