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EVALUATION OF ANTIBACTERIAL STUDY OF INDIAN SPICES AND ITS COMPOUNDS CHARACTERISATION – A COMPARATIVE STUDY

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ABSTRACT: The antibacterial activity of the four Indian spices namely cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), pepper (*Piper nigrum*) and turmeric (*Curcuma longa*) were studied using various extract (methanol and petroleum ether) in the concentration of 1000ppm and 2000ppm. Antibacterial activity was carried out against *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae,* the bioactive compound was identified primarily by UV-Visible study and the best extract was selected based upon the peak and was further analyzed to study the bioactive compounds using GCMS.

KEYWORDS: Spices, UV-VIS, Antibacterial study, GC- MS.

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1. INTRODUCTION

The term spices refer to aromatic or pungent vegetable substances used for flavoring foods and have several commercial uses. Since ancient times people have used spices for preventing food deterioration and pathogenic diseases. Spices have become today as an integral part of our daily diet and many of the spices are widely used to flavor food beverages, for food preservations, medicinal preparations, as masalas and also included in the prescriptions of cosmetics, perfumery, bakery goods and various products. Even today spices are used as an ingredient in drug preparations in Unani, Homeopathy and Ayurveda systems of medicine [1]. Nature has been a source of medicinal agents for thousands years and since the beginning of mankind. Extraction of bioactive compounds

from medicinal plants has been permitted the demonstration of their physiological activity by medicinal researcher. Therefore, there is need to search for plants with medicinally valuable and excellent extracts produced from widely varying substrates. *Piper nigrum* is famous as the spices king due to its pungent quality [2]. Phytochemicals are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health. Phytochemicals have antioxidant or hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues. Black pepper (*Piper nigrum*) is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion and fever. It has been shown that piper has antimicrobial activity and some have already produced compounds effective against antibiotic resistant strains of bacteria [3]. Turmeric (*curcuma longa* L.) is a therapeutic plant that belongs to family *Zingiberaceae*. It is moderately tall, perennial plant with underground rhizomes. Rhizomes are mostly ovate, pyriform oblong and often short-branched. It is widely consumed as a flavoring, preservative and colouring agent in South Asia, Indian and China [4]. Turmeric has healthy influence on digestive system and it also enhances the mucin secretion in the digestive tract. In classical literature several actions of turmeric have been specified like antibacterial, antithelmintic, anticancer, antiparasitic, antiseptic, anti-oxidative, anti-inflammatory, anti-rheumatic,anti-tumor, anti-phlegmatic, antiviral, antitringent, aromatic, blood purifier, clear skin colour, remove wound maggots, hepatoprotective, stop liver obstruction, heals wound, stimulant and sedative in the food industries, as a coloring agent as well as an additive to important flavor in curries. Turmeric is a prompt source of bioactive compounds like antioxidants, polyphenols and flavonoids, which may be the substitute of antibiotics used in food and food products [5]. *Syzygium aromaticum* (*S.aromaticum*) Commonly known as clove, is a median size tree [6]. Clove is one of the major vegetal sources of phenolic compounds such as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxi phenylpropens. The main bioactive compound of clove is Eugenol. This plant has been used for centuries as food preservatives and as medicinal plants mainly as antioxidant agents and has antimicrobial activities. Recently, many reports confirmed the antibacterial, antifungal, antiviral and anticarcinogenic properties of this plant. Clove in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices [7]. Cloves are used in the natural treatment for indigestion, loose stools, flatulence and nausea. In fact cloves are also effective in gaining relief from vomiting, gastric irritability and diarrhoea. The present study was done to identify the antibacterial activity of the spices using different solvent system with different concentration by well diffusion method, characterization of the spices was examined primarily by UV-Visible spectrophotometer and the best extract was selected to find the compounds by GCMS study.

2. MATERIALS AND METHODS

2.1. Collection of samples

The spices of black pepper, clove, cinnamon and turmeric were procured from local market of Coimbatore, Tamilnadu, India. The materials were sun dried for one to two days and powdered. The powdered samples were sealed in separate polythene bag for the extraction and used for further analysis.

