PREPARATION OF POLYHERBAL FORMULATION FOR COMMON COLD AND TOXICITY ANALYSIS IN ZEBRA FISH MODEL

Roja B[1], V.Mahesh Priya[2], Angel Jemima E[3], Muthusamy C[4]

Department of Biotechnology, DhanalakshmiSrinivasan College of Arts And Science For Women (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli (Nationally Re-Accredited with 'A' Grade by NAAC),Perambalur – 621212

ABSTRACT

Polypharmacy often referred as Polyherbalism is the combination of several medicinal herbs to achieve therapeutic effectiveness. Polyherbalism tends to provide larger benefits in a synergistic interaction. This investigation aims to study the phytochemical characterization of Vapcozam polyhedral formulation extract, and their anti-inflammatory and anti-oxidant activity in in-vitro condition followed by toxicity analysis using the zebra fish model. Thepolyherbal formulation was prepared from tendifferent plant materials using Soxhlet apparatus. The phytochemical characterization of Vapcozam polyhedral formulation was carried out and their anti-inflammatory activity was attained by the albumin denaturation assay.The anti-oxidant activity of polyhedral formulation was studied using the hydrogen peroxide assay.A dose response curve was plotted to determine the IC50 values using graph pad prism software. The traditional medicinal poly herbal plants formulation gave promising results for the treatment of cough and common cold. Phytochemical characterization results confirmed the presence of tannins, flavanoids, carbohydrates, and glycosides obtained from the Vapcozam polyhedral formulation. A maximum anti-inflammatory activity was obtained at 46.79µg/ml. The antioxidant results showed 117 µg/ml of IC50 value using hydrogen peroxide assay. Toxicity analysis using zebra fish model highlighted the efficacy of the drug usage for the treatment of common cold. Taken together, these results showed that the Vapcozam polyhedral formulation could be a effective treatment method for common cold.

Keywords: Vapcozam, cough syrup, Soxhlet, Phytochemical, Polyherbalism

1. INTRODUCTION

A cold is an infection of the upper respiratory system affects the noseand throat. A cold virus gets inside our body and makes us sick. The rhinovirus is the most common cold virus, but more than 200 viruses can cause colds.The common cold spread either by direct contact with infected secretions from contaminated surfaces or by inhaling the airborne virus after individuals sneezes or coughs. Person-to person transmission often occurs when an individual who has a cold blows or touches their nose and further passes on

to other individuals.A cough can be the result of respiratory tract infection such as the common cold, acute bronchitis, pneumonia, pertussis, or tuberculosis. This typically is a dry, non –productive cough that produces no phlegm. Symptoms may include a tightness in the chest, and a tickle in the throat.A post-Viral cough is a lingering cough that follows a viral respiratory tract infection, such as a common cold or flu and lasting up to eight weeks patients usually experience repeated ofpost viral cough.It is a clinically recognized condition represented within the European medical. An Available treatment method such asa humidifier or cool mist vaporizer can decrease cold symptoms, such as a sore throat, cough, and congestion. Theself-treatmentmethods includes decongestant sprays usage to unblock the nose and ease breathing, moreover cough drops could be used to soothe the throat. Some drugs, such as riboflavin and ferrous sulphate, are more efficiently absorbed in particular region of the gastrointestinal tract and therefore controlled-release tablets are not very useful, because they release the drug throughout the intestinal tract.

The disadvantages of the available treatment method includes Accidental poisoning with poor formulated controlled-release where the entire drug being released at once where cost of controlled release tablets is more per unit dose than conventional dosage forms. Dosage forms dose present special treatment. During the course of the reaction, water is liberated from the bicarbonate, which autocatalysis the reaction. Need adequate protection of effervescent tablets in the hands of the consumer where the moisture to which tablets are exposed after opening the container result in a rapid loss of product quality.

Health benefits of herbs plants includes strengthening the immune system, help reduce blood sugar level& cholesterol. It also provide relief from toothache& bad breath. They also help to relief from cold and cough and flu by maintaining healthy skin& hair. Reducing of cancer and Alzheimers disease is also achieved.

The Alternative medical treatment encompasses of Ayurvedic and Sidda system. Medicines Alternative medicines are being used by about 60 percent of the world's population. These medicines are not only used by the rural masses for their primary health care in developing countries but are also used in developed countries where modern medicines dominate . The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments. The

alternative medicines in the traditional systems are derived from herbs, minerals, and organic matter, while for the preparation of herbal drugs only medicinal plants are used.

