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"DEVELOPMENT AND STANDARDIZATION OF POLYHERBAL SYRUP FOR ANTIBACTERIAL SCREENING"

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ABSTRACT

Between 150 million and 156 million children under the age of five worldwide lose their livesto pneumonia every year; the majority of these deaths occur in underdeveloped countries. Theobjective of the current study is to determine how the antibacterial activity of vitex negundo leaves and zingiber officinale rhizomes, which are used to manufacture syrup, may be increased. This composition will be standardised as part of the current study's effort to developa polyherbal syrup for the treatment of pneumonia. The test batch with the best viscosity was chosen after several were created using various sugar and flavouring dosages. The produced product satisfies the requirements, however more in vitro testing is needed to verify and assessits efficacy. Using the microdilution method, the in vitro antibacterial activity of plants was tested against both Gram-positive and -negative pathogens to determine their MIC and MBC values. The MIC and MBC values of extracts from various plant species were tested against test bacteria.

Keywords: pneumonia, polyherbal syrup, extract, antibacterial activity.

INTRODUCTION

A buildup of pus and other fluids in the lung air sacs is a characteristic of the pneumonia, a frequent lung illness (alveoli). Lung air sacs are a group of organs that allow the exchange of oxygen and carbon dioxide. As a result of an infection, breathing in foreign substances, or being exposed to radiation, the lungs may swell up and become inflamed. Although bacteria, particularly species of Streptococcus and Mycoplasma, are the most frequent causes ofpneumonia, viruses and fungi can also cause the disease. While viral pneumonia can happen, it does so more commonly because it weakens the lung, leaving room for bacterial pneumoniato subsequently develop. Yet, it typically affects hospitalised patients who have a diminished ability to fight off infections due to lowered immunity. A serious case of fungal pneumonia canarise very quickly.

More people worldwide are affected by pneumonia than by conditions like cancer, diabetes, HIV/AIDS, malaria, and many other maladies combined.

MATERIALS:

Green *Vitex negundo* leaves and *Zingiber officinal* root extract was purchased from S.A.Herbal Pvt. Limited. Mumbai-400703

A. METHODS:

1. Phytochemical Screening for Raw Materials

To determine the presence of various phytoconstituents like mucilage, alkaloids, steroids, terpenoids, anthraquinone glycosides, flavonoids, tannins and phenolic compounds, steroids, carbohydrates, proteins, and amino acids, petroleum ether, chloroform, ethanol, and aqueous extracts were subjected to preliminary phytochemical screening. The following tests were used to determine the various phytoconstituents that were found in allof the extracts.

2. Prepration of polyherbal syrup

a. Method of preparation of decoction

The seven dried raw materials were coarsely powdered. The dried powder wasmixed with 400 ml of water and the mixture was boiled until the total volume become

b. Method of preparation of simple syrup

35 gm of sugar was weighed and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 100 ml.

c. Preparation of the Polyherbal syrup

One part of decoction was mixed with five parts of simple syrup (1:5). Requiredquantity of methyl paraben, propyl paraben, was added to the above mixture. Solubility was checked by observing the clarity of the solution visually. The final herbal syrup was then subjected to evaluation of product quality as per official standards.

Batch	*Vitex negundo* drug (mg)	*Zingiber officinale* drug (mg)	Glycerol & CMC ratio (ml)	Sucrose	Methyl paraben (g)	Propyl paraben (g)	Flavo-ring oil	Distilled water
F1	600 mg	600 mg	30	66.7	0.2	0.1	q.s.	q.s.
F2	600 mg	600 mg	20	66.7	0.2	0.1	q.s.	q.s.
F3	600 mg	600 mg	20	66.7	0.2	0.1	q.s.	q.s.
F4	600 mg	600 mg	20	66.7	0.2	0.1	q.s.	q.s.
F5	600 mg	600 mg	20	66.7	0.2	0.1	q.s.	q.s.
F6	600 mg	600 mg	30	66.7	0.2	0.1	q.s.	q.s.
F7	600 mg	600 mg	20	66.7	0.2	0.1	q.s.	q.s.
F8	600 mg	600 mg	10	66.7	0.2	0.1	q.s.	q.s.
F9	600 mg	600 mg	20	66.7	0.2	0.1	q.s.	q.s.
F10	600 mg	600 mg	30	66.7	0.2	0.1	q.s.	q.s.

