Contents lists available at Science-Gate

International Journal of Advanced and Applied Sciences

Journal homepage: http://www.science-gate.com/IJAAS.html

Antibacterial activity of *Sphaeranthus indicus* and *Helianthus annus* against multi-drug resistant (MDR) clinical isolates

Harish. C. Chandramoorthy *

Department of Microbiology & Parasitology, College of Medicine, King Khalid University, Abha, Kingdom of Saudi Arabia

ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form
25 January 2016
Accepted 26 January 2016

Keywords:
Sphaeranthus indicus
Helianthus annus
Antibacterial activity
Herbal drugs
Bioactivity of herbs

ABSTRACT

In the current investigation the traditional aqueous and ethanolic extraction products from leaves of *Sphaeranthus indicus and* flowers of *Helianthus annus* were evaluated for antibacterial activity against multi drug resistant (MDR) bacterial isolates from the clinical samples. Standard tube dilution and disc diffusion methods were employed to assess the antibacterial activity. The results showed, ethanolic *sphaeranthus* extract at a minimum inhibitory concentration (MIC) of 3mg/ml by tube dilution & 1.8 mg/ml by disc diffusion method inhibited the growth of MDR *Staphylococcus and Klebsiella*. Further the MDR *E.coli* and *pseudomonas* were effectively inhibited by the ethanolic extract of *Helianthus* at a concentration of 4 mg/ml and 2.5 mg/ml by tube dilution and disc diffusion methods respectively. The aqueous extracts of both the herbs showed a transient antibacterial activity against MDR staphylococcus isolate, while there was no observable activity against MDR *E.coli*, *pseudomonas* or *klebsiella* isolates. Collectively targeting the MDR isolates may pave a way for future class of drugs from natural products in way they have been extracted and used for generations.

1. Introduction

In the recent decades, modern research methodologies are developed and aim at authenticating the traditional herbs for its potential anti-infectious activities regardless of the agents associated with the cause (Kowalska, 2015; Awan et al., 2013; Komolafe OO, 2003). In most of the studies, use of complex extraction methods, processing and formulations of the plant products have been introduced with varied success. However the traditional extraction methods or the classical formulations are still available today with the oriental system of medical practitioners, which are less attended by these authenticating studies and investigations. Though there are very few such evaluation studies done in past, there are no recommendations for oriental plant based medicines to be included in the drug lists used in modern medicine (Chen et al., 2015). There are no scientific data available on bioactivity of herbal drugs to the oriental medical practitioner, with very few exceptional entries of translational research data in recent past. The use and misuse of anti-microbial agents especially antibiotics resulted in the evolution of MDR organisms posing a threat to healthcare systems. The need for broad-spectrum drugs has pushed the researchers to look for new agents from natural products, herbs, small molecules to combat the growing infections due of emergence of MDR organisms (Komolafe OO, 2003; Mellon et al., 2001). There are good number of herbs identified in traditional, folklore and tribal medicine prescribed to combat diseases which will resemble the bacterial infections and modern defined diseases, while many preliminary works for evaluating such herbal extracts against bacterial isolates has been successful without any further translational approach towards bedside in modern medicine (Gromek et al., 2015; Tulunay et al., 2015). The use of the *Sphaeranthus* as a drug is mentioned in ayurvedic system of medicine for varied clinical conditions like Jaundice & leprosy (Ramachandran, 2013). The herb is rich in sesquiterpene and all parts of the plants are used in oriental medicine, while leaves play a prominent part in the drug formulations and preparations. Few studies have documented *sphaeranthus* for its antibacterial properties (Basu and Lamsal, 1946; Galani et al., 2010; Selvi et al., 2011; John and Tamilmaraiselvi, 2011). Sunflower plants are widely studied for biological and chemical activities. *Helianthus annuus* is widely grown as commercial crop, whole plant and leaf extracts has been traditionally used as anti-inflammatory, anti-tumor agent and antibacterial agents (Rajakannu and Sritharan, 2012; Ukiya et al., 2003; Sechi et al., 2001). The sunflower oil (Oleozon)

* Corresponding Author.
Email Address: ccharishjabali@gmail.com

extracted from flowers has been used as food and an additive of drug and cosmetic formulations (Casetti et al., 2011).

