Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

https://doi.org/10.36673/AJPCR.2020.v08.i04.A20

ISOLATION AND CHARACTERIZATION OF ANTHELMENTIC COMPONENT-COUMARIN FROM LEAVES OF *SESBANIA GRANDIFLORA LINN*

Neethu Sara Varghese*[1], R. Arunkumar[1], M. L. Lal Prasanth[1], Shibu Prasanth[2]

[1*]Dm Wims College of Pharmacy, Naseera Nagar, Meppadi Po, Wayanad, Kerala, India.
[2]St. Joseph's College of Pharmacy, Cherthala, Kerala, India.

ABSTRACT

Sesbania grandiflora belonging to the family Leguminoseae, considering various medicinal properties of the plant, were collected and studied for the anthelmintic property of the leaves. The present study involves the isolation and characterization of coumarin from the aqueous extract of the leaves of *Sesbania grandiflora*, experiments were conducted to evaluate the possible anthelmintic effects of various concentrations of the extract/ isolated compound in both earthworms and eggs of *Ascaridia galli* and *Dipylidium caninum*. The coumarin isolated from *Sesbania grandiflora* showed dose depending anthelmintic activity and was compared to the standard drug albendazole.

KEYWORDS

Sesbania grandiflora, Anthelmintic, Coumarin, *Megascolex mauritti, Ascaridia galli* and *Dipylidium caninum.*

Author for Correspondence:

Neethu Sara Varghese,

Dm Wims College Of Pharmacy,

Naseera Nagar, Meppadi Po,

Wayanad, Kerala, India.

Email: neethusaravarghese1@gmail.com

INTRODUCTION

Over 1 million people among the world tropical countries suffer from intestinal parasitic infection[1]. It refers to the complex condition caused by nematodes such as hook worms or round worms. The common source of infections includes poor hygiene, poor sanitation, malnutrition and crowded condition. The parasitic infection contributes to the prevalence of anaemia, malnutrition, eosinophilia and pneumonia resulting in social and economical deprivation leading to huge economic impact in tropical countries[2]. These parasites are hence known as poverty promoting parasites[1]. Most diseases caused by the helminths are chronic in

nature causing severe morbidity to people including pregnant women and children[3]. Some of the parasitic infections that lead to severe morbidity are lymphatic filiarisis, onchocerciasis and schistisomiasis. Although these parasitic infections are increasing day by day, only few drugs are available to treat them. Among these drugs none of them have optimum efficacy. Furthermore, there is increased possibility of development of resistance towards these drugs. For practical reasons, only a drug is used at a time for Single Dose Mass Administration (MDA)[4]. Therefore, there is an urgent need to develop new anthelmintic drug with optimum efficacy. Parasitic worms also affect livestocks as well as crops, which results in a negative impact on the economy of the developing countries. Plants or plant products offer a better alternative to the existing anthelmintics that is both sustainable and environmentally acceptable. In future such plants or plant products can play an important role in controlling helmenthiasis in tropical countries.

By different mechanisms such as chronic inflammation, host immune system regulation, inhibition of intracellular contact, disruption of proliferation-antiproliferation pathways, activation of genomic instability and stimulation of progeny of malignant stem cells, Helminth infection can promote carcinogenesis[5]. Helminth infections if controlled, can act as a preventable cause of cancer[6] and thereby decrease about 16 % of global cancer burden.

It is recognized that the leaves of Sesbania grandiflora have anthelmentic, alixeretic, apierent, tonic, diuretic and laxative properties. They are reported in kaphaja diseases, pruritis, skin disorders, night blindness, asthma, gout, ophthalmia, headache and nasal catarrh, as therapeutically beneficial[7].

METHODS

Plant material

The plant *Sesbania grandiflora* was brought from various regions of Alappuzha district, Kerala, India in the month of August, 2012, The plants were authenticated from the Botany Department of Sacred Heart College of Science, Ernakulam by Dr. Dominic VJ.

Preparation of extracts and saponin containing fraction

The leaves of *Sesbania grandiflora* (L) were washed, dried in shade and powdered into coarse powder.

Powdered leaves of *Sesbania grandiflora* (L) were extracted with hexane using Soxhlet apparatus. The hexane was completely removed under vacuum by rotary flash evaporation.

The water extract was prepared by extracting the plant powder with distilled water with constant stirring for 4 hrs, filtered and the residue was again extracted with distilled water. The extracts were combined and lyophilized to get dry powder.

