Volume 64, Issue 1, 2020
Journal of Scientific Research
Institute of Science,
Banaras Hindu University, Varanasi, India.

Evaluation of Antimicrobial Activity of *Cissus quadrangularis* L. stem extracts against Avian Pathogens and Determination of its Bioactive Constituents using GC-MS

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Abstract: **The current study evaluates the antimicrobial activity and characterizes the chemical constituents of stems of *Cissus quadrangularis* L. using Gas Chromatography Mass Spectrophotometry (GC-MS). The disc diffusion method was employed to determine the antimicrobial activity of methanol, ethanol, aqueous and petroleum ether extracts of *Cissus quadrangularis* stems against avian microorganisms viz. *Escherichia* sp., *Klebsiella* sp., *Salmonella* sp, *Staphylococcus* sp., *Pasturella* sp. and *Aspergillus* sp. were isolated from layer chicken. Among the various extracts, methanol extract showed potential antimicrobial activity against avian microorganisms especially *Escherichia* sp. and least activity was noted against *Aspergillus* sp. followed by ethanol, petroleum ether and aqueous extracts also showed activity against the tested avian pathogens. Among all the bioactive compounds identified in the current study, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, E-10-Pentadecenol, Cyclopentaneundecanoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, Docosanoic acid, ethyl ester, phytol, squalene, Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-,4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-, 2H-Pyran, 2-(7-heptadecynyloxy) tetra hydro- were represented in the *C. quadrangularis* extracts . It is concluded that the presence of various bioactive compounds as revealed by the GC-MS analysis in *C. quadrangularis* may have possessed the significant antimicrobial activity against the avian microorganisms.**

***Index Terms*: Antimicrobial activity, Avian, Bioactive compounds, *Cissus quadrangularis*, GC-MS**

I. INTRODUCTION

The commercial poultry farms are being challenged by outbreak of infectious diseases which is the major factor causing heavy mortality of chicken. Among the diseases, Fowl cholera, Colibacillosis, Salmonellosis and Aspergillosis are responsible for causing high mortality and morbidity in layer poultry farms.

Colibacillosis caused by *Escherichia coli* is an important septicemic and respiratory disease among the commercial poultry all around the world. It causes high percentage of mortality among the chicken (Barens & Gross, 1997). The poultry layer farms are facing heavy economic losses due to high mortality or significant reduction in egg and meat production caused by Salmonellosis (Haider & Rahman, 2004). Fowl cholera or avian Pasteurellosis caused by *Pasteurella multocida* is an infectious disease affecting all avian species (Rimler & Glisson, 1997). Staphylococcosis caused by avian strains of *Staphylococcus* sp. is an important systemic disease of commercial layer birds (Devriese, 1998).

Klebsiella pneumoniae is a common saprophyte causes embryonic mortality and excess losses in young chickens and turkeys affected with respiratory diseases (Sandra & Duarte, 2018). Aspergillosis, caused by saprophytic genus *Aspergillus*, in particular *Aspergillus fumigatus* is an economically important pathogen due to its severe mortality in chicks. The growth of broilers and layers mainly depended upon the use of antibiotics in feed but this proved to have negative effect on health of human beings on consumption of animal byproducts. The indiscriminate usage of antibiotics results in deleterious effects viz., hypersensitivity, allergy reactions, immune suppression and also the evolution of antibiotic resistant pathogens (Ahmad et al, 1998). Further in a recent research report revealed the presence of pencillin V, amoxicillin, enrofloxacin, ciprofloxacin, thiamphenicol and tylosin and the presence of penicillin V was more comparing to other antibiotics which insisted the need of

alternate therapy instead antibiotics (Chiesa et al, 2018). Chicken manure compost contains the antibiotic resistant organism (Awasthi, 2019) insisted the need to find safe alternate strategies to tackle the problem.

Herbal plants consist of high source of secondary metabolites such as antiviral, antifungal and antibacterial agents. Screening of bioactive molecules from plants leads to the development of new therapeutic drugs that not only shown efficient activity against various disease causing pathogens, also gives a ray of hope to treat deleterious disease including cancer and Alzheimer's disease (Sheeja & Kuttan, 2007). Further investigation of the antimicrobial activities of the medicinal plants will expose the plants as potential source of therapeutic agents.