All these medical herbs have been documented and studied for its phytochemical constituents. However, we are not sure on the bioavailability of the individual secondary metabolites (the phyto-active chemicals) activity against bacteria or tumor cells, when it comes as isolated and purified component. It has been known that many modern extraction procedures reduces the phytochemicals into unavailable forms or in many cases compromise the bioactivity or generally documented as synergistic in action (Yang et al., 2014). As early as 1946, there were reports on the drug resistance to penicillin and speed at which the single drug resistance to the bacteria turned to MDR was not predictable (Barber, 1947). Mechanism of the action for MDR is a dynamic process and over the decades it has developed into additional types of the mechanisms of drug tolerance or resistance. Drug binding proteins, drug modification by enzymes, mutated drug targets, altered membrane permeability and enhanced efflux mechanism are some the recent modifications observed in the MDR strains. Many of the mechanisms are through adaptive changes which alters the genetic makeup of the bacterial strains. Recently drug resistance plasmids, transposon like complex, conjugative, bacteriophage and integron are some of the adaptations which conferred drug resistance and pose a great threat to the general health (Alekshun et al., 2007). Today antibiotic abuse has revived the authentication of the herbal and traditional drugs with multiple numbers of drug resistance strains. In many developing countries there are no proper antibiotic policies or monitoring system aiding more on MDR infections. Collectively emerging new strains of MDR organisms in an alarming rate has pushed the pharmaceutical industries to look for alternative and new drugs (WHO, 2000). The current lacuna in the search is that many screening reports or studies have no clear-cut validation and reproducibility on the bioactivity, especially with clinical isolates, which forms a larger part of MDR organisms. Further we do not find enough evidence from the literature on use of aqueous and ethanolic extracts of the herbs directly on the clinical isolates that are clinically relevant than the commensals and non-virulent strains that are frequently used in these studies (Kumar et al., 2006).

In the current study we have authenticated two widely used herbs *Sphaeranthus indicus* and *Helianthus annus*, which were screened primarily for antibacterial activities on common laboratory bacterial strains and MDR strains isolated from clinical specimens.

2. Materials and Methods

2.1. Plants (Herbs)

2.1.1. Sphaeranthus indicus and Helianthus annuus

Sphaeranthus indicus is distributed widely in hilly and damp landscape of tropical regions. The herb belongs to the family Asteraceae. The whole plant is used in the Ayurveda and Siddha system of the oriental medicine (Ramachandran, 2013). *Helianthus annuus* is an important cash crop (oil seed) widely cultivated throughout the tropical regions. It belongs to the family of Asteraceae (Rajakannu and Sritharan, 2012). The flower and seeds are used in many folklore and traditional system of medicine (Thiyagarajan, 2006). The leaves of sphaeranthus were collected from the social forest areas in around Chennai and leaves of the *helianthus* were collected form the farm lands of the rural north Tamil Nadu, a Taxonomist identified the herbs.

2.2. Use in traditional medicine

Sphaeranthus indicus:

The leaves of *sphareanthus* was demonstrated to have ovicidal, antitussive, wound healing, anxiolytic, neuroleptic, immunomodulatory, antifeedant, antihelmintic and analgesic/antipyretic activities.

Apart from the above said medicinal properties this herb has been documented to have antidiabetic/ antihyperlipidemic/ antioxidant activities along with few documented studies on antimicrobial activities. Further sphaeranthus leaves have been used in the both Siddha (as Veezhi Ennai or Veezhi oil) and Ayurveda (Navaratnaraja and Guduchi taila) system of medicine (Ramachandran, 2013; Basu and Lamsal, 1946; Galani et al., 2010).

Helianthus annuus:

It has been documented for anti-inflammatory, antimalarial, anti-asthmatic, anti-oxidant, anti-tumor and antimicrobial agent. In the traditional system and folklore medicine it has been widely used to treat catarrh, blindness, sinusitis, diarrhea, dysentery, hemorrhoids, and scorpion stings /snake bite etc. (Sechi et al., 2001; Rajakannu and Sritharan, 2012; Ukiya et al., 2003).

2.3. Bacterial cultures

The multi-drug resistant isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *and Klebsiella pneumoniae* were obtained from city hospitals and were identified using recommended morphological and biochemical tests (data not shown).

The cultures were grown in Muller Hinton broth at 37^0C and maintained in nutrient agar slopes at 4^0C. Antibiotic sensitivity pattern were determined by Kirby baur method (Baur et al., 1966).

2.4. Preparation of the leaf extracts

The freshly collected leaves were cleaned, shade dried for two weeks, pounded to coarse powder in

bender, stored in an airtight container for further extraction processes. Briefly for the preparation of aqueous and ethanolic extract, 50 g of the leaf powder was mixed with 300ml of water and ethanol respectively. The homogenate was kept in orbital shaker for 48hrs and filtered through muslin cloth. The supernatant was dried at 55^0C and stored in airtight vessel at 4^0C. The sediments were re-extracted and processed as described above. The working solutions were prepared by dissolving 10mg/1ml of the extract dissolved in phosphate buffered saline (PBS) for aqueous and 0.25% dimethyl formamide (DMF) in case of ethanolic extract. For disc diffusion known concentration of the extract were poured into the small discs of filter paper and dried. The discs were stored in a labeled ziplock bags at 4^0C. For the tube dilution technique, the appropriate amount of extract was added to the testing medium (Muller Hinton Broth) to make the final concentration from1mg/ml to 10mg /ml. Appropriate controls were included in the assay (Harish CC et al., 2010).

