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***Research Article** (Open Access)*

STUDY ON SYNERGIC EFFECT OF *PSIDIUM GUAJAVA* WITH *MANGIFERA INDICA* LEAVES EXTRACT AGAINST THE HUMAN HEPATOMA G2 (HEPG2)

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ABSTRACT
Cancer is a multifaceted and heterogeneous disease leads people death vigorously. Amongst males in sub-Saharan Africa and Southeast Asia liver cancer is more common type. Still many of them depends on medicinal plants as a form of therapy and now researchers are focusing on new therapeutic agents with less side effects. The current study is aimed to evaluate the combined effect of *Psidium guajava* (Guava) and *Mangifera indica* (Mango) leaves powder was successively extracted with Ethanol Extract-GMEE, Ethyl Acetate -GMEA and water-GMDW to examine the antioxidant activity (DPPH, FRAP) and anti-inflammatory activity using albumin denaturation and membrane stabilization assay. The efficacy of these extracts was tested for anti-cancer potential against the liver cancer cell line (Hep G2) through MTT assay. Phytochemicals were analyzed using standard method. Significant activity displayed by GMDW exhibited strong antioxidant potential in free scavenging activity at high concentration 100 µg/mL in DPPH and FRAP (IC50 value; 57.29±7.519 µg/mL and 71.6±9.760µg/mL respectively). The lowest IC50 value (31.508±2.406 µg/mL and 46.071±6.079 µg/mL respectively) observed in the albumin denaturation and membrane stabilization assay. The results of anticancer activity with GMDW working on HepG2 revealed that the cell viability increased in low concentration 15.625 µg/mL with 93.402% at high concentration 250 µg/mL with 19.873% the viability of cell decreases (IC50 value 64.395±11.67 µg/mL). Phytochemical study showed good sources of bioactive compounds. This study is evident that combined leaf extract of guava and mango with distilled water has strong potential antioxidant, anti-inflammatory and anticancer activity with lesser side effects.

Keywords: *Psidium guajava, Mangiferaindica,* Phytochemicals, GCMS, Antioxidants, Anti-inflammatory, Anticancer.

INTRODUCTION
Cancer is a progressive accumulation of multiple genetic mutations cause of death worldwide. The raising burden of cancer globally calls for an alternative solution against currently available treatment include surgery, radiation and drug or medication therapy since these have several undesired side effects. Intake of synthetic chemotherapy drug wield good effect on cancer cells and also causes harmful side effects. Liver cancer is one of the most prevalent malignity across the world in cancer-related mortality due to the lack of standard treatments and the severe side effects associated with the existing therapies have made it compulsory to explore novel and more effective anticancer. Still research is carried out to search for better drug from naturally occurring compounds help to suppress or prevent the process of carcinogenesis (Lesetja R Motadi, *et al.*, 2020).
Plants have firmly bounded active substances that counteract this life-threatening disease. Natural compounds derived from medicinal plants play a central part in the health care and drug development in classical as well as advanced systems of medicine. Therefore, World Health Organization (WHO) insist the researchers to screen the medicinal plant and its materials for the presence of biologically active compounds used as anticancer drugs should destroy the cancer cell without damaging normal cells.

The population of developing countries use the medicinal plants have long been effective in both traditional and modern medicine as nutraceuticals as well as food supplements for the treatment of ailments (Muhammad Zeeshan Bhatti, *et al.,* 2015). In the last few years there has been an exponential growth in the field of herbal medicine gaining wide attention against cancer.

Several, studies have previously demonstrated the cytotoxic activities of *Psidium guajava* (Guava) and *Mangifera indica* (Mango) leaves extract in cancer cells type. Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population and supply a major source for traditional and modern medicines. Guava are cheap and easily available in all seasons and is important tropical fruit widely grown in Taiwan, Hawaii, Thailand, Philippines and Malaysia. Guava extracts purified from leaf have many bio-active molecules such as beta-caryophyllene oxide, vitamins, tannins, phenolic compounds, flavonoids and triterpenoid acids for anti-cancer activities(Neeta Chaudhary and Shalini Tripathi 2014). Mango is one of the most important tropical plant grows in tropical and subtropical regions in the world. Mango is rich in various polyphenolic compounds. Mangiferin compound in mango leaf has inhibitory potential on key enzymes involving glucose metabolism, that is, α-amylase and α-glucosidase against tested cancer cell lines. Currently, many studies are investigating in the effects of combination therapy using various medicinal plants for anticancer drugs to overcome this issue with low concentration usage of biological compound gives more effective than individual plant concentration effect(Girish Sharma , *et al.,* 2004).

