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SYNERGISTIC ANTIBACTERIAL EFFECT OF ESSENTIAL OILS OF *NIGELLA SATIVA* AND *SALVADORA PERSICA* ON HUMAN PATHOGENS

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ABSTRACT
Essential oils are aromatic oils extracted from vegetative as well as reproductive organs of plants such as stem, bark, root, flowers, fruits, seeds *etc*. Traditionally, these oils have been used as natural preservatives in medicine, food and cosmetics. The essential oils are a rich source of biologically active components and are shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. In recent times, there is a considerable interest in these essential oils due to the emergence and spread of new drug resistant human pathogens to existing antimicrobials. The emergence of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. This calls for a renewed effort to identify agents effective against pathogenic bacteria to current antimicrobials. The mode of action of essential oils likely involves several targets in the cell due to large number of active components and also their hydrophobicity enables them to partition in the lipids of the cell membrane, rendering them permeable and leading to leakage of cell contents. In this context, two essential oils from traditional medicinal plants, Black cumin (*Nigella sativa*) and miswak (*Salvodora persica*) were used alone or in combination to assess their antibacterial efficacy against both gram positive and gram negative bacterial clinical isolates such as *Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Entercocccus fecalis, Salmonella typhi and Salmonella paratyphi.* The antimicrobial test was done by agar disc diffusion method. Although, both the oils were found to be effective in inhibiting pathogens to varying degrees to the tested organisms, the black cumin oil is found to be more effective than miswak. Among the pathogens, *E. fecalis* was highly susceptible to both the oils. When both oils were used in combination, they have shown strong synergistic effect against all the pathogens tested in the present study.

KEYWORDS: *Nigella sativa L., Salvadora persica*, Essential Oils, Antibacterial Activity, Agar Disc Diffusion Method, Synergistic Effect.

INTRODUCTION

The problem of microbial confrontation, resistance and degenerative diseases are growing and the stance for the use of synthetic drugs without unsympathetic effects in the future is still doubtful. Medicinal plants are important therapeutic aids for various ailments and the use of those that are native to India in various traditional system of medicine are awe inspiring (Abdel-Fattah *et al*., 2000). The improved interest in alternative natural substances is driving the research community to find novel uses and applications of these plants. *Salvadora persica L.* commonly known as the miswak tree occurs in shrub Savannah from northwestern India, Pakistan, and Sri Lanka to Africa. *Salvadora persica L.* (Salvadoraceae family), an endangered economic plant which is an oil-yielding medicinal and multipurpose tree (Alhaj *et al*., 2008).The ethnobotanical literature reveals it as a versatile medicinal plant used to treat different human and livestock ailments. It has been reported as anti-inflammatory, analgesic, hypolipidemic activities, antimicrobial, diuretic, anti-plaque, astringent, aphrodisiac, and anti-pyretic. *Salvadora persica L.* have great medicinal uses in the healing of toothache, bronchitis, tumors, gonorrhea, piles, leucoderma, scurvy, scabies and boils (Darmani *et al*., 2006). These species contain important phyto-constituents such as salvadorine, salvadourea, vitamin C, trimethylamine, tannins, cyanogenic glycosides, alkaloids, saponins and salts mostly as chlorides. The leaves of *S. persica L.* are purgative, used as a cure for cough (Dua'a A *et al*., 1996). The fruit is alexipharmic, appetizer, and laxative, carminative, useful in piles, disease of the spleen and ascites. The oil obtained from the seeds is used in painful rheumatic infection and after child-birth, seed fat of *S. persica L.* can be used for making candles and soap. This research discusses the current knowledge of synergistic antibacterial effects on the seven human pathogens. *Nigella sativa L.* (Ranunculaceae family), known commonly as "black cumin", is an herbaceous plant that grows in Mediterranean countries. Recently, many biological activities of *Nigella sativa* L. seeds have been reported, including: antioxidant, anti-inflammatory, anticancer and antimicrobial. In this study seed oil from *Nigella sativa L.* have been tested for antibacterial activity. Most of the bacterial pathogens are resistant to existing synthetic antibacterial agents demanding an increasing effort to seek effective photochemical as antibacterial

