

Full Length Research Paper

# Antimicrobial and free radical scavenging activity of extracts of some Indian medicinal plants

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**Antimicrobial and antioxidant potency of aqueous and organic solvent extracts of five Indian medicinal plants (*Prosopis cineraria*, *Capparis decidua*, *Tinospora cordifolia*, *Carissa carandas* and *Cordia dichotoma*) was investigated. The acetone and ethanol extracts exhibited highest antimicrobial activity (60 to 80% and 40 to 60%, respectively) against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. Acetone extracts showed inhibitory zones ranging from 11.2 - 19.8 mm whereas no inhibitory effect was observed for aqueous extracts. During NBT assay of acetone and ethanol extracts of all the plants, maximum antioxidant activity was noticed in *C. carandas* (63.5 and 61.0% for acetone and ethanol extracts, respectively). The inhibitory potential when compared with known antioxidant (L-ascorbic acid), it was observed that IC<sub>50</sub> values of acetone extracts of *C. carandas* and *T. cordifolia* (93 and 97 µg/ml) were very close to L-ascorbic acid (81 µg/ml). The results suggest that *C. carandas* has promising antimicrobial and antioxidant activities.**

**Key words:** Antimicrobial activity, antioxidant activity, microbial susceptibility index, NBT, medicinal plants.

## INTRODUCTION

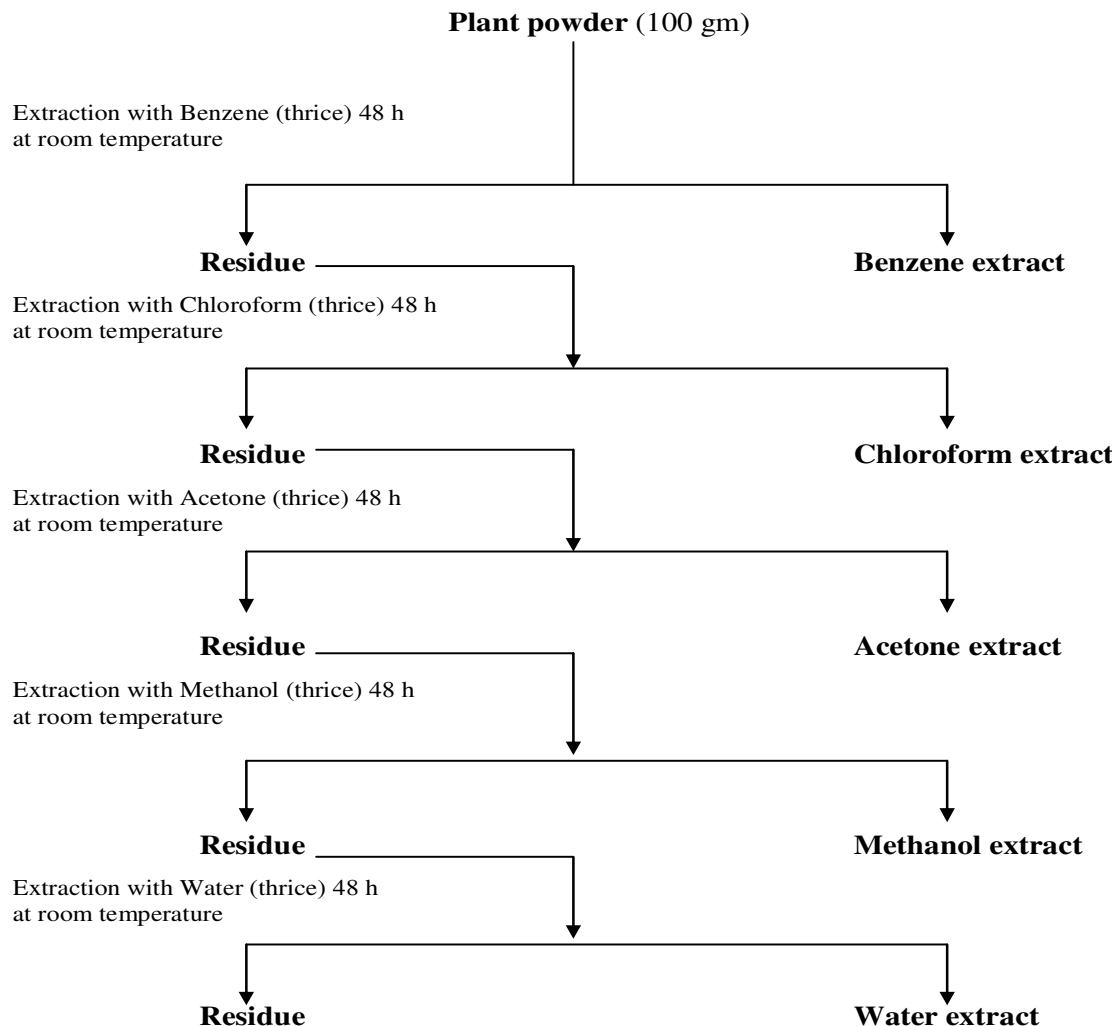
Free radicals are a major cause of oxidative stress that may lead to DNA strand breakage, *gene* mutation and DNA-DNA and DNA-protein cross links. Free radicals are known to be a product of normal metabolism. When oxygen is supplied in excess or its reduction is insufficient, reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and H<sub>2</sub>O<sub>2</sub> are generated (Aruoma, 1999). ROS are involved in an organism's vital activities including phagocytosis, regulation of cell proliferation, intracellular signaling and synthesis of biologically active compounds (Halliwell and Gutteridge, 1989; Miquel and Romano-Bosca, 2004). ROS have been implicated in several diseases including carcinogenesis, malaria, heart diseases, arteriosclerosis, diabetes and many other health problems related to ageing (Duh, 1998; Honda et al., 2004; Tanizawa, et al., 1992; Uchida, 2000).