The present investigation was designed to determine the phytochemical constitutes, antioxidant, anti-inflammatory activity and anticancer potentials of the active synergistic effect of guava with mango leaf extract against the liver cancer cell line HepG2.

MATERIALS AND METHODS

Selection of the plant

The present study is to evaluate the anticancer activity of *Psidium guajava* and *Mangifera indica* based on the literature review .

Collection and authentication of plant material

The leaves of *Psidium guajava* and *Mangifera indica* were collected from Madhavaram horticulture, Chennai, Tamil Nadu, India. The plant material was identified and authenticated by Prof. Jayaraman, Taxonomist, Plant Anatomy Research Centre (PARC), Chennai with ref no. PARC/2022/4626 and PARC/2022/4627 .

Chemicals:

Ethanol, Ethyl acetate, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallic acid, aluminum chloride, potassium acetate, sodium acetate, ascorbic acid, phosphate buffer, potassium ferricyanide, ferric chloride Trichloro Acetic acid (TCA), dimethyl sulfoxide (DMSO), bovine serum albumin (BSA), aspirin, and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT).All reagents and chemicals were of analytical grade.

Preparation of leaf extract

The collected fresh leaves of *Psidium guajava* and *Mangiferaindica* were taken to the laboratory and removed dust from samples with running tap water then further washed with distilled water and allowed to drip. The samples were chopped into small pieces and then kept in the shade dry for 15 days at room temperature and then grounded into fine powder using laboratory blender. The powdered samples were sieved using 90 micron sieve and store in airtight container for further process. About 20 g of each powdered samples were weighed and extracted for with 200 ml of ethanol, ethyl acetate and Distilled water at 60-100^{o} C in Soxhlet apparatus for 4 hrs. The extract was then concentrated at reduced pressure using rotary evaporator and stored in vials at 4°C until further analysis. (Jaikumar K, *et al.,* 2017)

Cell culture:

The human liver cancer cell lines and normal VERO cell line were obtained from ***National Centre for Cell Science*** (NCCS), an autonomous organization aided by the Department of Biotechnology, Government of

India, Pune , India The cells were maintained in Minimal Essential Medium supplemented with 10% Fetal Bovine Serum (FBS), Penicillin (100 µg /ml), and Streptomycin (100 µg/ml) in a humidified atmosphere. They were incubated at 37°C in a 5% (v/v) CO_2 atmosphere.

Preliminary Phytochemical Analysis of the Leaves Extracts

Phytochemical analysis for the screening and identification of bioactive chemical constituents such as flavonoids, terpenoids, alkaloids, glycosides, steroids, saponins and tannins of the leaves extracts were determined using standard procedures. The components analyzed were carbohydrates, tannins, saponins, flavonoids, alkaloids, terpenoids, phenols and steroids

Total phenolic content

Total phenol content was analyzed using the Folin-Ciocalteu colorimetric method with some modifications (Chlopicka J, *et al.,* 2012). 0.3 ml aliquot of plant extract was mixed with Folin-Ciocalteu phenol reagent (2.25 ml). After 5 minutes 6% sodium carbonate (2.25 ml) was added and the mixture left at room temperature for 90 minutes. The absorbance of the mixture was measured using a spectrophotometer at 725 nm. A standard curve for gallic acid ranged from 20 to 80 µg/ml and the results were expressed in mg of gallic acid equivalents (GAE) per gram of extract.

Total flavonoid content

Total flavonoid content was measured using the aluminum chloride colorimetric method with some modifications (Chang Cc, *et al.,* 2002). A standard curve for quercetin was range from 20-80 µg/mL. 0.5 ml of Plant extract and standard were placed in separate test tubes and mixed with 10% aluminum chloride (0.1ml), 1M potassium acetate (0.1ml), 80% methanol (1.5ml) and distilled water (2.8 ml) was added and mixed. 0.5 mL of distilled water used as Blank. All tubes were incubated at room temperature for 30 minutes and absorbance read at 415 nm. Flavonoid concentrations were expressed in mg of quercetin equivalents (QE) per gram of extract. Each plant extract was prepared in triplicate.

