Research Article**A comparative study of bactericidal effect of wild collected plant and commercially available powder of *Vernonea cinerea* and *Cardiospermum halicacabum*****P. Jasmin Beula¹, S. K. Sundar², S. Rajan^{2*}**¹Department of Microbiology, Noorul Islam College of Arts and Science, Kumarakoil, Kanyakumari, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, TamilNadu, India²PG and Research Department of Microbiology, M. R. Government Arts College, Mannargudi – 614 001, TamilNadu, India

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Abstract

Background: *Vernonea cinerea* and *Cardiospermum halicacabum* are important herb which are endemic to south India and used for the treatment of inflammatory disorders induced by microorganisms. The dried plant powders of these two plants are available in market also. **Objective:** The aim of the present study is to assess the efficiency of collected wild and pulverized whole plant powder and compare the antimicrobial activity with same plant powder available in the market. **Material and Methods:** The following techniques were used to assess antibacterial effect which includes disc diffusion assay, MIC, MBC, % inhibition and IC50 were used to assess antibacterial efficiency of these plants. In the present study, only ethanolic extracts of VCWEE, VCCEE, CHWEE and CHCEE were used for assessing antimicrobial activity. **Results:** All the powder preparations showed comparitively good antimicrobial activity when compared to the commercial antibiotics. The zone of inhibition ranges from 8.3±0.6 to 21.7±3.2mm percentage inhibition and IC₅₀ also ranges from 49.8% to 97.3% and 102.7µg/ml to 200.8 µg/ml concentration respectively. **Conclusion:** This study confirms antimicrobial pattern of *Vernonea cinerea* and *Cardiospermum halicacabum* ethanolic extracts and recommends the use of fresh plant powders for medicinal purposes.

Keywords: Bacterial pathogens, antibacterial, percentage inhibition, *Vernonea cinerea*, *Cardiospermum halicacabum*

Introduction

Medicinal plants have become a best source of health care, which is used often by country people as well as city population, used by old and modern citizens of India. Now a days, medicinal plants are available commercially as raw drug and solvent extracted drug in siddha, ayurveda and homeopathic medicinal stores. People procure these items from these stores and use it for medicinal purpose. Despite a various advancements in modern medicine, the prevalence of infectious disease and development of multidrug resistance among human pathogens, haphazard use of synthetic drugs and side effects created by the modern medicine drives the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects (Prusti et al., 2008). To compare stored

market drug with wild collected plant material, the present study was undertaken and screened for antimicrobial activity against seven common bacterial species. In this study *Vernonea cinerea* and *Cardiospermum halicacabum* were selected for screening antimicrobial activity. Novel drugs are made from Medicinal plants. *Vernonea cinerea* is an annual plant belonging to the family Asteraceae. It is commonly called as Mukuthipundu in Tamil, Sahadevi in Sanscrit, Puuvankirutala in Malayalam and little iron weed in English (Abirami and Rajendran, 2012). The whole plant is edible and used for the treatment of inflammation, bleeding, swelling, asthma, wound healing (Lakshmi Prabha, 2015; Guanrui and Chiao Song, 2013; Thiagarajan et al., 2014). *Cardiospermum halicacabum* is an annual climber belonging to the family Sapindaceae (Malpaniet al., 2016). This plant has been used for the treatment of rheumatism (Suresh et al., 2012). It also shows an analgesic, antipyretic and antifilarial activity (Razoet al., 2013). It is commonly called as heartwine in English and Mudakaruthaan in Tamil (Shareefet al., 2012). Both these plants are used for the

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treatment of inflammatory disorders like arthritis, bronchitis etc as an anti-inflammatory agent. Stored powder of these plant were selected along with wild collected plant were used for screening the antimicrobial activity and its efficiency was compared with reference to MIC, MBC, % inhibition and IC_{50} .

