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# TLC FINGERPRINT OF ANTIOXIDANT AND CYTOTOXIC ACTIVE EXTRACTS OF *ARTEMESIA MONOSPERMA* DELILE AND *ARTEMESIA HERBA ALBA* ASSO.

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**Abstract**

*The present study was performed to investigate the antioxidant and cytotoxic activity, as well the phytochemical constituents of two species of the Astraceae family. The percentage yield of total and successive extracts was determined and showed that the total extract were (14.75 % and 21.36 %) for Artemesia monosperma and Artemesia herba alba respectively, while the highest percent of the successive extracts were methanol 50 % (10.39 %) in Artemesia monosperma, while petroleum ether (12.20 %) in Artemesia herba alba. The preliminary phytochemical screening showed that the two plant contained carbohydrates and/or glycosides, proteins, amino acids flavonoids, saponins, tannins, unsaturated sterols and/or terpenoids and absence of alkaloids. Investigation of the antioxidant activity proved that the most active extracts of the two plants were the ethyl acetate one with $IC_{50}$ (28.59 and 37.15 µg/ml), respectively. These extracts showed inhibition of the human colon (HCT) carcinoma cell lines. The TLC finger print of the ethyl acetate extracts showed that Artemesia monosperma contained 10 spots while, Artemesia herba alba contained 16 spots, mainly flavonoid compounds.*

**Key words**: *Artemesia monosperma, Artemesia herba alba, antioxidant activity, cytotoxic activity, colon carcinoma cell line, TLC finger printing*

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## INTRODUCTION:

However oxygen is very important for the biochemical pathways in any human body, 5% of the oxygen intake is transformed into reactive oxygen species, which play an important role in physiological disorders leading to many diseases. The presence of free radical scavenging agent declares our bodies from these harmful species. This protects the living bodies from diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders and in the process of aging [1].

The antioxidant constituents of plant materials provide protection from cancer and protect the body from damage caused by free radical induced oxidative stress. Recently, more interest has been given in medicinal plants as antioxidant agents in reducing free radical induced tissue injury. The synthetic antioxidants have restriction for use, as they are suspected to be carcinogenic [2].

The plants under investigation *Artemesia monosperma* and *Artemesia herba alba* belong to family Astraceae, genus *Artemesia*. The genus ***Artemisia*** contains between 200 and 400 species. It contains plants known for their volatile oils. Plants of this genus range from dwarf herbaceous plants to tall shrubs. These are mostly perennial plants and are frequently aromatic [3].

They grow in temperate climates, usually in dry or semi-dry habitats. They can be found from mountain areas to the dry deserts, and dominate the steppe communities of Asia, South Africa, and the New World [3].

The genus *Artemisia* is known to contain many bioactive compounds; artemisinin exerts not only antimalarial activity but also profound cytotoxicity against tumor cells [4] and arglabin is employed for treating certain types of cancer in the former USSR [5].

Thus, it encouraged the idea of the present study to investigate two plants of family Astracea for their antioxidant and cytotoxic activity as well as to study their phytochemical constituents.

## MATERIALS AND METHODS:

**Plant material:**

The aerial parts of *Artemesia monosperma* and *Artemesia herba alba* were collected from Mersa-Mattruh during March 2015. The plants were identified in the Herbarium of the Desert Research Centre. They were kept fresh for the further investigation.

**Extraction of total extract:**

Powdered aerial parts of *Artemesia monosperma* and *Artemesia herba alba* (100 gm) were extracted with 70% methanol using

soxhlet apparatus. The obtained residue from each plant was dried and weight.

**Successive extraction technique:**

One hundred grams of *Artemesia monosperma* and *Artemesia herba alba* were extracted with successive selective organic solvents using soxhlet apparatus, in order of increasing polarity including petroleum ether (b.p. 40-60 ºC), chloroform, ethyl acetate, methanol and 50% methanol. The obtained residue from each solvent was dried and weight.