2.5. Disc diffusion method

The multidrug resistant clinical isolates were surface swabbed in Muller-Hinton agar plates with 100µl of the logarithmic phase bacteria at a density adjusted to; 0.5 McFarland turbidity standard (10^8 cfu/ml). The prepared *sphaeranthus* and *helianthus* discs were placed into the medium with 2 cm spacing respectively. The plates were incubated at 37^0C for 24-48 hours. The lowest concentration that prevented visible growth "zone of clearance" was measured as MIC (Harish et al., 2010).

Serial dilutions 10mg/ml to 1mg/ml of the leaf extracts were prepared in 2.6 ml of Muller Hinton broth to make final volume of 3.6 ml. Initial OD were measured at 590nm, to which 0.4ml of the bacterial suspension containing $1X10^6$ cfu/ml was added to 3.6ml of the susceptibility test broth. The final medium volume was 4 ml. The test was performed in triplicates. The tubes were incubated at 37^0C for 24-48hours. The Final OD of the incubated tubes was measured. The difference between the initial and final OD determined the bacterial inhibition. A blank tube with only media and one with the test bacteria served at the negative and positive internal controls respectively. The concentrations showing fall in OD compared with positive control was noted as MIC of the drug (Harish et al., 2010; Manavathu et al., 1996).

3. Results

Results depicted in Table 1 confirm the multi-drug resistance and antibiotic sensitivity pattern of the clinical isolates. The results of the bioactivity of the aqueous and ethanolic extracts of *Sphaeranthus indicus* and *Helianthus annuus* are summarized for disc dilution method in Table 2 & Fig. 1, 2 & 3, while fall in the OD were tabulated in Table 3 for tube dilution method and results are summarized in Table 4 for convenience. The residual toxicity for ethanolic extracts (though well dried) were measured (data not shown) with routine alcohol identification test, that resulted negative. The overall study results showed both *S.aureus* and *E.coli* were susceptible to the ethanolic extracts of *S.indicus* and *H.annuus*. The aqueous extract of the both the herbs had relatively fair activity over these clinical isolate.

2.6. Tube dilution Method

Table 1: Antibiotic susceptibility pattern of the clinical isolates

Clinical Isolates / Antibiotics	*S.aureus*	*K.pneumoniae*	*E.coli*	*P.aeruginosa*
Ampicillin (A)	NA	0	0	NA
Amoxyclov (Ac)	NA	0	0	NA
Amikacin (Ak)	NA	12	0	NA
Ceftazidime (Ca)	NA	0	0	0
Cephotoxime (Ce)	NA	0	0	5
Ciproflaxicin (Cf)	NA	0	0	6
Cefuroxime (Cu)	NA	0	0	NA
Cefazolin (Cz)	NA	0	NA	NA
Gentamycin (G)	13	0	0	0
Imipeneum (I)	NA	14	20	0
Nalidixic acid (Na)	0	0	0	NA
Nitrofurantoin (Nf)	18	4	0	NA
Netillin (Nt)	NA	0	4	NA
Norfloxacin (Nx)	NA	0	0	0
Penicillin (P)	0	0	0	NA
Rifampicin [R]	0	NA	NA	NA
Vancomycin (Va)	0	NA	NA	NA

The zone of clearance or inhibition zone (IZ) was measured in mm; Value 0 refers to nil zone of clearance, NA - Not Applicable

Staphylococcus aureus: The clinical isolate was sensitive only to nitrofurantoin and gentamycin, while resistant to almost all antibiotics tested (Table 1).

Table 2: Antibacterial activity of aqueous and ethanolic extracts of *Sphaeranthus indicus* and *Helianthus annuus* by disc diffusion method

Herb	Nature of extracts	Clinical Isolates	Zone of inhibition (IZ) in mm					
			10mg/ml	5mg/ml	4mg/ml	3mg/ml	2mg/ml	1mg/ml
S.indicus	Aqueous	*S.aureus*	7	2	0	0	0	0
		K.pneumoniae	0	0	0	0	0	0
		E.coli	25	18	13	7	2	0
		P.aeroginosa	0	0	0	0	0	0
	Ethanolic	*S.aureus*	22	19	14	12	10	7
		K.pneumoniae	17	15	9	2	0	0
		E.coli	33	27	22	15	12	9
		P.aeroginosa	0	0	0	0	0	0
H.annuus	Aqueous	*S.aureus*	19	15	7	4	0	0
		K.pneumoniae	0	0	0	0	0	0
		E.coli	12	5	0	0	0	0
		P.aeroginosa	0	0	0	0	0	0
	Ethanolic	*S.aureus*	25	22	17	15	11	6
		K.pneumoniae	8	0	0	0	0	0
		E.coli	22	17	13	8	5	2
		P.aeroginosa	15	7	0	0	0	0

The zone of clearance or inhibition zone (IZ) was measured in mm; Value 0 refers to nil zone of clearance.

