Cytotoxicity Potential of Essential Oils and Extracts of Oleo-Gum Resins from *Boswellia papyrifera* (Tarak tarak) Grown in Different Regions of the Sudan

Ghada Ibrahim Mustafa[1], Abdalla Abdelsamad Abdalla[2*], Khojali Elnour Ahmed[3], Aisha Zoheir Al-magboul[4] and Eltayeb Fadul[4]

[1]*Department of biochemistry and food science, Faculty of natural Resources and Environmental Studies, University of Kordofan.*
[2] *Department of Food Technology, Faculty of Agricultural Technology and Fish Sciences, Al-Neelain University.*
[3]*Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum.*
[4]*Institute of Medicinal and Aromatic Plants and Traditional Medicine Research, National Centre for Research, Khartoum, Sudan*

ABSTRACT

In this study, three samples of oleo-gum resins of *Boswellia papyrifera* (Tarak tarak) grown in different regions of the Sudan were used (*Kordofan*, *Damazine* and *Nagawa*). Solvent extracts, acid fractions and essential oils of the three samples were evaluated for their cytotoxicity potential via the brine shrimp lethality assay. Results revealed a dose–dependent response in mortality, and where the degree of lethality was directly proportional to the concentration of the extracts. Among all tested materials, the best cytotoxic activity was exhibited by the methanolic extracts and acid fractions of the three samples, non acid fractions from *Kordofan* and *Damazine* olibanum as well as the petroleum extract of *Damazine* sample. All examined materials, excluding the volatile oils of *Damazine* and *Nagawa* samples, showed high cytotoxicity with LD_{50} values less than 20 µg /ml; thus can be used to predict anti-carcinogenic activity.

Keywords: *Boswellia papyrifera*; cytotoxicity; essential oils; extracts; resins; olibanum;

.

INTRODUCTION

The resins of *Boswellia spp*. family Burseraceae are commonly recognized as frankincense or olibanum. *B. serrata*, *B. papyrifera*, *B. frereana* and *B.carterii* are reported to be the most important commercial sources of olibanum in Arabia, India and the eastern coast of Africa (Hairfield *et al*, 1989). Sudanese olibanum is obtained from *B. papyrifera* (Del.) which occurs on rocky slopes between altitudes of 950 and 1800 m (Dekebo *et al*., 1999). Olibanum is used as incense and it is also used in folk medicine in the treatment of cough and asthma, as an ingredient of embalming fluid, a diuretic stimulant and an emmenagogue, but its essential oil and absolute oil are used as fixatives in perfumes, soaps, creams, lotions and

*Corresponding author E-mail: abdgha@yahoo.com

detergents (Abdel Wahab *et al*., 1987). The oil derived from *Boswellia carterii* Birdw demonstrated immunostimulant activity which is an added value to the previously reported anti-inflammatory, immunomodulatory and anti-leukotriene activities of the resin. This encourages the use of olibanum oleo-gum resin in several immune disorders (Mikhaeil *et al*., 2003).

Cytotoxicity is the quality of being toxic to cells. Treating cells with the cytotoxic compound can result in a variety of cell fates (Riss and Moravec, 2004). The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis). A substance is considered to be cytotoxic if it inhibits vital metabolic processes or it causes disorders in living organisms resulting in perversion of behavior or death. A product is only considered cytotoxic if it can prevent important metabolic processes from occurring in an organism on exposure. It is equally cytotoxic if it causes anomalies in the organ systems which may result in abnormal behavior or death of the organism (Kamanja *et al*., 2018). The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxicity and anti-tumor properties (Carballo *et al*., 2002; Hazra and Chatterjee, 2008; Indabawa, 2009). The plant extracts *of Boswellia serrata*, exhibited significant brine shrimp lethality with LD_{50} value of 18 µg/ml (The dose at which 50% lethality was observed). The degree of lethality was found to be directly proportional to the concentration of the extract (Krishnarajua *et al*., 2005). Low LD_{50} (that is, < 20 µg/ml) found for this plant extract shows the possibility of potent antitumor and insecticidal compounds presence in the plant (Krishnaraju *et al*., 2005).

Objectives

There are no known reports concerning the cytotoxicity of the essential oils as well as the extracts of oleo-gum resins from *Boswellia papyrifera* grown in different locations in the Sudan. So the proposed study aimed at evaluating the toxic effect of volatile oil and extracts from *B. papyrifera* on brine shrimp (*Artemia salina*).

