Ethiopian Journal of Science and Sustainable Development

e-ISSN 2663-3205 *Volume 7 (2), 2020*

Journal Home Page: www.ejssd.astu.edu.et

Research Paper

Chemical Constituents and Anti-proliferative Activity of Resin of *C. sphaerocarpa* against Four Human Cancer Cell Lines

Worku Dinku[1], Sang Un Choi[2], Sang-Ho Lee[2], Young-Sik Jung[2], Aman Dekebo[1,*]

[1]*Department of Applied Chemistry, School of Applied Natural Science, Adama Science and Technology University, P. O. Box 1888, Adama, Ethiopia*

[2]*Bio and Drug Discovery Division, Korea Research Institute of Chemical Technology, Republic of Korea*

Article Info

Article History:
Received 1 February 2020
Received in revised form 14 March 2020
Accepted 24 March 2020

Keywords:
Anti-proliferative activity
Commiphora sphaerocarpa
GC-MS
ovarian cancer
pancreatic cancer
stomach cancer
SRB assay

Abstract

Myrrh, a resin derived from the *Commiphora* genus, has traditionally been used for treatment of various human diseases, such as amenorrhea, ache, tumors, fever, and stomach pains. Despite widespread traditional medicinal use, the pharmacological activity and chemical composition of many *Commiphora* species are not well known. The current study pursues to evaluate the cytotoxicity activity of the *n*-hexane and chloroform fractions of *C. sphaerocarpa*. Both the anti-proliferative effect and chemical compositions were correlated. The compositions of the chemical constituents were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The *in vitro* cytotoxicity activity of crude extracts were evaluated in cellular lines of non-small cell lung cancer A549, ovarian cancer A2780, pancreatic cancer MIA-PaCa-2, and stomach cancer SNU-638 by SRB assay. The *n*-hexane fraction showed better apparent cytotoxicity on A549, A2780, and SNU-638 cancer cell lines with IC_{50}value ranging from 9.62 µg/mL to 10.30 µg/mL with dose-dependent relationship *in vitro* compared to the chloroform fraction. This efficacy might be correlated with the presence of pentacyclic triterpenes such as lupeol, urs-12-en-3-one, α-amyrin, β-amyrin, and sesquiterpenes: α-copaene, caryophyllene, humulene, alloaromadendrene, γ-muurolene, γ-selinene, β-humulene and caryophyllene oxides in resin of the plant. Our findings indicated components of *n*-hexane fraction of *C. sphaerocarpa* might be useful to treat aforementioned selected cancers after further studies.

1. Introduction

Cancer, a cellular malignancy that results in the loss of normal cell-cycle control, such as unregulated growth the lack of differentiation, can develop in any tissue of any organ, at any time (Chang and Kinghorn, 2001). The global cancer burden was estimated 18.1 million new cases and 9.6 million deaths in 2018. Cancers of the lung, female breast, and colorectal cancer are the top three cancer types in terms of incidence, are ranked within the top five in terms of mortality (first, fifth, and second, respectively). Together, these three cancer types are responsible for one third of the cancer incidence mortality burden worldwide (Bray et al., 2018). In Ethiopia, cancer has become the second leading cause of death next to cardiovascular diseases in the adult population (Memirie et al., 2018). The most common adult cancers were: cancers of the breast cervix, colorectal cancer, non-Hodgkin lymphoma, leukemia, cancers of the prostate, thyroid, lung, stomach, and liver (Memirie et al.,

* *Corresponding author, e-mail: amandekeb@gmail.com*

https://doi.org/10.20372/ejssdastu:v7.i2.2020.194

2018). Natural products have proven to be an important source of anticancer drugs (Cragg and Newman, 2005), identification of phytochemical cytotoxic entities that are effective against a range of cancer cell lines, less active or non-toxic against the normal cell population is an effective method for selection of fractions.

The genus *Commiphora* (Burseraceae) comprises over 150 species, most of which are confined to Eastern Africa, with few species also occurring in southern Arabia, India and South America (Shen et al. , 2012; Shen and Lou, 2008; Xu et al., 2011). According to Soromessa et al. (2013), South east lowland of Ethiopia is characterized by its high diversity of *Acacia* and *Commiphora* species and the same study revealed that out of total species in the genus *Commiphora* about half of them are endemic to the small area of south eastern Ethiopia, north eastern Kenya and Somalia. Over 50 *Commiphora* species are known to occur in Ethiopia, of which 14 (25%) species are endemic (Vollesen et al., 1989). The resin of *C. sphaerocarpa* is sold under the product name myrrh. Ethnobotanical notes on herbarium specimens of *Commiphora* species deposited at the National Herbarium in Addis Ababa University also revealed various traditional uses of resins by different communities in Ethiopia. For example, the resin of *C. sphaerocarpa* is used against cough, diarrhea, headache, and against ticks of cattle. Additionally, the note indicated fruits of this plant are edible. This resin is sometimes found in true myrrh as adulterants. Previous study revealed that petroleum ether extract of *C. sphaerocarpa* afforded six sesquiterpenes of which one of them *i.e.* (1E)-8,12-epoxygermacra-1,7,10,11-tetraen-6-one was novel (Dekebo et al, 2002). We hereby report chemical composition of *n*-hexane fraction of *C. sphaerocarpa* which was analyzed by GC-MS along with anticancer activity of crude methanol extracts, *n*-hexane, and chloroform fractions, obtained by liquid-liquid partitioning from methanol extracts, against four human cancer cell lines: A549, A2780, MIA-PaCa-2 and SNU-638 using SRB assay under *in vitro* conditions.

