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Screening of phytochemical constituents and potential antioxidant, antibacterial and anticancerous activities of *Carpesium nepalense* seeds oil

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Abstract: The pharmacological importance and ecofriendly nature of medicinal plants holding a unique edge in the arena of pharmaceutical industries. Therefore, the current research was aimed to evaluate the phytochemical constituents and potential antioxidant, *in vitro* anticancer and antibacterial activity of *Carpesium nepalense* seeds essential oil. The analysis performed through Gas chromatography/Mass spectroscopy confirmed the presence of different types of biologically active compounds. At the concentration of 500µg/mL, *n*-hexane fraction of *C. nepalense* showed highly significant ($P<0.001$) antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid and superoxide assays with the percentage inhibitions of 86.60±1.6%, 82.55±1.0% and 80.50±1.0% respectively. The extract also produced highly significant anticancerous activity against different cell lines at 500µg/mL. The significant antibacterial activity of extract was observed against bacterial strains with the zone of inhibitions of 24.3±0.8, 28.20±0.10, 22.33±0.11 and 33.22±0.10 mm respectively. The significant damage in bacterial cell membranes was also observed in atomic force microscopic analysis. In the light of obtained findings, it is concluded that *C. nepalence* proved to be a potential candidate as an alternative medicinal agent.

Keywords: *Carpesium nepalense,* GC/MS, antioxidant, anticancer, antibacterial, AFM technique.

INTRODUCTION

Several herbs are in practice as a therapeutic agent globally for centuries. Hundreds of biologically active molecules and drugs that are currently used against various diseases have been developed from natural resources (Jardak *et al.,* 2017, Rehman *et al.,* 2019). Plants are naturally capable to synthesize secondary metabolites holding potential biological activities. Traditionally, plants grab much attention to cure different diseases with negligible toxicity (Rehman *et al.,* 2020). Synthetic drugs in the field of treatment of cancer and bacterial infections are frequently used and considered as backbone of chemotherapy. However, the intrinsic toxicity of conventional anticancer drugs and resistance to existing antibiotics compromised their efficiency (Ijaz *et al.,* 2018, Lin *et al.,* 2015). This results in the utilization of broad spectrum antibiotics which leads to an increase in the ratio of multi-resistant pathogens as well as cost of therapy (Asghar *et al.,* 2020, Mumtaz *et al.,* 2017). Therefore, the demand for medicinal plants has been progressively increased. Hence, the development of new drugs, particularly chemotherapeutic agents from natural sources is considered to be a unique idea.

The genus *Carpesium*, which belongs to the family *Compositae* has been reported with enriched antifungal and antibacterial activities (Lin *et al.,* 1996). This genus comprises twenty-five different species worldwide, in which most of them are habitats in Europe, Asia, Southwest China. Many species of this genus have been traditionally used for the treatment of different diseases like fevers, bruises, colds and snake bites. This genus has been therapeutically reported with detoxifying, analgesic, vermifuge, hemostatic, anti-inflammatory and antipyretic properties (Zhang *et al.,* 2015). Sesquiterpenoid lactones compounds, such as germacranolides and eudesmanolides are the chief constituents found in this genus. Previous studies specified that sesquiterpenoid having aα-methylene-γ-lactone moiety which possess cytotoxic potential against human cancer cells (Zhang *et al.,* 2015, Yang *et al.,* 2003). Recently, six sesquiterpenoid lactones having cytotoxic potential were obtained from the genus *Carpesium* (Yang *et al.,* 2014). In China, *Carpesium divaricatum* is use traditionally for the management of different diseases such as bruises, fevers, insect bites, colds and inflammatory diseases.

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Carpesium nepalense (*C. nepalense*) is widely distributed in Azad Kashmir Pakistan and is traditionally use in the treatment of different diseases. In China traditionally *C. nepalense* has been reported to use as an alternative to *C. abrotanolides* plant in hepatitis (Lin *et al*., 1996). Owing to the significant role of *Carpesium* species as an antioxidant, antibacterial and cytotoxic agent, the current research was designed to evaluate the *C. nepalense* essential oil phytochemically for the presence of pharmacologically active components. Furthermore, antioxidant, anticancer and antibacterial activities of *C. nepalense* essential oil was also evaluated.

MATERIALS AND METHODS

Plant sample collection

C. nepalense plant was collected from Azad Kashmir, Pakistan in June 2019. The plant after the collection was authenticated by Pharmacognosist associated with the Department of Botany, University of Karachi. A voucher specimen of *C. nepalense* (SB/07/20-CN) was submitted to the Faculty of Pharmaceutical Sciences, Federal Urdu University, Karachi, Pakistan. The seeds were separated from the arial parts of the plant.

Seeds oil extraction

About 30g of *C. nepalense* seeds were minced in to fine powder and soaked in *n*-hexane. After 15 days the seeds were subjected to an oil extraction process using a Soxhlet extractor at 70^{o}C for 6 h. After extraction, the oil-*n*-hexane mixture was passed through the layer of magnesium sulfate (anhydrous) using Whatman filter paper. Further, the solvent was evaporated at 40^{o}C using a rotary evaporator (Buchi 490) (Szentmihályi *et al*., 2002). The obtained oil with an extracted yield of 26% (7.8g) was flushed with nitrogen and stored at -10^{o}C till further use.

