Ayurline
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AYURLINE

e-ISSN: 2456-4435
July- Sept. 2021
Vol. 05th Issue:3rd

International Journal of Research in Indian Medicine

Evaluate the efficacy of Antimicrobial activity of aqueous extract of *Nigella sativa* seeds powder on *Staphylococcus aureus*

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Abstract—

Siddha system of medicine is the main traditional medicine which is very popular in south India and north and east Sri Lanka. In this system herbal, mineral and metal are mainly use for the medicine preparation and to treat the diseases. Each and every part of the herbs can be used for various, multiple ailments because of their different phyto constituents. In Siddha medicine system has been classified into *Aga marunthu* (Internal) and *Pura marunthu* (External) having 32 forms of medicines in each. The external remedies are very useful for topical ulcers, skin diseases like eczema, psoriasis bacterial and fungal infections. The purpose this study was Evaluate the efficacy of Antibacterial activity of aqueous extract of *Nigella sativa* seeds powder against *Staphylococcus aureus* by used disk diffusion method. The result has been evaluated as the diameter of the zone of inhibition of microbial growth showed that the different concentration (100%, 50%, 25%) of *Nigella sativa* seed powder's aqueous extract more effective against *Staphylococcus aureus.* Mean value of the hot extract 100%, 50%, 25% respectively 12.19±1.91mm, 7.63±2.32 mm and 3.78±1.73mm and the mean value of Cold extract were 9.45±1.32mm, 4.21±0.92mm and 2.71±.64mm. Standard maintained 18mm of zone of inhibition .Among the hot and cold extract, 100% of hot extract was showed more significant affect against *Staphylococcus aureus* which was 12.19mm. Finally this study was concluded that the *Nigella seeds powder* can be used as an antibacterial agent.

Keywords— *Nigella sativa*, Hot and cold aqueous extract, Different concentration, disk diffusion method, *Staphylococcus aureus.*

INTRODUCTION

Herbal plants are being an effective source of both traditional and modern medicines. They are genuinely useful for primary healthcare. The medicinal plants produce wide range array of bioactive molecules and rich source of medicines (Agharkar, 1991). *Nigella sativa* belongs to the family Ranuculacea. It is 35-50cm tall and commonly known as black cumin. The seeds have traditionally been used for thousands of years in Middle East, Far East and Asian as a food additive and as a herbal health aid. Also its uses extended for carminative, diuretic, lactogogue and vermifuge, fever, common cold, Headache, asthma,

rheumatic disease, warts, strings of scorpion, bite of snake (Eman, 2009). The black cumin seeds and its oil extract act as antimicrobial, anthelmintic, immune stimulant, antihypertensive, anti-inflammatory, anti-cancer, anti-oxidant, hypoglycemic, spasmolytic and bronchodilator (Jayawera, 2006). One of the central theme of success in human therapeutics in the 20th century was the discovery and development of antimicrobial and antibiotic agents for the treatment of micro-organism infections. A huge array of antimicrobial agent has been introduced and antibiotics can use effectively to treat major infectious disease (Eman, 2009). According to the publication of WHO in 2017, *Staphylococcus aureus* is one of the anti-biotic resistant microorganism and considered to be a multi drug organism as well (WHO, 2017).

MATERIAL AND METHODS

The working bench was sterilized and decontaminated using 70% ethanol from 95% ethanol. Before every research work proceedings glassware were wrapped with foil clear papers and autoclaved for 15 minutes at 120^{0}C.

Preparation of plant extract:

Nigella sativa seeds were collected from local shop at Trincomalee district and taxonomically authenticated. After that it was washed thoroughly with tap water and dried in sun shed. Then powdered by using grinder. Finally stored in air tight glass container and labelled.

1. Cold water extraction

Ten (10) mg of powder was added to 10ml of distilled water and crushed well by using motor and pestle. Then it was centrifuged for 10 minutes in 10000rpm. The supernatant was separated carefully and stored at room temperature (Khan *et al*., 2013).

2. Hot water extraction

Ten (10) mg of powder was added to 10ml of distilled water and crushed well by motor and pestle. Then it was placed in water bath (100^{0}C) for 5 minutes. After allowing it to cool, it was centrifuged for 10 minutes in 10000rpm. The supernatant was separated carefully and stored at room temperature (Khan *et al*., 2013).

Different concentration were prepared in order to identify the optimum concentration in Hot and cold extracts (25mg/10 ml, 50 mg/10 ml, and 100 mg/10 ml).

