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Evaluation of synergistic antimicrobial activity of Gemifloxacin with *Linum usitatissimum* seed oil

Yogesh Joshi, Ruchika Garg, Divya Juyal*

Yogesh Joshi
Himalayan Institute of Pharmacy & Research, Rajawala, Dehradun, Uttarakhand, India

Ruchika Garg
Himalayan Institute of Pharmacy & Research, Rajawala, Dehradun, Uttarakhand, India A

Divya Juyal
Himalayan Institute of Pharmacy & Research, Rajawala, Dehradun, Uttarakhand, India

Correspondence:
Yogesh Joshi
Head of Department & Assistant Professor, Himalayan Institute of Pharmacy & Research, Rajawala, Dehradun, Uttarakhand, India
Email:
yogeshjoshi1583@rediffmail.com

Abstract

Gemifloxacin and Linseed (*Linum usitatissimum* L.) oil has individual in-vitro antimicrobial activity against a wide range of gram-negative and gram-positive microorganisms. To reduce dose dependent side effects and the development of drug-resistance by an antibiotic when used alone, in accordance with the maintenance of the effectiveness, the alternative approach nowadays is to go for combinations of antibiotic required for the treatment along with any other suitable agent for minimization of the above problems. This study involved an initial approach to evaluate the synergistic antimicrobial effects of gemifloxacin with linseed oil. The physicochemical properties of the oil determined were color, odor, specific gravity, acid value, iodine value and saponification value. Phytochemical analysis revealed that Linseed oil contains fats, flavonoids, glycosides, phenols and tannins. Combined antimicrobial effects of both gemifloxacin and linseed oil were then analyzed in different combinations using the microbial strains of *Staphylococcus aureus*, *Bacillus subtillus*, *Escherichia coli* and *Shigella boyedi*. From the results, it was concluded that linseed oil synergies the antimicrobial potential of gemifloxacin when used simultaneously in various combinations.

Keywords: Gemifloxacin, Linseed oil, Antimicrobial activity, Synergistic.

Introduction

Gemifloxacin is a synthetic broad-spectrum antibacterial agent for oral administration. It is a compound related to the fluoroquinolone class of antibiotics, is available as the mesylate salt in the sesquihydrate form and then called gemifloxacin mesylate. Gemifloxacin kills a variety of bacteria, and prevents their reproduction and is often used for the treatment of infections all over the body. It has shown potent antibacterial activity against clinical isolates and reference strains in both *in vitro* studies and experimental models of infection in animals. Gatifloxacin, gemifloxacin, and moxifloxacin are the newer fluoroquinolones and show excellent in vitro activity against a wide variety of respiratory tract pathogens, many gram-negative aerobic organisms, and *Bacteroides fragilis*. Chemical nomenclature of gemifloxacin is (R,S)-7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid (Figure 1).[1]

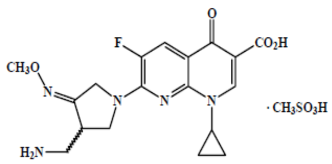

Figure 1: Chemical Structure of Gemifloxacin

Gemifloxacin has *in vitro* activity against a wide range of gram-negative and gram-positive microorganisms. Gemifloxacin is bactericidal with minimum bactericidal concentrations (MBCs) generally within one dilution of the minimum inhibitory concentrations (MICs). Gemifloxacin acts by inhibiting DNA synthesis through the inhibition of both DNA gyrase and topoisomerase IV (TOPO IV), which are essential for bacterial growth. It is rapidly absorbed from the gastrointestinal tract. Binding of gemifloxacin to plasma proteins in healthy subjects is approximately 60 to 70%, and after repeated doses plasma protein binding in healthy subjects from 55% to 73%. Gemifloxacin is metabolized to a limited extent by the liver.[2-4]

Flax (also known as common flax or linseed), with the binomial name: *Linum usitatissimum*, is a member of the genus Linum in the family Linaceae. It is an upright annual plant growing to 1.2 m (3 ft 11 in) tall, with slender stems. The leaves are glaucous green, slender lanceolate, 20–40 mm long and 3 mm broad. The flowers are pure pale blue, 15-25 mm diameter, with five petals; they can also be bright red. The fruit is a round, dry capsule 5-9 mm diameter, containing several glossy brown seeds shaped like an apple seed, 4-7 mm long (Figure 2).[5]