Phytochemical investigation

The GC and GCMS analysis was carried out according to the method described by Canli et al. (Canli *et al*., 2016). Briefly, the extracted crude oil of *C. nepalense* seed was diluted in *n*-pentane with a 1:1000 ratio. Then, 1.0 µL v/v sample was injected to GC (Agilent USB 393752, USA) capillary column of HHP-5MS (5%) phenylmethyl siloxane (30m 0.25mm 0.25µm) equipped with FID detector. The GC/MS of crude extract of *C. nepalense* essential oil was analyzed with similar conditions outfitted with selective mass detector (Agilent HM-5973 USA) ionization energy (70eV) in electron impact mode. The obtained spectra and principal ingredients were authenticated by comparing their retention time with authentic literature in Wiley and NIST library (Burki *et al*., 2019).

Antioxidant activity

DPPH scavenging activity

The free radical scavenging potential of *C. nepalense* was performed using 1,1-diphenl-2-picrylhydrazyl (DPPH) assay (Saeed *et al*., 2012). Briefly, about 0.2 mM solution of DPPH was prepared in methanol and was incubated in dark for 2 h before analysis. The crude essential oil of *C. nepalense* seeds were dissolved in respective extraction solvent at various concentrations of 100-500µg/mL. From the prepared solutions, 2mL was poured into each 96-well plate. To each well, 1mL of DPPH was added using a multichannel pipette followed by incubation for 15 min. After incubation, the obtained absorbance was recorded at the wavelength of 517 nm. The assay was performed in triplicate while ascorbic acid was used as a standard. The DPPH scavenging activity was calculated as (Saeed *et al*., 2012);

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ABTS scavenging activity

The 2,2-Azino-Bis-(3ethylbenzothiazoline-6-sulphonic acid (ABTS) activity was carried out using a freshly prepared ABTS reagent by mixing 7 mmol of ABTS with 2.45 mmol of $K_2S_2O_8$. The resultant mixture was incubated for 16 h in dark, followed by diluting the solution with distilled water to obtained 0.7 ± 0.005 absorbance at 734 nm. In other containers, different concentrations (100-500µg/mL) of crude essential oil of *C. nepalense* seeds were prepared and mixed with ABTS stock reagent in the ratio of 0.1:0.9. After a few min of the reaction, the absorbance of the resultant solution was measured at 734nm. The procedure was preform in triplicate using ascorbic acid as a standard. The percent free radical scavenging was estimated using the following formula (Abu *et al*., 2017).

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Superoxide scavenging activity

The anionic superoxide-free radical scavenging activity was accomplished using the system of riboflavin-light-NBT. In brief, a 1mL sample of *C. nepalense* was used for the preparation of different concentrations i.e. 100-500 µg/mL. To the prepared samples, 0.3mL riboflavin (50 mM), 0.5mL of phosphate buffer (50mM, pH 7.6), 0.1 mL of NBT (0.5mM) and 0.25mL of PMS (20mM) were added. The reaction was started under fluorescent light illumination (Agilent, USA). After 20 min, the obtained absorbance of the mixture was recorded at the wavelength of 560 nm using ascorbic acid as a standard. The observed absorbance was used to obtain the percent inhibition using the following formula (Beauchamp and Fridovich, 1971);

$$\text{Percentage inhibition (\%)} = \frac{\text{1–Sample absorbance}}{\text{ontrol absorbance}} \times 100$$

Anticancer activity

The anticancer activity of *n*-haxane fraction of *C. nepalense* was carried out against human breast epithelial

and adenocarcinoma cell lines (MCF-7 and MDA-MB-231) and normal mouse fibroblast 3T3 cell line using MTT assay (Burki *et al*., 2019). Briefly in DMEM containing FBS (10%), penicillin and streptomycin (100 units/mL); the treated cancerous cells were cultured in a controlled humidified atmosphere containing CO_2 (5%) at 37°C. Cells were seeded into 96-well plates at a density of 8×10^4 cells/well in a culture medium (100µL). After 24h of incubation, the original medium was replaced by a fresh medium (200µL) having concentrations of (30, 60, 120, 240 and 500µg/mL) test samples and allowed to be grown for further 24h. To each well, 200µL MTT solution of 0.5mg/mL in PBS was added and incubated the cells for 4h. After 24h incubation, the medium having unreacted dye was removed. The obtained crystals of purple formazan were thawed in 100µL DMSO per well and the obtained absorbance was measured on the microplate reader (Spectra Max Plus, USA) at 570 nm. The % inhibition was calculated as (Burki *et al*., 2019):

$$\% \text{ cell survival} = \frac{\text{absorbance of extract treated cells}}{\text{absorbance of control}} \times 100$$

Antimicrobial assay

Bacterial strains and culture media

The antibacterial bioassay was carried out on Gram-negative bacteria: *Escherichia coli*: ATCC No. 10536, *Pseudomonas aeruginosa*: ATCC No. 10145, MDR *Salmonella enterica* Serovar typhi *S. typhi*: ATCC No. 19430 and Gram-positive bacteria: *Bacillus subtilis* (ATCC No. 6051). The bacterial strains were procured from Jinnah Postgraduate Medical Center (JPMC), Pakistan. The strains were verified using the 16s RNA technique. Bacterial strains were then cultured on nutrient agar slants. After incubation at 37°C, bacterial cultures were stored at 4°C.