Table 3: Antibacterial activity of aqueous and ethanolic extracts of *Sphaeranthus indicus* and *Helianthus annuus* by tube dilution method

Herb	Nature of extract	Clinical Isolates	Positive control OD (nm)	Concentration (mg/ml) of the extract versus growth OD (nm) values					
				10	5	4	3	2	1
S.indicus	Aqueous	*S.aureus*	1.84 ± 0.2	0.46 ± 1.3	0.55 ± 0.2	0.66 ± 0. 1	0.74 ± 0.2	0.91 ± 0.2	1.42 ± 0.2
		K.pneumoniae	0.79 ± 0.4	0.80 ± 0.2	0.77 ± 0.2	0.80 ± 0.2	0.78 ± 0.1	0.79 ± 0.3	0.74 ± 0.2
		E.coli	1.34 ± 0.3	0.52 ± 0.1	0.59 ± 00	0.66 ± 0.2	0.68 ± 0.2	0.91 ± 0.2	0.96 ± 0.2
		P.aeroginosa	0.94 ± 0.3	0.91 ± 0.3	0.96 ± 0.2	0.89 ± 0.2	0.96 ± 0.1	0.92 ± 0.2	0.93 ± 0.2
	Ethanolic	*S.aureus*	1.84 ± 0.2	0	0	0	0.18 ± 0.3	0.26 ± 0.2	0.56 ± 0.2
		K.pneumoniae	0.79 ± 0.4	0.47 ± 0.2	0.40 ± 0.1	0.43 ± 00	0.45 ± 0.2	0.76 ± 0.2	0.79 ± 0.2
		E.coli	1.34 ± 0.3	0	0	0.33 ± 0.2	0.41 ± 0.2	0.52 ± 0.2	0.51 ± 0.2
		P.aeroginosa	0.94 ± 0.3	0.99 ± 0.1	0.96 ± 0.2	0.92 ± 0.2	0.98 ± 0.2	0.99 ± 0.1	0.93 ± 0.4
H.annuus	Aqueous	*S.aureus*	1.84 ± 0.2	0.57 ± 0.1	0.74 ± 0.3	0.81 ± 0.2	0.92 ± 0.2	1.78 ± 0.2	1.76 ± 00
		K.pneumoniae	0.79 ± 0.4	0.76 ± 0.2	0.74 ± 1.2	0.72 ± 0.2	0.81 ± 0.5	0.78 ± 0.2	0.83 ± 0.2
		E.coli	1.34 ± 0.3	0.36 ± 0.2	0.41 ± 0.2	0.52 ± 0.1	0.53 ± 0.5	0.89 ± 0.2	1.51 ± 0.2
		P.aeroginosa	0.94 ± 0.3	0.91 ± 1.2	0.92± 0.2	0.99 ± 0.1	0.96 ± 0.2	0.98 ± 0.2	0.99 ± 0.2
	Ethanolic	*S.aureus*	1.84 ± 0.2	0	0.26 ± 0.3	0.48 ± 0.3	0.40 ± 0.1	0.52 ± 0.2	0.56 ± 0.4
		K.pneumoniae	0.79 ± 0.4	0.44 ± 0.2	0.63 ± 0.1	0.71 ± 0.2	0.72 ± 00	0.71 ± 0.2	0.79 ± 0.3
		E.coli	1.34 ± 0.3	0	0	0.15 ± 0.1	0.26 ± 0.2	0.38 ± 0.1	0.49 ± 0.2
		P.aeroginosa	0.94 ± 0.3	0.45 ± 0.1	0.51± 0.2	0.91 ± 0.6	0.94 ± 0.2	0.98 ± 0.2	0.92 ± 0.2

Growth is measured by difference in OD at 590 nm due to the turbidity and color change of bacterial growth. CV = initial OD - final OD compared to OD of the positive control (without extracts) at 590nm

The results of the disc diffusion and tube dilution method were varied as ethanolic extract of *S.indicus* showed a significant activity over staphylococcus at a concentration as low as 2 mg/ml effectively (Table 2 & 3, Fig. 1a). The effective dose (ED) was 3 mg/ml. On the other hand the aqueous extract of the *S.indicus* did not show clear-cut activity by disc diffusion compared to the tube dilution method (Table 2 & 3 and Fig. 1a). The results aqueous and ethanolic extracts of the *H.annuus* were highly significant with ED as low as 3 mg/ml (Table 2, 3 and Fig. 1b) over MDR *Staph aureus*.