**Preliminary phytochemical screening:**

The total and successive extracts were used to perform the phytochemical screening for the detection of the presence of carbohydrates and/or glycosides, proteins (Biuret test); amino acids (Ninhydrin test) according to Rodwell [6]; alkaloids and/or nitrogenous bases Harborne [7], flavonoids and/or phenolics [8], saponins (Frothing test) according to Kumar [9], tannins (Ferric chloride reagent) using the methods described by Trease and Evans [10]; unsaturated sterols and/or triterpenes using Libermann-Burchard test and using Salkowiski's test [11] and terpenoids [12].

**Determination of Free Radical Scavenging Activity for Aerial Parts of *Artemesia monosperma* and *Artemesia herba alba***

Different concentrations (125, 250, 500 and 1000 µg/ml) of each tested extracts of *Artemesia monosperma* and *Artemesia herba alba* and ascorbic acid were prepared in 80% (v/v) ethanol. A volume of 3 ml from each extract and ascorbic acid concentrations were mixed with 1ml of 1mM of DPPH radical. A control tube was prepared by mixing 3 ml of 80 % ethyl alcohol with 1 ml of alcoholic solution of DPPH radical. The tubes were kept at room temperature in the dark for 30 minutes. The degree of disappearance of purple color was measured against blank (80% ethyl alcohol) at 517nm [13].

Reagents obtained from Sigma company: 1 mM 1, 1 Diphenyl-2-picrylhydrazyl (DPPH) and Ascorbic acid (vitamin C).

**Calculation**

$$(\%)\ \text{Scavenged DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

$IC_{50}$ was calculated by means of Graph Pad Prism software (Ver.7).

**In vitro assay of cytotoxic activity**

The most active antioxidant active extracts of the two plants were tested for their cytotoxic activity against colon carcinoma cell line (HCT). Cytotoxicity of the different extracts and $IC_{50}$ were obtained using the method described by Skehan *et al [14]*.

**TLC fingerprint of ethyl acetate extracts:**

Volumes of 10 µl of extracts were applied in the form of a spots on the silica gel 60 $F_{254}$ TLC plate of 0.2 mm thickness. The plates were developed in solvent system chloroform: methanol (9: 1).

## RESULTS AND DISCUSSION:

**Total extract (70% methanol):**

The obtained data show that total extract residues (70% methanol) of of *Artemesia monosperma* and were *Artemesia herba alba* 14.75 % and 21.36 %, respectively as shown in table (1).

**Successive extracts**

Data presented at table (1) show that the highest yield was the methanol 50 % extract residues (10.39 %) in *Artemesia monosperma*, while petroleum ether extract (12.20 %) in *Artemesia herba alba*.

**Preliminary phytochemical screening:**

The phytochemical screening of various phytoconstituents in the total and successive extracts of the aerial parts of *Artemesia monosperma* and *Artemesia herba alba* revealed the presence of carbohydrates and/or glycosides, proteins, amino acids flavonoids, saponins, tannins, unsaturated sterols and/or triterpenes terpenoids and absence of alkaloids.

Out of these extracts 95% ethanol and 50% ethanol extracts showed maximum number of plant constituents such flavonoids, tannins, coumarins, carbohydrate, glycosides, protein and amino acids and saponins and absence of terpenes and alkaloids. Ethyl acetate extract showed the presence of flavonoids and tannins as well as trace amounts of caumarins and saponins in both plants. The results are presented in table (2).

**Table 1: Total and successive extracts residues (%) of *Artemesia monosperma* and *Artemesia herba alba* aerial parts.**

| Solvent used | Residue percentage (%) | |
|---|---|---|
| | ***Artemesia monosperma*** | ***Artemesia herba alba*** |
| Total extract | 21.36 | 14.75 |
| Petroleum ether | 9.47 | 12.20 |
| Ethyl acetate | 6.81 | 4.70 |
| Chloroform | 7.01 | 2.18 |
| Methanol | 4.72 | 8.27 |
| Methanol 50 % | 10.39 | 6.12 |