Traditional Indian medicine (or ayurveda) is becoming increasingly popular in Europe, with many chronic conditions responding to it well. While conventional medicine dominates many fields in this market, it does not always outperform traditional ayurvedic approaches.

Conventional medicine frequently relies on lifelong medication, on which patients come to depend. Many medications have side-effects, and withdrawal symptoms that, if the medications are later discontinued, can become problematic. In such circumstances, ayurveda has much to offer. Patients generally respond well to ayurvedic treatments, experiencing a reduction, and sometimes even a cessation, of their symptoms. Most patients begin to take conventional medications as soon as their diagnoses are made, so ayurvedic treatments are usually undergone alongside and/or after conventional medical approaches. Patients therefore tend to experience ayurveda once their conditions have progressed. Despite this, much can be done to minimize conditions' symptoms and control their progress. Ayurveda can help improve patients' symptoms by reducing their cortisone and analgesic usage, thereby enhancing their quality of life.(H. P. T. Ammon and M. A. Wahl et al., 1991). The aim of the present study was to prepare polyhedral formulation and toxicity analysis using zebra fish model.

2.COMMON COLD AND ITS CAUSES

The common cold, also known simply as a cold, is a viral infectious disease of the upper respiratory tract that primarily affects the nose. (Arrolet al., march 2011) The throat, sinuses, and larynx may also be affected. Cold and cough medications are among the top 20 substances leading to death in children younger than five years. (Bronstein AC ,et.al 2012) In 2008, the U.S. Food and Drug Administration recommended that over-the-counter cough and cold medications be avoided in children younger than two years Inhaled Corticosteroids. Some children with viral cold symptoms also develop wheezing. Arroll B, Kenealy 2005) Sedating and nonsedating antihistamines are ineffective for cough and other cold symptoms.(Smith SM, et.al 2008)

Pathology

The most commonly implicated virus is a rhinovirus (30–80%), a type of picornavirus with 99 known serotypes.[Palmenberg AC et.al 2009,] Other commonly implicated viruses include human coronavirus(≈ 15%),[Pelczar 2010], Russell La Fayette cecil; Lee Goldman ; Andrew I. Schafer2012) influenza viruses (10–15%),[Michael Rajinik; Robert W Tolan 2013]adenoviruses (5%), humanrespirator ysyncytialvirus (orthopneumovirus), enter oviruses other than rhinoviruses, human parainfluenza viruses, and human metapneumovirus. Frequently more than one virus is present. In total over 200 viral types are associated with colds

Spread

Children Cold and cough medications are among the top 20 substances leading to death in childrenyounger than five years. (Bronstein AC ,et.al 2012) In 2008, the U.S. Food and Drug Administration recommended that over-the-counter cough and cold medications be avoided in children younger than two years Inhaled Corticosteroids. Some children with viral cold symptoms also develop wheezing. Although low-dose corticosteroids are ineffective in these children, one review of high-dose inhaled corticosteroids found a trend toward decreased frequency of wheezing episodes that require oral corticosteroids, the duration of episodes, and the number of physician visits.

Post infection

Post-infectious chronic cough (Ryan NM, et.al 2012). Cough considered post infectious when a patient complains of cough that lasts greater than 3 weeks but less than 8 weeks after an acute upper respiratory tract infection and chest x-ray is nor- mal (Irwin RSI, et.al 2006, and Huliraj N. 2014). The frequency of postinfectious cough has been reported between 11% up to 50% during outbreaks of Mycoplasma pneumoniae and Bordetella pertussis infections (Braman SS2006, and Kwon NHI, et.al 2006). H1N1 influenza infection is also is a risk factor for persistent cough that in one study has been reported in 43% of pa- tients (Ryan NM et.al 2012.

Available treatment method of cough and cold

A cold is a viral illness, antibiotics often are inappropriately prescribed to patients, even when bacterial complications (e.g., pneumonia, bacterial sinusitis) are not present. Studies of antibiotics for the treatment of the common cold focus on cure rate, symptom persis- tence, prevention of secondary

bacterial complications, and adverse effects. Systematic reviews have shownthat antibiotics have no role in the treatment of the common cold.(Kenealy T 2005)This is because antibiotics are ineffective at reducing symptom duration or severity and because of the risk of adverse gastrointestinal effects, cost of treatment, and increased resistance of bacteria to antibiotics(Fahey T. 1998).

