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Antibacterial and extracellular enzyme activities of gut actinobacteria isolated from *Gallus gallus domesticus* and *Capra hircus*

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ABSTRACT

The aim of the present study was to isolate the actinobacteria from the faeces of domestic animals and to explore their biotechnological properties. A total of 87 actinobacterial cultures were isolated from different faeces of goat and chicken collected from various locations in Pudukkottai and Tiruchirappalli Districts, Tamil Nadu. Among the 87 actinobacterial isolates, 45 isolates were selected for the screening of antibacterial activity and extracellular digestive enzyme production based on distinct morphological features and growth rate. The actinobacterial isolates were varied one among them in their antibacterial activity and digestive enzyme producing abilities. Out of 45 isolates, 7 (15.6%) isolates showed multiple inhibitory activities against all the tested bacterial pathogens and 26 (57.8%) displayed excellent production of industrially important extracellular digestive enzymes.

Keywords*:* Animal faeces, actinobacteria, antibacterial activity, extracellular digestive enzymes.

INTRODUCTION

Actinobacteria are a significant and interesting group of gram positive bacteria belong to the actinomycetales subdivision of the prokaryote. They hold a prominent position as targets in screening programs due to their diversity and their proven ability to produce novel metabolites and other molecules of pharmaceutical importance [1]. Secondary metabolites produced by the actinobacteria have a major biotechnological contribution from antibiotics to enzymes and anticancer agents to various alkaloids. They are extensively studied in soils, where they play an important ecological role in soil nutrient turnover. They also inhabit all types of substrates in the most diverse ecosystems ranging from decomposing organic materials to living plants, fresh water, marine sediments and organisms among others [2, 3]. Though high number of bioactive compounds discovered from the existing actinobacteria, more than 50% of that are produced by the members of the genus *Streptomyces* and *Micromonospora.* At present, the isolation rate of novel actinobacteria is decreasing due to the searching of routine ecosystems. This problem can be overcome by exploitation of less explored ecosystems which can pave the way for discovery of novel actinobacteria and bioactive metabolites.

The gastrointestinal tract of the vertebrates and invertebrates are one of the less explored natural ecosystems in which some actinobacteria forms intimate associations as a gut microflora. They can be found as commensals and symbionts which colonize the gastrointestinal tract and actively influence the digestive system of animals including ruminants. Symbiotic interactions are essential mainly for the survival and reproduction because they play a crucial role in nutrition, detoxification of certain compounds, growth performance and protection against pathogenic bacteria. Further earlier studies have shown that some symbiotic actinobacterial species, i.e. probiotics, control bacterial diseases in livestock, poultry [4] and aquaculture [5, 6]. They also take part in host health by converting the feedstuffs into microbial biomass and fermentation end products that can be utilized by the animal host. In the absence of this microbial fermentation, calories present in a diverse array of complex dietary glycans would be unavailable to the host [7]. Thus, the knowledge of gastrointestinal tract actinobacterial ecology is essential for the

isolation of novel bioactive actinobacteria and to ascertain their role in animal health performance. In this view, the present study was carried out in order to isolate the gut actinobacteria from the domestic animals chicken, goat and to study their biotechnological properties.

EXPERIMENTAL SECTION

2.1. Sample collection

The faecal samples of *Capra hircus* (goat) and *Gallus gallus domesticus* (chicken) were collected from different locations in Pudukkottai and Tiruchirappalli Districts, Tamil Nadu, India. The desirable quantity of the faecal samples was collected in a sterile pre-labelled polythene bags. At the time of faeces collection the sterilized polythene bags were fitted to the animals for a few minutes to ensure the collection of a sufficient amount of faeces. The faecal samples collected without any soil contamination were brought to the laboratory immediately for further processing.

2.2. Isolation of actinobacteria

The actinobacteria were isolated from the faecal samples by following the method of Tan *et al.,* [3] with slight modifications. Totally 24 different faecal samples (Indigenous chicken-6, Broiler-6 and Indigenous goat-6, Farm goat-6) were collected for actinobacteria isolation. Each faecal sample (0.5 g) was homogenized in 4.5 ml of sterilized water and stirred for 5 min. An aliquot of 0.1 ml suitably diluted samples were spread over the surface of starch casein agar (SCA) medium [g/L: 10-soluble starch, 2-KNO_3, 2-NaCl, 0.3-casein, 2-K_2HPO_4, 0.05-$MgSO_4.7H_2O$, 0.02-$CaCO_3$, 0.01- $FeSO_4.7H_2O$, 15-agar, pH-7.2) supplemented with streptomycin and cycloheximide (50µg/ml). The inoculated plates were incubated at 41±2°C and monitored periodically over 7-10 days for actinobacterial growth. After incubation the colonies showing actinobacterial morphology were enumerated and purified using streak plate technique. Growth characteristics of the selected actinobacterial cultures such as colony size, consistency, pigmentation, colour of aerial and substrate mycelium were also recorded.