The role of ROS in the etiology and progression of

several clinical manifestations has led to the suggestion that the antioxidants can be beneficial as prophylactic agents. Nevertheless, all aerobic organisms, including humans, have antioxidant defences that protect against oxidative harm and repair damaged molecules. However, the natural antioxidant mechanisms can be insufficient, the supply of antioxidants through dietary ingredients, is of great interest for a healthy life (Duh, 1998; Espin et al., 2000; Greenwald et al., 2001; Scalbert and Williamson, 2000; Terao et al., 1994).

A number of plants have been documented for their antimicrobial (Ahmad and Beg, 2001; Polambo and Semple, 2001) and antioxidant activities (Gajera et al., 2005). Drugs from plant origin are relied upon by 80% of the world's population. In India, the use of herbal drugs is an important component of the traditional system of medicine. Knowing the fact that several diseases have been treated by the administration of plant extracts from medicinal plants (Borek, 1997), the present investigation was aimed at evaluating the antimicrobial and antioxidant potential of the acetone and methanol extracts of five different medicinal plants [*Prosopis cineraria* (Mimosaceae), *Capparis decidua* (Capparidaceae),

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**Figure 1.** Extraction by maceration of plant powder by increasing order of solvent polarity.

*Tinospora cordifolia* (Menispermaceae), *Carissa carandas* (Apocynaceae) and *Cordia dichotoma* (Boraginaceae)] by employing various assays. All these plants have commonly been used to treat gastrointestinal disorders and as anti-inflammatory, anti-diabetic, antimalarial and anti flatulent agents (Ambasta, 2000). To the best of our knowledge no positive reports are available on the antimicrobial and antioxidant activities of organic solvent extract of some these plants.

## MATERIALS AND METHODS

### Chemicals and culture media

Nutrient Agar (NA), Nutrient Broth (NB) and Czapek Yeast Extract Agar (CYA) used to culture the microorganisms were purchased from Hi-Media Laboratories Pvt. Ltd. Mumbai. Tris-HCL buffer, b-

nicotinamide adenine dinucleotide (reduced for NADH), nitroblue tetrazolium chloride (NBT), Phenazin methosulfate (PMS) were obtained from GalaxoSmithKline, Mumbai. All other chemicals including organic solvents used for the extraction of the plant metabolites were procured from Central Drug House (CDH), Mumbai were of analytical grade.

### Preparation of plant extracts

Preparation of plant extracts initiated with benzene, a less polar solvent followed by chloroform, acetone, methanol and water. A 100 g quantity of finely powdered plant material was soaked for 48 h in each solvent (3 ml x 300 ml). After recovering the supernatant, the respective solvents were added twice to the residue. The three supernatants obtained with each solvent were pooled and dried. Extraction in each solvent was done thrice (Figure 1). The crude chloroform, acetone and methanol extracts, which were used in the present study, were obtained after extracting the plant powder in benzene. The extracts obtained by extraction of the plant powder in the respective solvents were

dried and weighed. Ethanol (EtOH) extracts of the plant powder (100 g) were extracted separately by maceration with ethanol (3 ml x 300 ml) on a rotary shaker overnight. The filtrate was concentrated to produce a crude extract which was dissolved in EtOH. The different concentrations (25 - 175 µg/ml) of the dried extracts were made after dissolving in respective solvents for checking antioxidant activity. All the extracts were kept in tightly stoppered bottles under refrigeration until used for biological testing.

