

Antimicrobial and biochemical properties of *Linum Usitatissimum* the flax seeds and *Syzygium Aromaticum*

Abstract

The current examination was directed to concerning the antimicrobial, anti-fungal and biochemical properties of *Linum Usitatissimum* the flax seeds and *Syzygium Aromaticum* the clove. Flax seeds are one of dietary sources containing significant measure of phenolics named lignans. *Syzygium Aromaticum* ordinarily called clove is generally significant and second important flavor in world exchange and is broadly developed in North Maluku Islands in Indonesia. Glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes, and other phytoconstituents have been shown to have pharmacological actions inferable from their phytoconstituents. *Syzygium Aromaticum* (clove) and *Linum Usitatissimum* (flax) are two common zings that have unique pharmacological effects and have been utilized for food safety. In this investigation the ethanolic, methanolic and refined water concentrate of *Linum Usitatissimum* the flax seeds and *Syzygium Aromaticum* the clove barks were utilized against the 3 bacterial strains and 2 contagious strains. Among the bacterial strains i.e. *E. coli*, *P. syringae* and *B. subtilis*, the best antimicrobial action was appeared by *Syzygium Aromaticum* against the *E. coli*, methanolic and refined water concentrate of *Linum Usitatissimum* and ethanolic concentrate of *S. aromaticum* shows a lot of hostile to microbial action against the *P. syringae*, and against the *B. subtilis* the best enemy of microbial action was appeared by the ethanolic concentrate of the *L. usitatissimum* and methanolic and refined water concentrate of *S. aromaticum*. With respect to the parasitic strains of *A. niger* and *F. solani*, the each of the 3 concentrates of *L. usitatissimum* shows the best outcomes against the *A. niger* and the ethanolic and methanolic concentrates of *S. aromaticum* shows the best enemy of contagious action against the *F. solani* strain. The refined water concentrates of both the plants have no impact against the *F. solani* strain. The phytochemical screening shows the presence of terpenoids, tannins, flavonoids, saponins and cardiovascular glycosides in the two plants.

Keywords: Anti-microbial, Anti-fungal, *Linum Usitatissimum*, *Syzygium Aromaticum*, *F. solani*, *A. niger*, *B. subtilis*, *E. coli*

Introduction

Plants have since a long time ago gave humanity a wellspring of therapeutic specialists, with regular items once filling in as the wellspring, all things considered (El-Maati, 2016). Reliance on plants as the wellspring of medication is predominant in agricultural nations where conventional medication assumes a significant part in medical services (Abo-EL-Sooud, 2018). The provincial populace of a nation is more arranged to conventional methods of treatment as a result of its simple accessibility and less expensive expense (Agostini *et al.*, 2012).

Home grown treatment, albeit still an unwritten science, is grounded in certain societies and customs, and has become a lifestyle in practically 80% individuals in country regions, particularly those in Asia, Latin America and Africa (Alfikri *et al.*, 2020). Therapeutic plants, which structure the foundation of conventional medication, have over the most recent couple of many years been the subject of extreme pharmacological examinations. This has been achieved by the affirmation of the worth of restorative plants as likely wellsprings of new mixtures of remedial worth and as wellsprings of lead compounds in drug advancement (Ali *et al.*, 2013; Ali *et al.*, 2014; Al-Marzoqi *et al.*, 2015).

Clove trees are composed up of leaves and buds (the tree's business component), and blooming buds appear four years after planting. As a result, they're put together by hand or with the help of a proprietary phytohormone during the pre-blooming stage (Ayoola *et al.*, 2008). Cinnamon, oregano, clove, thyme, and mint have been shown to have antibacterial, antiviral, anticarcinogenic, and antifungal properties in a couple of studies. Clove, on the other hand, has sparked a lot of interest among other flavors due to its remarkable antibacterial and malignant growth predictor abilities (Alma *et al.*, 2007). The fundamental oil of *S. aromaticum* buds is broadly utilized in gainful applications, especially in dentistry (Bachiega *et al.*, 2012). The focal oil is compelling against oral microscopic organisms that cause dental pits and periodontal sickness, just as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Salmonella enteritidis*, *Campylobacter jejuni*, and *Staphylococcus aureus* (Banerjee *et al.*, 2020; Batiha *et al.*, 2019; El-Saber Batiha *et al.*, 2020; Batiha *et al.*, 2020).

