RESEARCH ANNALS OF XAVIERS AHMEDABAD

A Multidisciplinary Journal

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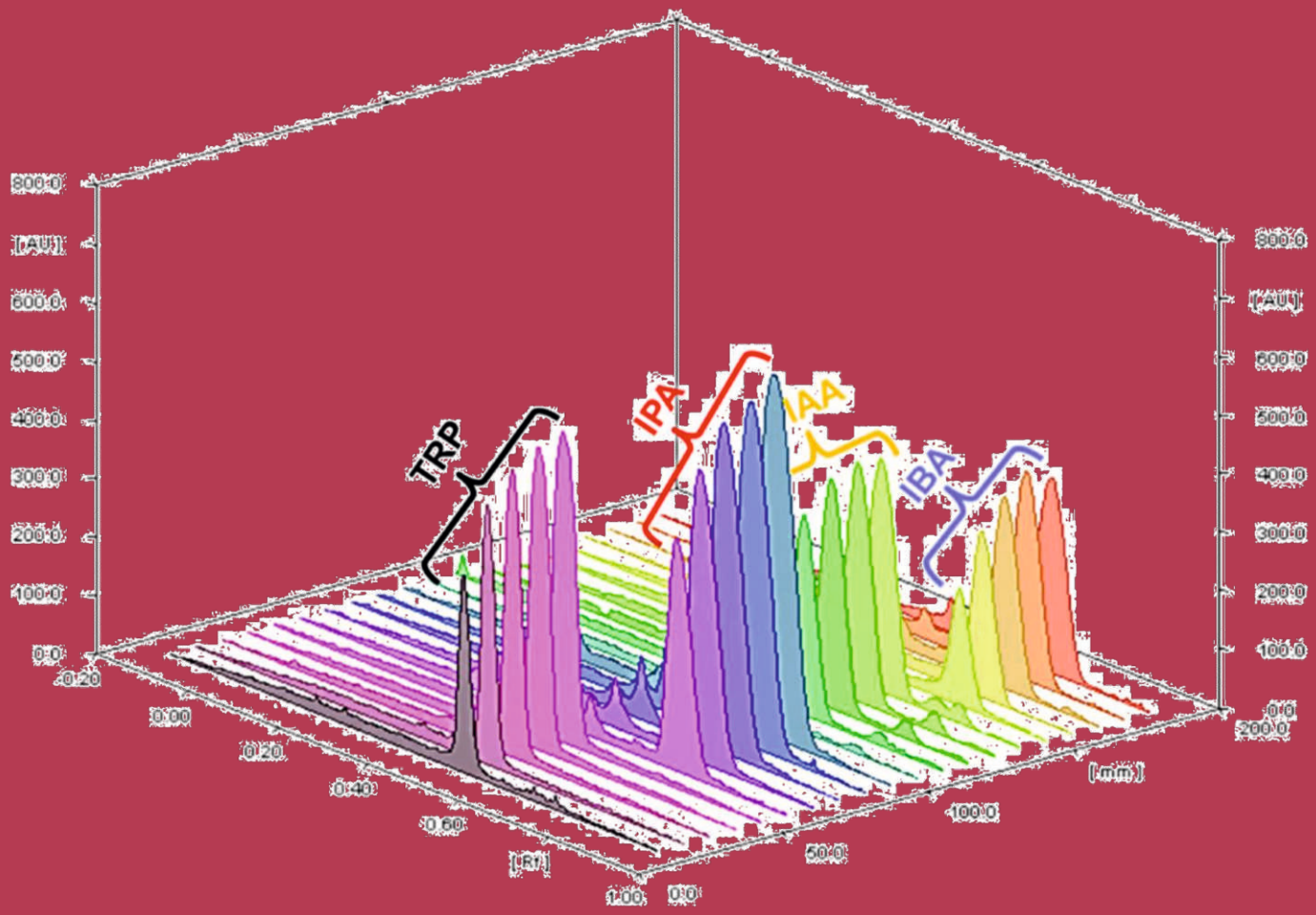

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RESEARCH ANNALS OF XAVIERS AHMEDABAD

A Multidisciplinary Journal

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Cover Page :
HPTLC 3D densitogram representing the standard curve of indolic-auxins

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Dear Readers and Colleagues,

This is a momentous occasion and a milestone in the journey that our institution has undertaken towards a path of excellence in the field of education. The College, in its 63rd year, has embarked on one more sojourn with its research wing taking flight with its new found wings heralding promise of quality research output. Let us all celebrate together the first issue of the Research Annals of Xaviers, Ahmedabad (RAXA). On behalf of RAXA, the Editorial Board and the Editorial Team of our journal, I would like to extend our gratitude to all authors, patrons and readers for enabling this.

Research has become an inherent component of today's education system as it is an experiential learning. We, at Xavier's, infused this research culture as early as the 1980s, with undergraduate research programme being offered by a few departments, and that now has burgeoned into every department offering select students to undertake a one year research project under the able guidance of our faculty as well as eminent scientists of other institutions, as collaborative partners. The outcome of this integration of research into curriculum has been very positive and some of the students have even got publications in reviewed journals. The evaluation of this programme has shown that the programme has enabled to build the capacity of students to perform better not only in their subjects of specialization but also in developing the personality of students.

The faculty has always been motivated to pursue research and annually, we have been conducting Staff Research Seminar. On this occasion, Research Annals of Xaviers Ahmedabad, from the year 2003 has been collated into a book. This practice now gets an exalted status by becoming a peer reviewed publication with an ISSN number. And this move also entails that we have the responsibility of doing quality research.

We look forward to contributions from all subjects as RAXA is a multidisciplinary journal. 2018 has marked the hiatus to a new status, which we will need to maintain. It will be our commitment and continuous effort to strengthen our publications and we seek the same vision from all of you. We wish and hope to see that gradually we will get contributions from many parts of the world. That is our dream and you can help us fulfil this dream.

Looking forward to a productive, challenging and successful year ahead.

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Paper should be typed in MS Word. Title should be followed by First name Last name
Abstract should not be more than 200 words
The paper should have an introduction, methodology, result, discussion and conclusion. It should be followed by conflict of interest and acknowledgement.

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Journal article (Format APA must be followed)

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South, J., & Blass, B. (2001). The future of modern genomics.

Book chapter

Patel, K., Goswami, D., Dhandhukia, P., & Thakker, J. (2015). Techniques to study microbial phytohormones. In *Bacterial metabolites in sustainable agroecosystem* (pp. 1-27). Springer, Cham

Online document

Cartwright, J., (2007) Big stars have weather too. IOP Publishing PhysicsWeb.
http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

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ARTICLE RESEARCH ANNALS OF XAVIERS AHMEDABAD

Overcoming interference of plasma phospholipids using Hybrid SPE for the determination of trimetazidine by UPLC-MS/MS

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Pravin G. Vanol [a] and Mallika Sanyal[a*]

Abstract: An improved, precise and reliable ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method has been developed for the quantification of trimetazidine, using trimetazidine-d8 as the internal standard (IS). Interference due to plasma phospholipids during sample preparation was overcome by using hybrid solid phase extraction-phospholipid ultra cartridge. The mean extraction recovery of trimetazidine (98.66 %) and trimetazidine-d8 (97.63 %) from spiked plasma was consistent and reproducible. Chromatographic analysis was performed on UPLC Ethylene Bridged Hybrid (BEH) C18 (50 × 2.1 mm, 1.7 µm) column with isocratic elution using acetonitrile-5 mM ammonium formate, pH 3.5 (40:60, v/v) as the mobile phase. The parent to product ion transitions for trimetazidine (m/z 267.1→181.1) and trimetazidine-d8 (m/z 275.2→181.1) were monitored on a triple quadrupole mass spectrometer with electrospray ionization functioning in the positive multiple reaction monitoring mode. The linearity of the method was established in the concentration range of 0.05-100 ng/mL for trimetazidine. The intra-batch and inter-batch accuracy and precision (% CV) ranged from 97.3-103.1 % and 1.7-5.3 %, respectively. Qualitative and quantitative assessment of matrix effect showed no interference of endogenous/exogenous components. The developed method was used to measure plasma trimetazidine concentration for a bioequivalence study with 12 healthy subjects.

Keywords: Trimetazidine; HybridSPE; Ultra-performance liquid chromatography-tandem mass spectrometry; Phospholipids; Bioequivalence; Human plasma

Introduction

Trimetazidine (TMZ), which displays anti-ischemic and cardioprotective effects, is a clinically effective anti-anginal agent. The efficacy of TMZ has been mainly attributed to its selective and specific fatty acid oxidation inhibition, metabolic effects and lack of haemodynamic effects in stable angina pectoris (Marzilli, 2003). It has been demonstrated that TMZ modulates energy metabolism via inhibitory effects on fatty acid oxidation and favours glucose oxidation due to its mitochondrial long chain 3-ketoacyl-coenzyme A thiolase inhibition (3KAT) (Chierchia, 2001; Kantor et. al., 2000; McCullough, 2005). As a consequence of this inhibition, patients treated with TMZ showed improvement in left ventricle dysfunction due to reduced demand for oxygen when glucose is utilized instead of free fatty acids (Napoli and Taccardi, 2009; Sigmund et. al., 2014). TMZ is more than 95 % non-ionized at physiological pH, permitting the drug to pass through lipoprotein membranes (Oulsnam et. al., 1984). Plasma protein binding is low and the volume of distribution is 320 L. In humans, it is rapidly and completely absorbed from the gastrointestinal tract. Four pathways of metabolism are known but metabolism is not extensive, with 51 % of unchanged drug eliminated in urine. Elimination is rapid (t1/2, 6h) and predominantly renal. No kinetic interaction is found with theophylline, digoxin or antipyrine and food intake does not modify TMZ (Edeki et. al., 1988).

Several methods are reported for determination of TMZ in different biological samples such as human plasma (de Jager et. al., 2001; Ding et. al., 2007; Grabowski et. al., 2012; Helmy and Mansoor, 2014; Jiao et. al., 2007; Khedr et. al., 2007; Liu et. al., 2012; Lv et. al., 2013; Medvedovici et. al., 2005; Mistri et. al., 2008; Ozbay et. al., 2012; Wang et. al., 2007; Zhang et. al., 2010; Zhou et. al., 2010; Zou et. al., 2008), human serum (Chowdhury et. al., 2011), rat plasma (Xiong et. al., 2011; Xiong and Yang, 2015), pig and dog plasma (Genissel et. al., 2004). Mainly, high performance liquid chromatography (HPLC) with fluorescence (Khedr et. al., 2007), electrochemical (Grabowski et. al., 2012) and UV (Chowdhury et. al., 2011; Helmy and Mansoor, 2014; Lv et. al., 2013) detection, liquid chromatography-mass spectrometry (LC-MS).

(de Jager et. al., 2001; Ding et. al., 2007; Jiao et. al., 2007; Ozbay et. al., 2012; Wang et. al., 2007) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Liu et. al., 2012; Medvedovici at.al., 2005; Mistri at.al., 2008; Xiong et.al., 2011; Xiong and Yang, 2015; Zhou et.al., 2010; Zou et.al., 2008) have been the preferred techniques for quantification of TMZ in these matrices. Besides, few other

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reports present use of gas chromatography with nitrogen-phosphorous (Barre et.al., 2003) and mass detector (Fay et. al., 1989). The HPLC based methods had the limitations of either long analysis time (Grabowski et. al., 2012; Helmy and Mansoor, 2014; Khedr et. al., 2007; Lv et. al., 2013), large sample volume for processing (Grabowski et. al., 2012; Khedr et. al., 2007; Lv et. al., 2013) or low sensitivity (Chowdhury et. al., 2011; Grabowski et. al., 2012; Helmy and Mansoor, 2014; Khedr et. al., 2007; Lv et. al., 2013). Methods with MS detection have achieved much higher sensitivity ranging from 0.1-2.5 ng/mL for TMZ in human plasma. A detailed summary of liquid chromatographic methods developed for TMZ in different biological matrices is given in Table 1.

An ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) has been reported for sensitive and rapid determination of TMZ in human plasma (Zhang et. al., 2010). The objective of the present work was to develop and optimize a rugged UPLC-MS/MS method with improved sensitivity and better performance compared to all existing methods for TMZ. Due to significant interference of plasma phospholipids with all conventional extraction procedures, hybrid solid phase extraction (SPE) was employed for almost complete removal of phospholipids. This approach provided a simple and faster solution to efficient sample clean-up. The method was successfully applied to a bioequivalence study with 12 healthy Indian males using 35 mg TMZ tablet formulation.

Experimental

Chemicals and materials

Reference standards of trimetazidine (TMZ, 98.56 %) and trimetazidine-d8 (TMZ-d8, 99.74 %) used as internal standard (IS) was procured from Clearsynth Labs (P) Ltd. (Mumbai, India). High performance liquid chromatography (HPLC) grade methanol and acetonitrile were procured from Mallinckrodt Baker, S.A.de C.V. (Estado de Mexico, Mexico). Ammonium formate and formic acid were obtained from Merck Specialties Pvt. Ltd., (Mumbai, India). Supelco HybridSPE®-Phospholipid ultra cartridges (1 cc, 30 mg) were from Sigma-Aldrich Co. (Bangalore, India). Water used in the entire analysis was prepared from Milli-Q A10 gradient water purification system procured from Millipore (Bangalore, India). Blank human plasma was obtained from Supratech Micropath (Ahmedabad, India) and was stored at –70°C until use.

