www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

GC-MS analysis of bioactive compounds and comparative antibacterial potentials of aqueous, ethanolic and hydroethanolic extracts of *Senna alata L* against enteric pathogens

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ABSTRACT

In this investigation, aqueous, ethanolic and hydroethanolic extracts of *Senna alata* was analysed for antibacterial property. For this, extracts of *Senna alata* was studied against selected enteric pathogenic bacteria includes *Bacillus subtilis* ATCC441, *Staphylococcus aureus* ATCC25923, *E.coli* ATCC25922, *Pseudomonas aeruginosa* ATCC2785, *Enterococcus feacalis* ATCC29212 and *Klebsiella pneumoniae* ATCC15380 and showed growth inhibition activity. The results of different extracts were compared based on zone of inhibition in mm and it was found that, hydroethanolic extract was found to be most effective. This hydroethanolic was further subjected into GC-MS analysis to identify the bioactive compounds present in the plant extract. We have found 7 different compounds are present in the extract.

Keywords: *Senna alata*; Antioxidant; Antibacterial; bioactive compounds; enteric pathogens.

INTRODUCTION

India is a country with lot of people who believes in traditional medicine practices for the treatment of wide range of diseases using herbals collected from the natural resources (Akana, 1992). The plant and its derived chemical compounds with valuable active bio compounds such as metabolites, antioxidants leads to development of new drugs against infections (Bnouham *et al*., 2006). The active bio compounds of medicinal plants can be used to treat diabetic conditions by evaluating it to development of active fractions against it (Mahesar *et al*., 2010).

Senna alata have been identified with proven therapeutical significances and examined for antimicrobial activity and found with strong inhibitory effect against P*ropionibacterium acnes* and *Staphylococcus epidermis* with MIC values of 0.625 and 2.5 µg/ml respectively (Chomnawang *et al*., 2005).

Senna alata plants were subjected to physic-chemical and microbiological activities. *Senna alata* was tested against many infectious agents and identified that MIC ranged from 3-10mg/ml for bacteria and minimum bactericidal concentration ranged 25-50 mg/ml for fungi respectively (Ehiowemwenguan *et al*., 2014).

The main objective of this study is to assess the antibacterial potential of aqueous, ethanolic and hydroethanolic extract of *Senna alata* against selected enteric pathogenic bacteria and the major bioactive compounds present in the most potential extract

MATERIALS AND METHODS

Plant materials

Senna alata used in this study were collected from Thirukkalukundram, Kanchipuram District, Tamil Nadu, South India. The plant was identified by the experts of Botanical Survey of India, Coimbatore, India. The collected plant sample was refluxed in running tape water for 1-2 h and shade dried at room temperature for 8 – 10 days. Aqueous, ethanolic and hydroethanolic extract of *Senna alata* was prepared (Mohanasundaram *et al*., 2016) using soxhlet apparatus for about 24h. The extract was distilled and concentrated in vacuo with addition of $CaCl_2$. Lyophilized fractions were further used for further studies.

Microbial cultures

Standard strains of *Bacillus subtilis* ATCC441, *Staphylococcus aureus* ATCC25923, *E.coli* ATCC25922, *Pseudomonas aeruginosa* ATCC2785, *Enterococcus feacalis* ATCC29212 and *Klebsiella pneumoniae* ATCC15380 *were* procured from Karpaga Vinayaga Institute of

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Received on: 05-12-2016
Revised on: 23-01-2017
Accepted on: 28-01-2017

Table 1: Antibacterial activity of aqueous extract of Senna alata L leaves

Concentration in µg		Diameter of the zone of inhibition (mm)					
		A	B	C	D	E	F
] Senna alata L	12.5	NI	NI	NI	NI	NI	NI
	25	NI	NI	NS	NS	NI	NI
	50	NS	NI	NS	NS	NI	NI
	100	NS	NS	11 ±0.3	NS	NI	NI
Streptomycin	10	10 ±0.8	11±0.8	13 ±0.3	12 ±0.4	12±0.9	12±0.3

NS – Non significant value (<10mm) NI – No Inhibition *A* - *Bacillus subtilis* ATCC441; B - *Staphylococcus aureus* ATCC25923; C - *E.coli* ATCC25922; D - *Pseudomonas aeruginosa* ATCC2785; E - *Enterococcus feacalis* ATCC29212 and F - *Klebsiella pneumoniae* ATCC15380