Cissus quadrangularis L., family *Vitaceae* has quadrangular stems, small leaves and also roots that all possess medicinal properties as revealed by the surveys and trials conducted by ethno-botanists and traditional herbal healers especially for the treatment of bone fractures and swelling. The stem is bitter in taste and commonly used as the oral and topical applications in bone fractures, complaints of the back and spine, asthma, heat burns, wounds and insect bites (Sharma et al, 2001).

Methanol extracts of stems of *C. quadrangularis* were analyzed using GC-MS for identifying the bioactive constituents in this plant species because of its high antimicrobial properties reported elsewhere (Costa et al, 2008). Screening of available literatures revealed that the information on antimicrobial effect of medicinal plants especially on avian pathogen is totally lacking. To the best of our knowledge as far as literature is concerned, this types of study represents avian pathogens may not be a common and hence this kind of studies are need in this hour due to emergence of multi drug resistant organisms in animals especially birds (Gibbs et al, 2007). Hence, the current research is proposed for evaluation of the antimicrobial activity of stems of *C. quadrangularis* on microorganisms isolated from Layer chickens reared in various commercial farms and also for the documentation of its phytochemical constituents using GC-MS analysis.

II. MATERIALS AND METHODS

A. Isolation and identification of Avian Pathogens

A total of 780 samples namely heart blood (260 No's), tracheal swab (260 No's), liver (60 No's), ovary (60 No's) and lung nodules (80 No's) have been collected from 460 layer poultry birds suffering from respiratory diseases, enteritis during post mortem examination at the farm premises of various commercial poultry farms located in and around Namakkal district in Tamilnadu. The samples were inoculated on to blood agar (Himedia) and Sabouraud's dextrose agar (SDA; Himedia, Mumbai) and incubated at 37 ^{o}C for 24-48 hours for bacterial and 22-28 ^{o}C for 72 -96 hours for fungal isolation. The basic tests like Grams staining, motility tests and other various biochemical properties of the isolated organisms have been performed as per standard protocols and for fungi lactophenol cotton blue staining and other features (Quinn et al, 1994) were carried out to identify the organisms upto genera level.

B. Preparation of plant extracts and Phytochemical screening

The stems of healthy, disease free plant, *C. quadrangularis* were collected and thoroughly washed in running water to clean the dust particles and second washing with distilled water. The shadow dried stems of *C. quadrangularis* were finely ground to obtain powder weighing 250 gm was subjected to extraction using 1000 ml of different solvents namely, methanol, ethanol, aqueous and petroleum ether. The extraction procedure was carried out for 24 hours at 4 ^{o}C. The collected solvent content was concentrated by evaporation followed by preservation in air tight containers.

C. Screening of Antimicrobial activity

1) Preparation of inoculum

The test microorganisms were obtained from nutrient agar slants were placed in the nutrient agar medium to raise individual colonies at 37 ^{o}C for 24 hours. Individual colonies (3-5) were identified and transferred into a test tube containing sterile nutrient broth (5 ml) and mixed thoroughly. The culture broth was kept in incubator at 37 ^{o}C and observed for turbidity of 10^{5}-10^{8} CFU/ml which is equivalent to 0.5 McFarland standard (8 hrs.) visually adjusting it by comparing the test. For fungus, inoculum prepared from SDA plates with the inoculum size of $1x10^{7}$ cell / ml.