Antioxidant Assay

Radical scavenging assay (DPPH method) :

The radical scavenging activity of three different samples was carried out DPPH method proposed by Harini et al. with minor modifications (Koksal E, *et al.,* 2011). Different concentrations of leaf extract were added to 5 ml of DPPH methanol solution (0.1 mmol/L), shaken vigorously, left at 27°C for 20 min, and absorbance was measured at 517 nm. . A pure DPPH solution was used as a control. Radical scavenging activity (RSA), expressed as percent inhibition of free radicals by the sample, was calculated using the following formula:

$\%RSA=[(Abs_{control} -Abs_{sample})/Abs_{control}]X100\%$

Where, $Abs_{control}$ is absorbance of control at 517 nm, Abs_{sample} is absorbance of sample at 517 nm.

Ferric reducing antioxidant potential (FRAP) assay :

The reducing power of plant extracts was measured using the Oyaizu method (Ivette Gonzalez Palma *et al.,* 2016). Different concentrations from 0.2 to 1 mg/ml were mixed with 2.5 ml phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50° C. for 20 minutes and 2.5 ml TCA (10%) was added. The mixture was centrifuged at 3,000 rpm/min for 10 minutes. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml), 0.5 ml of ferric chloride (0.1%) was added, and the absorbance of the test sample was measured at 700 nm.

Anti-inflammatory activity

Inhibition of protein denaturation method

Inhibition of protein denaturation was measured by the method of Mizushima *et al.,* 1968 with some changes. The reaction mixture contained various concentrations of the test extract and 1% BSA (in water). 1N HCl was used to adjust the pH of the reaction mixture. The sample was heated at 37°C for 20 minutes, he then heated it at 57°C for 20 minutes and then allowed to cool. The turbidity of the samples was measured at

660 nm. Experiments were performed in triplicate. Percent inhibition of protein denaturation was calculated as:

Percentage inhibition= (AC of control-AC of test sample)×100/AC)

where A_C and A_S are the absorbance (at 600 nm) of the control and sample, respectively.

Human red blood cell (HRBC) membrane stabilization test

Fresh human blood (10 ml) was drawn and centrifuged at 3000 rpm for 10 minutes before being thoroughly rinsed thrice with normal saline solution. According to Sadique J, et al. (1989) measured the blood volume and reconstituted it as a 10% v/v suspension with normal saline. The reaction mixture (2 ml) contained 1 ml of 10% red blood cell suspension and 1 ml of methanolic plant extract. Saline was used as the control instead of plant extract. Aspirin served as the reference medication (positive control). The samples were centrifuged at 2500 rpm for 5 minutes after being incubated at 56 °C for 30 minutes to measure the supernatant's absorbance at 560 nm. The experiment was carried out three times. The formula in Inhibition of Protein Denaturation Method was used to compute the percentage of membrane stabilisation activity, and the following formula was used to get the percentage of protection:

Percent of protection=100−AS/AC×100

where A_C and A_S are the absorbance (at 560 nm) of the control and sample, respectively.

MTT Assay:

The cytotoxicity effect of the sample was tested against HepG2 cell line by MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (T Mosmann 1983). The cells were seeded in 96 well microplates ($1x10^6$ cells/well) and incubate at 37oC for 48 h in 5% Co2 incubator and allowed to grow 70-80% confluence. Then the medium was replaced and the cells were treated with different concentration of samples and incubated for 24 h. The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (40X magnification) after 24 h an photographed. The cells were then washed with phosphate- buffer saline (PBS, pH-7.4) and 20 µL of (MTT) solution (5 mg/mL in PBS) was added to each well. The plated were then stand at 37oC in the dark for 2 h. The formazan crystals were dissolved in 100 µL DMSO and the absorbance was read spectrophotometrically at 570 nm. Percentage of cell viability was calculated using the formula,

Cell viability (%) = (Absorbance of sample/Absorbance of control) x 100

Cell control and sample control were included in each assay to compare the full cell viability assessments.

STATISTICAL ANALYSIS:

The data obtained were subjected to SPSS package. Mean and Standard Deviation was carried out.

RESULT AND DISCUSSSION

From the beginning of human history most plants have been used for medicinal purposes for various diseases and those plants are basis of modern medicine. Thus, frequently biologically active compound obtaining from natural sources are always being a great interest for scientist working on infectious diseases.

