

## ANTIMICROBIAL ANALYSIS OF POLYHERBAL FORMULATION LINKUS COUGH SYRUP

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### ABSTRACT

The Herbal products has widely use throughout the globe since 5,000 years. The objective of this study is to determine the antimicrobial analysis of Polyherbal formulation Linkus cough syrup and its comparison with the other antibiotics recommended. Linkus syrup tested for anti-microbial activity against Staphylococcus Aureus, Streptococcus pyogenes, Salmonella and Pseudomonas Aeruginosa. Mc Farland principles were adopted for maintaining the turbidity in bacterial microorganism. TSA is recommended for clinical laboratories for susceptibility testing procedures and maintenance of microbial culture. Antimicrobial testing were performed on agar and well diffusion method. For agar disc diffusion method, the disc were 0.7 cm

and well was prepared with the help of a cork-borer were 0.85 cm. The results of the study showed that the linkus cough shows strong sensitivity against Staphylococcus Aureus, Streptococcus pyogenes and Salmonella in both disc and well diffusion method and showed resistivity against Pseudomonas Aeruginosa. However with the comparison of amoxicillin and ampicillin, linkus showed more sensitivity against Salmonella and Streptococcus pyogenes. The poly herbal blend of Linkus syrup can be used as an anti-microbial agent on cough and associated symptoms related to upper respiratory tract infections.

**KEYWORD:** Antimicrobial analysis, polyherbal formulation.

## 1. INTRODUCTION

Including other diseases, infectious diseases are the foremost cause of death worldwide.<sup>[1]</sup> Antibiotics resistance has increasing throughout the globe.<sup>[2]</sup> Cough is the severe seasonal symptoms in children.<sup>[3]</sup> It cause unadorned discomfort for parents and children both. For preventing the distress, over the counter medications are using frequently.<sup>[4]</sup> For taking the serious concern about safety, the food drug administration and health Canada amend the positioning of the product in 2008.<sup>[5,6]</sup> A Cochrane meta-analysis include 25 clinical studies having 8 pediatric clinical trial in which 4000 individuals and more than 600 children inducted. This review assessed the safety concerns of over the counter medications in ambulatory care setup. No evidence found to be against on over the counter medication either pediatric and adult population with different associated symptoms of cough.<sup>[7]</sup> Beside all the achievement in modern science and technology, traditional medicine have shown minimum side effects.<sup>[8]</sup> The traditional medicinal practice is renown in health care division both in rural and urban areas of Pakistan.<sup>[9]</sup> OTC most selling product cough should be with experimental support against the indication.

The dilemma of antimicrobial resistance is increasing all around the world especially in developing countries and the risk burden is continuously upsurge. The multidrug resistance therapies, antifungal and antimicrobial uses increase the development of new therapies for decreasing the disease burden.<sup>[10]</sup> Traditionally use herbs and medicines are the excellent source of antimicrobial agents.<sup>[11]</sup> Linkus is the outstanding blend of poly herbal formulation include *Alpinia galangal*, *Adhatodavasica*, *Hyssopus officinalis*, *Glycyrrhizaglabra*, *Piper longum* and *Viola odorata*. Published studies supported the ingredients present in the formulation on against microbial activity.<sup>[12-14]</sup> Lack of the data found on antimicrobial activity on herbal formulation present in the herbal traditional market. Due to the reference, current study has establish on evaluation of antimicrobial activity on poly herbal formulation Linkus syrup.

## 2. MATERIAL AND METHOD

### 2.1. Apparatus

High-efficiency particulate arrestance (HEPA) filter used with air cleaning specification, ISO 4644-1(Class 3), EC-GMP (A), FS-209E (Class 1) with H 14 (>99.999%) efficacy, Sanyo lab Auto clave used with MLS-3780-SV, Japan. Vertical pressure steam sterilizer with vessel volume 35L (Working pressure 0.22 Mpa and temperature 134C), Oven 55 LTR (MMM)

Germany with 5C above ambient temperature up to 250 C (stainless steel, mat No.14301 (AISI 304), Incubator 55 LTR (MMM) Germany with 5C above ambient temperature up to 70.0 C/99.9 C,. Laminar flow with H14 filters with micromesh downstream, lightning >800 lux, Volumetric flask, metallic borer, Petri dish and all glass ware were type A pre sterilized. Temperature tank (Digital constant) with RPM 60-230 +10 by Germany used in experiment.

## 2.2. Test microorganism

For the establishment of antimicrobial activity, Gram positive organisms were the part of the experiment including *Staphylococcus Aureus*, *Streptococcus pyogenes* and *Salmonella*. However the gram negative bacterial culture was *Pseudomonas Aeruginosa* used for the polyherbal antimicrobial activity. All the cultures were obtained from Dr. Ehsanullah Lab.