2. Materials and Methods

2.1. Plant material collection

Resin of *C. sphaerocarpa* Chiov (Burseraceae) and other botanical specimens were collected from Sof Omar Bale zone, Oromia regional state, Ethiopia in October, 2016. The specimens have been identified by botanist Shambel Alemu and deposited at the National Herbarium, Biology Department, Addis Ababa University, Ethiopia (Voucher number: 072820).

2.2. Extraction and Liquid-Liquid fractionation

The resin sample was air dried left under shadow till experimental work. The powdered resin of *C. sphaerocarpa* (95.1 g) was extracted with methanol (1 L x 3) for three days at 25°C. The crude methanolic extract was evaporated under vacuum to afford a yellow solid (34.8 g, 36.6%) which was then suspended in water (500 ml) and successively partitioned with *n*-hexane (500 ml x 3) followed by chloroform (500 ml x 3) to afford 12.1 g (34.8%) and 2.5 g (7.1%) yields for *n*-hexane and chloroform fractions, respectively.

2.3. GC-MS analysis of n-hexane fraction

GC-MS analysis was done by using a GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). The GC had an HP 5MS column (non-polar column, Agilent Technologies), 30 m × 250 μm internal diameter (i.d.) 0.25 μm film thickness. The carrier gas was helium flowing at a rate of 1 mL/ min. The injector temperature was 230°C and the injection mode was split mode with split ratio 4:1. The initial oven temperature was 40°C held for 3 min. It was raised to 70°C at 4°C/min held at this temperature for 3 min. The oven temperature was then raised 80°C at 1°C/min with no holding time, 100°C at 4°C/min with no holding time then to 180°C at 10°C/min, then with no holding time 20°C/min until it reached 280°C held at this temperature for 2 min. The total run-time was 60 min. Mass spectra were recorded in EI mode at 70 eV, scanning the 33-500 m/z range. The identification of the volatile compounds was performed by comparing the mass spectra of the compounds with those in the database of NIST11 and literature data.

2.4. Anti-proliferative assessment

The cancer cell lines including the human non-small cell lung cancer cell line (A549), ovarian cancer cell line (A2780), pancreatic cancer cell line (MIA-Paca-2) stomach cancer cell line (SNU-638) were used for evaluation of anticancer activity. All cell lines were maintained using RPMI1640 cell growth medium (Gibco, Carlsbad, CA), supplemented with 5% fetal

bovine serum (FBS) (Gibco), grown at 37°C in a humidified atmosphere containing 5% CO_2. The cytotoxicity of the crude methanol extract its organic solvent fractions of the plant against cultured human tumor cell lines were evaluated on the already developed sulforhodamine B (SRB) method (Schols et al., 1988).

2.5. Treatment of cell lines

Stock solutions of the crude MeOH extract, *n*-hexane and chloroform fractions dissolved in DMSO were prepared in the corresponding medium at different concentrations of 0.1, 0.3, 1.0, 3.0, 10.0 and 30 µg/mL to determine percentage of growth inhibition the and for the 50% growth inhibition (IC_{50}). Each tumor cell line was inoculated over standard 96-well flat-bottom micro plates then incubated for 24 h at 37°C in a humidified atmosphere of 5% CO_2. The attached cells were then incubated with serially diluted each samples. After continuous exposure to the compounds for 72 h, the culture medium was removed from each well and the cells were fixed with 10% cold trichloroacetic acid at 4°C for 1 h. After washing with tap water, the cells were stained with 0.4% SRB dye incubated for 30 min at room temperature. The cells were washed again and then, the cell bound SRB was solubilized with 10 mM buffered Tris base solution of pH 10.5. The absorbance was measured spectrophotometrically at 520 nm with a micro titer plate reader. Each experiment was conducted in triplicate. The IC_{50} values of crude MeOH extract and fractions were calculated by the nonlinear regression analysis. The IC_{50} was expressed as the concentration of drug reducing the plaque number by 50% as compared to mock-treated controls. It was calculated from a dose–response line obtained by plotting the percentage plaque reduction, with respect to the control plaque count, versus the logarithm of compound dose. Triplicate wells were utilized for each drug concentration tested.

