International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 2336-2340

Received: 22-01-2018
Accepted: 24-02-2018

D Jain
Assistant professor, Department of Animal Nutrition, CVAS, Bikaner, Rajasthan, India

RK Dhuria
Professor, Department of Animal Nutrition, CVAS, Bikaner, Rajasthan, India

T Sharma
Professor, Department of Animal Nutrition, CVAS, Bikaner, Rajasthan, India

Tara bothra
Assistant professor, Livestock Production Management, CVAS, Bikaner, Rajasthan, India

Correspondence
D Jain
Assistant professor, Department of Animal Nutrition, CVAS, Bikaner, Rajasthan, India

Phytochemical analysis of ethyl acetate extract of *Tinospora cordifolia* by mass spectrometry

D Jain, RK Dhuria, T Sharma and Tara bothra

Abstract
The present study was carried out to investigate the presence of phytochemical constituents in ethyl acetate extract of *Tinospora cordifolia* stem using GC-MS**.** In the GC-MS analysis, 45 numbers of phytochemicals were identified. Out of the 45 compounds obtained, 09 compounds had the higher matching probability above 40% with the standard compounds of the NIST Library database namely (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol (47.67%); l-(+)-Ascorbic acid 2,6-dihexadecanoate (59.49%); Octadecanoic acid (65.96 %); Columbin (1,4-Etheno-3H,7H-benzo[1,2-c:3,4-c']dipyran-3,7-dione, 9-(3-furanyl) decahydro-4-hydroxy-4a,10a-dimethyl-, [1R-(1à,4á,4aà,6aá,9á,10aá,10bà)]-) (83.88 %); Campesterol (Ergost-5-en-3-ol, (3á,24R)-) (65.36 %); Stigmasterol (Stigmasta-5,22-dien-3-ol, (3á,22E)-) (62.76 %); ç-Sitosterol (Stigmast-5-en-3-ol, (3á,24S)-) (85.21 %); 9-Ethyl-3-hydroxy-10,11b-dimethyl-1,2,3,4,6,6a,6b,7,8,9,11a,11b-dodecahydrobenzo[a]fluoren-11-one (53.57 %) and á-Amyrin (Olean-12-en-3-ol, (3á)-) (49.54%). Presence of various phytochemicals indicates the antioxidant, antimicrobial, antiviral, antinflammatory, antidiabitic, analgesic, hepatoprotective and flavouring agent property of *Tinospora cordifolia*. Hence, the presence of phytochemicals reported in the present study indicates that the *Tinospora cordifolia* could serve as "herbal feed additive" in poultry industry in the coming years.

Keywords: ethyl acetate, gc-ms analysis, phytochemicals, *Tinospora cordifolia*.

1. Introduction
Antibiotic growth promoter has been widely and successfully used to promote the growth and protect health of the poultry birds. With increasing public issues about bacterial resistance to antibiotics, the use of antibiotics in therapeutic or sub-therapeutic doses in poultry feed has been banned in many countries, so herbal growth promoters could be important component of the health care system at global level. Plants and plant derived products being natural, nontoxic or residue free have multiple effects on animal including appetite stimulator, enhanced secretary activity of GIT, immuno-stimulant, antiviral, antioxidants, bactericidal and are termed as phytogenics (Hashemi and Davoodi 2011) [1]. *Tinospora cordifolia* is one of the most extensively used herbs from the ancient medicinal history of Ayurveda as a medicine. *Tinospora cordifolia* is also called as amrita or nectar of life. It belongs to family Menispermaceae. It is extremely useful in strengthening the immune system of the body and keeping the functions of its various organs in harmony (Desai *et al.,* 2002) [2]. It is large deciduous climbing shrub distributed throughout India, Sri Lanka, Bangladesh and China (Raghu *et al.,* 2006) [3]. Therefore, the present study of GCMS analysis of *Tinospora cordifolia* stem was planned to investigate the presence of phytoconstituents in ethyl acetate extract of *Tinospora cordifolia* stem which may further support the utilization of geloi herb *(Tinospora cordifolia)* as feed additive.

2. Material and Methods
2.1 Collection of plant materials
The dried stem was collected from reputed herbal shop from Bikaner. It was identified and authenticated by the Department of Botany, Govt. Dungar College, Bikaner (Rajasthan).