2) Disc Diffusion Method

The disc diffusion method was used to study the patterns of antimicrobial sensitivity of the plant extracts (Bauer et al, 1966). The 6 mm sterile discs prepared from Whatman's filter paper (No.1) were used for absorbing 500 µg of the plant extract samples (Isenberg, 1998). The positive control used in this study includes the standard reference antimicrobial discs of ciprofloxacine (5 mcg/ml) for bacteria and Ketoconazole (10 mcg/ml) for fungi were used. In 1ml of DMSO about 100 mg of crude extract was dissolved to obtain stock solution. Nutrient and Sabouraud dextrose broths were inoculated with the respective organism and load (inoculum size is $1x10^{8}$ CFU/ml (McFarland standard) and fungal (inoculum size is $1x10^{7}$ cell/ml) of inoculums were adjusted. The isolated cells were spread over the Muller-Hinton agar and Sabouraud's dextrose agar medium using sterile cotton swab horizontally and vertically. The discs with plant extracts (1000 µg- 4000 µg) placed using sterile forceps were mounted on top layer of the inoculated plates. All the plates with discs were kept under incubation at 37 ^{o}C for 18-24 hours and 25 ^{o}C for 48 hours during which the activity was confirmed by the clear inhibition

zone appearing around each discs and triplicates were maintained.

D. GCMS analysis

Twenty grams of finely ground powder of stems from *C. quadrangularis* was equilibrated with 200 d/m of methanol for 24 hrs. The volume was further reduced to 2 d/m by careful constant heating and also by separating the supernatant. The concentrated methanolic extract of *C. quadrangularis* was tested using the GC Clarus 500 GCMS (Perkin Elmer) equipment. This equipment employed the fused silica column equipped with Elite-1 (100% dimethyl polysiloxane, 30 nm x 0.25 nm ID x 1 µm df) and the bioactive compounds were separated by using Helium (99.999%) as a carrier gas at a regular flow of 1 ml/min. The 2 µl of extract was injected and detected by the Perkin Elmer - Turbo gold mass detector with the aid of Turbo mass 5.1 software. During the GC extraction process the temperature of oven was maintained at 110 ^{o}C at 36th minute, with 2 min holding time. The temperature of injector was maintained at 250 ^{o}C (mass analyzer). The other parameters involved in the operation of Clarus 500 MS, were standardized i.e. inlet line temperature was set as 200 ^{o}C and the source temperatures 200 ^{o}C. Mass spectra were obtained at 70 eV with scan intervals of 0.5 second and fragments from 45 to 450 Da. The total GC running time was 36 minutes.

E. Identification of components

The mass spectrum was interpreted with the aid of the database of National Institute Standard and Technology, WILEY8 and FAME which possess 65,000 plus patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The name of the component, structure, molecular formula and molecular weight of the tested samples were documented. The relative percentage amount of components was estimated by comparing their average peak areas to total areas. The software utilized to handle Mass Spectra and chromatograms was a GCMS solution version. 2.53.

III. RESULTS AND DISCUSSION

A. Phytochemical analysis of extracts of C. quadrangularis

On the basis of therapeutic potential of secondary metabolites, the phytochemical characters of the *C. quadrangularis* extract in aqueous, ethanol, methanol and petroleum ether were investigated and represented in Table I. The detailed phytochemical analysis of stems of *C. quadrangularis* indicated the existence of saponin, tannin, phenol, flavonoid, terpenoid, alkaloids in aqueous, ethanol and methanol extracts while phlobatannin and cardiac glycoside were absent in all the extracts and the absence of above two phytochemical components were reported earlier by Mujeep et al (2014).

Table I. Phytochemical analysis of *Cissus quadrangularis* in aqueous, methanol, ethanol and petroleum ether extracts

Secondary compounds	Aqueous	Methanol	Ethanol	Petroleum ether
Tannin	+	+	+	-
Phlobatannin	-	+	-	-
Saponin	+	+	+	+
Steroids	-	+	+	-
Terpenoids	+	+	+	+
Cardiac glycoside	-	-	-	-
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
(+) Present (-) Absent				

B. Antimicrobial activity of different extracts of Cissus quadrangularis

Based on the colony characteristics, cultural morphology and biochemical properties and isolation methods, the isolates have been confirmed as *Escherichia* sp. (60 Nos.), *Staphylococcus* sp. (16 Nos.), *Pasteurella* sp. (42 Nos.), *Salmonella* sp. (3 Nos.), *Klebsiella* sp. (3 Nos.) and *Aspergillus* sp. (40 Nos.).

