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DEVELOPMENT OF A POLYHERBAL FORMULATION FOR THE TREATMENT OF ULCERS, A NOVEL THERAPEUTIC APPROACH

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ABSTRACT

Herbal plants such as *Musa paradisiaca* leaves *Moringa oleifera* leaves *Raphanus sativus* fruit *Momordica cymapalaria* leaves and *Sesbania grandiflora* leaves which have long been known to be a very important source of pharmaceutical properties. The study examined the anti ulcers effect of polyherbal gel *in vitro*. Denaturation of proteins is the main cause of inflammation. The anti-inflammatory properties of polyherbal extract were studied at 500, 250, 100, 50 and 5 µg/mL using an albumin denaturation assay and proteinase inhibition assay. The free radical scavenging activity of polyherbal extract was evaluated by DPPH assay. The results showed that the anti-inflammatory activity of a gel based polyherbal extract shows a strong inhibition value at of IC_{50} 64.89µg/ml, and. This study provides an evidence that the polyherbal gel possesses anti-inflammatory and antioxidant activity. That can be used for the treatment of Rheumatoid arthritis.

KEYWORDS: Polyherbal formulation, anti-inflammatory, albumin denaturation and DPPH assay.

INTRODUCTION

Ulcer is a very conventional disease in the world. Statistics from all sources indicate 10% or more of adult population are affected within their life time. Peptic ulcer disease refers to the defect in the mucosal surface of the stomach or duodenum called peptic and duodenal ulcer. Peptic ulcer occurs more often in individuals from 20 to 60 years of age with males. The symptoms of peptic ulcer include abdominal pain, nausea, epigastric gnawing, heartburn, acid eructations, hemorrhage, anemia, weight loss, and vomiting (Vyawahare *et al*., 2009, Asali *et al*., 2018).

In general, gastric ulcers are the results of an imbalance in the equilibrium between mucosal damaging (acid and pepsin) and protecting (mucous, PGE2, and PGI2) mechanism of the gastric mucosa. Acid secretion is a physiologically important function of the stomach, as gastric acid (HCl) induces pepsinogen activator that helps to initiate digestive process (Sung *et al*., 2009). Several factors are responsible for gastric ulcers such as consuming several marketed nonsteroidal anti-inflammatory drugs (nimesulide and aceclofenac), steroidal drugs (prednisolone), tobacco smoking, psychological stress, alternative lifestyle, and alcohol abuse (Malfertheiner 2009).

The major causative factor is inflammation due to *Helicobacter pylori*, which damages the gastric mucosa via excessive acid secretion from parietal cells by increasing the parietal cell mass due to its inflammatory effects on parietal cells of gastric mucosa Velmishi *et al*., 2013. If supposed to understand that *H. pylori* was the causative agent for an ulcer, numerous investigation and animal models were established to come up with the mechanism via which *H. pylori* established itself in gastric environment and is responsible for the pathogenesis of gastric ulcer. Although more than half of the population of the world is affected by *H. pylori*, only 5–10% develops ulcer (Testerman *et al*., 2014, Salih 2009).

H. pylori is the main cause of stomach ulcers, was first identified by the two Australian scientists in 1982 .*H. pylori* is a gram negative *bacillus*, motile, microaerophilic, flagellated and spiral shaped bacteria. Type I strains of *H. pylori* possess a pathogenic activity, that encodes the effectors protein cytotoxin - associated gene A (cagA) (Harsh Mohan 2009). After Translocation into the host cell, cag A effects cell shape, increases cell motility, disturbs cell junctional activity and thus responsible for gastric carcinomas and gastric ulcers. *H. pylori* causes increases expression of cytokines such. TNF-α in gastritis. Further, IL -1 β is too over expressed in the *H. pylori* – induced gastritis. *H. pylori* - infected gastric mucosa

showed infiltration of polymorphonuclear leukocytes, lymphocytes, monocytes and plasma cells in the lamina propria, and intraepithelial severe neutrophil infiltration (Graham *et al*., 1999).

The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. As there are approximately 5,00,000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds. Many plant derived medicines used in traditional medicinal systems have been recorded in pharmacopeias as agents used to treat infections and a number of these have been recently investigated for their efficacy against different diseases. It has been found that over 36% Americans use natural therapy for the treatment of different disorders and the trend is on rise in the present days (Kwoh *et al*., 1996). Traditional Chinese medicine, Ayurvedic medicine and Homeopathic medicine are the major contributors to the natural products consumed by patient populations.

