

COMPARITIVE ASSESSMENT OF ANTIMICROBIAL STUDIES OF CLOVE AND  
NEEM OILSSimmi Singh\*<sup>1</sup>, Lavkush Tiwari<sup>2</sup>, Punj Kulshrestha<sup>1</sup>, Shikant Sharma<sup>1</sup> and Paramsukh<sup>1</sup><sup>1</sup>S.R.A.M College of Pharmacy, Tundla, Firozabad, U. P.<sup>2</sup>Nalanda College of Pharmacy, Nalgonda, Telangana State.

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## ABSTRACT

Essential oils are widely used in the treatment of skin disease. They are also important as a flavouring agent in the food industry. Clove essential oil is used traditionally as flavouring agent and antimicrobial material in food. Neem oil is beneficial to the skin. The study was done on antibacterial and antifungal activities of extracted and readymade essential oils of clove spice and Neem herb. The further study of total phenolic content of essential oils. The essential oils of spice and herb showed antibacterial and antifungal activities which were tested against Gram negative bacteria such as *Escherichia coli*(ATCC 9961), *Proteus vulgaris*(ATCC 25933), *Salmonella typhi* (ATCC 23564), *Pseudomonas aeruginosa*(ATCC 19154), Gram positive bacteria *Staphylococcus aureus*(ATCC 6538) and filamentous fungi *Aspergillus niger*(ATCC 934), *Mucor sp.*(ATCC 1279), *Penicillium sp.*(ATCC 28682). Agar well diffusion assay was performed to study the antibacterial and antifungal activities of essential oils. Total phenolic content of essential oils was measured by Folin-Ciocalteu method. Highest antibacterial activity of clove oil observed at 100µl concentration, gave 19 mm zone of inhibition against *Pseudomonas aeruginosa*. Highest antifungal activity of clove oil observed at 100µl concentration, gave 31 mm against *Aspergillus niger* and *Penicillium sp.* And also found that clove essential oil have 23.44 mg/g highest phenolic compound. Phenolic compound is most important compound which is responsible for antibacterial and antifungal activities. The study reported important and comparison of spice and herb essential oils and their used as antibacterial and antifungal agent in pharmaceutical and food industry.

**KEYWORDS:** Essential oil, Clove, Neem, Antimicrobial activity, Total phenolic content.

## 1. INTRODUCTION

Microbial contamination is the important factor for developing food-borne diseases and food spoilage. Multidrug resistance of these microorganisms is a major medical problem. It is important to search for new natural alternative having antimicrobial activity such as plant extracts and essential oils.<sup>[1]</sup> Over the past two or three decades many beneficial effects of the common food spices and herbs on the health have been understood.<sup>[2]</sup> Essential oils obtained from plants are used against many skin diseases. Essential oils are mixture of volatile compounds. These volatile compound acts as protective substances against microorganisms. Phenolic compounds and terpenes are the main biological constituents in the essential oil.<sup>[3,4,5]</sup> Essential oils are used as carminative, stomachic, stimulant, and aromatic, antiseptic.<sup>[6]</sup> and as a flavouring agent in beverages, foods, cosmetics, and household products.<sup>[7]</sup> Clove essential oil is obtained from *Syzygium aromaticum*. Neem essential oil is obtained from *Azadirachta indica*. In this study, we investigated the *in-vitro* antimicrobial effects of two essential oils of spice and herb against 5

bacteria *Escherichia coli*(ATCC 9961), *Staphylococcus aureus*(ATCC 6538), *Proteus vulgaris*(ATCC 25933), *Salmonella typhi*(ATCC 23564), *Pseudomonas aeruginosa* (ATCC 19154) and 3 fungal *Aspergillus niger*(ATCC 934), *Mucor sp.*(ATCC 1279), *Penicillium sp.*(ATCC 28682) respectively and further analysed the total phenolic content of 2 essential oils.

## 2. MATERIALS AND METHODS

## 2.1 Collection and Preparation of spice mixture

Clove spice and readymade essential oil of Neem herb were purchased from local store (in Valsad, Gujarat, India).The spice was milled to fine powder for extraction of essential oil. 100g of the spice powder was taken, mixed with 500ml of sterile distilled water and allow it soaked for overnight.

## 2.2 Microorganisms

Standard strains of *Escherichia coli* (ATCC 9961), *Staphylococcus aureus*(ATCC 6538), *Proteus vulgaris* (ATCC 25933), *Salmonella typhi* (ATCC 23564), and *Pseudomonas aeruginosa*(ATCC 19154)microorganisms

were used for detection of the antibacterial activity. *Aspergillus niger*(ATCC 934), *Mucor sp.*(ATCC 1279), *Penicillium sp.*(ATCC 28682)were used for detection antifungal activity. Bacteria and fungi were stored on Nutrient agar medium and Sabouraud dextrose agar medium till the analysis.

