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**Original Article**

# Chemical and Bio-guided Evaluation of Some Natural Resins as Potential Antitumor Agents

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## ABSTRACT

Naturally occurring resins have beneficial pharmaceutical and nutraceutical properties. Based on the Ethnopharmacological literature, several species of natural resins used in traditional medicine were collected. In this study eleven commonly available natural resins were screened for their chemical and biological activity. Phytochemical screening of selected resins showed the presence of carbohydrates and/or glycosides, sterols and or triterpenes, flavonoids, Volatile oil and tannins. Brine shrimp lethality bioassay for the methanol extracts of different tested resinous material revealed that Olibanum, Mastic, Myrrh and Colophony were the most active with potential cytotoxic activity ($LC_{50}$) at 231.0±2.02, 261.3±1.05, 575.8±1.13 and 696.2±3.05, respectively. The bio-guided biological assays revealed that Myrrh and Mastic proved to have the highest protective activity against DNA damage induced by bleomycin-iron complex with absorbance at 0.003±0.041 and 0.002±0.014 respectively. This study indicated that Olibanum, Mastic and Myrrh retained the most biological activity of all of the tested resins as potential natural antitumor agents.

**Keywords:** Resins, Brine shrimp, Cytotoxicity, DNA, Bleomycin, Mastic, Olibanum.

## INTRODUCTION

Resins are natural or induced solid or semi- solid exudations from plants or from insects feeding on plants. They are characterized by being insoluble in water, mostly soluble in alcohol or ether, often un crystallisable, and softening or melting at moderate heat forming adhesive fluid without volatilization or decomposition. They range in specific gravity from 0.9 to 1.25. They burn in the air with a smoky flame, owing to their high carbon content in their molecules. They are considered as the oxidized terpenes of the volatile oils of plants and owing to their insolubility in water, have little taste. Resins, when pure are usually translucent; when they contain water they are opaque, and no longer hard and fragile. They cannot conduct electricity, but when rubbed they become negatively electrified.

**Resin combinations**:

**A – Oleoresins**: Natural oleoresins are mixtures of volatile oils and resins and therefore they are liquids or semi liquids substances depending on the amount of the volatile present. Turpentine, Copaiba and Canada balsam are examples of this group.

**B - Gum – Resins**: They are natural mixtures of gum and resin, usually obtained as exudations from plants, as myrrh.

**C- Oleo-gum- resins**: Resin may occur in combination with volatile oil and gum e.g. asafetida.

**D- Glycoresins**: Resin may be combined in a glycosidal way with sugars as the resin of the convolvulaceae being called glycoresins which are found in Ipomea, Jalap and Podophyllum.

**E- Balsams**: resinous substances that contain the aromatic balsamic acids i.e. benzoic acid or cinnamic acid or both or esters of these acids. Balsams usually contain small amount of volatile oil.

Some natural oleogum resins are valuable source of developing new drugs for treatment of different diseases [1, 2, 3]. Boswellia-Curcumin preparation was investigated clinically for treatment of knee osteoarthirits [4]. Meanwhile, glycyrrhizin, curcumin and *Boswellia carterii* formula exhibited a hepatoprotective effect and used as endogenous interferon inducer [5]. The combination of boswellia, curcumin, and glycyrrhizin exhibited the highest activity against *Herpes simplex* virus [6]. Some natural resins (Olibanum and Myrrh) have

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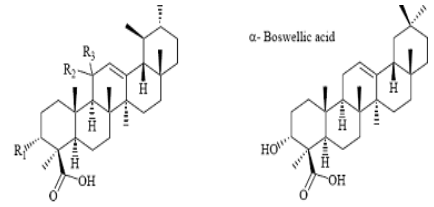
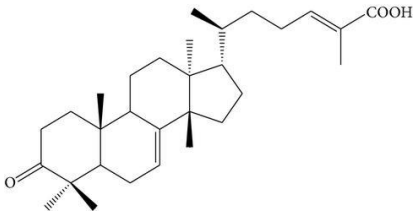
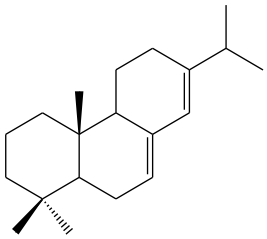
proven to be a bountiful reservoir of numerous biologically active components with advanced antioxidant and antitumor activities that attracted the attention of researchers to Olibanum [7, 8] and Myrrh [9, 10, 11]. The aim of the present study was to promote interest in the discovery of alternative natural compounds with cytotoxic activity.




**α- Masticadienonic acid**



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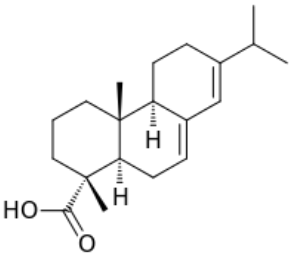

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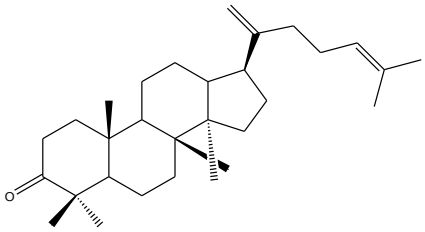
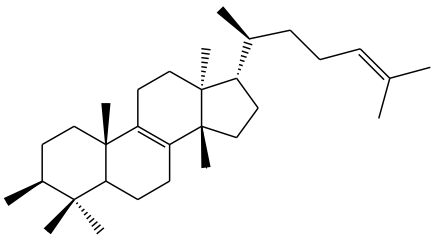
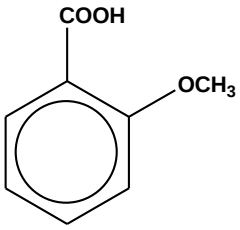