2.2. Extract preparation

The extract were prepared by dissolving spices in solvents in a concentration of 1:4 (W/V) and kept at room temperature at 37˚C for 24 hours in a sterile beaker covered with aluminium foil, after incubation filtered through What man No. 1 filter paper to get the powder free extract. The stock solution of this extract prepared by diluting the dried extracts with 50% of respective solvents of petroleum ether and methanol separately. After the preparation of the extract it was diluted as (1000ppm, to 2000ppm). The extract was used for analysis.

2.3. Antibacterial activity

The antibacterial activity of the extract was done against four bacteria such as *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The isolates were cultured into Mueller Hinton agar plate. The bore well method was used to assess the antibacterial activity of the extract against the bacteria. The isolates were inoculated in to 10 ml of sterile Nutrient broth, and incubated at 37˚C for 24 hrs. After incubation the turbidity of culture was compared with Mac Farland standard number II. Then the cultures were subjected to antibacterial activity on Mueller-Hinton agar plates by bore well method. 100 µl of different dilution (1000ppm, 2000ppm) of the extracts was added to the wells of the culture inoculated plates. 50% of the methanol and 50% of the petroleum ether added in to the well. The plates were incubated at 37˚C for 24 hrs and the zone of inhibition was measured and expressed in millimeters (mm).

2.4. Identification and characterization of compounds

2.4.1. UV-Visible study

A relative measurement accumulation of UV Visible reading for the identification of the bioactive compounds were carried out by using petroleum ether extract, which is showing better activity in antibacterial activity. After filtration the extract was scanned by using UV-Visible spectrophotometer in the nm of 200-900nm. The presence of plasmon peak was denoted the presence of different bioactive compounds [8].

2.4.2. GCMS

GCMS analysis was done to detect the active constituents which are present in the clove extract. The purified petroleum ether extract was checked by THERMO GC-TRACE VER : 5.0 instrument, using the column of DB 35- MS CAPILLARY STANDARD NON – POLAR COLUMN.

3. RESULTS AND DISCUSSION

3.1. Antibacterial activity

Herbs improve the function of various internal organs of the body which results in correction of the hormonal imbalances. Their regular use improves the function of the immune system which results in reduced cases of seasonal infection like common cold or cough. They also soothe the mucous membranes which reduce inflammation and internal pains on the hole; they strengthen the body in several ways. The health benefits of this of mainly due do presence of certain phytochemicals and antioxidant properties. It has been proven by many published scientific researches to contain a lot of active constituents that can help to cure the diseases.

Table 1: Antibacterial activity of Black pepper

Name of organism	Zone of inhibition (mm)			
	Petroleum ether extract		Methanol extract	
	1000ppm	2000ppm	1000ppm	2000ppm
E.coli	11 ± 0.81	11.8 ± 0.62	11.2 ± 0.21	11.8 ± 0.20
Staphylococcus aureus	11.06 ± 1.55	12.26 ± 0.36	10.3 ± 0.94	11.3 ± 0.94
Pseudomonas aeruginosa	10.7 ± 0.55	11.8 ± 0.22	10.6 ± 1.24	11.8 ± 1.02
Klebsiella pneumoniae.	11.5 ± 0.40	12 ± 0.40	10.5 ± 0.40	10.9 ± 0.29

Table 2: Antibacterial activity of Clove

Name of organism	Zone of inhibition (mm)			
	Petroleum ether extract		Methanol extract	
	1000ppm	2000ppm	1000ppm	2000ppm
E.coli	13.3 ± 0.47	15.5 ± 0.70	11.1 ± 0.23	11.6 ± 0.24
Staphylococcus aureus	12.5 ± 0.40	13.9 ± 0.70	11 ± 0.81	11.5 ± 0.49
Pseudomonas aeruginosa	12.8 ± 0.61	14.3 ± 1.44	10 ± 0.64	11.1 ± 0.47
Klebsiella pneumoniae.	13.1 ± 0.62	16.8 ± 1.22	11 ± 0.40	11.6 ± 0.47