Determination of Zone of inhibitions (ZIs)

The antimicrobial potential of *C. nepalense* was assessed via the disc diffusion method (Shafiq *et al*., 2020, Mumtaz *et al*., 2019). The Mueller Hinton agar (BD Difco, USA) was transferred into a 90 mm sterile petri dish up to the depth of 4.0±0.5 mm. The 6 mm diameter sterile antimicrobial disks were loaded with 10, 20, 30, 40 and 60mg/mL of *C. nepalense* essential oil. All disks were subjected to drying at 30 °C in aseptic conditions to prevent any residual solvent, which may affect the results. The prepared sterile disks were placed on the inoculated agar. For positive control, standard ciprofloxacin (10 µg disc) was used, while DMSO was used as a negative control. The petri dishes containing inoculated bacteria and antibacterial disks were incubated for 24h at 37°C, followed by ZIs measurement in mm. The antimicrobial assay was performed in triplicate using empty sterile discs and extraction solvent as a negative control.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC and MBC of *C. nepalense* were determined using the broth dilution method (Asghar and Asghar, 2020a). First, the extract with a desired concentration of 50 mg/mL was prepared in the freshly prepared nutrient broth, then the serial dilution method was used to make several dilutions up to 0.1 mg/mL. All bacterial isolates were adjusted to the Mcfarland standard concentration i.e. 1×10^6 cfu/mL. After incubation of inoculated microbial plates for the period of 12 h, optical densities (ODs) were recorded using an ELISA reader (Infinite 200; USA) at the wavelength of 600 nm. In addition, MBCs of extract was estimated by spreading the already incubated tubes solution on nutrient agar plates and incubated for 24 h at 37°C. After the defined incubation period at controlled environment, bacterial colonies were observed on each inoculated plate. Each experiment was performed in triplicates and mean ± SD data of each test were reported.

Bacterial cell morphology by Atomic Force Microscope (AFM)

The size and morphological changes using AFM were only studied in those bacterial cells which were treated with *C. nepalense* following the method reported by (Burki *et al*., 2019). Briefly, the bacterial cells after treating with *C. nepalense* were harvested from the petri dishes. Each extract treated bacterial cell culture was washed three times with phosphate buffer saline (PBS). One drop of each washed bacterial cell was applied on a fresh mica slide. Images of each *P. aeruginosa*, *E. coli*, MDR *S. typhi* and *B. subtilis* were recorded on (AFM, Agilent, Technologies, 5500, USA) in ACAFM mode.

STATISTICAL ANALYSIS

The obtained data from the present study experiments were expressed as their mean ± SEM. One-way ANOVA was used using Graph Pad Prism software version 6.01 for inferential analysis on obtained experimental results. For multiple comparisons studies among various treatment, standard and control groups, *posthoc* test was applied. The obtained results were considered as statistically significant at $p<0.05$ and highly statistically significant at $p<0.005$.

RESULTS

GC analysis and GCMS analysis

The essential oils from the aerial parts of *C. nepalense* seed were obtained using *n*-hexane as an extraction medium. The obtained oil with the aromatic odor was subjected to GC and GC/MS analysis. The obtained spectra (fig. 1) were matched with Wiley and NIST library. In the essential oil of *C. nepalense* about 66 compounds were identified, out of which the important compounds include α- pinene, camphene, γ-terpinene, 2, 3

Table 1: Zone of inhibition of *C. nepalense* seed extract against different bacterial strains

Test solutions	Concentrations	Zone of inhibition (mm) (Mean ± SEM)			
		P. aeruginosa	*E. coli*	MDR *S. typhi*	*B. subtilis*
Control (Distilled water)	——	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00
C. nepalense n-hexane extract (mg/mL)	10	7.3 ± 0.3	7.88 ± 0.14	6.34 ± 0.11	9.51± 0.13*
	20	10.1 ± 0.4*	13.75 ± 0.13*	9.36 ± 0.09*	15.65±0.15**
	30	14.8 ± 0.5*	17.49 ± 0.13**	13.31 ± 0.15*	20.44±0.17**
	40	19.4 ± 0.2**	21.00 ± 0.09**	17.72 ± 0.1**	26.46±0.19**
	60	24.3 ± 0.8**	28.20 ± 0.10**	22.33 ±0.11**	33.22±0.10**
Ciprofloxacin (10 µg disc)	10	14.41 ± 0.05*	13.52 ± 0.05*	15.39 ±0.09**	16.80±0.07**

Results are presented as mean ± SEM, the test was performed in triplicated (n=3).
*$p \leq 0.05$ significant as compared to control, **$p \leq 0.005$ highly significant as compared to control