## Antimicrobial assays

### Microorganisms

Fungal and bacterial strains were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Two Gram positive strains *Staphylococcus aureus* (MTCC - 87), *Staphylococcus epidermidis* (MTCC - 435), one Gram negative strain *Escherichia coli* (MTCC - 41), fungus *Aspergillus niger* (MTCC - 404) and an yeast *Candida albicans* (MTCC - 854) were used in the present study as test organisms for investigating antimicrobial activity.

### Antimicrobial activity

Antimicrobial activity of the above mentioned extracts was determined, using agar well diffusion assay method (Perez et al., 1990) and as described previously (Salar and Suchitra, 2009). Briefly, 20 ml of molten and cooled media (NA and CYA) were poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination. The bacterial test organisms (*S. aureus*, *S. epidermidis*, *E. coli*) were grown in nutrient broth for 24 h. A 100 µl nutrient broth culture of each bacterial organism was used to prepare bacterial lawns on the agar plates. For *A. niger*, spore suspension of the fungus was prepared in 2 ml sterilized distilled water in a test tube and 100 µl of spore suspension was spread on CYA plates.

After 5 min of inoculation of test organisms, wells of 5 mm diameter were prepared in the agar plates with the help of sterilized stainless steel cork borer. One well in each plate was loaded with 60 µl of crude extract of test plant. One well loaded with extraction solvent served as control and other wells were loaded with 60 µl of standard antibiotics viz., streptomycin (10 µg/ml), ampicillin (25 µg/ml), tetracycline (30 µg/ml), penicillin (10 µg/ml), for bacterial strains and standard antifungals viz., fluconazole, clotrimazole and ketoconazole (each 10 µg/ml) for fungal strains were used as positive controls for comparison. The plates were then aerobically incubated at 37°C for 24 h (for *E. coli* and *S. epidermidis*) and at 30°C (for *S. aureus*) and at 25°C for 72 h for yeast and fungal test organisms. All the tests were performed in triplicates.

The antimicrobial activity was assessed on the basis of diameter of zone of inhibition which was measured at cross-angles after 24 h of incubation and compared with the zone of inhibition of the standard antimicrobials. Strains with a clear zone of inhibition of more than 12 mm were considered as sensitive. The mean of three evaluations is shown in Table 1.

### Quantitative evaluation of antimicrobial activity

Antimicrobial activity of crude plant extracts may be expressed in different ways based on the method used. The agar diffusion technique is commonly used for preliminary screening of plant extracts for antimicrobial activity. The present study also employs percent activity values that demonstrate the total antimicrobial

potential of a particular extract and the microbial susceptibility index (MSI) which is used to compare the relative susceptibility among the microbial strains (Bonjar, 2004; Fabri et al., 2009; Rangasamy et al., 2007). These values were calculated as follows:

Percent activity (%):

$$\text{Activity(\%)} = \frac{\text{No. of susceptible strains to a specific extract} \times 100}{\text{Total no. of tested microbial strains}}$$

The percent activity depicts the total antimicrobial potency of particular extracts. It shows the number of microbial strains found susceptible to a particular extract.

Microbial Susceptibility Index (MSI):

$$\text{MSI} = \frac{\text{No. of extracts effective against each microbial strain} \times 100}{\text{Total no. of samples}}$$

MSI is used to compare the relative susceptibility among the microbial strains. MSI values ranges from '0' (resistant to all samples) to '100' (susceptible to all samples).

### Antioxidant activity (NBT superoxide radical scavenging assay)

The scavenging activity of the plant extracts towards superoxide anion radicals was measured by following the method of Liu et al. (1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In the experiment, the superoxide anion was generated in 3 ml of Tris-HCL buffer (100 mM, pH 7.4) containing 750 µl of NBT (300 µM) solution, 750 µl of NADH (936 µM) solution and 300 µl of different concentrations (25 - 175 µg/ml) of acetone/ methanol extracts). L-Ascorbic acid was used as a positive control. The reaction was initiated by adding 750 µl of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance was measured at 560 nm in a spectrophotometer (Systronics) against blank. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percent NBT decolorization of the sample was calculated by the equation:

$$\text{Per cent inhibition} = (A_0 - A_1/A_0) \times 100$$

Where,  $A_0$  is the absorbance of the negative control and;  $A_1$  is the absorbance of the reaction mixture. The  $IC_{50}$  values (the amount required to inhibit superoxide radical formation by 50%) of plant extracts were calculated and compared with that of L-Ascorbic acid used as positive control. All experiments related to antimicrobial and antioxidant activities were repeated at least thrice. Results are reported as means  $\pm$  SD (not shown in the graphs).  $IC_{50}$  values were also calculated.