Klebsiella pneumoniae: The clinical isolate was susceptible to amikacin and imipeneum, while mild sensitivity observed towards nitrofurantion (Table 1).

The aqueous extract of *S.indicus* did not show any activity, however the ethanolic extracts were active

at a concentration of 5 mg/ml by disc diffusion and 3 mg/ml by tube dilution method (Table 2, 3 and Fig. 3 a). Aqueous extract of *H.annuus* did not show any significant activity, while ethanolic extract had an observable activity except at higher concentration.

Table 4: Summary of results of antibacterial activity by tube dilution technique

Herbs	Concentration of the extracts in mg/ml	Clinical Isolates			
		S.aureus	*K.pneumoniae*	*E.coli*	*P.aeroginosa*
Aqueous *S.indicus*	10	Sensitive	Resistant	Sensitive	Resistant
	5	Sensitive	Resistant	Sensitive	Resistant
	4	Sensitive	Resistant	Sensitive	Resistant
	3	Sensitive	Resistant	Sensitive	Resistant
	2	Mild	Resistant	Mild	Resistant
	1	Mild	Resistant	Mild	Resistant
Ethanolic *S.indicus*	10	Highly Sensitive	Sensitive	Highly Sensitive	Resistant
	5	Highly Sensitive	Sensitive	Highly Sensitive	Resistant
	4	Highly Sensitive	Sensitive	Sensitive	Resistant
	3	Sensitive	Sensitive	Sensitive	Resistant
	2	Sensitive	Resistant	Sensitive	Resistant
	1	Sensitive	Resistant	Sensitive	Resistant
Aqueous *H.annuus*	10	Sensitive	Resistant	Sensitive	Resistant
	5	Sensitive	Resistant	Sensitive	Resistant
	4	Sensitive	Resistant	Sensitive	Resistant
	3	Sensitive	Resistant	Sensitive	Resistant
	2	Intermediate	Resistant	Mild	Resistant
	1	Intermediate	Resistant	Resistant	Resistant
Ethanolic *H.annuus*	10	Highly Sensitive	Sensitive	Highly Sensitive	Sensitive
	5	Sensitive	Sensitive	Highly Sensitive	Sensitive
	4	Sensitive	Resistant	Sensitive	Resistant
	3	Sensitive	Resistant	Sensitive	Resistant
	2	Sensitive	Resistant	Sensitive	Resistant
	1	Sensitive	Resistant	Sensitive	Resistant

Highly sensitive – No growth compared to the positive control, Sensitive – very low growth, Mild & Intermediate – low growth observed

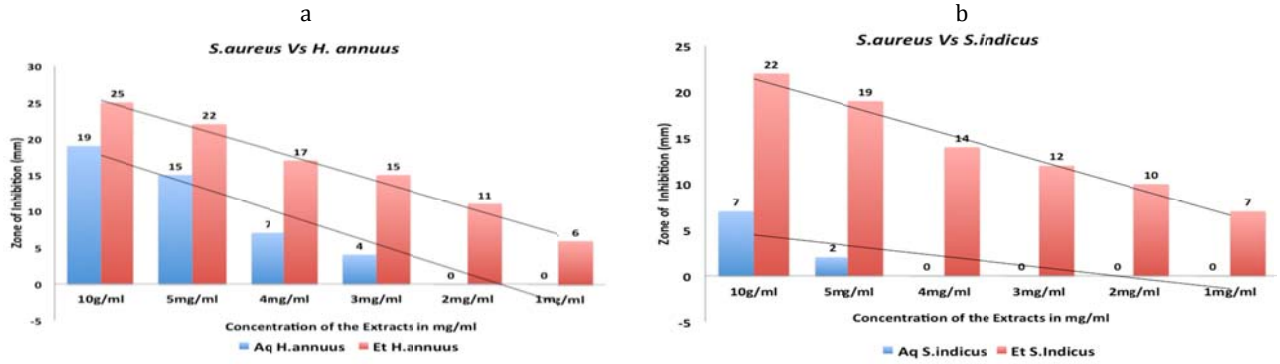

Fig. 1: Effect of aqueous & ethanolic extracts of 1a. *Sphaeranthus indicus* and 1b. *Helianthus annuus* on multi drug resistant *Staphylococcus aureus* by disc diffusion method. The line connecting the bars shows the concentration dependent clearance (Linear) of the bacterial growth

