Biological Activities of Iraqi Fig (Ficus carica) Crude Ethanol and Total Flavonoids Extracts

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Abstract

The present study focuses on the biological strategy for treating cancer and parasitic infections, such as leishmaniasis antiparasitic activity, for the crude alcoholic extract of Ficus leaves and their extracted total flavonoids with a comparison between their effects. The flavonoids were extracted from the leaves of the mature Ficus carica using the reflux extraction method. Total flavonoids were detected qualitatively by TLC technique which demonstrated that the plant was rich in indifferent flavonoids, especially Rutin, Quercetin, Kaempferol, luteolin and others. Quantitatively, the plant total flavonoids content was 337.3 mg / 100 g fig leaves calculated as rutin. The biological effects of the crude and purified total flavonoid on cell lines (L-20B and MCF7) and two parasites (Leishmania tropica, Leishmania donovani) were investigated. Maximum growth inhibition rates for the total flavonoids on the cell lines L20B and MCF7 reached 43 % at the concentration of 0.169 mg/ml and 28% at the concentration of 2.7 mg/ml, respectively, in comparison with the negative control. The ethanolic crude extract had a low effect on L20-B cell line, while the inhibition rate for MCF-7 cell line reached 34% at a concentration of 0.084 mg/ml. For Leishmania tropica, the total flavonoid and crude plant extract caused maximum inhibition rates of 48% and 56%, respectively, at a concentration of 2.7 mg/ml for both. Cytotoxicity value on Leishmania donovani was 20% for the crude extract at 1.35 mg/ml concentration, whereas it was 11% for the total flavonoids at a concentration of 0.169 mg/ml. In conclusion, the differences in anticancer and anti-parasitic activities are attributed to different compounds present in each extract.

Keywords: Ficus carica leaves, L20-B cell line, MCF-7 cell line, Leishmania tropica, Leishmania donovani.

الفعالية الحيوية لمستخلص الكحولي الخام ومستخلص الفلافنيدات الكلية لنبات التين العراقي

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الخلاصة

انكرت الدراسة الحالية على الاستراتيجية الحيوية لمعالجة السرطان والبكتيريا بالخلايا المضادة لنمو مطيانية الشماني باستخدام مستخلص الكحولي الخام لفواكه التين علامة على مستخلص الفلافنيدات الكلية لنبات وعمل مقارنة بين تأثيرهما، حيث تم استخلاص الفلافنيدات الكلية لفواكه التين.

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Introduction

Cancer is the second cause of death in the world. In 2015, it was responsible for 8.8 million deaths. Genetic influences have been long suspected[1].

Parasitic diseases such as malaria, leishmaniasis, and trypanosomiasis represent a significant global burden and pose a great challenge to drug discovery and delivery, due to their intracellular nature and disseminated locations. Moreover, the poor rate of discovery in the anti-parasitic segment seen in last few decades has necessitated effective management of the existing drugs by modulating their delivery[2].

Medicinal plants are gaining importance in these fields of research. *Ficus carica* (Fig), family Moraceae, is one of the medicinal plants used in different illness conditions. It has been cultivated from the Middle East and West Asia and spread to many regions in the world. Products of *F. carica* are widely used as food sources and medicine to treat various diseases[3]. The plant is rich in minerals such as copper, magnesium, potassium, calcium, and vitamin K. It is also a good source of flavonoids and polyphenols, including gallic acid, chlorogenic acid, syringic acid, catechin, picatechin, and rutin[4]. Fig fruits are astringent to bowels, tonic, and useful in the treatment of leucorrhoea, blood discharges, leprosy, menorrhagia, and intestinal worms. While leaves have an astringent use in urinary disorders and bronchitis. Bark is useful in asthma and piles, whereas latex is applied externally on chronic infected wounds to alleviate edema and pain and to promote the healing[5].

