Preliminary Biological Screening of Some Marine Algae Collected from Karachi Coasts of Arabian Sea

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Summary: The antibacterial assay using the ethanol extracts of the studied algae showed significant activity against bacteria while the water and chloroform extracts were found inactive. Most of the algae showed very significant activity against *Corynebacterium diptheriae* and *Staphylococcus aureus*. The results of the antifungal assay showed that all the extracts i.e. Ethanol, chloroform and water extracts are capable to inhibit the growth of human, animal and plant pathogens. Most of the extracts showed inhibitory activity, however few of the extracts were found to promote the growth of certain fungi.The ethanol extracts of the most of the three classes of algae had antifungal activity against human pathogens. An interesting observation was made regarding the ethanol, chloroform and water extracts of *Iyengaria stellata*, all of which were found active against animal pathogen *Microsporum canis*. From all the algae tested, the ethanol extract of *Codium iyengarii* showed very significant antifungal activity against all types of pathogens. The brine shrimp lethality bioassay showed that most of the ethanol, chloroform and water extracts were active but the LD_{50} values of most of the extracts were higher than 1000 µg/ml. The ethanol extracts of green algae did not show significant results.The water extracts of the three classes of algae were active at higher doses.The results of the *Lemna* bioassay showed that some of the ethanol extracts of the algae inhibit while others help to promote the growth of *Lemna aequinoctiatls welv*. The water extracts of different alga were found to promote the growth of the tested plant. A very interesting result was observed from the ethanol extract of *Sargassum teneriumum* which gave 100 % inhibition of the fronds at highest concentration i.e 500 ppm. Among the chloroform extracts the most active was the chloroform extract of green algae *Codium iyengarii*. This extract significantly inhibited the growth in all three concentrations. From all the algae tested for the phytotoxic activity, the extracts (ethanol, chloroform and water) of brown alga *Iyengaria stellata* were found to inhibit the growth of the fronds while the extracts (ethanol, chloroform and water) of the red alga *Melanothamnus somalensis* promoted the growth of *L. aequinoctiatls*.

Introduction

The significance of algae as a source for biologically active natural products is well known. Marine algae have long been recognized for their antimicrobial, antitumor, anticoagulant and cytotoxic activities. This profile encouraged us to biologically screen the Arabian Sea algae belonging to classes Rhodophyceae, Phacophyceae and Chlorophyceae. For biological screening four activities were selected which are:

1. Antibacterial Activity.
2. Antifungal Activity.
3. Brine shrimp Bioassay.
4. *Lemna* Bioassay.

For the biological screening all the algae were collected from the Karachi coasts named as Manora,Paradise Point, Buleji, Mubarak Village , Sandspit and Hawkes Bay of Arabian Sea. The collected algae were:

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Brown Algae	Red Algae	Green Algae
Iyengaria stellata	*Hypnea musciformis*	*Caulerpa racemosa*
Sargassum binderi	*Botryocladia leptopoda*	*Codium iyengerii*
Jolyna laminarioides	*Laurencia pinnatifida*	*Codium flabellatum*
Padina tetrastromatica	*Sarconema filiforme*	*Enteromorpha intestinalis*
Sargassum tenerimum	*Melanothamnus somalensis*	

The collected algae were identified by Prof. Dr. Mustafa Shameel, Department of Botany, University of Karachi, where the voucher specimens are deposited in the herbarium. The fresh material was thoroughly washed with water to remove sea salt and dried under shade for a period of ten days. The dried material was soaked in ethanol for two weeks, the extract was evaporated under reduced pressure to yield a gummy residue. The ethanol extracts of *Iyengaria stellata, Padina tetrastromatica, Melanothamnus somalensis, Botryocladia leptopoda* and *Codium iyengarii* were further partitioned between chloroform and water. The chloroform and water layers were separated and evaporated under reduced pressure. The three extracts i.e water, ethanol and chloroform thus obtained were subjected to biological screening.

