Study of Antimicrobial Activities and Preliminary Phytochemical Screening on the Seed Extracts of *Linum Usitatissimum*

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Abstract- The present study aimed to determine in vitro antimicrobial activities and phytochemical analysis of Linum usitatissimum which is commonly used as traditional edible oil and ayurved medicine in Betul M.P.,India. Sensitivity of bacterial and fungal species against plant seed extracts were determined using Disc Diffusion method. In this method, an microorganism is placed on agar, it will not grow near the disk if it is susceptible to the chemical. The study also evaluated phytochemical compounds present in the seed Linum usitatissimum. It was revealed that extract from Linum usitatissimum seed extract showed maximum inhibition against selected bacterial isolates Streptococcus mutans i.e. 17 mm, 19mm, 12mm and 23mm, Enterococcus fecalis i.e. 13, 16 18 and 22, Escherichia coli i.e. 13, 16 17 and 19, Streptococcus aeruous i.e. 12,14,20 and 22 and pseudomonas aeruginosa i.e. 16,17,18 and 20 respectively. The preliminary phytochemical analysis revealed presence of various phytochemicals namely alkaloids, flavonoid, Quinones, tannin, terpenoid and Steroids. It was concluded that presence of large number of phytochemicals in the plant seed extracts may be associated with pharmacological properties of G. abyssinica therefore this study provide alternative to synthetic antimicrobial agents.

Indexed Terms- Linum usitatissimum, antimicrobial, phytochemical, microorganism

I. INTRODUCTION

Plants have been an important Source of medicine for thousands of years. Plants produce an amazing variety of metabolites that are gaining importance for their therapeutic and biotechnological applications (Quiroga *et al.,* 2001). The evolution of new genes to synthesize novel secondary products in plants is an ongoing process that might account for most differences in gene function among the plant genomes. Among the thousands of metabolites, only a few are part of the "primary" metabolic pathways and the rest are termed as secondary as they have no specific function in the plants (Pichersky & Gang 2000). Primary metabolites are produced as a result of photosynthesis and are additionally involved in cell component synthesis (Ramawat *et al.,* 2009). The therapeutic use of herbs is as old as human civilization and has evolved along with it. Primary metabolites are involved directly in growth and metabolism, viz. carbohydrates, lipids and proteins. The vast majorities of people on this planet still rely on their indigenous system of medicine and use herbal drugs. The Indian and Chinese systems of medicine are well established with written records going back around 3000 years. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, malaria, cardiovascular diseases and neurological disorders. According to the report of The World Health Organisation, (WHO) 80% of the worlds population rely mainly on the traditional therapies which involve the use of plant extracts or their active substance (World Health Organisation, 1993).

Flax (Linum usitatissimum) plant growing to one m tall. The seeds are oval, 2.5-9.5 cm long and 1-3.5 cm. thin shiny in experienced depilatory with a black and a brief stalk regarding are 1-1.8 cm long seeds of Linum usitatisamum plant are used medication for treatment of Rheumatism, Dyspepsia, stomach upset, Dysmenorrheal, Diabetes, Cardiovascular disease, Cancer, Expelling disorders, Skin diseases, Trauma

symptom and has sedative and antiviral properties. The seeds and alternative Components of Linum usitatissimum plant periwinkle exhibit inhibitor properties. Therefore, phenoplast compounds have chemical reaction properties that act as reducing agents, chemical element donors.It's multiple applications in foods, cosmetics and Pharmaceutical industries. Besides inhibitor activity, these compounds exhibit antiallergic, medicinal drug, antimicrobial antithrombotic cardio protecting and vasodilatory effects.

II. MATERIALS AND METHODS

2.1. Materials

Ethanol (99.7%) purchased from Avantor Perfomance Ltd, India. Dimethyl sulphoxide (DMSO) (99.9%) and ethyl acetate (85.5%) purchased from RFCL Ltd, Haryana, India. Nutrient broth and Nutrient agar were supplied by HIMEDIA Laboratories Pvt. Ltd, India. Five bacterial strains namely Streptococcus aureus (ATCC 29213), Enterococcus fecalis (ATCC 51559), Streptococcus mutans (ATCC BBA-854), Escherichia coli (ATCC 25922), pseudomonas aeruginosa (ATCC 7006) respectively were obtained from the department of Microbiology.