## 2.3. Reference Adjustment

McFarland principles were adopted for maintaining the turbidity in bacterial microorganism. For preparing the standards, Barium chloride and sulfuric acid were added for obtaining the precipitates of barium Sulphate by maintain the bacterial quantity inside suspension. For matching the bacterial cell density ( $1.5 \times 10^8$  CFU/ml), BCl 0.5 ml and H<sub>2</sub>SO<sub>4</sub> 99.5ml were taking to make 100 ml. Absorbance have been noted by Spectrophotomer (Spekol 2000 series, Analytikjena) (0.124 AU).<sup>[15,16]</sup>

## 2.4. Remel Tryptic Soy Agar (TSA) for qualitative procedure

TSA is recommended for clinical laboratories for susceptibility testing procedures and maintenance of microbial culture.<sup>[17-20]</sup> By provided specification (OXOID, USA) medium were prepared and substance were inoculated after sterilization. Culture were prepared at 36°C ± 1°C over nightly and diluted with sterile physiological solution (108 CFU/ml).

## 2.5. Antimicrobial Assay

The antimicrobial assay was performed by two methods viz. Agar Disc Diffusion Method and Agar Well Diffusion Method. Before the microbial assay we performed the sterility test.

## 2.6. Sterility test

The sterility test performed on, MacConkey Agar, & Brain Heart Infusion Broth i.e. inoculated with the given samples and poured into the Petri plate & by streaking on Blood Agar plate and place in an incubator for incubation at 37 c for 48 hr. After incubation no microbial growth observed on plates.

### 2.7. Agar Disc Diffusion Method

The molten Mueller Hinton agar was inoculated with 100 µl of the inoculums (1 x 10<sup>8</sup> cfu/ml) of (*Staphylococcus Aureus*, *Streptococcus pyogenes*, *Pseudomonas Aeruginosa*, *Salmonella*) and poured into the Petri plate (Hi-media). For agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 10 µl (0.6mg) of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate.

### 2.8. Agar Well Diffusion Method

For antimicrobial analysis agar well diffusion method were performed.<sup>[21,22]</sup> A well was prepared in the plates with the help of a cork-borer (0.85 cm). 100 µl (6 mg) of the test compound was introduced into the well. The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of zone of inhibition. The result was obtained by measuring the zone diameter. The experiment was done three times for mean values.

## 3. RESULTS

Linkus is the excellent blend of polyherbal formulation for cough and related symptoms. The main herbs of Linkus syrup is mentioned in below table 01. The mentioned herbs were using for cough since decays. For maintaining the flavors acceptability and palatability have established with the help of sugar, peppermint oil, citric acid and clove oil.

Antimicrobial activity of the Linkus was evaluated on different microorganisms included Gram positive and gram negative. The method which was adopted for antimicrobial analysis was well and disc diffusion method as shown in figure no 1. Sensitivity on both methods are clearly shown in the below diagram.

The test micro-organism included *Staphylococcus Aureus*, *Streptococcus pyogenes*, *Salmonella* and *Pseudomonas Aeruginosa* was checked on Linkus cough syrup. The antimicrobial analysis was performed on sample A and B. Both test drugs became sterile on auto-clave. Gram positive micro-organisms included *Staphylococcus Aureus*, *Streptococcus pyogenes* and *Salmonella* however gram negative micro-organisms included *Pseudomonas Aeruginosa* were appraised as shown in table 2 and 3. On every sample analysis (test product A & B Linkus), the test was performed 3 times for minimizing the human errors on both disc and well diffusion method. The test drug Linkus is compared with the given MIC concentration of standard amoxicillin and ampicillin. As it Figure number 01 Linkus shows

more sensitivity on *Streptococcus pyogenes* and *Salmonella* as compared to the other marked generic which is recommended for the upper respiratory tract infections and cough. However it was evaluated that *Pseudomonas Aeruginosa* showed resistant in both disc diffusion and agar method.

