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Research Article

Chemical Compositions, Potential Cytotoxic and Antimicrobial Activities of *Nitraria retusa* Methanolic Extract Sub-fractions

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ABSTRACT

Nitraria retusa is an edible halophyte, used for several traditional medicine purposes. In this work, 6 sub-fractions of methanolic extract of *N. retusa* aerial parts were investigated for their cytotoxic and antimicrobial activities. For cytotoxic activity, the n-hexane (N-He) sub-fraction exhibited the highest cell growth inhibition against human breast carcinoma cells (MCF-7) (98.5±0.7%), followed by hepatocellular carcinoma cells (HEPG-2) (96±0.8%) as compared to the other sub-fractions. For antimicrobial activities, the N-He sub-fraction had the highest antimicrobial activity among all other sub-fractions. Moreover, the N-He sub-fraction at 1000 µg/ml concentration inhibited *Escherichia coli* and *Pasteurella hemolitica* growth by 85.4±0.12% and 85.8±0.18%, respectively after 24 h of incubation. Gas chromatography/mass spectrometry (GC/MS) analysis revealed the presence of different compounds in N-He and N-De sub-fractions. The above results revealed that different sub-fractions of *N. retusa* could be considered as a potential source of compounds with cytotoxic and antimicrobial effects.

Keywords: Antimicrobial activity, cancer cells, cytotoxicity, GC/MS, *Nitraria retusa*.

INTRODUCTION

Medicinal plants are believed to harbor potential antimicrobial, antioxidant, and anticancer agents that can act through various mechanisms and this may be an excellent alternative strategy for developing future effective and safe drugs. Moreover, medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[1]. Cancer is a dreadful disease and combating this disease is of great importance to public health. There is a necessity for search of new compounds with cytotoxic effects as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem cytotoxicity to the normal cells. Phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of plausible anticancer compounds[2]. Chemical studies of Egyptian medicinal plants provide a valuable material base for the discovery and development of new drugs of natural origin[3]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases[4]. They are of great importance to the health of individuals and communities. *Nitraria retusa*, locally known as Ghardaq, is one of the native perennial halophyte species that belong to the botanical family Nitrariaceae. It is distributed in North Africa and restricted to Algeria, Egypt and Tunisia. This plant is shrub, grows along shallow and hummocks on saline grounds near the coastal areas and produces fleshy red fruits from which a tasty and refreshing juice may be extracted[5]. Prior studies have shown the presence of several classes of secondary metabolites in *N. retusa* including sterols, fatty acids, alkaloids and flavonoids derivatives[6,7,8]. The leaves, fruits and seeds of some Nitraria species are often used in folklore medicine as an antispasmodic, antineuropathic, and anti-arrhythmic agent[9]. Moreover, anticancer, antioxidant and antiviral activities of *N. retusa* have been reported[8,10]. The aim of this study is to evaluate the cytotoxic effect and antimicrobial activity of n-hexane (N-He), diethyl ether (N-De) , dichloromethane (N-Dm) , ethyl acetate (N-Ea), n-butanol(N-Bu) and methanol (N-Me) sub-fractions of the hydro-methanolic extract of *N. retusa* aerial parts. Besides, the GC/MS analysis of n-hexane (N-He) and diethyl ether (N-De) sub-fractions was studied.

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MATERIALS AND METHODS

Plant collection

N. retusa aerial parts were collected from the region of Cairo-Ras Sudr road (30 km) North Sinai- Egypt. Samples were identified by Dr. S.R. Hussein at the Herbarium of Phytochemistry and Plant Systematic Dept., National Research Centre - Egypt.

Preparation of the hydro-alcoholic crude extract and its sub-fractions

The aerial parts of the plant were air dried at room temperature and pulverized. The pulverized plant material (1.2 Kg) was macerated with 70 % methanol on an orbital shaker (Heidolph Unimax 2010) for 48 h at room temperature. After filtration through Whatman No.4 filter paper, plant residue was re-extracted twice with the same solvent. The pooled hydro-alcoholic extracts were concentrated under reduced pressures at 40°C using a rotary evaporator (Heidolph-Germany) to remove the methanol completely from the extract and the rest water portion was lyophilized to obtain the crude extract (N-Me, 196.3g). The lyophilized crude extract (184.8g) was re-dissolved in 400 ml of distilled water then partitioned with different polarity solvents ; n-hexane, diethyl ether, dichloromethane, ethyl acetate and n-butanol successively. Each sub-fraction was concentrated to dryness under reduced pressures at 40°C using a rotary evaporator (Heidolph-Germany) to yield; hexane sub-fraction (N-He, 1.4g), diethyl ether sub-fraction (N-De, 1.7g), dichloromethane sub-fraction (N-Dm, 2.9g), ethyl acetate sub-fraction (N-Ea, 4.3g) and n-butanol sub-fraction (N-Bu, 19.7g).

