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In vitro haemolytic, antioxidant and antibacterial (ESBLs and MRSA) activity of *Datura metel* L. flower and leaf extracts

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Prevalence of antibiotic resistant bacteria is an issue of concern as it affects the efficiency of antibiotics significantly. It makes the drug manufacturers continuously search for newer molecules that are safe and effective. To meet this challenge, researchers look towards natural resources, particularly plants, for possible new molecules that can be potentially tapped for medicinal purposes. In this context, here, we investigated *Datura metel*, commonly called the Angel's trumphet as it is known for medicinal properties. We studied the hot water and methanol extracts of its flowers and leaves for antibacterial, antioxidant and antihemolytic activities. We used disc diffusion, phospho-molybdate and spectrophotometric methods. The bacteria tested were Methicillin resistant *Staphylococcus aureus* (MRSA) and β lactamase (ESBL) producing *E. coli*. The zone of inhibition (ZoI), as a measure of bacterial growth inhibition, was checked and found to range from 1.2-17.5 mm at the tested concentrations; ESBL producing *E. coli* was more subtle than MRSA. Total antioxidant capacity measured at 695 nm showed an average score of 17.4 µg/mL, displaying better activity of methanol extract. Antihemolytic property was tested on 0.5% RBC and percentage hemolysis was measured at optical density of 540 nm. Both the extracts showed considerably similar activity, though hot water lowered the hemolytic activity. Over all, the results suggest that *D. metel* could be exploited for its potential to inhibit drug resistant bacteria; free radical scavenging activity; and anti-hemolytic properties.

Keywords: Angel's trumpet, Bactericidal, Drug resistance, Hemolysis, Oxidative stress

Drug discovery has always been challenging in the context of rising cost and low success rate. Discovery of new molecules by researchers costs approximately two billion US$ with an average period of 10-15 years before it reaches the pharmacy[1]. The analysis of new drug sources dating from 1981 reveals that almost half of the approved drugs were based on natural products[2]. In recent times, the use of natural products as drug components has decreased in the pharmaceutical industry but the "influence of natural product structures" is unchanged as it holds the best choice to find novel agents/active templates[3]. Natural product research continues to be a productive source in various applications providing commercially useful compounds[4].

The bacterial infections are key cause of illness and death in many countries[5]. Further, discovery of novel antibacterial agents is necessary due to emergence of drug- resistance in both Gram-positive and Gram negative organisms[6,7]. Among several other drug-resistant bacteria, Extended Spectrum Beta Lactamases (ESBLs) producing *E. coli* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) are highly prevalent in the hospital acquired infection[8,9]. Their tolerance levels exceed from the existing antibiotics. Oxidative stress is been considered as main cause of development and progression of several diseases with varied metabolic processes. The effects of reactive oxygen species (ROS) inducing oxidative damage can be combated by supplementation of exogenous or endogenous antioxidants. Plants have innate ability to synthesize large number of antioxidants[10]. The intake of exogenous antioxidants would help to terminate the damage occurred by oxidative stress and by acting as free radical scavengers[11]. Recently, plant secondary metabolites like flavonoids, terpenes, alkaloids, α-tocopherol, and carotenoids have gained major attention due to their antioxidant property[12]. Erythrocytes are the blood cells that deliver oxygen to the body tissues[13]. Owing to this principal role, erythrocytes are under continuous stress by oxygenation and deoxygenation cycles, narrowing blood vessels, high content of polyunsaturated fatty acid (PUFA) in their membranes and reactive oxygen species (ROS) (endogenous generation) during their 120-day life span[14,15]. Hemolysis is the impairment of a variety of host defense mechanisms, including macrophage and neutrophil dysfunction; and impaired adaptive immune responses[16]. Hence, the role of

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herbs with antihemolytic activity is also beneficial to the society.

Datura metel Linn. (Fam. Solanaceae) is a biennial to perennial herb commonly found in the different tropical and subtropical regions of Asia, Africa and Europe. The contents of the plant acts as anaesthetic, antiproliferative, antidiarrheal, antiasthmatic, antispasmodic, antitumor, antimicrobial effects and also in curing skin disorders, ulcers, bronchitis, jaundice, heart disease, fever and so on[17].