Table 2: Antibacterial activity of ethanolic extract of Senna alata L leaves

Concentration in µg		Diameter of the zone of inhibition (mm)					
		A	B	C	D	E	F
] Senna alata L	12.5	NI	NI	NI	NI	NI	NI
	25	NI	NI	NS	10 ±0.9	NI	10 ±0.1
	50	10 ±0.1	NI	10 ±0.5	11 ±0.1	10 ±0.2	11 ±0.6
	100	10 ±0.3	11 ±0.2	11 ±0.6	11 ±0.9	10 ±0.9	12 ±0.7
Streptomycin	10	10 ±0.7	11±0.6	13 ±0.4	12 ±0.7	12±0.7	13±0.4

NS – Non significant value (<10mm) NI – No Inhibition *A* - *Bacillus subtilis* ATCC441; B - *Staphylococcus aureus* ATCC25923; C - *E.coli* ATCC25922; D - *Pseudomonas aeruginosa* ATCC2785; E - *Enterococcus feacalis* ATCC29212 and F - *Klebsiella pneumoniae* ATCC15380

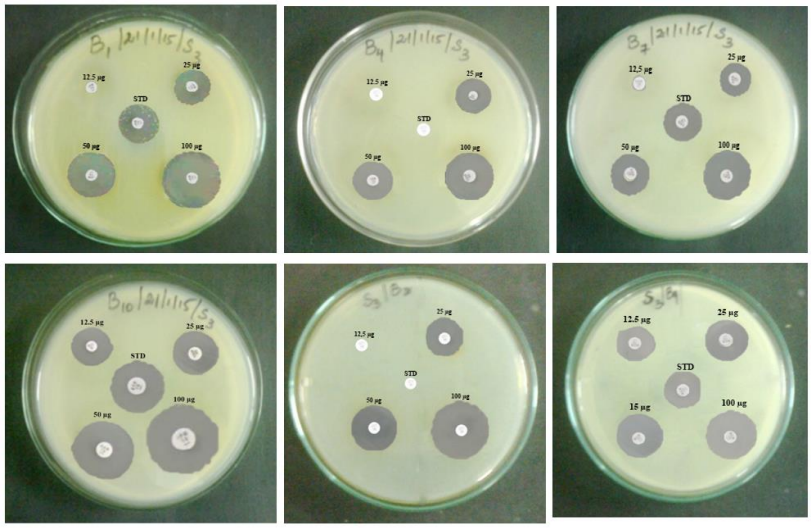

Figure 1: Antibacterial activity of 50% hydroethanolic extract of Senna alata L leaves

B1 - *Bacillus subtilis* ATCC441; B4 - *Staphylococcus aureus* ATCC 25923; B7 - *E. coli* ATCC25922; B10 - *Pseudomonas aeruginosa* ATCC2785; B2 - *Enterococcus feacalis* ATCC 29212; B9 - *K. pneumoniae* ATCC 15380