Materials and Methods

1. Plant collection and classification

The samples of Fig leaves were collected from the Al Haweja area/Kirkuk-Iraq and classified in the Faculty of Agriculture, Baghdad University. The samples were taken to the laboratory after cleaning well from suspended soil. They were dried at room temperature (25°C) and manually ground, then packaged in sterile containers and kept away from direct light until use.

2. Samples preparation

2.1. Preparation of leaves crude ethanolic extract

About 50 g dried powdered fig leaves were macerated in 400 ml of 70% ethanol for 3 days at room temperature then filtered. The filtrate was dried by rotary evaporate at 45°C till dryness. The residue from the crude extract was weighted and kept in a dark container for further chemical and biological assays[6].

2.2. Extraction of the total flavonoids from fig leaves

About 30 g powdered plant samples were placed in a 500 ml glass flask and then 300 ml distilled water with 10% v/v HCl was added. Reflux extraction was performed for 8 hours continuously to ensure that the cleavage and breakdown of the glycoside linkage between the flavonoids and the aglycone part was obtained. The plant extract was filtered and cooled. The aglycone portion that
possesses biologic effects was extracted by ethyl acetate in a portion of 1:1. The extraction was repeated three times using a separating funnel. The acetate layer was combined and washed with distilled water to remove HCl residues. Finally, the acetate layer was dried with a rotary evaporator at 45 °C. The residue was weighted and kept for further analysis [7].

2.3- Preparation of stock solution

0.4 g of the crude ethanolic residue and total flavonoids residue were re-dissolved, each in 75 ml distilled water to obtain a final concentration of 5.33 mg/ml.

3. Phytochemical tests for the crude extract

For the purpose of these tests, about 10 ml of the crude extract solution with a concentration of 5.33 mg/ml was used to identify the leaves chemical components, as follows [8]:

A. Detection of Tannins

Few drops of 1% lead acetatesolution were added to the plant extract. A white precipitate indicated the presence of tannins.

B. Detection of reduced sugar

An aliquot of 1 ml plant extract was mixed with 2 ml of Benedict reagent. The mixture was placed in a boiling bath for 5 minutes and left to cool. The red deposit indicated the presence of reduced sugar groups.

C. Detection of alkaloids

According to Dragangroff test, about 60 mg of bismuth subnitrate was dissolved in 0.2 ml HCL to obtain a solution. Solution B contained 600 mg potassium iodide in 1 ml distilled water. The combined solution [A + B] was added to the plant extract, forming an orange-brown precipitate which indicated the presence of alkaloids.

D. Detection of the saponins

Formation of a foam at the top when shaking the plant extract indicated the presence of saponins.

E. Detection of flavonoids

Based on the alkaline reagent test, sodium hydroxide 2N solution was mixed with a few amount of plant extract solution and left for few minutes. The yellow color obtained indicated the presence of flavonoids.

F. Detection of Polyphenolic compounds

Adding ferric chloride 1% solution to the plant extract solution caused the formation of a green-brown deposition, which is an indication of polyphenolic compounds.

4. Determination of Fig leaves’ Total Flavonoids

4.1. Qualitative assay

Standard flavonoids, namely rutin, quercetin, kaempferol, and luteolin, were prepared in ethanol. Thin layer chromatography (TLC) was carried out using a silica coated 60 plate with a thickness of 0.1 mm, while the mobile phase contained toluene: ethyl acetate: formic acid in a ratio of 36:12:5.

The types of flavonoids separated could be detected in corresponding to standard flavonoid spots, whereas the Rf value was calculated as in the following equation:

\[ Rf \, value = \frac{Distance \, traveled \, by \, each \, flavonoid}{Distance \, traveled \, by \, the \, mobile \, phase} \]

Each flavonoid could be detected separately after exposure of the silica plate to the UV light at 254 nm wavelength [9].