3. Result and Discussion

Analysis of *n*-hexane fraction comprised of 38 components among which 23 constituents were identified (Figure 1 and Table 1). Pentacyclic triterpenes were the major compounds with a high content of which, **1**: (1S,6R,9S)-5,5,9,10-tetramethyltricyclo[7.3.0.0(1,6)]dodec-10(11)-ene, **2**: 2(1H)naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-, **3**: β-amyrin (13.42 %), **4**: urs-12-en-3-one (14.39%), **5**: lupeol (15.91%), **6**: hop-22(29)-en-3.β-ol (7.89%) and **7**: α-amyrin (10.96 %) (Figure 2).

Terpenoids have been previously reported to show antimicrobial activity (Field and Lettingam 1992). For example, β-amyrin has been shown to exhibit various pharmacological activities *in vitro* and *in vivo* against inflammation, microbial, fungal, viral infections and cancer cells (Vázquez et al., 2012).

Sesquiterpene hydrocarbons such as α-copaene (1.16%), caryophyllene (0.96%), humulene (0.12%), alloaromadendrene (0.12%), γ-muurolene (0.03%), γ-selinene (0.12%), β-humulene (0.04%) and oxygenated sesquiterpene caryophyllene oxide (0.50%) were found as minor constituents in the n-hexane fraction (Table 1).

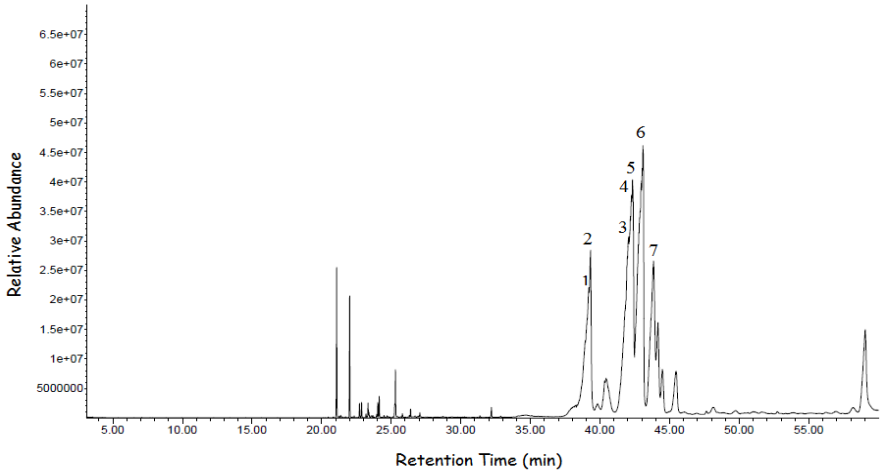

Figure 1: GC of *n*-hexane fraction of *C. sphaerocarpa*, **1**: (1S,6R,9S)-5,5,9,10-Tetramethyltricyclo [7.3.0.0(1,6)] dodec-10(11)-ene, **2**: 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-, **3**: β-amyrin (13.42 %), **4**: urs-12-en-3-one (14.39%), **5**: lupeol (15.91%), **6**: Hop-22(29)-en-3.β-ol (7.89%) **7**: α-amyrin (10.96 %).

Table 1: Compounds identified in the *n*-hexane fraction of *C. sphaerocarpa* and their percentage area

Name of Compounds [a]	RT	Mol.Wt	Mol. Formula	% composition
α–Copaene	21.1	204.19	$C_{15}H_{24}$	1.16
Caryophyllene	22.0	204.19	$C_{15}H_{24}$	0.96
Humulene	22.7	204.19	$C_{15}H_{24}$	0.12
Alloaromadendrene	22.9	204.19	$C_{15}H_{24}$	0.12
γ–Muurolene	23.2	204.19	$C_{15}H_{24}$	0.03
γ–Selinene	23.3	204.19	$C_{15}H_{24}$	0.12
β–Humulene	23.4	204.19	$C_{15}H_{24}$	0.04
α-Amorphene	23.9	204.19	$C_{15}H_{24}$	0.03
α–Panasinsen	24.0	204.19	$C_{15}H_{24}$	0.12
δ–Cadinene	24.1	204.19	$C_{15}H_{24}$	0.18
Caryophyllene oxide	25.3	220.18	$C_{15}H_{24}O$	0.50
(+)-epi-Bicyclosesquiphellrene	26.4	204.19	$C_{15}H_{24}$	0.07
(1S,6R,9S)-5,5,9,10-Tetramethyltricyclo[7.3.0.0(1,6)]dodec-10(11)-ene (**1**)	39.2	218.20	$C_{16}H_{26}$	9.59
2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (**2**)	39.3	218.17	$C_{15}H_{22}O$	4.83
Olean-12-ene	40.3	410.39	$C_{30}H_{50}$	1.23
Olean-12-en-3-one	40.4	424.37	$C_{30}H_{48}O$	2.39
β.-Amyrin (**3**)	42.0	426.39	$C_{30}H_{50}O$	13.45
Urs-12-en-3-one (**4**)	42.3	426.39	$C_{30}H_{50}O$	14.39
Lupeol (**5**)	43.0	426.39	$C_{30}H_{50}O$	15.91
Hop-22(29)-en-3 β-ol (**6**)	43.1	426.39	$C_{30}H_{50}O$	7.89
α–Amyrin (**7**)	43.8	426.38	$C_{30}H_{48}O$	10.96
Taraxasterol	44.2	426.39	$C_{30}H_{50}O$	3.72
A'-Neogammacer-22(29)-en-3-one	48.1	424.37	$C_{30}H_{48}O$	0.34

[a] Compounds listed in order of elution from a HP 5MS column; RT = Retention time; Mol.Wt = Molecular weight; Mol. Formula = Molecular formula.