In the present study, we explored the hot water and methanol extracts of flowers and leaves of *D. metel* for possible antibacterial activity against antibiotic resistant bacteria; and prevention of oxidative damages; and also its potential to inhibit hemolysis.

Materials and Methods

Extraction of plant material

The plant material (flowers and leaves of *D. metel*) were collected from the local household regions of Kolar, India and were identified based on the morphological characters and information as given in the Flora of Madras 2[18]. The characters used for identification were as follows: green hollow stem, simple, alternate, glabrous leaves, solitary cyme inflorescence; flowers are large, bracteate, ebracteolate, pedicellate, complete, dichlamydeous, pentamerous, regular, actinomorphic, bisexual, and hypogynous; cup shaped gamocephalous and persistent calyx; corolla is white to creamy white, wide mouthed gamopetalous with 5 petals; and the fruit, spiny capsule with persistent calyx. Further, the specimen herbarium was authenticated by Horticulture College, Kolar, India, and later submitted to Dr. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. Given voucher specimen number is 1142.

Flowers and leaves were separated and examined for insects or insect bites, washed with distilled water, shade dried, finely ground, sieved (12-20 mesh) and stored in dark till extraction. Hot water and methanol extractions were made from the established procedures. Briefly, the hot water extractions were prepared by soaking 10 g of *D. metel* powder in 160 mL of distilled water and continuously boiled for 24 h until its volume is reduced to one fourth. The boiled extracts were cooled and centrifuged to remove non-soluble compounds, and then strained, filtered through Whatman No. 1 paper to attain a clear filtrate. The filtrates were later evaporated and dried at 40°C in an incubator under sterile condition[19].

The methanol extractions were made by mixing 10 g of *D. metel* powder with 100 mL of methanol, allowed to dissolve the compounds by keeping it on a rotary shaker for 24 h. Clarified and the collected supernatant was filtered using Whatman no. 1 paper and the filtrates were kept for evaporation at room temperature (37°C) under sterile condition[20,21]. The yielded extracts after drying for about 20 days were collected, weighed and further stored in airtight bottles at 4°C. The percentage extract yield was calculated using the formula

$$\text{Extract yield \%} = R/S$$

where R is the weight of extracted plant residues and S, the weight of plant raw sample.

Isolation of pure culture

MRSA and ESBL producing *E. coli* were acquired from the Department of Microbiology, R.L. Jalappa Hospital and Research Center, Kolar, India. Glycerol stocks were made for the cultures and stored for further analysis. Well isolated colonies of MRSA and ESBLs producing bacteria were obtained by quadrant streaking of the cultures on the nutrient agar plate (pH 7). Further, the species were confirmed by its morphological characteristics and various biochemical tests. Antibiotic proneness test and minimum inhibitory concentration analysis were done using Muller-Hinton agar to confirm the antibiotic resistance of the bacteria[22,23].

Antimicrobial susceptibility test: Disc diffusion method

Modified Kirby-Bauer disk diffusion method was followed to evaluate the antibiotic susceptibility of the MRSA and ESBLs producing isolates at different concentration of crude extracts. The stock solution (50 mg/mL) was prepared with hot water and methanol extracts made with working concentrations (250 μg^{-1}, 500 µg with 250 variations) and were loaded on the previously sterilized filter paper discs (8 mm in diameter). The cell density was adjusted to 0.5 Mc Farland standards, equivalent to 1×10^8 CFU/mL of bacteria[24,25]. The surface of Muller-Hinton agar plates were evenly spread with the organisms using a sterile "L" shaped spreader. By means of sterile forceps, the extract loaded discs were placed on the surface of the inoculated agar. The maintained three biological replicate plates were kept at 4°C for 2 h to permit the plant extracts to diffuse into the agar and subsequently incubated overnight at 37°C. The inhibition zone was observed and measured.