**Table 2: Phytochemical screening of the total and successive extracts of aerial parts *Artemesia monosperma* and *Artemesia herba alba***

| | Total extract | | Petroleum ether | | Chloroform | | Ethyl acetate | | 95 % ethanol | | 50% ethanol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A.m. | A.h.a | A.m. | A.h.a | A.m. | A.h.a | A.m. | A.h.a | A.m. | A.h.a | A.m. | A.h.a |
| Terpenoids | + | + | + | + | - | - | - | - | - | - | - | - |
| Carbohydrates and/or glycosides | + | + | - | - | - | - | - | - | + | + | + | + |
| Flavonoids | + | + | - | - | + | + | + | + | + | + | + | + |
| Tannins | + | + | - | - | + | + | + | + | + | + | + | + |
| Saponins | + | + | - | - | - | - | ± | ± | + | + | + | + |
| Coumarins | + | + | - | - | ± | ± | ± | ± | + | + | + | ± |
| Proteins | + | + | - | - | - | - | - | - | + | + | + | + |
| Alkaloids | - | - | - | - | - | - | - | - | - | - | - | - |

A.m. *Artemesia monosperma* A.h.a. *Artemesia herba alba*

(+) present (-) absent (±) traces

**Free Radical Scavenging Activity of *Artemesia monosperma* and *Artemesia herba alba* aerial part different extracts:**

Percentage scavenging activity of all extracts shown in table (3) and illustrated in figure (1 and 2).

**Table 3: The percentages of scavenging for total and successive extracts residues of *Artemesia monosperma* and *Artemesia herba alba* aerial parts at concentration (1000 µg/ml).**

| Extract | | % Scavenging activity |
|---|---|---|
| *Artemesia monosperma* | Total extract | 74.156 |
| | Petroleum ether extract | 14.097 |
| | Chloroform extract | 89.644 |
| | Ethyl acetate extract | 90.645 |
| | Methanol extract | 89.044 |
| | Methanol 50% extract | 77.767 |
| *Artemesia herba alba* | Total extract | 71.889 |
| | Petroleum ether extract | 15.640 |
| | Chloroform extract | 75.822 |
| | Ethyl acetate extract | 86.122 |
| | Methanol extract | 76.811 |
| | Methanol 50% extract | 12.110 |

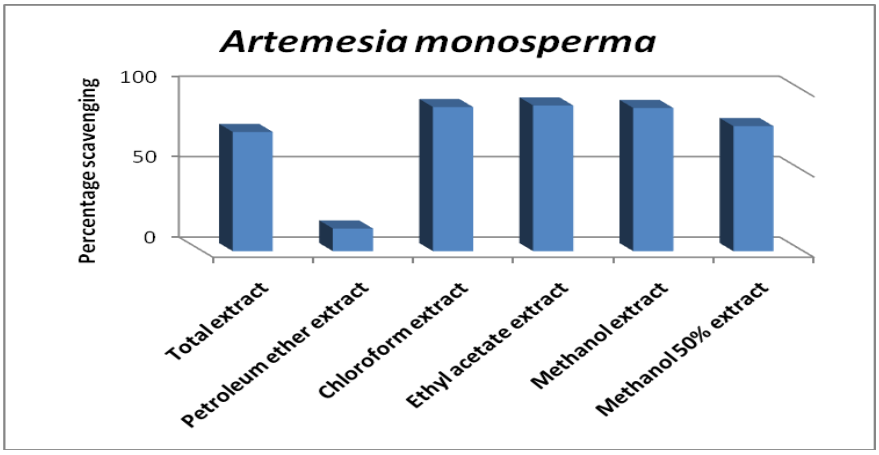



**Fig 1: The percentages of scavenging for total and successive extracts residues of *Artemesia monosperma* aerial parts at concentration (1000 µg/ml).**

