Antimicrobial and biochemical properties of *Linum usitatissimum* the flax seeds and *Syzygium aromaticum*

Abstract

Introduction: The current research was performed to evaluate antimicrobial, anti-fungal and biochemical properties of *Linum usitatissimum* (flax seeds) and *Syzygium aromaticum* (clove). Flaxseeds is one of dietary sources containing phenolics, named lignans. *Syzygium aromaticum* ordinarily called clove is generally considered second important flavor in world and is broadly developed in North Maluku Islands in Indonesia. Glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes, and other phytochemical constituents have been shown to have pharmacological actions in different activities. *Syzygium aromaticum* (clove) and *Linum usitatissimum* (flax) have unique pharmacological effects and have been utilized for food safety. **Materials and Methods**: In this study, ethanolic, methanolic and aqueous extracts of *Linum usitatissimum* and *Syzygium aromaticum* were evaluated against the 3 bacterial strains and 2 contagious fungal strains viz. *A. niger* and *F. solani*. **Results**: Among the bacterial strains i.e., *E. coli*, *P. syringae* and *B. subtilis*, antimicrobial action was noted by *Syzygium aromaticum* against *E. coli*, methanolic and aqueous extract of *Linum usitatissimum* and ethanolic extract of *S. aromaticum* showed antimicrobial action against the *P. syringae* and *B. subtilis*. Best antimicrobial action was observed by the ethanolic extract of the *L. usitatissimum* and methanolic and aqueous extract of *S. aromaticum*. Regarding parasitic strains of *A. niger* and *F. solani*, 3 extracts of *L. usitatissimum* showed satisfactory results against *A. niger* strain and 2 extracts (ethanolic and methanolic) of *S. aromaticum* against *F. solani* strain, whereas aqueous extracts of both the plants had no effect against *F. solani* strain. The phytochemical screening showed presence of terpenoids, tannins, flavonoids, saponins and cardiovascular glycosides in both plants. **Conclusion**: Flaxseed and clove extracts were found potent antimicrobial agents.

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

Introduction

Plants have been used for different therapeutic purposes since ancient times [1]. Most of agricultural nations rely on plants for medicinal needs as well as plants are also part of conventional medications [2]. More than half of world population use plants as medicine due to their easy access and cheap price [3]. Plants are also part of many home remedies in societies and customs of Asia, Latin America and Africa, which is not documented in any scientific forum [4]. Many therapeutic plants have been evaluated for their different pharmacological actions against many illnesses. This has been achieved by complete screening of medicinal plants as likely new mixtures of lead compounds in drug advancement were found [5-8].

Clove plants produce phytohormone during the pre-blooming stage [9]. 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 [5]. The fundamental oil of *S. aromaticum* buds is broadly utilized in gainful applications, especially in dentistry [10, 11]. The focal oil is compelling against oral microscopic organisms that cause dental pits and periodontal sickness, caused by *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Salmonella enteritidis*, *Campylobacter jejuni* and *Staphylococcus aureus* etc. [12-15]. 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 [16]. Clove oil has antimicrobial properties against a variety of infectious microorganisms, including those that cause urogenital illness. Clove oil has been discovered to have potent antifungal activity in case of pathogenic bacteria and fungi like *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. Eugenol from cloves is the primary component responsible for its antifungal activity [17-20].

Linseed has very old history of medicinal potential, with its primary effects as being diuretic as well as pain relieving, demulcent, emollient, purgative, pectoral and resolvent [20-24]. This makes flaxseed oil extremely helpful for shivering just as for nerve sicknesses such as in Parkinson' sand Alzheimer's infection. This exceptionally valuable in curing knowledge affiliated issues, for example, ADHD, bipolar confusion, melancholy, menopausal indications, etc [24-26]. Several studies revealed that phytoestrogens derived from flaxseed can effectively stimulate estrogen production in MCF7 breast cancer cells. The impact of flaxseed proteins on a variety of gram positive and gram negative microbes has been studied [27].

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 samples of both were taken and ground to the powdered form.

Extracts of plant material

Ethanolic, methanolic 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 was taken out in petri-dishes and left

on the shelf so that the remaining solvent was evaporated and pure extract was obtained in dried form. Dried solid material left on petri-dishes, was 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 [28].