EVALUATION OF SYRUP

After scaling up, physical constants, phytochemical screening, heavy metals, and microbiological load analyses were performed on the chosen batch of Polyherbal syrup. The polyherbal syrup's pH, specific gravity, viscosity, colour, odour, and taste were all measured.

- **Determination of pH:** A pH metre was used to calculate the pH of the polyherbal syrup. In order to get consistent readings, the pH metre was calibrated using buffer (atpH 4 and 9) and distilled water.
- **Determination of specific gravity:** Pycnometer was used to determine the specific gravity at 25°C. It was determined dividing the weight of sample (expressed in gm) bythe weight of water (in ml).
- **Determination of viscosity:** Ostwald viscometer was used to determine the viscosity of polyherbal syrup. The method was followed as per the standard procedure.
- **Stability Study:** The stability study conducts by ICH guideline. It showed No significance change in properties of the optimized formulation. Short term stability studies were performed in a Stability chamber over a period of 3 weeks (21 days) on the promising vitex negundo and zingiber officinalis leaf syrup. Sufficient quantity of syrup formulation were packed in stability container and kept in a Stability chamber atTemperature 45°c & RH 75% Samples were taken on 21st day for the pH, viscosity, density and microbial studies were performed to determine the stability profile.

Analytical Evaluation:

- **Differential scanning calorimetric (DSC) Analysis:**

  DSC scans of the powdered sample of *Vitex negundo* leaf extract, *Zingiber officinale* root extract and a mixture of excipients with the drug. DSC analyses of powders were recorded using DSC- Shimadzu 60 with TDA trend line software. The pans were positioned on the sample pan holder of a DSC 60. The thermal traces were obtained byheating from 50°C to 300°C at a heating rate of 20°C per minute.

Thermograms were obtained by the DSC 60 thermal analyzer program and recorded a chart speed of 1 inch/min. The thermogram, transition temperature range, the onset of peak transition and the maximum peak of transition were recorded.

- **Infrared spectroscopy:**

  This technique is based upon the simple fact that the substance shows marked selectiveabsorption in the infrared region. After absorption of 18 radiations, the molecules of the chemical substance vibrate at many rates of vibration, giving rise to done-packed absorption bands, called as I absorption spectrum which may extend over a wide wavelength range. Various hands will be present in IR spectrum which will correspondto the characteristic functional groups and bonds present in the chemical substance. It is used to establish the structure of unknown compound and analysis of functional group. The sample was analyzed between 4000-600 cm

- **Antibacterial activity:**

  The inoculum of the microorganion was prepared from the bacterial cultures 15ml of strict agar (Hi modis) medium was poured in clean sicriftred Petri plates and allowed to cool and solidify 100 µl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading red till it dried properly. Wells of 6mm in diameterwere bered ung a sterile cork borer Solutions of all the compounds (5µl/ml and 105µl/ml) in DMSO west prepared 100µl of sample polyherbal syrup solutions was added to the wells. The petri plates incubated at 37°C for 24 h streptomycin (Imp/ml) was prepared as a positive conmel DMSO was taken as negative control Antibacterial activity was evaluated by measuring the diameter of the zone of inhibitions (1) all the determination were performed in triplicates.

- **Stability Study:**

  The stability study conducts by ICH guideline. It showed No significance change in properties of the optimized formulation. Short term stability studies were performed ina Stability chamber over a period of 3 weeks (21 days) on the promising *vitex negundo*and *zingiber officinalis* leaf syrup. Sufficient quantity of syrup formulation were packedin stability container and kept in a Stability chamber at Temperature 45°c & RH 75% Samples were taken on 21st day for the pH, viscosity, density and microbial studies were performed to determine the stability profile.