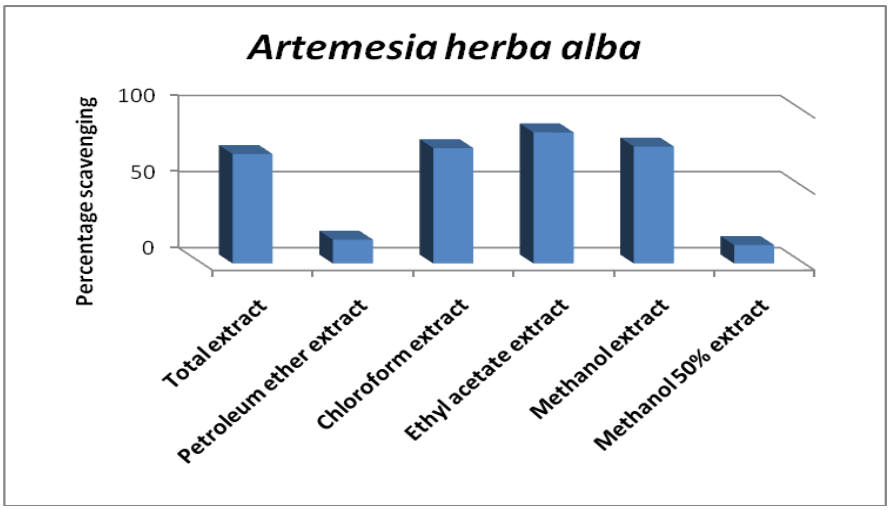



**Fig 2: The percentages of scavenging for total and successive extracts residues of *Artemesia herba alba* aerial parts at concentration (1000 µg/ml).**

The scavenging percentage of different concentrations for *Artemesia monosperma* and *Artemesia herba alba* extracts were illustrated in table (4 and 5) and figure (3 and 4). The $IC_{50}$ of extracts exceeding 60% scavenging activity were determined and shown in table (6) and illustrated in figure (5)

**Table 4: The percentages of scavenging for total and successive extracts residues of *Artemesia monosperma* aerial parts.**

| Concentration (µg/ml) | % sacvenging of total extract | % scavenging of chloroform extract | % scavenging of ethyl acetate extract | % scavenging of methanol extract | % scavenging of 50% methanol extract | % scavenging of ascorbic acid |
|---|---|---|---|---|---|---|
| 1000 | 74.156 ± 0.084* | 89.644 ± 0.575 | 90.645 ± 0.267 | 89.044 ± 0.967 | 77.767 ± 0.088 | 97.767 ± 0.088 |
| 500 | 71.511 ± 0.926* | 84.433 ± 0.924 | 87.067 ± 0.067 | 82.967 ± 0.606 | 73.5 ± 1.048 | 96.3 ± 0.033 |
| 250 | 41.367 ± 0.066* | 75.511 ± 0.051 | 70.078 ± 0.051 | 62.933 ± 0.067 | 66.456 ± 0.083 | 94.078 ± 0.051 |
| 125 | 30.078 ± 0.102* | 60.744 ± 0.084 | 48.700 ± 0.033 | 37.467 ± 0.034 | 54.478 ± 0.135 | 89.744 ± 0.084 |

*Values are given as mean ± S. D. (n=3). *Significant at $p < 0.05$, p-value was calculated by comparing with standard (ascorbic acid) by ANOVA followed by Dunnett's test.*

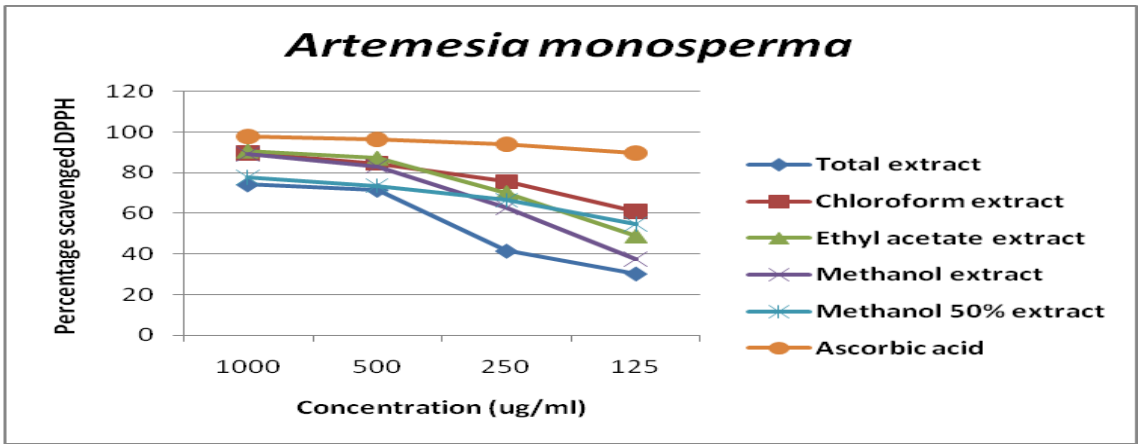



**Fig 3: The percentages of scavenging for total and successive extracts residues of *Artemesia monosperma* aerial parts**

