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Antioxidant and antimicrobial properties of seed oil of *Datura metel*

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Authors Info

R.K. Bachheti[1], I. Rai[1], V.K. Mishra[2]* and A. Joshi[3]

[1]Department of Chemistry, Graphic Era University, Dehradun-248 002, India

[2]Department of Biotechnology, Doon P.G. Paramedical College, Dehradun-248 991, India

[3]Department of Environmental Science, Graphic Era University, Dehradun-248 002, India

*Corresponding Author Email : mishravkbhu@gmail.com

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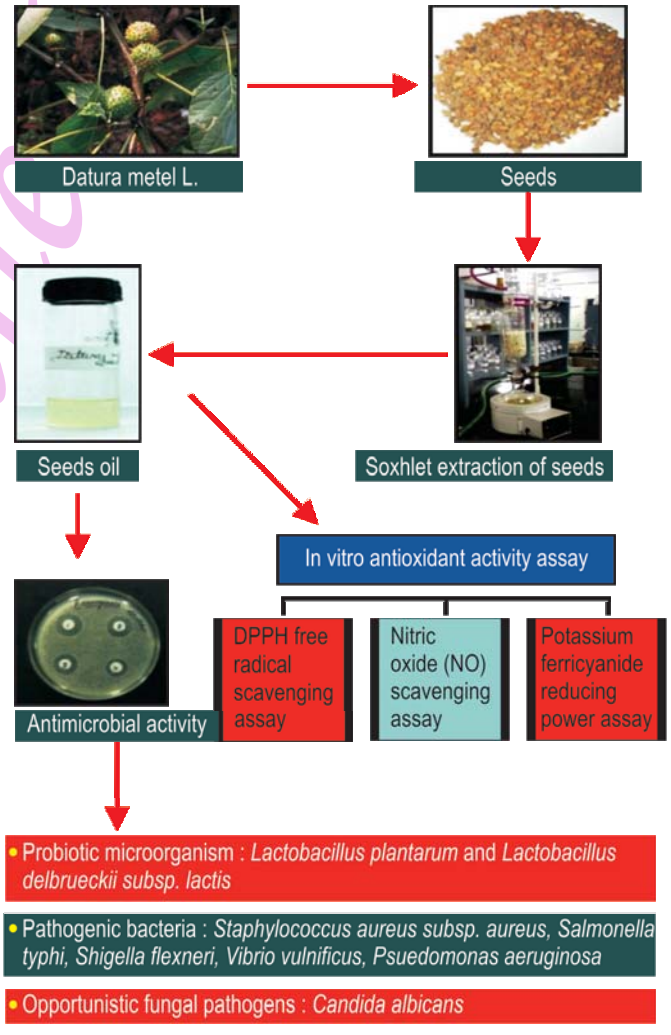
Abstract

Aim : *Dature metel* is a valuable medicinal plant in traditional and contemporary systems of medicines in India, China and Africa. The present study aimed to evaluate antioxidant and antimicrobial properties of seed oil of *Datura metel*.

Methodology : The seeds of *D. metel* were extracted in Soxhlet apparatus with petroleum ether. The seed oil obtained was used for assessing the antioxidant capacity using radical scavenging (DPPH and nitric oxide) and reducing power. The antimicrobial potential of seed oil was assessed by agar diffusion and microdilution methods.

Results : The IC_{50} of seed oil for nitric oxide radical scavenging was at par with standard antioxidant, curcumin (88 µg ml^{-1} and 80 µg ml^{-1}) The reducing power ability of oil was higher than the reference ascorbic acid (IC_{50} 8 and 22 µg ml^{-1}). The study also showed significant antibacterial and anti-candidial activities of seed oil.

Interpretation : Seed oil of *Datura metel* exhibited significant antioxidant and antimicrobial activities.