agents against such pathogens. *Nigella sativa L.* (black cumin) seeds play an important role in folk medicine and some of its major constituents are reported to be pharmacologically active. The study was designed to examine the antimicrobial activities of essential oils of these plants by the disc diffusion and minimum inhibitory concentration (MIC) methods. Essential oil (EO) samples of *Nigella sativa* and *Salvadora oleoides* were selected to determine antimicrobial activity against a wide range of disease borne gram-positive and gram-negative bacteria (K.alwas *et al*., 1997) .Oils obtained were evaluated for their antimicrobial potential against seven bacterial strains (*Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, E. fecalis, Salmonella paratyphi, Salmonella typhi*). EOs and their components show proficient activities against many disease-borne pathogens and spoilage microorganisms when tested in vitro (Sanogo *et al*., 1999). Use of combinations of EOs and their isolated components are thus new approaches to increase the efficacy of EOs, taking benefit of their synergistic and additive effects. The purpose of this research is to offer an overview on the antimicrobial efficacy of these combinations (Sevil Toroglu *et al*., 2007). Both essential oils when used together exhibited higher MIC (Minimum inhibitory concentration) values towards all seven micro-organism especially, *E. faecalis* than their respective individual concentration.These results suggested the occurrence of synergism effects between the different oil constitutes a higher rate of antibacterial activity. The study was carried out with an objective to investigate the, antimicrobial activity of *Nigella sativa* and *Salvadora persica* oil against seven human clinical isolates either alone or in combination. As microorganism are becoming resistant to present day antibiotics, our study focuses on antimicrobial activity and future prophylactic potential of this essential oil and to find out the minimum inhibitory concentration of seven human clinical isolates.

MATERIALS & METHOD

Source of oil

- Commercially available oil of *Nigella sativa* and *Salvadora persica* (Fig 1 & 2) from AL-BARAKH 19/2 MG. Layout Bangalore-560062.INDIA

Fig-1 Oil: *Nigella sativa* (Seed)

Fig-2 Oil: *Salvadora persica* (Root Cut)

❖ Source of pathogen

- Seven human clinical pathogen *Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Entercocccus fecalis, Salmonella typhi and Salmonella paratyphi.* Were obtained from Sagar Apollo Hospital, Bangalore, INDIA.

METHODS

Agar-Disc Diffusion Method And Preparation of Microbial Cultures: 3X 10^{5} CFU/ml

The assay was conducted by agar disc diffusion method. About 25 to 30 ml of Nutrient agar medium was poured in the sterilized petridishes and allowed to solidify. Bacterial strains were cultured overnight in Nutrient agar (HiMedia, Mumbai) at 37±2°. Overnight grown culture of microorganisms was used for inoculums preparation. A loopful of isolated colony was inoculated in 4ml of peptone water (HiMedia, Mumbai) at 37°C for 2h. The turbidity of resulting suspension was compared to 0.5 McFarland turbidity standards (Dickert *et al*., 1981). The level of turbidity was corresponding to approximately 3.0 × 10^{5} cfu/ml[14]. The Nutrient Agar media (HiMedia, Mumbai) solidified, was then inoculated with microorganism suspended in peptone water, using sterile swab stick, standardized inoculate of each isolate was swabbed onto the surface of Nutrient Agar in separate petridishes. Discs of the extracts were placed to the surface of the inoculated media (Rao *et al.*, 1996). The plates were inverted and allowed to stand for 30 minute for the extract to diffuse into the agar after which the plates were incubated. The experiment was carried out in triplicates to get rid of any error (Sofrata *et al*., 2008). The Petri dishes were incubated for 24 h at 37±2°C for bacteria. The antimicrobial activity was calculated by measuring the diameter of zone of inhibition in millimeters around the well of the discs, shown in table -1, Fig-1 A below. However the robust combinational effect of the extract can be seen more effective in the entire microorganism such as *E. coli, S. typhi, S. aeurus, E. faecalis K. pneumonia, P. aeruginosa and S. para typhi.* [All the microbial strains are highly sensitive to ampicillin with clavulanic acid proving their pathogenicity] (*Medical Formulation 2009-2013*)

Determination of minimal inhibitory concentration (MIC)

