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Phytochemical screening, antimicrobial and cytotoxic potential of *Datura stramonium* seeds: growing wild in Kashmir Himalayas

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ABSTRACT

The accumulative evidences suggesting medicinal importance of Datura stramonium coupled with ethnopharmacological importance were the basis to select this plant.The qualitative phytochemical examination of ethanolic extract of Datura stramonium seeds was executed by carrying out standard tests for carbohydrates, flavonoids, amino acids, saponins, proteins, tannins, starch, alkaloids, terpenoids, reducing sugars, steroids, anthraquinones, and glycosides. The antibacterial (Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa and Escherichia coli) and antifungal (Candida albicans, Aspergillus clavatus and Aspergillus niger) analysis of the extract was performed using disk-diffusion method. The cytotoxic effects of Datura stramonium seeds were examined using MTS proliferation assay.The results of this investigation showed significant phytochemical composition of ethanolic extract of Datura stramonium seeds like the presence of alkaloids, terpenoids, steroids, anthraquinones, carbohydrates and Flavonoids. The ethanolic extract induced significant antibacterial effects against S. aureus, S. pyogenes, P. aeruginosa and E. coli with MIC values of 0.85, 0.97, 0.43 and 0.76 µg/ml, respectively. Further, remarkable antifungal effects were induced by the ethanolic extract against C. albicans, A. clavatus and A. niger with MIC values of 0.30, 0.44 and 0.25 µg/ml, in comparison to that of reference control nystatin (MIC=0.001), respectively. Additionally, strong cytotoxic effects of ethanolic extract of Datura stramonium seeds against Hep-G2 liver carcinoma cells with an IC_{50} concentration of 50.95µg/ml. In conclusion, the ethanolic extract exhibited remarkable phytochemical content, antibacterial and antifungal properties against the selected pathogens. Moreover, the extract exhibited protective effects against liver cancer by inducing cytotoxic effects to Hep-G2 cells.

Keywords: *Medicinal plants, Datura stramonium, phytochemicals, antimicrobial, cytotoxicity*

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INTRODUCTION

Medicinal plants are a pool source of pharmacodynamic chemical entities possessing tremendous biological applications [1]. The plant based chemical entities (Phytochemicals) show additive and synergistic therapeutic efficacies against a number of metabolic distortions found in human beings [2]. Maximum of the pharmaceutical drugs have been developed based on either phytochemicals or phytochemicals themselves [3]. Phytochemicals serve as templates in manufacturing or designing many pharmaceutically active compounds. The development of phytochemical drugs and their implications to different metabolic distortions have cut the financial burden which was laid by the synthetic drugs. Phytochemical based drugs are cheaper, low toxic and minimum side-effects. According to a statement of WHO, 80% of the world population are still relying upon medicinal plants to fulfill their medicinal demands [4]. Further, medicinal plants have long served in traditional medicine systems like Ayurveda, Unani medicine and Traditional Chinese Medicine and folk medicine [5,6]. The different classes of natural products found in medicinal plants include terpenoids, flavonoids, alkaloids, saponins, steroids, coumarins, glycosides, etc [7,8]. All these classes of natural products show significant pharmacology including antioxidant, anti-inflammatory, antipyretic, antimicrobial, antiviral and anticancer effects [9]. Several natural products and drugs based on them have made it to clinical trials and some have been approved to use [10]. Over 70% of the drugs currently in clinical stage for chemotherapy are either natural products or derived from them.

Datura stramonium belongs to the class of medicinal plants and has been found with numerous biological activities [11]. The plant is an annual and is a member of family of *Solanaceae*. The plant grows up to 3-5 feet tall forming a bush like structure with several branches and erect physical appearance [12]. The plant origin lies in America but is found globally in abundance like Europe, Asia, America and Africa. The plant