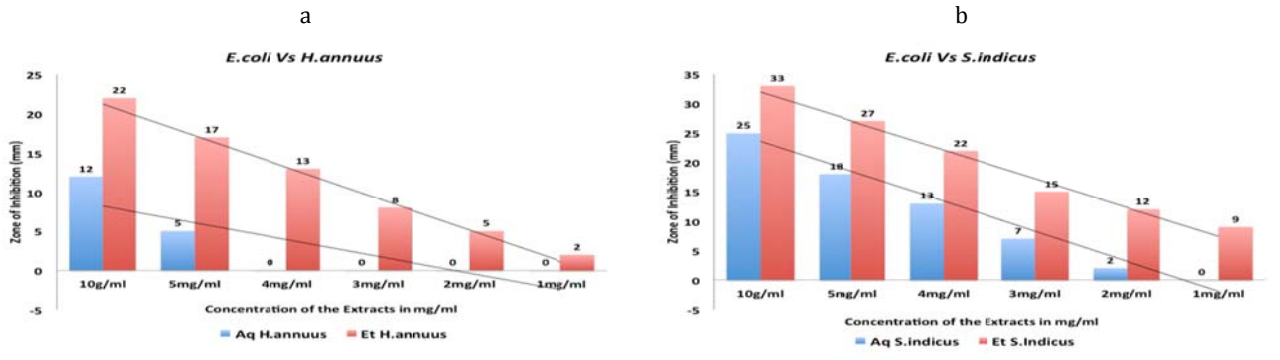

Fig. 2: Effect of aqueous & ethanolic extracts of 2a. *Sphaeranthus indicus* and 2b. *Helianthus annuus* on multi drug resistant *Escherichia coli* by disc diffusion method. The line connecting the bars shows the concentration dependent clearance (Linear) of the bacterial growth.

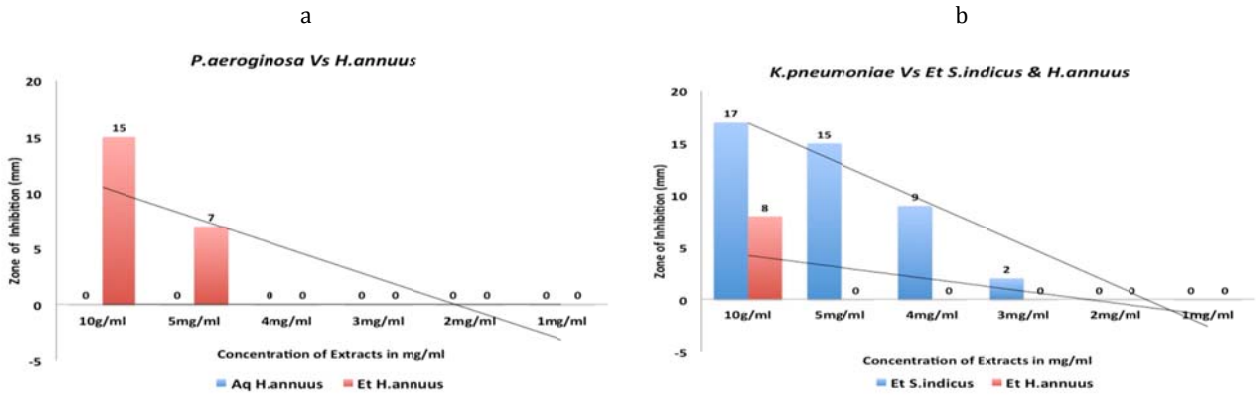

Fig. 3: Effect of aqueous & ethanolic extracts of *Sphaeranthus indicus* and *Helianthus annuus* on multi drug resistant *Klebseilla pneumoniae* by disc diffusion method. The line connecting the bars shows the concentration dependent clearance (Linear) of the bacterial growth.

Escherichia coli:

Isolate was sensitive only to imipeneum and mildly responded to netillin (Table 1). The aqueous and ethanolic extracts of the *S.indicus* showed an observable anti-E.coli activity at a lower concentration of 2 mg/ml and 1mg/ml respectively (Table 2, Fig. 2a) by disc diffusion method. The aqueous extracts of *H.annuus* was active at higher concentration, while ethanolic extract showed anti *E.coli* activity at a concentration as low as 1mg/ml by tube dilution method (Table 2 &3, Fig. 2b). The results of the tube dilution method were more promising and mostly on par with results of disc diffusion for both the herbs. Both aqueous and ethanolic extracts of *S.indicus* and *H.annuus* exhibited a significant antibacterial activity over *E.coli* with an effective dose of 2 mg/ml over MDR *E.coli.*

Pseudomonas aeruginosa:

The clinical isolate showed a mild sensitivity to cephotoxime and ciproflaxicin (Table 1) and resistant to all other drugs. We did not observe any observable/significant activity with aqueous and ethanolic extract of *S.indicus* or aqueous extracts of *H.annuus* (Table 2, 3). *P.aeruginosa* was sensitive to ethanolic extracts of *H.annuus* at a higher concentration of 10 mg/ml by both disc and tube dilution method (Table 2, 3, 4 & Fig. 3b).

