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Bioactive potential of plants and spices extracts against human bacterial pathogens

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Abstract: *The aim of this study was to screen antibacterial and antioxidant activity of four different plants and six spices extracts along with phytochemical constituents. Antibacterial activity of the crude extracts of four plants revealed that, plant Vitex negundo had high activity against all the pathogens tested followed by garlic and ginger in spices. It was found that presence of different kinds of chemical groups such as flavonoids, phenolic compounds, saponins, tannins, alkaloids and glycosides in all the four plants. The aqueous extract of V.negundo exhibited DPPH free radical scavenging activity with highest IC_{50} value with concentration of 100 µg/ml followed by methanol. In the case of garlic, methanol extract showed good antioxidant activity with highest IC_{50} value with concentration of 100 µg/ml followed by aqueous extract. The crude extracts of V. negundo and garlic were partially purified using thin layer chromatography. The highest Rf value 0.75 and 0.69 were found in methanol solvent system of for V. negundo and garlic extracts respectively. The results obtained in the present investigation suggest that the extracts of V. negundo leaves and garlic had promising antibacterial and antioxidant activity against free radicals. This will be of more useful in therapeutic of bacterial infections and possible replacement of synthetic antioxidants.*

Keywords: *antibacterial, antioxidant, garlic, phytochemical, V. negundo*

I. Introduction

Antibiotic therapy in recent years has faced difficulties due to the rapid emergence of multidrug resistance among bacteria causing several life threatening infectious diseases and this in turn, making the future management of infectious diseases uncertain. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic infectious agents has led the screening of several medicinal plants for potential antimicrobial activity and the plant extracts found to have promising activity against microorganisms (Mandel *et al*., 2007). According to a study conducted by the World Health Organization (WHO), more than 80% of the world population relies on traditional medicine for primary healthcare needs (WHO, 2013). Medicinal plants would be the best source to obtain a variety of drugs and active compounds. Therefore, such plants should be investigated to better understand about their properties, safety and efficiency (Gautam *et al.*, 2012). There is no doubt that increasing our intake of these spices is one of the most effective, convenient and economical ways in which we can fortify ourselves against infectious diseases and related cancers (Gull *et al.*, 2012). Many infectious diseases have been identified to be treated with herbal products right through the history of mankind (Wadud *et al*., 2007). Natural products provide huge opportunities for the development of new drugs, particularly antimicrobials, which can have therapeutic potential to treat infectious diseases. There is a continuous need to discover new antimicrobial compounds with suitable chemical structures and novel mode of actions against pathogens. Antimicrobial compounds of plant origin have an enormous therapeutic potential to treat many infectious diseases (Mukherjee and Wahile, 2006). In developing countries, due to the cost of efficient antimicrobials, a large proportion of the population utilizes medicinal plants and spices for the treatment of infectious diseases. It is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs (Melvin Joe *et al.,* 2009) Many types of molecules with antibacterial activity have been isolated from plants (Boonnak *et al.,* 2009 and Mahabusarakam *et al.,* 2008). Medicinal plants contain active principles which can be used as an alternative to cheap and effective herbal drugs against common bacterial infections (Kareru *et al.,* 2008). Spices occupy a prominent place in the traditional culinary practice and are essential part of daily diets of millions of people all over the world. They are basically flavoring agent used in small amounts and are reported to have both beneficial effect and antimicrobial properties (Papachan karur sofia *et al.,* 2007). In the area of cancer prevention, plants consumption such as spices and their constituents as potential chemo preventive agents remains an extensive research topic. Numerous studies have been published in regards to the relation between plants utilization, antimicrobial effects, cancer prevention, and overall protection of human health (Bhattacharjee and Sengupta, 2009). In our

study, we have evaluated the antibacterial effect of the extracts of four medicinal plants in India such as *Andrographis paniculata* (Green Chirayta), *Vitex negundo* (Horse shoe vitex), *Ervatamia coronaria* (moon beam) and *Citrus medica* (Citron) and also widely used spices such as *Syzygium aromaticum* (Clove),*Cinnamomum verum* (Cinnamon), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger) and *Piper nigrum* (Pepper) against various human bacterial pathogens.