Due to its pharmacologic properties, Psidium guajava L. is used as a traditional medicine in subtropical regions all over the world (Deguchi Y And Miyazaki K , 2010). It is commonly known that guava is frequently to treat a variety of illnesses, such as treating diarrhea and lowering fever, dysentery, gastroenteritis, hypertension, diabetes, caries, pain relief and wounds (Anand V, *et al.*, 2016) . Commercially mangoes are grown more than 111 countries but well-known in China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria, and the Philippines where India ranks first in both production and plantation area (Kumar *et al.*, 2021).Mango leaves has been used to treat gastrointestinal tract infections, diarrhea, dysentery, mouth infection in children, typhoid, sore throat, scurvy and many human aliment. (Baishakhi Ghosh *et al.*, 2022).

These Plants plays an important roles to discover new beneficial therapeutic agents and have received significant focus because of their bioactive substances like antioxidants, antimicrobial . Hence an investigation was carried out to study the antioxidant, anti-inflammatory and anticancer activities of fruit medicinal plant, *Psidium guajava* (Guava) and *Mangifera indica* (Mango) leaves. The medicinal importance

of *these plants* was well appreciated and reported from an ethnobotanical perspective but the biological activities of these plants collected from many recent papers (CM Noorjahan and T. Saranya , 2018)

Phytochemical Analysis

Phytochemicals in plant play a role to discover a new drug for the treatment of the diseases and as base for the development of compounds, which act as a natural blue print for the development of the new drug. (Abdullahi Adamu, 2021) Phytochemical screening provides the information about the secondary metabolites which is responsible for therapeutic potential of plants. These include Tannins, Saponins, Flavonoids, Alkaloids, Proteins, Steroids, Quinones, Terpenoids, Cardio glycosides and Phenols. (Deepika and CM Noorjahan, 2016)**.** The preliminary phytochemical analysis of the individual leaf extract selected plant contain carbohydrate, flavonoid, tannin, phenol, steroid, terpenoids in individual plant extract of *Psidium guajava* (Guava) .In leaves of *Mangifera indica* revealed the presence of alkaloids, carbohydrates, phytosterols, tannins, fixed oils and fats, resins, phenols, flavonoids, proteins; and absence of glycosides and amino acids (Suchada Jongrungraung chok et., al 2023). Phytochemical analysis of guava and mango leaves with Ethanolic extract showed the presence of carbohydrates, Tannins, flavonoids, phenol, steroid, Glycosides and terpenoids Since there was no significant changes in saponins and aminoacid but in Distilled water extract showed reactant in all test results as positive. In Ethyl acetate extract showed the presence of alkaloids, flavonoid, phenol and terpenoids and no changes found in steroid, saponins tannin, carbohydrate, Glycosides and aminoacid indicates to be negative**.** Flavonoid and phenol compound are much stronger in GMDW and GMEE than GMEA as shown. (Table 1)

Table 1: Major Phytochemical Test in Guava with Mango Leaves Extract in Different Solvents:

Phytochemical test	GMEE	GMEA	GMDW
Alkaloids	++	+	+
Flavonoid	+++	+	+++
Phenol	+++	-	+++
Steroid	+	-	+
Saponins	-	-	++
Terpenoids	++	++	++
Tannin	+++	+	++
Carbohydrate	++	-	++
Glycosides	++	-	++
Amino Acid	-	-	+

GMEE, Guava and mango Ethanol Extract; GMEA, Guava and mango Ethyl Acetate Extract; GMDW, Guava and mango Distilled Water Extract

+++ Strongly positive; ++ Moderately positive;+ Weakly positive; - Negative

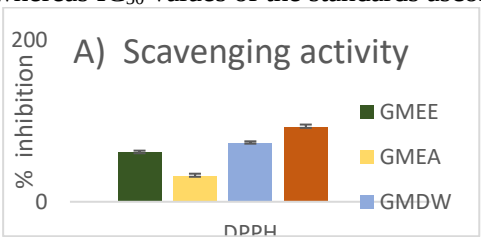
Phenolic compounds and flavonoids are highly found in guava leaves(Nighat Gull *et al.*, 2022). The result of quantitative analysis revealed that GEDW exhibited the maximum phenolic and flavonoid contents (42.55 and 76.31 mg/g, respectively), followed by GMEE (42.55 and 71.5 mg/g). The minimum phenolic and flavonoid contents were observed in the GMEA (Table 2). Many researchers reported that Flavonoids possess many useful properties, including antiinflammatory, estrogenic, enzyme inhibition,antimicrobial, antiallergic, antioxidant, vascularand cytotoxic antitumour activity.(Dipali O.Somkuwar and Vilas A Kamble, 2013)