has been recognized with significant biological activities both in the form of extracts and isolated bioactive constituents like antibacterial, anti-asthmatic, antiepileptic, antimicrobial, analgesic, antioxidant, insecticidal, organophosphate protective, repellent and anticancer effects [13-16]. Moreover, several classes of the phytochemicals have been identified and isolated from different parts of the plant like flowers, roots, leaves and seeds including proteins, carbohydrates, atropine, alkaloids, lignin, tannin, saponins, scopolamine, phenol, glycosides, sterols and fats [17,18].Hepatocellular carcinoma is a life threatening malignancy effects all sects of people globally. It has been ranked among top 10 cancers in accordance with the associated incidence and death rates [19]. Long time exposure to infectious diseases like Hepatitis B virus (HBV) and Hepatitis C virus (HCV) and increasing alcohol abuse remain the high rated risk factors for its development [20]. Organ transplantation, cutting of infected area, chemotherapy, radiotherapy and combination therapies are the primary treatment modalities in hepatocellular carcinoma [21]. Despite of the recent increase in awareness campaigns, and advancements made towards the treatment of hepatocellular carcinoma, the satisfactory five year survival rates have been not achieved yet. Therefore, there is a need for the search of novel methodologies and chemopreventive agents that could lead us with better outcome, improved survival, elimination of relapses, and lower side-effects.

Keeping in view the rich phytochemistry, medicinal value and bioactivity of *Datura stramonium* plant, we designed the present study to investigate the phytochemical composition of seeds, antimicrobial and *in vitro*cytotoxic activities.

MATERIALS AND METHODS

Collection of plant material

The plant material was collected from District Budgam, Jammu and Kashmir in the month of September, 2020. The plant was authenticated by the Department of Botany, Madhyanchal Professional University, Ratibad, Bhopal, Madhya Pradesh and a specimen was deposited there.

Extract preparation

The seeds of the plant were directly collected by the opening of seed capsule and placed in clean plastic bags. After collection the seeds were shade dried in open air for 4 weeks followed by crushing to powder. The 200 g of the powder was placed on refluxing with 70% ethanol for 72h. The solution was then filtered using Whatman's filter paper no. 1 to remove solid particles. The filtrate was then placed to rotatory evaporation for 20 min to discard the remaining solvent. The crude extract was dissolved in DMSO and different experimental concentrations were prepared.

Qualitative phytochemical screening

Amino acids:The 2 ml of seed extract were added with Ninhydrin reagent (0.2%) followed by 5min of heating. The appearance of blue color confirms presence of amino acids.

Saponins: The 2 ml of the seed extract were mixed thoroughly with 2 ml of distilled water within a graduated cylinder. The formation of foam/froth confirms the presence of saponins.

Proteins:Crude extract of 0.5 mg was added to a solution of equal volumes of 1% copper sulphate and 40% NaOH. Violet color confirms proteins.

Tannins:To the 1 ml extract was added 2 ml of 5% ferric chloride. The greenish black or dark blue color confirms tannins.

Starch:5 ml of the extract was boiled with 5% KOH (1 ml) for some time followed by the addition of sulphuric acid. Appearance of yellow coloration confirms starch.

Alkaloids:2 ml of conc. HCL were added to the 2 ml of extract followed by the addition of Mayer's reagent (few drops). White precipitate or green coloration confirms alkaloids.

Terpenoids:1 ml of extract was placed in ethanol followed by the addition of 1 ml acetic acid. To this solution was added conc. sulphuric acid and the appearance of color from pink to violet confirms terpenoids.

Reducing sugars:1 g of the extract was dissolved in distilled water and then filtered. The filitrate was gentally heated with 5 ml of Fehling's solution (equal volumes of A and B). Formation of red precipitate confirms reducing sugars.

Steroids:About 0.5 g of the extract was added with 2 ml of acetic acid followed by cooling in a cold ice bath. Then conc. sulphuric acid was added to the solution and appearance of coloration from violet to bluish-green or blue confirms steroids.

Anthraquinones:10 ml of benzene were added to 0.5 g of the extract followed by filtration and addition of 5 ml of 10% ammonia. This mixture was vigorously shaken and appearance of pink, red or violet color confirms anthraquinones.

Glycosides:1 ml of water was added to 0.5 mg of the seed extract followed by addition of NaOH solution. Yellow color appearance confirms glycosides.

Carbohydrates:0.5 mg of the seed extract was diluted in water and then added with few drops Molisch reagent. Then this solution was added with 1 ml conc. sulphuric acid followed by standing upto 2 min. The formation of red color or dull violet color after addition of 5 ml distilled water to the solution confirms carbohydrates.