Table 3: Antibacterial activity of Cinnamon

Name of organism	Zone of inhibition (mm)			
	Petroleum ether extract		Methanol extract	
	1000ppm	2000ppm	1000ppm	2000ppm
E.coli	10.6 ± 1.24	11.2 ± 1.22	10.6 ± 1.24	11.06 ± 0.96
Staphylococcus aureus	11.8 ± 0.85	12.4 ± 0.75	11.8 ± 0.85	12.26 ± 0.87
Pseudomonas aeruginosa	11.6 ± 1.24	12.4 ± 0.99	10 ± 3.4	11.8 ± 2.46
Klebsiella pneumoniae.	10.3 ± 1.24	11 ± 1.08	9.3 ± 0.94	10.16 ± 0.84

Table 4: Antibacterial activity of Turmeric

Name of organism	Zone of inhibition(mm)			
	Petroleum ether extract		Methanol extract	
	1000ppm	2000ppm	1000ppm	2000ppm
E.coli	10.23 ± 1.57	10.73 ± 1.59	9.43 ± 1.01	9.9 ± 0.64
Staphylococcus aureus	11 ± 0.81	11.6 ± 1.02	9 ± 1.63	10 ± 1.47
Pseudomonas aeruginosa	10.5 ± 0.40	11.06 ± 1.41	9.8 ± 1.63	11 ±1.65
Klebsiella pneumoniae.	10.6 ± 0.47	10.9 ± 0.55	9.93 ± 0.82	10.5 ± 0.81

All the used spices contain phytochemical constituents and different type of essential compounds, nutrients, vitamins and minerals and the quantity is differ. The present study showing good antibacterial activity against all the pathogens of *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia,* for the different solvents and the result given in tables (1 to 4). Pepper, curcumin, clove and cinnamon of different solvents showing better zone of inhibition for four spices in the different concentration and in the different solvent of petroleum ether and methanol extract. Investigation about turmeric - A promising spice for phytochemical and antimicrobial activities [9], also describing antimicrobial and antifungal activity of clove (*Syzygium aromaticum*). Various studies showed that turmeric and curcumin can be tolerated at a high dosage without any risk of toxicity. Turmeric oil a byproduct of curcumin was also found active against *B.coagulans*, *S. aureus*, *B.subtilis*, *P.aeruginosa* and *E.coli* [10]. Studies have reported that spicy tang of pepper is the due to the presence of piperamides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit. The results showed significant activity of piperine and suggesting its use as natural antimicrobial agent [11]. [12] Done investigation about Antimicrobial activity of Indian spices against pathogenic bacteria. All extracts of spices showed good antibacterial property. The ethanolic extract of clove showed highest potential activity against *E.coli* (25.0±0.81mm), methanolic extract against *Alcaligenes* (18.3±0.24mm) and aqueous against *Proteus* shows18.7±0.47mm with methanolic extract against *salmonella* (12.6±0.47mm) and aqueous extract against *pseudomonas* showing 19.6±0.47mm. The ethanolic extract of turmeric showed highest potential against *E.coli* and showed 29.3±0.47mm. The ethanolic extract of black pepper showed highest potential against *E.coli* 19.5±0.47mm. The ehanolic extract of cinnamon exhibited maximum antibacterial property against *E.coli* was 21.3±0.7mm. While methanolic extract against *Alcaligenes spp.* shows 12.6±0.47mm and aqueous extract against *Pseudomonas* giving 18.3±0.47mm. The mechanism behind the antimicrobial action of different spices involves the hydrogen bonding of various phenolic compounds to the membrane damage, distribution of the electron transport chain and cell wall distraction. Based on the findings of [13], comparing with curcuminoid and the oil showed good antibacterial activity against different *Bacillus* Spp. and