Table 2: Total phenolic and flavonoid content in Guava- Mango Leaves Extract with Different Solvents:

Samples	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
GMEE	42.55 ± 2.72	71.5 ± 2.3
GMEA	19 ± 0.39	15 ± 1.28
GMDW	42.83 ± 1.57	76.31 ± 1.95

Antioxidant Activity:

Antioxidants are found naturally in numerous plant parts, such flowers, stems, barks, pods, leaves, fruits, roots, wood, seeds, and pollen. Vitamins, phenolic compounds, and carotenoids are common antioxidant composites.(Alsarhan A, *et al.,* 2014). Guava is highly rich in antioxidants which are helpful in decreasing the incidences of degenerative diseases such as brain dysfunction, inflammation, heart disease, cancer, arteriosclerosis and arthritis (Koo Mh Mohamed S, 2001) . Quercetin, quercetin-3-O-glucopyranoside and morin can be isolated from leaves contain richest anti-oxidant activity helps has free radical balancing activity. It is considered as most active and strong antioxidant in the leaves of guava (Nantitanon W, *et al.,* 2012). Polyphenols is the main biological property of almost all the *M. indica* indicates high antioxidant activity (Dreosti IE, 2000). The DPPH assay is commonly used to determine the antioxidant potential of plant extracts/compounds by measuring their ability to act as free radical scavengers. IC_{50} (the substrate concentration that causes 50% loss of DPPH) is used to interpret the assay results. Test plant extracts (25–100 µg/mL) inhibiting the scavenging activity and reducing power. IC_{50} values of scavenging DPPH radicals for GMDW, GMEE and GMEA extracts were 57.29±3.519 µg/mL, 61.3±2.935 µg/mL 124.76±5.671 µg/mL respectively; their scavenging ability was found to be lower than that of ascorbic acid (6 ± 0.4 µg/mL) (Fig. 1 A). The DPPH scavenging activity showed very low concentration in Plant extract with Distilled water. As per the FRAP assay, the presence of e- donating compounds results in the reduction of Fe3+(ferricyanide) into Fe2+(ferrous). The result showed in the fig 1 B was revealed that GMDW extract displayed strong reducing activity than GMEE and GMEA (IC_{50} 71.6±9.760µg/mL , 93.1±5.817µg/mL and 184.2±4.301µg/mL respectively) whereas IC_{50} values of the standards ascorbic acid 11.5 ± 0.63.



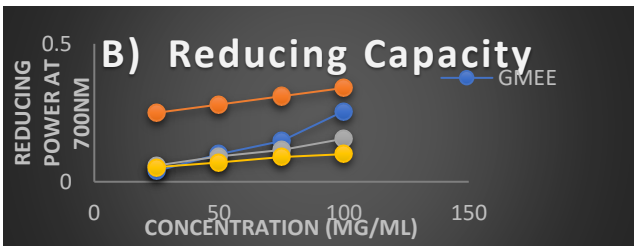


Fig 1 Antioxidant Activity of Guava- Mango with Ethanol (GMEE), Guava- Mango with Ethyl Acetate(GMEA), Guava- Mango with Distilled Water(GMDW) A) DPPH free Radical Scavenging activity B) FRAP Reducing Capacity.

Anti-Inflammatory Assay:

Phenol is an important compound which is present in guava and dependable for the anti-allergic and anti-inflammatory activity (Denny C, *et al.*, 2013). Therefore in mango leaves gives production of a large amount of proinflammatory cytokines (IL-1, 2 and 6 and TNF) increase the expression of enzymes such as COX-2 and iNOS which are associated with anti-inflammation (Mercurio F, *et al.*, 1997). From this study test plant extracts (25–100 µg/mL) inhibited albumin denaturation and hemolysis of HRBCs has shown (Table 3). Albumin denaturation inhibition at the highest concentration of 100 µg/mL was found in GMDW > GMEE > GMEA. The IC_{50} of GMDW was 78.11 µg/mL but in hemolysis of HRBCs exhibited the maximum inhibition in GMEE than GMDW and GMEA.(IC_{50} of GMEE was 44.65 µg/mL)

Table 3: IC 50 value of Albumin denaturation and membrane protection/stabilization potential of three extract of the selected plant species.