Flavonoids:Boiling of about 0.5 mg of seed extract was done in distilled water followed by filtration and then addition of few drops of 10% $FeCl_3$. The appearance of violet or greenish-blue confirms flavonoids.

Disc-diffusion technique

To evaluate the effects of ethanolic extract of *Datura stramonium* seeds on selected bacterial and fungal strains, we performed disk diffusion analysis. In brief, several concentrations of ethanolic extract of *Datura stramonium* seeds were prepared using DMSO viz 0, 20, 40, 80 and 160 µg/ml. These test extract concentrations were stored till use. The bacterial strains (*S. aureus, S. pyogenes, P. aeruginosa and E. coli)* and fungal strains (*C. albicans, A. clavatus and A. niger*) with a concentration of 10^4 CFU/mL were arranged over Mueller-Hinton agar using a sterile cotton bud, separately. Then, the discs of 6 mm diameter were cleaned and sterilized at 110°C for 10 min. Afterwards, each extract concentration was loaded to these discs. Ciprofloxin and Ampicilin, and Nystatin were used as reference control in antibacterial and antifungal analysis, respectively. The culture bearing plates were placed in close contact of sterilized and dried discs. Each area was labeled followed by 48 h of incubation. The experimental procedures for individual extract concentrations were replicated in triplicates. The minimum inhibition concentration (MIC) was that concentration at which no evident bacterial or fungal growth is seen.

Cytotoxic assay

Cell culture and conditions

The hepatocellular carcinoma Hep-G2 cell line was obtained from American Type Culture Collection; ATCC (Manassas, VA, United States). The cells were plated in RMPI-1640 medium (Sigma-Aldrich) carrying 10% fetal bovine serum (Gibco). Streptomycin and penicillin were antibiotics to prevent growth of pathogens. These cells were maintained in a humid environment bearing 37°C of temperature and 5% CO_2.

MTS assay

To study the cytotoxic effects of ethanolic extract of *Datura stramonium* seeds, we used 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium inner salt (MTS) assay. In brief, Hep-G2 cells were plated with different concentrations of ethanolic extract viz 0, 20, 40, 80 and 160 µg/ml within 96-well plates containing 3×10^4 cells/well. After culturing for 48 h at 37°C, cells were added with MTS solution(Promega, Madison, WI, United States) followed by further 3 h of incubation. Thereafter, the viable cell percentage was recorded by measuring absorbance with the help of an ELISA microplate reader (Awareness, Palm City, FL, United States) at 490 nm. IC_{50} concentration was also determined and each experimental concentration was experimented in triplicates.

Statistical analysis

The experimental procedures for individual extract concentrations were replicated in triplicates and data was illustrated as mean ± SD. One way ANOVA was implemented for determination of statistical analysis followed by Student's t-test. The p values of lower than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Phytochemical composition of ethanolic extract of *Datura stramonium* seeds

Medicinal plants are considered as rich source of bioactive phytochemicals and several drugs serving in modern medicine have been either isolated or based on phytochemicals present in medicinal plants [22]. The *Datura stramonium* belongs to the group of medicinal plants identified with rich phytochemistry. In this study, we found rich phytochemical content in *Datura stramonium* with the presence of bioactive classes of natural products. The list of phytochemicals tested and present in ethanolic extract of*Datura stramonium*seeds are listed in **Table 1**.

Antibacterial activity of ethanolic extract of *Datura stramonium* seeds

Pathogens like bacteria are both beneficial as well as infectious in nature. Infectious bacteria are responsible for triggering of a wide spectrum of health conditions including mild, moderate and acute [23]. To treat the bacterial infections in human beings is a great challenge because bacteria have occupied almost every habitat on earth. Therefore, it is challenging to develop efficient drugs to treat bacterial infections. Despite, huge number of antibiotics has been approved for use but still the development of drug-resistance leads to their failure and lower efficacy [24]. Medicinal plants have been used since early times to treat bacterial infections in human beings. The plant *Datura stramonium* is a medicinally active and phytochemicals rich species. It has been previously shown to possess remarkable antibacterial activities [15].