Azotobacter. In this study larger zone of inhibition was observed against *B.subtilis*. [14] Clove and cinnamon extract of aqueous and ethanol extract against food borne pathogens an *in-vitro* study showing better activity. [15] Investigated the antibacterial activity of clove and garlic on different pathogenic bacteria using well diffusion method and maximum zone of inhibition was observed in 2000ppm. [16] and [17] reports occurrence of antibacterial activity against *S.aureus*, *E.coli*, *B. megaterium*, *B.sphaericus*, *B.polymixa*. The variation in the zone of inhibition among the gram negative bacteria and gram positive bacteria is due to cell membrane and cell wall composition [18].

3.2. UV-Visible study

The identification and characterisation of the bioactive compounds was analyzed by using UV-Visible study for all the selected spices. The better solvent was selected for this study based upon the result of antibacterial screening. In antibacterial activity all the sample showing higher activity in petroleum extract compared with methanol extract. The results were given in figure 1 to 4.

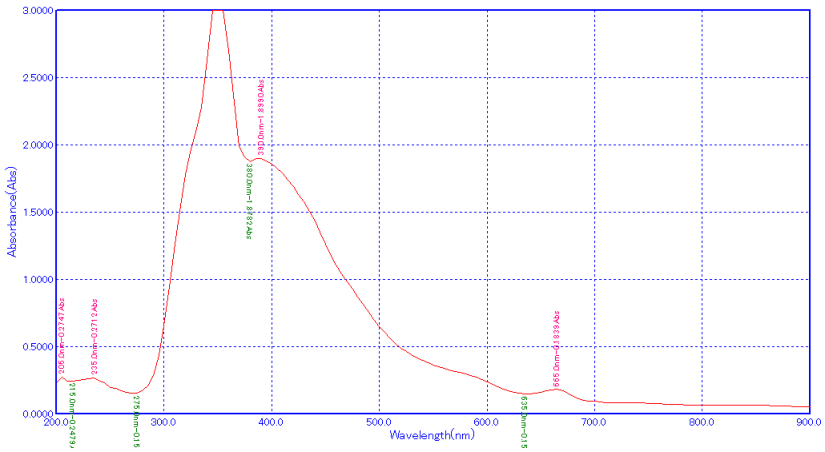