2.2. Extraction process

Studied plant seeds name Linum usitatissimum is collected from Betul districts. The voucher specimens coded GA-001 for Linum usitatissimum is kept at the Department of Chemistry Laboratory, Govt.J.H.CollegeBetul. Collected plant seeds were air dried and crushed. For non-polar and medium polar extraction, crushed samples of 250 g were macerated in Ethanol. Second, dried seeds were coarsely used with a mortar and pestle and then a mechanical blender was used to ground them further. 30gm 340 ml of organic Solution of Methanol & D.W. were collected from the sample extraction at Soxhlet. The extraction was completed in 8 days at 65°c. In order to form a paste, extract were then evaporated at 45^0C and further transfer to sterile and refrigerated once used.

2.3. Determination of antimicrobial activities

In this study, The disc diffusion method (Kirby Bauer *et al*., 1966) was used to test antibacterial activity of the extractives against 5 bacterial species. Grow the culture overnight in liquid broth. Maintain OD at 660 nm (1 x 10 6). Spread the culture on NAM agar medium. Place 6 mm Whatman filter paper. Disc 75 mg / mL dilution of different concentrations of plant extracts (25,50,75,100%). Incubate at 37 ° C for 8 hours. Measure the area of inhibition in mm using the zone scale (Hi-Media). (Daughry et al 2008).

2.4. Phytochemical screening

Preliminary phytochemical screening of the Linum usitatissimum seed extracts were tested for alkaloid, flavonoid, tannin , terpenoid, and Steroids. The choice of these extracts based on their highest potency against antimicrobial activity. The results are indicated as (+) for the presence and (-) for the absence of phytochemicals.

2.5. Test for alkaloid

Few drops of Dragendroff's were applied to a test tube of about 1 ml of extract and colour change was detected. Occurrence of an orange colour was a sign of the presence of alkaloids (Firdouse and Alam, 2011).

2.6. Test for flavonoids

In this test, 2 ml of plant extract was treated with 5 drops of dilute sodium hydroxide (NaOH), followed by diluted hydrochloric acid (HCl). A yellow solution with NaOH turned colorless with dilute HCl indicated existence of flavonoids (Onwukaeme et al.,2007).

2.7. Test for tannin

About 2 ml of tested plant extract was stirred with 3 ml of distilled water and five drops of ferric chloride ($FeCl_3$) were added. Formation of dark blue precipitate was the indication of tannins (Kumar et al., 2007).

2.8. Test for terpenoid

About 5 ml of plant extract was applied to a 3 ml of chloroform and 2 ml of concentrated sulphuric acid (H_2SO_4). The presence of terpenoids was observed by reddish brown colour (Edeoga et al.,2005).

2.9. Test for Steroids

In this test, about 5 ml of tested extract was shaken with 5 ml of distilled water in a test tube. The

formation of foam was considered as an indication of the presence of saponins (Parekh and Chanda, 2007).

2.10. Test for Quinones

About 0.5 gm of plant extract was taken and extra 1 c.c. of extract and 1cc of con. H_2SO_4 was extract formation of red color shows the presence of quinones. One drop of ethanol take a look at resolution is placed on a filter paper, followed by one drop of 0.2% ethanolic phenylacetonitrile resolution and one drop of 0.1 N hydroxide. A positive response is indicated by the appearance of a blue or violet stain edged by a yellow ring.
(Parekh and Chanda, 2007).

