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EVALUATION OF PHYTOCHEMICAL, ANTIBACTERIAL AND ANTIOXIDANT ANALYSIS OF *LINUM USITATISSIMUM* L. SEED EXTRACT

Auxilliya Anthonisamy [*1], Revathi Mathiyazhagan [1], Manonmani Rajasekaran [1] and A. Asha Monica [2]

Department of Botany [1], Holy Cross College, Tiruchirappalli - 620002, Tamil Nadu, India.
Department of Biotechnology [2], St. Joseph's College, Tiruchirappalli - 620002, Tamil Nadu, India.

Keywords:

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Correspondence to Author:
Dr. Auxilliya Anthonisamy

Assistant Professor,
Department of Botany,
Holy Cross College, Tiruchirappalli - 620002, Tamil Nadu, India.

E-mail: ashamonica7@gmail.com

ABSTRACT: Plants have been widely adopted as the source of all drugs; this field has gradually broadened in developing countries where traditional medicine is essential in health care. Flaxseed is the seed from the plant *Linum usitatissimum*, which is an annual plant. This plant has attracted man's attention for its advantages for human health. Plant materials contain a lot of bioactive phenolics, which demonstrate biological activity, including anti-radical, antioxidant, anti-microbial, and anti-cancer effects. Antioxidants are synthetic or natural compounds that can delay some types of cell damage and quench reactive radical species formed during oxidative reactions in metabolism. Our aim is to collect the seed of *Linum usitatissimum L.*, screen the antibacterial activity of the solvent extracts of this plant against some selected bacteria, and identify the bioactive chemical compounds from the plant extracts using preliminary phytochemical analysis. To investigate the antioxidant activity of methanol, ethyl acetate, and chloroform extracts of *Linum usitatissimum L.*

INTRODUCTION: In the past several decades, plant-placed products have played an important role in preventing or reducing disease progression [1]. One of the major topics to be investigated in this field is focused on identifying antibiotics from natural sources. This is due to resistance in pathogenic bacteria against common antibiotics [2]. Chemical analysis of flaxseed averaged 30–40% oil, 20–25% protein, 20–28% fiber, 4–8% moisture and 3–4% ash. Research on flaxseed composition suggests that lignans within flaxseed have a potential role as antimicrobial and antioxidants agent [3]. Recent studies suggest that Lignan extracts was most efficient antibacterial against the Gram positive bacteria [4].

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The overall goal of this work was to investigate new natural compounds with promising antibacterial activities. Flaxseeds, scientifically known as *Linum usitatissimum* L. belong to Linaceae family [5], is an annual or biannual plant, one of the most useful crops, cultivated as a commercial plant in over thirty countries all over the world. It has been cultivated for oil and fiber [6]. Flaxseed is being cultivated in more than 50 countries; the majority of the mare in the northern hemisphere. Canada is the main Flax producer, followed by China, United States and India [7].

Flaxseed is rich in fat, protein and dietary fiber. Chemical analysis of flaxseed averaged 30 to 40% oil, 20 to 25% protein, 20 to 28% total dietary fibre, 4 to 8% moisture and 3 to 4% ash. The oil contains vitamins A, B, D and E, minerals and amino acids, by the presence of physiologically active food components that may provide health benefits beyond basic nutrition [8]. Day by day, the incorporation of flaxseed in of and food products has increased due to its high content of essential

omega-3 fatty acid, alphalinolenic acid (ALA), dietary fiber and natural phenolic antioxidants. Flaxseed is emerging as one of the key sources of phytochemicals [9]. These phytochemicals (phenolic acids, cinnamic acids, flavonoids and lignins) are antioxidants and affect the cell growth and viability. Flaxseed is an essential source of high quality protein and soluble fiber and has considerable potential as a source of phenolic compounds [10]. Plant materials contain a lot of bioactive phenolics, which demonstrate biological activity including antiradical, antioxidant, antimicrobial and anticancer effects. These chemical classes have many beneficial health effects and prevent some chronicle diseases [11]. Natural antioxidants have been associated with cancer development generally opted by consumers because of their safety [12]. Therefore, the identification and the corresponding importance of natural antioxidant sources need to be researched from the stand point of health-improving properties [13].