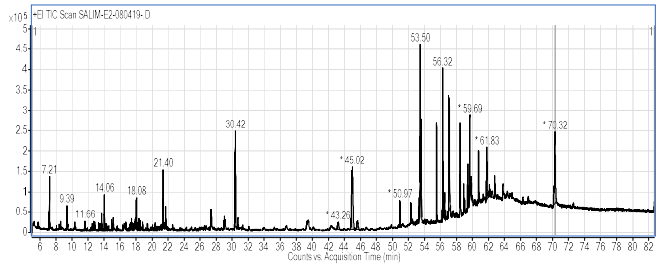


Fig. 1: GC/MS spectra of *C. nepalense* seeds essential oils

Table 2: MIC and MBC of crude seeds extract of *C. nepalense*

Clinical Isolates	MIC (mg/mL)	MBC (mg/mL)
P. aeruginosa	6.0 ± 0.62	6.5 ± 1.52
E. coli	6.0 ± 0.45	7.0 ± 0.71
S. typhi	8.5 ± 0.61	9.5 ± 1.40
B. subtilis	5.0 ± 0.80	6.0 ± 2.20

dihydroaromomectin, telekin, ethyl palmitate, linoleic acid, myricitrin, α-tocopherol, stigmasterol, carabrone, nepalolide A, 1-butanamine, n-butyl-, oxalic acid, 2-ethylhexyl isohexyl ester, pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters, 2-propenoic acid, oxybis (methyl-2,1-ethanediyl) ester, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester and 20-Hydroxy-5alpha-pregnan-18-oic acid. However, the structures of biologically active important compounds identified in the GC/MS of essential oils of *C. nepalense* are presented in fig. 3.

Antioxidant activity of C. nepalense seeds against DPPH, ABTS and superoxide

The activities (absorbance) linearly increase with the increasing concentration of extract. The α-diphenyl-β-picrylhydrazyl (DPPH) scavenging activity of *C. nepalense* seed extract was found to be highly significant (*P<0.005*) i.e. 86.60±1.6% at 500µg/mL. The IC_{50} against DPPH was achieved at 300µg/mL, which was comparable with ascorbic acid (ASA). The maximum free radical scavenging potential of *C. nepalense* crude *n*-hexane extract against 2,2'-azino-bis(3-ethylbenzothi-azoline-6-sulfonic acid) (ABTS) was found to be 82.55±1.0% at 500µg/mL with IC_{50} of 300µg/mL. At 500µg/mL, *C. nepalense* crude *n*-hexane extract inhibited superoxide free radical (80.50±1.0%) in the highly significant manner (*P<0.005*), but this was relatively lower than that of ASA as depicted in (fig. 2). The results were comparable with standard ascorbic acid.

Anticancer activity

The *in vitro* anticancer activity was performed on human breast epithelial and adenocarcinoma cancer cell lines (MDA-MB-231 and MCF-7). The results were compared with normal mouse fibroblast (3T3) cells. All the cell lines were exposed to different concentrations of crude *n*-hexane extract of *C. nepalence* and presented varying results. (fig. 4A) shows that at 500µg/mL of *C. nepalence*, highly significant inhibition of cells growth was observed i.e. 73.96±2.0% while the IC_{50} against MDA-MB-231 was achieved at 120µg/mL. However, the crude *n*-hexane extract showed more pronounced results against MCF-7 cells with the percentage inhibition of 83.76±1.2% at 500µg/mL. The IC_{50} of plant extract against MCF-7 cells was also found at 120µg/mL (fig 4B). Remarkably, the *C. nepalence* crude extract was also inhibited (57.66±1.4%) normal mouse fibroblast 3T3 cells at 500µg/mL (fig. 4C). The results were comparable with standard drug docetaxel.

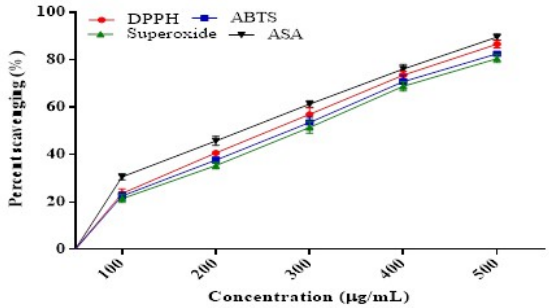


Fig. 2: Antioxidant activity of *C. nepalence* crude *n*-hexane extract (CnHE) against DPPH, ABTS and superoxide.

Antibacterial activity

The antibacterial activity of *C. nepalense* seed extract was evaluated through the disc diffusion method against human pathogenic bacterial strains i.e. *P. aeruginosa, E. coli,* multi-drug resistance (MDR) *S. typhi and B. subtilis.* The obtained ZIs of *C. nepalense* against all strains are summarized in table 1 while the MICs and MBCs of plant extract were given in table 2. The zone of inhibition against *P. aeruginosa, E. coli,* MDR *S. typhi and B. subtilis* were 24.3±0.8, 28.20±0.10, 22.33±0.11 and 33.22±0.10 mm at a concentration of 60mg/mL.

Morphological changes observation using AFM

The extract treated bacterial culture was observed under an atomic force microscope for morphological changes in bacterial cells. After treatment with 60 mg/mL extract of *C. nepalense,* uneven rough surfaces were observed in all bacterial cells membrane. For instance, the cell wall of *P. aeruginosa* was remarkably ruptured (fig. 5a) while the *E. coli* bacterial cell shape was also changed with damaged cell wall and leaked cytoplasm (fig. 5b). Similarly, the MDR *S. typhi* bacterial cell shape was changed with a partial damaged cell wall (fig. 5c). However, the Gram-positive *B. subtilis* cells were significantly melted with complete irreversible cytoplasm leakage as presented in fig. 5d.

