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3D Printing Technology in Pharmaceutical Dosage forms: Advantages and Challenges

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REVIEW ARTICLE

3D Printing Technology in Pharmaceutical Dosage Forms: Advantages and Challenges

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Abstract: Three Dimensional (3D) printing is a promising method for quick prototyping and manufacturing of any material. It is similar to photocopy or printing, where the new materials are formed on layers (3D) like their mother component. Following its growth and advancement in the 1980s, its application in pharmaceuticals is still limited. It has become one of the most innovative and influential tools serving as a technology for developing dosage forms from the last decade. The potential of 3D printing to produce drugs for precise measurement customized to specific patients' needs has shown the possibility of developing personalized medicines to novel dosage forms. The breakthrough allows the clear perception of the dosage structures on different shapes, sizes, surfaces and the associated challenges in delivering them by using such designed conditions. There are different difficulties related to the correct utilization of 3D imprinting in the pharmaceuticals, which have a strong impact on the scope of this technology. Recent advancements in the field of 3D printing technology used in the pharmaceutical industry mainly focused on different techniques for the fabrication of different dosage forms. The Food and Drug Administration's (FDA) recent approval of the first 3D prescription highlights possibilities for 3D printing innovation in the field of pharmaceutical drug supply. This analysis assesses 3D printing advancement possibilities, particularly in the area of custom prescriptions. This technology can be regarded as the future produced on demand, low-cost solid dosage forms and helps minimize side effects due to overdose.

Keywords: 3D Printing, Fused Deposition Molding, Inkjet Printing, Stereolithography, Personalized Medication.

1. INTRODUCTION

Three Dimensional Printing (3DP) has become one of the most innovative developments in the area of pharmaceuticals. The development of drug delivery has increased dramatically over the last decade [1]. The 3D print refers to a different approach for fabricating solid objects, known as the additive manufacturing process. In 3D printing, successive similar layers of the mother component are formed using sophisticated control systems [2]. 3DP is now one of the fastest-developing technologies in the creative and engineering industries, expanding its application [3]. International Standard Organization (ISO) describes 3D technology as manufacturing items by depositing a printed head, Nozzle, or other printer technology content [4]. The 3D model is used for the preparation of parts by layer in materials connection in this technique. 3DP is also used in the latest drug delivery method for viable tablet growth. The tablets were thus produced to satisfy regulatory tests and commercial tablet standards [5].

In the last decade, the focus was on developing patient--driven medicines that remained a novel type of dosage form [6]. The vital progress of personalized drug communications is used to drive interest in custom devices combined with the expansion of technological innovation. For example, the generation of small, exclusively selected doses and custom prosthesis meets the patient's anatomical needs. Inside numerous disclosures brought into the pharmaceutical and biomedical market, 3DP is the foremost progressive and compelling. This strategy has a versatile instrument of tangible assembling of various gadgets. It is an advancement to create new dosage formulations, engineering tissues and organs, and model diseases. The use of 3D printers has increased significantly in the last decade. Global sales of consumer printers have increased by more than 33% [7]. Various 3DP technologies were found to generate the most common, distinct, and modern solid dosage types [8, 9]. Additional production is a subset of quick prototyping, involving techniques for the fast development of models and prototypes, but now it is a scalable production process [10]. 3D

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printers and other additive manufacturing technologies have increased steadily over the last thirty years in the engineering industries across biomedical applications [11]. The utilization of those technologies for improving drug delivery systems has been investigated and more recently realized with the FDA approval of the 3D printed or dispersible tablet. Moreover, supporting the skills of 3D printing to fabricate complex and customized dosage forms is also exploited [12]. The advantages of utilizing additive production technology for drug delivery means the ability to track the spatial dispersal of an active pharmaceutical ingredient (API) accurately within a dosage form, creates complicated geometries, stores modest API numbers, reduces rapidly growing variability [13]. The pharmaceutical industry can reduce traditionally complex, lent, and expensive supply chains, minimize the production and stock waste, and allow individual methodizing methods with no significant amounts to be generated [14]. 3D printing has also quickly developed and revolutionized the healthcare sector [15]. The medical applications of 3DP include customized prosthetics, body tissue, manufacture of organs, dental modeling, pharmaceutical research on the forms of medicinal dosage, delivery of medicines, and discovery [16]. The principle behind a 3D printer is similar to a regular printer [17, 18]. The 3D printer is a horizontally moving extruder that moves on a double axis, allowing it, on the x-y level, to move back and forth to form the object base [19]. Both axes are fixed to the printer ends. The only difference is that the 3D printer is based on the z-axis to generate the layers above the object vertically [20]. The extruder remains at the top and only moves in Two Dimensional (2D) while printing the first layer. The base containing the substratum decreases in height to allow for the next layer. The cycle is repeated in computerized formulation until the object is layer by layer built [21]. This method is known as the development of additives, Rapid Prototyping (RP), or solid freeform (SFF) technology. For printing various controlled chemicals, interconnected porosity and specific porous scaffold forms are employed by 3D printers. These graphics are biologically degradable and are ideal for supplying drugs [22]. This technique allows some of the highly complex structures containing live cells to become more frequent and accessible in cancer treatment [23].

2. HISTORY OF 3D PRINTING

3DP covers a wide variety of processes and techniques, providing a full assortment of manufacturing capabilities in various materials for parts and goods. The principles of 3DP processing by layer compared with conventional production methods that involve subtractive processes or molding processes are the same [24]. 3DP applications emerge almost daily, and as these technologies continue to penetrate the industrial, manufacturing, and consumer sectors more broadly and more profoundly, they will only grow. Many well--known experts in this technology field believe that the true potential of 3D printing has only just started to appear [25]. The 3D Printing industry, a credible 3D print media outlet, brings new perspectives, process innovations, and applications as they arise in this exciting field [26]. This review article aims to provide the 3DP industry with a comprehensive backgrounder on 3D printing [27].

The early 3D printer technology known as RP technology was the first technology to become apparent in the late 1980s [28]. Initially, the processes were designed to create prototypes for product development as a fast and cost-efficient method [16].

The 3D printers concentrated on improving the design and functional prototyping of prototypes, which were designed expressly as office-orientated and economically efficient devices, the prelude to today's mobile computers. However, all the contrivances for industrial applications were also widely used. On the lower end of the market, a mid-range price war today, with rapidly improving print accuracy, speed, and materials, emerged. In 2007, 3D systems had the first $10,000 system in the industry, but this never really reached the mark, partly because of the system itself and certain other factors. The Holy Grail would then have a 3D printer less than $5000 - many business experts, consumers, and commentators saw the catalyst for opening 3D pressing technology for a broader audience [32]. The organization went wrong during the launch and it came to nothing. With the IP 3D Systems acquired from Desktop Factory and its information, Cathy Lewis, in 2008, and everything was slightly lost. “Dr. Bowyer invented an open-source, self-replicative, 3D printer idea of RepRap in 2004” and the following years. In particular, Vik Oliver and Rhys Jones used the elimination of the 3D printer technology to refine and create prototypes. The shooting started in 2007, and this early development in open-source 3D printing became more apparent.

Nonetheless, the first commercially available, kit-based 3D printer based on RepRap was not released until January 2009 [33]. In April of the same year, the creators of MakerBot Industries were very interested in creating RepRap until, after significant investment, they abandoned the open-source philosophy. A host of similar film printers have grown since 2009 and continues to do so with marginal unique selling points (USPs). The fascinating dichotomy here is that while RepRap's trend has created a whole new business field for entry-level 3D printers, RepRap's ethos culture is about using open sources for 3D printing and keeping marketing under control! 2012 marked the beginning of market entry for alternative 3D printing processes. In 1984, Charles Hull first created the technology for the printing of digital 3D physical objects. He called the process a “stereolithography and, in 1986, secured a patent”. Stereolithography systems were popular in the late 1980s, but related technologies, such as fused deposition machines and selective laser sintering, were introduced. The process, called “3-dimensional printing techniques,” was patented by the Massachusetts Institute of technology in 1993, was close to inking-jet technology used in 2D printers. Three primary devices, Stratasys “Genisys,” 3D Systems “Actua 2100,” and Z Corporation's “Z402” were launched in 1996. In the first high definition 3D printer on the market, Z Corp. released an interruptive Spectrum Z50 in 2005. The launch of an open-source project called Riprap, to create a free 3D printer, was another advance in 3D printing in 2006.

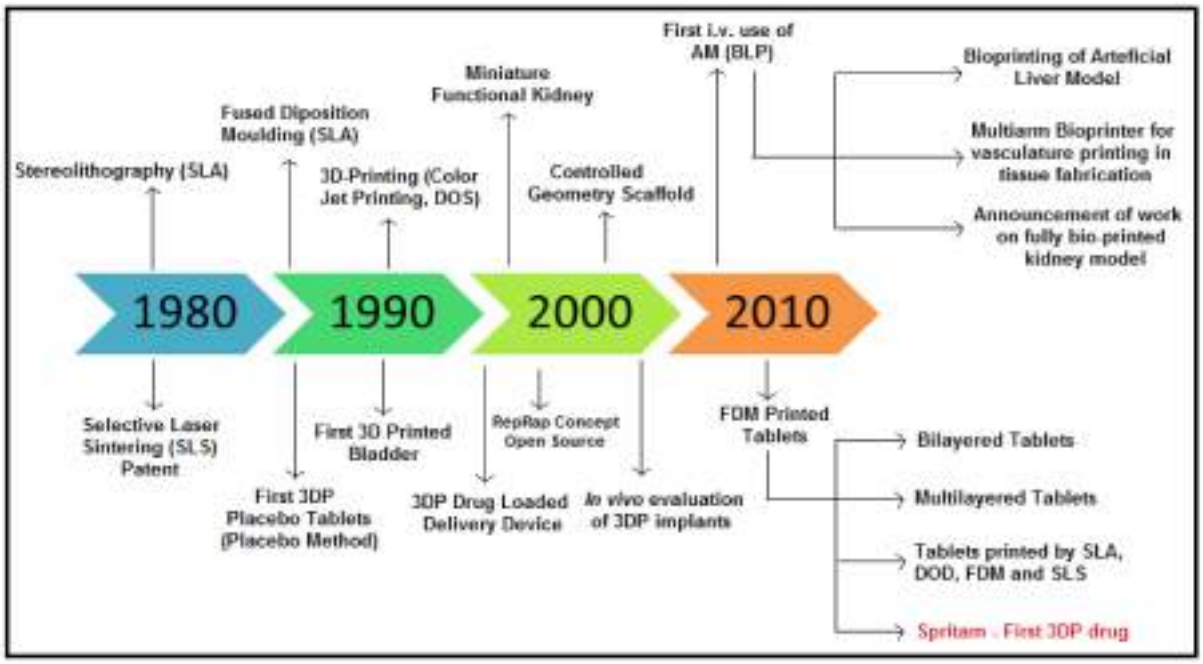

Fig. (1). History of 3D printing in pharmaceutical dosage forms.

3D printed pyramid-shaped tablets, for example, can become famous as a quick-acting treatment method, and that drugs are released quicker than standard dosage forms. The US FDA launched Spritam, the world's first 3D drug, to treat epileptic seizures in adults and infants. The drug developed with the patented Zip Dose technology of Aprecia Pharmaceuticals based on current 3DP analysis to create materials for quick melting. The technology would play a crucial role in advancing small volumes, custom pharmaceutical medical innovations, and making dosage formats more versatile. A move forward could encourage physicians to improve care upon request by creating personalized dose schemes in clinics and pharmacies. Further, patients can utilize customized medicine with various colours, tastes, and forms to improve adhesion. Fig. (**1**) demonstrates the most impressive improvement in the field of pharmaceuticals by 3DP.

3. 3D PRINTING MATERIALS

The technology would play a crucial role in advancing small quantities, custom pharmaceutical innovations, and making dosage formats more versatile. A move forward could encourage physicians to improve care upon request by creating personalized dose schemes in clinics and pharmacies [34]. Nylon or polyamide plastics (powder or filament form) commonly used in fused deposition printing. The 3DP technology was proved reliable for robust, versatile, and durable plastic materials. It is naturally white, but it can be coloured-pre- or post-printed [35]. This material (in powder format) can also be mixed with powdered aluminium to produce another regular 3D printer for sintering aluminide [36]. Another common 3D-printing material, Acrylonitrile Butadiene Styrene (ABS), is commonly used in filament form in 3D input stages of fused deposition machines. It comes in several colours and is especially strong in plastic. ABS can be collected from a variety of non-owned sources in the form of a filament. Polylactic acid is a biodegradable plastic material and became popular for 3D printing [37]. Polylactic acid can be used for stereolithography and the fused deposition machine cycle in the resin format. It is available in various colours, including transparent, proven useful in some 3D printing applications. It is not as durable or versatile as ABS. However, Lay wood is a 3D printing medium designed specifically for 3D extrusion printing at the entry-level. The wood or polymer combination is available in filament form [38].

In the industrial 3D printing sector, an increasing number of metals and metal composites are used. Aluminium and cobalt products are two of the most popular materials used in 3D printing. Stainless steel is one of the strongest and most used metals in 3D printing in powder for sintering, melting, and eating. It is, of course, silver, but it can be plated with other materials to give a bronze or gold effect [39]. The organization's business model is radically different from that of other 3D printing firms, which allows a system to obtain an average cost, but emphasizes a readily available cost--effective supply of material that can be bought on the market. 3D printed paper models are safe, eco-friendly, easy to recycle, and do not require post-processing. A tremendous amount of research is conducted into the potential of 3D printing biomaterials for a host of medical (and other) applications. Various leading organizations improve live tissue, including human transplant systems and external body substitute tissue printing [40].

4. THE PRINTING CYCLE

3D printing is a simple, highly automated process [41]. The air inside the printer warms initially to create an ideal 3D printing operating environment. The machine immediately fills the building chamber with a powder coating of one ounce (3.18 mm) to make it simple to remove the components. The computer can also perform an automated head alignment routine. This procedure consists of a template printing onto the material, electronic eye reading of the template, and subsequent aligning of its imprint heads [42].

Printing-once the pre-build routine is complete, the printer immediately begins printing the layers created in the "ZPrint" software. This machine stores powder in the machine's back from the trigger, spreading the thin layer of 0.004 inches (0.1 mm) over the building platform forward. The print carriage is then moving over that sheet, depositing binder in the template for the first slice sent from "ZPrint" (and various inks for the colour model). Within that cross--section, the binder consolidates the powder and leaves the remaining powder dry to be recycled. The piston lowers under the chamber and prepares for the next layer, lowering the pulverizing bed by 0.004 inches.

Depowering or recycling-when finished, the model is suspended in powder to cure. The unit automatically extracts most powder from the model at the end of the curing operation by applying vacuum and vibration pressure to the building chamber base. Pneumatically, the loose powder is transferred through the device and collected into the hopper for further use. Any powder filling, removal, and recycling system require an airborne particle-containing device supporting a closed-loop system with constant negative pressure. 3D printing works when the user clicks "3D Print," warms the printer and fills the building chamber with building content. The printer begins making a pattern and deposits a powder film. The print wagon travels in the first slice pattern through this sheet that deposits binder (and colour inks). Through cross-section, the binder solidifies the material, allowing the remaining material to be recycled dry. The piston lowers the powder pad and prepares for the next layer after each layer below the building chamber. The process goes on to the completion of the pattern. The cycle continues until the model is complete. After completion, the model is suspended in powder to cure. At the end of curing time, the machine automatically vacuums most of the powder from around the model and recycles it for subsequent builds.

If all powder traces of the components are removed, they can be used in post-process treatment to strengthen further or improve the finish. This process is called infiltration, and it can be decided if or how to infiltrate by using the model. All is free, clean, fast, and efficient to infiltrate our items. Infiltrates are secondary resin material, usually drizzled or broken onto the model sheet. This infiltrate fills the microscopic pockets of the model, screens its surface, improves colour saturation, and improves the model's mechanical characteristics [43].

5. ADDITIVE MANUFACTURING TECHNOLOGIES

5.1. Laser-Based Writing Systems

It works on the principle of photo-polymerization, which releases free radicals after interaction with UV light from a photoinitiator [44]. Photo-polymerization requires ultraviolet or other reliable sources of light for exposure of liquid resins to polymerization reactions. The main restriction of this technique is the need for photopolymerizable raw materials, which are relatively rare in pharmaceutical production. The uncured content is chemically distinct from the printed product and may also contain functional groups plausibility of structural warnings to genotoxicity—the residual Resin, which poses a toxicology risk.

5.1.1. Stereolithography

The enterprise in the late 1980s was stereolithography (Fig. **2**) as a reliable free-form production technology [45]. The stereolithography technique involves the curing of photosensitive materials to create 3D artifacts (photo-polymerization) [46]. SLA uses the digital mirroring tool for scanning a concentrated ultraviolet (UV) layer over a photopolymerizable liquid, causing a photopolymer to gel in an exposed area; the chemical reaction is initiated [47]. The whole layer of the object is assembled repeatedly by this method. However, this method poses a health risk in the form of possible carcinogenic resins. It is also a long process [48]. The SLA presses consist of an ultraviolet light pulse, such as a laser that transfers energy to a liquid resin [49].

A UV lamp, XYZ point, shutter, lens, and computer are used in this device. To create a 3D microstructure, thin cross-sectional spliced forms of the final product are constructed using the transparent Z-stage UV beam based on photopolymers. They are lined up to build the real 3D layers [50]. The lifting step slips to another liquid pitch layer's tallness when a layer setting starts the technology again before the assembly of the 3D product finished layer-by-layer [51]. The thickness of the deposits here depends on the strength of the UV light that discloses tar [52].

5.1.2. Continuous Layer Interface Production

This is a technical advancement in terms of printing speed [53]. However, the process comprises the non-layered development of the 3D structure. The Resin tank is containing oxygen that allows photo-polymerization more straightforward and guaranteed [54]. It is similar to jetting material where a powder bed is not needed [55]. There is no need for a powder pad. Inkjet solidifies the masses by the drop-in method to form the structures [55]: molten waxes, UV resins, and complex multi-component fluids used as jetted materials. For jetting and rapid solidification, the whole kit should be formulated [56]. Wang *et al*. used the stereolithography for fabricating 4-aminosalicylic acid and paracetamol-loaded tablets for modified-drug release characteristics using polyethylene glycol diacrylate a resin and diphenyl (2,4,6-trimethyl benzoyl) phosphine oxide (DPPO) as a photoinitiator. Tablets with different quantities of polyethylene

glycol 300 (PEG 300) were successfully printed, and an improvement in the content of PEG300 revealed a 10-hour improvement in drug release studies [25].

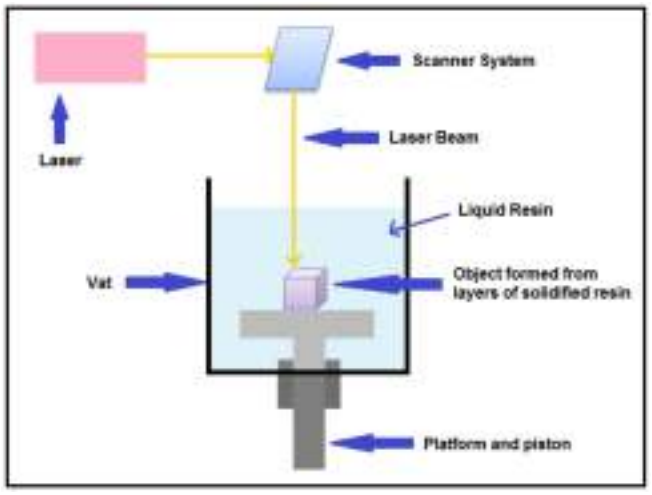

Fig. (2). A representation of stereolithography printer. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

5.2. Powder Based 3D Printing

This technique uses powder jetting or a powder bed to spread thin layers of powder simultaneously with the aid of inkjet printers [57]. The tint (binders and APIs, or binders) is distributed over a 2-dimensional powder bed to create the finished product in layer after layer [58]. It is simpler for this technology to be adapted to pharmaceutical production than other technologies, as powder and binder solutions are commonly used in the pharmaceutical industry [59]. This approach often has its inconveniences. To remove solvent residues, additional drying is required. During printing, excess powder builds up and contributes to wastage. Also, because of the porous powder structure, the drug delivery system's mechanical strength is reduced [60]. This approach can be used for the manufacturing of Orodispersible dosage forms in the pharmaceutical industry.

5.3. Nozzle-Based Deposition Systems

Nozzle-based systems (Fig. **3**) comprise pre-3D printing blending of medicines, polymers, and other solids [61]. Dust is passed through which the three-dimensional object starts, layer by layer. In the case of the type of material used, there are three styles of printing: inkjet printing, fused deposition manufacturing with melted parts, and micro-assisted pressing syringes without using molten materials [62].

5.3.1. Inkjet Printing

This technique begins with a similar method of a personal computer (PC) operating inkjet printing—custom drug treatment [63]. The ink supplants with a pharmaceutical system containing medicines and ordinary paper with edible sheets called substrates was adjusted for pharmaceutical application [64]. Dose modifications by modifying the number of layers in the printed zone area. The drug and excipients are formulated in such a way that they can print on a rich substratum as microdots [65]. Warm inkjet printers and piezoelectric inkjet printers are the two significant print styles used under inkjet printing [66]. Two kinds of techniques are implemented in print-based inkjet frameworks: Continuous inkjet printing (CIP) and Drop-on-Demand Printing (DOD) [67].

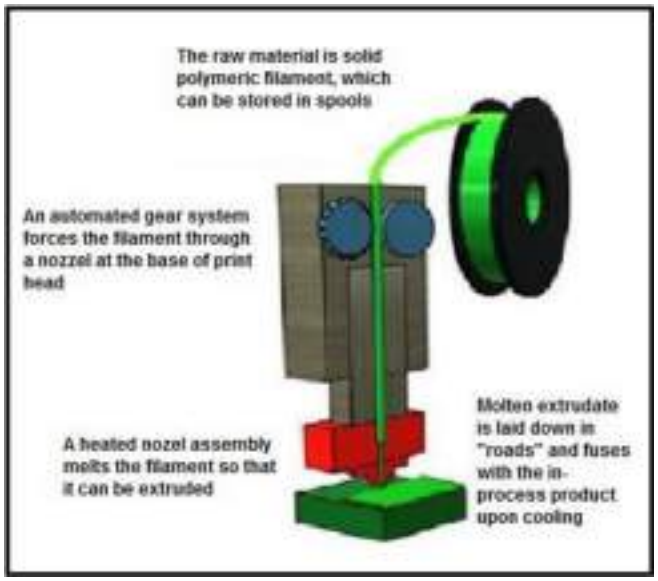

Fig. (3). A representation of nozzle based 3D printer. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The liquid ink is guided through an aperture with a diameter of 50-80 μm to produce a continuous flow of ink while printing continually [68]. The fluid flows and breaks down at a certain speed and scales with a piezoelectric crystal at regular intervals. Electrostatic regulates the formation of the 3D object. To reduce electrostatic repulsion, the droplets are filled and separated by "guard droplets." The induced electrostatic field leads the loads to the substratum [69].

The drop-on-demand technique includes several heads and contains two types of translators, a thermal head or piezoelectric crystal. The thermal head is only limited to gases, and the piezoelectric cuts a wide variety of fluids [70]. The thermal head often reaches temperatures to 300°C, which means it will cause degradation of bioactive compounds due to high vapour pressure. This limitation of the use of thermal heads in drug use [71]. The piezoelectric crystal changes quickly, but this can lead to a sudden change in volume [72]. All heads can produce drops between 10 and 50 μm, equivalent to an amount between 1 and 70 pL. Due to their ability to work at room temperature using less volatile and compliance fluid, Piezoelectric Printing Technology is best suited to design medicines [71].