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Introduction

The genus Datura belongs to family solanaceae comprise of nine species. The term Datura is derived from Sanskrit Dustura or Dahatura (Mann, 1996). *Datura metel* is a wild growing shrub. The alkaloids present in solanaceae plants have been an age old source of herbal anticholinergic drugs, scopolamine and hyosciamine (Alebiowu *et al.,* 2007; Gaire and Subedi, 2013), but their overdose and concurrent use may cause toxicity. Almost all parts of *Datura metel* including seeds, seed oil, flower and leaves find applications in traditional medicine (Duke and Ayensu, 1985; Maheshwari *et al.,* 2013). Previous studies on extracts of *Datura metel* have reported many phytochemicals such as alkaloids, flavonoids, phenols, saponins, sterols and tannins (Donatus and Ephraim, 2009). A growing body of evidence suggest that *Datura metel* extract possess antioxidant (Alabri *et al.,* 2014; Roy *et al.,* 2016), antibacterial (Hossain *et al.,* 2014; Akharaiyi 2011), antifungal ; (Rajesh and Sharma, 2002; Dabur *et al.,* 2005), anti-proliferative and immunosuppressive properties (Bellila *et al.,* 2011; Yang *et al.,* 2014). In contrast, very little is known about seed oil of *Datura metel*.

The lesser known oilseeds including *Datura metel* represent untapped resource for lipids that otherwise would go waste if not utilized properly. Recently, there has been growing interest in antioxidants and antimicrobial property of these plants (Ozcan *et al.,* 2010; Atanasov *et al.,* 2015; Pawar *et al.,* 2017). A number of researchers have reported antioxidant and antimicrobial potential of seed oil from some plants such as apple (Cervantes-Cardoza *et al.,* 2010, Tian *et al.,* 2010), black cumin (Nair *et al.,* 2005), basil (Hussain *et al.,* 2008), grape (Garavaglia *et al.,* 2016), olive (Rigacci and Stefani, 2016), Pongamia (Sajid *et al.,* 2012), pumpkin (Nawirska-Olszańska *et al.,* 2013), sesame (Borjian *et al.,* 2016) etc. The seed oil of *Datura metel* is rich in polyunsaturated fatty acids, lipid soluble antioxidants and polyphenols (Ramadan *et al.,* 2009). So far antioxidant and antimicrobial properties of seed oil of *Datura metel* has not been comprehensively studied. Therefore, the aim of the present investigation was to assess antioxidant and antimicrobial properties of seed oil of *Datura metel*.

Materials and Methods

Plant material : *Datura metel* seeds of were collected from the Dugadda forest (latitude 29°48'25.04"N and longitude 78°36'33.74"E), Uttarakhand (India). Firstly, the seeds were dried with in shade for 14 days, then oven-dried at 40^{0}C for 48-72 hrs. The dried seeds were powdered in a blender, sieved through 1 mm mesh screen and soxhlet extracted using petroleum ether for 8 hrs. Subsequently, the solvent was kept off using rotary evaporator at 40^{0}C under reduced pressure. Seed oil was then collected in capped tubes and refrigerated at 4^{0}C for further study.

Microorganism and growth media : The microbial pathogens, Gram-positive bacteria: *Lactobacillus delbrueckii subsp lactis* MTCC 911, *Lactobacillus plantarum* MTCC 2621, *Staphylococcus aureus* MTCC 737 and Gram-negative bacteria: *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhi* MTCC 531, *Shigella flexneri* MTCC 1457, *Vibrio vulnificus* MTCC 1145; and the *Candida* species, *Candida albicans* MTCC 227 and *Candida tropicalis* MTCC 230 were procured from microbial type culture collection (MTCC), Chandigarh, India and stored on agar slants at 4°C in a refrigerator until further use.

DPPH radical scavenging activity : DPPH free radical scavenging activity of seed oil of *Datura metel* was assessed following the method of Cuendet *et al.* (1997). The reaction consisted of 0.5 ml of oil samples (10-1000 µgml^{-1}), 2.5 ml of distilled water and 3 ml DPPH (0.1 mM) in ethanol. The resulting solution was kept for 30 min in dark at room temperature. Following this, the absorbance of solution was recorded at 517 nm against control (ethanolic DPPH without any sample/ or standard). Curcumin was used as reference. The percent DPPH free radical scavenging was calculated by comparing the absorbance of control and test sample.