## RESULTS AND DISCUSSION

### Antimicrobial activity

Percent activity values of different crude extracts of the

**Table 1.** Antimicrobial activity of acetone extracts of various plants compared with standard antibiotics.

Microorganism	Control	Extracts <sup>a</sup>						Antibiotics (µg/ml) <sup>b</sup>					
		Pc	Cad	Tc	Cc	Cod	S10	P10	A25	T30	F10	C10	K10
<i>E. coli</i>	8.2±1.67	17.2±0.40	14.1±0.46	13.5±0.45	+	-	16±.1	18±.4	17±.2	20±.1	ND	ND	ND
<i>S. aureus</i>	7.9±1.06	19±.21	11.2±.71	+	16.1±.45	19.1±1.34	15±.2	17±.3	16±.1	22±.2	ND	ND	ND
<i>S. epidermidis</i>	6.7±1.06	15±1.04	-	14.5±.89	19.8±.85	-	13±.7	15±.2	16±.4	17±.3	ND	ND	ND
<i>A. niger</i>	6.4±2.14	-	11.8±.83	13.1±1.21	13.2±.77	15±.82	ND	ND	ND	ND	14±.2	16±.3	13±.2
<i>C. albicans</i>	7.4±1.73	-	11.7±.91	15.1±.72	14.8±.83	18.5±.75	ND	ND	ND	ND	15±.3	17±.2	14±.1

- = No zone of inhibition; + = Zone of inhibition < 10 mm; ±, standard deviation; ND = Not Determined; <sup>a</sup> Pc, *Prosopis cineraria*; Cad, *Capparis decidua*; Tc, *Tinospora cordifolia*; Cc, *Carissa carandas*; Cod, *Cordia dichotoma*; <sup>b</sup> S10, streptomycin; P10, penicillin; A25, ampicillin; T30, tetracycline; F10, fluconazole; C10, clotrimazole; K10, ketoconazole.

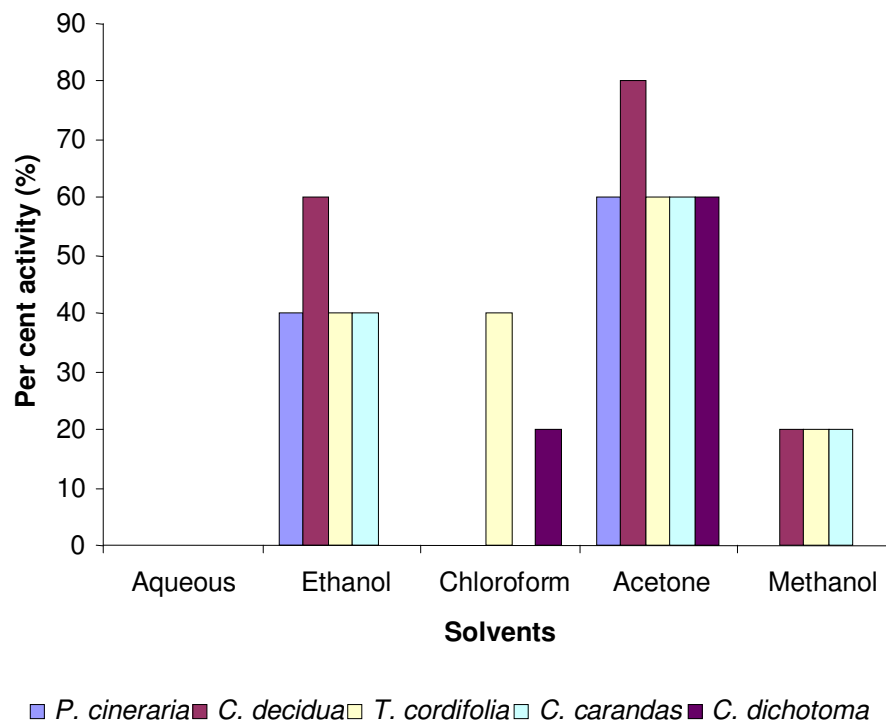
plants used in the present study depicting the total antimicrobial potency is presented in Figure 2. It is clear from this figure that acetone extracts were the most effective of all the extracts. Consequently, data pertaining only to acetone extracts of different plants that showed inhibitory activities against all the tested bacterial, fungal and yeast species is shown in Table 1. Extracts of other solvents showed moderate activity against the tested organisms. Water extracts of the investigated plants did not show any antimicrobial activity against all the tested microorganisms (Figure 2). Acetone extract of *C. carandas* was the most effective against the tested microorganisms closely followed by *T. cordifolia*, *C. dichotoma*, *P. cineraria* and *C. decidua*. Among the microorganisms, *S. aureus* was the most sensitive organism, followed by *S. epidermidis* towards acetone extracts. Extracts of acetone of different plants give inhibitory zones ranging from 11.2 - 19.8 mm. Data pertaining to acetone extracts is shown in Table 1. The results of the present study are encouraging as all the investigated plants showed antimicrobial potential, although the method of extraction and the plant part used affected their antimicrobial

