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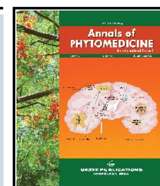

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Chrysopogon nodulibarbis (Hochst. ex Steud.) Henrard: A pharmacotherapeutical approach

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Abstract

The paper aimed to explore the bioactive potential of *Chrysopogon nodulibarbis* (Hochst. ex Steud.) Henrard. The shade dried plant powder (whole aerial part) was extracted with ethanol, ethyl acetate, acetone and water. Phytochemical screening and GC-MS analysis were carried out using standard procedures. Antioxidant activity was conducted using 2, 2, Diphenyl-1-picryhydrazyl (DPPH) assay. Antimicrobial evaluation was performed using the agar Well-diffusion method. The anticancer activity was evaluated on oral cancer cells. Phytochemical screening exhibited the presence of alkaloids, flavonoids and phenols in the ethanol extract. GC-MS analysis reflected 25 chemical constituents. Potential antioxidant activity with highest value 82.4 ± 0.91 at 16 mg/ml of ascorbic acid equivalent was recorded. Ethanol extract exhibited antimicrobial activity against bacteria and fungi with minimum inhibitory concentrations (MICs) were 1.5 mg/ml, 4 mg/ml, 0.13 mg/ml and 0.25 mg/ml. The anticancer activity of ethanolic extract against cancerous cell lines with a very low cell survival rate observed in 500, 250 and 125 µg/ml concentration. The conclusively established anticancer activity was also validated by *in silico* receptor binding predicted energy levels and receptor-site docking feasibility.

1. Introduction

Natural medicines join the basis for pharmaceutical medicine. The primary source of natural medicine is the plant, owing to its chemical and structural diversity and the biodiversity of its components. The most significant plant bioactive chemicals have been identified as flavonoids, alkaloids, tannins and phenolic compounds. Higher levels of flavonoids and phenolics of various structural templates as constituents of plant extracts have abundantly revealed varying roles of antioxidant activity of plants in hostile environments for their bio action against microorganisms. They have been associated with lowering the risk of cancer by postponing or reversing the process of carcinogenesis by preventing or inhibiting COX-1, COX-2 and DNA topoisomerase 1 enzymes (Ritesh and Sanmati, 2010).

C. nodulibarbis belongs to the Poaceae or Gramineae family which is the fourth largest flowering plant. The genus *Chrysopogon* with about 45 species is mostly distributed in the Old World tropics. In India, the genus (including *C. zizanioides*) is known to have 16 species. The fresh rhizome paste of *C. aciculatus* is known to cure stomach ache and gastric disorder (Harasourav and Subrata, 2015). The stem decoction of *C. zizanioides* is effective against treating urinary tract infections. By working as a nervine tonic and easing anxiety, the essential oil soothes the neurological and circulatory systems. A recent study revealed that *C. zizanioides* extract has antiageing qualities and enhances skin moisture and shine (Madhuri *et al*., 2021). Till date, no studies have been carried out in *C. nodulibarbis* species. Hence, it remains necessary to exploit the bioactive potential of this species.

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2. Materials and Methods

2.1 Plant sample

The plant *C. nodulibarbis* used in the present study was collected from Udhagamandalam during the month of December. The plant was identified, authenticated and voucher specimen is submitted to the Department of Botany, PSG College of Arts and Science, Coimbatore, Tamilnadu, India

2.2 Preparation of extract

The plant was shade dried and reduced to coarse powder using a mechanical grinder. The powdered plant (20 g) was extracted for 8 h over heat with 250 ml of ethanol, ethyl acetate, acetone and water using Soxhlet apparatus. The crude extracts were then dried using a rotary evaporator and stored at – 20°C until further use.

2.3 Phytochemical analysis of the extract

The phytochemical screening of the *C. nodulibarbis* extracts was performed according to the standard procedures - Wagner's test for alkaloids, Molisch's test for carbohydrates, alkaline reagent test for flavonoids, ferric chloride test for phenolic compounds, foam test for saponins, Braymer's test for tannins, Salkowski tests for steroids and terpenoids, Millon's test for proteins and quinines test (Harborne, 1998; Kokate, 2005).