RESULT AND DISCUSSION:

Phytochemical Screening for Raw Materials

Sr no.	Phytochemical test	*Vitex negundo*	*Zingiber officinale*
1.	Carbohydrates	+	–
2.	Glycosides	+	+
3.	Anthraquinones	–	+
4.	Saponins	+	+
5.	Steroids	–	–
6.	Flavonoids	+	+
7.	Tannins	+	–
8.	Alkaloid	+	+
9.	Phenols test	+	–

Different formulation of polyherbal syrup

Table no.1 Different formulation of polyherbal syrup

Formulation	X1 (ml)	X2 (0^0C)	X3 (min)	Viscosity (cP)
1.	30	60	45	15.31
2.	20	30	15	19.28
3.	20	90	15	19.03
4.	20	60	30	22.5
5.	20	30	45	16.7
6.	30	60	15	11.2
7.	20	60	30	12.9
8.	10	60	45	12.82
9.	20	60	30	24.60
10.	30	90	30	23.16

Evaluation of syrup

Table no.2 Evaluation of syrup

Sr. No.	Specifications	Observations
1	Physical appearance	Viscous liquid
2	Colour	Yellowish brown
3	Odour	Sweet
4	Taste	Sweet aromatic

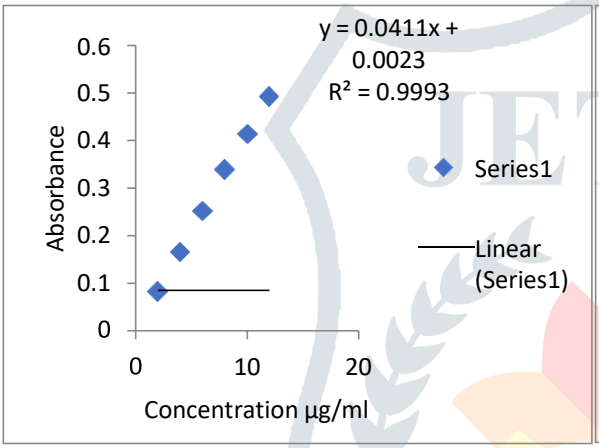
Analytical Evaluation : Estimation by UV spectroscopy

Determination of λmax: The concentration 100 µg/ml *vitex negundo* extract in methanol was found to be 269nm and the concentration 100 µg/ml *zingiber officinale* extract in methanol wasfound to be 280nm.

Table no.3 Absorbance value

Sr no.	Concentration (ug/ml)	Absorbance (µg/ml) of vitex negundo	Absorbance (µg/ml)
1.	2	0.082	0.397
2.	4	0.165	0.542
3.	6	0.252	0.685
4.	8	0.338	0.846
5.	10	0.413	1.004
6.	12	0.492	1.118

Calibration curve:



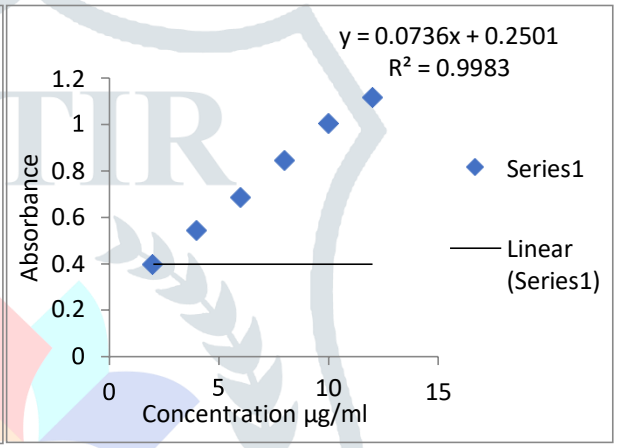


Fig no.2 Calibration curve of *vitex negundo* Fig no.3 Calibration curve of *Zingiber officinale*

Compatibility study by FTIR:

The compatibility study between drugs and was carried out using the FTIR spectrometer. Thepeak numbers of the drug exhibiting O-H, C=C, C-O, C-H, C-C, C=O stretching were observedand are depicted as below.

FTIR of *vitex negundo* leaf extract:

From the above observation table, the FTIR study of Pure *vitex negundo* leaf extract compatibility was studied to observe their peak compared to the standard frequency of FTIR.