Isolation of active fraction from aqueous extract of *sesbania grandiflora* (AF1)

5g of the aqueous extract was dissolved in sufficient amount of distilled water (10ml) and to this 10ml of alcoholic KOH was added. The mixture was heated in a boiling water bath for half an hour. The resulting hot alkaline solution was cooled and extracted with 20ml of ether. The alkaline fraction and ether soluble fraction were separated. Both the fractions were screened for its anthelmintic activity. The alkaline fraction (positive activity) was acidified and further extracted with 20ml of chloroform. The acid fraction and chloroform fraction were separated using separating funnel. Both the final fractions were evaporated to dryness. The acid fraction yielded a brown amorphous solid mass whereas the chloroform fraction on evaporation yielded white powder (which is further denoted as AF1), which showed better activity than the acid fraction.

The solubility of both the fractions was determined and TLC study was performed. The fractions were also tested for its biological activity. The preliminary phytochemical screening was performed to identify the type of compound present in the fraction.

Isolation of active compound (SG-1) from anthelmintic fraction (AF1)

The preparative TLC was carried out using glass plates (20 x 20cm). These were coated with well stirred suspension of silica gel G in distilled water (50gm in 120ml) to get a thickness of about 1mm. After drying for 2 hours at room temperature, the plates were heated at 105°C for 1 hour, cooled and then used for spotting the AF1. The AF1 was dissolved in methanol and used for preparative TLC. The plates were developed in a glass development chambers previously saturated with the mobile phase of toluene: ether (1:1, saturated with 10% acetic acid). After running the plates in the mobile phase of toluene: ether (1:1), the plates were visualized in UV light and the area which showed fluorescent band was scratched and soaked overnight in absolute methanol and next day sonicated for 10 minutes and then filtered to separate the isolate from the silica[8].

Drugs and Chemicals

All the reagents used were of analytical grade. The standard reference drugs Piperazine citrate (Triveni chemicals) and Albendazole (Micro Pharma) were used at a concentration of 10mg/ml.

Statistical Data

Graph pad Instat v-3.36 Statistical Package was used to conduct the statistical analysis. The data is expressed as mean ± SEM in tables, statistics and text. Differences between groups have been investigated by one-way variance analysis.

Anthelmentic Activity

Anthelmintic activity of aqueous extract was studied on *Megascolex mauritii* (Megascolecidae). *M. mauritii* was collected from Cherthala, Alapuzha district. The model organisms collected were of equal size. 7 groups of 6 organisms each were added into 10ml of normal saline. Then the test animals were introduced to the respective category with albendazole, piperazine citrate, varying concentrations of the extracts in normal saline. Observations were made on the paralysis and death of individual worms for the time taken.

When no movement of any kind could be detected, even when the worms were actively shaken, time for paralysis was noted. Death was concluded when the worms lost their motility, followed by fading away of colours from their body when dipped in warm water at 500ºC[9].

Egg hatch assay

The eggs of *Ascaridia galli* and *Dipylidium caninum* were collected from stool samples obtained from Veterinary poly clinic, Cherthala. 4g of stool sample was triturated in a mortar and pestle with a little amount of water. The liquid was then centrifuged and the supernatant liquid was collected. The supernatant liquid containing eggs was identified and quantified under the supervision of Dr. Salim Chellapan.

The Egg hatch assay was performed with minor modifications by the otherwise mentioned method[9]. 5 small test tubes were taken and one ml (100 eggs ml^{-1}) of egg suspension was added to each test tubes. To the first test tube 500µl of 0.1 % NaCl was added and considered as control. Tubes 2, 3 and 4 were added with 500µl of different concentrations of SG-1 (0.5, 1 and 10mg/ml). To the 5th test tube500µl of albendazole was applied, as a positive control. At 27°C for 48 hours, the test tubes were incubated. After incubation, two drops of Lugol's iodine was added. The number of viable eggs were counted using McMaster counting technique.

With the supernatant liquid, both sides of the Mc Master counting chamber were filled. For around 5 minutes, the chamber was permitted to stand. At 10x10 magnification capacity, the chamber was examined under a microscope. The eggs were counted within the engraved region of the two chambers. The number of eggs per gram of fecal matter is measured[10].

Studies of cytotoxicity with the active compound

Trypan blue exclusion method

The effect of AF2 and SG-1 on short-term cell viability was evaluated by incubating 1x106 DLA cells in 1ml of vehicle-containing phosphate buffered saline (PBS) (0.1 percent DMSO) and various AF2/SG-1 concentrations for 3 hours in a 37ºC CO2 incubator, 5 percent CO2, 95 percent air and 95 percent relative humidity. Cell viability was

evaluated by the Trypan blue exclusion[10]. A cytotoxic effect on AF2/SG-1 and curcumin (10-400µg/ml) DLA cells was assessed[11].