2.2 Preparation of plant extract
- 15 grams±0.1 of sample was taken in a 50 ml polypropylene tube.
- 15 ml water was added and sample was extracted at least for 5 min.
- 15 ml of 1% formic acid was added in acetonitrile and shake well.

- 6 gram of anhydrous magnesium sulphate and 1.5 gram of sodium chloride was added.
- Hand shake vigorously and vortex for 3 minutes.
- Centrifuge the tube at 5000rpm for 5 min.
- Pipette out 1.5 ml of the supernatant into 2ml tube already consist of 150 mg of anhydrous magnesium sulphate and 50 mg of PSA.
- Vortex for one minute, centrifuged at 5000 rpm for 5 minutes.
- Pipette out 1 ml and filter with 0.22µl nylone syringe filter into 2ml vial.

2.3 GC-MS Full Scan analysis

The ethyl acetate extract of *Tinospora cordifolia* stem was subjected to GCMS full scan analysis of the instrument TSQ GCMSMS (Thermo Fisher Scientific) with TG-5MS
Analytic column. The GC-MS full scan analysis of ethyl acetate extract of *Tinospora cordifolia* stem was carried out as analytical column-TG-5MS, 30mx0.25mm IDx0.25µm; injection volume-2µl; flow rate (He gas)-1.0 ml/min.; mode-Full scan mode; inlet temperature-280^{0}C; source temperature-220^{0}C; transfer line temperature-300^{0}C; pre run timeout 10.00min.;equilibration time-0.50 min.; max temperature-350^{0}C; injector port-B; plunger stroke-3; sample vial depth-30mm and pre injection dwell time-2.0. The spectra were recorded for mass range 50-550 m/z for about 50 minutes. The separated compounds were identified by comparing their mass spectra with the mass spectral data of the compounds present in the NIST library data base.

3. Results and Discussion

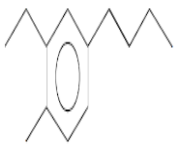
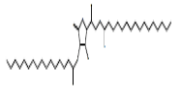
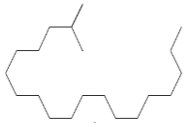
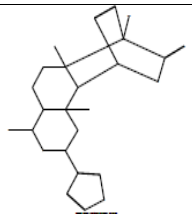
GCMS full scan analysis was carried out on the ethyl acetate extracts of *Tinospora cordifolia* stem using the instrument GCMS-MS (Thermo Fisher TSQ with Trace Finder Software. The sample volume was 2.0µL. The sample of ethyl acetate extract of stem of *Tinospora cordifolia* was run for 50 minutes. The results revealed the presence of 45 different phytocompounds, which has been presented with their retention time (RT), molecular formula, molecular weight; direct matching factor (SI) and reverse matching factor (RSI) in Table 1. Out of the 45 compounds obtained, nine compounds had the higher matching probability above 40% with the standard compounds of the NIST Library database namely (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol (47.67%); l-(+)-Ascorbic acid 2,6-dihexadecanoate (59.49); Octadecanoic acid (65.96 %); Columbin (1,4-Etheno-3H,7H-benzo[1,2-c:3,4-c']dipyran-3,7-dione,9-(3-furanyl) decahydro-4-hydroxy-4a,10a-dimethyl-, [1R-(1à,4á,4aà,6aá,9á,10aá,10bà)]-) (83.88 %); Campesterol (Ergost-5-en-3-ol, (3á,24R)-) (65.36 %); Stigmasterol (Stigmasta-5,22-dien-3-ol, (3á,22E)-) (62.76 %); ç-Sitosterol (Stigmast-5-en-3-ol,(3á,24S)-) (85.21%); 9-Ethyl-3-hydroxy-10,11b-dimethyl-1,2,3,4,6,6a,6b,7,8,9,11a,11b-dodecahydrobenzo[a]fluoren-11-one (53.57 %); á-Amyrin (Olean-12-en-3-ol, (3á)-) (49.54%) and presented here in Table 2 with their structure, nature of compounds and medicinal uses. Five compounds of the remaining 36 compounds match the library Bank at the range 20 to 40% and all the remaining 31 compounds of the 45 compounds obtained in ethyl acetate extract of *Tinospora cordifolia* stem were found to match with the data bank compounds at the range between 1.02-20%. The chromatogram (Fig. 1) showed prominent peaks in the retention time ranging 8.27 – 47.64 minutes. Further,
More research is needed to identify properties of these compounds