4. Discussion

The antibacterial effects of aqueous and ethanolic extracts of *S.indicus* and *H.annuus* were effective over three out of four MDR isolates by both disc diffusion and tube dilution methods. However *P.aeruginosa*, did not show up susceptibility to both the herbal extracts.

It is evident that the ethanolic extracts of the *S.indicus* and *H.annuus* had relatively higher activity over the MDR *S.aureus*, *E.coli* and *K.pneumoniae* than the aqueous extracts of the same. This may be due to the presence of the more secondary metabolites soluble in ethanol. It may be further noted that various solvents of higher polarity, that has been used to extract plant/leaves represents a good array of phytochemicals available as bioactive constituents (Selvi et al., 2011; Ukiya et al., 2003). Interestingly in the current investigation, the aqueous extracts showed significant antibacterial activity at a higher concentration like 5 or 10 mg/ml compared to the activities ethanolic extracts at a bare minimum concentration. This can be attributed to the ethanol soluble metabolites of the herbs augmenting the bioactivity compared to the aqueous extract. Further these observations very well coincided with the literature evidences of long term use of aqueous extracts in the treatment schedule over the extracts of ethanol or methanol origin (Malini et al., 2013).

The aqueous and ethanolic extract of *S.indicus* exhibiting anti-*Staph.aureus* activity was well in agreement with previously published studies. However these studies have used different subspecies for their evaluation, collectively *S.indicus* comprises the active phytochemicals, which has more evolved action and present throughout this family (Ramachandran, 2013). Same results were observed with the *E.coli*, *Klebsiella,* which were in good agreement with previous studies, but not with *Pseudomonas*, which varied from previous studies (John and Tamilmaraiselvi, 2011). Many studies with *Helianthus* and its oil or isolated secondary metabolites exhibited bioactivity against wide range of bacterial species. In our study too, the only herb, which was active to all the MDR isolates, was ethanolic extract of *H.annuus*. Though the activity against *Pseudomonas* was not very convincing as the activity observed only at higher concentrations with ethanolic extract completely differed from previous studies which had shown *Pseudomonas* to be susceptible to sunflower extracts (Rajakannu and Sritharan, 2012). We presume that phytoactive agent of the *H.annuus* might share the mechanism of action of the antibiotics over *Pseudomonas*.

Many studies done with plants and natural products mostly try to pinpoint the secondary metabolite (phytochemical constituent) abundantly

present in the extracts. However many such pure phytochemicals have not shown satisfactory antibacterial activity with few or more exceptions but still to hit the market? Further in our case of the herbs selection, there has been some literature on the isolated and purified phytochemicals showing antibacterial activity however there has not been subsequent follow up on these phytochemicals over normal or MDR strains. On the other hand still in the classical medicine practice, these extracts are used as therapeutic intervention, with varied success and poor documentation. Put all these results together, we do have some medicinal herbs potent to fight against the MDR strains that can be easily transmitted through hospitals, feco-oral route etc.

5. Conclusion

Both aqueous and ethanolic extracts of *S.indicus* and *H.annuus* showed antibacterial activity against MDR strains of *Staphylococcus, Klebsiella* and *E.coli* with varying degree of activity. The ethanolic extracts showed more potent activity even at low concentrations like 2 and 1 mg/ml. The results of both disc diffusion and tube dilution methods were in good agreement with previously published literatures. In our investigation we observed that *S.indicus* did not show any observable activity over *Pseudomonas* while ethanolic extract of *H.annuus* inhibited the growth of *Pseudomonas* at higher concentrations, was very much different from published sources. In conclusion more studies have to be undertaken to identify herbs and medicinal plants to test MDR strains, which will open the gate for new class of antibacterial agents for the therapeutic adaptation.

Acknowledgement

I sincerely thank Dr. A.Saffiullah, S.S. Lab (Research and Development Wing), Royapettah, Chennai, Tamil Nadu with India for his help with the clinical isolates and reagents therein.

References

Alekshun MN and Levy SB (2007). Molecular mechanisms of antibacterial multidrugresistance. Cell, 128(6):1037-50.

Awan UA, Andleeb S, Kiyani A, Zafar A, Shafique I, Riaz N, Azhar MTand Uddin H (2013). Antibacterial screening of traditional herbal plants and standard antibiotics against some human bacterial pathogens. Pak J Pharm Sci, 26(6): 1109-16.

Barber M (1947). Staphylococci infection due to penicillin-resistant strains. *BMJ*, 2(4534): 863–865.

Basu NK and Lamsal PP (1946). A chemical investigation of *Sphaeranthus indicus* Linn. J Am Pharm Assoc Am Pharm Assoc, 35: 274.