Figure 1: UV-Visible analysis of Cinnamon

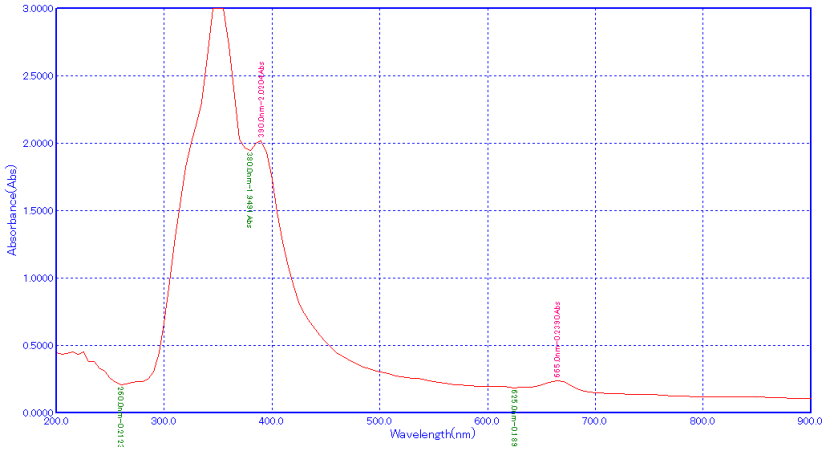

Figure 2: UV-Visible analysis of Pepper

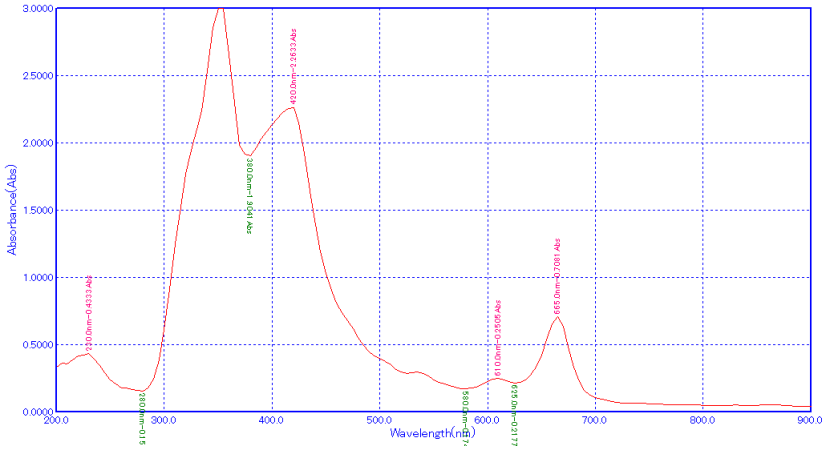

Figure 3: UV-Visible analysis of Clove

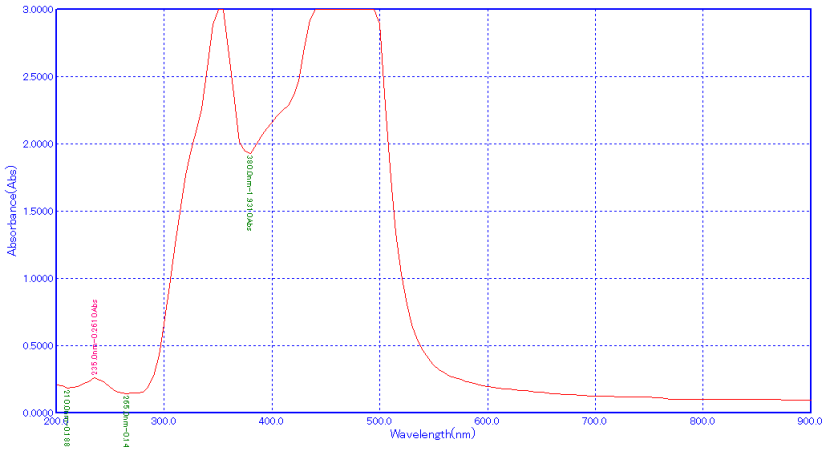

Figure 4: UV-Visible analysis of Turmeric

From the UV-Visible study which is easy to identify the presence of compounds based upon the peak, each sample showing different peak in each nanometer. Cinnamon extract showing peak at 205nm, 215nm, 235nm, 275nm, 380nm, 390nm, 635nm, 665nm. Pepper showing peak at 260nm, 380nm, 390nm, 625nm, 655nm. Clove showing peak at 250nm, 280nm, 380nm, 420nm, 580nm, 610nm, 625nm, 665nm.In turmeric 210nm, 235nm, 265nm, 380nm, each peak denoting in different absorbance reading. The similar study was done with clove extract [19] and which is showing peak in the nanometer of 224nm, 268nm, 348nm, 350nm for ethanol extract.

3.3 GCMS Study

GCMS study was done with the selected extract by the screening of better activity in antibacterial and also from the UV-Visible study. Clove petroleum extract was used for this study and which is given in figure 5.