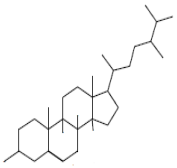
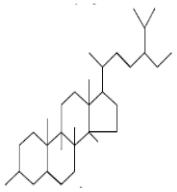
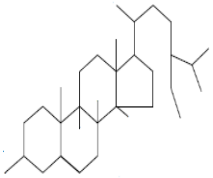
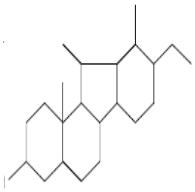
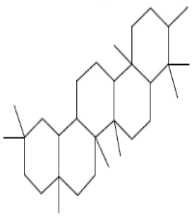
Table 1: Phyto-components identified in ethyl acetate extract of geloi (*Tinospora cordifolia*) stem

S. No.	Compound name	Ethyl acetate extract of geloi (*Tinospora cordifolia*) stem						
		Molecular formula	Molecular weight	RT (Min.)	Scan	Probability	SI	RSI
1.	Vanillin (Benzaldehyde, 4-hydroxy-3-methoxy-)	$C_8H_8O_3$	152	19.18	4228.00	25.70	876	888
2.	Benzaldehyde, 3-hydroxy-4-methoxy-	$C_8H_8O_3$	152	19.18	4228.00	14.80	860	872
3.	Vanillin, acetate (Benzaldehyde, 4-(acetyloxy)-3-methoxy)	$C_{10}H_{10}O_4$	194	19.18	4228.00	11.34	853	866
4.	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	25.96	6252.00	47.67	887	910
5.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	25.96	6252.00	37.47	881	912
6.	4-(1-Hydroxyallyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	25.96	6252.00	7.69	832	891
7.	Nootkaton-11,12-epoxide	$C_{15}H_{22}O_2$	234	29.16	7205.00	22.35	740	745
8.	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	$C_{15}H_{22}O_2$	234	29.16	7205.00	21.48	739	742
9.	5-Isopropylidene-4,6-dimethylnona-3,6,8-trien-2-ol	$C_{14}H_{22}O$	206	29.16	7205.00	5.39	704	737
10.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	33.38	8465.00	59.49	834	835
11.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	33.38	8465.00	14.48	797	814
12.	Palmitic anhydride	$C_{32}H_{62}O_3$	494	33.38	8465.00	11.38	791	796
13.	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	36.84	9496.00	31.40	859	868
14.	(Z)-18-Octadec-9-enolide	$C_{18}H_{32}O_2$	280	36.84	9496.00	14.30	839	871
15.	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336	36.84	9496.00	4.13	811	838
16.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	36.92	9518.00	19.05	845	885
17.	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	36.92	9518.00	7.53	832	842
18.	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	36.92	9518.00	7.24	831	843
19.	Octadecanoic acid	$C_{18}H_{36}O_2$	284	37.16	9590.00	65.96	826	845
20.	Octadecanoic acid, 2-(2-hydroxyethoxy)ethylester	$C_{22}H_{44}O_4$	372	37.16	9590.00	12.36	769	804
21.	L-Ascorbic acid, 6-octadecanoate	$C_{24}H_{42}O_7$	442	37.16	9590.00	9.71	763	763
22.	Octanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]c yclodecen-6-yl ester,[1aR-(1aà,2à,5á,5aá,6á,8aà,9à,10aà)]-	$C_{28}H_{42}O_6$	474	39.02	10145.00	23.60	735	738