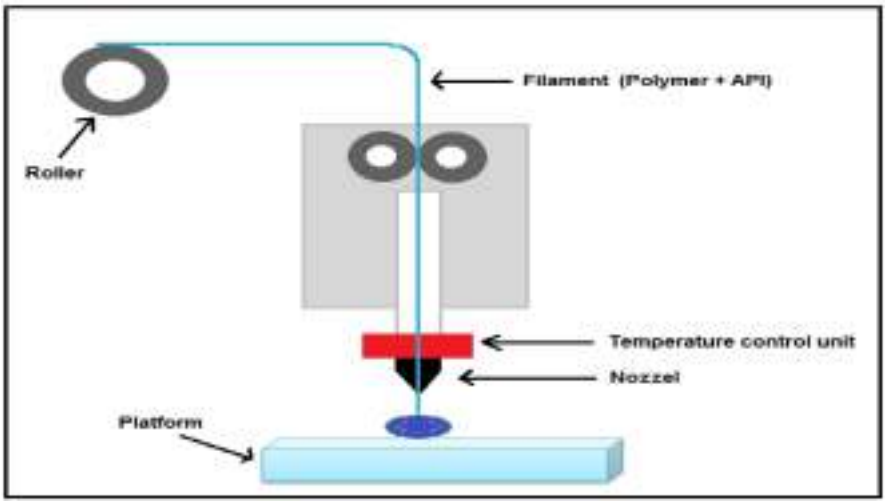

Fig. (4). A representation of fused deposition modeling 3D printer. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

5.3.1.1. Inkjet Printing Ideal for Minimal Doses of the Therapeutic Agent

For the multi-layer print in a specific region, it is challenging to formulate higher doses through this technology in the form of a longer drying time. The rising Part to address this problem would also increase the dosage type size. So, by using this technology, unique dosage forms are manufactured in the pharmaceutical industry [73].

5.3.2. Fused Deposition Modelling

In this technique, extrude a thermoplastic filament into a semi-solid layer by layer fusion filament *via* a high-temperature nozzle shown in Fig. (**4**) [74]. The item consists of layers of molten or blocked thermoplastic filaments extruded to different directions by computer software from the printer [75]. The material is then heated just above its softening point, extracted by a piston, and layer by layer is deposited and solidified in a second. Due to this fused filament, drug loading is also called filament incubation, and poor filling of medicines can reduce its use to lower dose medicines [76]. Xuyu Chai *et al*. used 3D modeling for fusion deposition to prepare to float intragastric domperidone tablets [77]. Using hot-melt extrusion, the medication is inserted in Hydroxypropyl Cellulose (HPC) filament. The filaments have then been printed in select tablets with modification of shell numbers and penetration estimates. Skowyra *et al*. have shown that the fusion deposition can be modeled in printing Polyvinyl Alcohol (PVA) filaments up to 24 hours after oral administration, with prolonged drug release of prednisolone [78]. The ability for extended-release fuse deposition modeling has been demonstrated for 5-Aminosalicylic acid (5-ASA) or 4-ASA. A significant limitation on the use of the fused deposition model is that the high temperature required for operations (~220°C) may degrade the potential of fusion deposition modeling for extended-release to a significant number of pharmaceutical excipients and active drugs. Pietrzak *et al*. bridged 3D extrusion technology to expand the variety of polymers that can be adapted for fused deposition modeling and increase the loading of drugs [79]. They showed that theophylline cells based on cellulosic or methacrylic polymer filaments could be printed immediately and extensively, with 50% of the drug loading. For processing to be possible, materials must have excellent rheological properties. Such properties are determined by the dust diameter, pressure drop, feeding rate, *etc*., which are related to the thermal properties of feed materials such as thermal conduction, density, or glass transition temperature.

5.3.3. Pressure-Assisted Microsyringe Technology

This technology employs a syringe extruder, which uses a pressure piston to deposit viscous material. These deposits in the default geometry layer by layer [80]. The main parameters which determine the robustness of the article are viscosity, viscoelasticity, and apparent elastic limits. Advantage: continuously flows and works at room temperature Disadvantage: the use of solvents could threaten the health and degrade the active ingredient of pharmaceuticals [81].

5.4. Semi-Solid Extrusion 3D Printing

Extrude semi-solids into a substance through the moving stage layer by layer. This technique uses a syringe tool head to inject layer after layer of semi-solid material. The semi--solid gel or paste is a polymer and solvent mixture in a ratio that makes the semi-circular consistency ideal for printing [82]. Okwuosa *et al*. manufactured tablets for immediate release through 3D printing to provide a powerful tool for personalizing the dosage type on demand. They also reported using pharmaceutically approved and soluble polymer in the manufacture of patient-sized tablets at a relatively low temperature (110^{o}C). This work confirms that Fused Deposition Modeling (FDM) 3D can be used in a broader range of temperatures for immediate releases when needed. The material

used should be in gel or paste form, as the method requires extrusion. During drying, the deformation can be induced or decreased. If the dosage layers are not sufficiently hardened, the following layers cannot be borne in weight during printing, causing the supply collapse [83].

6. DESIGN OF VARIOUS DRUG DELIVERY SYSTEMS USING 3D PRINTERS

3D printing techniques are used in the production of several dosage forms, from the immediate release to the distribution of the osmotic drug, such as conventional manufacturing technology. Below are some recent attempts to develop different types of oral dosage by 3D print.

6.1. Immediate-Release Tablets

For immediate release of the pill, a drug filament and a hydrophilic polymer are prepared with or without plasticizers. The widely used hydrophilic polymers can be picked like povidone, hydroxypropyl-methylcellulose, or newly used graffiti polymers like Soluplus ®. These filaments are fed to an instant release tablet using an FDM-based 3D printer [26]. Okwuosa *et al*. manufactured polyvinyl-pyrrolidine, triethyl citrate as a plasticizer, and talc as a filler tablet at ratios of 10%, 50%, 12.5%, and 27.5% wt., for direct releasing of theophylline and dipyridamole. More than 90% of the drug was dissolved in 30 minutes, showing the usefulness of 3D printing, informing the immediate-release tablet for both drugs with a 10% load [83].

6.2. Orodispersible Films

Jamróz *et al*. manufactured Orodispersible aripiprazole firms using polyvinyl alcohol as a polymer through FDM-based 3DP technology. The decay time for placebo tablets was 43.00 ± 1.00 s as opposed to 27.50 ± 4.23 s. In contrast, to cast films, the conversion of Aripiprazole into morphine and a high surface of printed films showed more substantial dissolution; however, the mechanical characteristics of coiled films were negligible. 3D films were printed better because of their permanent foundation [84].

6.3. Floating Drug Delivery System (FDDS)

Chai *et al.* developed floating drug delivery systems by FDM-based 3DP [20]. FDM 3D printers' critical parameters are shells and infills, which describe the outline shape and structure of the object's emotional support. At least one shell is required to print an item, and extra shots add strength and weight to the body but take more time and material to print. Likewise, the infill rate is another parameter that can be adjusted from 0% to 100% to generate the entity from a hollow structure to a solid structure. In this study, the optimized 0.77 g / cm^3 tablet configuration with its two shells and 0% infill had dissolution capabilities above 10 hours, while the more than 20 percent coated shells had densities above 0.9 g / cm^3, which caused them to sink in less than 1 hour. The sample was more than 10 hours after dissolution. Dissolution tablets over 12 hours, and no significant effects were observed in the number of coats or the infill stage [77].

6.4. Monolithic Sustained-Release Tablets

Sustainable release tablets of 5-ASA manufactured with medicinal filled filaments of Polyvinyl Alcohol. Filaments were produced by loading drugs from their ethanolic solutions on the market, all available polyvinyl alcohol filaments. The dissolution in the Auto pH System controlled bicarbonate buffer tablets 5-ASA (pH 6.8) showed a 100% release over four hours in tablets with an infill rate of 90 percent. Reduced infill percentage increased the manufacture of goods. During the preparation of the tablet, 50% of 4-ASA degraded to PVA filament due to high extrusion (210°C). Consequently, thermolabile medication this approach cannot be appropriate. A lower extrusion temperature alternative polymer can help to minimize product degradation due to temperatures [85].

6.5. Pulsatile Drug Release Tablets

ChronoCap® is a pulsatile delivery system based on capsular design. Capsules with various densities fabricated using hydrophilic polymers with a technique for molding injections, which creates different degrees with a time lag [86]. Through 3D HPC filament printing, such capsular devices are possible too. Melochhi *et al.* studied the behaviour of these 3D print and injection molding capsular devices [87]. It was reported that a lag was seen in 3D printed applications before the drug was released in the targeted area. Also, morphological changes corresponded to the technique of injection molding. The work also revealed that 3DP could be used instead of using traditional injection molding technology.

6.6. Bilayer Tablets

Khaled *et al*. prepared two-layered guaifenesin tablets using 3D printing to equate them to the bi-layer tablet brand--Mucinex®, which is available on the market. The two-layered tablet was created with immediate release and a continuous release layer. Immediate-release layer (IR) is printed with microcrystalline cellulose (MCC) as a diluent and sodium starch glycolate (SSG) as a super disintegrant polymer. They were sustaining release layers printed with Hydroxypropyl Methylcellulose (HPMC 2208) and poly (acrylic acid) (PAA) polymers. 3DP tablets with two printing heads were used to make the tablets. Formulation of 14% w/w of hydrophilic polymer HPMC 2208 with 2% as binder showed a dissolution profile faster than but not significantly different from that of Mucinex®. Increased dissolution of HPMC 2208 showed a decrease due to the formation of a thicker hydration gel barrier that reduces drug release [88].

6.7. Multi-Active Solid Dosage Forms

Shaban *et al*. fabricated a tablet that contains nifedipine, captopril, and glipizide using room temperature extrusion-based 3D printing with separate controlled release profiles [89]. The same research group has recorded a single tablet that immediately releases aspirin and hydrochlorothiazide and continues to release atenolol, pravastatin, and ramipril *via* extruder processes [26].

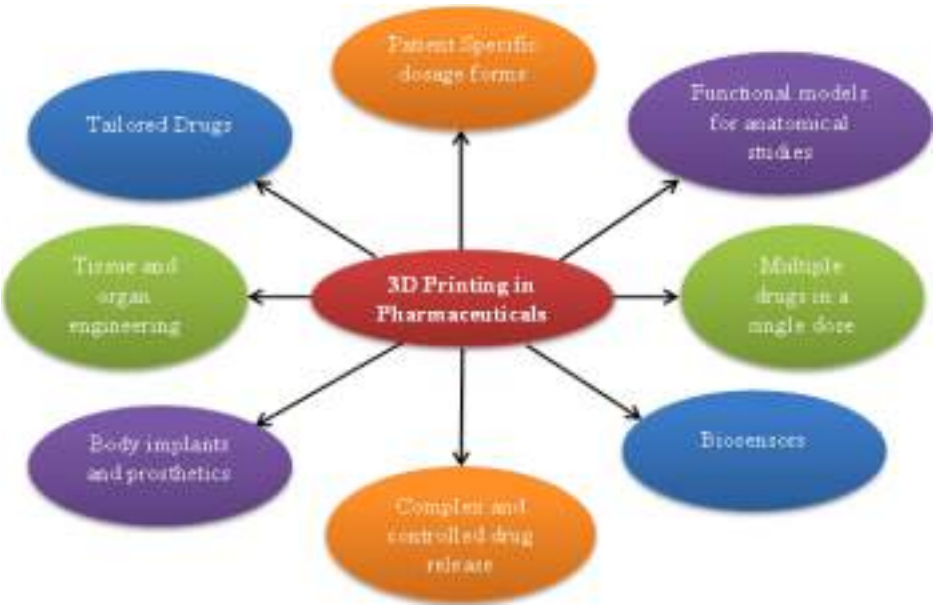

Fig. (5). Application of 3DP in the area of pharmaceutical sciences. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

6.8. Fast-Disintegrating Tablets

Yu *et al.* prepared a rapidly disintegrating tablet having loosely bound powder in the core area of the printed binder system. Three phases of printing created the hollow core area by printing binder solutions: a rugged circular area for a basis, various ring layers for a cave, and a solid circular area to be finally covered. There were just 21.8 s with a hardness of 54.5 N/cm^2 during the disintegration [90].

6.9. Nano Capsule-Based Formulation

3D-equipped printed tablets were developed by Gazzaniga *et al.* with a Nano capsule deflazacort 138 nm particle size. They were using the commonly used FDM. In filaments Poly (ti-caprolactone) (PCL) and Eudragit RL100, they are channeling agents with or without mannitol. Subsequently, tables were soaking into a particular volume (2 ml per unit) and dry for 24 hours at 30 degrees C for polymer nanoparticles. For 24-hour drinking of the bottle, up to 0.62% of the drug load was reached [91].

6.10. Transdermal Delivery Systems

It can be useful to prevent first-pass metabolism or pH--mediated degradation or to facilitate the smooth administration of patients with chronic diseases like diabetes. 3D printing techniques can easily be used layer by layer to produce multi-layered transdermal film patches; for a manufacturer's transdermal pharmaceutical microneedles, 3D technology offers a unique advantage. The micro-adhesives are typically less than 500 mm high and will penetrate the corneal stratum (10-15 mm) to provide active agents [92]. The insert forces of 0.1 to 3.0 n were measured, and the use of 150 mm microneedles with 26-gage needle controls showed little to no discomfort; these needles should not be enough for the epidermis but should not be too difficult to cause pain and irritation [93]. Biodegradable polymers are preferred for the manufacture of needles if the tip breaks and is impregnated in the skin. The size, nature, and number of microneedles render making and painting a complicated geometry but suited to 3D printing techniques [93].

Boehm *et al*. The preparation of drug-loaded micro-needles used two additive fabrication processes. SL has been used to manufacture polymers, biodegradables polymers, and inkjets, used as an active agent for the model for the quantum dot needles [94]. Boehm *et al*. later applied stereo and inkjet techniques for generating amphotericin B packed, miconazole-laden antimicrobial-clogging micro-needles and demonstrated adequate transdermal resistance [95]. There have been some improvements in the surface and structure in micro-needles caused by printing of the ink wetting of the micro-needles, covered by a quantum dot and amphotericin B solutions. The deposition focused on the end of the needle than on the needle top, and the Miconazole tests had less influence on the structure of microneedle [96].

7. PHARMACEUTICAL APPLICATIONS FOR 3D PRINTING

3DP pharmaceutical applications are fast expanding, and health care should revolutionize. 3DP technologies implemented in pharmaceutical research and development. The advantages of 3DP include accurate control of the droplet size and dose, high reproductivity, and complex drug-release profiles to produce dosage forms. The technology of 3DP facilitates the standardization, accessibility, and feasibility of complex drug manufacturing processes. In the development of personalized medicine, 3DP technology is also a powerful resource. The applications of 3DP are listed in Fig. (**5**).

7.1. Personalized Medicine

The aim of the medicines' development should be to enhance effectiveness and decrease the risk of adverse reactions. The objective is to produce personalized drugs with 3D printing. Oral tablets are the most common type of medication because of their simpler manufacturing, pain relief, exact dosage, and strong patient adherence. However, there is no workable way of manufacturing customized, solid dosage forms like tablets regularly. Oral tablets are usually produced by well-known methods, such as grinding, milling, and dry and wet granulation through pumping or molding ingredients into tablets [101]. These steps can lead to problems, such as degradation of drugs and changes in shape, which can lead to formulation problems or batch failures.

Moreover, these conventional methods of manufacturing are ineffective for personalized medicines. It reduces the ability to create custom dosage forms with highly complex geometries, new drug release profiles, and longer-run stability to create custom dosage shapes [77]. Clients who are known to have pharmacology or are using medicines with limited therapeutic indices may benefit from personalized 3D drug prints. Pharmacists may evaluate the pharmacogenetic patient profile and other factors such as age, breed, and sex to determine an optimal dose of medication. An automated 3D printing system is used to print and dispense personalized medicines. The dosage could be further modified based on the clinical response, if appropriate. Customized drugs may also be made with entirely new formulae such as multi--active combinations or complex written multi-layer or multi--reservoir tablets, such as multiple active ingredients. Patients with many chronic illnesses could have their medication printed at the point of care in a single multi-dose form. Patients will theoretically boost patient compliance with a precise, customized dosage of several medicines in one tablet.

7.2. Complex Drug-Release Profiles

The creation of drugs with complex drug release profiles is one of the most researched applications in 3D printers. Traditional dosage shapes are mostly made from a homogeneous combination of active and inactive ingredients and are mostly limited to a specific pharmaceutical discharge profile [48]. However, in layers typically 200 micrometres thick, 3D printers can print binder onto the powder bed of a matrix to make a barrier between the active ingredients easier to release controlled drugs. 3D-printed dosage shapes can also develop into complex and porous geometries with various medications, surrounded by barrier-modifying layers [84].

7.3. Orodispersible High-Dose Medications

3DP allows the manufacturing of High-Dose medicines Orodispersible (up to 1000 mg) without compression or conventional molding. With the use of an aqueous fluid, the 3D printer stitches various layers of pulverized medicines, creating a porous, water-soluble matrix that quickly breaks with a sip of liquid [103].

7.4. Unique Dosage Forms

Unique dosage forms produced by using "injection-based 3D processing of medicines" and inkjet printers are used to spray medicine formulation and binders onto a substratum in tiny droplets at exact speeds, movements, and sizes. Cellulose, coated or uncoated paper, micropores, glass scaffolding, metal alloys, and potato starch films are among the most common substrates in use [104]. This technique has been further developed by spraying uniform "black" droplets on the liquid film encapsulating the material and forming microparticles and nanoparticles. These matrices are suitable for the supply of small hydrophobic molecules and growth factors. In the "powder-based 3D drug development," the inkjet printer head sprays the "ink" on the powder-based base. If the ink reaches the powder, it will harden and produce a solid, layer by layer dosage shape. The ink may also contain active ingredients, binders, and other inactive ingredients. After the 3D-printed dosage form is dry, the solid object is removed from the surrounding powder substratum. It also enables the creation of unlimited forms of dosage that are likely to challenge traditional drug manufacture. A significant number of novel dosing forms were already being used by 3D printers, including microcapsules, synthetic extracellular hyaluronic matrices, paper-printed micro-antibiotic designs, and bioplasm scrapers, nanosuspensions, and multi-layered drug delivery systems [97].

7.5. Drugs with Complex Geometries

In dry conditions, the shape is used to prepare medicinal tablets in various uncommon forms that are difficult to manufacture by conventional methods. Pharmacy Scientists and FabRx, Inc. University College London (UCL). Scholars - Researchers Five tablets, each of which has a distinctly different design, [*i.e.*, the ring/donut], were designed in their study using auto Computer-Aided Design (CAD) software — the cube, pyramid, cylinder, sphere, and torus. The device used to produce tablets of stable surface (275 mm2), the volume ratio of surface area (1:1), or weight (500 mg). However, each type has been preserved in all cases by its length, width, and height ratio. Each tablet was finally published with a "MakerBot Replicator 2X Desktop 3D Printer," using a drug-infused filament. The dissolution testing of each pill was performed after printing the tablets [90]. The drug release rates in the pyramids were found to be the highest, followed by the torus, cubes, body, and finally, the ring, where the tablets' surface area continuously held. The pyramid tablet and the low cylinder limit are directly associated with the tablet surface/volume ratio. This led the researchers to believe that a tablet's geometric form influences its profile of the release of drugs [105].

7.6. Increased Cost Efficiency

The essential advantage of 3D printing is the ability to manufacture low-cost products. The traditional method of medication is not as cost-effective as 3D printing technologies because the conventional method uses a large number of processes such as mixing, frying, dry or wet granulation,

compression, molding, *etc*. For instance, a 10 mg pharmaceutical tablet may probably be custom-made as a 1 mg tablet on request. Some medicine may also be printed in a more cost-effective and easier-to-use dosage format [106].

7.7. Enhanced Productivity

In 3D printing, the conventional process of drug processing uses different methods, including mixing, grinding, dry or wet granulation, compaction, or molding that makes it time-consuming, is much faster than the traditional process. Other qualities like solving, precise, reliable, and repeatable 3D printing technologies improve as well as speed [107].

7.8. Environment-friendly

3D printing technology appears to have more sustainable benefits than conventional medicines that require vast quantities of pill manufacturers [107].

8. REGULATORY CONSIDERATIONS

A further significant obstacle to the widespread pharmaceutical use of 3D printing is the safeguarding of regulatory approvals. Until then, the approval of the FDA has been given to only one dosage form printed by Aprecia pharmaceuticals. However, meeting more demanding regulatory requirements from the FDA could represent an obstacle to the availability of large-scale 3D printing medicines [97]. Their similarity sells these products to conventional medical devices under the new FDA rules. Despite over a decade of 3D printing technology developed over 150 licensed medical devices, the application for the manufacture of medicines is still in its infancy. The USFDA has taken the first 3d print pharmaceutical-Spiritam® significant steps forward with the approval [12].

The research and review unit at the USFDA's CDER Pharmaceutical Quality Unit focuses on outstanding problems relating to the implementation of this technology. To create regulations for printed 3D products, several unanswered questions about the CMC (Chemistry, Manufacture, and Control) and clinical aspects must be addressed. CMC problems include a description of critical process parameters, critical and in-process attributes of the material, and finished test requirements for the product [98]. The determination of the connection between geometry and live performance, as well as patient acceptability, the impact on the in--vivo performance of different 3D printer models, and so on, is other challenges. The CDER reviews, in collaboration with the FDA Centre for Devices & Radiological Protection, to ensure the operational quality of the 3D Printers, printer equipment, and intermediate products [99].

8.1. Challenges and Perspectives

The advances in technology in the field of pharmaceuticals continuously improve and offer different possibilities for satisfying the needs of personalized medical treatment. 3DP technology has infinite potential as technical progress continues while manufacturing patient-specific drug delivery devices (DDDs) and dosage types. Besides, fast-moving 3D printed DDD work has helped to recognize numerous problems associated with the development and marketing of customized drug delivery systems [100]. *Via* 3DP, prototypes of drug delivery systems have been produced with varying complexity, and it is shown that drug products can be adapted. Patient-specific potential drug delivery is likely to be improved with printing technology. New scientific principles, interdisciplinary research, and established regulatory guidelines would encourage and enhance 3DP perspectives as a choice in the manufacturing of medical products [72]. In the last 35 years, 3DP has been one kind. Its various advantages and its ability to manufacture large, solid doses with high precision and accuracy can revolutionize drug delivery.

3DP can produce robust, variable densities and diffuse dosage forms, complex internal geometries, and several medicinal products.3DP effectively addresses problems relating to the poor delivery of water-soluble medicines, peptides, active drugs, and multidrug releases. However, difficulties limit 3DP's commercial applications on the market, including the lack of appropriate binders, excipients, and final product pharmacy. If 3DP is successfully combined with a new drug delivery system, further progress in the process is required [101]. 3DP includes a variety of techniques with advantages and open problems each one. The manufacture of drug products was mainly affected by the solidification of powder, extrusion, and stereolithography. The biggest obstacle in using them for custom pharmacologic therapy is likely to be regulatory issues and designing production models to efficiently turn clinical criteria for specific patients into limited amounts of suitable drug products that meet predetermined quality specifications [102]. 3DP developed to address patients ' needs as a supporting and potential medicinal tool. It is the product of personalized medicine. It provides many benefits, such as improved cost efficiency and production speed, because rapid prototyping (RP) is possible within minutes. However, there are still significant obstacles to ensuring that the efficacy, protection, and durability of 3D printed drugs are the same as those that the pharmaceutical industry typically manufactures. Due to traditional requirements in the pharmaceutical industry, it is challenging to develop laws, regulations, quality processes, and the safety and implementation of 3D printed medications products by national regulators. National regulatory authorities applying various printing technologies offer potentially customized medicine solutions and adapted dosage forms that meet the requirements of future treatments in particular. Many types of dosage form scenarios are available, including the accurately deposited doses of pharmaceutical substances at the simplest level. Also, computer design provides countless possibilities for creating suitable geometries with a tailor-made purpose and complexity to monitor the release characteristics of one or more drugs. It takes some time to turn these technical advances in the printer industry into improved treatments for patients because challenges exist. However, printing methods are adapting rapidly to build innovative drug delivery systems and cellular functions for human treatments using versatile materials—printing technologies [86].