PREPARATION OF MŬELLER-HINTON AGAR

Mŭeller-Hinton agar were prepared from a commercially available dehydrated base according to the manufacturer's instructions, immediately after autoclaving, allowed it to cool down in a 45-50^{0}C water bath. Poured the freshly prepared and cooled medium into glass flat bottomed petri dished on a level, horizontal surface to give a uniform depth of approximately 4mm. This was corresponds to 60 to 70ml of medium for plates with diameters of 150mm and 25-30ml for plates with a diameter of 100mm.The agar medium allowed to at room temperature and, unless the plates were stored in a refrigerator (2 to 8^{0}C).

SELECTION AND SOURCE OF MICRO-ORGANISM

To assess the antimicrobial activity of the extracts, two bacterial cultures were obtained from Batticoloa Teaching Hospital, Batticaloa. The bacterial strain was of a gram-positive *Staphylococcus aureus*. The culture was sub cultured on Mŭeller-Hinton agar and was left to incubate at 37°C for 24hours. The sub

cultures were then stored at 5°C, until further use.

PREPARATION OF INOCULUM

During the disk diffusion assay, bacteria from the sub culture were dissolved in saline solution under aspetic conditions. This was compared against the 0.5 MacFarland standard to obtain a bacterial concentration between 1-2 x 10^8 colony forming units per milliliter (CFU/ml) and was streaked against petri dishes contained with Mŭeller-Hinton agar, under sterile condition. Thereafter, filter paper disk were placed. Amoxicillin was used as positive control and distilled water was used as negative control.

PREPARATION OF POSITIVE CONTROL

Amoxicillin was prepared from 500mg tablets suspended in distilled water to a final stock concentration of 10mg/ml and filter sterilized.

STATISTICAL ANALYSIS

Inhibition zone were measured by using Vernier caliper. All available data were entered into a database using the SPSS statistical software (SPSS for windows, version 21.0, IBM Corporation, NY, USA) and were analyzed using one-way analysis of variance (ANOVA) to compare the mean inhibitory zones and Independent Sample T test. The values were considered to be statistically different at $p<0.05$ mean +/- standard deviation.

RESULTS

DISK DIFFUSION ASSAY

The antibacterial activity of the aqueous extract (Hot/Cold) of *N.sativa* were investigated against *Staphylococcus aureus.* The results indicated that both extracts displayed antibacterial activity against bacterial strains at concentrations of 10mg/10ml, 5mg/10ml and 2.5mg/10ml. Table 5.1 displayed the mean inhibitory zones for the bacterial strains, ranging from (2.71±0.64mm) to (12.19±1.91mm). The maximum inhibitory zone was exhibited by the positive control (18.33±2.08). There were no inhibition zones recorded for the negative control.

The extracts displayed zones of inhibition in a dose-dependent manner, with an exception for the hot extract tested against *Staphylococcus aureus.* Results from the hot aqueous extract tested against *Staphylococcus aureus*, exhibited the maximum zone of inhibition for 10mg/10ml (12.19±1.91mm followed by 5mg/10ml (7.63±2.32mm) and 2.5mg/10ml (3.78±0.1.73mm) than compared to the cold extract 10mg/10ml (9.45±1.32mm) followed by 5mg/10ml (4.21±0.92mm) and 2.5mg/10ml (2.71±0.64mm).

Table 1: Zones of inhibition for *N.sativa* hot and cold seed extracts against *Staphylococcus aureus*.

Staphylococcus aureus		
	Mean Inhibitory zones (mm)	
Concentration (mg/ml)	***N.sativa* Hot extract**	***N.sativa* Cold extract**
10mg/10ml	12.19±1.91 mm	9.45±1.32 mm
5mg/10ml	7.63±2.32mm	4.21±0.92 mm
2.5mg/10ml	3.78±0.1.73 mm	2.71±0.64 mm

Data expressed as mean±SD

Table 2: p value of Cold extract of Nigella sativa among different concentration

ANOVA					
Inhibition					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1014.181	4	253.545	103.896	.330
Within Groups	48.808	20	2.440		
Total	1062.989	24			

Results from the one-way ANOVA statistical analysis, indicating that there was no statically significant difference ($p<0.05$) between the *N.sativa* hot extract inhibitory zones and each concentration against *Staphylococcus aureus*.

Table 3 p value of hot extract of Nigella sativa among different concentration

ANOVA					
Inhibition					
	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	1070.387	4	267.597	420.670	.000
Within Groups	12.72	20	.636		
Total	1083.110	24			

Results from the one-way ANOVA statistical analysis, indicating that there was no statistically significant difference ($p<0.05$) between the *N.sativa* hot extract inhibitory zones and each concentration against *Staphylococcus aureus*.