2.4 DPPH free radical scavenging activity

The free radical scavenging activity of *C. nodulibarbis* extracts was evaluated using 2,2, Diphenyl-1-picryhydrazyl (DPPH) assay described by Ara and Nur (2009). About 100 µl of extracts were taken in different test tubes, followed by 3.7 ml of absolute methanol. 200 µl of DPPH reagent was added to the test tubes including blank. The mixture was vortexed for 15-30 sec and incubated at dark for 30 min at room temperature and its absorbance was read at 517 nm using UV-Visible spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The radical scavenging activities of the tested samples percentages were calculated according to the following equation:

% inhibition = [(control absorbance – absorbance of sample)/ control absorbance] × 100

where A_A and A_B are the absorbance values of the test and the blank samples. From a per cent inhibition concentration curve, the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value. The results are presented as means ± SEM of three independent experiments.

2.5 Antimicrobial activity test

The antimicrobial activity of the *C. nodulibarbis* extracts was determined using the agar Well-diffusion bioassay method described by Jahir and Naveen (2011). Mueller Hinton agar plates were seeded with 0.1 ml bacterial suspension (equivalent to 10^7–10^8 CFU/ml) and sabouraud dextrose agar plates seeded with fungal strain (0.5-2.5×10^3). A standard antibiotic was used as positive control and 100 µl of respective solvents as negative controls were added in each well. The seeded plates were incubated for12 h at 37°C, allowed to set. Subsequently, the agar plates were grown on Mueller-Hinton for 18-24 h and verified for the presence of pure colonies. The diameters of zones of inhibition were measured and compared to 0.5 McFarland range as standards. The results are presented as mean ± SEM of three independent experiments.

2.6 Determination of minimum inhibition concentration

The MIC values were determined using the Micro-dilution method described by Teh *et al.,* (2013). Nutrient broth culture (for bacteria) and 4 days of incubation culture (for fungus) was utilized. The culture was adjusted to obtain turbidity comparable to that of the turbidity of 0.5 McFarland standards. Under aseptic conditions, 96 well micro titer plates were used for Resazurin based Micro titer dilution assay. The first row of the micro titer plate was filled with different concentrations of test materials in 10% (v/v) DMSO or sterile water. All the wells of micro titer plates were filled with 100 µl of nutrient broth and 10 µl of indicator resazurin solution was added in each well. Finally, a volume of 10 µl was taken from bacterial and fungal suspension and then added to each well to achieve a final concentration of 5×10^6 CFU/ml. The plates were incubated in a temperature controlled incubator at 37°C for 24 h. A change in color from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value.

2.7 Cell culture

The ethanol extract of *C. nodulibarbis* was evaluated against cytotoxic capacity of the front oral cancer cell line obtained from National Center for Cell Science (NCCS), Pune. Cells were cultured in Dulbecco's modified Eagles medium (DMEM) with 10% v/v Fetal Bovine Serum (FBS) with Penicillin G sodium (100 IU/ml) and Streptomycin (100 µg/ml) as a complete growth medium (CGM).

2.8 Antiproliferative (cytotoxic) activity

Antiproliferation (cytotoxic activity) of plant extract on KB cells was evaluated by the MTT (3- [4, 5-dimethyl- 2-thiazolyl]-2, 5-diphenyl -2H tetrazolium bromide) assay (Mosmann, 1983). The KB cells in the logarithmic phase were seeded in to 96 well flat bottom micro titer plates at their optimal cell density of approximately 1.2 x 10^4 cells per well, and allowed for cell attachment overnight at 37°C. The medium was then discarded and the cells were treated with fresh medium containing different concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.12 µg/ml) of the ethanol extract of the plant dissolved in DMSO and incubated for 48 h at 37°C. DMSO (0.2%) solvent used as negative control. Cells were incubated for 48 h at 37°C and then 20 µl of MTT (5 mg/ml) was added to each well. The cells were maintained at 37°C for a further 4 h. After incubation, the medium in the supernatant was removed and 100 µl of DMSO was added to each well to dissolve the insoluble purple formazan crystals and mixed well. The cell viability was determined by measuring the optical density (OD) of each well at 540 nm by a micro plate reader. All experiments were performed in triplicates. The concentration of the extract causing 50% inhibition of cancer cell growth was considered as IC_{50}. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by 100% relative viability. Cell survival was calculated by the following formula:

Cell viability% = (Test OD/Control OD) × 100/Cytotoxicity % = 100 – Viability %.

2.9 Gas Chromatography and Mass Spectrophotometric (GC-MS) analysis

GC-MS analysis of ethanolic extract was carried out the method described by Ragavi *et al.,*(2010) in *C. nodulibarbis*. The chromatograph interfaced to a mass spectrometer (GC-MS–Perkin-Elmer) equipped with an elite-1, fused silica capillary column (30 m 0.25 mm ID × 1 m df, composed of 100% dimethy lpolysiloxane). For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 ml was employed (Split ratio of 10:1) with injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 2000°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a turbo mass.