CONCLUSION

3DP has become a valuable and future resource for the pharmaceutical industry that contributes to customized medicine that meets the need of patients. This analysis demonstrates 3DP technology's versatility that is well-suited for customized/personalized medication when it comes to advanced drug delivery with integrated functionality. Third--party manufacturing revolutionizes the design and composition of pharmaceutical development. However, a significant barrier still exists in ensuring 3D printed pharmaceuticals in the pharmaceutical sector as effective, safe, and stable as traditional medications. Regulators face a unique challenge, given the conventional requirements of the pharmaceutical industry, several obstacles in establishing guidelines, regulations, quality systems, the safety of use, and the use of 3D printed medicines. Initial thoughts on technical aspects related to the process and recommendations for the testing and characterization of devices that involve at least one additive production stage are provided in the FDA Guideline "Technical considerations for additive manufactured devices." Shortly, a 3DP approach was used to produce and develop different new dosing forms. Evolving personalized drugs and optimized dosage release, compacting or preventing drug incompatibilities and biomolecules during the manufacture, and constructing multiple dosage forms for medicines and different dosing forms will be transformed into the new era of 3D printing technologies. While the commercial production of such new dosage forms continues to be challenging.

LIST OF ABBREVIATIONS

3DP = 3-Dimensional Printing
ABS = Acrylonitrile Butadiene Styrene
AM = Additive Manufacturing
API = Active Pharmaceutical Ingredient
ASA = Aminosalicylic Acid
CAD = Computer-Aided Drug Design
CDER = Center for Drug Evaluation Research
CIP = Continuous Inkjet Printing
CMC = Chemistry Manufacture and Control
DOD = Drop on Drop
DOS = Drop on Solid
FDA = Food and Drug Administration
FDM = Fused Deposition Modeling
HME = Hot-melt extrusion
HPMC = Hydroxypropyl Methylcellulose
IR = Immediate Release
ISO = International Organization of Standardization
MCC = Microcrystalline Cellulose
ODF = Orodispersible Film
PAA = Polyacrylic Acid
PC = Personal Computer
PCL = Polycaprolactone
PEG = Polyethylene Glycol
PLA = Poly Lactic Acid
PVA = Polyvinyl Alcohol
PVP = Polyvinylpyrrolidone
R & D = Research and Development
RP = Rapid Prototyping
SLA = Stereolithography
SLM = Selective Laser Melting
SLS = Selective Laser Sintering
SSG = Sodium Starch Glycolate
US = United States
UV = Ultra Violet

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Effects of different monosaccharides on thermal stability of phycobiliproteins from *Oscillatoria* sp. (BTA-170): Analysis of kinetics, thermodynamics, colour and antioxidant properties

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ABSTRACT

Phycobiliproteins (PBPs) are natural colourants and antioxidants derived from cyanobacteria. The purity indexes of extracted C-phycocyanin (C-PC), allophycocyanin (A-PC), and phycoerythrin (PE) were 0.98-1.23, 0.78-0.0.96, and 0.85-0.99, respectively. To investigate thermodynamic characteristics, degradation kinetics, colour, and antioxidant capabilities of *Oscillatoria* sp. (BTA-170) extract powder, the PBPs were thermally treated with various monosaccharides such as glucose, fructose, glucose, and lactose. In comparison to other monosaccharides that can stabilize the degradation of C-PC, A-PC, and PE at higher temperatures, glucose was found to be the most essential supplement. At 85 °C, glucose enhanced the half-life of C-PC from 2.09 to 5.37 h, whereas glucose increased the half-life of A-PC from 4.9 to 13.51 h and PE from 5.57 to 15.77 h. While glucose was added, entropy (S) for C-PC was reduced from −177.82 to −183.25 J/Mol K, for A-PC from −178.24 to −169.61 J/Mol K, and for PE from −176.28 to −170.97 J/Mol K. However, the value of enthalpy (H) was enhanced from 52.37 to 53.20 KJ/Mol, while the values of A-PC and PE were raised from 40.63 to 40.56 KJ/Mol and 40.32 to 41.43 KJ/Mol, respectively. Gibbs free energy (G*) was found in the range of 111.48–118.81KJ/Mol for C-PC, 94.49–101.28 KJ/Mol for A-PC, and 95.79–102.63 KJ/Mol for PE when glucose was added, showing a higher degree of protein stability. When fructose was added, the ΔE values of PBPs were reduced from 13.31 to 6.62 at 85 °C, with the least amount of colour degradation among the monosaccharides. At 85 °C, glucose reduced the IC 50 of PBPs from 30.45 mg/ml to 17.33 mg/ml. The thermal tolerance of monosaccharides for PBPs suggested that they could be a potential source of PBP stabilization in the food industry.

1. Introduction

Food companies had excess synthetic colourant around 1800 BCE, but there was no control over its usage in food goods. Currently, there is a legislative body in place to regulate the use of colourants in food items (Downham & Collins, 2000). The consumer's concern about the safety and health risks associated with the use of synthetic colourants has grown in the last decade. The use of synthetic colourants in food has been questioned due to their harmful impact on human health (Faieta et al., 2020). Simultaneously, the food industry is looking for an alternate supply of E-numbered chemicals, such as natural additives, to replace them (Khazaei et al., 2014). The Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have placed restrictions on the use of synthetic colourants in food, beverages, and confectionary due to their association with cancer cell development or allergic reaction stimulation. Natural colourants are concentrated extracts derived primarily from algae, vegetables, and plants that have vivid colour qualities and have been shown to be beneficial to human health. These pigments, on the other hand, differ from synthetic pigments in that they are extremely susceptible to stress (temperature,

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light, etc.) and storage conditions, and may bleach or degrade the attractive appearance and final quality of the products to which they are added (MacDougall, 2002). As a result, more study is needed to stabilize this colourant for use in stressful environments, particularly in the food industry.

Natural blue colourants are in high demand in the drinks and beverage industry, but they are extremely rare in nature, forcing producers to rely on manufactured colourants. As a result, the food sector has expressed an interest in finding, using, and stabilizing natural blue colourants. Color extraction using cyanobacteria such as *Spirulina* sp. for phycocyanin, *Haematococcus* sp. for astaxanthin pigments, and *Dunaliella* sp. for carotene pigments is becoming more popular in the commercial world (Dufossé et al., 2005). Proteins, particularly PBPs, which are light-harvesting proteins and are regarded a natural source of blue colourant, make up the majority of cyanobacteria (Sharma et al., 2020). C-phycocyanin (C-PC), allophycocyanin (A-PC), and phycoerythrin (PE) are PBPs that are blue, bluish-green, and reddish in colour respectively (Tiwari et al., 2019b). C-PC, A-PC, and PE have optimum absorption ranges of 610–620 nm, 650–655 nm, and 540–570 nm, respectively (Kannaujiya & Sinha, 2016b). C-PC, A-PC, and PE had maximal absorption at wavelengths of 615 nm, 652 nm, and 562 nm, respectively (Bennett & Bogorad, 1973). PBPs are classed as high (PE), intermediate (C-PC), or low (A-PC) energy pigments based on their energy capacity. Phycocyanin's molecular weight varies from 44 to 260 kDa (MacColl, 1998). PBPs have a high water solubility and account for 40% (w/w) of the accessible water-soluble protein in cyanobacteria (Sharma et al., 2020; Tiwari et al., 2020). The intensity of light, the light to days ratio (L:D), and the cyanobacteria's nutrition availability were all factors that affected the formation of PBPs (Norena-Caro & Benton, 2018; Tiwari et al., 2019a, 2019d). Biochemical engineers will need kinetic and thermodynamic analyses of PBPs in order to design and develop a bioreactor for industrial use. Phycobiliproteins (PBPs) pigments as a colourant have an anticipated total commercial value of roughly US$60 million, while the cost of phycocyanin (C-PC) is around 500$-1000,00$ per kilogramme (Borowitzka, 2013).

Cyanobacteria are also thought to be the ecosystem's primary biomass producer (Rastogi & Sinha, 2009; Sardar et al., 2018; Tiwari et al., 2019c). PBPs are deemed Generally Recognized as Safe (GRAS) substances by the Food and Drug Administration for use as an antioxidant in functional foods (Borowitzka, 2013). PBPs, on the other hand, have certain health-promoting qualities, such as radical scavenging, anti-inflammatory, and hepatoprotective capabilities, and are frequently used in food and pharmaceuticals (Castro-García et al., 2018). As a result, food manufacturers' goals are to enhance the usage of PBPs as natural food additives. PBPs are more appealing in the food business because of their vivid bright hue (Morais, 2018). The *Oscillatoria* sp. was utilised to make butylated hydroxytoluene (BHT), which is used in food additives (Babu & Wu, 2008). Phycocyanin isolated from *Spirulina* sp. has also been reported to have chemopreventive effects (Chen & Wong, 2008). Because it possesses a tetrapyrrole chain, phycocyanin has high antioxidant qualities because it absorbs free radicals by donating hydrogen atoms (Romay et al., 2003). Despite the fact that it has positive characteristics, C-PC is extremely sensitive to both extrinsic (light and temperature) and intrinsic (pH) factors, resulting in protein denaturation in *Spirulina* extract (Kupka & Scheer, 2008). The key issue in the food industry is the stability of PBPs pigment because to its susceptibility to temperatures, pH, and light. To some extent, phycocyanin encapsulation with alginates could inhibit heat deterioration (Yan et al., 2014). The most commonly employed preservatives for C-PC for analytical purposes are dithiothreitol and sodium azide, which are not suitable for human consumption (Mishra et al., 2008).

To maintain the structure of the protein, polyols such as sorbitol, xylitol, and mannitol have been utilised (Petersen et al., 2004). It has previously been reported on the influence of polyols and mono- or disaccharides on the stability of PBPs when exposed to light, heat, and freezing (Kannaujiya & Sinha, 2016b). Sugar also inhibited the degradation of the C-phycocyanin blue colour derived from spirulina sp. (Martelli et al., 2014). Because it is a cheap and readily available material, sugar is frequently added to proteins as a stabilizer to reduce protein heat denaturation. Monosaccharides and disaccharides are mostly utilised to stabilize proteins. Glucose, fructose, galactose, and lactose are monosaccharides, while lactose, maltose, and sucrose are disaccharides. Sugar interacts to protein primarily through an N-linked glycosidic bond, which helps to prevent protein breakdown during heat processing (Imamura et al., 2003). Sugar's stabilizing impact has been extensively studied in the food sector to reduce the degradation of anthocyanin in raspberry juice (Martelli et al., 2014). Sugar, according to Vikram et al. (2005), can reduce the breakdown of nutrients in orange juice (Vikram et al., 2005). Sadilova et al. (2009) investigated the effect of sugar addition in fruit juice on anthocyanin pigment stability (Sadilova et al., 2009). As a result, sugar can be considered the primary stabilizer in preventing PBP heat deterioration.

According to our findings, there is insufficient research on the stability of PBPs employing sugar as a stabilizing agent. The majority of the experiments were carried out in order to quantify PBPs. Therefore, the manuscript is emphasized on evaluating the influence of sugar on the stability of PBPs following thermal treatment in order to extend the use of PBPs.

2. Materials and methods

2.1. Chemicals

Chemicals from Hi-Media India Ltd were used in this study, and they are analytical grade. All of the trials in this investigation were done in triplicate. The results were presented with their mean values and standard deviation (SD) of triplicate trials.

2.2. Culturing of cyanobacteria and maintenance

The cyanobacterium *Oscillatoria* sp.(BTA-170) was collected from the Indian Agricultural Research Institute (IARI, New Delhi).The cyanobacterium employed in this work was selected from a pool of different strains based on its ability to produce high yields of phycobiliproteins in BG 11 medium (Nath et al., 2020, pp. 1–11; Tiwari et al., 2019b). In BG 11 solid media, a pure culture was developed using a conventional procedure. BG-11 medium was used for maintenance of *Oscillatoria* sp. (BTA-170) (Tiwari et al., 2019b).

2.3. Collection of powder from cyanobacteria

The seed cultivation of *Oscillatoria* sp. (BTA-170) was done in a conical flask (250 ml) with 100 ml BG-11 media. The seed culturing was done by transferring a loop of pure culture into BG-11 media and incubation was done at 28 °C for 5 days to the fixed light intensity at 4k Lux with a photoperiod of 12:12 (Light: Day) ratio. The manual agitation of the culture was at 24 h interval of time. The production of cyanobacterial biomass was carried out for 30 days in the second phase, in which 10% of the seed culture was incubated in a conical flask (2 L) using BG-11 (1 L) media as described for seed culture. After 30 days, the biomass was separated by centrifugation at 10,000 rpm for 10 min at 4 °C. To collect the dry biomass, the precipitate was collected and dried in a tray dryer at 40 °C for 96 h, then pulverised in a mortar and pestle to collect the powder. For further experimentation, the powder was maintained in an airtight aluminium pouch.

2.4. Sample preparation and thermal treatment

The dried powder was collected and PBPs samples were prepared for heating. The 60 mg of dried powder and sugar as (15 w/w%) was mixed into 60 ml of 0.05 M phosphate buffer (pH-7). The conical flask

containing the sample was surrounded by aluminium foil to get protected from light to avoid the PBPs degradation. The conical flask was kept in the water bath and heated for 40 min and the samples were withdrawn after 10 min interval. The thermostat was used to set the temperature at 45 °C, 55 °C, 65 °C, 75 °C, and 85 °C. Before the heat treatment, sample PBPs were measured using a UV–Vis spectrophotometer (Bennett & Bogorad, 1973) and absorbance was recorded. The colour parameters as well as the scavenging activity of the fresh and heated samples were measured on the same day. To avoid protein degradation, the samples were kept in the refrigerator at 4 °C until further analysis was finished.

2.5. PBPs measurement

The PBPs of the sample were determined using a UV–Vis spectrophotometer (LABMAN, LMSP UV1900). The 0.05 M phosphate buffer (pH-7) was used for calibration and about 3 ml of samples were poured into cuvettes. The absorbance was taken at 615, 652, and 652 nm. The C-PC,A-PC, and PE were calculated from equations (1)–(3) (Bennett & Bogorad, 1973). The PBPs were measured in milligram per litter of BTA-170 culture.

$$C-PC(mg/L)=\frac{A_{615}-0.474(A_{652})}{5.34} \quad (1)$$

$$A-PC(mg/L)=\frac{A_{652}-0.208(A_{615})}{5.09} \quad (2)$$

$$PE(mg/L)=\frac{A_{562}-2.41(C-PC)-0.849(A-PC)}{9.62} \quad (3)$$

2.6. Purity index

The purity Indexes of PBPs were measured by the spectrophotometric method (Kannaujiya & Sinha, 2016a) and calculated from eq. (4) (5) &(6). This ratio signifies the specific proteins to contaminated proteins. The purity Indexes of C-PC, A-PC, and PE were determined at wavelengths of 615, 652, and562 nm, respectively to the total soluble protein concentration at 280 nm.

$$(C-PC)=\frac{A_{615}}{A_{280}} \quad (4)$$

$$(A-PC)=\frac{A_{652}}{A_{280}} \quad (5)$$

$$(PE)=\frac{A_{562}}{A_{280}} \quad (6)$$

When the purity Index ≥0.7, PBPs represented as food-grade, and when the ratio is ≥ 4, the PBPs are fit for the analytical grade.

2.7. Thermal kinetics of PBPs with stabilizing agent

The deactivation study of PBPs was done under various temperatures and the effect of sugar as the stabilizing agent was evaluated in the deactivation process. The deactivation of PBPs was observed, and the deactivation process was assumed to follow first-order kinetics.

$$P_A=P_D$$

P_A and P_D signify the active and inactive state of PBPs. The assumptions were made that intermediates were not formed significantly at the time of the deactivation process. The process of deactivation is presented in eq. (7)

$$\frac{dP_A}{dt}=-k_d[P_A] \quad (7)$$

Were, k_d represent the deactivation rate constant. Integration of Eq. (7) gives

$$\alpha=\exp.(-k_d.t)$$

$$\text{Were, } \alpha=\frac{P_D}{P_A}$$

The calculation of k_d was done by finding the slope by plotting ln(α) versus t. Half-life was determined and it is the time required to deactivate the C-PC, A-PC, and PE to half amount and calculated using Eq. (8).

$$t_{1/2}=\frac{0.693}{k_d} \quad (8)$$

Thermodynamic properties of PBPs after thermal treatment were evaluated by finding parameters like enthalpy, entropy, Gibb's free energy, and activation energy and evaluated the mechanism of the deactivation process (Naidu & Panda, 2003). The temperature dependence of the deactivation rate constant can be expressed as shown in eq. (9)

$$k_d=\frac{kT}{h}e\frac{\Delta S}{R}e\frac{-H}{RT} \quad (9)$$

or

$$Ln\left(\frac{k_d}{T}\right)=Ln\frac{k}{h}+\frac{\Delta S}{R}-\frac{\Delta H}{RT} \quad (10)$$

where ΔH is enthalpy and ΔS represents the entropy values. R denotes the gas constant and T is temperature. The h is the plank's constant and k represent the Boltzmann constant. The plot was generated to ln(k_d/T) versus 1/T and calculated the enthalpy and entropy values of C-PC, A-PC and PE as shown in Fig. 1a,b & 1c.

The Eq. (11) was used to calculate Gibb's free energy (ΔG)

$$\Delta G=\Delta H-T\Delta S \quad (11)$$

Similarly, the Arrhenius equation was used to determine the activation energy (E_a) as shown in Eq. (12)

$$k_d=k_o e\frac{(-E_a)}{RT} \quad (12)$$

or

$$\ln(k_d)=\ln(k_o)-\left(\frac{E_a}{R}\right)\frac{1}{T} \quad (13)$$

The graph was plotted to ln (k_d) versus 1/T to find the value of E_a of C-PC, A-PC, and PE as shown in Fig. 2a, b & 2c.

2.8. Colour degradation measurement

Hunter lab colorimeter (HunterLab, MSXP-4500L) was used to determine the colour of PBPs before and after heat treatment with the addition of various sugars. The sample was produced using the same procedure as for the measurement of PBPs. The calibration of the colorimeter was done with a white reference tile. The 60 μl of samples were poured into a transparent glass having a dimension of 10 cm × 15 cm and the color parameters were evaluated. The lab coordinates values including L*, a* & b* were measured and signified the lightness, red to green, and yellow to blue color of PBPs. The measurement was done by CIE scale, and change of color (ΔE*) was evaluated with Eq. (14)

$$\Delta E^*=\sqrt{\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2}} \quad (14)$$

2.9. Antioxidant activity

The antioxidant activity of PBPs was evaluated by using the free radical DPPH (Chan et al., 2018). 1 g of PBPs was dissolved in 25 ml of methanol to make the stock solution. 80 mg of DPPH was dissolved in

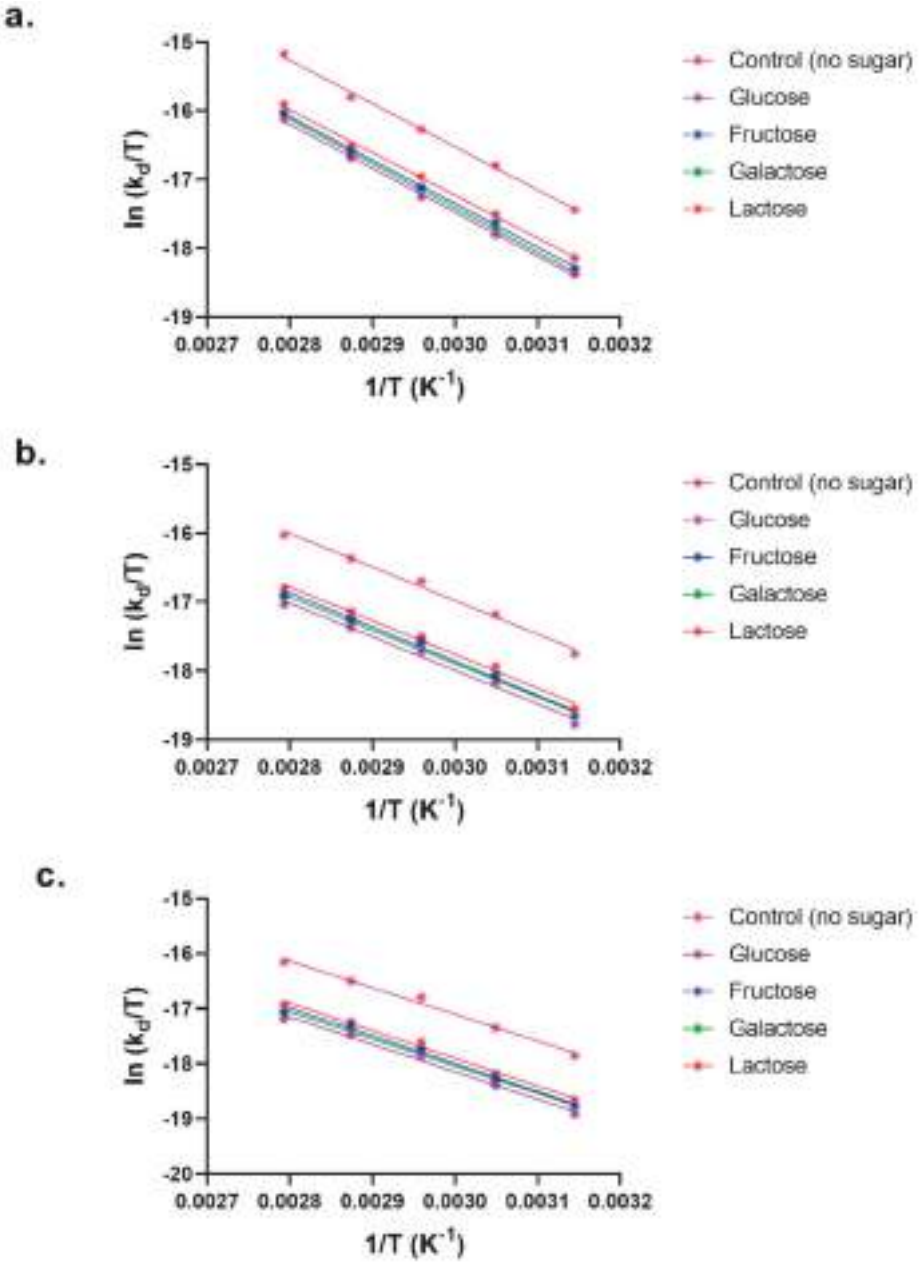

Fig. 1. Plot to determine enthalpy and entropy of (a) C-PC, (b) A-PC, and (c) PE during thermal treatment.