Sr no.	Functional group	Standard frequency	Peak observed
1.	O-H	3000-4000	3292.90
2.	C=O	1000-1700	1336.81
3.	C-C	500-1000	523.28
4.	C-O	1000-1300	1008.17
5.	C=C	1000-1500	1074.61

FTIR of *zingiber officinale* rhizomes extract

From the above observation table, FTIR study of Pure *zingiber officinale* root extractcompatibility was studied and to observe their peak comparing to standard frequency of FTIR.**FTIR of *Zingiber Officinale***

Sr no.	Functional group	Standard frequency	Peak observed
1.	O-H	3000-3500	3291.04
2.	C=O	1220-1760	1398.12
3.	C=C	650-1000	863.29
4.	C-H	700-900	843.41

FTIR of polyherbal syrup

Sr no.	Functional group	Standard frequency	Peak obseved
1.	O-H	3000-3500	3272.08
2.	C=O	1500-2000	1643.25
3.	C-H	1000-1300	1275.82
4.	C=C	1000-1500	1453.04
5.	C-C	500-1000	924.22

From the above observation table, FTIR study of syrup formulation of polyherbal extract and other excipients like CMC, Glycine, they are compatible to each other and to observe their peakand comparing to standard frequency of FTIR.

Differential Scanning Calorimetry (DSC):

From this result, it clears that there is no interaction in between *vitex negundo*, *zingiber Officinale*, polyherbal syrup and excipients.