In Korea, clove oil (*Syzygium aromaticum*) is widely used as a fragrance and spice, as well as a pharmaceutical for the treatment of asthma and other negatively vulnerable conditions, and in clinical dental treatments as a general sterile (Chaudhry *et al.*, 2012). Clove oil has antimicrobial properties against a variety of infectious microorganisms, including those that cause urogenital illness. Clove oil be discovered to have potent antifungal activity in case of pathogenic bacteria like *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*. Eugenol from cloves is the primary component responsible for its antifungal activity (Chaves *et al.*, 2011; Condò *et al.*, 2020; Cortés-Rojas *et al.*, 2014; de Oliveira *et al.*, 2020).

Linseed has a long history of medicinal work, with its primary effects being diuretic as well as medicine that alleviates bothered tissues, controls hacking and diminishes torment moreover of being pain relieving, demulcent, emollient, purgative, pectoral and resolvent (de

Souza *et al.*, 2020; Saeed *et al.*, 2021; Carvalho *et al.*, 2021; El-Kady *et al.*, 2019; Abdalla *et al.*, 2016). This makes flaxseed oil extremely helpful for deadness and shivering just as for forestalling genuine nerve sicknesses such as Parkinson's and Alzheimer's infection. This exceptionally valuable in curing knowledge affiliated issues, for example, ADHD, Bipolar confusion, melancholy, menopausal indications (Faujdar *et al.*, 2020; Goel & Makkar, 2012; Goodrich *et al.*, 1984). Several studies looked into whether phytoestrogens derived from flaxseed can effectively stimulate estrogen production in MCF7 breast cancer cells. It additionally noticed a guidance of In MCF7 cells following treatment, receptor (Er β) articulation and PR articulation were down-guided. The impact of flaxseed proteins on a variety of gramme positive and gramme negative microbes has been studied (Gülçin *et al.*, 2012)

Material and Methods

Sample collection

The both plants *Linum Usitatissimum* and *Syzygium Aromaticum* are widely used spices and are available in every home and departmental store. The both samples are taken and grinded to the powdered form.

Extracts of plant material

Ethanollic, methanollic and distilled water extracts of the *Linum Usitatissimum* and *Syzygium Aromaticum* were obtained by soaking 20g of the powdered sample in 100 ml of the solvent. After 2 days the soak samples were filtered and the filtrate was concentrated by using rotary apparatus. The remaining thick solution after rotary will be taken out in petri dishes and left on the shelf so that the remaining solvent will evaporate and pure extract is obtained. Now on the petri dishes there will be the solid dried material left which will be converted to semi solid mass by the addition of few drops of DMSO (Dimethyl sulfoxide). Then the concentrated extract was stored in the Eppendorf's for further use.

PHYTOCHEMICAL SCREENING

The qualitative chemical tests for terpenoids, flavonoids, saponins, tannins and cardiac glycoside were performed for each extract of both plants by using standard procedures (Ayoola *et al.*, 2008).

Anti-microbial activity

For the anti-microbial testing the nutrient agar media was made by dissolving 5g peptone, 8g NaCl, 3g yeast extract and 15g agar in 1ltr distilled water. The media was poured in petri

dishes and all the petri dishes were placed upside down once the media was solidified. Now by using the disc diffusion method, the plates were streaked, disc were placed and the extracts were poured in the amount of 5µl, 10µl and 15µl and then incubated in upside down position for 48 hrs. The zone of inhibition was measured on all the plates and the results were recorded.

Results

The table 1 shows the phytochemical screening of the concentrates of *Syzygium Aromaticum* and *Linum Usitatissimum*. In the methanolic concentrate of fennel terpenoids, flavonoids, tannins, saponins and cardiovascular glycosides were available. In the ethanolic concentrate of fennel terpenoids, flavonoids, tannins and saponins, heart glycosides were available. Table 1 in the refined water concentrate of flax terpenoids, heart glycosides, tannins and saponins were available yet no flavonoids. In the ethanolic concentrate of clove terpenoids, flavonoids and saponins were available however no tannins and heart glycosides. In the methanolic concentrate of clove terpenoids, flavonoids, heart glycosides, tannins and saponins were available yet not present in flavonoids test 2. In the refined water concentrate of clove terpenoids, saponins, tannins and heart glycosides were available however there were no flavonoids.