The data presented in Table 5-1, 5-2 and 5-3. indicated that the *Nigella sativa* seed hot extract exhibited the highest zone of inhibition at 10mg/10ml (12.19±1.91mm) for *Staphylococcus aureus* which subsequently reduced with the decrease in concentration of the extract, 5mg/10ml (7.63±2.32mm) and 2.5mg/10ml (3.78±0.1.73mm). The comparison of means were displayed a statistically significant difference ($p=0.03$, $p<0.05$) at each concentration of *Nigella sativa seed* hot extract against *Staphylococcus aureus*, among *Nigella sativa* seeds cold extract the highest zone of inhibition was also exhibited at 10mg/10ml (9.45±1.32mm) followed by 5mg/10ml (4.21±0.92mm) and 2.5mg/10ml (2.71±0.64mm). The mean inhibitory zones for the hot extract displayed a statistically significant difference ($p=0.00$, $p<0.05$) with each concentration. There was no significant difference between the *Nigella sativa* hot and cold extract at a concentration of 10mg/10ml against *Staphylococcus aureus*.

Table 4 Independent t- test Analysis of 25% of Hot and cold extract

T-Test

Extract	**N**	**Mean**	**SD**	**Std.error Mean**

Inhibition Hot	5	3.788	1.73024	0.77379
Inhibition Cold	5	2.776	0.64562	0.28873

Independent Sample test

	Levene's Test for Equality of Variances		t-Test for Equality for Mean						
	F	Sig.	t	df	Sig. (2 tailed)	Mean Differences	Std. Error Difference	95%Confidences Interval of the Differences	
								Lower	Upper
Equal variances assumed	4.544	0.066	1.255	8	0.255	1.0122	0.82559	-0.892533	2.916533
Equal variances not assumed			1.255	5.09	0.27	1.0122	0.82559	-1.09947	3.123477

Results from the independent sample t test for 25% of Hot and Cold aqueous extracts of *N.sativa* elicits that since the Significant (2-tailed) values 0.255, 0.274 are > p=0.05. Therefore, there is no significant difference between the mean variances of both hot and cold extracts. Also from the analysis it could be concluded that 95% confidence that the actual competency between mean variances of hot and cold extracts lies between (-0.89253 to 2.92) and (-1.09947 to 3.12). Also the mean value of hot extract is much higher 3.78±1.73 than that of the cold extract 2.77±0.64.

Table 5 Independent t- test Analysis of 50% of Hot and cold extract

T-Test

Extract	**N**	**Mean**	**SD**	**Std.error Mean**
Inhibition Hot	5	7.6340	2.32143	1.03817
Inhibition Cold	5	4.2100	0.92906	0.41549

Independent Sample test

	Levene's Test for Equality of Variances		t-Test for Equality for Mean					
	F	Si	t	d	Si	Mea	Std.	95%Confidenc

		g.		f	g. (2 tailed)	nDifferences	Error Difference	es Interval of the Differences	
Equal variances assumed	0.649	0.444	2640	8	0.030	2.74800	1.04110	Lower 0.34746	Upper 5.14854
Equal variances not assumed			2640	7.110	0.033	2.74800	1.04100	0.29413	5.20187

Results from the independent sample t test for 50% of Hot and Cold aqueous extracts of *N.sativa* elicits that since the Significant (2-tailed) values 0.016, 0.026 were < p=0.05. Therefore, there is significant difference between the mean variances of both hot and cold extracts. Also from the analysis it could be concluded that 95% confidence that the actual competency between mean variances of hot and cold extracts lies between (0.84-6.00) and (0.59-6.25). Also the mean value of hot extract was much higher 7.63±2.32 than that of the cold extract 4.21±0.93.