The antimicrobial activity of different extracts of *C. quadrangularis* was tested against the above avian pathogens. Antibiotic sensitivity tests against the isolates showed the high sensitivity to Ciprofloxacin, Enrofloxacin and Gentamicin. These isolates were resistance to Streptomycin, Penicillin, Ampicillin and Amoxicillin. Antibiotic sensitivity test against *A. fumigates* isolate revealed sensitivity to Ketaconazole. Based on these results, Ciprofloxacin discs (5 mcg/ml) were used as a positive control for these bacteria and Ketoconazole (10 mcg/ml) for fungi while evaluating the antimicrobial effect of different solvent extracts of stems of *C. quadrangularis*.

The antimicrobial activity stem extracts of *C. quadrangularis* in different solvents against isolated avian microorganisms is presented in Table II. The highest inhibition zone of *E.* coli and *K. pneumoniae* were observed in methanol extract while the lowest activity was noticed in aqueous extract in higher dose. Similarly the highest inhibition zones of *Salmonella* sp., *Pasteurella* sp., *Staphylococcus* sp. and *Aspergillus* sp. were observed in methanol extracts while the lowest activity was observed in petroleum ether extract in higher doses. The antimicrobial activity of *C. quadrangularis* observed was directly proportional to the concentrations. The studies on chloroform, aqueous, methanol and ethyl acetate extracts of *C. quadrangularis* by Mishra et al (2009) recommended that this plant as an important source of naturally available useful antimicrobial agent.

The highest activity of *C. quadrangularis* stem extract is close to the standard antimicrobial agent. These results are in agreement with the studies on ethyl acetate and methanolic

extracts for its antimicrobial activity against clinically important strains of bacteria at different concentrations of 100µg/disc by disc diffusion method that reported significant antimicrobial activity against the avian pathogens (Kumar et al, 2017).

Table II. Antimicrobial activity of different extracts of *Cissus quadrangularis* against avian microorganisms

S.No.	Microorganisms	Aqueous (µg /disc)				
		1000µg	2000µg	3000µg	4000µg	Positive
1	*Escherichia sp.*	3.80±0.26	6.40±0.44	8.50±0.59	9.60±0.70	12.30±0.86
2	*Klebsiella sp.*	3.70±0.25	6.20±0.43	8.30±0.58	9.80±0.71	12.10±0.84
3	*Salmonella sp.*	3.35±0.23	5.85±0.40	7.90±0.55	9.80±0.68	11.75±0.82
4	*Pasteurella sp.*	3.10±0.21	5.75±0.40	7.65±0.53	9.70±0.67	11.65±0.81
5	*Staphylococcus sp.*	3.60±0.25	6.05±0.42	8.00±0.56	10.05±0.70	12.00±0.84
6	*Aspergillus sp.*	2.55±0.17	4.95±0.34	6.90±0.48	9.10±0.63	11.00±0.77
		Ethanol(µg /disc)				
1	*Escherichia sp.*	4.00±0.28	6.50±0.45	8.65±0.60	10.40±0.74	12.50±0.87
2	*Klebsiella sp.*	3.90±0.27	6.35±0.44	8.40±0.58	10.30±0.72	12.35±0.86
3	*Salmonella sp.*	3.40±0.23	6.00±0.42	8.05±0.56	10.00±0.70	12.00±0.84
4	*Pasteurella sp.*	3.20±0.22	5.90±0.41	7.95±0.55	9.80±0.68	11.75±0.82
5	*Staphylococcus sp.*	3.80±0.26	6.20±0.43	8.10±0.56	10.20±0.71	12.15±0.85
6	*Aspergillus sp.*	3.10±0.21	5.50±0.38	7.20±0.50	9.50±0.66	11.20±0.78
		Methanol (µg /disc)				
1	*Escherichia sp.*	4.10±0.29	6.80±0.47	8.95±0.62	10.80±0.75	12.60±0.88
2	*Klebsiella sp.*	4.05±0.28	6.70±0.46	8.55±0.59	10.75±0.75	12.55±0.87
3	*Salmonella sp.*	3.50±0.24	6.10±0.42	8.30±0.58	10.25±0.71	12.20±0.85
4	*Pasteurella sp.*	3.35±0.23	6.00±0.42	8.15±0.57	10.05±0.70	12.10±0.84
5	*Staphylococcus sp.*	3.90±0.27	6.50±0.45	8.40±0.58	10.30±0.72	12.40±0.86
6	*Aspergillus sp.*	3.30±0.23	5.65±0.39	7.60±0.53	9.80±0.68	11.50±0.80
		Petroleum ether (µg /disc)				
1	*Escherichia sp.*	3.50±0.24	6.10±0.42	8.35±0.58	10.15±0.71	12.05±0.84
2	*Klebsiella sp.*	3.35±0.23	5.80±0.40	8.10±0.56	10.00±0.70	11.90±0.83
3	*Salmonella sp.*	3.10±0.21	5.50±0.38	7.80±0.54	9.55±0.66	11.45±0.80
4	*Pasteurella sp.*	3.00±0.21	5.40±0.37	7.50±0.52	9.35±0.65	11.30±0.79
5	*Staphylococcus sp.*	3.20±0.22	5.60±0.39	8.00±0.56	9.85±0.68	11.60±0.81
6	*Aspergillus sp.*	0.50±0.04	4.50±0.31	6.60±0.46	8.50±0.59	10.90±0.76