Cytotoxic activity

Cell lines and culture conditions

Five human carcinoma cell lines obtained from the Karolinska Institute, Stockholm, Sweden viz human lung carcinoma (A-549), human colon cancer (HCT-116), human breast cancer (MCF-7), human prostate cancer (PC-3) and human liver carcinoma (HEPG-2) were used in this assay. All cells were maintained in RPMI 1640 medium (Lonza Biowahittkar, Belgium), except for the MCF-7 and PC-3 cancer cells which were maintained in DMEM medium (Lonza Biowahittkar, Belgium). Media were supplemented with 1% antibiotic-antimycotic mixture (10,000 Uml^{-1} potassium penicillin, 10,000 µg/ ml streptomycin sulphate, 25 µg/ ml amphotericin B and 1% L-glutamine).

MTT assay

Cell viability was investigated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as published previously by El-Sawy et al.[11]. Cell lines were incubated in an incubator with 5% CO_2 at 37 °C (Sheldon, TC2323, Cornelius, OR, USA). Cells were placed into 96-well microplates at a concentration of (10^4 cells / well) and allowed to stand for 24 h. Medium was aspirated and fresh medium (without serum) was added to the cells with different concentrations (1.25-100 µg/ml) of N-Me, N-He, N-De, N-Dm, N-Ea and N-Bu of *N. retusa* extracts dissolved in dimethyl sulfoxide (DMSO). After 48 h incubation, medium was aspirated and 40 µl MTT solution (2.5 µg/ml DMSO) was added to each well and incubated for further 4 h. The final concentration of DMSO was less than 0.2%. The formazan crystals formed was dissolved and the reaction was stopped by adding 200 µl of 10% sodium dodecyl sulphate (SDS) to each well for overnight at 37°C. The amount of formazan produced was measured at 595 nm with a reference wavelength of 620 nm as a background using a microplate reader (Bio-Rad Laboratories, model 3350, USA). For the untreated cells (negative control), medium was added instead of the tested sub-fractions extracts. A positive control Adrinamycin® (doxorubicin) was used as a known cytotoxic natural agent giving 100% inhibition. Data were expressed as growth inhibition (%) using the following formula;

Growth inhibition (%) = 100- $A_{sample}/A_{control}$ x100.

Where, A_{sample} is the absorbance of treated cells with sub-fractions extract, and $A_{control}$ is the absorbance of untreated cells

Antimicrobial activity

Microbial strains

The microorganisms were obtained from the American type culture collection (ATCC; Rockville, MD, USA). The three gram-positive bacteria; *Staphylococcus aureus* (ATCC- 47077), *Bacillus cereus* (ATCC- 12228), *Listeria monocytogenes* (ATCC- 35152), Three gram-negative bacteria; *Escherichia coli* (ATCC- 25922), *Pasteurella hemolitica* (ATCC- 33396), *Pseudomonas aeruginosa* strain OS4; two yeasts; *Saccharomyces cerevisiae* (ATCC-9763), *Candida albicans* (ATCC- 10231) and one fungi *Aspergillus niger* (ATCC- 16888) were used.

Culture medium and inoculums

The stock cultures of microorganisms used in this study were maintained on plate count agar slants at 4°C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient agar broth and was incubated at 37°C for 24 h. About 60 µl of bacterial suspensions adjusted to 10^6-10^7 colony forming units (CFU)/ml were taken and poured into Petri plates containing 6 ml sterilized nutrient agar medium. Bacterial suspensions were spread to get a uniform lawn culture.

Antimicrobial assay

The agar well diffusion method was applied to detect antimicrobial activity[12]. Wells of 6mm diameter were dug on the inoculated nutrient agar medium using sterilized cork borer and 50 µl of the following extracts ; N-Me, N-He, N-De, N-Dm, N-Ea and N-Bu were dissolved in dimethylsulfoxide (DMSO) at concentration (1000 µg/ml), then added in each well. The wells introduced with 50 µl of DMSO were used as a negative control. The plates were allowed to stand at 4°C for 2 h before incubation to prevent evaporation of tested extracts. The plates were incubated at 37°C overnight and examined for the inhibition zone. The diameter of the inhibition zone was measured in mm.

