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## The antimicrobial vulnerability testing of *Linum flavum* hydrocolloids against pediatric surgical MRSA isolates with qualitative bio-phytochemical analysis quantified by GC-MS-UV-Vis spectrophotometry

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**ABSTRACT**

The primary goal of this review was to decide the adequacy of *L. flavum* medicinal oil hydrocolloids against MRSA by anti-microbial weakness testing and decide its phytochemical properties. *Linum flavum* (Golden Flax seed) is an herbaceous plant that has long been used since ancient past to combat pathogens particularly those caused by bacterial, viral and fungal agents. This study attempted to rediscover the forgotten past by treating the plant derivative in the form of the hydrocolloids obtained from *L. flavum* and testing its antimicrobial vulnerability testing against pediatric surgical isolates of MRSA and comparing its data with the standard antibiotics available on the market. The qualitative Kirby-Bauer disc diffusion techniques and the quantitative standard antimicrobial techniques employed in this study. The qualitative bio-phytochemicals of *L. flavum* hydrocolloids was carried out using standard biochemical tests to detect the presence of phytochemical components, which were then quantified using GC-MS-UV-Vis spectrophotometry. The bio-phytochemical nature of *L. flavum* hydrocolloids was assessed and the results were promising, along with MRSA vulnerability test data, proving to be an asset for the phytol-pharmaceutical industries. This study was to analyze the antibacterial effect of *L. flavum* oil hydrocolloids against pediatric surgical isolates of *Staphylococcus aureus* resistant to

methicillin antibiotics (MRSA). This study was also designed to analyze the bio phytochemical properties of *L. flavum* oil hydrocolloids in order to provide adequate future drug availability for combating dangerous pathogens.

**Keywords:** Antimicrobial, MRSA, GC-MS UV-Vis spectrophotometry, *Linum flavum*, Flax Seed

## 1. INTRODUCTION

*Linum flavum* in the Linaceae family is a perennial herbaceous species possessing  $n = 15$  haploid chromosomes (Dijoux et al., 2006). Colchicine medicines to have caused tetra ploidy, phenotypic changes such as sterility with lower seed oil along with the iodine content in the seeds of *L. flavum* with lower seed size and plant stature in fiber (Nyarko et al., 2012). Thousands of years of differential selection of *L. flavum* have given rise to similar species, fibers and types, but differing greatly in morphology (Sofowora et al., 1984), life structure, physiology and agronomic performance (Hayes et al., 2002). For seed oil extraction the species of *L. flavum* developed in are generally short plants. Taller and less branched, in contrast to types developed for fiber obtained from stems.

Organic produce are five carpel shells that can hold up to 10 seeds. 4 Seeds contain 35-45% oil and 20-25% protein (Kong et al., 2016). *L. flavum* oil has medicinal properties mainly due to its high content of omega-3 alpha-linoleic acid (55-57%) attributed to its unique unsaturated fat structure (Maqbul et al., 2020). Hydrocolloids found in *L. flavum* shown health-promoting properties, including antibacterial properties (Muazzam et al., 2020). The purpose of this study was targeted to analyze the antimicrobial efficacy of *L. flavum* oil hydrocolloids towards the pediatric surgical isolates of *Staphylococcus aureus* which are resistant towards Methicillin antibiotic (MRSA) (Gouse et al., 2017). This study was also designed to analyze the bio-phytochemical nature of *L. flavum* oil hydrocolloids such in order to provide adequate medicine availability to combat dangerous pathogens in the future (Maqbul et al., 2020). Staph microorganisms, as different types of microscopic organisms live as a normal flora causing no problems (Muazzam et al., 2020). However, if these microorganisms become resistant to antimicrobials, they can cause real diseases (Hasson et al., 2009). This is because MRSA strains of staph do not respond well to many basic anti-microbial used to eliminate microscopic organisms (Cavalheiro et al., 2019). When methicillin and other anti-infection agents fail to kill the microbes that cause disease, it becomes increasingly important to eliminate the contamination (Adukwu et al., 2012). When anti-toxins are used repeatedly or incorrectly (Barzani et al., 2016), MRSA microscopic organisms are bound to develop. Given enough time, microbes can evolve to the point where these anti-microbial no longer function admirably (Heydorn et al., 2003). These superbugs emerge as a result of antimicrobial resistance (Muazzam et al., 2022).