Liquid chromatographic and mass spectrometric conditions

Acquity UPLC system from Waters Corporation (Milford, Massachusetts, USA) consisting of binary solvent manager, sample manager and column manager was used for reversed-phase liquid chromatographic conditions. The analysis of TMZ and TMZ-d8 was performed on Acquity UPLC Ethylene Bridged Hybrid (BEH) C18 (50 × 2.1 mm, 1.7 µm) column from Waters Corporation (Milford, Massachusetts, USA), which was maintained at 40°C with an alarm band of ±5ºC in the column oven. For isocratic elution of TMZ and TMZ-d8, a mobile phase consisting of acetonitrile-5 mM ammonium formate, pH 3.5 adjusted with 0.1% formic acid (40:60, v/v) was used and delivered at a constant flow rate of 0.300 mL/min. The pressure of the system was 6000 psi.

Ionization and detection of TMZ and TMZ-d8 was carried out on a Quattro Premier XE mass spectrometer from Waters Corporation (Milford, Massachusetts, USA), equipped with ion spray interface and operating in positive ionization mode. Quantitation was performed using multiple reaction monitoring (MRM) mode to monitor precursor to product ion transitions at m/z 267.1 to 181.1 for TMZ and m/z 275.2 to 181.1 for TMZ-d8. The optimized mass parameters were, capillary volts: 4.00 kV; extractor volts: 4.00V; source temperature: 100ºC; desolvation temperature: 400ºC; cone gas flow: 100L/h; desolvation gas flow: 800 L/h. The compound dependent parameters like cone potential and collision energy were set at 22.0V and 15.0eV for TMZ and 22.0V and 18.0eV for IS, respectively. Quadrupole 1 and 3 were maintained at unit mass resolution. The dwell time was kept at 100ms for TMZ and TMZ-d8. Data collection, peak integration and all calculations were performed using Mass Lynx software version 4.1 from Waters Corporation (Milford, Massachusetts, USA).

Standard stock, calibration standards and quality control sample preparation

The standard stock solution of TMZ (1.0 mg/mL) was prepared by dissolving its requisite amount in methanol. Calibration standards (CSs) and quality control (QC) samples were prepared by spiking blank plasma with stock solution. CSs were made at 0.05, 0.10, 0.50, 1.00, 2.00, 5.00, 10.0, 20.0, 50.0 and 100 ng/mL concentrations while QC samples were prepared at 80.0 ng/mL (HQC, high quality control), 40.0/2.50 ng/mL (MQC-1/2, medium quality control), 0.15 ng/mL (LQC, low quality control) and 0.05 ng/mL (LLOQ, lower limit of quantification). Stock solution (200 µg/mL) of the IS was prepared by dissolving 2.0 mg of TMZ-d8 in 10.0 mL of methanol. Its working solution (1.0µg/mL) was prepared by appropriate dilution of the stock solution in methanol. Standard stock and working solutions used for spiking plasma samples were stored at 5°C, while CSs and QC samples in plasma were kept at -70°C until use.

Sample extraction procedure

Prior to analysis, all frozen subject samples, CSs and QC samples were thawed and allowed to equilibrate at room temperature for 30 min. To an aliquot of 100µL of spiked plasma/subject sample, 50µL of IS solution and 300µL of 1.0 % ammonium formate in methanol was added and vortex mixed for 2.0 min. Thereafter the samples were centrifuged at 3200 g for 5 min and were applied to HybridSPE-phospholipid extraction cartridges. The analyte and IS were collected in pre-labelled vials by apply positive pressure for 4.0 min and 5 µL was used for injection in the chromatographic system.

Bioanalytical method validation

Method validation was performed following the US Food and Drug Administration (FDA) guideline (FDA, 2001) and the procedures were similar to our previous work (Shah and Shrivastav, 2017; Sharma et. al., 2015).

Bioequivalence study

The design of the study comprised of "An open label, balanced, randomized, two treatment, two period, two sequence, single dose, two way crossover bioequivalence study of test formulations of trimetazidine dihydrochloride (35 mg modified release tablets from a Generic company, India) and a reference formulation 'Vastarel' (35 mg modified release tablets from Les Laboratories Servier, France) in 12 healthy adult subjects under fasting". Each subject was judged to be in good health through medical history, physical examination and routine laboratory tests. Written consent was taken from all the subjects after informing them about the objectives and possible risks involved in the study. An independent ethics committee constituted as per Indian Council of Medical Research approved the study protocol. The study was conducted strictly in accordance with guidelines laid down by International Conference on Harmonization (ICH), FDA (FDA, 1996) and European Medicines Agency (EMA) (EMA, 2011). The subjects were orally administered a single dose of test and reference formulations with 240 mL of water after recommended wash out period of 5 days. Blood samples were collected at 0.0 (pre-dose), 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 7.00, 8.00, 10.0, 12.0, 14.0, 16.0, 24.0 and 36.0 h after oral administration of the dose for test and reference formulation in labelled K_3EDTA-vacuettes. Plasma was separated by centrifugation and kept frozen at -70 °C until analysis. During study, subjects had a standard diet while water intake was unmonitored. The pharmacokinetic parameters of TMZ were estimated by non-compartmental model using WinNonlin software version 5.2.1 (Certara, Princeton, NJ 08540, USA). The primary target variables of the study were maximum plasma concentration (Cmax), area under the plasma concentration-time curve from zero hour to 36 h (AUC0–36h), and area under the plasma concentration-time curve from zero hour to infinity (AUC0-inf), which were analysed using the confidence interval (CI) approach. The secondary end points of the study included time point of maximum plasma concentration (Tmax), half life of drug elimination during the terminal phase (t1/2) and elimination rate constant (Kel). To determine whether the test and reference formulations were pharmacokinetically equivalent, Cmax, AUC0–36h, and AUC0-inf and their ratios (test/reference) were assessed using log transformed data; their means and 90 % CIs were analysed by using SAS® software version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The drug formulations were considered pharmacokinetically equivalent if the difference between the compared parameters was statistically non-significant ($P \geq 0.05$) and the 90 % CIs for these parameters were within 80 to 125 %.

To test the accuracy of incurred samples, 10% of total subject samples (58 samples) which included samples near the Cmax and the elimination phase in the pharmacokinetic profile of TMZ were reanalysed as reported previously (European Medicines Agency, 2011; Yadav and Shrivastav, 2011). The acceptance criterion requires that the concentration obtained for the initial analysis and the concentration obtained by reanalysis should be within 20% of their mean for at least 67% of the repeats.

Results and discussion

Method development

Sample preparation is a key step in analyzing small molecules from biological fluids due to the presence of endogenous matrix components. Literature presents variety of extraction procedures for TMZ which includes protein precipitation (PP) (Jiao et. al., 2007; Medvedovici et. al., 2005; Xiong et. al., 2011; Zhou et. al., 2010; Zou et. al., 2008), hollow fiber-based liquid phase microextraction (Lv et. al., 2013), LLE (Chowdhury et. al., 2011; Ding et. al., 2007; Grabowski et. al., 2012; Helmy and Mansoor, 2014; Ozbay et. al., 2012; Wang et. al., 2007; Zhang et. al., 2010;), LLE followed by precolumn derivatization (de Jager et. al., 2001; Khedr et. al., 2007), salting-out-assisted LLE (Xiong and Yang, 2015) and SPE (Mistri et. al., 2008). Majority of the methods have utilized LLE for selective extraction of TMZ from human plasma, however, the recovery obtained using different solvent systems was in the range of 57-75 %. In one of our previous work, SPE on DVB-HL cartridge gave highly precise and quantitative recovery 97.36 %. However, during our initial trials with LLE based on previous reports there were significant issues related to matrix effect. LLE under alkaline conditions was tried using diethyl ether, methyl-tert butyl ether, dichloromethane, n-hexane and ethyl acetate as extracting solvents along with their combinations. The recovery obtained in all these solvent systems was poor (22-57 %) and inconsistent with considerable interference due to plasma phospholipids. Extracts obtained from LLE with ethyl acetate showed an interfering peak near the retention time of TMZ (1.08 min) at 1.12 min, which corresponded to lysophosphatidylcholine-1 (496.0/184.0), while lysophosphatidylcholine-2 (524.0/184.0) eluted subsequently at 1.38 min (Fig. 1a). During these trials chromatography was performed on Hypersil Hypurity C18 (50 × 4.6 mm, 5µm) column based on our previous work using 2.0 mM ammonium acetate (pH 3.5)-acetonitrile (40:60, v/v) as the mobile phase (Mistri et. al., 2008). Similar results were obtained with other solvent systems where TMZ and the phospholipids co-eluted within 1.0-2.0 min. Changing the mobile composition to 20:80 (v/v) resulted in early elution of TMZ and the phospholipids with no separation (Fig. 1b). Thus, SPE was tried on Oasis HLB (1cc, 30 mg) cartridges which provided much improved performance compared to LLE by separating bulk of the residual phospholipids; however, it was difficult to remove their interference completely (Fig. 1c). Lysophosphatidylcholine-1 eluted ahead of TMZ peak but lysophosphatidylcholine-2 which had greater retention could interference during subsequent injections. These results on

conventional extraction techniques prompted us to recreate the extraction and chromatography to eliminate their interference. Moreover, the analyte response was also not adequate to achieve the desired sensitivity. Subsequently, to overcome the interference of phospholipids, hybrid SPE cartridges were used which completely remove proteins as well as phospholipids from the biological sample. This hybrid SPE-phospholipid technology involves a simple PP step together with a fast and robust SPE that is customized to remove phospholipids. The phosphate moiety of phospholipids is a strong Lewis base that interacts with the zirconium atoms coated on the silica surface and is retained on the cartridge. This procedure helped in completely removing the interference of phospholipids and provided highly consistent recovery of TMZ across all QC levels.

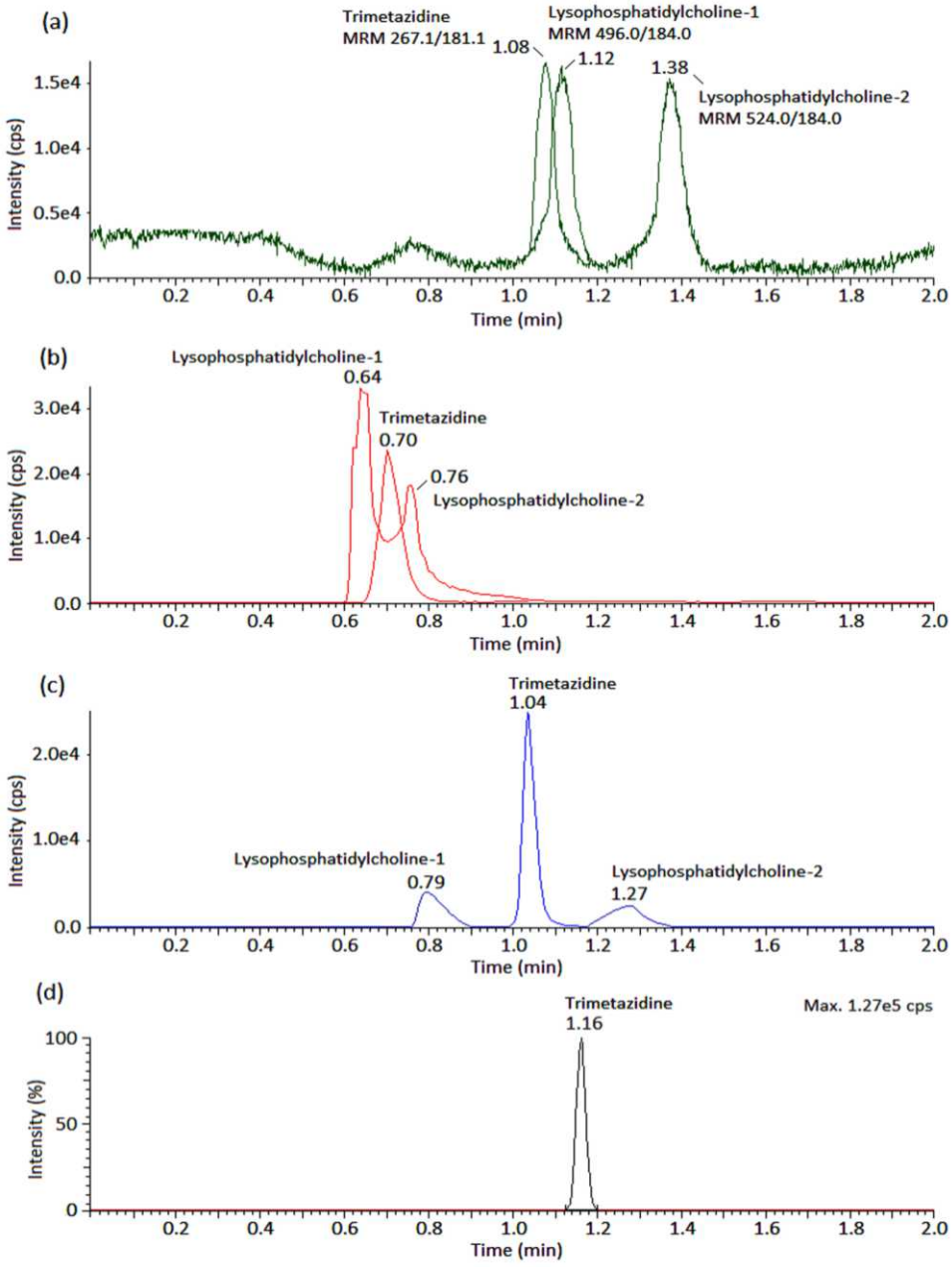

Figure 1 Chromatograms showing presence of phospholipids on Hypersil Hypurity C18 (50 × 4.6 mm, 5 µm) column using 2 mM ammonium acetate (pH 3.5)-acetonitrile as the mobile phase in (a) 40:60 (v/v) and (b) 20:80 (v/v) ratio during liquid-liquid extraction with ethyl acetate, (c) after solid-phase extraction on Oasis HLB cartridge using 2 mM ammonium acetate (pH 3.5)-acetonitrile (30:70, v/v) as the mobile phase and (d) absence of plasma phospholipids on UPLC BEH C18 (50 × 2.1 mm, 1.7µm) column after extraction from Hybrid SPE-phospholipid cartridge using acetonitrile-5mM ammonium formate, pH 3.5 (40:60, v/v) as the mobile phase.

