ABSTRACT
Background: Evaluation of herbal formulation is essential in order to assess the quality of the drugs, based on the concentration of their active principles. This article reports on the Phytochemical analysis of Septilin drops, an Ayurvedic Polyherbal formulation used in upper and lower respiratory tract infections, skin infections and as an immunomodulator in children. It is well known for its therapeutic actions like Anti-inflammatory, Anti-bacterial, Hepatoprotective and Immunomodulatory activities. Objectives: The experiment was carried out with the aim of evaluating various chemical constituents of Septilin Drops. Methods: A Septilin drop was procured from The Himalaya Drug Company and same used for the analysis. Evaluation of the polyherbal formulation is possible by following modern scientific quality control procedures for the finished product. The prepared “Septilin drops” is evaluated phyto-chemically by subjecting it to various tests like physio-chemical, qualitative analysis and TLC. The present formulation has been used for clinical study, showed efficacy, safety and immunomodulation in upper respiratory tract infections of infants and children. Results: The Qualitative tests on Septilin Drops revealed the presence of sugars, proteins, alkaloids, flavanoids and tannins. The richness of various chemical constituents in the formulation is confirmed by the presence of various Rf values in the TLC study. Conclusion: The present phytochemical evaluation of Septilin Drops showed the presence of various chemical constituents.

Key words: Polyherbal, anti-inflammatory, Alkaloids, Septilin, Rf

INTRODUCTION
A respiratory tract infection comprises infections associated with upper as well as lower respiratory tract. Upper respiratory infections composed of tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media and the common cold. Upper respiratory infections are the common cause of hospital visits among paediatric population. Among respiratory infections, 87.5% constitutes upper respiratory infections only [1]. Due to their anatomical peculiarities and immature immunological responses children are more susceptible to respiratory infections very recurrently [2]. A high incidence of respiratory tract infections in young infants and pre-school going children are due to defects in the immune system [3]. Recent studies also have demonstrated the role of host defence
mechanism and hypothesized the impact of immunomodulation in URTI's.

Plants are preliminary source of phyto-chemicals used to treat various disorders. Septilin is one such herbal formulation containing herbs with anti-inflammatory, anti-bacterial, hepato-protective, anti-tussive and immune-modulatory properties and indicated as an immune-modulator in upper and lower respiratory tract infections, allergic disorder of the upper respiratory tract, skin and soft tissue infections, dental and periodontal infections, bone and joint infections, urinary tract infections, as an adjuvant to anti-infective therapy in children. Drugs used in this formulation are having properties like Shothhara (~Anti-inflammatory), Kaphavatashamaka (~Expectoarant), Jantughna (~Anti-microbial) and indicated in diseases like Kasa (~Cough), Shwas (~Dyspnœa), Hikka (~Hiccough) and Swarabheda (~Altered voice) etc.

The composition of Septilin Drops constitutes extracts Commifera mukhul (Hook ex Stocks)(purified), Maharasnadi kvatha, Tinospora cordifolia (Wild) Miers ex Hook.f., Rubia cordifolia Linn, Trikatu (Piper longum Linn, Zingiber officinale Rosc, Piper nigrum Linn, Inula racemosa Hook, F. Emblica officinalis Linn, Glycyrrhiza glabra Linn. (Table1).

Previous studies on Septilin tablets and syrup reporting its analgesic, anti-inflammatory, and wound-healing properties. And its utility in treating gram-positive as well as gram-negative infections. The Clinical trials on Septilin reported that, it is effective in chronic URTI, tonsillitis, tropical eosinophilia, skin infections, and dental infections.

Clinical Studies have been conducted on Septilin Drops showed efficacy in upper respiratory tract infections as well as lower respiratory tract infections in children and experimental studies like acute toxicity studies on Wistar rats showed its safety in oral dose. A Septilin drop is a unique formulation, as it differs from Septilin syrup by change in their composition and strength of drugs. As it is a poly herbal formulation, very rich in chemical constituents and no phyto-chemical studies have not been found evaluated yet and hence it was thought to evaluate phyto-chemically.

AIMS AND OBJECTIVES:
1. Evaluation of Phytochemicals of formulation through preliminary phyto-chemical screening.
2. Evaluation of phyto-chemicals through thin layer chromatography.

MATERIALS AND METHODS
The clinical study of Septilin Drops has been approved by the Institutional Ethical Committee (IEC) with IEC no: BMK/PG/11/19.

