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

Abstract

The current research is dealt with 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 lignans which belong to the class of phenolics. *Syzygium aromaticum* ordinarily called clove is significant and is second important flavor in world exchange. It is broadly cultivated in North Maluku Islands of Indonesia. Glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes and other phytoconstituents were found to exhibit pharmacological actions inferable from their phytoconstituents. *Syzygium aromaticum* (clove) and *Linum usitatissimum* (flax) are two common plants having unique pharmacological effects and are 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 three bacterial strains and two contagious strains. *E. coli*, *P. syringae* and *B. subtilis* were the bacterial strains used for the present study and the best antimicrobial action was exhibited by *Syzygium aromaticum* against *E. coli*, methanolic and refined water concentrate of *Linum usitatissimum.* Ethanolic concentrate of *S. aromaticum* shows lot of susceptibility to microbial action against the *P. syringae* and *B. subtilis* is resistant to 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*, 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 reveals the presence of terpenoids, tannins, flavonoids, saponins and cardiovascular glycosides in selected plants which might be responsible for antimicrobial action.

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

Introduction

Plants since a long time are therapeutic specialists to mankind (El-Maati, 2016). Reliance on plants for medication is predominant in agricultural nations where conventional medication plays a significant part in medical services (Abo-EL-Sooud, 2018). The population of a nation is more dependent to conventional methods of treatment due to its simple accessibility and its low cost (Agostini *et al.*, 2012). Home grown treatment is an unwritten science, which is grounded in certain societies and customs, and has become a lifestyle in

practically 80% individuals, particularly those in Asia, Latin America and Africa (Alfikri *et al.*, 2020). As plants have therapeutic properties, they structure the foundation of conventional medication and recently they are subjected to extreme pharmacological examinations. This proves the worth of restorative plants for new pharmacological mixtures worth 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 the blooming buds appear four years after planting. They are collected 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 previous studies(reference). Clove, on the other hand, has gained lot of interest among other flavors due to its remarkable antibacterial and malignant growth inhibition abilities (Alma *et al*., 2007). The essential oils of *S. aromaticum* buds is broadly utilized in several applications, especially in dentistry (Bachiega *et al*., 2012). The oil works 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 for fragrance and as spice. It is pharmaceutically used for the treatment of asthma and for dental problems (Chaudhry *et al.*, 2012). Clove oil has antimicrobial properties against a variety of infectious microorganisms, including those that cause urogenital illness. Clove oil is discovered to have potential 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 in medicinal work, with its primary effects being diuretic and secondarily it 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 (references). It additionally noticed a guidance of In MCF7 cells following treatment, receptor (Erβ) articulation and PR articulation were down-guided. The impact of flaxseed proteins on a variety of gram positive and gram 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

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 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(reference). In the ethanolic concentrate of fennel terpenoids, flavonoids, tannins and saponins, heart glycosides were available(reference). 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 but flavonoids were absent. test 2. In the refined water concentrate of clove terpenoids, saponins, tannins and heart glycosides were present but 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_2O Extract	Clove Methanolic Extract	Clove Ethanolic Extract	Clove dH_2O 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
E. coli	1.96cm	2..9cm	2.7cm	2.23cm	2.63cm	2.7cm	0.9cm	1.3cm	1.5cm
P. syringae	1.53cm	1.2cm	1.93cm	1.3cm	1.63cm	1.93cm	1.26cm	1.7cm	1.86cm
B. subtilis	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*
E. coli	0.76cm	0.8cm	0.96cm	0.93cm	1.4cm	.96cm	1cm	1.03cm	*0.9cm*
P. syringae	1.36cm	1.66cm	2.6cm	1.26cm	1.46cm	1.48cm	1.13cm	1.30cm	*1.46cm*
B. subtilis	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(At which concentration). 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*

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	0.001cm	0cm	0.9cm	0cm	0cm	0cm	0cm	0cm	0cm

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 the 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* prove that the phytochemicals present in those plants were responsible for 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 monocyte* testing microorganisms and all strains at the same time as previous tests. Lee *et al*(*year of referrence*) 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. year of referrence* showed that the relationship of clove oleoresin with concentrated sugar has a strong fungicidal effect against *A. niger*. Ahmad *et al. year of referrence* 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 *referrence*. The antifungal development of essential clove oil obtained by *S. aromaticum* was thought in Pinto *et al year of referrence*. 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** . *year of referrence* 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 but 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 hereby 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 gyrase ativity. 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 ochraceous* 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.

References

El-Maati, M. F. A., Mahgoub, S. A., Labib, S. M., Al-Gaby, A. M., & Ramadan, M. F. (2016). Phenolic extracts of clove (*Syzygium aromaticum*) with novel antioxidant and antibacterial activities. *European Journal of Integrative Medicine*, *8*(4), 494-504.

Abo-EL-Sooud, K. (2018). Ethnoveterinary perspectives and promising future. *International journal of veterinary science and medicine*, *6*(1), 1-7.