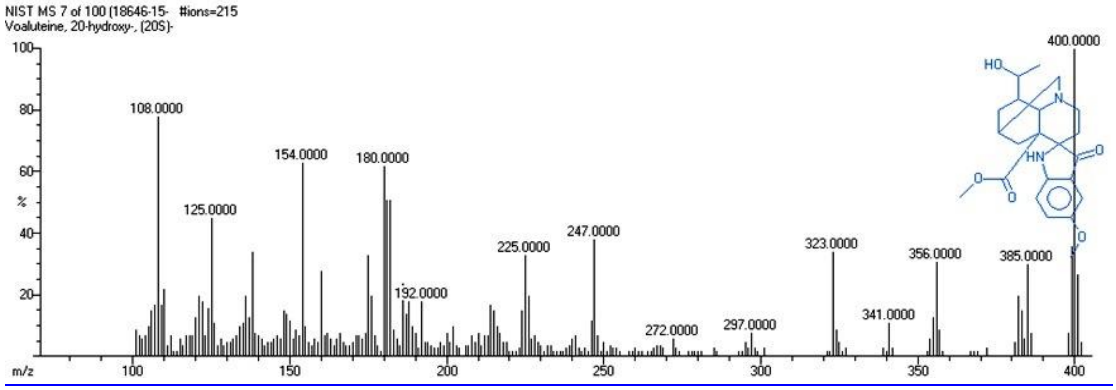

Figure 2: GC-MS spectrum of Compound 1

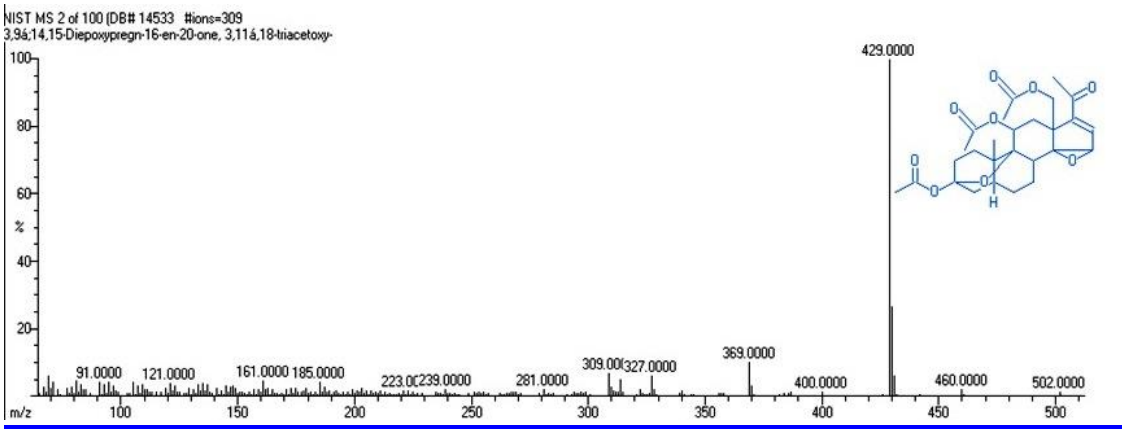

Figure 3: GC-MS spectrum of Compound 2

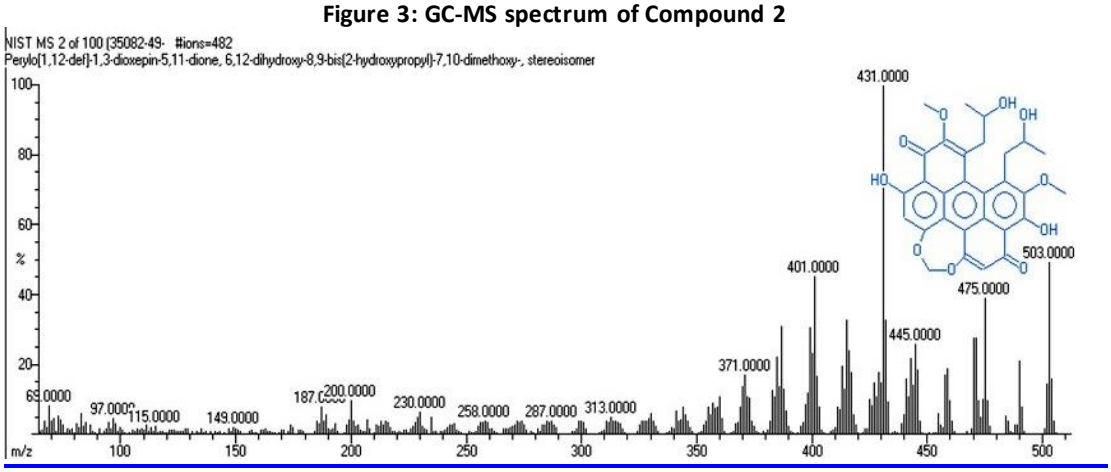

Figure 4: GC-MS spectrum of Compound 3

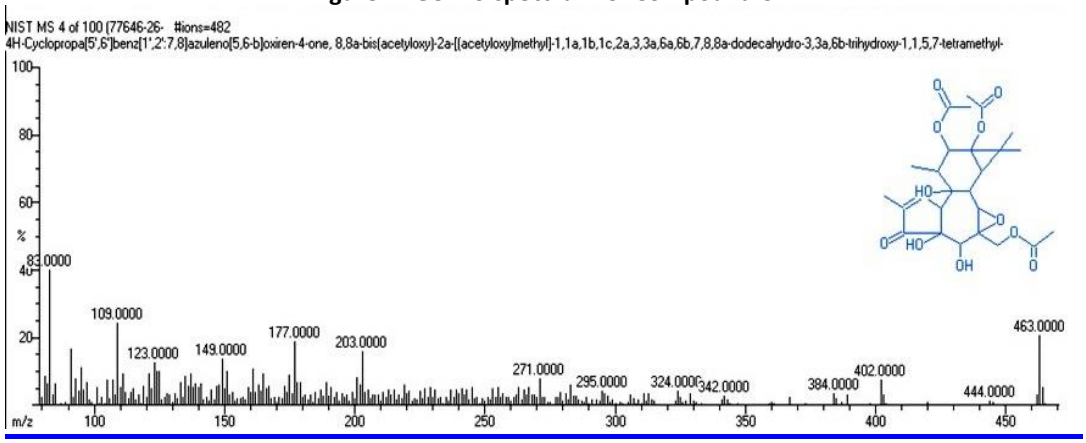

Figure 5: GC-MS spectrum of Compound 4

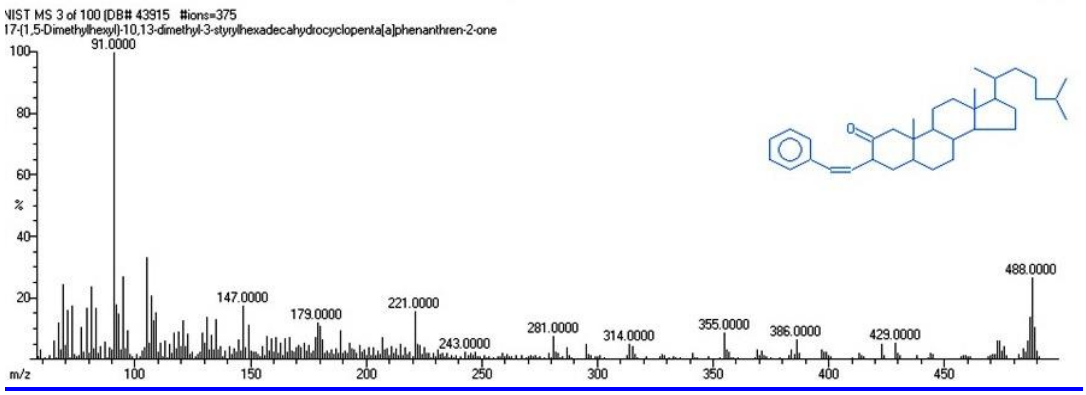

Figure 6: GC-MS spectrum of Compound 5

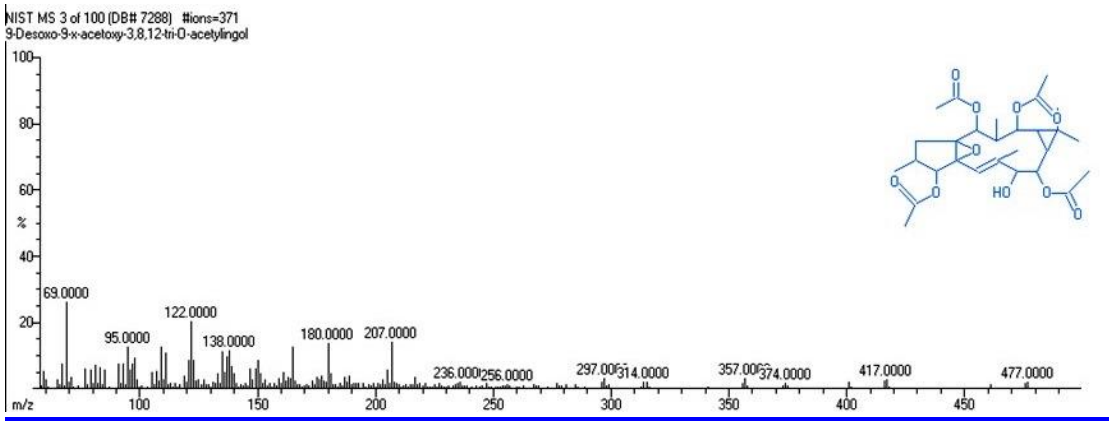

Figure 7: GC-MS spectrum of Compound 6

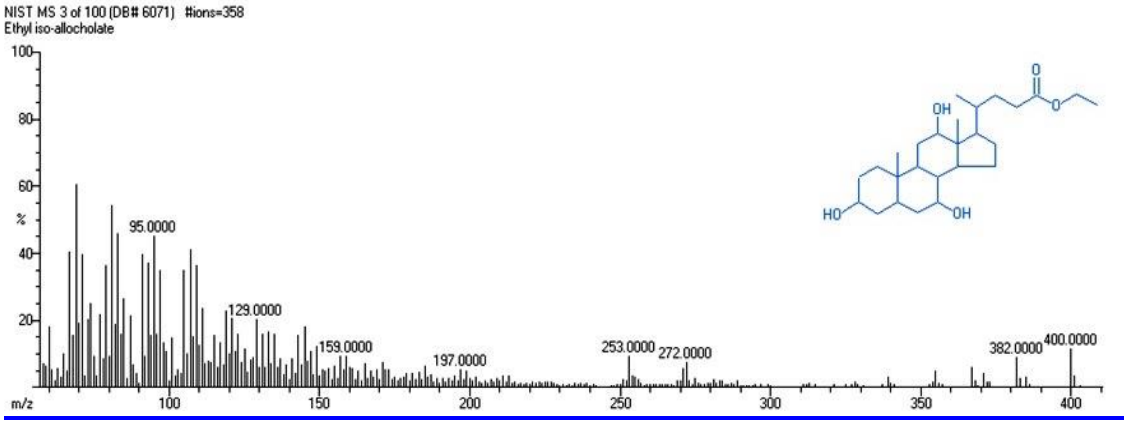

Figure 8: GC-MS spectrum of Compound 7

Table 3: Antibacterial activity of 50% hydroethanolic extract of Senna alata L leaves

Concentration in µg		Diameter of the zone of inhibition (mm)					
		A	B	C	D	E	F