4.2. Quantitative Assay

Rutin standard flavonoid solutions (0.3125, 0.15625, 0.625, 1.25 mg/ml) were prepared in ethanol. About 1 ml of stock flavonoids extract solution (5.33 mg/ml) was transferred to a glass tube, and 1 ml rutin standard solution from each concentration was placed in separated glass tube, then 1 ml of 5% sodium nitrite solution was added to all tubes, mixed and left at room temperature for 5 minutes. To each tube, 2 ml of aluminum chloride 10% was added, mixed and left for another 5 minutes at room temperature. Finally, 5 mL of 1N NaOH solution was added and the resulting color was read with spectrophotometer at 510 nm wavelength. A standard curve was generated based on the absorbance of each standard solution against each concentration. The straight line equation was detected to calculate the total amount of flavonoids in the extracted plant [10].
4.3. Anticancer activity in vitro

In this study, the general protocol reported by Chliet et al. and Freshen y[11,12] was applied against two types of cancerous cell; the L20B cells and MCF-7 breast cancer cells. Briefly, different concentrations of (0.084, 0.169, 0.338, 0.675, 1.35 and 2.7 mg/ml) of the crude ethanolic extract and total flavonoids solution were prepared in a medium. Two plates were separately seeded with 100 µl of one kind of cancer cells suspended in growth medium and incubated for 24 hours for monolayer confluent. An aliquot of 100 µl from each plant extract concentration was added to a number of cultured wells in duplicate manner and incubated after all additions at 37°C overnight for 24 hours. A volume of 10 µl of the freshly prepared MTT coloring reagent (5 mg/ml) was added to all wells. The plate was incubated at 37°C for at least three hours. Finally, about 50 µl of DMSO was added to all wells and incubated for 10 min. The control was indicated as cell culture suspended in medium without plant extract. The absorbance of the treated and untreated wells was measured at 620 nm with an ELISA reader. The growth inhibition ratio was calculated as follows:

Growth Rate inhibition % = \[ \frac{\text{Control} - \text{Treated cell}}{\text{Control}} \times 100 \] [11]

4.4. Anti Leishmanial activity in vitro

The anti-leishmanial activity of Ficus caricaextracted flavonoids and the crude ethanolic leave extract was studied against two species of Leishmania parasite in the promastigote form; L. Tropica and L. donovani. A colorimetric method described by Mahmoud et al.[13] was applied. Initially, about 100 µl from both species of Leishmania promastigotes was suspended in all of the 96 wells of a tissue culture plate in a concentration of 10^6 parasites/ml. Then, 100 µl of various concentrations (0.084, 0.169, 0.338, 0.675, 1.35 and 2.7 mg/ml) of each extract solution were prepared in RPMI medium. Aliquots of 100 µl of each prepared treatment solution at different concentrations were added to the two seeded well plates and incubated at 26°C for 24 hours. About 10 µl of the freshly prepared solution of the MTT coloring reagent (5 mg/ml) was added to all wells. The plate was incubated at 26°C for at least three hours. Finally, about 50 µl of DMSO was added to all wells and incubated for another 10 min. The control was indicated as promastigotes cultured in complete medium without plant extract. The absorbance of treated and untreated wells was measured at 620 nm with an ELISA reader. The growth inhibition ratio was calculated as follows:

Growth inhibition Rate % = \[ \frac{\text{Control} - \text{Treated cell}}{\text{Control}} \times 100 \] [13]

Results and Discussion

1. Plant Classification:
The plant was classified at the Faculty of Agriculture, Baghdad University, as Ficus carica.

2. Plant Extract Yields
The crude ethanolic extract of 50 g of the dried Ficus crica leaves yielded about 8.5 g residue, while the total flavonoids extracted from 30 g of the dried plant leaves yielded about 0.42 g residue.

3. Phytochemical tests for the crude extract:
Table-1 indicates the main active groups that present in the crude alcoholic extract of plant leaves.

| Table 1-The main active groups in the crude alcoholic extract of fig leaves |
|------------------------|-----------------|-----------------|
| TEST                  | RESULT | COMMENTS         |
| Reduced sugar         | +++    | Heavy Red precipitate |
| Alkaloids (Draganroff)| +      | little brown precipitate |
| Saponines             | ++     | Foam formation    |
| Flavonoids            | +++    | Bright yellow color |
| Polyphenolic compounds| +++    | Brown precipitate  |
4. Determination of Fig leaves’ Total Flavonoids

4.1. Qualitative Assay

Flavonoids were extracted from the plant and were identified by thin layer chromatography (TLC). Spots were obtained by UV exposure of the plate to a 254nm wavelength, in comparison with standard flavonoids(Figure-1).