Among the various extracts, methanol extract showed potential antimicrobial activity against avian microorganisms followed by ethanol, petroleum and aqueous extract. Methanolic extracts of important medicinal plants showed higher antibacterial activity on human pathogenic bacteria than the aqueous extracts (Mahesh & Satish, 2008). In addition there are many publications regarding the comparison of different solvents used for extraction of phytochemical compounds and most of them recommended methanol as a best solvent because of its high extractability of wide range of polar and non-polar phytochemical compounds.

C. GC-MS analysis

The results of GCMS analysis revealed nineteen compounds identified from the methanolic extract of stems of *C. quadrangularis*. The names of various compounds with their retention time, molecular formula and compound nature are presented in the Table III. Identification of the compounds was done using Dr. Duke's Phytochemical and Ethno-botanical Database. The results of GCMS spectrum profile (Fig. 1) confirmed the existence of nineteen components having the Retention Time as 12.38, 6.87, 14.42, 29.90, 8.71, 23.05, 13.71, 30.78, 10.69, 2.31, 27.49, 31.35, 13.56, 16.21, 32.04, 11.13, 10.95, 11.68 and 20.17, respectively.

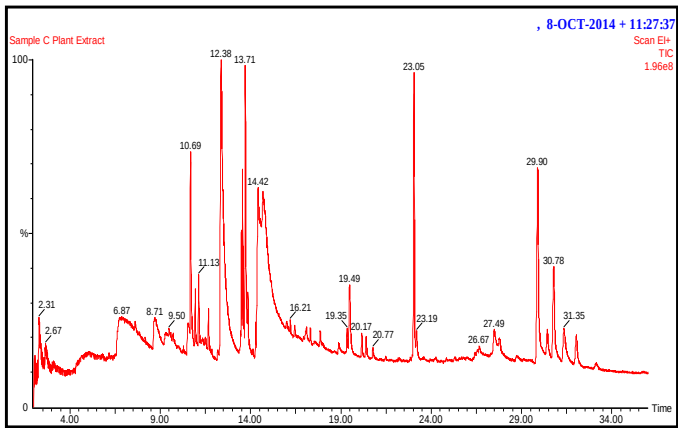

Figure 1. GC-MS chromatogram of methanolic extract of *Cissus quadrangularis*

The results clearly indicated the presence of n-Hexadecanoic acid (23.42%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (18.64%), 9,12-Octadecadienoic acid, methyl ester (12.05%),

Urs -12 -en -24 -oic acid, 3 -oxo-, Methyl ester, (+)- (6.73%), 4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)- (3.74%), 3,7,11,15 -Tetramethyl -2-hexadecen -1-ol (3.30), Propane, 1,1,3-triethoxy- (2.37%), Vitamin E (2.33%), 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro- (2.23%), 2(1H) Naphthalenone, 3,5,6,7,8,8a –hexahydro -4, 8a -dimethyl-6-(1-methylethenyl)- (1.35%), 9-Octadecenoic acid (Z)-, methyl ester (1.69%), 1,2-15,16-Diepoxyhexadecane (1.64%), E-10-Pentadecenol (1.10%), E-2-Tetradecen-1-ol (0.80%), Cyclopentaneundecanoic acid, methyl ester (0.64%) and Docosanoic acid, ethyl ester (0.44%).