DISCUSSION

In this research, the essential oils from *C. nepalense* seeds were phytochemically analyzed using GC and GC/MS.

The crude *n*-hexane extract of *C. nepalense* was further evaluated for antioxidant, anticancer and antibacterial activity. The bacterial cells treated with plant extract were further observed for morphological changes under an atomic force microscope (AFM). According to our literature survey, it is the first study on *C. nepalense* specie belongs to *Carpesium* genus for the evaluation of antioxidant, anticancer and antibacterial activities. Previously four new sesquiterpene lactones napalolides A−D, were isolated from *C. nepalense* (Lin *et al*., 1996). The principal constituents identified in GC/MS analysis of essential oils of *C. nepalense* were α- pinene, camphene, γ-terpinene, 2, 3 dihydroaromomectin, telekin, ethyl palmitate, linoleic acid, myricitrin, α-tocopherol, stigmasterol, carabrone, nepalolide A, 1-butanamine, *n*-butyl-, oxalic acid, 2-ethylhexyl isohexyl ester, pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters, 2-propenoic acid, oxybis (methyl-2,1-ethanediyl) ester, 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester and 20-Hydroxy-5alpha-pregnan-18-oic acid.

These compounds were previously reported for potent oxidative stress like camphene, α-pinene, β-pinene enantiomers and telekin possess significant anticancer activity in hepatocellular carcinoma. α-Tocopherol also possesses anticancer activity (Ghaffari *et al*., 2019). Linoleic acid has been reported for anticancer activity in colorectal cancer (Cheng *et al*., 2019). α-Pinene was reported for the activity against breast cancer cell line (MDA-MB-231, MCF-7) (Abu-Dahab *et al*., 2014). 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester have pronounced anticancer activity against liver cancer cell line HepG2, but are less toxic against 3T3 (Selvakumar *et al*., 2019). Napalolide A previously reported inhibition of inducible nitric oxide synthase expression in C6 glioma cells in the rat (Zhang *et al*., 2018). However, ethyl palmitate, methyl palmetate, myricitrin and stigmasterol have been reported for anti-inflammatory, antioxidant and antibacterial activities (Jin *et al*., 2010).

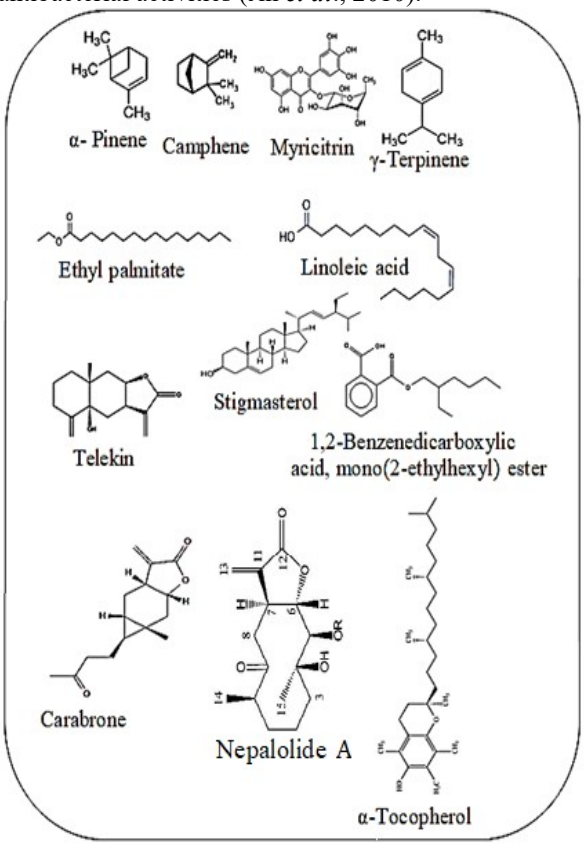


Fig. 3: Structures of important compounds identified in GC/MS of oils of *C. nepalense* seeds

Antioxidants are tremendously important substances that exhibit the ability to protect the body from damages and stress caused by free radicals. The free radical scavenging activity of *C. nepalense* was measured by the ability to scavenge DPPH, ABTS and superoxide comparing with ascorbic acid as standard. The present study results demonstrated the highly significant antioxidant activity of *C. nepalense* against DPPH, ABTS and superoxide in a pattern of DPPH>ABTS>superoxide. This significant result might be due to the presence of a variety of phytochemicals constituents particularly myricitrin and stigmasterol (Moresco *et al*., 2016). The results were comparable with standard vitamin C.