Figure 2: Linseed Plant

Flax is grown for its use as edible oil, as a nutritional supplement, and as an ingredient in many wood finishing products. It is also grown as an ornamental plant in gardens. Flax fibers are used to make linen. Flax seeds come in two basic varieties (Figure 3):

1. Brown.
2. Yellow or golden (also known as golden linseeds).

Most all flax seeds have similar nutritional characteristics and equal numbers of short-chain omega-3 fatty acids while yellow flax called Solin, which has a completely different oil profile and is very low in omega-3 fatty acids. Although brown flax can be consumed as readily as yellow, and has been for thousands of years, it is better known as an ingredient in paints, fiber and cattle feed. Flax seeds produce a vegetable oil known as flaxseed or linseed oil, which is one of the oldest commercial oils, and solvent-processed flax seed oil has been used for centuries as a drying oil in painting and varnishing.[6, 7]

Figure 3: Flax seeds in different color

Linum usitatissimum seeds have been used in the traditional Austrian medicine internally (directly soaked or as tea) and externally (as compresses or oil extracts) for treatment of disorders of the respiratory tract, eyes, infections, cold, flu, fever, rheumatism and gout.[8, 9] *In-vitro* antimicrobial activity of *Linum usitatissimum* fixed oil was evaluated against a number of microorganisms by agar disc diffusion method and MIC determination. The *in-vitro* antimicrobial activity of the oil against Staphylococcus aureus, Streptococcus agalactiae and Escherichia coli was comparable to that of cefoperazone while the antimicrobial activity against Enterococcus faecalis, Micrococcus luteus and Candida albicans, was greater than that of cefoperazone.[10]

In recent years, the uses of antibiotics are most important for the treatment of the various bacterial diseases and disorders. Various side effects arise commonly after the antibiotic use and development of drug resistance also comes as a bigger problem. To reduce dose dependent side effects and the development of drug-resistance in accordance with the maintenance of the effectiveness, the alternative approach nowadays is to go for combinations of antibiotic required for the treatment along with a suitable agent for minimization of the above problems. Therefore, the study was designed to evaluate the synergistic antimicrobial effects of gemifloxacin with *Linum usitatissimum* seed oil.

Materials and Methods

Physicochemical Characteristics of Linseed Oil

Physicochemical characteristics provide a baseline for suitability of oils. The physicochemical properties of the oil determined were color, odor, specific gravity, acid value, iodine value and saponification value.

Phytochemical Screening

Phytochemical screening was performed to test the presence of alkaloids, carbohydrates, tannins, amino acids, proteins, flavonoids, phenols, fats, glycosides etc by chemical methods.

Evaluation of Antimicrobial Activity

Antimicrobial activity was evaluated as a measure of minimum inhibitory concentration (MIC) by determining the zone of inhibition using agar well plate assay method. Standard concentration of gemifloxacin was prepared and different dilutions in the dose range of 0.004-0.16 ml was then evaluated to determine the MIC range of gemifloxacin using the microbes *Staphylococcus aureus*, *Bacillus subtillus*, *Escherichia coli* and *Shigella boyedi*. Standard concentration of flaxseed oil was also prepared and different doses like 0.25, 0.5, 1.0 and 2.0 ml was then evaluated to determine the MIC range of flaxseed oil using the same microbes as used in case of gemifloxacin. Combined synergistic antimicrobial effects of both gemifloxacin and flaxseed oil were then analyzed using same microbial strains.

Results and Discussion

Physicochemical properties of linseed oil showed that the color of oil is yellow-brown with a pleasant odor. Specific gravity of oil was determined to be 0.94 gm/ml. The acid value of linseed oil was determined to be 0.87 mg KOH/gm oil. This value measures of the amount of free fatty acids present and is an indicator for the edibility of oil and suitability for industrial use. The low free fatty acid value suggested that linseed oil is stable. The saponification value was found to be 185.5 mg KOH/gm oil and iodine value was 167.5 gm I_2/100gm oil. The iodine value indicates a high level of unsaturated fatty acid is an asset in nutrition as high content of saturated fatty acids is implicated in cardiovascular diseases (Table 1).