Minimum inhibitory concentration (MIC)

A bacterial suspension (10^6-10^7 CFU/ml) of each tested microorganism was spread on the nutrient agar plate. The wells (6 mm diameter) were dug on the agar plate, and 50 µl of tested samples, dissolved in DMSO at different concentrations (from 200 to 1000 µg/ml) were delivered into them. The plates were allowed to stand at 4°C for 2 h before incubation to prevent evaporation of tested extracts.

The plates were incubated at 37ºC for 24 h under aerobic conditions then followed by the measurement of the diameter of the inhibition zone expressed in millimeter. MIC was taken from the concentration of the lowest dosed well visually showing no growth after 24 h.

Determination of bacterial cell growth

To inspect the bacterial cell growth in the presence of two *N.retusa* extracts (N-He and N-De sub-fractions), *E. coli* and *P. hemolitica* cells were grown in 50 ml of mineral salt medium (MSM) broth medium[13] amended with 1000 µg/ml of each extract (DMSO was used as a negative control and MSM without any additives as a positive control) at 37°C with continuous agitation at 120 rpm.

After 24 h of incubation, bacterial cell growth was measured as optical density (OD) at 600 nm using spectrophotometer. Data were expressed as bacterial cell growth inhibition (%) using the following formula;

Bacterial cell growth inhibition (%) = $[OD_{control} - OD_{sample} / OD_{control}] X 100$

Where, $OD_{control}$ is the absorbance of positive control (MSM without any additives) and OD_{sample} is the absorbance of MSM amended with extract.

Gas chromatography/mass spectrometry (GC/MS) analysis

The GC/MS profile of *N. retusa* N-He and N-De sub-fractions were performed using a Thermo Scientific capillary gas chromatography (model Trace GC ULTRA) directly coupled to ISQ Single Quadruple MS and equipped with TG-5MS non polar 5% phenyl methylpolysiloxane capillary column (30 m × 0.25 mm ID × 0.25 µm). The operating condition of GC oven temperature was maintained as: initial temperature 40ºC for 3 min, programmed rate 5ºC/min up to final temperature 280ºC with isotherm for 5 min. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as a carrier gas at a constant flow rate of 1.0 ml/min. 1 µl of each sub-fraction was injected automatically in the splitless mode. Detection was performed in the full scan mode from 40 to 500 m/z. The quantification of the components was based on the total number of fragments (total ion count) of the metabolites as detected by the mass spectrometer. The identification of the chemical components was carried out based on the retention time of each component (R_t) compared with those of the Wiley 9 and NIST 08 mass spectra libraries[14].

Statistical analysis

All data are presented as means ± SD; the mean values were calculated based on the data taken from at least three independent experiments conducted on separate days using freshly prepared reagents.

RESULTS AND DISCUSSION

MTT cytotoxic activity

Five human cancer cell lines A-549, HCT-116, MCF-7, PC-3 and HEPG-2 were used to investigate the *in vitro* cytotoxic effects of the *N. retusa* sub-fractions using MTT assay. The net growth inhibition of cancer cell lines at 100 µg/ml concentration of all the sub-fractions were presented in Table (1). The results prove that the hexane sub-fraction (N-He) exhibited the highest cell growth inhibition against MCF-7(98.5±0.7%), followed by HEPG-2 (96±0.8%), HCT-116 (47.5±0.9%) and A-549 (15.1±0.6%) as compared to the other sub-fractions, but it has no effect on PC-3 cell lines.

The present results are in analogous with that of Boubaker et al.[7] who reported that hexane extract of *N. retusa* leaves showed the highest growth inhibition of Human chronic myelogenous leukemia cell line (K562) with IC_{50} value (9300 µg/ml) as compared to chloroform and methanol extracts, and they attributed the highest activity of hexane extract to the presence of sterols in the extract. Anticancer agents destroy or inhibit the growth of cancer cells and most of the formerly used anti-cancer agents are natural components or derived from natural sources[15]. In general, anti-cancer agents seem to act through multiple mechanisms of action, including inhibition of carcinogen production, cancer-cell growth, angiogenesis, invasion and metastasis, and through the promotion of apoptosis of cancerous cells stress[16].