Plants are a very good source of herbal drugs. Many of the medicinal plants have been found to have extensive therapeutic application against various ailments. The phytochemical constituents present in the plants are accountable for their therapeutic properties. A single herb may even contain more than one phytochemical constituent, which works synergistically with each other in producing pharmacological action (Meena *et al*., 2009). The beneficial effects of various Indian herbs are reported by various researchers.

Traditional therapeutic herbal approach exploits the combination of several medicinal plants to accomplish additional healing effectiveness, generally known as polypharmacy or polyherbalism (Parasuraman *et al*., 2014). Scientific studies have revealed that the plants of varying effectiveness when combined may theoretically produce a better consequence, as compared to specific use of the plant and the combination effect is greater than their individual outcome. This occurrence of positive herb-herb interaction is known as synergism. Due to synergism, polyherbalism advises some aids not available in single herbal formulation. It is obvious that better therapeutic effect can be achieved with a single multi-constituent formulation. In addition, a lower dose of the herbal formulation may accomplish desirable pharmacological action, hence reducing the risk of deleterious side-effects.

MATERIALS AND METHODS

Collection of plant materials

The selected medicinal herbs such as *Musa paradisiacal, Moringa oleifera, Raphanus sativus, Momordica cymapalaria* and *Sesbania grandiflora* were collected from Virudhunagar District, Tamil Nadu. The dried herbs were grinded using mortar and pestle.

Musa paradisiaca leaves – 10 g, *Moringa oleifera* leaves – 10 g, *Raphanus sativus* fruit – 40 g, *Momordica cymapalaria* leaves – 40 g, *Sesbania grandiflora* leaves - 10 g.

Weigh the appropriate quantity of powdered leaves and finally reached 50 g of polyherbal leaves powder.

Formulation of polyherbal leaves

50 grams of polyherbal leaves were taken and subjected into cold extraction process using hydroalcoholic solvent mixture which comprises of distilled water and ethanol at 1:1 ratio. Then, the polyherbal extract was obtained after evaporation process at 78°C. The polyherbal formulation was stored in suitable container.

Plate – 1 Plant habitat

Fig: 1 *Musa paradisiaca.*

Fig: 2 *Moringa oleifera.*

Fig: 3 *Raphanus sativus.*

Fig: 4 *Sesbania grandiflora.*

Plate – 2 Plant materials

Fig: 5 *Musa paradisiacal* flower.

Fig: 6 *Moringa oleifera* leaf.

Fig: 7 *Raphanus sativus* rhizome.

Fig: 8 *Sesbania grandiflora* Leaf.

Fig: 9 *Momordica cymapalaria.*

DPPH Radical scavenging activity

Principle

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure 1, below, shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the

few stable and commercially available organic nitrogen radicals. The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.

Material Required

0.1mM DPPH solution, Ascorbic acid, Methanol.

0.1mM DPPH solution

Dissolve 39 mg of DPPH in 100 ml of methanol and store at -20^{o} C until needed.

Ascorbic acid (Standard)

1mg/ ml of Ascorbic acid.

Procedure

Prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of Poly herbal formulation sample at different concentration (500, 250, 50, 10, and 5 µg/mL). The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical can be calculated by using the following formula. DPPH scavenging effect (% inhibition) = [(absorbance of control- absorbance of reaction mixture)/absorbance of control] X 100

Acid Neutralizing Capacity (Anti-ulcer activity)

Material Required

Plant sample, Distilled water, hydrochloric acid, Sodium hydroxide pellets and Phenolphthalein indicator solution.

Procedure

Acid Neutralizing Capacity

The acid neutralizing capacity value for Hydroalcoholic solution of Poly herbal mixture using different concentration (500, 250, 100, 50 and 10 µg/ml) was compared with the standard antacid Aluminium hydroxide + Magnesium hydroxide (50 mg/ml). To the 5 ml quantity of this mixture, water was added to make up the total volume 70 ml and then mixed for one minute. There after 30 ml of 1.0 N HCl was added into standard and test preparation and stirred for 15 minutes, drops of phenophthalein solution was added and mixed. The excess HCl was immediately titrated with 0.5 N Sodium hydroxide solution drop wise until a pink color is attained.