Zebra fish, the topical fish model

The zebra fish (Daniorerio) is a fresh water fish belong to the minnow family native to south Asia. Its popular aquarium fish frequently sold under the trade name zebra Danio often it's called as a 'TROPICAL FISH'.(HankemeierT, Spaink HP, Van der Graaf PH et al., 2017). "Systems pharmacology of hepatic metabolism in zebrafishlarvae"Zebrafish (Daniorerio) is an animal originating from Myanmar, Srilanka, and India.

Homology between human and zebra fish

Zebrafish is an important model system for analysis of vertebrate development (Kimmel 1989; Drieveret al.1996) and an emerging model system for human disease (Zon 1999). Understanding the relationship between the zebrafish and human genomes will help identify roles for human genes from zebra fish mutations, and help identify zebra fish models for genes identified by human disease (Brownlieet al. 1998)..Zebra fish genome found strikingly similar to humans.

Medicinal Research

Immune system

In research into acute inflammation, a major underpinning process in many of the disease, This approach allows detailed study of the genetic controls of inflammation and the possibility of identifying potential of new drugs. The zebra fish has been extensively used as a model organism to study vertebrate innate immunity.

Infectious disease

The immune system is relatively conserved between zebra fish and human, many human infection disease can be modeled in zebra fish .the transparent early life stage are well suited for in vivo imaging and genetic dissection of host-pathogen interactions. Zebra fish model for wide range of bacterial viral and parasitic pathogens have already its established

2.3.3 Scope of Indian traditional medicinal system

Indian is known for its traditional medicinal system Ayurveda, siddha, and unani. Medicinal systems are found

mentioned even in the ancient Vedas and other scriptures. The Ayurvedaconcept appeared and developed between 2500 and 500 BC in India. It's an interactive system that is used friendly and educational. Ayurveda is not a nutritional system for those seeking an escape or excuse to further abuse their body or mind. It's a system for empowerment, a system of freedom, and long life.Verma V. Ayurveda: A way of life. York beach:Weiser Books; 1995.

Virus

Cold and cough caused many types of virus can cause a common cold; rhinoviruses are the most common culprit. A cold virus enters your body through your mouth, eyes or nose. The virus can spread through droplets in the air when someone who is sick coughs, sneezes or talks.

Bacteria

The common cold can be caused by more than 200 different viruses. Around 50 percent of colds are caused by rhinoviruses. Bacteria are one celled organisms that multiply & are linked to ear, throat, & sinus infections, as well as bronchitis, pneumonia, & whooping cough.

Allergies

The common symptoms of a cold, flu & allergies are stuff or a runny nose, sneezing, a sore throat, a cough, a headache, or even fatigue. Common allergy triggers are pollen, dust, mold, pet dander.

Ayurveda

Ayurveda is system of healing based on homeopathy and naturopathy, with an extensive use of herbs Ayurveda is a system of traditional medicine native to the Indian subcontinent originated>5000yrs ago and practiced in the other parts of the world as a form of alternative medicine. In Sanskrit, the world Ayurveda consists of the word ayus, meaning life.India is the largest producer of medicinal plants.

The Ayurveda combines the maintaining good health.Ayurveda aids in medical system that has been the traditional system of health care in India for more than 5000 years, and is one of the world's oldest medical systems. The process of detoxification without harming its medicinal properties (gunas) is referred to as the process of 'Shodhana' or 'ShodhanaPrakriya' in Ayurveda.The concept of shodhana treatment.

Siddha

Traditionally, it's taught that the siddhars laid the foundation for this system of medication. Siddhars were spiritual adepts who possessed the astasiddis, or the eight supernatural powers. Agastya is considered the first siddha and the guru of all siddhars.The siddha science is a traditional treatment system generated from Dravidian culture. The siddha flourished in the period of Indus valley civilization. Palm leaf manuscripts say that the siddha system was first described by lord Shiva to his wife Parvathi. They are many types of herbal plats are used to cold and cough and flu and other disease to treated in to use for the traditional medicinal plants