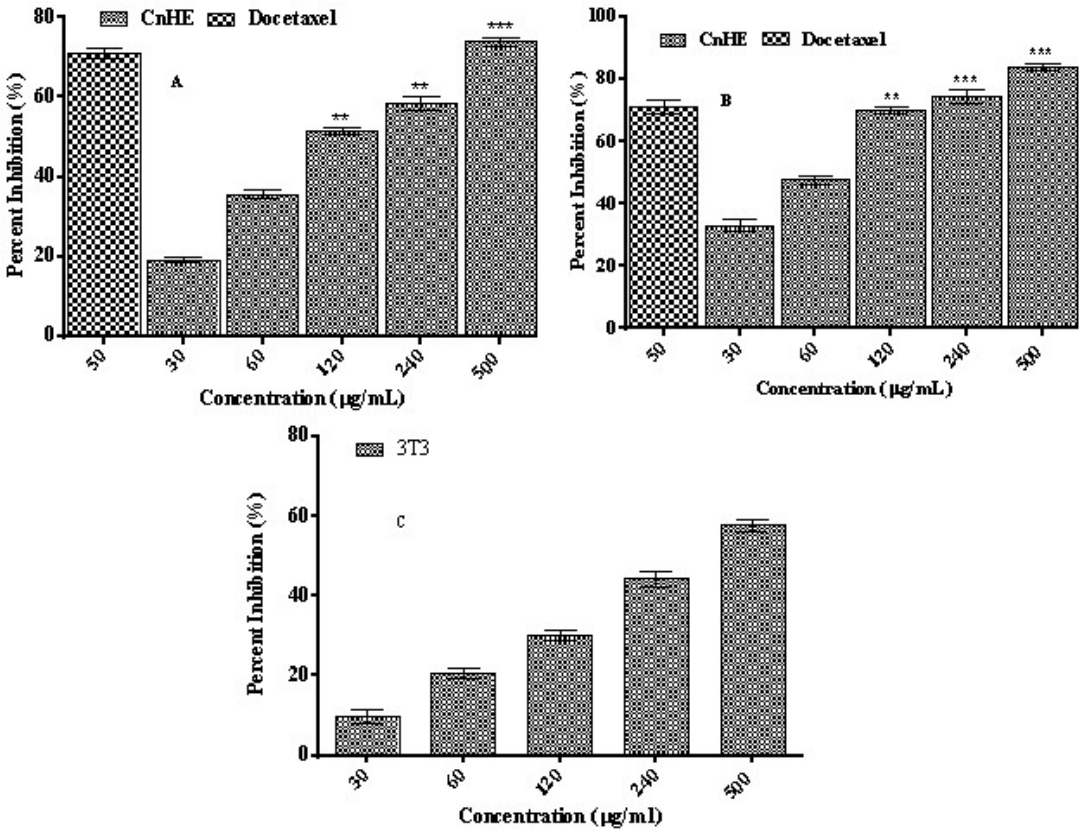
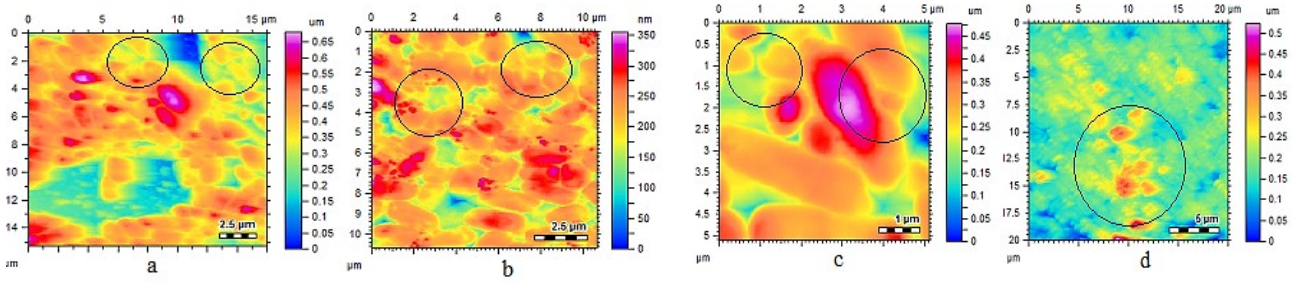


Fig. 4: Cytotoxicity bioassay against breast cancer cell line (A) MDA-MB-231(B) MCF-7 and normal mouse fibroblast cell line (C) 3T3 cells (*p<0.05, **p<0.001, ***p<0.0001)



*Circles show significant changes in cells shape with damaged cell walls

Fig. 5: AFM images of (a) *P. aeruginosa*, (b) *E. coli*, (c) *MDR S. typhi* and (d) *B. subtilis* treated with *C. nepalense* seed extract.

C. nepalense exhibited significant inhibitory action against both MDA-MB-231and MCF-7 breast cancer cell lines in dose dependent manner. Surprisingly the effect on normal mouse fibroblast 3T3 cell line was significantly low at 500µg/mL. This selective anticancer nature of *C. nepalense* may be due to the presence of different distinctive compounds like α-pinene. The underlying mechanism might be due to the inhibition of TNFα dependent MMP-9 mRNA by α-pinene which is involved in tumor invasions and metastasis. In addition, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester is another compound identified in *C. nepalense* seed which was previously reported for selective anticancer activity against MCF-7 and HepG2, while less toxic against 3T3 cell line (Krishnan *et al*., 2014).