Materials and methods

Collection and preparation of plant material

Whole plant of *Vernonea cinerea* and *Cardiospermum halicacabum* were collected as wild from Keezha Puthalam Village, Kanyakumari district and stored market powder of these plants were procured from local siddha medicinal store, Nagercoil, Kanyakumari. The wild collected plants were washed thoroughly with water and shade dried. After drying, the plant materials were ground into powder and then sieved using a sieve. Five hundred grams of powdered plant were transferred into airtight containers and stored at room temperature. Plant materials after extraction were coded as VCWEE (*Vernonea cinerea* Wild ethanolic extract), CHWEE (*Cardiospermum halicacabum* wild ethanolic extract), VCCEE (*Vernonea cinerea* Commercial ethanolic extract) and CHCEE (*Cardiospermum halicacabum* Commercial ethanolic extract).

Extraction of plant powder

Active components of the plant were extracted using the cold extraction method (Fransworth, 1988). Ethanol was used for the extraction. Pure ethanol was added to 50g of the plant powder in sterile conical flasks individually and allowed to soak at room temperature for 48 hours. A shaker set at 120 rpm was used to improve extraction of phyto-chemicals. The filtrate was obtained by means of a vacuum filter pump. Filtering was repeated for three times with same plant material until the solution was clear. The filtrate was evaporated in a weighed flask, with a water bath set at 40°C. A small proportion of dry extract was stored for phyto-chemical analysis. Remaining portion of the extract was used for antibacterial assay. Extracts were reconstituted by re-dissolving in DMSO. The final filtrate was filter-sterilized by using syringe filter with a pore size of 0.45µm. Sterile extracts obtained were stored separately in labelled, sterile capped bottles in a refrigerator at 4°C.

Antibacterial activity

Antimicrobial activity was performed by standard methods like the disc diffusion method on Mueller Hinton agar (Bauer et al., 1966) and MIC, MBC were calculated using modified drug dilution methods (Kowser and Fatena, 2009). Cells used for antibacterial assays were harvested at log phase while they are most active.

Assessment of MIC, MBC and IC_{50}

It was performed by making use of the method of Kowser and Fatena, (2009) with few modifications.

Preparation of Turbidity standard for inoculum

Inoculum for the assay of MIC and MBC were prepared at 0.5 level of McFarland standard. The approximate cell density corresponding to 0.5 McFarland is 1×10^8 CFU/ml.

Inoculum preparation

Overnight Mueller Hinton broth cultures of uropathogenic *E. coli* was prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of McFarland 0.5 standard and then further diluted to 1: 40 in Mueller Hinton broth. The inoculums thus prepared expected to obtain 103 CFU/ml.

Determination of MIC

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. A MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Various concentrations of the extracts, antibiotics were prepared as illustrated below. Fourteen tubes were placed in a rack and labeled each 1 through 11, one tube marked as B (blank), one tube was labeled as AC (Antibiotic Control) and other tube was labeled as G.C (Growth Control). Different volumes of Mueller Hinton broth was added in each test tube as described in table 4.3 and plugged with cotton. All the tubes were sterilized at 121°C for 15 minutes. 100 µl of 0.1% antibiotic solution was added to test tube A.C and hundred µl of plant extract from stock was added to the tube no. 1 and mixed properly. Similarly the volume of extracts ranges from 90 µl to 5 µl for the tube no. 2 through 11 was added. The tube G.C received no extract and served as a growth control. A.C labeled test tube served as an antibiotic control. Each tube was inoculated (including the growth control except blank and antibiotic control) with 100 µl of the culture of respective organism. All the tubes were incubated at 37°C for 24 hours. The tubes were examined for visible growth (cloudy) and was recorded {visible growth as (+) and no growth as (-)}. The concentration at which no visible growth was described as the MIC of the extract.

Determination of MBC

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium completely. It can be determined from broth dilution of minimum inhibitory concentration (MIC) tests by sub culturing to agar plates that do not contain the test agent. The MBC is identified by determining the lowest concentration of antibacterial agent

that reduces the viability of the initial bacterial inoculums by $\geq 99.9\%$. Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC. Select MIC tubes for determining MBC based on the visible growth. Last two non-visible tubes to the 1st two visible tubes were selected to screen MBC. One ml of culture (grown in Mueller hinton Broth) from MIC tube were serially diluted upto 1:1000 dilution and inoculated 1:100 and 1:1000 diluted samples into Mueller Hinton agar containing Petri plate by spread plate technique. GC tube containing culture was serially diluted upto 10⁻⁸ and plated 10⁻⁴, 10⁻⁵, 10⁻⁶ and 10⁻⁷ dilution as on respective properly labeled nutrient agar plate to check total viable count of the initial inoculums used to determine percentage inhibition. The number of bacterial colonies in each plate were counted properly and recorded. The dilution at which no colonies counted was considered as the MBC of the extract.