On the other hand, it was reported that support using natural antioxidant additives decreases oxidative stress levels and chronic disease [14]. Also, it was reported that synthetic antioxidants had shown side effects, including mutagenic, carcinogenic and toxic impacts. For this reason, the usage of these synthetic compounds has been restricted due to their undesired effects [15, 16].

Because of the restrictions, interest has been given to the antioxidants from natural sources. It was well-known that plant constituents have antioxidant activity and free radicals scavenging effects. So, there is an increasing demand in safer and natural antioxidants for food, biological and pharmaceutical systems. Also, there are increasing trends in consumer preference towards natural and safer antioxidants from plant origin [17]. In the present study, we have concentrated on the Phytochemical Screening, antioxidant and antimicrobial activity of *Linum usitatissimum L*.

MATERIAL AND METHODS:

Plant Collection: The Plant seed were collected from an ayurvedic shop in Trichy.

Preparation of Plant Powder: The Plant seeds were ground to a fine powder using an electronic blender, and the powders were stored in a closed container at room temperature for further use.

Plant Extraction: 150 g of dry powder of plant seeds were taken in the 500ml beaker. Around 500ml of 70% ethanol, chloroform, and methanol was added to that and soaked at room temperature 36 hrs.

The final extract was filtered using the normal filter paper, and again the filtrate was filtered through the Whatman filter paper (double filtration process). This was repeated for two more days and similar extracts were pooled together. The final filtrate was subjected to qualitative screening, antibacterial and antioxidant activities

Phytochemical Analysis: The extracts used for antimicrobial screenings were used for all the phytochemical analyses of the present study. The preliminary phytochemical screening was carried out by following Harborne [18].

Antibacterial Screening and Antioxidant Screening: Antibacterial and antioxidant activity was carried out [19].

RESULTS AND DISCUSSION:

Phytochemical Analysis: Phytochemical analyses of experimental plants have shown that the numerous compounds in plants traditionally used for medicinal purposes have chemical properties which are effective at treating the illness. The knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but also for disclosing the new resources of relatively chemical substances.

In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies [20]. The qualitative phytochemical tests revealed the presence of various phytochemicals in chloroform, ethanol and methanol leaf extract of *Linum usitatissimum L*.

Table 1 The methanol and ethanol leaf extract showed a high amount of alkaloids, followed by carbohydrates, glycosides, tannins, phenolic compounds, saponins and steroids.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *LINUM USITATISSIMUM L*

Name of the Chemical Test	Inference		
	Methanol	Chloroform	Ethyl Acetate
Alkaloids detection	+	+	+
Carbohydrate detection	+	+	+
Glycoside detection	-	-	-
Saponins detection	+	-	-
Protein detection	+	+	+
Amino acid detection	-	-	-
Fixed oils and fat detection	+	+	-
Phenolic compounds detection	-	-	+
Lead acetate detection	+	-	+
Flavonoids compounds detection	+	+	+
Terpenoid detection	+	+	+
Lignins detection	-	-	-
Anthocyanin detection	-	-	-
Betacyanin detection	+	+	-

Antibacterial Assay: The *in-vitro* antibacterial activity of methanol, chloroform and ethyl acetate extract of different concentrations of *Linum usitatissimum* L. was evaluated by well diffusion assay against five bacterial pathogens. The bacteria include both Gram-positive and Gram-negative. The antibacterial activity of the plant extracts and their potency were qualitatively assessed by the presence or absence of an inhibition zone and the inhibition zone was measured in diameter (mm) respectively given in **Table 2**. The antibacterial effect was compared with that of the standard broad spectrum antibiotic Gentamicin (30mg/disc).