Samples	Albumin Denaturation	Membrane Protection
GMEE	41.165±4.3872	44.65±2.106356
GMEA	201.51±10.0635	176.48±9.7514
GMDW	31.508±2.406	46.071±6.079
Aspirin	9.2±1.2	11.8±1.3

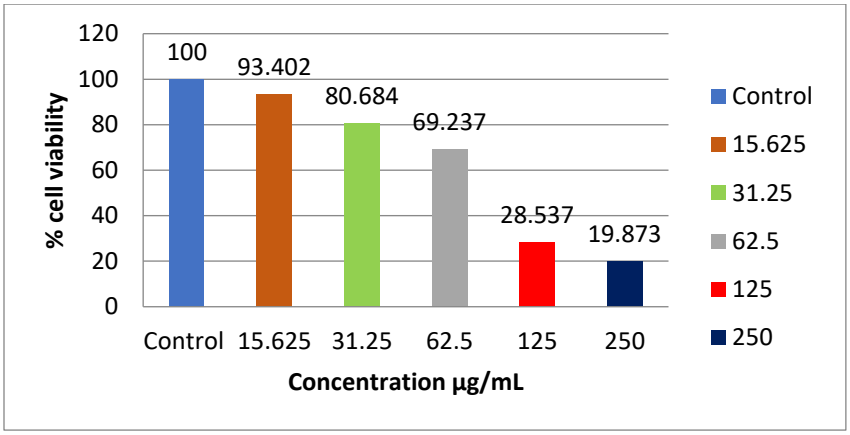


Fig. 1: Percentage viability of HepG2 cell lines after the exposure with the leaf extract of Guava-Mango with Distilled Water

Anti Cancer Activity:

Many research studies are investigated in guava as well as in mango leaves extract inhibition concentration approximately less than 200 µg/mL. Several cancer cell lines, including MCF-7 and MDA-MB-231 (breast cancer)[5, COLO320DM (colon cancer)[6, PC-3, DU 145, and LNCaP (prostate cancer), KB (nasopharyngeal cancer), and HeLa (cervical cancer), were inhibited by the leaves of P. guajava. (Bronwyn Lok , *et al.*, 2020). Guava extracts purified from leaf have many bio-active molecules such as beta-caryophyllene oxide, vitamins, tannins, phenolic compounds, flavonoids and triterpenoid acids for anti-cancer activities. (Hsiao-Chun Liu, *et al.*, 2020 and Sumra Naseer, *et al.*, 2018). Mango leaf extract and its active compound, mangiferin showed in vitro inhibitory potential on key enzymes involving glucose metabolism, that is, α-amylase and α-glucosidase against tested cancer cell lines.(Arunachala G, et.al, 2017 and Qian Zhang, *et al.*, 2019). Due to the tight relationship between tumour progression and inflammation and oxidative stress, a combination of antioxidants with anti-inflammatory characteristics can serve as an effective anti-cancer treatment. (Zahra Barmoudeh , *et al.*, 2022) From the above assay reveals that Guava-