Total antioxidant capacity (TAC) by phosphomolybdate assay

For the antioxidant assay, the 1.0 mg extract was dissolved in 1.0 mL of respective solvent. Concentration dependent dilutions were made in the α-tocopherol (1.0 g/mL) stock solution. The spectrophotometric TAC method was carried out by phosphomolybdenum method[26]. This is based on the molybdenum (VI) reduction to Molybdenum (V) by the analyte of the sample. This results in the development of green phosphate/molybdenum (V) complex at acidic pH. About 0.1 mL of extract was shaken with 1.0 mL of phosphomolybdate reagent containing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The sample was further incubated at 90°C for 90 min and allowed to cool. The absorbance was measured at 695 nm against phosphomolybdate reagent as blank. The amount of total antioxidant capacity was expressed in mM α-tocopherol acetate equivalent/g dry mass.

Antihemolytic activity

The antihemolytic potential of the extracts were inspected by the spectrophotometric procedure[15]. After obtaining ethics clearance from the institution (DMC/KLR/IEC/23/2017-18), 05 mL of human blood sample was collected from a healthy volunteer in a heparin tube, clarified at 1500 rpm for 5 min and used for preparing 2% erythrocyte suspension in sterile Phosphate Buffer Saline (PBS) (0.2M, pH 7.2). The pellet obtained after centrifugation was washed in PBS (pH 7.2) and re-suspended in 0.5% PBS. From the solvent extracts, 1.0 mg/mL stock and 125-1000 µg/mL working stocks were made. To perform the assay, 1:1 ratio of 0.5% RBC and extracts were mixed thoroughly to initiate oxidative degradation of membrane lipid, followed by incubation at 37°C for half an hour and clarified at 1500 rpm for 10 min. The resultant supernatant was collected and measured the absorbance at 540 nm. The comparative hemolysis was assessed by comparing the H_2O_2-treated (negative control) and PBS treated (positive control) samples. Experiments were performed in triplicates and percent inhibition of hemolysis was calculated by the formula:

$$\% \text{ Hemolysis} = \frac{A_t - A_n}{A_c - A_n} \text{ X } 100$$

where A_t is the absorbance of the test sample, A_n is the absorbance of the control (positive control) and A_c is the absorbance of the control (negative control).

$$\% \text{ Protection} = 100 - (\text{Hemolysis})$$

Statistical analysis

Mean and standard errors were calculated from the triplicates. Antibacterial activity results with methanol and hot water extractions of leaf and flower were analyzed by one tailed *t* test and *P* value <0.05 is found significant.

Results & Discussion

Plant extract yield

Antibiotic resistant bacteria were characterized by a few biochemical tests (Table 1) for MRSA and ESBLs, such as IMViC test and TSI as the most special tests to identify them.

Regarding presence or absence of catalase and oxidase enzymes, both the organisms were catalase positive and oxidase negative respectively. In motility test, it was found to be non-motile cocci and motile rods (Table 2 and Fig. 1 A-D).

Data of the employed plant and its percentage of extract yields were tabulated (Table 2). From both hot water and methanol, the total leaf and flower extract residues ranged from 0.43 g to 1.2 g. Leaf extract yielded more with methanol of 1.2 g and hot water of 0.96 g; whereas, flower extract yielded more with hot water extract of 0.43 g.

Table 1 — Characteristics of isolated antibiotic resistant bacteria

Basic characteristics	*Staphylococcus aureus*	*Escherichia coli*
Gram staining	Gram positive	Gram negative
Shape	Cocci	Rod
Catalase	+	+
Oxidase	-	-
Indole	-	+
Methyl Red	+	+
Voges Proskauer	+	-
Citrate	+	-
Triple Sugar Iron	+	+
Gas production	-	+
Urease	+	+
Glucose fermentation	+	+
Sucrose fermentation	+	-
Mannitol fermentation	+	+
Lactose fermentation	+	+

[Biochemical and sugar fermentation tests were performed for *Staphylococcus aureus* and *Escherichia coli*, such as IMViC test and TSI as the most special tests to identify them. Regarding presence or absence of catalase and oxidase enzymes, both were catalase positive and oxidase negative respectively. Motility test was applied to the isolated bacteria by which it was found to be non-motile cocci and motile rods]