III. RESULTS

The antimicrobial activities of Linum usitatissimum seed extrct showed maximum inhibition against selected bacterial isolates Streptococcus mutans i.e. 17 mm, 19mm, 12mm and 23mm, Enterococcus fecalis i.e. 13, 16 18 and 22, Escherichia coli i.e. 13, 16 17 and 19, Streptococcus aeruous i.e. 12,14,20 and 22 and pseudomonas aeruginosa i.e. 16,17,18 and 20 respectively. The observations were comparable with that of other studies. The flavonoids and tannins present in the seed are responsible for their antibacterial properties[Fig.1 table-1].

Zone of inhibition was noted that of 50 and 100 mg/ml should be interpreted as strong antibacterial potential. Zone of inhibition obtained in this study are within the range of what is considered significant for plants and even purified extracts. The study clarified a complex ethno pharmacological picture in terms of extraction. Firstly, results showed the importance of extraction, the method of extraction can also change the results. For the antimicrobial activity, the plant extracts were active against both Gram-positive and Gram negative bacteria. Activity depends from the bacterial strain, the plant organ, their maturation state and the nature of the extraction.

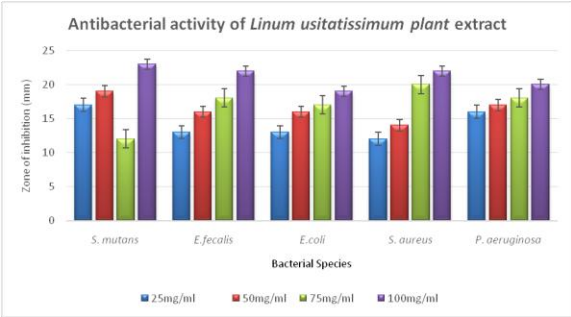

Fig.1: Antibacterial activity of *Linum usitatissimumplant* seed extract

Table.1: Antimicrobial Susceptibility (*Linum usitatissimumplant* seed)

S.No	Sample ID	Concentration			
		25mg/ml	50mg/ml	75mg/ml	100mg/ml
1.	*S. mutans*	17mm	19mm	12mm	23mm
2.	*E.fecalis*	13mm	16mm	18mm	22mm
3.	*E.coli*	13mm	16mm	17mm	19mm
4.	*S. aureus*	12mm	14mm	20mm	22mm
5.	*P. aeruginosa*	16mm	17mm	18mm	20mm

The phytochemical screening of Linum usitatissimum seeds revealed presence of main categories of phytochemical compounds which are alkaloids, flavonoid, Quinones**,** tannin, terpenoid, and Steroids as shown in Table 2.

Table.5: Phytochemical screening of Ethanolic extracts of *Linum usitatissimumplant* seeds

S. No.	**Phytochemical test**	***Linum usitatissimumplant* Seed extract**
1.	Alkaloids	Positive
2.	Flavonoids	Positive
3.	Quinones	Negative
4.	Steroids	Negative
5.	terpenoid	Positive

IV. DISCUSSION

Justification of ethnomedical information of plants used as herbal medicines has been a strategy in the discovery of bioactive phytochemicals (Bonjar, 2004). In spite of this, some plant species have not been screened for their biological capabilities. The antimicrobial screening showed interesting activities at all concentrations: Every extract was active. Plant extracts are generally a crude mixture of no active and active compounds and their Zone of inhibition must be interpreted accordingly.

We noted that Zone of inhibition of less than 50mg/ml should be interpreted as strong antibacterial potential. Zone of inhibition obtained in this study are within the range of what is considered significant for plants and even purified extracts. The study clarified a complex ethnopharmacological picture in terms of extraction. Firstly, results showed the importance of extraction, the method of extraction can also change the results. For the antimicrobial activity, the plant extracts were active against both Gram-positive and Gram-negative bacteria. Activity depends from the bacterial strain, the plant organ, their maturation state and the nature of the extraction.

The ethanolic extract of *Linum usitatissimumplant seed* had promising MIC value against all human bacteria especially *S. mutans, A. viscosus, and E. faecalis*. Although in some studies, it has been reported that *Guizotia abyssinica seed* extract has antibacterial activity against several bacteria such as *S. aureus, E. faecalis,* and *E. coli,* In this report, antibacterial activity of this plant against *A. viscosus* evaluated for the first time.