In the present study, the highest cytotoxic effects of N-He can be clarified partly by the presence of high concentration of some phenolic compounds such as butylated hydroxyltoluene and 3-tert-butyl-4-hydroxy anisole besides may be to the presence of 17-pentatriacontene and docosane that identified in the N-He sub-fraction by GC/MS analysis presented in Table (5). The butylated hydroxyltoluene and 3-tert-butyl-4-hydroxy anisole are known to possess cytotoxic activity[17,18]. Different plant extracts that contain 17-pentatriacontene and/or docosane showed cytotoxic effects against different cell lines[19,20].

Antimicrobial activity

The antimicrobial activities (zone of inhibition and MIC values) of each sub-fraction extract are summarized in tables (2 and 3). On the whole, all the sub-fractions extract at 1000 µg/ml concentrations showed variable inhibition effect against gram-positive and gram-negative bacteria except N-Bu sub-fraction extract showed no effect among all the tested bacteria. Also, all sub- fraction extracts showed no effect against the tested fungi at the same concentrations. It was noted that, the *N. retusa* N-He sub fraction extract exhibited the largest inhibition zone (14±1.3mm) against *P. hemolitica* followed by N-De sub fraction extract (10±1.2mm). Whereas, the *N. retusa* N-De sub- fraction extract exhibited the largest inhibition zone (15±1.7mm) against *E. coli* followed by N-He sub fraction extract (13±1.5mm) as presented in Table (2).

The present results are in a partial agreement with the previous results of Bouaziz et al.[21], who concluded that the non-polar extracts exhibited more antimicrobial activity than the polar ones and when the hexane extracts are active their specters are generally larger than that of other solvents. In the present study, ethyl acetate (N-Ea) sub-fraction extract showed the antibacterial activity against *S. aureus*, *P. aeruginosa*, *E. coli* and *B. cereus*, but it has no activity against *C. albicans*. Similarly, different polar extracts from different Nitraria species have been shown to possess variable levels of antimicrobial activity[21,22].

Table 1: Cytotoxic activity of *N. retusa* sub-fractions at 100 µg/ml concentration.

Plant extracts	Growth inhibition (%) of human carcinoma cell lines				
	Lung (A-549)	Colon (HCT-116)	Breast (MCF-7)	Prostate (PC-3)	Liver (HEPG-2)
N-He	15.1±0.6	47.5±0.9	98.5±0.7	0	96±0.8
N-De	18.8±0.9	16.6±0.2	0	6.9±0.5	0
N-Dm	24.8±0.4	16.2±0.7	6.8±0.3	20.1±0.8	19.4±0.4
N-Ea	8.2±0.5	0	0	0	0
N-Bu	10.66±0.8	0	0	0	0
N-Me	10.5±0.5	6.3±0.2	9.1±0.4	5.64±0.1	0

The values are expressed as means ±SD.

Table 2: Antimicrobial activity of *N. retusa* sub-fractions at 1000 µg/ml concentration by agar well diffusion method

Microbes	Inhibition zone (mm)					
	N-He	N-De	N-Dm	N-Ea	N-Bu	N-Me
B. cereus	11±1.1	11±1.3	8±0.8	10±0.9	NI	NI
S. aureus	12±1.3	10±1.2	8±1.1	11±1.4	NI	10±1.3
L. monocytogenes	10±1.1	10±1.3	9±0.5	12±1.3	NI	NI
E. coli	13±1.5	15±1.7	11±1.1	10±1.1	NI	7±0.5
P. hemolitica	14±1.3	10±1.2	8±0.6	9±0.9	NI	8±0.5
P. aeruginosa	10±1.1	10±1.4	8±0.9	8±0.5	NI	NI
S. cerevisiae	NI	NI	NI	NI	NI	NI
C. albicans	NI	NI	NI	NI	NI	NI
A. niger	NI	NI	NI	NI	NI	NI

The diameter of the well (6mm) is included. NI: No inhibition zone.
The values are expressed as means ±SD.

Table 3: Minimal Inhibitory Concentration (MIC) of *N. retusa* sub-fractions.