Moles of acid neutralized

Moles of acid neutralized = (vol. of HCl ×Normality of HCl) - (vol. Of NaOH × Normality of NaOH)

Acid neutralizing capacity (ANC) = moles of HCl neutralized per gram of antacid / Grams of Antacid/Extract

Physical parameters

pH

The pH of the polyherbal formulation was measured by an electronic portable pH meter. The pH meter was calibrated with phosphate buffer of known pH. At a constant temperature, a pH change was observed in the electrical property of the solution. This change was read by the electrode and the accuracy was obtained in the middle pH ranges.

TDS (Total dissolved solids)

Total dissolved solid levels of polyherbal formulation samples were analyzed using digital TDS meter.

EC (Electrical conductivity)

The electrical conductive ability of polyherbal formulation was assessed using a digital conductivity meter.

Turbidity

Similarly, the turbidity of polyherbal formulation was tested using using turbidity meter.

Temperature

Similarly, the temperature of the polyherbal formulation was tested using *thermometer*.

Chemical parameters procedure

Calcium hardness

The presence of calcium (fifth most abundant) in water results from passage through or over deposits of limestone, dolomite, gypsum and such other calcium bearing rocks. Calcium contributes to the total hardness of water and is an important micro-nutrient in aquatic environment and is especially needed in large quantities by molluscs and vertebrates. It is measured by EDTA titrimetric method. Small concentration of calcium carbonate prevents corrosion of metal pipes by laying down a protective coating. But increased amount of calcium precipitates on heating to form harmful scales in boilers, pipes and utensils.

Principle

When EDTA (Ethylene-diamine tetra acetic acid) is added to the water containing calcium and magnesium, it combines first with calcium. Calcium can be determined directly with EDTA when pH is made sufficiently high such that the magnesium is largely precipitated as hydroxyl compound (by adding NaOH and iso-propyl alcohol). When murexide indicator is added to the solution containing calcium, all the calcium gets complexed by the EDTA at pH 12-13. The end point is indicated from a colour change from pink to purple.

Apparatus required: Burettes, pipette, conical flask, beakers and droppers.

Reagents

Sodium hydroxide (8%) 8g of sodium hydroxide is dissolved in 100ml of distilled water.

Murexide indicator (ammonium purpurate) 0.2 g of murexide is ground well with 100g of sodium chloride thoroughly.

Standard EDTA titrant, 0.01M: 3.723 g of EDTA (disodium salt) is dissolved in distilled water and made up to 100ml with the same.

Procedure

A known volume (5 ml) of the polyherbal formulation sample is pipetted into a clean conical flask, to which 1ml of sodium hydroxide and 1ml of iso-propyl alcohol is added. A pinch of murexide indicator is added to this mixture and titrated against EDTA until the pink color turns purple.

Calculation

$$\text{Calcium as Ca} = \frac{T \times 400.5 \times 1.05 (mg/L)}{\text{Sample taken, ml}}$$

Where, T= volume of titrant, ml

$$\text{Calciumhardness (mg/L as } CaCO_3) = \frac{T \times 1000 \times 1.05}{\text{Sample taken, ml}}$$

Total hardness

Hardness is predominantly caused by divalent cations such as calcium, magnesium, alkaline earth metal such as iron, manganese, strontium, etc. The total hardness is defined as the sum of calcium and magnesium concentrations, both expressed as $CaCO_3$ in mg/L. Carbonates and bicarbonates of calcium and magnesium cause temporary hardness. Sulphates and chlorides cause permanent hardness.

Hardness Chart *(for drinking water)*

Soft	0 – 60 mg/L
Medium	60 –120 mg/L
Hard	120 - 180 mg/L
Very Hard	> 180 mg/L

Principle

In alkaline conditions EDTA (Ethylene-diamine tetra acetic acid) and its sodium salts react with cations forming a soluble chelated complex when added to a solution. If a small amount of dye such as Eriochrome black-T is added to an aqueous solution containing calcium and magnesium ions at alkaline pH of 10.0 ± 0.1, it forms wine red colour. When EDTA is added as a titrant, all the calcium and magnesium ions in the solution get complexed resulting in a sharp colour change from wine red to blue, marking the end point of the titration. Hardness of water prevents lather formation with soap rendering the water unsuitable for bathing and washing. It forms scales in boilers, making it unsuitable for industrial usage. At higher pH>12.0, Mg^{++} ion precipitates with only Ca^{++} in solution. At this pH, murexide indicator forms a pink color with Ca^{++} ion. When EDTA is added Ca^{++} gets complexed resulting in a change from pink to purple indicating end point of the reaction.