100 mL of methanol to make the stock solution of DPPH. By diluting the sample with the required amount of methanol, different PBP concentrations were obtained. An equal volume of sample was mixed with an equal amount of DPPH stock solution to achieve various PBP concentrations. The mixed sample was incubated for 30 min at room temperature. The absorbance of mixture were determined by spectrophotometer (LABMAN, LMSP UV1900) at 517 nm (Chan et al., 2018). The inhibition percentage was calculated by equation (15)

$$\text{Inhibition}(\%) = 1 - \frac{(\text{Abs.}_{\text{sample}})}{(\text{Abs.}_{\text{control}})} \times 100\% \quad (15)$$

3. Results and discussions

3.1. Kinetics and thermodynamic properties of PBPs with sugar

In the present investigation, the C-PC, A-PC, and PE content of *Oscillatoria* sp. (BTA-170) were determined to be 287.03 mg/L, 254.23 mg/L, and 82.34 mg/L, respectively, which is quite similar to our prior findings (Tiwari et al., 2019b). The purity index of C-PC, A-PC, and PE were determined to be 0.98–1.23, 0.78–0.0.96, and 0.85–0.99, respectively. Tables 1–3 show the results of evaluating the deactivation rate constant (k_d) for C-PC, A-PC, and PE at various temperatures and estimating the effect of sugar on the deactivation rate constant (k_d). Temperature has long been known to play a significant impact in protein deactivation (Bhunia et al., 2013). Results showed that, the k_d value for PBPs was found to be low in the temperature range of 45 °C–55 °C, but as the temperature goes up, the k_d value increased. The k_d value of C-PC was found to be 0.331 at 85 °C, with glucose lowering the value to 0.129 at the same temperature. Fructose, galactose, and lactose can all lower the K_d value for C-PC to 0.142, 0.136, and 0.159 at 85 °C, respectively. Sugars were successful in lowering the k_d values for C-PC, A-PC, and PE at temperatures higher than 55 °C. Glucose, among the sugars, reduced the k_d values to the lowest and highest k_d values held by lactose, for

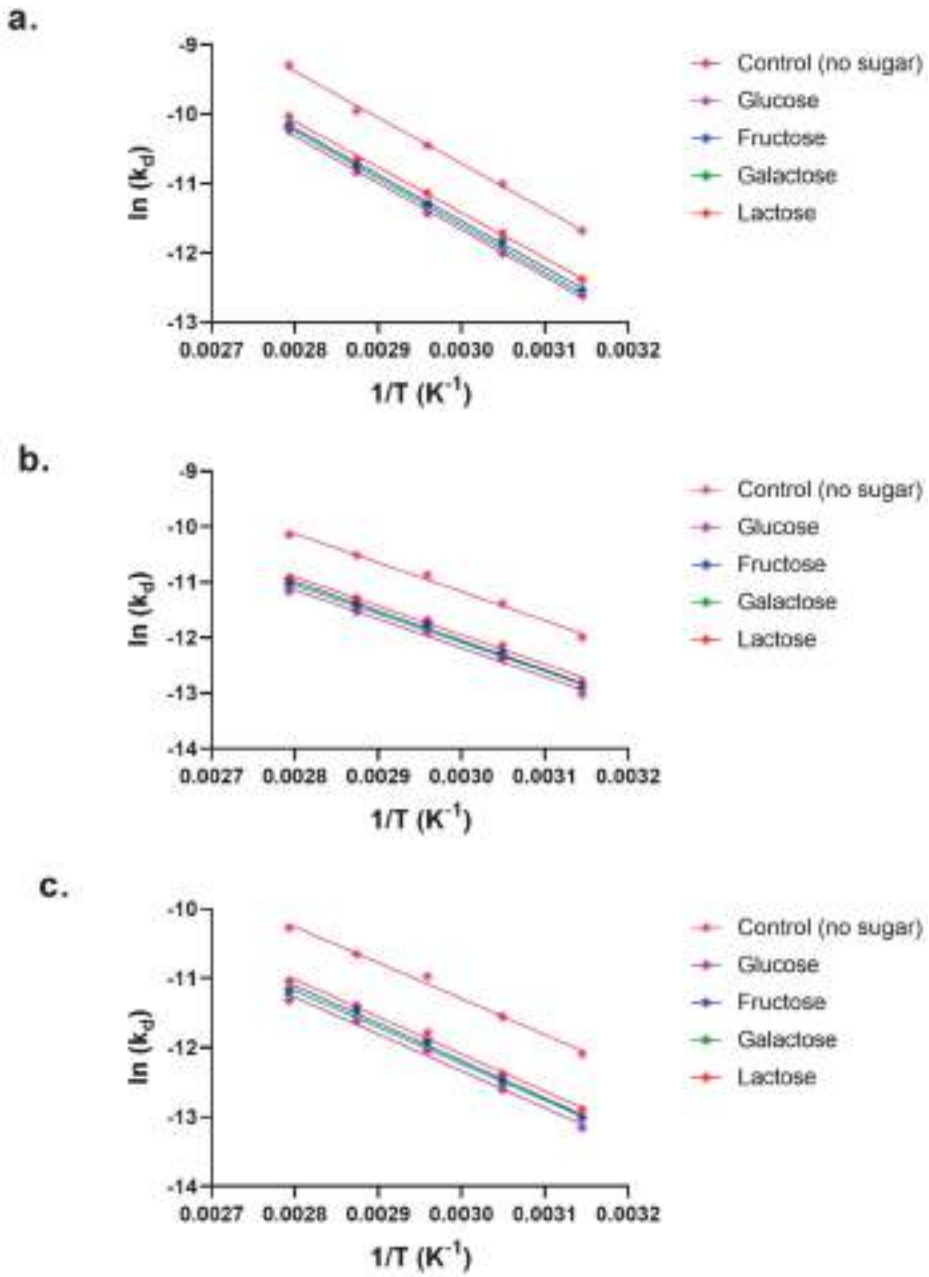

Fig. 2. Arrhenius plotting during thermal treatment to determine the activation energy of (a) C-PC, (b) A-PC, and (c) PE.

Table 1
Effect of temperature and different sugar on deactivation rate constant (k_d) for C-PC.

k_d values (hr^{-1})					
Temperature (°C)	Control (no sugar)	Glucose	Fructose	Galactose	Lactose
45	0.030	0.012	0.013	0.012	0.015
55	0.060	0.022	0.026	0.024	0.029
65	0.104	0.039	0.045	0.044	0.052
75	0.172	0.071	0.079	0.075	0.084
85	0.331	0.129	0.142	0.136	0.159

Table 2
Effect of temperature and different sugar on deactivation rate constant (k_d) for A-PC.

k_d values (hr^{-1})					
Temperature (°C)	Control (no sugar)	Glucose	Fructose	Galactose	Lactose
45	0.022	0.008	0.009	0.009	0.010
55	0.041	0.015	0.017	0.016	0.019
65	0.073	0.024	0.028	0.027	0.030
75	0.098	0.036	0.041	0.039	0.045
85	0.141	0.051	0.060	0.057	0.065

C-PC, A-PC, and PE, as shown in Tables 1–3. At 85 °C, the k_d value of A-PC was calculated to be 0.141. Glucose was shown to be the most effective sugar for A-PC, with the lowest kd value (0.060), followed by galactose (0.057), fructose (0.060), and lactose (0.065). The k_d value of PE was 0.124 at 85 °C, but when glucose is present, it drops to 0.044 at 85 °C. At 85 °C, the k_d value of PE lowers to 0.051, 0.048, and 0.058 in the presence of fructose, galactose, and lactose, respectively. It was found that C-PC degraded at 6.71%, 10.83%, and 19.82% at 65, 75 &

Table 3
Effect of temperature and different sugar on deactivation rate constant (k_d) for PE.

k_d values (hr^{-1})					
Temperature (°C)	Control (no sugar)	Glucose	Fructose	Galactose	Lactose
45	0.020	0.007	0.008	0.008	0.009
55	0.035	0.012	0.014	0.013	0.015
65	0.062	0.021	0.024	0.023	0.027
75	0.086	0.033	0.039	0.037	0.041
85	0.124	0.044	0.051	0.048	0.058

85 °C respectively while heating for 40 min. The glucose treatment reduced the C-PC degradation to 2.55%, 4.65%, and 8.24% at 65, 75 & 85 °C respectively. The half-life of PBPs was used to assess the effect of sugar on their degradation rate. The glucose, fructose, galactose, and lactose elevate the half-life of C-PC from 2.09 h to 5.37 h, 4.90 h, 5.10 h, and 4.36 h respectively at 85 °C. Similarly, A-PC degraded at 4.45%, 6.30% and 9% at 65, 75 & 85 °C respectively while heating for 40 min. Patel et al. (2004) reported that C-PC is unstable above 55 °C (Patel et al., 2004), and according to Chronakis (2001), the denaturation temperature of PC is over 67 °C (Chronakis, 2001). According to several publications, PC solubility decreased in acidic conditions, with aggregation starting at pH 3 and denaturation happening at pH 5–7 when heated over 45 °C (Chaiklahan et al., 2012). In the present investigation, the PBPs were stable up to 55 °C at 7 pH and instability raised with increasing the temperature up to 85 °C. The glucose, fructose, galactose, and lactose increase the half-life of A-PC from 4.90 h to 13.51 h, 11.60 h, 12.25 h, and 10.65 h respectively at 85 °C. The PE was least degraded PBPs which degraded at 4.04%, 5.54% & 7.96% at 65, 75 & 85 °C respectively. The glucose, fructose, galactose, and lactose increase the half-life of PE from 5.57 h to 15.77, 13.51 h, 14.40 h, and 12.03 h respectively at 85 °C. The PE was found to be the most stable PBP, followed by A-PC and C-PC, and a similar finding was found in a prior publication (Tiwari et al., 2019b).

In present study, glucose increased the thermal stability of PBPs at all temperatures tested. Miyawaki et al. (2016) reported that trehalose was found to be efficient in improving the heat stability of phycocyanin pigments (Miyawaki et al., 2016). The differences in thermal stability amongst sugars may be related to distinct solvent/protein interaction processes (Barbiroli et al., 2017). While formulating Spirulina sp. to liquid solutions, food-grade substances such as monosaccharide, organic acids, and salts have been beneficial in reducing the effect of heat on phycocyanin (Faieta et al., 2020). The mechanism for thermal stabilization of proteins by monosaccharides in aqueous phase is due to alteration in interaction between water and protein molecules (Barbiroli et al., 2017). The bonding of protein molecules controls the orientation of the secondary, tertiary, and quaternary structure of the protein, and protein deactivation happened after modifying the structure at various temperatures and pH levels. Rastogi et al. (2015) investigated the functioning and stability of pure PBPs at different temperatures and pH levels (Rastogi et al., 2015). Tables 4–6 demonstrate the variation of entropy (S) of C-PC, A-PC, and PE after heat treatment in the presence of sugar. It is evident that the values of enthalpy (H) and entropy (S) altered with temperature and were also affected by the type of sugar used. Finding H and S can be used to determine the degree of denaturation of a protein thermodynamically. Protein randomness is denoted by entropy (S). The positive symbol of S denotes a higher amount of randomness and represents protein denaturation, whereas the negative symbol denotes a lower degree of protein denaturation (Bhunia et al., 2013).

The entropy (S) of C-PC was calculated to be −177.82 J/Mol K before the addition of sugar. Glucose elevated the entropy value to −183.25 J/Mol K. As demonstrated in Table 4, all sugars have a reduced S value, with lactose having a particularly low S value. Fructose, galactose, and lactose, on the other hand, reduce the entropy value of C-PC to −183.74, −181.27, and −185.60 J/Mol K, respectively. In the absence of sugar, the S value of A-PC is determined to be −178.24 J/Mol K, but when glucose is added, the value drops to −169.61 J/Mol K. As indicated in Table 5, fructose, galactose, and lactose were also prominent in lowering the S value. In the current study, the S value of PE was determined to be −176.28 J/Mol K in the absence of sugar, with glucose increasing the value to −170.97 J/Mol K. As seen in Table 6, all sugar lowers the S value to a satisfactory level for PE. It is evident that the PE had the lowest S value among the PBPs, followed by C-PC and A-PC. The drop in entropy value after adding sugar was attributed to less breakdown of PBPs as a result of the formation of linkages between protein and sugar molecules.

Enthalpy (H) signifies the amount of energy required to denature the protein. As a result, a greater enthalpy value suggests that the protein is more stable (Marangoni, 2003). Tables 4–6 show the variation of H values of C-PC, A-PC, and PE after heat treatment, as well as the effect of sugar on enthalpy values. In the absence of sugar, the H values of C-PC were determined to be 52.37 KJ/Mol. The enthalpy value increased to 53.20 KJ/Mol when glucose was added. Fructose, galactose, and lactose, on the other hand, increased the values of H to 52.70, 53.69, and 51.73 KJ/Mol, respectively. The H value for A-PC was calculated to be 40.63 KJ/Mol in the absence of sugar, and fructose increased the value to 41.31 KJ/Mol. Similarly, all sugars were prominent in increasing H levels in PE, with lactose being the most efficient, as indicated in Table 6. Because the free energy is reliant on the values of enthalpy and entropy as given in eq. (10), the value of Gibb's free energy is used to determine the protein's stability (Bhunia et al., 2013). The G* value in the negative signifies the higher degree of deactivation and more denaturation of the protein. Tables 4–6 show the evaluated G values of C-PC, A-PC, and PE under sugar treatment conditions. In the absence of sugar, the G* value of C-PC varies from 108.92 to 116.03 KJ/Mol. In the addition of glucose, the G* value increased to 111.48–118.81 KJ/Mol. Similarly, when fructose, galactose, lactose were introduced, the G* value increased to 111.12–118.47 KJ/Mol, 111.33–118.58 KJ/Mol, and 110.76–118.18 KJ/Mol respectively. C-PC had the highest G* values among the PBPs, followed by A-PC and PE. To identify the minimal energy required to activate the degradation process of PBPs, the activation energy (Ea) on thermal degradation of C-PC, A-PC, and PE was investigated. The activation energy (E_a) was computed from the slope of the graph which was plotted ln (k_d) versus 1/T. Fig. 2a, b, and 2c show Arrhenius plot during thermal treatment to calculate the activation energy for C-PC, A-PC, and PE. As shown in Table 4, the E_a value of C-PC before adding sugar was 55.17 KJ/Mol, and glucose raise activation energy to 56.00 KJ/Mol. Similarly, E_a values of A-PC and PE were computed as 43.43 KJ/Mol and 43.12 KJ/Mol respectively. Fructose raises activation energy of A-PC to 44.11 kJ/mol, whereas glucose raises activation energy of PE to 44.23 kJ/mol. At 15% concentration, sugars were dominant in increasing activation energy of PBPs, as indicated in

Table 4
Evaluated thermodynamic properties during the heating process of C-PC.

Thermodynamic parameters	Control (no sugar)	Glucose	Fructose	Galactose	Lactose
H (KJ/Mol)	52.37	53.20	52.70	53.69	51.73
S (J/Mol K)	−177.82	−183.25	−183.74	−181.27	−185.60
E_a (KJ/Mol)	55.17	56.00	55.50	56.40	54.54
G (kJ/Mol)	108.92–116.03	111.48–118.81	111.12–118.47	111.33–118.58	110.76–118.18

Table 5
Evaluated thermodynamic properties during the heating process of A-PC.

Thermodynamic parameters	Control (no sugar)	Glucose	Fructose	Galactose	Lactose
H (KJ/Mol)	40.63	40.56	41.31	40.34	40.77
S(J/Mol K)	−178.24	−169.61	−172.98	−169.76	−172.17
E_a (KJ/Mol)	43.43	43.36	44.11	43.14	43.58
G (kJ/Mol)	97.31–104.44	94.49–101.28	96.32–103.24	94.32–101.11	95.52–102.41

Table 6
Evaluated thermodynamic properties during the heating process of PE.

Thermodynamic parameters	Control (no sugar)	Glucose	Fructose	Galactose	Lactose
H (KJ/Mol)	40.32	41.43	42	40.97	41.78
S(J/Mol K)	−176.28	−170.97	−173.93	−170.45	−174.05
E_a (KJ/Mol)	43.12	44.23	44.81	43.77	44.58
G (kJ/Mol)	96.37–103.42	95.79–102.63	97.32–104.28	95.17–101.99	97.13–104.09

Tables 4–6.

3.2. Influence of heat treatment and sugar on the color parameters of PBPs

The color of the fresh PBPs was deeper blue and the CIE scale and coordinates values were, L* = 36.60, a* = 2.43, and b* = −6.8. Fig. 3a, b & 3c, show that ΔL*, Δa*, and Δb* values increased slowly up to 55 °C and increased significantly from 65 °C as the temperature raised and the values were comparable to the literature (Chaiklahan et al., 2012). With the addition of sugar, the fructose retained the deeper blue color when compared with the other sugar samples with giving lower ΔE* values shown in Fig. 3d. As ΔE* increased, the discoloration was also clearly visible. Normally, the colour of PBPs with sugar added was darker blue when heated at 45 °C and 55 °C, but at 65 °C, the colour became lighter blue and discoloration grew dramatically until 85 °C. The ΔE* values

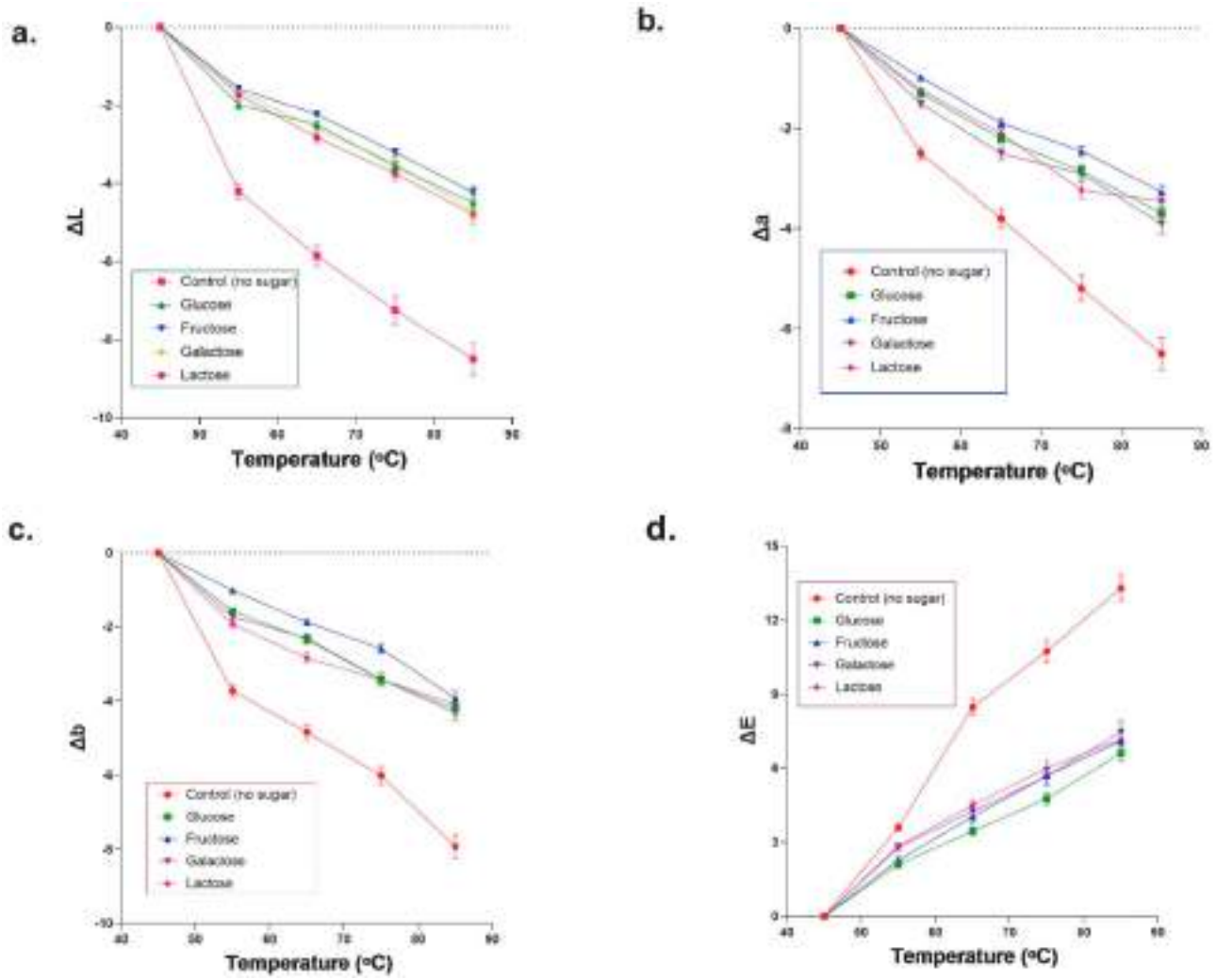

Fig. 3. Relation of (a) Δ L, (b) Δ a, (c) Δ b, and (d) Δ E of PBPs over heat treatment at 45°C, 55°C, 65°C, 75°C & 85°C.

were highest when the PBPs were heated in absence of sugar and the addition of sugar reduced the ΔE* values significantly. The overall change in ΔE values of PBPs for control (no sugar), glucose, fructose, galactose, and lactose were 13.31, 7.11, 6.62, 7.47, and 7.17 respectively. Chen and Wong (2008) also found that adding fructose to phycocyanin reduced colour deterioration at 80 °C (Chen & Wong, 2008). The change in colour of phycocyanin from dark blue to light blue could be due to the creation of bonds between phycocyanin and sugar or the denaturation of PBPs. Moreira et al. (2012) investigated the stability of the purple colour of Nostoc sp. PBPs in yoghurt and observed that b* values varied, indicating a loss of blue colour (Moreira et al., 2012). The difference was owing to C-instability PC's at lower pH. In our case, the experiment was carried out at pH 7 in order to maximise the stability during heat treatment. C-PC is mostly stable at 5–7 pH, according to Sadilova et al. (2009), and it starts losing colour values above and below that pH range (Sadilova et al., 2009). However, no research on the influence of sugar following heat treatment on PBP colour values has been published to far. PBPs mixed with fructose showed a deeper blue colour than those mixed with other sugars, indicating that fructose slowed the hydration reaction on phycocyanin active sites (Huang, 1956). Adding the excess sugar also degraded the color of phycocyanin due to its association with microorganisms (Garzón & Wrolstad, 2001).

3.3. Influence of heat treatment and sugar on the antioxidant activity of PBPs

PBPs were tested for their ability to scavenge DPPH radicals at concentrations of 2, 4, 6, 8, and 10 mg/ml at 45, 55, 65, 75, and 85 °C. Fig. 4a, b, 4c, 4d, and 4e show the effect of sugar treatment on inhibition percentage at various temperatures. In fresh conditions, the PBPs inhibited DPPH radicals by 65.82 percent, but this changed dramatically following heat treatment, and the change in inhibition percentage was studied by changing the sugar molecules. The variation in inhibition percentages of PBPs depends on the amount and compositions of PBPs in the solvent, therefore the variation in absorbance was seen during the experiment. There were no significant changes in inhibition percentage at temperatures below 55 °C, but it declined dramatically as the temperature was increased. At 85 °C, the inhibition percentages changed from 65.82 percent to 16.45 percent, and glucose could raise the inhibition to 46.07 percent with a sample concentration of 10 mg/ml at 85 °C.