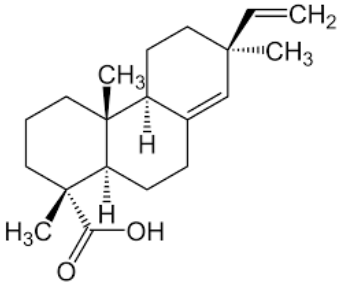
**Tirucallol**



**Dammaradienone**




**Guaiconic acid**




**pimaric acid**

**Fig. 1 Common chemical structures of some natural resins**

## MATERIAL AND METHODS

**Unorganized sample collection**: the unorganized samples (250 gm of each) used in this study consists of the following: Olibanum, Mastic, Colophony, Galbanum, Benzoin, Asafetida, Ammoniacum, Aloe, Copaiba, Guaiacum, and Myrrh purchased from a spice store (Al Nekity-Mansoura, Egypt).

**Extract preparation**: The resins are grounded in a tooth miller grinded to a coarse powder, 100 gm of each powdered unorganized drugs extracted by 70% MeOH until exhaustion and filtered. The Filtrates were concentrated under vacuum and subjected to activity studies.

**Preliminary Phytochemical Screening**

The dried extracts of tested resins (Aloe, Ammoniacum, Asafetida, Benzoin, Colophony, Copaiba, Guaiacum, Mastic, Myrrh, and Olibanum) were subjected to general phytochemical screening for volatile oil [12], carbohydrates and / or glycosides [13], tannins [14], flavonoids[15], oxidase enzyme [16], alkaloids and/ or nitrogenous bases [17], unsaturated sterols and/ or tri-terpenes [18] and cardenolides [19].

**Biological Screening:**

**Brine shrimp assay:** [20]

Brine shrimp eggs were obtained from a pet store (Mansoura, Egypt), artificial sea water was prepared from sea salt (40 g/L) suspension supplemented with 6 mg/ L dried yeast.

The eggs of Brine shrimps were suspended in artificial salt water and the hatchery was oxygenated with an aquarium pump. After 48 hours incubation in warm room (22-29°C), nauplii (brine shrimp larvae) were collected with a Pasteur pipette after attracting the organisms to one side of the hatchery with a light source. The nauplii were separated from the eggs and egg shells by pipetting them 2-3 times in small beakers containing sea water. Tested material were dissolved in 50 µl DMF prior to adding artificial sea water (1 mg/mL). Serial dilutions (1000, 100, 10 µg/mL) were made in the wells in triplicate in 100 µl sea water. A suspension of nauplii containing 10-60 organisms (100 µl) was added to each well and the plate was incubated at 22-29° C for 24 hours. The Plate was then examined under a binocular microscope and the number of dead nauplii in each well was counted. MeOH (100 µl) were then added to each well and after 15 minutes, the total numbers of shrimp in each well were counted. $LC_{50}$ values were calculated using Microsoft Excel XP.

**Bleomycin dependent DNA damage assay** [21]

EDTA, DNA (Salmon testes) and Thiobarbituric acid were from Sigma, USA. Bleomycin sulfate were purchased from Cipla, India. $MgCl_2$ and $FeCl_3$ were from El-Nasr, Egypt. L-ascorbic acid were purchased from Memphis Pharmaceutical Co., Egypt).

The reaction mixtures contained in a final volume of 1.0 mL, the following reagents at the final concentrations stated: DNA (0.2 mg/mL), bleomycin (0.05 mg/mL), $FeCl_3$ (0.025 mM), magnesium chloride (5 mM), $KH_2PO_4$-KOH buffer pH 7.0 (30 mM) and ascorbic acid (0.24 mM) or the tested fractions diluted in MeOH to give a concentration of (0.1 mg/mL). The reaction mixtures were incubated in a water-bath at 37˚C for 1 hour. At the end of the incubation period, 0.1 mL of 0.1 M EDTA was added to stop the reaction (the iron-EDTA complex is unreactive in the bleomycin assay). DNA damage was assessed by adding 1 mL 1% (w/v) thiobarbituric acid (TBA) and 1 mL 25% (v/v) hydrochloric acid (HCl) followed by heating in a water-bath maintained at 80°C for 15 min. The chromogen formed was extracted into butan-l-ol and the absorbance was measured at 532 nm.


Fig. 2 Brine shrimp nauplii as they appear in micro well plate under a binocular microscope