Results and Discussion

Antibacterial Activity:

The antibacterial activity was determined by measuring diameter of the zones (mm) showing inhibition and growth inhibition was calculated with reference to control. The results were compared with the control.The antibacterial assay using the ethanol extracts of the algae showed significant activity against bacteria while the water and chloroform extracts were found inactive.The ethanol extracts of chlorophyceae, phaeophyceae and rhodophyceae exhibited activity against both the gram positive and gram negative bacteria. None of the three classes of algae showed any selective activity against gram positive or gram negative bacteria. Most of the algae showed significant activity against *Corynebacterium diptheriae* and *Staphylococcus aureus*. They were also active against *Klebsiella penumonia*. *Sargassum binderi* was very active against *Corynebacterium diptheriae* while inactive against rest of the gram positive organisms. It showed inhibitory activity against gram negative organisms *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The ethanol extract of *Jolyna laminarioides* was inactive against most of the organisms showing activity against only *Streptococcos pyogenes* and *Klebsiella pneumonia*. The red alga *Botryocladia leptopoda* was the most active alga among rhodophytes showing significant activity against *Corynebacterium deptheriae, Staphylococcus aureus, Bacillus subtilus, Klebsiella penumonia* and *Shigella bodii*. *Sarconema filiforme* gave inhibitory activity against *Corynebacterium diptheriae, Staphylococcus aureus, Pseudomonas areuginosa* and *Salmonella typhii, Laurencia pinnatifida* showed activity against just one organism i.e. *Bacillus subtillus, Hypnea musciformis* exhibited antibacterial activity against *Streptococcus pyogenes* and *Bacillus subtillus*. The ethanol extract of *Melanothamnus somalensis* was found active against *Corynebacterium diptheriae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The alga, *Caulerpa racemosa* belonging to the class chlorophyceae showed better antibacterial activity than the rest of the green algae. It was active against *Corynebacterium diptheriae, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Shigella boydii*. The ethanol extract of *Enteromorpha intestinalis* also showed antibacterial activity against *Corynebacterium deptheriae*. Among the ethanol extracts of Phaeophytes, *Podina tetrastromatica* showed significant activity against both gram positive and gram negative bacteria, it was found active against *Corynebacterium diptheriae, Staphylococcus aureus, Klebsiella pneumonia* and *Shigella bodyii*. *Sargassum tenerimum* showed activity against *Corynebacterium diptheriae, Staphylococcus aureus, Klebsiella pnemonia* and *Salmonella typhii*. *Iyengaria stellata* also exhibited most significant activity against gram positive *Corynebacterium diptheriae*. It also inhibited the growth of *Staphylococcus aureus, Klebsiella pneumonia*. Among all the algal extracts tested against *Corynebacterium diptheriae*, the ethanol extract of *Codium iyengerii* gave the biggest zone of inhibition. It also inhibited the growth of *Klebsiella pneumonia* and *Salmonella typhi*. Another green alga, *Enteromorpha intestinalis* showed better antibacterial activity against both gram +ve and gram -ve bacteria. It was found active against *Corynebacterium deptheriae, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhi*.

Antifungal Activity:

The results of the bioassay show that all the extracts i.e. Ethanol, chloroform and water extracts posses antifungal activity against human, animal and plant pathogens. Most of the extracts showed inhibitory activity, however few of the extracts were found to promote the growth of certain fungi.The ethanol extracts of the most of the three classes of algae had antifungal activity against human pathogens but few promoted the fungal growth. The ethanol extract of red alga *Sarconema filiforme* showed most significant antifungal activity against human pathogens. It suppressed the growth of all human pathogens against which it was tested. The most promising results were observed against *Tricophyton longifusis*. The ethanol extract of green alga, *Padina tetrastromatica* showed complete inhibition of the human pathogen *Tricophyton longifusis*. The ethanol extract of *Melanothamnus somalensis* belongs to class Rhodophyceae promoted the growth of almost all human pathogens against which it was tested. It enhanced the growth of *Aspergillus niger* and *Tricophyton schoenleinii* most significantly. The chloroform extracts of some of the algae when screened for antifungal activity on human pathogens did not show as promising results as shown by their ethanol extracts. However, the water extract of brown alga, *Iyengaria stellata* was found to inhibit most of the tested fungi. Its antifungal activity was more prominent against *Tricophyton longifusis* and *Pseudallescheria boydii*. The water extract of green alga *Codium iyengarii* also showed antifungal activity which was most prominent against *Aspergillus flavus*. The fungicidal activity of *Iyengaria stellata* against *human pathogens* was observed from its water and ethanol extracts, the chloroform extract was not found very active against those organism. The same profile is observed from the extracts of *Codium iyengarii*. The ethanol extracts screened against various plant pathogens had shown fungicidal activity but in few cases growth promotion was also observed. The ethanol extract of *Padina tetrastromatica* showed most significant activity against plant pathogens which was highest against *Fusarium oxysporum var Lycopersici*. Its activity was also prominent against *Fusarium solani var. Lycopersici* and *Macrophomina phaseolina*. None of the chloroform extracts showed promising activity against plant pathogens, whereas the water extracts of the same algae showed better results. As mentioned above the ethanol extract of *Padina tetrastromatica* showed significant activity against plant pathogens, the water extract of the same alga also exhibited antifungal activity. The chloroform extract of *Melanothamnus somalensis* showed better antifungal activity against plant pathogens as compared to its ethanol and water extracts. It was highly active against *Fusarium solani var. Lycopersici*. The extracts of another red alga *Botryocladida leptopoda* repeated the same profile against plant pathogens. Though the ethanol extract of *Padina tetrastromatica* was most active against plant pathogens, its chloroform extract was not found to have such good antifungal activity. However, its water extract showed antifungal activity that was most significant against *Fusarium oxysporum var. Lycopersici*. Most of the ethanol extracts of the three classes of algae showed very significant antifungal activity against animal pathogens, a very low growth promoting activity was also observed in some of the cases. Most promising antifungal activity was observed from the ethanol extracts of *Codium iyengarii*. Its antifungal activity was most significant against animal pathogen *Microsporum canis* and *Tricophyton mentagrophytes*. The chloroform extracts of the three classes of algae did not give as good results but significant antifungal activity was observed from the water extracts of the same algae. The antifungal activity of the water extract of *Iyengaria stellata* was most promising in this regard. It showed significant activity against *Microsporum canis* and *Tricophyton simii*. An interesting observation is made regarding the ethanol, chloroform and water extracts of *Iyengaria stellata*, all of which were found active against animal pathogen *Microsporum canis*. From all the algae tested, the ethanol extract of *Codium iyengarii* showed very significant antifungal activity against all types of pathogens i.e. human, animal and plant.

Brine Shrimp Lethality Bioassay :

The brine shrimp lethality bioassay showed that most of the ethanol, chloroform and water extracts were active but the LD_{50} value of most of the extracts was higher than 1000 µg/ml. Among all the ethanol extracts tested, the extract of brown algae *Padina tetrastromatica* showed very significant results having very low LD_{50} values. Another brown alga *Jolyna leminaroides* was also found to have

significant activity. From all the ethanol extracts of the red algae, only the extract of *Sarconema filiforme* showed significant lethality of the brine shrimps. The ethanol extracts of green algae did not show significant results. The chloroform extracts of red and brown algae showed activity at higher doses. However the LD_{50} value of green alga *Codium iyengarii* was most significant. It was found to have LD_{50} value at very low concentration. The water extracts of the three classes of algae were active at higher doses. The LD_{50} values of all the samples were higher than 1000 mg/ml.

Lemna Bioassay :

The results of the *Lemna* bioassay shows that some of the ethanol extracts of the algae inhibit while others help to promote the growth of *Lemna aequinoctiatls welv*. The ethanol extracts showed both the inhibition and the proliferation of the fronds. The chloroform extracts of most of the algae showed significant frond inhibition however, an extract (*Melanothamnus somalensis*) was found to promote the growth of *Lemna aequinoctiatls welv*. The water extracts of different alga were found to promote the growth of the tested plant. The ethanol extract of red alga, *Hypnea musciformis* significantly inhibited the growth of *L. aequinoctiatls* in all the three concentrations. A very interesting result was observed from the ethanol extract of *Sargassum teneriumum* which gave 100 % inhibition of the fronds at highest concentration i.e 500 ppm. The growth of the fronds was promoted when ethanol extract of red alga *Melanothamnus somalensis* was used. The chloroform extract of most of the algae showed frond inhibition. Most active was the chloroform extract of green algae *Codium iyengarii*. This extract significantly inhibited the growth in all three concentrations. The chloroform extract of *Sargassum tenerimum* gave very significant inhibition at two higher concentrations but was found inactive at least concentration i.e 5 ppm. It showed 100 % inhibition at 500 ppm. Among all the chloroform extracts, the extract of green algae *Melanothamnus somalensis* promoted the growth of *Lemna aquinoctiatls* very significantly. Its water extract also showed the growth promotion. The water extract of red alga *Botryocladia leptopoda* also promoted the growth of the tested plant. From all the algae tested for the phytotoxic activity, the extracts (ethanol, chloroform and water) of brown alga *Iyengaria stellata* were found to inhibit the growth of the fronds while the extracts (ethanol, chloroform