Agar well Diffusion Method: The data pertaining to the antibacterial potential of the seed extract of *Linum usitatissimum L.* are presented in **Table 2**, **Fig. 1**. The results were observed in terms of inhibition zones around each well or whole caused by diffusion of antibacterial properties from the plant extract into the surrounding medium. As results obtained from **Table 2**, the chloroform seed extract had a strong and broader spectrum of antibacterial activities compared with methanol and ethyl acetate. The results indicated that the chloroform seed extract showed significant antibacterial activities towards the gram-positive and gram negative bacteria followed by methanol and ethyl acetate. In addition to that the zones of inhibition provided by standard antibiotic drug (Gentamicin, 300µg/ml) are also compared with the experimental results. It was observed that the inhibition zones of the plant extracts to be either less than or greater than or equal to the standard antibiotics. The chloroform seed extract showed high degree of inhibition against Enterobacter aero genes (20 mm), whereas moderate antibacterial activity was associated with *Staphylococcus aureus* (19 mm), *Bacillus subtilis* (18mm), *E. coli* (18mm). Low degrees of inhibitions were associated with *Pseudomonas aeruginosa*.

Methanol extract showed a high degree of inhibition against *Enterobacter aerogenes* (18mm) and *Staphylococcus aureus* (17mm), whereas moderate antibacterial activity was associated with *Bacillus subtilis* (16mm) and *E. coli* (16mm). Low degree of inhibition was associated with *Pseudomonas aeruginosa* (12mm). Infections induced by *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Ethylacetate extract also showed high degree of inhibition against *Enterobacter aerogenes* (18mm), *Bacillus subtilis* (14mm) especially those with multidrug resistance are among the most difficult to treat with conventional antibiotics (NNIS, 1999; Abu Shanab, 2004). In our study, the growth of *Staphylococcus aureus* was remarkably inhibited by the chloroform seed extract in which diameter of inhibition zone is more or less equal to the standard antibiotics. Hence, the activity is significant.

In general, the data obtained by the agar well diffusion method revealed that, among the five bacterial species tested *Enterobacter aerogenes* and *Staphylococcus aureus* were the most susceptible bacteria. In addition, *Bacillus subtilis* and *E. coli* were also susceptible to the plant extracts. This is agreed with the susceptibility of the pathogenic bacteria to different plant extracts reported by several researchers [21, 22].

This could be explained according to the cell wall of gram-positive bacteria is less complex and lack in the natural sieve effect against large molecules due to the small pores in their cell envelop that may lead to easier penetration through gram-positive bacterial cell [23, 24].

TABLE 2: ANTIBACTERIAL ACTIVITY OF SAMPLE

Sample	Extract 100µl added and Zone of inhibition (mm/ml)			
	LU-Ethyl Acetate	LU-Chloroform	LU-Methanol	Control
Staphylococcus aureus	14	19	17	20
Bacillus subtilis	14	18	16	20
Enterobacter	18	20	18	20
E. coli	12	18	16	20
Pseudomonas	12	16	12	20

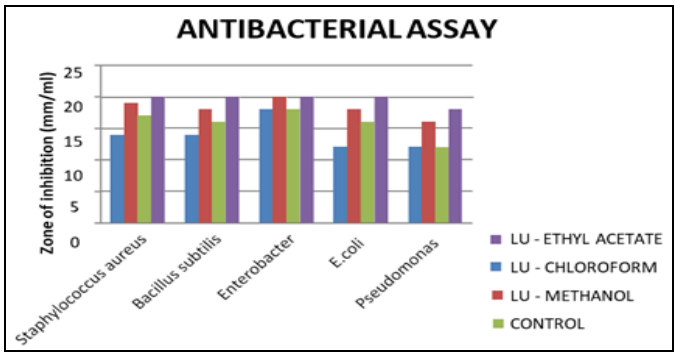

FIG. 1: ANTIBACTERIAL ACTIVITY OF SEED EXTRACT OF *LINUM USITATISSIMUM L.* (WELL DIFFUSION METHOD)

Antioxidant Activity: Antioxidants protect cells against damage caused by molecules known as free radicles. The antioxidant effect of plant extracts are mainly due to the presence of Phenolic compounds such as flavonoids, phenolic acids and tannins.