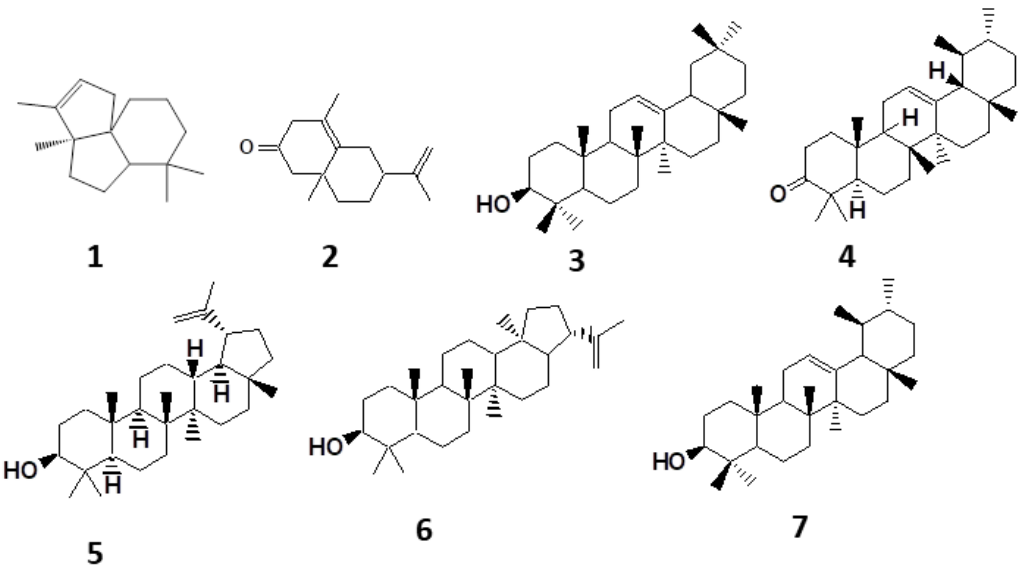

Figure 2: Structures of some major compounds identified in *n*-hexane fraction of the resin of *C. sphaerocarpa*

GC-MS analysis results indicated that β-amyrin had a shorter retention time than α-amyrin (Table 1) which was consistent with the earlier reports (Burnouf-Radosevich et al., 1985). Some constituents such as α-copaene, α-humulene, β-selinene, and δ-cadinene were identified in our previous study from the essential oil of *C. sphaerocarpa* resin (Dekebo et al., 2002).

Sesquiterpenes, which are one of the most common terpenes, are a class of natural products with a diverse range of attractive industrial properties (Wang et al., 2011; Scalcinati et al., 2012). Several biological activities are attributed to sesquiterpenes, such as antimicrobial (Wu et al., 2012), antibacterial (Stojanović-Radić et al., 2012) antioxidant, antifungal (Al-maskri et al., 2011; Conforti et al., 2008) and antigenotoxic (Anter et al., 2011) activities. Nishida et al. (2000) reported that α-copaene is not genotoxic and it increases the antioxidant capacity in human lymphocyte cultures. Fernandes et al., (2007) reported pronounced oral anti-inflammatory effects for the sesquiterpenes isolated from the essential oil of *Cordia verbenacea*. Their oral anti-inflammatory properties are probably related to an important inhibition of the activation and/or release of different inflammatory mediators such as bradykinin, platelet activating factor, histamine, IL-1β, TNFα and PGE_2. The anti-inflammatory effects of these compounds seem to be closely associated with their ability to inhibit the up-regulation of both COX-2 and iNOS enzymes. These workers suggest that sesquiterpenes isolated from the essential oil of *C. verbenacea*, α-humulene and (−)-*trans*-caryophyllene, might constitute a relevant therapeutic alternative for the treatment of inflammatory diseases (Fernandes et al., 2007). In vivo disease resistance assays, using ZmTps21 and Zmtps21 near-isogenic lines, supported the endogenous antifungal role of selinene-derived metabolites involved in the biosynthesis of nonvolatile antibiotics, ZmTps21 exists as a useful gene for germplasm improvement programs targeting optimized biotic stress resistance (Ding et al., 2017).