Herein, we tested the ethanolic seed extract of *Datura stramonium* against four bacterial strains including *S. aureus, S. pyogenes, P. aeruginosa and E. coli*. The standard reference drugs used were ciprofloxin and

ampicillin. The various concentration of the ethanolic extract tested were 0, 20, 40, 80, 160 µg/ml. Results demonstrated that ethanolic extract inhibition the bacterial growth significantly against all the four selected bacteria strains. Against the *S. aureus*,*S. pyogenes*and *E. coli*, the ethanolic extract showed inhibition zones of 8.14 mm, 10.39 mm, 13.94 mm and 19.21 mm, 7.21 mm, 9.58 mm, 14.23 mm and 17.69 mm, and 9 mm, 11.87 mm, 16.76 mm and 21.34 mm, respectively. The highest inhibition was recorded against the *P. aeruginosa* bacteria with inhibition zones of 10.3 mm, 12.92, mm, 17.64 mm and 22.12 mm (**Table 2**). The inhibition zones in case of reference controls were;20.22 mm, 23.31 mm, 25.15 mm and 27.33 mm, 19.69 mm, 21.42 mm, 23.73 mm and 25.67 mm, 21.73 mm, 24.33 mm, 27.97 mm and 30.12 mm, 21.19 mm, 23.99 mm, 26.73 mm and 29.44 mm, in ciprofolxin treated groups while ampicillin treated group showed 11.77 mm, 13.93 mm, 15.70 mm and 18.52, 13.45 mm, 15.22 mm, 17.99 mm and 19.21 mm, 16.27, 18.87, 21.63 and 23.6, and 15.45, 17.95, 20.47 and 22.47, respectively (**Table 3**). The efficient antibacterial activity of the ethanolic extract of *Datura stramonium* seeds was recorded against *P. aeruginosa* with an MIC value of 0.43 µg/ml.

Table 1: The tests performed and phytochemicals present in ethanolic extract of *Datura stramonium* seeds.

Phytochemicals present in ethanolic extract of *Datura stramonium* seeds			
S.No.	**Phytochemicals**	**Test**	**Presence (+)/Absence (-)**
1.	Amino acids	Ninhydrin test	**(+)**
2.	Saponins	Frothing test	**(+)**
3.	Proteins	Biuret test	**(+)**
4.	Tannins	Ferric chloride test	**(+)**
5.	Starch	Potassium hydroxide and Sulphuric acid	**(-)**
6.	Alkaloids	Mayer's reagent	**(+)**
7.	Terpenoids	Acetic anhydride and Sulphuric acid	**(+)**
8.	Reducing sugars	Fehling's test	**(-)**
9.	Steroids	Liebermann-Burchard test	**(+)**
10.	Anthraquinones	Borntrager's test	**(+)**
11.	Glycosides	Sodium hydroxide test	**(-)**
12.	Carbohydrates	Molisch's test	**(+)**
13.	Flavonoids	Ferric chloride test	**(+)**

Table 2: Antibacterial activity and MIC values of ethanolic extract of *Datura stramonium* seeds against selected bacteria.

S.No.	**Concentration (µg/ml)**	**Antibacterial effects of ethanolic extract of Datura stramonium seeds**			
		Inhibition zone (mm)			
		S. aureus	***S. pyogenes***	***P. aeruginosa***	***E. coli***
1.	**0**	-	-	-	-
2.	**20**	8.14	7.21	10.3	9
3.	**40**	10.39	9.58	12.92	11.87
4.	**80**	13.94	14.23	17.64	16.76
5.	**160**	19.21	17.69	22.12	21.34
MIC values		**0.85**	**0.97**	**0.43**	**0.76**

Table 3: Antibacterial activity and MIC values of reference against selected bacterial strains

Drug	**Concentration (µg/ml)**	**Antifungal activity of reference controls**			
		Inhibition zone (mm)			
		S. aureus	***S. pyogenes***	***P. aeruginosa***	***E. coli***
Ciprofloxin	**0**	-	-	-	-
	20	20.22	19.69	21.73	21.19
	40	23.31	21.42	24.33	23.99
	80	25.15	23.73	27.97	26.73
	160	27.33	25.67	30.12	29.44
MIC value		**0.007**	**0.002**	**0.097**	**0.019**
Ampicillin	**0**	-	-	-	-
	20	11.77	13.45	16.27	15.45
	40	13.93	15.22	18.87	17.95
	80	15.70	17.99	21.63	20.47
	160	18.52	19.21	23.6	22.19
MIC values		**0.005**	**0.0093**	**0.01**	**0.0023**

Table 4: Antifungal activity and MIC values of ethanolic extract of *Datura stramonium* seeds against selected fungal strains.