activity. Despite the tremendous progress in human medicines, infectious diseases are still a major threat to public health. Their impact is intense in the developing countries particularly due to relative unavailability of the medicines and the emergence of drug resistance (Okeke et al., 2005). In case of immunocompromised, AIDS and cancer patients, drug resistant bacteria, virus and fungal pathogens have further complicated the treatment of infectious diseases (Diamond, 1993). Thus, in the present scenario of multiple drug resistance to infectious pathogens, it is important that the search for new antimicrobial substance from alternative sources including plants be initiated (Ahmed and Beg, 2001). Contrary to synthetic drugs, antimicrobials from plants are safe and possess effective therapeutic potential to treat several infectious diseases (Iwu et al., 1999).

Percent (%) activity and the microbial susceptibility index (MSI) were calculated for all the samples. Percent activity values (Figure 2) revealed that acetone extracts of different plants were the most active against the different microorganisms used (60 to 80% activity) followed by ethanol (40 to 60%). MSI values (Table 2) were useful in evaluating the degree

of susceptibility of the different microbial strains towards the plant extracts investigated. *S. aureus* and *C. albicans* were the test organisms found to be most susceptible to the samples investigated showing mean MSI of 44 and 36, respectively. Percent activity and MSI are of pharmacological interest (Rios and Recio, 2005).

The higher sensitivities of *S. aureus* and *C. albicans* could be attributed to physiological and strain differences. In an earlier study (Kaur and Arora, 2008) both aqueous and acetone extracts of different plants belonging to family Umbelliferae have shown marked antibacterial activity. In the present study, aqueous extracts were not very much effective. Such differences could be attributed to the plant material, strain differences and protocols used. In the face of increasing drug resistance of pathogens, the use of traditional medicinal plants is supported for their use to treat diseases including nosocomial infections (Junaid et al., 2008). Microbial pathogens such as *E. coli* are associated with gastrointestinal disorders. The present study is noteworthy in the wake of increasing drug resistance of pathogens against commonly used antibiotics.



**Figure 2.** Per cent activity values of the crude extracts of different plants depicting the total antimicrobial potency of the extracts.

**Table 2.** Microbial susceptibility index (MSI) calculated for various strains of microorganisms used for screening of the acetone extracts of different plants.

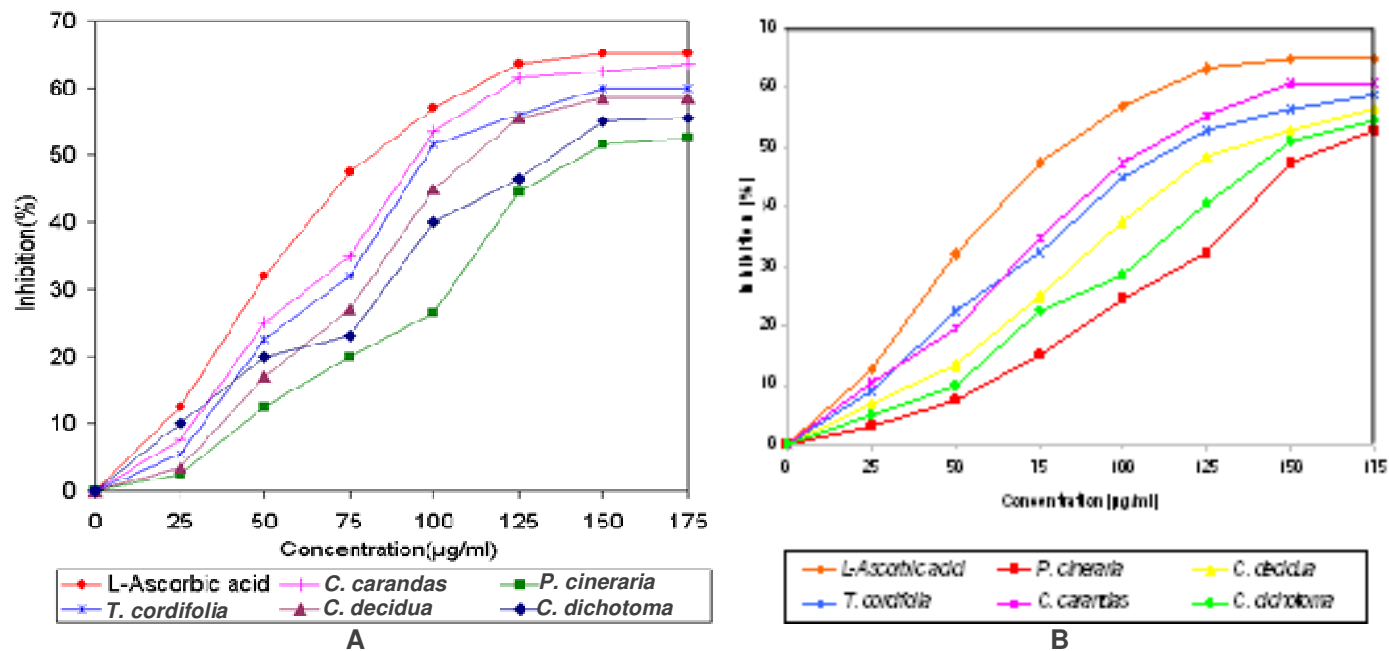
Microorganisms	Extracts <sup>a</sup>					Mean	S.D.
	Pc	Cad	Tc	Cc	Cod		
<i>E. coli</i>	40	60	60	0	0	32	30.331
<i>S. aureus</i>	20	40	40	40	80	44	21.909
<i>S. epidermidis</i>	20	20	40	80	0	32	30.331
<i>A. niger</i>	20	20	20	40	40	28	10.954
<i>C. albicans</i>	0	20	40	80	40	36	29.665