Table 2 — pH and Percentage yield of plant extract

Extraction	Plant part used	Extract pH	Extract yield (%)
Methanol	Leaf	7.2	12
	Flower	7.0	6.1
Hot water	Leaf	6.8	9.6
	Flower	6.9	4.3

[Extract yield % = R/S, where R is the weight of extracted plant residue and S is the weight of plant raw sample]

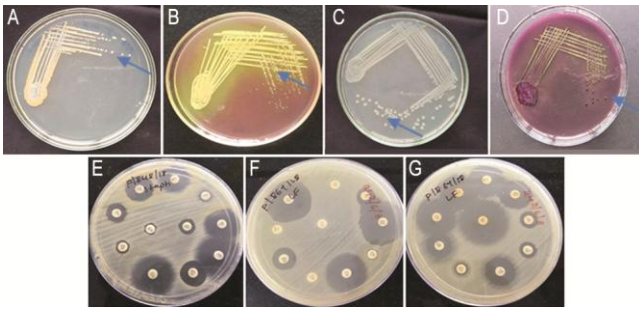

Fig. 1 — Colony morphology of *Staphylococcus aureus*. (A) Small golden pigmentation of colonies on nutrient agar plate; (B) Yellow colonies due to acid production by fermentation on Mannitol Salt Agar (MSA) plate, Colony morphology of *E. coli*; (C) Thick, greyish white, moist, smooth, opaque colonies on Nutrient agar; (D) Small pink colonies with metallic green sheen due to lactose fermentation on Eosin Methylene Blue (EMB) agar plate, Confirmation of Antibiotic resistance bacteria; (E). MRSA; and (F-G) ESBLs producing *E. coli*.

Antibacterial Activity

The extracts were investigated to evaluate its antibacterial potency against MRSA and ESBL producing *E. coli* by disc diffusion method. The antibacterial activity of the extract was measured by the zone of inhibition (ZoI) around the discs impregnated with various concentrations of extract.

Methanol leaf extract showed a ZoI at 250 µg/5 µL against both MRSA and ESBL producing *E. coli* and there was a gradual increase in ZoI with increasing concentration of the compound against both the bacteria. The maximum ZoI was 11 mm against MRSA and 17.5 mm against ESBL producing *E. coli*. Though there was no inhibition at 250 µg/5 µL with methanol flower extract on MRSA and ESBL producing *E. coli*, there was a gradual increase in ZoI on dose dependent manner. The maximum ZoI was 9.5 mm against MRSA and 10.2 mm against ESBL producing *E. coli.* When compared to leaf, flower showed less activity against ESBLs. When analyzed statistically, methanol extractions were found to be significant with *P* value <0.05. Hot water flower and leaf extracts showed negligible inhibition between 250 and 1,250 µg/5 µL and maximum ZoI was observed at 1500 µg/5 µL (3.1 mm for leaf and 2.2 mm for flower) against MRSA. Hot water leaf and flower extracts showed ZoI from 750 µg/5 µL to 1500 µg/5 µL against ESBL producing *E. coli*; the maximum ZoI was 7.3 mm and 6.6 mm for leaf and flower extracts, respectively (Figs 2 and 3).

Total antioxidant capacity (TAC)

Total antioxidant capacity of the extracts was determined by a quantitative spectrophotometric (695 nm) phosphomolybdate assay. The results reveal that