2.3. Determination of extracellular enzyme activities

2.3.1. Screening for amylase by starch iodine plate assay

The screening of actinobacterial isolates for amylase production was studied by following the method of Selvam *et al.,* [8] with slight modification. The starch agar plates were prepared [g/L: 5-peptone, 3-beef extract, 5-NaCl, 4-starch, 15-agar] and sterilized in autoclave at 121°C, 15psi pressure for 15 mins. The isolates were inoculated on to the media and incubated at 41±2°C for 4-7days. After that gram's iodine stain (0.25g iodine crystals, 2.5g potassium iodide, 125ml of water) was spread over the plates and left for 5 min. Then the plates were observed for the zone of clearance or decolorization against the blue colour back ground.

2.3.2. Screening for lipase by tributyrin agar plate assay

The actinobacterial isolates were screened for lipase enzyme production by inoculating them on tributyrin agar according to the method of Priya *et al.,* [9] with slight modification. Initially, the tributyrin agar plates were prepared [g/L: 5-peptone, 3-beef extract, 5-NaCl, 10ml-tributryin, 15-agar] and sterilized in autoclave at 121°C, 15psi for 15 mins. The plates were then inoculated with the actinobacterial isolates and incubated at 41±2°C for 4-7days. After incubation, the plates were observed for the zone of clearance around the actinobacterial growth.

2.3.3. Screening for protease by casein agar plate assay

The proteolytic activity of the actinobacterial isolates were screened as per the protocol of Gopalakrishnan *et al.,* [10] with slight modification. Casein agar plates were prepared [g/L: 10-casein, 1-K_2HPO_4, 0.5-$CaCl_2$, 5-NaCl, 15-agar] and sterilized in autoclave at 121°C, 15psi for 15 mins. The actinobacterial cultures were streaked on casein agar and incubated at 41±2°C for 4-7days. At the end of the incubation, the plates were observed for halo zone around the actinobacterial growth which indicates the production of protease.

2.3.4. Screening for cellulase by cellulose agar plate assay

The cellulolytic activity of the actinobacterial isolates were screened by using modified cellulose agar medium of Hans [11]. The cellulose agar plates were prepared [g/L: 0.5-K_2HPO_4, 0.5-KH_2PO_4, 1.0-$(NH_4)_2SO_4$, 0.1-$MgSO_4.7H_2O$, 0.1-$CaCl_2$, 6.0-NaCl, 0.1-yeast extract, 10-carboxy methyl cellulose, 15-agar] and sterilized in autoclave at 121°C, 15psi for 15 mins. Each actinobacterial isolate was inoculated onto the cellulose agar medium and incubated at 41±2°C for 4-7 days. Then the agar plates were flooded with 1.0% congo red and allowed to stand for 30 minutes followed by counter staining with 1M NaCl solution for 10-15 minutes. The plates were then examined for zone of clearance around the actinobacterial growth [12].

2.4. Antibacterial activity

Antibacterial activity of the actinobacterial isolates against clinical pathogens was determined by adopting the method of Valli *et al.,* [13] with slight modification. The pathogenic bacteria used were *Escherichia coli, Salmonella typhi, Enterobacter aerogenes, Staphylococcus aureus, Klebsiella* sp. and *Pseudomonas* sp. Initially a single streak of actinobacterial isolates were made on nutrient agar medium [g/L: 5-peptone, 3-yeast extract, 5-NaCl, 15-agar] and incubated at 41±2ºC for 5 days. After observing a good ribbon like growth of the actinobacteria, the plates were inoculated with 18 hours old test bacterial pathogens in perpendicular manner. The inhibition of bacterial pathogens (mm) were measured and recorded after 24 hours of incubation at 37ºC. A control plate was also maintained without inoculating the actinobacteria to assess their normal growth.