Trial Drug Details
Materials used in this formulation are mentioned in the Table1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Part used</th>
<th>Qty in mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guggulu (Commifera mukhul)</td>
<td>Oleo-gum resin</td>
<td>47.4</td>
</tr>
<tr>
<td>Maharasnadi kvatha</td>
<td>-</td>
<td>17.8</td>
</tr>
<tr>
<td>Trikatu (Zinziber officinalis Rosc, Piper longum Linn and Piper nigrum Linn)</td>
<td>Fruit and rhizome</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Table 1: Shows Composition of Septilin Drops
**Method of Preparation**
The drug was prepared from The Himalaya Drug Company as follows. The Extracts of Guggul (Oleo-resin), Maharasnadi kvatha, Yastimadhu, Guduchi, Amalaki, trikatu, Manjista and Pushkar moola were mixed thoroughly, approved preservative and sweetener were added and mixed thoroughly, the obtained mixture is finished into liquid form and filled in the bottles.

**The Organoleptic Characteristics of Septilin Drops**
The study of organoleptic characters includes the examination of the sample for appearance, colour as well as consistency by visual inspection.

**Physico-chemical study**
**pH** \[13\]
The pH of the formulations was measured by taking 1% aqueous solution of the formulation using digital pH meter.

Weight variation, specific gravity and pH evaluation were done as per the standard methods preliminarily and then it is subjected to chemical evaluation.

**Preliminary Phytochemical Screening**
The sample was subjected for qualitative tests for reducing sugar, monosaccharide, pentose sugar, proteins, steroids, tannins and flavanoids were carried out by following standard methods \[13\].

**Thin Layer Chromatography**
In TLC study, chloroform extract of formulation was run on a pre-coated silica gel 60F 254 plate by using Toluene: Ethyl acetate: Methanol (7:3:1) and Toluene: Ethyl acetate (6:4) as a solvent system. The solvent was allowed to run up to 8cm distance and plate was observed as such under long(366) and short(254) Ultraviolet (UV) rays and the fluorescent spots resolved were noted down. The Rf values from the developed coloured spots resolved were noted down and results are mentioned in Table 3.

**RESULTS**

**The Organoleptic Characteristics of Septilin Drops**
The present sample from formulation was assessed for organoleptic characters and results are mentioned in Table 2.

**Analysis on Physico-Chemical Parameters**
The prepared formulation was assessed for routine physico-chemical tests showed specific gravity 1.12 and 29.27 % of total solids, with 1.46 Refractive index and pH value of 4.77.(Table 3).

**Analysis on Routine Qualitative Parameters**
Qualitative tests revealed the presence of tannins, alkaloids, saponin, flavonoides, carbohydrate, proteins and glycoside (Table 4).

**Thin Layer Chromatography of Chloroform Extract**
1) Toluene: Ethyl acetate: Methanol solvent
TLC revealed five resolved spots (Rf: 0.5, 0.58, 0.67, 0.76, and 0.82) in long UV and Four spots (Rf: 0.43, 0.5, 0.62 and 0.68) in Short UV.
2) Toluene: Ethyl acetate TLC analysis of the extract showed four resolved spots (Rf: 0.41, 0.47, 0.56 and 0.75) in long UV and four spots (Rf: 0.31, 0.38, 0.43 and 0.67) in short UV. Indicating the presence of alkaloids in it (Figure 1).

Table 2: Organoleptic Characteristics of Septilin Drops

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Blackish</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Taste</td>
<td>Sweet</td>
</tr>
<tr>
<td>Touch</td>
<td>Smooth</td>
</tr>
</tbody>
</table>

Table 3: Physicochemical Parameters of Septilin Drops

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Gravity</td>
<td>1.12</td>
</tr>
<tr>
<td>Total solids</td>
<td>29.27%</td>
</tr>
<tr>
<td>Refractive Index</td>
<td>1.468</td>
</tr>
<tr>
<td>pH</td>
<td>4.77</td>
</tr>
</tbody>
</table>

Table 4: Qualitative Parameters of Septilin Drops

<table>
<thead>
<tr>
<th>Components</th>
<th>Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin</td>
<td>Dil. HNO3</td>
<td>Positive</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>Dragendorff’s reagent</td>
<td>Positive</td>
</tr>
<tr>
<td>Saponin</td>
<td>Foam test</td>
<td>Positive</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>With neutral Lead</td>
<td>Positive</td>
</tr>
<tr>
<td>Hexose Sugar</td>
<td>Selvinoff’s test</td>
<td>Positive</td>
</tr>
<tr>
<td>Proteins</td>
<td>Million’s reagent test</td>
<td>Positive</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Test for tyrosine</td>
<td>Positive</td>
</tr>
<tr>
<td>Anthroquinine</td>
<td>Borntragers test</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Figure 1: TLC plates of Chloroform extract of Septilin Drops
DISCUSSION

Though considerable advances are made in the pharmaceutical sciences, especially in synthetic chemistry, plants and their derivatives continue to maintain their significance in medicines. Increased interest in natural drugs than synthetic is because of a high degree of adverse side effects caused by the latter [14].

