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The Synergistic Anticancer Effect of Some Plant Extracts in Combination against Human Liver Cancer Cell Line

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ABSTRACT

Background: Cancer is still a major health problem worldwide, despite the big development in treatment and diagnostic methods. For this reason, this study aims to find new drugs with low cost and fewer side effects on cancer cells continue. Medical plants represent a suitable candidate for discovering new materials for cancer treatment.

Methods: Ten plants usually used in traditional medicine were used to evaluate their effectiveness as anti-cancer. These plants were divided into two mixtures according to traditional usage, named mixtures A and B. The anticancer effect against HepG2 was determined by MTT assay. The IC_{50} was also calculated.

Result: Mixture A showed cytotoxicity reached 66.98 %, while mixture B showed 63.66% by using 400 µg/ml at 48h. The IC_{50} for mixture A was 53 µg/ml, while for mixture B was 44.29 g/ml. All these results compare to normal cell line HnFd cells. In conclusion, mixing medical plants can effectively increase the anticancer activity of the synergistic effect between the phytochemicals. Future studies will be done to scan these effects.

Key words: Anticancer activity, Cancer, Cytotoxicity, Medicinal plants, Phytochemicals.

INTRODUCTION

Cancer is a major public health problem worldwide and the leading cause of death in developed and developing countries (Ferlay *et al*., 2018). Moreover, cancer incidence and mortality are quickly growing worldwide for different reasons, including aging, population growth and socioeconomic development (Gersten and Barbieri, 2021). Cancer is uncontrolled cell growth caused by several genetic and epigenetic changes in genes that control cell proliferation or regulate cell death (Garcia-Oliveira *et al*., 2021). The methods of treating and diagnosing cancer have greatly advanced over the past decade.

In recent years, the pharmacological effects of medicinal plants have been under-focused and herbal medicine has gained a lot of attention as promising medicine for many diseases, including cancer (Aung *et al*., 2017). The reason for the demand for herbal medicine return to grow knowledge of natural products that are non-toxic, have minimal side effects and have low expenses and a variety of plants that have not been studied yet (Iqbal *et al*., 2017).

The therapeutic efficacy of plants returns to their secondary metabolites, which are term as phytochemicals considered pharmaceutical active (Das *et al*., 2018a). Many of these phytochemicals were revealed to be strong anticancer effects by their impact on the proteins and enzymes and signaling pathways that participate in the initiation or development of cancers (Tariq *et al*., 2017). These phytochemicals include Alkaloids, phenolic, flavonoids, tannins, oils, glycosides, resin, gums and derivatives. But the phytochemicals that are found naturally in plants in trace amounts while it's required in high doses to give their therapeutic effect, so there is a suggestion to combine two or more plants to increase their doses (Lewis and Tollefsbol, 2017).

Medical plants represent a suitable candidate for discovering new materials for cancer treatment, so this study aims to find new drugs with low cost and fewer side effects on cancer cells continue.

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MATERIALS AND METHODS

Medicinal plants

Ten plants (*Saussurea costus, Teucrium polium, Linum usitatissimum, Artemisia annua, Commiphora molmol, Zingiber officinale, Syzygium aromaticum, Curcuma longa, Cinnamomum verum* and *Allium sativum*) were used in this study to determine their synergistic effect as anticancer. These plants have been known to use in folk medicine for many decades. These plants are commonly used individually, but sometimes by mixing two or more plants for different therapeutic purposes.

Table 1 shows the medicinal plants used in the current study and the types of their phytochemicals mentioned, which were revealed to be responsible for their effect according to previous studies.

Plants extraction

All plants were obtained from the local markets of Baghdad city/Iraq. Mixture A contains ten plants (*Saussurea costus*, *Teucrium polium, Zingiber officinale, Artemisia annua, Linum usitatissimum*, *Cinnamomum verum*, *Syzygium aromaticum, Allium sativu, Commiphora molmol* and *Curcum longa*). In contrast, mixture B consists of five of ten plants (*Saussurea costus, Teucrium polium, Linum usitatissimum, Commiphora molmol* and *Artemisia annua*). Two grams from each crude plant were taken and mixed, then distilled water was added in a ratio of 1:4 and boiled for 15 min. The concentrated extracts were filtered and left at room temperature until all the water evaporated.