RESULTS AND DISCUSSION

3.1. Isolation of actinobacteria

The gastrointestinal tract is the major digestive and absorbing organ and it plays a crucial role in health and well-being of the animals. This complex ecosystem often harbours a diverse bacterial community contributes to nutrient digestion and forms a layer on mucosal surfaces that protects them from the overgrowth of pathogens. Moreover, it is well known that faecal samples are a good reflection of changes in the gut microflora [14]. Thus, the faecal samples of chicken and goat were selected for the present study to investigate their gut actinobacterial population.

Table 1. Isolation of actinobacteria from different chicken faeces

S. No.	Sampling location	Type of sample	Total actinobacterial population (CFU gfw^{-1})	Number of actinobacterial isolates
1.	Kulathur, Pudukkottai District	Indigenous	$4.6x10^5$	6 (15.38%)
2.	Mandaiyur, Pudukkottai District	Indigenous	$3.6x10^4$	3 (7.69%)
3.	Nallur, Pudukkottai District	Indigenous	$1.2x10^4$	4 (10.26%)
4.	Suriyur, Tiruchirappalli District	Indigenous	$1.3x10^6$	9 (23.07%)
5.	Puthu Theru, Tiruchirappalli District	Indigenous	$2.4x10^4$	2 (5.13%)
6.	Gundur, Tiruchirappalli District	Indigenous	$6x10^4$	7 (17.95%)
7.	Kulathur, Pudukkottai District	Broiler	$1.4x10^4$	4 (10.26%)
8.	Mandaiyur, Pudukkottai District	Broiler	-	-
9.	Nallur, Pudukkottai District	Broiler	$1.2x10^4$	3 (7.69%)
10.	Suriyur, Tiruchirappalli District	Broiler	-	-
11.	Puthu Theru, Tiruchirappalli District	Broiler	-	-
12.	Gundur, Tiruchirappalli District	Broiler	$2x10^4$	1 (2.56%)

Table 2. Isolation of actinobacteria from different goat faeces

S. No.	Sampling location	Type of sample	Total actinobacterial population (CFU gfw^{-1})	Number of actinobacterial isolates
1.	Kulathur, Pudukkottai District	Indigenous	$6x10^2$	11 (22.92%)
2.	Mandaiyur, Pudukkottai District	Indigenous	$4x10^3$	9 (18.75%)
3.	Nallur, Pudukkottai District	Indigenous	$8.8x10^4$	10 (20.83%)
4.	Suriyur, Tiruchirappalli District	Indigenous	$8x10^3$	4 (8.33%)
5.	Puthu Theru, Tiruchirappalli District	Indigenous	$10x10^2$	5 (10.42%)
6.	Gundur, Tiruchirappalli District	Indigenous	$4x10^2$	2 (4.17%)
7.	Nitherson goat farm, Tiruchirappalli District	Farm goat	$3x10^2$	4 (8.33%)
8.	Nitherson goat farm, Tiruchirappalli District	Farm goat	-	-
9.	Nitherson goat farm, Tiruchirappalli District	Farm goat	-	-
10.	S.M.S goat farm, Tiruchirappalli District	Farm goat	$4x10^2$	3 (6.25%)
11.	S.M.S goat farm, Tiruchirappalli District	Farm goat	-	-
12.	S.M.S goat farm, Tiruchirappalli District	Farm goat	-	-

Totally twenty four fresh faecal samples (six samples of each from indigenous chicken, goat, broiler and farm goat) were collected from different locations in Pudukkottai and Tiruchirappalli Districts, Tamil Nadu, India and processed in starch casein agar medium for the isolation of actinobacteria. The total actinobacterial population found was varied from $3x10^2$ to $1.3x10^6$ CFU gfw^{-1} (gram fresh weight). Moreover, a total of 87 actinobacterial isolates (48 from goat faeces and 39 from chicken faeces) were recorded from 24 different faecal samples (Table 1, 2). Out of 48 goat actinobacterial isolates 27 isolates were selected based on distinct morphological features and growth rate and marked as LD1-LD27. Similarly, 18 isolates were selected from 39 chicken actinobacterial isolates and named as JD1-JD18 (Plate 1).