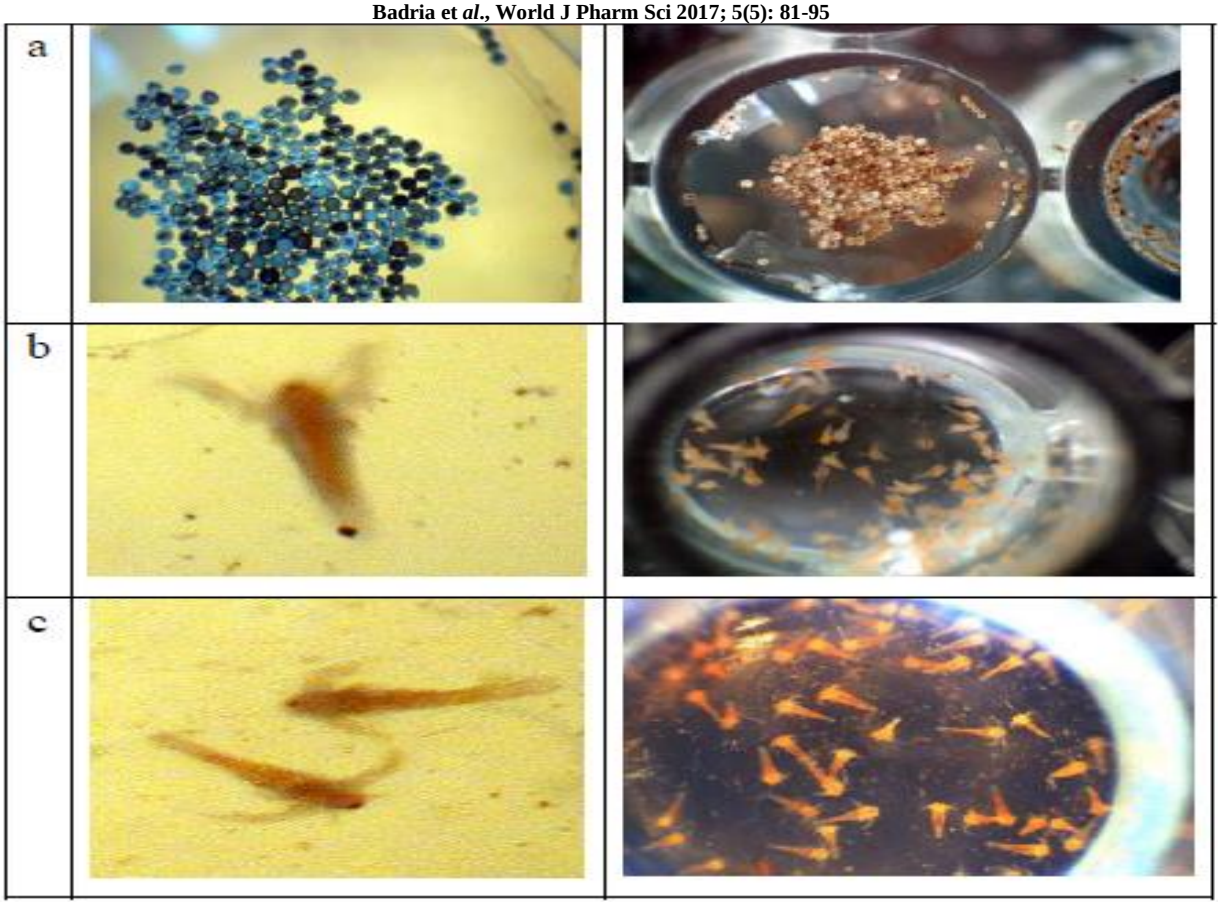



**Fig. 3** Brine shrimp stages of growth: a. eggs of brine shrimp, b. living brine shrimp (moti), c. dead shrimps (non-motile, their front appendages are extended, easily counted).

## RESULTS

The Phytochemical screening of selected natural resin revealed the presence of volatile oil, carbohydrates, tannins, unsaturated sterols and flavonoids as shown in Table 1. The brine shrimp cytotoxicity assay showed that Olibanum, Mastic, Colophony, Asafeitda, Copaiba, Guaiacum and Myrrh have the most cytotoxic activity as presented in Table 2. It was revealed that the number of dead nauplii is directly proportional to the incubation time. Dead nauplii was counted in each well at different times, after15 minute, 24 hours, 48 hours and the results were indicated in Table. 3.

**Table. 1 Phytochemical screening of tested natural resins**

| Extract | Col | Gua | Cop | Asa | Myr | Amm | Oli | Mas | Ben | Alo |
|---|---|---|---|---|---|---|---|---|---|---|
| **Volatile Oil** | + ve | - ve | + ve | + ve | + ve | + ve | + ve | + ve | - ve | - ve |
| **Carbohydrates** | + ve | - ve | + ve | + ve | + ve | - ve | + ve | + ve | + ve | + ve |
| **Flavonoids** | - ve | - ve | - ve | - ve | + ve | - ve | - ve | + ve | - ve | - ve |
| **Alkaloids** | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve |
| **Oxidase enzyme** | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve |
| **Tannins** | + ve | + ve | + ve | - ve | + ve | + ve | - ve | - ve | - ve | + ve |
| **Unsaturated sterols** | + ve | + ve | + ve | + ve | + ve | + ve | + ve | + ve | + ve | + ve |
| **Cardenolides** | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve | - ve |

- Ve: not detected, + Ve: detected

Col: Colophony, Gua: Guaiacum, Cop: Copaiba, Asa: Asafetida, Myr : Myrrh, Amm: Ammoniacum, Oli: Olibanum, Mas : Mastic, Ben : Benzoin, Alo : Aloe.