RESULTS AND DISCUSSION

The aqueous fraction was subjected to preliminary anthelmintic screening on *Megascolex mauritii* owing to their resemblance both anatomically and physiologically with intestinal round worm parasite found in human beings. The aqueous extract at a concentration of 40mg/ml showed comparable activity with the standard drug albendazole and piperazine citrate. The active fraction (AF1) showed better activity at a concentration of 20 mg/ ml compared to the standard drug albendazole and piperazine citrate. The effect of the active fraction and the isolated compound are summarized in Table No.1.

The AF1 isolated from aqueous extract showed good anthelmintic activity. The percentage activity of the active fraction (AF1) was compared with the standard drugs albendazole (10mg/ml) and piperazine citrate (10mg/ml). Figure No.1 displays the percentage operation graphically, and Figure No.2 shows the anthelmintic screening conducted on *Megascolex mauritii*.

Characterization of SG-1

The structure of the isolated compound (SG-1) was determined following various spectral analysis like UV, IR, NMR, GC-MS etc. the results are depicted below.

SG-1 was obtained as a white crystalline powder from AF1 isolated from aqueous extract with melting point 69-72ºC with 0.009% yield from dried leaf powder. The compound gave a positive fluorescence test for coumarins. The UV spectrum in $CHCl_3$ showed absorption maxima (λmax) at 310nm. The IR spectrum in KBr showed the presence of pyrone carbonyl group (1707cm^{-1}), C=C (1600cm^{-1}), aromatic ring (1448). The GC-MS spectrum of SG--1 revealed the molecular ion peak at *m/z* 146, a base peak at *m/z* 118. Besides the molecular ion peak the mass spectrum showed other prominent fragment ion at *m/z* 90.

The ^1H NMR spectrum of SG-1 indicated resonances for an H3 proton at δ 6.48 (dd). The H4 proton was observed at δ 7.36 (m).

^{13}C NMR spectral data of SG-1 indicated resonances for nine carbon atoms. The spectrum showed the presence of a carbonyl carbon at δ 159.93ppm and other carbons at δ 153.48, 144.22, 131.95, 128.43, 124.48, 118.72, 116.25, 116.18ppm.

X axis- concentration of AF1; Y axis- % activity; AF1- Active fraction-1; ALB- Albendazole; PIP- Piperazine; concentration of albendazole and piperazine citrate was kept constant at 10 mg/ ml

The anthelmintic activity of SG-1 was performed on eggs of *Ascaridia galli* and *Dipylidium caninum* (Egg hatch assay). The compound SG-1 showed better activity compared to albendazole at a concentration of 10mg/ml. The results are tabulated in Table No.2.

Cytotoxicity of SG-1

The cytotoxicity of the isolated compound (SG-1) was studied on DLA cells. The compound 2 *H*-chromen-2-one showed slight cytotoxicity. The percentage cell death of DLA cells corresponding to the various concentration of the isolated compound was reported and summarized in Table No.3.

Discussion

The anthelmintic potential of the aqueous extract of S. grandiflora was studied and found to possess potential activity, a promising anthelmintic fraction was isolated from aqueous extract. A potent anthelmintic compound was isolated from the fraction and the compound showed better activity compared to standard drug albendazole. The compound showed moderate cytotoxity to DLA cells. The compound is reported to be non-toxic to normal cells[9]. From the spectral data the compound isolated was found to be 2 *H*-chromen-2-one and the structure of the compound is given in Figure No.9.

Table No.1: Effect on *Megascolex mauritii*

S.No	Extract/ Compound	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
1	Aqueous extract	10	44.8 ± 0.75	86.7 ± 1.2
		20	23.1 ± 1.02	43.1 ± 0.92
		30	14.1 ± 0.58	25.7 ± 0.61
		40	8.9 ± 0.38	17.5 ± 0.45
2	Anthelmintic Fraction (AF1)	0.5	12 ± 0.45	18.4 ± 0.86
		1	9.3 ± 0.41	14.6 ± 0.58
		10	4.8 ± 0.26	10.92 ± 0.38
		20	3.1 ± 0.2	8.7 ± 0.41
3	Piperazine citrate	10	10.5 ± 0.7	14.3 ± 0.6
4	Albendazole	10	4.1 ± 0.2	12.7 ± 1.3