Similarly, with 10 mg/ml of PBPs, fructose, galactose, and lactose increase by 45.94%, 19.2%, and 45.96% at 85 °C, respectively. The degradation of PBPs resulted in a decrease in the inhibition percentage as the temperature was increased. The degradation of PBPs reduced the inhibition percentage of PBPs. The addition of sugars could increase the inhibition percentage due to the stability of PBPs by the sugar molecules. As shown in 4c, 4d, and 4e, glucose, fructose, and lactose were the most effective sugars in increasing the inhibition percentage at 65 °C and higher temperatures, whereas galactose was less effective. According to Christwardana et al. (2018), adding glucose to phycocyanin increased its scavenging capacity while decreasing the IC50 to 18.47% for *Spirulina* sp., and a similar result was seen in present case. (Christwardana et al., 2018). The 50% free radical scavenging activity (IC_{50}) of PBPs was determined by graphing the graph of PBP concentration vs inhibition percentage (see Table 7). The effect of thermal treatment and sugar on IC_{50} was evaluated in the present study. PBPs had an IC50 of 9.52 mg/ml, which was enhanced to 30.45 mg/ml by thermal treatment at 85 °C. Sugar was found to be beneficial in lowering the IC50 at all temperatures, as indicated in Table 7. The glucose, fructose, and lactose were productive to decrease the value from 30.45 mg/ml to 17.33 ml/ml,18.30 ml/ml, and 17.68 mg/ml respectively at 85 °C. In comparison to PC and PE, Rastogi et al. (2015) found that A-PC isolated from *Phormidium rubidium* showed increased sensitivity to free radicals (H_2O_2) (Rastogi et al., 2015). The presence of free radicals accelerates the ageing process and causes tissue damage in the body, which can lead

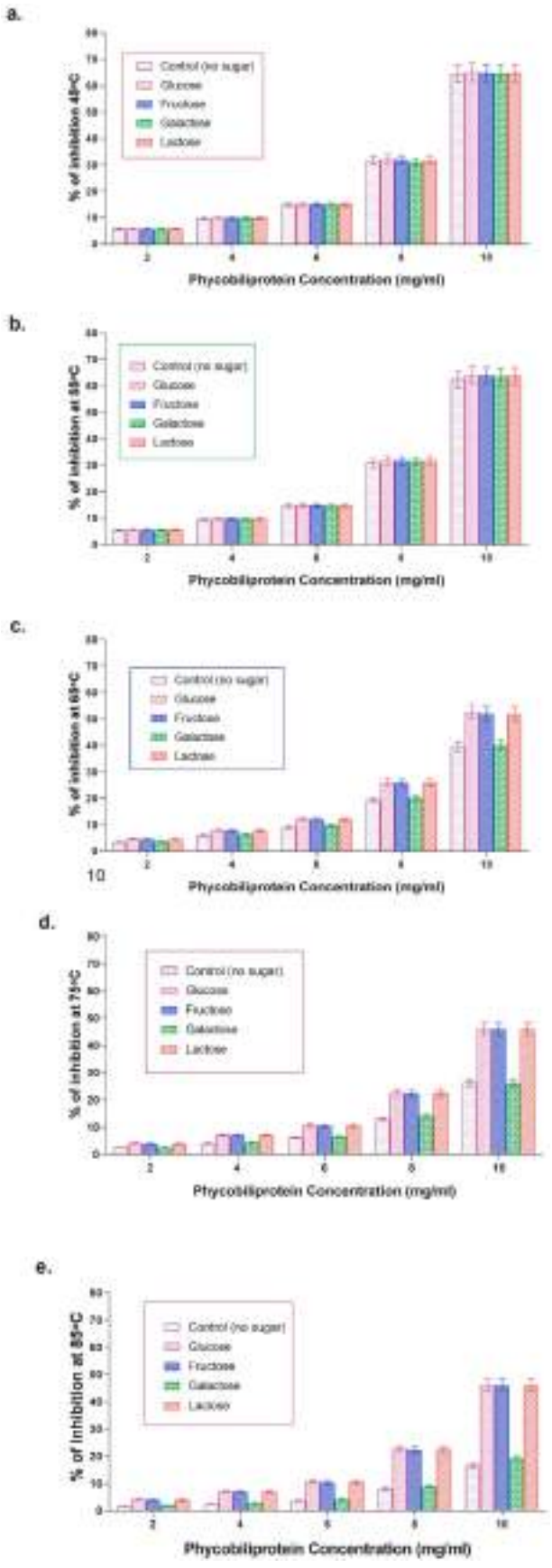

Fig. 4. Variation of inhibition % of PBPs at (a) 45°C, (b) 55°C, (c) 65°C, (d) 75°C, and (e) 85°C.

Table 7
Effect of temperature and different sugar on IC50 values of PBPs in BTA 170.

Temperature (°C)	Control (no-sugar) (mg/ml)	Glucose (mg/ml)	Fructose (mg/ml)	Galactose (mg/ml)	Lactose (mg/ml)
45	9.52	9.46	9.49	9.51	9.50
55	9.74	9.56	9.58	9.62	9.63
65	14.06	11.14	11.24	13.79	11.25
75	19.92	12.39	12.46	19.74	12.42
85	30.45	17.33	18.30	30.10	17.68

to the development of a variety of diseases. The present findings indicate that PBPs from *Oscillatoria* sp. could be useful food additives for scavenging free radicals.

4. Conclusions

In the present investigation, the effect of different monosaccharides such as glucose, fructose, glucose, and lactose, on the thermal stability of phycobiliproteins of *Oscillatoria* sp. (BTA-170) was examined and thermodynamic properties, degradation kinetics, color, and antioxidant properties of PBPs was evaluated. Glucose was determined to be the most important monosaccharide in comparison to other sugars that can stabilize the degradation of C-PC, A-PC, and PE at higher temperatures. When glucose was added to PBPs, the degradation rate (kd) for C-PC, A-PC, and PE was drastically reduced when compared to other sugars. The PE had the lowest S value out of all the PBPs, followed by C-PC and A-PC. When sugars were added to PBPs at a concentration of 15% (w/w), entropy (S) was reduced and enthalpy (H) was enhanced, resulting in less breakdown of PBPs. In the presence of all sugars, the G* value was found to be positive, indicating that PBPs were deactivated or denaturized less during heat treatment. PBPs treated with fructose showed less discoloration than those treated with other sugar molecules. The most important stabilizers for maintaining the antioxidant activity of PBPs at 65 °C and higher temperatures were glucose, fructose, and lactose. The above investigation indicates that PBPs may be stabilized by sugars while preserving antioxidant activity, and colour characteristics and the usage of synthetic colourants in the food industry can be replaced by PBPs.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Nutritional Impact of Foods Made from Spirulina on Children of Selected Anganwadis of Siddipet District in Telangana State in India

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ABSTRACT

In the present investigation, we sought to study the impact of introducing spirulina based nutritional supplementation to the children of Siddipet District in Telangana, India. It was observed that beneficiaries are the social community who need help, support and awareness. An integrated, spectrum and holistic study approach was made to reach the beneficiary community. Several attempts were made to find out the answers to questions raised in the concurrent development of malnourished child health status in two ICDS projects, with 30 anganwadis in Bharat Nagar and 34 anganwadis in Cheriyal under Siddipet District, with the guidance and help of District Collector and team of company, Sukrutha Organics. The Study encompasses both primary and secondary source of information, covering anganwadis of ICDS, Bharat Nagar and Cheriyal, out of which 2119 children each were selected for the study by using information collected by anganwadi teachers scheduled as tool for data collection. Peanut Chikkis (Brittle) and Biscuits made by the addition of Spirulina were distributed among the test group and no supplement was given in the control group of children who were enrolled under Anganwadis of Bharat Nagar and Cheriyal ICDS of Siddipet District. Descriptive analysis was made to draw inferences. The study has come out with some major findings, in spreading the knowledge about the ill effects of malnutrition and benefits of spirulina and foods made with spirulina.

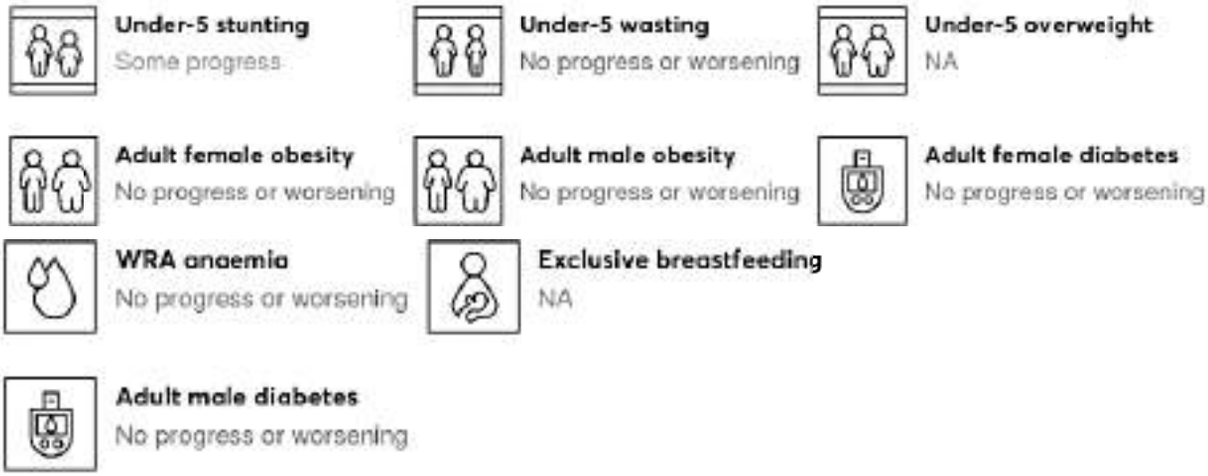
KEYWORDS: Malnutrition; Spirulina supplementation; healthy snacking.

Introduction

India's malnutrition problem results not from calorie intake but from dependence on a carbohydrate-based diet, low in protein and fat. We compromise on the intake of protein, fat and vitamins – all of which are essential for growth and inducing disease-fighting immunity at a young age. It is vital that Indian children get a balanced and nutrient-rich diet which includes all micro- and macronutrients need to bring about a healthy growth.

Poor maternal health and anemia during pregnancy is another reason for induction of stunting in children. This could have roots from adolescent anemia (Uliyar et al., 2000). This compromises resistance to diseases and nutrition value of breast milk. Poor pre-pregnancy body-mass index (BMI) and insufficient weight gain during pregnancy are common, as is blood and urine micronutrient deficiency. All of these, cause low birth weight, damaging the physiological development of a child. In many Indian households, women are taught to eat last, even when expecting.

Malnutrition or malnourishment is a condition that results from eating a diet in which nutrients are either not enough or are too much such that the diet causes health problems. It may involve calories, protein, carbohydrates, vitamins or minerals (Sandhu et al., 2010; Liu et al., 1991, Zhang and Liu, 1999). Not enough nutrients are called undernutrition or undernourishment while too much is called overnutrition. Malnutrition is often used specifically to refer to under nutrition where there is not enough a calorie, protein, or micronutrients. If under nutrition occurs during pregnancy, or before two years of age, it may result in permanent problems with physical and mental development. Extreme under nourishment, known as starvation, may have symptoms that include: a short height, thin body, very poor energy levels, and swollen legs and abdomen. People also often get infections and are frequently cold. The symptoms of deficiencies depend on the micronutrient that is lacking.



(*Source: UNICEF/WHO/World Bank Group: Joint child malnutrition estimates, UNICEF global databases: Infant and young Child feeding, NCD risk Factor Collaboration, WHO Global Health Observatory*).

Fig. 1. India's Progress against global nutrition targets in the year 2018.

Nutritional problems like protein energy malnutrition (PEM), anemia and vitamin A deficiency continue to plague a large proportion of Indian children. The major nutritional problems are protein energy malnutrition (PEM), vitamin A deficiency (VAD) and iron deficiency anemia (IDA). The main objective is to fight malnutrition and enhance the habit of healthy eating in all possible age groups.

In those with malnutrition, some of the signs of dehydration differ. Children; however, may still be interested in drinking, have decreased interactions with the world around them, have decreased urine output, and may be cool to touch.

TABLE 1

Signs of malnutrition.

Site	Sign
Face	Moon face (kwashiorkor), simian facies (marasmus)
Eye	Dry eyes, pale conjunctiva, Bitot's spots (vitamin A), periorbital edema
Mouth	Angular stomatitis, cheilitis, glossitis, spongy bleeding gums (vitamin C), parotid enlargement
Teeth	Enamel mottling, delayed eruption
Hair	Dull, sparse, brittle hair, hypopigmentation, flag sign (alternating bands of light and normal color), broomstick eyelashes, alopecia
Skin	Loose and wrinkled (marasmus), shiny and edematous (kwashiorkor), dry, follicular hyper-keratosis, patchy hyper- and hypopigmentation, erosions, poor wound healing
Nail	Koilonychia, thin and soft nail plates, fissures or ridges
Musculature	Muscles wasting, particularly in the buttocks and thighs
Skeletal	Deformities usually a result of calcium, vitamin D, or vitamin C deficiencies
Abdomen	Distended - hepatomegaly with fatty liver, ascites may be present

Spirulina is a natural food supplement. It is a blue-green alga (an ancient cyanobacterium) well known worldwide (Becker 1994a, 1994b; Fox 1996)

- Addresses several important micronutrient deficiencies among children;
- Approved as a food supplement & nutraceutical ingredient by the Government of India
- It is low cost, easy to produce locally, long shelf life, easy to store and transport.
- Quality standards are prescribed, and labs exist to test.

A study conducted by MCRC Madras, (volume 36 monograph, 1991) under Department of Bio Technology GoI with 5000 children with 1 gram of spirulina supplementation for 150 days has concluded that 'under prolonged under nutrition, food supplementation alone is not enough to combat malnutrition and there is requirement for micronutrients'. Today, India's national institutions like CFTRI, NIN, NDRC, are recommending Spirulina as a micronutrient supplement, a "Magic wand", enhancing the food absorption among children.

The study intended to report the social impact of intervention by the Sukrutha Organics team with the support of Anganwadi teachers to fight malnourishment among children between the age group of 1 year -5 years. In tune with the objectives mentioned above, the present study is based on detail and extensive survey of sample selected unit for collecting the data needed for the study.

Materials and Methods

Preparation Spirulina Chikki and Biscuit

Spirulina are blue-green microorganisms that was grown in fresh water and was procured from Spirulina Entrepreneurs, Kurnool. Spirulina powder was used in the preparation of the biscuits and chikkis. The chikkis were manufactured at Kriswa industries, Hyderabad, with the recipe and spirulina given as per the requirement and biscuits were manufacture at Sumo Biking foods, Hyderabad, as per the recipe given.

Peanuts, jaggery and liquid glucose were easily procured from local market. Peanut seeds have to be

roasted to golden brown colour (120–130°C), de-husked, de-germed and crushed into small bits of about 2.8 mm. Jaggery is generally crushed and made into syrup with addition of water and 10% weight of liquid glucose and warming and filtered through a nylon mesh of ~ 30 mesh to remove extraneous matter. The clear jaggery syrup is then heated until the temperature reached 145°C and immediately pre-weighed, roasted and de-husked peanuts are added and mixed thoroughly till the nuts get coated with jaggery syrup. This hot mass was then transferred on to a wooden board or clean platform, which was smeared with oil. The addition of required amount of spirulina was done. The product is then spread uniformly by rolling it with the help of a roller. Vertical and horizontal lines are marked with a cutter to make individual slabs then cooled to room temperature (27 ± 2°C) and are packed in polythene pouches.

Locally made hand biscuits are generally very popular among rural children. With a simple change in the recipe involving addition of micronutrients can take care of the wellness of the local community. There are primarily four stages of making the biscuit in a factory – mixing, forming, baking, and cooling. In the mixing stage, ragi flour, whole wheat flour, fat, sugar, water and spirulina ingredients were mixed together in the right proportion in large mixers to form the dough. The mixing time is carefully managed to achieve uniform distribution of ingredients and the right dough consistency. Convection ovens were used with belt operators maintaining a baking temperature on the belt and then cooling and packaging them to wrappers.

Analysis of the Products for their Nutritional Values

All the ingredients were approved as a food supplement & nutraceutical ingredient by the Government of India in their individual monographs. The products were tested for their nutritional values and approved in a NAAC accredited Lab (Vimta Lab, Hyderabad) as per the prescribed data.

TABLE 2

Ingredients and Nutritional value of the products used in the study.

S. No.	Products	Ingredients	Nutritional Value per 100gm
1	Spirulina Peanut Chikki	Spirulina, Peanut, Jaggery, Liquid Glucose	Calories 486, Calories from fat 213, Protein 16.62, Carbohydrates 51.71, Total fat 23.64, Saturated Fat 3.33, Trans Fat <0.1, Calcium 103.84, Iron 6.20, Dietary Fibre 11.71
2	Spirulina Ragi Biscuit	Spirulina, Ragi flour, Whole wheat, Edible Vegetable fat, Sugar, Milk Solids, Glucose, Raising Agents.	Calories 486, Calories From Fat 183, Protein 8.33, Carbohydrates 20.33, Total 20.33, Saturated Fat 11.07, Trans fat <0.1, Calcium 167.01, Iron 6.62, Dietary Fibre 7.64

Study Design

According to a study conducted by Ministry of health and welfare the malnutrition in the districts of Telangana was as follows in the year 2017. Siddipet was a new district formed after division of Medak district and it can be observed from the table that the Stunting and wasting is relatively higher. Hence, this place was selected for the study.

TABLE 3

District wise Stunting, wasting and underweight details of children in Telangana State.

District	Children Under 5 who are stunted (%)	Children under 5 who are wasted (%)	Children under 5 who are under weight.
Adilabad	38.3	22.1	35.8
Hyderabad	15.7	14.1	16.8
Karimnagar	24.3	19.3	25.4
Khammam	26.5	13.7	22.2
Mahbubnagar	37.1	18.6	34.5
Medak	33.4	20.7	37.0
Nalgonda	28.9	23.1	34.1
Nizamabad	36.6	22.0	36.3
Ranga Reddy	26.2	14.8	25.8
Warangal	26.6	16.6	29.1
TELANGANA	**28.1**	**18.0**	**28.5**

Source: Press Information Bureau, Government Of India, Ministry of Health and Family Welfare, (11 August 2017 16:45 IST).

The multi-stage single blind random sampling method was implemented for the present study. Sampling has been selected from 30 Anganwadis of Bharatnagar ICDS project and 34 anganwadis of Cheriyal ICDS projects of Siddipet district. The list of children enrolled is as follows.

TABLE 4

Selection of Study groups.

S. No.	Selection	Name of the ICDS selected	Number of Anganwadis selected	Number of children Enrolled
1.	Control Group	Cheriyal (CH)	34	2119
2.	Test Group	Bharat Nagar (BN)	30	2119

TABLE 5

Study design details.

S. No	Detail	Spirulina Peanut Chikki	Spirulina Ragi Biscuit
1.	Bite Size	~25gm	~20gm
2.	Amount of spirulina	~500 mg/piece	~500mg/piece
3.	No of pieces per day	1	1
4.	Duration of the day	In addition to break-fast or evening snack alternatively	In addition to breakfast or evening snack alternatively
5.	No of days per month	25 days	25 days
6.	Data collected	Every 25 days for test and control groups	Every 25 days for test and control groups

TABLE 6

Number of children enrolled in the Anganwadis.

S. No	Name of the Anganwadi under Cheriyal ICDS, Siddipet, Telangana	No of children enrolled (Control Group), CH	Name of the Anganwadi under Bharath Nagar ICDS, Siddipet, Telangana	No of children enrolled (Test Group) BN
1	Cheiral - I	50	Barimam 1	71
2	Cheiral - II	83	Barimam 2	60
3	Cheiral - III	59	Bharath nagar 1	71

TABLE 6 *Contd…*

S. No	Name of the Anganwadi under Cheriyal ICDS, Siddipet, Telangana	No of children enrolled (Control Group), CH	Name of the Anganwadi under Bharath Nagar ICDS, Siddipet, Telangana	No of children enrolled (Test Group) BN
4	Cheiral - IV	56	Bharath nagar 2	76
5	Cheiral - V	49	Bharath Nagar 3	72
6	Cheiral - VI	75	Bharath Nagar 4	86
7	Gunturpally -VII	70	Charavadan 1	72
8	Cherial-VIII (Pochamma Vedhi)	62	Charavadan 2	80
9	Kadaverugu - I	76	Dogbangla	66
10	Kadaverugu - II	62	Erukalawada 1	59
11	Kadaverugu - III	75	Erukalawada 2	66
12	Ramsagar	61	Hanuman Nagar 1	69
13	Pothireddipally - I	50	Hanuman Nagar 2	93
14	Pothireddipally - II	61	Kanchariwada 1	60
15	Peddaraiupeta	75	Kanchariwada 2	57
16	Nagapuri - I	56	Kanchit Chowrastha	58
17	Nagapuri - II	66	Khadeerpura 1	69
18	Nagapuri - III	75	Khadeerpura 2	74
19	Nagapuri - IV	48	Khadeerpura 3	85
20	Balbajiguda	62	Moinpura 1	58
21	Gandikunta	72	Moinpura 2	57
22	Chuchannakota - I	75	Moinpura 3	72
23	Chuchannakota - II	58	Moinpura 4	64
24	Chuchannakota - III	70	Murshad Gadda 1	72
25	Mustyala - I	67	Murshad Gadda 2	75
26	Mustyala - II	41	Murshad Gadda 3	70
27	Mustyala - III	60	Nasarpura 1	77
28	Veerannapet - I	65	Nasarpura 2	80
29	Veerannapet - II	62	Nasarpura 3	68
30	Veerannapet - III	75	Ramnagar	82
31	Rampur	54	-	-
32	Kasigudiselu	62	-	-
33	Kommuravelli - I	47	-	-
34	Kommuravelli - II	40	-	-
		2119	Total	2119

Anganwadi teachers having experience from their concurrent field work were visited personally for primary data collection. All the selected anganwadis were covered in this study. The children of Bharat Nagar ICDS were supplied with the chikkis and biscuits made from spirulina in addition to their normal routine diet given by anganwadis and the other group was not provided with any supplementation but were following the normal routine diet given by anganwadis. Approximately 25gm peanut chikki and approximately 20gm ragi spirulina biscuit were given to the children. Both were given to the children alternatively during breakfast as additional supplement and during snacking in the evening. The Protein Energy Malnutrition levels were considered and the deficit of 300kcal and 10gm (Daily Nutritional requirements of Indians, NIN) of protein was calculated and the dosage was designed accordingly.

Study Base Line and End Point

The data such as social background, economical background, gender, height, weight and age of the children were collected prior to the supplementation, during and after the supplementation at regular intervals. The anganwadi helpers were trained and were interviewed and instruction on data collection.

To monitor the growth of children in terms of height, weight, appetite (Observations on the scale of 1-10), physical activities (Observations on the scale of 1-10) and cognitive development (using VSMS tool) over a period of 32 weeks. Number of students enrolled in the each Anganwadi is given in the table below.

Results and Discussion

Data has been analyzed and given in the tabulated form and analyzed based on the obtained frequencies. The table gives clear picture that around 35.67% in Cheriyal and 33.84% of the respondents belongs to the Scheduled caste (SC) and 32.08% of the respondents belongs to Scheduled Tribes (ST), 49.25% of the respondents belongs to the other backward (OBC) Classes and lastly 1.83% of the respondents belongs to the other categories.

TABLE 7

Caste of the enrolled children.

S. No.	Caste	Frequency CH	%	Frequency BN	%
1	SC	758	35.67	717	33.84
2	ST	609	28.77	527	24.86
3	OBC	713	33.69	823	38.90
4	Others	39	1.87	52	2.45
Total		**2119**	**100%**	**2119**	**100%**

The caste-based enrollment was similar in the two ICDS projects selected.

The table below tells that 56.2% of the respondents were male and 43.8% of the respondents were female in Bharat Nagar ICDS, whereas 54.55% were male and 45.44% female children in Cheriyal ICDS.