The starter biochemical examination was followed by GC MS examinations to assess the bio-phytochemical nature of the *L. flavum* oil, while the antimicrobial movement towards MRSA was accomplished by performing the standard antimicrobial test procedure for the pediatric surgically separated species by contrasting and the standard antimicrobial plates accessible (Muazzam et al., 2019).

## 2. METHODS

### Materials

Pediatric surgical isolates of MRSA from the hospital, Standard Chemical solutions, Standard Microbiological Culture Media, GC Ultra/ISQ Single Quadruple MS, TG-5MS, UV-Vis spectrophotometer, Standard Hi Media and Standard antibiotic discs were used. For these research studies, analytical grade chemicals were used.

### Preparation of seed extract of *L. flavum*

Extraction was performed with ethanol water (70:30, v/v), (CH<sub>3</sub>)<sub>2</sub>CO: Water (70:30, v/v), methanol water (70:30, v/v) and seed powder water, 100 g, 2-liter water soluble, with shaking twice daily for 5 days. After 5 days, supernatants were separated by separation on permeabilized tissue. Excess plants were absorbed in an additional litre of solvent for 5 days, after which the supernatants were pooled and all sample isolates were dried.

### Qualitative bio- phytochemical analysis of *L. usitatissimum*:

The qualitative phytochemical screenings of *L. flavum* concentrates (glycosides, emodins, alkaloids, phenols, flavonoids, steroids, terpenoids, tannins, saponins and anthocyanins) were completed using standard method biochemical test procedures.

**Quantitative bio- phytochemical analysis of *L. flavum*:**

The quantitative analysis study of *L. flavum* achieved by performing the determination of total polyphenols content, assurance of complete flavonoids content and assurance of complete antioxidant agent and the results were recorded for the interpretation.

**Determination of Total polyphenols content**

The Folin-Ciocalteu reagent was used to resolve absolute polyphenols (TP). The alignment bend was created using a standard of arrangement of Gallic corrosive ranging 0.01 to 0.05 mg ml<sup>-1</sup> and apportions were conveyed at 760nm utilizing a UV-Vis spectrophotometer. All investigations were carried out in duplicate. As an alignment standard, Gallic corrosive was used and the results were communicated in milligrams of identical Gallic corrosive dry concentrate.

**Assurance of complete flavonoids content**

The flavonoid content of each concentrate was determined by mixing 0.5 mL of test solution (5 g/L) with 1.5 mL of methanol, followed by 0.1 mL of 10% potassium acetate derivative and 2.8 mL of purified water was estimated using the presented strategy and the mixture was grilled at room temperature for 30 minutes. A spectrophotometer was used to calculate the absorbance at 415 nm. The results were reported in milligrams concentrate (mg QE/g remove). The standard bend was established by quercetin in various concentrations (5-50 mg/L).