MATERIALS AND METHODS

Materials

Material collection

Two authenticated samples of oleo-gum resins of *Boswellia papyrifera* were obtained from Elobied Agricultural Research Station, North Kordofan State (*Kordofan* sample) and Eldamazine Agricultural Research Station, Blue Nile State (*Damazine* sample), in addition to a commercial sample (*Nagawa*) kindly offered by an oleo-gum exporter in Khartoum were used in this investigation. Samples were identified by experts from the Forestry Department, Ministry of Agriculture and Forestry as well as Agricultural Research Stations of Elobied and Eldamazine.

Preparation of extracts

Solvent extracts were prepared according to Mothana *et al*. (2006). Each time, the air dried and powdered oleo-gum resin (50 g) was extracted under shaking at room temperature separately with petroleum ether (petroleum ether extract, PE), 90% methanol (methanolic extract, ME) and hot (70 °C) distilled water (water extract, WE). For each solvent the extraction was carried overnight (12 hours) and repeated 3 times. Extracts obtained were filtered and the solvents were then evaporated using rotary evaporator or freeze dryer in the case of the water extracts to give the crude dried extracts.

Isolation of the acid fractions:

The acid fraction (AF) of the resin was isolated by 2% KOH extraction according to the method described by Basar (2005).

olibanum (10 g) was extracted by shaking with 50 ml methanol for 12 hours. After filtration the extract was concentrated using rotary evaporator to nearly 30 ml until it becomes a thick solution. The concentrated solution was dissolved in 100 ml of 2% KOH aqueous solution and extracted five times with 30 ml ethyl acetate. Every time the aqueous phase was separated from the organic phase (non acidic fraction, nAF) using separating funnel. The aqueous phase was then neutralized with 2% HCl to pH 6. The acid fraction was isolated from the aqueous phase by extraction five times with 30 ml ethyl acetate. Every time the organic phase (acidic fraction) was collected separately. Finally the two fractions were washed with distilled water, dried over with anhydrous Na_2SO_4 and the solvent as evaporated to dryness.

Extraction of the essential (volatile) oil

The oleo-gum resin (500 g) was subjected to hydro-distillation using Clevenger's apparatus until complete exhaustion. The obtained colorless oil was collected, dried over with magnesium sulphate and kept at 4 °C for analysis (Al-Harrasi and Al-Saidi, 2008).

Methods

Brine-shrimp lethality assay

The brine shrimp lethality assay was carried out according to McLauglin (1988) and McLauglin *et al*. (1991).

Hatching of shrimp eggs

Some sea water (Red Sea) was placed in a small plastic container with perforated dividing dam which was fabricated from a plastic soap case. Some shrimp eggs were added to one side of the divided dam tank. This side was darkened by covering it with a plastic lid while the other compartment was exposed. The set-up was left for 48h for the shrimp eggs to hatch and mature as nauplii. Mature nauplii usually swim to the exposed compartment**.**

Preparation of vials for testing

A stock solution from each extract was prepared by dissolving 20 mg of the sample in 2 ml methanol. For the essential oil, the stock solution was prepared by dissolving 20 mg essential oil in 2 ml DMSO (1%) according to the solution method modified by Krishnaraju *et al.* (2005).

To obtain the desired final concentrations (1000 µg/ml, 100 µg/ml and 10 µg/ml), 0.5 ml, 0.05 ml and 0.005 ml of the stock were transferred into the three vials respectively. The solvent was then evaporated by leaving the vials in vacuum desiccators for 24 hours. Ten shrimp nauplii were counted into each vial (i.e. 30 nauplii per dilution). The total volume of solution in each vial was adjusted to 5 ml by adding the sea water (5 ml/vial). The vials were maintained in the laboratory with normal fluorescent illumination and the set-up left for 24 hours. The number of survivors usually swimming was counted with the aid of a magnifying lens for each of the vials at the end of 24 hours. Thus, the number of the dead was calculated.

Determination of the lethal dose (LD_{50}**)**

LD_{50} is the dose at which 50% lethality was observed. The LD_{50} values in ppm with 95% confidence intervals were determined using the Finney probit analysis software (Meyer *et al*., 1982). LD_{50} values below 200 ppm are generally considered as significant according to Oladimeji *et al*. (2006).