### 2.3 Extraction of essential oil

The essential oil of spice was extracted by steam distillation method (modified), using 100 g of spice powder was mixed with 500 ml sterile distilled water and subject to steam distillation process for 3-4 hours. After completion of process two separate layer of oil and water was observed and separation of oil was carried out with use of separating funnel. Extracted oil was kept in the desiccator for the dehydration of remaining water for 24 to 48 hours and stored it at 4°C until used.<sup>[9]</sup>

### 2.4 Evaluation of antimicrobial activity using agar well diffusion technique

Antibacterial and Antifungal activity was determined by agar well diffusion method.<sup>[8]</sup> Antibacterial activity of extracted essential oil was studied using Muller-Hinton agar medium (HiMedia, India), and the antifungal activity of the essential oils were studied using Sabouraud dextrose agar medium. Using pour plate method, one tube of melted Muller-Hinton agar was taken and 0.1 ml of bacterial inoculum ( $6 \times 10^5$  CFU/ml) was inoculated in melted agar tube. It was then mixed and poured into empty sterile petri dish, allowed to solidify. After it was solidified, 6 mm diameter well was bored on the surface of all selected sterile agar plates. Antimicrobial activity using diffusion assay, different concentrations like 50, 75, 100, 150, 200, 300 and 400  $\mu$ l of essential oil was taken and reconstituted with 0.25 ml of dimethylsulfoxide (DMSO) and 0.1 ml tween 80 mixtures. From the mixture, 20  $\mu$ l was pipetted into wells. Plates were allowed to stand for 30 min for prediffusion of reconstituted essential oil. Plates were incubated at 37°C and room temperature for 24h. Plates were examined and zone of inhibition was measured in millimetre (mm). DMSO used as negative control to check that DMSO itself does not give any zone of inhibition.

### 2.5 Estimation of total phenolic content

The total phenolic content present in essential oils were estimated by Folin-Ciocalteu method with slight modification.<sup>[9]</sup> Gallic acid was used as a reference standard for plotting calibration curve.<sup>[10]</sup> A different volume in millilitre of essential oils of spices and herbs were taken in the separate sterile tubes and adjust volume to 1 ml by adding ethanol. Then tubes were incubated at 70°C in water bath for 20 mins. After the incubation 1 ml Folin-Ciocalteu reagent (diluted 1:10 with DMSO (modified) was added in all tubes. After 3 min, 1 ml of Na<sub>2</sub>CO<sub>3</sub> solution (2%) was added and mixture was allowed to stand for 2h with intermittent shaking for colour development. The absorbance of the resulting blue colour was measured at 760 nm.<sup>[11]</sup> The total phenolic content was determined from liner equation of standard. The content of total phenolic compound was expressed as mg Gallic acid equivalent/g (mg GAE/g) of dry mass.

## 3. RESULTS AND DISCUSSION

### 3.1 Extraction of essential oil

Essential oils are complex mixture of wide variety of compound and have been recognized for their antibacterial, antifungal and antioxidant properties. Essential oil was extracted from the previously prepared fine powder and overnight soaked clove by the steam distillation process in the laboratory. Table 1 indicated extraction of essential oil.

**Table 1: Essential oil extracted by steam distillation from clove spice.**

Sample	Yields of essential oils (%)
Clove	2.6

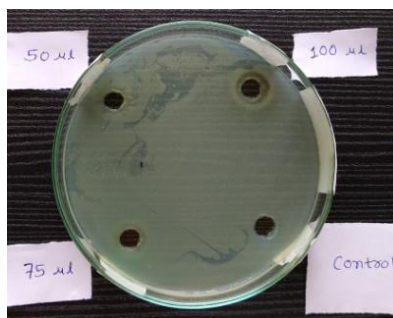
According to table 1 we were obtained 2.6% yield of essential oil from 100 g of each Clove.

### 3.2 Evaluation of antimicrobial activity

Antibacterial and Antifungal activity was determined by agar well diffusion method. Table 2 and 3 clearly shows that Clove spice and Neem herb essential oils were effective against the bacteria and fungi that were used in study.

**Table 2: Antibacterial activity of essential oils at 50, 75, 100, 150 $\mu$ l by agar well diffusion assay.**

Zone of inhibition (mm)										
Sample	Clove				Neem					
	Concentration	Organisms	50 $\mu$ l	75 $\mu$ l	100 $\mu$ l	150 $\mu$ l	50 $\mu$ l	75 $\mu$ l	100 $\mu$ l	150 $\mu$ l
		<i>Escherichia coli</i>	12	12	12	13	12	12	13	-
		<i>Salmonella typhi</i>	-	12	11	13	-	-	-	-
		<i>Staphylococcus aureus</i>	-	11	11	12	-	-	-	-
		<i>Proteus vulgaris</i>	12	13	11	13	13	-	14	-
		<i>Pseudomonas aeruginosa</i>	-	12	19	-	-	-	-	-