**Assurance of complete antioxidant agent**

The cell reinforcement exercises of the (CH<sub>3</sub>)<sub>2</sub>CO (70%), methanol (70%), ethanol (70%) and water separates were surveyed by estimating free extremist searching movement using the free extremist 1,1 diphenyl-2-picrylhydrazyl staining of these solvents (DPPH). Water assemblies of 2 milliliters of (CH<sub>3</sub>)<sub>2</sub>CO (70%), methanol, ethanol (70%) and various concentrations (1–64 g/mL) of each test material and methanol assemblies used as controls. 2 ml of DPPH (25 mg/l) was dissolved in methanol and the reaction mixture was shaken vigorously and left in the dark for 30 minutes. Finally, the absorbance of the mixture was measured against pure methanol at 517 nm using a T80 UV/Vis spectrophotometer. The degree of innovative rooting effect was calculated using the provided formula (%) = ((A<sub>0</sub> - A<sub>1</sub>/A<sub>0</sub>) / (A<sub>0</sub>)). where A<sub>0</sub> represents control absorbance and A<sub>1</sub> represents sample distance absorbance. The half inhibitory fixation esteem (IC<sub>50</sub>) is shown as the powerful grouping of the example required to sSample-7ch half of the DPPH free extremists.

**Recognizable proof of methanol separate utilizing gas chromatography-mass spectrometry (GC/MS)**

GC/MS analysis was performed in a mass range laboratory using a scientific thermos followed by a GC Ultra/ISQ single quadruple MS, TG-5MS, braided, hairy silica segments (30 μm, 0.25 mm, 0.1 mm film thickness) was performed. An electron ionization scaffold with ionization energy of 70 eV was used for GC/MS identification and helium gas applied as the transport gas at a constant flow rate of 1 mL/min. The injector and MS stirring line temperatures were set to 280°C. Ratings of multiple recognized parts were considered relative to percentages. Conditional identification of mixtures was performed using overall maintenance time and mass spectra and NIST correlations, WILLY GC/MS framework library information.

**Antimicrobial vulnerability testing**

Standard antimicrobial circles of limited pediatric surgical specimens for MRSA and antimicrobial susceptibility testing on prepared *L. flavum* extraction plates were performed using the usual standard antimicrobial testing technique, the Kirby-Bauer circular dispersion strategy. Conducted and noted that bacterial detachment was ineffective. Immunized independently on Mueller-Hinton agar plates and incubated next to impregnated plates at 37 °C for 24 h. Results are ordered for understanding. MIC and minimum bactericidal concentration (MBC) values for sufficient antimicrobial transfer to bacteria on *L. flavum* plates were evaluated by performing a standard cylinder decay method. Various conditions of assembly were inoculated for corrosive weakening in peptone water and growth for 24 h at 37 °C to observe the lack of turbidity that determined bacterial susceptibility. Results were ordered for translation. MBC was determined independently of attenuation by immunizing MIC attenuation on a different agar plate for each house. Immunizations were incubated at 37° C for 24 hours and no growth was observed to determine bacterial susceptibility. The MIC and MBC test methods have been reprocessed for standard infection control circles, but are configured differently. Data were cleaned and calculated for interpretation of observations with results.

### 3. RESULTS AND DISCUSSION

Proximate examination of *L. flavum* medicinal oil hydrocolloids performed the normal qualities for the chemical properties by the bio-phytochemical analysis. Each value mentioned in the mean of SD (n=3). The fiber content in *L. flavum* a seed was 23.5%, 34.1% absolute lipid, 19.2% protein, 5.77% debris, 5.02% dampness and 7.47% starch. The dampness in *L. flavum* seed was 7.04% lower in this study. Whereas the debris content in this was 3.72%. In this study, the protein content of *L. flavum* seed was 21.22%, the unrefined fat content of *L. flavum* seeds was 50.2% and the rough fiber content of *L. flavum* seed was 23.5% (Table 1).

**Table 1** Proximate examination of *L. flavum*

Components of <i>L. flavum</i> extract	Percentage
Unrefined lipid	50.2%
Absolute lipid	34.1%
Protein	19.2%
Fiber content	23.4%
Starch content	7.47%
Dampness	5.02%
Residual Debris	5.77%

The presence of phytochemical constituents in seeds was discovered through subjective examination and the results are summarized. It demonstrates the steroids, terpenoids, anthocyanins, emodins, glycosides, flavonoides and phenols in (CH<sub>3</sub>)<sub>2</sub>CO, methanolic, ethanolic and water extracts, but not tannins. Saponins could not be found in ethanol. The inferences of phytochemical components in *L. flavum* extract as demonstrated by a series of qualitative analyses (Table 2)