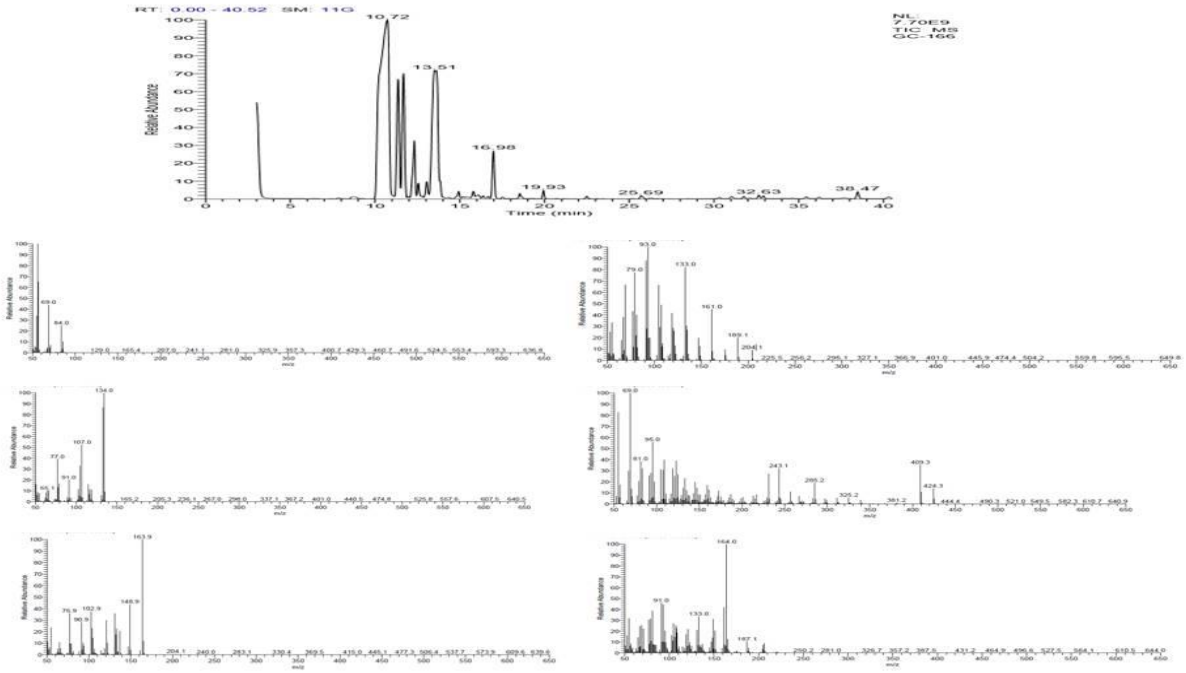

Figure 5: GCMS analysis of clove extract

GC-MS analysis of the petroleum ether extract of clove showed the presence of volatile compounds. More than 25 volatile compounds were identified in this study. A significant antimicrobial activity observed in the petroleum ether extract of clove with the highest anti-bacterial activity for further GC-MS analysis to detect the active constituents in mediating the clove's anti-bacterial effect. The GC-MS analysis showed that the probability of caryophyllene 33.29, Eugenol 28.27, Stigmasterol 75.94, farnesyl acetate etc. Were the major components present in clove and thus are probably the major contributors to the antibacterial effect of the sample. As reported in earlier study the following components have found to be the active ingredients responsible for the antibacterial effect [20 & 21]. [19] Done GCMS analysis with clove extract and that result is showing similarity with our GCMS study. Seven chemical components were identified qualitatively in the essential clove oil as follows: 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl), Phenol 4-(2-propenyl), 3-Allyl-6-methoxyphenol, caryophyllene, Allpha-caryophyllene, euginol and 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester. This is in agreement with the previous studies results that showed similar components in the essential clove oil cultivated in Turkey, Bangladesh and sultanate of Oman [22].

4. CONCLUSION

Spices are using from the ancient time because of its mechanical importance especially, antilarval activity, diabetic properties, antiulcer activity, asthma, obesity, antiseptic, anticancer, antiviral, antibacterial, antifungal activity etc. In the present study clove, cinnamon, turmeric, and pepper extract showed high antibacterial activity against the pathogens of *E.coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In antibacterial activity clove showed better

activity compared with other spices in the used concentration. Characterization was done by using UV-visible study and followed by GCMS, the particular study showed the presence of caryophyllene, Eugenol, Stigmasterol, 2-methyl-5-(1-methylethenyl), Phenol,4-(2-propenyl), 3-Allyl-6-methoxyphenol, farnesyl acetate are found as a major compounds in the clove extract.

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CONFLICT OF INTEREST

The authors declare who have no conflict of interest.

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