2.10 *In silico* activity

The 3D protein structure for transferase of oral cancer was recovered from the protein data bank database (PDB ID: 4I DV). Dynamic web page region was anticipated utilizing ligsite online apparatus. The

concoction mixes from the referenced plants were recovered from the Pub Chem database. Lipinski rule of 5 helped in distinguishing between drug like and non-drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules: Molecular mass less than 500 Dalton, High lipophilicity (expressed as Log P less than 5) and less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, Molar refractivity must between 40-130.

2.11 Molecular docking study

MGL tools with AutoGrid4 and AutoDock4 were used to set up and to perform blind docking calculations between the ligands and protein. Receptor (protein) and ligand (complex) files were prepared using auto dock tools. The protein was enclosed in a box with grid points in x, y and z directions and a grid spacing of 0.375 Å. The center of the grid is set to – 6.516, 30.278 and – 1.951 Å. Lamarckian genetic algorithms, as implemented in auto dock, were employed to perform docking calculations. All other guidelines are default settings. For every individual the docking cases, the lowest energy docked conformation, according to the auto dock scoring function and number hydrogen bonds was selected as the binding mode. The output from auto dock was rendered with PyMol.

3. Results

In preliminary phytochemical screening flavonoids and phenols were present in all extracts whereas alkaloid and terpenoids in ethanol extract. Alkaloids were also observed in ethyl acetate fraction. *C. nodulibarbis* exhibited good antioxidant activity, where 16 mg/ml of ethanolic extract promoted the highest value (82.4 ± 0.91), followed by aqueous extract (22.2 ± 0.75) compared to standard ascorbic acid with value of 92.21 ± 0.82. The results of antimicrobial activity in *C. nodulibarbis* were observed. Among the other tested extracts, ethanolic extract exhibited highest activity at the concentration of 10 mg/ml against bacterium and fungi stains with MIC values between 0.13 to 1.5 mg/ml using 0.5 McFarland as standard.

3.1 Antiproliferative activity of *C. nodulibarbis* ethanol extract

The antiproliferative activity of *C. nodulibarbis* ethanolic extract was tested against KB (Oral cancer) cell line (Figure 1). The % of viability of the plant extract was measured by the MTT assay. Low cell viability was observed at 500, 250 and 125 µg/ml of extract against KB cell line (Oral cancer) respectively, indication the plant's potent ability to fight cancer. (Table 1). However, the percentage of cell viability was found to be higher for the ethanol extracts when compared to the control cells.

Table 1: Antiproliferative activity of *C. nodulibarbis* ethanolic extract against KB cell line

Concentration µg/ml	Absorbance 540 nm	% cell viability
500	0.07	5.8
250	0.12	10
125	0.29	24
62.5	0.56	46.6
31.2	0.87	72.5
15.6	1.13	94.1
DMSO	1.2	100
Control Cells	1.2	100

3.2 Identification and profiling of *C. nodulibarbis* constituents by GC-MS

GC/MS analysis was done to determine the highly potential active phytocompounds in the ethanol extract of *C. nodulibarbis* (Figure 2). 25 peaks with relative retention times were observed from a chromatogram. Each peak's mass spectrum was examined and contrasted with chemicals from the NIST collection. A list of 25 chemicals was compiled (Table 2).