Determination of % inhibition

It is a calculation of inhibitory effect of extracts at particular concentration by making use of total viable count value of GC tube and dilution tubes. It was calculated by making use of the following formula.

% Inhibition =

$$\frac{\text{Number of colonies in tube GC} - \text{Number of colonies in dilution tube}}{\text{Number of colonies in tube GC}} \times 100$$

Determination of IC₅₀

According to the FDA, IC₅₀ represents the concentration of a drug that is required for 50% inhibition *In Vitro*. It is obtained from the %inhibition and the concentration of extract used. IC₅₀ was calculated by using the formula.

$$IC_{50} = \frac{\text{Concentration of Extract}}{\% \text{ inhibition}} \times 50$$

Statistical Analysis

All the results were expressed as mean $\pm$ S.E.M. The data were statistically analyzed by one way analysis of variance (ANOVA).

Results

The antibacterial assay was carried out for the ethanolic extracts of test plants from wild and market available plant powders of *Vernonea cinerea* and *Cardiospermum halicacabum* against seven potential diseases causable microorganisms. Disc diffusion method was adopted to screen anti-bacterial activity. Zone of inhibition produced by four extracts of two plants ranges from 7.0 $\pm$ 4.4mm (VCCEE – *Pseudomonas aeruginosa*) to 21.7 $\pm$ 2.5 mm (VCWEE – *Salmonella typhi*). VCWEE produced higher activity against *Salmonella typhi* followed by CHWEE against *Streptococcus pyogenes* (21.7 $\pm$ 3.2) and *Staphylococcus aureus* (21.0 $\pm$ 0.6). The negative controls didn't show any inhibitory effect on bacterial growth. There was a significant difference in the mean zone of inhibition between positive control and plant extracts (Table 1). Among the two plant powder, obtained from wild showed more activity than the commercial plant powder whereas all the plant extracts showed antibacterial activity against all the test organisms.

Wild collected fresh plant materials showed comparatively better activity when compared to the stored powdered plant materials (Table 2). CHWEE inhibited effectively the growth of *Pseudomonas aeruginosa* with MIC at 116.7 $\pm$ 14.4 μ g/ml concentration. Similarly VCWEE also produced MIC at 116.7 $\pm$ 28.9 μ g/ml concentration against *Klebsiella sp.*, whereas CHCEE produced good effect at 125.0 $\pm$ 25.0 μ g/ml concentration against *Salmonella typhi*, VCCEE produced MIC at 166.7 $\pm$ 57.7 concentration against *Klebsiella sp.*

Table 1. Antibacterial nature of alcoholic extracts of *Vernonea cinerea* and *Cardiospermum halicacabum* wild as well as stored powders

S. No	Microorganisms	Tetracycline	Zone of inhibition in mm			
			VCWEE	CHWEE	VCCEE	CHCEE
1	<i>Escherichiacoli</i>	R	13.7 $\pm$ 0.6	13.3 $\pm$ 1.5	13.0 $\pm$ 6.8	8.7 $\pm$ 0.6
2	<i>Shigella sp.</i>	R	12.0 $\pm$ 1.0	13.0 $\pm$ 3.0	13.7 $\pm$ 9.8	8.3 $\pm$ 0.6
3	<i>Klebsiella sp.</i>	R	17.0 $\pm$ 3.6	13.0 $\pm$ 2.0	9.7 $\pm$ 0.5	11.3 $\pm$ 0.6
4	<i>Salmonella typhi</i>	R	21.7 $\pm$ 2.5	16.3 $\pm$ 5.5	11.7 $\pm$ 1.2	11.0 $\pm$ 2.7
5	<i>Pseudomonas aeuroginosa</i>	R	16.7 $\pm$ 4.7	15.3 $\pm$ 2.1	7.0 $\pm$ 4.4	11.3 $\pm$ 0.6
6	<i>Streptococcus pyogenes</i>	R	15.0 $\pm$ 2.0	21.7 $\pm$ 3.2	13.7 $\pm$ 1.2	8.3 $\pm$ 0.6
7	<i>Staphylococcus aureus</i>	R	14.7 $\pm$ 0.58	21.0 $\pm$ 0.6	15.3 $\pm$ 0.5	11.30.6