RESULTS

The bioactivity of different extracts from *Kordofan* oleo-gum resin against the brine shrimp (*A. salina*) is illustrated in figure 1. At the maximum concentration used (1000 ppm), all brine shrimps died after 24 h when treated with the acid fraction, which gave 100% mortality. Essential oil, methanol extract and non acid fraction showed high mortality percentage in the range of 90% - 96.66%. Slightly lower values of 60% and 80% were recorded for water and petroleum extracts, respectively. At the medium level applied (100 ppm), the highest mortality (96.67%) was exhibited by the acid fraction. Lesser values of 90% and 83.33% were exerted by methanol extract and non acid fraction, respectively. Water and petroleum extracts as well as the volatile oil showed inferior cytotoxicity towards the brine shrimp (43 - 50%) compared to the remaining materials. At the minimum concentration of 10 ppm the acid fraction still has the uppermost lethality power (93.33% mortality). Similarly, methanol extract and non acid fraction maintained the same moderate activities (83 and 70%, respectively), while the other substances showed less than 50% mortality.

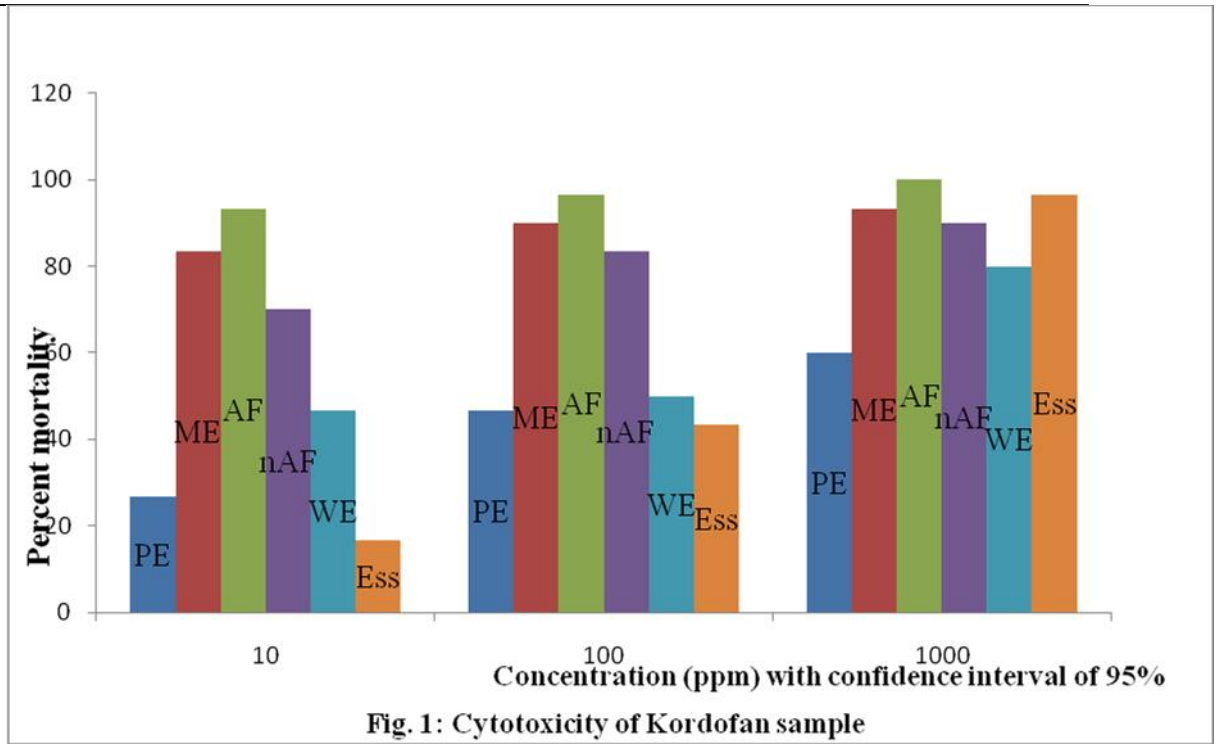

Fig. 1: Cytotoxicity of Kordofan sample

The acid fraction has the highest toxicity of all, even at 10 ppm, the dose at which LD_{50} was 0.0004 ppm (Tab. 1). Methanol extract was close in toxicity with LD_{50} value of 0.0024 ppm, while non acid fraction gained slightly higher LD_{50} of 0.0038 ppm. In contrast, water extract and essential oil has much greater LD_{50} values of 28.7552 and 81.2413 ppm, respectively. However, petroleum extract has the least mortality with highest LD50 value of 219.906 ppm.

