Analysis Of Antitumour And Antioxidant Activity Of A Formulation Of Trigonella Foenum Graecum, Aloe Barbadensis Miller, Apis Mellifera And Zingiber Officinale By Using Ags Cell Line

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Abstract:

Background:. Gastric carcinoma is the world's second leading cause of cancer-related deaths, with the epidemiology changing over the last few decades. The effect of increased hygiene standards, conscious nutrition, and Helicobacter pylori eradication, which constitute primary prevention, has resulted in a steady decline in gastric cancer incidence rates. Surgery, chemotherapy form the mainstay of treatment. Still many side effects continue to stop the progress of management. As a result, finding new agents to enhance the antioxidant and anticancer effects becomes critical in order to overcome these complications.. We improvised the same to a mixture of extracts and the efficacy of the same in the process of antitumour activity. As a result, the purpose of this study was to evaluate the phytochemical screening, antioxidant activity, and antitumour activity of a formulation of herbal extracts of Trigonella foenum graecum, Aloebarbadensis miller, and Apis mellifera and Zingiber officinale. **Methods:**. This combination of herbal extract was subjected to phytochemical screening and experiments were done using using AGS CELL LINE on MTT, LDH and Trypan blue assay**.** **Results:** Our results revealed that the combination had adequate phytoconstituents viz., alkaloids, flavonoids, glycosides, Tannins, Quinones and phenolic compounds. Herbal extract at concentration ranging from (2.5µg to 12.5 µg /ml was used to find its inhibitory effect on DPPH , (1mg to 5mg) on FRAP ((100µg to 500 µg) on ABTS to facilitate antioxidant effect. A significant antioxidant activity was noticed. It also shows good effect for anti hemolytic activity. 15.625 µg to 250 µg concentration was used to find the percentage of cytotoxicity by LDH and MTT assay, which showed favourable cytotoxic profile. **Conclusion:**. A mixed formulation of herbal extracts of Trigonella foenum graecum,

Aloebarbadensis miller, and Apis mellifera and Zingiber officinale had collaborative phytoconstituents and more than individual contents. This mixture had antioxidant and anti-cancer activity on AGS cell lines. We accept that the anti-tumour effect was not compared with individual contents. This is the first such report of herbal mixtures.

Keywords: cancer stomach , herbs, trigonella, apis, zingiber , AGS cell line

Introduction:

Gastric cancer is still one of the most common and lethal cancers in the world, particularly among older men. According to GLOBOCAN 2018, stomach cancer is the fifth most common neoplasm and the third most lethal cancer, with an estimated 783,000 deaths in 2018. Gastric cancer incidence and mortality vary greatly by region and are heavily influenced by diet and Helicobacter pylori infection. While advances in the prevention and treatment of Helicobacter pylori infection have reduced the overall incidence of gastric cancer. Innumerable chemotherapy options are available.[1] These side effects may confuse the original disease progression. Hence herbal extracts may come into play. *Trigonella Foenum-Graecum* otherwise known as fenugreek has been used for multiple illness including diabetes[2]. *Aloe barbadensis miller* originates from the Arabian Peninsula but it grows wild in all around the world. It will accelerate wound healing and it also contains antioxidant and antibacterial properties. Honey is an antioxidant and gastric softening agent. *Zingiber officinale* is widely used as a folk medicine and spice and it is a flowering plant. It has many powerful medicinal properties that can treat many forms of nausea, morning sickness and help in weight loss.[3,4] It will lower the blood sugars and help to treat osteoarthritis. We have tried a combination of herbal extracts to find out the anti-oxidant and anti-cancer activity of the combination.

Aims and objectives:

The present study is to investigate the phytochemical constituents, Antiulcer and Antioxidant activity of the formulation of *Trigonella Foenum Graecum, Aloe Barbadensis Miller, Apis Mellifera* and *Zingiber Officinale* using AGS cell line.

Materials And Methods:

Trigonella foenum graecum, Aloebarbadensis miller, Apismellifera and Zingiber officinale are purchased from Trichy, Tamil Nadu, India. AGS ulcer cell lines were purchased from The National Centre for Cell Science (NCCS), Pune. The collected sample Trigonella foenum

graecum is finely powdered, the Zingiber officinale, Aloebarbadensis miller was washed peeled and crushed with pestle and mortar and it was filtered through filter paper and the pure extract was taken. Preliminary phytochemical screening of various extracts and test sample was carried out as per the standard textual procedure. Then all the four samples were mixed thoroughly in the ratio of 4:3:2:1 and stored in an air tight container. The antioxidant activity was assessed by .2, 2-Diphenyl-picrylhydrazyl (DPPH) FRAP and ABTS assays as per the described and established methodology[5,6,] The antihemolytic assay was measured based on the erythrocyte hemolysis. The decrease in absorption was read at 410nm.