**Table 5: The percentages of scavenging for total and successive extracts residues of *Artemesia herba alba* aerial parts.**

| Concentration (µg/ml) | % sacvenging of total extract | % scavenging of chloroform extract | % scavenging of ethyl acetate extract | % scavenging of methanol extract | % scavenging of ascorbic acid |
|---|---|---|---|---|---|
| 1000 | 71.889 ± 0.572** | 75.822 ± 0.277* | 86.122 ± 0.549 | 76.811 ± 2.155* | 97.767 ± 0.088 |
| 500 | 64.38 ± 0.055** | 73.678 ± 0.051* | 82.056 ± 0.051 | 70.322 ± 0.051* | 96.3 ± 0.033 |
| 250 | 41.578 ± 0.084** | 59.378 ± 0.051* | 67.589 ± 0.084 | 47.277 ± 0.049* | 94.078 ± 0.051 |
| 125 | 38.522 ± 0.107** | 47.033 ± 0.034* | 45.011 ± 0.084 | 40.267 ± 0.067* | 89.744 ± 0.084 |

*Values are given as mean ± S. D. (n=3). *Significant at $p < 0.05$, p-value was calculated by comparing with standard (ascorbic acid) by ANOVA followed by Dunnett's test.*

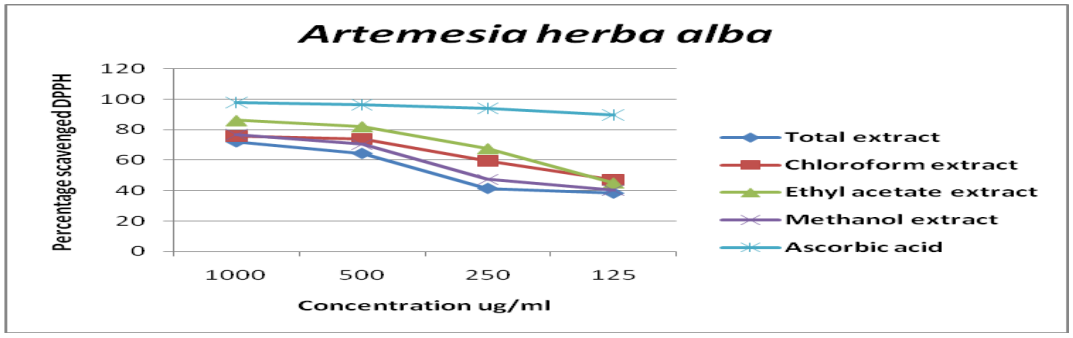



**Fig 4: The percentages of scavenging for total and successive extracts residues of *Artemesia herba alba aerial* parts**

**Table 6: $IC_{50}$ (µg/ml) for total and successive extracts residues of *Artemesia monosperma* and *Artemesia herba alba* aerial parts**

| Extract | $IC_{50}$ of *Artemesia monosperma* | $IC_{50}$ of *Artemesia herba alba* |
|---|---|---|
| Total extract | 192 | 296 |
| Chloroform extract | 41.93 | 75.39 |
| Ethyl acetate extract | 28.59 | 37.15 |
| Methanol extract | 42.64 | 337.6 |
| Methanol 50% extract | 40.11 | -- |
| Ascorbic acid | 22.63 | 22.63 |

From the previous tables it could be concluded that the most active extract as free radical scavenging agent was ethyl acetate extract in both plants with $IC_{50}$ (28.59 and 37.15 µg/ml) for *Artemesia monosperma* and *Artemesia herba alba* respectively. This lead to the study of this extract activity as cytotoxic agent on human colon carcinoma cell line (HCT).