Table 1: Physicochemical Properties of Linseed Oil

S. No.	Physicochemical property	Observation
1.	Color	Yellow-brown
2.	Odor	Pleasant
3.	Specific gravity (gm/ml)	0.94
4.	Acid value (mg KOH/gm oil)	0.87
5.	Saponification value (mg KOH/gm oil)	185.5
6.	Iodine value (gm I_2/100gm oil)	167.5

The linseed seed is good source of nutrition. Phytochemical analysis revealed that Linseed *(Linum usitatissimum L.)* oil contain fats, flavonoids, glycosides, phenols and tannins which could make the plant useful for the synthesis of various drugs of human use (Table 2).

Table 2: Phytochemical Analysis of Linseed Oil

S. No.	Phytochemical Constituent	Test	Result
1	Carbohydrate	Molish test Fehling's test Benedict'test Iodine test	All Negative
2	Protein	Biurete test Millon test	Both Negative
3	Amino acid	Ninhydrin test	Negative
4	Fats and oil	Solubility test	Positive
5	Alkaloid	Dragendroff's test Mayes's test Hagger's test	All Negative
6	Glycoside: Cardiac glycoside Anthraquinone glycosides Saponin glycosides Steroidal glycosides	Legal's test Baljeet test Borntrager's test Foam test Salkowski test	All Positive
7	Phenols/ tannins	Ferric chloride test Bromine water test Gelatin test Lead acetate test Iodine test	All Positive
8	Flavanoids	Shinoda test	Positive

Standard concentrations of gemifloxacin in the dose range of 0.004-0.16 ml were evaluated to determine the zone diameter of inhibition using the microbes *Staphylococcus aureus, Bacillus subtillus, Escherichia coli* and *Shigella boyedi* as a measure of antimicrobial activity as shown in Table 3. Figure 4 showed the bacterial culture plates with gemifloxacin inhibition.

Table 3: Antimicrobial Activity of Gemifloxacin

S. No	Dose (ml)	Selected Microbes (Zone Diameter in mm)			
		Bacillus subtillus	***Staphylococcus aureus***	***Escherichia coli***	***Shigella boyedi***
1.	0.16	14.33±0.58	13.67±0.58	9.33±0.58	12.67±0.58
2.	0.016	12.67±1.15	12.33±1.15	7.00	10.33±0.58
3.	0.032	8.33±0.57	10.33±1.15	5.33±1.15	8.67±1.15
4.	0.08	7.67±1.15	9.00±1.00	2.00	5.00
5.	0.008	5.33±0.58	8.33±1.15	1.00	5.33±0.58
6.	0.04	5.00	7.67±0.58	0.00	2.00

* All values are in mm units and value = mean ± s.d.

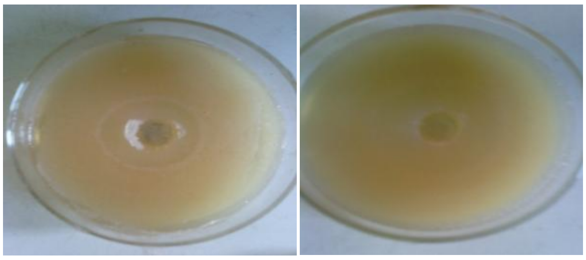

Figure 4: Bacterial culture plates with gemifloxacin inhibition

Standard concentrations of flaxseed oil i.e. 0.25, 0.5, 1.0, 2.0 ml were then evaluated to determine the zone diameter of inhibition using the same microbes as used in case of gemifloxacin as a measure of antimicrobial activity as shown in Table 4. Figure 5 showed the bacterial culture plates with linseed oil inhibition.

Table 4: Antimicrobial Activity of Linseed Oil

S. No	Dose (ml)	Selected Microbes (Zone Diameter in mm)			
		Bacillus subtillus	***Staphylococcus aureus***	***Escherichia coli***	***Shigella boyedi***
1.	0.25	1.00	3.67±0.58	0.00	0.00
2.	0.5	2.33±0.58	4.33±0.58	1.00	1.33±0.58
3.	1.0	4.00	5.67±0.58	2.33±0.58	3.00
4.	2.0	7.33±0.58	6.67±0.58	4.67±0.58	5.33±0.58

* All values are in mm units and value = mean ± s.d.