In the present study, this plant showed antibacterial activity against *S. aureus* but it is interesting that *Linum usitatissimumplant seed* extract did not show any antimicrobial activity when tested against this microorganism in another study (Nirmala *et al.*, 2011). one of the most important substances in this plant, had antibacterial activities against some strains and it was more active against gram positive strains than gram negative. In the present study, seed extract of this plant exhibited the highest MIC value against *E. coli*, so maybe antibacterial activity of *Linum usitatissimumplant seed* against gram positive bacteria was more than gram negative bacteria.

CONCLUSION

The extracts of *Linum usitatissimum* exhibit varying degrees of antimicrobial activities against *S. aureus, E. faecalis,* and *E. coli* Majority of plant extracts possessed antimicrobial potency and therefore the current study supports sustainable utilization of herbal medicine. Additionally, the presence of large number of phytochemicals in these plant seeds, justifies its use for the management of various diseases. In this study, the correlation of some biochemical properties and antimicrobial activity of herbal extracts was observed. However, this experiment was found that no correlation between *E. coli* inhibition and antioxidant activity, but correlated with some chemical properties including acidity and pH, in particular roselle and licorice root extracts. However, isolation of the active phytochemicals and develop antimicrobial drugs from these plants remained unveiled and therefore needs further investigations.

REFERENCES

[1] Anani, K., de Souza, C., Akpagana, K., Tower, G.H.N., Arnason, J.T., Gbeassor, M.,Hudson, J.B.,. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. J. Pharma Biol. 2000; 38 (1), 40–45.

[2] Bhumi G, Savithramma N. Screening of pivotal medicinal plants for qualitative and quantitative phytochemical constituents. Int J Pharm Pharm Sci, 2014; 6: 63-5.

[3] Govindasamy C. and Srinivasan R. (2012) In vitro antibacterial activity and phytochemical analysis of *Linumusitatissimum* (Linn.) G.Don.Asian Pacific Journal of Ttopical Biomedicine,S155-158

[4] Jigna P, Sumitra CV. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol, 2007; 31: 53-8.

[5] Kiran Kumari SP, Sridevi V, Chandana Lakshmi MVV. Studies on phytochemical screening of aqueous extract collected from

fertilizers affected two medicinal plants. J Chem Biol Phys Sci, 2012; 2(3):1326-32.

[6] Mhuji Kilonzo, David Munisi, Antimicrobial activities and phytochemical analysis of Harrisonia abyssinica (Oliv) and Vepris simplicifolia (Verd) extracts used as traditional medicine in Tanzania. Saudi Journal of Biological Sciences , 2021 (28) 7481–7485

[7] SainM.andSharmaV.(2013)*Linumusitatissimum plant* A Review of Potential Therapeutics Properties. Int. J. PureApp. Biosci.1(6): 139-142

[8] Savithramma N, Linga Rao M, Suhrulatha D. Screening of medicinal plants for secondary metabolites. Middle East J Sci Res, 2011; 8(3):579-84

[9] ShaliniS. And SampathkumarP.(2012) Phytochemical screening and anti microbial activity of plant extracts

[10] Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol, 2011; 3(12): 10-4.

[11] Xie, D.W., Dai, Z.G., Yang, Z.M., Tang, Q., Deng, C.H., Xu, Y., Wang, J., Chen, J., Zhao,D.B., Zhang, S.L., Zhang, S.Q., Su, J.G., 2019. Combined genome-wide association analysisand transcriptome sequencing to identify candidate genes for flax seed fatty acid metabolism.Plant Sci.286, 98–107.

[12] Zhang, J.P., Xie, Y.P., Dang, Z., Wang, L.M., Li, W.J., Zhao, W., Zhao, L., Dang, Z.H., 2016.Oil content and fatty acid components of oilseed flax under different environments in China.Agron.J. 108 (1), 365–372.