Nitric oxide radical scavenging activity : Sodium nitroprusside (SNP) initiates spontaneous formation of nitric oxide in solution at physiological pH (Marcocci *et al.,* 1994) that on reacting with oxygen forms nitrite ions which was measured by Griess Illosvoy reaction (Garrat, 1964). Two millilitre sodium nitroprusside (SNP) (10 mM) in phosphate buffer saline (PBS) (0.5 mM, pH 7.4) was aliquoted into 0.5 ml of seed-extract (10-1000 µgml^{-1}, prepared in ethanol) and incubated at 25^{0}C for 2 hrs. After incubation, 0.5 ml of extract was mixed with 0.5 ml of Griess reagent. The absorbance of the chromophore was measured at 540 nm against control (10 mM SNP in PBS without seed-extract/ or standard). Here in the assay, curcumin was used as reference. The percent inhibition of nitric oxide was measured by comparing the absorbance of control and test sample.

Potassium ferricyanide reducing power assay : The Fe^{3+} reducing power of the extract was determined following Oyaizu (1986). 0.1 ml of seed oil of *Datura metel* (10-1000 µgml^{-1}) was mixed with 2.5 ml potassium ferricyanide (1% w/v) and 2.5 ml phosphate buffer (0.2M, pH 6.6). The resulting solution was incubated in a water bath at 50°C for 20 min. The reaction was stopped with 2.5 ml of 10% trichloroacetic acid. Thereafter, the solution was centrifuged at 4000 rpm for 10 min. A 2.5 ml supernatant was mixed with equal volume of distilled water and then 0.5 ml of 0.1% ferric chloride was immediately added to the above solution. The absorbance was measured at 700 nm. Ascorbic acid was used as positive control.

Agar diffusion method : Assessment of antimicrobial potential of seed oil of *Datura metal* was carried out by using agar diffusion method as recommended by clinical and laboratory standards institute-approved standard (CLSI), CLSI M02-A11 and CLSI M27-A2. Amikacin (10µg) was used as standard antibiotic for

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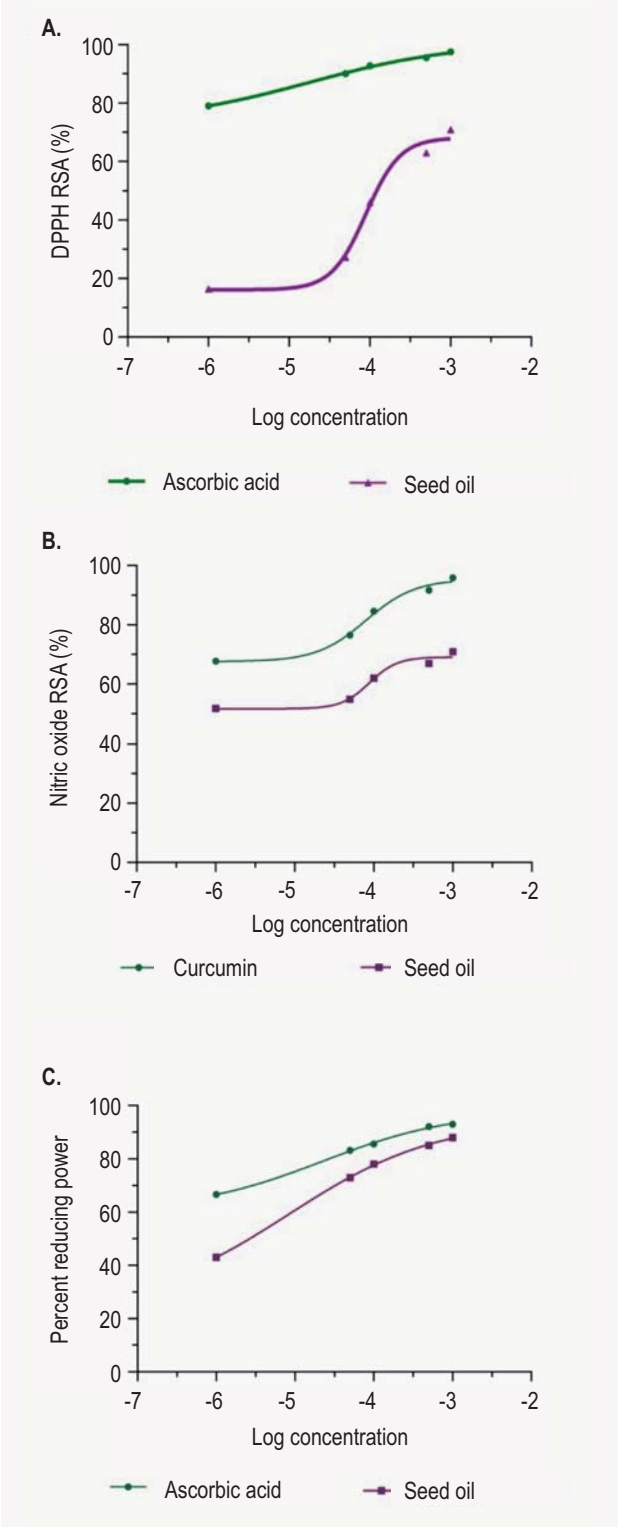