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism after 18 to 24 h (Agarwal *et*

al., 1979). The extracts that showed antibacterial activity by agar well diffusion method were subjected to serial micro broth dilution technique to determine their minimum inhibitory concentration by using turbidometric evaluation. In this MIC was determined by the liquid dilution method, dilution series were set up with 180µl of nutrient broth medium, to each microtitre well 10µl of standard suspension of bacterial colony was added and 10µl of diluted extract was added and incubated at 37 for 24 hours. The lowest concentration which did not show any growth for the tested bacteria after microscopic evaluation was determined as MIC (Eloff *et al*., 1998). Based on the evaluation *S. typhi, E. fecalis, K. pneumonia* is showing the MIC at 1: 200 dilutions while the one which is being inhibited least is *S. paratyphi* that is at 1:180 dilutions. The below mentioned table-1 shows the individual minimal inhibitory concentration (MIC) of the microorganisms, mentioned in table -3

RESULT & DISCUSSION

Nigella sativa and *Salvadora persica* have the immense medicinal value (Holy Quran 2006). The tested bacterial strains showed different pattern of inhibition zone. Readings were recorded in tabular form (Table 1). The oil of *Nigella sativa* showed more antimicrobial activity than *Salvadora persica.* The result of antibacterial screening by agar disc diffusion method (Table 1, Fig. 3) indicates that highest zone of inhibition was shown by the oil *Nigella sativa* for *E. fecalis* 45 mm/2.5 µl and lowest for *Salmonella typhi* 14mm /2.5 µl. The *Salvadora persica* extract highest zone of inhibition for *Staphylococcus aureus* 24mm/2.5 µl and lowest for the *Klebsiella pneumonia* 13mm/2.5 µl. Robust combinational effect were observed, when the oils were used in combination for the *E.coil,31mm* , *Staphylococcus aureus* 35mm, *P. Mirabilis* 32mm, *Salmonella paratyphi* 35mm and *K.pneumonia* 23mm however for the microbial strain of *E. fecalis 45mm and Salmonella typhi 22mm* there is no significant combinational effect (Table-2, Fig.4) . A more generally accurate method of assessment is the broth dilution technique. In this study, therefore, the broth dilution method was used in determining the activities measured as MIC by turbidometric evaluation. The range of MIC values for all the microbial strains correlated well with the results obtained by using the agar disc diffusion method. The minimum inhibitory concentration is highest for the *Enterococcus fecalis* (MIC-1:190), *Salmonella typhi* (MIC-1:200 and *K. pneumonia* (MIC-1:200) dilution and lowest for the *Staphylococcus aureus that is* (MIC-1:170) dilution (Table-3, Fig.5). Therefore due to the antimicrobial activities of these plants there are several reasons that people use plants for medication. This includes progress of health following herbal treatment, low cost of the drugs, non availability of synthetic drugs particularly in the countryside areas, where available were either fake or expired drugs and in some cases the people are more familiarized to and comfortable with traditional healing.