The concentrations of crude methanol extract which inhibit 50% of cell growth (IC_{50}) was found to be less than 30 μg/mL (Table 2, Figure 3a) against most of the cancer cell lines tested, which is within the limit of criteria set by the American National Cancer Institute for further purification (Radovanovic, 2015). Our anti-proliferative activity data indicated that the crude extract and fractions are far less than etoposide (Figure 3d). The *n*-hexane fraction (Table 2, Figure 3b) showed better anti-proliferative activity than the crude methanol extract and $CHCl_3$ fractions (Table 2, Figure 3c) against all cell lines tested. The chloroform fraction has comparable activity to that of the crude methanol extract (Figures 3a and 3c). The *n*-hexane fraction showed apparent bioactivities on A549, A2780, and SNU-638 cancer cell lines with IC_{50} value ranging from 9.62 μg/mL to 10.30 μg/mL with dose-dependent relationship *in vitro* (Figure 3b and Table 2). These results suggest that *n*-hexane fraction is responsible for the observed high anti-proliferative activity.

The cytotoxicity of *C. sphaerocarpa* crude methanol extract, *n*-hexane and chloroform fractions have not been studied before, but the cytotoxicity of some compounds identified in the *n*-hexane fraction extracted from a variety of plant species showed different pharmacological activities. *In vivo* studies have identified that lupane type pentacyclic triterpenoid have strong antitumor and anti-inflammatory effects (Saleem, 2009). Experimental results showed that, in the mouse skin carcinogenesis model, local application of lupeol for 28 weeks can inhibit the growth of tumor prolongs the latency of tumor cells. The mechanism might be related to the nuclear factor kappaB (NF-κB)/phosphatidylinositol 3-kinase (PI3K)/proteinkinase B (Akt) signaling pathway (Saleem et al., 2004). Aratanechemuge et al (2004), reported that hypodiploid apoptotic peak can be detected after the lupane type pentacyclic triterpenoid treatment on HL-60 leukemia cells, with time-dose-dependency (Aratanechemuge et al., 2004). Gallo and Sarachine (2009) reviewed that lupeol and some of its analogues have been shown to possess a wide range of biological activities such as anti-cancer, hepatoprotective, anti-microbial, cardioprotective, anti-melanoma, etc. Therefore, lupeol and its derivatives have a potential to be consumed as a dietery supplement to prevent cancer, coronary and hepatic disease.

Though, the sesquiterpenes such as caryophyllene and caryophyllene oxide found in trace amount in *n*-hexane fraction, essential oils or fractions with high amount of the sesquiterpenes possessed higher cytotoxic activity against animal human tumor cells (El Hadri et

al., 2010). The sesquiterpene hydrocarbon caryophyllene and its corresponding oxygenated sesquiterpene caryophyllene oxide showed anti-inflammatory effect on LPS induced paw edema rat with a mechanism of action by reduction of neutrophil migration inhibition of NF-κB (Medeiros et al., 2007) and inhibition of 5-LOX catalyzed leukotrienes rat (Jin et al., 2011). The study reported by Da Silva et al. (2016) has shown that *n*-hexane extract of *I. coccinea* flowers led to the isolation of a mixture of α and β-amyrin. The mixture was found to be moderately cytotoxic towards B16-F10 and HepG2 cancer cell line with IC_{50} 23.21 and 24.09 µg/mL, respectively, and non-cytotoxic with IC_{50}> 25 µg/mL against HL60, K562 and PBMC cancer cell lines (Da Silva et al., 2016). The mixture of α- and β-amyrin from *Protium heptaphyllum* demonstrated peripheral and central analgesic effects independent of the opioid system, and also showed a potent anti-inflammatory activity. The anti-inflammatory activity was potentiated by both indomethacin and thalidomide, suggesting a potential involvement of prostaglandins and TNF-alpha inhibitions (Aragao et al, 2008). Urs-12-en-3-one,(3β)-27-[(Z)-feruloyloxy]-3-hydroxyurs-12-en-28-oic acid reported form the stem bark of *Plumeria obtusa* showed anti-mutagenic activity (Siddiqui et al., 2004).

In conclusion, our results for the *n*-hexane fraction revealed moderate cytotoxic activity, which might be attributed to the presence of pentacyclic triterpenes and sesquiterpenes. The cytotoxicity to the three cancer cell lines by the *n*-hexane fraction needs further research on the underlying molecular mechanisms of action.

Table 2: Cytotoxic activity of the crude MeOH extract and solvent fractions from *C. sphaerocarpa* resin against four human cancer cell lines using SRB assay

Extract/Fractions	Cell lines IC_{50} (µg/ml)			
	A549	A2780	MIA-PaCa-2	SNU-638
MeOH	11.03	11.32	23.51	11.56
n-Hexane	9.64	9.62	17.21	10.3
$CHCl_3$	11.23	11.06	24.57	10.84
Etoposide, µM	0.34	0.58	0.72	0.24

(Inhibition of cell growth by 50%). Data was generated by experiments performed in triplicates.