**Table 2** Presence of phytochemical components of *L. flavum* extract

Qualitative analysis	Inference	Result
Steroids	The upper layer turns red and sulphuric corrosive layer indicated yellow with green fluorescence	Positive
Alkaloids	Creamy Color formation	Positive
Phenols	Development of an extreme tone	Positive
Anthocyanin	Presence of pink-red which goes to blue-violet	Positive
Glycosides	earthy colored ring formation	Positive
Flavonoids	Development of extraordinary yellow shading that got dry on expansion	Positive
Tannins	Development of yellowish tone	Positive
Terpenoids	Arrangements of blue green ring	Positive
Emodins	Red tone appearance	Positive
Saponins	Formation of froth	Positive

As Gallic corrosive counterparts, the total phenolic substance of *L. flavum* separate ranges from (11.5mg-15.5 mg/g) (GAE). Water concentrates and (CH<sub>3</sub>)<sub>2</sub>CO extricate had the lowest complete phenolic acids content (11.5 and 12mg/g, respectively), whereas methanol concentrate and ethanol remove had the highest absolute phenolic acids content (13.5 and 15.5mg/g).

The DPPH technique deployed for antioxidant agent movement of ethanol, methanol and (CH<sub>3</sub>)<sub>2</sub>CO and water concentrates of seeds. The DPPH technique is a consistent natural free revolutionary with an assimilation most extreme band around 515-528 nm and is commonly evaluate the cell reinforcement capability of mixtures (Hadjilouka et al., 2016). The results demonstrate the restraint of *L. flavum* seed separates. Watery methanol (70%) remove had the highest hindrance level of 62.10%, followed by 70% ethanol extricate (48.06%), 70% fluid (CH<sub>3</sub>)<sub>2</sub>CO separate (40.26%) and the H<sub>2</sub>O with 2,6,6-Trimethylbicyclo (3.1.1) hept-2-ene was discovered to be the following fundamental mixtures (10.21%) and hexadecanoic corrosive, ethyl ester (CAS) was recognized as the third constituents (5.67). Furthermore, the fourth component was Phenol, 2, 6-bis (1, 1-dimethylethyl) - 4-methyl-(CAS) (3.0%) and there were various other components under 3.0%.

The data of *L. flavum* methanol extract was scrutinized by employing GC/MS. The data shown seven mixtures as a primary component that were isolated from *L. flavum* extricate and distinguished by GC-MS spectroscopy. The significant compound was identified as octadecatrienoic corrosive, ethyl ester, (Z, Z, Z) - at 24.94%. *L. flavum* is a multi-purpose harvest and its use is beneficial to human health (Muazzam et al., 2020). The current study discovered that methanol is the more effective dissolvable to remove the complete phenolic mixtures and flavonoids from *L. flavum*.

