INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 4, p. 221-228, 2016

RESEARCH PAPER OPEN ACCESS

In vitro evaluation of synergistic antimicrobial activity of *Trachyspermum ammi* and *Syzygium aromaticum*

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Key words: *Syzygium aromaticum* (Cloves), *Trachyspermum ammi* (ajwain), *Staphylococcus aureus*, *Streptococcus pyogenes, Escherichia coli.*

http://dx.doi.org/10.12692/ijb/9.4.221-228 Article published on October 27, 2016

Abstract

A remarkable Increase in antibiotic toxicity and multidrug resistance among several group of microorganism are the major challenge worldwide. These new emerging trends led for development of different strategies. Cloves (*Syzygium aromaticum*) and ajwain *(Trachyspermum ammi)* are plant species widely used in traditional medicine to treat various infectious diseases. The main objective of this research study is to investigate the antimicrobial effects of acetone extract of the seed of *Syzygium aromaticum* and *Trachyspermum ammi individually and combination of the both (1:1ratio)* via disc diffusion method against three bacterial culture of clinical significance like (*Staphylococcus aureus*, *Streptococcus pyrogenes and, Escherichia coli)*. Both the extract was found active against almost all the bacterial strain tested. Greater sensitivity was observed in Gram positive bacteria as compared to gram negative bacteria. Synergetic effect evaluations demonstrate they have synergetic as well as no antagonist properties. Due to their therapeutic potential they can be used against multidrug resistant microorganisms in combination therapy.

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Introduction

In the past few decades there is a significant increased record of bacterial and fungal infections. The major reasons are development of remarkable increase resistance in pathogenic bacteria species against the several conventional antibiotics**.** These drugs resistance pathogens are the major concerned subject to the successful treatment of microbial infections. These situations can lead fetal diseases and ultimately the death. So there is a dire need to investigate the new, efficient and cost effective source for the control of infectious diseases. Plant extracts are being utilized since ancient times. The plant extract, essential oils and some other novel drugs isolated from different plant source have been proved significantly effective are also an alternative option for the treatment of different infectious diseases (Tepe *et al*., 2004). These extracted crude extracts, oils and drug possess significant inhibitory properties against the infectious microorganism (Burt, S. 2004). The plant origin remedies are not associated with side effects and have a significant therapeutic potential to treat certain infectious diseases (Ravikumar *et al*., 2010). While the importance is the recognition of specific infectious micro-organisms against the extract is effective (Ravikumar *et al*., 2010). A vast number of active components of plant extract have been found to possess significant activity against certain types of microbial infections and used for therapeutic purposes (Upadhyay *et al*., 2010). Therapeutic prospective of some sort of medicinal plant extracts are extensively studies all over the worlds, but merely little data is available that is studies in systemic manner (Ahmad *et al*., 1998 & Rojas *et al*., 2006). However, due to lack of any scientific evidence of their efficiency, the authenticity of their uses remains doubtful and restricted. Cloves (*Syzygium aromaticum*) are unopened floral aromatic dried buds; belong to the family *Myrtaceae* used as spice and flavoring agents in all over the world (Shyamala *et al*., 2003). Due to their aromatic, carminative and stimulant properties are best remedial agent for gastric irritations and atonic dyspepsia.

Clove buds extracts and oils have been recognized to have a number of antimicrobial and antioxidant properties (Fu *et al*., 2007). GC-MS analysis of extract and essential oils revealed that eugenol acetate, eugenol and caryo-phyllene are the main components. While eugenol acetate, and eugenol the principal constituents of the clove oil mainly attribute the antimicrobial and antioxidant activity (Nassar *et al*., 2007).

While *Trachyspermum ammi* (ajwain) a well reputable medicinal plant spices belongs to the family Apiaceae widely distributed and cultivated in different country of like Iraq, Iran, Afghanistan, Pakistan, and India (*Bairwa et al*., 2012). *Trachyspermum ammi* a traditional therapeutic potential herb is widely used for curing of the numerous infectious diseases in human and animals. The use of *Trachyspermum ammi* as analogous and substitute remedy for infectious agents has been increased considerably during last few years (Rios *et al*., 2005). Due to their stimulant, carminative properties used as important curative representative for the abdominal pain, diarrhea, piles. It also used to cure, asthma, lack of appetite (Rios *et al*., 2005). *Trachyspermum ammi* also exhibit anti-inflammatory and antioxidant activity (Umar *et al*., 2013). Essential oil investigation of the *Trachyspermum ammi* revealed that they possess the antimicrobial and antifungal activities (Syed *et al*., 1986; Ruberto *et al*., 2000 & Mahran *et al*., 1991**)**. Essential oil mainly contains the major component about 30-60% thymol that is strong germicide, antispasmodic and fungicide agent, also used in tooth paste and perfume industry (Zarshenas *et al*., 2013). The present research aim was to evaluate the combine antibacterial consequence of the extract from *Trachyspermum ammi* and *Syzygium aromaticum* against clinical significant strain like *Escherichia coli*, *Staphylococcus aureus*, and *Stapylococcous pyrogenes*. The activities were evaluated by determining minimum inhibitory concentration (MIC) of the extract by using disc method.