Table (1): Brine shrimp lethality (LD_{50} ppm with 95% confidence interval)

Sample	PE	ME	AF	nAF	WE	Ess
Kordofan	219.9060	0.0024	0.0004	0.0386	28.7552	81.2413
Damazine	0.0002	0.0020	0. 0452	0.0002	ND	723.0950
Nagawa	14.3919	0.0237	0.0063	0.9672	104.8150	4431.9100

LD_{50}: The dose at which 50% lethality was observed

PE: petroleum ether extract ME: methanol extract

AF: acid fraction nAF: non acid fraction

WE: water extract Ess: essential oil

Regarding *Damazine* frankincense (Fig. 2) at concentration of 1000 ppm, all the tested materials gave 96.67% mortality. When brine shrimp was manipulated with different extracts at concentration of 100 ppm, the highest percent mortality (93.33%) was given by petroleum and methanol extracts as well as the non acid fraction. A lower percentage of 86.67% was obtained by the acid fraction. However, the essential oil showed low lethal effect (36.67%). At 10 ppm, petroleum extract, methanol extract and non acid fraction revealed similar percent mortality (90%), whereas acid fraction showed lower activity (83.33%). The lethal effect of *Damazine* volatile oil was only 10% mortality.

From Table 1, it is obvious that petroleum, methanolic and non acid fractions exhibit the highest toxicity even at the lowest concentration (10 ppm), with LD_{50} value of 0.0002 ppm. The acid fraction was next in toxicity with 0.052 ppm LD_{50}. The essential oil gave the least lethality with the highest LD_{50} value of 723.095 ppm.