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 [9].

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

Table 1 shows the phytochemical screening of the concentrates of *Syzygium aromaticum* and *Linum usitatissimum*. Terpenoids, flavonoids, saponins and cardiac glycosides were present in both methanolic and ethanolic extracts of *L. usitatissimum* but not tannins. On the other hand, flavonoid was present only in the ethanolic extract of *S. aromaticum* but not in methanolic extract. 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. The distilled water extract of *S. aromaticum* contained no flavonoids and that of *L. usitatissimum* did not contain flavonoids (Test 2), tannins and cardiac glycosides (Table 1).

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_2O Extract	Clove Methanolic Extract	Clove Ethanolic Extract	Clove dH_2O Extract
Terpenoids	+	+	+	+	+	+

Flavonoids (*S. aromaticum*)	+	+	+	+	+	_
Flavonoids (*L. usitatissimum*)	+	+	_	_	+	_
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 (inhibition zone in 'mm') 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
E. coli	19.6	29	27	22.3	26.3	27	9	13	15
P. syringae	15. 3	12	19.3	13	16.3	19.3	12.6	17	18.6
B. subtilis	14	16.3	19.6	12.5	13.3	24.6	13.6	17.6	16. 6

Table 3. Antibacterial activity (inhibition zone in 'mm') of various extracts of *Linim usitatissimum*

Micro-Organism	Ethanolic extract			Methanolic extract			Distilled water extract		
	5µl	10µl	15µl	5µl	10µl	15µl	5µl	10µl	15µl
E. coli	7.6	8	9.6	9.3	14	9.6	10	10.3	9
P. syringae	13.6	16.6	26	12.6	14.6	14.8	11.3	13	14.6
B. subtilis	0	9.9	10.3	8.6	10.3	17	6	7.4	12

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 (inhibition zone in 'mm') 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
A. niger	0	0	0	0	0	0	0	0	0
F. solani	0.001	0	0.9	0	0	0	0	0	0

Table 5. Anti-fungal activity (inhibition zone in 'mm') 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	20	25	28	20	24	26	6.6	12.6	14.3
F. solani	22	23	26.5	25	26	21	14.4	10.6	12.6

Discussion

Oil from the leaf, bud and stem of *Syzygium aromaticum* was effective against 23 of 25 *Listeria monocyte* testing microorganisms and all strains at the same time. Lee *et al.* clarified the antimicrobial activity in which *M. gypsum* polluted eugenol and nerolidol removed from Japanese cypress oil [29-30]. Their findings suggested that eugenol and nerolidol might be used as beneficial antifungal agents. 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* [31]. The phytochemical analysis of the *Syzygium aromaticum* and *Linum usitatissimum* extracts was performed in current study. Flavonoids, tannins, saponins and cardiac glycosides have been accessible in the methanol concentrate of fennel terpenoids. Cardiac glycosides were present in the ethanolic extracts. The antifungal screening of essential clove oil of *S. aromaticum* was also evaluated by Pinto *et al.* The main oil and eugenol were also inhibitory against many tested strains [32]. The results of Uchôa Lopes were found as shown by clove EO. They did chromatographical TLCs and fragments to check strong anti-fungal property. They mentioned eugenol as the best antifungal component of clove oil against *T. mentagrophytes* and *M. canis dermatophytes* [33]. According to current study findings, aqueous extract of clove contains terpenoids, cardiac glycosides, tannins and saponins, however flavonoids were not found. In ethanolic extract of clove, terpenoids, flavonoids and saponins were found, however tannins and cardiac glycosides were not present. In methanolic extract of clove, terpenoids, flavonoids, cardiac glycosides, tannins and saponins were present, however flavonoids were not found as evidenced by test 2 [34]. The ethanolic, methanolic and aqueous extracts of *L. usitatissimum* and *S. aromaticum* have the phytochemicals that has antimicrobial property. Previous studies showed the presence of phytochemicals in plants that displayed antimicrobial potential like tannins, phenols, alkaloids, saponins, flavonoids and glycosides [35-37]. In ethanolic extracts, tannins, phenols and flavonoids were found, while saponins and glycosides were absent.

Conclusion

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 that have anti-microbial and anti-fungal properties and thus can be used as potential medicine.

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