TABLE 8

Gender of the enrolled children.

S. No.	Gender	Frequency CH	%	Frequency BN	%
1	Male	1156	54.55	1191	56.2
2	Female	963	45.44	928	43.8
	Total	**2119**	**100%**	**2119**	**100%**

The table 9 tells that children enrolled under the control group were not given the supplements at all but in the test group, about 96.33% of the respondents have consumed the spirulina chikkis and biscuits regularly, 3.25% of the respondents have not consumed the spirulina chikkis and biscuits regularly and only 0.42% of the respondents have not taken spirulina biscuits and chikkis. It is because they were hesitant towards the smell of spirulina after consuming. So, they could not continue the dosage. 3.25% of the respondents have not taken spirulina regularly because they have been out of the station, migration, change of their houses from one place to another place. So, they could not take the dosage regularly.

TABLE 9
Consumption of spirulina biscuits and chikkis regularly.

S. No.	Consumption of Spirulina biscuits and chikkis regularly	Frequency CH	%	Frequency BN	%
1	Regular	0	0	2041	96.33
2	Irregular	0	0	69	3.25
3	No	2119	100%	9	0.42
	Total	2119	100%	2119	100%

The table 10 expresses the observation of anganwadi teachers of the children after taking spirulina-based biscuits and chikkis and the ones without the supplementation. Only 54.9% of children from the control group and 79.42% of the respondent from the Test group have replied that the child's appetite has increased. With respect to the physical activity, teachers observed that 62.74% children have shown improvement in their physical activities in the control group whereas, 81.92% of the respondents from test group have shown improvement in their physical activities. Regarding cognitive development of the children, teachers told that as less as 45.27% children have shown an improvement in the control group whereas 79.75% of the respondents have shown positive response towards cognitive development in the test group.

The below graph represents the improvement of the increase in appetite, physical activities and cognitive development over the period of the study. This shows that there was a considerable increase in the children with supplementation when compared to the children without supplementation.

The difference in the increase in weights and heights of the students were calculated and have been recorded for every 25 days. After 32 weeks, it was found observed that over 82.64% of the children had an increase of their weight of more than 900gm in the test group when compared to only 68.39% children of control group. Also, the increase in height was calculated and analyzed. About 85.04% of the children gained height more than 5cm in the group with the supplementation whereas, only 71.79% of the children gained height above 5cm.

The increase in heights and weights was summarized every month and the growth in the control group was much lesser than that of the test group. Apart from the daily activities the children have shown good development in their BMI.

The graphical representation of the above work is represented in figure 3 here under.

TABLE 10
Anganwadi teacher's observation after intervention.

Sl. No	Teachers Observation after intervention	Yes (CH)		No (CH)		Yes (BN)		No (BN)	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
1	Increase in Appetite	1163	54.90	956	45.1	1690	79.75	429	20.24
2	Physical activities	1329	62.74	790	37.26	1736	81.92	383	18.07
3	Cognitive Development (Response)	959	45.27	1160	54.73	1683	79.42	436	20.58

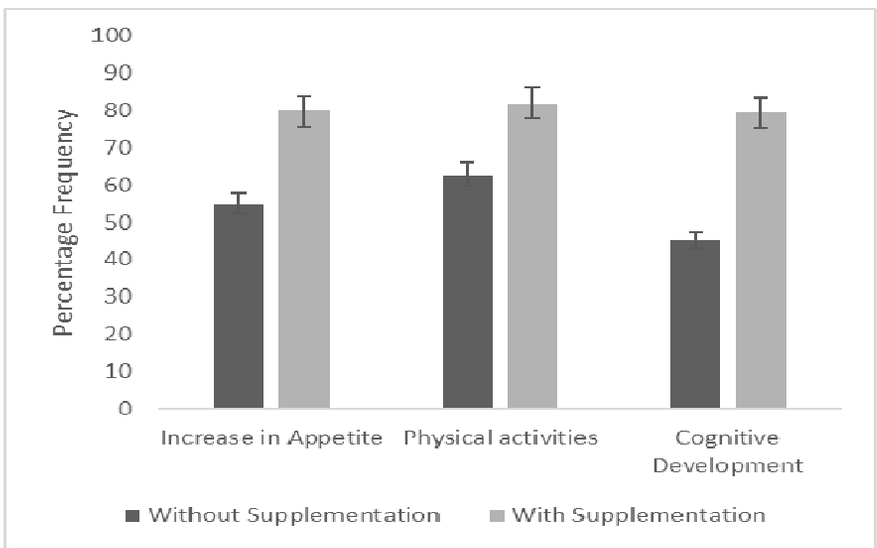


Fig. 2. Graphical representation of the recorded data in control and test groups.

TABLE 11

Calculation of differences in heights and weights after the intervention.

S. No	Data summarized after 32 weeks of intervention	Yes (CH)		No (CH)		Yes (BN)		No (BN)	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
1	Increase in Weight (>900gm)	1449	68.39	670	31.61	1751	82.64	386	17.36
2	Increase in Height (>5cm)	1522	71.79	597	28.21	1802	85.04	317	14.96

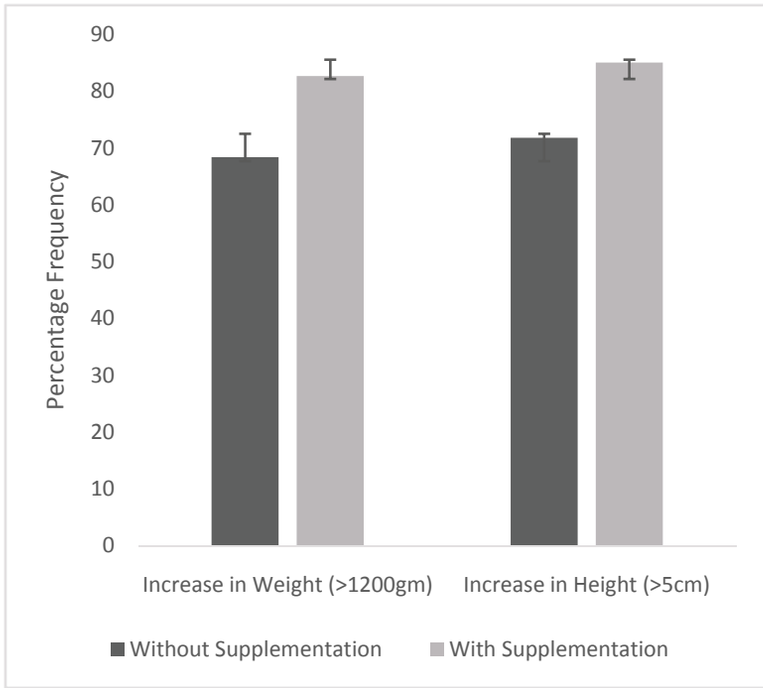


Fig. 3. Graphical representation on increase in heights and weights of the children with and without the spirulina supplementation.

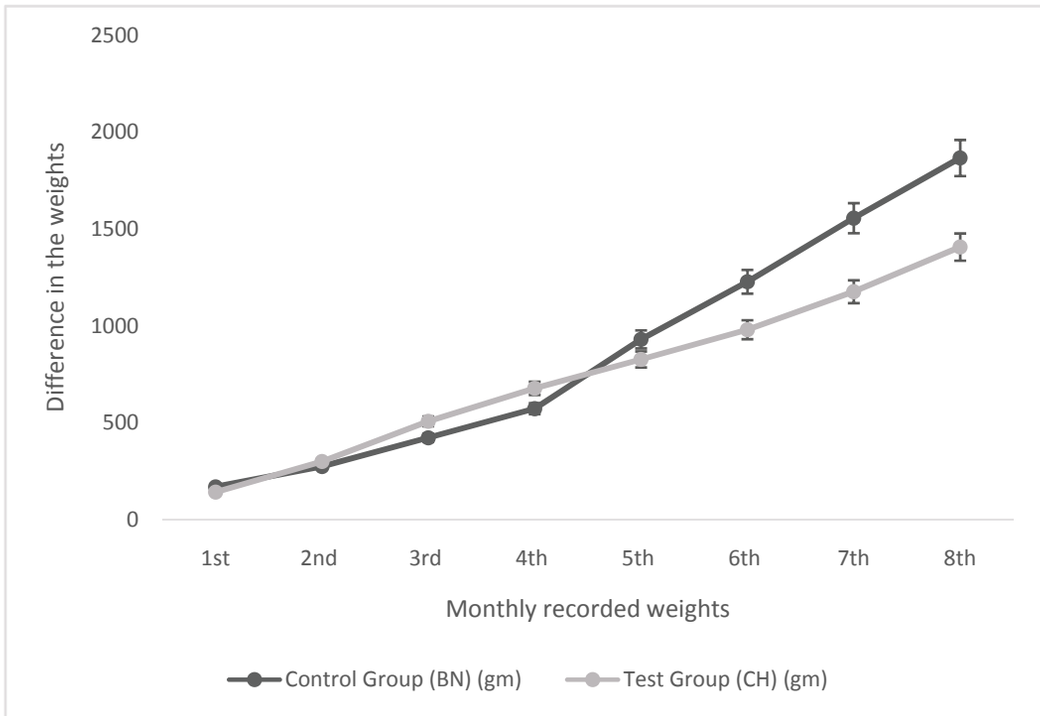


Fig. 4. The monthly increase in the weights of the children.

Also, the taste of the spirulina biscuits and chikkis was much appreciated by the children and the parents of the respondents. The anganwadi teachers referred to an increase in the regularity of the children after the intervention was started. It was also reported by Anganwadi teachers that there were no adverse effects recorded.

TABLE 12

Number of respondents wish to continue at Bharath Nagar ICDS.

Sl. No.	Wish to continue to feed Spirulina chikkis and biscuits	Frequency	Percentage
1	Yes	2080	98.16
2	No	39	1.84
	Total	**2119**	**100%**

Conclusions

From the above study, it was found that majority of the parents of the children enrolled under the program at ICDS Bharatnagar and Cheriyal were ignorant about their child's nutritional requirements. The data collected have given major findings on how micronutrients like spirulina can be given to children by masking its taste and odour. This way the intake was regular and consistent. The study also suggested that the children had benefitted in their height, weight, appetite, physical activities and their psychomotor skills. The average deficit of energy and protein, including the essential micronutrients like iron, calcium, dietary fiber etc., were provided with this healthy snacking. Considering the successful implementation at Siddipet, it can be expanded to other area where severe malnourished children are present. To enhance the scalability of the process and decentralized production of Spirulina, chikkis and biscuits, it would be better to involve rural women and build a capacity to encourage them to initiate small and micro enterprises so that the women and the entire community can produce and made available in the villages at a very reasonable cost to sustainability.

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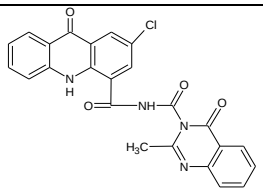
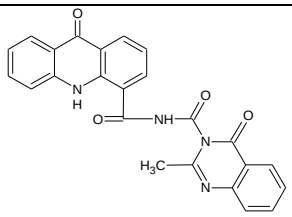
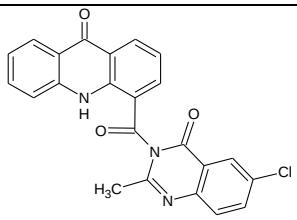
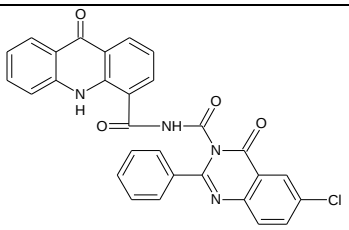
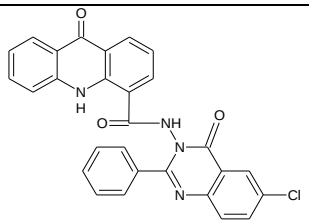
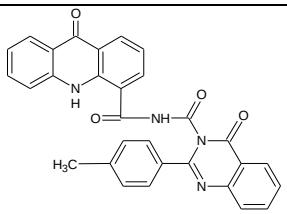
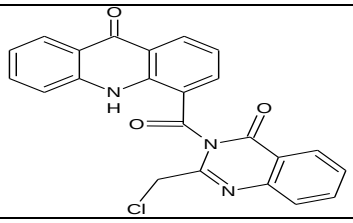
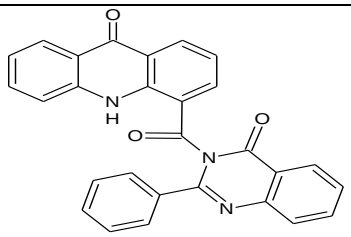
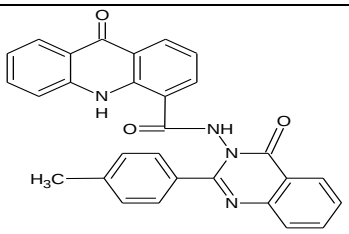
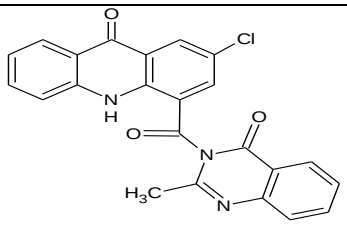
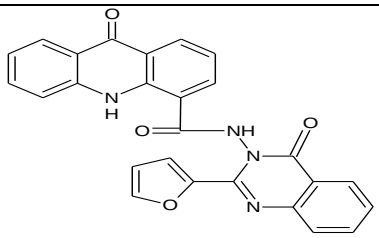
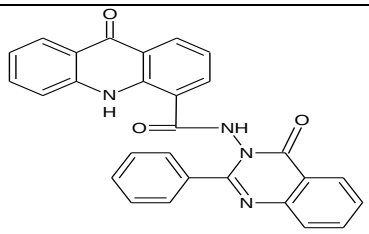
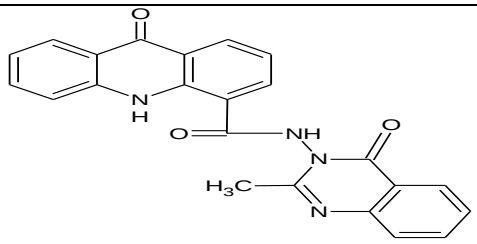
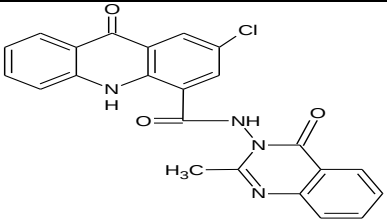
Development of nitric oxide releasing quinoline derivatives as inhibitors of doxorubicin resistance in cancer cells

Protein ligand interactions of the chlorine containing acridones with calmodulin dependent *c*AMP – Phosphodiesterase (PDE1c) enzyme were studied by employing an efficient docking protocol, GLIDE XP. Initially, a theoretically built digitalized structure of the protein PDE1C was retrieved from the protein databank with PDB ID: 1LXS. Structure of the protein was corrected by adding hydrogens to satisfy the valence and optimized by using OPLS-2005 force field (optimized potentials for liquid simulations). Binding pockets were identified by using the SITEMAP tool.Receptor grid generation was accomplished using Glide docking protocol and ligands were docked by employing XP mode of Glide. Best pose of each ligand was ranked according to the E-model energy.The docking score from Glide (Glide Score) is entirely based on Chem Score. It also includes a steric-clash term, adds polar terms featured by Schrodinger to correct electrostatic mismatches.

GScore = 0.065 x Van der Waals energy + 0.130 x Coulomb energy + Lipophilic term (Hydrophobic interactions) + H bonding + Metal binding + BuryP (Penalty for buried polar groups) + RotB (Penalty for freezing rotatable bonds) + Site (Polar interactions in the active site)

Table 1: Structures and Docking result

Compound	Structure	Dock Score
31		-6.95975
27		-6.50991
22		-5.43411
15		-5.26858
13		-5.1381
7		-5.12026
34		-5.1168

2		-5.07641
5		-4.94562
30		-4.85447
17		-4.8065
1		-4.59469
25		-4.50395
29		-4.39855

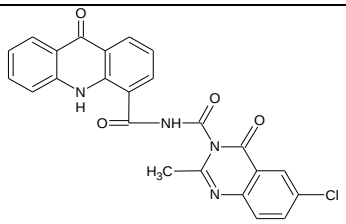
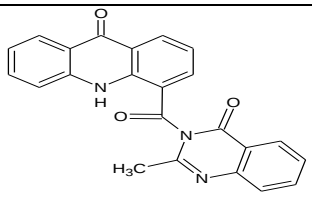
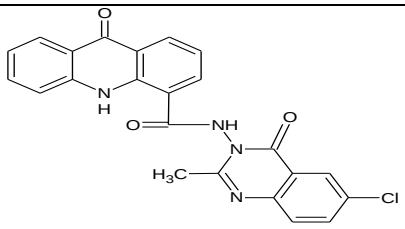
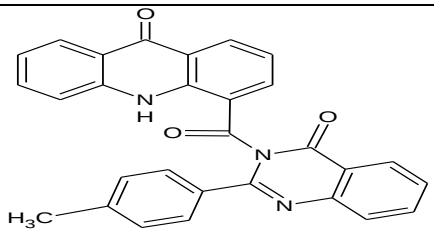
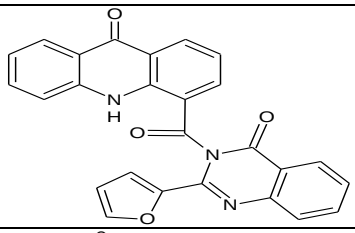
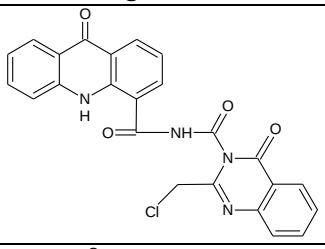
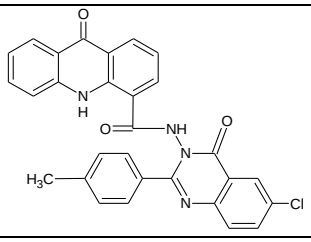
23		-4.35577
26		-3.88481
21		-3.80914
6		-3.78187
18		-3.63449
35		-3.56668
9		-1.06874

Table 2: Molecular Properties

Molecule	MW	Dipole	SASA	FOSA	FISA	PISA	WPSA	Volume
31	458.86	11.039	658.349	70.563	157.026	359.325	71.436	1230.942
27	424.415	8.66	629.351	77.161	151.305	400.884	0	1183.541
22	415.835	6.198	656.94	79.116	122.556	383.686	71.582	1176.015
15	520.931	6.794	747.129	0	152.374	536.597	58.158	1423.571
13	492.92	9.201	707.719	0	123.415	512.658	71.646	1337.1
7	500.512	7.376	767.625	88.161	150.923	528.54	0	1450.461
34	415.835	6.487	650.65	47.848	111.776	430.403	60.623	1168.089
2	443.461	7.605	728.319	0	97.518	630.801	0	1310.486
5	472.502	7.522	732.85	82.362	123.984	526.504	0	1361.525
30	415.835	6.966	663.243	76.295	110.553	407.046	69.348	1180.372
17	448.437	7.167	721.472	0	129.216	592.256	0	1295.234
1	458.475	6.013	723.914	0	108.808	615.106	0	1335.377
25	396.404	6.763	671.211	73.812	153.163	444.236	0	1214.115
29	430.849	6.856	675.721	73.187	155.319	408.472	38.741	1227.895
23	458.86	7.6	651.175	77.096	164.548	350.14	59.391	1222.537
26	381.39	6.457	644.47	76.561	109.386	458.523	0	1139.713
21	430.849	4.657	669.92	57.369	131.282	409.689	71.58	1207.827
6	457.487	6.821	732.655	88.049	121.623	522.983	0	1349.379
18	433.422	5.903	681.657	0	113.21	568.447	0	1246.227
35	458.86	8.331	680.817	45.996	161.111	411.045	62.665	1230.239
9	506.947	9.143	689.689	56.737	124.562	436.846	71.543	1338.67

Recommended range: MW – molecular weight (130-725), dipole (1-12.5), SASA- solvent accessible surface area (300-1000), FOSA – hydrophobic component of SASA (0-750), FISA – hydrophilic component of SASA (7-330), PISA -π (carbon and attached hydrogen) component of the SASA (0.0 – 450.0), WPSA - Weakly polar component of the SASA (halogens, P, and S) (0.0 – 175.0),volume (500-2000).

Table 3:Predicted Pharmacokinetic (ADME) profiles of compounds

Molecule	CNS	QPlog Po/w	QPlogS	QPlog HERG	QPP Caco	QPlog BB	QPP MDCK	QPlog Kp	QPlog Khsa	% Human Oral Absorption
31	-1	3.628	-5.438	-4.4	240.341	-0.792	356.954	-3.052	0.356	90.802
27	-1	3.135	-4.549	-4.338	268.094	-0.861	165.928	-2.8	0.218	88.761
22	0	4.163	-5.936	-6.271	681.864	-0.536	806.72	-2.331	0.518	100
15	-1	5.074	-6.918	-5.643	268.846	-0.928	336.946	-2.245	0.873	74.222
13	0	4.887	-6.294	-6.738	669.197	-0.638	791.177	-1.7	0.727	100
7	-2	5.028	-7.071	-5.797	280.164	-1.101	167.433	-2.247	0.951	74.274
34	0	4.199	-5.769	-6.426	862.827	-0.445	906.119	-1.967	0.495	100
2	0	5.23	-6.813	-7.854	1177.977	-0.585	590.528	-0.903	0.866	100
5	-1	4.778	-6.442	-7.107	660.931	-0.875	316.199	-1.662	0.791	100
30	0	4.293	-6.045	-6.476	886.179	-0.438	1041.151	-2.027	0.531	100
17	-1	4.162	-6.169	-7.65	589.582	-0.915	279.47	-1.623	0.526	100
1	-1	4.819	-6.278	-7.538	920.605	-0.723	452.395	-1.07	0.718	100
25	-2	3.537	-5.479	-6.563	349.506	-1.076	158.81	-2.585	0.424	93.178
29	-1	3.716	-5.73	-6.382	333.431	-0.999	246.037	-2.751	0.463	93.863
23	-1	3.465	-5.251	-4.269	204.091	-0.873	256.757	-3.223	0.332	88.573
26	0	3.832	-5.387	-6.651	909.058	-0.585	446.264	-1.824	0.413	100
21	0	3.88	-5.767	-6.419	563.58	-0.687	656.569	-2.304	0.406	100
6	-1	5.191	-6.896	-7.165	695.904	-0.805	334.322	-1.727	0.979	95.256
18	0	4.386	-5.83	-7.13	836.237	-	407.753	-	0.588	100

						0.615		1.507		
35	-1	3.622	-5.96	-5.102	236.863	-0.958	290.196	-2.945	0.354	90.655
9	0	4.806	-5.965	-6.015	652.642	-0.587	769.041	-1.988	0.737	92.504

Recommended range: CNS Predicted central nervous system activity on a –2 (inactive) to +2 (active) scale; QPlogPo/w: Predicted octanol/water partition coefficient (–2.0 - 6.5); QPlogS: Predicted aqueous solubility (-6.5 – 0.5); QPlogHERG: Predicted IC50 value for blockage of HERG K+ channels (below -5); QPPCaco: Predicted apparent Caco-2 cell permeability in nm/sec. Caco- 2 cells are a model for the gut-blood barrier (<25: poor, >500: great); QPlogBB: Predicted brain/blood partition coefficient (-3 – 1.2); QPPMDCK: Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier (<25: poor, >500: great); QPlogKp: Predicted skin permeability, log Kp (–8.0 – –1.0); QPlogKhsa: Prediction of binding to human serum albumin (–1.5 – 1.5); %Human- Oral Absorption (>80% is high, <25% is poor).