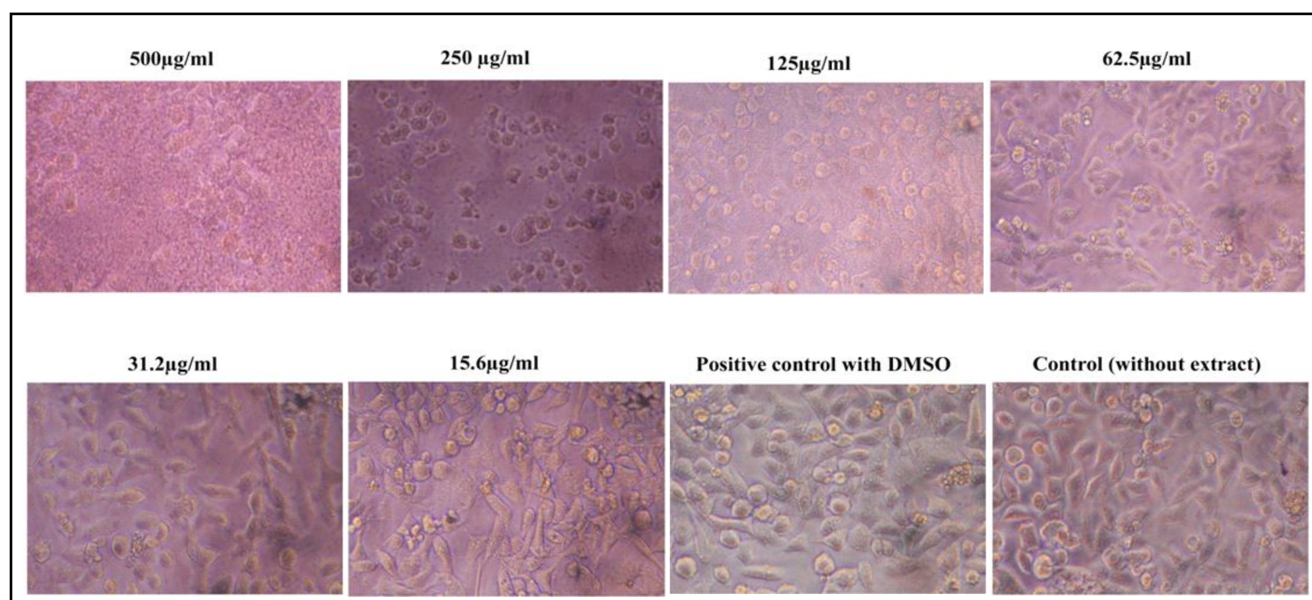

Figure 1: Antiproliferative activity of *C. nodulibarbis* ethanol extract against KB cell line.

Table 2: GCMS analysis of ethanol extract of *C. nodulibarbis*

RT	Compound name	M.W	M. formula
5.398	O-[n-Propylcarbamoyl]-3-exo-dimethylaminomethyl-2-norbornanone oxime	267.37	$C_{14}H_{25}N_3O_2$
7.164	Phosphoric acid, diethyl pentyl ester	224.24	$C_9H_{21}O_4P$
8.675	Benzamide, N,N-dioctyl-2,6-difluoro-	381.543	$C_{23}H_{37}F_2NO$
11.042	6-Quinolinamine, 2-methyl-	158.2	$C_{10}H_{10}N_2$
11.142	Cycloheptasiloxane, tetradecamethyl-	519.078	$C_{14}H_{42}O_7Si_7$
11.653	Phenol, 2,4-bis(1,1-dimethylethyl)	206.324	$C_{14}H_{22}O$
11.908	Lup-20(29)-en-21-ol, 3,28-bis[(tetrahydro-2H-pyran-2-yl)oxy]-, 3,3-dimethylbutanoate, (3.beta.,21.beta.)-	626.9	$C_{40}H_{66}O_5$
12.519	1,2-Benzenedicarboxylic acid, dihexyl ester	334.45	$C_{20}H_{30}O_4$
12.819	Dodecanoic acid,1-methylpropyl ester	256.425	$C_{16}H_{32}O_2$
13.53	Hexacosane	366.707	$C_{26}H_{54}$
14.186	Cyclononasiloxane, octadecamethyl-	667.386	$C_{18}H_{54}O_9Si_9$
14.763	1,4-Eicosadiene	278.5	$C_{20}H_{38}$
14.997	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	278.344	$C_{16}H_{22}O_4$
15.397	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276.371	$C_{17}H_{24}O_3$
15.808	Dibutyl phthalate	278.344	$C_{16}H_{22}O_4$
17.007	Phytol	296.531	$C_{20}H_{40}O$
17.618	1,1,1,5,7,7,7-Heptamethyl-3,3,5-tris(trimethylsiloxy)tetrasiloxane	533.147	$C_{16}H_{48}O_6Si_7$
19.485	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	577.2	$C_{16}H_{50}O_7Si_8$
19.574	5-Methyl-2-phenylindolizine	207.27	$C_{15}H_{13}N$
19.752	Di-n-decylsulfone	346.614	$C_{20}H_{42}O_2S$
19.862	Benzo[h]quinoline, 2,4-dimethyl-	207.27	$C_{15}H_{13}N$
21.007	2-Methyl-7-phenylindole	207.27	$C_{15}H_{13}N$
21.285	4-Methyl-2-trimethylsilyloxy-acetophenone	222.35	$C_{12}H_{18}O_2Si$
22.851	Cyclotrisiloxane, hexamethyl-	222.462	$C_6H_{18}O_3Si_3$
23.584	1,2-Bis(trimethylsilyl) benzene	222.47	$C_{12}H_{22}Si_2$