23.	1,25-Dihydroxyvitamin D3, TMS derivative	$C_{30}H_{52}O_3Si$	488	39.02	10145.00	7.61	711	790
24.	Docosanoic acid, 1,2,3-propanetriyl ester	$C_{69}H_{134}O_6$	1058	39.02	10145.00	7.60	710	712
25.	Columbin (1,4-Etheno-3H,7H-benzo[1,2-c:3,4-c']dipyran-3,7-dione, 9-(3-furanyl)decahydro-4-hydroxy-4a,10a-dimethyl-, [1R-(1à,4á,4aà,6aá,9á,10aá,10bà)]-)	$C_{20}H_{22}O_6$	358	40.99	10733.00	83.88	879	895
26.	Pregnan-20-one, 3-(acetyloxy)-5,6-epoxy-,(3á,5á,6á)-	$C_{23}H_{34}O_4$	374	40.99	10733.00	1.64	703	703
27.	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate,[1aR (1aà,1bá,4aá,5á,7aà,7bà,8à,9á,9aà)]-	$C_{26}H_{36}O_8$	476	40.99	10733.00	1.38	699	699
28.	1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl	$C_{13}H_{20}O$	192	41.41	10857.00	21.70	713	754
29.	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-but-3-en-2-one oxime	$C_{13}H_{21}NO$	207	41.41	10857.00	16.62	706	738
30.	2,2-Dimethyl-4-(2-methyl-1-propenyl)-5-oxo-2,5-dihydro-3-furancarboxylic acid	$C_{11}H_{14}O_4$	210	41.41	10857.00	4.10	670	708
31.	Campesterol (Ergost-5-en-3-ol, (3á,24R)-)	$C_{28}H_{48}O$	400	47.13	12565.00	65.36	899	903
32.	5-Cholestene-3-ol, 24-methyl-	$C_{28}H_{48}O$	400	47.13	12565.00	15.41	860	882
33.	Ergost-5-en-3-ol, (3á)-	$C_{28}H_{48}O$	400	47.13	12565.00	10.88	850	922
34.	Stigmasterol (Stigmasta-5,22-dien-3-ol, (3á,22E)-)	$C_{29}H_{48}O$	412	47.64	12716.00	62.76	844	858
35.	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	$C_{29}H_{48}O$	412	47.64	12716.00	12.53	792	832
36.	9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-,3-acetate	$C_{31}H_{52}O_3$	472	47.64	12716.00	3.21	758	760
37.	ç-Sitosterol (Stigmast-5-en-3-ol, (3á,24S)-)	$C_{29}H_{50}O$	414	48.73	13041.00	85.21	899	910
38.	á-Sitosterol (Stigmast-5-en-3-ol, (3á)-)	$C_{29}H_{50}O$	414	48.73	13041.00	5.11	784	857
39.	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	$C_{29}H_{50}O$	414	48.73	13041.00	1.02	732	739
40.	9-Ethyl-3-hydroxy-10,11b-dimethyl-1,2,3,4,6,6a,6b,7,8,9,11a,11b-dodecahydrobenzo[a]fluoren-11-one	$C_{21}H_{30}O_2$	314	49.00	13122.00	53.57	694	729
41.	Acetamide,N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-	$C_{14}H_{22}N_2O_3$	266	49.00	13122.00	10.21	638	702
42.	5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate	$C_{22}H_{36}O_2$	332	49.00	13122.00	3.42	614	700
43.	á-Amyrin (Olean-12-en-3-ol, (3á)-)	$C_{30}H_{50}O$	426	49.53	13280.00	49.54	843	876
44.	à-Amyrin (Urs-12-en-3-ol, (3á)-)	$C_{30}H_{50}O$	426	49.53	13280.00	13.50	812	844
45.	24-Noroleana-3,12-diene	$C_{29}H_{46}$	394	49.53	13280.00	9.26	801	834

Table 2: Activity of phyto-components identified in ethyl acetate extract of geloi (*Tinospora cordifolia*) stem

S. N.	Compound name	Ethyl acetate extract of geloi (*Tinospora cordifolia*) stem		
		Structure	Nature of compound	Uses
1.	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol		Alcohol	Antimicrobial
2.	l-(+)-Ascorbic acid 2,6-dihexadecanoate		Sugar acid (Vitamin derivative)	Antioxidant
3.	Octadecanoic acid		Stearic acid	Antiviral, Anti-inflammatory activities, Flavoring agents and Hypocholesterolemic
4.	Columbin (1,4-Etheno-3H,7H-benzo[1,2-c:3,4-c']dipyran-3,7-dione, 9-(3-furanyl)decahydro-4-hydroxy-4a,10a-dimethyl-, [1R-(1à,4á,4aà,6aá,9á,10aá,10bà)]-)		Diterpenoid lactone	Antimicrobial and Anti-inflammatory