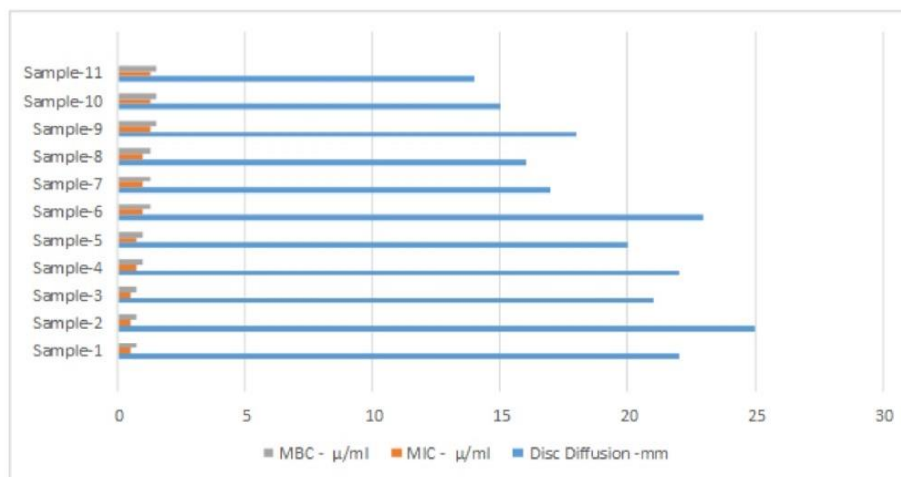
The *L. flavum* essential oil hydrocolloid was found to have a normal circular extension of the 21 mm zone of restriction obtained by performing the Kirby-Bauer strategy with a normal MIC estimate of 0.88/mL and a normal MBC estimate, all showed positive antibacterial exercise results against pediatric surgical isolates 1.13/ml. The best surgical MRSA containment in children was independently observed with a zone spread of 25 mm, an MIC of 0.5/mL and an MBC of 0.75/mL in swab testing of sample 2 against *L. flavum*. The least vulnerable pediatric MRSA surgical isolation study was sample 11 with a zone extension of 14 mm, a MIC of 1.25/mL and an MBC of 1.5/mL in the L direction but outperforms standard synthetic antitoxins in similar instances, except for weakness to vancomycin. Other surgical MRSA specimens are for children B. Sample-6 showed an estimated *L. flavum* zone of 23 mm in the plate distribution method with a MIC of 0.5µ/ml and an MBC of 0.75µ/ml, indicating a defect in removal of *L. flavum*, although the sample test of 3 showed helplessness. In a test demonstrating the vulnerability of samples 1 and 4 to *L. flavum*, we removed *L. flavum* with zone coverage of 21 mm with a circular scattering strategy using an MIC of 0.5µ/ml and an MBC of 0.75µ/ml., zone width in mm. A test, a test of sample 9, a model of sample 10 and a cautious model are shown separately against *L. flavum* with MICs from 0.75 µ/ml to 1.25 µ/ml and MBCs from 1.0 µ/ml to 1.5 µ/ml with zone intervals of 14 mm to 20 mm by the circle spread method showed weakness, which also fundamentally yielded microbial results that were infinitely superior to the standard material style. However, the lack of MRSA pediatric isolates to the standard antidote vancomycin showed surprisingly stable results. The antibacterial effect of *L. flavum* eliminated larger than individual cases, indicating better and more promising susceptibility outcomes (Muazzam et al., 2020). Delayed results of other standard microbial adversary frailty assessments for MRSA infantile protection isolates were much lower than *L. flavum* impotence assessments obtained for retreats. The evaluation found that the vancomycin, most of the standard antidotes showed conflicting results. Because they showed antimicrobial compatibility only for certain models against others, the unreliable impact of all MRSA pediatric breakpoints reached certain contaminations. The standard serum toxin vancomycin and other standard microbial adversaries were found in certain models and the standard immunotoxin methicillin showed safe results in all pediatric MRSA isolates (Benzaid et al., 2019). This evaluation showed that the impact of standard anti-microbial on MRSA pediatric isolation was limited with high MIC and MBC values ranging from 0.5µ/ml to 2.5µ/ml. Therefore, the higher MIC and MBC values suggest that despite the clear separation from the microbial standard foe, even the unprotected version from the specific contaminant foe, the MRSA pediatric contaminant. It reveals the need for another level of disease assessment as a motivation for complete destruction illness. *L. flavum* discredited the standard antidote venom failure assessment because all pediatric MRSA isolates were weak, with zones ranging from 14 mm to 27 mm. Normal MIC and MBC characteristics were significantly lower than standard adversarial MRSA pediatric isolates of the organism, with typical values of 0.88µ/ml and 1.33 µ/ml, respectively. MIC and MBC scores for *L. flavum* ranged from 0.5µ/mL to 1.5µ/mL for all pediatric MRSA products, indicating low estimates of *L. flavum* clearance, unlike those of standard anti-microbial was found to be adequate for children. Complete containment or eradication of the disease (Naika et al., 2010). Thus, a commonly available antibacterial-rich *L. flavum* cleanse showed much better results than standard serum venoms against the resulting MRSA pediatric cleanse (Rios et al., 2005). The information collected for antimicrobial potency testing of pediatric MRSA compounds, including cautious compounds against *L. flavum* hydrocolloids, was very well organized (Table 3 and Figure 1).