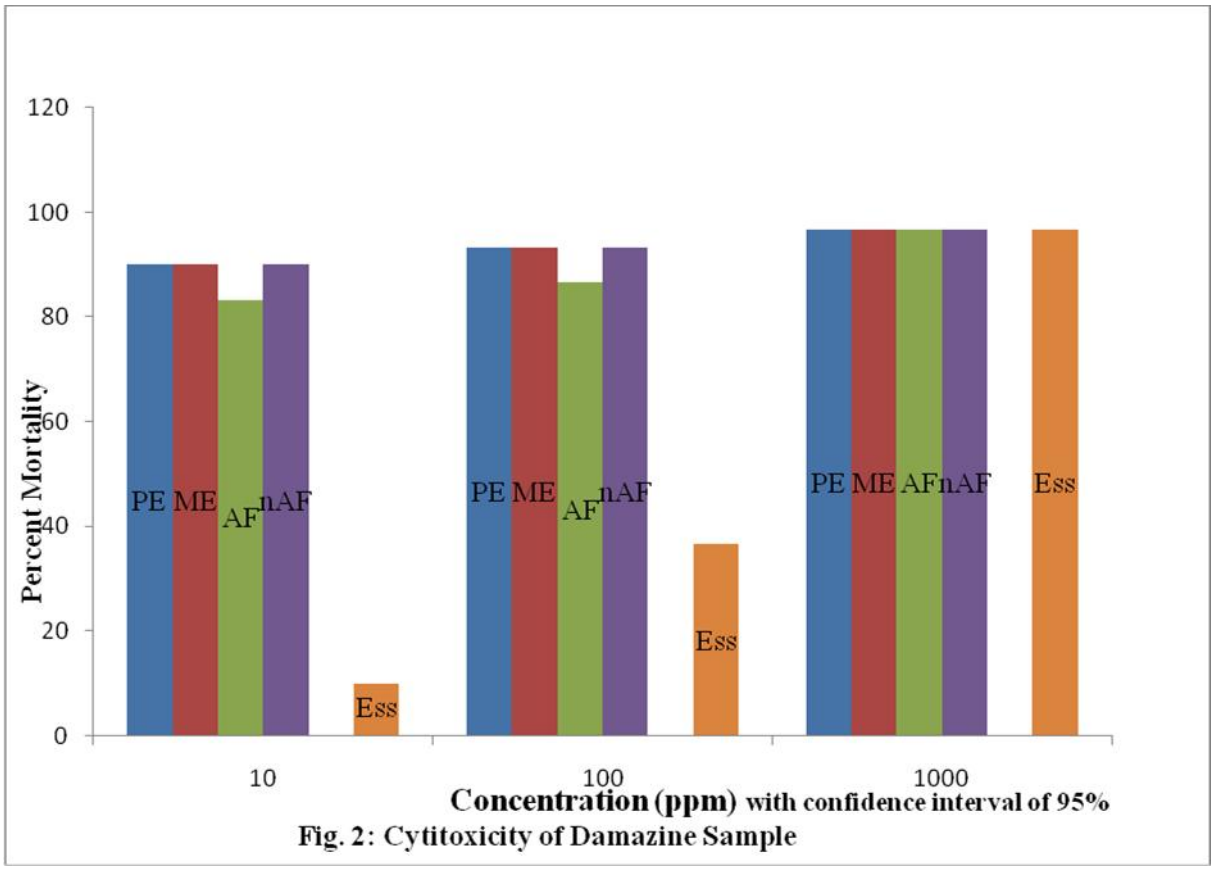

Fig. 2: Cytitoxicity of Damazine Sample

The cytotoxic influence of *Nagawa* samples was depicted in figure 3. Acid and non acid fractions possessed the highest mortality (96.67%) when applied at level of 1000 ppm. On the other hand, methanol and water extracts showed the same mortality percent (90%). Trivially lower mortality of 86.67% was observed for petroleum extract. However, *Nagawa*

essential oil maintained the least percent mortality (40%). At 100 ppm, the highest mortality was given by the acid fraction (86.67%). Both petroleum and methanol extracts showed mortality of 83.33%. The non acid fraction gave moderate mortality of 76.67%, while the water extract as well as the volatile oil possessed inferior mortality of 36.67 and 30%, respectively. At 10 ppm concentration the acid fraction retained nearly the same highest mortality 83.33%, whereas the essential oil showed a very low lethal power of 16.67%. The remaining tested materials gave percent mortality range between 20 and 76.67%.

Clearly, according to table 1, the acid fraction and the methanol extract from *Nagawa* sample had the highest toxicity even at the lowest concentration (10 ppm), with LD_{50} values of 0.0063 and 0.0237 ppm, respectively. The non acid fraction as well as the petroleum extract showed moderate toxicity with 0.9672 and 14.3919 ppm LD_{50}, respectively. The water extract and the essential oil gained the least lethality with the high LD_{50} values of 104.8150 and 4431.9100 ppm, respectively.