**In vitro assay of cytotoxic activity**

The cytotoxic activity was estimated for the ethyl acetate extracts of both *Artemesia monosperma* and *Artemesia herba alba* on Human colon (HCT) Carcinoma Cell Lines and as shown in (table 7 and figure 5) the percentage of survival cells decreased on increasing the concentration of the extract concentration. The $IC_{50}$ was (22.0 and 24.5 µg/ml) for the two plants respectively (table 8 and figure 6).

**Table 7: Cytotoxic Activity of Ethyl acetate Extract of *Artemesia monosperma* and *Artemesia herba alba* on Human colon (HCT) Carcinoma Cell Lines**

| Concentration (µg/ml) | Percentage of Survival Cells | | |
|---|---|---|---|
| | *Artemesia monosperma* | *Artemesia herba alba* | Doxorubicin |
| 0.0 | 100 | 100 | 100 |
| 5.0 | 84.4 | 85.3 | 52.7 |
| 12.5 | 76.1 | 72.4 | 40.0 |
| 25.0 | 41.0 | 49.1 | 37.5 |
| 50.0 | 40.0 | 48.3 | 37.2 |

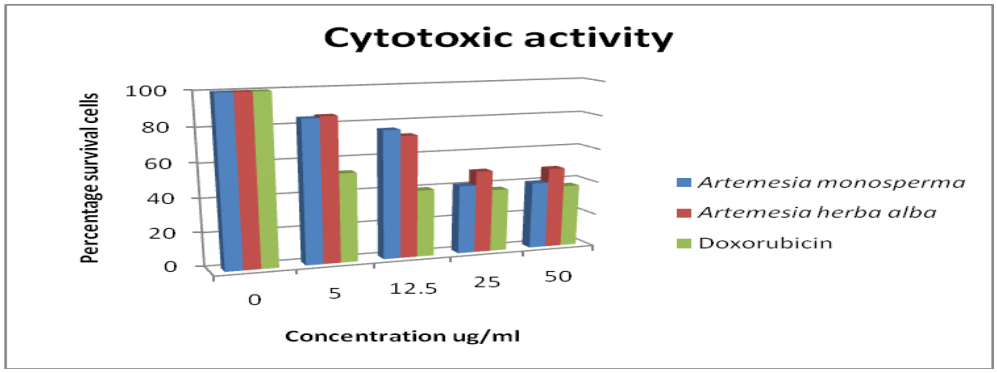



**Fig 5: Cytotoxic Activity of Ethyl acetate Extracts of *Artemesia monosperma* and *Artemesia herba alba* on Human colon (HCT) Carcinoma Cell Lines**

**Table 8: $IC_{50}$ of Ethyl acetate Extract of *Artemesia monosperma* and *Artemesia herba alba* on Human colon (HCT) Carcinoma Cell Lines**

| Extract | IC $_{50}$ (µg/ml) |
|---|---|
| *Artemesia monosperma* | 22.0 |
| *Artemesia herba alba* | 24.5 |
| Doxorubicin | 6.6 |