Apparatus required Lab glassware-burette, pipette, conical flask, beakers etc.

Reagents

Buffer solution: 16.9 g of ammonium chloride and 1.25g of magnesium salt of EDTA is dissolved in 143ml of concentrated ammonium hydroxide and diluted to 250ml with distilled water.

Eriochrome black-T indicator: 0.5 g of Eriochrome black-T indicator is dissolved in 100g of triethanolamine.

Standard EDTA titrant: 0.01M or Ng AR grade EDTA is dissolved in distilled water and diluted to 1000ml and is standardised against standard calcium solution, 1ml = 1mg $CaCO_3$.

Standard Calcium Solution: 1.0g of AR grade $CaCO_3$ is weighed into a 250ml conical flask, to which 1+1 HCl is added till all $CaCO_3$ is dissolved completely. 200ml of distilled water is added and boiled to expel carbon-di-oxide, and diluted to 1000ml.
1ml = 1mg $CaCO_3$.

Procedure

Exactly 5 ml of the well-mixed polyherbal formulation sample is pipetted into a conical flask, to which 1ml of ammonium buffer and 2-3 drops of Eriochrome black -T indicator is added. The mixture is titrated against standard 0.01M EDTA until the wine red colour of the solution turns pale blue at the end point.

Calculation

$$\text{Total hardness} = \frac{(T)X(1000)mg/l}{V}$$

Where, T = Volume of titrant

V = Volume of sample

Magnesium hardness

Magnesium is a relatively abundant element in the earth's crust, ranking eighth in abundance among the elements. It is found in all natural waters and its source lies in rocks, generally present in lower concentration than calcium. It is also an important element contributing to hardness and a necessary constituent of chlorophyll. Its concentration greater than 125 mg/L can influence cathartic and diuretic actions.

Principle

Magnesium hardness can be calculated from the determined total hardness and calcium hardness.

Calculation

Magnesium = (T - C) x 0.243(as mg/L)

where, T = Total hardness mg\L (as $CaCO_3$)

C = Calcium hardness mg\L (as $CaCO_3$)

High concentration of magnesium proves to be diuretic and laxative, and reduces the utility of water for domestic use while a concentration above 500 mg/L imparts an unpleasant taste to water and renders it unfit for drinking. Chemical softening, reverse osmosis and electro dialysis or ion exchange reduces the magnesium hardness to acceptable levels.

Potassium

Potassium ranks seventh among the elements in order of abundance, behaves similar to sodium and remains low. Though found in small quantities (<20mg/L) it plays a vital role in the metabolism.

Principle

Trace amount of potassium can be determined by direct reading of flame photometer at a specific wavelength of 766.5nm by spraying the sample into the flame. The desired spectral lines are then isolated by the use of interference filters or suitable slit arrangements. The intensity of light is measured by the phototube.

Working principle of Flame photometer: The emission of characteristic radiations by alkali and alkaline earth metals and the correlation of the emission intensity with the concentration of the element form the basis of flame photometry. The principle of the flame photometer depends on the "Emission Spectroscopy" in which the electrons of the metals after absorbing energy get excited from ground state to higher energy level and return back to the ground state with emission of light. The sample under test is introduced into flame in solution by means of atomizer. The radiation from the flame enters a dispersing device and isolates it (radiation) from the flame to the desired region of the spectrum. The phototube measures the intensity of isolated radiation, which is proportional to the concentration of the element present in the sample.

Apparatus required: Flame photometer, lab glassware and Whattman filter paper.

Reagents

Deionised distilled water

Stock potassium solution: 1.907g of dried Potassium chloride, is dissolved in 1000ml of distilled water, to give 1ml = 1mg of potassium

Working Potassium solution**:** Working standards of suitable strengths are prepared from the stock solution.

Procedure

The filter of the flame photometer is set at 766.5nm (marked for Potassium, K) the flame is adjusted for blue colour. The scale is set to zero and maximum using the highest standard value. A standard curve of different concentration is prepared by feeding the standard solutions. The sample is filtered through the filter paper and fed into the flame photometer. The concentration is found from the standard curve or as direct reading.