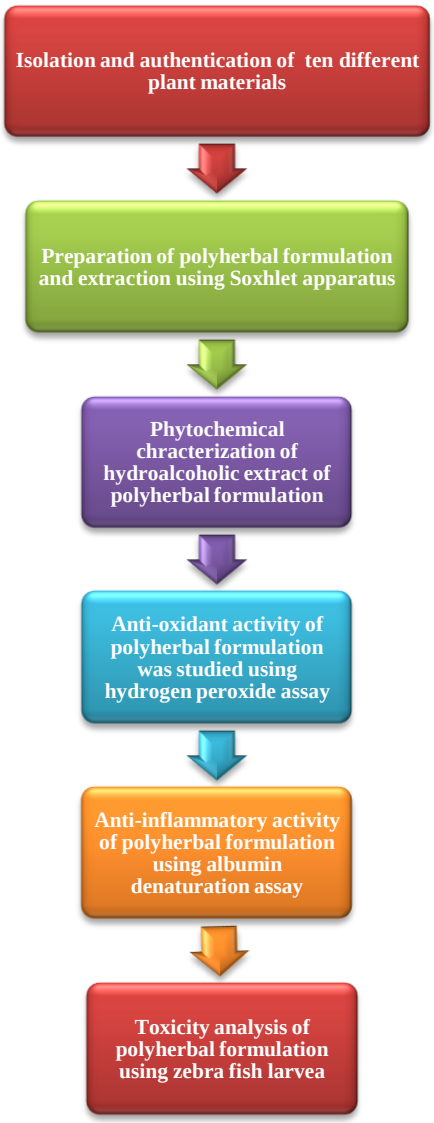
Plectranthusamboinicus

TheScientific name of Plectranthusamboinicus is a semi-succulent perennial plant in the family Lamiaceae belonging to the Plectranthus genus. The origin is native to Africa, and India where it grows in woodland, on rocky slopes and loamy or sandy flats at low elevations.The leaves are taken internally in the treatment of a range of digestive problems such as dyspepsia, indigestion, and diarrhea. The plant has cytotoxic and anti-tumour promoting activity and can be used in the treatment of cancer. Thus, Traditional therapeutics based on herbal medicinal principles are time tested and widely accepted across various cultural and socioeconomic strata.

LIST OF HERBALS MEDICINAL PLANTS

- Black pepper (Piper nigrum)
- Garlic (Allium sativum)
- Ginger (Zingiber official)
- Nochi (Vitexnegundo)
- Vetrilai (Piper betel)
- Turmeric (Curcuma longa)
- Pudina(Menthe spicata)
- Tulsi (Acimumtenuiflorum
- Musumusukai (Aukiamaderaspatana)
- Karpooravalli (Plectranthusamboinicus)

Work flow of the present study



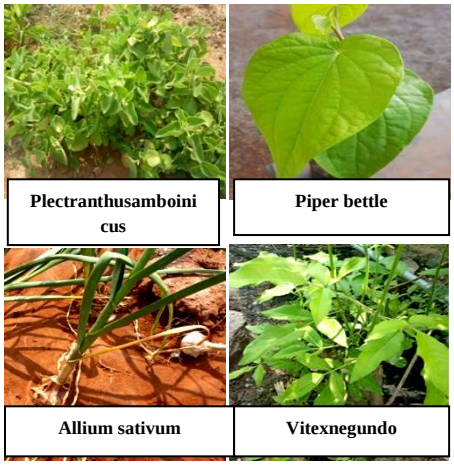
3. MATERIALS AND METHODS

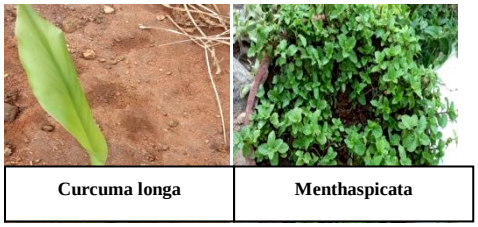
3.1.Chemicals required

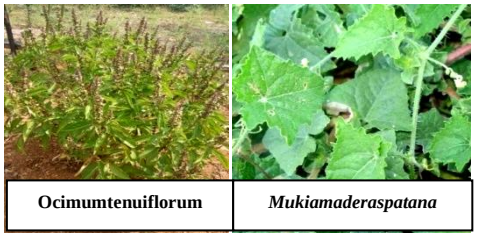
PBS(Phosphate buffer saline),95% of Ethanal,10ml of Egg albumin solution, Cuso4(Copper sulfate pentahydrate), 2ml Sodium bicarbonate solution, 2ml of HCL(Hydrogen chloride), 5 ml of chloroform, H2SO4(Sulfuric acid), 4ml of ammonia, 0.5ml of Benedict reagent , sodium Hydroxide, 5ml of brad ford reagent, 5% of Ferric chloride solution, 2ml Of absolute alcohol, Hydrogen peroxide, sodium phosphate buffer were purchased from Merk, USA.