Table 6 Independent t- test Analysis of 100% of Hot and cold extract

T-Test

Extract	N	Mean	SD	Std.error Mean
Inhibition Hot	5	12.1980	1.91514	0.85648
Inhibition Cold	5	9.4500	1.32310	0.59171

Independent Sample test

	Levene's Test for Equality of Variances		t-Test for Equality for Mean						
	F	Sig.	t	df	Sig. (2 tailed)	Mean Difference	Std. Error Difference	95%Confidences Interval of the Differences	
Equal variances assumed	8.016	0.022	3.062	8	0.016	3.42400	1.11823	Lower 0.8453	Upper 6.0024

								6	6
Equal variances not assumed			3.062	5.249	0.026	3.4240	1.11823	0.59008	6.25792

Results from the independent sample t test for 100% of Hot and Cold aqueous extracts of *N.sativa* elicits that since the Significant (2-tailed) values 0.03, 0.033 were < p=0.05. Therefore, there is a significant difference between the mean variances of both hot and cold extracts. Also from the analysis it could be concluded that 95% confidence that the actual competency between mean variances of hot and cold extracts lies between (0.34-5.15) and (0.29-5.21). Also the mean value of hot extract was much higher 12.19±1.92 than that of the cold extract 9.45±1.32.

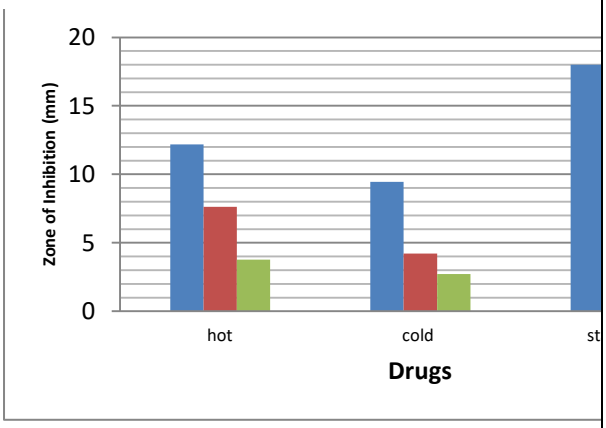

Figure 1 Effectiveness of *Nigella sativa* against *Staphylococcus aureus* in different concentration of hot and cold extract

Hence, according to the above histogram analysis standard value of inhibition for the both extract were between 18m. Compared with the cold extract hot extract was more efficient for inhibit the growth of *staphylococcus aureus.* Among the three different concentrations (10mg/10ml, 5mg/10ml and 2.5 mg/10ml) 10mg/10ml was elicited the highest mean rate of inhibition for both the extracts.

DISCUSSION

Nigella sativa is one of the special traditional herbal plants in siddha system of medicine which is very effective for all kinds of skin disorders and respiratory disorder. The antibacterial activities of Nigella sativa was tested against bacterial strain of *Staphylococcus aureus* in these studies.

Antibacterial and antimicrobial activity of medicinal plant extract tested was more pronounced against Gram positive bacteria than Gram negative bacteria (Bourgou, Pichette, Marzouk, & Legault, 2012). Many research findings elicits that Thymoquinone and Thymohydroquinone were the main chemical components present in N.*Sativa* responsible for the anti-bacterial activity (Halawani, 2009; Randhawa *et al.*,2017; and Hossienzadeh, Bazzaz, & Haghi, *et al.*, 2007). Further antibacterial activity of Nigella sativa stated on their studies by (Halawani, 2009; Arshad, 2008; Salman *et a*l, 2008; L.B *et al.*, 2015; Bakathir & Abbas, 2011; and Jashothan, 2012 *et al).*

The antibacterial activity of *Nigella sativa* study against *Staphylococcus aureus* presented by elicit that aqueous plant extract of *Nigella sativa* showed significant antimicrobial and antifungal activity (Jeyasee,2012; Salman et al.,2008; Hussain, Hannan, Absar, & Butt, 2017; and Chaudhry, Fatima, &Ahmad, 2015 *et al).*

The antimicrobial activity of hot and cold queues extract of *Nigella* seeds powder has not been previously reported. As it already reported in previous studies, that *Nigella sativa* has an antimicrobial activity and this study suggest that hot extract of *Nigella* seeds powder show more significant effect than cold extract of *Nigella sativa* seeds powder.

This study was investigated that antimicrobial effect of hot and cold aqueous extract of *Nigella sativa* on *Staphylococcus aureus*. The highest zone of inhibition against *Staphylococcus aureus* was (12.19±1.9mm) was observed at 100% of hot aqueous extract of *Nigella sativa* (10mg/10ml). 100% cold extract of *Nigella sativa* seed has moderate antibacterial effect on *Staphylococcus aureus.* In this study minimum inhibitory concentration was not carried out, however zone diameter of different concentration, hot and cold extract were reported here in (Table-5-2).