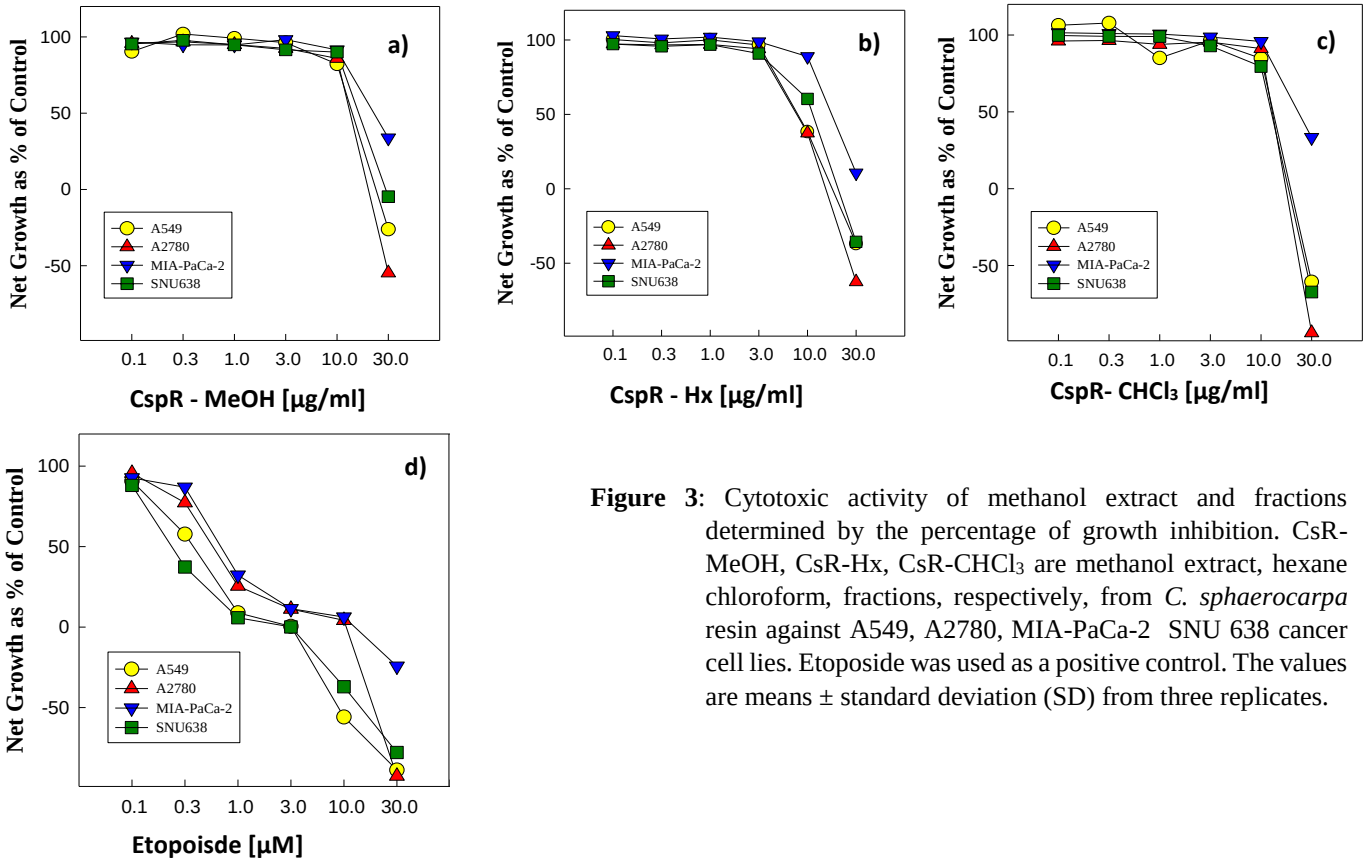

Figure 3: Cytotoxic activity of methanol extract and fractions determined by the percentage of growth inhibition. CsR-MeOH, CsR-Hx, CsR-$CHCl_3$ are methanol extract, hexane chloroform, fractions, respectively, from *C. sphaerocarpa* resin against A549, A2780, MIA-PaCa-2 SNU 638 cancer cell lies. Etoposide was used as a positive control. The values are means ± standard deviation (SD) from three replicates.

The net growth as percent control or cell viability expressed in percentage of the crude MeOH extract, n-hexane and chloroform fractions showed concentration-dependence (Figure 3), which decreased with an increase in crude extract and solvent fraction concentration. The A549 and A2780 cell line were the most sensitive to *Commiphora sphaerocarpa* resin *n*-hexane fraction with IC_{50} value 9.64 and 9.62 µg/mL The *n*-hexane fraction showed a significant dose dependent in reducing the proliferation of A549 and A2780 cancer cells at 0.1, 0.3, 1.0, 3.0, 10.0, and 30.0 µg/mL (Figure 3b). Particularly, the cell viability of the cancer cell line decreased sharply at higher concentration, i.e., at 10 and 30 µg/ml with more than 60% and 100% inhibition. Slight inhibitory activity was observed by the MeOH crude extract and chloroform fraction in the range of 10.84 - 11.23 against A549, A2780 and SNU-638 cancer cell line (Figures 3a and 3c). The activity presented by the crude MeOH extract, *n*-hexane and chloroform fractions was least potent against the MIA-PaCa-2 cell line with IC_{50} value 23.51, 17.21 and 24.57 µg/mL respectively (Figures 3).