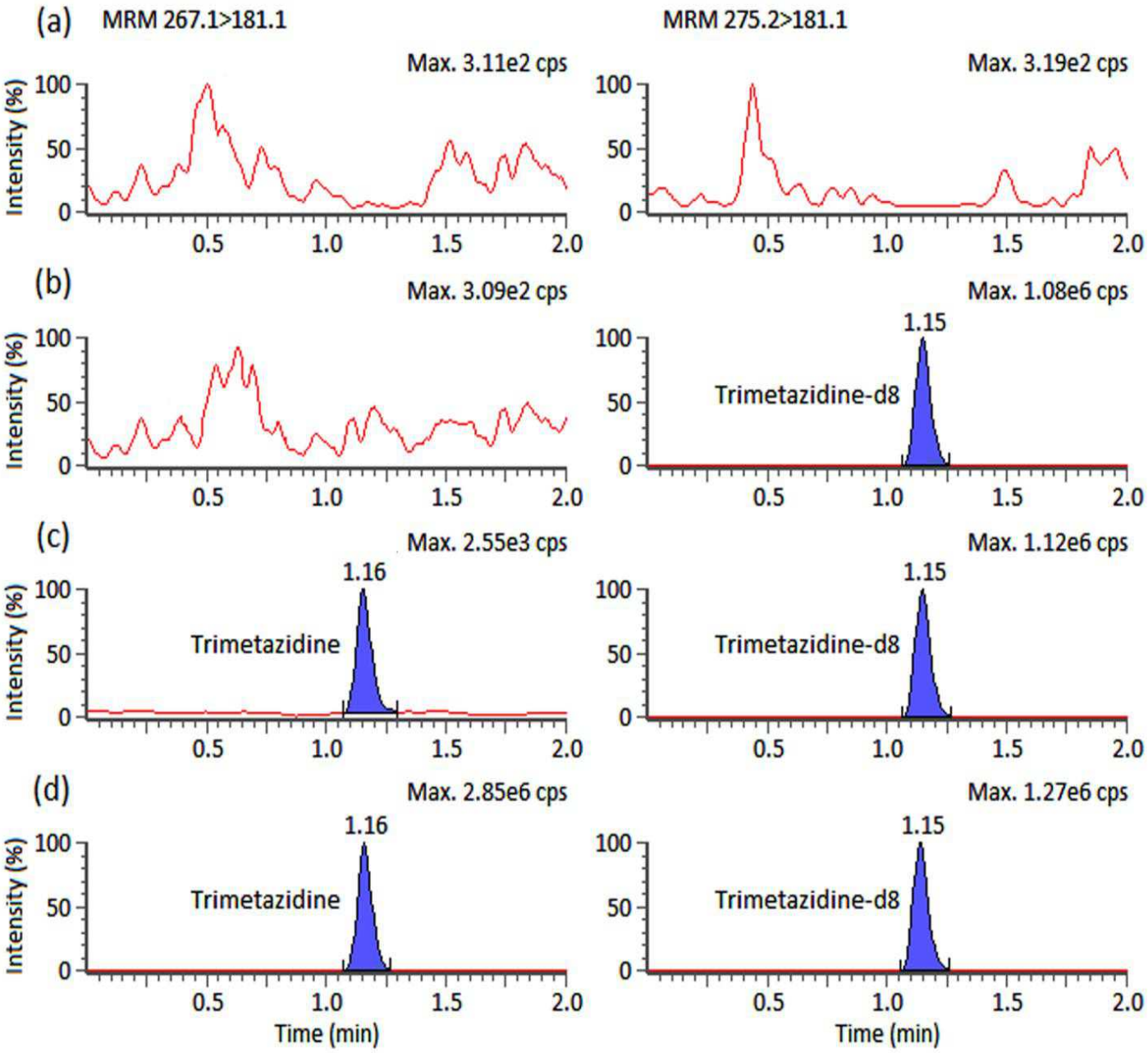

Figure 2. MRM ion-chromatograms of (a) double blank plasma (without analyte and IS) (b) blank plasma with trimetazidine-d8 (IS), (c) trimetazidine (0.05ng/mL) and IS (d) subject sample at Cmax after oral administration of 35 mg dose of trimetazidine.

After successful removal of phospholipids the chromatography was tested on UPLC BEH C18 (50 × 2.1mm, 1.7µm) column instead of Hypurity C18 (50 × 4.6 mm, 5 µm) used in our previous method (Mistri et. al., 2008). This was mainly due to inadequate response and peak shape at LLOQ and LQC concentration. Initially, 2.0 mM ammonium acetate (pH 3.5)-acetonitrile (40:60, v/v) was used as the mobile phase on BEH C18 column; however, the peak shape was rather broad for TMZ. Thus, acetonitrile/methanol together with ammonium formate/formic acid buffer (pH 3.0-6.0) with different ionic strengths (2-10mM) was tried. The organic content was also varied from 20 to 50 % and flow rate from 0.200-0.350 mL/min. The best conditions were established using the mobile phase consisting of acetonitrile-5mM ammonium formate, pH 3.5 adjusted with 0.1 % formic acid (40:60, v/v) at a flow rate of 0.300 mL/min as shown in Fig. 1d. These conditions enabled a run time of 2.0 min per analysis with acceptable peak shape, adequate retention and analyte response. The retention time for TMZ and TMZ-d8 was 1.16 and 1.15 min, respectively. The reproducibility in the measurement of retention times of TMZ,

expressed as % CV was ≤0.82 % for 100 injections on the same column. The chromatograms of double blank plasma, plasma spiked with TMZ-d8, TMZ at LLOQ and a subject sample at Cmax demonstrates the selectivity of the method to differentiate and quantify the analyte from endogenous components (Fig. 2).

To establish the mass spectrometry conditions, MRM studies were carried out to investigate the fragmentation of TMZ and TMZ-d8 precursor ions into their characteristic product ions and to establish optimum collision energies. The electrospray ionization in the positive mode gave protonated precursor ions at m/z 267.1 and 275.2 for TMZ and TMZ-d8, respectively in the parent ion spectra. The most abundant product ions at m/z 181.1 were found by applying 15 and 18 eV collision energy for TMZ and TMZ-d8, respectively. This fragment can be attributed to the loss of piperazine moiety from their precursor ions as shown in Fig. 3. For unequivocal identification of TMZ, the fragment ion at m/z 166.0 was also monitored. Furthermore, a dwell time of 100ms gave adequate data points across the peaks for reproducibility in the quantitative measurements.

Assay performance and validation

Carry-over evaluation was performed in each analytical run so as to ensure that it does not affect the accuracy and precision of the method. There was no significant carryover observed during autosampler carryover experiment. No enhancement in the response was observed in double blank plasma after subsequent injection of highest calibration standard at the retention time of TMZ and TMZ-d8, respectively. For selectivity assessment, nine independent sources of blank plasma (6-K_3EDTA, 1-lipemic, 1-heamolyzed and 1-heparinized) were monitored for endogenous interferences at the same retention time of TMZ and TMZ-d8. The interference observed for TMZ was less than 1.5 % of the LLOQ concentration. Negligible interference (<0.84 % of LLOQ) was observed for commonly used medications (paracetamol, chlorpheniramine maleate, caffeine, acetylsalicylic acid and ibuprofen) by human volunteers The calibration curves of the peak area ratio of TMZ to TMZ-d8 against the nominal concentration were constructed using 1/x2 weighted linear least-square regression model. The assay was found to be linear over the whole concentration range of 0.05-100 ng/mL (r2 ≥ 0.9992). The mean linear equation for five calibration curves was y = (0.9977 ± 0.0263)x + (0.0771 ± 0.0373). The lowest concentration in the standard curve was obtained at a signal-to-noise (S/N) ratio ≥ 14. The intra-batch and inter-batch precision (% CV) was between 1.7 and 5.3, while the accuracy ranged from 96.2 to 103.1 % for TMZ (Table 2).

The extraction recovery for TMZ and TMZ-d8 at different QC levels was highly consistent, precise and reproducible as evident from Fig. 4a. The mean recovery for TMZ and TMZ-d8 was 98.66% and 97.63%, respectively. Absolute matrix effects expressed as matrix factors (MFs) were evaluated at four QC levels (Fig. 4b). The IS-normalized MFs were calculated by dividing the MF of TMZ with that of TMZ-d8 and the values ranged from 0.964 to 1.013 across QC levels. Furthermore, the relative matrix effect was assessed in nine different plasma sources (6 normal K3EDTA, 1 lipemic, 1 haemolysed and 1 heparinized). The precision (% CV) in the measurement of slope of calibration curves for TMZ was 2.11, which is within the acceptance criteria of 3.0%. Post column infusion experiment was performed to investigate potential matrix effects. MRM chromatograms showed no regions of ion suppression or enhancement at the retention time of TMZ and TMZ-d8 as evident from Fig. 5.

The results for stability of TMZ at two QC levels are shown in Fig. 4c. TMZ in control human plasma (bench top) at room temperature was stable for at least 24h at 25 °C and for minimum of five freeze and thaw cycles at –20°C and –70°C. Processed sample stability of the spiked QC samples maintained at 5 °C was determined up to 55h without significant drug loss. Spiked plasma samples stored at –20°C and –70°C for long term stability experiment were found stable for a minimum period of 72 days. The % change in the concentration for all stability experiments was within ± 5.0 % of the corresponding stability samples. The drug and IS stock solutions were stable for 24h at 25°C and for 20 days at refrigerated temperature below 8 °C.

The dilution integrity experiment was performed with an aim to validate the dilution test to be carried out on higher analyte concentration above the upper limit of quantification (ULOQ), which may be encountered during real sample analysis. The precision values for dilution integrity of 1/5th and 1/10th dilution of 250 ng/mL concentration of TMZ were 2.7% and 2.2%, while the corresponding accuracy results were 97.2% and 94.6% respectively.

Method ruggedness was evaluated by analysing two precision and accuracy batches on two different columns of the same make and also with different analysts. The precision (% CV) and accuracy values for two different columns ranged from 2.3 to 5.4% and 94.9 to 98.7% respectively at all QC levels. For the experiment with different analysts, the results for precision and accuracy were within 2.3 to 5.4% and 96.7 to 99.5% respectively.

Application to a bioequivalence study

The validated method was successfully used to quantify TMZ from human plasma samples after oral administration of a single 35 mg oral dose of TMZ to healthy subjects. **Fig. 6** shows the plasma concentration vs. time profile of TMZ in human subjects under fasting. The method was sensitive enough to monitor the TMZ plasma concentration up to 36h. The values obtained for Cmax and AUC0-t were in good agreement with a similar study using 35 mg modified-release TMZ in healthy Turkish volunteers under fasting (Ozbay et. al., 2012). However, except for Cmax which were higher compared to the present study, all other values of pharmacokinetic parameters were somewhat lower for an identical study in healthy Bangladeshi subjects (Chowdhury et. al., 2011). These differences in pharmacokinetic

parameters may be due to gender type (body size and muscle mass), type of food etc.

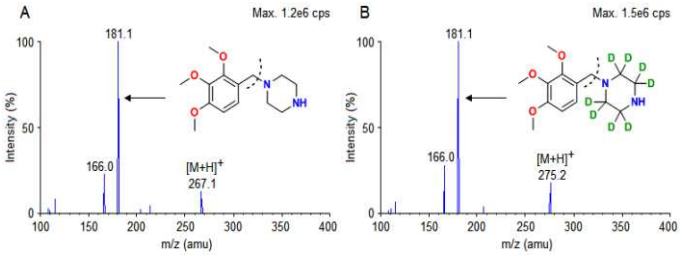

Figure 3 Product ion mass spectra of (A) trimetazidine (m/z 267.1 → 181.1) and (B) internal standard trimetazidine-d8 (m/z 275.2 → 181.1), scan range 100-400amu in the positive ionization mode.