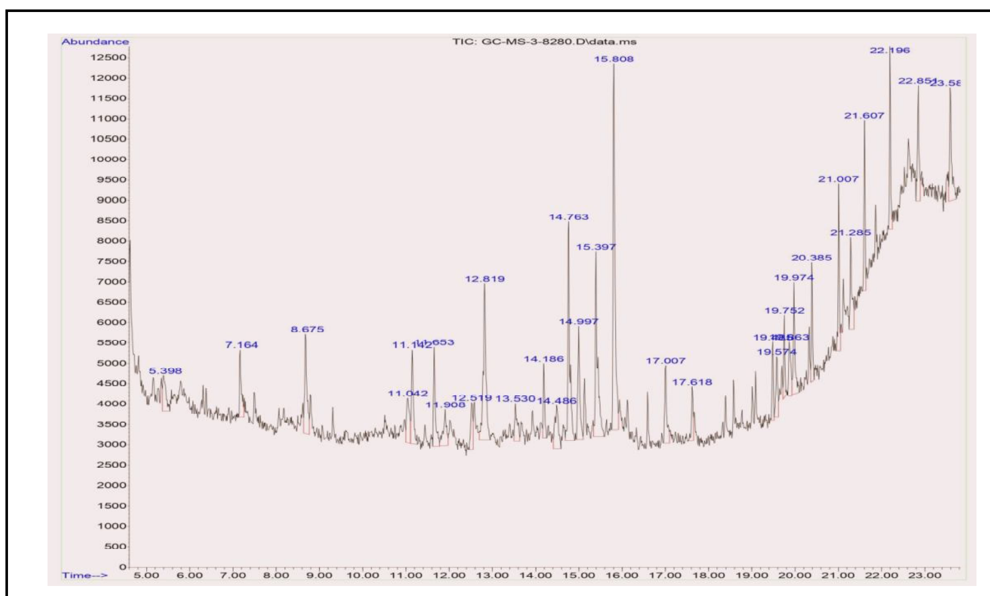

Figure 2: GC-MS analysis of ethanol extract of *C. nodulibarbis*.

3.3 Docking simulations of the major constituents as anticancer agents

The simulations of docking were carried out by MGL tools with AutoGrid4 and AutoDock4, to target human oral cavity protein transferase (PDB ID: 4IDV) compared to the recognized cancer-controlling compound Doxorubicin. The results of docking studies are recorded (Table 3). The best binding energy with 4IDV was recognized for the compound 1,1-dioxo tetrahydro thien-3-yl 2,6-difluoro benzyl carbamate, – 7.33 kcal/mol. The interactions residue were observed with ALA-445, THR-597, with a bond length of 2.3Å, and 3.1Å, respectively. The compounds 2,4-di-tert-butylphenol and N-Methyl-1-adamantane acetamide had binding energy of -5.43 and – 5.15 kcal/mol, respectively. The control drug doxorubicin predicted binding energy of – 4.71. The phytocompounds in the ethanolic extract have also shown potential binding preferences, such as geometric arrangements in relation to the target ligand molecule with the anticancer protein. Studies on receptor docking and binding energy needs suggested that the products phytoconstituents may work together synergistically to display potent anticancer bio-effects.

Table 3: Interaction of *C. nodulibarbis* phytoconstituents with 4IDV

Name of ligand	Binding energy	Residues interacted	Bond length (Å)	No. of bonds formed
1,1-dioxo tetrahydro thien -3-yl 2,6-difluorobenzoyl carbamate (4543607)	-7.33	ALA-445(H-O)	2.3	2
		THR-597(O-O)	3.1	
2,4-Di-tert-butylphenol (7311)	-5.43	GLY-52(H-O)	2.5	7
		ALA-350(O-N)	2.9	
		GLN-445(O-O)	3.0	
		LEU-447(O-N)	3.0	
		ARG-509(O-N)	2.9	
		(O-N)	2.8	
		ARG-601(O-O)	3.3	
N-Methyl-1-adamantane acetamide (610088)	-5.15	ALA-350(O-N)	2.9	6
		ARG-601(O-O)	3.3	
		LEU-447(O-N)	3.0	
		ALA-445(O-O)	3.0	
		ARG-509(O-O)	2.9	
		(O-N)	2.9	
1-(2,6-dimethylphenyl) -3-methyl imidazolidin -2-one52420-37-8(613243)	-4.77	ARG-601(O-O)	3.3	10
		ALA-350(O-N)	2.9	
		ALA-445(O-O)	3.0	
		LEU-447(O-N)	3.0	
		ARG-509(O-N)	2.8	
		(O-N)	2.9	
		(O-N)	3.3	
		(O-N)	3.4	
		TYR-505(O-O)	2.9	
		(O-O)	2.9	
		ALA-350(O-N)	2.9	
1H-2-Benzopyran-3-one, 7-ethoxy-4-hydroxy-4-methoxycarbonyl-(610141)	-4.26	ARG-601(O-O)	3.3	9
		ALA-445(O-O)	3.0	
		LEU-447(O-N)	3.0	
		ARG-509(O-N)	2.9	
		(O-N)	2.8	
		TYR-505(O-O)	2.9	
		SER-508(O-O)	3.0	
		(O-O)	2.8	
		ALA-445(H-O)	2.3	