The values were given as data ± SD; n= 6; p< 0.05

Table No.2: Egg hatch assay

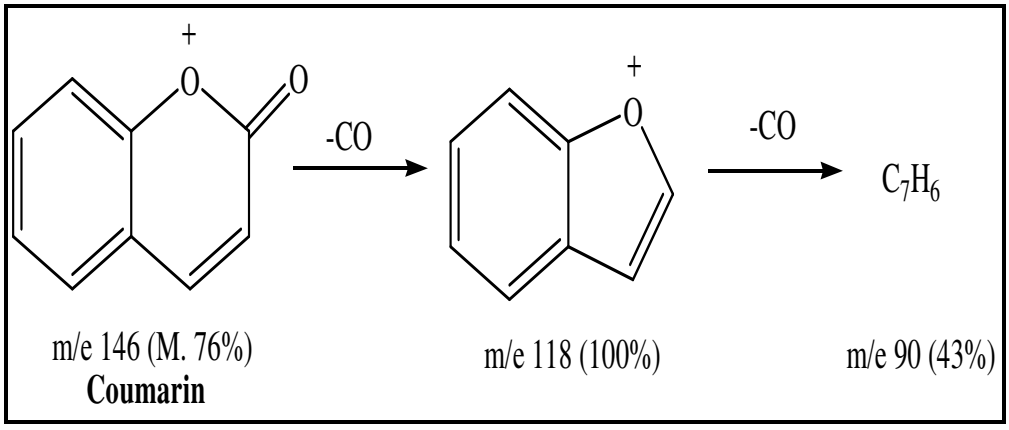
S.No	Extract/ compound	Concentration	Percentage Death of Eggs
1	SG-1	0.5	52.5 ± 2.2
		1	82 ± 2.3
		10	93.6 ± 2.3
2	Albendazole	10	83.3 ± 2.3

The values are given in data ± SD; n = 3

Table No.3: Cytotoxicity of SG-1

S.No	Concentration	% Cell Death of Dla Cells
1	62.5	18.7±0.58
2	125	35±1
3	250	54.7±2.5
4	500	79.7±2.1