Location of the experiment

Lab experiments were done in the biological labs/ Institute of Biological Sciences/ University of Malaya, Malaysia.

Cell culture

Human liver cancer (HepG2) and primary dermal fibroblast normal (HdFn) cell lines were used to evaluate the anticancer effect of the plant mixture extract. The cells were cultured (Freshney, 2015) in 75 cm^2 tissue culture flasks under humidified 5% CO_2 atmosphere at 37°C in RPMI-1640 medium (Sigma chemicals, England) with 10% fetal bovine serum (FBS) and penicillin-streptomycin 1% (100 U/ mL penicillin and 100 µg/mL streptomycin).

Cytotoxicity of plants mixture A and B on HepG2 and HdFn cell lines

The MTT assay was used to determine the cytotoxicity where the cell line was treated with six concentrations (400, 200, 100, 50, 25 and 12.5 µg/ml) of mixture A and B for 48 h., were incubated with 10 µL of MTT (37°C, 2 h). After the incubation, media in the cells was aspirated, followed by the addition of DMSO (100 µL) in each well. The absorbance was recorded in a microtiter plate reader at 570 nm.

Statistical analysis

All data were analyzed with a one-way analysis of variance (ANOVA) expressed as the mean± standard deviation of three replicates. Additionally, the best fit method and the regression equation were used for calculating the half-maximal inhibitory concentration (IC_{50}) values.

RESULTS AND DISCUSSION

People in Asia and Africa have used traditional or folk medicine for a long time, depending on using plants according to practice and theories (WHO, 2015). Many modern medicines depend on plant phytochemicals or derivatives used in folk medicine (Tanaka and Kashiwada, 2021). In our study, several plants consumed by people as traditional medicine were used to evaluate their anticancer effects against the HepG2 cancer cell line compared to their effect against HdFn normal cell line at 48 h. The results of the A mixture were illustrated in (Table 2) where it showed a concentration-dependent effect against HepG2, whereas the 400 and 200 µg/ml revealed the highest cytotoxicity by 66.98

Table 1: The phytochemicals of the used medicinal plants.

Plant name	Phytochemicals	Reference
Saussurea costus	Sesquiterpene lactones	(Lin *et al.*, 2016)
Teucrium polium	mono- and sesquiterpenes, flavonoids, phenolic, saponins terpenoids, alkaloids and terpenoids.	(Salhab, 2017); (Sharifi-Rad *et al.*, 2022)
Linum usitatissimum	Phenolic acids, flavonoids, lignan	(Lazić *et al.*, 2018); (Garros *et al.*, 2018)
Artemisia annua	Artemisinin, coumarins, flavones, flavonols, phenolic acids, sesquiterpenes.	(Lang *et al.*, 2019)
Commiphora molmol	volatile oil, tannins, phenols, steroids, terpenoids, carbohydrates, resins, gums	(AL-Samarrai, 2017)
Zingiber officinale	Sesquiterpene, gingerols	(An *et al.*, 2016)
Syzygium aromaticum	sesquiterpenes, monoterpenes, hydrocarbon and phenolic compounds. Eugenyl acetate, eugenol, β-caryophyllene	(Cortés-Rojas *et al.*, 2014)
Curcuma longa	tannins, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides and phenolic acids (curcumin)	(Singh and Madan, 2019)
Cinnamomum verum	Cinnamaldehyde, eugenol, caryophyllene, cinnamyl acetate and cinnamic acid, glutathione	(Singh *et al.*, 2021)
Allium sativum	alliin, allicin, ajoenes, vinyldithiins, flavonoids	(El-Saber *et al.*, 2020)

and 60.65%, respectively. In comparison, the same concentration shows 30.44% cytotoxicity against control cells. Moreover, the IC_{50} for mixture A shown in (Fig 1) and was 53.0 µg/m against HepG2, while its effect on normal cells HdFn was ineffective in a few concentrations.