S.No.	Concentration (µg/ml)	Antifungal effects of ethanolic extract of *Datura stramonium* seeds		
		Inhibition-zone (mm)		
		C. albicans	*A. clavatus*	*A. niger*
1.	0	-	-	-
2.	20	25.61	14.23	27.19
3.	40	32.67	27.11	44.83
4.	80	55.94	46.66	61.21
5.	160	61.34	57.33	72.21
MIC values		0.30	0.44	0.05

Table 5: Antifungal activity and MIC values of reference control against selected fungal strains.

S.No.	Concentration (µg/ml)	Antifungal activity of reference control Nystatin		
		Inhibition-zone (mm)		
		C. albicans	*A. clavatus*	*A. niger*
1.	0	-	-	-
2.	20	40.12	37.69	43.14
3.	40	51.70	49.31	56.71
4.	80	69.32	63.32	79.33
5.	160	87.22	75.53	91.3
MIC values		0.12	0.09	0.01

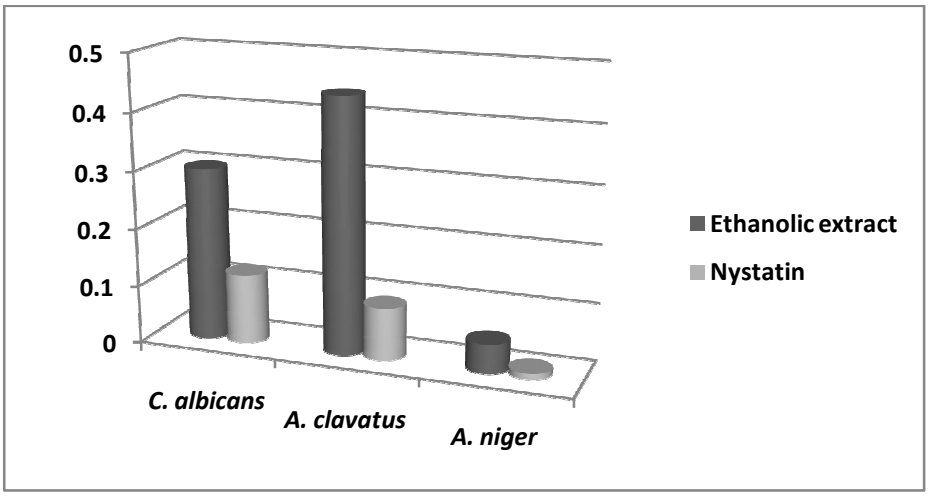

Figure 1. Relative MIC values for antifungal potential of the ethanolic extract and reference control.

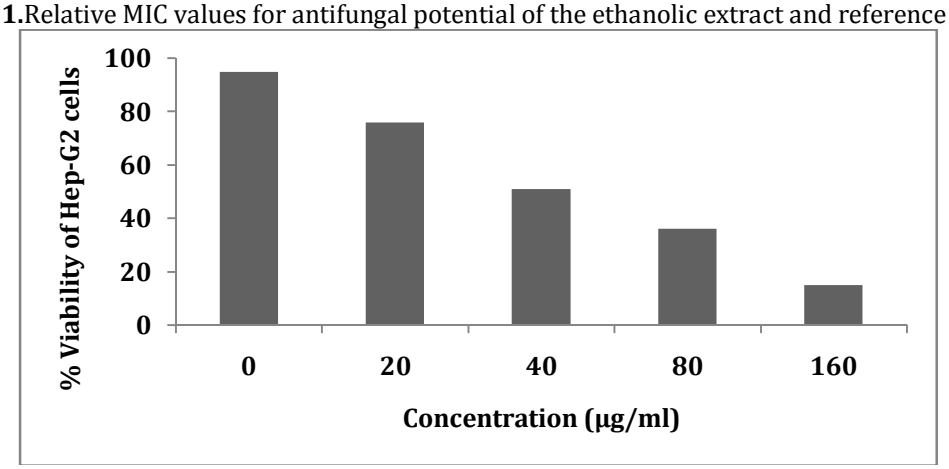

Figure 2. Cytotoxic activity of ethanolic extract of *Datura stramonium* seeds against Hep-G2 cells. The experimental procedures for individual extract concentrations were replicated in triplicates and data was illustrated as mean ± SD.