LD1 LD8 JD5

Plate 1. Pure cultures of actinobacterial isolates

The total count of actinobacterial population showed that they were significantly high in the faeces of indigenous chicken and goat when compared to the broiler and farm goats. In the cases of broiler and farm goats, less or no actinobacterial colonies were observed which may be due to the difference in the nutritional intake/food habits, birthing process, excessive hygienic condition, usage of disinfectants and administration of antibiotics in the farm animals. Growth characteristics of the actinobacterial isolates in starch casein agar medium revealed that they were varied in the formation of aerial and substrate mycelium, colony size and consistency. The aerial and substrate mycelium of the actinobacterial isolates ranges from white, grey, yellow, brown, green etc. The size of the colonies were varied from 2 – 8 mm and the consistency of the isolates were almost same i.e. powdery in nature, however some were produced leathery, dry and mucoid colonies (Table 3). Starch casein agar was reported as a suitable medium for the isolation of actinobacteria from soil, water and less explored ecosystems such as mountain, forest, desert ecosystems [15], thus starch casein agar medium was chosen for the isolation of actinobacteria from the faecal samples. Similar kind of actinobacterial isolation from goat faeces was carried out by Tan *et al.,* [3], but they used CM agar medium supplemented with 25 mg L^{-1} potassium dichromate inhibitor. They have found the actinobacterial population of $1.4x10^7$ CFU gfw^{-1} and streptomycete like strains of $3.3x10^3$ CFU gfw^{-1} which were high in number compared with our results. The variation in actinobacterial population may be due to the difference in the variety of goat, habitat, isolation medium and incubation temperature.

Table 3. Growth characteristics of goat and chicken actinobacterial isolates on starch casein agar medium

S. No.	Characteristics	Growth pattern	Number of isolates
1.	**Aerial mycelium**	Grey	20
		White	17
		Green	4
		Yellow	2
		Pink	2
2.	**Substrate mycelium**	White	21
		Grey	11
		Yellow	8
		Pink	2
		Brown	2
		Green	1
3.	**Consistency**	Powdery	42
		Mucoid	1
		Leathery	1
		Dry	1
4.	**Pigment production**	Brown	1
		Green	1
		Yellow	1
		Pink	1

3.2. Determination of extracellular enzyme activities

The selected actinobacterial isolates were screened qualitatively for the production of different extracellular enzymes namely cellulase, amylase, lipase and protease using agar plate assays. Among the 45 actinobacterial isolates, 26 (57.8%) isolates showed the production of 4 industrially important extracellular digestive enzymes. Out of 45 actinobacterial cultures, 41 (91.1%) isolates exhibited amylolytic and proteolytic activity, 38 (84.4%) isolates displayed lipolytic activity and 37 (82.2%) isolates demonstrated cellulolytic activity (Plate 2).

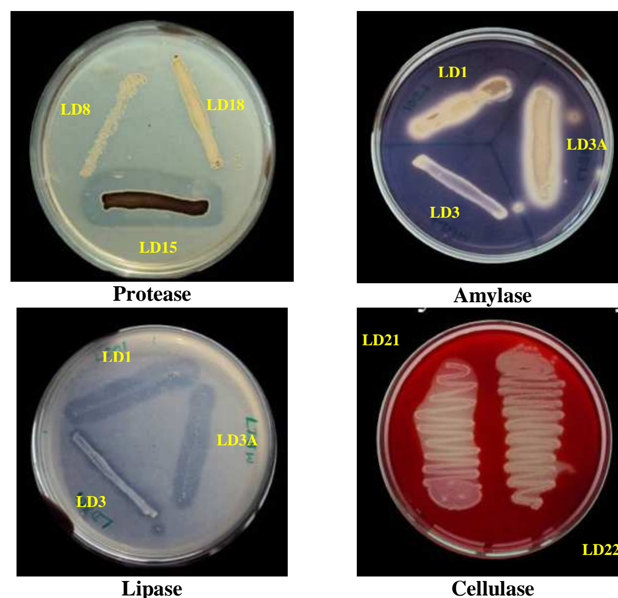

Plate 2. Extracellular enzyme activity of actinobacterial isolates

The results revealed that the animal faeces were potential source for various digestive enzymes producing actinobacteria. Moreover, it can be an imperative resource for bio prospecting novel actinobacterial species which could yield valuable bioactive molecules. Taking into account of the fact that domestic animals consumes a diet high in fibre, sugars, starch and protein, a variety of cellulolytic, amylolytic, proteolytic actinobacteria should be found in the faeces of goat and chicken. The results of the present study also confirmed the presence of multiple extracellular enzyme producing actinobacteria in the faeces of domestic animals. Besides, they clearly demonstrated that high number of animal gut actinobacteria was biochemically active and they can play a significant role in digestion of nutrients. Similar study conducted by Tan *et al.,* [3] revealed that the actinobacterial isolates of goat have the ability to produce enzymes which can degrade common components of the goat diet.