![Figure1-TLC chromatogram of the extracted total flavonoids(F) corresponding to standard flavonoids; Rutin(R), Quercetin(Q), Luteolin(L), Kaempferol(K).](image)

As shown in Figure-1, the total flavonoids extracted from the plant leaves contained different types, namely quercetin, kaempferol, luteolin and others, the levels of which were detected by the calculation of R_f values shown in Table-2.

**Table 2**- The values of R_f for different flavonoids and the extracted flavonoids.

| Flavonoid Type | Rutin | Quercetin | Luteolin | Kaempferol | Extracted flavonoids |
|----------------|-------|-----------|----------|------------|----------------------|
| R_f value      | Base line | 0.28 | 0.2 | 0.53 | Base line, 0.2, 0.28, 0.53 and 0.9 |

4.2. Quantitative Assay

The amount of flavonoids found in *F. caricas* leaves was estimated using the standard rutin curve. Table-3 shows the absorption values of the standard flavonoid at different concentrations as well as of the extracted total flavonoids, where the straight line equation was obtained as shown in Figure-2.

**Table 3**- Absorption values of the standard flavonoids (rutin) at different concentrations and the extracted total flavonoids of plant extract.

| Absorption at (510 nm) | Rutin standard solution (mg/ml) |
|------------------------|---------------------------------|
| 0.135                  | 0.15625                         |
| 0.341                  | 0.3125                          |
| 0.602                  | 0.625                           |
| 1.003                  | 1.25                            |
| 2.750                  | 2.5                             |
| 1.144                  | Total flavonoids of the plant extract |
From the equation of the straight line, the concentration of the total flavonoids in the extract was calculated as follows:

\[ Y = 0.847X \]

\[ X = Y / 0.847 \]

\[ X = 1.144 / 0.847 \]

X = 1.35 mg/ml total flavonoids in each 5.33 mg residue that dissolved in 75 ml yielded from the extraction of 30 g plant Total flavonoids / 100 g * ficus carica * dried leaves = 1.35 mg/ml x 75 ml X 100 g / 30 g = 337.5 mg.

4.4. Anti-Cancer Activity on L20-B Cell line

As shown in Figure-3, different cytotoxic effects were obvious for both *F.carica* leaves ethanolic crude extract (Red) and the total flavonoids (Green) on L20-B cell line.

**Figure 2**- Rutin standard curve. From the equation of the straight line, the concentration of the total flavonoids in the extract was calculated as follows:

\[ y = 0.847x \]

\[ R^2 = 0.9777 \]

**Figure 3**- Cytotoxic effect for both crude ethanolic fig leaves extract (Red) and the total flavonoids (Green) on L20-B cell line.
As shown in Figure 3, the extracted total flavonoids showed a more potent effect on the cell growth than the crude. Each of the individual flavonoids extracted in the present study possess important roles in controlling and treating different kinds of cancers in the three stages, as flavonoids act as free radical scavengers and potent antioxidants [14]. The growth inhibition rate for L20-B cancerous cell line was constant in almost all plant crude concentration. While in the case of *F. carica* total flavonoids, the cytotoxic effect against this cell line appeared even in small concentrations, reaching a maximum inhibition rate (43%) at a total flavonoid concentration of 0.169 mg/ml.

These effects could be mediated through phenolic acids, chlorogenic acids, flavones, and flavonols that are present in *F. carica*. Quercetin compounds are the main phenolic compounds found in *F. carica*. Quercetin has the ability to stimulate the apoptosis of many cancer cells by stimulating the release of cytochrome c from the mitochondria [16]. *F. carica* also contains fibers, vitamin A, vitamin C, calcium, magnesium, and potassium which are needed by the body. Other bioactive compounds of *F. carica* are arabinose, β-amirin, β-carotene, glycosides, β-sitosterol, and xanthol, which are antioxidant compounds [17,18].