RESULTS AND DISCUSSION

The obtained yield of the hydroalcoholic polyherbal extract was found to be 4.61 g.

Today a large section of world's population relies on traditional remedies to treat plethora of diseases due to their low cost and less side effects. Peptic ulcer disease is a serious gastrointestinal disorder and is common in India. It has multifactorial causes in its pathophysiology including free radical generation and inflammation and hence requires a well-targeted therapeutic strategy. It is suggested that compounds containing antiulcer, antioxidant activity can prove effective in peptic ulcer diseases Czinner *et al*., 2001. Some of the phytoconstituents now possess antiulcer activity for eg flavonoids, saponins, tannins, and terpenoids.

DPPH Radical scavenging activity

OD Value at 517 nm

Control Mean OD value: 1.471

Table 1: DPPH Radical scavenging activity.

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	1.493	1.522	1.398
2.	500 µg/ml	0.278	0.206	0.190
3.	250 µg/ml	0.130	0.343	0.253
4.	50 µg/ml	0.337	0.300	0.267
5.	10 µg/ml	0.300	0.301	0.344
6.	5 µg/ml	0.328	0.326	0.375
7.	Ascorbic acid	0.08	0.11	0.12

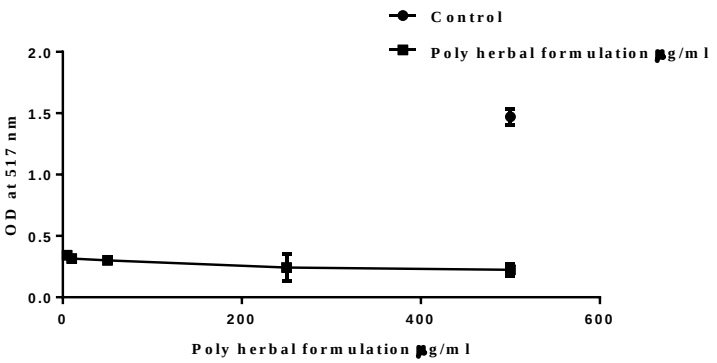


Fig. 10: Polyherbal formulation.

Table 2: Percentage of inhibition.

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Control	100	100	100	100
2.	500 µg/ml	81.23	85.99	87.08	84.76
3.	250 µg/ml	91.16	76.68	82.8	83.54
4.	50 µg/ml	77.09	79.6	81.84	79.51
5.	10 µg/ml	79.6	79.53	76.61	78.58
6.	5 µg/ml	77.7	77.83	74.5	76.67
7.	Ascorbic acid	94.56	91.84	92.52	92.97

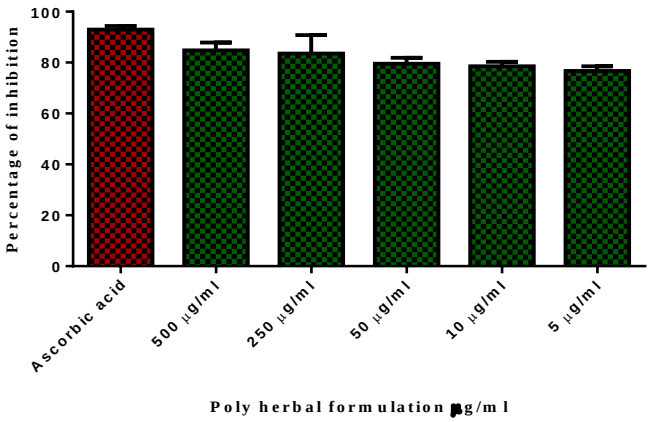


Fig. 11: Polyherbal formulation µg/ml.

Table 3: IC_{50} Value of tested sample: 64.89µg/ml.

log(inhibitor) vs. normalized response --Variable slope	
Best-fit values	
LogIC50	1.812
HillSlope	-1.213
IC50	64.89
Std. Error	
LogIC50	0.2916
HillSlope	0.7999
95% Confidence Intervals	
LogIC50	1.182 to 2.442
HillSlope	-2.941 to 0.5148
IC50	15.22 to 276.7
Goodness of Fit	
Degrees of Freedom	13
R square	0.4616
Absolute Sum of Squares	23804
Sy.x	42.79
Number of points	
Analyzed	15

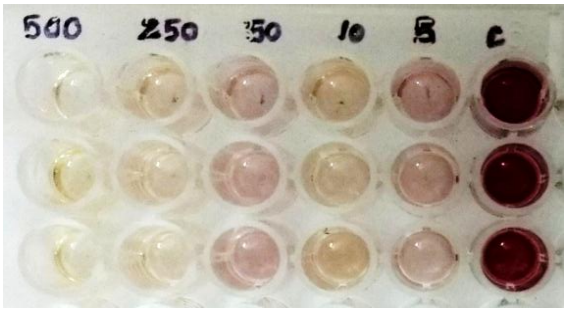


Fig. 12: DPPH Radical scavenging activity.