Table-1 Antibacterial Activity

Ampicillin	**19**	**25.5**	**16.5**		**16**	**14**	**24**	**23**
Amoxicillin	**19**	**30**	**15**		**30**	**15.5**	**30**	**25**
Cefuroxime	**15.5**	**16.5**	**14.5**		**17**	**14.5**	**17**	-
Alga	**C.d**	**S.a**	**S.p**	**B.s.**	**K.p**	**P.a**	**S.t**	**S.b**
Iyengaria stellata	11.0	10.0	-	-	8.0	-	-	-
	(7.0)	(-)	(-)	(-)	(-)		(-)	
	[-]	[-]	[-]	[-]	[-]		[-]	
Sargassum binderi	12.5	-	-	-	8.5	9.0	-	-
Jolyna laminarioides	-	-	8.0	-	4.5	-	-	-
Padina tetrastromatica	11.0	9.0		8.0	8.5	-	-	10.0
	(-)	(-)	(-)	(-)	(-)		(-)	
Sargassum tenerimum	11.5	9.0	-	-	7.0	80.0	-	-
Hypnea musciformis	-	-	7.0	8.0	-	-	-	-
Botryocladia leptopoda	11.0	8.5	-	8.5	8.5	-	-	9.5
	(-)	(-)	(-)	(-)	(-)		(-)	
	[-]	[-]	[-]	[-]	[-]		[-]	
Laurencia pinnatifida	-	-	-	7.5	-	-	-	-
Sarconema filiforme	11.0	8.0	-	-	-	8.5	9.0	-
Melanothamnus somalensis	12.0	-	-	-	8.5	8.5	-	-
	(-)	(-)	(-)	(-)	(-)		(-)	
	[-]	[-]	[-]	[-]	[-]		[-]	
Caulerpa racemosa	11.0	9.0	-	7.0	8.0	8.5	-	9.0
Codium iyengerii	14.0	-	-	-	9.0	-	8.5	-
	(6.0)	(-)	(-)	(-)	(-)		(-)	
	[-]	[-]	[8.5]	[-]	[-]		[-]	
Codium flabellatum	-	-	6.5	-	-	-	-	-
Enteromorpha intestinalis	12.5	10.0	-	-	9.5	10.0	9.0	-

C.d = *Corynebacterium deptheriae*, S.a = *Staphylococcus aureus*, K.p = *Klebsiella pneumonia*,
B.s = *Bacillus , subtilis*, P.a =*Pseudomonas aeruginosa*, S.p = *Streptococcus pyogenes* ,S.t = *Salmonella typhi*,
S.b = *Shigella boydii*, values show zone of inhibition in mm ; Conc. of samples: 200µg/100µl DMSO;
Values in ( ) refer inhibition caused by chloroform extracts; Values in [ ] refer inhibition caused by water extracts.

and water) of the red alga *Melanothamnus somalensis* promoted the growth of *L. aequinoctiatls.*

Biological Screening

Antibacterial Bioassay:

The antibacterial bioassay was performed against both gram positive and gram negative bacteria using agar well diffusion technique (sea table-1). The pure bacterial cultures were inoculated in nutrient broth and incubated at 37 °C for 2-8 hr. till the turbidity developed. Turbidity of the nutrient broth in the test tubes was compared with McFarland turbidity standard. Test samples of concentration 200 µg/100 ml DMSO were added in their respective wells. The zones of inhibition were measured in mm and compared with the control i.e. DMSO [1]. The results of the bioassay are given in Table-1.

Antifungal Activity:

The antifungal bioassay was performed on human, animal and plant pathogens (sea table-2) using ethanol, water and chloroform extracts of the algae. The method used for the screening was agar tube dilution bioassay. DMSO and reference antifungal drugs miconazole and ketokenazole served as negative and positive control, respectively. The test tubes were incubated at 27-29°C for 7-10 days. Growth in the medium containing the extract was determined by measuring linear growth (mm) and growth inhibition was calculated with reference to negative control [2,3]. In this bioassay. The results of the bioassay are given in Table -2.