<sup>a</sup> Pc, *Prosopis cineraria*; Cad, *Capparis decidua*; Tc, *Tinospora cordifolia*; Cc, *Carissa caranda*; Cod, *Cordia dichotoma*; S.D., standard deviation.

### Antioxidant activity

Antioxidant activity of acetone and ethanol extracts (as these give most encouraging results of microbial inhibition) of all the five medicinal plants was evaluated using nitroblue tetrazolium NBT superoxide scavenging assay with L-ascorbic acid as a positive control. The ethanol/ acetone extracts quenched NBT superoxide anion in a dose dependent manner because, as the concentration of extracts increased, the NBT quenching activity also increased (Figure 3). The order of effectiveness of the acetone extracts of different plants was: *C. carandas* (63.5%) > *T. cordifolia* (60.0%) > *C.*

*decidua* (58.5%) > *C. dichotoma* (55.5%) > *P. cineraria* (52.5%) at 175 µg/ml concentration. Similarly, the order of effectiveness of the ethanol extracts of these plants was *C. carandas* (61.0%) > *T. cordifolia* (59.0%) > *C. decidua* (56.5%) > *C. dichotoma* (54.5%) > *P. cineraria* (53.0%) at 175 µg/ml concentration. The ability of L-ascorbic acid to scavenge NBT superoxide anion became almost stable after 150 µg/ml concentration and there was no increase in scavenging activity with further increase in concentration to 175 µg/ml. The observed antioxidant activity of the extracts may be due to the neutralization of superoxide anion character of NBT either by transfer of electron or hydrogen atom.



**Figure 3.** Superoxide scavenging activity of ethanol (A) and acetone (B) extracts of different plants measured using NBT assay. L - Ascorbic acid was used as a positive control.

**Table 3.** IC<sub>50</sub> values of acetone and methanol extracts of various medicinal plants in Nitroblue Tetrazolium (NBT) superoxide radical scavenging assay.

S. no.	Plant name	Plant part used in present assay	IC <sub>50</sub> value (µg/ml)	
			Acetone	Methanol
	L-ascorbic acid (control)	-	81.0	81.0
1.	<i>P. cineraria</i>	Pods	143.0	162.5
2.	<i>C. deciduas</i>	Fruits	117.0	132.0
3.	<i>T. cordifolia</i>	Stem	97.0	112.5
4.	<i>C. carandas</i>	Fruits	93.0	107.5
5.	<i>C. dichotoma</i>	Fruits	131.0	149.0

Figure 3 depicts the results of acetone and ethanol extracts obtained in increasing order of solvent polarity (Figure 1) of different plants. It is clear from the results that though there was not much difference in the antioxidant potential of the acetone and ethanol extracts but, in general, the acetone extracts showed more inhibitory activity than did the ethanol extracts. Among all the plants, the acetone extracts of *C. carandas* showed maximum (63.5%) inhibition. In the PMS-NADH-NBT system superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT (Gulcin et al., 2005). The decrease in absorbance at 560 nm with acetone/ethanol and antioxidants indicates the consumption of superoxide anion in the reaction mixture. The degree of discoloration indicates the scavenging potential of the plant extracts.