It was found that in Clove spice essential oil showed the highest antibacterial and antifungal activities than Neem herb essential oil. In antibacterial activity, clove essential oil shows 19mm highest zone of inhibition against *Pseudomonas aeruginosa* at 100µl concentration of oil. While clove oil with other studied bacteria were also gave highest antibacterial activity at the increase concentration to 150µl. Other three gram negative

bacteria *Escherichia coli*, *Salmonella typhi* and *Proteus vulgaris* were inhibited by clove essential oil and shows decreased inhibition zone size to 13 mm. Gram positive bacteria *Staphylococcus aureus* showed less inhibition zone size of 12 mm. Neem essential oil was more effective against *Proteus vulgaris* and gave 14 mm zone of inhibition at 100µl concentration of oil. *Escherichia coli* was also inhibited at 100µl concentration and give 12 mm zone of inhibition. While remaining other bacteria *Salmonella typhi*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were showed resistance towards Neem oil. The antibacterial activity of spice essential oil obtained by agar well diffusion assay in our study were higher than to the results obtained by El Kady et al; (1993).The antibacterial activity of herb essential oil obtained by agar well diffusion assay were less than to the results obtained by K. Upadhyay et al; (2010).

**Table 3: Antifungal activity of essential oils at 50, 75,100µl by agar well diffusion assay.**

Zone of inhibition (mm)						
Sample	Clove			Neem		
Concentration Organisms	50µl	75µl	100µl	50µl	75µl	100µl
<i>Aspergillus niger</i>	12	30	31	-	-	-
<i>Mucor sp.</i>	11	27	28	-	-	-
<i>Penicillium sp.</i>	27	30	31	-	-	-

In antifungal activity, *Aspergillus niger* and *Penicillium sp.* were showed highest (31 mm) zone of inhibition with clove essential oil at 100µl concentration. While clove oil was tested against *Mucor sp.*, gave 28 mm zone of

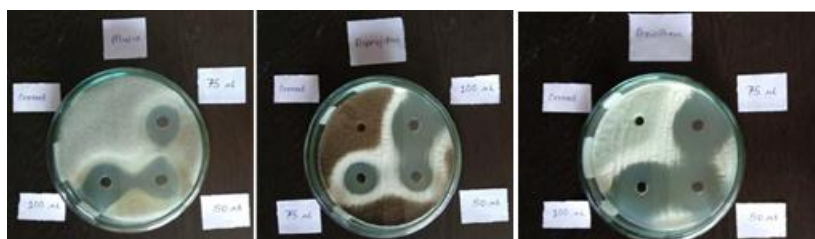
inhibition at 100µl concentration. All fungi were resistance against Neem essential oils at 50µl, 75µl and 100µl concentration. Therefore same assay was run with higher concentration.

**Table 4: Antifungal activity of Neem essential oil at 200µl, 300µl and 400µl done by agar well diffusion assay.**

Zone of inhibition (mm)			
Sample	Neem		
Concentration Organisms	200µl	300µl	400µl
<i>Aspergillus niger</i>	-	-	-
<i>Mucor sp.</i>	10	10	11
<i>Penicillium sp.</i>	-	-	-

Table 4 showed Antifungal activity of Neem essential oils at 200µl, 300µl and 400µl concentration. Neem

essential oil showed antifungal activity only against *Mucor sp.* at 400µl concentration.



**3.3 Estimation of total phenolic content**

Biological activities related to antibacterial and antifungal activities may be correlated with total phenolic content.<sup>[8]</sup> The results of the present study showed that clove essential oil had highest activity in compare to Neem herb essential oil.

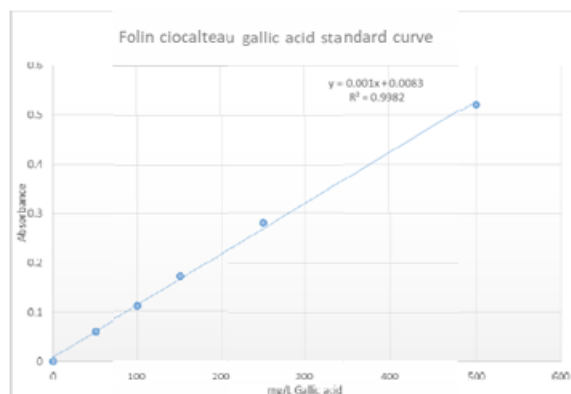


Figure 1: Gallic acid standard curve.

Figure 1 showed standard Gallic acid curve.<sup>[10]</sup> On the basis of the graph and equation total phenolic content of samples find out.

Table 5: Total phenolic content of samples.

Samples	Total phenolic content(mg/g)
Clove	23.44
Neem	3.08

In table 5 clearly showed that Clove essential oil has more phenolic content than Neem essential oil. Clove and Neem essential oils have 23.44 mg and 3.08 mg total phenolic content respectively.

## CONCLUSION

The results obtained from this study showed that spice and herb essential oils can be considered good source of natural compound for antibacterial and antifungal activities. The present study was showed Clove essential oil have more antibacterial and antifungal activities and total phenolic content than Neem essential oil. Therefore, it can be concluded that Clove essential oil has more effective against few pathogenic and commensal microorganisms and also use as a flavouring agent in beverages, foods, cosmetics, and household products.

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