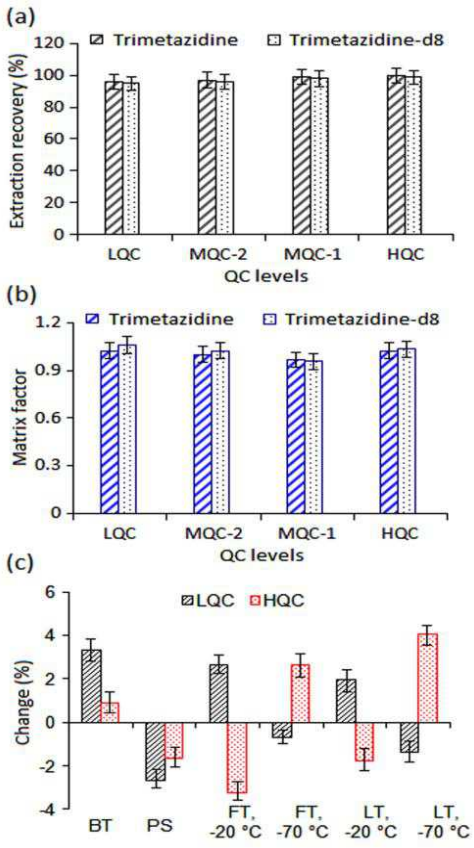

Figure 4. Graphical representation of (a) mean extraction recovery and (b) matrix factors at four quality control levels and (c) stability results of trimetazidine under different conditions

Further, the test/reference ratios of the mean natural log-transformed and 90 % confidence intervals for Cmax, AUC0-36h and AUC0-inf varied from 89.36 to 106.17 % for TMZ, which is within the acceptance criterion of 80-125%. Further, there was no adverse event during the course of the study. The % change in 58 reanalysed subject samples for assay reliability was within ±12 %, which reinforces confidence in the method for subject sample analysis. The mean extraction recovery for these methods varied from 57.53 to 98.25%. However, none of the existing methods have reported interference of plasma phospholipids during sample preparation which was observed in the present work. Hybrid-SPE-phospholipid technology which is designed to remove interferences due to endogenous proteins and phospholipids provides a simple one-step sample preparation approach. So far there is only one UPLC-MS/MS method which was validated in the concentration range of 0.25-100 ng/mL using 500 µL human plasma (Zhang et. al., 2010). The present UPLC-MS/MS method is five times more sensitive compared to this method and utilizes only 100 µL plasma for processing. Furthermore, all existing methods have used a general IS whereas a deuterated analogue, TMZ-d8 was used in the present work which provided better control of extraction conditions and ionization efficiency. Additionally, incurred sample reanalysis experiment is also performed which verifies the utility of the method for subject sample analysis.

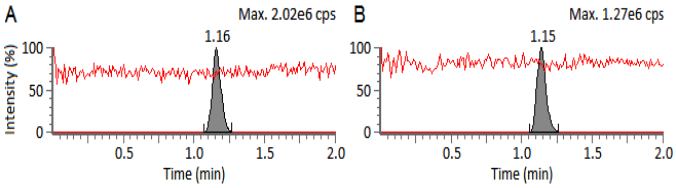

Figure 5 Representative post-column infusion MRM LC-MS/MS chromatograms for (A) trimetazidine (40.0 ng/mL) and (B) trimetazidine-d8.

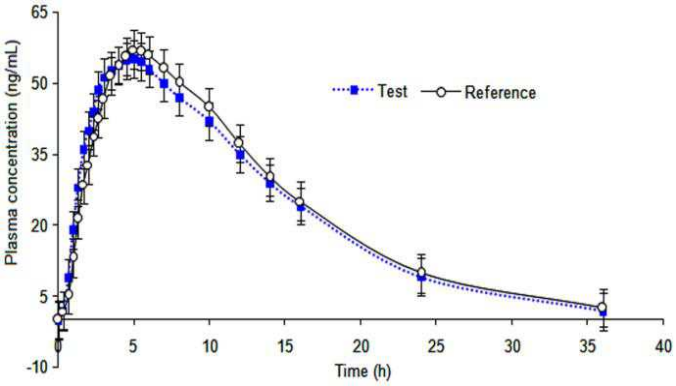

Figure 6. Mean plasma concentration-time profile of trimetazidine after oral administration of 35 mg (test and reference) modified release tablet formulation to 12 healthy volunteers.

Comparison of liquid chromatographic methods developed for TMZ in biological samples

Majority of the reported methods are based on LC-MS/MS analysis with limit of quantitation ranging from 0.1-1.5 ng/mL using LLE or PP as the extraction technique.

Conclusion

This study outlines a validated UPLC-MS/MS method for the analysis TMZ in human plasma. The method provides excellent results for accuracy and precision even at very low concentration (0.05 ng/mL) in plasma samples. Moreover, this is report on the use of hybrid SPE-phospholipid technology for the extraction of TMZ from human plasma with highly precise and quantitative recovery (98.66 %). The method demonstrates a simple, quick and reproducible sample preparation protocol which overcomes matrix effect due to plasma phospholipids observed with conventional extraction techniques. This technology provides sufficiently clean sample which can be applied for routine testing and analysis. Additionally, the method offers several advantages in terms of lower sample requirements, simplicity of extraction process, higher sensitivity and overall analysis time. Finally, the current method was successfully applied to determine TMZ in a clinical study with acceptable accuracy, precision and reproducibility.

Acknowledgement

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Developing a novel HPTLC protocol for analysing indolic auxins produced by Rhizospheric *Aspergillus* strains

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Abstract: Fungi are known to interact with plants by several known mechanisms, one of which is through the production of phytohormones, auxins. Indolic auxins such as indole-3-acetate (IAA), indole-3-butyrate (IBA) and indole-3-pyruvate (IPyA) are most widely produced by the strains of fungi through which they interact with plants. Fungal strains produce these indolic auxins by Tryptophan (Trp) dependent and independent pathways. Under present study, we found *Aspergillus flavus* strain PGFW, *Aspergillus niger* strain BFW and *Aspergillus caespitosus* strain DGFW as the most efficient IAA producing strains from the rhizosphere of healthy wheat plant which was determined by spectrophotometric method that uses Salkowski reagent. This method though has a flaw that it is not specific to IAA but develops colour by reacting with all the other indolic derivatives. We found that, for the spectrophotometric method the absorption maxima (λmax) of the mixture containing indolic compounds tend to shift when compared to pure standard. To overcome this limitation, High performance thin layer chromatography (HPTLC) based protocol has been developed to precisely detect and quantify IAA and IBA in the range of 100 to 1000 ng per spot ignoring other Trp derivatives. HPTLC analysis showed that all the three strains under study could produce indolic auxins by Trp dependent and independent pathways but the production of indolic auxins were enhanced in presence of Trp. These strains may act as phytoaugmentor or phytopathogen as they produce various indolic auxins, which can be profiled by the novel method described in this paper.

Keywords: HPTLC, *Aspergillus* spp., Indolic compounds

Introduction

Fungi residing in the rhizosphere are known to produce auxins like Indole-3-acetic acid (IAA) and benefits plant growth (De-Palma et al., 2016). Such fungi live symbiotically with plants and supports plant growth while in return plant provides sugars and amino acids to the fungi for their survival. Such fungi are named plant growth promoting fungi (PGPF) (Meiners et al., 2017). The microbial biosynthesis of auxins has undergone intense investigation which has deduced that several strains of fungi produce IAA from tryptophan (Trp) and while many fungi produced IAA even in absence of Trp (Goswami et al., 2015).

Spectrophotometric technique is the most conventionally used to distinguish indolic subordinates produced by microorganisms and parasites. The spectrophotometric strategy utilizes the conventional Salkowski reagent ($FeCl_3$ broken down in perchloric acid/sulfuric acid) which reacts with the indolic subordinates to create color complexes (Szkop and Bielawski, 2013). This procedure is simple but has an ambiguity as it gives non specific colour complex with all the indolic derivatives; thus, the exact amount of IAA can not be estimated. High performance thin-layer chromatography (HPTLC) is recently reported to be very reliable in distinguishing indolic units created by microbes (Goswami et al., 2015).

Here, this study reports the method development to analyze indolic molecules from fungi using HPTLC. For the study, we chose three efficient IAA producing strains from the rhizospheric soil of wheat plant. We also compared the sensitivity of HPTLC method with routinely used Spectrophotometric method.

Experimental

Materials and reagents

IAA (99.0%) and IBA (99.0%) were procured from Hi-Media (Mumbai, India). Iso-propanol, n-butanol, methanol and ammonia solution (25% v/v) were purchased from Merck Ltd. Aluminum backed silica gel 60F254 TLC foils with 0.25-mm thickness were purchased from Merck (Darmstadt, Germany).

Instrumentation

The HPTLC instrument used in the experiments was purchased from CAMAG (Muttenz, Germany) which basically consisted of two parts: Linomate 5 which is an automatic applicator fitted with a Hamilton syringe (100 µl) for accurate loading of samples

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onto TLC foils; and a Scanner 3 for the scanning of HPTLC foils after development.

Standard solutions

Standard solutions of IAA and IBA were prepared in absolute methanol in a concentration of 100 µg/ml.

Strains of PGPF used for study

Three strains, *Aspergillus flavus* strain PGFW (KY964054), *Aspergillus niger* strain BFW (KY964055) and *Aspergillus caespitosus* strain DGFW (KY964056) capable to produce IAA producing strains from the rhizospheric soil of *Triticum aestivum* (wheat) plant (22°61'N, 72°93'E).

Estimation of the IAA produced by PGPF strains using the traditional spectrophotometric method

The initial IAA producing ability of these strains was determined using conventional spectrophotometric method which involves the use of Salkowski reagent (Goswami et al., 2013) Fungal isolates were grown in the potato dextrose broth supplemented with Trp (1mg/ml of broth) at 25 ± 2 °C for 168 h. Culture supernatants from each of these isolates were mixed with Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution) in the ratio of 1:1. Development of pink color indicates production of IAA and its optical density was recorded at 530 nm. Concentration of the IAA produced was estimated against the standard curve of IAA (Hi-media) in the range of 10–100 µg/ml.

Extraction of indolic derivatives

Indolic derivatives were extracted from culture supernatants as described by (Goswami et al., 2015). Fungal strains were grown for 168 h in the potato dextrose broth supplemented with Trp (1mg/ml of broth) at 25 ± 2 °C. Culture supernatants were acidified to pH 2.5 using 1 N HCl and extracted thrice using equal volume of ethyl acetate. A fraction of ethyl acetate was air dried and re-dissolved in one tenth volume of methanol (Goswami et al., 2015). These methanolic extracts were used for HPTLC analysis.

HPTLC procedure

Sample application

Sample loading on TLC foils (sized 10 × 15 cm) was performed using a Linomat 5 applicator (CAMAG, Muttenz, Germany) which contained Hamilton 100 µl syringe.

Calibration curves

Calibration curves of IAA and IBA (100 µg/ml) were prepared as per the technique previously developed by Goswami et al. (2015) within the range of Limit of detection (LOD) and limit of quantitation (LOQ). Each of these standards were loaded ranging from 4 µl to 20 µl was separately loaded in the form of bands on TLC (sized 10 × 10 cm). After development, TLC plate was air dried for 10 min, and the developed spots were visualized under UV chamber with a short wavelength of 254 nm. Such TLC loaded with standards were developed in pre-saturated twin trough chamber containing mobile phase Iso-propanol:n-Butanol:Ammonia:Water [10:6:3:1 (v/v)]. TLC plates were air dried and the developed spots were scanned using Scanner 3 (Camag) in absorbance–reflectance mode at 254 nm.

Densitometric analysis of chromatogram

For quantitative densitometric analysis, peak area of developed spots for respective sample was quantified by linear scanning at 254 nm using the Camag TLC Scanner 3 with deuterium source at a scanning rate of 20 mm/s. The slit dimension setting was 6-mm length × 0.45-mm width, and data resolution was 100 µm per step using filter factor Savitsky-Golay 7, baseline correction was set to the lowest slope and display scaling was set to automatic.

Determination of IAA from extracted indolic derivatives from fungal cell free supernatant

Fungal methanolic extract (containing indolic derivatives produced by fungal strains) and standards (IAA and IBA) were loaded on the same TLC plate. On the TLC plate (sized 15 × 10 cm), 16 bands were loaded in total, where 2 bands loaded with each standard, 2 bands loaded with mixture of standards (IAA and IBA) and 2 bands loaded with each fungal methanolic extract in different volumes. For Aspergillus flavus strain PGFW, *Aspergillus niger* strain BFW and *Aspergillus caespitosus* strain DGFW, the methanolic extracts loaded per band were 20 µl and 30 µl respectively. TLC plate was developed in pre-saturated twin trough chamber containing mobile phase Iso-propanol:n-Butanol:Ammonia:Water [10:6:3:1 (v/v)]. On development, TLC plates were air dried and spots develop were scanned using Scanner 3 (Camag) in absorbance–reflectance mode at 254 nm. Densitometric analysis was performed to detect and quantify IAA and IBA in the methanolic fungal extracts; quantified values were compared with the values obtained using the spectrophotometric method.

Results

IAA production by PGPF strains analyzed using spectrophotometric method

Spectrophotometric analysis suggested that all the 3 strains under study could produce indolic derivatives under both the conditions (culture medium with and without supplementation of Trp).

Maximum values of IAA produced by these strains are represented in Table 1.