Table 2. Antibacterial nature of *Vernoneacinerea* and *Cardiospermum halicacabum* wild as well as stored powder with reference to MIC

S. No	Microorganisms	Minimum Inhibitory Concentration in µg/ml			
		VCWEE	CHWEE	VCCEE	CHCEE
1	<i>Escherichiacoli</i>	266.7±28.9	366.7±28.9	266.7±57.7	316.7±28.9
2	<i>Shigellasp.,</i>	250.0±50.0	366.7±57.7	233.3±57.7	333.3±28.9
3	<i>Klebsiellasp.,</i>	116.7±28.9	325.0±25.0	166.7±57.7	216.7±28.9
4	<i>Salmonella typhi</i>	158.3±38.2	283.3±28.9	175.0±43.3	125.0±25.0
5	<i>Pseudomonas aeuroginosa</i>	183.3±52.0	116.7±14.4	233.3±76.4	216.7±28.9
6	<i>Streptococcus pyogenes</i>	158.3±38.2	125.0±25.0	208.3±38.2	225.0±25.0
7	<i>Staphylococcus aureus</i>	166.7±28.9	283.3±28.9	275.0±90.1	166.7±14.4

Table 3. Antibacterial nature of *Vernonea cinerea* and *Cardiospermum halicacabum* wild as well as stored powder with reference to MBC

S. No	Microorganisms	Minimum Bactericidal Concentration in µg/ml			
		VCWEE	CHWEE	VCCEE	CHCEE
1	<i>Escherichiacoli</i>	325.0±25.0	275.0±25.0	391.6±14.4	358.3±52.0
2	<i>Shigellasp.,</i>	261.6±12.6	308.3±38.2	383.3±14.4	383.3±28.9
3	<i>Klebsiellasp.,</i>	236.6±23.1	350.0±25.0	241.6±14.4	366.7±28.9
4	<i>Salmonella typhi</i>	233.3±28.9	275.0±25.0	241.6±14.4	275.0±25.0
5	<i>Pseudomonas aeuroginosa</i>	250.0±25.0	308.3±14.4	250.0±25.0	333.3±28.9
6	<i>Streptococcus pyogenes</i>	275.0±25.0	308.3±28.9	300.0±25.0	258.3±38.2
7	<i>Staphylococcus aureus</i>	283.3±28.9	233.3±28.9	291.6±14.4	341.7±28.9

Minimum bactericidal concentration represents complete killing of the bacterial cells, which is a direct detection of antimicrobial potential of the extract. Here also wild collected plant materials showed potent effect than market procured powdered plant materials (Table 3). *Vernonea cinerea* and *Cardiospermum halicacabum* wild plant extracts produced effective MBC at 233.3±28.9 µg/ml concentrations for bacterial pathogens like *Salmonella typhi* (VCWEE) and *Staphylococcus aureus* (CHWEE). Similarly commercial powder also showed good effect of MBC at 241.6±14.4 µg/ml concentrations for *Klebsiella* and *Salmonella typhi*.

Extracts showed different percentage of inhibition against different bacterial agents. More than 90% of bacterial cells are killed by VCWEE and CHWEE at 200µg/ml concentrations. Commercially available powder exhibited different percentage of inhibition when compared to wild. Percentage of inhibition showed by commercial plant powder was ranged from 49.8% to 84.5%, whereas wild plant showed slightly good percentage of inhibition. It ranges from 59.6% to 97.3% of inhibition at 200µg/ml concentrations with best activity against

Streptococcus pyogenes (Figure 1).

The half maximal inhibitory concentration which is otherwise called as IC₅₀. It can be defined as the measure of the effectiveness of a compound in inhibiting biological or biochemical function. This is the first of this kind of study indicating the IC₅₀ values exhibited by the plant extracts along with percentage of inhibition. Concentration required for killing 50% of *Streptococcus pyogenes* by CHWEE was at 102.7 µg/ml concentration, followed by *Staphylococcus aureus* at 103.2 µg/ml concentration. VCWEE produced best activity against *Klebsiella* sp., at 105.7 µg/ml concentration. Powder from market exhibited variable IC₅₀ value, which was higher than wild plant powder (Figure 2).