Fig. 1 : (A) Log dose response curve of 1,1-diphenyl 2-picrylhyorazyl free radical scavenging activity of seed oil of *Datura metal* compared with ascorbic acid; (B) Log dose response curve of nitric oxide free radical scavenging activity of seed oil of *Datura metal* compared with curcumin; (C) Log dose response curve of potassium ferricyanide reducing power of seed oil of *Datura metal* compared with ascorbic acid

bacteria, while fluconazole (10µg) as standard antifungal agent for fungi. Dimethylsulfoxide (10%) was used as negative control. Mueller-Hinton Agar was used for bacteria while Sabouraud Dextrose Agar was used for fungi. Agar plates were seeded with 100 µl indicator microorganisms (equivalent to 0.5 Mac Farland turbidity standard, ~1× 10^6 CFU ml^{-1}) and 6 mm well were filled with 100 µl of extracts at 250, 500, 1000 and 2000 µg ml^{-1} concentrations. The plates were incubated for 24 hrs at 37ºC for bacteria and 48 hrs at 28ºC for fungi. The antimicrobial capacity of seed oil was assessed against indicator microorganisms by measuring zone of inhibition around well.

Micro-dilution assay : Minimum inhibitory concentration (MIC) of seed oil was determined by micro-dilution assay as recommended by CLSI M02-A11 and CLSI M27-A2. Mueller-Hinton broth was used for bacteria, while Sabouraud dextrose broth was used for fungi. The amikacin was used as reference antibiotic for bacteria, while fluconazole as reference antifungal agent for fungi. The seed oil (500 mg) was dissolved in 10% DMSO (10 ml) to have a stock concentration of 50 mg ml^{-1}. Pre-sterilized 96-well micro-titer plates were used for micro-dilution assay. The serial dilution of seed oil was performed with broth media. Thereafter, 100 µl of microbial cell suspension was added to each well. The micro-titer plates were incubated at 37° for 24 hrs for bacteria and 28° for 48 hrs for fungi. The minimum inhibitory concentration of seed oil was scored as the lowest concentration giving 100% growth inhibition.

Statistical analysis : The data on antibacterial and antifungal activities of seed extract of *Datura metel* were examined as mean of three replicates ±SD. The IC_{50} values were calculated using four parameters logistic curve (Hill equation) (GraphPad Prism 6.00).

Results and Discussion

Rai *et al.* (2013) reported the fatty acid composition of *Datura metel* seed oil. The major fatty acids were linoleic acid (C18:2) (55.11%), oleic acid (C18:1) (26.13%) and a low amount of palmitoleic acid (C16:1) (0.95 %). Fig. 1A shows percent DPPH scavenging of seed oil of *Datura metel* with increasing concentration in the range of 10 –1000 µg ml^{-1}. The IC_{50} of seed oil was 81 µg ml^{-1}, while IC_{50} of reference ascorbic acid was 18 µg ml^{-1}. Leaf extract *Datura metel* in non-polar solvent showed DPPH scavenging potential lower than reference ascorbic acid (Sangeetha *et al.*, 2014). Similarly, seed oil of Malvaceae species *viz.,* such as *M. sylvestris*, *M. sylvestris* var. *mauritiana* and *A. officinalis* extracted with petroleum ether also showed 28-53 times weaker DPPH scavenging activity that butylated hydroxytoluene (Tesevic *et al.*, 2012). The result of the present investigation showing weaker antioxidant activity of seed oil are consistent with those of other studies (Tesevic *et al.*, 2012; Sangeetha *et al.*, 2014) and suggest that polarity of the solvent significantly affect extractable biomolecules. However, when