**Table. 2 Results of Brine Shrimp assay of different resin and resin combinations extracts.**

| Extract | Log conc | Conc (µg / mL) | Initial nauplii | Mean | No. of dead nauplii after 24 h. | % Dead after 24 h. | % Dead mean | %Dead mean corrected to control* |
|---|---|---|---|---|---|---|---|---|
| **Olibanum** | 1 | 10 | 5 | 8.6 | 2 | 40.00% | 51.42% ±2.52 | 44.28% ± 9.38 |
| | | | 7 | | 6 | 85.71% | | |
| | | | 14 | | 4 | 28.57% | | |
| | 2 | 100 | 20 | 11.6 | 4 | 20.00% | 34.04% ±2.52 | 26.90% ± 9.38 |
| | | | 8 | | 2 | 25.00% | | |
| | | | 7 | | 4 | 57.14% | | |
| | 3 | 1000 | 8 | 12.3 | 5 | 62.5% | 66.61% ±2.52 | 59.47% ± 9.38 |
| | | | 11 | | 9 | 81.8% | | |
| | | | 18 | | 10 | 55.55% | | |
| **Mastic** | 1 | 10 | 13 | 12 | 4 | 30.76% | 26.49% ±4.74 | 19.35% ± 19.10 |
| | | | 14 | | --------- | --------- | | |
| | | | 9 | | 2 | 22.22% | | |
| | 2 | 100 | 12 | 11 | 1 | 8.30% | 31.33% ±4.74 | 24.19 % ± 19.10 |
| | | | 7 | | 4 | 57.14% | | |
| | | | 14 | | 4 | 28.57% | | |
| | 3 | 1000 | 10 | 12.3 | 9 | 90.00% | 86.07% ±4.74 | 78.93% ± 19.10 |
| | | | 10 | | 8 | 80.00% | | |
| | | | 17 | | 15 | 88.23% | | |
| **Colophony** | 1 | 10 | 13 | 12 | 1 | 7.69% | 7.69% ± 2.40 | 0.55% ± 10.10 |
| | | | 11 | | -------- | ------- | | |
| | | | 12 | | -------- | ------- | | |
| | 2 | 100 | 12 | 11 | 5 | 41.66% | 38.38% ± 2.40 | 31.24% ± 10.10 |
| | | | 4 | | 2 | 50.00% | | |
| | | | 17 | | 4 | 23.50% | | |
| | 3 | 1000 | 11 | 9 | 3 | 27.27% | 59.88% ± 2.40 | 52.74% ± 10.10 |
| | | | 9 | | 6 | 66.66% | | |
| | | | 7 | | 6 | 85.71% | | |
| **Galbanum** | 1 | 10 | 13 | 17 | 2 | 15.38% | 17.82% ± 1.68 | 10.68 % ± 10.40 |
| | | | 24 | | 4 | 16.66% | | |
| | | | 14 | | 3 | 21.42% | | |
| | 2 | 100 | 12 | 13.6 | 1 | 8.33% | 7.42% ± 1.68 | 0.28% ± 10.40 |
| | | | 16 | | 1 | 6.25% | | |

| Extract | Log conc | Conc (µg / mL) | Initial nauplii | Mean | No. of dead nauplii after 24 h. | % Dead after 24 h. | % Dead mean | %Dead mean corrected to control* |
|---|---|---|---|---|---|---|---|---|
| | | | 13 | | 1 | 7.69% | | |
| | 3 | 1000 | 12 | 10 | 6 | 50.00% | 42.59% ± 1.68 | 35.45% ± 10.40 |
| | | | 9 | | 3 | 33.33% | | |
| | | | 9 | | 4 | 44.44% | | |
| **Benzoin** | 1 | 10 | 16 | 14.6 | 6 | 37.50% | 37.5% ± 2.01 | 30.36% ±7.12 |
| | | | 12 | | ------- | ------- | | |
| | | | 16 | | ------- | ------- | | |
| | 2 | 100 | 9 | 15.3 | 3 | 33.33% | 25.72% ± 2.01 | 18.58% ±7.12 |
| | | | 18 | | 6 | 33.33% | | |
| | | | 19 | | 2 | 10.52% | | |
| | 3 | 1000 | 8 | 11.3 | 2 | 25.00% | 50.39% ± 2.01 | 43.25% ±7.12 |
| | | | 14 | | 6 | 42.85% | | |
| | | | 12 | | 10 | 83.33% | | |
| **Asafetida** | 1 | 10 | 15 | 15.6 | 2 | 13.33% | 11.9% ± 1.26 | 4.76% ± 3.10 |
| | | | 13 | | ------- | ------- | | |
| | | | 19 | | 2 | 10.50% | | |
| | 2 | 100 | 13 | 16.3 | 3 | 23.07% | 21.85% ± 1.26 | 14.71% ± 3.10 |
| | | | 20 | | 6 | 30.00% | | |
| | | | 16 | | 2 | 12.50% | | |
| | 3 | 1000 | 14 | 15.6 | 4 | 28.57 % | 20.42 % ± 1.26 | 13.28% ± 3.10 |
| | | | 13 | | 1 | 7.69 % | | |
| | | | 20 | | 5 | 25.00 % | | |
| **Ammoniacum** | 1 | 10 | 16 | 17 | ------- | ------ | 27.27% ±1.89 | 20.13% ± 4.60 |
| | | | 13 | | ------- | ------ | | |
| | | | 22 | | 6 | 27.27% | | |
| | 2 | 100 | 18 | 12.3 | 2 | 11.11% | 11.11% ±1.89 | 3.97% ± 4.60 |
| | | | 10 | | ------ | ------ | | |
| | | | 9 | | 1 | 11.11% | | |
| | 3 | 1000 | 13 | 12.3 | 7 | 53.84% | 37.75% ±1.89 | 30.61% ± 4.60 |
| | | | 11 | | 4 | 36.36% | | |
| | | | 13 | | 3 | 23.07% | | |
| **Aloe** | 1 | 10 | 19 | 15.6 | ------ | ------ | 9.09% ±1.17 | 1.95% ±1.80 |
| | | | 17 | | ------ | ------ | | |