II. Materials And Methods

Collection of Plants and Spices

Four plants and six spice samples were selected and used for the study. Plants were collected in and around Bharathidasan University, Trichy, Tamil Nadu and the spices were procured from the shops in Tiruchirappalli. All the four plants were identified with the help of plant science department in our university.

Test Organisms

Twelve test organisms namely *Aeromonas hydrophila, Acinetobacter baumanii, Bacillus subtilis,* Entero Toxigenic *Esherichia coli (ETEC), Pseudomonas aeroginosa, Esherichia coli, Salmonella* Typhi, *Shigella flexneri, Proteus mirabilis, Klebsiella pneumoniae, Staphylococcus aureus* and *Vibrio cholerae* were used for the study. All the cultures were obtained from MTCC and the stock cultures were maintained at room temperature in agar slants.

Preparation of Plant and Spice Extracts

The air dried and powdered plant spice materials (1 g of each) were extracted with 100 ml of methanol and water. The preparations were kept in rotary shaker at 160 rpm at room temperature for 24 hours. The obtained extracts were filtered by using Whatman No.1 filter paper. The dried extracts were stored at 4˚C until used for further study**.** All dried extract samples were dissolved in methanol and separately to the final concentration of 100 mg/ml. The extract solutions were stored at 4°C. (Jigna Parekh *et al* 2006).

Culture Preparation

All the test organisms were grown in nutrient broth for overnight at 37˚C separately before performing antimicrobial assay. For antimicrobial susceptibility testing, the turbidity of bacterial suspension was adjusted equivalent to 0.5 McFarland standards at 560nm using UV-Vis spectrophotometer.

Determination of Antibacterial Assay

Disc Diffusion Method

The antibacterial activity of the plants and spices were analyzed by disc diffusion method (Kirby-Bauer *et al.*, 1996). Muller–Hinton agar (MHA - Hi-Media, India) was prepared, sterilized by autoclaving and poured into the sterile petri plates. After solidification, the plates were swabbed with test organism by using sterile cotton swabs. Sterile paper discs of 6 mm diameter were concentrated with 20 µl of all extracts and air dried. The discs were placed on the surface of the MHA agar plates inoculated with test organisms. All the plates were incubated at 37˚C for 24 h and the zone of inhibition was measured at the end of incubation period.

Agar Well Diffusion Method

MHA was prepared, sterilized and poured into the sterile petri plates. The plates were swabbed with the test organism using sterile cotton swab after solidification. Then, the wells were made with 6 mm sterile cork borer and filled with 20 µl of plant extracts. All the plates were incubated at 37˚C for 24 h and the zone of inhibition was noted.

Antioxidant Assay

Antioxidant activity was checked for the potential of the extract by DPPH (Diphenyl Picryl Hydroxide) free radical scavenging assay (Braca *et al*, 2001). Freshly prepared DPPH (0.004% w/v) solution was taken in test tubes and the extracts were added followed by serial dilution (10, 50,100,500 and 1000 µg/ml) to every test tubes, so that the final volume was 1 ml and after 30 minutes , the absorbance was read at 517 nm using UV visible Spectrophotometer. Ascorbic acid was used as a positive control. Control sample was prepared which has the same volume without the addition of any extract and Standard. The inhibition percentage of DPPH free radical was measured by using the following equation.

$$\% \text{ of radical scavenging activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Phytochemical Screening

Phytochemical screening was carried out using crude extract to access the qualitative chemical composition of crude extract using commonly employed precipitation and colouration methods to identify the major natural chemical groups such as steroids, reducing sugars, alkaloids, phenolic compounds, saponins, tannins, flavonoids, amino acids and cardiac glycosides. General reactions in these analyses revealed the presence or absence of these compounds in the extract (Harboune, 1998).