HPTLC

Calibration curves

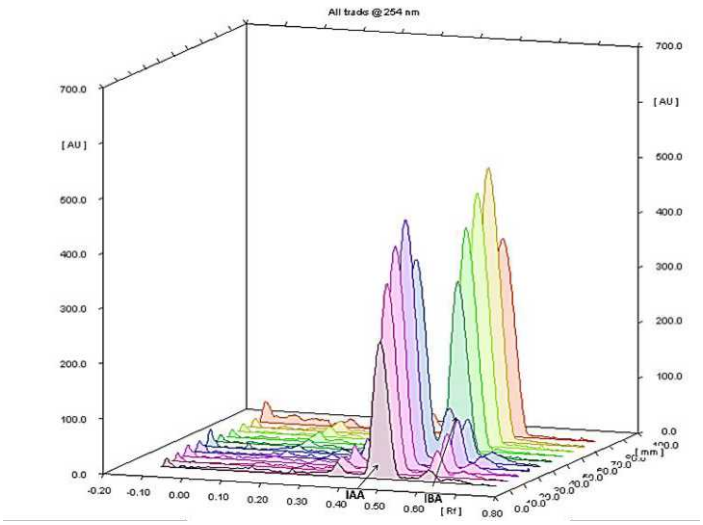

Figure 1 Shows the 3D densitogram of the same TLC foil where intensity of peak describes the area under the curve. It can be observed that as the concentration of standard IAA and IBA increases as the area under the curve for corresponding spot also increases.

TLC plate loaded with standards were allowed to developed in twin trough chamber pre-saturated with mobile phase Iso-propanol:n-Butanol:Ammonia:Water [10:6:3:1 (v/v/v)] for 75 min. On development, the TLC plate was air dried and analyzed for retention factor (Rf) values under short UV (254 nm) where, IAA showed a Rf value of 0.63 and IBA showed a Rf value of 0.70. For preparing calibration curve, each standard was loaded individually ranging from 400 to 2000 ng per band. Densitometric analysis deduced that Trp possessed linear correlation with concentration of standard loaded and peak area of the developed spot in the range of 100 to 1000 ng per spot. IAA and IBA showed similar linearity in the range of 100 to 1000 ng per spot and 100 to 500 ng per spot respectively. Comparative visualization of standard IAA and IBA standards on single TLC is shown in Figure 1. Further, LOD and LOQ were determined for each standard from the calibration curve where, which were 96.1 ng and 291.2 ng per spot respectively for IAA and 83.4 ng and 252.7 ng per spot respectively for IBA.

Determination of IAA and IBA from bacterial cell free supernatant containing indolic derivatives

On accurate calibration, this method was used to detect and quantify IAA and IBA from bacterial extracts. Image of TLC after development is shown in Figure 2. Track 1 and 2 are loaded with standard IAA (20 µl and 30µl respectively), track 3 and 4 are loaded with standard IBA (20 µl and 30µl respectively), track 5 and 6 are loaded with extracted indolic derivate from A. flavus in absence of Trp in media (20 µl and 30µl respectively), track 7 and 8 are loaded with extracted indolic derivate from A. flavus strain PGFW in the presence of Trp (20 µl and 30µl respectively), track 9 and 10 are loaded with extracted indolic derivate from A. niger strain BFW in absence of Trp in media (20 µl and 30µl respectively), track 11 and 12 are loaded with extracted indolic derivate from A. niger strain BFW in the presence of Trp (20 µl and 30µl respectively), track 13 and 14 are loaded with extracted indolic derivate from A. caespitosus strain DGFW in absence of Trp in media (20 µl and 30µl respectively), track 15 and 16 are loaded with extracted indolic derivate from A. caespitosus strain DGFW in the presence of Trp (20 µl and 30µl respectively). Area under the curves of IAA and IBA produced by fungal isolates are shown in figure 3. These values are used to calculate IAA produced by fungal isolates which is represented in table 1

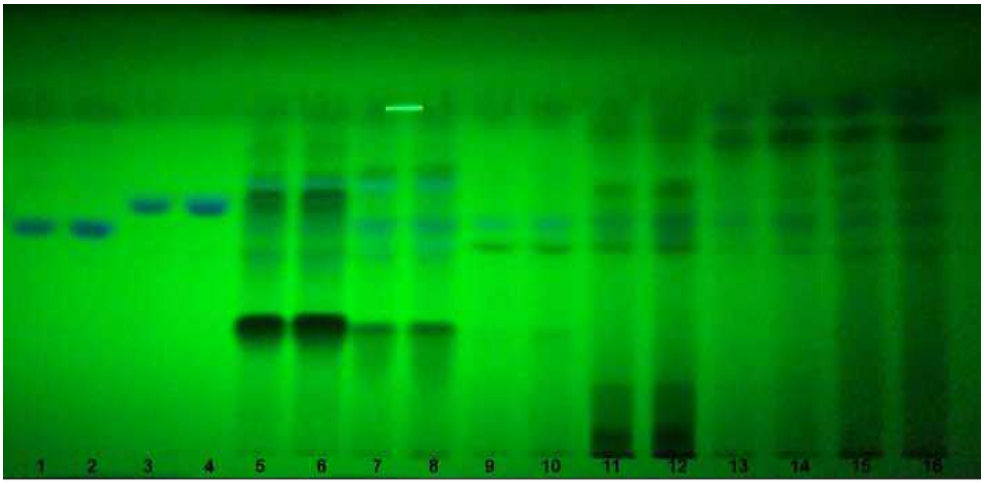

Figure 2 This figure shows the image of TLC foil under 254 nm UV light.

Table 1 *Table 1 HPTLC analysis of IAA from three Aspergillus spp.*

Strains	Culture Media	Sample loaded	Area under curve	ng of IAA per spot	ng of IAA per ml sample	µg of IAA per sample	µg of IAA per ml of broth	µg of IAA per ml of broth	Spectrophotometric results showing production (µg of IAA per ml of broth)
A.flavus strain PGFW	without Trp	20	10173.00	3681.30	184065.01	184.07	18.41	17.58 ± 1.17	85.67 ± 4.33
		30	13519.10	5024.04	167467.90	167.47	16.75		
	with Trp	20	16628.90	6271.95	313597.51	313.60	31.36	30.10 ± 1.78	112.29 ± 8.34
		30	22566.50	8654.61	288487.16	288.49	28.85		
A.niger strain BFW	without Trp	20	7551.90	2629.49	131474.72	131.47	13.15	12.26 ± 1.26	82.67 ± 3.22
		30	9498.60	3410.67	113689.14	113.69	11.37		
	with Trp	20	12703.60	4696.79	234839.49	234.84	23.48	23.25 ± 0.33	139.72 ± 7.44
		30	18205.00	6904.41	230147.14	230.15	23.01		
A.caespitosus strain DGFW	without Trp	20	8534.50	3023.80	151189.81	151.19	15.12	14.55 ± 0.81	106.62 ± 6.44
		30	11445.90	4192.09	139736.49	139.74	13.97		
	with Trp	20	13552.10	5037.28	251863.96	251.86	25.19	26.54 ± 1.92	156.08 ± 5.44
		30	21855.60	8369.34	278978.06	278.98	27.90		

It was found that the strains produced IAA or IBA or both. Their actual production detected by spectrophotometric method is much greater than the HPTLC derived values. This is because fungal isolates produce several other molecules other than IAA and IBA (Figure 2) which may be falsely detected as IAA by the spectrophotometric method.

Discussion

Spectrophotometric method is the most widely used to detect indolic derivatives from Trp among the existing approaches. The spectrophotometric method uses the conventional Salkowski reagent ($FeCl_3$ dissolved in perchloric acid/sulfuric acid) which responds to the indolic derivatives to develop colour (Glickmann and Dessaux, 1995; Akbari et al., 2007; Karnwal, 2009). This technique is simple but highly imprecise as it gives a non-specific colour reaction with all the indolic derivatives produced by fungi and enables detection of total indole content relatively than precise detection of IAA and IBA individually. Thus, the spectrophotometric method gives inaccurate quantities of IAA/IBA produced by PGPFs (Szkop and Bielawski, 2013). Other than the spectrophotometric method, thin layer chromatography (TLC) (Hartmann et al., 1983; Robinson et al., 1998; Swain et al., 2007; Goswami et al., 2013) and high performance liquid chromatography (HPLC) methods are also used (Szkop and Bielawski, 2013). TLC provides merely qualitative detection of indolic compounds while, HPLC is very sensitive (Beni et al., 2014), but needs highly purified samples which make process of sample preparation tedious. HPLC analysis also requires longer time durations for detection and calibration (Dhandhukia and Thakkar, 2008; Dhandhukia and Thakker, 2011).

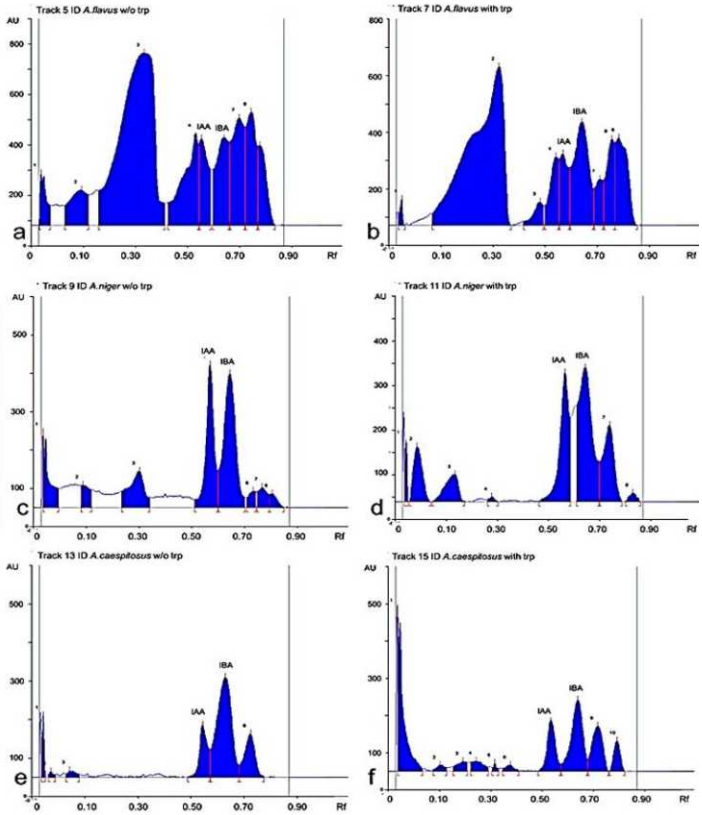

Figure 3 Shows 2D densitogram of bacterial extracts containing indolic compounds (a) track loaded with extract of *A. flavus* strain PGFW without Trp (track 5 of figure 3), (b) track loaded with extract of *A. flavus* strain.

Conclusion

Thus, in this paper we report the use of high performance thin-layer chromatography (HPTLC) for the simultaneous detection

and quantification of IAA produced by several PGPFs. We have also provided comparison of its sensitivity with traditional spectrophotometric method. Thus this study has developed a methodology to quantitate IAA and IBA precisely from fungal isolates using HPTLC.

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Internet, Gaming and the Role of Counselling

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Abstract: The internet has taken the world by storm by bringing people from all corners of the world onto one platform. The internet has made its mark by reaching out to people in the remotest of towns and smallest of villages combined. The power that it holds over people can never be measured. But with its emergence, cybercrime has started taking place virtually. Cybercrimes are on the rise with not only money being looted of the people but also their identity. Gaming is another sport that is considered to be childish but is one of the most popular activities, not just amongst children but also amongst young men and women. People's lives have been revolving around the screen, be it of mobile phones, laptops, personal computers or televisions. In order to bring a certain amount of sanity to this insane madness of being active in the virtual world and losing touch with reality, counselling is the best option. Counselling would not only be helpful to the people involved in the activity but also people associated with them from different walks of life.

Keywords: Internet, gaming, counselling, role of counselling

Introduction

Taking the definition from Wikipedia, the most used website online, the internet is defined as a "global system of interconnected computer networks that use the Internet protocol suite to link devices worldwide." It is also described as something that "carries a vast range of information resources and services, such as the internet linked hypertext documents and applications of the World Wide Web (WWW), electronic mail, telephony and file sharing.

Apart from that, other definitions also come up with similar explanations where only the theoretical aspect of the term 'internet' is explained. The main idea about internet is lost in these definitions. The reason why we need to define 'internet' properly, is because there are many aspects to it which are quite unknown as well as scary.

The internet has great many uses. It has made this world a smaller place by bringing everything to your finger-tips. From booking holidays to buying groceries, the internet is one thing that has made it all possible. In order to exactly define what the 'internet' is and what are its functions can be difficult because of the varied things it indulges in. But then since most of us know what the internet is capable of doing, it becomes very easy to discuss about it. Most of us are only aware of the good that the net is capable of doing. We look at the advantages of it and become very impressed. In simple term, we could say that the internet has helped in making our lives very easy.

What we fail to see is how challenging the net can become. It never occurs to us that when we get so much in return, we are robbed of one thing that is the closest to us: privacy. When you are given a key to get all the information you need, you are doing so by giving up your personal details. When you provide those personal details to these tech giants, they may use the information to study you, your ways of living, your choices and also indirectly to watch on you.

The most debated topic in the U.S. right now is the right to privacy. People are objecting to their governments not to spy on them. China has already built a firewall in order to avoid the Western influence. This kind of spying into peoples' lives is called an act to maintain the national security by the governments. But the question that needs to be asked is at what cost. The internet just doesn't spy on people suspected to be a threat to the nation but on the general public.