Antioxidant activity of the polyherbal extract was perfomed by DPPH radical scavenging effect. The results shows that polyherbal extract exhibits concentration dependent antioxidant activity. The maximum inhibition of antioxidant activity was found to be 84.76% and it was observed at 500 µg/ml. The IC50 value for the polyherbal formulation was found to be 64.89 µg/ml. Taamalli *et al*., 2019 have been reported that the plant natural products possess antioxidant activity. Among the phytochemicals present, phenolic compounds, with flavonoids being one of the major phenolics, play an important role in contributing to this activity by acting as an electron donor, hydrogen donor and by chelating metal ions (Zeb 2020). It has been widely known that plant natural products possess antioxidant activity. Among the phytochemicals present, phenolic compounds, with flavonoids being one of the major phenolics, play an important role in contributing to this activity by acting as an electron

donor, hydrogen donor and by chelating metal ions (Khatun *et al*., 2021). scavenge free radicals. Over the last few decades, numerous methods have been advanced to evaluate the antioxidant potential of various samples. However, performing a single assay is usually insufficient to fully understand the full antioxidant potential of samples due to differences in the reaction mechanisms, radicals produced, and assay parameters used. Hence, Schlesier et al. strongly advise that at least two different methods should be used (Schlesier *et al*., 2009). Single Herbal versus Polyherbal Formulation Ayurvedic Medication Formulation is Based on Two Principles using more than one drug (PHF) and using more than one drug as a single drug. This important traditional therapeutic herbal approach, also known as polypharmacy or polyherbalism, makes use of the interaction between many therapeutic plants to increase therapeutic efficacy. Historically, the Ayurvedic literature "Sarangdhar Samhita" dated centuries ago in 1300 A. D. has highlighted the concept of polyherbalism in this ancient medicinal system Srivastava *et al*., 2012. In the traditional system of Indian medicine, plant formulations and combined extracts of plants are chosen rather than individual ones. It is known that Ayurvedic herbals are prepared in a number of dosage forms, in which mostly all of them are PHF Parasuraman *et al*., 2014 and Parasuraman *et al*., 2010.

Siddeeg *et al*., 2021 reported that the equation of the standard curve was y = 0.0109 x – 0.0087 (r2 = 0.9991). Consistent with ABTS and DPPH assays, among the extracts and polyherbal tested, the FRAP assay revealed that *C. longa* (12.92 ± 1.03 µM FeSO4/ µg) exhibited the highest antioxidant activity, followed by *P. nigrum* (6.79 ± 0.95 µM FeSO4/ µg) or *Z. officinale* (6.36 ± 0.29 µM FeSO4/ µg) and TC-16 (12.92 ± 1.03 µM FeSO4/ µg). These three assays demonstrated that no significant improvement in the antioxidant activity was yielded by the mixture of herbs in TC-16 as compared tothe individual herb *C. longa, Z. officinale* and *P. nigrum*.

Table 4: Acid Neutralizing Capacity (Anti-ulcer activity.

S.NO	Name of the sample concentration	Reading a burette	Moles of acid neutralized	Acid neutralizing capacity (ANC) / antacid (g)
1.	500 µg/ml	2.7	1.65	37
2.	250 µg/ml	2.5	1.75	37
3.	100 µg/ml	2.4	1.8	36
4.	50 µg/ml	2.3	1.85	35
5.	10 µg/ml	2.3	1.85	33
6.	Control	2.6	1.7	34