O-[n-Propylcarbamoyl]-3-exo-dimethylaminomethyl-2-norbornanone oxime (9601676)	-4.11	THR-597(O-O)	3.1	2
		THR-597(O-O)	3.1	
Doxorubicin	-4.71	SER-508(O-O)	3.0	8
		ARG-509(H-O)	2.1	
		(O-N)	2.8	
		(O-N)	2.9	
		ARG-601(O-O)	3.3	
		ALA-445(O-O)	3.0	
		LEU-447(O-N)	3.0	
		ALA-350(O-N)	2.9	

4. Discussion

In this study, phytochemical screening of *C. nodulibarbis* denoted the presence of simple and polyphenolic active compounds. When compared to other family members, few studies have evaluated the pharmacological and phytochemical characteristics of grasses, and only a small number of isolated chemicals have been found in grass extracts (Stepp and Moerman, 2001). Nevertheless, grasses possess antimicrobial, antimutagenic, antitumor, antidiarrheal, antidiabetic, anti-inflammatory, antiplasmodial, diuretic, hepatoprotective and antioxidant activities. Several grass species used in traditional South African medicine have more than 100 secondary metabolites that have been discovered and isolated (Gebashe *et al.,* 2019). These compounds include steroid derivatives, phenol aldehydes (benzaldehyde and cinnamaldehyde derivatives), benzoxazinoid derivatives, chiral monoterpenes, aldehydes, fatty acids, and volatiles, and they have shown potential for anticancer, antimicrobial, antiinflammatory, and antioxidant effects (Kahkonen *et al.,* 1999; Middleton *et al.,* 2000; Tundis *et al.,* 2008).

Recently, Zakaria *et al.* (2011), reported the active compounds as disease aversion with antimicrobial activity and to reduce the risk of cancer (Marjorie, 1999). The studied strains of bacteria and fungus were significantly resistant to the ethanolic extract's antimicrobial activity. The antiproliferative activity of this study vigorously accedes with the report on lemon grass extracts which exhibited its efficacy in inducing cell death in human prostate cancer cells are affected by time and dose (Christopher, 2019) and also this study supports the reports of Sayed *et al.* (2016), where the edible grains of *Echinocolon crusgalli* ethanolic extract shown significantly considerable cytotoxic action against four human cancer cell lines.

Compounds identified from GC-MS such as Di-n-decylsulfone possess several biological activities such as antimicrobial and anticancer. Phenol, 2, 4-bis (1, 1-dimethylethyl) has antimitotic, antiproliferative, antimicrobial and antioxidant activities (Vijayakumari *et al.*, 2019). Phytol has antimicrobial, anti-inflammatory and anticancer activity (Santos *et al.,* 2013). Previous study proved that the compound phytol exhibits antioxidant and antinociceptive effects (Gnanavel and Saral, 2013) and additionally Sayed *et al.* (2016) reported phytol , precursor of synthetic vitamin E and vitamin K, was discovered to be cytotoxic against breast cancer cell lines (MCF-7). Most of the compounds identified from the plants *C. nodulibarbis* have anticancer activity. The result of this study additionally corroborates that the *C. nodulibarbis* ethanol extract has vigorous anticancer activity on the KB cell line.

5. Conclusion

In conclusion, profiling of chemical components by GC-MS analysis of *C. nodulibarbis* was performed, with twenty five compounds belonging to various chemical classes. Planned extension to bioassay-based purification of components from extracts has led to the identification of active components as potent anticancer molecular leads, providing new drug candidates and new molecular templates that show promise given prospects. The potential for structural modification is a further impetus for culminating in new natural product-based anticancer agents.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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