3.2. Collection and authentication of plant materials

The traditional herbalplants were collected from Chathiramanai, (Latitude and Longitude; 11.1818`N, 78.7760`E) Tamil Nadu. The plant materials includes*Ocimumtenuiflorum, Plectranthusamboinicus, Curcuma longa ,Zingiberofficinase,Pippernigurum, Piper bettle, Vitexnegundo, menthe spicata , Mukiamaderaspatana , Allium sativum.*







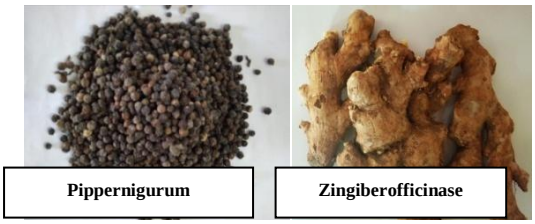


Figure 1. Collection and identification of medicinal plants

3.3.Preparation of polyherbal formulation

The leaves of The crude powdersof*Piper nigrum, Allium sativum, Zingiber official, Vitexnegundo, Piper betel,Curcuma longa, Menthe spicata,Acimumtenuiflorum, Aukiamaderaspatana, Plectranthusamboinicus* were collected and washed thoroughly in running tap water to remove soil particles and other debris. **Preparation of extracts by Soxhlet extraction method**

The crude powdersof Piper nigrum, Allium sativum, Zingiber official, Vitexnegundo, Piper betel,Curcuma longa, Menthe spicata,Acimumtenuiflorum, Aukiamaderaspatana, Plectranthusamboinicusweremixed with differnt proportions as shown in Table 1. After defating, the extraction was carried out using 1000 mL of 50% ethyl alcohol and 50% distilled H20(75.0°c) for 4 h.

After extraction, the samples were evaporated at the rotary evaporator to remain with important ingredients.

S.NO	HERBAL PLANTSNAME (SCIENTIFIC NAME)	QUANTITY
1	*Ocimumtenuiflorum*(Tulsi)	7 gm
2	*Plectranthusamboinicus*(karpuravalli)	5 gm
3	*Curcuma longa*(Turmeric)	2 gm
4	*Zingiberofficinase* (Ginger)	5gm
5	*Piper niguram* (Black pepper)	7gm
6	*Piper betel* (Vetrilai)	3gm
7	*Vitexnegundo* (Nochi)	3gm
8	*Mentaspicata (*Pudina)	3gm
9	*Mukiamaderaspatana*(Musumusukai)	10gm
10	*Allium sativum* (Garlic)	5gm
	TOTAL	**50gm**

Table:1 Composition of poly herbal medicinal plants

4.TEST AND ASSAY

Phytochemical test

The crude extract obtained were used for qualitative phytochemical characterization for the identification of the various classes of active chemical constituents, using standard prescribed methods where the tests were noted as absent (-), weak (+), moderate (++), and strong (+++) in (Table :2).

Detection of resins: To 0.5 of plant extract, 3ml of cuso4 solution is added. Shake for about 1-2 min formation of green color precipitation indicates the presence of resins.

Detection of Tannins: To 2ml of plant extract, 2-3ml of 10% HCL is added and boiled for 5-6 min. Formation of red color indicates the presence of tannins.

Detection of Steroids:To 0.5 ml extract, 5ml of chloroform is added and equal amount of conc. H2so4 is added. In the upper layer formation of red coloris and in the lower layer, yellow with green color is formation indicates the presence of steroids.

Detection of flavonoids: To 0.5 ml extract, add 4ml of 1% ammonia and to this add 1ml of conc. H2so4. Formation of yellow color indicates the presence of flavonoids.

Detection of Carbohydrates: To 0.5ml of extract, 0.5ml of Benedict reagent is added ad boiled for 2 min. Color changes and ppt is formed. It indicates the presence of carbohydrate.

Detection of Glycosides Saponification test: To 1 or 2ml of normal sodium hydroxide, 2mlof extract is added and boiled for 2 minutes. Formation of soap or fat indicates the positive test for saponification.

Detection of Proteins Estimation of Brad Ford Method: To 500 and 1 of extract, add 5mlof brad ford reagent, Take OD at 575nm.

Detection of Phenol Ferric Chloride Test: To 50 mg of extract, 5ml distilled water, few drops of 5% ferric chloride solution, dark green color.Indicates the presence of phenol.

Biuret Test: To 2ml of extract, 1 drop of 2% cuso4 solution. Add 1 ml of s95% ethanol,then add 2 to 3 sodium hydroxide pellets.Formation of pink color indicates the test is positive.

SaponTest: To 50 mg of extract,20 ml of distilled water. Shake vigorously for 15 min, at 2 cm layer of foam formation indicates the presence of saponins.