Table 4: Binding energy predictions using MMGBSA calculations

Compound	Prime MMGBSA Ligand Energy	Prime MMGBSA Receptor Energy	Prime MMGBSA Complex Energy	Prime MMGBSA DG bind
31	10.614942	-12400.48218	-12461.73689	-71.869657
27	31.020423	-12400.48218	-12436.37635	-66.914599
22	26.875696	-12400.48218	-12417.01052	-43.404036
15	24.361114	-12400.48218	-12438.3363	-62.215234
13	12.897055	-12400.48218	-12457.31489	-69.729772
7	35.067764	-12400.48218	-12418.86285	-53.44844
34	29.497186	-12400.48218	-12429.6953	-58.710309
2	44.695166	-12400.48218	-12422.17456	-66.387554
5	19.412305	-12400.48218	-12437.80588	-56.736014
30	17.752203	-12400.48218	-12450.17266	-67.442686
17	21.947572	-12400.48218	-12431.30236	-52.767752
1	22.618603	-12400.48218	-12441.6908	-63.827227
25	12.295664	-12400.48218	-12436.40754	-48.221029
29	-7.729278	-12400.48218	-12461.50121	-53.289759
23	18.010575	-12400.48218	-12445.86514	-63.39354
26	38.163555	-12400.48218	-12417.89096	-55.572336
21	-4.800136	-12400.48218	-12470.05472	-64.772412
6	36.72764	-12400.48218	-12389.21948	-25.464948
18	40.025004	-12400.48218	-12398.78225	-38.325076
35	25.292569	-12400.48218	-12403.00388	-27.814275
9	10.790788	-12400.48218	-12437.26277	-47.57138

The Prime MM-GBSA approach is used to predict the free energy of binding for a receptor anda set of ligands. MM-GBSA is an acronym for a method that combines OPLS molecularmechanics energies (EMM), an SGB solvation model for polar solvation (GSGB), and a nonpolarsolvation term (GNP) composed of the nonpolar solvent accessible surface area and van derWaals interactions. The total free energy of binding is then expressed as:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

where

$$G = E_{MM} + G_{SGB} + G_{NP}$$

The ligand in the unbound state is minimized in SGB solvent but is not otherwise sampled. Inthe calculation of the complex, the ligand is minimized in the context of the receptor. Theprotein is currently held fixed in all calculations. The following descriptors generated by thePrime MM-GBSA approach:

MM-GBSA_DG_bind: Ligand binding energy, ΔG_{bind}

MM-GBSA_E_complex: Energy of the complex, $G_{complex}$

MM-GBSA_E_protein: Energy of the receptor without the ligand, $G_{protein}$

MM-GBSA_E_ligand: Energy of the unbound ligand, G_{ligand}

Table 5: **Embrase calculations for ligand binding energy**

Compound	MBAE Complex Total Energy-OPLS-2005	MBAE Rec Total Energy-OPLS-2005	MBAE Lig Total Energy-OPLS-2005	MBAE Del Total Energy-OPLS-2005
31	-4464.179668	-4365.702312	93.740479	-192.217834
27	-4363.533169	-4365.702312	176.034424	-173.86528
22	-4453.182247	-4365.702312	149.049454	-236.529388
15	-4465.980438	-4365.702312	155.900314	-256.17844
13	-4486.879223	-4365.702312	70.90551	-192.08242
7	-4442.784595	-4365.702312	193.26413	-270.346413
34	-4412.546429	-4365.702312	166.637756	-213.481873
2	-4328.60968	-4365.702312	218.52951	-181.436878
5	-4477.749912	-4365.702312	97.529282	-209.576881
30	-4387.141613	-4365.702312	109.180939	-130.620239
17	-4420.547894	-4365.702312	110.716797	-165.562378
1	-4470.624641	-4365.702312	98.782898	-203.705227
25	-4580.207062	-4365.702312	50.148129	-264.652878
29	-4675.097286	-4365.702312	-26.948503	-282.44647
23	-4449.573925	-4365.702312	130.186203	-214.057816
26	-4316.43198	-4365.702312	193.465561	-144.195229
21	-4694.094612	-4365.702312	-12.645243	-315.747057
6	-4308.456825	-4365.702312	209.598465	-152.352978
18	-4363.897919	-4365.702312	178.150131	-176.345737
35	-4335.764233	-4365.702312	162.9133	-132.97522
9	-4540.806538	-4365.702312	58.089577	-233.193802

Embrace calculates ligand-receptor binding energies by molecular mechanics energy minimization of the complex and the separated receptor and ligand, with or without continuum solvation. The Embrace calculation is run in energy difference mode. The following descriptors aregenerated from the calculation:

Embrace_Total_Energy_without_constraints: Ligand binding energy

Embrace_Valence_Energy: Valence energy difference

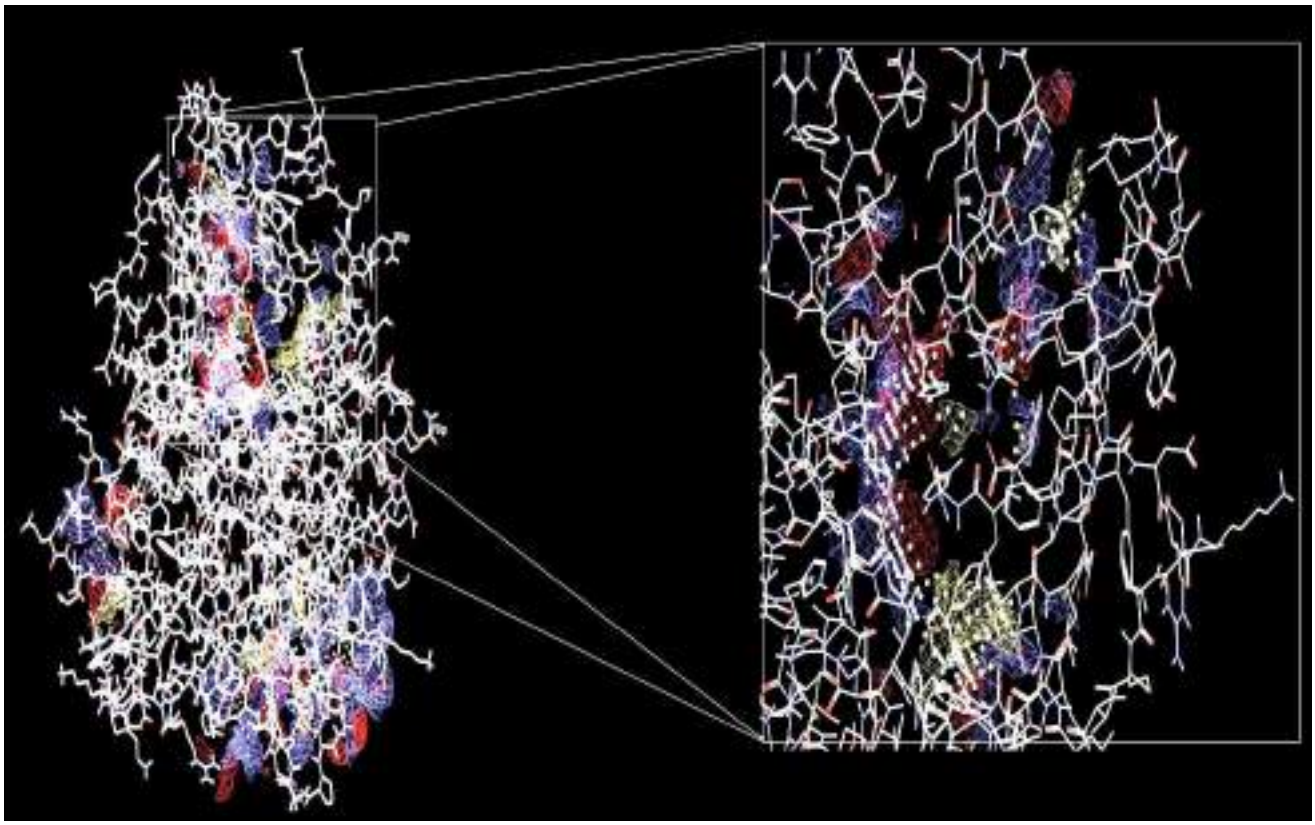
Embrace_vdW_Energy: van der Waals energy difference

Embrace_Electrostatic_Energy: Coulomb energy difference

Embrace_Solvation_Energy: Solvation energy difference

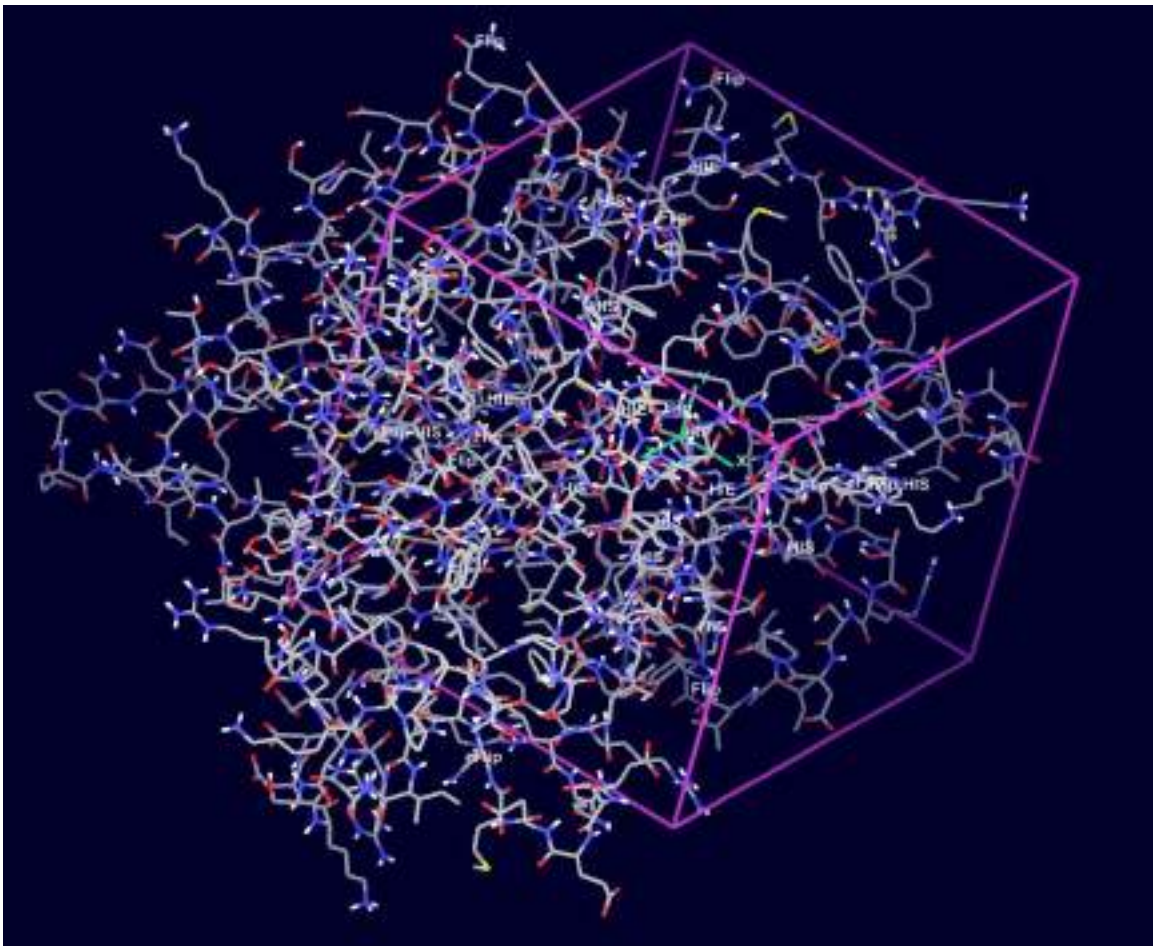
Embrace_Constraint_Energy: Constraint energy difference

Figure 1: site map calculations for the protein 1LXS.



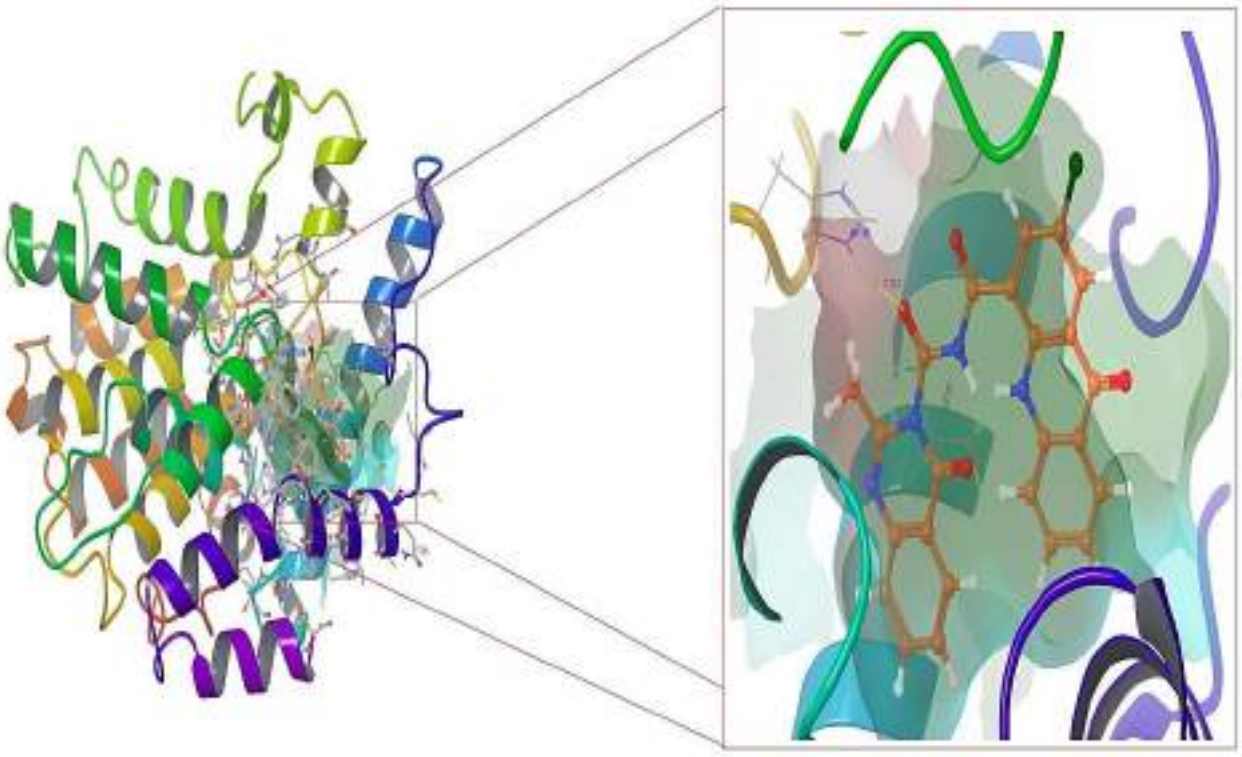
- Site map calculation identifies the possible receptor pockets along with their area and volumes.
- Site map is useful for binding site characterization and also in SBDD.
- Red colour: hydrogen bond acceptor region (high conc of oxygen)
- Blue color: hydrogen bond donor region (high conc of nitrogen)
- Yellow colour: hydrophobic region.

Figure 2: 1LXS Grid position for docking



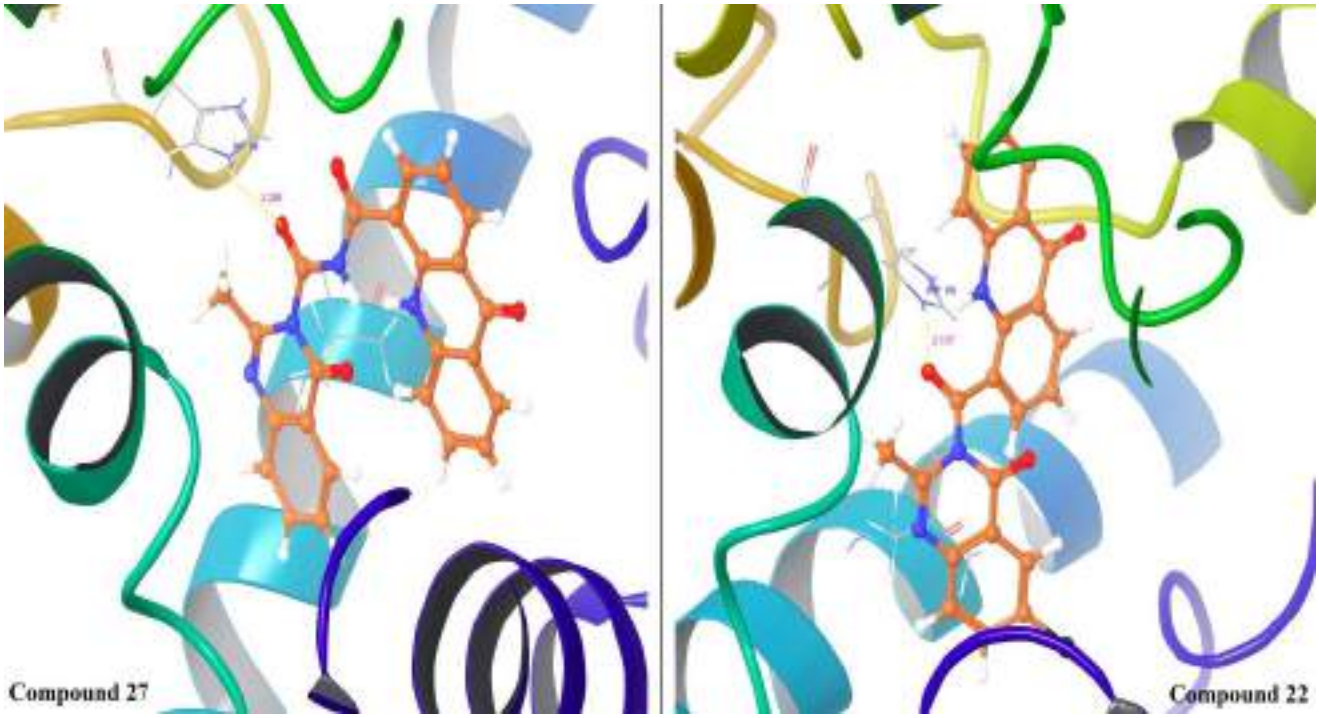
- Magenta colour cube represents the grid established for docking.
- Ligands with maximum of 20Å can fit into the grid.

Figure 3: protein ligand interactions of the docked PDE1c enzyme and ligand 31 complex.



- 1 hydrogen bond with Histidine 66 with a bond length of 2.132 Å
- Hydrogen bond is represented in yellow dotted lines.
- Magnified image shows the ligand conformational fitting into the receptor pocket.

Figure 4: Docking conformations of compound 27 and 22.



- Compound 27, and 22 interact with Histidine 66 residue of protein at hydrogen bond distance of 2.269Å and 2.107 Å respectively.

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This is to certify that **Miss. AFREEN KHANAM (HT.NO.18DH1S1201)** pursuing her M. Pharmacy in **VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH** has carried out her project work in our Organization entitled **"SIMULTANEOUS ESTIMATION OF NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DOLUTEGRAVIR AND LAMIVUDINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY"** in the department of Pharmaceutical Analysis from **2-MAY-2019 TO 03-OCTOBER-2020.**

During her tenure she was sincere, hardworking and Punctual in her Project work.

We wish her to success in her future career.

Authorized Signatory

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Study No: GMC2018-002
Sample Type (Matrix): Serum
Assay Kit: 25(OH) Vitamin D Kit: Krishgen Biosystems Cat# KBH501

Animal No.	Sample ID	25(OH) Vit-D Concn. (ng/ml) in Serum [Final Day (Day 84)]
1	S1	31.72
2	S2	46.23
3	S3	23.27
4	S4	24.38
5	S5	26.42
6	S6	21.49
7	S7	24.50
8	S8	25.95
9	S9	24.44
10	S10	23.10
11	S11	20.79
12	S12	24.69
14	S13	20.26
16	S14	26.42
17	S15	24.26
18	S16	23.91
19	S17	23.85
20	S18	21.75
21	S19	16.43

Prepared by:

(Mr Y. Vishwanadham)

Authorized by:

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INVOICE

Date: 13.02.2020

S.No	Particulars	No. of Samples	Amount in Rs.
1	Charges for Elisa tests conducted in our Laboratory	36	50,000-00
	TOTAL		**50,000-00**
	(Rupees Fifty Thousand Only)		

For Vishnu Institute of Pharmaceutical Education & Research

13.2.2020

Authorized Signatory

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Date 13.02.2020

To,

Head'
Cell & Molecular Biology,
R&D Centre,
Lila Nutraceuticals
Vijayawada
Andhra Pradesh

Dear Sir,

Sub: Request for payment of Charges for Research work done at our Laboratory - Reg.

***

We are happy to support Elisa testing for biological samples at our Research Laboratory i.e. Centre for Molecular Cancer Research (CMCR). The testing charges for the same is amounting Rs.50,000/- We request for the payment of **Rs. 50,000/-(Fifty thousand Rupees only)**.

With regards,

PRINCIPAL

Encl: Invoice

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This is to certify that **Miss. CHAPALA UMARANI (HT.NO.18DH1S1203)** pursuing her M. Pharmacy in **VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH** has carried out her project work in our Organization entitled **"SIMULTANEOUS DETERMINATION OF DARUNAVIR AND COBICISTAT IN BULK FORM AND COMBINED MARKETED FORMULATION BY USING REVERSE PHASE-HPLC METHOD"** in the department of Pharmaceutical Analysis from **2-MAY-2019 TO 03-OCTOBER- 2020.**

During her tenure she was sincere, hardworking and Punctual in her Project work.

We wish her to success in her future career.

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During her tenure she was sincere, hardworking and Punctual in her Project work.

We wish her to success in her future career.

Authorized Signature

Mrs.Rajini Sura

Managing Director

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During her tenure she was sincere, hardworking and Punctual in her Project work.

We wish her to success in her future career.

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Ref: 03-09/5/21

CERTIFICATE

This is to certify that Miss. **MUTHARI MAMATHA (HT.NO.1808E51209)** pursuing her M. Pharmacy at **VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH** has carried out her project work in our Organization entitled **"SIMULTANEOUS ESTIMATION OF ESCITALOPRAM AND CLONAZEPAM IN NATURAL SHAPE AND ADVERTISED PHARMACEUTICAL DOSAGE FORM THROUGH THE USAGE OF REVERSE PHASE-HPLC APPROACH"** in the department of Pharmaceutical Analysis from **2 MAY-2019 TO 03-OCTOBER-2020.**

During her tenure she was sincere, hardworking and Punctual in her project work.

We wish her to success in her future career.

Authorized Signatory

Mr. Ra[illegible]

Managing Director

4th Floor, S.S. Towers, Beside Chandana Brothers, Dilsukhnagar, Hyderabad - 500 060, T.S., India.
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Date: 03-10-2020.

CERTIFICATE

This is to certify that **Miss. R.MANASA (HT.NO.18DH1S1213)** pursuing her M. Pharmacy in **VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH** has carried out her project work in our Organization entitled **"A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE DETERMINATION OF SITAGLIPTIN AND SIMVASTATIN IN BULK FORM AND COMBINED MARKETED PHARMACEUTICAL DOSAGE FORM BY USING REVERSE PHASE-HPLC"** in the department of Pharmaceutical Analysis from **2-MAY-2019 TO 03- OCTOBER-2020.**

During her tenure she was sincere, hardworking and Punctual in her Project work.