5.	Campesterol (Ergost-5-en-3-ol, (3á,24R)-)		Phytosterol	Antimicrobial, Antioxidants, Hypocholesterolemic, Antiatheroscleroic, Antiasthma and Antiartritic
6.	Stigmasterol (Stigmasta-5,22-dien-3-ol, (3á,22E)-)		Phytosterol	Antimicrobial, Antioxidants and Cholesterol-lowering properties.
7.	ç-Sitosterol (Stigmast-5-en-3-ol, (3á,24S)-)		Steroid	Antimicrobial, Anticancer, Antiarthritic, Antiasthma, Diuretic and Antiinflammatory
8.	9-Ethyl-3-hydroxy-10,11b-dimethyl-1,2,3,4,6,6a,6b,7,8,9,11a,11b-dodecahydrobenzo[a]fluoren-11-one		NA	NA
9.	á-Amyrin (Olean-12-en-3-ol, (3á)-)		Triterpine	Hepatoprotective, Prevent oxidative stress, Apoptosis, Anti-inflammatory and Anti-fibrogenic agent

Note: NA means not found in Cas

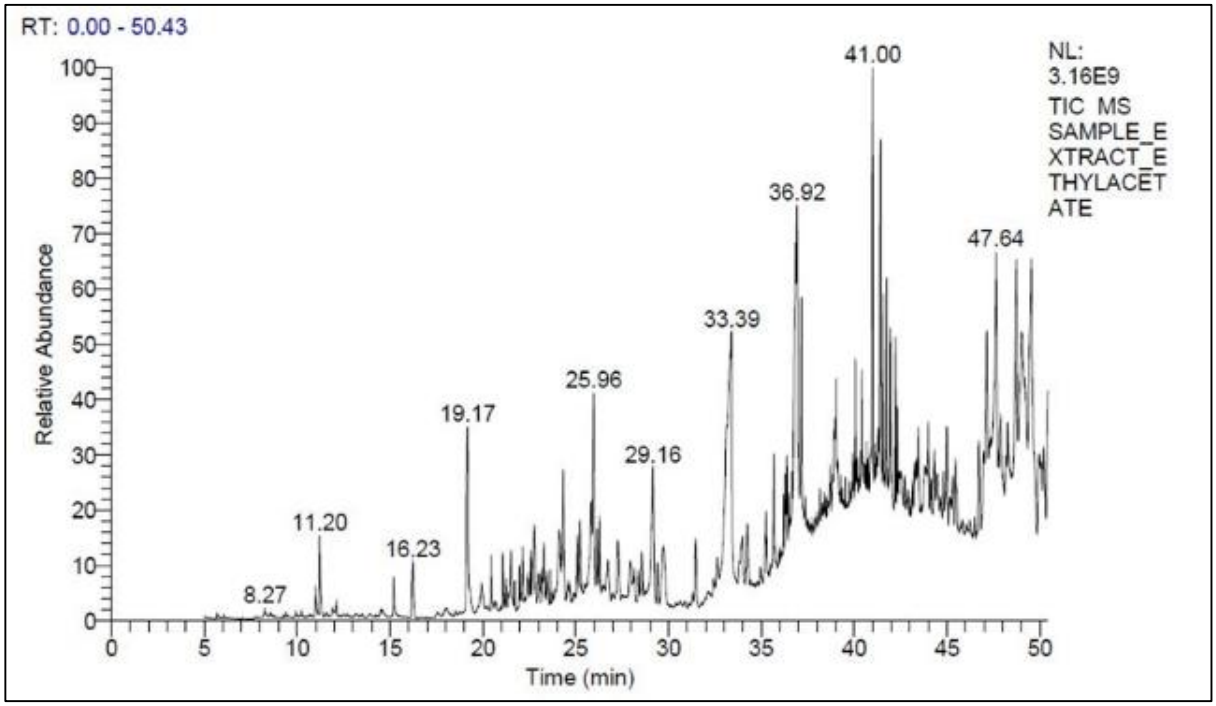


Fig 1: GCMS chromatogram of ethyl acetate extract of geloi (Tinospora cordifolia) stem

Many of the infectious diseases are still a major challenge to health issues all over the world. The emergence of resistance to antibiotics has further compounded the problem (Alli *et al.*, 2011) [4] because of extensive use of antibiotic growth promoters in broiler production. Consumers demand antibiotic free chicken so, there is vast scope for herbal feed additive for broiler production. Present study indicated that presence of phytocompounds in ethyl acetate extract of