Not everyone likes to be kept under surveillance. It acts as something that curbs a person's freedom. When we discuss the dangers of the internet, the main issue that must concern us is that nothing remains private once you come online. Even your mails and passwords to E-mail IDs are never safe.

Originally the internet was first started as Web 1.0. A noteworthy difference between both the versions is how the flow of information takes place. The old 1.0 version followed a rigid structure where there were the webmasters and they were the only souls responsible for what went online. Since a very few determine what goes online, this was considered as quite a communist idea.

Then the upgrade of Web 2.0 revolutionized everything. It came up from a capitalistic idea. The Web 2.0 was a place where everyone owned it and everyone could put up what they felt like. The control of the content was now in the hands of everyone who had an internet connection and that itself shows the capitalistic idea that everyone has the right to say what they like. This came about, once the social networking sites took to the publics' interest. Websites like Orkut and Facebook are part of this revolution and they are the ones who gave momentum to it.

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But what is to come is something that could also be the end of humanity. The dynamic applications of the World Wide Web will soon include Web 3.0. This could enable machine-to-machine interaction. The 3.0 enabled computers will start reading and interpreting information, like humans do. It will also generate and distribute content which will be useful and tailored according to the needs of its users. The internet has brought the world together on one platform where everyone can be heard.

Disadvantages of the Net

The communication dynamics in the world started changing with the advent of the internet. One could claim that overseas communication became quite cheap. The world started becoming smaller and people from one end of the world could connect a little faster to someone who was in the other corner. But this also brought with it an interference into personal space.

Commodification of Users

Discussing about how peoples' mail ids are sent to other sellers so that they can sell their items to us, Google is doing nothing but selling their users ids to online sellers. This can also be referred to, as the commodification of users. It is indeed thought provoking how our personal details and choices have become just a commodity for search engines to sell and get paid for it.

Google Sells 'Likes'

Google, the big tech giant is known to have been in unimaginable controversies. Google knows all and sees all. Everything you do is tracked by Google. You feel it is okay because there is no much harm. But to have someone know what you search and what you prefer can be quite dangerous. It is like tracking what you like and then selling things based on your preferences. If you think it is helpful, then you might be wrong. When Google knows what you like, they start taking your personal details and then bombard you with similar choices on any device that you log into. This in a way is also referred to as a breach of privacy. Mails are personal, and they need to be kept that way. Google has bugs that crawl through your mails and read through the content getting to know not only what you have said but also what your friends have to say and what items are being sold to you. This way, the details of the choices you make are then sent by Google to other sellers who bombard you with mails about buying things.

The internet has not only brought your far-away relatives closer to you but has also ended up getting random people who aren't even remotely related, to come and talk to you. The social media has opened up the realm to a new experience but it can also be ugly, and that was something which wasn't expected.

Dark Web

The dark web is a place that is not known to the normal world. While many believe there exists a dark web where all the unhealthy activities take place, many feel it is just a conspiracy to make people scared. The dark web is just like maybe Google but its functions would not be that noble. The dark web is blamed to have been indulging in selling of drugs, guns, information etc. When major companies are hacked into, their details and everything is leaked over to the dark web where people can then buy and sell information as well. Apart from that, even mail ids and passwords, as well as credit card details of many people are also sold online.

Gaming

According to a paper 'Psychological and Physical Effects of Action Video Games' by Arjun Banerjee, it states that video games are considered as time wasters by many on this planet. Many studies also show that by playing action video games, children tend to witness violent and antisocial behaviour. At the same time, a lot many studies also show how video games help in building the brains physical structure. The same studies also talk about how the generation of kids who were open to gaming, were smarter than the previous generation. In this world of contradictions, it is highly essential that we discuss the amount of exposure to gaming. Everything in limits is fine. Problems take place when things are abused and not used justly. Having children stuck on TV screens and computer screens for a long time can make them like the person they see most of the times.

Children pick up from their surroundings. They tend to copy what they see and therefore it becomes very important to surround them with things that are socially acceptable and also morally right. The video games open up a world of gruesomeness that children sometimes tend to follow. Children of small age, when exposed to a lot of violence, it tends to start showing up in their behaviour.

We are all aware of how the Blue Whale game inspired so many people to commit suicide. The game was part of the dark web. No one could just install it and play it. People didn't approach the game, but the game approached youngsters. Blue Whale was played by completing a series of tasks and each task was more gruesome than the other. Then there were also tasks that asked people to listen to sad songs and watch horror films. This game would be played over a span of few weeks and slowly brainwash their victims till they would fall to their death. The person behind the game would always pry on the emotionally unstable people who were not happy with life. A social networking site was the original source where the gamer would look into profiles of people who were weak.

Counselling

According to Carl Rogers, "Effective counselling consists of a definitely structured permissive relationship which allows the client to gain an understanding of himself to a degree which enables him to take positive steps in the light of his new orientation."

Patterson describes counselling as "…the process involving interpersonal relationships between a therapist and one or more

clients by which the former employs psychological methods based on systematic knowledge of the human personality in attempting to improve the mental health of the latter". Prashantam defines counselling as, "… a relationship between the counsellor and the client characterized by trust and openness, in a one-to-one or a small group relationship, whereby the client is helped to work through her or his interpersonal or intrapersonal problems and crisis". According to Prem Pasricha, "Counselling can be defined as a process which takes place in a one-to-one relationship between an individual who is troubled by problems and a professional person whose training and experience have qualified him to help others reach solutions to various types of difficulties".

The Role of Counselling in the Present Scenario

With the world shrinking, everything is connected and one is able to reach anyone they wish at a click of a button. But before the internet, there were crimes that only took place in the physical world. With the emergence of the internet, crimes started taking place even virtually which is referred to as 'cyber-crime.' With several cases of online pranking, abuse, stealing of identity and money, the world is now in stressful times, where the people are being abused by technology as well. It can be stressing because not many may want to report such cases. Dealing with them can be disturbing. This is when counselling comes to the rescue. Emotions cannot be supressed, because when they blow up they take the form of something horrific. Counselling at such times is the best remedy to help people deal with such disturbances.

Apart from that, schools need to be equipped with not only a good psychologist but also keep regular sessions where they open up discussions about the effects of the internet, gaming, etc. Schools must host talk shows where the students as well as the teachers need to be told about how the internet causes more problems than solving them. The children also need to be told about how playing video games is not the only thing that they should be getting involved in. Playing outdoors and socialising with everyone is equally important and more.

Due to the emergence of social media and gaming it is observed that a lot of people have started isolating themselves from social interactions. Sometimes this can also result in psychological anomaly which retards the overall progress of the individual.

College students also need to be counselled upon how people are scammed as well as cheated for money through emotional blackmailing. Counsellors must also talk about how work and studies are affected when more time is invested on the net. The trend of uploading selfies and reaching new levels in the video games has caught like wildfire. What needs to be addressed is how it affects the psyche and their relationship with the outside world.

For young and college-going students, parents are considered as the most important people in their lives because they get to interact with them on a daily basis. Therefore, parents/ guardians also need to be made aware of counselling as a tool of helping their children to overcome this virtual addiction. Most of the times, parents don't realise that it is important to see how their child is growing and interacting with their surroundings. Parents must see what their children are getting involved in. Teenagers specially need to keep an eye upon because they do tend to act impulsively without knowing the consequences. Parents are the best hope to take their child to a counsellor and help them enhance their child's life.

Counselling is one of the most effective ways of dealing with all kinds of personal as well as social issues. A lot many people feel that mentally unstable people go to psychologists; But that is not true. Sometimes, even mentally sound people require a little help in solving problems. Thus, the stereotypical way of having a myopic vision of counselling definitely needs to be addressed and changed. Only then, can the world be helped from being stuck in the virtual world.

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Stressful life events and psychological distress in college students

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Profaina K. Christian[a]

Abstract: The present study examined the stressful life events and subjective distress among college students. The sample consisted of 120 college students (60-boys and 60-girls) from different colleges of Ahmedabad city. Personal datasheet and stressful life events inventory developed by Revathi (1986) were used for data collection. The result found that on an average, students experienced about six life events in a one year life span. Majority of the events experienced were in the area of education. A positive correlation was found between life events and subjective distress.

Keywords: Stress, College students

Introduction

Life events are those events which cause significant change. They can be either positive or negative, but they all demand adjustment to new circumstances. It is also called stressors, and stressors are those events which cause distress. Stressful life events have an illness producing role within the individuals. Young people are characterized by changes in their biological functioning, cognitive development, social roles and social environments (Hamburg, 1974).

College students are in a transitional phase where adjustment to changing patterns of life and social expectations happen. They are also under pressure to achieve academically. A college year, according to Chine's writing, has a dual meaning "crisis" as well as "opportunity". Whether the new student is an 18-year-old who just passed High School, experiences challenges as an "opportunity" or a "threat", a chance to practice wellness or worseness. The college years present a wonderful opportunity to apply many of the ideas and skills.

The issue of college students' mental health has garnered considerable attention in recent years. Today's college students have been portrayed as generally afflicted with more serious mental health problems than students in the past. Experiencing a greater number of traumatic events across the lifespan is associated with worse self-reported health, greater health care utilization, functional disability, arthritis, greater number of acute and chronic illnesses, and mortality (Gawronski, Kim, & Miller, 2014; Keyes et. al., 2013). But is this an accurate characterization of today's students? Some evidence does suggest that growing numbers of college students are experiencing emotional problems of a serious nature, but much of the evidence is based on the perception of college counsellors (Ramesh Chaturved, 2007; Profaina Christian, 2011).

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Since the 1980s, college counsellors have been reporting a steady rise in the number of students seeking counselling. Many college counsellors claim that they see fewer cases of students with traditional developmental struggles and many more cases of students presenting serious psychological problems. High rates of students' mental health problems and treatment-utilization are a major concern for all types of institutions of higher educations (Sarah et.al., 2015). These types of issues have received considerable attention in the counseling literature. The lack of research evidence regarding the trend of increasing problem-severity may be due to inadequacies in the research.

A life event is indicative of, or require a significant change in the ongoing life pattern of the individual. These events can occur in a variety of domains (family, health, education, finance bereavement), and may be age-graded, (school, marriage, retirement); history graded (war and depression) or normative (illness and divorce). Most of the adolescent and adult literature reflect the sociological tradition of assessing the impact of life events as tradition, age related, status gains and losses and so and so forth. When events are regarded positively, people tend to assume that they had influence or control over those events.

Others have suggested that generating positive feeling may enhance an individual's capacity to adapt to stress. Negatively related event are also correlated with adjustment.

College students are in such a transitional phase; this is a period of adjustment to changing patterns of life and social expectations. They are also under enormous pressure to achieve academically. The life style they develop now will set the future pattern of their lives because life without purpose or hope can lead to depression, insecurity, loss of self-esteem and confidence, boredom, frustration, feeling of failure and discontentment-all are breeding ground for stress and distress (Zeena, Kiran Rao, 1990).

One conclusion that we can make at this point is that there has been a perception among college counselors that the severity of

problems has been on the rise. Although the question of whether the mental health problems of today's students are more serious that they were a decade or two ago remains unclear, some indications do suggest that today's students might be particularly susceptible to certain forms of distress. Even the transition and adjustment to college, a common developmental struggle during the first year at college, may be becoming more stressful than ever for many students**.** Clinically insomnia has been statistically significant more frequently in first year students. Students who classified as poor-sleepers experienced more problems with physical and psychological health (Choueirly et.al., 2016).

As a result, serious stress related illness such as anxiety, depression, migraine and eating and sleeping disorders are seen as a new syndrome or pattern of processes and symptoms that characterize a disease called "Student –Shock". This syndrome is emerging not only among young people with long time problems but even among those who were formally well adjusted (Gottschalk, 1983).

The youth of today are unable to cope with stress in the form of life events that they face in daily life. This certainly causes distress in them. In this context it is essential to conduct a study on life event and related distress in college students. The aim of the present investigation is to examine stressful life events and subjective distress in college students.

Objectives

1. To examine the number and type of stressful life events and distress experienced by college students over a period of one year,
2. To study gender difference on stressful life events,
3. To find out the relation between stressful life events and subjective distress.

Hypotheses

1. There is no significant difference on stressful life events between college going boys and girls.
2. There is no significant difference on subjective distress between college going boys and girls.
3. There is no relation between number of stressful life events and subjective distress.

Methodology

Sample

For the purpose of the present investigation a sample consisted of 120 students (60 boys and 60 girls) were selected randomly from various colleges of Ahmedabad city. The sample was purposive and participation was on a voluntary basis.

Tools

1. Personal data sheet: A personal data sheet prepared by the investigator was used to collect basic information about student like gender, age, year of study etc.
2. Stressful life event inventory: Stressful life event inventory modified by Revathi (Revathi, 1986) was used for collecting the data. It is an open-ended and consisting of 56 events covering 7 different areas.

The mean age of total group is 20 years. Statistical analysis: frequency, percentage, t-test and correlation were used for statistical analysis.

Table 1: Mean, SD and t-value of two groups on life events.