| Extract | Log conc | Conc (µg / mL) | Initial nauplii | Mean | No. of dead nauplii after 24 h. | % Dead after 24 h. | % Dead mean | %Dead mean corrected to control* |
|---|---|---|---|---|---|---|---|---|
| | | | 11 | | 1 | 9.09% | | |
| | 2 | 100 | 9 | 16.6 | 1 | 11.11% | 14.78% ±1.17 | 7.64% ±1.80 |
| | | | 19 | | 2 | 10.52% | | |
| | | | 22 | | 5 | 22.72% | | |
| | 3 | 1000 | 13 | 11.3 | 1 | 17.60% | 14.75% ±1.17 | 7.61% ±1.80 |
| | | | 6 | | 1 | 16.66% | | |
| | | | 15 | | 3 | 20.00% | | |
| **Copaiba** | 1 | 10 | 8 | 13.3 | 1 | 12.50% | 13.4% ± 3.20 | 6.26% ± 7.33 |
| | | | 11 | | 2 | 18.18% | | |
| | | | 21 | | 2 | 9.52% | | |
| | 2 | 100 | 22 | 20.6 | 8 | 36.36% | 38.35% ± 3.20 | 31.21% ± 7.33 |
| | | | 21 | | 11 | 52.38% | | |
| | | | 19 | | 5 | 26.30% | | |
| | 3 | 1000 | 16 | 13 | 5 | 31.25% | 30.11% ± 3.20 | 22.97% ± 7.33 |
| | | | 11 | | 1 | 9.09% | | |
| | | | 12 | | 6 | 50.00% | | |
| **Guaiacum** | 1 | 10 | 19 | 18.6 | 2 | 10.52% | 8.75% ±1.39 | 1.61% ±12.50 |
| | | | 16 | | 1 | 6.25% | | |
| | | | 21 | | 2 | 9.50% | | |
| | 2 | 100 | 16 | 19 | 1 | 6.25% | 8.68% ±1.39 | 1.54% ±12.50 |
| | | | 18 | | 2 | 11.11% | | |
| | | | 23 | | ------- | ------- | | |
| | 3 | 1000 | 12 | 11.6 | 10 | 83.33% | 46.21% ± 1.39 | 39.07% ±12.5 |
| | | | 12 | | ------ | ------ | | |
| | | | 11 | | 1 | 9.09% | | |
| **Myrrh** | 1 | 10 | 8 | 10.6 | 3 | 37.50% | 42.02% ±1.71 | 34.88% ± 1.30 |
| | | | 14 | | 4 | 28.57% | | |
| | | | 10 | | 6 | 60.00% | | |
| | 2 | 100 | 15 | 13 | 5 | 33.33% | 41.6% ± 1.71 | 34.46% ± 1.30 |
| | | | 11 | | 5 | 45.45% | | |
| | | | 13 | | 6 | 46.15% | | |
| | 3 | 1000 | 11 | 16.6 | 4 | 36.36% | 44.48% | 37.34% |

| Extract | Log conc | Conc (µg / mL) | Initial nauplii | Mean | No. of dead nauplii after 24 h. | % Dead after 24 h. | % Dead mean | %Dead mean corrected to control* |
|---|---|---|---|---|---|---|---|---|
| | | | 20 | | 11 | 55.00% | ±1.71 | ± 1.30 |
| | | | 19 | | 8 | 42.10% | | |

*Each experiment was carried out in triplicates and expressed as mean ± S.D

Table. 3 Differences in the number of nauplii at different times.