Material and methods

Collection and identification of the samples

Seed of the *Syzygium aromaticum and Trachyspermum ammi* was purchased from the local market of the Lahore city. The sample was identified by Botany Department University of the Punjab Lahore. The seeds were further dried in shade and grind using pestle mortar fill in air tight bottle stored in refrigerator for further use.

Preparation of acetone extract

Extract from the *Syzygium aromaticum and Trachyspermum ammi* was obtained by simple extraction method. 20 gram of the fine grind powder of each *Syzygium aromaticum and Trachyspermum ammi* seeds were transferred into 250ml conical flask. Then added the 40ml acetone in each flask mix well closed with aluminum foil and placed both flasks at dark place for maximum 7 days. The crude acetone extract was filtered by passing through the whatman filter paper no.1 and solvent was evaporated from the crude extract under reduced pressure using rotary evaporator. The residual extracts were resuspended in equal volume of DMSO and stored in small aliquots for further investigation.

Test microorganism

The known bacterial isolates *E.Coli, Staphylococcus aureus*, *Streptococcus pyogenes* were obtained from the chugtai lab Lahore. There cultural, morphological and biochemical methods were studied and identified bacterial isolates with API staph, API strep and API 20 Kit of BioMerieu (Lorian, V., 1996).

Determination of antibacterial activity

To evaluate the Antibacterial activity of the acetone extract of *Syzygium aromaticum and Trachyspermum ammi* disc diffusion method was applied. The acetone extract were tested against, *Staphyllococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*. Nutrient agar plates were prepared for investigation of the *Syzygium aromaticum and Trachyspermum ammi* acetone extract. Microbial suspension of the each selected bacterium was uniformly streaked over the nutrient agar plates with the help of sterile cotton swab.

In the disc diffusion method, disc made saturated by 15uL of each sample of *Trachyspermum ammi* and *Syzygium aromaticum* acetone extract. Then disc was placed to dry. The dried saturated disc was placed on the upper layer of agar plate and incubates them overnight. The bacterial growth produced by a bacterial strain, and then measured by the diameter of the zone of inhibition.

Synergistic activity

The crude acetone extracts of both *Syzygium aromaticum and Trachyspermum ammi* that individually show optimum antimicrobial activity were mixed with a ratio 1:1 (*Syzygium aromaticum and Trachyspermum ammi*) and tested there combine activity against the Gram-positive (*Staphyllococcus aureus, Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*) pathogenic bacteria by disc diffusion method. Nutrient agar plates were prepared for investigation of combine acetone extract. Microbial suspension of the each selected bacterium was uniformly streaked over the nutrient agar plates with the help of sterile cotton swab. For the purpose, the disc of (0.7cm) was saturated by adding 15uL of crude acetone extract of *combine Syzygium aromaticum and Trachyspermum ammi acetone extract* samples. Then allowed the disc to air dry. After that this saturated disc placed on the upper layer of Nutrient agar and incubated overnight. The zone of inhibition was measured after incubation.

Results

The anti-microbial activity of the crude acetone extract of *Syzygium aromaticum and Trachyspermum ammi* was evaluated against the clinical significant Gram-positive and Gram-negative pathogenic bacteria species summarized if the Table 1 and Figure 1. The result of the conducted study revealed that the crude acetone extract showing the antimicrobial activities of different values. The zone inhibition of microbial growth above 7 mm diameter was considered as the positive results.

Syzygium aromaticum crude acetone extract was found to be effective against all the bacterial strain tested and exhibit significant inhibitory effect against, *Staphyllococcus aureus*, *Streptococcus pyogenes* with inhibition zone (18 mm,16 mm), and against *E.Coli* with inhibition zone (10 mm).

While in the present study *Trachyspermum ammi* crude acetone extract exhibit considerable inhibitory effect against *Staphyllococcus aureus*, *Streptococcus pyogenes* with inhibition zone (18 mm,17 mm), and against (*Escherichia coli,)* with inhibition zone (10mm).

Table 1. Inhibition zone of, *Syzygium aromaticum and Trachyspermum ammi* on bacterial strains.