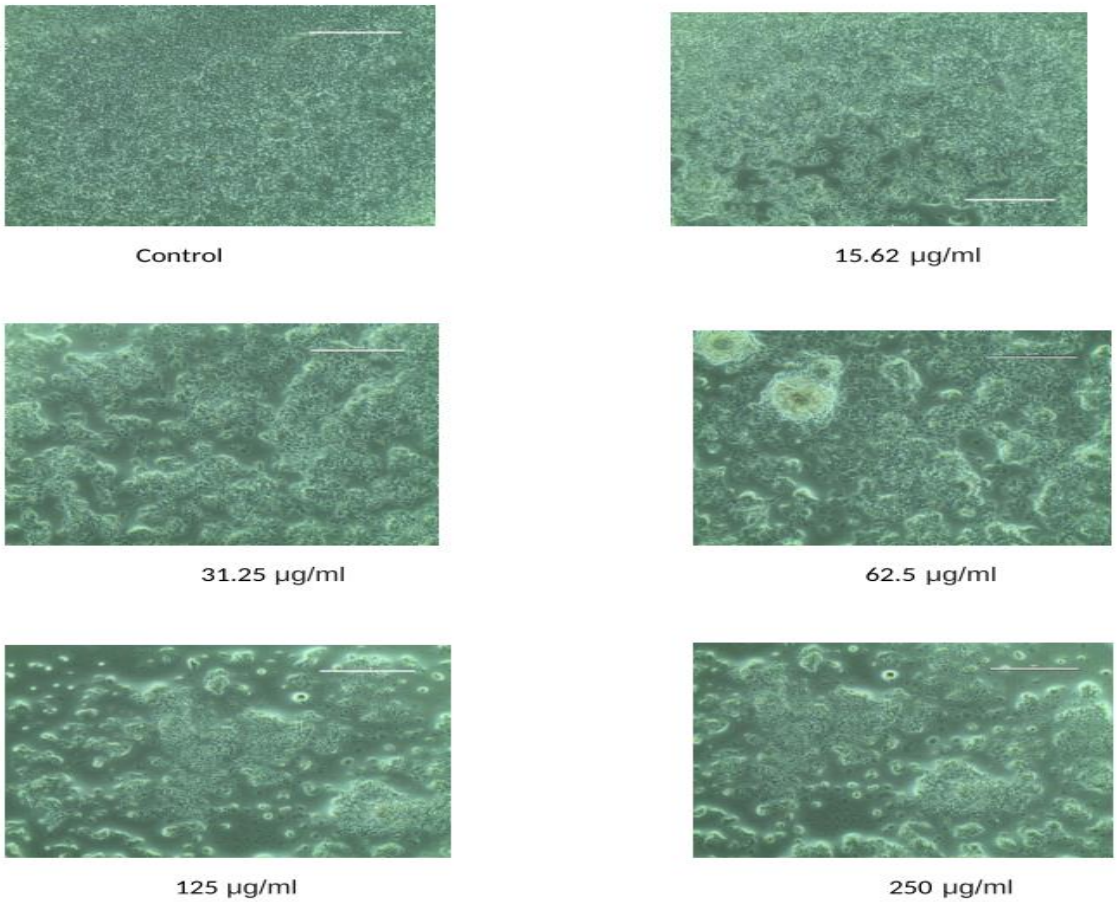


Fig 2: Cell Viability Test for Guava- Mango with Distilled Water against Hepatoma G2(Hep G2)

Table 5:Anticancer activity of Guava- Mango with Distilled Water leaves extract on Hepatoma G2(Hep G2)Cancer cell lines

Concentration Unit: µg		**Incubation: 24hrs**					
	BLANK	**CONTROL**	**15.625**	**31.25**	**62.5**	**125**	**250**
Sample	0.05	0.632	0.595	0.512	0.436	0.188	0.128
Mean		0.628	0.587	0.507	0.435	0.179	0.125
Standard Deviation		0.00495	0.010607	0.006364	0.000707	0.012021	0.004243
Standard Error		0.0035	0.0075	0.0045	0.0005	0.0085	0.003
Viability %		100	93.402	80.684	69.237	28.873	19.873

Mango with Distilled water extract had high potential activity in antioxidant. The present findings indicated that the Distilled water extract of Guava- Mango extract finalized to proceed for anticancer activity. The percentage of the cells were determined by calculating the optical density of the cells treated with the extracts against the blank containing only cells as a control at 540nm as shown in fig 2. The result of Guava-Mango

with Distilled water extract illustrated higher cytotoxicity in 250 µg concentrations activity against the liver cancer cell line HepG2 revealed that the cell viability increased in low concentration 15.625 µg/mL with 93.402% at high concentration 250 µg/mL with 19.873% the viability of cell decreases (IC50 value 64.395±11.67 µg/mL).The present study suggesting that the GMDW extract will be high potent therapeutic agent and for the development of novel drug for the treatment of liver cancers.

CONCLUSION

Based on the results obtained, it can been concluded that combination of guava- mango leaf extract remains with tremendous potential significant in antioxidant, anti-inflammatory and anticancer activity than the individual extract of *Psidium guajava* (Guava) and *Mangifera indica* (Mango) was reviewed with previous research papers. From this present study clearly demonstrated pharmacological effect of this plants particularly of GMDW combination against the human liver cancer cell line has high potent therapeutic agent. This study will prolong for further research investigation for cheap and new drug for the treatment of cancer cell types.

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