Superoxide anion is one of the most representative free radicals. In cellular oxidation reaction, superoxide radicals are normally formed first, however, their effects can be magnified because they produce other kinds of cell damaging free radicals and oxidizing agents (Liu and NG, 2000). Table 3 shows the IC<sub>50</sub> values of the plant extracts, which were found to vary from 93.0 - 143.0 µg/ml for acetone extracts and 107.0 - 162.0 µg/ml for ethanol extracts. In the present investigation, the data obtained show that acetone extracts are free radical scavengers and may act as primary antioxidants which can react with free radicals by donating hydrogen. As expected, the lower the IC<sub>50</sub> values the higher the percentage of NBT radical inhibition of the samples.

The most potent activities with IC<sub>50</sub> values of 93 and 97 µg/ml were observed with acetone extracts as

compared to ethanol extracts (107 and 112 µg/ml) of *C. carandas* and *T. cordifolia*, respectively. It is worth to note that superoxide scavenging activity of acetone extracts of these two plants is comparable to that of L-ascorbic acid (81 µg/ml) used as positive control. This study is in conformity with the observations made in the literature (Sravanan et al., 2007) who reported the antioxidant activity of *C. carandas* fruit extracts with IC<sub>50</sub> value of 92 µg/ml. It has been reported that there is an inverse relationship between dietary intake of antioxidant-rich food and the incidence of human diseases (Demir et al., 2009; Gordon, 1990; Hall and Cuppett, 1997; Nieto et al., 1993). The inhibitory potential for antimicrobial and antioxidant activity was also positively correlated in the present investigation.

## Conclusion

In conclusion, all these plants exhibited antimicrobial and antioxidant activities, though to a varied extent. Some extracts exhibit least activity to tested microorganisms (as the zone of inhibition is much less compared to others) under *in vitro* conditions. The results further revealed that among all the plants evaluated for their antimicrobial and antioxidant potential, *C. carandas* could be an interesting source of antimicrobial and antioxidant compounds for potential use in different fields viz., food, cosmetics and pharmaceuticals. The results of the present investigation also indicate that the solvents and extraction procedure may modify the final results to get maximum antimicrobial and antioxidant activity. In the current study, all the plants were selected on their relevant ethnomedicinal use and they provide difference in their antimicrobial and antioxidant activity when their active constituents were extracted with different solvent systems. Further studies are needed to isolate and characterize the active principles to elucidate their different antimicrobial and antioxidant mechanism and the existence of possible synergism, if any, among the compounds.