Tinospora cordifolia stem, which has antioxidant, antimicrobial, antiviral, antinflammatory, hepatoprotective, antidiabitic, analgesic and flavouring agent. The presence of stigmasterol and campesterol impart antioxidant and antimicrobial activities and prevent the fatty liver syndrome by lowering the cholesterol level. Stigmasterol is also a precursor of anabolic steroid boldenone and Vitamin D3 (Kametani and Furuyama, 1987) [5]. Oliveira *et al*. (2005) reported the hepatoprotective potential of alpha- and beta-amyrin against toxic liver injury [6]. Antibiotics as feed supplement are seriously criticized due to global concern of their harmful effects like development of microbial resistance to the pathogens (Rahmatnejad *et al.,* 2009) [7]. Different pharmacological activities of *Tinospora cordifolia* is also reported by many researchers such as antioxidant (Patel *et al.,* 2009) [8], immunomodulator (Singh *et al.,* 2004) [9], antiviral (Mamidala *et al.,* 2012) [10], antimicrobial (Manjusha *et al.,* 2011) [11] and hepatoprotective activity (Dahanukar *et al.,* 2004) [12]. Considering the presence of various phytochemicals of medicinal uses in *Tinospora cordifolia*, it could be alternative as antibiotic growth promoter and would be ultilized as feed additive in broiler production.

4. Conclusion

The present investigation revealed the presence of 45 medicinal bioactive compounds by GC-MS and out of which 09 has probability more than 40 %. There has been an increase in demand for the phytopharmaceuticals all over the world because of the fact that the allopathic drugs have more side effects. The presence of phytochemicals reported in the present study indicates that the *Tinospora cordifolia* could serve as "herbal feed additive" in poultry industry in the coming years. In this regard, further studies need to be carried out to explore *Tinospora cordifolia* for its potential not only for alternative growth promoter but also in preventing and treating diseases.

5. Acknowledgement

We gratefully acknowledge the help offered by Thermo scientific laboratory for providing GCMS analysis. We also acknowledge the help offered by Head, Department of Botany, Govt. Dungar College, Bikaner (Rajasthan) for identification and authentication of *Tinospora cordifolia*.

6. References

1. Hashemi SR, Davoodi H. Herbal plants and their derivatives as growth and health promoters in animal nutrition. Veterinary Research Communication. 2011; 35:169-180.
2. Desai VR, Kamat JP, Sainis KB. An immunomodulator from *tinospora cordifolia* with antioxidant activity in cell-free systems. Proceedings of Indian Academy of Science. 2002; 114(6):713-719.
3. Raghu AV, Geeta SP, Martin G, Ravindran PN. *In vitro* Cellular and Developmental Biology Plant. 2006; 42:584-588.
4. Alli AI, Ehinmidu JO, Ibrahim YKE. Preliminary phytochemical screening and antimicrobial activities of some medicinal plants used in Ebiraland. Bayero Journal of Pure and Applied Science. 2011; 4:10-16.
5. Kametani T, Furuyama H. Synthesis of Vitamin D3 and related compounds. Medicinal Research Reviews. 1987; 7(2):147-171.
6. Oliveira FA, Chaves MH, Almeida FR, Lima RC, Silva RM, Maia JL, Brito GA, Santos FA, Rao VS. Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. Journal of Ethnopharmacology. 2005; 98(1-2):103-108.
7. Rahmatnejad E, Roshanfekr H, Ashayerizadeh O, Mamooee M, Ashayerizadeh A. Evaluation the effect of several non-antibiotic additives on growth performance of broiler chickens. Journal of Animal and Veterinary Advances. 2009; 8:1757-1760.
8. Patel SS, Shah RS, Goyal RK. Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats. Indian Journal of Experimental Biology. 2009; 47:564-570.
9. Singh N, Singh SM, Shrivastava P. Immunomodulatory and antitumor actions of medicinal plant *Tinospora cordifolia* are mediated through activation of tumor-associated macrophages. Immunopharmacology and Immunotoxicology. 2004; 26:145-162.
10. Mamidala Estari, Lunavath Venkanna, Annem S Reddy. *In vitro* anti-HIV activity of crude extracts from *Tinospora cordifolia*. BMC Infectious Diseases, 2012.
11. Manjusha GV, Rajathi K, Alphonse JKM, Meera KS. Antioxidant potential and antimicrobial activity of *Andrographis paniculata* and *Tinospora cordifolia* against pathogenic organisms. Journal of Pharmacy Research. 2011; 4-2:452-455.
12. Dahanukar SA, Rege NN, Oak MA. In-vitro effect of *Tinospora cordifolia* on leukemia cell proliferation. Indian Journal of Pharmacology. 2004; 95:130-135.