The compounds having multiple biological activities identified in this study are represented in Table III. The present observations by GC-MS analysis of stem extracts of *C.quadrangularis,* revealed the presence of useful chemical compounds viz. diterpene, triterpene, lauric acid ester, aldehyde, flavonoid, ether, vitamins, palmitic acid and phenolic fractions. All these active principles were found to have multiple biological properties as per the previous ethanopharmacological studies. The compound Squalene is suggested as triterpene which might be useful as antioxidant, antimicrobial, anticancer, antitumour (Konovalova et al, 2013) and also used as an adjutant in vaccines. 2-Furancarboxaldehyde, 5 -(hydroxymethyl)- is suggested as an aldehyde having antimicrobial activity (Sathyaprabha et al, 2010). Urs-12-en -24- oic acid, 3- oxo-, Methyl ester, (+) - is reported to be a Bosewellic acid and might exert antimicrobial, anticancer and anti-inflammatory activities. The Phytol is considered to be diterpene alcohol in methanol fraction and can be used as an antiinflammatory, antimicrobial, anticancer and diuretic. It is considered as an important acyclic diterpene alcohol, one of the precursor of vitamin E and K, important antioxidant and used as a suppressant on breast cancer induced by epoxide. It is reported to possess no adverse autoimmune effects when used as effective vaccine adjuvant (Daniet et al, 2011). Phytol is usually mixed with other sugars or corn syrups as a hardener in preparing candies.

The 3,7,11,15 – Tetramethyl -2- hexadecen -1- ol is proven to be Terpene alcohol and it may act as antimicrobial agent (Srinivasan et al, 2014). Docosanoic acid, ethyl ester is considered to be lauric acid esters that could be used as antimicrobial and antioxidant. The n-Hexadecanoic acid methyl ester is an aliphatic acid ester reported to arrest growth of cancer cells but induced apoptosis human gastro-intestinal tumours (Anandan et al, 2012). It is also known as Methyl palmitate or palmitic acid in methanolic fraction that might act as nematocide, hypocholesterolemic, lubricant, pesticide and antiandrogenic. Vitamin E is employed as analgesic, antiinflammatory, antidiabetic, antidermtitic, anticancer, anti-leukemic, antioxidant and hepatoprotective. Reports also revealed that the stems of *C. quadrangularis* has antibacterial activity against *E. coli*, *S. aureus*, *K. pneumonia,* and antifungal property against *A. flavus*, *C. albicans* and *Fusarium* sp. (Merinal & Boi, 2012).

The current investigation on GCMS analysis exposed the presence of multivarious bioactive compounds with diversified chemical structures in stems of *C. quadrangularis*. Among all the bioactive compounds identified in the current study, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, E-10-Pentadecenol, Cyclopentaneundecanoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, Docosanoic acid, ethyl ester, phytol, squalene, Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-,4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-, 2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro- have the effective antimicrobial property as evidenced by the literature survey (Manas & Raka, 2011; Ramos-Nino et al, 1998). The methanol extract of *C. quadrangularis* with high antibacterial activity against the Gram positive and Gram negative bacteria (Ampai et al, 2007) is therefore promoted the worldwide use of its stems for various ailments by natural and traditional healers even today.