| Extract | Conc (µg /mL) | Initial Nauplii | Mean | No. of dead nauplii after 15 min | No. of dead nauplii after 24 h. | No. of dead nauplii after 48 h* |
|---|---|---|---|---|---|---|
| **Olibanum** | 10 | 5 | 8.6 | --- | 2 | 5± 3.1 |
| | | 7 | | --- | 6 | 7± 3.1 |
| | | 14 | | 2 | 4 | 14± 3.1 |
| | 100 | 20 | 11.6 | 1 | 4 | 15± 3.1 |
| | | 8 | | 1 | 2 | 5± 3.1 |
| | | 7 | | 1 | 4 | 4± 3.1 |
| | 1000 | 8 | 12.3 | 1 | 5 | 6± 3.1 |
| | | 11 | | 1 | 9 | 10± 3.1 |
| | | 18 | | 9 | 10 | 15± 3.1 |
| **Mastic** | 10 | 13 | 12 | --- | 4 | 5± 1.5 |
| | | 14 | | --- | --- | 6± 1.5 |
| | | 9 | | --- | 2 | 3± 1.5 |
| | 100 | 12 | 11 | --- | 1 | 9± 1.5 |
| | | 7 | | --- | 4 | 5± 1.5 |
| | | 14 | | --- | 4 | 11± 1.5 |
| | 1000 | 10 | 12.3 | --- | 9 | 10± 1.5 |
| | | 10 | | 5 | 8 | 10± 1.5 |
| | | 17 | | --- | 15 | 17± 1.5 |
| **Colophony** | 10 | 13 | 12 | --- | 1 | 4± 2.5 |
| | | 11 | | --- | --- | --- |
| | | 12 | | --- | --- | 5± 2.5 |
| | 100 | 12 | 11 | --- | 5 | 12± 2.5 |
| | | 4 | | --- | 2 | 4± 2.5 |
| | | 17 | | --- | 4 | 15± 2.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 1000 | 11 | 9 | 1 | 3 | 11± 2.5 |
| | | 9 | | --- | 6 | 9± 2.5 |
| | | 7 | | --- | 6 | 7± 2.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Galbanum** | 10 | 13 | 17 | --- | 2 | 9 ±1.4 |
| | | 24 | | --- | 4 | 15±1.4 |
| | | 14 | | --- | 3 | 7±1.4 |
| | 100 | 12 | 13.6 | --- | 1 | 4±1.4 |
| | | 16 | | --- | 1 | 11±1.4 |
| | | 13 | | --- | 1 | 11±1.4 |
| | 1000 | 12 | 10 | --- | 6 | 6±1.4 |
| | | 9 | | --- | 3 | s8±1.4 |
| | | 9 | | --- | 4 | 8±1.4 |
| **Benzoin** | 10 | 16 | 14.6 | --- | 6 | 9 ±1.0 |
| | | 12 | | --- | --- | 5±1.0 |
| | | 16 | | --- | --- | 6±1.0 |
| | 100 | 9 | 15.3 | --- | 3 | 8±1.0 |
| | | 18 | | --- | 6 | 15±1.0 |
| | | 19 | | --- | 2 | 8±1.0 |
| | 1000 | 8 | 11.3 | 1 | 2 | 7±1.0 |
| | | 14 | | --- | 6 | 12±1.0 |
| | | 12 | | --- | 10 | 12±1.0 |
| **Asafetida** | 10 | 15 | 15.6 | 1 | 2 | 8 ±1.8 |
| | | 13 | | --- | --- | 6±1.8 |
| | | 19 | | --- | 2 | 9±1.8 |
| | 100 | 13 | 16.3 | --- | 3 | 10±1.8 |
| | | 20 | | --- | 6 | 16±1.8 |
| | | 16 | | --- | 2 | 12±1.8 |
| | 1000 | 14 | 15.6 | 1 | 4 | 14±1.8 |
| | | 13 | | --- | 1 | 13±1.8 |
| | | 20 | | --- | 5 | 20±1.8 |
| **Ammomiacum** | 10 | 16 | 17 | --- | --- | 2± 2.6 |
| | | 13 | | --- | --- | 6± 2.6 |
| | | 22 | | --- | 6 | 16± 2.6 |
| | 100 | 18 | 12.3 | --- | 2 | 8± 2.6 |
| | | 10 | | --- | --- | 3± 2.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 9 | | --- | 1 | 8± 2.6 |
| | 1000 | 13 | 12.3 | --- | 7 | 11± 2.6 |
| | | 11 | | 1 | 4 | 9± 2.6 |
| | | 13 | | --- | 3 | 6 ± 2.6 |
| **Aloe** | 10 | 9 | 12.3 | --- | --- | 9 ± 1.3 |
| | | 17 | | --- | --- | 6 ± 1.3 |
| | | 11 | | --- | 1 | 4± 1.3 |
| | 100 | 9 | 16.6 | --- | 1 | 5± 1.3 |
| | | 19 | | --- | 2 | 8± 1.3 |
| | | 22 | | --- | 5 | 12± 1.3 |
| | 1000 | 13 | 11.3 | --- | 1 | 9± 1.3 |
| | | 6 | | --- | 1 | 6± 1.3 |
| | | 15 | | --- | 3 | 14± 1.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Copaiba** | 10 | 8 | 13.3 | --- | 1 | 2 ±3.1 |
| | | 11 | | --- | 2 | 4±3.1 |
| | | 21 | | 2 | 2 | 8±3.1 |
| | 100 | 22 | 20.6 | --- | 8 | 12±3.1 |
| | | 21 | | --- | 11 | 21±3.1 |
| | | 19 | | --- | 5 | 10±3.1 |
| | 1000 | 16 | 13 | 1 | 5 | 10±3.1 |
| | | 11 | | --- | 1 | 9±3.1 |
| | | 12 | | --- | 6 | 8±3.1 |
| **Guaiacum** | 10 | 19 | 18.6 | --- | 2 | 10 ±1.2 |
| | | 16 | | --- | 1 | 5±1.2 |
| | | 21 | | 1 | 2 | 9±1.2 |
| | 100 | 16 | 19 | --- | 1 | 8±1.2 |
| | | 18 | | --- | 2 | 15±1.2 |
| | | 23 | | --- | --- | 20±1.2 |
| | 1000 | 12 | 11.6 | 2 | 10 | 12±1.2 |
| | | 12 | | --- | --- | 7±1.2 |
| | | 11 | | --- | 1 | 6±1.2 |
| **Myrrh** | 10 | 8 | 10.6 | --- | 3 | 3±3.1 |
| | | 14 | | --- | 4 | 7±3.1 |
| | | 10 | | --- | 6 | 7±3.1 |
| | 100 | 15 | 13 | 1 | 5 | 14±3.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 11 | | 2 | 5 | 11±3.1 |
| | | 13 | | 1 | 6 | 11±3.1 |
| | 1000 | 11 | 16.6 | 2 | 4 | 8±3.1 |
| | | 20 | | 5 | 11 | 20±3.1 |
| | | 19 | | 2 | 8 | 19±3.1 |