**Table 3** Antimicrobial vulnerability testing data of MRSA pediatric surgical isolates towards *L. flavum* hydrocolloids

Sample	Disc Diffusion -mm	MIC - µ/ml	MBC - µ/ml
Sample-1	22	0.5	0.75
Sample-2	25	0.5	0.75
Sample-3	21	0.5	0.75
Sample-4	22	0.75	1
Sample-5	20	0.75	1
Sample-6	23	1	1.25
Sample-7	17	1	1.25
Sample-8	16	1	1.25
Sample-9	18	1.25	1.5



Sample-10	15	1.25	1.5
Sample-11	14	1.25	1.5



**Figure 1** Comparative chart of the antimicrobial vulnerability testing data of MRSA pediatric surgical isolates towards *L. flavum* hydrocolloids

#### 4. CONCLUSION

The antimicrobial vulnerability testing of *L. flavum* hydrocolloids against pediatric surgically isolated MRSA specimens yielded excellent results versus the antibiotics commercially available. Though the commercially available standard antibiotic Vancomycin demonstrated the best data among the other standard antibiotics used against pediatric surgical isolates of MRSA, *L. flavum* hydrocolloids outperformed that data against all pediatric surgical isolates of MRSA. When compared to the standard antibiotic test data, the *L. flavum* hydrocolloids showed promising results against all pediatric surgical isolates of MRSA in terms of disc diffusion zone value, MIC and MBC values. The qualitative bio-phytochemical analysis of *L. flavum* hydrocolloids revealed significant chemical constituents. The qualitative bio-phytochemical analysis of *L. flavum* hydrocolloids revealed significant chemical components, which serve as potential antimicrobial properties. A detailed study was conducted using the quantified technique, which shown enormous biochemical activity of the chemical components, determining the antimicrobial properties of the *L. flavum* hydrocolloids. Thus, it was a minimal attempt to rediscover ancient hydrocolloids used in the past against many ailments, which will be useful in the future for the phytol-pharmaceutical industries in combating many dangerous pathogens. This study's long-term goal is to use these hydrocolloids for post-surgical intervention, particularly in the pediatric population, to keep them from developing infections.

#### Ethical Approval

This research study entitled "The antimicrobial vulnerability testing of *Linum flavum* hydrocolloids against pediatric surgical MRSA isolates with qualitative bio-phytochemical analysis quantified by GC-MS-UV-Vis spectrophotometry" was approved by the Institutional Human Ethics Committee with ethical approval IBC Ref No.H-11-30042019 of the Ibn Sina National College Research Review Board with Protocol identification number 042MP0808652019.

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#### Authors' contributions

All authors have made equal contribution to the work and approved it for publication. MSM: Conception, literature search, design, Supervision and organized the work, and final approval. RNS & FAS: Conception and design of the work, revisions and final approval. AJH & MAS: Writing, critical review of final draft and final approval. SYW, IKA & RGN: Investigation, conceptualization, methodology, wrote the original draft of the manuscript; AMH, WAH & RMA: Conceptualization, methodology, co-wrote and

organized the original draft of the manuscript. MHE & TMA: Methodology, writing, reviewing, aligning and editing. All authors critically review and approve the final draft and are responsible for the content of the manuscript and the similarity index

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This study has not received any external funding.

### Conflict of interest

The authors declare that there is no conflict of interests.

### Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

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