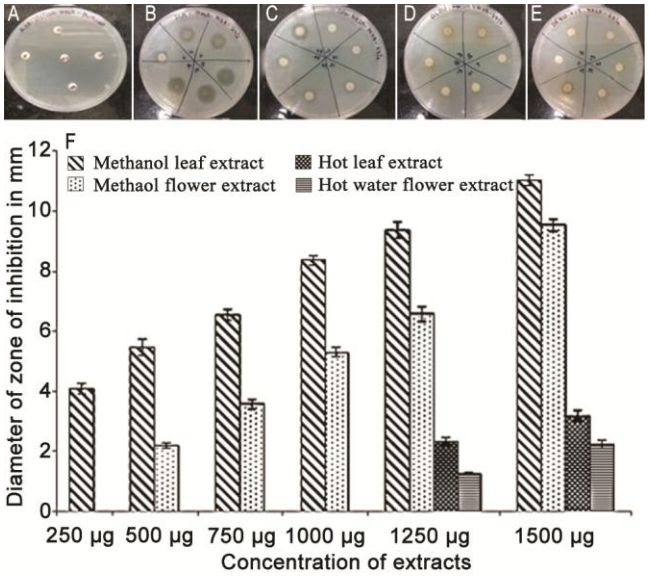

Fig. 2 — Growth inhibition of *S. aureus* by various concentrations of plant extract by disc diffusion method. (A) Methanol (Control); (B) Methanol leaf extract; (C) Methanol flower extract; (D) Hot water leaf extract; (E) Hot water flower extract; and (F) Minimum inhibitory concentrations of plant extracts against MRSA (Concentrations of extracts in µg/mL).

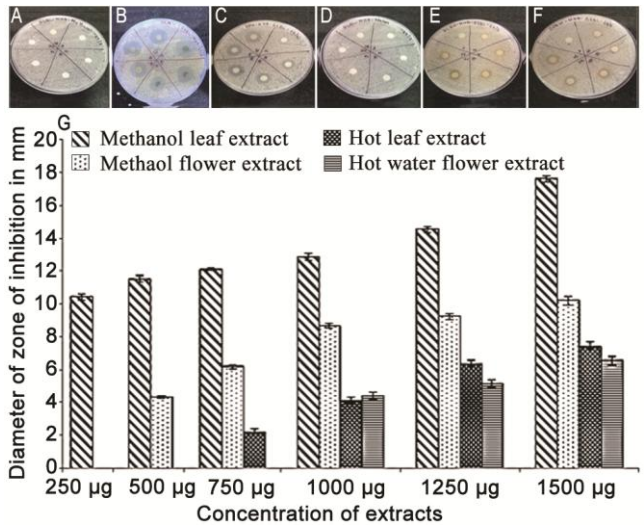

Fig. 3 — Growth inhibition of *E. coli* by various concentrations of plant extract by disc diffusion method. (A) Methanol (Control); (B) Methanol leaf extract; (C) Methanol flower extract; (D) Water (Control); (E) Hot water leaf extract; (F) Hot water flower extract; and (G) Minimum inhibitory concentrations of plant extract against ESBLs producing *E. coli.* (*P* <0.05)* indicates significant inhibition.

D. metel methanol leaf extract displayed the antioxidant capacity of 17.45 µg/mL; the values of other extracts were 17.26, 17.42 and 17. 44 µg/mL for methanol flower extract, hot water leaf extract and hot water flower extract, respectively from Tocopherol standard graph (Table 2).

Antihemolytic activity

In this study, we have also determined the antihemolytic effect of *D. metel* preventing the oxidative damages to erythrocyte membrane. Hemolytic activity of the plant is stated in percentage hemolysis. The extracts of the plant displayed a differential pattern of anti-hemolytic activity. Though showed hemolysis at higher concentrations, the lower percentage was noted with leaf and flower hot water extract than the methanol extracts (Table 3) of leaf and flower.

Traditionally, for testing pharmacologically important phytomedicine, water decoction (hot/cold) or infusion is usually used. In the present study we dried the hot water extracts and compared that with methanol extracts. Methanol has low boiling point and most of the polar and semi-polar constituents blend well with methanol. Extracts with water are rich with water soluble proteins, glycoproteins, small peptides, a few amino acids, nucleotides, sugars, polysaccharides, metals, ions, hydrophilic compounds. Methanol's polarity index is 5.1 and the polar phytochemicals (eg., tannins, alkaloids, glycosides, phenolics, etc.) can easily be extracted. Though not completely soluble, a few non-polar phytochemicals can fairly be soluble in methanol, making methanol a commonly used extraction solvent. Moreover, among all the organic solvents, methanol has a low boiling point of 65°C. Solvent extraction is purely based on polarity and solubility of the material of interest. The least polar to most polar order is as follows: hexane < chloroform < ethyl acetate < acetone < methanol < water[27].