*Each experiment was carried out in triplicates and expressed as mean ± S.D

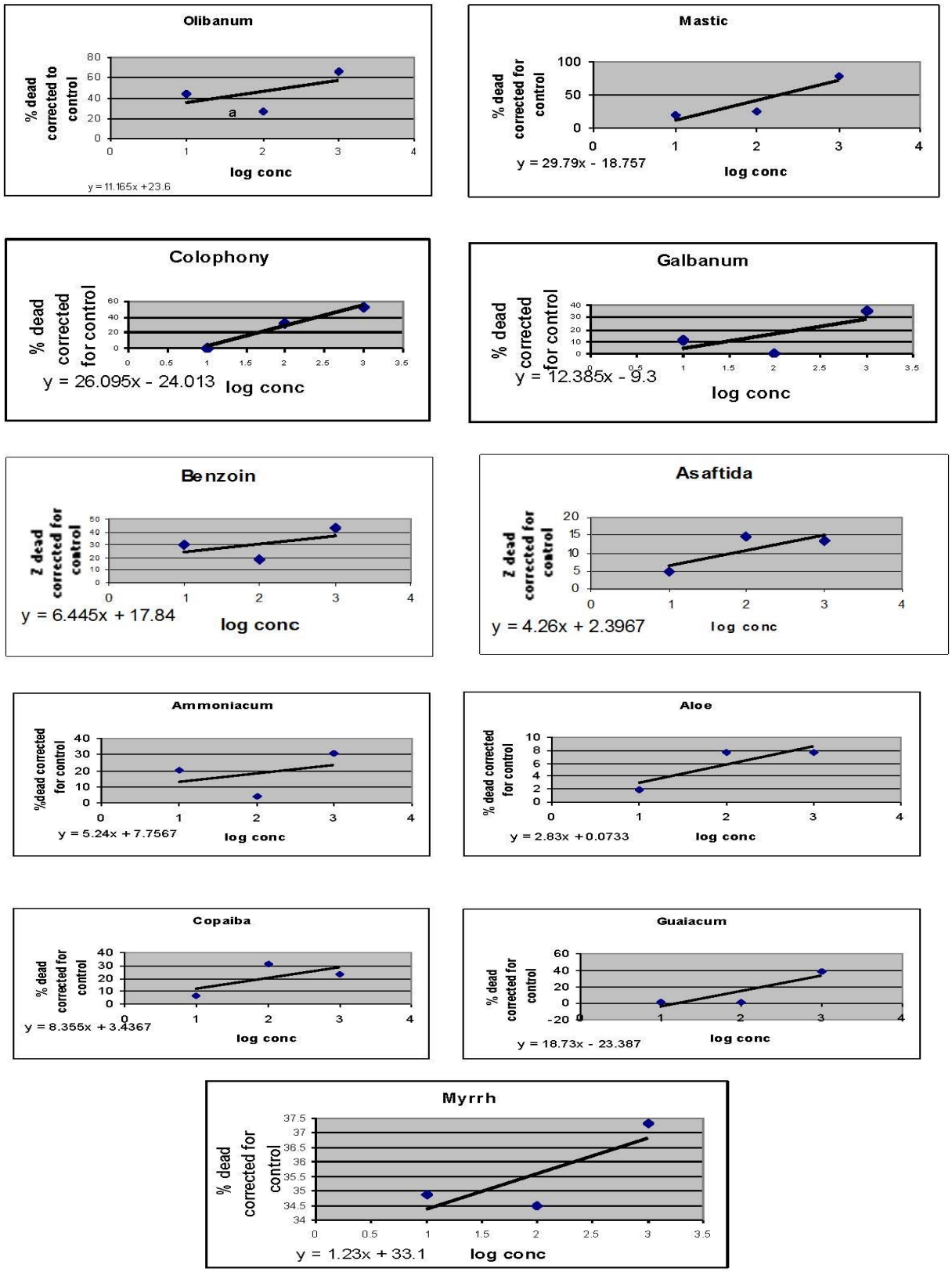



**Fig. 4 Graphical representation of log conc. of the different extracts of some natural resins (X-axis) and % death of nauplii (Y- axis).**