Purification of the Compound

Thin layer silica gel plates were prepared by using silica gel slurry to separate compound through thin layer chromatography (TLC) technique. After drying, the plates were kept in hot air oven for 3 hours at 110˚C. All the extracts were spotted on the TLC plate and it was run by different solvent system including methanol, methanol: chloroform (5:5) and methanol: chloroform: water (75:21:4). Spots were developed with iodine vapor and Rf value was calculated.

III. Results And Discussion

The complete profile of four plants and six spices used for this study are shown in Table 1. All the plants were collected in and around Tiruchirappalli, Tamil Nadu, India. Out of four plants tested for their antibacterial activity, 1 plant and 1 spice showed more activity inhibiting one or more organisms.

Table 1 Profile of plants and spices used for the study

Name of the Plants and spices	Local and common name	Family name	Part used
Andrographis paniculata	Siriyanangai, Green chirayta	Acanthaceae	Leaf
Vitex negundo	Nochi, five leaved chaste tree, Horse shoe virtex	Verbenaceae	Leaf
Ervatamia coronaria	Nathiya vattai, Grape jasmine, Moon beam	Apocyanaceae	Leaf
Citrus medica	Narthai citron	Rutaceae	Leaf
Syzygium aromaticum	Kirambu, Clove	Myrtaceae	Dried flower buds
Cinnamomum verum	Pattai, Cinnamon	Lauraceae	Bark
Allium sativum	Poondu, Garlic	Amaryllidaceae	Root bulb
Zingiber officinale	Inji, Ginger	Zingiberaceae	Rhizome
Piper nigrum	Milagu, Black pepper	Piperaceae	Dried fruit
Curcuma longa	Manjal, Turmeric	Zingiberaceae	Rhizome

Methanol and aqueous extract of all the plants were tested for their antibacterial activity against important human pathogens and the results are shown in Table 2. All the extracts showed variable degree of antibacterial activity on different plant extracts against the tested pathogens. Among the entire plants tested against the pathogens, *Vitex negundo* showed the inhibition against all the pathogens. In methanol extract, the maximum inhibitory activity was found against ETEC (14 mm) followed by *Salmonella* Typhi (12 mm) and the minimum least activity was against *Staphylococcus aureus* (10 mm). From the results, it is clearly indicated that the gram negative bacterium inhibited more efficiently than gram positive one. *Citrus medica* showed the inhibitory activity only against the *Salmonella* Typhi (10 mm). In aqueous extract, *Vitex negundo* showed high activity against ETEC (13 mm) and least was against *Staphylococcus aureus* (9 mm).

Table 2 Antibacterial activity of methanol and aqueous extracts of plants by disc diffusion method

Test pathogen	*A. paniculata*		*V. negundo*		*E. coronaria*		*C. medica*	
	Met	Aqu	Met	Aqu	Met	Aqu	Met	Aqu
ETEC	11	10	14	13	10	10	-	-
S. Typhi	10	11	12	11	-	-	10	10
P. aeroginosa	10	-	11	11	-	-	-	-
K. pneumoniae	-	-	10	11	10	9	-	-
P. mirabilis	-	-	11	10	-	-	-	-
S. flexineri	-	-	12	9	-	-	-	-
S. aureus	-	-	10	9	-	-	-	-
B. subtilis	-	-	11	10	-	-	-	-
A. hydrophilia	-	-	10	9	-	-	-	-
A. baumanii	10	9	11	10	-	-	-	-
E. coli	10	9	10	10	-	-	-	-