Inhibition zone(mm) Bacteria	*Syzygium aromaticum* (A)	*Trachyspermum ammi* (B)	*1:1Ratio (Syzygium aromaticum and Trachyspermum ammi)* (A+B)
Staphylococcus aureus	18 mm	18 mm	21mm
Streptococcus pyogenes	16 mm	17 mm	20 mm
Escherichia coli	10 mm	10 mm	14 mm

As compare to their individual inhibitory effect the synergetic effect with combine ratio 1:1 (*Syzygium aromaticum and Trachyspermum ammi*) was demonstrated in the present study. the combine effect also indicate the significant inhibitory effect against the all tested bacterial strain with inhibition zone (21 mm,20 mm), against *Staphyllococcus aureus*, *Streptococcus pyogenes*, and against (*Escherichia coli,)* with inhibition zone (14 mm) but not showing any antagonistic effect against the tested clinical important bacterial strains.

Discussion

Increase in antibiotic resistance is a global concern for all the developed and developing countries. To manage these issues of drug resistance a lot of alternative approaches are being evaluated worldwide. It might be the use of different antibiotic combination which shows synergistic antimicrobial activity. Other alternative approaches are the use of combining plant extracts with antibiotics. To look for and isolation of such compound that can be individually or combine with antibiotic exhibit broad-spectrum antimicrobial activity may help in overcoming antibiotic resistance to an extent.

In the present study, crude acetone extract of *Syzygium aromaticum* and *Trachyspermum ammi* was found to be sensitive to *Staphyllococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*. *Syzygium aromaticum* acetone extract was found to be more sensitive as compared to the *Trachyspermum Ammi*. *Syzygium aromaticum* and *Trachyspermum ammi* extract shows maximum activity against Gram positive bacteria as compare to gram negative bacteria. Different results, observations have been reported by many researchers. In an earlier reports acetone and aqueous extract *Trachyspermum ammi* was found to be sensitive against *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella typhimurium* and *Shigella flexneri* evaluated by using agar diffusion assay (Kaur *et al*., 2008). Oil extract of Iranian *T. ammi* was analyzed through GC-FID and GC-MS and components like thymol (67.4%), *p*-cymene (17.9%) and γ-terpinene (11.3%) were analyzed to be major one. Different studies, like antimicrobial, antioxidant activity, cytotoxicity and induction of lymphocyte proliferation were performed. The prominent antimicrobial effect was taken against *Staphylococcus aureus* and *Candida albicans* (34.7 and 54.3 mm, respectively) using an agar disc diffusion method with respect to the reference antibiotics (Vitali *et al*., 2015). Abdel-Hameed *et al*. (2014) reported the antimicrobial and anticancer activity of *Trachyspermum ammi* L. They used the n-hexane and oil extract against five microorganisms like *Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica*, *Staphylococcus aureus* and *Candida albicans*.

They also reported the cytotoxic activity against HepG2 cells. Tariq *et al.* (2014) extracted the ethanolic extract of *Trachyspermum Ammi* and analyzed its synergistic activity with ampiclin against the ESBL and MBL producing uropathogens.

Their results show the treatment of infectious diseases caused by multiple drug resistant pathogens with *Trachyspermum Ammi* extract in combination therapy. Acetone extracts of *Trachyspermum ammi* exhibit antimicrobial activity against *K. pneumonia* and *Staphylococcus aureus* (Hassanshahian *et al.*, 2014).