*F. carica* has previously been demonstrated to inhibit the growth of HeLa cancer cells and MDA-megabyte (MB)-231 breast cancer cells [19].

### 4.5. Anti-Cancer Activity on MCF-7 Cell line

Figure 4 shows the different effects for both plant extract on the breast cancer cell line MCF-7, as indicated by the values of inhibition rate.

![Figure 4](image-url)

**Figure 4**-Cytotoxic effect for both crude ethanolic *F. carica* leaves extract (Red) and the total flavonoids (blue) on MCF-7 cell line.

Both types of extracts affected the growth of the breast cancer cell line MCF-7 in a different manner. As shown in Figure 4, the crude ethanolic extract (Red) inhibited the cell growth in all concentrations, where the most potent effect appeared at the lower concentration (0.084 mg/ml) which caused a maximum inhibition rate of 34%. Total flavonoids extracted from fig leaves resulted in MCF-7 growth inhibition (blue) from 0.084 mg/ml which gave a rate inhibition 20% up to 28% at concentration 2.7 mg/ml.

One study published in 2018 [20] indicated that breast cancer has substantially higher incidence than any other cancer diseases in women, and are categorized into three basic groups: human epidermal growth factor receptor-2 (HER2/ERBB2), estrogen receptor (ER) positive, and triple-negative breast...
It was found that there are some key molecules in breast cancer that are tightly involved in proliferation or apoptosis of breast cancer cells. These include GATA3, p53, Bax, p21, ELF5, and cyclin-dependent kinases (CDKs), which can affect the viability of cancer cells by repairing damaged DNA, influencing the cell cycle, or inducing apoptosis[21-23]. The main cause of death in breast cancer is metastasis, and many molecules are involved in the process, including MPP2, TIMP1, and TIMP2[24-26].

Another study investigated the molecular mechanisms of the effects by analyzing the expression of key breast cancer biomarkers which are crucial to cell proliferation and cell cycle. Migration of the extracts from F. carica leaves indicated that the leaf components have anticancer effects on triple-negative breast cancer MDA-MB-231 cells (TNBC) cell line, which was the most difficult subtype of breast cancer to treat. The reports also suggested that F. carica leaves might be a good source to develop drugs for suppressing cancer-cell growth and migration and to treat TNBC cancer[27].

4.6. Antiparasitic Activity against Leishmania tropica

Figure 5 shows in vitro effects of the F. carica crude extract (blue) at different concentrations and the total flavonoids (Red) against Leishmania tropica.

Both figures show that the extracts affected the growth of Leishmania tropica at all concentrations and in a dose dependent manner. The maximum cytotoxic effect appeared at the concentration of 2.7 mg/ml for both the crude and total flavonoids extract, reaching 56% and 48%, respectively. There are only a few studies about antileishmaniasis activity for Ficus carica different plant parts. A review [28] noted that the milky sap and ethanolic extract for two species, Ficus carica and Ficus religiosa, had antiparasitic effects against Aedes aegypti and Pheretima posthuma parasites, respectively. Recently, the number of researches on antileishmanial agents significantly increased for two reasons. Firstly, several treatments such as antimony derivatives remain toxic and expensive. Secondly, several Leishmania species showed the resistance against synthetic molecules, and therefore the emergence and reemergence of infectious diseases. These two situations have oriented pharmacological researches on antileishmanial drugs to screen plants components that possess a selective efficacy and tolerable safety. Medicinal plant secondary metabolites such as volatile oil, flavonoids, polysaccharides, alkaloids and others, showed several pharmacological properties including antibacterial, antioxidant, and anticancer ones, which enhanced the researches to project them as antiparasitic compounds [28].