Agostini, P. S., Sola-Oriol, D., Nofrarías, M., Barroeta, A. C., Gasa, J., & Manzanilla, E. G. (2012). Role of in-feed clove supplementation on growth performance, intestinal microbiology, and morphology in broiler chicken. *Livestock Science*, *147*(1-3), 113-118.

Alfikri, F. N., Pujiarti, R., Wibisono, M. G., & Hardiyanto, E. B. (2020). Yield, Quality, and Antioxidant Activity of Clove (*Syzygium aromaticum* L.) Bud Oil at the Different Phenological Stages in Young and Mature Trees. *Scientifica*, *2020*.

Advancements in Life sciences 1. Ali, Q., Ahsan, M., Kanwal, N., Ali, F., Ali, A., Ahmed, W., Ishfaq, M., and Saleem, M. (2016). Screening for drought tolerance: comparison of maize hybrids under water deficit condition. Advancements in Life Sciences 3, 51-58.

Ali, Q., Ahsan, M., Ali, F., Aslam, M., Khan, N. H., Munzoor, M., Mustafa, H. S. B., and Muhammad, S. (2013). Heritability, heterosis and heterobeltiosis studies for morphologicaltraits of maize (Zea mays L.) seedlings.

Ali, Q., Ali, A., Ahsan, M., Nasir, I. A., Abbas, H. G., and Ashraf, M. A. (2014). Line× Tester analysis for morpho-physiological traits of Zea mays L seedlings. Advancements in Life sciences 1, 242-253.

Al-Marzoqi, A. H., Al-Khafaji, K. R., and Kadhim, A. (2015). Influence of the crude Phenolic, Alkaloid and Terpenoid compounds extracts of Cardaria draba (Lepidium draba L.) on Human Pathogenic Bacteria. World Journal of Pharmaceutical Research 4, 456-460.

Ayoola, G. A., Coker, H. A., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., & Atangbayila, T. O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research, 7(3), 1019-1024.

Alma, H. M., Ertas, M., Nitz, S., & Kollmannsberger, H. (2007). Research on essential oil content and chemical composition of Turkish clove (*Syzygium aromaticum* L.). *BioResources*, *2*(2), 265-269.

Bachiega, T. F., de Sousa, J. P. B., Bastos, J. K., & Sforcin, J. M. (2012). Clove and eugenol in noncytotoxic concentrations exert immunomodulatory/anti-inflammatory action on cytokine production by murine macrophages. *Journal of Pharmacy and Pharmacology*, *64*(4), 610-616.

Banerjee, M. B., Chowdhury, S. R., Roy, R. B., Tudu, B., Ghosh, M., Pramanik, P., & Bandyopadhyay, R. (2020). Development of a Low-Cost Portable Gas Sensing System Based on Molecularly Imprinted Quartz Crystal Microbalance Sensor for Detection of Eugenol in Clove Oil. *IEEE Transactions on Instrumentation and Measurement*, *70*, 1-10.

Batiha, G. E. S., Beshbishy, A. M., Tayebwa, D. S., Shaheen, H. M., Yokoyama, N., & Igarashi, I. (2019). Inhibitory effects of *Syzygium aromaticum* and Camellia sinensis methanolic extracts on the growth of Babesia and Theileria parasites. *Ticks and tick-borne diseases*, *10*(5), 949-958.

Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). *Syzygium aromaticum* L.(Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, *10*(2).

El-Saber Batiha, G., Magdy Beshbishy, A., El-Mleeh, A., Abdel-Daim, M. M., & Prasad Devkota, H. (2020). Traditional uses, bioactive chemical constituents, and pharmacological and toxicological activities of Glycyrrhiza glabra L.(Fabaceae). *Biomolecules*, *10*(3), 352.

Chaudhry, A. S., & Khan, M. M. H. (2012). Impacts of different spices on in vitro rumen dry matter disappearance, fermentation and methane of wheat or ryegrass hay-based substrates. *Livestock Science*, *146*(1), 84-90.

Chaves, A. V., Dugan, M. E. R., Stanford, K., Gibson, L. L., Bystrom, J. M., McAllister, T. A., ... & Benchaar, C. (2011). A dose-response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livestock Science*, *141*(2-3), 213-220.

Condò, C., Anacarso, I., Sabia, C., Iseppi, R., Anfelli, I., Forti, L., ... & Messi, P. (2020). Antimicrobial activity of spices essential oils and its effectiveness on mature biofilms of human pathogens. *Natural product research*, *34*(4), 567-574.

Cortés-Rojas, D. F., de Souza, C. R. F., & Oliveira, W. P. (2014). Clove (*Syzygium aromaticum*): a precious spice. *Asian Pacific journal of tropical biomedicine*, *4*(2), 90-96.

de Souza, K. A., Ramos, T. R., Bonin, E., Carvalho, V. M., Guerrero, A., Bagaldo, A. R., ... & do Prado, I. N. (2020). Performance and immune response of steers Nellore finished in feedlot and fed diets containing dry leaves of Baccharis dracunculifolia. *Research, Society and Development*, *9*(10), e339107776-e339107776.