The results revealed that the essential oils efficiently suppressing the growth of human pathogenic bacteria in dose dependent manner. *C. nepalense* presented highly significant activity against both Gram positive and Gram

negative bacterial strains. The antibacterial activity of *C. nepalense* is highly significant due to the presence of different previously reported anti-bacterial compounds such as stigmasterol and pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters in seeds oil (Omoregie *et al*., 2018). Similarly, Shafiq *et al.,* 2021, Burki *et al.,* 2021 and Mumtaz *et al*., 2017 reported similar antibacterial activity of ethanolic extract of *Nerium oleander, Ajuga parviflora* and *Sphaeranthus indicus* respectively. The antibacterial activity of *C. nepalense* was further studied for its morphological changes in bacterial cells. The extract treated bacterial cells were observed under an atomic force microscope for morphological changes. The cells of human pathogenic Gram positive bacteria *B. subtilis* after being treated with extract were severely damaged. On the other side, the Gram negative *P. aeruginosa* and *E. coli* cell walls were also abrasive with leaked cytoplasm. The obtained images also predicted that *P. aeruginosa* and *E. coli* may regain and maintain cellular integrity after few days. The MDR *S. typhi* were partially damaged while the cellular shape was disturbed. Furthermore, it is evident that the MDR *S. typhi* overlap each other due to which the flagella could not be seen, or either the flagella may be detached from the cell. Thus results confirmed the antibacterial effects of *C. nepalense*. In future studies, it is necessary to explore its bioactive constituents so that it can be used for therapeutic purposes.

CONCLUSION

In the present study, sixty-six compounds were identified by comparing the GC/MS spectra of *Carpesium nepalense* essential oil with reliable literature in Wiley and NIST library. These compounds possess an unarguably decisive role in the health care system. Plant essential oil in this study greatly contributed to antioxidant, *in vitro* anticancer and antibacterial activities. Based on significant results it is deduced that *Carpesium nepalense* should be a part of the health care system as a natural alternative source.

REFERENCES

Abu-Dahab R, Kasabri V and Afifi FU (2014). Evaluation of the volatile oil composition and antiproliferative activity of *Laurus nobilis* L.(Lauraceae) on breast cancer cell line models. *Rec. Nat. Prod.,* **8**(2): 136-147.

Abu F, Taib M, Norma C, Moklas M, Aris M and Mohd Akhir S (2017). Antioxidant properties of crude extract, partition extract, and fermented medium of dendrobium sabin flower. *Evid. Based. Complement. Alternat. Med.,* 2017: 1-9.

Asghar, MA and Asghar MA (2020a). Green synthesized and characterized copper nanoparticles using various new plants extracts aggravate microbial cell membrane damage after interaction with lipopolysaccharide. *Int. J. Biol. Macromol.,* **160**: 1168-1176.

Asghar MA, Yousuf RI, Shoaib, MH and Asghar MA (2020). Antibacterial, anticoagulant and cytotoxic evaluation of biocompatible nanocomposite of chitosan loaded green synthesized bioinspired silver nanoparticles. *Int. J. Biol. Macromol.,* **160**: 934-943.

Beauchamp C and Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.,* **44**(1): 276-287.

Burki S, Burki ZG, Jahan N, Muhammad S, Mohani N, Siddiqui FA and Owais F (2019). GC-MS profiling, FTIR, metal analysis, antibacterial and anticancer potential of *Monotheca buxifolia* (Falc.) leaves. *Pak. J. Pharm. Sci.,* **5**(Special): 2405-2413.

Burki S, Burki ZG, Asghar MA, Ali I and Zafar S (2021). Phytochemical, acute toxicity and renal protective appraisal of *Ajuga parviflora* hydromethanolic leaf extract against CCl_4 induced renal injury in rats. *BMC Complemen Med. Ther.*, **21**(1): 1-14.

Canli K, Altuner EM, Akata I, Turkmen Y and Uzek U (2016). In vitro antimicrobial screening of *Lycoperdon lividum* and determination of the ethanol extract composition by gas chromatography/mass spectrometry. *Bangladesh J. Pharmacol.,* **11**(2): 389-394.

Cheng G, Zhang X, ChenY, Lee RJ, Wang J, Yao J, Zhang Y, Zhang C, Wang K and Yu B (2019). Anticancer activity of polymeric nanoparticles containing linoleic acid-SN38 (LA-SN38) conjugate in a murine model of colorectal cancer. *Colloids. Surf. B: Biointerfaces,* **181**: 822-829.

Ghaffari T, Kafil HS, Asnaashari S, Farajnia S, Delazar A, Baek SC, Hamishehkar H and Kim KH (2019). Chemical composition and antimicrobial activity of essential oils from the aerial parts of Pinus eldarica grown in Northwestern Iran. *Molecules,* **24**: 3203.

Ijaz S, Akhtar N, Khan MS, Hameed A, Irfan M, Arshad MA, Ali S and Asrar M (2018). Plant derived anticancer agents: A green approach towards skin cancers. *Biomed. Pharmacother.,* **103**: 1643-1651.

Jardak M, Elloumi-Mseddi J, Aifa S and Mnif S (2017). Chemical composition, anti-biofilm activity and potential cytotoxic effect on cancer cells of *Rosmarinus officinalis* L. essential oil from Tunisia. *Lipids. Health. Dis.,* **16**: 190.