4.7. Antiparasitic Activity against Leishmania donovani

The effects of Ficus carica crude extract and total flavonoids on the growth of Leishmania donovani is shown in Figure 6.
Crudeethanolic extract for Ficus carica leaves(Red) possessed potent antileishmaniasis effects in all concentration,giving a maximum inhibition rate(20%) at the concentration of 1.35mg/ml. While, Fig total flavonoids showed potent cytotoxic effect (11%) in a low concentration (0.169 mg/ml). The differences in the biological activity for both crude and total flavonoids of Ficus plant were due to the differencesin the active constituents present in each one. Studies reported that the major components in the fig leaves ethanolic extract were thefuranocoumarins including psoralen and bergapten[29]in addition totriterpenes such as lupeol acetate[30]. Other valuable ingredients are phenols, anthocyanins, fructose, glucose, and sucrose were identified from the figs [31], while anotherfinding reported that the fruit has phyto-sterols [32].

**Conclusions**

The differences in anticancer and anti-parasitic activitieswere attributed to differing compounds present in each extract.

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**References**

1. Amin,K.M, Eissa.A.M, Abbr-seri.S.M, Awadallah.F.M , Hassan,G.Y. 2013. Phytochemical Profiling of Fig Fruit Ficus Racemosa Extract. Medichrem.J., 60: 187-198.
2. Arshad, H. R. and Yousef, H. 2017. Ficus Carica And Its Constituents Role In Management Of Diseases. Asian Journal of Pharmaceutical and Clinical Research: June, 10(6).
3. Barolo, M.I. Mostacero, N.R. and López, S.N. 2014. Ficus carica L. (Moraceae): an ancient source of food and health. Food Chem.; 164: 119–127.
4. Vaya, J. and Mahmood, S. 2006. Flavonoid content in leaf extracts of the fig (Ficus carica L.), carob (Ceratoniasiliqua L.) and pistachio (Pistacia lentiscus L.). Biofactors , 28: 169-75.
5. Gilani, A.H., Mehmood, M.H., Janbaz, K.H., Khan,A.U. and Saeed, S.A. 2008. Ethnopharmacological studies on antispasmodicand antiplatelet activities of Ficus carica. JEthno pharmacol, 119: 1-5
6. Harborne, J.B. 1973. phytochemical methods, A guide to modern techniquos of plant analysis . First Edition, chapman and Hall, London.
7. Harborne, J.B. 1984. phytochemical methods, A guide to modern techniquos of plant analysis . Second edition, chapman and Hall, London, pp. 169-172.
8. Richird, I.P. 2000. Natural products Isolation. Fourth edition New Jersey.
9. Kato, M., Mizuna, K., Fujimura, T., Wama, M.:Irie, M., krozier, A., Ashihara, H. 1999. Purification and characterization and of caffeine syntheses from tea leaves. Plant Physiology. 12: 579-586.
10. Marcia, M.S., Vesna, R., Mirza,B. and Zelijan, M. 2012. From functional food to Medicinal product systematic approach in analysis of polyphenolic , from popolis and wine ; Nutrition journal, 8: 33.

11. Chi, P.L., Wei, J.T., Yang, L.L. and Yuh, C.K. 2004. The extract from Nelumbonucifera suppress cell cycle progression, cytokine genes expression ,and cell proliferation in human blood priferial mononuclear. Life Science. 75: 699-716.

12. Freshney, R.I. 2012 .Cultureofanimal cell, sixth Edition .Wily-Liss, NewYork.

13. Mahmoud, H., Ezzatekhah , F. Sharififar, F. 2015 , Antileishmanial and Cytotoxic effects of essential oil and methanolic extract of Myrtus communis L. Korean J.Parasitol., 53(1): 21-27.

14. Ignas, G.I.D. and Vilma, P. 2017. Relationship between Antioxidant and Anticancer Activity of Trihydroxyflavones. Molecules. vol.7, December; 22(12): 2169.

15. Santoro, M., Guido, C., de Amicis, F. 2016 . Bergapten induces meta–bolic reprogramming in breast cancer cells. Oncol Rep. ;35(1): 568–576.