Differential Scanning Calorimetry

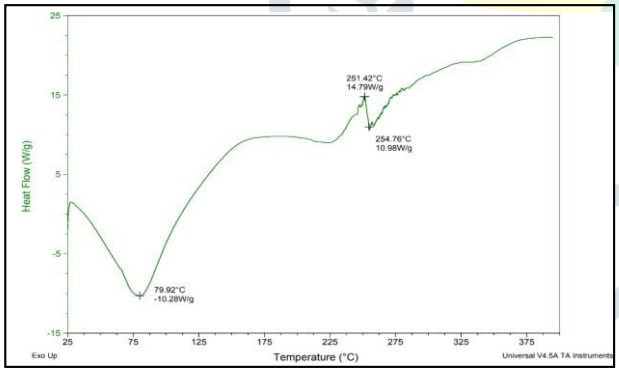


Fig no.7 DSC of *Vitex Negundo* extract

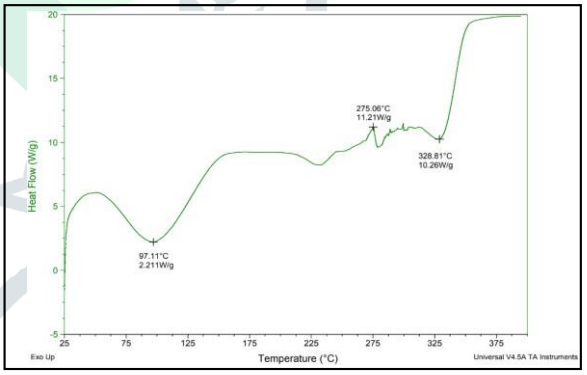


Fig no.8 DSC of *Zingiber officinale* extract

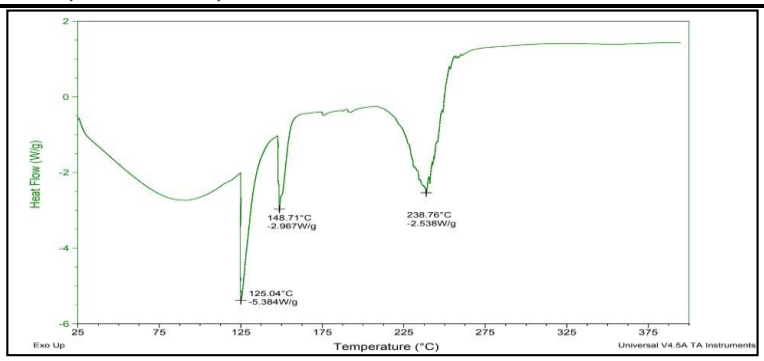


Fig no.9 DSC of Polyherbal syrup formulation

X-Ray Diffraction

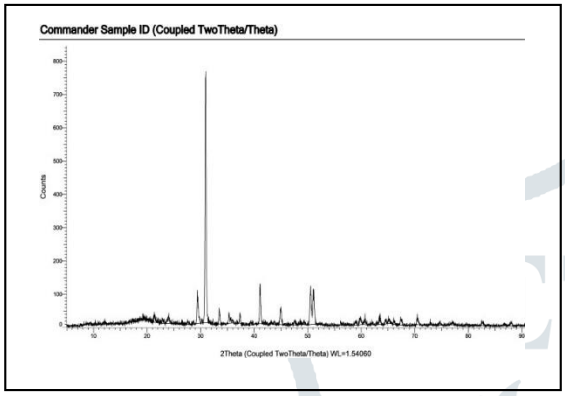


Fig no.10 X-Ray of *vitex negundo*

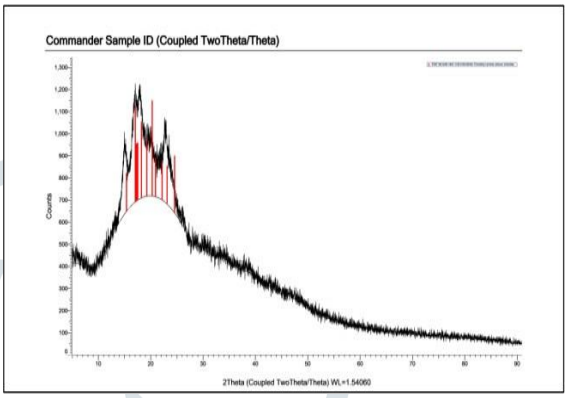


Fig no.11 X-Ray of *zingiber officinale*

***In vitro*Antibacterial activity**

Samples	**Concentration (mg/ml)**	**Zone in inhibition (mm)**
Control	-	0
Standard (Streptomycin)	1	28
Stap.Aureus	100µl	14
	200µl	20

Fig no.12 Antimicrobial Activity of polyherbal syrup against *Stap.Aureus* **RESULT:** At the concentration 200µL the sample of polyherbal syrup shows moderateantibacterial (20 mm) activity against *Stap.Aureus* as compare to std. Streptomycin (28 mm)

Stability studies :

Accelerated Stability Study data of polyherbal syrup

Sr no.	Test	"0"	1 month	3 month
1	Temperature	40°c	40°c	40°c
2	RH	75%	75%	75%
3	Physical apperance	Light brown	Yellowish brown	Dark brown
4	Texture	Smooth	Smooth	Smooth
	Colour	Brown	Brown	Brown
	Odour	Aromatic	Aromatic	Aromatic
	Ph (4 to 60	5.2	5.4	5.5

CONCLUSION:

In the present study, the work was an attempt to carry out standardization of active constituentsof *Vitex negundo* leaf and *Zingiber officinale* root. Also to formulate and evaluate polyherbal syrup. All formulations were checked for PH, viscosity, density and specific gravity. The FTIRstudies revealed that the formulated product is a mixture of the drug and the polymers used butnot the reaction product with the excipients used. DSC studies of the above-mentionedformulations realized that during the process of formulation, a chemical reaction is not taken place. The extract of *Vitex negundo* and *Zingiber officinale* had antibacterial activity attempt was made to develop polyherbal syrup to achieve better viscosity with improved bioavailabilityby oral route. The syrup formulation contains the Carboxymethyl cellulose, viscosity builder, which forms a stable complex and improves the bioavailability of extract of *Vitex negundo* and*Zingiber officinale*. It can be concluded that the polyherbal syrup formulation oral of *Vitex negundo* and *Zingiber officinale* showed viscosity. Thus, the prepared polyherbal syrup couldbe a better alternative for rapid oral bioavailability in antibacterial activity and better absorption. Different formulations of polyherbal syrup evaluation parameters results were observed, F9 and F10 formulation was found to be the best formulation as per viscosity.

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