] Senna alata L	12.5	0NS	**0**NI	NS	13±0.2	NS	12±0.3
	25	11±0.2	11±0.3	10±0.2	15±0.2	11±0.1	15±0.2
	50	15±0.1	14±0.2	13±0.1	19±0.3	13±0.2	17±0.3
	100	18±0.1	17±0.1	15±0.3	22±0.2	15±0.3	19±0.2
Streptomycin	10	12±0.2	NS	11±0.2	17±0.1	NS	12±0.1

NS – Non significant value (<10mm) NI – No Inhibition *A* - *Bacillus subtilis* ATCC441; B - *Staphylococcus aureus* ATCC25923; C - *E.coli* ATCC25922; D - *Pseudomonas aeruginosa* ATCC2785; E - *Enterococcus feacalis* ATCC29212 and F - *Klebsiella pneumoniae* ATCC15380

Table 4: Compounds identified in GC-MS analysis of 50% hydroethanolic extract of Senna alata through NIST Library interpretation

S.No	RT	Compound
1	14.23	Voaluteine, 20-hydroxy, (20s)
2	16.95	3, 9a; 14,15 diepoxypregn-16-en-20-one, 3,11a, 18 triacetoxy
3	17.65	Perylo (1,12-def)-1,3-dioepin-5,11-dione, 6,12, dihydroxy-8,9-bis (2-hydroxy propyl)-7,10-dimethoxy stereoisomer
4	18.05	4H-cyclopropa(5',6')-benz (1',2',7,8) azuleno (5,6'-b)oxiren-4-one, 8,8a-bis (acetloxy)-2a-[(acetyloxy)methyl]-,1a,1b,1c,2a,3,3a,6a,6b,7,8, 8a-dodecahydro-3,3a,6b trihydroxy-1,1,5,7 tetramethyl
5	19.1	17-(1,5-dimethylhexyl)-10,13-dimethyl-3'-styryl hexa deca hydro cyclo penta (a) phenanthren-2'-one
6	19.73	9-desoxo-9-x-acetoxy-3,8,12 tri-o-acetylingol
7	20.47	Ethyl–iso-allocholate

Medical Sciences, Padalam, Kanchipuram – Dist, India. Solvent and other chemicals which were used during this study were purchased from Himedia, Merck and s.d. Fine-Chemicals, Mumbai.