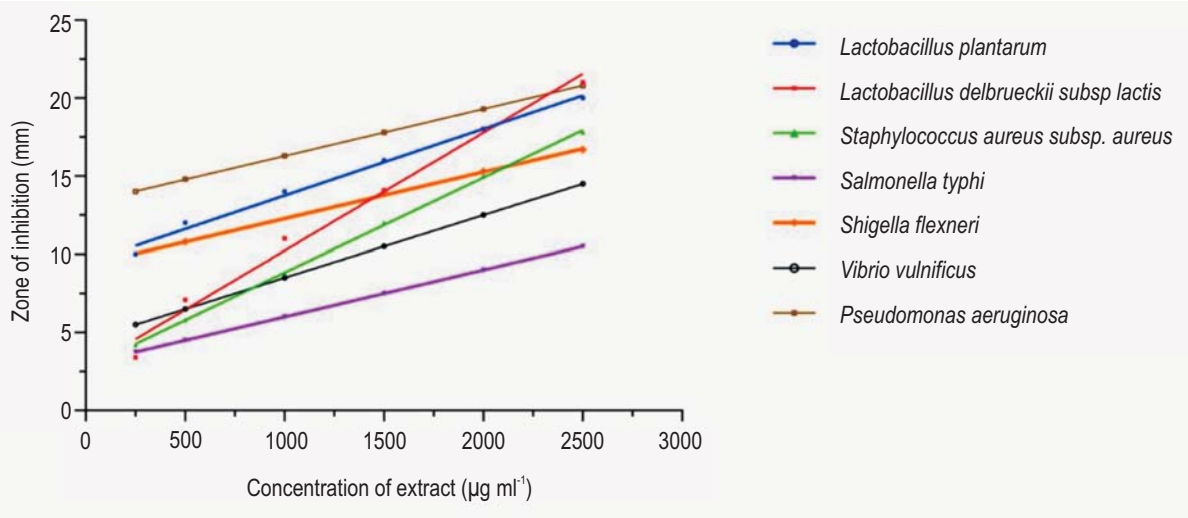

Fig. 2 : Linear regression analysis for susceptibility of different test bacteria correlating with zone of inhibition with increasing concentration of seed oil of *Datura metel*

Table 1 : Antibacterial activities of seed oil of *Datura metel* and standard antibacterial drug, amikacin against bacterial test organisms

	Microorganisms	Zone of inhibition (mm) Concentration of seed oil *D. metel** (µgml^{-1})					Positive control Amikacin ** (100 µg ml^{-1})
		125	250	500	1000	2000	
Gram positive bacteria	*Lactobacillus plantarum* MTCC 2621	9 ± 0.5	10 ± 0.6	13 ± 0.5	14 ± 0.6	17 ± 0.6	17 ± 0.8
	Lactobacillus delbrueckii subsp lactis MTCC 911	ND	6 ± 0.5	7 ± 0.6	11 ± 0.7	19 ± 0.7	17 ± 0.7
	Staphylococcus aureus subsp.aureus MTCC 737	ND	6 ± 0.5	8 ± 0.6	12 ± 0.7	16 ± 0.6	15 ± 0.6
	Enterococcus faecalis MTCC 439	ND	ND	ND	ND	ND	16 ± 0.7
Gram negative bacteria	*Escherichia coli* MTCC 443	ND	ND	ND	ND	ND	18 ± 0.8
	Salmonella typhi MTCC 531	ND	6 ± 0.4	7 ± 0.4	8 ± 0.5	10 ± 0.6	17 ± 0.7
	Shigella flexneri MTCC-1457	9 ± 0.5	10 ± 0.6	12 ± 0.6	13 ± 0.5	16 ± 0.7	18 ± 0.8
	Vibrio vulnificus MTCC-1145	ND	ND	7 ± 0.4	8 ± 0.5	13 ± 0.6	24 ± 0.7
	Pseudomonas aeruginosa MTCC-424	12 ± 0.6	14 ± 0.7	16 ± 0.6	17 ± 0.7	18 ± 0.6	17 ± 0.7

ND= not detected; (*100µl of the seed extract of respective concentrations (125, 250, 500, 1000 and 2000 µg ml^{-1}) was added to each well **100 µl of amikacin of concentration 100 µg ml^{-1} was added in well (positive control; values are mean of replicates ± S.D.)