Table 1. Phytochemical constituents of ethanolic, methanolic and distilled water extract of *Linum Usitatissimum* and *Syzygium Aromaticum*

TESTS	Flax seed Methanolic Extract	Flax seed Ethanolic Extract	Flax seed dH ₂ O Extract	Clove Methanolic Extract	Clove Ethanolic Extract	Clove dH ₂ O Extract
Terpenoids	+	+	+	+	+	+
Flavonoids (Test1)	+	+	+	+	+	—
Flavonoids (Test 2)	+	+	—	—	+	—
Saponins	+	+	+	+	+	+
Tannins	—	—	—	+	+	+
Cardiac glycoside	+	+	—	+	+	+

Among the bacterial strains i.e *E. coli*, *P. syringae* and *B. subtilis*, the best antimicrobial activity was shown by *Syzygium Aromaticum* against the *E. coli*, methanolic and distilled water extract of *Linum Usitatissimum* and ethanolic extract of *S. Aromaticum* shows much anti-microbial activity against the *P. syringae*, and against the *B. subtilis* the best anti-microbial

activity was shown by the ethanolic extract of the *L. Usitatissimum* and methanolic and distilled water extract of *S. Aromaticum* (table 2 and 3).

Table 2. Anti-bacterial activity of Ethanolic, methanolic and distilled water extract of *Syzygium Aromaticum*

Micro- organism	Ethanolic extract			Methanolic extract			Distilled water extract		
	5µl	10µl	15µl	5µl	10µl	15µl	5µl	10µl	15µl
<i>E. coli</i>	1.96cm	2..9cm	2.7cm	2.23cm	2.63cm	2.7cm	0.9cm	1.3cm	1.5cm
<i>P. syringae</i>	1.53cm	1.2cm	1.93cm	1.3cm	1.63cm	1.93cm	1.26cm	1.7cm	1.86cm
<i>B. subtilis</i>	1.4cm	1.63cm	1.96cm	1.25cm	1.33cm	2.46cm	1.36cm	1.76cm	1.66cm

Table 3. Anti-bacterial activity of ethanolic, methanolic and distilled water extract of *Linum Usitatissimum*

Micro-organism	Ethanolic extract			Methanolic extract			Distilled water extract		
	5μl	10μl	15μl	5μl	10μl	15μl	5μl	10μl	15μl
<i>E. coli</i>	0.76cm	0.8cm	0.96cm	0.93cm	1.4cm	.96cm	1cm	1.03cm	0.9cm
<i>P. syringae</i>	1.36cm	1.66cm	2.6cm	1.26cm	1.46cm	1.48cm	1.13cm	1.30cm	1.46cm
<i>B. subtilis</i>	0	0.99cm	1.03cm	0.86cm	1.03cm	1.7cm	0.6cm	0.74cm	1.2cm

As for the fungal strains of *A. niger* and *F. solani*, the all 3 extracts of *Linum Usitatissimum* shows the best results against the *A. niger* and the ethanolic and methanolic extracts of *Syzygium Aromaticum* shows the best anti-fungal activity against the *F. solani* strain. The distilled water extracts of both the plants have no effect against the *F. solani* strain (table 4 and 5).

Table 4. Anti-fungal activities of ethanolic methanolic and distilled water extract of *Linum Usitatissimum*

[illegible]

F. solani	0.001cm	0cm	0.9cm	0cm	0cm	0cm	0cm	0cm	0cm
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Table 5. Anti-fungal activity of ethanolic, methanolic and distilled water extract of *Syzygium Aromaticum*

Micro- organism	Ethanolic extract			Methanolic extract			Distilled water extract		
	5µl	10µl	15µl	5µl	10µl	15µl	5µl	10µl	15µl
A. niger	2cm	2.5cm	2.8cm	2cm	2.4cm	2.6cm	0.66cm	1.26cm	1.43cm
F. solani	2.2	2.3	2.65	2.5	2.6	2.1	1.44	1.06	1.26

While performing the phytochemical testing, it was to be assured that in tests that involve concentrated H_2SO_4 , pour the H_2SO_4 in such a way that each drop slides down with the wall of test tube to reduce splashing and any burning incident. There will be no splashing when the H_2SO_4 is mixed with chloroform. From the results shown in tables 3, 4, 5 the ethanolic, methanolic and distilled water extracts of *L. Usitatissimum* and *S. Aromaticum* have the phytochemicals as well as the anti-microbial and anti-fungal properties and thus can be used as potential medicine.