Antibacterial activity assessment

The antibacterial activity of aqueous, ethanolic and hydroethanolic extracts of *Senna alata* L was evaluated by a proven method (Mohanasundaram *et al*., 2011) with slight modification. Agar medium was prepared with a uniform depth of 4 mm. The bacterial cultures were inoculated by spread plating technique. In this study, standard strains of *Bacillus subtilis* ATCC441, *Staphylococcus aureus* ATCC25923, *E.coli* ATCC25922, *Pseudomonas aeruginosa* ATCC2785, *Enterococcus feacalis* ATCC29212 and *Klebsiella pneumoniae* ATCC15380 were used and the various concentrations from 12.5 to 100µg of extracts were added. The control experiment was carried out with streptomycin (10µg). The inhibition zone formation in mm was measured, compared among different extracts and analyzed.

Gas Chromatography - Mass Spectrometry

The hydroethanolic solvent extract was analyzed further by Gas chromatography and Mass spectrometry. For this, the oven temperature programs to start at 35^{o}C, hold for 2 minutes, and then ramp at 20^{o}C per minute to 300^{o}C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode). Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Mass spectrometry library search

Identification of the components of the purified compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software.

RESULTS AND DISCUSSION

To understand the efficacy, aqueous, ethanolic and hydroethanolic extracts, were studied separately and the results were tabulated (Table 1, 2 and 3). From the results, we have found that, *Senna alata* L is having significant antibacterial effect and the results are shown in Table 1, 2 and 3. Among others, hydroethanolic extract showed most significant inhibitory effect on the growth of bacteria.

The hydroethanolic extract of *Senna alata* leaves extract inhibits the growth of human pathogens at various concentration by well diffusion method and the zone of inhibition observed was at the maximum of 22±0.2 mm and least of 10±0.2 mm for the different test species processed. From the results (Figure 1), it was found that, *Pseudomonas aeruginosa* ATCC2785 is highly sensitive to *Senna alata* extract and *E.coli* ATCC25922 is highly resistance among others. Our results are in line with a study stating that the aqueoeus and ethanolic extracts of *Senna alata* leaves and barks were studied for antimicrobial properties against *E.coli, Staphylococcus aureus, Candida albicns, Aspergillus fumigatus* by well diffusion method showed significant inhibition and zones were observed in the ranges between 10-17mm and ethanolic extracts were more active than the aqueoeus extracts of *Senna alata* (Somchit *et al.,* 2003). The minimum inhibitory concentration values were evaluated against *Staphylococcus aureus, Streptococcus feacalis, Micrococcus luteus, Bacillus subtilis* and *Pseudomonas putida* using broth dilution method and found to be 500 µg/ml of the stock concentration and the preliminary phytochemicals screening also shows the presence of secondary metabolites likes of steroids, glycosides, anthraquinones, volatile oils and tannins (Adedayo *et al.,* 2001).

In qualitative screening of phytochemicals, we have found (Doss VA *et al*., 2016), *Senna alata*, as a rich source of alkaloids, triterpenoids, steroids, flavonoids, Tannins, Phenols, Phlobatannins, anthraquinones, Quinones, Cyanins, reducing sugars and proteins which might play significant role in pharmacological value of this plant.

From the compounds we identified (Table 4 and Figures 2 – 8), the pharmacological significance and medicinal values for all the compounds should be studied further to under the exact mechanism.

CONCLUSION

The research carried shows the potentials of hydroethanolic extracts of *Senna alata* leaves and its pharmacological properties. The development of phytocompounds as drugs for the treatment of many diseases by replacing the synthetic drugs in order to avoid side effects is the main objective of this study. The further research on its valuable compounds responsible for the biological activity and its potential has to be performed.

ACKNOWLEDGEMENT

The authors grateful to Research and Development Centre, Bharathiar University, Coimbatore for approving this project. We are very much thankful to Mrs. Meenakshi Annamalai, Director, Karpaga Vinayaga College of Engineering and Technology for the continuous support throughout the completion of this project successfully.

CONFLICT OF INTEREST

We declare that no conflict of interest.

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