Saeed, M., Khan, M. S., Alagawany, M., Farag, M. R., Alqaisi, O., Aqib, A. I., ... & Ramadan, M. F. (2021). Clove (*Syzygium aromaticum*) and its phytochemicals in ruminant feed: an updated review. *Rendiconti Lincei. Scienze Fisiche e Naturali*, 1-13.

Carvalho, V. M., Ávila, V. A. D., Bonin, E., Matos, A. M., do Prado, R. M., Castilho, R. A., ... & do Prado, I. N. (2021). Effect of extracts from baccharis, tamarind, cashew nut shell liquid and clove on animal performance, feed efficiency, digestibility, rumen

fermentation and feeding behavior of bulls finished in feedlot. *Livestock Science*, *244*, 104361.

El-Kady, A. M., Ahmad, A. A., Hassan, T. M., El-Deek, H. E., Fouad, S. S., & Althagfan, S. S. (2019). Eugenol, a potential schistosomicidal agent with anti-inflammatory and antifibrotic effects against Schistosoma mansoni, induced liver pathology. *Infection and drug resistance*, *12*, 709.

Abdalla, E. El-Hadary & Mohamed F. Ramadan Hassanien (2016): Hepatoprotective effect of cold-pressed *Syzygium aromaticum* oil against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats. *Pharma. Biol*, *54*(8), 1364-1372.

Faujdar, S. S., Bisht, D., & Sharma, A. (2020). Antibacterial activity of *Syzygium aromaticum* (clove) against uropathogens producing ESBL, MBL, and AmpC beta-lactamase: Are we close to getting a new antibacterial agent?. *Journal of family medicine and primary care*, *9*(1), 180.

Goel, G., & Makkar, H. P. (2012). Methane mitigation from ruminants using tannins and saponins. *Tropical animal health and production*, *44*(4), 729-739.

Goodrich, R. D., Garrett, J. E., Gast, D. R., Kirick, M. A., Larson, D. A., & Meiske, J. C. (1984). Influence of monensin on the performance of cattle. *Journal of animal science*, *58*(6), 1484-1498.

Gülçin, İ., Elmastaş, M., & Aboul-Enein, H. Y. (2012). Antioxidant activity of clove oil–A powerful antioxidant source. *Arabian Journal of chemistry*, *5*(4), 489-499.

Ayoola, G. A., Coker, H. A., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., & Atangbayila, T. O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research, 7(3), 1019-1024.

Silva, R. B. D., Pereira, M. N., Araujo, R. C. D., Silva, W. D. R., & Pereira, R. A. N. (2020). A blend of essential oils improved feed efficiency and affected ruminal and systemic variables of dairy cows. *Translational Animal Science*, *4*(1), 182-193.

Russell, J. B., & Houlihan, A. J. (2003). Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS microbiology reviews*, *27*(1), 65-74.

Uddin, M. A., Shahinuzzaman, M., Rana, M. S., & Yaakob, Z. (2017). Study of chemical composition and medicinal properties of volatile oil from clove buds (Eugenia caryophyllus). *International Journal of Pharmaceutical Sciences and Research*, *8*(2), 895.

Uchôa Lopes, C. M., Saturnino de Oliveira, J. R., Holanda, V. N., Rodrigues, A. Y. F., Martins da Fonseca, C. S., Galvão Rodrigues, F. F., ... & da Costa, J. G. M. (2020). GC-MS Analysis and Hemolytic, Antipyretic and Antidiarrheal Potential of Syzygium aromaticum (Clove) Essential Oil. *Separations*, *7*(2), 35.

Ugbogu, E. A., Elghandour, M. M., Ikpeazu, V. O., Buendía, G. R., Molina, O. M., Arunsi, U. O., ... & Salem, A. Z. (2019). The potential impacts of dietary plant natural products on the sustainable mitigation of methane emission from livestock farming. *Journal of Cleaner Production*, *213*, 915-925.

Van, T. T. H., Yidana, Z., Smooker, P. M., & Coloe, P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. *Journal of global antimicrobial resistance*, *20*, 170-177.

Zhou, R., Wu, J., Lang, X., Liu, L., Casper, D. P., Wang, C., ... & Wei, S. (2020). Effects of oregano essential oil on in vitro ruminal fermentation, methane production, and ruminal microbial community. *Journal of dairy science*, *103*(3), 2303-2314.

ZHONG, R. Z., Yi, F. A. N. G., SUN, H. X., Min, W. A. N. G., & ZHOU, D. W. (2016). Rumen methane output and fermentation characteristics of gramineous forage and leguminous forage at differing harvest dates determined using an in vitro gas production technique. *Journal of integrative agriculture*, *15*(2), 414-423.