V. cholerae	-	-	11	11	-	-	-	-

Met- Methanol, Aqu- Aqueous

The antibacterial activity of different spices used in this study is given in Table 3. Out of six spices tested against the pathogens, garlic showed good activity against all the pathogens followed by ginger. In methanol extract of garlic showed promising activity against the ETEC (14 mm) followed by the gram positive bacteria *S. aureus* (13 mm), *S.* Typhi (11 mm), *P. aeruginosa* (11 mm) and the least activity was noted against the gram negative bacteria *P. mirabilis* (9 mm). Extract of ginger also inhibited all the bacteria except *K. pneumoniae*. Other spices also had the ability to inhibit 2 to 3 test pathogens, the extract of clove inhibit the growth of *P. mirabilis* (9 mm) and *S. flexineri* (12 mm). *P. mirabilis* and ETEC was sensitive against the cinnamon extract of both methanol and aqueous.

Table 3 Antibacterial activity of methanol and aqueous extracts of plants by well diffusion method

Test pathogen	*A. paniculata*		*V. negundo*		*E. coronaria*		*C. medica*	
	Met	Aqu	Met	Aqu	Met	Aqu	Met	Aqu
ETEC	11	10	15	13	12	10	-	-
S. Typhi	11	11	13	12	-	-	11	10
P. aeroginosa	11	11	12	10	-	-	-	-
K.pneumoniae	-	-	10	11	11	10	-	-
P. mirabilis	-	-	11	10	-	-	-	-
S. flexineri	-	-	12	7	-	-	-	-
S. aureus	-	-	10	10	-	-	-	-
B. subtilis	-	-	10	10	-	-	-	-
A. hydrophilia	-	-	11	10	-	-	-	-
A. baumanii	11	10	11	10	-	-	-	-
E. coli	10	10	10	11	-	-	-	-
V. cholerae	-	-	10	10	-	-	-	-

All the plant crude extracts of methanol and aqueous were also tested against the bacterial pathogens by agar well diffusion method and the results were presented in Table 4. Out of 4 plants tested, *V. negundo* had the ability to inhibit all the human pathogens. Gram negative bacteria ETEC was sensitive against both methanol extract and aqueous extract. Similarly, *S.* Typhi inhibithed by *A. paniculata* and *C. medica* extracts. Interestingly, gram positive bacteria were found to resistant against *A. paniculata*, *E. coronaria* and *C. medica* of methanol and aqueous extracts.

In spices, garlic extract inhibited all the pathogens like ETEC, *S.* Typhi, *P. aeruginosa*, *S. aureus*, *K. pnemoniae*, *B. subtilis* and *P.mirabilis* (Table 5). Extracts of ginger showed the activity with maximum inhibition (14 mm) against *S. Typhi* and the least was observed against *P. mirabilis*. However, no activity was observed against *K. pnemoniae*. Other spices such as cinnamon, clove, pepper and turmeric inhibited the most of the gram negative bacteria but not gram positive bacteria except turmeric which had inhibitory effects on *B. subtilis*.

Table 4 Antibacterial activity of methanol and aqueous extracts of spices by disc diffusion method

Test pathogen	Clove		Cinnamon		Garlic		Ginger		Pepper		Turmeric	
	Aqu	Met	Aqu	Met	Aqu	Met	Aqu	Met	Aqu	Met	Aqu	Met
ETEC	-	-	10	7	14	13	12	10	-	-	-	-
S. Typhi	-	-	-	-	11	10	14	12	-	-	10	11
P.aeroginosa	-	-	11	10	11	11	11	11	11	10	-	-
K.pneumoniae	-	-	-	-	10	10	-	-	-	-	-	-
P. mirabilis	11	10	10	11	12	10	10	7	-	-	-	-
S. flexineri	12	11	-	-	10	11	-	-	-	-	-	-
S. aureus	-	-	-	-	13	12	11	10	-	-	11	10
B. subtilis	-	-	-	-	10	11	10	10	10	7	-	-
A.hydrophila	-	-	-	-	12	10	11	10	10	7	-	-
A. baumanii	-	-	-	-	12	11	10	7	11	7	-	-
E. coli	-	-	-	-	10	11	10	10	-	-	-	-
V. cholerae	-	-	-	-	11	10	11	10	-	-	-	-