Groups	N	Mean	SD	t-test	Sig.
Boys	60	7.37	1.30	11.68	0.01
Girls	60	4.8	1.19		

According to table 1, the t-ratio of 11.68 is significant at 0.01 levels which is indicating that the two groups differ significantly on number of life events. The mean score of boys is 7.37 which is more than the mean score of girls which is 4.8. Thus, the null hypotheses no-1, is rejected. According to the mean score of both group, boys experienced on an average 7 life events and girls students experienced 5 events. The result found that on an average, students experienced about six life events in an year life span. Life events are events that cause significant changes in life. It also precedes a number of physical and psychological health problems. They can trigger psychological disorder and may lead to clinical anxiety or depression.

Table 2: Mean, SD and t-value of two groups on subjective distress.

Groups	N	Mean	SD	t-test	Sig.
Boys	60	16.62	1.69	5.39	0.01
Girls	60	18.83	2.70		

Table 2 indicates the results of two groups on subjective distress of life events. The t-ratio of both groups is 5.39 which is significant at 0.01 level of confidence. That means both groups are differing on subjective distress. Thus, the null hypothesis no-2 is rejected. The mean score of boys are 16.62 and girls are 18.83. It can be concluded that the girls experienced more subjective distress than the boys.

Table 3: Frequency, percentage and mean of associated distress of life events.

Stress area	Frequency	Percentage	Mean distress	SD
Education	190	26.03	5.45	0.76
Heterosexual	80	10.96	8.87	0.92
Health	180	24.67	6.08	1.03
Finance	120	16.44	6.25	1.25
Bereavement	80	10.96	4.2	0.82
Family	60	8.22	3.28	0.66
Miscellaneous	20	2.74	1.3	0.63

Table 3 shows the frequency and mean score of all seven areas of stressful life events and subjective distress experienced by students.

The number of events range from 1 to 56 with a mean of 6.1. The distribution of responses were as follows: 26.03% of events were experienced in the area of educations; and 24.67% in the area of health. The number of responses in each area can be found in table 3. Maximum number of events were experienced by students in the area of education (26.03%). It can be seen that the area of education is not subjectively distressing to the students. This may be because the events like appearing for an examination and the change of a teacher or college were perceived by them as being part of their college life. In this area, failure in examination and a loss experienced in any competition are distressing for some students.

There are two areas that are more distressing. Health related events like minor physical illness requiring few days off from college and illness in close family member or friend. In the area of finance, like losing a significant amount of money, or borrowing money from friends (mean-6.08 and 6.25 accordingly) are being more distressing.

In the area of heterosexual relations, the events experienced by students is comparatively less but subjectively perceived as very distressing (mean-8.87 SD-0.92). For example, breaking of a serious relationship, terminating a friendship because of an argument, are more distressing to students.

Gender difference in the experiencing of the events is shown table 1. Male population experienced more events than girls. Events like losing personal belonging/money, borrowing money from friends, sustaining injuries due to an accident, start smoking/ alcohol etc., could reflect higher risk-taking behaviour in boys as compared to girls. But we can also say that the girls are more distressed than the boys by a break in a serious relationship, or terminating a friendship, having a minor physical illness and by the death of relatives.

In the present study a high correlation (0.82, $p< 0.01$) was found between life events and subjective distress. Students feel pressure because the function of education is closely related to the expectation of pupils, parents, teachers, and society. Adjustment is the most common problem they experience.

Generally, college students place the ambition at a very high level and when they fail to achieve their ambitious goals, they become emotionally maladjusted and frustrated. In short, they face psychological problems and distress.

Conclusions

1. Boys experienced, on an average 7 events and girls experienced 5 events.
2. Girls experienced more subjective distress compared to boys.
3. A positive correlation found between life events and subjective distress.

Suggestions

1. It is important to stress the need for intervention programs to be designed to address the stress of college students.
2. Arrange seminar/workshop on conflict management, time management and stress management.
3. Counselling through the counsellor to help the students to establish a positive attitude towards life and learn to develop effective coping styles.
4. Parents should update their knowledge, pay attention to the children's mental abilities and to help them with their career planning and emotional problems. Parents must give love, support and time. And most importantly they must trust them.

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ARTICLE RESEARCH ANNALS OF XAVIERS AHMEDABAD

Flora of Tungareshwar Wildlife Sanctuary, Vasai Taluka (Maharashtra), India

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Abstract: Vasai taluka is situated in the western part of Maharashtra. It lies below the Tropic of Cancer on the North of Konkan region. To its eastern side are Bhiwandi and Wada taluka and to its west lies the Arabian Sea. It is separated from Palghar taluka by Vaitarna River and in the south from Mumbai city by Bassein creek. There is a Tungareshwar Wildlife Sanctuary (TWS - area measuring 8,570 hectares) situated at the eastern boundary of the taluka. Although several taxonomists have done floristic work of Mumbai and Thane districts in past, there were many localities in Vasai taluka which remained unexplored floristically. Thus the floristic work of Vasai taluka was undertaken to study the vegetation types and to record the plant species occurring in Vasai taluka. Frequent field visits were conducted in different seasons of the year from 2012 to 2015 to study the flora of regions of Vasai taluka and Tungareshwar wildlife sanctuary. The outcome of the work is the enumeration of 1845 taxa of Vascular plants. It includes 934 genera and 187 families. About 129 rare species were recorded during the work.

Keywords: Flora; Vasai Taluka; Tungareshwar Wildlife Sanctuary, Maharashtra

Introduction

Vasai taluka is situated between 72.48 - 72.54 E latitudes and 19.24 - 19.28 N longitudes. Its width is 26.5 km from east to west and 32.8 km from North to South. Vasai Taluka has a personality of its own, due to its historical, mythological, social and cultural importance. Its topography shows variations with small hillocks scattered in the eastern and north-eastern part of the region and the western and south western part are considerably plain. The city falls in the Deccan lava terrain. The area can be classified in to three broad topographic zones comprising of (i) Coastal belt; (ii) Plain region; and (iii) The central undulating area. There are 3 main rivers in Vasai taluka, viz. Ulhas, Vaitarna and Tungar. The taluka shows rich mineral deposits like bauxite, common salt and ground water. The soil of Vasai taluka can broadly be divided into two major types, viz the coastal saline and alluvial soils and red and grey soil (occupying most part of the taluka). The climate on the whole is very humid throughout the year. Average annual rainfall in this area is 2000–2500 mm and humidity is 61-86. Tungareshwar Wildlife Sanctuary is situated at the eastern boundary of the taluka (Fig. I. 5). Sanctuary is exposed to Wada taluka in the east and Palghar taluka in the north. It is separated from Palghar taluka by Vaitarna River. To its south is Bassein creek. The western side is habitant to human life followed by Arabian Sea. It is situated between 19° 23' 38" N latitude and 72°58'9" E longitude. An area measuring 8,570 hectares (85.7 sq. km) of Tungareshwar reserved land was declared as Tungareshwar Wildlife Sanctuary on 24th October, 2003 (Gazette Notification) 21,iii and an additional 10 sq. km land was proposed to be declared as reserved and as restored landiii. The forest area of TWS is now about 95.70 sq km. The Sanctuary lies to an altitude of about 2200 feet. The only river that flows through the hills is the Tungareshwar River.

Several Indian taxonomists had done extensive floristic work on the Western part of the country such as T. Cooke (1901-1908), Graham (1839), Dalzell & Gibson (1861), Talbot (1909-1911) and Blatter and McCann (1935). N.Y. Das (1959-62) had made intensive collections from Tungar hills. G. M. Ryan (1902-1905), a forest officer, also made collections from Mandvi and Gokhivare area. In spite of the floristic work done by many known taxonomists in past, many localities in Vasai taluka having TWS remained unexplored floristically. Hence the floristic work of Vasai taluka was undertaken to – I) study the vegetation and II) to list the plant species in this region.

Methodology

Field visits were conducted to explore the area under study in different seasons from 2012 to 2015. A note was made on the common plant species along with other field observations. Specimens (twigs with flowers and fruits) were collected in the cases where plants could not be identified in the field. Photographs and GPS readings were taken for important plants.

Results and discussions

The total number of vascular plants recorded in Vasai taluka was 1845 taxa belonging to 934 genera and 187 families out of which 866 species where reported from TWS.

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Floristic account of TWS

1) The number of families, genera and species reported in Tungareshwar Wildlife Sanctuary from each group is given below:

a. No. of Angiosperm: 116 Families; 493 Genera; 848 Species.
b. No. of Gymnosperm: 02 Families; 02 Genera; 02 Species.
c. No. of Pteridophytes: 09 Families; 12 Genera; 16 Species.

The data analysis shows that:

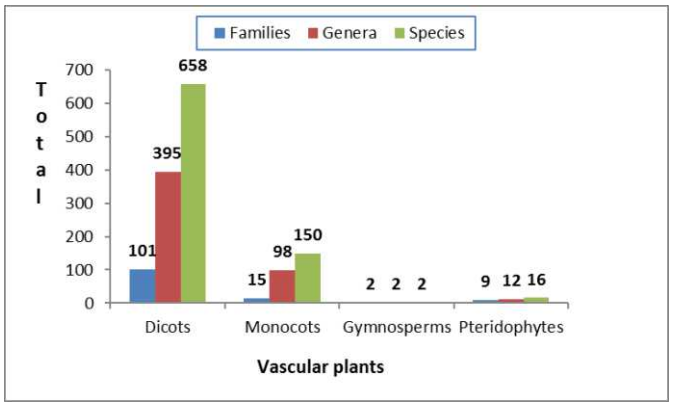

Figure 1 Total number of families, genera and species representing vascular plants.

The above figure shows that the highest number of families (101), genera (395) and species (658) occurred in TWS are from Dicots, followed by Monocots (15, 98, and 190) and Pteridophytes (9, 12, and 16). Gymnosperms recorded in TWS are also very low (2, 2, and 2). They are observed in plantation near the Ashram. TWS shows similar pattern of distribution of Angiosperm as seen in the flora of Vasai taluka.

2) Following chart represents total number of vegetation spectrum of Vasai taluka and TWS to Maharashtra.

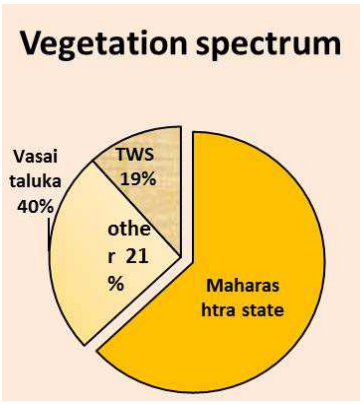

Figure 2 Total plants represented from of TWS to Vasai taluka and Vasai taluka to Flora of Maharashtra.

Out of the total species of Angiosperm recorded in Maharashtra state, 40% (1821) is found in Vasai Taluka and TWS represents 19 % (848) of the of Angiosperm found in Maharashtra state. About 47 % of the vegetation of Vasai taluka is found in TWS.

3) Floristic Spectrum of Vascular plants.

The chart represents total number of species found in Vasai taluka with blue line and TWS with red line:

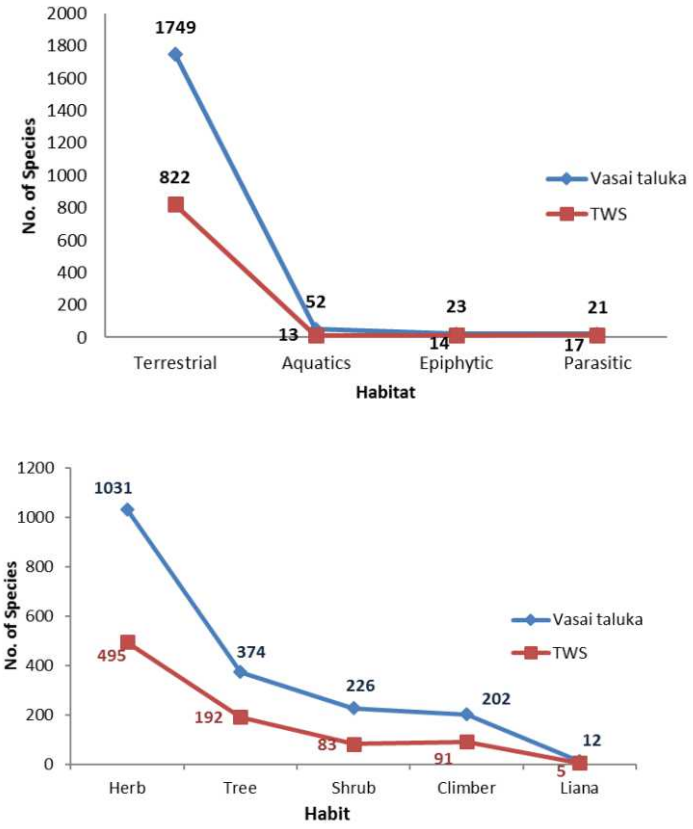

Figure 3 Habitat and Habit distribution in Vasai taluka and TWS

Most of the species recorded from Vasai taluka are terrestrial contributing 95 % of the total flora, aquatic about 3 %, epiphytic 1 % and parasitic plants 1%. As seen in the Vasai taluka.

The plants were segregated based on their habit such as herbs, shrubs, trees, climbers and lianas, represents no. of species per habit for Vasai taluka is given in blue line and red line indicates TWS. It is observed that 56% of total plants recorded are herbs, trees represent 20%, whereas climbers and shrubs are almost equal in distribution (i.e. 11 % and 12%). Lianas are the most contributing only 1% to Vasai taluka. The vegetation of TWS also show similar pattern of vegetation with respect to habit as that of Vasai.