Table 2 : MIC of seed oil of *Datura metel* and standard antibiotic, amikacin against test bacteria

	Test bacteria	MIC (µg ml^{-1}) Seed oil *D. metel*	Amikacin
Gram positive bacteria	*Lactobacillus plantarum* MTCC 2621	3125	25
	Lactobacillus delbrueckii subsp lactis MTCC 911	1562	25
	Staphylococcus aureus subsp.aureus MTCC 737	6250	13
	Enterococcus faecalis MTCC 439	-	13
Gram negative bacteria	*Escherichia coli* MTCC 443	-	6
	Salmonella typhi MTCC 531	12500	13
	Shigella flexneri MTCC-1457	3125	6
	Vibrio vulnificus MTCC-1145	1562	3
	Pseudomonas aeruginosa MTCC 424	781	13

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Table 3 : Linear regression equation between zone of inhibition with increasing concentration of seed oil of *Datura metel* for different bacterial strains

Bacteria	Regression equation	R^2
Lactobacillus plantarum MTCC 2621	y = 0.004x + 9.500	0.9029
Lactobacillus delbrueckii subsp lactis MTCC 911	y = 0.007x + 3.609	0.9954
Staphylococcus aureus subsp.aureus MTCC 737	y = 0.006x + 2.783	0.9739
Salmonella typhi MTCC 531	y = 0.003x + 3.000	0.9979
Shigella flexneri MTCC-1457	y = 0.003x + 9.292	0.9465
Vibrio vulnificus MTCC-1145	y = 0.004x + 4.500	0.7417
Pseudomonas aeruginosa MTCC 424	y = 0.003x + 13.290	

Table 4 : Antifungal activity of seed oil of *Datura metel* and standard antifungal drug, fluconazole against bacterial test organisms

Microorganism	Zone of inhibition (mm)						MIC (µg ml^{-1})	
	Seed oil *D. metel* (µg ml^{-1})					Fluconazole (µg ml^{-1})	Seed oil *D. metel*	Fluconazole
	125	250	500	1000	2000	10		
Candida albicans MTCC-227	ND	ND	D	ND	10± 0.3	14± 0.4	781	30
Candida tropicalis MTCC-230	ND	ND	ND	9± 0.2	11±0.3	13±0.4	1562	60

ND = not detected; ; values are mean of replicates ± S.D.

polar fractions (mainly polar lipids and phenolics) were found in high levels, extract would have strong radical scavenging activities. The extract obtained in non-polar solvent has weaker antioxidant activity extract obtained in polar solvent. However, these components can act synergistically with primary antioxidants (Ramadan *et al*., 2009). The DPPH radical scavenging activity of plants oils was highest in *D. innoxia* followed by *D. tatula*, *H. niger*, *H. muticus*, *D. metel* and least in *D. stramonium* (Ramadan *et al*., 2007). Several studies have reported that antioxidant potential of seed oil may be attributed to tocopherols, phytosterols, carotenoids and phenol (Anwar *et al.,* 2008; Cheikh-Rouhou *et al*., 2008; Nehdi *et al*., 2010; Jorge *et al*., 2016).

The effect nitric oxide free radical is stronger when it reacts with superoxide to form peroxynitrite, a potent oxidant with cytotoxic effect. In the present study, seed oil of *Datura metel* exhibited dose-dependent NO scavenging activity with IC_{50} value of 88 µgml^{-1}, which was at par with standard antioxidant, curcumin (IC_{50} value 80 µg ml^{-1}) (Fig. 2B). Previous study suggest that tocopherols, in addition to their chain breaking abilities, react with nitric oxide and protect against peroxynitrite (Joseph *et al*., 2009) and other reactive nitrogen species formation. Excess production of nitric oxide is implicated in several physiological and pathological processes (Förstermann and Sessa, 2012). The radical scavenging activities of the antioxidant may assist in protecting lipid membranes from attack by nitric oxide/peroxynitrite. In the study, it is implicated that seed oil antioxidants of *Datura metel* might compete with oxygen and inhibit generation of peroxynitrite.

In addition, seed oil has effective reducing power, IC_{50} 8 µg ml^{-1} which was lower than reference ascorbic acid (IC_{50} 22 µg ml^{-1}) (Fig. 1C). The results of the study (Fig. 1C) revealed that seed oil of *Datura metel* has concentration-dependent response towards Fe^{3+} reducing power. Thus, seed oil of *Datura metel* can reduce ferric ion to ferrous ion by donating an electron.