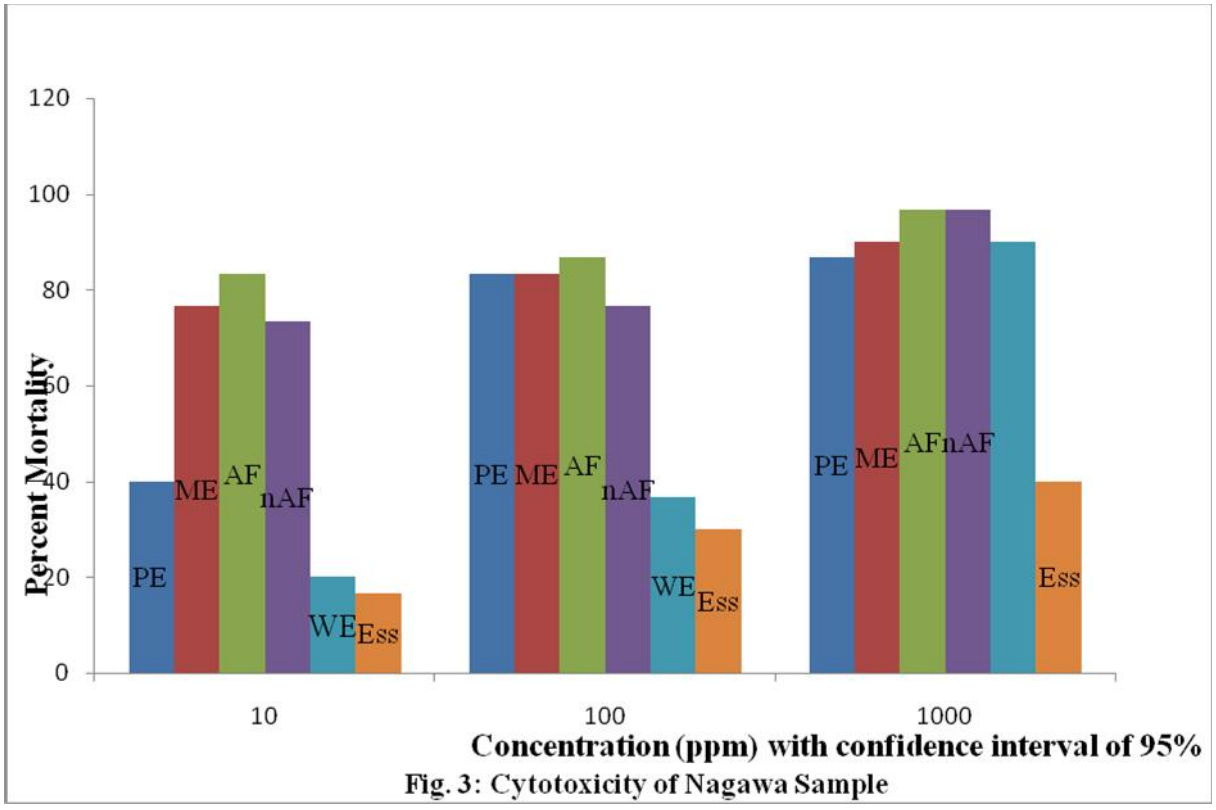

Fig. 3: Cytotoxicity of Nagawa Sample

DISCUSSION

It seems that the degree of lethality was directly proportional to the concentration of the extracts. Maximum mortalities took place at 1000 ppm and least mortalities were at 10 ppm. Comparatively, a dose–dependent response in mortality was observed for the *M.*

aeruginosa extract from Taiwan against brine shrimp (Metcalf *et al.*, 2002). Equally, Chou *et al*. (2004) conducted a brine shrimp lethality assay with the extract of *M. aeruginosa* from central Europe, the results showed that the tested animals were killed at various doses of the extract. Moreover, Indabawa (2009) stated that the extracts of *M. aeruginosa* were toxic to brine shrimp at 24 hours in a dose dependent manner in which the tested animals were killed at the highest dose of 100 µg /ml in most of the samples.

Among all tested materials, the best cytotoxic activity was exhibited by the methanol extracts and acid fractions of the three samples, non acid fractions from *Kordofan* and *Damazine* olibanum as well as the petroleum extract of *Damazine* sample. Present results were in conformity with Ogunnusi and Dosumu (2008). Krishnaraju *et al*. (2005) reported LD_{50} value of 18 µg /ml for *Boswellia serrata* aqueous extract against brine shrimp. However, *Commiphora wightii* and *Commiphora myrrha* Oleo-gum resins which belong to the same family (Burseraceae) showed high LD_{50} of 1,600 and >5,000, respectively. Also, the methanolic extracts of *B. socotrana* and *B. dioscorides* manifested a considerable cytotoxic effect with LD_{50} values between 18 and 29 µg/ml. Boswellic acids, which may represent a considerable part of the chemical content, could contribute to this observed effect (Mothana *et al*., 2009). Recent toxicological studies indicated that *Commiphora swynnertonii* resin extract affected brine shrimps with LD_{50} of 15.8 µg /ml (Bakari, 2012).

The significance of low LD_{50} value is indicative of the presence of potent cytotoxic and insecticidal compounds (Ogunnusi and Dosumu, 2008), which supports the earlier findings of Rieser *et al*. (1996) who concluded that extracts resulting in LD_{50} values of less than 250 µg/ml were considered significantly active and deserve further investigations. Since all examined extracts in this study, excluding all water extracts and petroleum extract of *Kordofan*, possessed high cytotoxicity with LD_{50} values less than 20 µg /ml; and according to Meyer *et al*. (1982) they could be used to predict anti-carcinogenic activity. Whoever, Plants found to be toxic to brine shrimp are likely to be good candidate for anti-cancer research (Ramachandran *et al*., 2011).