4) Species distribution in TWS:

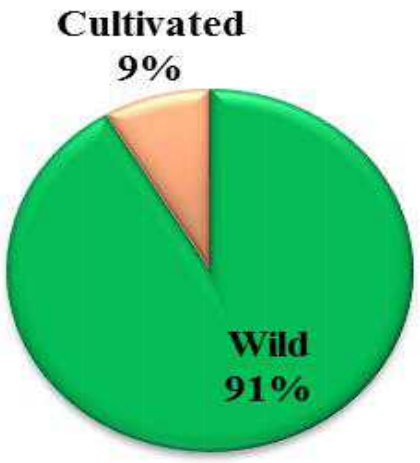

Figure 4 Percentage of cultivated and wild species found in TWS.

The vegetation of TWS represents 91 % of wild species and 9 % of cultivated species. These cultivated species are found near the ashram and some species which are planted by forest department. It is observed that TWS contributes 43% of wild plants to the vegetation of Vasai taluka.

5) IUCN Categorisation of the species represented in Vasai taluka and TWS.

The plant species when categorized as abundant, frequent, occasional, rare and threatened following results were obtained: IUCN categorization of the species found in Vasai taluka and TWS are as follows:

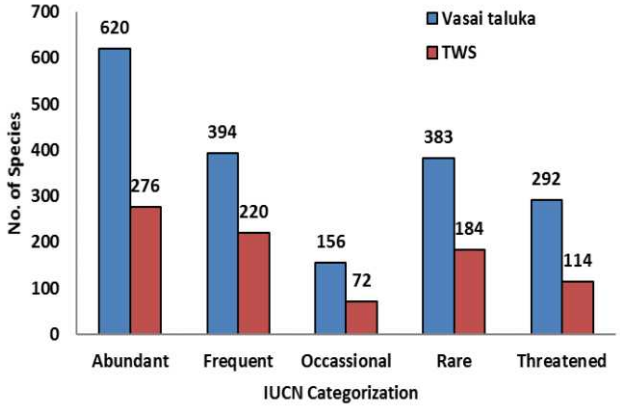

Figure 5 Number of species abundant, frequent, occassional, rare and threatened.

33.6 % of the species are abundant, 21.3 % as frequent, 20.7 % are rare. About 15.8 % of the vegetation is threatened, whereas 8.5 % of the species is found to be occasionally distributed throughout the taluka. About 45 % of rare plants of Vasai taluka are contributed by TWS. Approximately 86% of the plants found in the taluka are economically important. Out of total economically important plants found in Vasai taluka, 29 % are medicinal, 25 % ornamental, 13 % are dye yielding, 12 % are edible plants, 9 % is timber plants, 6 % are aromatic, 3 % are oil yielding and 3 % are fiber yielding plants. It may be noted that 43 % of the timber yielding are present in TWS. Almost 1/3 of the medicinal plants of Vasai taluka occur in TWS.

Conclusion

Vasai taluka is one of the most important talukas of present Palghar (old Thane) district in Maharashtra state, because of its rich vegetation and biodiversity. To the best of our knowledge present treatise is the first comprehensive information on the unexplored wealth of the taluka. The data presented in this work is an outcome of my intensive studies on the vegetation and Flora of Vasai Taluka with special focus on Tungareshwar Wild life Sanctuary (TWS). Vasai Taluka contributes about 1/3 of the flora of Maharashtra. The vegetation represents about 80 % of Dicots and 81 % of Monocots families found in Maharashtra state. TWS contributes 43% of the wild plants to the vegetation of Vasai taluka.

The most dominant genus in TWS is *Ficus Moraceae* - Dicot, among Monocots the most dominant genus in TWS is Cyperus and Eriocaulon respectively. Some of the rare and important species observed in TWS are *Ancistrocladus heyneanus* Wall. ex J.Graham, *Capparis rotundifolia* Rottl., *Cassine glauca* (Rottb.) Kuntze, *Christisonia lawii* Wt., *Clitoria annua* Graham. var. emarginata S.Yadav. & P.B.Dhanke, *Crinum woodrowii* Baker, *Eriolaena quinquelocularis* (Wt. & Arn.) Wight, *Ficus pubinervis* Blume, *Gymnosporia puberula* Law., *Hydrophylax maritima* L., *Mammea longifolia* (Wt. ex Graham) Planch & Triana, *Memecylon umbellatum* Burm.f., *Moringa concanensis* Nimmo, *Pecteilis gigantea* (Sm.) Rafin., *Sageraea laurina* Dalz., *Toona hexandra* (Roxb.) Roem. The endemic species to Tungareshwar Wildlife Sanctuary are *Capparis rotundifolia* Rottl. and *Chukrasia tabularis* Juss. var. velutina (W. & A.) King.

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Density functional approaches to understand the Be and Mg monolayer material

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Abstract: We study density functional theory (DFT) based electronic properties like stabilities, band structure, density of states (DOS), partial density of states (PDOS) of two dimensional (2D) Be and Mg monolayered material. By calculating lattice constant for minimum energy, we have calculated self-consistent field (SCF), non-self-consistent field (NSCF), and calculated band structure. The outcomes of electronic band structure suggest that both are metallic in nature. The PDOS show that both s and partial p-orbitals of Be and Mg contributed near the Fermi energy level.

Keywords: Flora; Vasai Taluka; Tungareshwar Wildlife Sanctuary, Maharashtra

Introduction

Two Dimensional (2D) materials, including graphene, and elemental monolayers, are being proposed for the next generation materials for future technologies via manipulation of their electronic properties. It is expected that various new materials in the form of nanoclusters, nanorods, can be derived by using these nanolayers as building blocks [1-4]. Monolayer structures made up purely one kind of atoms possess many novel properties different from their bulk structures. The best example is graphene, which is honeycomb monolayer of carbon. Since the discovery of graphene, two dimensional layer materials have triggered considerable attentions in nano science and condensed matter physics. The other group IV elements such as silicon, and germanium also form a honeycomb monolayer known as silicene, germanene respectively. Their geometric structures are bulked so their properties are different from their monolayer structures. Electronic, magnetic, optical and mechanical properties of 2D materials have also been reviewed for their emerging applications in the area of catalysis, electronic, optoelectronic and spintronic devices; sensors, high performance electrodes and nanocomposites. Graphyne is another 2-dimensional carbon allotrope whose structure is similar to graphene's. It can be seen as a lattice of benzene rings connected by acetylene bonds. Borophene is a proposed crystalline allotrope of boron. Germanene is a two-dimensional allotrope of germanium, with a buckled honeycomb structure [5-8]. Stanene is a predicted topological insulator that may display dissipation less currents at its edges near room temperature. It is composed of tin atoms arranged in a single layer, in a manner similar to graphene. In the present work, we have discussed the prospect of obtaining the structure and its band structure of 2D monolayer Be and Mg monolayered material.

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Computational Details

We have used Quantum Espresso package for performing a fully self-consistent DFT calculations [9-10]. We have relaxed the structure and then taken lattice constant for the minimum energy. Investigation of various properties and most stable form of molecules or solid the computational methods are required. Different types of computational methods are available, some are based on the wave-function like, Hatree-fock method and MP2 (Moller plesset perturbation); these approximation methods are utilised for the determination of the wave-function and the ground state energy of a quantum many body system in a stationary state. Another method which is based on density like, is the density functional theory (DFT) [9-12]. Compared to other method dealing with the quantum mechanical multi-body problems, LDA give satisfactory results with experimental data. For solid state calculations the pseudopotential is utilized which is different from the exchange-correlation functions. DFT is now a leading method for electronic structure calculations in many areas. The kinetic energy cut-off for plane wave basic set was taken to be 600 eV. The Brillion zone is sampled by using (21×21×1). The convergence criteria for energy in self-consistent field cycle was taken to be 1×10-7 eV. The supercell is periodic in the XY-plane and separated by 20Å slab along the z-direction to avoid the interaction between adjacent planes. Energy minimization in each case was carried out using the standard conjugate gradients (CG) technique.

Results and Discussion

We have optimized 2D Be and Mg structures having bond angle of 120° which is same as graphene structure as shown in Fig 1. However, bond length varies with the choice of the material. Bond length between Be-Be is 2.0207 Å, while bond length between Mg-Mg is 3.8913 Å; which is greater

than graphene C-C bond length 1.42 Å. Lattice constant of Be structure is 3.5Å and Mg structure is 5.33 Å which is also greater than lattice constant of graphene structure at 2.4612 Å as shown in Table 1. The electronic band structures and partial and total density of states (DOS) of Be and Mg with 2 atoms per unit cell, respectively are shown in Fig. 2. The electronic band structure of 2D Be and Mg shows metallic behaviour [4-8]. The band lines crossing Fermi energy level is attributed to quantum conductance in the units of $G_0=2e2/h$, where, e is unit of charge and h is Planck's constant. In the band structure of 2D Be, there are 8 bands lines crossing the Fermi energy level; so its quantum conductance is 8 G_0. Whereas, in case of Mg, four bands lines are crossing the Fermi energy level, which ascribed quantum conductance of 4 G_0. (G_0 = 12.9(kΩ) −1). In the case of Be, the density of states in the valance band is contributed by 2s, partial 2p-orbitals, while conduction band and near the Fermi level is mainly contributed by s-orbitals. But in the case of Mg, the density of states in the valance band is contributed by 3s-orbital, near the Fermi level is mainly contributed by 3s, 2p-orbitals, while the conduction band it is mainly contributed by 3s-orbital

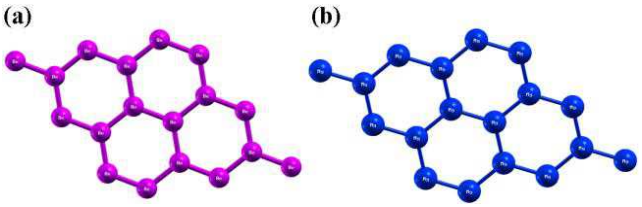

Figure 1 Top view structure of optimized (a) 2D Be monolayer and (b) 2D Mg monolayer.

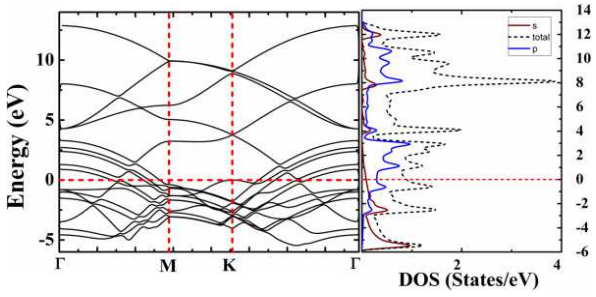

Figure 2 The calculated electronic band structure of Be monolayer and its corresponding partial and total density of states (DOS).

Table 1 Table for calculated lattice parameters of 2D monolayer Be and Mg.

Material	Bond Length	Bond Angle	Lattice constant
Graphene	1.42 Å	120°	2.4612 Å
Be	2.0207 Å	120°	3.5 Å
Mg	3.8913 Å	120°	5.33 Å

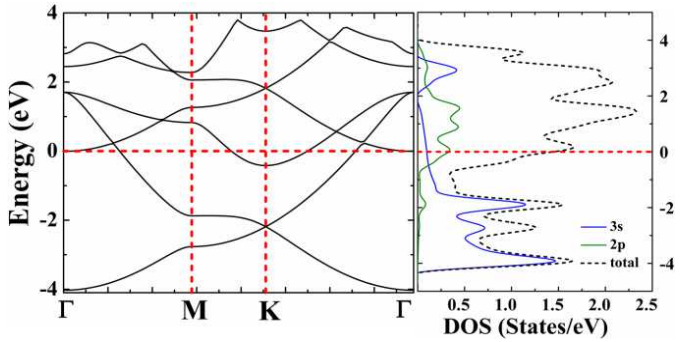

Figure 3 The calculated electronic band structure of Mg monolayer and its corresponding partial and total density of states (DOS).

Figure 4 shows the charge contours of Be and Mg monolayers. From the charge density plots, we are able to distinguish the charge transfer according to their colour. The red colour indicates the charge depletion and pink colour shows the charge accumulation. The iso-lines show the equipotential surface.

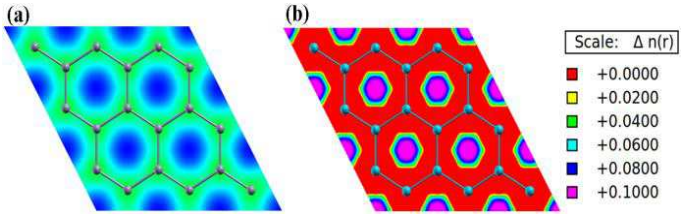

Figure 4 the electronic charge distribution of (a) Be (b) Mg

Conclusion

The electronic and structural properties of Be and Mg two-dimensional graphene like hexagonal material were studied. The electronic band structures show these materials are metallic in nature due to their bands lines crossing the Fermi level. We have observed that in Be 2s-orbital's and partial p-orbital contribution is there near the Fermi energy level whereas in Mg 3s and 3p-orbitals contribute near the Fermi energy level. As a result, pure 2D Be and Mg monolayer system shows superior potential for light harvesting and metallic contacts especially to be effective in enhancing the light absorption.

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