Microbes	MIC (µg/ml)					
	N-He	N-De	N-Dm	N-Ea	N-Bu	N-Me
B. cereus	450	350	800	850	>1000	>1000
S. aureus	200	300	750	250	>1000	200
L. monocytogenes	400	350	800	800	>1000	>1000
E. coli	350	300	400	800	>1000	800
P. hemolitica	200	800	800	800	>1000	850
P.aeruginosa	400	750	750	800	>1000	>1000
S. cerevisiae	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans	>1000	>1000	>1000	>1000	>1000	>1000
A. niger	>1000	>1000	>1000	>1000	>1000	>1000

Table 4: Bacterial growth inhibition (%) of *E. coli* and *P. hemolitica* in the presence of *N. retusa* N-He and N-De sub-fractions at 1000 µg/ml concentration after 24 h of incubation.

Microbes	Parameters	OD at 600 nm	Bacterial growth inhibition (%)
E.coli	Control	0.666±0.003	0.0
	DMSO	0.631±0.002	5.2±0.18 %
	N-He	0.097±0.001	85.4±0.12 %
	N-De	0.241±0.003	63.8±0.27 %
P.hemolitica	Control	0.600±0.005	0.0
	DMSO	0.591±0.004	1.5±0.15%
	N-He	0.085±0.002	85.8±0.18 %
	N-De	0.205±0.005	65.8±0.51 %

The values are expressed as means ±SD.

Pertaining to, Minimum Inhibitory Concentrations (MIC) presented in Table (3), It was observed that N-He sub-fraction extract exhibited the highest activity with the lowest MICs, (200 µg/ml) against *P. hemolitica* and *S. aureus* and (400 µg/ml) against *P. aeruginosa*. Also, N-Me sub fraction extract exhibited the lowest MIC (200µg/ml) for *S. aureus.* The N-De sub- fraction extract exhibited the highest activity with the lowest MICs, (300 µg/ml) against *E. coli* and (350 µg/ml) against *B. cereus* and *L. monocytogenes*.

The present results confirmed that the *N. retusa* sub-fraction extracts were more active against gram-positive bacterial strains as compared to gram negative bacteria. This is in agreement with the fact that gram positive

Table 5: GC/MS chemical profile of *N. retusa* hexane sub-fraction (N-He).