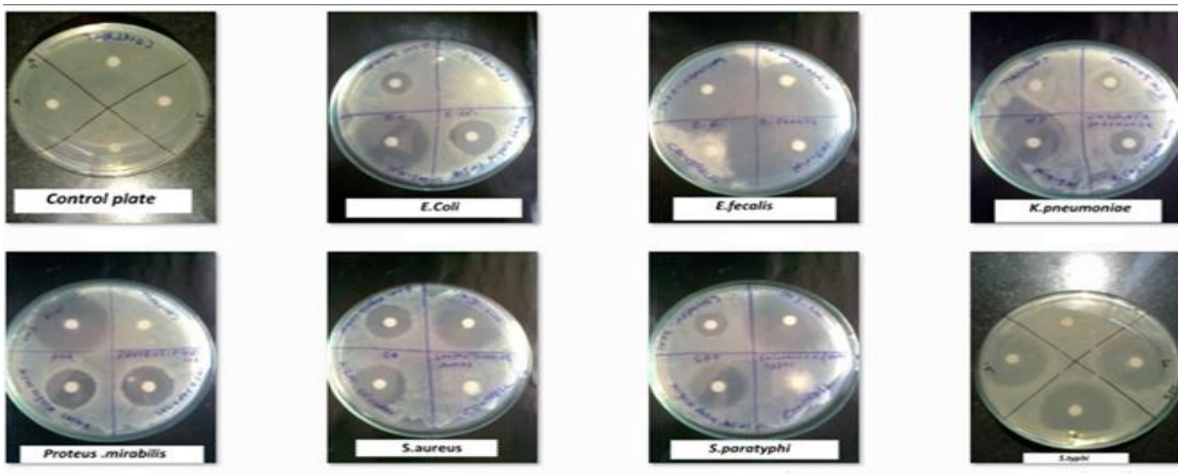

FIGURE 3. Zone Of Inhibition

TABLE 1: Zone Of Inhibition

Organism Name	Oil 1: *Nigella sativa* Zone of inhibition (mm)	Oil 2: *Salvadora oleoides* Zone of inhibition (mm)	Mixed:Oil1 and Oil2 Zone of inhibition (mm)
	2.5 µl	2.5 µl	5.0 µl
E. coli	20	14	31
S. typhi	14	16	22
S. aeurus	22	24	35
E. fecalis	45	22	45
P. mirabilis	21	22	32
S. para typhi	22	16	35
K. pneumonia	15	13	23

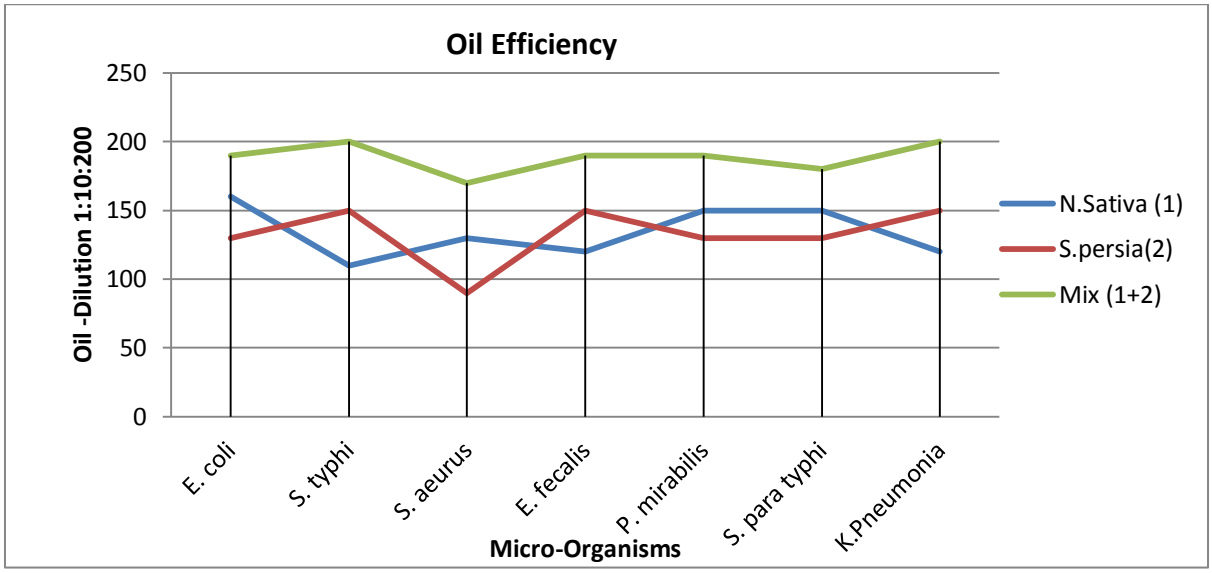

FIGURE 4. Oil Efficiency

Oil Dilution Table			
Organism	*Nigella Sativa (1)*	*Salvadora Persia(2)*	*NS+SP*
E. coli	160	130	190
S. typhi	110	150	200
S. aeurus	130	90	170
E. fecalis	120	150	190
P. mirabilis	150	130	190
S. para typhi	150	130	180
K.Pneumonia	120	150	200