Cytotoxic activities of plant extract on AGS cells were studied by Trypan blue method[7]. The tumor cell line was cultured in culturing flask it was trypsined and transferred in centrifuge tube for centrifugation at 2500 rpm for 5 minutes. After centrifugation the cells are separated at the bottom and supernatant at the bottom was discarded. Then 2 ml of media is added and mixed well. Different concentrations of the plant extract were incubated at 37^0C for 3 hours. The cell number was determined using a hemocytometer and adjusted to 1 x 10 4 cells /0.5 ml. For the cytotoxicity assay, 0.5ml of cell suspension was transferred into each 6 well plate and incubated for 24 Hrs at CO2 incubator. Then added 0.5 ml different concentrations of the extracts (100 – 500 µg/ml) were added to each well and incubated for 3 Hrs. Then, in each well, 0.5 mL trypsin is added and incubated for 5 minutes. Then, in an isotonic solution, 20l of 0.2 percent Trypan blue dye is added. The medium without the plant extract worked well as a control. A hemocytometer was used to count the number of viable (unstained) and non-viable (stained) cells. Percentage of Growth Inhibition = Total cells counted – Total Viable Cells / Total cells counted x 100

The MTT (3-(4, 5-dimethylthazolk-2-yl)-2,5-diphenyl tetrazolium bromide) assay and the Lactate Dehydrogenase assays were also done on the extract of the mixture as per described protocols.

Results:

Phytochemical screening was performed on extracts of Trigonella foenum graecum, Aloebarbadensis miller, Apismellifera, and Zingiber officinale. Pure extracts of each constituent in the formulation were used for the phytochemical analysis. Table 1 displays the phytochemical properties of Trigonella foenum graecum, Aloebarbadensis miller, Apismellifera, and Zingiber officinale extracts, indicating the presence or absence of major

phytoconstituents. Alkaloids, flavonoids, terpenoids, glycosides, phenols, tannins, and saponins were found in pure extracts of Trigonella foenum graecum, Aloebarbadensis miller, Apismellifera, and Zingiber officinale.

Table 1 showing the phytochemical constituents

	Aloevera	Fenugreek	Honey	Ginger
Carbohydrate	+	+	+	+
Glycoside	+	+	+	+
Cardiac glycoside	+	+	+	+
AMINO ACIDTEST				
Ninhydrin	+	+	+	+
Xanthoprotein	+	+	+	+
Folin	+	+	+	+
ALKALOIDTEST				
Dragendroff	_	+	+	+
FLAVONOIDTEST				
Alkaline	+	+	+	+
NH_4 OH	+	+	+	+
PHENOLIC COMPOUND	+	+	+	+
TEST Tannin	-	+	-	-
Quinone	+	+	_	+
Terpenoid	+	+	+	+
saponin	+	+	_	+
Phenol	+	+	+	+
Tannin	-			
Phlobotannin	_	+	_	_
Anthracyanin	_	_	_	_

The DPPH assay was used to determine the scavenging activity of plant extracts against free radicals. The violet colour has been replaced with yellow. At 580nm, the decreased intensity is measured. The results showed that as the concentration of the test drug increased, so did

the DPPH (Diphenyl-2-picrylhydrazyl) scavenging. When the concentration of plant extract is increased, so is the percentage of inhibition. At 12.5 mic.g, the maximum absorption is 91.10 percent. When compared to the standard, the percentage of inhibition was nearly equal to the standard value obtained at 10g concentration. Ascorbic acid is used as the standard here, and it inhibits at 94.07 percent at 10 mic.g. The Ic50 value was noticed to be 5.970mic.g/ml.

Table 2 showing FRAP assay results.

CONCENTRATION OF PLANT EXTRACT mg	**% of inhibition**
1mg	9.61
2mg	30.85
3mg	37.99
4mg	45.45
5mg	51.58
0.5mg (std)	50.13

Considering the FRAP assay, the maximum percentage of inhibition is 51.58 is seen at 5mg concentration. When compared to standard the plant extract was almost tothe herbal extracts. The Ascorbic acid is used as the standard here and it shows 50.13 percentage of inhibition at 0.5mg concentration. The IC50 value was found to be 4.401mg/ml.

Table 3 showing results of DPPH assay !

Concentration of plant extract µg	**% of inhibition**
2.5 µg	**37.77**
5.0 µg	**56.29**
7.5 µg	**71.85**
10.0 µg	**82.55**
12.5 µg	**91.10**
10 µg (std)	**94.07**