Guggulu (oleo-gum resin) being one of the main ingredient in Septilin drops, which is very effective in the management of upper respiratory infections [15] due to its anti-inflammatory, anti-oxidant properties [16] and anti-bacterial activity [17]. It regulates the secretion of mucus from the respiratory tract by the virtue of laghu (~lightness), ruksha (~dryness) and Kapha-vata shamaka properties.

Rasna is the main component of Maharasnadi kvatha, having potent analgesic, antiphlogistic and anti-pyretic properties, well known in the treatment of rheumatism and arthritis [18].

Guduchi, by activating the Macrophages it does shows immuno-modulatory effects, as macrophages are known to represent the first line of defence against invading microorganisms or in a state of altered self [19].

Manjista (Rubia cordifolia) is having jwara (~fever)hara, Kapha pitt shamaka action and researches showing its anti-inflammatory activity [20], Hepato protective effect [21], analgesic effect [22], anti-pyretic effect, anti-viral activity [23] and Nephroprotective effect [24].

TrikatuChurna is one of the digestive tonic for the assimilation of food in the body. It plays an essential role in the treatment of wide variety of conditions, eliminates the aggravated Kapha in the respiratory tract as well as in the digestive channel.

The flavonoids present in the ethanolic extract of Inula racemosa is active in the Type-I allergic conditions because of their ability to inhibit the release of mediators from mast cells and thus influence the course of the disease by preventing the harmful effects of the released mediators and thereby possess in-vivo anti allergic activity [25]. Plants containing flavonoids have been reported to possess antihistaminic, anti-allergic and mast cell degranulation properties [26, 27].

Yastimadhu and Amalaki both are well known for its rasayana (rejuvenating) action. Even though Yastimadhu is Madhura (sweet) in rasa its possessing kanthya effect by the virtue of Anti-inflammatory, immuno-modulatory, Anti-tussive activity [28]. Glycyrrhizan from Glycyrrhiza glabra potentiates the reticuloendothelial system, enhances immunostimulation [29].

According to the principles of Ayurveda, drugs possess augmentation in their properties when similar property of drugs used in combination. A Septilin Drops is such formulation which is having drugs of Kapha-vata shamaka (~ Sabsides Kapha and Vata Together), Ushna Veeryal (~Hot potency) properties. Hence, in present study we have made an attempt to find out the phyto-chemicals in the formulation. So that to correlate the efficacy of formulation with clinical relevance.

The colour of the Formulation attributed to the Guggulu, being main ingredient and sweetish due to Sugar, which is added to the formulation to reduce the Teekshanata of extracts of drugs as well as for good palatability in children.

In phyto-chemical analysis, shows low pH (4.77) i.e. acidic in nature which is helpful in combating micro-organisms like bacteria, viruses. The
refractive index, total solids and specific gravity are under normal limits of sugar based formulations.

The preliminary phytochemical analysis of Septilin Drops was showed the presence of sugars, tannins, saponins, flavanoids, glycosides, proteins and alkaloids.Further, multiple compounds were detected in the thin layer chromatographic analysis of chloroform extract of Septilin Drops, which having their Rf values: 0.5, 0.58, 0.67, 0.76, and 0.82 in long wave and: 0.43, 0.5, 0.62 and 0.68 in short wave by using mobile phase as Toluene:Ethyl acetate:Methanol(7:3:1) as in Figure 1.

Upper respiratory infections are very common due their high incidence in paediatric age group and recurrence also is more because of low immunity. Currently available options of treatment for the management of Upper respiratory tract infections are Decongestants, analgesics and Antibiotics. However, these agents show certain limitations, either due to poor clinical efficacy, drug resistance or due to the compliance issues. Furthermore, these drugs are unable to prevent recurrence. Septilin drops is a polyherbal formulation having tannins, alkaloids and rich in chemical constituents and showed anti-inflammatory and immuno-modulatory activity\(^4\), is best fit to the above matrix. Wide arrays of natural products from botanicals are traditionally in use over several hundred years. Plant kingdom is a gold mine for novel and affordable immunomodulation acting through novel mechanisms against pathogens.\(^{10}\) To overcome, ailments natural formulations are widely used. Hence the above formulation was come into presence.

CONCLUSION

Qualitative assessment in Septilin Drops by different tests reveals the presence of Sugars, Tannins, Alkaloids, Saponins, Flavanoids, Proteins and Glycosides. The resolution of multiple spots on TLC indicates the richness of different chemical constituents within it.

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