Interestingly, octyl acetate (the main component in the essential oil of the three samples) was found to be the major compound in *Heracleum sphondylium* (Husnu, 2002). This plant was proved to have cytotoxic and phytotoxic effect (Weimarch and Nilsson, 1980 and Ugur, 1998). Thus octyl acetate was suggested to be the cytotoxic agent in *Heracleum persicum* (Moshafia *et al*., 2009). According to Rieser *et al*. (1996), this suggestion could be true only for *Kordofan* essential oil which exhibited LD_{50} value less than 250 µg/ml.

CONCLUSION

The cytotoxicological investigation using the brine shrimp assay revealed a dose–dependent response in mortality, that the degree of lethality was directly proportional to the concentration of the extracts. Among all tested materials, the best cytotoxic activity was exhibited by the methanolic extracts and acid fractions of the three samples as well as the non acid fractions from *Kordofan* and *Damazine* olibanum in addition to the petroleum extract of *Damazine* sample. Since all examined materials, excluding the volatile oils of *Damazine* and *Nagawa* samples, possessed high cytotoxicity with LD_{50} values less than 20 µg /ml; they could be used to predict anti-carcinogenic activity.

REFERENCES

Abdel Wahab, S.M.; Aboutabl, E.A.; El-Zalabani, S.M.; Fouad, H.A.; DePooter, H.L. and El-Fallaha, B. (1987). The Essential Oil of Olibanum. Planta Medica 53: 382-384.

Al-Harrasi, A. and Al-Saidi, S. (2008). Phytochemical analysis of the essential oil from botanically certified oleogum resin of *Boswellia sacra* (Omani Luban). Molecules 13 (9): 2181-2189.

Bakari, G.G.; Max, R.A.; Mdegela, H.R.; Phiri, E.C. and Mtambo, M.M. (2012). Effect of crude extracts from *Commiphora swynnertonii* (Burtt) against selected microbes of animal health importance. Journal of Medicinal Plants Research 6 (9): 1795-1799.

Basar, S. (2005). Phytochemical investigations on *Boswellia species*. Comparative studies of the essential oils, pyrolysates and boswellic acids of *B. carterii*, *B. serata*, *B. frereana*, *B. neglecta* and *B. rivae*. Dr. rer. Nat. Thesis, universitat Hamburg, Germany.

Carballo, J.L.; Hernandez-Inda, Z.L.; Perez, P. and Garcia-Gravalos, M.D.A. (2002). Comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. BMC Biotechnology 2: 17-21.

Chou, N.H.; Chen, J.Y. and Bai, R.H. (2004). Protein phosphatase inhibition assay for screening of Toxic *Microcystis*. An abstract of the 6 th Asian Fisheries Forum.

Dekebo, A.; Zewdu, M. and Dagne, E. (1999). Volatile oils of frankincense from *Boswellia papyrifera* (Bull). Bulletin of the Chemical. Society of Ethiopia 13 (1): 93–96.

Duncan, B.D. (1955). Multiple range and multiple F-test. Biometrics 11: 1 - 42.

Hairfield, E.M.; Hairfield, H.H. and McNair, H.M. (1989). GC, GC/MS, and TLC of β-Boswellic Acid and O-Acetyl-β-Boswellic Acid from *B. serrate*, *B. carteii*, and *B. papyrifera*. Journal of Chromatographic. Science 27 (3): 127–133.

Hazra, A.G. and Chatterjee, P.A. (2008). Nontoxic antitumour compound from the leaves of *Bauhinia scandens* characterized as 1-*O*-alkyl glycerol by gas–liquid chromatography and evaluation of

its antitumour property by Brine Shrimp bioassay. Industrial Crops and Products 27 (1): 39–43.

Husnu, C.B.E. (2002). Aromatic biodiversity among the flowering plant taxa of Turkey. Pure Applied Chemistry 74: 527-45.

Indabawa, I.I. (2009). Detection and toxicological study of Microcystin by Brine shrimp assay from *microcystis aeruginosa* isolated from Kano Burrow pits. Bioscience Research Communications 21 (5): 209–214.

Kamanja, I.T.; Mbaria J.M.; Gathumbi, P.K.; Mbaabu, M.; Kabasa, J.D. and Kiama, S.G. (2018). Cytotoxicity of selected medicinal plants extracts using the brine shrimp lethality assay from Samburu County, Kenya. The Journal of Medical Research, 4(5): 249-255.