No.	[a]R_t	Compounds name	Area (%)[b]	Molecular formula
1	22.77	4-Hexen-1-ol	0.13	$C_6H_{12}O$
2	22.88	1-Propylundecylacrylate	0.17	$C_{17}H_{32}O_2$
3	26.05	8-Heptadecene, 9-octyl-	0.65	$C_{25}H_{50}$
4	26.99	Tetraneurin-A-diol	0.15	$C_{15}H_{20}O_5$
5	27.16	2,6,11-Trimethyldodecane	0.86	$C_{15}H_{32}$
6	27.30	2,6-di-butyl-2,5-cyclohexadiene-1,4-dione	6.38	$C_{14}H_{20}O_2$
7	27.61	3-tert-Butyl-4-hydroxy anisole	47.16	$C_{11}H_{16}O_2$
8	28.15	R-Limonene	0.91	$C_{10}H_{16}O_3$
9	28.75	Butyl Hydroxy Toluene	13.34	$C_{15}H_{24}O$
10	29.26	Iso-aromadendrene epoxide	2.39	$C_{15}H_{24}O$
11	29.60	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	0.58	$C_{18}H_{30}O_2$
12	29.95	2-Cyclohexen-1-one,3-(3-hydroxybutyl)-2,4 ,4-trimethyl-(CAS)	2.20	$C_{13}H_{22}O_2$
13	30.43	4,4'-Dinitro-3,3",5,5"-tetraphenyl-1,1': 4',1"-terphenyl	0.24	$C_{42}H_{28}N_2O_4$
14	31.18	Lucenin 2	0.16	$C_{27}H_{30}O_{16}$
15	31.29	6,7-dihydro- Neblininane,	0.17	$C_{23}H_{32}N_2O_2$
16	31.70	24,25-Dihydroxycholecalciferol	0.13	$C_{27}H_{44}O_3$
17	32.04	3,3-Dimethyl-4-(3,3,4,4-tetramethyloxetan-2-ylidene) butan-2-one	0.68	$C_{13}H_{22}O_2$
18	33.09	Pentalene,octahydro-1-(2-octyldecyl)(CAS)	0.93	$C_{26}H_{50}$
19	33.51	Cyclobutanone-3-d, 2,2-dimethyl-,(S)-(CAS)	0.12	C_6H_9DO
20	33.65	Dimethoxyglycerol docosyl ether	0.12	$C_{27}H_{56}O_5$
21	34.20	3-Oxo-20-methyl-11-à-hydroxyconanine-1,4-diene	0.17	$C_{22}H_{31}NO_2$
22	35.93	5á-Cholestan-3-one,cyclic ethylene acetal	0.12	$C_{29}H_{50}O_2$
23	37.96	Hexamethylenediamine-N,N,N',N'-tetraacetic acid	0.45	$C_{14}H_{24}N_2O_8$
24	39.78	Pipradrol	1.30	$C_{18}H_{21}NO$
25	40.14	8-Acetoxy-5-b-methylperhydrocyclobuta[n] phenanthrene-11-ol-2,3-dione-2-a-propanoic acid lactone	0.14	$C_{22}H_{28}O_6$
26	41.05	Villalstonintriol	0.12	$C_{40}H_{52}N_4O_3$
27	42.30	d-Allo-dec-2-enonic acid, 5,8-anhydro-2,3,4,9-tetradeoxy-8-C(hydroxymethyl)-3-methyl-7,8-O-(1-methylethylidene), methyl ester,10-acetate,(E)-	0.87	$C_{18}H_{28}O_8$
28	43.18	Docosane (CAS)	1.96	$C_{22}H_{46}$
29	43.58	Pregan-20-one,2-hydroxy-5,6-epoxy-15-methyl-	0.46	$C_{22}H_{34}O_3$
30	44.00	Nonacosane (CAS)	0.94	$C_{29}H_{60}$
31	46.36	Oleoamide	6.77	$C_{18}H_{35}NO$
32	49.34	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	0.41	$C_{28}H_{40}O_{10}$
33	50.00	7-Tetradecenyl-1-(N-acetyl)amine	1.60	$C_{16}H_{31}NO$
34	50.05	Ergosta-2,24-dien-26-oic acid,27-(acetyloxy)-5,6-epoxy-22-hydroxy-1-oxo,ëlactone (CAS)	0.96	$C_{30}H_{40}O_6$
35	50.10	17-Pentatriacontene (CAS)	2.70	$C_{35}H_{70}$
36	50.51	Oleic acid	1.26	$C_{18}H_{34}O_2$
37	53.08	Bis(2-ethylhexyl) phthalate	0.80	$C_{24}H_{38}O_4$
38	57.16	N-[4-(3-Hydroxy-1-pyrrolidinyl)-2-butynyl]-N-methylacetamide	0.16	$C_{11}H_{18}N_2O_2$
39	57.75	Rhodopin	0.14	$C_{40}H_{58}O$
40	61.66	17á-Acetoxy-1',1'-dicarboethoxy-1á,2á-dihydro-17à-methyl-3'-H-cycloprop[1,2]-5à-androst-1-en-3-one	1.20	$C_{29}H_{42}O_7$
		Total	100	
		Oxygenated hydrocarbons	80.98	
		amine compounds	10.98	
		Hydrocarbons	8.04	

[a]R_t: retention time (min).

[b]The percentage composition was computed from the gas chromatography peak areas.

bacteria lack an outer membrane containing lipopolysaccharide but are surrounded by layers of peptidoglycan which is not enough to enable bacteria to survive in their different environments[23].

Table 6: GC/MS chemical profile of *N. retusa* diethyl ether sub-fraction (N-De).