Jin KS, Bak MJ, Jun M, Lim HJ Jo, WK and Jeong WS (2010). α-pinene triggers oxidative stress and related signaling pathways in A549 and HepG2 cells. *Food. Sci. Biotechnol.,* **19**(5): 1325-1332.

Krishnan K, Mani A and Jasmine S (2014). Cytotoxic activity of bioactive compound 1, 2-benzene dicarboxylic acid, mono 2-ethylhexyl ester extracted from a marine derived Streptomyces sp. VITSJK8. *Int. J. Mol. Cell Med.,* **3**(4): 246.

Lin J, Nishino K, Roberts MC, Tolmasky M, Aminov RI and Zhang L (2015). Mechanisms of antibiotic resistance. *Front. Microbiol.,* **6**: 34.

Lin YL, Ou JC, Kuo YH, Lin JK and Lee KH (1996). Napalolides A-D, four new sesquiterpene lactones from *Carpesium nepalense*. *J. Nat. Prod.,* **59**(10): 991-993.

Moresco HH, Colla G, Cavalcante ID, Queiroz GS, Pizzolatti MG and Brighente IM (2016). Chemical constituents of *Eugenia catharinae* and their antioxidant activity. *Nat. Prod. Res.,* **30**(22): 2624-2628.

Mumtaz N, Asghar MA, Naqvi SBS, Asghar MA, Raz ML and Rehman AA (2019). Time kill assay and bactericidal mechanism of action of ethanolic flowers extract of *Sphaeranthus indicus*. *RADS J. Pharm. Pharmaceut. Sci.,* **7**(1): 27-33.

Mumtaz N, Naqvi SBS, Asghar MA and Asghar MA (2017). Assessment of antimicrobial activity of *Sphaeranthus indicus* L. against highly resistant pathogens and its comparison with three different antibiotics. *J. Dis. Glob. Health,* **10**: 67-73.

Omoregie GO, Ovuakporie-Uvo O and Idu M (2018). Phyto-composition and antimicrobial activities of the ethanol seed extracts of *Buchholzia coriacea*. *Afr. J. Pharmaco. Therap.,* **7**(2): 53-58.

Rehman AA, Riaz A, Asghar MA, Raza ML, Ahmed S and Khan K (2019). *In vivo* assessment of anticoagulant and antiplatelet effects of *Syzygium cumini* leaves extract in rabbits. *BMC Complement Altern. Med.,* **19**(1): 1-8.

Rehman AA, Riaz A, Asghar MA, Sikandar B and Baig M (2020). Screening of anxiolytic and antidepressant of methanolic leaves extract of *Syzygium Cumini* in mice. *RADS J. Pharm. and Pharmaceut. Sci.,* **8**(2): 91-97.

Saeed N, Khan MR and Shabbir M (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement Altern. Med.,* **12**(1): 221.

Selvakumar JN, Chandrasekaran SD, Doss GPC and Kumar TD (2019). Inhibition of the ATPase domain of human topoisomerase IIa on HepG2 cells by 1, 2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester: Molecular docking and dynamics simulations. *Curr. Cancer Drug Targets,* **19**(6): 495-503.

Shafiq Y, Asghar MA, Ali H, Abedin S, Rehman AA and Anser H (2020). *In vitro* assessment of antimicrobial potential of ethanolic and aqueous extract of *Phlomis umbrosa* aagainst some ighly resistant pathogens. *Ann. Jinnah Sindh Med. Uni.,* **6**(1): 3-9.

Shafiq Y, Naqvi SBS, Rizwani GH, Asghar MA, Bushra R, Ghayas S, Rehman AA and Asghar MA (2021). A mechanistic study on the inhibition of bacterial growth and inflammation by *Nerium oleander* extract with comprehensive *in vivo* safety profile. *BMC Complement Med. Ther.,* **21**(1): 1-19.

Szentmihályi K, Vinkler P, Lakatos B, Illés V and Then M (2002). Rose hip (*Rosa canina* L.) oil obtained from waste hip seeds by different extraction methods. *Bioresour. Technol.,* **82**(2): 195-201.

Yang C, Yuan C and Jia Z (2003). Xanthanolides, Germacranolides, and Other Constituents from *Carpesium longifolium*. *J. Nat. Prod.,* **66**(12): 1554-1557.

Yang YX, Shan L, Liu QX, Shen YH, Zhang JP, Ye J, Xu XK, Li HL and Zhang WD (2014). Carpedilactones AD, four new isomeric sesquiterpene lactone dimers with potent cytotoxicity from *Carpesium faberi*. *Org. Lett.,* **16**(16): 4216-4219.

Zhang JP, Wang GW, Tian XH, Yang YX, Liu QX, Chen LP, Li, HL and Zhang WD (2015). The genus Carpesium: A review of its ethnopharmacology, phytochemistry and pharmacology. *J. Ethnopharmacol.,* **163**: 173-191.

Zhang T, Si JG, Zhang QB, Chen JH, Ding G, Zhang HW, Jia HM and Zou ZM (2018). Three new highly oxygenated germacranolides from *Carpesium divaricatum* and their cytotoxic activity. *Molecules,* **23**(1): 1078.