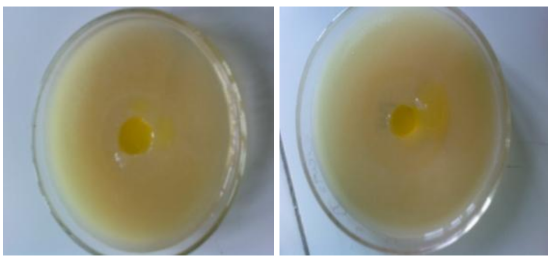

Figure 5: Bacterial culture plates with linseed oil inhibition

Combined antimicrobial effects of both gemifloxacin and flaxseed oil were then analyzed using same microbial strains in different combinations (Table 5). By comparing the zone diameter of inhibition obtained as combined effects with their individual antimicrobial effects, it was found that linseed oil synergizes the antimicrobial potential of gemifloxacin when used simultaneously in various combinations (Figure 6). After increasing the dose of linseed oil in different combinations, results gets improved and evoked out to show the synergistic effects of combination.

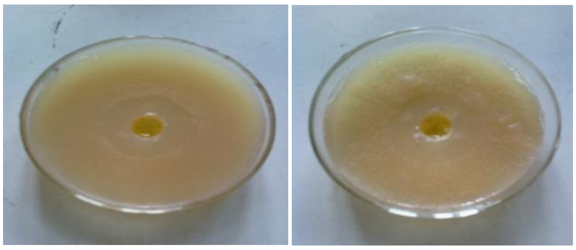

Figure 6: Bacterial Culture Plates with Gemifloxacin and Linseed Oil Inhibition

Table 5: Synergistic Antimicrobial Activity of Gemifloxacin and Linseed Oil

S. No	Drug Dose (ml)	Linseed Oil (ml)	Selected Microbes (Zone Diameter in mm)			
			Bacillus subtillus	***Staphylo-coccus aureus***	***Escherichia coli***	***Shigella boyedi***
1.	0.16	0.5	13.33±1.15	12.67±0.58	8.33±1.15	7.67±0.58
2.	0.016	0.5	12.67±0.58	11.67±1.15	7.33±0.58	7.33±1.15
3.	0.032	0.5	9.33±0.58	10.33±0.58	5.67±0.58	6.67±0.58
4.	0.08	0.5	7.67±0.58	9.33±0.58	2.00	4.00
5.	0.008	0.5	5.33±1.15	7.33±1.15	0.00	1.00
6.	0.04	0.5	4.67±0.58	7.00	0.00	0.00
7.	0.16	1.0	18.67±0.58	20.33±0.58	10.67±0.58	9.67±0.58
8.	0.016	1.0	16.33±1.15	17.67±0.58	8.33±0.58	8.33±0.58
9.	0.032	1.0	11.33±0.58	16.67±0.58	7.33±0.58	7.33±1.15
10.	0.08	1.0	9.33±0.58	12.33±1.15	5.33±0.58	6.33±1.15
11.	0.008	1.0	8.33±0.58	9.67±0.58	2.00	5.00
12.	0.04	1.0	7.33±0.58	8.67±1.15	0.00	2.33±0.58

* All values are in mm units and value = mean ± s.d.

Conclusion

Nowadays, we have been using the various types of medicines and antibiotics for the purpose to prevent the microbial infection or to treat the diseases, but many times these antibiotics are very less effective individually as well as shows various unwanted adverse effects at high doses along with the problem of the development of drug resistance specially for antibiotics. So, to induce potency and reduction of adverse effect, combined antimicrobial approach is the necessity. Finally, it was concluded that linseed oil was individually a good antimicrobial agent and also confirms its potency to synergize the antimicrobial effects when analyzed in combination with gemifloxacin. This study could be a vital path for researchers to go for such kind of combinations and this idea could also be better utilized in future in many aspects of treatment alternatives through in-vitro or in-vivo experiments for the benefit of mankind.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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