Brine Shrimp Bioassay:

The brine shrimp bioassay was performed using the larvae (naupli) of *Artemia salina.* The approach in this assay was that toxicology is simply pharmacology at a higher dose, thus a simple bioassay might lead to a new pharmacologically active agent. The stock solution was prepared by taking 20 mg of crude extract in 2 ml of methanol. Transfer 500 µl, 50 µl and 5 µl of the stock solution to the vials corresponding to make 1000, 100 and 10 µg/ml, respectively. Five replicates of each dose level were used. Copper sulphate was used as control. The solvent was allowed to evaporate from the vials overnight. Placed 10 larvae (nauplii) of *Artemia salina* in each vial. These larvae were 2 days old. The volume was raised to 5 ml with syringe, by adding sea water. It was incubate at 27 °C for 24 hrs. under illumination. After 24 hrs the number of survivors were recounted and recorded. The data was analyzed with Finney program to determine LD_{50} values and 95 % confidence intervals [4,5]. The results of this bioassay are given in Table-3.

Lemna Bioassay :

The Lemna bioassay was aimed used to study the phytotoxic activity of the different extracts i.e.,

Table-2 Antifungal Activity

Alga	A.f	E.f	T.s	T.l	C.a	A.n	F.s	M.p	F.o	R.s	M.c	T.s	T.m
Iyengaria steyllata			31.37	66.15	5.00	25.45	33.82	-17.28	24.10		56.92		
			(0)	[83.07]	(0)	(9.09)			(5.50)	(8.59)	(43.10)		
Sargassum binderi	37.50			9.23	15.00	-5.45	11.76	7.40	1.78		55.38	50.98	
Jolyna laminerioides	3.61		-3.84	-70.00		3.40	-78.50	22.41	25.92				
Padina tetrastromatica	0			100	15.00	34.54	48.52	32.09	85.71		13.84	-1.96	
			(7.69)										
			[69.20]		(2.80)	(0)			(1.10)		(32.09)	(7.14)	
Sargassum tenerimum	-25.00			6.00	2.00	49.09	41.17	32.09	33.03		29.23	45.09	
Hypnea musciformis	25.01			70.76	6.00	41.81	48.52	13.58	8.92		27.69	23.52	
Botryocladia leptopoda		-15	(1.53)	38.46	4.00	30.90	0.00	-12.34	24.10		46.15	25.49	
			[46.66]										
	[33.33]			(0)	(0)	(5.45)			(22.22)	(8.69)	(22.20)	(2.85)	
Laurencia pinnatifida	12.50			15.38	4.00	43.63	7.69	65.43	1.78		66.5	70.53	
Sarconema filiforme	37.50		11.76	83.07	30.00	43.63	11.76	-17.28			60.00		
Melanothamnus	[-1.25]	-2.5	-	-10.00	14.77	-142.85	1.72	14.81			31.25	41.44	
somalensis			101.92	[39.21]	(0)	(3.63)				(6.65)	(0)	(8.5)	
			(0)						(4.40)				
Caulerpa racemosa	27.04		57.33		15.68	6.86	-1.12	51.57			-9.75		75.82
Codium iyengari	9.90		76.00		9.80	59.90	39.32	46.31	18.51		89.02		83.87
	[62.50]		(0)		(0)	(0)			(69.00)				
										(3.00)		(4.28)	
Codium flabellatum			50.00		0	38.40	50.00	36.30	38.40		33.30	53.80	
Enteromorpha intestinalis	32.50			7.69	2.00	67.27		-12.34		-15.38	58.46	45.09	

A.f = *Aspergillus flavus,* E.f = *Epidermophyton floccosum,* T.s = *Trichophyton schoenleinii,* T.l = *Trichophyton longifusis,* C.a = *Candida albicans,* A.n = *Aspergillus niger,* F.s = *Fusarium solani var. lycopersici,* M.p = *Macrophomina phaseolina,* F.o = *Fusarium oxysporum var. lycopersici,* R.s = *Rhizoctonia solani,* M.c = *Microsporum canis.,* T.s = *Tricophyton Simii.* T.m = *Tricophyton mentagrophytes;* Std.drugs: Miconazole, Ketokenazole; Values refer inhibition in % ; Inhibition by std.drugs:100% ; Incubation temp. :27-29 °C; Incubation time:10 days.