Numerous studies with Plant Phytochemicals with antioxidant activity may reduce risk of cancer and improve health. Methanolic extract of *Linum usitatissimum* L. and standard ascorbic acid tested for *in-vitro* antioxidant activity using the DPPH–assay method. The IC_{50} was calculated in the graph obtained by plotting the percentage scavenging against concentrations used. The result is shown in **Fig. 2** & **3**. The free radical scavenging effect of extracts and standard on the DPPH was found to be concentration dependent and was in the order of ascorbic>seed extracts. The sample showed the antioxidant activity of IC_{50} value of 88.496±0.076 µg/ml. The IC_{50} value of ascorbic acid was 25.358±0.053µg/ml. The result indicates that the antioxidant activity of the sample is higher than the standard ascorbic acid.

TABLE 3: ANTIOXIDANT ACTIVITY

S. no.	Concentration (µg/ml)	Standard Absorbance	Sample Absorbance	Sample % of DPPH Scavenged	Standard % of DPPH Scavenged
1	20	0.539	0.212	46.192 ± 0.193	0.369 ± 0.027
2	40	0.411	0.156	60.406 ± 0.113	14.391 ± 0.038
3	60	0.284	0.145	63.197 ± 0.069	25.645 ± 0.012
4	80	0.198	0.138	64.974 ± 0.219	41.697 ± 0.055
5	100	0.078	0.125	68.274 ± 1.116	61.254 ± 0.040
IC_{50} value	25.358 ± 0.053µg/ml	88.496 ± 0.07µg/ml			

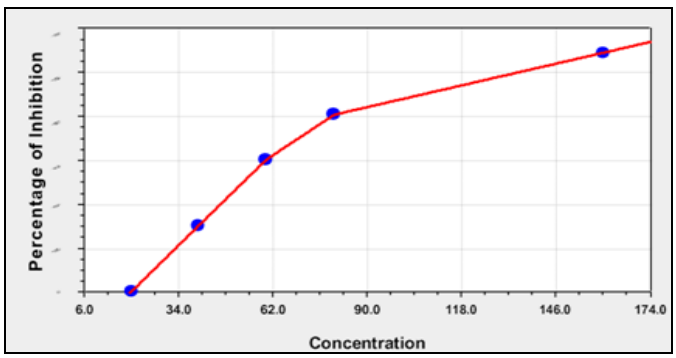

FIG. 2: ANTIOXIDANT ACTIVITY IMAGE OF STANDARD ASCORBIC ACID BY DPPH ASSAY

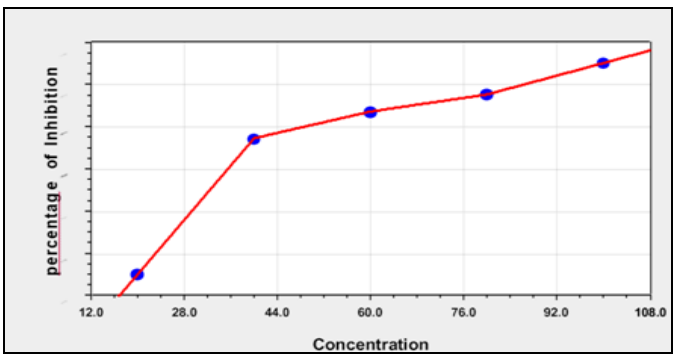

FIG. 3: ANTIOXIDANT ACTIVITY IMAGE OF SAMPLE BY DPPH ASSAY

CONCLUSION: The medicinal properties of plants have been the center of attraction for researchers in recent scientific developments worldwide, due to their potent Phytochemistry, antimicrobial and antioxidant properties. Based upon the results of the present investigation, it can be concluded that Ethyl acetate and methanol are the most effective solvents for recovering phytochemical compounds, antimicrobial and antioxidant components from flaxseed. It is recommended to optimize the extraction efficacy of these solvents using different extraction techniques. The medicinal properties of flaxseed extract may be due to the abovementioned phytochemicals rendering them a potential source for isolating compounds for developing nutraceuticals.

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CONFLICTS OF INTEREST: Nil

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