3.3. Antibacterial activity of actinobacterial isolates

The actinobacterial isolates were also screened for antibacterial activity by cross streak method. Out of 45 actinobacterial isolates, 28 isolates (62.2%) have antibacterial activity and 17 isolates (37.8%) have no activity against the tested bacterial pathogens. Among the 28 positive isolates, 16 isolates (57.1%) showed activity against more than two bacterial pathogens (Table 4) and remaining 12 isolates (42.9%) showed activity against one or two bacterial pathogens. The isolates namely, LD4, LD5A, LD10, JD2, JD6, JD10 and JD16 showed multiple inhibitory activities against all the tested bacterial pathogens with the inhibition range of 3-33 mm. The pathogen *S. aureus* was highly inhibited by 21 actinobacterial cultures and maximum inhibition of 33 mm was observed for the isolates LD2, JD16 against *Pseudomonas* sp. Comparatively, the goat actinobacterial isolates showed more antibacterial activity than the chicken actinobacterial isolates.

The results were in agreement with the report of Tan *et al.,* [3]. They have screened 126 streptomycete like strains and other actinobacterial cultures isolated from the faecal samples of goat for antibacterial and antifungal activity. They found that above 50% of streptomycete like strains have antibacterial activities and other actinobacteria had no activity against *Pseudomonas aeruginosa* and *Escherichia coli*.

Similar type of antibacterial activity studies were carried out by Dhanasekaran *et al.,* [16], for the actinobacterial cultures isolated from the Vellar Estuary soil. In that, the isolate *Streptomyces* sp. DPTD-5 showed a broad-spectrum of antimicrobial activity against yeast, gram positive and gram negative bacteria with the maximum inhibition zone of 30 mm. Krishna *et al.,* [17] also studied the antibacterial activity of the actinobacteria isolated from the molluscans samples, who reported that the tested ten actinobacterial isolates were active against gram positive and gram negative bacteria. Among them, 80% of strains were active against *S. aureus*, 70% were active against *K. pneumoniae*, *E. coli*, *S. pyogenes* and 60% were active against *B. cereus*, *P. mirabilis*. These reports revealed that the actinobacterial cultures isolated from the different sources having antagonistic activities against gram positive and gram negative bacterial pathogens. However, the variation in the production of antibacterial compound might be due to the differences in the substrates, habitats, genera and species.

Table 4. Antibacterial activity of the actinobacterial isolates

S. No.	Isolate identity	Activity against the bacterial pathogens (inhibition zone in mm)					
		E. coli	*Klebsiella* sp.	*Pseudomonas* sp.	*S. typhi*	*E. aerogenes*	*S. aureus*
1.	LD1	4	12	-	9	7	26
2.	LD2	-	-	33	-	-	-
3.	LD2A	-	-	28	-	10	-
4.	LD3	-	-	-	-	-	-
5.	LD3A	-	-	-	-	-	10
6.	LD4	27	22	7	26	30	29
7.	LD5	11	15	-	14	19	12
8.	LD5A	11	14	12	14	18	16
9.	LD6	19	18	-	19	27	24
10.	LD8	-	-	-	-	-	-
11.	LD10	18	16	3	21	25	22
12.	LD11	-	-	-	-	6	-
13.	LD13	-	-	-	-	-	-
14.	LD14	-	-	-	32	10	-
15.	LD15	12	12	-	15	-	17
16.	LD16	-	-	-	-	16	3
17.	LD17	-	-	-	-	13	14
18.	LD18	4	6	-	7	6	5
19.	LD19	-	-	-	-	-	-
20.	LD20	1	-	-	-	-	-
21.	LD21	-	-	-	15	-	-
22.	LD22	-	-	-	-	-	-
23.	LD23	-	-	-	-	-	-
24.	LD24	17	12	-	16	22	20
25.	LD25	-	-	-	-	-	-
26.	LD26	-	-	-	-	-	-
27.	LD27	-	-	31	-	9	8
28.	JD1	-	-	32	-	-	8
29.	JD2	17	20	15	17	30	20
30.	JD3	-	-	29	-	-	-
31.	JD4	-	-	-	-	-	-
32.	JD5	-	-	-	-	-	-
33.	JD6	25	22	31	29	25	25
34.	JD7	-	-	-	-	-	-
35.	JD8	-	-	30	-	15	15
36.	JD9	-	-	-	-	-	-
37.	JD10	21	25	25	27	31	30
38.	JD11	5	5	4	2	-	5
39.	JD12	-	-	-	-	-	-
40.	JD13	-	-	-	-	-	-
41.	JD14	-	-	15	-	-	3
42.	JD15	-	-	-	-	-	-
43.	JD16	20	20	33	20	31	22
44.	JD17	-	-	-	-	-	-
45.	JD18	-	-	-	-	-	-