Plants contain an enormous number of bioactive phyto-constituents with potential therapeutic properties. In the present investigation, hot water and methanol extracts of *D. metel* flower and leaf were used to know their significant therapeutic values. The examined ZoI reveals its antimicrobial properties as discussed earlier from other literatures against various pathogenic bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. Controlling microbial load with minimum concentration is important and we use as low concentrations as possible. Both ESBL producing *E. coli* and MRSA were sensitive to the extracts, the first one being more sensitive. From the study, it is established that *D. metel* extracts help to combat emerging drug resistant issues. Different plant extracts will have different mechanisms to inhibit or control gram positive and gram negative bacteria as they have varied structural dissimilarities[28].

In recent times, the focus is on natural antioxidants with tremendous health benefits. To prevent and treat various complex diseases science relies on antioxidant drug formulations. Low (ascorbic acid, tocopherol, reduced glutathione and their derivatives) and high molecular weight compounds (polyunsaturated macrocyclic aromatics) form a bond with transition metal ions, referred as, "catalytic antioxidant mimetics"[29]. They mimic the functions of endogenous antioxidant enzymes. In our study, significant antioxidant activity was determined by the methanolic fractions of *D. metel*. Earlier investigations revealed that methanol extracts phytochemicals *viz.*, alkaloids, phenolics, carbohydrates, flavonoids and terpenoids. Our results correlate with the other similar studies stating that catechins, polyphenols and other phytochemicals would be responsible for significant antioxidant potential[20,21,30].

The most plentiful cells in the human body with copious morphological characteristics are erythrocytes. Erythrocytes have been used as a model system by a number of researchers to study the interaction of drugs with membranes. Polyunsaturated fatty acids target the erythrocytes as they have redox-active oxygen transportation feature. This results in hemolysis of erythrocyte membrane lipids and proteins that are multiplied by oxidation. This activity leads to osmotic fragility in diabetic patients which is positively related to glycated hemoglobin. As the available drugs cause varying side effects, medicinal plants with affordable and negligible side effects came into practice. The reports of *Allium sativum* bulb and seeds of *Persia americana* are proved to reduce

Table 3 — Optical density of plant extracts to measure percentage inhibition of hemolysis

Conc. (µg/mL)	Positive control	Negative control	Optical Density at 540 nm			
			Methanol extract		Hot water extract	
			Leaf	Flower	Leaf	Flower
125	0.007	0.176	0.047±0.09	0.037±0.01	0.034±0.0	0.023±0.02
250	0.007	0.176	0.066±0.4	0.128±0.03	0.033±0.02	0.053±0.0
500	0.007	0.176	0.098±0.4	0.146±0.01	0.054±0.0	0.062±0.02
1000	0.007	0.176	0.111±0.0	0.144±0.03	0.082±0.08	0.088±0.01

glycaemia which are proven safe and effective. The outcome of this study presents the occurrence of antioxidants with antihemolytic effect. This data suggests the non-toxic effects of the *D. metel* extract makes it suitable for the preparation of drugs to treat various diseases. Therefore, further studies have to be carried out to prove the safety usage of these extracts.

Conclusion

In this work, the therapeutic properties of *Datura metel* were investigated for antibacterial, antioxidant and antihemolytic activity. The employed standard disc diffusion method showed that *D. metel* has high potential antibacterial activity against drug resistant bacteria at lower concentrations. Analysis of free radical scavenging might lead to the replacement of synthetic antioxidant drugs with natural agents. The fractions possess low hemolytic activity indicating a positive sign to look for antihemolytic agent from an alkaloid rich plant. The limitation of the present study is to use various other solvents and compare their effectiveness by *in vitro* and *in vivo* models.

Conflict of Interest

Authors declare no competing interests.

References

1 Berdigaliyev N & Aljofan M, An overview of drug discovery and development. *Future Med Chem*, 12 (2020) 939.

2 Sarita Manandhar, Shisir Luitel & Raj Kumar Dahal, *In vitro* Antimicrobial activity of some medicinal plants against human pathogenic bacteria. *J Trop Med*, (2019) 1.