4. Conclusion

Among the two solvent fractions tested for cytotoxicity activity, the *n*-hexane fraction showed better apparent cytotoxicity against A549, A2780 and SNU-638 cancer cells with dose-dependent relationship *in vitro* compared to the chloroform fraction. Chemical constituents of the most active *n*-hexane fraction was analyzed by GC-MS and the result revealed that pentacyclic triterpenes and sesquiterpenes were found to be major components of the fraction which might be responsible for the observed anti-proliferative effects. The findings of this study suggest that the components of *n*-hexane fraction of *C. sphaerocarpa* might be useful to treat aforementioned selected cancers after further *in vivo* and clinical studies.

Conflict of interest

The authors declare no conflicts of interests.

Acknowledgment

Financial support for this work was provided by Adama Science and Technology University and National Research Foundation of Korea (No. 2016K1A3A1A09939937 in support of staff development program.

Reference

Al-Maskri A.Y., Hanif M.A., Al-Maskari M.Y., Abraham A.S., Al-sabahi J.N., Al-Mantheri O. (2011). Essential oil from *Ocimum basilicum* (Omani Basil): a desert crop. *Natural Product Communications, 6*(10):1487-90.

Anter J, Romero-Jiménez M., Fernández-Bedmar Z., Villatoro-Pulido M., Analla M., Alonso-Moraga A., Muñoz-Serrano A. (2011). Antigenotoxicity, cytotoxicity, and apoptosis induction by apigenin, bisabolol, and protocatechuic acid. *Journal of Medicinal Food, 14*(3):276-83.

Aragao, G.F., Pinheiro, M.C.C., Bandeira, P.N., Lemos, T.L.G. and Viana, G.S.D.B. (2008). Analgesic and anti-inflammatory activities of the isomeric mixture of alpha-and beta-amyrin from *Protium heptaphyllum* (Aubl.). *Journal of herbal pharmacotherapy, 7*(2): 31-47.

Aratanechemuge, Y., Hibasami, H., Sanpin, K., Katsuzaki, H., Imai, K., and Komiya, T. (2004). Induction of apoptosis by lupeol isolated from mokumen (Gossampinus malabarica L. Merr) in human promyelotic leukemia HL-60 cells. *Oncology reports, 11*(2): 289-292.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians, 68*(6): 394-424.

Burnouf-Radosevich, M., Delfel, N.E. and England, R., (1985). Gas chromatography-mass spectrometry of oleanane-and ursane-type triterpenes—application to Chenopodium quinoa triterpenes. *Phytochemistry, 24*(9): 2063-2066.

Chang, L., and Kinghorn, A. (2001). Flavonoids as cancer chemopreventive agents. *Bioactive Compounds from Natural Sources*, 159.

Conforti F., Menichini F., Loizzo M.R., Statti A.G., Rapisarda A., Menichini F., Houghton P.J. (2008).Antioxidant, alpha-amylase inhibitory and brine-shrimp toxicity studies on *Centaurea centaurium* L. methanolic root extract. *Natural Product Research, 22*(16):1457-66.

Cragg, G. M., and Newman, D. J. (2005). Biodiversity: A continuing source of novel drug leads. *Pure and applied chemistry, 77*(1): 7-24.

Da Silva, T.B., Costa, C.O.S., Galvão, A.F., Bomfim, L.M., Rodrigues, A.C.B.D.C., Mota, M.C., Dantas, A.A., dos Santos, T.R., Soares, M.B. and Bezerra, D.P. (2016). Cytotoxic potential of selected medicinal plants in northeast Brazil. *BMC complementary and alternative medicine, 16*(1): 199.

Dekebo, A., Dagne, E., and Sterner, O. (2002). Furanosesquiterpenes from *Commiphora sphaerocarpa* and related adulterants of true myrrh. *Fitoterapia, 73*(1): 48-55.

Ding, Y., Huffaker, A., Köllner, T.G., Weckwerth, P., Robert, C.A., Spencer, J.L., Lipka, A.E. and Schmelz, E.A., (2017). Selinene volatiles are essential precursors for maize defense promoting fungal pathogen resistance. *Plant physiology*, *175*(3): 1455-1468.

El Hadri, A., del Rio, M.G., Sanz, J., Coloma, A.G., Idaomar, M., Ozonas, B.R., González, J.B. and Reus, M.I.S.,(2010). Cytotoxic activity of α-humulene and transcaryophyllene from Salvia officinalis in animal and human tumor cells. *An R Acad Nac Farm*, *76*(3): 343-356.

Fernandes, E.S., Passos, G.F., Medeiros, R., da Cunha, F.M., Ferreira, J., Campos, M.M., Pianowski, L.F. and Calixto, J.B., (2007). Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *European journal of pharmacology*, *569*(3): 228-236.

Field, J.A.; Lettinga, G. (1992).Toxicity of tannic compounds to microorganisms. Plants polyphenols: Synthesis, properties, significance. *Basic Life Sciences*, 59: 673–692.