16. Panno, M.L. and Giordano, F. 2014 . Effects of psoralens as anti-tumoral agents in breast cancer cells. World J Clin Oncol.; 5(3): 348–358.

17. Wang, X., Cheng, K., Han, Y. 2016 . Effects of psoralen as an anti-tumor agent in human breast cancer MCF-7/ADR cells. Biol Pharm Bull. : 9(5): 815–822.

18. Wu, C., Sun, Z., Ye, Y., Han, X., Song, X. and Liu, S.(2013) Psoralen inhibits bone metastasis of breast cancer in mice. Fitoterapia.; 91: 205–210.

19. Risa, P., Dwi, W., Adita, A. P., Eva, A. Suhailah, H. and Win, D. 2018. Anticancer Activity of Methanol Extract of Ficus carica Leaves and Fruits Against Proliferation, Apoptosis, and Necrosis.Cells.Cancer Inform. 18.

20. Del, P. L. and Peccatori, F.A. 2018. Is ovulation induction with letrozole in breast cancer patients still safe even if it could increase progesterone levels. Eur Rev Med Pharmacol Sci. ; 22(1): 246–249.

21. Sun, J., Guo, Y. and Fu, X. 2016. Dendrobium candidum inhibits MCF-7 cells proliferation by inducing cell cycle arrest at G2/M phase and regulating key biomarkers. Onco Targets Ther.; 9: 21–30.

22. Malumbres, M. and Barbacid, M. 2009. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer.; 9(3): 153–166.

23. Choi,Y.S., Chakrabarti, R., Escamilla-Hernandez, R. and Sinha, S. 2009. Elf5 conditional knockout mice reveal its role as a master regulator in mammary alveolar development: failure of Stat5 activation and functional differentiation in the absence of Elf5. Dev Biol.; 329 (2): 227–241.

24. Sun, J., Fu, X. and Wang, Y. 2016. Erianin inhibits the proliferation of T47D cells by inhibiting cell cycles, inducing apoptosis and suppressing migration. Am J Transl Res.; 8(7): 3077–3086.

25. Gomes, L.R., Terra, L.F., Wailemann, R.A., Labriola, L. and Sogayar, M.C. 2012. TGF-B1 modulates the homeostasis between MMPs and MMP inhibi–tors through p38 MAPK and ERK1/2 in highly invasive breast cancer cells. BMC Cancer ; 12: 26.

26. Lansky, E.P., Paavilainen, H.M., Pawlus, A.D. and Newman, R.A. 2008. Ficus spp. (fig): ethnombotany and potential as anticancer and anti-inflammatory agents. J Ethnopharmacol.; 119(2): 195–213.

27. Yu, Z., Youzhong, W., Bo, H., Boyuan, L., Yue, J. and Xin, H. 2018. Extracts and components of Ficus carica leaves suppress survival, cell cycle, and migration of triple-negative breast cancer MDA-MB-231 cells. OncoTargets and Therapy.11: 4377–4386.

28. Ali, E. 2016. Antiparasitic effects of medicinal plants (part 1)- A review, IOSR Journal of Pharmacy Version. 3, 6(10): 51-66.

29. Abu-Mustafa, E.A., Tawil, E. and Fayez, E.1964. Constituents of local plants-IV; Ficus carica L., F. sycomorus L. and F. salicifolia L. leaves. Phytochem.; : 3: 701-3.

30. Ahmed, W., Ahmed, Z. and Malik, A. 1990. Triterpenes from the leaves of Ficus carica.Fitoterapia; 61: 373-5.

31. Çalışkan, O. and Polat, A.A. 2011. Phytochemical and antioxidant properties of selected fig (Ficus carica L.) accessions from the eastern Mediterranean region of Turkey.Sci Hortic; 128: 473-8.

32. Jeong, W.S. and Lachance, P.A. 2001. Phytosterols and fatty acids in fig (Ficus carica, var. Mission) fruit and tree components. Food Chem Toxicol.; 66: 278-281.