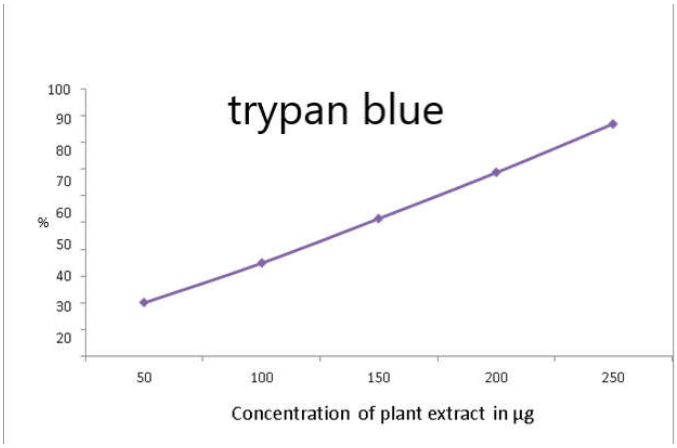
Regarding the ABTS assay , the maximum percentage of inhibition is 72.43 is seen at 500µg concentration. The Ascorbic acid is used as the standard here and it shows 76.27 percentage of inhibition at 400 µg concentration. The decreased O.D shows the maximum of absorption. The IC50 value was found to be 328.9474 µg/ml.the results are tabled below

Table 4: concentration and absorption of ABTS assay using herbal extract

CONCENTRATION OF PLANT EXTRACT µg	% OF INHIBITION
100 µg	12.17
200 µg	24.99
300 µg	55.76
400 µg	62.17
500 µg	72.43
400 µg	76.27

The Trypan blue test was performed to determine changes in the viability of each cell line after being exposed to the formulation of products. The assay was performed during 48 h of treatment and 24 h of recovery time after treatment with formulation of Trigonella foenum graecum, Aloebarbadensismiller, Apismellifera and Zingiber officinale. Here the greatest decrease in the viability in stomach cells was observed 48hrs of treatment in those of AGS cells. The viability of the cell lines decreased in a concentration dependent manner during the treatment with the formulation of Trigonella foenum graecum, Aloe barbadensismiller, Apismellifera and Zingiber officinale. The IC50 Value for herbal extract is 144.50 µg.

Fig 1 - The percentage of inhibition is depicted below in the picture.



Formulation of Trigonella foenum graecum, Aloebarbadensismiller, Apismellifera and Zingiber officinale showed notable cell death against the AGS cancer cell line. The percentage of cell viability was measured by MTT assay using different concentrations of the mixture such as15.625µg/ml, 31.25µg/ml, 62.5µg/ml,125µg/ml and 250µg/ml. The IC 50 value of herbal extract is found to be 184.50 µg.

The cytotoxic effect of formulation of Trigonella foenum graecum, Aloebarbadensis miller, and Apismellifera and Zingiber officinale in AGS Cell Line By Lactate Dehydrogenase Assay is given below

Table 5- Cytotoxicity effect of formulation of *Trigonella foenum graecum, Aloebarbadensis miller, and Apismellifera and Zingiber officinale* in AGS CELL LINE BY LACTATE DEHYDROGENASE ASSAY:

Concentration of Plant Extract (µg)	**% of Cytotoxicity**
15.625 µg	**16.10**
31.25 µg	**28.16**
62.5 µg	**39.98**
125 µg	**50.61**
250 µg	**61.66**

Discussion:

The plant extracts of different species like of *Trigonella foenum graecum, Aloebarbadensis miller*, and *Apis mellifera* and *Zingiber officinale* separately have been well described. We wanted to make a combination of extracts and decided to study the antioxidant and antitumour effects of the mixture on AGS cell lines. Alkaloids, flavonoids, terpenoids, glycosides, phenols, tannins, and saponins were found in pure individual extracts of *Trigonella foenum graecum*, *Aloebarbadensis miller, Apismellifera,* and *Zingiber officinale*. The pure extract of combined herbal mixtures of *Trigonella foenum graecum, Aloebarbadensis miller, Apismellifera* and *Zingiber officinale* showed the presence of alkaloids, flavonoids, terpenoids, glycosides, phenols, tannins and saponins. Wang et al [8]have described the presence of antioxidant chemicals in aloe plant extracts which is similar to our studies. Li Z[9] have established the saponins in *Zingiber officinale* but not in honey. Even though there are studies which establish benefits in individual extracts, we are the pioneers to

prove the synergistic benefit with mixtures. The MTT assay and the FRAP assay clearly demonstrates the antioxidant effect of the mixture. All the supplementary assays of antioxidant have proved the presence of antioxidants in our herbal extract. Formulation of *Trigonella foenum graecum*, *Aloebarbadensismiller, Apismellifera* and *Zingiber officinale* showed notable cell death against the AGS cancer cell line. Suchitra et al have described anti-cancer activities in AGS cell lines in many plant extracts[10]. Mohan verma et al [11]have used AGS cell lines to demonstrate anti cancer activities in many herbs. The most unique nature of our study is the activity of mixture of herbal extracts on AGS cell line. The most important limitation is that the study does not include comparative analyses of each of the components with the mixture.

Conclusion:

A mixed formulation of herbal extracts of Trigonella foenum graecum, Aloebarbadensis miller, and Apis mellifera and Zingiber officinale had collaborative phytoconstituents and this was more than a combination of individual contents. This mixture had antioxidant and anti-cancer activity on AGS cell lines. We accept that the anti-tumour effect was not compared with individual contents.

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