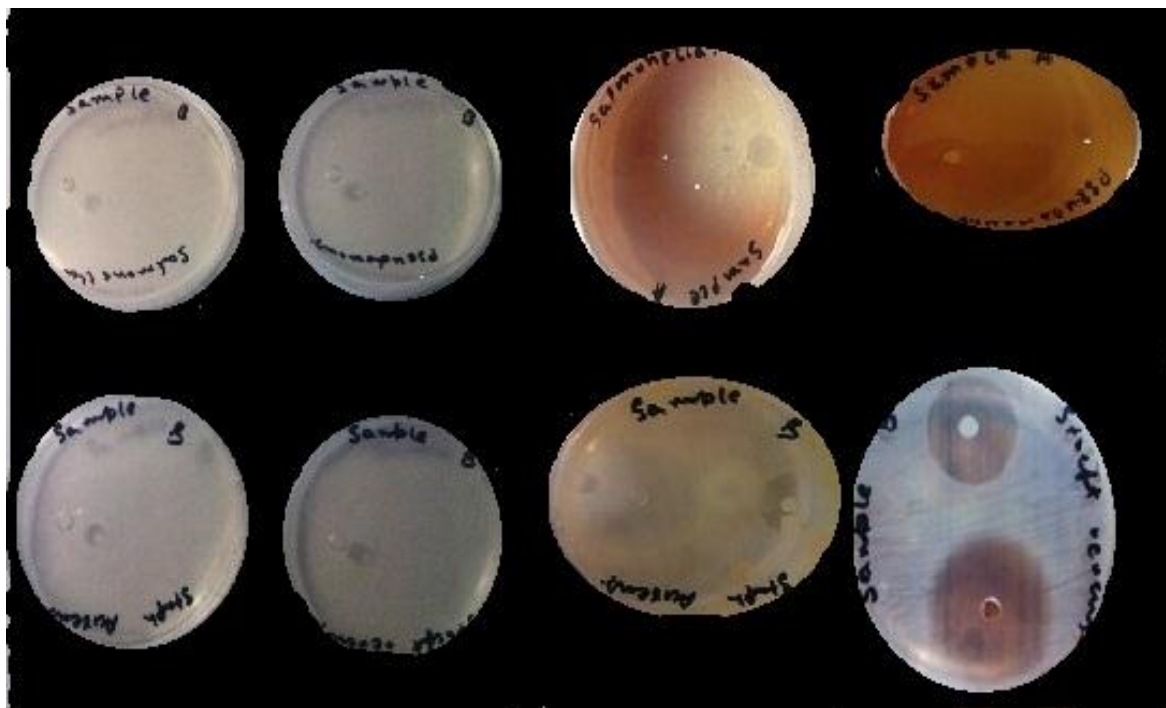


Figure 1: Zone of Anti-microbial inhibition of Linkus verses Ampicillin and Amoxicillin.

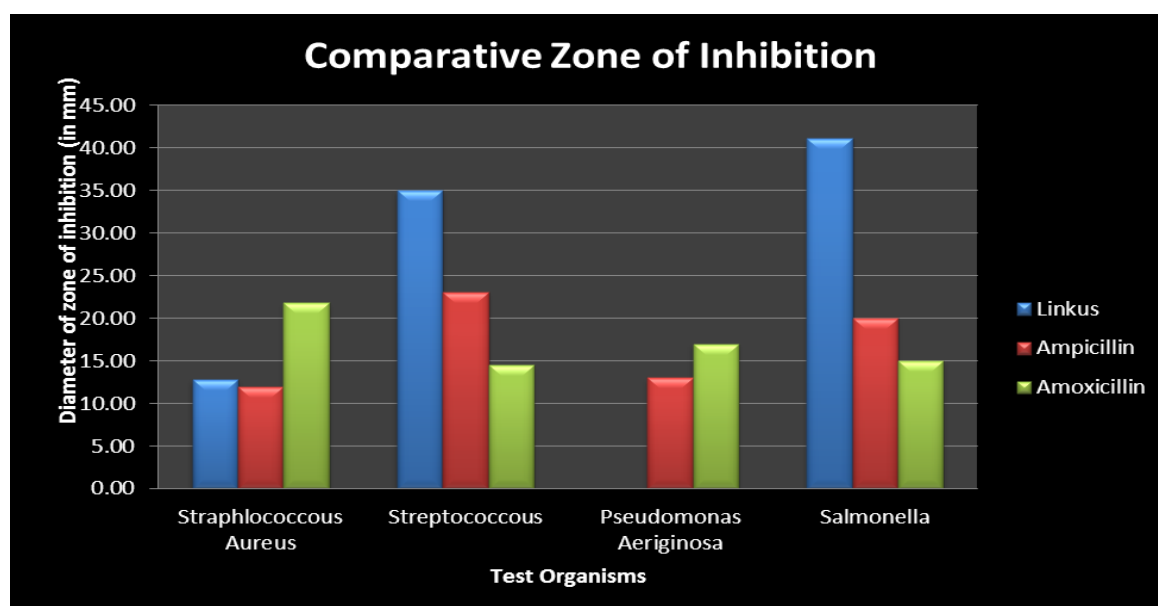


Figure 2: Comparative Zone of Anti-microbial inhibition of Linkus verses Ampicillin and Amoxicillin.