3 Newman DA-O & Cragg GA-O, Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J Nat Prod*, 83 (2020) 770.

4 Duggan BM. Editorial to the Special Issue-"Technology for Natural Products Research". *Molecules* (Basel, Switzerland), 25 (2020) 327.

5 Bilinski J, Grzesiowski P, Sorensen N, Madry K, Muszynski J & Robak K, Fecal Microbiota Transplantation in Patients with Blood Disorders Inhibits Gut Colonization With Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. *Clin Infect Dis*, 65 (2017) 364.

6 Meryem Tasc, Ozlem Temiz-Arpaci, Fatma Kaynak-Onudag & Suzan Okten, Synthesis and antimicrobial evaluation of novel 5-substituted-2-(p-tert-butylphenyl) benzoxazoles. *Indian J Chem*, 57B (2018) 385.

7 Bilal Aslam, Wei Wang, Muhammad Imran Arshad, Mohsin Khurshid, Saima Muzammil, Muhammad Hidayat Rasool, Muhammad Atif Nisar, Ruman Farooq Alvi, Muhammad Aamir Aslam, Muhammad Usman Qamar, Muhammad Khalid Farooq Salamat & Zulqarnain Baloch, Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist*, 10 (2018) 1645. doi: 10.2147/IDR.S173867. PMID: 30349322; PMCID: PMC6188119.

8 Hui Liu, Weilong Shang, Zhen Hu, Ying Zheng, Jizhen Yuan, Qiwen Hu, Huagang Peng, Xinyu Cai, Li Tan, Shu Li, Junmin Zhu, Ming Li, Xiaomei Hu, Renjie Zhou, Xiancai Rao & Yi Yang, A novel SigB (Q225P) mutation in *Staphylococcus aureus* retains virulence but promotes biofilm formation. *Emerg Microbes Infect*, 7 (2018) 72. doi:10.103/s41426-018-0078-1.

9 Weilong Shang, Yifan Rao, Ying Zheng, Yi Yang, Qiwen Hu, Zhen Hu, Jizhen Yuan, Huagang Peng, Kun Xiong, Li Tan, Shu Li, Junmin Zhu, Ming Li, Xiaomei Hu, Xuhu Mao, Xiancai Rao, β-Lactam Antibiotics Enhance the Pathogenicity of Methicillin-Resistant *Staphylococcus aureus* via SarA-Controlled Lipoprotein-Like Cluster Expression. *mBio*,10 (2019) 1. doi:10.1128/mBio.00880-19.

10 Kasote DM, Katyare SS, Hegde MV & Bae H, Significance of antioxidant potential of plants and its relevance to therapeutic applications. *Int J Biol Sci*, 11 (2015) 982. doi: 10.7150/ijbs.12096. PMID: 26157352; PMCID: PMC4495415.

11 Ganna Petruck, Rita Del Giudice, Maria Manuela Rigano & Daria Maria monti, Role of Antioxidants in the protection from aging-related diseases. *Oxid Med Cell Longev*, 1 (2018) 11.

12 12.Yuhang Chen, Xuerong Zhang, Qiaosheng Guo, Liping Cao, Qin Qin, Chen Li, Miao Zhao & Wenming Wang, Plant morphology, physiological characteristics, accumulation of secondary metabolites and antioxidant activities of *Prunella vulgaris* L. under UV solar exclusion. *Biol Res*, 52 (2019) 17. doi.org:10.1186/s40659-019-0225-8.

13 Boris GK Azantsa, Mary-Ann A Mbong, Guy RN Takuissu, Rosane S Matsinkou, Imelda LN Djuikoo, Janvier F Youovop, Judith L Ngondi & Julius E Ob, Anti-hemolytic, Anti-lipid peroxidation and Antioxidant properties of three plants locally used to treat metabolic disorders: *Allium sativum*, *Persea americana* and *Citrus sinensis*. *J Food Res*, 8 (2019) 89.