We wish her to success in her future career.

Authorized Signature

Mrs.RajiniSura

Managing Director

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Date: 03-10-2020.

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This is to certify that **Miss. NAZMA BEGUM (HT.NO.18DH1S1210)** pursuing her M. Pharmacy in **VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH** has carried out her project work in our Organization entitled **"A NEW RP-HPLC METHOD AND IT'S VALIDATION FOR THE ANALYSIS OF ARTESUNATE AND MEFLOQUINE IN BULK AND PHARMACEUTICAL DOSAGE FORM AS PER ICH GUIDELINES"** in the department of Pharmaceutical Analysis from **2-MAY-2019 TO 03-OCTOBER- 2020.**

During her tenure she was sincere, hardworking and Punctual in her Project work.

We wish her to success in her future career.

Authorized Signature

SURA LABS (Regd.No.3524) DILSUKHNAGAR

Mrs.RajiniSura

Managing Director

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Date: 03-10-2020.

CERTIFICATE

This is to certify that **Miss. P.BHAVANA (HT.NO.18DH1S1212)** pursuing her M. Pharmacy in **VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH** has carried out her project work in our Organization entitled **"DEVELOPMENT AND VALIDATION OF A SIMPLE AND NOVEL RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF LEVODOPA AND CARBIDOPA IN BULK FORM AND PHARMACEUTICAL DOSAGE FORM ACCORDING TO ICH GUIDELINES"** in the department of Pharmaceutical Analysis from **2-MAY-2019 TO 03- OCTOBER- 2020.**

During her tenure she was sincere, hardworking and Punctual in her Project work.

We wish her to success in her future career.

Authorized Signature

Mrs.RajiniSura

Managing Direct

SURA LABS (Regd.No.3524) DILSUKHNAGAR, HYDERABAD

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Pellets
Pharma Limited

20th November 2020.

TO WHOM SO EVER IT MAY CONCERN

This is to certify that Ms.CH. Shailaja, (Regd. No: 18DH1S1204, of (Vishnu Institute of pharmaceutical education and research), has undergone industrial training in **"Analytical Research and Development Department" in "Analytical Method Development and Method Validation of Stability Indicating Related Substances by RP-HPLC for Levomilnacipran Pellets"** at **Pellets Pharma Limited** from 01st October 2019 to 31st July 2020.

During this period, we found that her conduct is satisfactory. We wish her all the best for her future endeavors.

For **Pellets Pharma Limited,**

Pellets Pharma Limited HR

Authorized Signatory,

Human Resource-Department

Pellets
Pharma Limited

30th November 2020.

TO WHOM SO EVER IT MAY CONCERN

This is to certify that Ms. Yarlagadda. Navya, (Regd. No: 18DH1S0310, of (Vishnu Institute of pharmaceutical education and research), has undergone industrial training in **"Formulation Research and Development Department" in "Formulation Development and Invitro Evaluation of Esomeprazole Magnesium Trihydrate Capsule"** at **Pellets Pharma Limited** from 01st October 2019 to 02nd June 2020.

During this period, we found that her conduct is satisfactory. We wish her all the best for her future endeavors.

For **Pellets Pharma Limited,**

Authorized Signatory,

Human Resource-Department

Admn. Office : 784, Vivekanandanagar Colony, Kukatpally, Hyderabad, Telangana, INDIA - 500 072.
Phone : + 91-40-23160194-95, www.pelletspharma.com
Factory : Plot No. 8 & 9, TSIIC-EPIP, Pashamylaram, Patancheru (Mandal), Sangareddy (Dist.)
Telangana (T.S), INDIA - 502 307.

HETERO LABS LIMITED

(FORMULATIONS R&D)

"Hetero Corporate", 7-2-A2, Industrial Estates, Sanath Nagar, Hyderabad - 500 018. Telangana, INDIA.
Tel : 91-40-23704923/24/25, Fax : 91-40-23704926, 23714250
E-mail : contact@heterodrugs.com URL : http//www.heterodrugs.com
CIN: U24110TG1989PLC009723

Date: 21-05-2020

TO WHOMEVER IT MAY CONCERN

This is to certify that Ms.Y.Girisha Reddy bearing roll no: 18DH1S1215 Student of M.Pharmacy from Vishnu Institute of Pharmaceutical Education & Research, Narsapur affiliated to JNTU-H University has been allotted a project on **"Method Development & Validation for the Estimation of Solifenacin Succinate in Bulk & tablet dosage form by RP - HPLC"** in Analytical Research & Development Department of our unit, as part of her curriculum.

She has successfully completed her project from 10th October, 2019 to 20th March, 2020.

We found her sincere and hardworking during the project period.

We wish her all the best in future career.

Authorized Signatory,

21-05-20

HR Department.

VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH
VISHNUPUR, NARSAPUR, MEDAK 502313.

GRANULES INDIA LIMITED

VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION & RESEARCH
Vishnupur, Narsapur, Medak District 502 313

GIL BSC 3 & 4 BATCH 1st YEAR I SEMESTER TIMETABLE

S.NO	DATE	9.00-11.00	11.00-1.00	1.00-3.00	3.00-5.00	5.00-7.00
1	16-10-2019	CHEMISTRY,LAKSHMANTH	MATHS,MOHTADAY	BREAK	CHEMISTRY,LAKSHMANTH	MATHS,MOHTADAY
2	23-10-2019	MATHS,MOHTADAY	MATHS,MOHTADAY		MATHS,MOHTADAY	MATHS,MOHTADAY
3	30-10-2019	MATHS,MOHTADAY	PHYSICS,N.SUSHMA		MATHS,MOHTADAY	PHYSICS,N.SUSHMA
4	06-11-2019	CHEMISTRY,FLAWERENCE	PHYSICS,N.SUSHMA		CHEMISTRY,FLAWERENCE	PHYSICS,N.SUSHMA
5	13-11-2019	CHEMISTRY,FLAWERENCE	PHYSICS,N.SUSHMA		CHEMISTRY,FLAWERENCE	PHYSICS,N.SUSHMA
6	19-11-2019	CHEMISTRY,FLAWERENCE	MATHS,MOHTADAY		CHEMISTRY,FLAWERENCE	MATHS,MOHTADAY
7	26-11-2019	CHEMISTRY,PURUSHOTTAM	PHYSICS,N.SUSHMA		CHEMISTRY,PURUSHOTTAM	PHYSICS,N.SUSHMA
8	03-12-2019	CHEMISTRY,PURUSHOTTAM	MATHS,MOHTADAY		CHEMISTRY,PURUSHOTTAM	MATHS,MOHTADAY
9	10-12-2019	CHEMISTRY,PURUSHOTTAM	MATHS,MOHTADAY		CHEMISTRY,PURUSHOTTAM	MATHS,MOHTADAY
10	17-12-2019	CHEMISTRY,PURUSHOTTAM	MATHS,MOHTADAY		CHEMISTRY,PURUSHOTTAM	MATHS,MOHTADAY
11	24-12-2019	CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM		CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM
12	31-12-2019	CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM		CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM
13	07-01-2020	CHEMISTRY,PURUSHOTTAM	PHYSICS,N.SUSHMA		CHEMISTRY,PURUSHOTTAM	PHYSICS,N.SUSHMA
14	14-01-2020	Holiday			Holiday	
15	21-01-2020	PRACTICALS			PRACTICALS	
16	28-01-2020	PHYSICS,N.SUSHMA	CHEMISTRY,PURUSHOTTAM		PHYSICS,N.SUSHMA	CHEMISTRY,PURUSHOTTAM
17	04-02-2020	MATHS,MOHTADAY	CHEMISTRY,PURUSHOTTAM		MATHS,MOHTADAY	CHEMISTRY,PURUSHOTTAM
18	11-02-2020	CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM		CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM
19	18-02-2020	CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM		CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM
20	25-02-2020	MATHS,MOHTADAY	CHEMISTRY,PURUSHOTTAM		MATHS,MOHTADAY	CHEMISTRY,PURUSHOTTAM
21	03-03-2020	CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM		CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM
22	09-03-2020	MATHS,MOHTADAY	MATHS,MOHTADAY		MATHS,MOHTADAY	MATHS,MOHTADAY
23	17-03-2020	PHYSICS,N.SUSHMA	PHYSICS,N.SUSHMA		PHYSICS,N.SUSHMA	PHYSICS,N.SUSHMA
24	24-03-2020	CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM		CHEMISTRY,PURUSHOTTAM	CHEMISTRY,PURUSHOTTAM
25	24-03-2020	MATHS,MOHTADAY	MATHS,MOHTADAY		MATHS,MOHTADAY	MATHS,MOHTADAY

COORDINATOR

PRINCIPAL

Principal
Vishnu Institute of Pharmaceutical
Education & Research
Narsapur, Medak Dist.-502313

VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH								
VISHNUPUR, NARSAPUR, MEDAK Dt-502313								
Sl.No	Batch No	Invoice Amount	TDS %	Amount Received	Amount Due	Expenditure paid	Expenditure to be paid	Balance retained in Pri. & AO A/c, VIPER
1	GIL BSC -1(FIRST BATCH)	282000	7.5%	260850	0	90600	0	170250
2	GIL BSC -2(FIRST BATCH)	282000	7.5%	260850	0	68200	0	192650
3	GIL BSC -3(FIRST BATCH)	282000	7.5%	260850	0	76800	0	184050
4	GIL BSC -1(SECOND BATCH)	282000	7.5%	260850	0	98400	0	162450
5	GIL BSC -2(SECOND BATCH)	282000	10%	253800	0	92800	0	161000
6	GIL BSC -3(SECOND BATCH)	282000	10%	253800	0	28000	0	225800
7	GIL BSC -4(FIRST BATCH)	282000	10%	253800	0	64000	0	189800
8	GIL BSC -5(FIRST BATCH)	282000	10%	253800	0	61600	0	192200
9	GIL BSC -6(FIRST BATCH)	282000	10%	0	253800	19200	0	-19200
10	GIL BSC -4(SECOND BATCH)	282000	10%	0	253800	0	54200	0
TOTAL		2820000		2058600	507600	599600	54200	1459000

Principal
Vishnu Institute of Pharmaceutical
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"Phytochemical screening and evaluation of acute oral toxicity on Sandhivaataari gutika"

Sandhivaataari gutika is a poly herbal formulation which posses many therapeutic effects on human body.

Preparation of Sandhivaataari Gutika

The purified hingulam, guggulu (commiphora mukul) and bola(Commiphora molmol) are made into powder. This powder is pounded in Godugdha(cow's milk)for one day followed by drying and powdering . Powder is made into pills in the dose of 125mg.

METHODOLOGY

Table no.1 Materials

Material	Supplier
Sandhivaataari gutika	Davasaz (herbal shops) Begum bazaar ,Hyderabad.
solvent	water
Animals	Mahaveer enterprises

Preparation of plant extract

MATERIALS AND METHODS:

S.no	Equipment	Manufacturer
1	Borosil Soxhlet extractor	Borosil Manfacture
2	Solvent evaporator	Chemi Tech
3	Analgesiometer	INCO

4	Eddy's hot plate	INCO
5	Digital balance	ELB 300- SHIMADZU
6	Syringes and needles	Local Market

PROCESS:

BOROSIL SOXHLET EXTRACTOR:

Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a *limited* solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Fruit extraction in progress. The sample is placed in the thimble.

Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser.

The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet

chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded[13]

Preparation of extracts:

First the powered drug was subjected to extraction. The extracts were prepared by using hot air percolation technique using soxhlet apparatus, a process of extraction of a drug with a solvent with several daily shakings. This method was based on the extraction of active constituents by simple hot air percolation using water as solvent.

50g of the powdered material was placed inside a thimble supported by cotton pads which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser.

The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

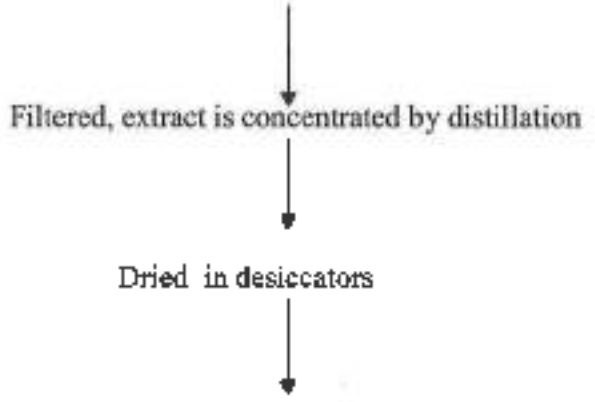
- The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask.

After 24 hrs, the water extract was filtered and the marc was repeated two more times with the same solvent for effective extraction. Extract was concentrated by open air drying. And the acquired extract was stored in a dessicator[14].

A Schematic Representation of Extraction

50g of powder was percolated with 500ml water as solvent for several times



4.2 Phytochemical screening of drug

PROCEDURE:

Test solution: Depending upon the type of natural drug under examination. the test solution may be a aqueous extract or alcoholic extract.

1. Alkaloids:

(a) **Dragendroff's reagent**: Alkaloids give reddish brown precipitate with Dragendroff's reagent (potassium bismuth iodide solution)

(b) **Mayer's reagent**: Alkaloids give cream colour precipitate with Mayer's reagent (potassium mercuric iodide solution)

(c) **Wagner's reagent**: Alkaloids give reddish brown precipitate with Wagner's reagent (Iodine potassium iodide solution)

(d) **Hager's reagent**: Alkaloids give yellow precipitate with Hager's reagent (saturated solution of picric acid)[15].

2. **Carbohydrates** (with aqueous test solution)

(a) **Molisch's test**: To the test solution few drops of alcoholic 1-napthol was added. Then few drops of conc.H_2SO_4 was added through sides of test tube; purple to violet colour ring appears at the junction.

(b) **Barfoed's test**: 1ml of the test solution is heated with 1ml of barfoed's reagent on water bath. appearance of red color, indicates the presence of monosaccharide. Disaccharides on prolonged heating (about 10min) may also cause reduction, owing to partial hydrolysis to monosaccharide.

(C) **Seliwanoff's test** : To the test solution, crystals of resorcinol and equal amount of conc.HCl were added and heated on a water bath, appearance of pink color indicates the presence of carbohydrates

(d) **Test for pentose**: To the test solution, equal volume of hydrochloric acid containing small amount of phloroglucinol were added and heated, red color indicates the presence of carbohydrates[16].

3 . Proteins:

(a) **Warming test**: The test solution was heated in a boiling water bath, proteins gets coagulated.

(b) **Test with trichloroacetic acid**: To the test solution, trichloro acetic acid was added, precipitate formation indicates the presence of proteins.

(c) **Biuret test**: To the test solution (2ml) violet colour indicates presence of proteins.

(d) **Hydrolysis test**: Hydrolyze the test solution with HCl or H_2SO_4.Then carry out for ninhydrin test for amino acids.

(e) **Xanthoproteic test**: To the (5ml) of test solution, 1ml of conc.HNO_3 was added and boiled, yellow precipitate is formed. After cooling it, 40% NaOH solution was added, orange colour is formed[17]

4. Flavanoids:

(a) **Shinoda test**: To the test solution, few magnesium turningswere added and conc.HCl was added drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

(b) **Alkaline reagent test:** To the test solution, few drops of sodium hydroxide solution were added, intense yellow is formed which turns to colourless on addition of few drops of dilute acid indicates presence of flavanoids.

(c) **Zinc hydrochloride test:** To the test solution, a mixture of zinc dust and conc.Hcl was added. It gives red colour after few minutes[18].

5. Cardiac glycosides:

(a) **Kedde's test:** The drug was extracted with chloroform and evaporated to dryness. One drop of 90% alcohol and 2 drops of 2% 3, 5-dinitrobenzoic acid in 90% alcohol were added. The solution was made alkaline with 20% NaOH solution, purple colour is produced. The colour reaction with 3,5 dinitro benzoic acid depends on the presence of a, b-unsaturated lactones in the aglycone.

(b) Keller-Killiani test (test for deoxy sugars):

The drug was extracted with chloroform and evaporated to dryness. 0.4ml of glacial acetic acid containing trace amounts of $FeCl_3$ was added. The solution was transferred to a small test tube and 0.5ml of conc.H_2SO_4 was added by the sides of the test tube. Acetic acid layer shows blue colour.

(c) **Raymond's test:** The test solution was treated with hot methanolic alkali, violet colour is produced.

(d) Baljet's test: The test solution was treated with picric acid or sodium picrate. orange colour is formed[19].

6. Anthra quinone glycosides:

a) **Borntrager's test:** The test material was boiled with 1ml of sulphuric acid in a test tube for five min. The solution was filtered while hot, the filtrate was cooled and shaken with equal volume of dichloromethane or chloroform followed by shaking it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer

b) **Modified borntrager's test:** 200mg of the test material was boiled with 2ml of dil.H_2SO_4.The solution was treated with 2ml of 5% aqueous $FeCl_3$ solution (freshly prepared) for 5min, shaken with equal volume of chloroform and continued as the test above. As some plants contain anthracene aglycone in a reduced form, if ferric chloride is used during the extraction, oxidation to anthraquinones takes place, which shows response to Borntrager's test.

c) **TEST FOR HYDROXY-ANTHRAQUINONES:** The sample was treated with KOH solution, red colour is produced[20].

7. SAPONIN GLYCOSIDES:

(a) **FROTH FORMATION TEST:** 2ml solution of drug in water is shaken in a test tube, stable froth (foam) is formed.

(b)**Haemolysis test**: 0.2ml of solution of Saponin was added(prepared in 15 normal saline) to 0.2ml blood in normal saline and mixed well. Centrifuge and note the red supernatant.

Compare with control tube containing 0.2ml of 10% blood in normal saline diluted with 0.2ml of normal saline[21].

8. TANNINS (PHENOLIC COMPOUNDS)

(a) **Goldbeater's skin test**: 2% HCl was added to a small piece of gold beater's skin, rinsed with distilled water and placed in the test solution for five minutes. Then wash was given with distilled water and transferred to 1% ferrous sulphate solution. A brown or black colour on the skin indicates of tannins.

(b) **Ferric chloride test**: The extract was treated with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

(c) **Phenazone test**: 0.5gm of sodium acid phosphatewas added to 5ml of aqueous extract. The solution was subjected to warming and filtered. To the filtrate 2% phenazone solution was added. bulky precipitate is formed which is often coloured.

(d) **Gelatin test**: To the test solution, 1% gelatin solution containing 10% sodium chloride was added. Precipitate is formed.

(e) **Test for catechin**: A matchstick was dipped in the test solution, dried and lastly moistened with concentrated **HCl**. Stick was placed near flame. The colour of the wood changes to pink due to phloroglucinol (phloroglucinol is formed when catechins are treated with acids)

(f) **Test for chlorogenic acid**: The test solution was treated with aqueous ammonia and exposed to air gradually, green colour is developed[22].

9 . STEROIDS AND TERPENOIDS:

(a) **Libermann – burchard test**: The extract was treated with few drops of acetic anhydride, boiled and cooled. Then conc.H_2SO_4 was added from the sides of the test tube, brown ring is formed at the junction two layers and upper layer turns

green which shows presence of steroids and formation of deep red colour indicates presence of tri terpenoids.

(b) **Salkowski test**: The extract was treated with few drops of conc.H_2SO_4, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of tri terpenoids.

(c) **Sulphur powder test:** Small amounts of sulphur powder was added to the test solution ,it sinks at the bottom[23].

Experimental animals:

Male Wistar rats (150-200g) were purchased from Mahaveer enterprises, hyderabad. The animals had free access to standard rodent pellet diet with water *ad libitum*. Animals were habituated to laboratory conditions prior to experimental protocol (22 ± 3°C temperature, 50-60% humidity). All the protocols and experiments were conducted in strict compliance according to guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals(CPCSEA/). The approval (Proposal No. IAEC/NCP/07/09) of the Institutional Animal Ethical Committee (IAEC) Vishnu institute of pharmaceutical education and research was taken prior to the experiments[24].

Grouping and dosing

Animals were divided into two groups of seven animals each. One group was administered with 2000mg/kg body weight and other group was administered with 5000mg/kg body weight.

Administration of doses,

Animals were fasted prior to dosing (food but not water should be withheld over-night). Following the period of fasting, the animals were weighed and the test substance was administered. The test substance was administered in a single dose by gavage using a stomach tube or a suitable intubation canula. After the substance has been administered, food was withheld for a further 3-4 hours in rats[25].

Table no.2 Administration of doses to first group

S.no	Weight of animal(g)	Weight of drug (mg/kg)	Volume of dose for each animal (ml)
1	175	0.35	0.71
2	180	0.36	0.73
3	170	0.34	0.69
4	168	0.33	0.67
5	180	0.36	0.73
6	170	0.34	0.69

n=6

Dose = 2000mg/kg body weight

Table no.3 Administration of doses to second group

S.no	Weight of animal(g)	Weight of drug (mg/kg)	Volume of dose for each animal (ml)
1	190	0.95	1.84
2	190	0.95	1.84
3	180	0.9	1.74
4	190	0.95	1.84
5	220	1.1	2.13

n=6
Dose = 5000mg/kg body weight

RESULTS

Table no.4 Phytochemical constituents of sadhaavataari gutika

CHEMICAL CONSTITUENT	OBSERVATION
ALKALOID	NEGATIVE
CARBOHYDRATES:	POSITIVE
PROTIENS	POSITIVE
FLAVANOIDS	NEGATIVE
CARDIAC GLYCOSIDES:	NEGATIVE
ANTHRA QUINONE GLYCOSIDES	NEGATIVE
SAPONIN GLYCOSIDES:	NEGATIVE
TANNINS &PHENOLS:	POSITIVE
STEROIDS	NEGATIVE

ACUTE TOXICITY STUDIES:

Administration of sadhavaataari gutika (2000mg/kg) showed no change in body weight of animals. Convulsions and tremorswere not found. Food and water intake were also found to be normal.

Administration of sadhavaataari gutika (5000mg/kg) showed no significant change in body weight of animals. Convulsions and tremors were absent . Food and water intake were also found to be normal.

DISCUSSION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Phytochemical screening is a process of tracing plant constituents. Sadhavaataari gutika was found to have carbohydrates, proteins and tannins as its phytochemical constituents.

It is clear that the strategy for toxicity testing has changed significantly over the years in order that early toxicology information can help support decisions on the best compounds to progress as potential human medicines. Lower dose (2000mg/kg body weight) has not shown changes in body weights of rats. It has not shown any convulsions or trenors. Food and water intake of animals was also found to be normal. Higher dose (5000mg/kg) has shown slight decrease in body weight of animals which was not significant. It has not shown any convulsions and tremors. Food and water intake of animals was also found to be normal.

CONCLUSION

Sadhavaataari gutika was found to have carbohydrates (aqueous extract), proteins (aqueous extract), tannins and phenols (aqueous extract) as their phytoconstituents. Lower dose of sadhavaataari gutika (2000mg/kg body weight) and higher dose (5000mg/kg body weight) did not produce any clinical signs of toxicity and death of animals.

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This is to certify that **Miss. G. SANDHYA (HT. No. 17DH1S0302)** pursuing her M.Pharmacy In **VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION & RESEARCH (VIPER)**she carried out her project work in our Organization entitled **"FORMULATION AND EVALUATION OF PALIPERIDONE SUSTAINED RELEASE TABLETS"** in the department of pharmaceutics from **19-NOV-2018 to 24-JUNE-2019.**

During her tenure she was sincere, hardworking and Punctual in her Project work.

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During her tenure she was sincere, hardworking and Punctual in her Project work.

We wish her to success in her future career.

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