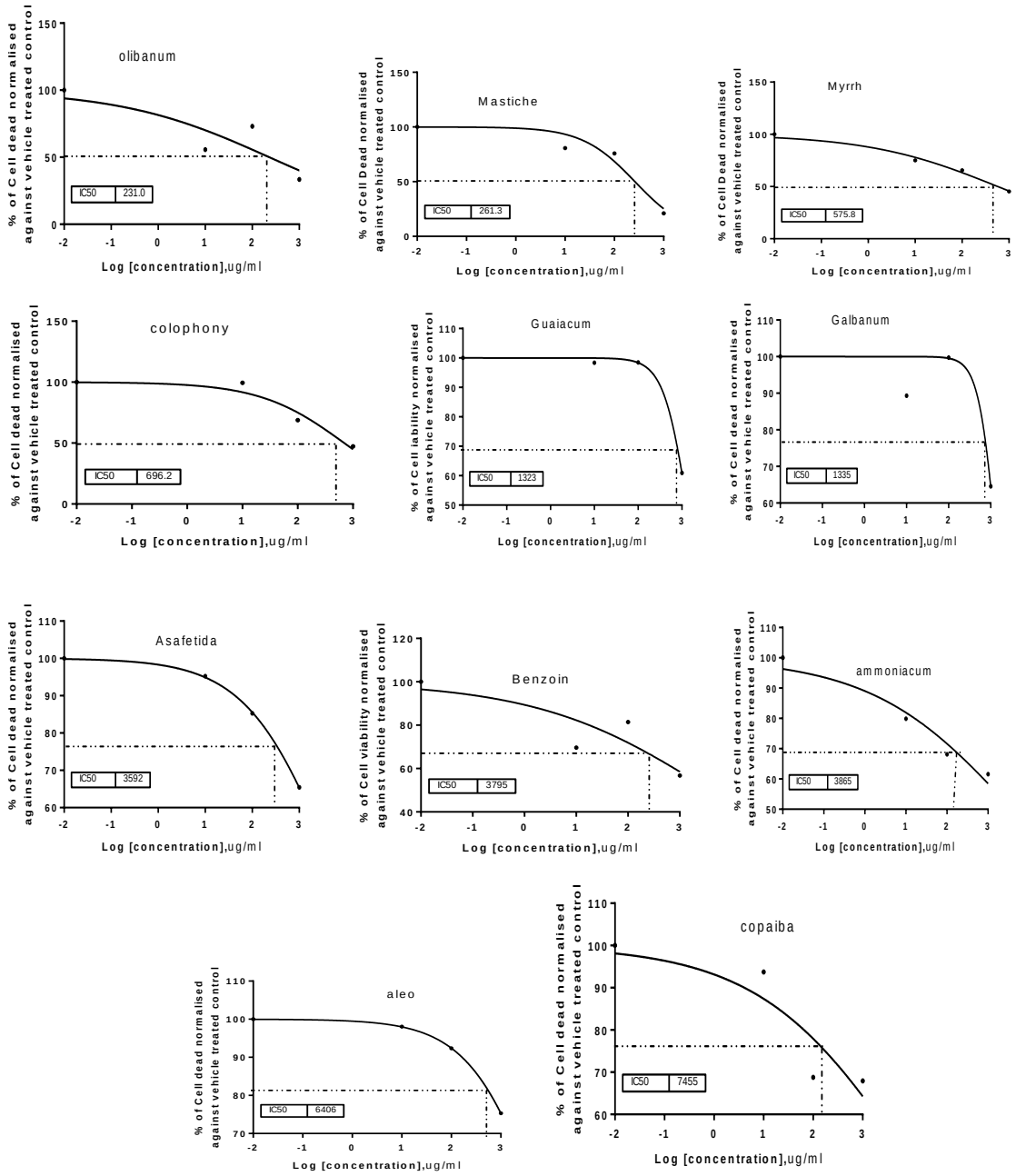



**Fig. 5** The descending order of $LC_{50}$ values (µg/mL) for different resin extracts and % death of nauplii

The results from the previous tables and graphical representations showed that olibanum and mastic were exhibited a potent activity ($LC_{50}$ = 231 ± 2.02 to 261.3 ± 1.05, respectively) then Myrrh showed moderate activity ($LC_{50}$ = 575.8± 1.13) compared to control (DMF =9000) and all results were summarized in table 4. On the other hand Myrrh and Mastic extracts were exhibited the highest protection against DNA damage as shown in Table 5.

**Table. 4** $LC_{50}$ (µg / mL) of different resin extracts of brine shrimp assay*

| **Extract** | **$LC_{50}$** * |
|---|---|
| Aloe | 6406.0 ± 9.18 |
| Ammoniacum | 3865.0 ± 9.17 |
| Asafetida | 3592.0 ± 7.15 |
| Benzoin | 3795.0 ± 5.09 |
| Colophony | 696.2 ± 3.05 |
| Copaiba | 7544.0 ± 6.09 |
| Galbanum | 1335.0 ± 8.54 |
| Guaiacum | 1323.0 ± 6.17 |
| Mastiche | 261.3 ± 1.05 |
| Myrrh | 575.8 ± 1.13 |
| Olibanum | 231.0 ± 2.02 |

*Each experiment was carried out in triplicates and expressed as mean ± S.D

**Table. 5** Results of bleomycin-dependent DNA damage assay of some natural resins

| Extract | Absorbance* (532 nm) |
|---|---|
| Ascorbic acid | 0.004 ± 0.021 |
| Asafetida | 0.017 ± 0.032 |
| Colophony | 0.571 ± 0.021 |
| Copaiba | 0.558 ± 0.021 |
| Guaiacum | 0.010 ± 0.11 |
| Mastic | 0.002 ± 0.014 |
| Myrrh | 0.003 ± 0.041 |
| Olibanum | 0.025 ± 0.012 |

*Each absorbance was carried out in triplicates and expressed as mean ± SD

## DISCUSSION

The brine shrimp lethality assay represents a rapid, economic and simple bioassay for testing extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties [22]. In the present study the brine shrimp lethality of extracts of 11 natural resins used in Egyptian markets was determined **Table1**. The degree of brine shrimp lethality was found to be directly proportional to the concentration of the extract. The $LC_{50}$ values of the resins extracts were obtained by a plot of percentage of the killed shrimp nauplii against the concentrations of the extracts **Fig. 4.** Alcoholic extract of olibanum showed the most prominent activity with $LC_{50}$ 231.0± 2.02 µg/mL. Mastiche, Myrrh, Colophony, exhibited significant brine shrimp lethality with $LC_{50}$ values 261.3±1.05, 558.7± 1.13 and 696.2 ± 3.05 µg/mL respectively. While Aloe and Copaiba showed the worst brine shrimp lethality with $LC_{50}$ values 6406.0 ± 9.18, 7455.0 ± 6.09 µg/mL respectively **(Fig.5**). This significant lethality of resins extracts to brine shrimp is an indicative of the presence of cytotoxic components which deserve further investigation.

On the other hand, the bleomycin assay has been adopted for assessing the pro-oxidant effects of food antioxidants. The antitumor antibiotic bleomycin binds iron ions and DNA. The bleomycin-iron complex degrades DNA that, upon heating with thiobarbituric acid, yields a pink chromogen. Added suitable reducing agents "antioxidants" compete with DNA and diminish chromogen formation. From **Table 5**, it was noted Myrrh and Mastic extracts showed the highest protection against DNA damage induced by bleomycin-iron complex thus diminishing chromogen formation between the damaged DNA and TBA.

## CONCLUSION

The brine shrimp lethality assay has proven to be a convenient system for monitoring biological activities of some resins that are used in the traditional medicine. Olibanum, Mastic, Myrrh and Colophony were the most active resins with potential cytotoxic activities. Also, the antitumor activity of Mastic and Myrrh was confirmed from the bleomycin-dependent degradation of DNA assay. Therefore, the present study concluded that Mastic, Myrrh and Olibanum are the most potential antitumor agent of all the tested resins.

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