- : No activity

CONCLUSION

Actinobacteria are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolites, notably antibiotics, enzymes and antitumor agents etc. Since, actinobacteria act as a good source for the novel bioactive secondary metabolites, a significant amount of effort has been focused on the successful isolation of novel actinobacteria from terrestrial sources for drug screening programs in the past fifty years. But the demand of chemically diverse compounds leads to the isolation of novel actinobacteria from unexplored or underexploited habitats as sources of novel bioactive secondary metabolites. The present study was also aimed with the same objective. The findings of the present study revealed that the faeces of goat and chicken acts as a rich source of cultivable actinobacteria with important biotechnological properties. Furthermore, the broad spectrum antibacterial activity and digestive enzyme producing ability of the actinobacterial isolates proved that which could be used in pharmaceutical industries and to develop novel feed enzyme additives for animal production.

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REFERENCES

[1] P Ellaiah; T Ramana; KVVSN Bapi Raju; P Sujatha; A Uma Sankar. *Asian J Microbiol Biotech Env Sci.,* **2004**, 6, 53–56.
[2] I Gonzalez; A Ayuso-Sacido; A Anderson; O Genilloud. *FEMS Microbiol Ecol.,* **2005**, 54, 01-415.
[3] H Tan; Z Deng; L Cao. *Lett Appl Microbiol.,* **2009**, 49, 248-253.
[4] KG Maciorowski; P Herrera; FT Jones; SD Pillai; SC Ricke. *Anim. Feed Sci. Technol.,* **2007**, 133, 109-136.
[5] S Das; PS Lyla; S Ajmal-Khan. *Israeli J. Aquacult.-Bamidgeh.,* **2006**, 58, 198-204.
[6] T Defoirdt; P Sorgeloos; P Bossier. *Curr. Opin. Microbiol.,* **2011**, 14, 251-258.
[7] HJ Flint; EA Bayer; MT Rincon; R Lamed; BA White. *Nat Rev Microbiol.,* **2008**, 6, 121–131.
[8] K Selvam; B Vishnupriya; VSC Bose. *Int J Pharm Biol Arch.,* **2011**, 2(5), 1481-1487.
[9] BS Priya; T Stalin; K Selvam. *Afr J Biotechnol.,* **2012**, 11(78), 14320-14325.
[10] S Gopalakrishnan; S Pande; M Sharma; P Humayun; BK Kiran; D Sandeep; MS Vidya; K Deepthi; O Rupela. *Crop Prot.,* **2011**, 30 (8), 1070-1078.
[11] RC Dubey; DK Maheshwari. Practical Microbiology. Chand and Company Ltd., Ram Nagar, New Delhi, **2008**, 385.
[12] CB Ogbonna; DP Berebon. *J Envi Sci, Toxicol and Food Technol.,* **2013**, 5 (4), 17-22.
[13] S Valli; SS Suvathi; OS Aysha; P Nirmala; P Vinoth Kumar; A Reena. *Asian Pac J Trop Biomed.,* **2012**, 2(6), 469-473.
[14] JM Campbell; GC Fahey; Wolf BW. *J Nutr.,* **1997**, 127, 130–136.
[15] S Radhika; S Bharathi; M Radhakrishnan; R Balagurunathan. *J Pharma Res.,* **2011**, 4(8), 2584-2586.
[16] D Dhanasekaran; S Selvamani; A Panneerselvam; N Thajuddin. *Afr J Biotechnol.*, **2009**, 8 (17), 4159-4162.
[17] SR Krishna; SR Sathish Kumar; L Meenambekha; M Madhusudhan. *Adv. Appl. Sci. Res.,* **2011**, 2 (4), 431-439.