No.	[a]R_t	Compounds name	Area (%)[b]	Molecular formula
1	27.30	Calarene epoxide	0.24	$C_{15}H_{24}O$
2	27.59	2,6-Di-(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	3.40	$C_{15}H_{24}O_2$
3	28.16	Docosane (CAS)	0.28	$C_{22}H_{46}$
4	28.75	Butyl Hydroxy Toluene	8.31	$C_{15}H_{24}O$
5	29.02	L-Serine, O-(phenylmethyl) (CAS)	0.21	$C_{10}H_{13}NO_3$
6	29.49	2-Propyldecan-1-ol	0.18	$C_{13}H_{28}O$
7	29.95	7-Hydroxy-6-keto-drimanol	0.27	$C_{15}H_{26}O_3$
8	30.34	Farnesol	0.20	$C_{15}H_{26}O$
9	32.17	Decanal (CAS)	0.16	$C_{10}H_{20}O$
10	34.00	Tetracosa-2,6,14,18,22-pentaene-10,11-diol, 2,6,10,15,19,23-hexamethyl-	0.16	$C_{30}H_{52}O_2$
11	34.11	2-Octadecoxyethanol	0.30	$C_{20}H_{42}O_2$
12	34.20	Heneicosane (CAS)	0.44	$C_{21}H_{44}$
13	35.36	2,2,4Trimethyl-1,3-pentanediol	0.16	$C_8H_{18}O_2$
14	36.63	Ceanothine C (CAS)	1.00	$C_{26}H_{38}N_4O_4$
15	36.68	9-Butyl-12-imino-10,11-dioxa-tricyclo[5.3.2.0(1,6)]dodecane-7,8,8-tricarbonitrile	0.72	$C_{17}H_{20}N_4O_2$
16	36.78	Dimethyl-4-tert-butyl-1,2-cyclopentanedicarboxylate	0.59	$C_{13}H_{22}O_4$
17	37.28	ar-Curcumene	0.73	$C_{15}H_{22}$
18	39.63	Pentacosane (CAS)	0.36	$C_{25}H_{52}$
19	40.94	Dodecanoic acid, 2-(acetyloxy)-1-[(acetyloxy) methyl] ethyl ester	0.20	$C_{19}H_{34}O_6$
20	41.85	Deoxyspergualin	0.69	$C_{17}H_{37}N_7O_3$
21	42.14	11-Octadecenal	1.54	$C_{18}H_{34}O$
22	43.59	9-Acetoxy-1-methyl-8-propyl-3,6-diazahomoadamantane	0.66	$C_{15}H_{26}N_2O_2$
23	44.10	E-9-Methyl-8-tridecen-2-ol, acetate	0.52	$C_{16}H_{30}O_2$
24	44.25	9-Octadecenamide, (Z)-	0.33	$C_{18}H_{35}NO$
25	44.51	Cis 9,10-Epoxyoctadecanamide	0.18	$C_{18}H_{35}NO_2$
26	45.05	Dimethoxyglycerol Docosyl Ether	0.23	$C_{27}H_{56}O_5$
27	45.69	9,12,15-Octadecatrienoic acid, (2-pheny-l1,3-dioxolan-4-yl) methyl ester (CAS)	0.36	$C_{28}H_{40}O_4$
28	46.01	N-Allyl-2-hydroxy-3-methylbutamide	38.03	$C_8H_{15}NO_2$
29	47.01	3-Tetradecanol	0.26	$C_{14}H_{30}O$
30	49.29	Prednisolone Acetate	0.21	$C_{23}H_{30}O_6$
31	49.70	Oleoamide	19.88	$C_{18}H_{35}NO$
32	49.95	Guanidine carbonate	1.95	$C_3H_{12}N_6O_3$
33	50.28	Hexanamide (CAS)	9.49	$C_6H_{13}NO$
34	51.09	Ethyl iso-allocholate	0.28	$C_{26}H_{44}O_5$
35	51.93	Methyl- 9,10,11,12-dimethyleneoctadecanoate	0.24	$C_{21}H_{38}O_2$
36	53.03	Dioctyl phthalate	1.07	$C_{24}H_{38}O_4$
37	54.04	Ethyl vinyl ether	3.45	C_4H_8O
38	54.40	(5á)Pregnane-3,20á-diol,14à,18à-[4-methyl-3-oxo(1-oxa-4-azabutane-1,4-diyl)], diacetate	0.31	$C_{28}H_{43}NO_6$
39	57.54	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-	2.09	$C_{28}H_{44}O_4$
40	57.96	Methyl linoleate	0.33	$C_{19}H_{34}O2$
		Total	100	
		Oxygenated hydrocarbons	24.74	
		amine compounds	73.45	
		Hydrocarbons	1.81	

[a]R_t: retention time (min).

[b]The percentage composition was computed from the gas chromatography peak areas.

The organic solvents were found to have marked influence on the extraction of the antibacterial compounds from the medicinal plants[24,25]. This activity may be due to different chemical compounds present in extracts including flavonoids, triterpenoids and natural phenolic compounds or free hydroxyl groups which are classified as active antimicrobial compounds[4,26]. Therefore, the present results suggested that hexane and diethyl ether are the effective solvents for the extraction of antibacterial compounds from *N. retusa*. It is concluded that the various extracts of *N. retusa* may be used for the treatment of various infectious diseases caused by the tested bacterial pathogen.