**Table 1: Content of Linkus Syrup and its anti-microbial effects**

S. NO	INGREDIENTS	QUANTITY / 120 ml	ANTIMICROBIAL ACTION
1.	Adhatodavasica – Bansa	7.2 g	Work against Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumonia <sup>[23]</sup>
2.	Piper longum – FilfilDaraz	1.2 g	Activity against S. aureus, E. coli, B. megaterium, B. sphaericus, B. polymyxa. <sup>[24,25]</sup>
3.	Cordialatifolia – Sapistan	1.2 g	Work against Staphylococcus aureus. <sup>[26]</sup>
4.	Glycyrrhizaglabra – Mulethi Extract	900 mg	Works against S. aureus, E. faecalis, and E. coli, A. viscosus and S. sanguis <sup>[27]</sup>
5.	Hyssopusofficinalis – Zufa	600 mg	Act against S. pyogenes, S. aureus, C. albicans and E. coli. <sup>[28]</sup>
6.	Alpinia galangal – Khulanjan	600 mg	Work against Escherichia coli, Salmonella enteritidis, Clostridium perfringens, Staphylococcus aureus, Campylobacter jejuni, Bacillus cereus and fungi such as Saccharomyces cerevisiae, Hansenula anomala, Mucormucedo and Candida albicans. <sup>[29]</sup>
7.	Onosmabracteatum – Gaozaban	1.2 g	S. aureus, P. aeruginosa, E. coli and S. pneumonia. <sup>[30,31]</sup>

**Table 2: Zone of Inhibition on Sample A (Linkus Cough Syrup).**

Sample "A"								
Sterility Test		Product is sterile.						
S.No	Staphylococcus Aureus		Streptococcus pyogenes		Pseudomonas Aeruginosa		Salmonella	
	Well Diffusion Method	Disc Diffusion Method	Well Diffusion Method	Disc Diffusion Method	Well Diffusion Method	Disc Diffusion Method	Well Diffusion Method	Disc Diffusion Method
	100ul (6 mg)	10ul (0.6 mg)	100ul (6 mg)	10ul (0.6 mg)	100ul (6 mg)	10ul (0.6 mg)	100ul (6 mg)	10ul (0.6 mg)
1	27mm	12mm	26mm	17mm	0 (R)	0 (R)	41mm	0 (R)
2	26mm	11mm	37mm	16mm	0 (R)	0 (R)	42mm	0 (R)
3	25m	11mm	35mm	16mm	0 (R)	0 (R)	43mm	0 (R)

Table 3: Zone of inhibition on Sample B (Linkus Cough Syrup).

Sample "B"								
Sterility Test		Product is sterile.						
S.No	Staphylococcus Aureus		Streptococcus pyogenes		Pseudomonas Aeruginosa		Salmonella	
	Well Diffusion Method	Disc Diffusion Method	Well Diffusion Method	Disc Diffusion Method	Well Diffusion Method	Disc Diffusion Method	Well Diffusion Method	Disc Diffusion Method
	100ul (6 mg)	10ul (0.6 mg)	100ul (6 mg)	10ul (0.6 mg)	100ul (6 mg)	10ul (0.6 mg)	100ul (6 mg)	10ul (0.6 mg)
1	26mm	10mm	37mm	18mm	0 (R)	0 (R)	40	0 (R)
2	25mm	10mm	38mm	16mm	0 (R)	0 (R)	40	0 (R)
3	27mm	10mm	37mm	18mm	0 (R)	0 (R)	41	0 (R)

## DISCUSSION

Alternative and herbal medications are widely used all over the world. Herbs all over the world including *adhatodavastica*, *Onosmabracteatum*, *Piper longum*, *Cordialatifolia*, *Alpinia galangal*, *Hyssopusofficinalis* and *Glycyrrhizaglabra*<sup>[23-31]</sup> all are works against microbial activity specifically *Staphylococcus aureus*, *Streptococcus pyogens*, *Salmonella* and *P. aeruginosa*. The composition of Linkus syrup based on above mentioned herbs with active constituents contains vasicinone, vasicine and glycyrrhizin which helps against cough, common cold and URI (upper respiratory tract infections) and serve as an expectorant.<sup>[32-35]</sup> *Piper longum* actively contains *piperlonguminine*, *piperine* and *piperlongumine* helps for cough and other URI.<sup>[36]</sup> *Hyssopusofficinalis*, *Alpinia galangal*, *Zingiberofficinale*, *Cordialatifolia* are used to control coughing and respiratory tract infections.<sup>[37-41]</sup> Cough is very frequent symptoms in all age groups and has been missed used all over in the world. The specifications and authenticity should be address. The poly herbal linkus syrup have proved its potential on anti-microbial activity against *Staphlococcous Aureus*, *Streptococcous pyogenes*, *Salmonella* and *Pseudomonas Aeruginosa*.

## CONCLUSION

The poly-herbal Linkus syrup exhibit excellent properties against pathogenic microbes therefore these herbs base (Linkus) able to be used as antimicrobial agent for treatment of various infectious diseases of gastrointestinal tract. Furthermore additives that are present in these formulations and the relevance of heat in the extraction method did not influence the antimicrobial activity.



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