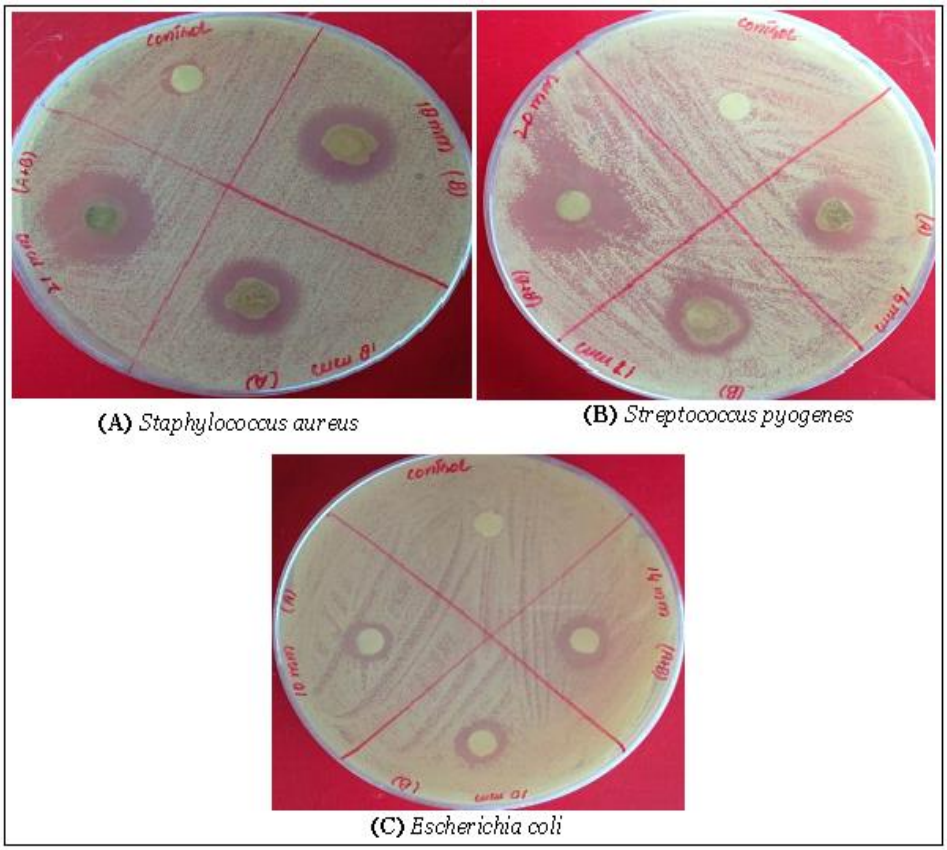

Fig. 1. Inhibition Zone of (A) *Staphylococcus aureus*, (B) *Streptococcus pyogenes*, (C) *Escherichia coli* based on disc diffusion method for the acetone extracts of *Syzygium aromaticum and Trachyspermum ammi.*

Similarly, Masih Usha *et al.*, 2012 evaluated the ethanol extract of *Trachyspermum ammi* and found to be sensitive against sensitive against *E.coli*, *B.subtilis* and *S.aureus*. Antimicrobial activity of the *Syzygium aromaticum* has been studies against different bacterial strains (Chopra & Chopra. 1933; Ueda *et al.*, 1982; Watanabe *et al.*, 1985; Briozzo *et al.*, 1989; Islam *et al.*, 1990 & Hoque *et al.*, 2008). Another study, Lopez *et al.* (2005) reported that clove oil found to be sensitive against *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Listeria monocytogenes*) and Gram-negative bacteria (*E. coli, Yersinia enterocolitica, Salmonella choleraesuis* and *P. aeruginosa*).

Betoni *et al.,* (2006) studied found *Syzygium aromaticum* extract exhibit inhibitory effect against *S. aureus*. In another study, *Syzygium aromaticum* seeds aqueous extract was taken and phytochemicals showed the oxidative stress and membrane permeability against the *Escherichia coli*, *Pseudomonas aeruginosa and Staphylococcus aureus* (Ajiboye *et al.*, 2016). A recent study showed the importance of ethanolic and aqueous extract of *Syzygium aromaticum* as an antioxidant and to control the microbes such like *Escherichia coli*, *Listeria monocytogenes* Scott A, *Salmonella enteritidis* PT4, *Serratia marcescens* and *Staphylococcus aureus* in food and

pharmaceutical products and further suggest the use of *Syzygium aromaticum* as a natural preservative (El-Maati *et al*., 2016). Saeed *et al*. (2013) utilized the methanolic and aqueous extract of *Syzygium aromaticum* as antimicrobial agents and suggest as an alternative to synthetic chemical additives into the food products.

They further propose that 1% clove extract is sufficient for bread for the inhibition of microbial load. Al-Saiym *et al*. (2015) studied the individual and the synergistic effect of methanolic extract of different medicinals plants like *Trachyspermum ammi* (Fruit), *Senna alexandrina* mill (Leaves) and *Vachellia nilotica* spp. nilotica (Fruit) against four standard bacterial strains. Their findings show the enhanced activity due to the synergistic effect of *T. ammi*, *S. alexandrina* and *V. nilotica* against standard bacterial strains.

In the present study the extract was further evaluated for synergetic interaction. The acetone extract of the both species that individually exhibit antimicrobial activity was selected for evaluation of synergetic interaction. The crude acetone extract of both *Syzygium aromaticum* and *Trachyspermum ammi* mixed in the ratio 1:1 and used for antibacterial activity. The mixed (ratio 1:1) crude acetone extract of the both exhibit high synergetic activity against the tested clinical significant bacterial strains.

Conclusion

In conclusion plant crude acetone extract tested in the present study had potential antibacterial against the all tested bacterial stain. Significant synergetic antibacterial activity was observed when tested in combination. Result of the present study revealed that the combine use of the *Syzygium aromaticum and Trachyspermum ammi* extract or with other antibiotic is useful for therapeutic purpose.

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