Additionally, the results of mixture B against HepG2 were shown in (Table 2), wherein the highest cytotoxicity appears in 400 and 200 µg/ml, which is 63.66 and 50.19% (Table 3 and Fig 2). Moreover, the IC_{50} against HepG2 was 44.29 µg/ml, while the effect on control cells was 229.6 µg/ml.

These results indicate that plant mixtures used in the present study have promised anticancer effects against cancer cell lines. These effects may return to their containing of phytochemicals, as mentioned in Table 1. These phytochemicals were investigated for their anticancer effects individually or combined with one or two chemicals. In Table 4, each plant is mentioned with previous studies that showed its anticancer efficacy and the type of cancer. Our hypothesis suggests that the synergistic effect of these phytochemicals may play a role in increasing their effects as therapeutic reagents as well as anticancer effects. Many mechanisms that can do these may increase the concentration bioavailability in the cancer cells, enhancing their effects or targeting the same molecular pathways that inhibit the proliferation of cancer cells or activate tumor suppressor genes that cause the programmed cell death leading to apoptosis. The synergistic effect of phytochemicals on HepG2 cells was studied in previous research by Leng *et al*. (2018). They found crocin, chlorogenic acid, geniposide and quercetin combination have therapy effects on hyperlipidemia and prevent complications of obesity.

In addition, the combination of two phytochemicals curcumin and Epigallocatchin-3-gallate (EGCG) from green tea has a more significant inhibitory effect on carcinoma than the individual effect of both phytochemicals (Jin *et al*., 2017).

Other studies revealed the prevention and antitumor effect of combining some phytochemicals (resveratrol, ellagic acid and grape seed extract) against skin cancer with higher efficacy than using each phytochemical alone. Also, the synergistic effect of different flavonoids increased the chemoprevention in prostate and breast cancer (Wang *et al*., 2014).

Table 2: The cytotoxicity effects of mixture a extract on HepG2 and HdFn cell line.

	HepG2		HdFn	
Concent. µg/ml	Cytotoxicity	SD	Cytotoxicity	SD
400	66.98	±3.45	30.44	±2.32
200	60.65	±3.25	13.97	±0.85
100	48.5	±2.12	7.87	±1.56
50	33.41	±4.82	3.82	±1.25
25	13.35	±6.48	3.05	±1.14
12.5	5.05	±0.24	5.09	±2.20

Table 3: The cytotoxicity effects of mixture B extract on HepG2 and HdFn cell line.

	HepG2		HdFn	
Concent. µg/ml	Cytotoxicity	SD	Cytotoxicity	SD
400	63.66	±2.05	30.17	± 2.84
200	50.19	±2.27	17.55	±7.22
100	48.5	±2.12	8.1	±1.01
50	35.84	±4.60	4.94	±0.37
25	11.96	±5.95	4.17	±2.24
12.5	6.48	±0.70	5.56	±1.62

Table 4: The used medicinal plants and cancer types that inhibited.