Table 5 Antibacterial activity of methanol and aqueous extract of spices by well diffusion method

Test pathogen	Clove		Cinnamon		Garlic		Ginger		Pepper		Turmeric	
	Met	Aqu	Met	Aqu	Met	Aqu	Met	Aqu	Met	Aqu	Met	Aqu
ETEC	-	-	11	10	14	13	12	11	-	-	-	-
S. Typhi	-	-	-	-	12	10	15	13	-	-	10	11
P. aeruginosa	-	-	10	11	12	11	11	10	12	11	-	-
K.pneumoniae	-	-	-	-	12	10	-	-	-	-	-	-
P. mirabilis	10	11	10	10	10	10	11	7	-	-	-	-
S. flexineri	12	10	-	-	10	11	-	-	-	-	-	-
S. aureus	-	-	-	-	12	10	12	10	-	-	10	11
B. subtilis	-	-	-	-	11	12	12	10	10	10	-	-
A.hydrophila	-	-	-	-	12	10	12	10	10	10	-	-
A. baumanii	-	-	-	-	13	11	11	10	10	10	-	-
E. coli	-	-	-	-	11	10	13	11	-	-	-	-
V. cholerae	-	-	-	-	12	10	11	11	-	-	-	-

Antioxidant activity for the potential plant and spice extracts were performed by the DPPH (Diphenyl Picryl Hydroxide) free radical scavenging assay and results are presented in Table 6. Results revealed that the *V. negundo* extracts showed the best radical scavenging activity than other plants extracts tested and the inhibition concentration was 50.3 (IC_{50}) at 100 µg/ml of both methanol and aqueous extracts. Among the spices used, garlic extracts also had better radical scavenging property than other spices. The IC_{50} values were 52.1 at 100 µg/ml of both methanol and aqueous extracts.

Table 6 Antioxidant property of *V. negundo* and garlic extracts

Concentration of the extract (µg/ml)	*V. negundo*		Garlic		Control (BHT)
	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract	
25	25.4	20.5	24.2	25.4	30.5
50	33.6	30.11	32.0	33.2	45.5
75	45.2	42.5	46.7	41.2	50.2
100	51.3	50.3	52.1	52.5	65.2
125	65.10	64.2	64.10	65.4	75.0
150	71.2	75.2	71.4	75.2	85.5

The analysis of different chemicals present in plant sample (*V. negundo*) and spice sample (garlic) were carried out and the results are presented in Table 7. Our results indicate the presence of various phytochemicals. The potential compounds such as alkaloids, phenolic compounds and flavonoids was found to present in the methanol extract of garlic and the compounds like alkaloids, phenolic compounds, tannins, flavonoids were present in the aqueous extract. It was also found that more yields were evident of phytochemicals in methanol extract than garlic extract and hence the methanolic extract was selected for the further studies.

Table 7 Phytochemical analysis of *V. negundo* and garlic extracts

Phytochemicals	*V. negundo*		Garlic	
	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract
Steroids	-	-	-	-
Reducing Sugars	-	-	-	-
Alkaloids	-	+	+	+
Phenolic compounds	-	-	+	+
Saponins	+	-	-	-
Tannins	+	-	-	+
Flavonoids	+	+	+	+
Aminoacids	-	-	-	-
Cardiac glysocides	-	-	-	-

Thin layer chromatography was performed for the extract and Rf value was determined. The highest Rf value was determined in the methanol solvent system of 0.75 and 0.69 in *V. negundo* and garlic methanolic extracts respectively. The values were decreased when the combination of the solvents were used, so the methanol can be the best mobile phase for the purification of compounds in the extracts and spots were identified both in *V. negundo* and garlic extracts.