Gum Test: To 100 mg extract. Dissolved in 2 ml of distilled water.2ml of absolute alcohol with constant stirring. White color

cloudy ppt indicates gums & mucilage's.**(figure:15), (Table:2).**

Albumin assay

Denaturation of proteins is the main cause of inflammation. Inhibition of protein denaturation was evaluated. 500 µl of 1% egg albumin was added to 100 µl of test sample.This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for20 minutes The experiment was carried out in triplicates for the concentrations from 100µl to 10 µl**(Figure16,17)**and percent inhibition for protein denaturation was calculated using**(Table:3)**

% Inhibition=100−((A1-A2)/A0)*100).

3.6. Hydrogen peroxide scavenging assay

The ability of plant extract to scavenging hydrogen peroxide was estimated according to the method. A solution of hydrogen peroxide (4.3Mm) is prepared in phosphate buffer (1M.PH7.4) different concentration of sample (2-10mg/ml)was added to a hydrogen peroxide solution. (0.6ml,43mM) absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The positive control used was hydrogen phosphate**.(Figure:18,19)**

Sodium phosphate was used standard the free radiation scavenging activity was determined by evaluating % inhibition as above % inhibition= [(control-test od)/control)] ×100.(**Table:4).**

Phytochemical analysis of polyherbal formulation

The Vapcozampolyherbal formulation was extracted using a Soxhlet apparatus as shown in figure 2.

Figure:2Soxhlet extraction of Vapcozampolyherbal formulation

S no	Phytochemical Test	Result
1	Resins	--
2	Carboxylic acid	--
3	Tannins	+++

4	Steroids	--
5	Flavonoids	+++
6	Carbohydrate	+++
7	Glycosides(saponification test)	+++
8	Protein (Bradford method)	+++
9	Phenol test(Ferric chloride test)	--
10	Biuret test	--
11	Sapon test	+
12	Gum test	--

Table:2. Phytochemical analysis of polyherbal formulation

Figure: 3. Phytochemical screening of polyherbal formulation

4.2.Anti-inflammatory activity

The anti-inflammatory activity of polyherbal formulation was studied using the albumin denaturation assay. These results showed that the polyherbal formulation exhibited concentration dependent anti-inflammatory activity.

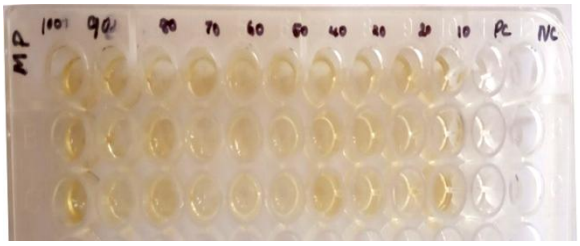
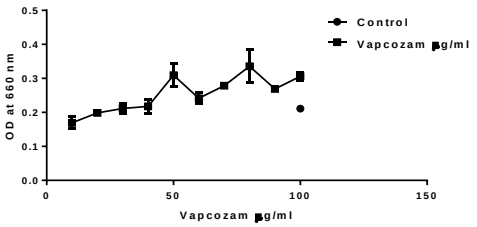


Figure4. Anti-inflammatory activity of polyherbal extract by albumin denaurationassay

A.OD value at 660nm

Control mean OD value:0.211

S.No	Tested sample concentration (µg/ ml)	OD value at 660nm (in duplicates)	
1	Positive control	0.211	0.211
2	Negative control	0.077	0.077
3	100µl/ml	0.298	0.314
4	90µl/ml	0.268	0.270
5	80µl/ml	0.370	0.301
6	70µl/ml	0.272	0.285
7	60µl/ml	0.253	0.230
8	50µl/ml	0.286	0.333
9	40µl/ml	0.233	0.202
10	30µl/ml	0.222	0.201
11	20µl/ml	0.202	0.195
12	10µl/ml	0.182	0.157



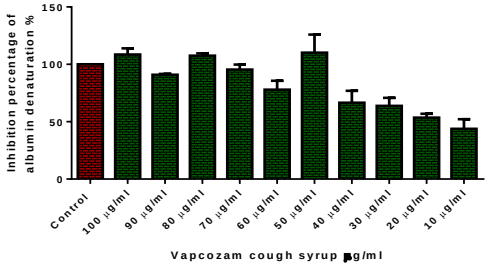
Graph 1. Concentration vs OD value of Vapcozam