Plant name	Cancer type inhibited	Reference
Saussurea costus	Breast, liver, colon Prostate, Lung, Gastric	(Tian *et al*., 2017); (Shati *et al*., 2020)
Teucrium polium	Glioblastoma, colon, melanoma, lung, breast	(Khazaei *et al*., 2018)
Linum usitatissimum	Prostate, breast, colon, skin	(Sharma *et al*., 2014); (Calado *et al*., 2018); (DeLuca *et al*., 2018); (Zhou *et al*., 2020)
Artemisia annua	Breast, colon, lung	(Rassias and Weathers, 2019); (Ko *et al*., 2020); (Jung *et al*., 2021)
Commiphora molmol	Liver, cervix	(Ramadan *et al*., 2017); (Anwar *et al*., 2021)
Zingiber officinale	Leukemia, prostate, breast, skin, ovarian, lung, pancreatic, colorectal	(Semwal *et al*., 2015); (Salehi *et al*., 2019)
Syzygium aromaticum	Breast, esophageal, cervical, colon, liver	(Liu *et al*., 2014); (Kumar *et al*., 2014); (Das *et al*., 2018b)
Curcuma longa	Prostate, colorectal, Head and neck, Breast, Brain, Glioblastoma, pancreatic	(Klinger and Mittal, 2016)
Cinnamomum verum	Cervical, colorectal, lymphoma, melanoma, cervix, prostate, ovarian	(Lin *et al*., 2016); (Sadeghi *et al*., 2019)
Allium sativum	Pancreas, lung, breast, prostate, colon, stomach, cervical, liver	(Chhabria *et al*., 2018); (Gore *et al*., 2021)

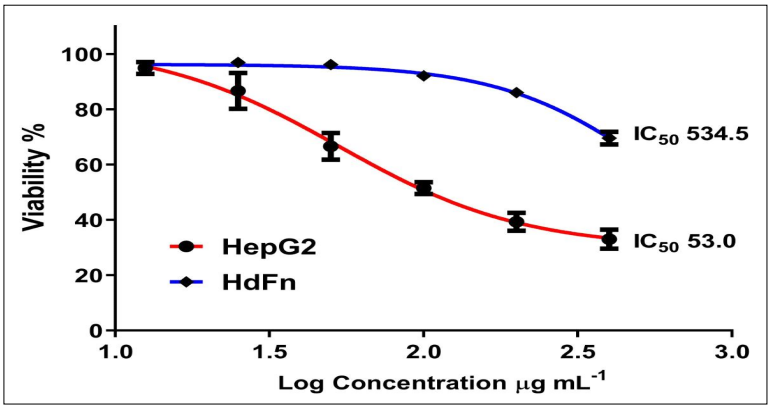

Fig 1: The IC_{50} of mixture A affected the HepG2 and HdFn cell line.

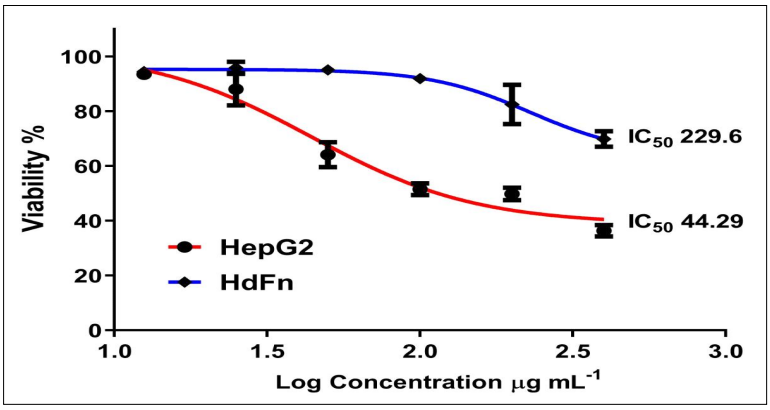

Fig 2: The IC_{50} of mixture B affected HepG2 and HdFn cell line.

CONCLUSION

The results obtained from the current study indicate that both mixtures of medicinal plants (A and B) show dose-dependent anticancer effects against the liver cancer cell line while not affecting a normal cell. Also, the results suggested that mixing traditional medicinal plants enhances their synergistic anticancer effect. Further studies will be established to find the *in vivo* effect and exact mechanisms of action, as well as to study the side effect (if found) of this mixing.

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Ethical statement

The study was done under the supervision of the Environment Research Center/University of Technology-Iraq and Al_Betar Research Center, Corporation of Research and Industrial Development, Ministry of Industry and Minerals, Baghdad, Iraq.

Conflicts of interest

The authors declare that there is no conflict of interest.

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