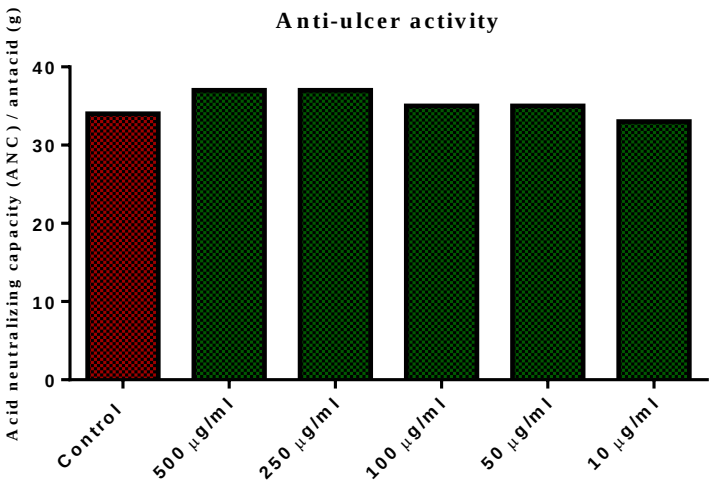
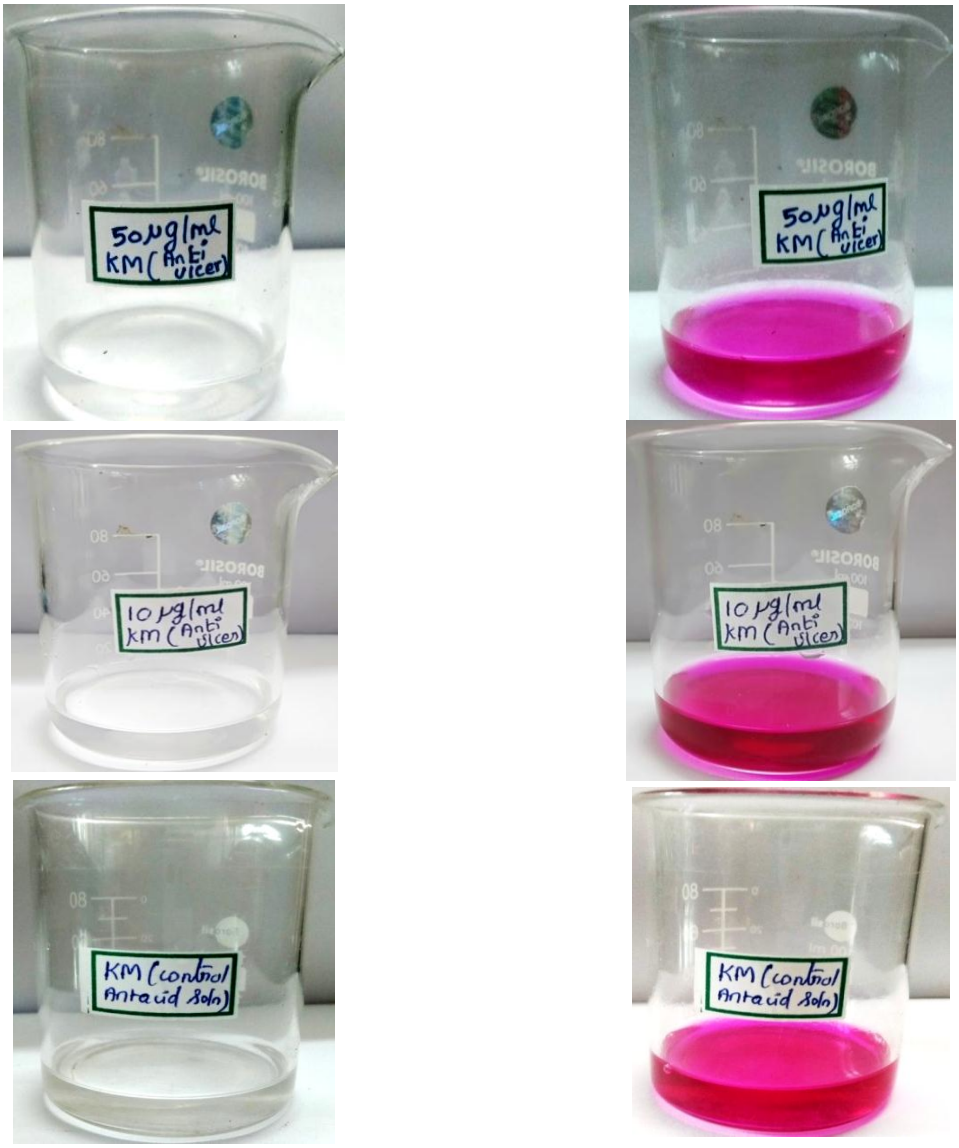


Fig. 13: Anti-ulcer activity.