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50	~ 3.520	1.670
HillSlope	~ 1.759	2.084
IC50	~ 3313	46.79
Std. Error		
LogIC50		0.05013
HillSlope		0.5546
95% Confidence Intervals		
LogIC50		1.565 to 1.775
HillSlope		0.9193 to 3.250
IC50		36.71 to 59.63
Goodness of Fit		
Degrees of Freedom	0	18
R square	1.000	0.6753
Absolute Sum of Squares	0.0	6897
Sy.x		19.57
Number of points		
Analyzed	2	20

B. Inhibition percentage of albumin denaturation (%)

Table:3Inhibition percentage of albumin denaturation

S. No	Tested sample concentration (µg/ml)	Inhibition percentage albumin denaturation (%) (in duplicates)		Mean Value (%)
1	Control	100	100	100
2	100 µg/ml	104.73	112.32	108.52
3	90 µg/ml	90.52	91.46	90.99
4	80 µg/ml	109	106.16	107.58
5	70 µg/ml	92.41	98.57	95.47
6	60 µg/ml	83.41	72.51	77.96
7	50 µg/ml	99.05	121.32	110.18
8	40 µg/ml	73.93	59.24	66.58
9	30 µg/ml	68.72	58.78	53.74
1	20 µg/ml	51.24	55.92	55.58
1	10 µg/ml	49.76	37.91	43.83



C. **IC50 Value of tested sample: 46.79 µg/ml**

4.3.Hydrogen peroxide scavenging assay

The anti-oxidant activity of polyherbal formulation was studied using the hydrogen peroxide assay.The maximum antioxidant activity was observed at the concentration of 117 µg/ml.

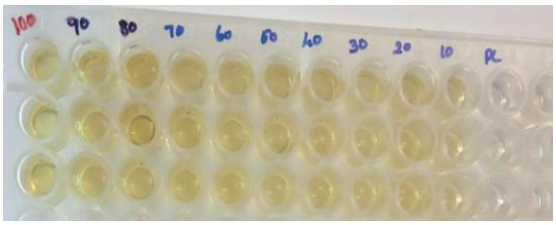
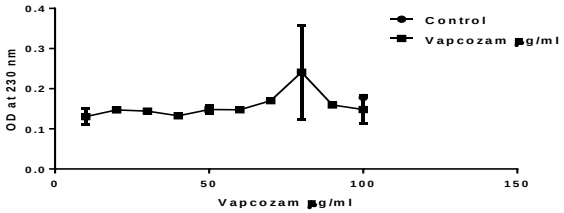


Figure. 5Anti-oxidant activity of Vapcozam cough syrup by Hydrogen peroxide assay

A. OD Value at 230 nm

Control Mean OD value: 0.178

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in duplicates)		Mean value (%)
1	Control	100	100	100
2	100 µg/ml	2.80	30.89	16.84
3	90 µg/ml	13.48	7.30	10.39
4	80 µg/ml	17.41	11.23	14.32
5	70 µg/ml	5.05	3.95	4.49
6	60 µg/ml	15.16	19.10	17.13
7	50 µg/ml	20.78	12.98	16.85
8	40 µg/ml	26.40	24.71	25.55
9	30 µg/ml	21.91	16.29	19.1
1	20 µg/ml	17.97	16.85	17.41
1	10 µg/ml	18.53	34.83	26.68



Graph 2. Concentration vs OD value of Vapcozam

B. Inhibition percentage of hydrogen peroxide scavenging assay(%)

Table: **Inhibition percentage of hydrogen peroxide scavenging assay**

33

C. IC50 Value of tested sample: 117.0 µg/ml

4.4. Toxicity analysis using the zebra fish model

The toxicity analysis of various concentrations of Vapcozam cough syrup was carried out using zebra fish larvae.

The results showed that the Vapcozam cough syrup was found to be non-toxic to zebra fish larvaeas shown in Fig 5.

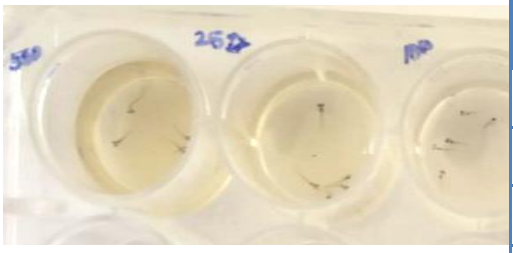


Figure. 6. Toxicity analysis of Vapcozam cough syrup in zebra fish larvae

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50	~ 3.542	2.068
HillSlope	~ 1.783	1.473
IC50	~ 3481	117.0
Std. Error		
LogIC50		0.2369
HillSlope		1.295
95% Confidence Intervals		
LogIC50		1.571 to 2.566
HillSlope		-1.247 to 4.193
IC50		37.20 to 368.0
Goodness of Fit		
Degrees of Freedom	0	18