Discussion

Vernonea cinerea and *Cardiospermum halicacabum* are annual plants which are commonly available throughout the year. Both of these plants are used for the treatment of inflammatory disorders like nephritis, neuritis, arthritis, wound healing etc. Microorganisms are the major player of

Figure 1. Antibacterial activity of *Vernonea cinerea* and *Cardiospermum halicacabum* wild as well as Stored powder with reference to % inhibition

Figure 2. Antibacterial activity of *Vernonea cinerea* and *Cardiospermum halicacabum* wild as well as Stored powder with reference to IC₅₀

inflammation, thereby effectiveness of these plants as an antimicrobial agent has been confirmed. *Streptococcus pyogenes* was more susceptible towards ethanolic extract of *Cardiospermum halicacabum* wild plant (21.7 ± 3.2) followed by *S. aureus* (21.0 ± 0.6). This study confirmed the effectiveness of *Cardiospermum halicacabum* against gram positive bacteria than gram negative bacteria. Anupama Singh et al. (2014) and Valsaraj et al. (1996) reported that *Vernonea cinerea* extracts were found against gram positive bacteria whereas the present study indicated that *Vernonea cinerea* found effective against

both gram positive and gram negative bacteria with higher activity against *Salmonella typhi* (Table 1). Though *Vernonea cinerea* extract inhibited higher activity against gram negative bacteria, *Cardiospermum halicacabum* extracts showed higher activity against gram positive bacteria like *Streptococcus pyogenes* and *Staphylococcus aureus* (More than 20mm zone of inhibition). Herbal extracts are a source of chemical diversity which could be considered as a safe phytotherapy against MDR pathogens (Suresh et al., 2012). He further indicated the presence of glycosides in

Cardiospermum halicacabum, which is not available in *Vernonea cinerea*. Tannins are the major compounds useful in the treatment of inflammatory activities. They coagulates the wall proteins along with lipids thereby prevents microbial viability (Prabha and Ramachandramurty, 2013). Ahsanul Haqueet al. (2012) confirmed the presence of glycosides, terpenoids and esters in *Vernonea cinerea*. Varshaet al. (2016) reported the antimicrobial potentials of this plant. This could be due to terpenoids and glycosides present in the plant. Vijayanand and Wesley (2014) also confirmed antimicrobial activity of this plant. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase (Li et al., 2003). They also serve as health promoting compounds as a result of their anion radicals (Hausteen, 1983). This result may suggest that all extracts possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in human. Extracts had an inhibition zone diameter between 10mm to 16mm, which was higher than to a standard antibiotic, hence we suggest their effectiveness as antimicrobials from the plant. The active components in the crude extract may be acting synergistically to produce antimicrobial effects (Elloff, 1998).

Effectiveness of these plants could be due to phytochemicals like phenolic compounds, tannins, flavonoids, sterols etc. Raza et al. (2012) confirmed that quercetin like compounds are prevalent in *Cardiospermum halicacabum*. They also stated that available phytochemicals may inhibits viral entry and also clean availability of HBV antigens. Ethanolic extract of *Vernonea cinerea* and *Cardiospermum halicacabum* effectively controls bacterial growth, which was evidence through the disc diffusion assay, MIC, MBC, % inhibition and IC_{50} assay (Table 1, 2, 3 and Figure 1 & 2). Susceptibilities of bacteria broadly depend on the type of barriers present on the surface of bacteria and type of phytochemicals present in the plant. Interactions of phytochemicals on microbes results in the antimicrobial susceptibility pattern.

In the present study efficiency of commercially available plant powders are not effective as the wild collected fresh plant materials, which also showed higher antimicrobial property. It was evident that IC_{50} value of stored powder ranges from 120 to 200 µg/ml concentrations, which is within the acceptable range.

Conclusion

This study confirms antimicrobial pattern of *Vernonea cinerea* and *Cardiospermum halicacabum* ethanolic extracts and recommends the use of fresh plant powders for medicinal purposes.

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