According to the test of homogeneity variance of hot and cold extracts of *N.sativa* the significant values were greater than that of the p value 0.05 (Hot: 0.560>p=0.05) (Cold: 0.690>p=0.05). Therefore the results were considered to be insignificant hence one way ANOVA was used to analyze the maximum inhibition of *N.sativa* in both hot and cold extracts of different concentrations against the growth of *Staphylococcus aureus.*

According this result the standard was maintained in the range of 18mm, in the zone of inhibition of hot and cold extract was gradually decreased depend on the concentration and also Mohammade, (2003) was elicited that aqueous plant extract of *Nigella sativa* showed significant antimicrobial and antifungal activity.

The data presented in Table 5-1, 5-2 and5-3 indicated that the *Nigella sativa* seed hot extract was exhibited the highest zone of inhibition at 10mg/10ml (12.19±1.91mm) for *Staphylococcus aureus* which subsequently reduced with the decrease in concentration of the extract, 5mg/10ml (7.63±2.32mm) and 2.5mg/10ml (3.78±0.1.73mm). The comparison of means was displayed a statistically significant difference (p=0.03, p<0.05) at each concentration of *Nigella sativa seed* hot extract against *Staphylococcus aureus*, among *Nigella sativa* seeds cold extract the highest zone of inhibition was also exhibited at 10mg/10ml (9.45±1.32mm) followed by 5mg/10ml (4.21±0.92mm) and 2.5mg/10ml (2.71±0.64mm). The mean inhibitory zones for the hot extract was displayed a statistically significant difference (p=0.00, p<0.05) with each concentration. There was no significant difference between the *Nigella sativa* hot and cold extract at a concentration of 10mg/10ml against *Staphylococcus aureus*.

Antibacterial activity of two main compounds of black cumin seeds which are Thymoquinone and Thymohydroquinone was investigated their interaction with some common antibiotics were stated by Halawani on his report. *Nigella sativa* Essential oil with high concentration of carvanol and Thymole are more sensible against bacteria. Both Thymoquinone and Thymohydroquinone showed synergism when combined with all tested antibiotics against the Gram positive and Gram negative *S.aureus* resistance in highly susceptible to TQ. In my studies also were showed more significant against 100% of hot concentration of *Nigella sativa.*

Mashhadan found that the aqueous extract was not show any effect but other extracts (methanol, chloroform) showed high inhibitory effect against all the tested microorganisms including *Staphylococcus aureus*, this was stated by Hussine (2011). But on my studies Hot and cold extract were showed significant effect

with different concentration which showed more significant on 10mg/10ml of hot extract of *Nigella sativa.*

Hanafy (1991) reported that different concentration showed that microgram concentrations (25400 &disc) of the ether extract of *Nigella sativa* seeds inhibited growth of several species of pathogenic bacteria representing Gram positive- *Staphylococcus aureus and* Gram Negative *Escherichia coli.* This gave more clues to my studies and 100% hot extract was showed more significant.

Mohammad Akram(2016) stated about that, Anaerobic bacteria are normal commensals and reside in human skin and mucous membranes, thus may cause endogenous infections, such as diarrhoea, aspiration pneumonia, lung abscess, brain abscess, and meningitis, TQ showed a significant antimicrobial activity against anaerobic bacteria although much weaker than metronidazole. So this study helped me in the way of select the amoxicillin as standard.

The extract displayed zones of inhibition in a dose-dependent manner, with an exception for the hot extract tested against *Staphylococcus aureus*. Results from the hot aqueous extract tested against *Staphylococcus aureus*, exhibited the maximum zone of inhibition for 10mg/10ml (12.19±1.91mm followed by 5mg/10ml (7.63±2.32mm) and 2.5mg/10ml (3.78±0.1.73mm) than compared to the cold extract 10mg/10ml (9.45±1.32mm) followed by 5mg/10ml (4.21±0.92mm) and 2.5mg/10ml (2.71±0.64mm).

CONCLUSION

Antibiotics that once readily cured a wide range of infections are becoming and developing antibiotic resistance. Scientist have realized an immense potential in natural products from medicinal plant to serve as an alternative source of combating infections in human beings which may be of lower cost and lower toxicity. This research finally concludes that aqueous extract of *Nigella sativa* seeds has significant antimicrobial activity against *Staphylococcus aureus.* Among that hot aqueous of *Nigella sativa* more significant against *Staphylococcus aureus.*

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Conflict of Interest: Non

DOI https://doi.org/10.52482/ayurline.v5i03.532

Source of funding: Nil

Cite this article:

Evaluate the efficacy of Antimicrobial activity of aqueous extract of Nigella sativa seeds powder on Staphylococcus aureus

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Ayurline: International Journal of Research In Indian Medicine 2021; 5(3):01-12