14 Mohamad S AlSalhi, Sandhanasamy Devanesan, Khalid E AlZahrani, Mashael AlShebly, Fatima Al-Qahtani, Karim Farhat & Vadivel Masilamani, Impact of Diabetes Mellitus on Human Erythrocytes: Atomic Force Microscopy and Spectral Investigations. *Int J Environ Res Public Health*, 15 (2018) 2368. https://doi.org/10.3390/ijerph15112368.

15 Hansiw N, Paradee N, Chotinantakul K & Srichairattanakool S, Anti-hemolytic, antibacterial and anti-cancer activities of methanolic extracts from leaves and stems of *Polygonum odoratum*. *Asian Pac J Trop Biomed*, 8 (2018) 580.

16 Kim Y, Park J & Kim M, Diagnostic approaches for inherited hemolytic anemia in the genetic era. *Blood Res*, 52 (2017) 84. doi: 10.5045/br.2017.52.2.84. PMID: 28698843; PMCID: PMC5503903.

17 Iqbal S, Sivaraj C & Gunasekaran K, Antioxidant and Anticancer Activities of Methanol Extract Seeds of *Datura stramonium* l. *Free Rad Antiox*, 7 (2017) 184.

18 Gamble JS, *Flora of the Presidency of Madras. Volume II,. Ranunculaceae to Caprifoliaceae*. (Govt. of India, Calcutta), 1956.

19 Parwata A, Manuaba P, Yasa S. The Potency of Flavonoid Compounds in water Extract *Gyrinops versteegii* Leaves as Natural Antioxidants Sources. *Biomed Pharmacol J*, 11 (2018) 1501.

20 Aita Rani Subba & Santosh Kumar Rai, Phytochemical screening, physico-chemical analysis and antioxidant activity of some ethnomedicinal plants from Sikkim Himalaya. *Indian J Nat Prod Resour*, 9 (2018) 235.

21 Uttam Das, Tanmay Saha & Subir Kumar Das, *Trianthema portulacastrum* L. extract protects against gamma radiation induced human red blood cell membrane damage *in vitro*. *Indian J Biochem Biophys*, 55 (2018) 321.

22 Karim M, Jabeen K, Iqbal S & Javaid A, Bioefficacy of a common weed *Datura metel* against *Colletotrichum gloeosporiodes*. *Planta Daninha*, 35 (2017) 1806

23 Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN & Bakri MM, Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J Biol Sci*, 25 (2018) 361.

24 Emily S Savela , Nathan G Schoepp , Matthew M Cooper, Justin C Rolando, Jeffrey D Klausner, Olusegun O Soge & Rustem F. Ismagilov, Surfactant-enhanced DNA accessibility to nuclease accelerates phenotypic β-lactam antibiotic susceptibility testing of *Neisseria gonorrhoeae*. *PLoS Biol*, 18 (2020) 1. e3000651. doi.org/10.1371/journal.pbio.300065.

25 David Alejandro Hernández-Marín, Fidel Guevara-Lara, Catalina Rivas-Morales, Jorge Armando Verduzco-Martínez, Sergio Arturo Galindo-Rodriguez & Eduardo Sánchez-García, Biological activity of *Nothoscordum bivalve* (L.) Britton and *Parthenium incanum* Kunth extracts. *Indian J Tradit Knowl*, 17 (2018) 699.

26 Khanday WI, Wani NA & Paulraj B, Antioxidant and cytotoxic potential of leaf extracts of *Costus igneus*. *J Nat Sci Biol Med*, 10 (2019) 157

27 Ammar Altemimi, Naoufal Lakhssassi, Azam Baharlouei, Dennis G Watson & David A Lightfoot, Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*, 6 (42) (2017) 1.

28 Radhika Parvataneni & Mahesh Pedireddi, Phytochemical analysis and antimicrobial evaluation of chloroform extracts of stem and roots of *Scoparia dulcis* L. *Indian J Exp Biol*, 57 (2019) 206.

29 Baunthiyal M, Singh V & Dwivedi S, Insights of Antioxidants as Molecules for Drug Discovery. *Int J Pharmacol*, 13 (2017) 874.

30 Cory H, Passarelli S, Szeto J, Tamez M &Mattei J. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Front Nutr*, 5 (2018) 5.