Gallo, M. B., and Sarachine, M. J. (2009). Biological activities of lupeol. International Journal of Biomedical and Pharmaceutical Sciences, *3*(1): 46-66.

Jin, J. H., Lee, D.-U., Kim, Y. S., and Kim, H. P. (2011). Anti-allergic activity of sesquiterpenes from the rhizomes of *Cyperus rotundus*. *Archives of Pharmacal Research*, *34*(2): 223-228.

Medeiros, R., Passos, G.F., Vitor, C.E., Koepp, J., Mazzuco, T.L., Pianowski, L.F., Campos, M.M. and Calixto, J.B.(2007). Effect of two active compounds obtained from the essential oil of Cordia verbenacea on the acute inflammatory responses elicited by LPS in the rat paw. *British journal of pharmacology*, *151*(5): 618-627.

Memirie, S. T., Habtemariam, M. K., Asefa, M., Deressa, B. T., Abayneh, G., Tsegaye, B., Rebbeck, T. R. (2018). Estimates of Cancer incidence in Ethiopia in 2015 using population-based registry data. *Journal of global oncology*, 4: 1-11.

Nishida, R., Shelly, T.E., Whittier, T.S. and Kaneshiro, K.Y. (2000). α-Copaene, a potential rendezvous cue for the Mediterranean fruit fly, *Ceratitis capitata*?. *Journal of Chemical Ecology*, *26*(1): 87-100.

Radovanovic, A. (2015). Evaluation of potential cytotoxic effects of herbal extracts. *Serbian Journal of Experimental and Clinical Research*, *16*(4): 333-342.

Saleem, M. (2009). Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer letters*, *285*(2), 109-115.

Saleem, M., Afaq, F., Adhami, V. M., and Mukhtar, H. (2004). Lupeol modulates NF-κB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice. *Oncogene*, *23*(30): 5203.

Scalcinati, G., Partow, S., Siewers, V., Schalk, M., Daviet, L. and Nielsen, J., (2012). Combined metabolic engineering of precursor and co-factor supply to increase α-santalene production by *Saccharomyces cerevisiae*. *Microbial cell factories*, 11:117.

Schols, D., Pauwels, R., Vanlangendonck, F., Balzarini, J., and De Clercq, E. (1988). A highly reliable, sensitive, flow cytometric/fluorometric assay for the evaluation of the anti-HIV activity of antiviral compounds in MT-4 cells. *Journal of immunological methods*, *114*(1-2): 27-32.

Shen, T., Li, G.-H., Wang, X.-N., and Lou, H.-X. (2012). The genus Commiphora: a review of its traditional uses, phytochemistry and pharmacology. *Journal of ethnopharmacology*, *142*(2): 319-330.

Shen, T., and Lou, H. X. (2008). Bioactive constituents of myrrh and frankincense, two simultaneously prescribed gum resins in Chinese traditional medicine. *Chemistry and biodiversity*, *5*(4): 540-553.

Siddiqui, B.S., Ilyas, F., Rasheed, M., Begum, S. (2004). Chemical constituents of leaves and stem bark of *Plumeria obtusa*. *Phytochemistry*, 65(14) 2077-2084.

Soromessa, T. (2013). Ecological phytogeography: a case study of Commiphora species. *Science, Technology and Arts Research Journal*, *2*(3), 93-104.

Stojanović-Radić, Z., Comić, L.J., Radulović, N., Blagojević, P., Denić, M., Miltojević, A., Rajković, J., Mihajilov-Krstev, T. (2012). Antistaphylococcal activity of *Inula helenium* L. root essential oil: eudesmane sesquiterpene lactones induce cell membrane damage. *European journal of clinical microbiology & infectious diseases* ,31(6):1015-1025.

Vázquez, L.H., Palazon, J., Navarro-Ocaña, A. (2012). The pentacyclic triterpenes α,β-amyrins: A review of sources and biological activities. In phytochemicals-A global perspective of their role in nutrition and health; InTech.

Vollesen, K., Hedberg, I., and Edwards, S. (1989). Flora of Ethiopia. *Addis Ababa, Ethiopia. National Herbarium*, 3: 442-478.

Wang, C., Yoon, S.H., Jang, H.J., Chung, Y.R., Kim, J.Y., Choi, E.S., and Kim, S.W. (2011). Metabolic engineering of *Escherichia coli* for α-farnesene production. *Metabolic engineering*, 13(6):*648-55*.

Wu ,Y.X., Chen, Y.J., Liu, C.M., Gao, K. (2012). Four new sesquiterpenoids from *Ligularia cymbulifera*. *Journal of Asian natural products research*, 14(12):1130-1136.

Xu, J., Guo, Y., Li, Y., Zhao, P., Liu, C., Ma, Y., Gao, J., Hou, W. and Zhang, T.,(2011). Sesquiterpenoids from the resinous exudates of Commiphora myrrha and their neuroprotective effects. *Planta medica*, *77*(18): 2023-2028.