The values are given in data ± SD; n = 3



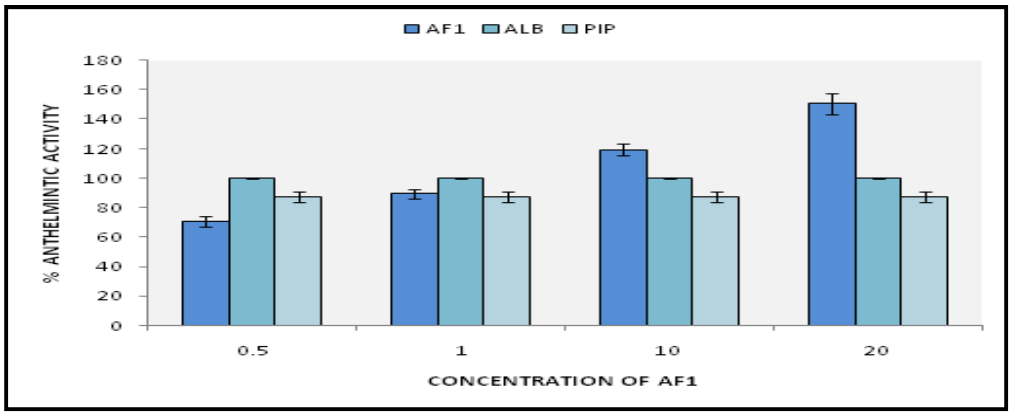


Figure No.1: Percentage anthelmintic activity of AF1

Figure No.2: Anthelmintic screening done on *Megascolex mauritii*

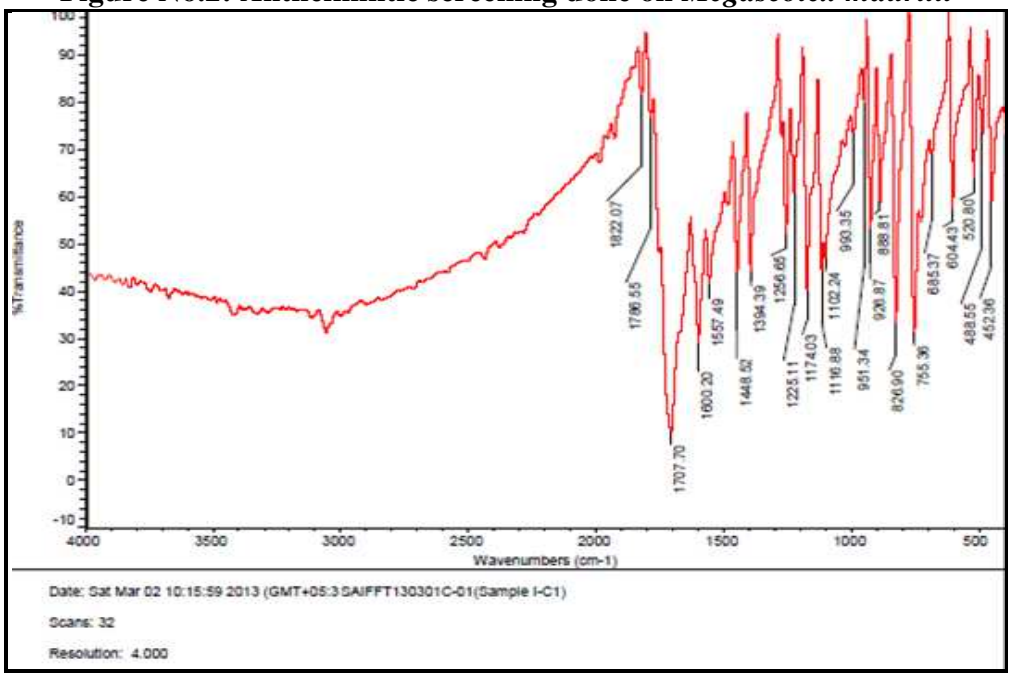


Figure No.3: IR spectra of SG-1

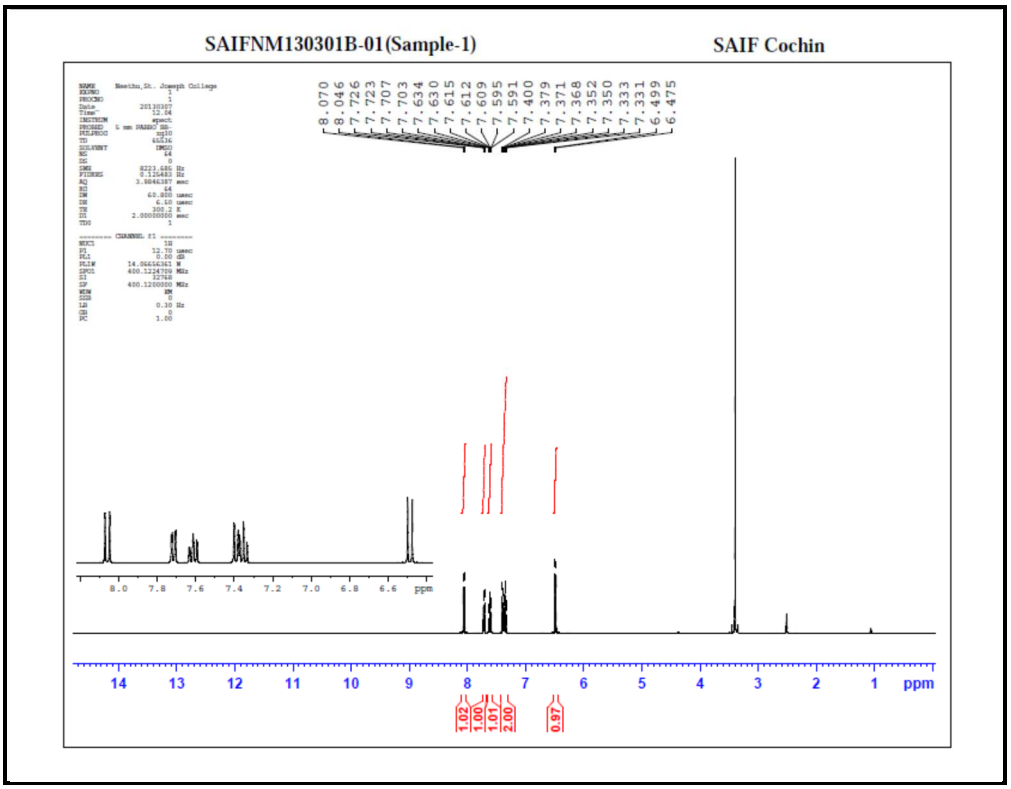


Figure No.4: H1- NMR SPECTRA

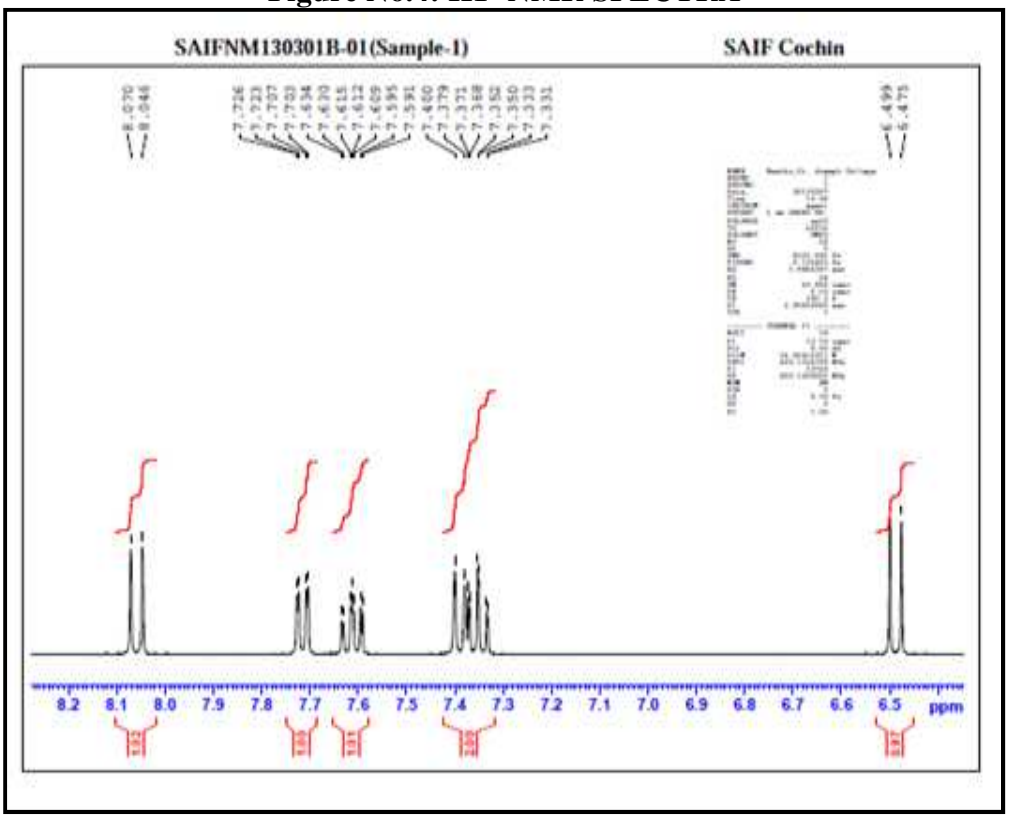


Figure No.5: H1- NMR SPECTRA

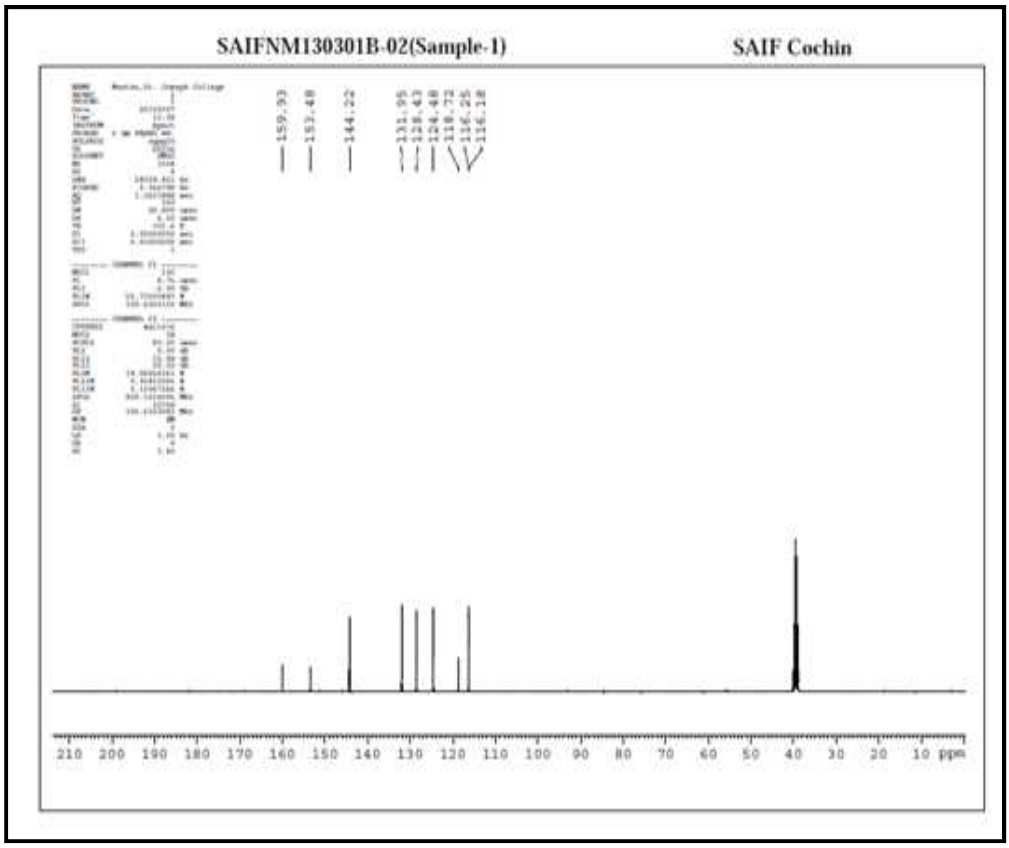


Figure No.6: C-13 NMR SPECTRA

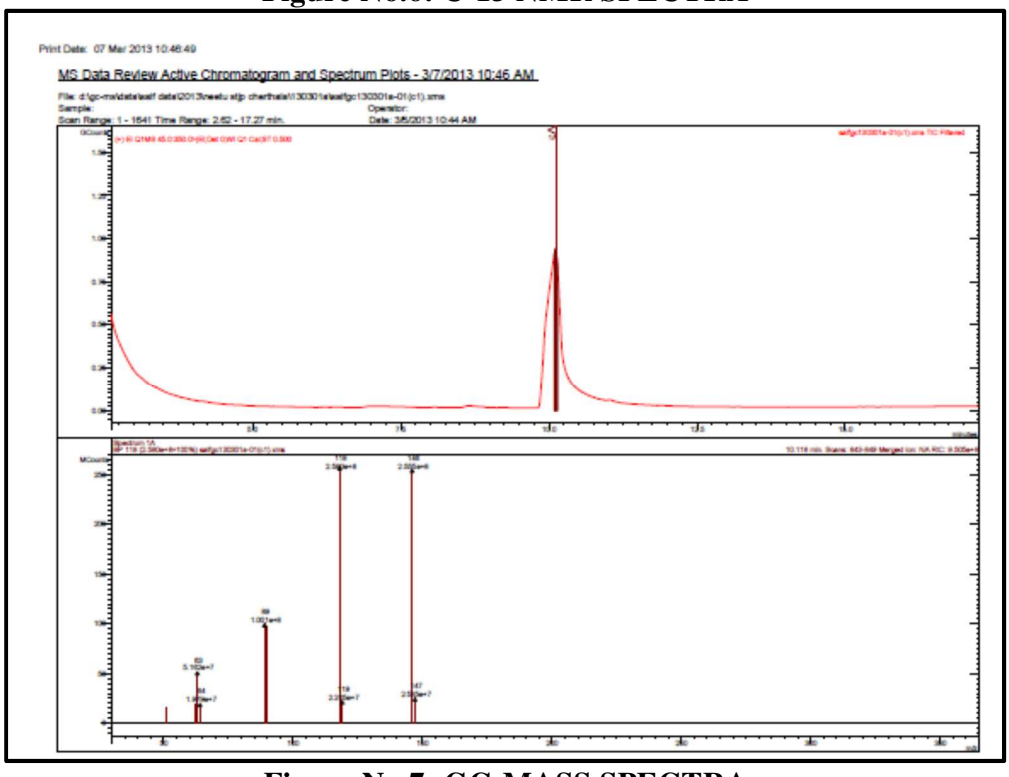


Figure No.7: GC-MASS SPECTRA

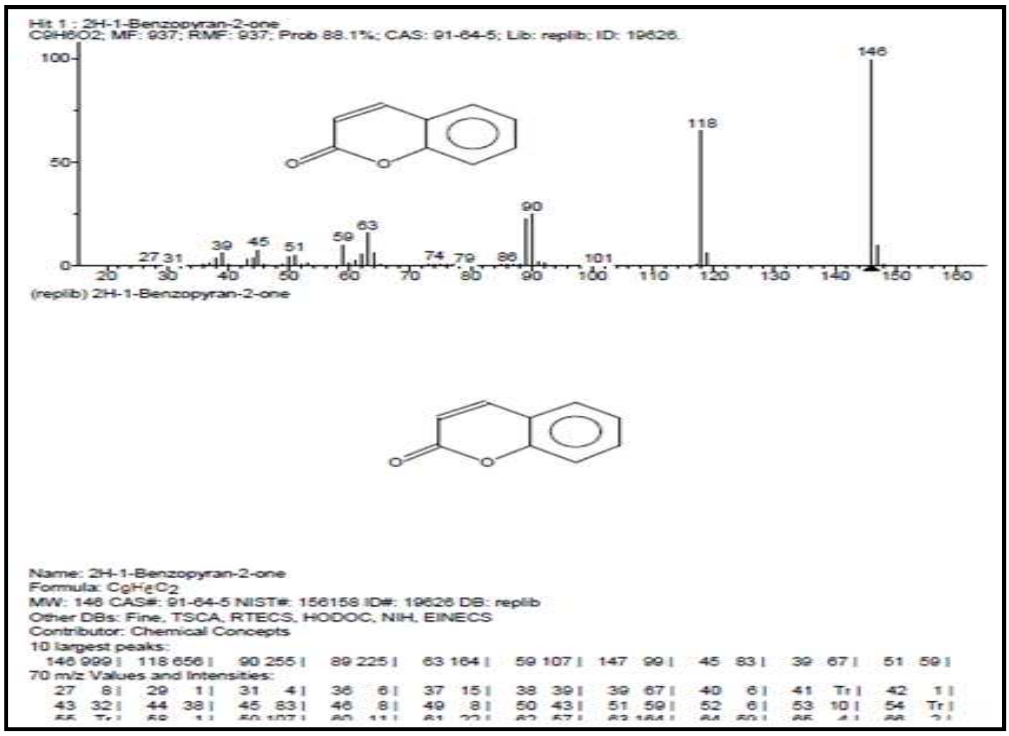


Figure No.8: Mass spectra

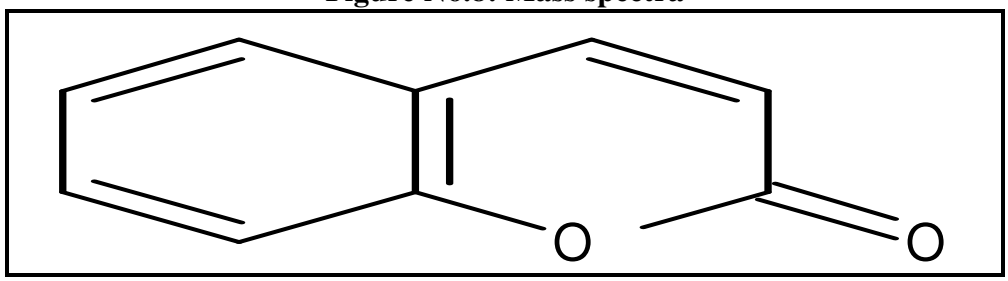


Figure No.9: *2H*-chromen-2-one

CONCLUSION

Helminth infection can promote carcinogenesis through various mechanisms[11]. Helminth infections if controlled, can act as a preventable cause of cancer[12] and thereby decrease about 16 % of global cancer burden. Helminthic infections are very common in