## REFERENCES

- Ahmed I, Beg AZ (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. *J. Ethnopharmacol.*, 74: 113-123.
- Ambasta SP (2000). The useful plants of India. National Institute of Science Communication, New Delhi, India, p.303
- Aruoma OI (1999). Free radicals, oxidative stress, and antioxidants in human health and disease. *J. Agric. Food Chem.*, 47: 397-492.
- Bonjar GHS (2004). New approaches in screening for antibacterials in plants. *Asian J. Plant Sci.*, 3: 55-60.
- Borek B (1997). Antioxidants and cancer. *Sci. Med. (Phila)*, 4: 51-62.
- Demir H, Acik L, Bali EB, Koc LY, Kaynak G (2009). Antioxidant and antimicrobial activities of *Solidago virgaurea* extracts. *Afr. J. Biotech.*, 8(2): 274-279.
- Diamond RD (1993). The growing problem by mycoses in patients infested with human immunodeficiency virus. *Rev. Infect. Dis.*, 13: 480-486.
- Duh PD (1998). Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free radical and active oxygen. *J. Am. Chem. Soc.*, 75: 269-277.
- Espin JC, Soler-rivas C, Wichers HJ (2000). Characterization of the total free radical scavenging capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *J. Agric. Food Chem.*, 48: 648-656.
- Fabri RL, Nogueira MS, Braga FG, Coimbra ES, Scio E (2009). *Mitracarpus frigidus* aerial parts exhibited potent antimicrobial, antileishmanial, and antioxidant effects. *Biores. Technol.*, 100: 428-433.
- Gajera HP, Patel SV, Golakiya BA (2005). Antioxidant properties of some therapeutically active medicinal plants – An overview. *JMAPS*, 27: 91-100.
- Gordon MH (1990). The mechanism of antioxidants action *in vitro*. In: Hudson BJJ (ed) *Food Antioxidants*. London, New York, pp. 1-18.
- Greenwald P, Clifford CK, Miner JA (2001). Diet and cancer prevention. *Eur. J. Cancer*, 37: 948-965.
- Gulcin I, Alici HA, Cesur M (2005). Determination of *in vitro* antioxidant and radical scavenging activities of propofol. *Chem. Pharm. Bull.*, 53: 281-285.
- Hall CA, Cuppett SL (1997). Structure-activities of natural antioxidants, In: Auroma OI, Cuppett SL (eds) *Antioxidant Methodology in vivo and in vitro concepts*. AOCS Press, pp. 141-170.
- Halliwell B, Gutteridge JMC (1989). *Free radicals in biology and medicine* (2<sup>nd</sup> ed). Japan Scientific Societies Press, Tokyo, Japan, p. 229-233
- Honda K, Casadesus G, Paterson RB, Perry G, Smith MA (2004). Oxidative stress and redox iron in Alzheimer's disease. *Ann. New York Acad. Sci.*, 1012: 179-182.
- Iwu MW, Duncan AR, Okunji CO (1999). New antimicrobials of plant origin. In: Janick J (ed) *Perspectives on New Crops and New Uses*. ASHS Press, pp. 457-462.
- Junaid SA, Abubakar A, Ofodile AC, Olabode AO, Echeonwu GON, Okwori AEJ, Adetunji JA (2008). Evaluation of *Securidaca longipendunculata* leaf and root extracts for antimicrobial activities. *Afr. J. Microbiol. Res.*, 2: 322-325.
- Kaur GJ, Arora DS (2008). *In vitro* antibacterial activity of three plants belonging to the family Umbelliferae. *Int. J. Antimicrob. Agents*, 31: 393-395.
- Liu F, NG TB (2000). Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci.*, 66: 725-735.
- Liu F, Ooi VEC, Chang ST (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci.*, 60: 763-771.
- Miquel J, Romano-Bosca A (2004). Oxidative stress and antioxidant diet supplementation in ageing, arterosclerotic and immune dysfunction processes. *ARS Pharm.*, 45(2): 91-109.
- Nieto S, Garrido A, Sanhuesa J, Loyola L, Morales G, Leighton F, Valenzuela A (1993). Flavonoids as stabilizers of fish oil: an alternative to synthetic antioxidants. *J. Am. Oil Chem. Soc.*, 70: 773-778.
- Okeke IN, Laxmaninarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A, Klugman KP (2005). Antimicrobial resistance in developing countries. Part 1: Recent trends and current status. *Lancet Infect. Dis.*, 5: 481-593.
- Pawar VC, Bagatharia SB, Thaker VS (2005). Antibacterial activity of *Aloe vera* leaf gel extracts against *Staphylococcus aureus*. *Indian J. Microbiol.*, 45 (3): 227-229.
- Perez C, Paul M, Bazerque P (1990). Antibiotic assay by agar well diffusion method. *Acta Biol. Med. Exp.*, 15: 113-115.
- Polambo EA, Semple SJ (2001). Antibacterial activity of traditional Australian medicinal plants. *J. Ethnopharmacol.*, 77: 151-157.
- Rangasamy O, Raelison G, Rakotoniriana FE, Cheuk K, Urverg-Ratsimamanga S, Quetin-Leclercq J, Gurib-Fakim A, Subratty AH (2007). Screening for anti-infective properties of several medicinal plants of the Mauritian flora. *J. Ethnopharmacol.*, 109: 331-337.
- Rios JL, Recio MC (2005). Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.*, 100: 80-84.
- Scalbert A, Williamson G (2000). Dietary intake and bioavailability of polyphenols. *J. Nutr.*, 130: 2073-2085.
- Salar RK, Suchitra (2009). Evaluation of antimicrobial potential of

- different extracts of *Solanum xanthocarpum* Schrad. and Wendl. Afr. J. Microbiol. Res., 3(3): 97-100.
- Sravanan K, Reddy HA, Aradhya SM (2007). Antioxidant activity of Karonda (*Carissa carandus* L.) fruits. NHBT, p. 241.
- Tanizawa H, Ohkava Y, Takino Y, Miyase T, Ueno A, Kageyama T, Hara S (1992). Studies on natural antioxidants in citrus species. I. Determination of antioxidative activities of citrus fruits. Chem. Pharm. Bull., 40: 1940-1942.
- Terao J, Piskula M, Ya Q (1994). Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipids bilayers. Arch. Biochem. Biophys., 308: 278-284.
- Uchida K (2000). Role of reactive aldehyde in cardiovascular diseases. Free Radic. Biol. Med., 28: 1685-1696.