Antifungal activity of ethanolic extract of *Datura stramonium* seeds

Like bacteria, several types of fungi are harmful to human health. These cause fungal infections in human body ranging from mild to severe [25]. Fungi are often ingested through food or grow on external soft and skin tissues. There are numerous antiseptic ointments and medicine available to treat fungal infections but the resistance and severity caused by these infections generate a need for medication with lower side-effects and higher efficacy. Medicinal plants have been recognized with tremendous potential to act against fungal infections in human beings and are a part of different traditional and folk medicine systems to treat fungal infections, globally [26]. The significant antifungal activity of medicinal plant has been attributed to their bioactive constituents.

Herein, we investigated the antifungal activity of ethanolic extract of *Datura stramonium* seeds using disc-diffusion method. The fungal strains used were *C. albicans, A. clavatus and A. niger*. These selected fungal strains were exposed to different ethanolic extract concentrations viz 0. 20, 40, 80 and 160 µg/ml. The nystatin drug was used as reference control. Results showed that ethanolic extract of *Datura stramonium* seeds induced concentration dependent antifungal effects against the selected fungal strains. The inhibition zones in ethanolic extract treated *C. albicans, A. clavatus and A. niger*, were 25.61 mm, 32.67 mm, 55.94 mm and 61.34 mm, 14.23 mm, 27.11 mm, 46.66 mm and 57.33 mm, and 27.19 mm, 44.83 mm, 61.21 mm and 72.21 mm, respectively (**Table 4**). In case of the reference control, the inhibition zones were 40.12 mm, 51.70 mm, 69.32 mm and 87.22 mm, 37.69 mm, 49.31 mm, 63.32 mm and 75.53 mm, and 43.14 mm, 56.71 mm, 79.33 mm and 91.3 mm, respectively (**Table 5**). The MIC value of ethanolic extract was efficient and comparable to that of reference control against *Aniger* (**Figure 1**).

Cytotoxic activity of ethanolic extract of *Datura stramonium* seeds

Medicinal plants serve as a pool of chemical entities that are potential drugs or serve as templates for future drugs. The currently used major chemotherapeutic drugs like taxol and vinblastine are the molecular isolations from medicinal plants [27]. In a few studies, *Datura stramonium* has been shown with significant cytotoxic activities [28]. Herein, the ethanolic extract of *Datura stramonium* seeds was examined for its cytotoxic potential against Hep-G2 cells using MTS assay. The Hep-G2 cells were treated with different seed extract concentrations viz 0, 20, 40, 80 and 160 µg/ml for 48h. Results showed that the ethanolic extract of *Datura stramonium* seeds induced concentration-dependent cytotoxicity to Hep-G2 cells (**Figure 2**). The viability of Hep-G2 significantly reduced from 95% to 76% after exposing them to 20 µg/ml of extract concentration. On further increasing the extract concentrations, it was observed that the ethanolic extract reduced viability to 15% at 160 µg/ml. The IC_{50} value of 50.95 µg/ml was obtained for the cytotoxicity of ethanolic extract of *Datura stramonium* seedsagainst the Hep-G2 cells. Therefore, these results show tremendous cytotoxic potential of *Datura stramonium* seeds against the hepatocellular cancer cells.

CONCLUSION

The outcomes of the present research investigation demonstrate the presence of bioactive phytochemicals in the ethanolic extract of *Datura stramonium* seeds. The ethanolic extract showed significant antibacterial and antifungal activities nonetheless higher antibacterial efficacy with low MIC was recorded. Moreover, the ethanolic seed extract of *Datura stramonium* showed strong cytotoxic effects against the Hep-G2 liver cancer cells. Therefore, this study could prove a platform for natural products researchers to guide them for future studies, isolations, drug design and drug discovery.

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CONFLICT OF INTEREST

None to declare

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