Table 8 The Rf values of the spots in thin layer chromatography plates with different solvent system

Solvent system	Methanol extract of *V. negundo*	Methanol extract of Garlic extract
Methanol	0.75	0.67
Methanol: Chloroform(5:5)	0.61	0.65
Methanol: Chloroform: Water (75:21:4).	0.67	0.63

This study was evaluated the antibacterial action of some plants and spices which are commonly consumed in India and probably other parts of the world. Both methanol and water were proved to be fine solvents in extracting inhibitory substances from tested plants and spices. In contrast, methanol was more efficient than water in phytochemical extraction. Investigation of this work shows that, methanol extracts of *V. negundo* and garlic showed high inhibitory against *ETEC* and low inhibition zones was observed against *B. subtilis* and *E.coli*. *V. negundo* and garlic extracts had high more inhibitory principles from various plants and spices tested. Otshudi *et al*. (2000) indicated that diethyl ether extracts of plants were inactive against bacteria compared to aqueous and methanol extracts. It was clearly stated that plant *V. negundo* contained microbial inhibitors (flavonoids) soluble in aqueous and methanol. The spices garlic contains alkaloids and phenolic compounds along with flavonoids which may be the reason for their inhibitory activity. Presence of the phytochemical constituents such as alkaloids, flavanoides, tannin, and phenolic compounds have been reported to be important compounds in numerous and believed to be of biomedical importance. The results of the present investigation, is in agreement with the findings reported elsewhere. In contrast, the extract of *V. negundo* has no activity against the bacterial pathogens like *B. subtilis, S. aureus, S. epidermidis, E. coli, Proteus vulgaris, S. typhimurium and P. aeruginosa* (Kumar *et al.*, 2006 and Ahmad *et al*., 1998). Valasraj *et al.*, (1997) studied the antibacterial activity of ethanol extracts of *V. negundo* leaf using agar dilution method against four bacteria *B. subtilis, S. epidermidis, E. coli* and *P. aeruginosa.* Panda *et al.* (2009) studied the antibacterial activity of *V. negundo* on bark and leaf of petroleum ether, chloroform, methanol and aqueous extracts against *B. subtilis, S. aureus, S. epidermidis, S. typhimurium, P.aeruginosa, V. cholerae,* and *V. alginolyteus* had little activity but the results was not taken into consideration because of the zone of inhibition is less and it may be effect of the solvent used. They concluded that antibacterial activities against Gram positive bacteria were more distinct than against Gram negative.

However, in contrast, the result of this study showed that the extracts of both *V. negundo* and garlic were very effective against gram negative bacteria too. The results indicated that different extracts of spices have broad spectrum antibacterial activity with variable degree of sensitivity of tested bacterial pathogens. Garlic methanol and aqueous extract exhibited highest antibacterial activity in all tested bacteria. The antimicrobial activity shown by garlic extracts in this study agrees with the findings of Yin MC *et al* (2002) and Bakht Je *et al* (2011). This has also been endorsed by Banerjee & Sarkar (2003), who also found aqueous extracts of garlic to possess potent bioactive principle against many bacteria. Arora & Kaur (1999) also found that the antimicrobial effect of garlic extract was noticeable within few hours of incubation. However, the results obtained in our study have enhanced inhibitory effects. The work of bioactive potential of plant parts and spices was fractionation based on the polarity of solvent and for their antimicrobial activity, at the double concentration. It is necessary to point out that the chemical compounds of any plant and spices significantly depend on geographical region, age of plant, local climatic and experimental conditions. Edible plants could be a potential source for inhibitory substances for some food borne pathogens.

IV. Conclusion

The extracts of these plants and could be a possible source to obtain a new and effective herbal medicine to treat a disease caused by multi-drug resistant strains in community. However, it is necessary to isolate the active constituents, and determine their toxicity, side effects and pharmaco-kinetic properties. Further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of the plant and spice extracts in treating various infectious diseases. In addition to these antibacterial activities, the data reported here is indicates that the extracts have best antioxidant activity which has more advantageous to the replacement of synthetic with natural antioxidants.

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