CONCLUSION

The evaluation of antimicrobial activity of aqueous, ethanol, methanol and petroleum extracts *C. quadrangularis* against the avian microorganisms isolated from commercial layer birds was carried out in the current study. The methanolic and ethanolic extracts were highly effective against Gram negative, Gram positive bacteria and fungal strains while petroleum and aqueous extracts showed minimum activity against the same strains of bacteria and fungi. The antimicrobial properties of different extracts of *C. quadrangularis* leaves may be because of the existence of multivarious active compounds, which exert their action by a different mechanism. The present investigation revealed that the type of solvent is also important in efficient extraction of bioactive compounds played the role in the inhibitory effect against the microorganisms. However, screening of individual bioactive phytochemical agents using NMR may further lead to discover a new drug as a broad spectrum antimicrobial agent against diseases among Layer chickens. The phytocompounds and their antimicrobial properties presented in the current study could help the pharmacology researchers to explore this plant to the next possible level. Future emphasis should be given on developing novel methods of isolation and production of secondary metabolites in the purest form from this plant that would result in disease free commercial layer chicken farming and further the present study yielded a platform to develop the plant based vaccine to control the avian pathogens.

Table III. GC-MS analysis showing phytochemical compounds and biological activities in methanol extract of *C. quadrangularis*

No.	RT	Peak Area %	Name of the compound	Molecular Formulae	Compound nature	Biological activity**
1.	2.31	2.37	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	Ether	Flavour
2.	6.87	18.64	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C6H6O3	Aldehyde	Antimicrobial preservative
3.	8.71	6.52	à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	C18H32O16	Sugar Moiety	Preservative
4.	10.69	3.30	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	Terpene alcohol	Antimicrobial
5.	10.95	0.80	E-2-Tetradecen-1-ol	C14H28O	Myristic acid	Good immunomodulator, Flavour
6.	11.13	1.10	E-10-Pentadecenol	C15H30O	Ether	Anticancer, Antimicrobial
7.	11.68	0.64	Cyclopentaneundecanoic acid, methyl ester	C17H32O2	Dihydro hydnocarpic acid	Antimicrobial
8.	12.38	23.42	n-Hexadecanoic acid	C16H32O2	Palmitic acid	Antioxidant, Hypocholestrolemic, Nematocide, Pesticide, Lubricant, Anti-androgenic
9.	13.56	1.69	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	Oleic acid Elaidic acid	Hypotensive, Anticancer
10.	13.71	4.95	Phytol	C20H40O	Diterpene	Antimicobial, Antiinflammatory, Anti-cancer, Diuretic
11.	14.42	12.05	9,12-Octadecadienoic acid, methyl ester	C19H34O2	Polyenoic acid	Hepato-protective, Antihistamine, Hypocholesterolemic, Anti-eczemic
12.	16.21	1.64	1,2-15,16-Diepoxyhexadecane	C16H30O2	Epoxide	Cytotoxicity
13.	20.17	0.44	Docosanoic acid, ethyl ester	C24H48O2	Lauric acid ester	Antimicrobial, Antioxidant
14.	23.05	6.07	Squalene	C30H50	Triterpene	Anticancer, Antimicrobial, Antitumor, Antioxidant
15.	27.49	2.33	Vitamin E	C29H50O2	Vitamin	Antidiabetic, Antioxidant, Antiinflammatory, Anticancer, Antidermatitic, Antileukemic, Analgesic, Hepatoprotective, Antispasmodic
16.	29.90	6.73	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	C31H48O3	Boswellic acid	Antiinflammatory, Antimicrobial, Anticancer
17.	30.78	3.74	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	C20H34O2	Tobacco compound	Antifungal, Antimicrobial
18.	31.35	2.23	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	C22H40O2	Flavonoid fraction	Antiinflammatory, Antimicrobial, Antioxidant
19.	32.04	1.35	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	C15H22O	Sesquiterpene / Ketone	Antiinflammatory

**Duke's Phytochemical and Ethno-botanical databases; www.ars-gov/cgi-bin/duke/, 2013

ACKNOWLEDGEMENT

The authors are thankful to the management of K.S.Rangasamy College of Arts & Science, Tiruchengode for providing all basic facilities to carry out the work. One among the author (Jaynathi Chenniappan) thanks the Director, Indian Institute of Food Processing Technology (IIFPT), Thanjavur, for the GC-MS analysis and its interpretation.

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