TABLE 2: Oil Dilution

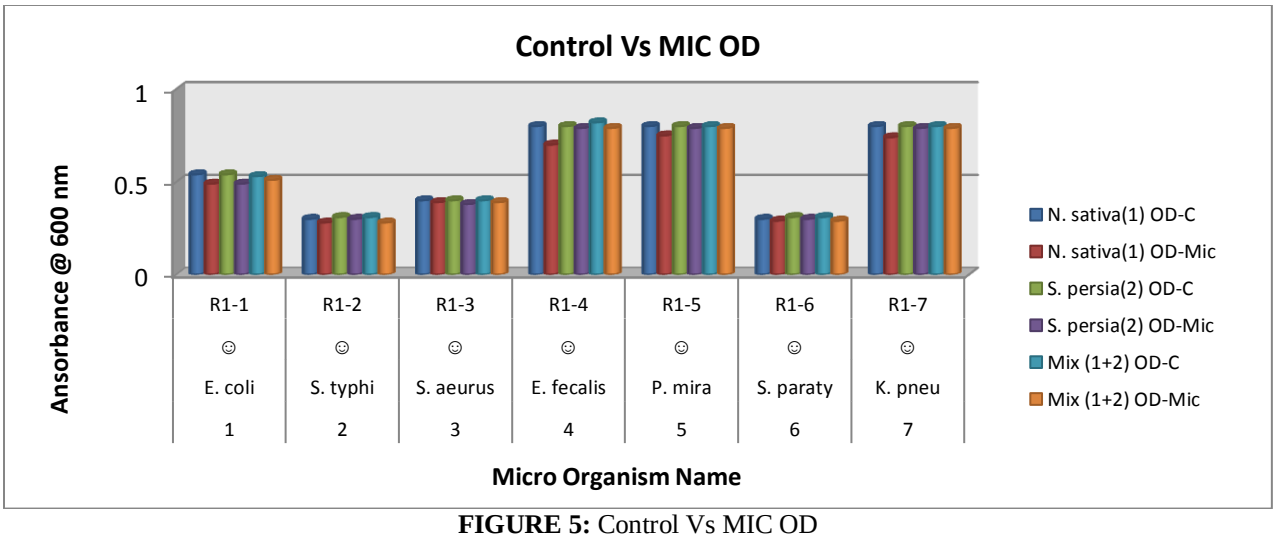

FIGURE 5: Control Vs MIC OD

Control OD Vs MIC OD				*N. sativa(1)*		*S. persia(2)*		Mix (1+2)	
Pos.	Organism	Quality	Rack-N	OD-C	OD-Mic	OD-C	OD-Mic	OD-C	OD-Mic
1	*E. coli*	☺	R1-1	0.54	0.49	0.54	0.49	0.53	0.51
2	*S. typhi*	☺	R1-2	0.30	0.28	0.31	0.30	0.31	0.28
3	*S. aeurus*	☺	R1-3	0.40	0.39	0.40	0.38	0.40	0.39
4	*E. fecalis*	☺	R1-4	0.80	0.70	0.80	0.79	0.82	0.79
5	*P. mirabilis*	☺	R1-5	0.80	0.75	0.80	0.79	0.80	0.79
6	*S. paratyphi*	☺	R1-6	0.30	0.29	0.31	0.30	0.31	0.29
7	*K. pneumonia*	☺	R1-7	0.80	0.74	0.80	0.79	0.80	0.79

TABLE 3: Control Vs MIC OD

CONCLUSION

In this context, two essential oils from traditional medicinal plants, black cumin (*Nigella sativa*) and miswak *(Salvodora persica*) were used alone or in combination to assess their antibacterial efficacy against both gram positive and gram negative bacterial clinical isolates. Among the pathogens, *E. fecalis* was highly susceptible to both the oils. When both oils were used in combination, they have shown strong synergistic effect against all the pathogens tested in the present study.

Future perspectives

Both the oils when used in combination show maximum activity against *E. fecalis* so it can be used to treat different diseases caused by *E. fecalis* species.

The oils can be used as hypoglycemic, hypo-cholesterolemic, and sedative, and effects in addition to be against gingival irritation. Moreover, the extracts and preparations from the plant, which are safe, exhibited various additional biological effects *e.g.* antioxidant, antifever, anti ulcerogenic, anti-caries, antiperiopathic activities.

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