R square	1.000	0.1341
Absolute Sum of Squares	0.0	23968
Sy.x		36.49
Number of points		
Analyzed	2	20

REFERENCE

1. Ahmed, I., and Beg, A.Z. "Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathologens." Journal of Ethnopharmacology, 2001.
2. Allan, GM; Arroll, B (18 February 2014). "Prevention and treatment of the common cold: making sense of the evidence". CMAJ : Canadian Medical Association Journal. 186 (3): 190–99.
3. Ammon and M. A. Wahl, "Pharmacology of *Curcuma longa*," *PlantaMedica*, vol. 57, no. 1, pp. 1–7, 1991
4. Ammon HPT, Wahl MA. PlantaMedica. 1991;5
5. Arrolet, B (March 2011). "Common cold". Clinical Evidence. 2011 (3): 1510
6. Arroll B, Kenealy T. Antibiotics for the common cold and acute purulent rhinitis. Cochrane Database Syst Rev 2005;(3):CD000247.
7. Arroll B, Kenealy T. Antibiotics for the common cold and acute purulent rhinitis. Cochrane Database Syst Rev. 2005;(3):CD000247.
8. Braman SS. Post infectious cough: ACCP ev- idence-based clinical practice guidelines. Chest. 2006;129:138-46
9. Bray F, Ferlay J, SoerjomataramI,Siegel RL, Torre LA, Jemal A . Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.
10. Brizee, S., van der Vaart, M., Walmsley, S. R., van Eeden, F. J., Renshaw, S. A., et al. (2013). Hypoxia inducible factor signaling modulates susceptibility to mycobacterial

11. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Giffin SL. 2009 annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 27th annual report. ClinToxicol (Phila). 2010;48(10):979-1178.
12. Brownlie, A., Donovan, A., Pratt, S.J., Paw, B.H., Oates, A.C.,Brugnara, C., Witkowska, H.E., Sassa, S., and Zon, L.I. 1998.Positional cloning of the zebrafish sauternes gene: A model for congenital sideroblasticanaemia.
13. Driever, W., Solnica-Krezel, L., Schier, A.F., Neuhauss, S.C., Malicki,J., Stemple, D.L., Stainier, D.Y., Zwartkruis, F., Abdelilah, S.,Rangini, Z., et al. 1996. A genetic screen for mutations affecting embryogenesis in zebrafish. Development 123:
14. Fahey T, Stocks N, Thomas T. Systematic review of the treatment of upper respiratory tract infection. Arch Dis Child 1998;79:225-30.
15. Frederick J Curley, Richard S Irwin, Melvin R Pratter, Diane H Stivers Am Rev Respir Dis 138, 305-1988
16. Hankemeier T, Spaink HP, Van der Graaf PH (2017). "Systems pharmacology of hepatic metabolism in zebrafish larvae".
17. Hankey A. Ayurvedic physiology and etiology: AyurvedoAmritanaam. The doshas and their functioning in terms of contemporary biology and physical chemistry. J Altern Complement Med. 2001;7:567–574.
18. Hemilä, Harri (January 2006). Do vitamins C and E affect respiratory infections (Thesis).University of Helsinki.hdl:10138/20335. ISBN 978-952-10-2837-3.
19. Irwin RS1, Baumann MH, Bolser DC, Boulet LP, Braman SS, Brightling CE, et al. Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. Chest. 2006;129:1-23.
20. Rossato SC, Leitao-Filho H, Gegossi A: Ethnobotany of Caicaras of the Atlantic forest coast (Brazil). Economic botany 1999, 53:387-395.
21. Russell La Fayette Cecil; Lee Goldman; Andrew I. Schafer (2012), Goldman's Cecil Medicine, Expert Consult Premium Edition (24 ed.), Elsevier Health Sciences, pp. 2103–ISBN 978-1-4377-1604- archived from the original on 4 May 2016
22. Smith SM, Schroeder K, Fahey T. Over-the-counter medications for acute cough in children and adults in ambulatory settings. Cochrane Database Syst Rev. 2008;(1):CD001831
23. Verma V. Ayurveda: A way of life. York beach: Weiser Books; 1995

24. World Health Organization Technical Package for Cardiovascular Disease Management in Primary Health Care. Hearts. 2016;76 doi: 10.1016/j.corteElks,
25. Zon LI, Peterson RT. In vivo drug discovery in the zebrafish. Nat Rev Drug Discov 2005.
26. Zon, L.I. 1999. Zebrafish: A new model for human disease.