and around Kerala. Therefore, the study can provide relief to the economically poor people living in coastal areas of Kerala since it can provide relief to both helminthiasis and cancer. The isolated compound may further act as lead molecule and provide new opportunities to develop new drugs and can be used for further research.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Dm Wims College of Pharmacy, Naseera Nagar, Meppadi Po, Wayanad, Kerala, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

BIBLIOGRAPHY

1. Hu Y, Xiao S H, Aroian R V. The New Anthelmintic tribendimidine is an l-type (levamisole and pyrantel) nicotinic acetylcholine receptor agonist, *PLoS Neg Tro Dis,* 3(8), 2009, e499.

2. Mohammed M S, Mohammed M, Yusuf O A, Joseph O A. Anthelmintic activity of the crude methanol extracts of *Xylopia* a*ethiopica* against *Nippostrongylus brasiliensis* in rats, *Veterinarski Arhiv,* 75(6), 2005, 487-495.
3. Keiser J and Utzinger J. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis, *JAMA,* 299(16), 2008, 1937-1948.
4. Smits H L. Prospects for the control of neglected tropical diseases by mass drug administration, *Expert Review of Anti Infective Therapy,* 7(1), 2009, 37-56.
5. Mayer D A and Fried B. The role of helminth infections in carcinogenesis, *Advances in Parasitology,* 65, 2007, 239-296.
6. Vennervald B J and Polman K. Helminths and malignancy, *Parasite Immunology,* 31(11), 2009, 686-696.
7. Venkateshwarlu V, Arunachalam G and Ravichan V. Anthelmintic potential of prosopis cineraria (Linn) druce stem bark, *Asian Journal of Plant Sciences and Research,* 1(2), 2012, 88-91.
8. Wagner H, Bladt S and Zgainski E M. Plant drug analysis, *Berlin, Heidelberg, Springer-Verlaag, New York,* 2nd Edition, 1996, 384.
9. Coles G C, Bauer C and Borgsteeded M. World association for the advancement of veterinary parasitology methods for the detection of anthelmintic resistance in nematodes of veterinary importance, *Veterinary Parasitology,* 44(1-3), 1992, 35-44.
10. Osman N H, Chong W K, Awang K & Manshoor N. Isolation and characterisation of l, l'-binaphthalene-2, 2'-diol, a new biaryl natural product from *Sesbania grandiflora* root, *Journal of Basic and Applied Sciences,* 8, 2018, 253-256.
11. Zarena A S, Gopal S and Vineeth R. Antioxidant, antibacterial and cytoprotective activity of a Protein isolated from Sesbania Grandiflora Linn. Leaves, *Asian Pacific Journal of Tropical Biomedicine,* 1, 2012, 1-7.
12. Ouattara B, Konate K, Kiendrebeogo M, Ouattara, Compaore M, Meda R, Millogo R J and Nacoulma O G. Antibacterial potential and Antioxidant activity of Polyphenols of *Sesbania Grandiflora, Current Research Journal of Biological Sciences,* 3(4), 2011, 351-356.

Please cite this article in press as: Neethu Sara Varghese. Isolation and characterization of anthelmentic component-Coumarin from leaves of *sesbania grandiflora Linn*, *Asian Journal of Phytomedicine and Clinical Research,* 8(4), 2020, 175-184.