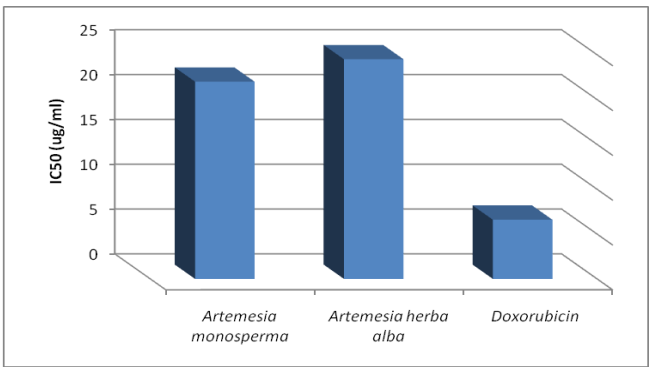


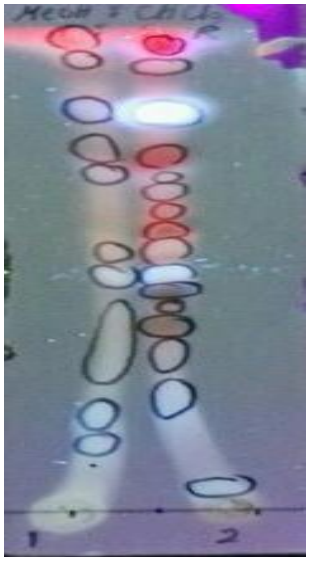

**Fig 6: $IC_{50}$ of Ethyl acetate Extracts of *Artemesia monosperma* and *Artemesia herba alba* on Human colon (HCT) Carcinoma Cell Lines**

From the previous data it is obvious that the ethyl acetate extracts of both plants inhibited the growth the colon carcinoma cell lines. This is probably due to the free radical scavenging activity. It has been found that free radicals in our bodies causes various diseases one of them is cancer [15]. Therefore, it is a worldwide demand to find naturally occurring antioxidants to overcome the harm of free radicals which are formed in our bodies due to internal and external pollution [16,17]. So by decreasing the effect of free radical by scavenging them with antioxidant agents this will prevent and overcome the risc of cancer development.

From the preliminary phytochemical screening of the ethyl acetate extract of both plants it was found that it contained flavonoids and tannins. Flavonoids are well known to have a great effect on both antioxidant and cytotoxic activities [18,19].

**TLC fingerprint of ethyl acetate extracts:**

The ethyl acetate extracts of whole plant of *Artemesia monosperma* and *Artemesia herba alba* were prepared. A large number of solvent systems were tried for extracts including different proportions of solvents in chloroform : methanol solvent system. The best resolution was observed in a solvent system, chloroform : methanol (9 : 1) (table 9 and figure 6). The TLC showed that *Artemesia monosperma* contained 10 spots while, *Artemesia herba alba* contained 16 compounds, seven spots are common in both plants.



*(1) Artemesia monosperma* (2) *Artemesia herba alba*

**Fig 7: TLC fingerprint profile of the ethyl acetate extracts of *Artemesia monosperma* and *Artemesia herba alba***

**Table 9: Major spots in the TLC of the ethyl acetate extracts of *Artemesia monosperma* and *Artemesia herba alba***

| Spot no. | Rf value | Spot color | *Artemesia monosperma* | *Artemesia herba alba* |
|---|---|---|---|---|
| 1 | 0.03 | Blue | - | + |
| 2 | 0.12 | Blue | + | - |
| 3 | 0.18 | Blue | + | - |
| 4 | 0.22 | Blue | - | + |
| 5 | 0.31 | Buff | - | + |
| 6 | 0.32 | Buff | + | - |
| 7 | 0.35 | Brown | - | + |
| 8 | 0.38 | Brown | - | + |
| 9 | 0.42 | Violet | - | + |
| 10 | 0.45 | Blue | + | + |
| 11 | 0.49 | Yellow | + | + |
| 12 | 0.54 | Orange | - | + |
| 13 | 0.58 | Orange | - | + |
| 14 | 0.62 | Blue | - | + |
| 15 | 0.66 | Blue | + | + |
| 16 | 0.71 | Red | + | + |
| 17 | 0.78 | Fluorescent blue | + | + |
| 18 | 0.89 | Orange | + | + |
| 19 | 0.94 | Red | + | + |

## CONCLUSION:

It could be concluded from the antioxidant activity test that the most active extraction was ethyl acetate in both plants. These two extracts were tested for their cytotoxic activity against colon cell lines and showed inhibition of the vital carcinoma cell with increase of the concentration indicating their cytotoxic activity with $IC_{50}$ (22 and 24.5 µg/ml) for *Artemesia monosperma* and *Artemesia herba alba* respectively. The preliminary phytochemical screening of the ethyl acetate extracts indicated the presence of flavonoids and tannins which are known of its antioxidant and cytotoxic activity. It is recommended to do further studies on these extracts to investigate its safety margins as well as its effect liver and kidney parameters on short and long term of treatment. It is also advised to establish an *in vivo* model to study its antitumor effect.

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