Table-3: Brine Shrimp Lethality Bioassay

Alga	LD_{50} mg/ml
Iyengaria stellata	>1000
	(>1000)
	[>1000]
Sargassum binderi	>1000
Jolyna laminarioides	235.466
Padina tetrastromatica	206.161
	[>1000]
Sargassum tenerimum	>1000
Hypnea musciformis	>1000
Botryocladia leptopoda	>1000
	(>1000)
	[>1000]
Laurencia pinnatifida	>1000
Sarconema filiforme	727.7016
Melanothamnus somalensis	>1000
	[>1000]
Caulerpa racemosa	>1000
Codium iyengarii	>1000
	(50.8973)
	[>1000]
Codium flabellatum	>1000
Enteromorpha intestinalis	>1000

Table-4 *Lemna* Bioassay

Name of alga	% Inhibition		
	500 ppm	50 ppm	5 ppm
Iyengaria stellata	47.4	20.0	10.0
	(82.2)	(64.4)	(-)
	[15.8]	[2.0]	[2.0]
Sargassum binderi	28.0	10.0	2.0
Jolyna lamenarioides	15.8	12.2	0
Padina tetrastromatica	60.9	0	0
	(15.5)	(15.0)	
Sargassum tenerimum	100.0	0	0
	(100)	(60.0)	(-)
Hypnea musciformis	68.5	55.2	40.3
Botryocladia leptopoda	-14.0	0	0
	(20.0)	(40.0)	(21.4)
	[-78.0]	[-20.0]	[-0.8]
Laurencia pinnatifida	7.1	0	0
Sarconema filiforme	-21.0	0.2	0
Melanothamnus somalensis	-28.0	-2.0	0
	(-73.0)	(-15.5)	
	[-50.0]	[-15.0]	[-0.001]
Caulerpa racemosa	31.5	25.2	15
Codium iyengarii	21.1	15.2	0
	(86.6)	(40.0)	(7.1)
	[5.3]	[0]	[0]
Codium flabellatum	-5.2	0	0
Enteromorpha intestinalis	5.3	0	0

Standard drug:Paraquat; Growth period: 7 days

ethanol, chloroform and water, of the algae. The plant *Lemna aequinoctiatls welv* was used to study this bioactivity. The stock solutions of the crude extracts were prepared by dissolving 15 mg of the crude extract in 1.5 ml of ethanol. Nine flasks, three for each concentration were inoculated with 1000 µl, 100 µl and 10 µl of the stock solution for 500, 50 and 05 ppm. The solvent was evaporated overnight in sterilized condition. To each flask, 20 ml of E-medium at pH of 5.5-6.0 was added. Then 10 plants of *Lemna aequinoctiatls welv* having a rosette of three fronds were added to each flask. Two other flasks were supplemented with solvent and reference plant growth inhibitor and promoter, serving as negative and positive control, respectively. For positive control, paraquat was used. The flasks were plugged with cotton and placed in growth cabinet for 7 days. On the 7^{th} days the number of fronds per flask were counted [6,7]. Interpretation of results was made by analyzing growth regulation in percentage, calculated with reference to the negative control. The results of this bioassay are shown in Table-4.

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References

1. R. Carran, A. Maran, J.M. Montero, L. Fernandozlago, A. Derminguez, Plantes Medicinal et *Phytotherapie*, **21**, 195 (1987).
2. H. Blank, G. Rewbell, *Arch. Derm.*, **92**, 322 (1965).
3. F.S. Davis, A. Villar, Real, J.R. Baur, I.S. Gold, Stein, (1972). Modified by S.S. Shaukat, N.A. Khan, F. Ahmed, *Pak. J. Bot.*, **12**, 97(1980).
4. N.B. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, P.E. Nicholas, J.L. McLaughlin, *Planta Medica*, **45**, 31(1982).
5. P.M. Dey, J.B. Harborne, *Meth. Plant. Biochem.*, **6**, 8(1991).
6. Atta-ur-Rahman, Studies in Natural Product Chemistry, p. 383, 9 (Part B), Elsevier Science Publishers, B.V., Netherlands (1991).
7. D.J. Finney, Probit Analysis, p. 333, 3rd ed., Cambridge University Press, Cambridge (1971).