Table 7: Anti-ulcer activity present in the plant sample (polyherbal formulation).



Acid neutralizing capacity analysis helps to determine the ani-ulcer activity for the polyherbal formulation. In ulceration condition, the gastric acid secretion will be increased. For the treatment of ulcer, the gastric acid (HCl) need to be neutralize by some chemical agent. In order to fulfill the strategy of treatment, acid neutralizing capacity was analysed to determine how much quantity of HCl get neutralized by the different concentrations of polyherbal formuation.

The results show that the maximum acid neutralizing capacity (37) observed at 500 µg/ml of polyherbal formulation. Also, this volumetric analysis results indicates that polyherbal formulation exhibits concentration dependent acid neutralizing capacity.

Table 5: Physical parameters.

S. No.	Physical parameters	Polyherbal formulation
1.	pH	6.3
2.	Electrical conductivity	0.432 µs/cm
3.	Total dissolved solids (TDS)	0.206 ppm
4.	Temperature	27.8^{o}C
5.	Turbidity	0.00

Chemical Parameters

Calculation

$$\text{Calcium} = \frac{T \times 400.5 \times 1.05}{\text{Sample taken}}$$

$$\text{Calcium} = \frac{0 \times 400.5 \times 1.05}{5}$$

Calcium (C) = 0

$$\text{Total hardness} = \frac{T \times 1000}{\text{Sample taken}}$$

$$\text{Total hardness} = \frac{0 \times 1000}{5}$$

Total hardness (T) = 0

Magnesium = (T – C) x 0.243

Magnesium = (0 – 0) x 0.243

Magnesium = 0

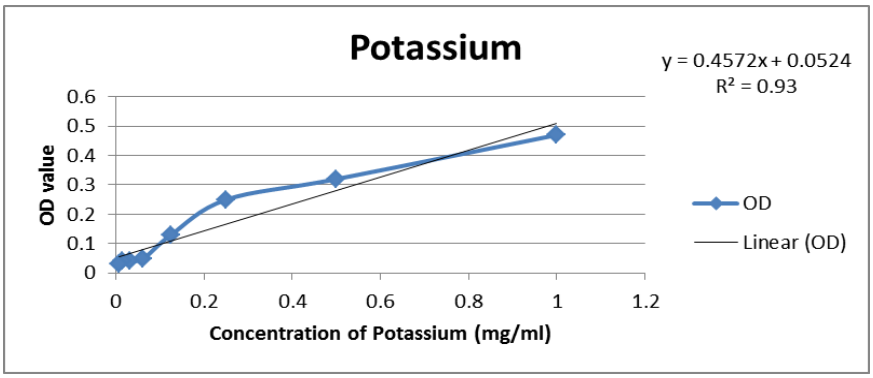


Fig. 14: Concentration of potassium.

y = mx + c

y = 0.457x + 0.052

OD value of the polyherbal formulation, y = 0.45

Potassium level present in the sample, x = (0.45-0.052) / 0.457= 0.87 mg/ml

The physicochemical properties for the prepared polyherbal formulation was analyzed with different parameters such as pH, total dissolved solids, electrical conductivity, temperature, turidity, calcium hardness, magnesium hardness, total hardness and concentration of potassium.

The clear polyherbal extract is in slightly acidic condition. The calcium, magnesium and total hardness for the test sample has resulted as zero and the concentration of potassium present in the formulated polyherbal extract was found to be 0.87 mg/ml.

CONCLUSION

Human health can be enhanced through certain traditional medicines. This includes selected herbs such as the leaves of *Musa paradisiaca*, *Moringa oleifera*, and *Momordica cymbalaria*, as well as the fruit of *Raphanus sativus* and the leaves of *Sesbania grandiflora*. These herbs can be processed using a cold extraction method. The antioxidant activity of the polyherbal formulation can be measured using the DPPH radical scavenging assay. Additionally, the acid-neutralizing capacity of the polyherbal extract was analyzed to determine its anti-ulcer activity. The results of both assays indicate that the formulated polyherbal extract exhibits concentration-dependent antioxidant and anti-ulcer properties. To further validate the pharmacological effects of this polyherbal formulation, preclinical (animal studies and alternatives) and clinical studies will be conducted to enhance patient health.

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