Discussion

Oil from the leaf, bud and stem *Syzygium Aromaticum* was dynamic against 23 of 25 *Listeria monocytogenes* testing microorganisms and all strains at the same time as previous tests. Lee *et al.* clarified the antimicrobial activity in which M. gypsum polluted eugenol and nerolidol removed from Japanese cypress oil (Silva *et al.*, 2020). Their findings suggest that eugenol and nerolidol might be used as beneficial antifungal experts. Nunez *et al.* showed that the relationship of clove oleoresin with concentrated sugar has a strong fungicidal effect against *A. niger*. Ahmad *et al.* declared clove oil to have strong antifungal development against *C. albicans*, *C. neoformans* and *A. treats* (Russell and Houlihan, 2003).

The phytochemical check-up of the *Syzygium Aromaticum* and *Linum Usitatissimum* concentrates was demonstrated by our study. Flavonoids, tannins, saponins and cardiac glycosides have been accessible in the methanol concentrate of fennel terpenoids. Heart glycosides were accessible in the ethanolic concentrates of fennel terpenoids, flavonoids, tannins and saponins. The antifungal development of essential clove oil obtained by *S. aromaticum* was thought in Pinto *et al.* They have concluded that the MIC is conducting a survey on the

antifungal activity of clinical and ATCC strains and its guideline fragment, eugenol, *Candida*, *Aspergillus* and *dermatophytes*. The main oil and eugenol were inhibitorous for all tested strains (Uddin *et al.*, 2017). The results of Uchôa Lopes were found as shown by clove EO. They did chromatographical TLCs and fragments to perceive the strong anti-fungal portions and their splitting. They envisaged eugenol as the best antifungal component of clove oil against *T. mentagrophytes* and *M. canis dermatophytes*. In our research (Uchôa Lopes *et al.*, 2020) The refined water concentrates of Alsi terpenoids, heart glycosides, tannins and saponins were available however no flavonoids. In the ethanolic concentrate of clove terpenoids, flavonoids and saponins were available however no tannins and heart glycosides. In the methanolic concentrate of clove terpenoids, flavonoids, heart glycosides, tannins and saponins were available however not present in flavonoids test 2. In the refined water concentrate of clove terpenoids, saponins, tannins and cardiovascular glycosides were available yet there were no flavonoids (Ugbogu *et al.*, 2019).

The ethanolic, methanolic and refined water concentrates of *L. Usitatissimum* and *S. Aromaticum* have the phytochemicals just as the counter microbial and against contagious properties and consequently can be utilized as possible prescriptions. Past examination showed the presence of phytochemicals like Tannins, Phenols, Alkaloids, Saponins and Flavonoids in hefty consumer and liquid focuses, On the other hand Glycosides was need the two concentrates. These results connect well with past assessment (Van *et al.*, 2020), prompted that alcoholic concentrate consolidate Tannins, phenols and Flavonoids, While Saponins and Glycosides were absent.

In every one of the counters microbial and against parasitic testing, the all plates placed upside down so that there will be less odds of tainting. Prior to streaking if the plates to be put away after the media was poured, the closures of the plates were fixed with biofilm so that there will be no development and defilement. The jobs of phenolic compound found in *Linum Usitatissimum* L is animate debasement of bacterial DNA just as deny the glyraseativity. The second justification antibacterial action of *Linum Usitatissimum* L is the lignans with bacterial cell divider along these lines, battle bacterial development (Zhou *et al.*, 2020). Past investigations point that linoleic corrosive specifically restrains as fundamental part of bacterial unsaturated fat union perceived as enoyl-acyl transporter protein re-teaches (FabI), a significant

segment of bacterial unsaturated fat union. Another unsaturated fat, for example, linolenic corrosive and oleic corrosive likewise displayed the hindrance of (FabI) (ZHONG *et al.*, 2016).

Our examination showed that playing out the phytochemical testing, it was to be guaranteed that in tests that include concentrated H₂SO₄, pour the H₂SO₄ so that each drop slides down with the mass of test tube to lessen sprinkling and any consuming episode. There will be no sprinkling when the H₂SO₄ is blended in with chloroform. The antimycotic properties of the clove oils were, regardless, inside and out more expressed than the antibacterial activity, suggesting that they are more powerful against eukaryotic animals. The two *Aspergillus* species used in the assessment had basic advancement limitation even at oil obsessions stock. Critical levels of advancement limitation of *Aspergillus ochraceus* would show likely applications for set aside cultivating things in warm and moreover sodden conditions, such things every now and again being powerless to spoiling by mycotoxigenic filamentous developments.

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