Krishnaraju, A.V.; Rao-Tayi, V.N.; Sundararaju, D.; Vanisree, M.; Tsay, H.S. and Subbaraju, G.V. (2005). Assessment of bioactivity of Indian medicinal plant using brine shrimp (*Artemia salina*) Lethality Assay. International Journal of Applied Science and Engineering, 3 (2): 125-134.

McLaughlin, J.L.; Chang, C.L. and Smith, D.L. (1991). Simple bench-top bioassays (brine-hrimp and potato discs) for the discovery of antitumour compounds. In: Kinghorn, A.D. and Balandrin, M.F. (Eds.), Human Medicinal Agents from Plants. American Chemical Society, Washington DC, pp. 112–137.

McLaughlin, J.L., 1988. Brine-shrimp and crown gall tumors: Simple bioassays for the discovery of plant anti-tumor agents. Proceedings of the NIH Workshop for the Discovery of Anti-Tumor and Antiviral Agents from Natural Sources, Bethesada, pp. 22-22.

Metcalf, J.S.; Lundsay, J.; Beattick, K.A.; Burminghams, S.M.L.; Torokne, A.K. and Codd, G.A. (2002). Toxicity of *M. aeruginosa* to the brine shrimp. Toxicon 42: 116–1120.

Meyer, B.N.; Ferrign, N.R.; Putnam, J.E.; Jacobson, I.B.; Nicholas, D.E. and MacLaughlin, J.L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica, 45: 31-34.

Mikhaeil, B.R.; Maatooq, G.T.; Badria, F.A. and Amer, M.M.A. (2003). Chemistry and immunomodulatory activity of Frankincense oil. Amernse Z. Naturforsch., 58c: 230-238.

Moshafi, M.H.; Sharififar, F.; Dehghan, G. and America, A. (2009). Bioassay screening of the essential oil and various extracts of fruits of *Heracleum persicum* Desf. and rhizomes of *Zingiber officinale* Rosc. using brine shrimp cytotoxicity assay. Iranian Journal of Pharmaceutical Research, 8 (1): 59–63.

Mothana, R.A.A.; Mente, R.; Reiss, C. and Lindequist, U. (2006). Phytochemical screening and antiviral activity of some medicinal plants from the Island Soqotra. Phytotherapy Research 20: 298–302.

Mothana, R.A.; Lindequist, U.; Gruenert, R. and Bednarski, P.J. (2009). Studies of the *in vitro* anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from

the island Soqotra. BMC Complementary and Alternative Medicine **9**: 7. Doi: 10.1186/1472-6882-9-7.

Ogunnusi, T.A. and Dosumu, O.O. (2008). Bioactivity of crude extracts of *Euphorbia kamerunica* Pax using brine shrimp (*Artemia salina*) lethality assay. Journal of Medicinal Plants Research 2 (12): 370–373.

Oladimeji, H.O; Nia, R. and Essien, E.E. (2006). In-vitro antimicrobial and brine shrimp lethality potential of the leaves and stem of *Calotropis procera* (Ait). African Journal of Biomedical Research 9: 205 - 211.

Ramachandran, S. Vamsikrishna, M. Gowthami, K. Heera, B. and Dhanaraju, M. (2011). Assessment of cytotoxic activity of Agave cantula using brine shrimp (Artemia salina) lethality bioassay. Asian J, Sci. Res., 4(1): 90 –94.

Rieser, M.J.; Gu, Z.M.; Fang, X.P.; Zeng, L.; Wood, K.V. and McLaughlin, J.L. (1996). Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata.* Journal of Natural Products 59: 100-108.

Riss, T.L. and Moravec, R.A. (2004).Use of multiple assay endpoints to investigate the effects of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays. Assay and Drug Development Technologies 2 (1): 51–62.

Snedecor, G.W. and Cochran, G.W. (1987). Statistical Method. 7th Edn., Lowa State University Press, Amers, IA, USA.

Ugur, M.S. (1998). Cytotoxicity assay and fibrinolytic evaluation of *Heracleum sphondylium* and *Ferula thirreana*. Fitoterapia 4: 338-380.

Weimarch, G. and Nilsson, E. (1980). Phototoxicity of *Heracleum sphondylium*. Planta Medica 38: 97-111.