Bacterial cell growth

The highest bactericidal action of *N. retusa* N-He and N-De sub-fractions encouraged us to study its effect on bacterial growth rates of *E. coli* and *P. hemolitica* at 1000 µg/ml concentration. The results (Table 4) showed that the growth rates of tested pathogens were reversely affected with addition of plant extracts compared with control media and DMSO. The N-He sub-fraction caused considerable reduction (85.4±0.12%) in growth rate of *E. coli* strain after 24 h of incubation. As similar trends, the N-He sub-fraction caused considerable reduction (85.8±0.18%) in growth rate of *P. hemolitica* strain after 24 h of incubation, while N-De sub-fraction caused 65.8±0.51% reduction in growth rate of *P. hemolitica* strain after 24 h of incubation. This is likely due to inhibit of bacterial growth by the two sub-fraction extracts and these extracts considered as antibacterial agents. In earlier studies, methanol fruit extract of *Nitraria sibirica* showed promising antibacterial and antifungal activities [27].

GC/MS analysis

The high cytotoxic and antimicrobial effects of *N. retusa* N-He and N-De sub-fractions encouraged us to study its chemical composition by GC/MS. The results of GC/MS analysis of *N. retusa* hexane sub-fraction (N-He) and diethyl ether sub-fraction (N-De) are listed in Tables (5 and 6), respectively, in which the percentage and retention times of components are given. The dominant compounds in the *N. retusa* hexane sub-fraction (N-He) were 3-tert-butyl-4-hydroxy anisole (47.16%), butyl hydroxy toluene (13.34%), oleoamide (6.77%) and 2,6-di-butyl-2,5-cyclohexadiene-1,4-dione (6.38%). Generally the *N. retusa* hexane sub-fraction (N-He) was characterized by the presence of oxygenated hydrocarbons (80.98%) followed by amine compounds (10.98%) and hydrocarbons (8.04%) as presented in Table (5). However, the major compounds in the *N. retusa* diethyl ether sub-fraction (N-De) were N-allyl-2-hydroxy-3-methylbutamide (38.03%), oleoamide (19.88%), hexanamide (9.49%) and butyl hydroxy toluene (8.31%).

The *N. retusa* diethyl ether sub-fraction (N-De) was characterized by the presence of amine compounds (73.75%) followed by oxygenated hydrocarbons (24.74%) and hydrocarbons (1.81%) as presented in Table (6). A number of different classes of secondary metabolites including sterols, fatty acids, alkaloids and flavonoids derivatives have been known in the different species of Nitraria[7,28]. In this context, Mohamed et al.[29] reported that the petroleum ether crude extract of *N. retusa* which exhibited antioxidant activity, proved hentriacontane (71.84%) and nonacosane (5.6%) as the major components (hydrocarbons) by GC/MS analysis, this is followed by eugenol (3.9%) and butyl hydroxy toluene (2.26%) as oxygenated hydrocarbon compounds**.** Also, the GC/MS analysis of the hexane extract of *N. schoberi* fruits exhibited fatty acids (68.86%) as the major groups of constituent in the extract followed by sterols (10.14%), hydrocarbons (7.7%), esters (1.8%), one chlorinated compound (such as 2- chloro ethyl linoleat) and one keton compound (such as farnesyl aceton) [30].

In the present study, different compounds that have been identified in the *N. retusa* sub-fractions by GC/MS analysis, previous studies proved that have different biological activities, such as the butylated hydroxyltoluene (presented in N-He and N-De extracts by 13.34% and 8.31%, respectively) has antioxidant, anticancer and antiviral activities[18,31,32]. Furthermore, 3-tert-butyl-4-hydroxy anisole and limonene (presented in N-He extract by 47.16% and 0.91%, respectively) have anticancer activity[17,33].

CONCLUSION

Several natural products can be explored for the huge needs of novel compounds having medicinal applications. Based on these results it can be concluded that *N. retusa* are abundant sources of several pharamaceutically important compounds which can be used as sources of new and useful antimicrobial and anticancer chemical entities. The n-hexane (N-He) sub-fraction exhibited the highest antimicrobial and anticancer activities among all other sub-fractions. The antimicrobial and anticancer activities of N-He sub-fraction may be attributed to the presence of 3-tert-butyl-4-hydroxy anisole, butyl hydroxyl toluene and limonene which have been identified by GC/MS analysis. Extra isolation and structure clarification of pure compounds which may be responsible for the antimicrobial and anticancer activities of *N. retusa* sub-fractions are needed.

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