In the present study, it was found that seed oil of *Datura metel* possessed antibacterial efficacy at least against seven bacterial strains tested, with zone of inhibition 6-19 mm (for reference amikacin 6-19 mm, at 1000 µg ml^{-1}) (Table 1) and MIC 781-12500 µg ml^{-1} (for reference amikacin MIC 3-25 µg ml^{-1}) (Table 2). The highest zone of inhibition and lowest minimum inhibitory concentration (MIC) was recorded against *Lactobacillus delbrueckii* subsp *lactis* MTCC 911 (19 mm) and *Pseudomonas aeruginosa* (18 mm) indicating susceptibility of strains to seed oil of *Datura metel*. The results of antibacterial study (Fig. 2) showed concentration-dependent response, the zone of inhibition increased with the increase in the concentration of seed oil. Linear regression equation with zone of inhibition with increasing concentration of seed oil is shown in Table 3. It is interesting to note that seed oil from this plant also sensitizes *S. aureus*, a strain commonly associated with the development of *methicillin resistance.* Okwu and Igara (2009) isolated a steroidal compound, 5', 7' dimethyl 6' hydroxyl amine -yne sitosterol from ethanolic extract of leaf of *Datura metel* that showed antibacterial potential against *S. aureus*, *P. aeruginosa*, *Proteus mirabis*, *S. typhi*, *B. subtilis* and *K. pneumonae* but the compound could not

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inhibit *E. coli*. In an another investigation, Okwu and Igara (2011) isolated a new β -carboline alkaloid (1, 7 dihydroxy-1-methyl 6, 8 dimethoxy β -carboline) from *D. metel* leaves that showed antagonistic effect against *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *P. mirabilis*, *E. coli*, *B. subtilis* and *S. typhi*. Various studies suggest that antimicrobial potential of extract is dependent on several factors such as the method of extraction, polarity of solvent, hydrophobicity of compounds, types of microorganisms and difference in the structure of outer membrane and antibiotic resistance pattern of test microorganisms (Bacon *et al.,* 2016; Ortega-Ortega *et al.,* 2017). Several reports are available that indicate the antibacterial capacity of *Datura metal* extract against bacteria (Sakthi *et al*., 2011; Vadlapudi and Kaladhar, 2012; Gachande and Khillare, 2013). However, only few studies have reported characterization of phytocompounds. In addition to antibacterial activity, seed oil of *Datura metel* also showed high antifungal potential against *Candida albicans* MTCC 227 (zone of inhibition 10 mm at 2000 µg ml^{-1} of seed oil) and *Candida tropicalis* MTCC 230 (zone of inhibition 11 mm at 2000 µg ml^{-1} of seed oil), which is comparable to the standard antifungal agent fluconazole (10 µg ml^{-1}) (Table 4). The MICs of seed oil for *Candida albicans* MTCC 227 and *Candida tropicalis* MTCC 230 was 781µg ml^{-1} and 1562 µg ml^{-1}, which was higher than fluconazole. The results based on MICs indicated that, although seed oil had anti-candidal activity but was less potent than standard antifungal agent, fluconazole (Table 4). Besides anti-candidal activity, few reports indicate antifungal activity of *Datura metel* extract against *Aspergillus* sp. (Dabur *et al.,* 2004; Fakai *et al*., 2016). It is reported that chloroform fraction of *D. metel* exhibited antifungal efficacy against *Aspergillus* sp. viz., *A. fumigatus*, *A. flavus* and *A. niger* but efficacy of extract was less than broad spectrum polyene antifungal agent, amphotericin B (Sharma, 2002). In a further investigation on leaf extract of *Datura metel,* Dabur *et al*. (2004) isolated a new pyrrole derivative, 2 beta-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1'-methylethyl pentanoate showing antifungal activity against *Aspergillus fumigatus*.

The findings suggest that seed oil of *Datura metel* exhibited antioxidant and antimicrobial potential. However, the weak antioxidant activity of the extract in comparison with reference may be due to low content of polyphenol in the oil. Several reports have attributed the antioxidant potential of seed oil due to tocopherols and phenolic compounds (Frankel, 1996; Sakai *et al*., 2010). The study also showed significant antibacterial and anti-candidial activities of seed oil. The secondary metabolites such as glycosides, alkaloids, steroids, flavonoids and tannins may contribute towards antimicrobial activity of seed oil of *Datura metel*.

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