Baur AW, Kirbuy WMM, Sherris KC & Turck M (1966). Antibiotic susceptibility testing by standardized single disc method. American Journal of Clinical Pathology, 45: 493.

Casetti F, Wölfle U, Gehring W and Schempp CM (2011). Dermocosmetics for dry skin: a new role for botanical extracts. Skin Pharmacol Physiol, 24(6):289-293.

Chen Y, Zhang C, Zhang M and Fu X (2015). Three statistical experimental designs for enhancing yield of active compounds from herbal medicines and anti-motion sickness bioactivity. Pharmacogn Mag, 11(43):435-443.

Galani VJ, Patel BG and Rana DG (2010). *Sphaeranthus indicus* Linn. A phytopharmacological review, Int J Ayurveda Res, 1 (4):247-253.

Gromek K, Drumond N & Simas P (2015). Pharmacovigilance of herbal medicines. Int J Risk Saf Med, 27(2):55-65.

Harish CC, Safiullah A, Shenbagaraman R, Shanta PV, Venkatraman SR and Arul Balaji V (2010). Antibacterial activity of Vicoa indica and Tridax procumbens against Multi-Drug Resistant (MDR) clinical isolates. J. Sci, Trans, Environ, Technov, 3 (3): 143-148.

John SA and Tamilmaraiselvi B (2011). Preliminary phytochemical investigation and antimicrobial activity of *Sphaeranthus indicus* Linn. AJMBES, 13 (2):251–256.

Komolafe OO (2003). Antibiotic resistance in bacteria - an emerging public health problem. Malawi Med J, 15 (2): 63-67.

Kowalska T and Cieśla Ł (2015). Assessment of Antioxidant and Antibacterial Potential of Medicinal Herbs and Botanical Preparations. J AOAC Int, 98(4): 847-849.

Kumar VP, Chauhan NS, Padh H and Rajani M (2006). Search for antibacterial and antifungal agents from selected Indian medicinal plants. J Ethnopharmacol, 107(2):182-188.

Malini M, Abirami G, Hemalatha V and Annadurai G (2013). Antimicrobial activity of Ethanolic and Aqueous Extracts of medicinal plants against wastewater pathogens. International Journal of Research in Pure and Applied Microbiology, 3(2): 40-42.

Manavathu EK, Alangaden GJ and Lerner SA (1996). A comparative study of both micro and macro dilution techniques for the determination of the in vitro susceptibility of *Aspergillus fumigatus*. Can J Microbiol, 42(9):960-964.

Mellon M, Benbrook C and Benbrook L (2001). Estimates of Antimicrobial Abuse in Livestock. Union of Concerned Scientists: Cambridge, MA, January. http://www.ucsusa.org/publications.

Subashini R & Rakshitha SU (2012). Phytochemical screening, antimicrobial activity and in vitro antioxidant investigation of methanolic extract of seeds from Helianthus annuus L. Chem Sci Rev and Lett, 1(1): 30-34.

Ramachandran S (2013). Review on *Sphaeranthus indicus* Linn. (Koṭṭaikkarantai): Pharmacogn Rev, 7(14): 157-169.

Sechi LA, Lezcano I, Nunez N, Espim M, Duprè I, Pinna A, Molicotti P, Fadda G and Zanetti S (2001). Antibacterial activity of ozonized sunflower oil (Oleozon). J Appl Microbiol, 90(2): 279-284.

Selvi BT, John SA and Theivandran G (2011). Preliminary phytochemical investigation and antimicrobial activity of *Sphaeranthus indicus* Linn: Ad Plant Sci, 24: 81–5.

Thiyagarajan R (2006). Gunapadam Thathu Jeeva Vaguppu. Sixth edition, published by Directorate of Indian Medicine and Hoemopathy, Chennai, India.

Tulunay M, Aypak C, Yikilkan H & Gorpelioglu S (2015). Herbal medicine use among patients with chronic diseases: J Intercult Ethnopharmacol, 4(3): 217-220.

Ukiya M, Akihisa T, Tokuda H, Koike K, Takayasu J, Okuda H, Kimura Y, Nikaido T and Nishino H (2003). Isolation, structural elucidation, and inhibitory effects of terpenoid and lipid constituents from sunflower pollen on Epstein-Barr virus early antigen induced by tumor promoter, TPA. J Agric Food Chem, 51(10): 2949-57.

World Health Organization (2000). WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food. Document No. WHO/CDS/CSR/APH/2000.4. http://www.who.int/emc-.

Yang Y, Zhang Z, Li S, Ye X, Li X & He K (2014). Synergy effects of herb extracts: pharmacokinetics and pharmacodynamic basis. Fitoterapia, 92: 133-47.